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ABSTRACT

BACKGROUND: Cutaneous melanoma in children and adolescents is a rare malignancy, and specific treatment guidelines have not been established for this patient group. Also, Spitzoid tumours in the young pose diagnostic challenges for the pathologists, and therefore influence on the planning of the treatments.

METHODS: Patients diagnosed with cutaneous melanoma at the age of 0–19 years diagnosed in 1990–2014 were retrieved from the Finnish Cancer Registry, and the primary tumour and metastasis samples of these patients were re-evaluated by two dermatopathologists. In addition, patients aged 15–39 years diagnosed with cutaneous melanoma in 1983–2011 were searched from the Turku University Hospital's database. Immunohistochemical analysis for BRAFV600E, ALK and PD-L1 were performed, and the clinical data of the patients was analysed.

RESULTS: The incidence of melanoma in 0–19-year-old patients increased approximately to 4-fold in Finland in 1990–2014. Melanomas in children and adolescents were often clinically amelanotic nodules. Melanomas in children and adolescents differed from each other and from melanomas in adults by their histopathological findings and genetic profiles (BRAFV600E, ALK) and PD-L1-positivity. Children have more often Spitzoid melanomas, but in adolescents, the number of other subtypes increases, and the melanomas in young adults resemble more of those described in adults. Despite more metastases found in the sentinel node biopsies, children and adolescent melanoma patients have better prognosis than adults. A subpopulation of adolescent and young adults with melanoma presented with other cancer or immunological disturbances.

CONCLUSIONS: Melanomas in children and adolescents differ from each other and from the melanomas in adults. Young adult melanoma patients may have pre-disposing conditions for melanoma.

KEYWORDS: Children, adolescents, young adults, Spitzoid melanoma, cutaneous melanoma

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TIIVISTELMÄ

TAUSTA: Ihomelanooma lapsella on harvinainen syöpäsairaus, eikä kyseiselle ryhmälle räätälöityjä hoitosuosituksia ole vielä laadittu. Lisäksi nuorilla tavattavien Spitzoidien kasvainten haastava tulkinta vaikeuttaa hoidon suunnittelua.

MENETELMÄT: Vuosina 1990–2014 diagnosoitujen 0–19-vuotiaiden ihomelanoomapotilaiden tiedot etsittiin Suomen syöpärekisteristä, ja kyseisten potilaiden kasvainten ja etäpesäkkeiden kudosnäytteet uudelleenanalysoitiin kahden ihopatologin toimesta. Lisäksi vuosina 1983–2011 diagnosoitujen 15–39-vuotiaiden potilaiden tiedot haettiin Turun yliopistollisen keskussairaalan tietokannasta. Näytteistä tutkittiin immunohistokemiallisesti BRAFV600E, ALK ja PD-L1 ilmentymät, sekä potilaiden hoitotiedot käytiin läpi.

TULOKSET: Havaitsimme ihomelanooman esiintyvyyden nousseen Suomessa noin nelinkertaiseksi vuosina 1990–2014 0–19-vuotiailla. Nuorilla potilailla melanoomat ovat myös usein kliinisesti pigmentittömiä (amelanoottisia) ja koholla olevia. Lasten ja teini-ikäisten melanoomat poikkeavat toisistaan ja aikuisten melanoomista histologialtaan ja BRAFV600E-, ALK- ja PD-L1-positiivisuudeltaan. Lapsilla tavataan aikuisia useammin Spitzoideja melanoomia, joilla vaikuttaa olevan ainakin toisinaan muita melanooman alatyyppejä parempi ennuste. Teini-iän myötä muiden alatyyppien osuus nousee, ja nuorten aikuisten melanoomat muistuttavat enemmän aikuisten melanoomia. Lasten ja nuorten melanoomien ennuste on aikuisilla kuvattua parempi huolimatta suuremmasta määrästä vartijaimusolmukeeseen metastasoineita tapauksia. Osalla teini-ikäisistä ja nuorista aikuisista melanoomapotilaista oli tiedossa myös toinen syöpäsairaus tai immuunijärjestelmän poikkeavuus.

JOHTOPÄÄTÖKSET: Lasten ja teinien ihomelanoomat poikkeavat toisistaan ja aikuisten melanoomista. Nuorilla aikuisilla melanooman taustalla voi olla altistavia tekijöitä.

AVAINSANAT: Lapset, teini-ikäiset, nuoret aikuiset, Spitzoidi melanooma, ihomelanooma

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Abbreviations

AJCC	American Joint Committee on Cancer
ALS	Amyotrophic lateral sclerosis
AYA	Adolescents and young adults
BOLD	Combination of dacarbazine, vincristine, lomustine, and bleomycin
CLND	Completion lymph node dissection
CSD	Cumulative sun damage
DFS	Disease free survival
DITC	Adjuvant combination therapy of IFN-α and Dacarbazine
ELND	Elective lymph node dissection
FCR	Finnish Cancer Registry
H&E	Haematoxylin and eosin
HR	Hazard ratio
IFN-α	Interferon alpha
IHC	Immunohistochemistry
NGS	Next-generation sequencing
NM	Nodular melanoma
OCD	Obsessive compulsive disorder
OS	Overall survival
SLE	Systemic lupus erythematosus
SLNB	Sentinel lymph node biopsy
SSM	Superficial spreading melanoma
TILs	Tumour infiltrating lymphocytes
TLND	Therapeutic lymph node dissection
TVEC	Talimogene Laherparepvec
WHO	World Health Organisation

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Rousi Emma, Koskivuo Ilkka, Kaarela Outi, Kääriäinen Minna, Kähäri Veli-Matti. Clinical and Pathological Aspects of Melanoma among Children in Finland. *Acta Dermato-Venereologica*, 2016; 96(5):718–20.

II Rousi Emma, Koskivuo Ilkka, Juteau Susanna, Talve Lauri, Hernberg Micaela, Vihinen Pia, Kähäri Veli-Matti. Different expression of BRAFV600E, ALK and PD-L1 in melanoma in children and adolescents: a nationwide retrospective study in Finland in 1990–2014. *Acta Oncologica*, 2021;60:165–72.

III Rousi Emma, Kallionpää Roope, Kallionpää Roosa, Juteau Susanna, Talve Lauri, Hernberg Micaela, Vihinen Pia, Kähäri Veli-Matti, Koskivuo Ilkka. Increased incidence of melanoma in children and adolescents in Finland in 1990–2014: nationwide re-evaluation of histopathological characteristics**.** *Annals of Medicine,* 2022;54:224–252.

IV Rousi Emma, Juteau Susanna, Talve Lauri, Kähäri Veli-Matti, Koskivuo Ilkka. Melanoma in Adolescents and Young Adults; Long Term Outcome, Co-Morbidities, Risk Factors and Pregnancy Status. *Manuscript.*

1 Introduction

Cutaneous melanoma in children and adolescents is a rare malignancy comprising of only 1% of all melanomas.[1,2] In young adults, melanoma becomes more common, but it is still not often encountered.[3–7] Paediatric melanoma has not been extensively studied, and therefore children and adolescents are treated similarly to adults.[1,2,8] Young adults, on the other hand, are found not to always benefit as much from the improvements in cancer treatments than adult patients.[9,10] Also, their melanomas are thought to resemble more melanomas of children and adolescents than those of adults.[9,11–14]

Melanoma in childhood was first time addressed in 1948, when a pathologist Sophie Spitz described a "juvenile melanoma", a melanocytic tumour resembling melanoma histologically but behaving less aggressively. Only one patient out of 13 died of the disease, which occurred in the sole of the foot of a 12-year-old girl. However, the tumour apparently did not involve the skin, and was described as a white lump excised from the plantar fascia.[15] This tumour has later been suspected to have been a clear cell sarcoma, a malignancy not yet described in the time of Sophie Spitz.[16,17] In this respect, in the set of 12 prepubertal patients there were no deaths due to "childhood melanoma".

Today, almost 80 years later, melanoma in children and adolescents is still challenging pathologists and clinicians.[1,17,18] Particularly difficult topic is distinguishing Spitzoid melanoma from its less aggressive mimic, atypical Spitz tumour. In the latest 2018 edition of WHO Classification of Skin Tumours, Spitzoid tumours are categorized in three classes of tumours; benign Spitz nevus, intermediately malignant atypical Spitz tumour and Spitz melanoma, also known as malignant Spitz tumor.[19] The Spitz melanoma described in WHO's latest classification is characterized mainly by kinase fusions, while other melanomas showing histopathologically Spitzoid features (Spitzoid melanomas) may possess known oncogenic driver mutations.[19,20] These Spitzoid melanomas do not appear to belong to the Spitz tumour -family, and resemble conventional melanomas by their more aggressive behaviour.[20] Since the diagnosis of Spitzoid melanoma is currently histopathological, and Spitz melanomas are impossible to distinguish histologically

from Spitzoid melanomas, the melanomas with Spitzoid features are referred as Spitzoid melanomas in this dissertation.

In this dissertation, melanoma in children, adolescents and young adults is studied with respect to epidemiology, clinical and pathological characteristics, potential therapeutic targets and its treatment and prognosis in Finland.

2 Review of the Literature

2.1 Cutaneous Melanoma

Cutaneous melanoma is a malignancy originating from melanocytes located in the skin. In healthy normal skin, melanocytes locate in the basal layer of the epidermis close to the dermo-epidermal junction. Melanocytes are responsible for the production of melanin, which causes skin and hair pigmentation and protects the skin from the UV-damage. Most of the melanin produced by the melanocytes is absorbed by the keratinocytes, and the extent of the pigmentation is dependent on the number of the melanosomes, whereas the number of the melanocytes is similar between individuals.[21] Mutated melanocytes proliferate first in the epidermis and superficial dermis (horizontal growth phase), and later penetrate deeper into the dermis (vertical growth phase) and, eventually, metastasise to the distant organs via lymphatic vessels, blood stream and perineural routes.[19] Most common site of metastasis is the skin, but also metastases in subcutaneous fat, distant lymph nodes, lung, liver, central nervous system and bone are often clinically present in patients with metastatic disease.[19,22] In autopsies, metastases in gastrointestinal tract, heart, pancreas, adrenal gland, kidney and thyroid gland are also commonly encountered.[22]

Incidence of cutaneous melanoma differs greatly between countries and continents. In Europe, the incidence of cutaneous melanoma is reported to be 13.8/100 000, and it has been increasing since the 1970's.[23] This increase in incidence is visible also in Finland, since the age-standardised incidence of cutaneous melanoma has increased from 12.1/100 000 in 1990–1994 to 24.9/100 000 in 2010–2014. The increase has been more prominent in men, and in 2015–2019 their incidence was already 35.3/100 000 compared to women whose incidence was 27.6/100 000. In 2015–2019, 8638 individuals were diagnosed with cutaneous melanoma in Finland, and 1093 persons died of metastatic melanoma during the same period. The incidence of cutaneous melanoma increases by age, and 64% of cases diagnosed in Finland in 2015–2019 occurred in patients over 60 years of age.[24]

Histological subtypes of cutaneous melanomas include superficially spreading melanoma (SSM), nodular melanoma (NM), Spitzoid melanoma, acral melanoma and lentigo maligna. Melanomas may occur also in the eye (uveal melanoma) or in

the mucosa (mucosal melanoma). The most common subtype in fair skinned populations is SSM comprising approximately 60% of all melanomas.[19] In the latest WHO classification, melanomas have been divided into nine categories based on their pathway of the pathogenesis. The first three pathways include I: superficial spreading melanoma (Low-Cumulative Sun Damage (CSD) melanoma), II: lentigo maligna melanoma (High-CSD melanoma) and III: Desmoplastic melanoma. These three melanomas arise in the sun exposed skin. The last six categories consist of IV: Malignant Spitz tumour (Spitz melanoma, a subclass of histologically Spitzoid melanomas), V: Acral melanoma, VI: Mucosal melanoma, VII: melanoma arising in congenital nevus, VIII: Melanoma arising in blue nevus and IX: Uveal melanoma. These melanomas are not known to be associated with UV-exposure. Nodular melanomas are melanomas of various pathways lacking the vertical growth phase in histopathological examinations.[19]

In Finland, melanoma is usually excised or biopsied by a general practitioner. After the histopathological diagnosis of melanoma is obtained, the patient is referred to a surgeon.[25] Surgical treatment includes an excision of primary tumour or biopsy scar with 1–2 cm margins according to the Breslow thickness of the tumour.[26] Sentinel node biopsy (SLNB) is routinely performed, if the Breslow thickness of melanoma is 1.0 mm or thicker. SLNB provides important prognostic information for staging purposes, yet SLNB or subsequent completion lymph node dissection (CLND) provide no survival benefit in patients with microscopic nodal disease.[27,28] Therefore, CLND has recently lost its role in sentinel-positive melanoma patients. In contrast, therapeutic lymph node dissection (TLND) is performed in patients with clinically detected lymph node metastases.[27–30] After surgery, further treatment plans and follow-up schedule are discussed in a multidisciplinary team (MDT) meeting including at least a surgeon, an oncologist, a pathologist, and a dermatologist.[25]

To obtain as accurate prognostic information as possible for each case, histopathological diagnosis report of the primary tumour according to Finnish Melanoma Group guidelines 2021 should include the following: Breslow thickness, Clark level, mitotic rate per mm^2, ulceration, excision margins, histological subtype, regression, tumour infiltrating lymphocytes (TILs), possible special features of invasiveness and the information on whether satellite or in transit metastases are present.[31] Melanoma staging is performed according to American Joint Committee on Cancer (AJCC) 8^{th} edition guidelines.[32] SLNB allows more precise nodal staging and personalised treatment decisions when planning medical adjuvant treatment for melanoma patients.[27–30] The most recent TNM classification of cutaneous melanoma according to AJCC 8^{th} edition is shown in Table 1, and melanoma staging system criteria is shown in Table 2 (both modified from Schadendorf et al. 2018).[23,32]

Table 1. TNM classification of cutaneous melanoma according to AJCC 8th edition criteria (modified from Schadendorf et al. 2018).

