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MALIGNANT MELANOMA AND OTHER MALIGNANCIES OF THE NASAL CAVITY AND THE PARANASAL SINUSES IN SWEDEN

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To my wonderful family and in loving memory of my father,
Christer Jangard

"Failure defeats losers, failure inspires winners"
Robert T. Kiyosaki

ABSTRACT

Background: Malignancies emerging in the nasal cavity and the paranasal sinuses are rare and accounts for 5% of all head and neck malignancies and 0.1% of all malignancies in Sweden. The incidence of sinonasal malignancy (SNM), except sinonasal malignant melanoma (SNMM), has been reported to decrease since 1960 in Sweden. Despite similar improvement in the prognosis of other malignancies, treatment of SNM still yields a poor survival outcome.

About 1–2% of all malignant melanomas originate from mucosal membranes in the genitourinary, digestive and the respiratory regions, whereas mucosal melanomas are most frequently located in the nasal cavity, followed by sites in paranasal sinuses in the head and neck region. The incidence of cutaneous malignant melanoma (CMM) continues to increase in many parts of the world, possibly due mainly to the effects of sun-related behaviour; however, the incidence of mucosal melanomas such as vulvar and ano-rectal melanoma display a more complicated pattern with a stable or decreasing incidence rate. We now know that the incidence of SNMM is increasing in Sweden, as we have documented one of the largest consecutively studied SNMM groups in the world. Nevertheless, the underlying mechanism remains unclear.
The treatment options for these patients have remained the same over the years; mainly radical surgery followed by radiotherapy. Alternatively, recent molecular-targeted therapy has become available for sub-groups of patients with malignant melanomas. Such therapeutic advances stress the importance of investigating the aetiology and molecular characteristics of SNMM, which are not yet well.

Aims: Given the rarity of SNM and SNMM, relevant knowledge is limited. Therefore, the overall aim of this thesis was to examine the clinical characteristics and features of SNMM and SNM and to determine the occurrence of molecular alterations. They include *KIT*, *NRAS* and *BRAF* mutation frequencies and mutation frequency of the *TERT* (Telomerase Reverse Transcriptase) promotor gene in SNMM.

Results: In the first project, we identified 3221 patients from the Swedish National Cancer Registry diagnosed with primary malignancies arising from the nasal cavity, paranasal sinuses, or both, during the period 1960 through 2011. The anatomical site, gender and age, incidence and survival were scrutinized. We found that the incidence of sinonasal malignancies decreased except for SNMM and adenoid cystic cancer during the study period. More than 50% of these malignancies involved the nasal cavity. The five-year relative survival was highest for patients with adenoid cystic cancer followed by adenocarcinoma. Those with SNMM and undifferentiated carcinoma had the poorest prognosis.

In the second project we identified 186 SNMM patients during the period 1960 through 2000 in Sweden from the National Swedish Cancer Registry (SCR). We investigated the incidence, gender, age, primary anatomical sites, geographic distribution, treatment and survival.
In this population the incidence of SNMM increased during the study period. The incidence for females was higher than for males, and the incidence increased with age for both genders. We found that about 70% of the tumours were clinically described as amelanotic. Surgery was the most common primary treatment. The five-year disease-specific survival rates were poor for both genders, but females had a better survival than males. The survival rate improved for both genders during the study period, regardless of therapeutic strategy. We conclude that the incidence of SNMM in Sweden increased significantly from 1960 through 2000 but not as rapidly as that of CMM.

In the third project, we analysed 56 primary SNMMs, the largest number, as far as we know, for mutations in *KIT* (exons 11, 13 and 17), *NRAS* (exons 1 and 2) and *BRAF* (exon 15) identified by using direct sequencing. Twelve of the 56 (21%) tumours contained mutations in these oncogenes, 2 tumours harboured *KIT* mutations, another 2 harboured *BRAF* mutation and 8 had *NRAS* mutations. We found a higher frequency of mutations in tumours originating from the paranasal sinuses compared to tumours from the nasal cavity (p=0.027).

In the fourth project we analysed 49 SNMM tumours for *TERT* promotor gene mutations, since former investigators found only a few driver mutations for these patients, who were never previously examined for this mutation. Recent studies of CMM have shown a high frequency (>70%) of driver mutations in this gene.
TERT promoter mutations occur at a moderate frequency in SNMM. We suggest that SNMM tumours should be included in molecular characterization, since these alterations probably will be therapeutic targets in the near future.

LIST OF PUBLICATIONS

I. Elliot A, **Jangard M,** Marklund L, Dickman P, Håkansson N, Hammarstedt-Nordenvall L and Stjärne P.
Sinonasal malignancies in Sweden 1960-2010; a nationwide study of the Swedish population.
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II. **Jangard M,** Hansson J, and Ragnarsson-Olding B.
Primary sinonasal malignant melanoma: a nationwide study of the Swedish population, 1960-2000.
Rhinology, 2013. **51**(1): p. 22-30.

III. Zebary A*, **Jangard***, Omholt K, Ragnarsson-Olding B, and Hansson J.
KIT, NRAS and *BRAF* mutations in sinonasal mucosal melanoma: a study of 56 cases.
Br J Cancer. 2013 Aug 6; 109:559-64.

IV. **Jangard M***, Zebary A*, Ragnarsson-Olding B, and Hansson J.
TERT promotor gene mutation in sinonasal mucosal melanoma.
Submitted for publication

**These authors contributed equally*

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LIST OF ABBREVIATIONS

ALM	Acral lentiginous melanoma
BRAF	v-RAF murine sarcoma viral oncogene homolog B1
CT	Computed tomography
CMM	Cutaneous malignant melanoma
ETS	E-twenty-six
DNA	Deoxyribonucleic acid
ICD	International classification of disease
LCM	Laser capture microdissection
LMM	Lentigo maligna melanoma
MAPK	Mitogen-activated protein kinase
MEK	MAP kinase extracellular signal regulated kinase
MLM	Mucosal lentiginous melanoma
MM	Malignant melanoma
MMM	Mucosal malignant melanoma
MRI	Magnetic resonance imaging
NCR	National cancer registry
NM	Nodular melanoma
NRAS	Neuroblastoma RAS viral (v-ras) oncogene homolog
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol 3-kinase
RAF	Rapidly accelerated fibrosarcoma
RTK	Receptor tyrosine kinase
SCR	National Swedish cancer registry
SNM	Sinonasal malignancy
SNMM	Sinonasal malignant melanoma
SNUC	Sinonasal undifferentiated carcinoma
SSM	Superficial spreading melanoma
TCF	Ternary complex factor
TERT	Telomerase reverse transcriptase
SCC	Squamos cell carcinoma
UICC	Union for International Cancer Control
UV	Ultraviolet

1 INTRODUCTION

Malignancies of the nasal cavity and the paranasal sinuses are rare, accounting for approximately 0.1% of all malignancies and 5% of all head and neck malignancies in Sweden(1). Moreover, the incidence is below one per 100 000 person years in Europe and in the USA(2, 3). These tumours usually present at an advanced stage, generally providing the patients poor prognosis(4).
Sinonasal malignancies (SNMs) are a biologically heterogeneous group. Our knowledge of tumour biology continues to evolve, but will most likely improve with the development of new treatment strategies. The majority of SNMs have an epithelial histology, with squamous cell carcinomas (SCCs) the most common type(5).

In the late 1960s and early 1970s, several reports described an association between SNM and occupational exposure to wood dust(6, 7). Further studies found an especially strong relationship between adenocarcinoma and exposure to dust from hardwood or leather(8, 9). Other important environmental factors associated with SNMs are tobacco and alcohol at least for squamous cell cancer(10, 11). Heavy air pollution was also suggested to be responsible for the increasing number of paranasal malignancies in Mexico City(12).

In Sweden, the incidence of SNM has been reported to decrease since 1960(13). In agreement, Kuijpen and colleagues and Youlden and colleagues described similar findings in two recent reports of a large series of patients with SNM(2, 14).

The first known descriptions of melanoma appeared in the manuscripts of Hippocrates in the 5th century, BC(15). In the 17th century, melanoma was described as a “fatal black tumour”, and in 1806, Professor R.Laennec used the term “melanosis”, to name this tumour entity before the Faculty of Medicine in Paris by (Laennec was also inventor of the stethoscope). Robert Carswell first termed melanoma the designated word for these pigmented malignant tumours in 1838(15, 16).

In 1869, A. Lücke was the first medical doctor to describe a patient with malignant melanoma of the nasal cavity(17). His reported depicted a melanotic sarcoma in the nasal mucous membrane of a 52-year-old man.

The term melanoma originates from the malignant transformation of a cell type called the “melanocyte”. Melanocytes are neuroectodermally-derived cells whose precursors, the melanoblasts, migrate during the embryogenesis from the neural crest to their final destinations in multiple parts of the body where they differentiate to melanocytes. Melanocytes are melanin-producing cells. Melanin is the pigment responsible for colouration of the skin and other tissues and also protects the skin from ultraviolet (UV) radiation. Melanocytes are mainly present in the skin, specifically in the basal layer of the epidermis and within hair follicles. Melanocytes are also present in the eye, the mucosal membrane of the gastrointestinal, genitourinary and the respiratory regions and in the central nervous system´s leptomeninges.

Malignant melanomas emerge from melanocytes that are present in the skin as well as in other tissues, for example the nasal mucosa(18). As is well established, the major carcinogen in the genesis of cutaneous malignant melanoma (CMM) is through ultraviolet (UV) light(19). However, for mucosal malignant melanoma (MMM) the aetiology and predisposing factors are still unknown.

The genetic background and phenotypic features of melanoma disease are heterogeneous. In recent years, the foundations of molecular classification have been established for CMM and several molecular genetic changes are now well described(20, 21). However, mucosal melanoma has not been characterized to the same extent in either molecular or genetic terms.

A now well-established characterization of CMM is that its activation occurs via differing signalling pathways. The most important are the mitogen protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. These pathways play a key role in the melanoma development by regulating cell survival and proliferation. Mutations of *BRAF*, *NRAS* and *KIT* oncogenes are the most common activation sources of these pathways in CMM(22-24). In contrast, mucosal melanomas do not have the same genetic background as cutaneous melanomas nor are they as well investigated as CMM.

Even though primary SNMMs are most commonly localised at diagnosis, surgical resection is difficult(25), and the postoperative radiotherapy, used in Sweden has not improved survival time(26-28). Therefore the development of other more effective treatment options is essential for patients with these tumours. Recently, molecular targeted therapy has become available for malignant melanoma patients carrying defined driver mutations. For example, selective BRAF inhibitors, such as vemurafenib and dabrafenib, in phase III trials have demonstrated improvement in overall and progression free survival in CMM patients with *BRAF* mutations compared with standard systemic chemotherapy(29, 30). Targeted therapy also produced favourable results for patients with KIT mutated melanomas in phase II trials and case reports(22, 31). Another phase II trial has demonstrated that treatment with MEK1/2 inhibitor may be beneficial for patients with NRAS mutated melanoma(32). These recent nonsurgical advances in the treatment of melanoma highlight the importance of investigating mutations in SNMM, which could serve as a possible target for drug therapy.

2 THE NASAL AND PARANASAL CAVITIES

The nasal and paranasal cavities extend from the mucosa in the nose all the way to the choanae (the posterior nasal aperture). The paranasal sinuses are air-containing, paired spaces in the facial bone of the skull. The maxillary sinuses surround the nasal cavity; the frontal sinuses are located above and in front of the eyes; the ethmoidal sinuses are located between the eyes and sphenoidal sinuses are located behind the nasal cavity and ethmoidal sinuses. All the paranasal sinuses are named after the locations of the facial bones in the skull. The paranasal sinuses evolve by forming small air-containing spaces that are fully developed at about the age of 14 years.

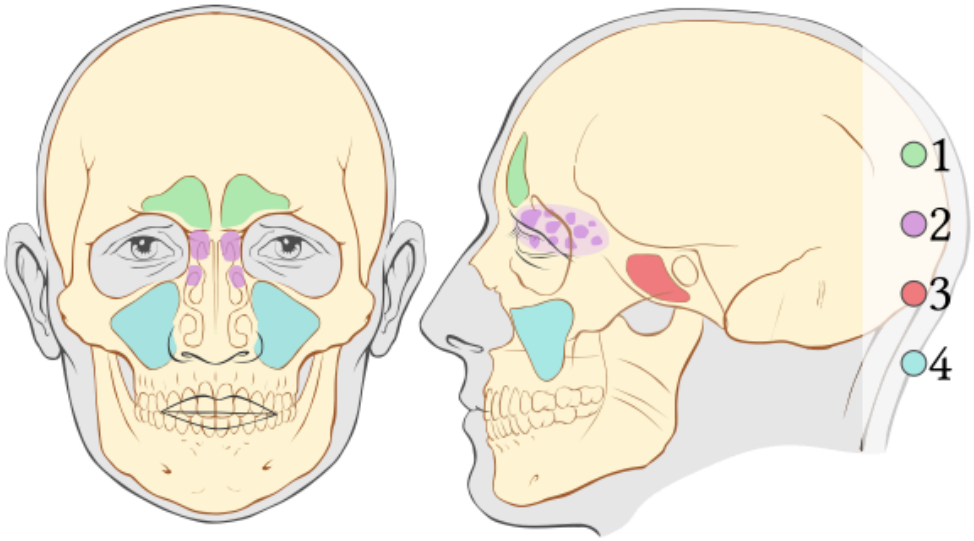


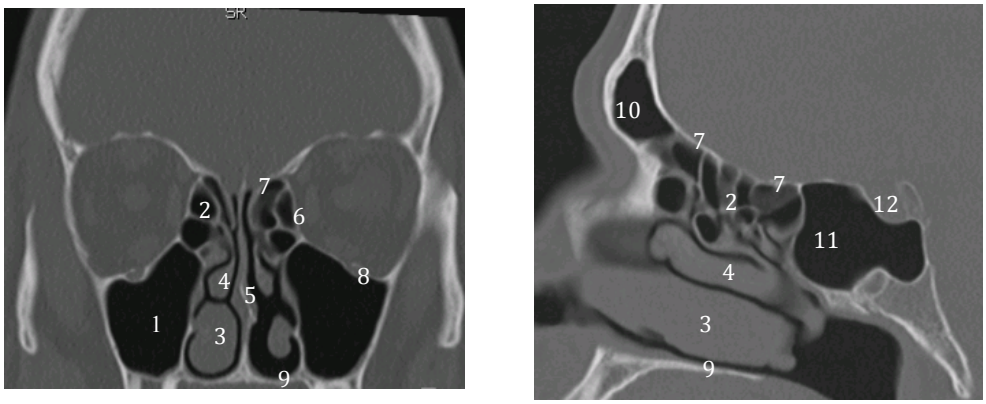
Figure 1
1. Frontal sinuses 2. Ethmoidal sinuses 3. Sphenoidal sinuses 4. Maxillary sinuses
(Source: Wikimedia commons – public domain)

The nasal and paranasal cavities are covered by 160 cm^2 mucosa that consists of respiratory and olfactory epithelium. Pigmentation of the oral mucosa is not uncommon; however benign pigmented lesions or melanosis of nasal and sinonasal cavities are extremely rare except for the olfactory mucosa, where paradoxically melanomas rarely occur(18). The majority of pigmented lesions in the sinonasal tract are melanomas(33).
The respiratory epithelium is columnar-ciliated with goblet cells and a layer of mixed glands. The olfactory mucosa (totally 200-400 mm^2), innervated by fibers of the olfactory nerve, covers the area of the olfactory cleft, the cribriform plate, and part of the superior turbinate.