Stage	T classification	N classification	M classification
Stage 0	Tis	N0	M0
Stage 1A	T1a or T1b	N0	M0
Stage 1B	T2a	N0	M0
Stage 2A	T2b or T3a	N0	M0
Stage 2B	T3b, T4a, or T4b	N0	M0
Stage 2C	T0	N1b and N1c	M0
Stage 3A	T1a–b to T2a	N1a of N2a	M0
Stage 3B	T0	N2b, N2c, N3b, or N3c	M0
Stage 3B	T1a–b to T2a	N1b–c or N2b	M0
Stage 3B	T2b–T3a	N1a–N2b	M0
Stage 3C	T1a–T3a	N2c or N3a–c	M0
Stage 3C	T3b–T4a	Any N ≥N1	M0
Stage 3C	T4b	N1a–N2c	M0
Stage 3D	T4b	N3a–c	M0
Stage 4	Any T and Tis	Any N	M1

Medical oncological adjuvant therapies available for the patients in Finland include adjuvant treatments with immuno- or targeted therapies, and in rare cases adjuvant radiotherapy. Patients with advanced melanoma may be treated with immuno- or targeted therapies, chemotherapies, and less frequently with isolated limp perfusion treatment or Talimogene Laherparepvec (TVEC) -therapies.[31]

The prognosis of melanoma is stage dependent, and therefore a rigorous diagnostic and staging approach is of utmost importance.[23] This applies also for the younger patients, in whom the diagnosis of melanoma is often challenging.[1,33] In this respect, new diagnostic tools such as reliable biomarkers are needed to ensure best possible treatment also for the youngest patients.

Table 2. Cutaneous melanoma staging according to AJCC 8th edition criteria (modified from Schadendorf et al. 2018).

	T classification	N classification	M classification
	T1	**N0**	**M0**
A	<0·8 mm thickness, no ulceration	No regional lymph nodes affected	No distant metastasis
B	0·8–1 mm thickness (<0·8 mm with ulceration)		
	T2	**N1a–c**	**M1a**
A	>1–2 mm thickness, no ulceration	One lymph node affected, micro-metastasis or macro-metastasis, or in-transit or satellite metastasis	Distant metastasis (skin)
B	>1–2 mm thickness with ulceration		
	T3	**N2a–c**	**M1b**
A	>2–4 mm thickness, no ulceration	Two to three lymph nodes affected, or at least one lymph node affected and in-transit or satellite metastasis	Distant metastasis (lung)
B	>2–4 mm thickness with ulceration		
	T4	**N3a–c**	**M1c**
A	>4 mm thickness, no ulceration	At least four lymph nodes affected or at least two lymph nodes affected and matted nodes	Distant metastasis (non-CNS)
B	>4 mm thickness with ulceration		
			M1c
			Distant metastasis (CNS)

2.2 Epidemiology of Melanoma in Children, Adolescents and Young Adults

2.2.1 Incidence

The incidence of melanoma in children and adolescents has been reported to increase in Europe and in the USA towards the 21st century, especially among adolescents.[1,2,34,35] The increase in the incidence has been rapid, and no evident reason for this change has been identified. In Sweden, the incidence of melanoma in adolescents was rising since the 1970's and started to decrease around millennium [35,36]. Also, in the United States, the increase was observed until 2004, but after that it has been reported to decline especially in adolescents.[37–39] Similar trend was observed also in the Netherlands.[34]

In adolescents and young adults aged 15–39 years (AYA), the incidence of cutaneous melanoma has also been increasing in especially females during last decades in the USA, but the incidence has started to decline after reaching its peak in 2004–2005.[3,39] The decrease in melanoma incidence is mostly seen in patients under 30 years old, whereas individuals aged 30–39 years have had a relatively stable incidence, and has even slightly increased.[39,40] In 2010–2015, the incidence of melanoma in young adults was approximately 30/1 000 000 in males and 70/1 000 000 in females among individuals aged 20–29 years, and approximately 90/1 000 000 in males and 140/1 000 000 in females among the 30–39 year olds based on the graphs presented by Paulson et al.[39]

The rates of the age-adjusted incidences of melanomas in children and adolescents vary between countries. In Sweden, the mean age-adjusted incidence for individuals aged less than 20 years decreased to 3.6/1 000 000 in 1993–2002 from 5.0/1 000 000 in 1983–1992.[36] In the USA, the age-adjusted incidence has been reported to be 5–6/1 000 000 among children and adolescents. [2,37,41,42] The incidence of melanoma among Dutch children and adolescents is similar to that of the USA.[34] In Germany, the incidence has not shown increasing or decreasing trends in 1983–2011.[43] However, the German study might have had underrepresentation of Spitzoid melanomas, since atypical tumours and tumours with uncertain malignant potential were excluded from the analysis, and the number of Spitzoid melanomas was relatively low.

In prepubertal children, melanoma occurs a bit more often in boys, but in adolescents, more often in girls.[35,44–48] However, one study reported male predominance in the adolescent group.[49] In AYA, melanomas are more often present in female patients.[3,5] Especially prepubertal patients are also more often non-white.[46,50]

Changes in protective behaviour towards sun exposure has been suggested as a possible reason for these changes in the incidence of melanomas in children and adolescents, as well as restrictions in the use of tanning beds in the United States.[8,35,36] The association with the increasing risk and UV-exposure has been demonstrated among adolescents. In a study based on the SEER-database, Hamre et al. (2002) showed increasing incidence of cutaneous melanoma among 10–19 years old fair skinned patients when their place of residence approached the southern parts of the USA.[42] However, the role of UV-exposure in melanoma development is unclear in smaller children. Another possible contributing factor for changing incidence levels could be evolving histopathological diagnostic criteria for Spitzoid melanomas, resulting into possible over- or under diagnostics of these rare tumours.[1,20,43]

2.2.2 Risk Factors

Several factors have been associated with an increased risk of melanoma in children and adolescents. These include fair complexion, red or blond hair, light eye colour, inability to tan and several sunburns, increased number of benign nevi, congenital nevi, family history of melanoma, disorders of DNA repair, immunosuppression, and a previous history of malignancy.[2,44,51–53] A Finnish study identified high nevus count, dysplastic nevi, family history of melanoma and previous melanoma as risk factors for melanoma in young adults, similarly to middle-aged individuals of the same study. However, sunburns and sun sensitive skin type were not as often reported in the young adults than among the middle-aged patients.[54]

Known risk factors for melanoma, such as UV-radiation seem to apply also to AYA patients, but detailed studies on possible co-morbidities or genotypic traits predisposing to melanoma have not been extensively studied within this population.[3,5–7,55,56] Female AYA patients are also in their child-bearing age, and possible effect of pregnancy in the development of melanoma is still in the need of further research.[57,58]

In a Californian study on melanoma patients aged 0–29 years, high birth weight and being born in a location of high UV-exposure was associated with increased risk for melanoma in this age group of non-Hispanic individuals. The risk of melanoma according to possible early-life UV-exposure was most evident in adolescents. In the same study, low birth weight was found to be a protective factor form melanoma, especially in females.[59] Also other studies performed with European population have demonstrated the risk of melanoma increasing by birth weight.[60–62]

In an Australian study, UV exposure in childhood was associated with increased number of melanomas in young adults.[63] Supporting the evidence of especially severe sunburns experienced during childhood being a risk factor for melanoma,

high UV-dose applied on the skin of transgenic mice neonates with skin resembling human skin induced the development on melanocytic tumours resembling human melanoma, but similar effect was not seen in adult transgenic mice.[64]

2.3 Clinical Characteristics of Melanoma in Children, Adolescents and Young Adults

2.3.1 Clinical Characteristics

Clinical appearance of paediatric melanoma differs from that of the adults, especially among the youngest patients. A large proportion of the melanomas in children are clinically amelanotic and elevated, and could resemble for example pyogenic granulomas.[65,66] Melanomas in prepubertal children locate most often in the extremities and in the head and neck area, whereas melanomas in adolescents occur more often in the trunk and in the extremities, similarly to young adults.[3,5,46,49,50,67]

In young adults, melanomas are more often conventional (SSM or NM), and challenges in clinical diagnosis of this population has not been reported to be particularly challenging.[3,5] However, in non-white individuals, melanomas often present in non-sun exposed and unconventional sites, such as in acral, subungual, mucosal areas or in the nails.[68,69] For example, plantar melanoma accounts for approximately 60% of melanomas of black individuals, but its incidence is not reported to be higher than in non-black patients, but the high percentage is rather explained by the rarity of melanomas in other body sites in this patient population.[69,70] Nevertheless, black individuals are reported to often experience diagnostic delays and misdiagnoses due to low suspicion of melanoma, which might have led to the fact that in many cases, melanoma has already metastasised at the time of diagnosis.[71–73] Since non-white patients compose a considerable part of paediatric melanoma patients, clinically atypical melanomas may be present more often in also young adult patients.

2.3.2 ABCDE-Criteria in Young Patients

The melanomas of children and adolescents do not often follow the traditional ABCD (A=asymmetry, B=Border irregularity, C=colour variegation, D=Diameter >6 mm, E=evolution) -criteria.[49,65] Large number of melanomas in the young are clinically amelanotic, skin coloured or pink nodules or bumps, which often appear *de novo*.[49,67] These features apply best for Spitzoid melanomas, which are often located in the limbs.[67] The use of modified ABDC -criteria of "A=amelanotic, B=bleeding, bump, C=Colour uniformity, D=De novo, any Diameter" together with the traditional ABCD -criteria have been suggested to be used in melanomas in

children and adolescents.[49] Recently, the letter "E" for evolution has been suggested to be included into the modified criteria.[18]

2.3.3 Dermoscopy

Dermoscopy is a method, in which skin can be observed and imaged via magnifying lens, facilitating the clinical diagnostics of e.g., melanocytic tumours. Dermoscopy is a common tool of the dermatologists. Specific dermoscopic patterns have been described in melanomas of children and adolescents.[67] Carrera et al. divided melanomas by their histopathological features into Spitzoid and non-Spitzoid melanomas, the latter consisting mostly of superficial spreading type melanomas. Non-Spitzoid melanomas are also known as adult- or conventional-type melanomas.[67,74,75] However, Spitzoid tumours can be impossible to distinguish from melanoma in dermoscopy, as shown by Lallas et al. (Fig. 1).[76] Spitzoid melanomas were diagnosed in younger patients, and they presented with atypical vascular patterns and shiny white lines, or with an atypical pigmented Spitzoid pattern in dermoscopy. Non-Spitzoid melanomas had often multicomponent or nevus-like patterns. Non-Spitzoid melanomas were also found in older patients than Spitzoid melanomas, and they had developed into pre-existing nevus in most cases.[67]

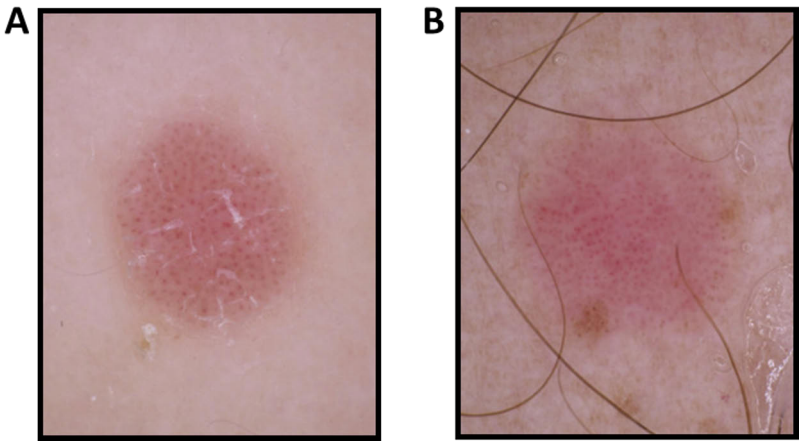

Figure 1. Dermoscopy image of A: amelanotic Spitz nevus and B: amelanotic melanoma may appear alike. (Modified from Lallas et. al. 2015, reprinted with the permission of Elsevier.)

2.4 Pathology and Genetics of Melanomas in Children, Adolescents and Young Adults

2.4.1 Spitzoid Melanoma

Cutaneous melanomas found in children and adolescents are Spitzoid melanomas, superficial spreading melanomas, nodular melanomas and rarely, acral melanomas.[1,2,17,18,20,36,49,67,77] At times, lentigo maligna melanomas can be found in young adults.[3] Spitzoid melanomas are the most common subtype in prepubertal children and are often found also in adolescents and young adults[1,2,78]. Spitzoid melanomas represent diverse group of often clinically nodular melanomas that are commonly amelanotic.[19,20] Spitzoid melanomas are often wedge-shaped and share cytologic features with Spitz nevus and atypical Spitz tumour, and often pose diagnostic challenges for pathologists.[19,20,33,77,79,80] However, several features associated with malignant behaviour are described in the 4th edition of WHO Skin Tumours, such as asymmetry, poor circumscription, ulceration, irregular and confluent nesting, extensive Pagetoid spread, effacement of epidermis, lack of cellular maturation, deep, marginal or atypical mitoses and tumour necrosis. In children, >6 dermal mitoses/mm^2 and >2 dermal mitoses/mm^2 in adults suggest for the diagnosis of Spitzoid melanoma over atypical Spitz tumour.[19]

Genetic profile of Spitzoid melanoma is still under investigation, but mutually exclusive kinase fusions of ROS, ALK, BRAF, NTRK1, NTRK3, MET and RET are often present in these melanomas. These kinase fusions are found more often in younger patients with Spitzoid melanoma.[19,81,82] BRAF, NRAS and HRAS mutations are reported to be rare in Spitzoid melanomas. Chromosomal copy number alterations, TERT promoter mutations and PTEN mutations are observed in these melanomas.[19,79,81,83] A distinct subtype of Spitzoid melanomas called Spitz melanoma has been defined and is regarded to be of the same lineage with other Spitz tumours. These tumours harbour gain-of-function mutation of HRAS and loss-of-function mutation in CDKN2A.[19,79] In Spitzoid melanomas, however, p16 is often expressed.[84] Spitzoid tumours with MAP3K8 fusions are reported to be often found in young adults.[85] MAP3K8 fusions are reported to be present in 33% of Spitzoid melanomas, and these often amelanotic nodules found also in children may be sensitive to MEK inhibitors.[86,87]

As evident, there are still many undiscoverable factors regarding Spitzoid melanoma and its diagnosis.[81] In the future, novel approaches such as micro-RNA profiling could assist pathologists and clinicians in their decisions for diagnosis and treatment of these rare tumours.[88] HE-staining of Spitzoid melanoma is shown in Figure 2.

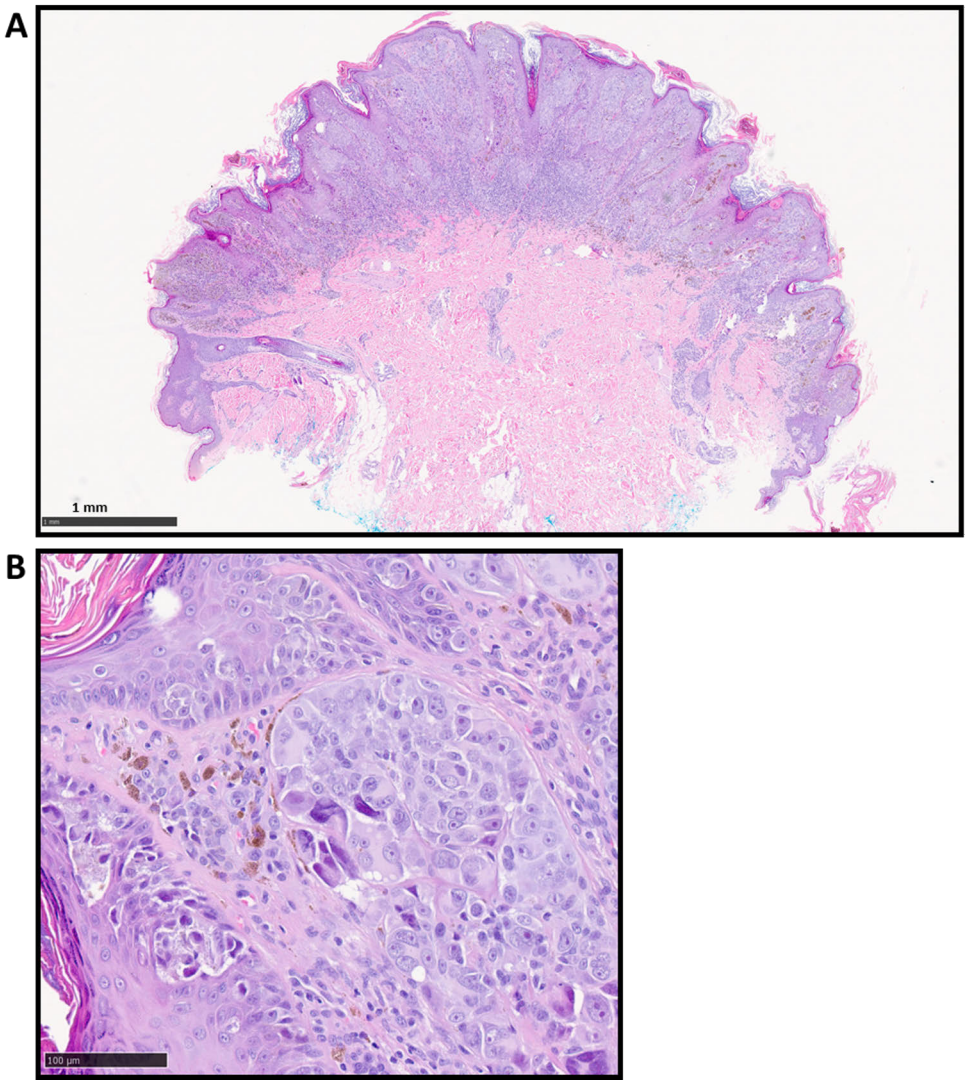

Figure 2. **A**: Melanoma developed into gluteal region of a prepubertal patient was of Spitzoid histology. **B**: 20x magnification of the same tumour. Haematoxylin and Eosin (H&E) staining. © Emma Rousi

2.4.2 Superficial Spreading Melanoma

Many, but not all of the melanomas are though to develop through mutations accumulating into benign precursor lesions, such as into benign melanocytic nevus.[74,75] This is most evident in the superficial spreading type, in which the association with benign melanocytic nevus is visible in histopathological examination in approximately 30% of cases.[19] SSMs are so-called low cumulative

sun damage (low-CSD) melanomas, and also the most common melanoma subtype in the adult population.[19,74,75] In children and adolescents, SSMs are more often found in the adolescent patients, and their mutational burden with many point-mutations refers to UV radiation exposure.[19,74,89] They usually arise in the intermittently sun exposed skin, and are thought to result from the accumulation of mutations caused by sunburns, especially by those experienced in the childhood.[19,74]

In histological examination, SSMs show pagetoid spread and nesting in the epidermis, and variable number of invasive melanocytes in the dermis forming an invasive radial growth phase of the tumour. The invasive vertical growth phase of the melanoma is seen as expansile nests with mitoses in the dermis. Variable number of TILs are visible, and microscopic satellite metastases may be detected in the dermis.[19]

BRAFV600E mutations, which activate MAPK-pathway are often found in SSMs, together with TERT promoter mutations. Mutations in TERT gene or in its promoter prevent telomere shortening in cell division, and by this mechanism result into extended lifespan of the mutated cell by preventing its senescence, thus allowing it to replicate and to obtain a rising number of mutations.[19,74,75,90,91] BRAFV600E mutations on the other hand are often found from benign melanocytic nevi, and are shown to develop in the early phase of melanoma development, resulting into development of a precursor lesion. When this precursor lesion, such as benign melanocytic nevus gains more mutations, it evolves into malignant melanoma through variable levels of dysplasia.[19,74,75]

Biallelic loss of familial melanoma syndrome associated CDKN2A, which encodes tumour suppressive protein p16, is commonly found in SSMs.[19,74,74,92] Other mutations found in the more advanced disease stages are PTEN and TP53.[19,74,75] Also losses in chromosomes 9, 10, 6q and gains of 1q, 6p, 7, 8q, 17q and 20q are frequent in SSMs.[19] Histological image of SSM is shown in Figure 3. Common genetic alterations of SSM and Spitzoid melanomas are shown in Table 3.

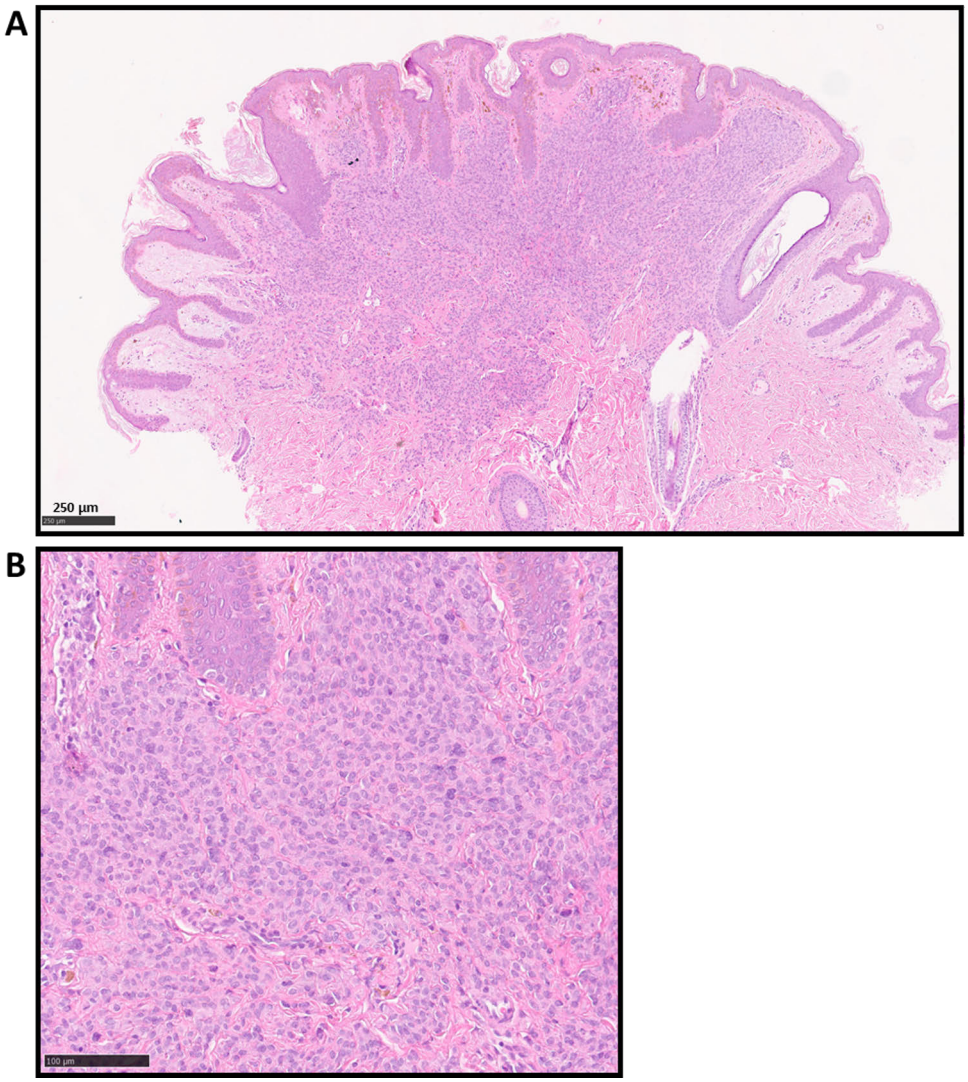

Figure 3. **A**: H&E staining of a superficial spreading melanoma located in the trunk of an adolescent patient. **B**: 20x magnification of the same tumour. © Emma Rousi

2.4.3 Nodular Melanoma

Nodular melanomas are histopathologically heterogenous group of melanomas, which lack the radial growth phase in histopathological examination. They are clinically and histopathologically diverse group with their appearance ranging from very dark, hyper-melanotic nodules to the amelanotic ones.[19] The genetic profile of nodular melanomas is diverse and overlapping with the other subtypes presenting histopathologically detectable radial growth phase, such as SSM. Therefore, it is

thought to represent an accelerated evolutional trajectory of the other melanoma subtypes rather than being a separate entity.[19,74,75] Nodular melanoma of an adolescent patient is shown in Figure 4.