The maxillary sinuses are the largest of all human sinuses, with an average size of 15 cm^3 each in adults. The superior wall of the maxillary sinus forms the floor of the orbit and the infraorbital nerve (one part of the trigeminal nerve (5^{th} cranial nerve): sensory nerve innervating the upper lip, cheek, nose and lower eyelid) runs within it. This nerve is often affected in facial trauma or tumour growth of the maxillary sinus.
The frontal sinus, which varies in size from 4-7 cm^3, forms the bottom part of the anterior orbital roof, and the posterior wall of the frontal sinus forms part of the anterior cranial fossa. When a tumour develops in the frontal sinus, orbital or intracranial growth must be considered.

The ethmoidal sinuses consist of six to ten air-containing cells of a total volume of 2-3 cm^3 on each side. In contrast to all the other sinuses the ethmoidal sinuses are fully formed at birth. The superior part of the ethmoid forms the anterior skull base and, laterally, the lamina papyracea; therefore, tumours evolving in the ethmoids have a pathway of spread intracranially and to the orbit.

The sphenoid sinuses are the most posterior of the sinuses. They vary individually in shape and size, from about 0.5 to 3 cm^3 on each side. The sphenoid sinuses start to develop after 6 years of age. Because, many vital structures such as the optic nerve, internal carotid artery and pituitary gland surround the sphenoid sinus, a tumour arising within may affect all these sites.



Coronal Sagittal

Figure 2. Coronal and sagittal view of computed tomography of the sinuses.

1=maxillary sinus, 2=ethmoidal sinus, 3=inferior turbinate, 4=medial turbinate, 5=nasal septum, 6=lamina papyracea, 7=skull base, 8=infraorbital nerve (N.V), 9=palate, 10=frontal sinus, 11=sphenoidal sinus, 12=pituitary gland
(Jangard 2014 with permission from patient)

The lymphatic drainage architecture consists of an anterior system, which collects the lymph from the nasal pyramid and drains to the submandibular superficial cervical lymph nodes, and a posterior system that drains the nasopharynx and the posterior nasal cavity to the retropharyngeal lymph nodes and the jugular nodes.

3 EPIDEMIOLOGY

Sinonasal malignancies consist of tumours evolving in the nasal cavity and paranasal sinuses, which include the maxillary, frontal, ethmoid and sphenoid sinuses. The epithelial tumours are the most frequent, and the majority of these tumours are SCCs followed by adenocarcinomas(5). Other tumour types of the sinonasal cavities are melanoma, adenoid cystic carcinoma, sinonasal undifferentiated carcinoma (SNUC), esthesioneuroblastoma, sarcoma, lymphoma as well as metastases from other organs. Malignancies of the nasal cavity and the paranasal sinuses are uncommon and account only for approximately 5% of all head and neck malignancies and 0.1% of all malignancies in Sweden(1).

The mean age at diagnosis varies among the malignant entities, although most SNMs are diagnosed in the sixth decade of life or later(2, 3, 14, 34). Patients with SNMMs have the highest average age at diagnosis of >71 years; in contrast, adenoid cystic carcinomas are diagnosed at the lowest mean age at diagnosis: <62 years in Sweden. The overall incidence of sinonasal malignancies is between 5 and 9 per million for males and 2 and 5 per million for females(2). Other sites of head and neck cancer, such as laryngeal cancer, oropharyngeal cancer, salivary gland cancer and oral cancer, are to the contrary, much more common, i.e., the incidence varies from 10 to 50 per million(35). Interestingly, in a recent study of several Western countries the highest incidence rates of SNM were in Denmark, where the incidence rates were 7 per million for females and 12 per million for males(2). Many studies have shown that SNM is more common among males than females except for sinonasal melanoma and adenoid cystic cancer. The incidence of SNM is decreasing overall for men, yet the incidence for women has remained stable and even increased in some studies(2, 3, 13, 14). Even though the incidence of sinonasal cancer has remained constant or decreased somewhat in whites, considerable decreases were noted in other ethnic groups, particularly blacks. These differences likely reflect changing demographics and socioeconomic development(3). Another noteworthy shift is to more tumours originating in the nasal cavity compared to the paranasal sinuses, as reported in our study in Sweden (Paper I) as well as other studies(2). This shift in location has been described to give better prognosis for patients with SNM(34).

As SNM does not have specific symptoms at initial presentation, the diagnosis is often mistaken and designated as for a benign disease such as nasal obstruction, nasal bleeding, tooth- and/or facial-pain. Other symptoms include cranial nerve involvement, tumour growth into the oral cavity, exophtalmus and loss of vision, facial swelling and lymph node metastasis to the neck and symptoms from distant metastasis.

Generally, patients with these tumours have a poor prognosis, as the diagnosis has often been delayed until a locally advanced stage was reached. Despite the different treatment strategies attempted, the 5-year survival rate is poor for patients with SNMs, especially for undifferentiated tumours and SNMMs. Owing to the scarcity of SNMs, treatment in Sweden as well as internationally has been based on local traditions. Therefore, the effect of such varied treatment modalities on the survival rate is still uncertain(3, 13, 36).

The incidence of CMM continues to rise worldwide, especially among Caucasian populations in the Western countries, and the annual increase of incidence is 3-7% (in Sweden over 5%)(37-40). In Sweden, CMM is the sixth most common tumour type and its incidence is increasing faster than that of any other malignancy(1). About 3300 new cases are diagnosed each year. The increased incidence of CMM might stem from such diverse factors such as changes in sun exposure, public awareness, skin screening programs and increased ability to diagnose thin melanomas(37).

New CMM cases and mortality per 100 000 per year in Sweden

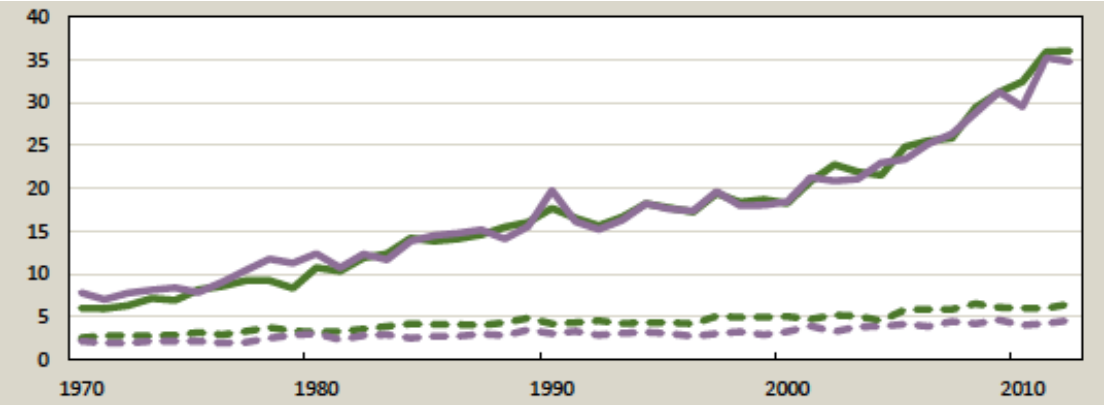


Figure 3. Age standardized (2000) incidence in Sweden. Green=incidence men; Purple=incidence women; Dotted green=mortality men; Dotted purple=mortality women. (Cancer Incidence Sweden, 2012)

In Europe, the highest incidence of melanoma are in Sweden, Norway, Denmark, Ireland, The Netherlands, Switzerland and Slovenia, see figure 4.

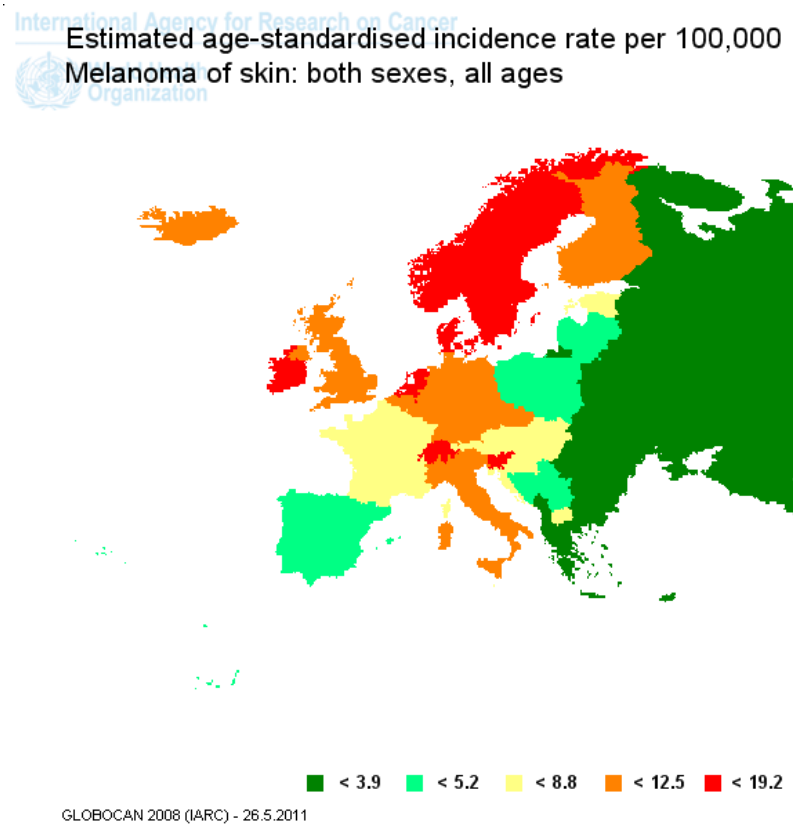


Figure 4. Age-standardized incidence of CMM in Europe. (Globocan 2008 (IARC))

In Europe, the highest incidence rates of melanoma are reported in the Northern countries; Denmark, Norway and Sweden has the highest incidence compared to Iceland and Finland, which have much lower incidence(38). The highest incidence rates of all occur in New Zealand and in Australia because of the greater exposure to UV radiation and the fact that much of the population has ancestors from Europe(41). Accordingly, light-skinned humans have a much higher incidence of melanoma than those with dark skin or of Asian origin(37, 42, 43). In the USA the lifetime risk of developing melanoma in Caucasians is 1 in 50, compared to the much lower incidence in African-Americans, 1 in 1000(44).

The average age at diagnosis of CMM in Sweden is 60 years for women and 64 years for men, whereas SNMM is diagnosed at a much later age. The 5-year survival rate for CMM in Sweden is 92% for women and 86% for men(45). However, for mucosal melanoma, incidence studies are rare and, for the most part, too few patients are included to draw any firm conclusions on the incidence rate.

The mucosal melanoma incidence for ano-rectal and vulvar melanomas, showed an inconsistent pattern with generally decreasing (46) or stable (47, 48) rates. In only one study did numbers for MMMs of the ano-rectal region tend to increase, especially for men(49). According to other recent studies, MMMs do not generally seem to be

increasing. In Sweden, MMMs account for about 2% of all melanomas(50) and the most common primary site, among MMMs registered in the National Cancer Registry from 1960 to 2009, was the vulva (35.6%), followed by the ano-rectal region (25.8%), and sinonasal cavities (SNMM) (19.7%).

Table 1

Cutaneous and Mucosal Melanoma in Sweden 1960-2009 (The Swedish National Cancer Registry)

Localization	Total	%	Men	Women
Cutaneous	58706		28722	29984
Mucosal	1240		317	923
Vulva	441	35,6		441
Ano-rectal	320	25,8	125	195
Sinonasal	244	19,7	104	140
Vagina	97	7,8		97
Oral	76	6,1	42	34
Penis	41	3,3	41	
Urethra	21	1,8	5	16

Uveal melanoma (3717) excluded

Of all the malignancies of the nasal and paranasal region, 1-12% consist of SNMM (13, 36, 51). Differing incidences have been reported for MMM among distinct ethnic groups, as well as for CMM, see above. For example, oral mucosal melanomas account for 7.5% of all melanomas among the Japanese population versus less than 1% for Caucasians(52, 53).

The prognosis is generally poor for patients with MMM considering that the 5-year survival rate has been reported as 25%(42). However, the numbers differ among sub-sites and the 5-year survival rate in Sweden for primary ano-rectal melanomas is only 18% and 35% for vulvar melanoma(46, 48). For SNMM, the 5-year survival rate varies from 20-28%(26, 54, 55), but has improved during the period from 1960-2000 in Sweden(55).

4 MELANOMA RISK FACTORS

Several studies have described an association between sinonasal malignancies and occupational exposure to wood dust(6, 7). Exposure to leather dust and wood dust is also known to be strongly related to the development of adenocarcinoma in the paranasal sinuses(8, 9). Other aetiologic possibilities including inhaled carcinogens (e.g. from smoking, formaldehyde, paints and lacquer) on the development of SNM could not be excluded. Additionally, high levels of air pollution have been described as a causative factor for SNM in general(9, 12). Tobacco and alcohol, at least for SCC, are also associated with SNMs(10, 11). Overall, the decreasing incidence of most histological types of SNM may be attributed to better working conditions and reduced air pollution over the years.

For SNMM the risk factors are mainly unknown, although those linked with the development of CMM are well established. UV radiation from either the sun or from indoor tanning devices is believed to initiate the tumour formation of CMM by damaging DNA and creating mutations in tumour suppressor genes and proto-oncogenes(56-58). The pattern of repeated sun light exposure is believed to play a crucial role in melanoma genesis, since intense and even intermittent exposure to UV radiation produces a high risk of melanoma development in humans(59). Moreover, individuals with a family or personal history of melanoma are at a greater risk of developing CMM(60). However, the heredity factors for SNMM are not known and have not yet been investigated.

We know that the nasal septum and the turbinates are the most frequently affected subsites where SNMM arises(55). That observation suggests that inhaled carcinogens could be responsible, since most inhaled air passes through the nasal cavity, during normal breathing. Medicine and carcinogens can be filtered by and conjugated to melanins, and also metabolized by melanocytes, which may induce carcinogenesis(61, 62). Occupational exposure for formaldehyde may also be a potential cause of SNMM, as is reported in a publication by Holmström et al(63).

5 DIAGNOSIS

The symptoms of the SNMM and SNM patients at their first visit to the clinician are heterogeneous and often nonspecific but usually referable to the tumour site. Moreover, the expansion of a SNM in the space of a large air-containing paranasal sinus allows tumour growth that is virtually asymptomatic. This explains why SNM often remains undiagnosed until an advanced stage of disease, when the patients still have only minor symptoms. In fact, some of the initial symptoms are difficult to distinguish from such common nasal complaints as congestion and epistaxis. Therefore, with symptoms that may be imprecise, these patients delay seeking medical care. Patients who actually had SNMM, but presented with epistaxis had a significantly better prognosis than those with only nasal congestion(55), probably because patients found epistaxis more alarming than nasal congestion and therefore looked for medical examination sooner.

These tumours can also present as a unilateral polyposis. Such lesions are easily mistaken for a benign disease, which clinicians should keep in mind to avoid a harmful "doctor´s delay". The most common symptom at diagnosis of SNM is facial swelling followed by nasal blockage and epistaxis(64) however for SNMM the most common symptom is epistaxis followed by nasal obstruction(55).

At diagnosis, patients with SNM rarely have cervical lymph node metastasis or distant metastasis; however the majority of the SNMs present at a clinical locally advanced stage(64, 65). For SNMM, the tumours tended to become evident at a more advanced stage over the years 1960 through 2000 in Sweden(55). Possibly, today's increasingly sophisticated techniques, including CT scanning, MRI, and PET/CT scanning, could account for the heightened number of more advanced tumours recently recorded.

The histopathology for SNM, especially SNMM, is challenging. As many as 20% or more SNMMs are initially misdiagnosed (e.g. as poorly differentiated squamous cell carcinoma, olfactory neuroblastoma, lymphoma, or sarcoma)(55). The diagnosis of melanoma is complicated. Not only do the several histological types of melanoma vary considerably, another difficult issue is that the melanin content ranges from large to small amounts of pigment. Lesions without pigment (amelanotic) in the sinonasal cavities may be diagnosed as other sinonasal malignacies(66-68). Fortunately, the histological diagnostics have improved over the years, thereby reducing the number of misdiagnosed patients. In analogy with the methods used for CMMs, the application of immunohistochemical stains, such as S-100, HMB-45 and Melan-A, can improve the diagnosis of these tumours(69). Histologically, most SNMM have an epithelioid or spindle cell like (sarcomatoid) growth pattern(66, 68), but considerable controversy remains in that individual studies report different results.

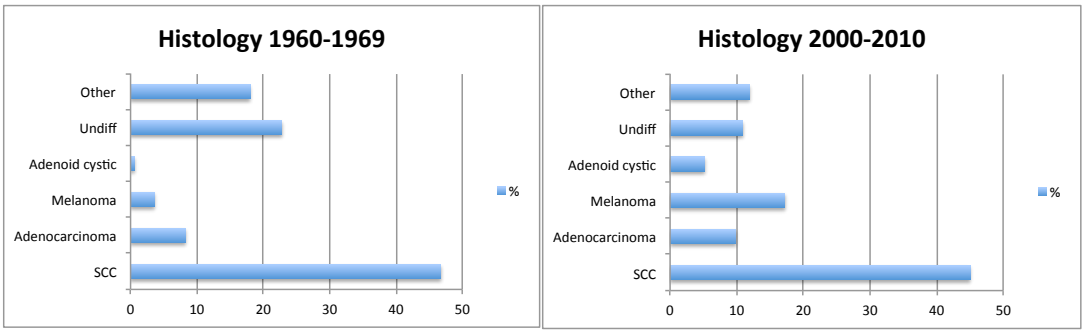
The most common site of SNM is the nasal cavity, especially for SCC and SNMM(1). An increasing proportion of tumours originating from the nasal cavity compared to the maxillary sinuses for all SNM over the study period was also found in a recent article by Youlden and colleagues(2). The reason for this is unknown. Perhaps the patients' and doctors' delayed recognition of tumour types in the 1960s resulted in the former

misclassifications compared to greater accuracy of the 2010s, when better diagnostic tools and earlier diagnoses provided more reliable information as to the origins and precise locations of theses tumours.

6 SINONASAL MALIGNANCY CLASSIFICATION

Epithelial tumours are the most common histology of SNM. Of the epithelial tumour types, the SCC predominates followed by adenocarcinomas. Melanoma is the most common non-epithelial pathology in Sweden. The most common types of SNM in Sweden from 2000-2010 were SCCs, followed by melanoma, adenocarcinoma, adenoid cystic cancer and undifferentiated cancer. Interestingly, there has been a significant shift in distribution of different histologic types from 1960 to 2010, see Table 2.

Table 2
Sinonasal malignancies in Sweden



The differences in distribution over time of the various histological types might reflect an actual increase and decrease of different histological types but also improved diagnostic methods.
SCC, the most common type of SNM, is more common in men than women (63 % men in Sweden)(1) and appears to be related to alcohol and tobacco use as well as inhaled carcinogens(10, 11). However alcohol and tobacco use as a risk factor is controversial, since other studies failed to show their association to SNM(70). A strong association between SCC and nickel exposure was also reported for workers at a nickel refinery in Norway(71). In Sweden, the most common localization for SNM is the nasal cavity, but many other reports refer to the maxillary sinus as the most common site of origin(65, 72). The majority of the patients with sinonasal SCC presents with a locally advanced disease (T3-T4)(65, 73). At presentation about 2-5% have nodal involvement and nodal metastasis at diagnosis is associated with poor prognosis(65, 72).
Adenocarcinoma has a strong relationship with occupational exposure to leather and wood dust, which probably explains the male predominance (76% in Sweden) and also the decreasing incidence as the working conditions improve over time. Lymph node engagement at diagnosis is lower than for SCC(65, 72).
Adenoid cystic carcinoma, which has a slight female predominance (53%), most commonly presents in the maxillary sinus(1, 74, 75). Diagnosis is often delayed as the tumours progress slowly and have a long clinical course during which the symptoms often mimic those of chronic rhinosinusitis and tend to include perineural extension(75, 76). Regional and distant metastases at diagnosis are rare(75, 77). The long-term survival is poor as the tumour spread (perineural spread) is often undetected and misjudged on radiologic scans(74).

7 CLASSIFICATION OF MELANOMA

7.1 CUTANEOUS MALIGNANT MELANOMA

For more than 40 years, CMM has been classified into multiple subtypes based on the tumour's anatomic location and growth pattern of the melanoma cells. The four main subtypes are superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM)(78). Over the years, other subtypes have been added such as spitzoid melanomas, nevoid melanomas, desmoplastic melanomas and others that are rare. The non-cutaneous melanoma subtypes consists of ocular and mucosal melanomas (Table 1).

7.1.1 Superficial spreading melanoma

SSM is the most common subtype accounting for about 81-84% of all melanomas in patients younger than 50 years and 60-63% in patients over 50 years. SSM is, in fact, the subtype that increases most of all melanomas(45). The growth pattern of SSMs is characterized by lateral spreading of melanoma cells within the epidermis, known as a pagetoid pattern. This subtype has the strongest correlation to areas with intermittent sun-exposure, such as the trunk in males and the extremities (mainly lower limbs) in females, and is also associated with the presence of pre-existing nevi(79). Notably, SSM is characterized by a high frequency of *BRAF* mutations(80).

7.1.2 Nodular melanoma

NM is the second (about 10-15%) most common subtype of CMM, is the most aggressive and is diagnosed mainly in patients older than those with SSM. Instead of arising from pre-existing nevi as for SSM, NM may appear in a spot where no lesion previously existed. The growth pattern is characterized by an invasion of the dermis, vertical growth phase, and lacking the radial growth phase. The *BRAF* mutation frequency is significantly lower in NM than for SSM; however, the *NRAS* mutation is more frequent in NM(80, 81).

7.1.3 Lentigo maligna melanoma

About 4-15% of all CMM is represented by LMM and is diagnosed most commonly on chronically sun-damaged skin. Most of this skin tumour class originates in the head and neck region. Patients younger than 40 years are rarely diagnosed with LMM(82-84). Histologically, LMM cells proliferate along the basal layers of the skin(85).

7.1.4 Acral lentiginous melanoma

ALM is the least common subtype of CMM accounting for 1-10% of all melanomas(42, 86). It occurs predominantly in distal sun-protected parts of the body, such as the palms, soles and nail beds. ALM is most frequently diagnosed in elderly people and their prognosis is poor compared to persons with other subtypes, partly

depending on delayed diagnosis(87). Histologically, there are no exact criteria that are necessary for the diagnosis of ALM, causing considerable difficulty, when attempting to determine the diagnosis(86).
ALM patients sometimes harbours *KIT* mutations, but *BRAF* and *NRAS* mutations are relatively rarely found among these patients(88).

7.2 NON-CUTANEOUS MELANOMA

Relative to melanoma of the skin, non-cutaneous melanomas are rare and represents about 8% of all melanomas in Sweden(1). Ocular (most common) and mucosal melanomas represent the majority of the non-cutaneous melanomas. The frequency of of non-cutaneous melanomas is higher among Caucasians compared to blacks, which is consistent with CMM(89). Most of the patients are elderly, and the prognosis is dismal compared to those with CMM. Owing to the tumour's rarity and the limited amount of relevant studies, information about the pathogenesis, risk factors and predisposing factors for non-cutaneous melanomas is still to a large extent lacking.

7.2.1 Ocular melanoma

Ocular melanoma is the most common type of non-cutaneous melanoma in Sweden, accounting for about 100 new cases every year(1). Most of these melanomas arise in the uveal tract, e.g. the choroid, ciliary body and iris. More than 80 % of ocular melanomas harbour activating somatic mutations in *GNAQ* or the *GNA11* oncogenes, which can lead to activation of the MAPK pathway(90, 91).

7.2.2 Mucosal melanoma

Less than 2% of all types of melanomas consist of primary malignant mucosal melanoma (MMM)(1, 42, 89). The most common primary site of MMMs in Sweden from 1960 to 2009 is the vulva, followed by the ano-rectal region, and sinonasal cavities (SNMM), see Table 1. Other primary sites included the vagina, oral cavity, penis and urethra, in that order. SNMM is the most common type of mucosal melanoma in the head and neck region(1, 92). The diagnosis of MMM usually occurs at an advanced stage and affects primarily the elderly. Their prognosis is poor with a 5-year survival of about 25%(69). UV-radiation and genetic risk factors such as family history of melanomas and high frequencies of melancytic nevus do not seem to be associated with mucosal melanoma. Histologically, MMM is classified as SSM, NM, and mucosal lentiginous melanoma (MLM). ALM and MLM are histologically very similar and sometimes identical, however ALM is not present in the in MMM(93, 94). In vulva melanoma, MLM is the predominating melanoma subtype(95). However, the histological classification of SNMM has not yet been investigated to my knowledge. The genetic background differs from CMM, whereas MMM is characterized by a higher frequency of *KIT* mutations and/or amplifications and a lower frequency of *BRAF* mutations(88, 96). A recent study of MMM revealed that *KIT* mutations in vulvar melanomas had a significantly higher frequency compared with tumours at other sites (35% vs 10%). This outcome implies that the *KIT* mutation rate in MMMs differs depending on anatomical sites(23).

8 STAGING SYSTEM FOR SNMM

Ballantyne established a staging system for CMM and MMM of the head and neck in 1970(97), which is still widely used. This system consists of stage I for local disease, stage II for regional disease, and stage III for distant disease. The prognostic assessment of this system has been a controversial, because the majority of the MMMs seem to fall into the Stage I category(98, 99). Moreover, Breslow thickness is often difficult to distinguish, because the SNMM tumours are commonly resected in pieces, and the Clark level is not applicable, because the mucosa and skin differ anatomically(100). As a result of this problem, Prasad and colleagues has proposed a micro-staging system according to an invasion into three tissue compartments(100), and Thompson and colleagues have described another staging system being histology- and site-specific(68). However, these improvements have unfortunately not resulted in using the new staging systems in the medical literature. The International Union Against Cancer (UICC 7th edition) has now established the TNM staging for melanoma in the upper aerodigestive tract and this system maybe more effective in classifying these highly malignant tumours(101). According to the 7th UICC, T1 and T2 as well as stage I and II are omitted when mucosal melanomas are considered to be aggressive tumours. Stage III (T3) is designated when a tumour is limited to the mucosa; stage IVa (T4a) applies when the tumour invades the deep soft tissue, cartilage, bone or overlying skin without lymph node involvement (N0) or T3 with lymph node involvement (N+), and stage IVb (T4b) depicts a tumour that invades the brain, skull base, cranial nerves or carotid artery with or without lymph node involvement. Stage IVc (any T, any N) is for melanomas with distant metastasis (M+). N indicates regional lymph nodes, and M indicates distant metastasis.

To form a comprehensive staging system, the prognostic factors involved in SNMM must be more thoroughly investigated and understood. However, for all the other types of SNM the 7th UICC system is used to classify tumours worldwide and does not, to the same extent, entail the same classification issues as those for SNMM.

9 TREATMENT

Although most of the SNMs are localised at diagnosis, the close distance of the brain and cranial nerves as well as the eye contributes to the difficulty of radical surgery and radiotherapy. Furthermore, these treatments, themselves, are associated with serious complications (25, 102).

Because of the rarity and the various biological behaviours of SNM, there is no consensus regarding treatment for these patients. Surgery alone, pre- or post-operative radiotherapy or radiotherapy alone are the foundation for treating these malignancies(65, 103, 104). Depending on the stage and histologic state of disease, some patients might benefit from adjuvant chemotherapy, e.g. individuals with sinonasal undifferentiated carcinoma (SNUC) and sarcoma.

Nearly 200 years ago the first resections of the maxilla were described(65, 105); however, since then, the surgical treatment for SNM has remained the same. Not until the endoscopic techniques were introduced for the surgical treatment of malignant tumours have new option arisen. Now we know that in selected patients, endoscopic surgery of SNM has an identical outcome as open surgery with, at the same time, a significantly lower rate of complications(26, 106).

Over the years, more sophisticated imaging techniques (e.g., magnetic resonance imaging (MRI), positron emission tomography (PET) and image guide surgery) have improved both pre- and post-operative illustrations of the tumours, thereby ameliorating the rate of tumour resection and improving the precision of radiotherapy. Presently, the main cause of treatment failure is local recurrence rather than distant disease for both SNM and SNMM(2, 55, 107).

Primary surgery followed by postoperative radiotherapy is the main standard for treatment of patients with SNMM in Sweden and in many other countries. Some studies indicate that postoperative radiotherapy increases the rate of locoregional control; unfortunately, though, the survival time has not been reported to improve(26-28). However, other studies emphasize the advantages of adjuvant radiotherapy for SNMM(108, 109). In contrast, some reports state that patients treated with adjuvant radiotherapy have a worse prognosis than those without radiotherapy. This is an obvious selection bias, of course, as non-radical or inoperable tumours are more likely to be treated with radiotherapy(99).

The well-known activating mutations in *BRAF*-, and *KIT*-genes in CMM now enable targeted therapy. Until now, this treatment has been an option mainly for CMM. Recent studies have shown that selective BRAF inhibitors (vemurafenib and dabrafenob) improve both progression-free and overall survival compared to the standard systemic chemotherapy(29, 30). For patients with *KIT* mutated melanomas, case reports and phase II trials have indicated promising effects of targeted therapy with imatinib and dasatinib(22, 31). Another phase II trial demonstrated that patients with *NRAS* mutated melanomas might benefit from treatment with MEK1/2 inhibitor(32). These new

therapeutic advances stress the importance of identifying mutations of these oncogenes in patients with SNMM.

Another major advance in recent years in the treatment of metastatic melanoma (including metastatic mucosal melanoma) is to target the immune system instead of the tumour. Ipilimumab, a monoclonal antibody, regulates the immune system by inhibiting cytotoxic T-lymphocyte-associated-antigen-4 (CTLA-4). This antibody is a negative regulator of T-cell activation that stimulates T-cell proliferation and infiltration thereby inducing tumour cell death. The result is improved overall survival time as well as long-term survival for patients with advanced metastatic melanoma(110-112).

10 PROGNOSIS

The prognosis for patients with SNM differs depending on the particular histological type each individual manifests. As SNM is a very rare disease, drawing indisputable conclusions about the prognostic factors and different treatment outcomes is difficult. As far as I know, there has been no randomized clinical trial of various treatments for SNM patients, and the possibility of accomplishing such a study is most unlikely.

Women diagnosed with SNM tend to have a better prognosis than men in Sweden. But for individuals with this tumour, regardless of its histological type, the 5-year survival time has improved over time from 1960–2010. Those with adenoid cystic cancer have the best chance of 5-year survival followed by patients with adenocarcinoma and SCC, whereas sufferers of SNUC and SNMM have the poorest prognosis. Patients with tumours arising from the nasal cavity have a significantly better prognosis than those with tumours originating from the paranasal sinuses(3, 36, 51).

The prognosis is generally poor when the tumour is SNMM, i.e., a 5-year survival rate of 20-28% (26, 54, 55), but has improved during 1960-2000 in Sweden(55). Women have a significantly better survival statistics than men. Moreover, patients with ano-rectal melanomas have the same gender-associated survival difference in Sweden(48), as also found for CMM in Sweden (113) and in other countries(42). Additionally, multifactorial analyses have shown that gender is an independent prognostic factor for CMM(114).

Tumours originating from the nasal cavity provide a better prognosis compared to tumours originating in the maxillary sinuses, in the ethmoid sinuses or overlapping sinonasal regions(28). The lesions in the nasal cavity cause earlier symptoms and are easier to visualize and detect, which offers an opportunity for earlier diagnosis and, therefore, improves treatment outcome. The well provided lymphatic and vascular systems of the submucosa ameliorate development of mucosal melanoma metastasis. Most commonly, MMMs relapses in their local environment, and unfortunately, this recurrence often serves as a marker of distant disease(99).