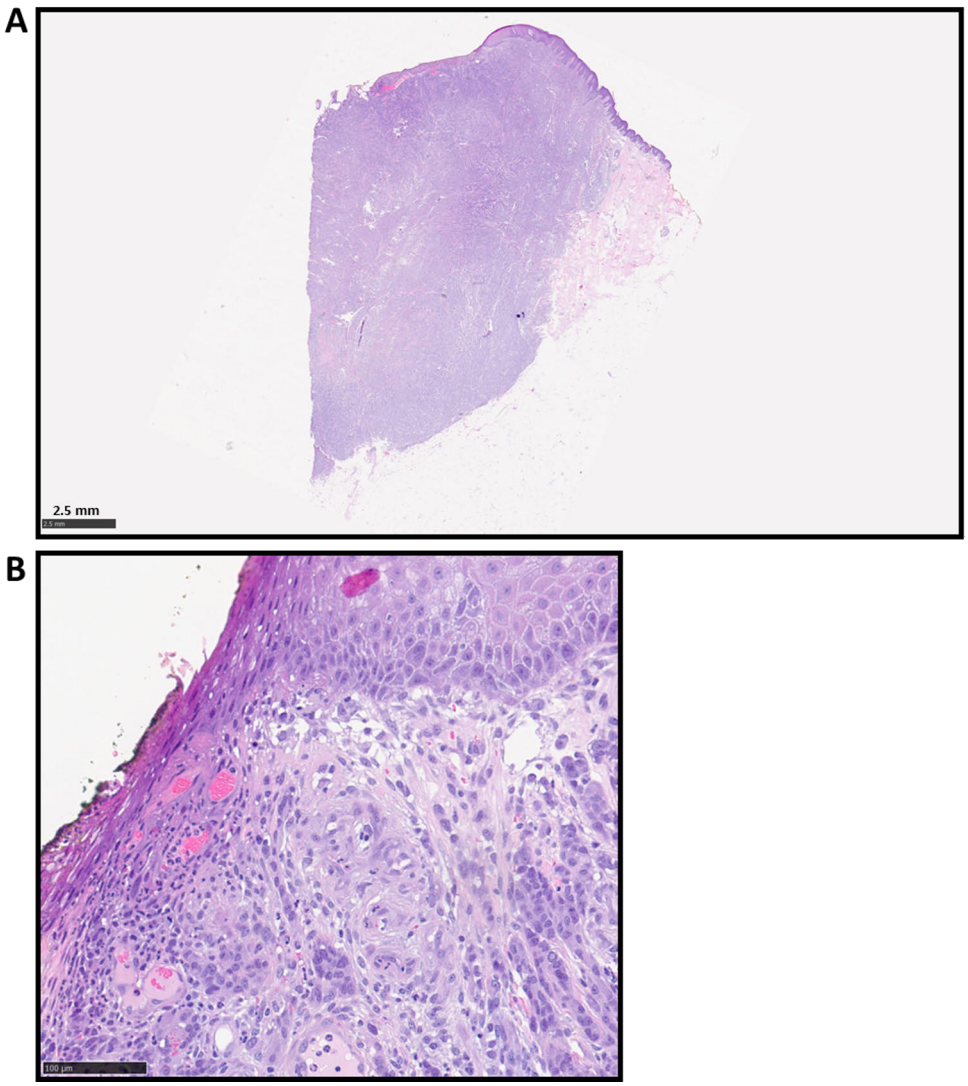

Figure 4. **A**: Thick, Breslow 15.3mm nodular melanoma in the lower limb of an adolescent shows extensive vertical growth phase without detectable radial growth phase. **B**: 20x magnification of the tumour. © Emma Rousi

Table 3. Common genetic aberrations found in superficial spreading melanomas (SSM) and in Spitzoid melanomas. (Modified from WHO Classification of Skin Tumours 4th edition, Elder at al. 2018)

	SSM	Spitzoid
Common mutations	BRAF NRAS TERT CDKN2A TP53 PTEN	HRAS TERT PTEN
Kinase fusions	Rare	MAP3K8 ALK ROS RET NTRK1 NTRK3 BRAF MET
Chromosomal abnormalities	losses at 9, 10, 6q and 20 gains of 1q, 6p, 7, 8q and 20q	May be observed

2.4.4 Melanoma Developed into Congenital Nevus

Melanomas developing into congenital nevi are often diagnosed in childhood or in adolescence and may occur together with a condition called neurocutaneous melanosis.[1,93] Congenital nevi arise from a mutation occurring *in utero* resulting into mosaicism of varying extent.[93] Approximately 90% of these mutations are NRAS mutations, which also act as driver mutations in melanoma. In contrast, cancer disposing mutations such as CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP and TERT in familial melanomas are germline mutations and are present in all the cells of the body.[94] Congenital nevi are classified as small (<1.5 cm), intermediate (1.5–20 cm) or giant (>20 cm).[19] Despite their benign nature, lifetime melanoma risk in congenital nevus is approximately 1%. However, the risk is even 10–15% in very large congenital nevi, and very low in the small nevi. Sometimes the proliferative nodules in congenital nevus may be challenging to differentiate from malignant melanomas. Children with multiple congenital nevi especially together with a neurological condition have increased risk for melanoma in childhood.[93]

2.4.5 Prognostic Factors

Breslow thickness (Tumour thickness in mm from top of the epidermis into the most invasive malignant cells measured from the deepest part of the melanoma in histopathological examination) of melanomas among young patients has been reported to be higher than in the adults.[45,50,65,66,89,95,96] However, this does not correlate as strongly with worsened prognosis as it would in adult melanoma

patients.[45,46,95,96] Similar discrepancy is also seen with the extent of the disease, since children and adolescents with higher stage of the disease have shown improved survival compared to adults with the same disease stage.[45,95–97] In contrast to this, Lange et al. noted in their study based on National Cancer Database of the USA, that patients aged 0–9 years had the worst prognosis.[46] This could be explained with the higher number of prepubertal non-white patients, who experience more often diagnostic delays. Since melanoma is far less common in non-white individuals, these children may have had a pre-existing condition such as giant congenital nevi, which are shown to have less favourable prognosis.[1,93,96,98,99]

Especially Spitzoid melanomas have been noted to have higher Breslow thickness and higher disease stages compared to other melanoma subtypes. These melanomas seem to metastasise more often into sentinel lymph nodes without necessarily developing distant metastases and worsening the prognosis.[1,17–19,100] This could be related to the fact that atypical Spitz tumours often metastasise into regional lymph nodes without worsening the prognosis.[101] On the other hand, conventional melanomas (SSM and NM) in children and adolescents seem to behave clinically similarly to adult melanomas.[1,89]

Mu et al. compared sentinel lymph node positivity and clinical characteristics of children and adolescent melanoma patients with those of young adults aged 20–24 years and noted that the younger group had more often positive sentinel node. In patients aged 0–19 years, 24% had positive SLNB, whereas 15% of patients aged 20–24 had a positive node. In non-ulcerated melanomas with 1.01–2.00 mm in thickness, the difference between paediatric and young adult patients was significant, 24% vs. 4%. Regional disease was found in 24% of patients in prepubertal children, in 15% in 10–19-year-olds and in 8% in 20–24-year-olds. Young adult patients had also significantly more often thin melanomas, and SSM was much more common subtype in the older patient group. In prepubertal children, 20% of the patients had ≥4 mm thick melanomas compared to 3% of the young adult group.[50]

Some genetic mutations in melanomas might have prognostic value. For example, biallelic loss of CDKN2A occurring together with MAPK-pathway activating mutations and TERT-mutation can suggest higher level of invasiveness. CDKN2A encodes INK4A, which acts as a tumour suppressor. Also, mutations in SWI/SNF chromatin-remodelling complex, such as ARID2 and ADRI1A are seen to emerge at the transition from the precursor lesion to invasive melanoma. SWI/SNF complex is suggested to be associated with DNA repair.[19,75]

2.5 Treatment of Melanoma in Children, Adolescents and Young Adults

2.5.1 Surgical Treatment

In the melanomas of the young, the first line treatment is surgical excision similarly to adult patients. In Finland, the excision is usually performed in general anaesthesia in the small children, and in local anaesthesia in the older children and adolescents. In the international literature, SLNB is often performed, and is possibly followed by the completion lymph node dissection (CLND).[18,102] Since the rate of local recurrence has been reported to lower in children than in adults, it has been suggested that the excision margins may be sometimes few millimetres smaller, if the surgery would compromise the esthetical or functional outcomes.[18,23,66] Paediatric patients have often been excluded from clinical trials, and therefore their surgical treatment is based on the adult guidelines.[1,18]

The standard surgical treatment for children and adolescents as well as for adults is the wide excision of melanoma with 1-2cm margins, corresponding the Breslow thickness of the tumour.[1,18,102–104] Also in young patients, the primary tumour should be first excised in narrow margins to obtain the diagnosis. Using this method, the lymph vessels of the region are preserved for possible SLNB. In case the narrow excision is challenging to perform for some reason, incisional biopsy of the tumour may be performed.[18] Schematic illustration of different excision types are shown in Figure 5.

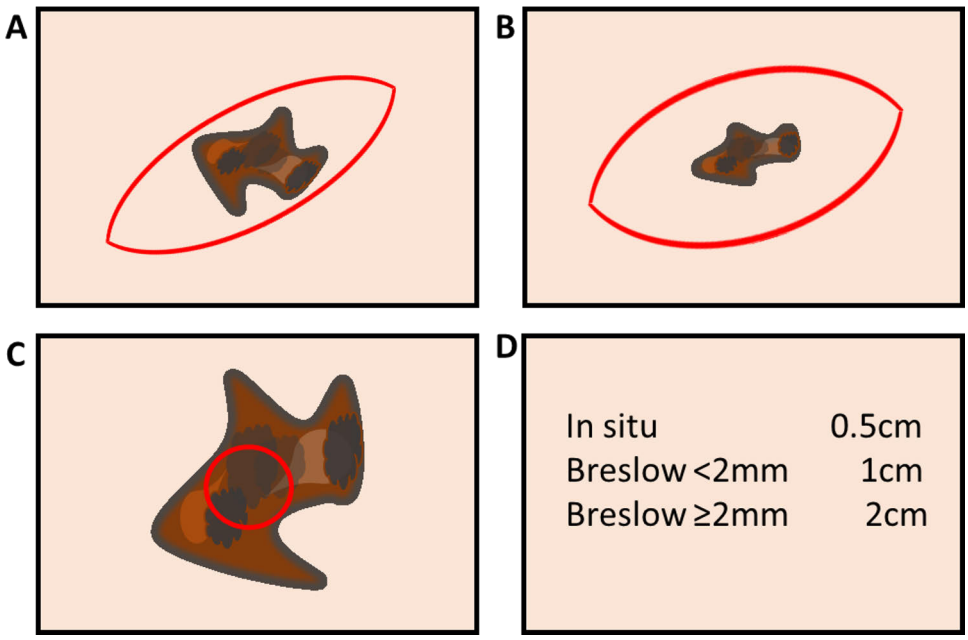

Figure 5. Different excision types in cutaneous melanomas. **A**: Narrow diagnostic excision allows the lesion to be analysed histopathologically as a whole and preserves the lymphatic vessels for possible SLNB. **B**: Wide local excision with 1–2 cm margins is performed after lymphoscintigraphy as a primary surgical treatment. **C**: If the lesion is very large and/or located in a challenging anatomical site, incisional (punch) biopsy may be obtained for diagnostic purposes. Excisional margins are chosen based on the Breslow thickness of the tumour (**D**) but may be slightly altered to ensure satisfactory functional and aesthetic outcome.

2.5.2 Sample Preparation Process

After its surgical excision in the clinics, the tumour sample is immediately fixated in formalin to be used in (histo)pathological analyses. The pathological sample preparation process starts with inspection of the sample as a whole and marking its borders with different colours in larger samples, which allows its spatial identification after sectioning. The sample is then dehydrated using increasing concentrations of ethanol and embedded in paraffin to obtain formalin fixed paraffin embedded (FFPE) block, which is then cut into thin sections with a microtome and mounted on a microscopic glass slide.[105,106] For immunohistochemical (IHC), the stainings are usually performed using an automated staining instrument and analysed by the pathologist with the light microscope. In addition to H&E staining, common IHC analyses performed include BRAFV600E, PD-L1, Melan-A, S-100, HMB-45, Ki-67 and p16.[106–108] For genetic analyses, next generation sequencing (NGS) panels are a common choice. These panels comprise of the known mutations in melanoma, such as BRAF, KRAS and NRAS-mutations. Along with targetable mutations, these

may include mutations with prognostic value. The samples are punched out from the FFPE block with 1mm puncher from the areas where the pathologist has marked to be of highest density of the tumour cells. From the punched sample, DNA is extracted and used for NGS analyses.[109,110]

2.5.3 Sentinel Lymph Node Biopsy

In children and adolescents, SLNB is recommended for the patients with ≥1 mm Breslow thickness according to the adult guidelines.[18,102,103] However, the higher rate of SLNB positive patients in the young poses treatment challenges for surgeons. It is not clear, which patients should undergo CLND, and who could be safely monitored with clinical examination and ultrasound.[1,18] In prepubertal children, there seem to be no difference in the overall survival (OS) based on the lymph node status, but in adolescents, the difference emerges.[111] Large SEER database based propensity-score matched analysis of children and adolescents with or without node biopsy/dissection performed showed no differences in survival rates.[112] Another large study of 310 patients younger than 20 years found also no difference in OSs of patients with or without SLNB.[113] However, the authors of these studies regard SLNB as an important staging tool in young patients, which aids the oncologists in the planning of medical oncological therapies.[112,113]

In a large MSLT-II trial with adult patients, CLND performed immediately after positive SLNB did not give survival benefit for the patients over dissecting the lymph nodes later if metastatic nodes are found during the follow-up. Therefore, follow-up with ultrasound and clinical examination is now recommended instead of routine CLND to reduce morbidity (e.g., swelling, pain, functional and aesthetic impairment) resulting from performing lymphatic surgery. In contrast, therapeutic lymph node dissection (TLND) is performed when clinically detected metastatic nodes are present.[28,114]