11 SIGNALLING PATHWAYS IN MELANOMA

Numerous regulatory pathways have been implicated in the melanoma development. Recent whole-genome analysis of melanomas has identified a higher mutation load in melanoma tumours compared with most other types of malignancies(115). Only a small number of these mutations, though, are thought to be involved in melanoma development.

The most important pathways in the initiation and progression of cutaneous melanomas are those known as RAS-RAF-MEK-ERK, or MAPK pathway, and the phosphatidylinositol 3-kinase (PI3K)-Akt-pathway. These pathways are activated in melanomas commonly through mutations in the *BRAF*, *NRAS* and *KIT* oncogenes(23, 24). Mucosal melanomas seem to have a different genetic background that differentiates them from cutaneous melanomas. The frequency of *BRAF* mutations are higher with respect to melanomas evolving in the trunk and skin without chronic sun damage compared to mucosal melanomas(80, 116, 117), whereas *NRAS* mutations are more frequent in melanomas arising in extremities and skin with chronic sun damage(80, 117). Former studies have shown that some mucosal melanomas harbour mutations and/or amplifications of the *KIT* gene, but very rarely contain *BRAF* mutations(88, 96).

11.1 MITOGEN ACTIVATED PROTEIN KINASE PATHWAY

MAPK pathway is a signalling pathway that becomes activated through extracellular growth factors, cytokines and hormones. This pathway regulates many different cellular functions including proliferation, survival, differentiation, growth and motility(118, 119). In many tumour cells, the MAPK pathway is hyper-activated as a result of different mutations involving the components of this pathway. The MAPK pathway consists of a multiple components as represented in figure 5.

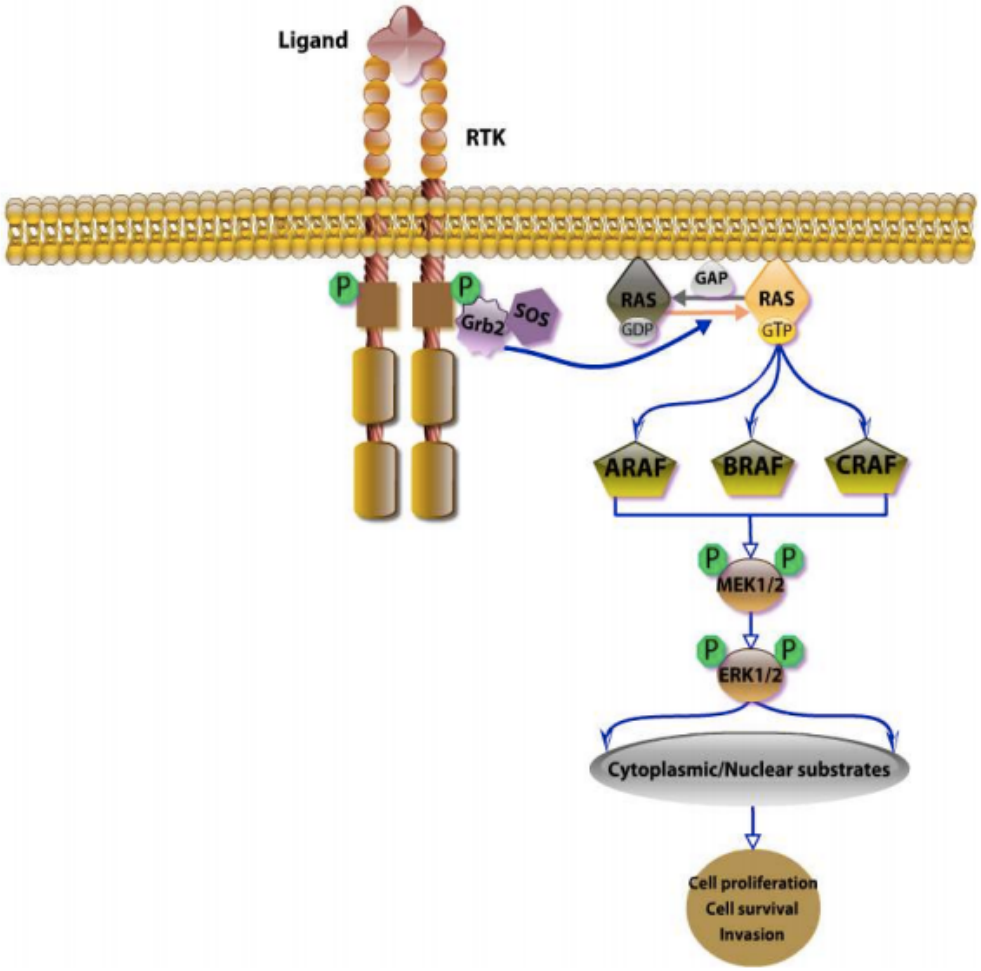


Figure 5. The MAPK pathway. three RAS (HRAS, KRAS and NRAS), three RAF (ARAF, BRAF, CRAF), two MEK (MEK1 and MEK2) and two ERK (ERK1 and ERK 2) proteins. (Zebary, 2013)

11.1.1 RAS

The RAS (rat sarcoma) protein is a GTPase located in the inner surface of the cell membrane. It transduces growth signals from the extracellular surface to downstream effectors. There are three isoforms of the *RAS* gene, *HRAS, NRAS* and *KRAS* that are commonly mutated in human cancers. Of these isoforms the *NRAS* gene is most frequently mutated in melanoma development (17% - 29% in primary CMM)(120-122) resulting in constitutionally activated RAS protein independent of the receptor tyrosine kinase (RTK e.g., KIT, PDGFR, EGFR) stimulation. This eventually stimulates cell proliferation and inhibits apoptosis by activating both MAPK and PI3K pathways.
In CMM, *NRAS* mutations are associated with older age at diagnosis, nodular type, high Breslow thickness as well as chronic sun-exposed areas and the extremities(80, 117, 122).

11.1.2 RAF

The three *RAF* (rapidly accelerated fibrosarcoma) genes are located on separate chromosomes and encodes for three serine/threonine kinases (ARAF, BRAF and CRAF). The *BRAF* mutations are relatively common in CMM (41% - 59%)(80, 122, 123) and are most frequently found in younger patients, in SSM-type, thin melanomas, intermittently sun-exposed areas and the trunk(80, 117, 122).

11.2 PHOSPHATIDYLINOSITOL 3-KINASE-AKT PATHWAY

The PI3K-Akt pathway is a well-known regulator of cell survival in melanoma and other cancers(124). See the pathway, including KIT receptor structure, presented in figure 6.
PI3Ks (three different classes) are lipid kinases that trigger the activation of Akt through phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol 3,4,5-bisphosphate (PIP_3), which in turn enables phosphorylation and activation of Akt. The PI3K-Akt pathway is activated in human cancers usually through mutation of receptor tyrosine kinases (RTK) for example KIT, upstream effectors (e.g. NRAS), inactivation of inhibitors (e.g. PTEN) or alteration in *Akt* and *PIK3CA* genes (coding for the catalytic subunit of PI3K)(125).

11.2.1 KIT

KIT is a protein encoded by the *KIT* gene, also known as *c-KIT* or *CD117*. The KIT receptor protein is a transmembrane RTK that is expressed on the surface of melanocytes and several other cell types. It consists of three domains: extracellular, transmembrane and intracellular (Figure 6). When KIT is activated extracellularly by stem cell factor (SCF, also known as c-kit ligand or steel factor), the intracellular domain phosphorylates and activates signal transduction of many downstream effectors such as MAPK and PI3K-Akt pathways(119).

The KIT receptor signalling is crucial for melanocyte development, proliferation and survival and is also important in melanoma pathogenesis(31). The *KIT* mutations is relatively common in CMM on chronically sun-damaged skin, in acral melanoma and in mucosal melanoma, whereas it is very rare in melanomas on skin without chronic sun-damage or in conjunctival melanoma(88, 96). In a recent investigation by Omholt and colleagues of various mucosal melanomas, it was reported a higher frequency of *KIT* mutations in vulvar melanomas in relation to other mucosal melanomas located elsewhere. They concluded that the frequency of *KIT* mutations in MMM varies with anatomical site(23). Although a recent Chinese report showed that *KIT* mutations adversely affected survival(126), we did not find any correlation between *KIT* mutation in SNMM patients and survival(127).

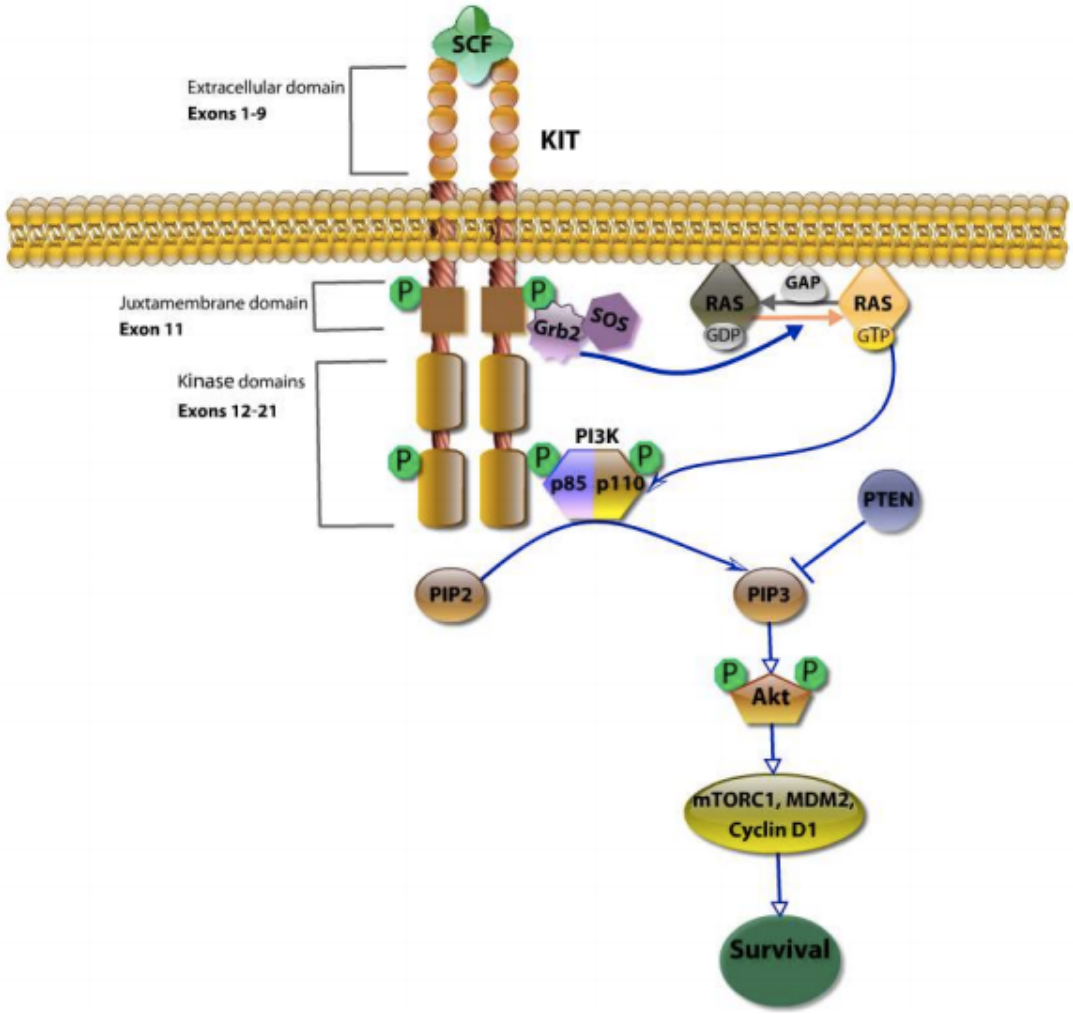


Figure 6. The KIT receptor structure and the PI3K-Akt pathway (Zebary 2013).

12 TELOMERASE REVERSE TRANSCRIPTASE PROMOTER

In 1985, Elizabeth Blackburn and Carol Greider were the first to discover telomeres. Telomeres are structures localized at the ends of the human chromosomes and are required for chromosomal protection(128, 129). With each cell division in normal cells, the telomeres are shortened. When they reach a critical length, DNA-damage responses are activated, which leads to senescence and apoptosis. However, cancer cells with chromosomal aberrations activate telomerase and prevent the shortening of telomeres despite the DNA-damage signal, which leads to uncontrolled growth and proliferation (Figure 7)(130, 131).

The telomerase reverse transcriptase gene (TERT) is located on the short arm of human chromosome 5. The core promoter spans 330 bp upstream of the translational start site(132, 133). Telomerase activity is important in the development of senescence and in obtaining unlimited growth potential in cancer cells. The activation of telomerase through *TERT* promoter mutations is thought to be an early event in developing cancer(133-135). Other potentially carcinogenic biological effects of telomerase protein include inhibition of apoptosis, enhanced cell proliferation and DNA damage response regulation(136), see figure 7. As is well established, telomerase plays an important role in tumourigenesis; on the other hand, the dysregulation in cancer cells is still not entirely understood, especially not for melanoma. The *TERT* promoter mutations have been reported to create E-twenty-six(ETS)/ternary complex factor (TCF) transcription binding sites. ETS transcription factors may become activated through impaired regulation of MAPK signalling. Therefore, some have hypothesized that *TERT* promoter mutations might increase gene expression(137).

The increased expression of telomerase, when for example *TERT* promoter mutations are present, may be useful as a target for therapy. Several strategies of therapeutic telomerase inhibition in numerous cancers that are now the subjects of clinical trials, including small molecular inhibitors (enzyme inhibition), antisense oligonucleotides (targeting RNA template of telomerase), immunotherapy (using TERT peptides to elicit immune responses), gene therapy (*TERT* promoter driven tumour cell lysis), and telomere- and telomerase-associated proteins (disrupting the telomerase assembly resulting in non-functional telomerase), G-quadruplex stabilizers (blocking telomerase access to telomeres) and a T-oligo approach (blocking of telomerase to induce DNA damage)(138).

Screening for promoter mutations represents a new way of finding significant alterations when mutations are missing in the coding sequences(137, 139, 140). Huang and colleagues recently reported a high prevalence of *TERT* promoter mutations in CMMs(137). Horn and colleagues also demonstrated a germline *TERT* promoter mutation in a kindred with familial CMM(139). Additionally, Egberts and colleagues have now found a low frequency of *TERT* promoter mutations in mucosal melanomas, but the frequency in SNMM was not specifically reported(133).

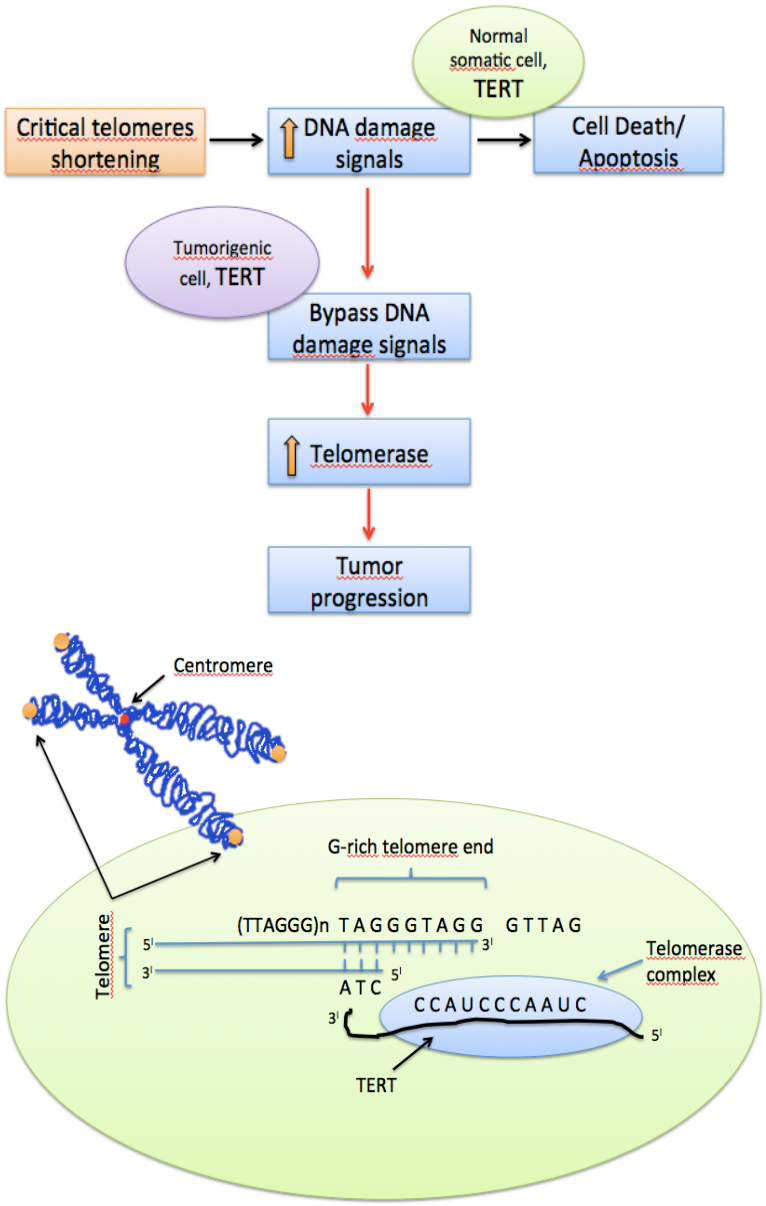


Figure 7. TERT in tumorigenesis (Ryott 2014).