2.5.4 Medical Oncological Therapies

Medical oncological therapies given in the past and in the present for children and adolescents with melanoma reported in the literature consist of adjuvant or non-adjuvant interferon alpha (INF-α) -treatment and of possible chemo-, targeted- and immunotherapies.[1,8,18,115–117] These treatments have been used also in adult melanoma patients.[31,102] As clinical studies in paediatric cancer patients show, the responses may differ from those observed in adults.[117] Regarding melanoma, several clinical trials are currently recruiting also children and adolescents, which may bring new treatments available also for the youngest patients.[1] Enrolling at least adolescents in the adult clinical trials have been suggested to deliver new treatments

faster also to the young, since low enrolment in clinical trials of the young patients due to rarity of melanomas in this age group challenges completing these studies. Also, the approval of novel therapies in adults leads to off-label use of those in children and adolescents, after which clinical trials are not feasible anymore.[118]

The survival of the patients with metastatic melanoma has improved significantly after 2011 along with the approvals of new targeted immunotherapies.[119–122] The discovery of BRAF mutations in approximately half of the primary melanomas enabled the development of BRAF/MEK inhibitors, which greatly increased the survival of the patients harbouring BRAFV600 mutations.[121,123] In addition to the BRAF/MEK inhibitors, the development of new checkpoint inhibitors, CTLA-4, PD-1 inhibitors further increased the survival of the melanoma patients in the following years.[119,120,122]

While combining several therapies may improve the patient's survival from melanoma, the side effects will also accumulate when using several therapies simultaneously.[120,121,123] Therefore, it is needed to develop suitable biomarkers for finding the right patients for each combination.[120]

2.5.4.1 BRAFV600E

B-Raf serine/threonine kinase, also known as BRAF proto-oncogene, is a part of the mitogen/activated protein kinase (MAPK) or extracellular signal regulated (ERK) pathway RAS/RAF/MEK/ERK. This pathway is activated by extracellular stimuli, such as growth factors, this way regulating the proliferation, differentiation, and the survival of the cells. Along with BRAFV600E mutation, B-Raf becomes constantly activated without external signals, and this leads to constitutive activation of ERK MAPK signalling pathway and in unbalanced proliferation of the mutation harbouring cell population.[123] However, the mutations in BRAFV600 site are common also in benign nevi.[124] Most common BRAF mutation is BRAFV600E, which is found from approximately 85% of BRAF mutated melanomas. The second most common BRAF mutation is BRAFV600K, accounting approximately 8% of BRAF-mutated melanomas.[125,126] A current opinion is, that in addition to MAPK-pathway mutations, the melanocytes need also another genetic or epigenetic changes, such as mutations in one of the tumour suppressor genes, to finally evolve into a malignant melanoma.[19,74,75,123]

Vemurafenib was the first BRAF inhibitor that showed significant clinical benefit in melanoma treatment, but complete response rates still remained low.[127–130] The second BRAF inhibitor in clinical use, dabrafenib, has also demonstrated with improved survival among patients with BRAF mutated melanomas.[131] When used in patients with BRAFV600E and BRAFV600K mutated melanomas, combination therapy of dabrafenib and MEK inhibitor trametinib have shown benefit over

monotherapy with dabrafenib.[132] In vemurafenib and dabrafenib therapies, BRAFV600E or BRAFV600K positivity is strongly associated with improved survival.[133,134] In this respect, IHC analysis of BRAFV600E in melanomas is important and feasible in clinical setting, and BRAF mutational status is also recommended for being routinely analysed from primary melanomas by Finnish Melanoma Group guidelines 2021.[31,135] Combining BRAF/MEK inhibitors with immunotherapies have benefitted at least a subgroup of patients with BRAF-positive melanomas despite increased toxicity, but clinical trials on this patient population are still ongoing. This might be beneficial approach to reduce treatment resistance commonly occurring in use of BRAF/MEK inhibitors, which is generally seen after one year after treatment initiation.[136,137] Schematic illustration on RAS/RAF/MEK/ERK pathway is shown in Figure 6 (Modified from Singh et al. 2016).[138]

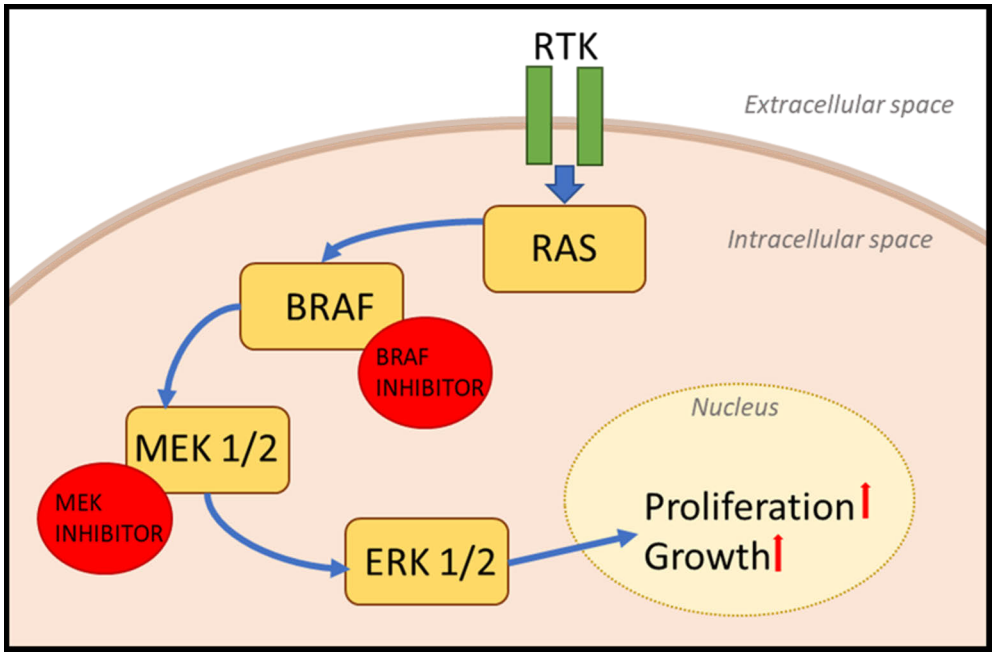

Figure 6. Schematic illustration of RAS/RAF/MEK/ERK pathway indicating the points of action of BRAF and MEK inhibitors. RTK = Receptor tyrosine kinase. (Modified from Singh et al. 2016)

2.5.4.2 ALK

In melanoma research, various Anaplastic lymphoma kinase (ALK) fusions have raised interest.[83,139–141] ALK gene, located in the short arm of chromosome 2 encodes a tyrosine kinase, and after mutated and genetically fused, it promotes cell division

and survival through Ras/ERK and JAK/STAT pathways.[142,143] ALK is normally involved in the nervous system development, but as an oncogene, its activation trough mutations an truncations in other tissues results in neoplasias.[142] Tumours with echinoderm microtubule-associated protein like 4 (EML4)-ALK fusion have responded well for ALK inhibitors.[142,144,145] ALK fusions have been found especially in Spitzoid neoplasms, but also in acral and cutaneous melanomas.[81,140,146,147]

In some melanomas, alternative ALK isoform, ALK^{ATI}, has been discovered, but in *in vitro* and *in vivo* experiments of its response to ALK inhibitors are controversial.[139,141] However, the latest study of Wiesner et al. demonstrated treatment response for ALK inhibitor crizotinib in ALK^{ATI}-positive melanoma murine model and in one patient with immunotherapy resistant ALK^{ATI}-positive metastasised melanoma.[148] Interestingly, knockdown or inhibition of ALK in BRAF inhibitor resistant cell lines restored their therapeutic sensitivity in vitro, and possibly ALK inhibitors may be beneficial in combination therapy with BRAF inhibitors in the future.[149] Also ALK^{ATI} can be detected in most cases with strong, diffuse expression in ALK IHC, making its analysis applicable in clinics along with other ALK isoforms. Stage III melanoma patients with ALK^{ATI} expressing tumours showed improved survival compared to patients with other ALK isoforms.[150] In this respect, analysing ALK IHC expression in melanomas in children, adolescents and young adults would be beneficial for both scientific interest and as a potential therapeutic target.

2.5.4.3 PD-L1

Programmed death receptor 1 (PD-1) and Programmed death ligand 1 (PD-L1) inhibitors have proven their potential in melanoma treatment, especially when used together with anti-CTLA-4 antibodies.[119,120,122] PD-1 receptors are located on the extracellular membrane of the effector T-cells. When attaching to its ligand, PD-L1 presented by the tumour cells, this coupling promotes immunotolerance towards the tumour cell. Blocking this coupling prevents the immune evasion of the tumour cells, thus promoting the immune system in tumour eradication.[120,151–153]

PD-1 inhibitors have clearly shown their efficacy in melanoma treatment, and their toxicity is reported lower than in anti-CTLA-4 antibodies.[154–156] Particularly PD-L1 negative patients have benefitted from the combination of PD-1 inhibitor and anti-CTLA-4 antibody treatment instead of monotherapy of those.[155,157,158] However, various patient subtypes have been selected for these studies, and oncological treatment decisions need to be planned using multiple patient parameters to obtain the best possible treatment results. Therefore, studies on new, additional biomarkers besides PD-L1 expression are ongoing.[154]

In PD-L1 immunohistochemistry of the primary melanoma samples and the metastases, the patients with ≥1% positivity in the tumour cells have been regarded as a positive for PD-L1 IHC.[159] In clinical studies, patients with higher PD-L1 IHC positivity have had a slightly better prognosis than the PD-L1 IHC negative patients.[160,161] In the recent study, however, patients with more than 5% PD-L1 expression in IHC responded better to combination therapy of PD-1 inhibitor and anti-CTLA-4 antibody treatment.[154] Nevertheless, patients with PD-L1-negative melanomas could also benefit significantly from anti-PD-L1 treatment.[120,154,155,157,158] Illustration of the working mechanisms of PD-1 inhibitors and anti-CTLA4-antibodies is shown in Figure 7 (Modified from Singh et al. 2016).[138]

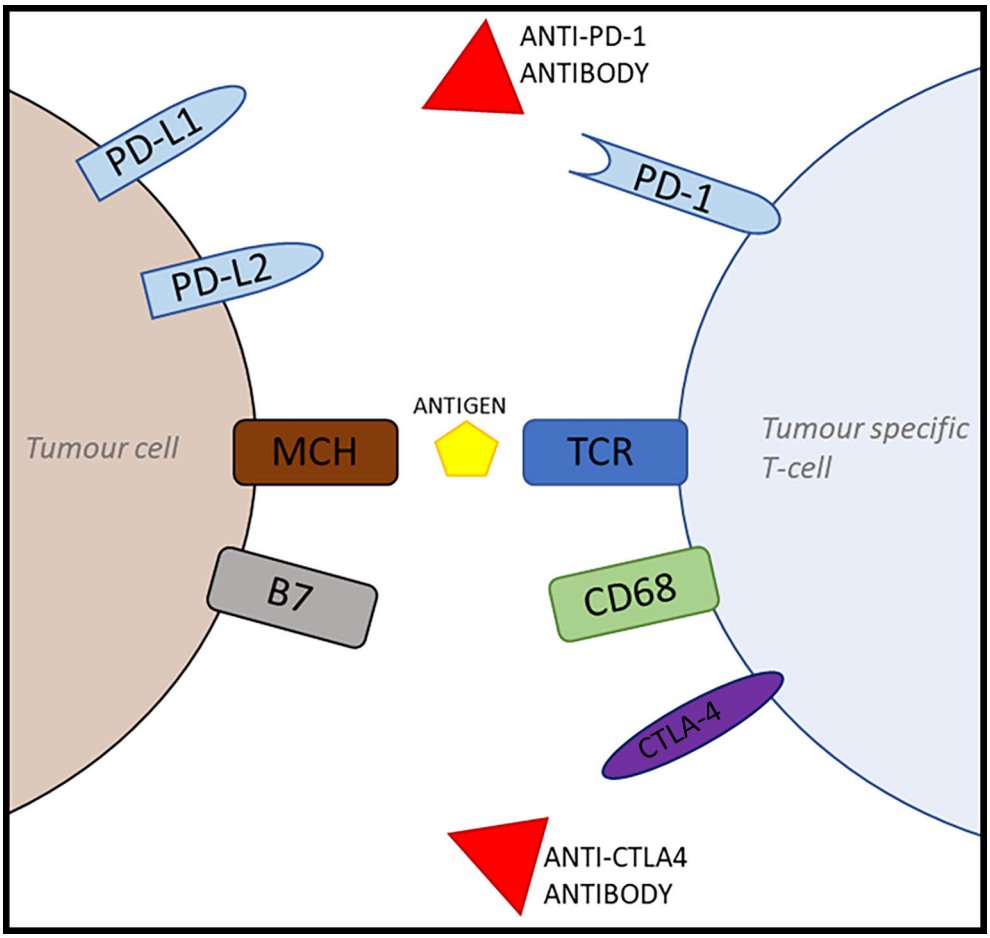

Figure 7. Schematic illustration of the mechanism of anti-PD-1-therapy and anti-CTLA4 therapy. TCR = T-cell receptor, MCH = Major histocompatibility complex. Modified from Singh et al. 2016.

2.6 Prognosis

The prognosis of primary melanoma in children and adolescents is relatively good when compared with the adults. Survival rates in Europe and in the USA are reported to vary from 70% to 94.8%. The prognosis depends highly on the melanoma subtype, the age of the patient and on the stage of the disease. In general, the prognosis is better in prepubertal children, in Spitzoid tumours and in a localised disease.[2,34,43,44,47,65,95,162,163]

Melanomas in young adults are thought to have similarities with paediatric and adolescent melanomas in both of their biological background and of the clinical course.[9,11–14] For example, the tumour microRNA expression profile differs by patient's age, and younger patients have more often Spitzoid melanomas compared with adults.[9,13,164] The prognosis of AYA is reported to be better than in adult melanoma patients. In a Swedish study, 5-year-OS in malignant melanoma was 94.6% in AYA, compared to 90.4% OS in patients aged 40–64 years, and 84.1% OS of patients over 65 years. This study did not find differences in 5-year-OS between sexes.[4] On the contrary, a large USA based study noted that non-Hispanic white male AYA patients had considerably worse prognosis compared to their female counterparts.[5] This difference persisted after correction for the prognostic parameters, such as Breslow thickness and primary tumour ulceration.[5,6] The 10-year-OS for male patients with localised disease was 94.3% and 97.0% in females, and in regional disease with metastases detected only in the regional lymph nodes and/or in-transit metastases 56.6% and 77.1% correspondingly. For patients with distant metastases, 10-year-OS was 14.5% in males and 25.2% in females. Male patients were 35–80% more likely to die compared to female patients aged 15–39 years depending on 5-year based age group.[5]

The difference in survival between sexes has been hypothesised to be a result of differences in the antitumour response since female melanoma patient are shown to have increased survival despite lymph node metastases.[5] Male patients have also noted to relapse and develop metastases more often than female patients.[165,166] Hormonal factors, such as oral contraceptives and hormonal replace therapy, have been hypothesised to contribute to melanoma development, but larger studies did not support this theory.[167,168] However, previous pregnancies have been suggested to decrease the risk for melanoma and also increased melanoma survival.[167,169]

Immune-related factors are also likely to play a role in differences in the survival observed in male and female melanoma patients.[5,170] Immune responses are known to be stronger in females, and females are also known to have higher prevalence of autoimmune diseases.[171–174] Also, X-inactivation in females is regarded to explain some of immune related differences between sexes, such as higher production of Toll like receptor 7 and Interferon alpha (INF-α).[173,175–177] Programmed Death Ligand 1 (PD-L1) may be partially responsive for the difference in antitumour

immunity between males and females.[170,178] Lin et al. demonstrated that female mice in PD-L1$^{-/-}$ murine model for melanoma were more resistant to disease progression than similar male mice. In addition, wild type female mice responded better when treated with PD-L1 inhibitor.[170] This was reported to be due to reduced regulatory T-cell (Treg) function in female mice, and occurred via different pathway than by its known ligands PD-1 or CD80.[170,179] Incubating Tregs of PD-L1$^{-/-}$ female mice with physiological concentration of oestrogen *in vitro* resulted in their nearly total functional elimination, which suggests oestrogen having pivotal role in Treg differentiation, and by this it may also influence to melanoma development.[170,180–182]

Females are also noted to have higher concentration of immunohistochemically (IHC) positive oestrogen receptor beta (ERβ) in their skin compared to males, which may affect to the immunological microenvironment of cutaneous melanoma.[183–185] Notably, the levels of ERβ IHC are reported to decrease along with increasing Breslow thickness and level of invasion in melanoma, two factors associated with worsened survival in melanoma.[185,186] However, many unknown factors still persist regarding the role of sex hormones and immunology in melanoma and other cancers.

3 Aims

The aim of this thesis is to characterise cutaneous melanoma in Finnish children, adolescents and young adults, and this aim is approached from a different aspect in each original publication.

- **Study I**: The aim of the study was to characterise melanomas in children in Finland by their clinical and pathological aspects.

- **Study II:** The aim of the study was to investigate melanoma in children and adolescents in the Finnish population for its incidence, clinical course, treatment, prognosis and BRAFV600E-, ALK- and PD-L1-positivity of the primary tumours.

- **Study III:** The aim was to examine the incidence of paediatric and adolescent melanomas in Finland in 1990–2014 and the associated clinical and histopathological characteristics to reveal temporal trends, such as changes in diagnostic sensitivity of Spitzoid melanomas.

- **Study IV:** The aim of the study was to investigate possible predisposing conditions for melanoma in AYA, which would aid the clinicians in screening, treating, and planning the follow up for this patient population.

The hypothesis is, that primary cutaneous melanomas in children and adolescents differ from the primary cutaneous melanomas in adults by their clinical characteristics, histopathological features, and by their prognosis. Discovering these differences would aid the pathologists and clinicians in diagnosing and treating these rare malignancies, as well as the patients and their families, who could become more informed on their malignancy.

4 Materials and Methods

4.1 Study I

4.1.1 Patients

The patients aged 0–15 years diagnosed with invasive cutaneous melanoma in Finland in 1990–2010 were included into the first original publication. The patient information was collected from Finnish Cancer Registry (FCR), and clinical patient and treatment information were obtained from the treating hospitals. Patients with invasive melanoma were included into this retrospective register study. FCR is a Finnish nationwide registry recording all cases of malignancies diagnosed in Finnish health care. It also receives information from the death certificates and comprises all diagnosed melanoma patients in Finland.

4.1.2 Statistical Analysis

Disease-free survival (DFS) was defined as the time from the primary surgical treatment until the first recurrence. Melanoma-specific OS was defined as the time from initial melanoma treatment until the disease-specific death due to metastatic melanoma. Follow up time ended in the last information on patient status, or death. Survival analyses were calculated using IBM SPSS version 21.0 for Windows.

4.2 Study II

4.2.1 Patients

Children and adolescents aged 0–19 years diagnosed with *in situ* or invasive cutaneous malignant melanoma in 1990–2014 were searched from the Finnish Cancer Registry (FCR) database. Primary tumour and metastasis samples and histopathological reports were collected from the institutions in which the patients had been diagnosed or treated by using the information derived from the FCR database. These institutions were hospitals, biobanks, and private laboratories across

Finland. The clinical data of the study patients was collected from the University hospitals where the patients had been treated.

4.2.2 Re-evaluation and Staging

The H&E-stained tumour tissue samples were independently reviewed by two dermatopathologists Lauri Talve and Susanna Juteau. Tumours diagnosed as malignant melanoma by at least one of the re-evaluating dermatopathologists together with the pathologists that had previously diagnosed the sample as malignant melanoma, were classified as malignant melanomas in this study. In tumours with only formalin-fixed paraffin embedded tissue blocks available, new sections were prepared and stained with HE. Tumours were staged at the time of diagnosis using AJCC 8th Edition criteria.[32] Melanoma subtypes were classified by the dermatopathologists according to WHO Skin Tumours 4th Edition criteria.[19]

4.2.3 Immunohistochemical Analysis

Immunohistochemical stainings for BRAFV600E (Roche, Cat# 790-5095, RRID: AB_2833072) and ALK (Roche, Cat# 790-4796, RRID: AB_2833073) were performed with BenchMark ULTRA IHC/ISH system using OptiView DAB IHC Detection Kit (Roche, Cat# 760-700, RRID: AB_2833075). PD-L1 stainings (Agilent, Cat# GE00621-2, RRID: AB_2833074) were made using the appliances and detection kit described above, and 1% threshold was used for positivity. Positive control slides were prepared according to the manufacturer's protocol.

4.2.4 Statistical Analysis

Statistical analyses were conducted IBM SPSS Statistics for Windows V26. Kaplan-Meier estimates for OS were calculated by the time between the diagnosis and death. Estimates for DFS were calculated from the time of the diagnosis to the first melanoma metastasis or recurrence. In case no melanoma related death or metastasis had occurred during the follow-up, the patient was censored. Follow up time ended in the last information on patient status, or death. Associations between age, stage and BRAFV600E-positivity and survival were analysed with log-rank test. Mann-Whitney U test, Chi-square test and Kruskal-Wallis test were used. *P*-values <0.05 were considered statistically significant.

4.3 Study III

4.3.1 Patients

The same patient material than in the study II comprising the children and adolescents diagnosed with cutaneous melanoma while under 20 years old in 1990–2014 were retrieved from the FCR database and re-evaluated as previously described above. Tumours of which both of the dermatopathologists regarded as non-malignant were considered as originally misclassified in the FCR data. Immunohistochemical analysis for BRAFV600E, ALK and PD-L1 were performed as described above. The endpoint of the follow-up was determined based on the last information on patient status, or death.

4.3.2 Statistical Analysis

The method described by Fay and Feuer was used for the calculations for the age-adjusted melanoma incidence and its confidence intervals.[187] The Finnish population in 2014 was used as the standard population, and the numbers of persons of 0–19 years of age were obtained from Statistics Finland in 1-year strata for years 1990–2014. Point estimates and 95% confidence intervals were used for the visualisation of the annual incidence per 1 000 000 individuals. For enhanced visual clarity, the data was smoothed using cubic splines. Incidence trends and annual percent change were calculated with the Joinpoint software (version 4.8.0.1) allowing for 0–2 joinpoints. In the Joinpoint analysis, one year (1994) was left out from the analysis since no melanomas were observed that year.

Calculations for the total observed incidence were based on the melanomas reported in the FCR. Also, another analysis was stratified by two age groups (0–10 and 11–19 years) to reveal potential differences between prepubertal children and adolescents. The true incidence of melanoma was estimated based on the proportions of confirmed melanomas in the available samples, since all the primary tumour samples were not obtained for the re-evaluation. To obtain misclassification-corrected incidence estimates, the study period was divided into 5-year intervals, and the proportions of confirmed melanomas was compared to the melanomas reported in the FCR. In addition, an analysis stratified by Spitzoid subtype was performed within the confirmed melanomas.

To observe differences in melanomas diagnosed at different times, the study period was divided into two sub-periods of 1990–2002 and 2003–2014, and primary tumour characteristics were compared between these two sub-periods within the confirmed melanomas. In addition, Spitzoid melanomas were compared with the other melanomas.

Comparisons for melanoma location, tumour type, SLNB result, and the extent of TILs were made using the Chi-squared test. Comparisons of sex, metastatic disease, deaths, ulceration, and BRAFV600E, PD-L1 and ALK expression was performed using Fisher's exact test. Mann-Whitney U test was used to examine differences in the age at diagnosis, Clark level, Breslow thickness, density of mitoses and in the tumour stage. Survival estimated were calculated using the Kaplan-Meier method. Hazard ratio (HR) for death was analysed with the Cox proportional hazards model. *P*-values <0.05 were considered statistically significant. Follow up time ended in the last information on patient status, or death.

All analyses apart from the Joinpoint analyses were performed with the R software for statistical computing (version 4.0.0) and packages dsrTest (version 0.2.1) and survival (version 3.1-12).

4.4 Study IV

4.4.1 Patients

Electronical patient charts of adolescents and young adults aged 15–39 years diagnosed in 1983–2011 registered into the melanoma research database were retrieved from Turku University Hospital database. Also, re-evaluated patients aged 15–19 years diagnosed with cutaneous melanoma in Finland in 1990–2014 were included from the study II materials to increase the number of patients. Clinical information on the patient survival status, primary tumour characteristics, co-morbidities, and possible risk-factors for melanoma was collected from the Turku University Hospital's electronical database. The information on tumour subtype was seldom recorded, and therefore not analysed. For female patients, also the information on pregnancies and deliveries was searched.

Information related to the melanoma treatment was recorded in the research database for all the patients. Comprehensive electronical clinical records including the information on possible co-morbidities and risk-factors were found for 164 (68%) patients. The information on possible pregnancies and deliveries was retrieved for 68 (49%) female patients.

4.4.2 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows V27. Kaplan-Meier estimates for OS were calculated by time between the diagnosis and death. Estimated for DFS were calculated from the time of the diagnosis to the time of the first melanoma metastasis or recurrence. If melanoma-related death or metastasis did not occur during the follow-up, the patient was censored. Follow up

time ended in the last information on patient status, or death. Log-rank test was utilised for discovering the associations between age, sex, or melanoma development in pre-existing nevus with survival, together with Mann-Whitney U test, Kruskal-Wallis test, and Chi-square tests. P-values <0.05 were considered statistically significant.

4.5 Ethical Aspects

This study was conducted under approval of the Institutional Review Board of Turku University Hospital, by the Ethics Committee of the Hospital District of Southwest Finland, and by the National Institute for Health and Welfare (licences: O45/12, TO6/001/16, ETMK:140/1803/2014 and THL/163/5.05.00/2012, THL/552/5.05.00/2016). Primary tumour samples were collected with the permission of Valvira National Supervisory Authority for Welfare and Health (License 6479/06.01.03.01/2016). Also, the research permit for the use of Helsinki University Hospital archives of clinical data was obtained (HUS/83/2019).

5 Results

5.1 Study I: Melanoma in Children in Finland

In the original publication I, 19 patients aged 0–15 years diagnosed with malignant melanoma in 1990–2010 were included into the study. From those, 13 were boys and six were girls. Four patients were under 11 years old, and 15 patients were 11–15 years old. Trunk was the most common anatomical location of melanoma, followed by the limbs. Melanoma was reported to have developed into a precursor lesion in 11 cases. Mean Breslow thickness was 2.4 mm (range 0.3–6.0 mm), and Clark levels III and IV were most common. Tumour ulceration was reported in five cases. On average, one paediatric melanoma case per year was diagnosed in Finland during the study period. Patient and primary tumour characteristics are shown in Table 4, and in the Table 1 of the original article.