13 AIMS OF THESIS

The overall aims of this thesis are to establish population-based trends for SNMM and SNM in Sweden and to contribute to the molecular characterization of SNMM by investigating the most commonly mutated oncogenes (*BRAF, NRAS* and *KIT*) in melanoma as well as to determining the *TERT* promoter mutation frequencies in SNMM.

Specific aims:

Paper I: The aim was to evaluate the incidence trends, survival rates and clinical features for all patients diagnosed with SNM in Sweden from 1960-2010.

Paper II: In this study our aim was to investigate incidence trends, prognosis, tumour specific survival as well as clinical features for all patients diagnosed with primary SNMM in Sweden from 1960-2000.

Paper III: The purpose of this study was to determine the frequency of mutations in the *BRAF, NRAS* and *KIT* oncogenes in SNMM and to investigate the association between mutations and clinical and histological characteristics.

Paper IV: The intent was to establish the *TERT* promoter mutation frequencies in SNMM and to correlate associations between mutations and clinical characteristics.

14 MATERIALS AND METHODS

14.1 PAPER I

14.1.1 Subjects

All patients in Sweden diagnosed with sinonasal malignancies from 1960-2010 were identified using the Swedish Cancer Registry (SCR). The SCR covers approximately 96% of all cancer cases diagnosed in Sweden. Since the reliability of the SCR was uncertain during the first two years, we used data from 1960(141, 142).

We included all malignant tumours in the sinonasal area, numbered 160.0, 160.2, 160.7-160.9 according to the ICD-7. Information obtained from the SCR included histological subtype, localization, patient's age and date at diagnosis and gender. Unfortunately, information on clinical stage is only available in the SCR for patients diagnosed from 2004 onward and could not be used to study long-term temporal trends.

Using the emigration-, immigration- and date of death registers from Statistics Sweden we were able to identify patients lost to follow-up and to analyse survival. For the survival analysis we included patients diagnosed up to 2010 with follow up to the end of 2012.
To calculate incidence rates, annual population- and gender distribution data were also retrieved from Statistics Sweden (Statistical Yearbook of Sweden).

14.1.2 Statistical analysis

The cancer patients were divided into 16 groups according to age at diagnosis, with a range of five-years. We divided the calendar period into five-year-periods. Incidence rates were calculated by dividing the number of cases in each calendar period by the total average population in each age group in respective calendar period. The rates were adjusted to the Swedish Standard Population of year 2000 as well as the World Standard Population of 1966.

We estimated relative survival ratios, where relative survival provides a measure of excess mortality as a result of cancer without relying on cause of death information (143). The definition is the observed all-cause survival in the patients divided by the expected survival of an equal group in the general population. We used the Ederer II method from Swedish population life-tables stratified by age, sex, and calendar period to estimate expected survival(144). We also used Poisson regression to adjust the effect of age at diagnosis, sex, and calendar year at diagnosis, on the excess mortality rate ratio. To estimate the age-standardized relative survival, we used age distribution (<55, 55-69, ≥ 70) at malignant diagnosis among all patients (145).

Statistical analysis was done in STATA, SAS 9.2 and Excel.

14.2 PAPER II

14.2.1 Subjects

We identified all the patients from 1960 - 2000 using the SCR and included patients according to the ICD-7 codes numbered 160.0, 160.2, 160.7, 160.8 and 160.9, and only patients with a specific histopathologic code of malignant melanoma were included. We analysed all patients for concomitant primary CMMs, which might indicate metastatic disease and no one was found. Clinical records and pathology reports were collected throughout Sweden, except for 11 patients whose medical records were not retrievable. Three patients were excluded due to incorrect diagnosis. Two patients were included, where the initial diagnosis mistakenly was coded as malignant melanoma of the palate and the nasopharynx. The medical charts revealed that those tumours originated in the nasal cavity. Altogether 186 SNMM cases were included. Before 1980, histology examination of melanoma tumours was commonly supplemented with the Masson silver staining to confirm presence of melanin pigment. After the 1980s immune markers like HMB45 and S-100 were used to verify the melanoma diagnosis. The histology sections were reviewed in 90 of the 186 cases. Information on standard demographic data such as age at diagnosis, gender, patient's residence, treating hospital, date of diagnosis as well as overall survival time were obtained from the SCR. We extracted diagnoses, symptoms, TNM (T=primary tumour size and nearby tumour invasion; N=regional lymph nodes; M=distant metastasis) classification and Stage, disease site, mode of treatment, five-year local control, and tumour-specific survival rate from the clinical records. We used the classification from the seventh edition of the TNM Classification of Malignant tumours from UICC (International Union Against Cancer) (2009) when comparing the outcome of the disease. Staging was done according to Ballantyne´s clinical system(97).

14.2.2 Statistical analysis

We divided the SNMM patients into eight groups according to age at diagnosis, each spanning ten years, except for the youngest (0 - 24 years) and oldest (≥ 85 years). The calendar period was divided into five-year-segments. Incidence was analysed by date and gender, using the age standardisation in Sweden, 1980-1984. We examined the changes in the annual age-standardized incidence rates by calculating the average yearly percentage change over a time period and straight lines were fitted to the standardised incidence by least squares regression. The slopes were estimated from these lines. We also analysed the incidence and population density in the 24 counties of Sweden by linear regression with the method of least squares. The definition of population density, as given by Statistics Sweden(146), is based on population centres. Survival rates were estimated with the life table method, taking censored observations into account. The cut-off date for follow up was 24th of February 2011. Tumour-specific survival was analysed.

14.3 PAPER III

14.3.1 Tumour samples

We collected tumour samples (formalin-fixed paraffin-embedded blocks), clinical records and pathology reports from SNMMs that were reported to the SCR throughout Sweden diagnosed between 1986 and 2011. We excluded five samples because the sections contained too few tumour cells. Altogether 56 primary SNMMs were included and 12 of these cases were part of a previously published data set, Omholt and colleagues(23).

14.3.2 Laser capture microdissection (LCM) and DNA extraction

5 mm sections were cut from the paraffin blocks and placed on plain glass slides. The sections were deparaffinised with two washes of xylene, rehydrated in increasing concentrations of ethanol, rinsed with deionised water, shortly stained with haematoxylin, rinsed with deionised water and dehydrated in decreased concentrations of ethanol and two washes of xylene. Tumour cells were microdissected from sections by laser capture microdissection (LCM) using the Arcturus PixCell LCM System (Arcturus Engineering, Mountain View, CA, USA) according to the manufacturer's recommendations. Samples were incubated overnight with proteinase K-enriched digestion buffer (PicoPure DNA Extraction KIT, Arcturus Engineering) to extract the DNAfrom the dissected cells. Proteinase K was then inactivated by heating samples at 95°C for 10 min.

14.3.3 Mutation analysis

The DNA from the tumour cells was subjected to first and second PCR to amplify *BRAF* (exon 15), *NRAS* (exons 1 and 2) and *KIT* (exons 11, 13 and 17) genes. In the first PCR, the DNA was amplified in a 10 ml mixture reaction containing 2.5mM deoxynucleotide triphosphate, 5U/µl platinumTaq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 50 pmol/µ1 of each primer, 10 x PCR buffer, 50mM $MgCl_2$ and 10 µg/µl bovine serum albumin. The PCR cycles: Initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds and then annealing at 54-63 °C (depending on exon examined) for 30 seconds, and then elongation at 72 °C for 30 seconds; and a final extension at 72 °C for 10 minutes. The two microlitres of the first PCR reaction was used as DNA template for the second PCR. The conditions for the second PCR were similar to that of the first PCR except that the numbers of cycles were reduced to 20. The DNA was extracted and purified from agarose gels by using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Sequencing reactions were performed in a final volume of 20 µl using BigDye Terminator V1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). The cycle sequencing conditions were as follows: 10 minutes at 95 °C, 25 cycles of 30 seconds at 96 °C, 5 seconds at 50 °C and 4 minutes at 60 °C. The sequencing products were purified by ethanol precipitation, and automated DNA sequencing was performed by ABI PRISM3130xl Genetic Analyzer (Applied Biosystems). All mutations were confirmed by a second independent PCR and sequencing reaction. The primers used for amplification and sequencing are described in the supplementary table in paper II.

14.3.4 Statistical analysis

We used Fisher's exact test to correlate the mutation status with clinicopathological features. Using Wilcoxon rank-sum test we compared the age at diagnosis between the mutated and wild-type group. We estimated the overall survival from the date of diagnosis to the date of death or last follow-up (1 November 2012). Multivariate Cox regression model, Log-rank test and Kaplan–Meier graphs were used to assess the association of anatomical site with overall survival.

14.4 PAPER IV

14.4.1 Tumour samples

We collected formalin-fixed paraffin-embedded tumour samples, clinical records and pathology reports of 54 SNMMs reported to the SCR from 1986 to 2011. The same patients were included in paper III (61 patients), except seven patients who were excluded because the sections left contained no tumour cells to analyse. Five samples were excluded since these sections contained too few tumour cells for analysis. Altogether 49 primary SNMMs were included in the study. The cut-off date for follow up was 31st of January 2013.

14.4.2 Laser capture microdissection (LCM) and DNA extraction

See 14.2.2. We used the same procedure as in paper II except for 20 patients whose, tumour cells were macrodissected and the DNA was extracted by using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA).

14.4.3 Mutation analysis

The *TERT* promoter region from base pair position +65 to −278 was screened for mutations using PCR and direct Sanger sequencing. Genomic DNA was subjected to initial and subsequently nested PCR to amplify *TERT* promoter regions. The first and second PCR was performed as described in 14.2.3.
The primers used for amplification and sequencing of *TERT* promoter were as described by Rachakonda and colleagues(147)(see Supplementary table 1 in paper IV).

15 RESULTS

15.1 PAPER I

15.1.1 Frequency of sinonasal malignancies

3221 patients with non-lymphoid malignant tumours of the sinonasal cavity and the paranasal sinuses was identified from the SCR. The majority were in the sixth decade or older when diagnosed. Patients with SNMM had the highest average age at diagnosis of 71.7 years in contrast to those with adenoid cystic cancer whose mean age at diagnosis was the lowest, 62.5 years.

The most common diagnosis was SCC followed by adenocarcinoma and SNMM. Of the patients diagnosed SCC 65% were male, however, for SNMM and adenoid cystic cancer, women predominated (55% and 53% respectively).

In total, 50.5% of the tumours involved the nasal cavity, and 31.7% affected the maxillary sinus. The remaining tumours were localized in the other sinuses or had a spread growth. Over the years we reviewed an increasing proportion of tumours originated in the nasal cavity compared to tumours arising in the maxillary sinuses. Individuals with SNMM had a higher frequency of tumours located in the nasal cavity compared to those with SCC.

15.1.2 Incidence of sinonasal malignancies

For SNM the overall incidence rate per 100 000 decreased from 1.19 in the 1960´s to 0.86 in the 2000´s. SCC as well as undifferentiated carcinoma decreased whereas the incidence of adenocarcinoma increased in the beginning of the study period and decreased at the end. SNMM is the only sinonasal malignancy that has increased in incidence during the study period (from 0.04 1960-1964 to 0.15 2005-2010).

15.1.3 Survival of patients with sinonasal malignancies

The women had a 4 % lower mortality rate than men, but the difference was not statistically significant (p=0.48). During the study period, the length of survival improved over time for both genders regardless of the histological diagnosis.

Patients with adenocarcinoma and SCC had a 5-year survival of 56% and 46% respectively. SNMM and undifferentiated carcinoma yielded a poorer prognosis, 27% and 37% 5-year survival, respectively. Patients with adenoid cystic cancer had the best 5-year prognosis, 58%. Moreover, patients whose tumours originated from the nasal cavity had a better prognosis (mean overall survival 84.2 months) than patients with tumours localized in the maxillary sinuses (mean overall survival 55.2 months) ($p<0.0001$).

15.2 PAPER II

15.2.1 Patients

Of the 186 patients represented here, 55% (102) were women and 45% (84) men. Their ages at diagnosis ranged from 31 to 93 years, with a median age of 72. All patients were Caucasians (as reported by the medical records). 168 of the 186 SNMM were classified according to TNM and Ballantyne stage. 18 patients had insufficient data for classification. 92.9% of the SNMM tumours presented in Stage I.

The mean age-specific incidence of SNMM increased with age peaking after the eightieth year in both genders. The age-standardized incidence increased significantly for both genders during the investigated time period. For females the average annual age-standardized incidence per million population increased from 0.54 1960-1964 to 1.08 during 1995 - 2000. The incidence increased more rapidly among males (0.25 to 0.67 per million per year) compared to females, however this difference was not statistically significant.
There was no correlation between population density and the incidence of SNMM. The island of Gotland, showed an unquestionable higher incidence of SNMM than all other counties.

15.2.2 Tumour site

44.1% of the tumours involved the nasal cavity, where the inferior turbinate followed by septum were the most frequent sites. About ¼ of the tumours affected the sinuses. 34.4% of the patients, was indeterminate due to growth both in the nasal cavity and sinuses or because the original site was not specified in the medical records.

15.2.3 Clinical and pathological features

The most frequent symptom at diagnosis was epistaxis followed by nasal obstruction. According to the medial charts 70.9% of the patients presented with an amelanotic tumour and 34.8%, initially had amelanotic polyps. 41.1 % presented as ulcerated tumours and only 4.6% of those manifested as macroscopically pigmented. Patients present with more advanced disease over the study period. Among the patients classified as Stage I (156 patients), 28.8% developed recurrent disease within one year of diagnosis. 57.8% of the recurrent disease remained localized. 17.8% developed regional metastases and 24.4% distant metastases. The median time to local recurrence was 23 months, and times to regional and distant metastasis were 11 and 8 months, respectively.

15.2.4 Histopathology

Over 1/5 of the patients were mis-diagnosed initially (e.g. olfactory neuroblastoma, lymphoma, poorly differentiated SCC or sarcoma). The mis-diagnoses patients were usually based on the small size of patients' biopsies. The initial diagnosis was revised after secondary extended surgery, when more tumour material was available for analysis.

15.2.5 Treatment

Surgery alone was the most common primary treatment performed in 53.1% of the patients, followed by surgery combined with pre- or postoperative radiotherapy in 32.0%. Only 2.7% received radio-chemotherapy combined with surgery. 17.8% of the patients underwent local excisions, whereas procedures for 47.4% were done according to Denker. Another 26.7% received a total maxillectomy, and 8.1% underwent maxillectomy and exenteration. In 12.2%, treatment was restricted to chemotherapy, radiation, or partial resection of the tumour, with palliative intent, or no antitumoural treatment. In about 1/5 of the patients, information regarding treatment was missing. Of the four patients in Stage II, one underwent neck dissection surgery and postoperative chemo/radiotherapy. The other 3 patients received palliative treatment as well as the patients in Stage III.