Table 3. Patient and primary tumour characteristics.

	0–10 years	**11–15 years**
Cases (n) (%)	4 (21.1)	15 (78.1)
Sex, n (%)		
Males	4 (100.0)	9 (60.0)
Females	0 (0.0)	6 (40.0)
Age at diagnosis, mean (range) (years)	6.8 (4–9)	12.9 (11–15)
Melanoma location, n (%)		
Head and neck	2 (50.0)	1 (6.7)
Trunk	0 (0.0)	6 (40.0)
Upper extremity	1(25.0)	4 (26.7)
Lower extremity	1 (25.0)	4 (26.7)
Clark level, n (%)		
1	0 (0.0)	0 (0.0)
2	0 (0.0)	2 (13.3)
3	2 (50.0)	4(26.7)
4	1 (25.0)	6(40.0)
5	1 (25.0)	0 (0.0)
Not available	0 (0.0)	3 (20.0)
Breslow thickness		
Mean (range) (mm)	2.4 (1.1–4.0)	2.5 (0.3–6.0)
Not available, n (%)	0 (0.0)	2 (13.3)
Ulceration, n (%)		
Yes	0 (0.0)	5(33.3)
No	3(75.0)	7 (46.7)
Not available	1 (25.0)	3 (20.0)
Sentinel lymph node biopsy, n (%)		
Performed	3(75.0)	6 (40.0)
Positive	3 (100.0)	3 (50.0)

Surgical treatment for all the patients was wide local excision with 1–3 cm margins depending on the location and on the guidelines applied at the time of diagnosis. Sentinel lymph node biopsy was performed in nine patients, of whom five had a positive node. Nine patients presented with metastasis at the time of diagnosis. Five of them were found with SLNB and four patients had macroscopic regional lymph node metastases and underwent therapeutic lymph node dissection (TLND). All SLNB positive patients underwent CLND and only the sentinel node was positive in all these patients. Interferon adjuvant therapy and/or chemotherapy were used for seven patients, in one patient for metastatic melanoma, and as in adjuvant therapy for the others. One patient received palliative radiation therapy. Clinical evolution and HE stained primary tumours of two patients with Spitzoid melanomas which metastasised into sentinel lymph nodes are shown in Figures 8 and 9, and in the Figure 1 of the original article. Both patients underwent CLND and remained disease free during the follow up.

Median follow-up time was 4.6 years, ranging from 0.6 to 10.0 years. Melanoma recurrence was observed in 5 patients, and 4 patients died of metastatic melanoma during the follow-up. First recurrences were discovered in a median time of 16 months, ranging from 4 to 191 months. The first recurrence was a local recurrence in one patient, regional lymph node recurrence in two patients, and manifested as distant metastases in two patients. One out of the five SLNB positive patient died of metastatic melanoma. Five-year-DFS of the patients was 81% and 5-year-OS was 83%.

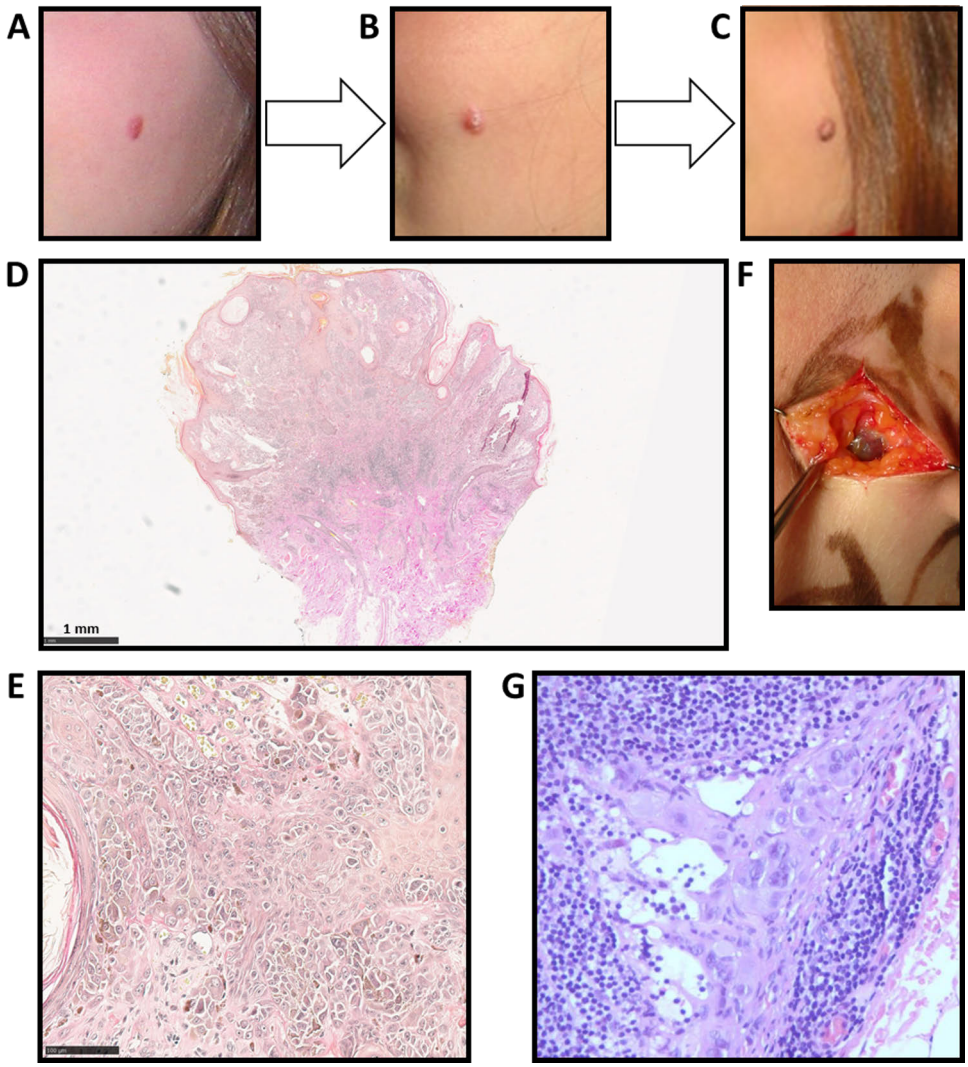

Figure 8. Patient with Spitzoid melanoma. Pictures obtained from the patients. **A**: 9 months before diagnosis, **B**: 6 months before diagnosis and **C**: 1 month before diagnosis show evolution of the lesion from small pink papule to more elevated with white lines present. Finally, the lesion is also pigmented from the edges. Sentinel lymph node biopsy revealed melanoma micrometastasis (**F,G**). Histopathological images (**D** and **E**) show typical, wedge-shaped architecture of Spitzoid melanoma. © Pictures A-C, F: Archives of Ilkka Koskivuo, Pictures D-E: Emma Rousi, Picture G: Lauri Talve.

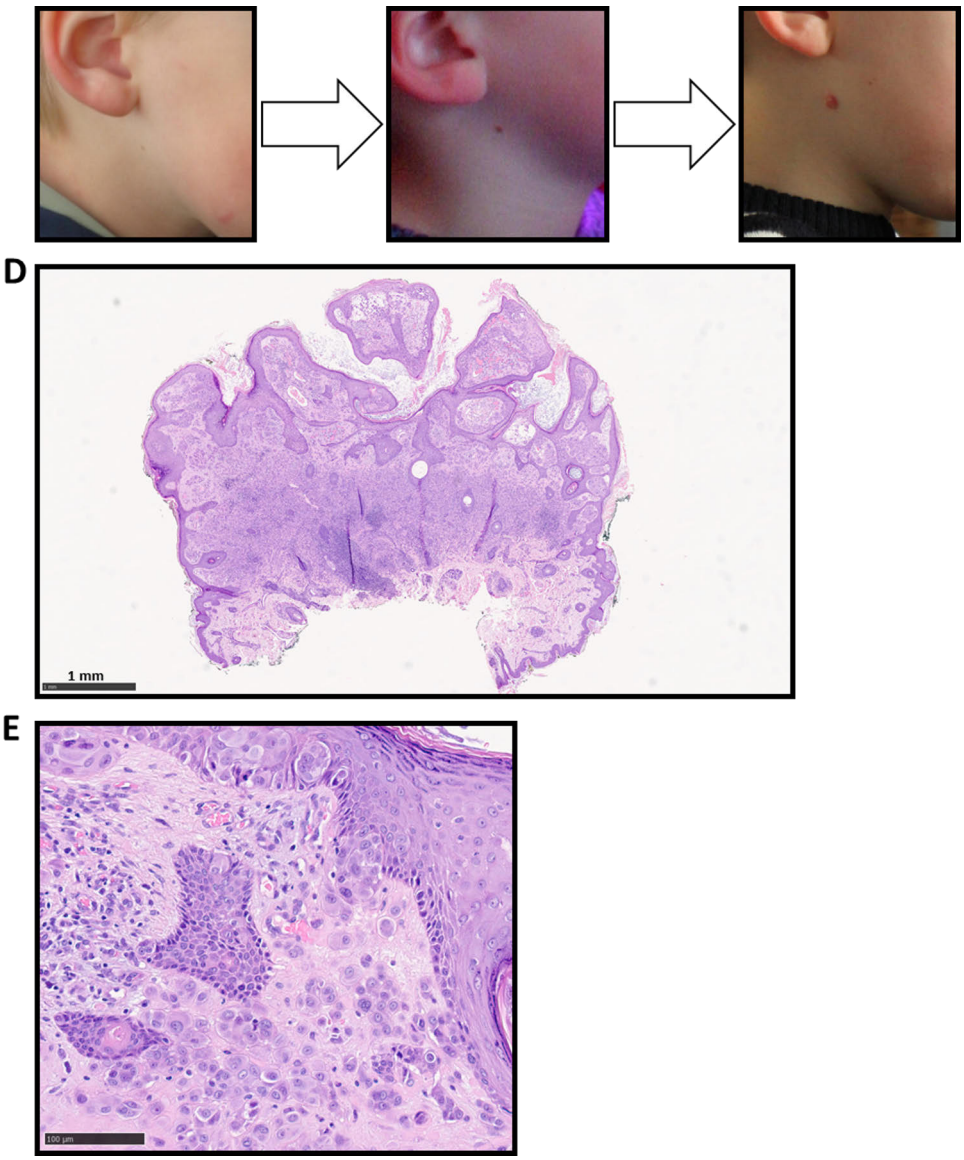

Figure 9. Young patient with slowly growing Spitzoid melanoma. Pictures obtained from the patient show the lesion **A**: 2,5 years and **B**: 2 years before diagnosis and **C**: 6 months before diagnosis. Histological image with HE staining (**D,E**) shows irregular nesting and pleiomorphic nuclei typical for Spitzoid melanoma. Picture of micrometastasis of the patient is shown in the original publication I, Fig 1. © Pictures A-C: Archives of Ilkka Koskivuo, Pictures D-E: Emma Rousi.

5.2 Study II: Different Expression of BRAFV600E, ALK and PD-L1 in Children and Adolescents

The original publication II comprised of 122 patients aged 0–19 years diagnosed with malignant melanoma were registered in the FCR in during 1990–2014. Seventy-three primary melanoma samples out of the 122 primary tumours were obtained for the re-evaluation, since not all the samples were found from the archives. In the independent re-evaluation of the two experienced dermatopathologists, 56 cases out of 73 were considered as malignant melanomas and were included into the study. Out of the 56 patients, the dermatopathologists gave discordant diagnoses in 16 cases.

Histopathological reports and H&E-slides were obtained from all the 56 patients. Primary tumour material was left for IHC analyses in 54 cases. Clinical data of 50 patients was obtained from the hospitals, where the patients had been treated. In four cases, the data was not found from the hospital databases of from the archives.

Melanomas distributed evenly between males and females (48.2% vs. 51.8%). Seven patients (12.5%) were <11 years old, from whom five were boys (71.4%). The mean age of the patients at diagnosis was 15.5 years. Most common anatomical locations of melanomas in adolescents were lower limbs (41%) followed by trunk (27%). In children <11 years old, head and neck and lower limbs (both in 43%) were most common locations.

Mean Breslow thickness of all the patients was 2.4 mm (range 0.2–15.3 mm), and it was similar in patients over and under 11 years. Clark levels III and IV were most common in children and adolescents. Ulceration was present in 29% of melanomas in children <11 years, and 14% in melanomas in adolescents. Most common histopathological subtype was Spitzoid melanoma (66%), followed by SSM (21%) and NM (7%). Three melanomas were *in situ*. In our cohort, there were no other melanoma subtypes and none of the melanomas had developed into congenital nevus.

The surgical treatment of primary melanoma in this patient cohort was wide excision with 1–3 cm margins based on the site of the tumour and on the guidelines applied on the time of the diagnosis. Two patients of the cohort had either distant metastasis or macroscopic lymph node metastasis at the diagnosis, and their primary tumours and metastases were excised mainly for diagnostic purposes. Twenty-seven patients (49%) underwent SLNB, and 12 of them had positive result (44%). CLND was performed for all the patients with positive SLNB, and two of them additional non-sentinel metastases (17%). Elective lymph node dissection (ELND) prior SLNB-era was performed in four cases (7%), and regional lymph node metastasis was found from one of them. TLND was performed for one patient with clinically evident lymph node metastases. The information on SLNB was absent in one case.