15.2.6 Survival

The follow-up time ranged from 0 to 283 months for patients who eventually died and 148 – 172 months for living patients. The tumour-specific, five-year survival rate was 20.4% for all patients. Women had significantly better survival rates than men (p=0.038). Patients over 60-years at diagnosis died significantly earlier than younger patients (p=0.040). However, there was no difference in the length of survival five years after diagnosis. Patients with epistaxis had a better prognosis than those with only nasal congestion (p=0.046). The choice of primary treatment for patients in Stage I did not have a significant impact on outcomes in terms of time to recurrent disease or tumour-specific survival. Survival increased significantly irrespective of therapy during the study-period of this survey(p=0.024).

15.3 PAPER III

15.3.1 Clinicopathological characteristics

The tissues examined were derived from 56 patients, 35 females and 21 males whose collective median age at diagnosis was 76 years. Of these, 34 tumours were located in the nasal cavity and 22 in the paranasal sinuses (10 in the maxillary sinuses, 6 in the ethmoid sinuses and 6 tumours invaded surrounding structures: 4 involved the orbit; one the skull base and another spread to the retromaxillary infratemporal fossa).

15.3.2 Mutation analysis

The analysis showed that 21% (12 of 56)) of the tumours harboured *KIT*, *NRAS* or *BRAF* mutations. *KIT* mutations were detected in 4% (2 of 56) of the cases. Both *KIT* mutations were identified in exon 11, L576P, however no mutations were observed in exons 13 and 17. According to a previous study by Omholt and colleagues(23) the frequency of *KIT* mutations in vulvar melanomas (35%) was much higher. NRAS mutations were identified in 14% (8 of 56) and BRAF mutations in 4% (2 of 56). Four of the *NRAS* mutations were found in exon 1 (G12C, G12D, G12A and G13D) and four in exon 2 (Q61K, Q61R and Q61H (n¼2). Of the two *BRAF* mutations one was V600E and one V600K change.

15.3.3 Association of mutations with clinicopathological features

The tumours with mutations had a higher frequency of paranasal localization compared to the wild-type tumours, which were more frequently found in the nasal cavity (p=0.045). However there was no difference between the mutated and the wild-type group when comparing gender, age at diagnosis, ulceration or tumour pigmentation.

15.3.4 Survival

The overall survival was significantly associated with anatomical site, clinical stage and age at diagnosis. Patients with tumours arising in the nasal cavity had a significantly better prognosis than those with tumours in the paranasal sinuses (median survival months, 39 vs 16 p=0.027). After adjusting for age at diagnosis, gender, ulceration, pigmentation and clinical stage, this effect remained significant (p=0.001). There was no association with the overall survival or differing mutation status.

15.4 PAPER IV

15.4.1 Clinicopathological characteristics

The samples analysed were from 49 patients, 31 females and 18 males whose overall median age at diagnosis was 76 years. Of these tumours, 30 were located in the nasal cavity and 19 in the paranasal sinuses (9 in the maxillary sinuses, 6 in the ethmoid sinuses and 4 that had invaded the surrounding structures: 3 involved the orbit; one spread to the retromaxillary-infratemporal fossa). When diagnosed, most of these patients were in stage III according to UICC 7 th edition. Only one patient had lymph node metastases in the neck region, and none had distant metastases at diagnosis.

15.4.2 Mutation analysis

Of the 49 primary SNMMs analyzed, 4 (8 %) harbored *TERT* promoter gene mutations and 5 (10 %) were wild type. All the others, 40 (82 %), had the -245G>A alteration, a known single nucleotide polymorphism (SNP), rs2853669 (Horn et al, 2013; Rachakonda et al, 2013). Among the identified promoter mutations two had the same mutations -124G>A (1295228) and -125G>A (1295229), one had the -146G>A mutation (1295250) and one 57T>G (1295161). All these mutations have been reported to create binding motifs for ETS/TCF, and are thus likely to be of functional significance(137, 139, 147).

15.4.3 Mutations in relation to clinicopathological features

Owing to the small number of patients with mutated tumours, no statistically valid comparison was possible between these and the remaining patients. Three females and one male were recorded among the patients with *TERT* promoter gene mutations. Two tumours were localized in the nasal cavity and 2 in the maxillary sinuses. The youngest patient was diagnosed at the age of 52 years and the oldest at 92 years. The survival range varied from 19-121 months.

16 DISCUSSION

The aim of this thesis was to investigate the epidemiology, clinicopathological features, pathogenetical aspects and prognostic factors for SNMM in Sweden and also the epidemiological aspects of SNM. Our unique material for both SNM and SNMM is one of the largest population-based sets of tumour studies of its kind. The results of our studies have enlightened the relatively poorly investigated patient categories SNM and, particularly, SNMM.

16.1 PAPER I

In this study of SNM, which embodied one of the largest population-based studies in recent years, we found an overall decreasing incidence of SNM in Sweden during the study period 1960 through 2010. Interestingly, SNMM is the only category of SNM tumours that has increased numerically to a significant extent during in that time period.
SCC was the most frequent SNM followed by adenocarcinoma during the study period. However, when comparing incidence data from 1960 and 2010, SCC was still the most frequently found tumour followed by SNMM, which was rare in 1960. An increasing incidence of SNMM was also observed in other recent studies(148, 149). The differences in distribution over time of the various histological types might reflect an actual change in relative incidence of different histological types but also denotes imoroved diagnostic methods. New diagnostic tools, such as immunohistochemical markers, have increased the accuracy of identifying certain tumours, whereas many of the "undifferentiated" tumours in 1960 now are likely to yield histologically specific diagnosis.

An association between SNMs and exposure to inhaled carcinogens such as wood dust has been reported in several investigations in the late 1960s and early 1970s(6, 7). Most notably, the adenocarcinoma is particularly strongly related to exposure to leather dust or dust from hard wood(8, 9). Moreover, excessive air pollution has been described as an etiological factor for SNM in general(9, 12). Therefore, the decreasing incidence in most histological types of SNM may reflect better working conditions and reduced air pollution over the years. Furthermore, the decreasing incidence of SCC might also be correlate with reductions in habitual smoking that we know is a crucial contributor to its pathogenesis(14).

The majority of tumours included here originated in the nasal cavity, as in other similar studies(2, 3, 14). We also found that an increasing proportion of tumours arose first in the nasal cavity compared to the maxillary sinuses for all SNM during this study period, in agreement with outcomes in a recently published article by Youlden and colleagues(2). Perhaps patients' and doctors' delays in the 1960s resulted in a misclassification of tumour, whereas by 2010, better diagnostic tools and earlier diagnoses provided more reliable information about tumours' sites of origin.

Some of the tumours in the nasal cavity, especially SCC and SNMM, may be misclassified and as they might, instead, have originated from the skin in vestibulum

nasi. Finally, differing the sites of origin for tumours found in the nasal cavity, ethmoid sinuses and maxillary sinuses would have been far more difficult before our modern diagnostic tools were available (CT and MRI).

We have found no significant improvement in the survival times of patients with SNM over the last 50 years. However, tumours originating in the nasal cavity enabled significantly longer survival periods compared to those at other locations, presumably because nasal cavity tumours present earlier with such straightforward symptoms as nasal blockage or epistaxis and are, therefore, diagnosed sooner. Yet, there was a slight increase in overall relative 5-year survival. Surprisingly, the new treatment strategies and modern diagnostic tools, which facilitate greater precision of therapy, have not significantly improved treatment outcomes. Our data do not show any clearcut difference in 5-year survival between men and women. Others have confirmed this conclusion in a long-term survival analysis(150), where survival was supposedly linked to stage at diagnosis, extent of spread and onset of symptoms rather than gender.

The strength of this present overviews, which is the largest European population-based study on SNM of its kind, is its basis on data from the nationwide Swedish cancer registry with its uniquely extensive coverage. Furthermore, our study is the first to describe the trends in incidence and survival of sinonasal malignancies in Sweden over a long period. The weakness is that, due to the rarity SNM and even more in some of the histological subtypes, conclusions from this material may be difficult to draw.

16.2 PAPER II

We found that the incidence of SNMM in Sweden has increased significantly from 1960 through 2000, although not at the same rate as CMM in the same time period. The reasons for this increased incidence are unknown, but the impact of improved diagnostic methods cannot be ruled out. New diagnostic tools such as immunohistochemical markers have increased the accuracy of identifying SNMM. Since over 30% of the patients with SNMM display non-pigmented tumours, identification of these markers is important for obtaining the correct diagnosis. In the samples assessed here, we found that about 70% manifested clinically amelanotic melanomas. We also documented that about 20% of the patients were primarily misdiagnosed as bearing low- or undifferentiated malignancies. We think that a reduction of misdiagnosis is a much too simple an explanation when there are a significant difference in incidence between males and females. There is a higher overall incidence of SNMM for females than males, but with a more rapid increasing incidence among males. In contrast, a female predominance was not seen in other sinonasal malignancies, where there is a higher incidence for men (except for adenoid cystic cancer, which also predominated in women, see paper I). However, the incidence of CMM in Sweden was similar in both genders(40).

We did not find any difference between the incidence of SNMM in either sparsely vs densely populated geographic regions of Sweden, which argues against a causative impact of air pollution in urban areas. Air pollution is otherwise an important factor to consider in the genesis of sinonasal malignancies. Consequently, a report from Mexico City described an increase of paranasal malignancies (including SNMM) suggesting

that the heavy air pollution could be responsible(12). Furthermore, inhaled carcinogens are known to induce SNM and SNMM. Environmental carcinogens and drugs (e.g. aflatoxin B1, benzidine, and polycyclic hydrocarbons, such as dimetylbenzanthracene and benzopyrene) are filtered by and conjugated to melanins, sometimes over a long time period(61). Human melanocytes are known to be capable of metabolizing the carcinogen benzopyrene and their metabolites that might be involved in the development of SNMM(62).

In the patient material examined here, the nasal septum and the turbinates were the most commonly affected subsites, which also suggests that an inhaled carcinogens could be a cause of tumour formation, since most inhaled air passes through the nasal cavity during normal breathing. Occupational exposure to formaldehyde may also contribute to SNMM as is suggested by Holmström and colleagues(63). Information on possible occupational and environmental factors was, unfortunately not available in our material.

For about 1/3 of the patients included here, the site of origin was not specified in the medical records because tumour growth was present in both the nasal cavity and the paranasal sinuses or because the original site was indeterminate. Presumably, patients' and/or doctors' delay in seeking diagnosis resulted in identifying tumours only at advanced stages so that the original location was difficult to determine. In the later part of the investigated time period more advanced tumours were identified. Today's more advanced techniques, including CT scanning, MRI, and PET/CT scanning, were not available during this study's initial period and might, therefore, account for the apparent increase of more advanced tumours over the years.

The prognosis is poor for SNMM patients of both genders. However, women had significantly better survival rates than men and the same gender-associated difference in survival has also been reported for patients with ano-rectal melanomas in Sweden(48). This gender difference is also described for CMM in Sweden(113).

During the first five years after diagnosis the older patients had a more lethal disease compared to younger patients. However, there was no difference in survival times five years or more after diagnosis between the age groups. Explanations for this calculation might be an age-related decline of immunologic defence or a choice not to institute standard therapy because of an elder's weak physical condition or refusal of treatment.

The nonspecific initial symptoms of SNMM, such as nasal congestion are probably the reason for a patient not to seek medical care in time. Accordingly, patients with the obviously more alarming early symptom of epistaxis undoubtedly sought medical examination sooner and thus had a better prognosis.

The SNMM tumours appeared in the majority of the cases as amelanotic and is frequently described as an amelanotic polyp, often mistaken for a benign lesion. To avoid misdiagnosis a solitary polyp in the nasal cavity always requires a biopsy for histopathological investigation.

Metastases to the regional lymph node at presentation were uncommon in our study (3.3%) compared to SCC(10%)(51) and to SNM in general(3). However, distant metastases were more frequents, which is due to the greater haematogenous dissemination of melanomas.

In this series the preferred treatment for patients with SNMM in Stages I-II (according to Ballantyne) was complete surgical tumour excision. The different treatment strategies did not have any impact on time to recurrence or disease-specific survival. However, the small numbers of patients, selection bias and that there were no systematic treatment or follow up for these patients makes it difficult to draw any firm conclusions on the outcome of different therapeutic strategies.

A study by Lund and colleagues in 2012, reported that radiotherapy did not improve the local control or survival of patients with SNMM. Those authors also found that cervical metastases were associated with a dramatically worse outcome(26). In contrast, another study reported that a higher total dose of postoperative radiotherapy improved loco regional control(98). Moreover, other reports emphasize the advantage of adjuvant radiotherapy(108, 109).

One must be aware of that when rare neoplasms, such as SNMM, are studied the analysis may be incomplete. However, our study examining many cases in a national population-based series, provides a far better opportunity to give us information on clinical features that the individual case study or case series may disregard.

16.3 PAPER III

Few published reports on SNMM exist, and those available include mutational data on only small numbers of tumours. In this study, the largest of its kind to our knowledge, we screened SNMM tumours for the most commonly mutated oncogenes in CMM. We found that *KIT* mutations are rare compared to other mucosal sites where for example vulva melanoma harbour a much higher frequency *KIT* mutation (35 %), reported by Omholt and colleagues(23). In a recent study by Schoenewolf and colleagues on sinonasal and vulvovaginal melanomas, no *KIT* mutations were found in 12 sinonasal tumours compared with 5 mutations in 11 vulvovaginal tumours (45%)(151). Moreover, Beadling and colleagues found a lower frequency of *KIT* mutations in mucosal melanomas of the head and neck (3 of 36; 8%) when compared with melanomas of the anorectum/vulva/vagina (4 of 9; 44%)(96).

BRAF mutations in mucosal melanoma are generally rare in mucosal melanomas and do not seem to vary significantly between different sites(23). In our study, *BRAF* mutations were also rare, which is similar values for other mucosal melanomas at other sites such as the vulva, vagina and ano-rectum(23, 88).

The frequency of *NRAS* mutations where higher compared to *KIT*- and *BRAF* mutations. The *NRAS* mutations seem to be corresponding to that in CMM(80, 122, 123). Yet, the types of *NRAS* mutations in SNMM differ from the types that are most commonly detected in CMM. Only two of eight *NRAS* mutated SNMM tumours

contained the two most common *NRAS* mutations in CMM(152). The different patterns of *NRAS* mutations in SNMM are consistent with other aetiology than UV-radiation.

We found that melanomas originating in the paranasal sinuses have a higher mutation frequency than those originating in the nasal cavity. Furthermore, patients with disease located in the paranasal sinuses had a worse prognosis than those with tumours located in the nasal cavity, which has also been reported in other studies(92, 153). The poor prognosis for those within paranasal tumours might be the consequence of more advanced tumour stage, as in six of these patients had tumours invaded the surrounding structures.

There were no difference in overall survival between patients with mutated melanomas and those with wild-type melanomas; however the power to detect a difference is low, since the numbers of tumours with mutations is small. In a previous study, *KIT* mutations as well as *NRAS* mutations were associated with poor survival(23). Another report has shown that *KIT* mutations adversely affected survival for both mucosal and CMM(126).

The relatively low proportion of *KIT* and *BRAF* mutations in SNMM tumours indicate that only a few SNMM patients may benefit from therapy with KIT- and BRAF-inhibitors. However, higher frequency of *NRAS*-mutated tumours suggest that it might be worth to perform studies on SNMM patients using MEK inhibitors, which have shown promising results in CMM with *NRAS* mutations(32).

16.4 PAPER IV

In this study we analysed a large number of primary SNMM for *TERT* promoter gene mutations, which are common in CMM(137, 139). To our knowledge this has not been analysed specifically in SNMM previously and never in as large a study as this one. We identified *TERT* promoter gene mutations in 8 % of the tumours, but because the number of cases was small, we could not analyse clinicopathological features in relation to the mutations. Three of the tumour associated mutations (-124G>A, -125G>A and -146G>A) are known to generate binding motifs for Ets transcription factors, resulting in increasing transcriptional activity from the *TERT* promoter by two- to four-fold(137). The -57T>G mutation is also known to increase transcriptional activity in the same way but only by 1.5-fold(139). The majority of the patients had the SNP rs2853669 (-245A>G). In previous studies this polymorphism was reported to disrupt an ETS binding site and was associated with low telomerase activity in patients with non-small cell lung cancer(154).
Rachaconda and colleagues have also found a reduction in promoter activity in urothelial carcinoma with the same SNP, and suggested that the rs2853669 polymorphism might have a protective effect on survival and recurrence(147).

17 CONCLUSION

The main conclusions are:

The incidence of sinonasal malignancies in Sweden have decreased from 1960 to 2010. However in the same time period sinonasal malignant melanoma incidence has increased and is now the second most common SNM.

There was no significant improvement in survival times for individuals with SNM over the last 50 years despite better diagnostic tools and treatment options.

The incidence of SNMM was significantly higher among women than men.
Despite the poor prognosis, although better for women than for men, the five-year, tumour-specific survival rate improved significantly for both genders during the period 1960 through 2000.

The SNMM patients with epistaxis as their initial symptom had a significantly better prognosis than those with only nasal congestion.

For the majority of the SNMM patients, the tumour appeared as a clinically amelanotic growth and often as an amelanotic polyp. This fact stresses the importance of biopsy for histological investigation when diagnosing a solitary polyp or growth in the nasal cavity.

The most common site of origin was the nasal cavity for both SNM and SNMM. For SNMM the most common site in the nasal cavity was the inferior tubinate followed by the nasal septum.

Patients with SNM and SNMM originating from the sinuses had a worse prognosis than those with tumours originating from the nasal cavity.

The proportion of advanced tumours increased for SNMM over the study period 1960 through 2000. The reason for this is not clarified, but may be due to more sophisticated diagnostic technology emerging over the years.

In our study, the different treatment strategies did not affect the time to recurrence or disease specific survival for SNMM.

KIT, *NRAS* and *BRAF* mutations occurred at low frequencies in SNMM, and the frequency of *KIT* mutation in mucosal melanoma mutations vary significantly between different anatomical sites.

Tumours from the paranasal sinuses have a higher frequency of *KIT*, *NRAS* and *BRAF* mutations than tumours from the nasal cavity.

TERT promoter mutations occurred at a moderate frequency in SNMM. We suggest that SNMM tumours should be included in molecular characterizations, since these alterations are likely be therapeutic targets in the near future.

18 FUTURE PERSPECTIVES

We found an increasing incidence of SNMM in Sweden from 1960 through 2010. The cause of this increase is at present unknown. Research has indicated that inhaled carcinogens and air pollution may be involved in the development of melanomas. However, although we have seen less air pollution and better working conditions over the years, our findings do not reflect those improvements. The aetiological factors still remain unknown and remain in need of future investigation.

There are relatively low frequencies of *KIT*, *NRAS* and *BRAF* mutations as well as a low frequency of *TERT* promoter gene mutation in SNMM. This indicates that SNMM harbours other mutations yet to be discovered.

Clearly, since the prognosis is so poor for patients with SNMMs, finding new treatment options continues to be of primary importance. But as one must acknowledge, identifying the pathogenesis is still an urgent research objective. Toward that end, revealing other driver mutations involved in the development of SNMM would benefit appreciably from whole genome mutation analysis.

Unfortunately, the new treatment strategies and modern diagnostic tools have not significantly improved treatment outcome for SNM; therefore, better options must be devised. Only when large-scale comparisons of diverse outcomes for SNM become available can we evaluate the optimal therapy for these patients.

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20 REFERENCES

1. The Swedish National Cancer Registry. Cancer incidence in Sweden, 1960-2010. Stockholm: National Board of Health and Welfare, annual publications 1960-2010.
2. Youlden DR, Cramb SM, Peters S, Porceddu SV, Moller H, Fritschi L, et al. International comparisons of the incidence and mortality of sinonasal cancer. Cancer epidemiology. 2013;37(6):770-9.
3. Turner JH, Reh DD. Incidence and survival in patients with sinonasal cancer: a historical analysis of population-based data. Head & neck. 2012;34(6):877-85.
4. Jakobsen MH, Larsen SK, Kirkegaard J, Hansen HS. Cancer of the nasal cavity and paranasal sinuses. Prognosis and outcome of treatment. Acta Oncol. 1997;36(1):27-31.
5. Franchi A, Miligi L, Palomba A, Giovannetti L, Santucci M. Sinonasal carcinomas: recent advances in molecular and phenotypic characterization and their clinical implications. Crit Rev Oncol Hematol. 2011;79(3):265-77.
6. Acheson ED, Cowdell RH, Hadfield E, Macbeth RG. Nasal cancer in woodworkers in the furniture industry. Br Med J. 1968;2(5605):587-96.
7. Acheson ED, Cowdell RH, Rang E. Adenocarcinoma of the nasal cavity and sinuses in England and Wales. Br J Ind Med. 1972;29(1):21-30.
8. Hayes RB, Gerin M, Raatgever JW, de Bruyn A. Wood-related occupations, wood dust exposure, and sinonasal cancer. American journal of epidemiology. 1986;124(4):569-77.
9. d'Errico A, Pasian S, Baratti A, Zanelli R, Alfonzo S, Gilardi L, et al. A case-control study on occupational risk factors for sino-nasal cancer. Occup Environ Med. 2009;66(7):448-55.
10. Hayes RB, Kardaun JW, de Bruyn A. Tobacco use and sinonasal cancer: a case-control study. Br J Cancer. 1987;56(6):843-6.
11. Zheng W, McLaughlin JK, Chow WH, Chien HT, Blot WJ. Risk factors for cancers of the nasal cavity and paranasal sinuses among white men in the United States. Am J Epidemiol. 1993;138(11):965-72.
12. Calderon-Garciduenas L, Delgado R, Calderon-Garciduenas A, Meneses A, Ruiz LM, De La Garza J, et al. Malignant neoplasms of the nasal cavity and paranasal sinuses: a series of 256 patients in Mexico City and Monterrey. Is air pollution the missing link? Otolaryngol Head Neck Surg. 2000;122(4):499-508.
13. Norlander T, Frodin JE, Silfversward C, Anggard A. Decreasing incidence of malignant tumors of the paranasal sinuses in Sweden. An analysis of 141 consecutive cases at Karolinska Hospital from 1960 to 1980. Ann Otol Rhinol Laryngol. 2003;112(3):236-41.
14. Kuijpens JH, Louwman MW, Peters R, Janssens GO, Burdorf AL, Coebergh JW. Trends in sinonasal cancer in The Netherlands: more squamous cell cancer, less adenocarcinoma. A population-based study 1973-2009. Eur J Cancer. 2012;48(15):2369-74.
15. Urteaga O, Pack GT. On the antiquity of melanoma. Cancer. 1966;19(5):607-10.
16. Rebecca VW, Sondak VK, Smalley KS. A brief history of melanoma: from mummies to mutations. Melanoma Res. 2012;22(2):114-22.

17. Lücke A. Die Lehre von den Geschwuksten in anatomischer und klinischer Beziehung in Handbuch d. allg u. spec. chir. Erlangen 1869:244.
18. Zak FG, Lawson W. The presence of melanocytes in the nasal cavity. Ann Otol Rhinol Laryngol. 1974;83(4):515-9.
19. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer. 2005;41(1):45-60.
20. Dahl C, Guldberg P. The genome and epigenome of malignant melanoma. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. 2007;115(10):1161-76.
21. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, et al. A landscape of driver mutations in melanoma. Cell. 2012;150(2):251-63.
22. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, et al. KIT as a therapeutic target in metastatic melanoma. JAMA : the journal of the American Medical Association. 2011;305(22):2327-34.
23. Omholt K, Grafstrom E, Kanter-Lewensohn L, Hansson J, Ragnarsson-Olding BK. KIT pathway alterations in mucosal melanomas of the vulva and other sites. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(12):3933-42.
24. Jovanovic B, Krockel D, Linden D, Nilsson B, Egyhazi S, Hansson J. Lack of cytoplasmic ERK activation is an independent adverse prognostic factor in primary cutaneous melanoma. J Invest Dermatol. 2008;128(11):2696-704.
25. Bradley PJ. Primary malignant mucosal melanoma of the head and neck. Curr Opin Otolaryngol Head Neck Surg. 2006;14(2):100-4.
26. Lund VJ, Chisholm EJ, Howard DJ, Wei WI. Sinonasal malignant melanoma: an analysis of 115 cases assessing outcomes of surgery, postoperative radiotherapy and endoscopic resection. Rhinology. 2012;50(2):203-10.
27. Krengli M, Jereczek-Fossa BA, Kaanders JH, Masini L, Beldi D, Orecchia R. What is the role of radiotherapy in the treatment of mucosal melanoma of the head and neck? Crit Rev Oncol Hematol. 2008;65(2):121-8.
28. Khan MN, Kanumuri VV, Raikundalia MD, Vazquez A, Govindaraj S, Baredes S, et al. Sinonasal melanoma: survival and prognostic implications based on site of involvement. International forum of allergy & rhinology. 2014;4(2):151-5.
29. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2012;380(9839):358-65.
30. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364(26):2507-16.
31. Woodman SE, Davies MA. Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. Biochemical pharmacology. 2010;80(5):568-74.
32. Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, et al. MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. Lancet Oncol. 2013.
33. Prasad ML. Update on pigmented lesions of the sinonasal tract. Head Neck Pathol. 2007;1(1):50-4.
34. Thorup C, Sebbesen L, Dano H, Leetmaa M, Andersen M, Buchwald C, et al. Carcinoma of the nasal cavity and paranasal sinuses in Denmark 1995-2004. Acta Oncol. 2010;49(3):389-94.

35. Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: a site-specific analysis of the SEER database. Int J Cancer. 2005;114(5):806-16.
36. Grau C, Jakobsen MH, Harbo G, Svane-Knudsen V, Wedervang K, Larsen SK, et al. Sino-nasal cancer in Denmark 1982-1991--a nationwide survey. Acta Oncol. 2001;40(1):19-23.
37. Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953-2008--are recent generations at higher or lower risk? Int J Cancer. 2013;132(2):385-400.
38. Garbe C, Leiter U. Melanoma epidemiology and trends. Clinics in dermatology. 2009;27(1):3-9.
39. Godar DE. Worldwide increasing incidences of cutaneous malignant melanoma. J Skin Cancer. 2011;2011:858425.
40. Mansson-Brahme E, Johansson H, Larsson O, Rutqvist LE, Ringborg U. Trends in incidence of cutaneous malignant melanoma in a Swedish population 1976-1994. Acta oncologica. 2002;41(2):138-46.
41. Liang JJ, Robinson E, Martin RC. Cutaneous melanoma in New Zealand: 2000-2004. ANZ J Surg. 2010;80(5):312-6.
42. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer. 1998;83(8):1664-78.
43. Qiu D, Marugame T. Comparison of time trends in skin cancer incidence (1973-97) in East Asia, Europe and USA, from Cancer Incidence in Five Continents Vol. IV-VIII. Japanese journal of clinical oncology. 2008;38(3):234-6.
44. Kabigting FD, Nelson FP, Kauffman CL, Popoveniuc G, Dasanu CA, Alexandrescu DT. Malignant melanoma in African-Americans. Dermatology online journal. 2009;15(2):3.
45. National standard of care program for malignant melanoma in Sweden, 2013. http://wwwcancercentrumse/Global/RCC Samverkan/Dokument/V%C3%A5rdprogram/NatVP_Malignt_melanom_130520_final%5Bl%C3%A5ng%5Dpdf.
46. Ragnarsson-Olding B, Johansson H, Rutqvist LE, Ringborg U. Malignant melanoma of the vulva and vagina. Trends in incidence, age distribution, and long-term survival among 245 consecutive cases in Sweden 1960-1984. Cancer. 1993;71(5):1893-7.
47. Weinstock MA. Malignant melanoma of the vulva and vagina in the United States: patterns of incidence and population-based estimates of survival. Am J Obstet Gynecol. 1994;171(5):1225-30.
48. Ragnarsson-Olding BK, Nilsson PJ, Olding LB, Nilsson BR. Primary ano-rectal malignant melanomas within a population-based national patient series in Sweden during 40 years. Acta oncologica. 2009;48(1):125-31.
49. Cagir B, Whiteford MH, Topham A, Rakinic J, Fry RD. Changing epidemiology of anorectal melanoma. Diseases of the colon and rectum. 1999;42(9):1203-8.
50. The Swedish National Cancer registry. Cancer incidence in Sweden, 1960-2000. National Board of Health and Welfare.
51. Harbo G, Grau C, Bundgaard T, Overgaard M, Elbrond O, Sogaard H, et al. Cancer of the nasal cavity and paranasal sinuses. A clinico-pathological study of 277 patients. Acta Oncol. 1997;36(1):45-50.
52. Batsakis JG, Regezi JA, Solomon AR, Rice DH. The pathology of head and neck tumors: mucosal melanomas, part 13. Head Neck Surg. 1982;4(5):404-18.

53. Takagi M, Ishikawa G, Mori W. Primary malignant melanoma of the oral cavity in Japan. With special reference to mucosal melanosis. Cancer. 1974;34(2):358-70.
54. Gal TJ, Silver N, Huang B. Demographics and treatment trends in sinonasal mucosal melanoma. The Laryngoscope. 2011;121(9):2026-33.
55. Jangard M, Hansson J, Ragnarsson-Olding B. Primary sinonasal malignant melanoma: a nationwide study of the Swedish population, 1960-2000. Rhinology. 2013;51(1):22-30.
56. Garibyan L, Fisher DE. How sunlight causes melanoma. Current oncology reports. 2010;12(5):319-26.
57. Lazovich D, Vogel RI, Berwick M, Weinstock MA, Anderson KE, Warshaw EM. Indoor tanning and risk of melanoma: a case-control study in a highly exposed population. Cancer Epidemiol Biomarkers Prev. 2010;19(6):1557-68.
58. Besaratinia A, Pfeifer GP. Sunlight ultraviolet irradiation and BRAF V600 mutagenesis in human melanoma. Hum Mutat. 2008;29(8):983-91.
59. Young C. Solar ultraviolet radiation and skin cancer. Occupational medicine. 2009;59(2):82-8.
60. Hansson J. Familial melanoma. Surg Clin North Am. 2008;88(4):897-916, viii.
61. Roberto A, Larsson BS, Tjalve H. Uptake of 7,12-dimethylbenz(a)anthracene and benzo(a)pyrene in melanin-containing tissues. Pharmacol Toxicol. 1996;79(2):92-9.
62. Agarwal R, Medrano EE, Khan IU, Nordlund JJ, Mukhtar H. Metabolism of benzo[a]pyrene by human melanocytes in culture. Carcinogenesis. 1991;12(10):1963-6.
63. Holmstrom M, Lund VJ. Malignant melanomas of the nasal cavity after occupational exposure to formaldehyde. Br J Ind Med. 1991;48(1):9-11.
64. Kazi M, Awan S, Junaid M, Qadeer S, Hassan NH. Management of sinonasal tumors: prognostic factors and outcomes: a 10 year experience at a tertiary care hospital. Indian journal of otolaryngology and head and neck surgery : official publication of the Association of Otolaryngologists of India. 2013;65(Suppl 1):155-9.
65. Dulguerov P, Jacobsen MS, Allal AS, Lehmann W, Calcaterra T. Nasal and paranasal sinus carcinoma: are we making progress? A series of 220 patients and a systematic review. Cancer. 2001;92(12):3012-29.
66. McLean N, Tighiouart M, Muller S. Primary mucosal melanoma of the head and neck. Comparison of clinical presentation and histopathologic features of oral and sinonasal melanoma. Oral oncology. 2008;44(11):1039-46.
67. Brandwein MS, Rothstein A, Lawson W, Bodian C, Urken ML. Sinonasal melanoma. A clinicopathologic study of 25 cases and literature meta-analysis. Arch Otolaryngol Head Neck Surg. 1997;123(3):290-6.
68. Thompson LD, Wieneke JA, Miettinen M. Sinonasal tract and nasopharyngeal melanomas: a clinicopathologic study of 115 cases with a proposed staging system. Am J Surg Pathol. 2003;27(5):594-611.
69. Patrick RJ, Fenske NA, Messina JL. Primary mucosal melanoma. J Am Acad Dermatol. 2007;56(5):828-34.
70. Jackson RT, Fitz-Hugh GS, Constable WC. Malignant neoplasms of the nasal cavities and paranasal sinuses: (a retrospective study). Laryngoscope. 1977;87(5 Pt 1):726-36.
71. Magnus K, Andersen A, Hogetveit AC. Cancer of respiratory organs among workers at a nickel refinery in Norway. Int J Cancer. 1982;30(6):681-5.

72. Cantu G, Bimbi G, Miceli R, Mariani L, Colombo S, Riccio S, et al. Lymph node metastases in malignant tumors of the paranasal sinuses: prognostic value and treatment. Arch Otolaryngol Head Neck Surg. 2008;134(2):170-7.
73. McKay SP, Shibuya TY, Armstrong WB, Wong HS, Panossian AM, Ager J, et al. Cell carcinoma of the paranasal sinuses and skull base. American journal of otolaryngology. 2007;28(5):294-301.
74. Sanghvi S, Patel NR, Patel CR, Kalyoussef E, Baredes S, Eloy JA. Sinonasal adenoid cystic carcinoma: comprehensive analysis of incidence and survival from 1973 to 2009. Laryngoscope. 2013;123(7):1592-7.
75. Rhee CS, Won TB, Lee CH, Min YG, Sung MW, Kim KH, et al. Adenoid cystic carcinoma of the sinonasal tract: treatment results. Laryngoscope. 2006;116(6):982-6.
76. Husain Q, Kanumuri VV, Svider PF, Radvansky BM, Boghani Z, Liu JK, et al. Sinonasal adenoid cystic carcinoma: systematic review of survival and treatment strategies. Otolaryngol Head Neck Surg. 2013;148(1):29-39.
77. Seong SY, Hyun DW, Kim YS, Cho HJ, Lee JG, Yoon JH, et al. Treatment outcomes of sinonasal adenoid cystic carcinoma: 30 cases from a single institution. Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery. 2013.
78. Clark WH, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969;29(3):705-27.
79. Elwood JM, Gallagher RP, Worth AJ, Wood WS, Pearson JC. Etiological differences between subtypes of cutaneous malignant melanoma: Western Canada Melanoma Study. J Natl Cancer Inst. 1987;78(1):37-44.
80. Lee JH, Choi JW, Kim YS. Frequencies of BRAF and NRAS mutations are different in histological types and sites of origin of cutaneous melanoma: a meta-analysis. The British journal of dermatology. 2011;164(4):776-84.
81. Greenwald HS, Friedman EB, Osman I. Superficial spreading and nodular melanoma are distinct biological entities: a challenge to the linear progression model. Melanoma Res. 2012;22(1):1-8.
82. McGuire LK, Disa JJ, Lee EH, Busam KJ, Nehal KS. Melanoma of the lentigo maligna subtype: diagnostic challenges and current treatment paradigms. Plast Reconstr Surg. 2012;129(2):288e-99e.
83. Cohen LM, McCall MW, Hodge SJ, Freedman JD, Callen JP, Zax RH. Successful treatment of lentigo maligna and lentigo maligna melanoma with Mohs' micrographic surgery aided by rush permanent sections. Cancer. 1994;73(12):2964-70.
84. Smalberger GJ, Siegel DM, Khachemoune A. Lentigo maligna. Dermatologic therapy. 2008;21(6):439-46.
85. Reed JA, Shea CR. Lentigo maligna: melanoma in situ on chronically sun-damaged skin. Arch Pathol Lab Med. 2011;135(7):838-41.
86. Stalkup JR, Orengo IF, Katta R. Controversies in acral lentiginous melanoma. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2002;28(11):1051-9; discussion 9.
87. O'Leary JA, Berend KR, Johnson JL, Levin LS, Seigler HF. Subungual melanoma. A review of 93 cases with identification of prognostic variables. Clinical orthopaedics and related research. 2000(378):206-12.
88. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2006;24(26):4340-6.
89. McLaughlin CC, Wu XC, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. Cancer. 2005;103(5):1000-7.

90. Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature. 2009;457(7229):599-602.
91. Van Raamsdonk CD, Griewank KG, Crosby MB, Garrido MC, Vemula S, Wiesner T, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med. 2010;363(23):2191-9.
92. Jethanamest D, Vila PM, Sikora AG, Morris LG. Predictors of survival in mucosal melanoma of the head and neck. Ann Surg Oncol. 2011;18(10):2748-56.
93. Clark WH Jr BE, Reed RJ, Kopf AW,. Acral lentiginous melanomas including melanomas of mucous membranes. In: Clark WH Jr., Goldman LI, Mastrangelo MJ, editors. Human malignant melanoma New York: Grune and Stratton, 1979:55-108.
94. McGovern VJ, Cochran AJ, Van der Esch EP, Little JH, MacLennan R. The classification of malignant melanoma, its histological reporting and registration: a revision of the 1972 Sydney classification. Pathology. 1986;18(1):12-21.
95. Ragnarsson-Olding BK, Kanter-Lewensohn LR, Lagerlof B, Nilsson BR, Ringborg UK. Malignant melanoma of the vulva in a nationwide, 25-year study of 219 Swedish females: clinical observations and histopathologic features. Cancer. 1999;86(7):1273-84.
96. Beadling C, Jacobson-Dunlop E, Hodi FS, Le C, Warrick A, Patterson J, et al. KIT gene mutations and copy number in melanoma subtypes. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008;14(21):6821-8.
97. Ballantyne AJ. Malignant melanoma of the skin of the head and neck. An analysis of 405 cases. Am J Surg. 1970;120(4):425-31.
98. Moreno MA, Roberts DB, Kupferman ME, DeMonte F, El-Naggar AK, Williams M, et al. Mucosal melanoma of the nose and paranasal sinuses, a contemporary experience from the M. D. Anderson Cancer Center. Cancer.116(9):2215-23.
99. Patel SG, Prasad ML, Escrig M, Singh B, Shaha AR, Kraus DH, et al. Primary mucosal malignant melanoma of the head and neck. Head Neck. 2002;24(3):247-57.
100. Prasad ML, Patel SG, Huvos AG, Shah JP, Busam KJ. Primary mucosal melanoma of the head and neck: a proposal for microstaging localized, Stage I (lymph node-negative) tumors. Cancer. 2004;100(8):1657-64.
101. Sobin LH, Compton CC. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. Cancer. 2010;116(22):5336-9.
102. Porceddu S, Martin J, Shanker G, Weih L, Russell C, Rischin D, et al. Paranasal sinus tumors: Peter MacCallum Cancer Institute experience. Head Neck. 2004;26(4):322-30.
103. Bristol IJ, Ahamad A, Garden AS, Morrison WH, Hanna EY, Papadimitrakopoulou VA, et al. Postoperative radiotherapy for maxillary sinus cancer: long-term outcomes and toxicities of treatment. Int J Radiat Oncol Biol Phys. 2007;68(3):719-30.
104. Khademi B, Moradi A, Hoseini S, Mohammadianpanah M. Malignant neoplasms of the sinonasal tract: report of 71 patients and literature review and analysis. Oral and maxillofacial surgery. 2009;13(4):191-9.
105. Syme J. Excision of upper jaw bones. Lancet. 1829; Lancet 1829(2):667-8.

106. Arnold A, Ziglinas P, Ochs K, Alter N, Geretschlager A, Ladrach K, et al. Therapy options and long-term results of sinonasal malignancies. Oral oncology. 2012;48(10):1031-7.
107. Robbins KT, Ferlito A, Silver CE, Takes RP, Strojan P, Snyderman CH, et al. Contemporary management of sinonasal cancer. Head Neck. 2011;33(9):1352-65.
108. Dauer EH, Lewis JE, Rohlinger AL, Weaver AL, Olsen KD. Sinonasal melanoma: a clinicopathologic review of 61 cases. Otolaryngol Head Neck Surg. 2008;138(3):347-52.
109. Kingdom TT, Kaplan MJ. Mucosal melanoma of the nasal cavity and paranasal sinuses. Head Neck. 1995;17(3):184-9.
110. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23.
111. Robert C, Thomas L, Bondarenko I, O'Day S, M DJ, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517-26.
112. Del Vecchio M, Di Guardo L, Ascierto PA, Grimaldi AM, Sileni VC, Pigozzo J, et al. Efficacy and safety of ipilimumab 3mg/kg in patients with pretreated, metastatic, mucosal melanoma. Eur J Cancer. 2014;50(1):121-7.
113. Cohn-Cedermark G, Mansson-Brahme E, Rutqvist LE, Larsson O, Johansson H, Ringborg U. Trends in mortality from malignant melanoma in Sweden, 1970-1996. Cancer. 2000;89(2):348-55.
114. de Vries E, Nijsten TE, Visser O, Bastiaannet E, van Hattem S, Janssen-Heijnen ML, et al. Superior survival of females among 10,538 Dutch melanoma patients is independent of Breslow thickness, histologic type and tumor site. Ann Oncol. 2008;19(3):583-9.
115. Hill VK, Gartner JJ, Samuels Y, Goldstein AM. The genetics of melanoma: recent advances. Annual review of genomics and human genetics. 2013;14:257-79.
116. Curtin JA, Fridlyand J, Kageshita T, Patel HN, Busam KJ, Kutzner H, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;353(20):2135-47.
117. Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, et al. Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res. 2011;17(2):229-35.
118. Shields JM, Pruitt K, McFall A, Shaub A, Der CJ. Understanding Ras: 'it ain't over 'til it's over'. Trends in cell biology. 2000;10(4):147-54.
119. Fecher LA, Amaravadi RK, Flaherty KT. The MAPK pathway in melanoma. Current opinion in oncology. 2008;20(2):183-9.
120. Omholt K, Karsberg S, Platz A, Kanter L, Ringborg U, Hansson J. Screening of N-ras codon 61 mutations in paired primary and metastatic cutaneous melanomas: mutations occur early and persist throughout tumor progression. Clin Cancer Res. 2002;8(11):3468-74.
121. Platz A, Egyhazi S, Ringborg U, Hansson J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Molecular oncology. 2008;1(4):395-405.
122. Edlundh-Rose E, Egyhazi S, Omholt K, Mansson-Brahme E, Platz A, Hansson J, et al. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res. 2006;16(6):471-8.

123. Omholt K, Platz A, Kanter L, Ringborg U, Hansson J. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin Cancer Res. 2003;9(17):6483-8.
124. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nature reviews Cancer. 2002;2(7):489-501.
125. Davies MA. The role of the PI3K-AKT pathway in melanoma. Cancer journal. 2012;18(2):142-7.
126. Kong Y, Si L, Zhu Y, Xu X, Corless CL, Flaherty KT, et al. Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011;17(7):1684-91.
127. Zebary A, Jangard M, Omholt K, Ragnarsson-Olding B, Hansson J. KIT, NRAS and BRAF mutations in sinonasal mucosal melanoma: a study of 56 cases. British journal of cancer. 2013;109(3):559-64.
128. Blackburn EH. Telomerase and Cancer: Kirk A. Landon--AACR prize for basic cancer research lecture. Molecular cancer research : MCR. 2005;3(9):477-82.
129. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. Nature medicine. 2006;12(10):1133-8.
130. Tian X, Chen B, Liu X. Telomere and telomerase as targets for cancer therapy. Applied biochemistry and biotechnology. 2010;160(5):1460-72.
131. Rankin AM, Faller DV, Spanjaard RA. Telomerase inhibitors and 'T-oligo' as cancer therapeutics: contrasting molecular mechanisms of cytotoxicity. Anti-cancer drugs. 2008;19(4):329-38.
132. Cong YS, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. Hum Mol Genet. 1999;8(1):137-42.
133. Egberts F, Kruger S, Behrens HM, Bergner I, Papaspyrou G, Werner JA, et al. Melanomas of unknown primary frequently harbor TERT-promoter mutations. Melanoma Res. 2014.
134. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
135. Daniel M, Peek GW, Tollefsbol TO. Regulation of the human catalytic subunit of telomerase (hTERT). Gene. 2012;498(2):135-46.
136. Mukherjee S, Firpo EJ, Wang Y, Roberts JM. Separation of telomerase functions by reverse genetics. Proc Natl Acad Sci U S A. 2011;108(50):E1363-71.
137. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957-9.
138. Ruden M, Puri N. Novel anticancer therapeutics targeting telomerase. Cancer treatment reviews. 2013;39(5):444-56.
139. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013;339(6122):959-61.
140. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546-58.
141. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27-33.
142. Mattsson B, Wallgren A. Completeness of the Swedish Cancer Register. Non-notified cancer cases recorded on death certificates in 1978. Acta Radiol Oncol. 1984;23(5):305-13.

143. Dickman PW, Adami HO. Interpreting trends in cancer patient survival. Journal of internal medicine. 2006;260(2):103-17.
144. Dickman PW, Sloggett A, Hills M, Hakulinen T. Regression models for relative survival. Stat Med. 2004;23(1):51-64.
145. Brenner H, Arndt V, Gefeller O, Hakulinen T. An alternative approach to age adjustment of cancer survival rates. Eur J Cancer. 2004;40(15):2317-22.
146. Statistical Yearbook of Sweden. Publication Services Statistics, Sweden, Annual Publications. Örebro.
147. Rachakonda PS, Hosen I, de Verdier PJ, Fallah M, Heidenreich B, Ryk C, et al. TERT promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. Proc Natl Acad Sci U S A. 2013;110(43):17426-31.
148. Marcus DM, Marcus RP, Prabhu RS, Owonikoko TK, Lawson DH, Switchenko J, et al. Rising incidence of mucosal melanoma of the head and neck in the United States. J Skin Cancer. 2012;2012:231693.
149. Koomen ER, de Vries E, van Kempen LC, van Akkooi AC, Guchelaar HJ, Louwman MW, et al. Epidemiology of extracutaneous melanoma in the Netherlands. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010;19(6):1453-9.
150. Sanghvi S, Khan MN, Patel NR, Yeldandi S, Baredes S, Eloy JA. Epidemiology of sinonasal squamous cell carcinoma: a comprehensive analysis of 4994 patients. Laryngoscope. 2014;124(1):76-83.
151. Schoenewolf NL, Bull C, Belloni B, Holzmann D, Tonolla S, Lang R, et al. Sinonasal, genital and acrolentiginous melanomas show distinct characteristics of KIT expression and mutations. Eur J Cancer. 2012;48(12):1842-52.
152. Hocker T, Tsao H. Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. Hum Mutat. 2007;28(6):578-88.
153. Lietin B, Montalban A, Louvrier C, Kemeny JL, Mom T, Gilain L. Sinonasal mucosal melanomas. European annals of otorhinolaryngology, head and neck diseases. 2010;127(2):70-6.
154. Hsu CP, Hsu NY, Lee LW, Ko JL. Ets2 binding site single nucleotide polymorphism at the hTERT gene promoter--effect on telomerase expression and telomere length maintenance in non-small cell lung cancer. Eur J Cancer. 2006;42(10):1466-74.