SLNB was adopted into clinical practice in Finland in the early 2000's. The SLNB samples were not re-evaluated.

SLNB positivity or negativity did not influence the survival in this study, since both groups had 100% melanoma specific OS and DFS rates. The Breslow thickness of the primary melanoma was higher in patients with positive SLNB compared to negative SLNB (mean 4.8 mm vs. 1.6 mm, P=0.00017). The mean age of the SLNB positive patients was lower than for the SLNB-negative patients (12 vs. 16 years, P=0.011).

Sixteen patients received medical oncological therapies, 11 of which had positive SLNB. One patient had negative SLNB, but she had a thick and deep melanoma with 2 mitoses/mm^2. These 12 patients were treated with adjuvant IFN-α treatment except for two patients, who were treated with combination adjuvant therapy. The other of those was treated with adjuvant combination therapy of IFN-α and Dacarbazine (DTIC), and the other received IFN-α and the combination of dacarbazine, vincristine, lomustine, and bleomycin (BOLD). Adjuvant radiation therapy together with IFN-α adjuvant therapy was used to treat one patient with highly invasive melanoma with increased mitotic rate and with non-sentinel metastases in CLND.

Four patients with metastatic melanoma were treated with a combination of chemo- and immunotherapies together with adjuvant or palliative radiotherapy. Two patients were given BRAF/MEK inhibitors. The other of them had weakly PD-L1 positive melanoma and was also treated with a combination therapy of CTLA-4 antibody and PD-1 inhibitor.

Immunohistochemical analysis for BRAFV600E, ALK and PD-L1 was performed for 54 primary tumours. BRAFV600E mutation was found in 26 melanomas (48%). Two BRAFV600E-positive tumours were also positive for ALK IHC. BRAFV600E-mutation was found in the primary tumours in 4/6 lethal cases in which primary tumour material was available for IHC analysis. One of these patients had also weak, <1% PD-L1-positivity in the tumour cells. The mean age of patients with BRAFV600E mutated melanomas was 17 years (range 11–19 years). The mean Breslow thickness of BRAFV600E positive tumours was 2.4 mm (range 0.2–7.0 mm). BRAFV600E-positivity was seen in all three *in situ* melanomas. Superficial spreading melanoma positive for BRAFV600E is shown in Figure 10.

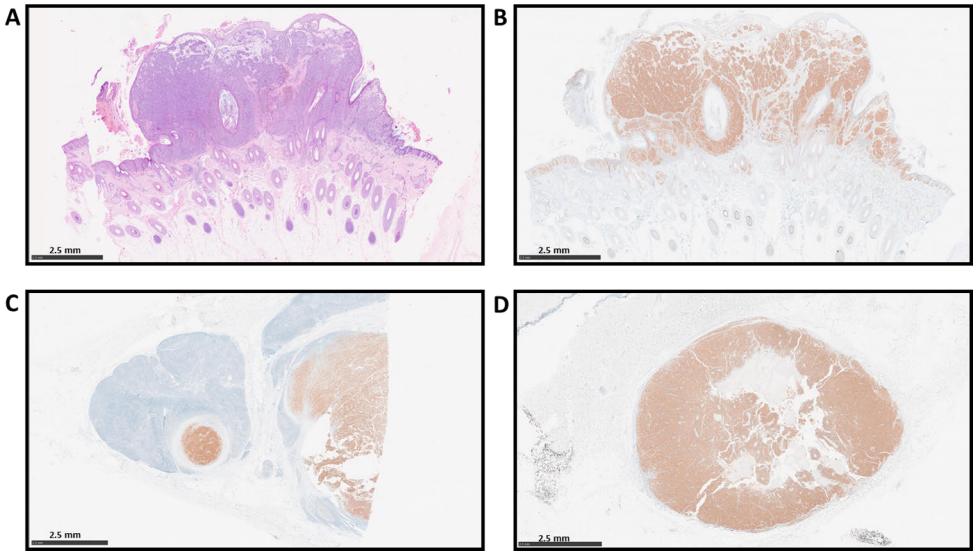

Figure 10. A: H&E staining of a deep superficial spreading melanoma developed into the head and neck area of an adolescent patient stained positive for BRAFV600E (**B**). Melanoma metastasis was found in the regional lymph nodes (**C**) and in the subcutis (**D**) half a year after initial diagnosis stained also positive for BRAFV600E. Patient died of metastatic melanoma. © Emma Rousi

ALK IHC was positive in five melanomas (9%). ALK-positive patients had the mean age of 14 years (range 9–18 years). All ALK-positive melanomas were of Spitzoid subtype. Mean Breslow thickness was 3.2 mm (range 1.2–6.0 mm). ALK positive Spitzoid melanoma is shown in Figure 11.

Only one adolescent male patient (2%) who had <1% PD-L1-positivity in the tumour cells. The patient had also BRAFV600E mutation in his tumour, and he died of the metastatic melanoma. The Breslow thickness of the tumour was high, 7.0 mm. The number of TILs in the stroma was low, and the PD-L1-positivity seen in non-tumour cells was virtually non-existing. Melanoma of the PD-L1 positive patient is shown in Figure 12.

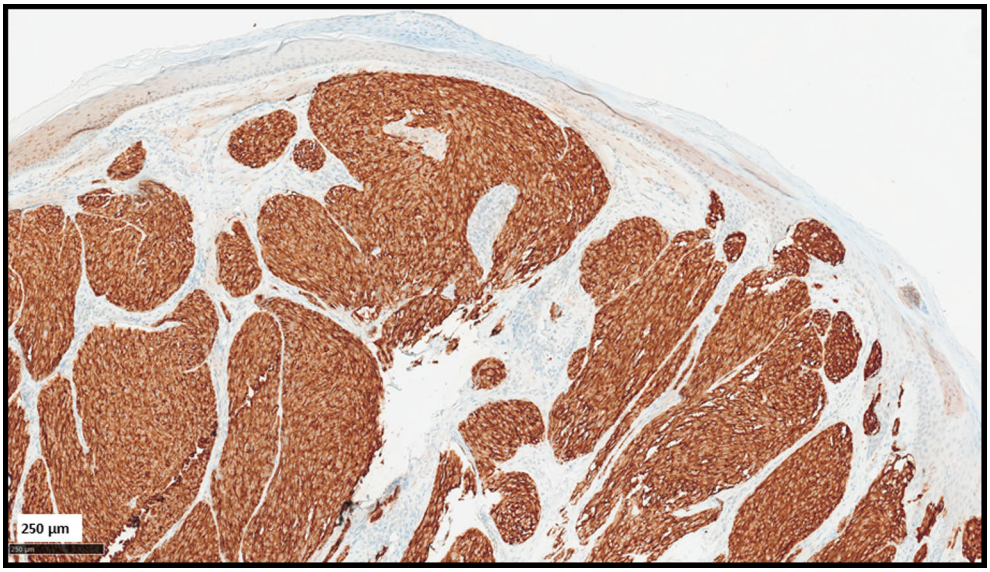

Figure 11. Spitzoid melanoma showing strong, diffuse cytoplasmic staining for ALK. © Emma Rousi

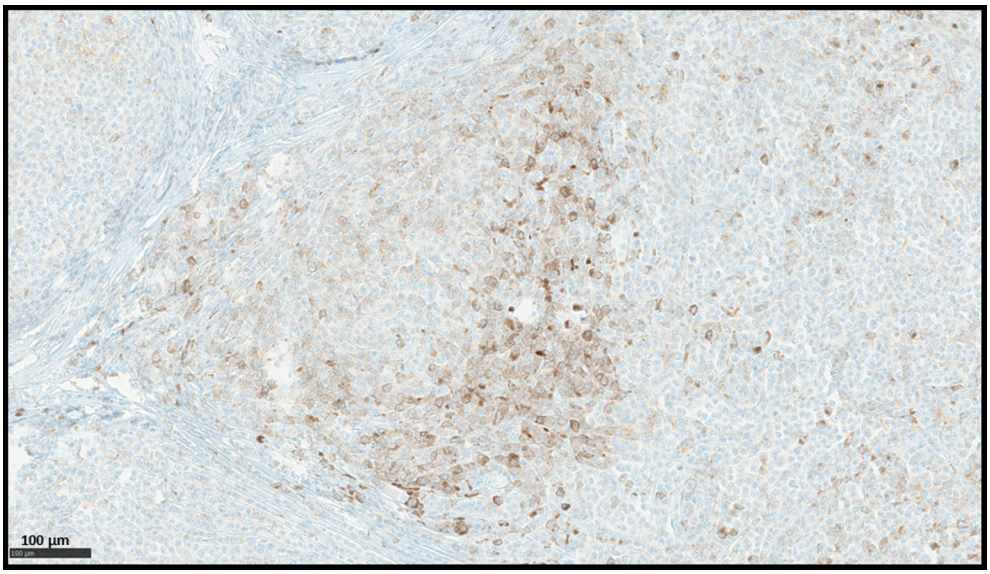

Figure 12. Only one adolescent patient with nodular melanoma showed weak, <1% PD-L1 -positivity in immunohistochemistry. © Emma Rousi

Nearly a half, 25 melanomas (46%) were named IHC negative after they stained negative for each BRAFV600E, PD-L1 and ALK. The mean age of IHC negative patients was 15 years (range 5–19 years), and their mean Breslow thickness was

2.6 mm (range 0.2–15.3 mm). Five out of six patients under 11 years old had IHC-negative primary tumours. One patient, who died of metastatic melanoma had IHC-negative melanoma. Also, one other patient with IHC-negative melanoma developed a cutaneous metastasis close to the primary melanoma site but remained disease free during the follow-up.

BRAFV600E IHC was analysed from the metastatic samples of the six patients who died of metastatic melanoma, and it was positive in all cases in which the primary tumour was also positive for BRAFV600E. Therefore, 5/6 (83%) of metastatic melanomas were positive for BRAFV600E.

Survival analyses were performed with stage I-IV patients (n=53), excluding the three *in situ* melanomas. Melanoma specific 1-year-DFS and OS in all patients were 90.6% and 96.2%, respectively. Five-year-DFS and -OS were 88.6% and 92.5%, respectively, and 10-year-DFS and OS were 86.8% and 88.7%, respectively (II: Fig.2). No melanoma recurrences or deaths due to metastasised melanoma occurred after 10 years of follow-up. Patients with BRAFV600E-positive primary tumour and/or metastasis had worse 10-year-OS compared with BRAFV600E negative patients, 80.0% vs. 96.4%. However, this was not statistically significant (P=0.081). (II: Figure 3.)

In children and adolescents, most common melanoma stages were I (46.4%) and III (26.8%). Paradoxically to the excellent 10-year-OS of the patients under 11 years old (100%), they had higher disease stages than the older patients in the 16–19 years age group (10-year-OS 92.9%). The mean age of the patients with stage III disease was different from the stage I patients, 12 vs. 17 years respectively (P=0.004). The patients in the age group of 11–15 years had the worst prognosis with 10-year-OS of 77.8%. (II. Figure 3.) The patient and the primary tumour characteristics are shown in Table 5, and in the Table 1 of the original article II.

Table 4. Patient and primary tumour characteristics. SSM = Superficial Spreading melanoma, NM = Nodular melanoma, Spitzoid = Spitzoid melanoma.

	0–10 years	11–15 years	16–19 years	*P*
Cases (n)	7	18	31	
Sex, n (%)				
Males	5 (71.4)	11 (61.1)	11 (35.5)	0.093
Females	2 (28.6)	7 (38.9)	20 (64.5)	
Melanoma location, n (%)				
Head and neck	3 (42.9)	4 (22.2)	5 (16.1)	0.795
Trunk	1 (14.3)	5 (27.8)	8 (25.8)	
Upper extremity	0 (0.0)	3 (16.7)	3 (9.7)	
Lower extremity	3 (42.9)	6 (33.3)	14 (45.2)	
Not available	0 (0.0)	0 (0.0)	1 (3.2)	
Clark level, n (%)				
1	0 (0.0)	0 (0.0)	3 (9.7)	0.098
2	0 (0.0)	1 (5.6)	5 (16.1)	
3	2 (28.6)	5 (27.8)	14 (45.2)	
4	5 (71.4)	10 (55.6)	9 (29.0)	
5	0 (0.0)	2 (11.1)	0 (0.0)	
Breslow thickness				
Mean (range) (mm)	2.4 (1-1 to 5.5)	3.4 (0.6 to 15.3)	1.9 (0.2 to 7.0)	0.133
Ulceration, n (%)				
Yes	2 (28.6)	2 (11.1)	5 (16.6)	0.608
No	5 (71.4)	16 (88.9)	26 (83.9)	
Mitoses / mm^2				
Mean (range)	1.6 (0 to 5)	3.3 (0 to 12)	2.0 (0 to 18)	0.394
Histological subtype n (%)				
In situ	0 (0.0)	0 (0.0)	3 (9.7)	0.209
SSM	0 (0.0)	3 (16.7)	9(29.0)	
NM	0 (0.0)	1 (5.6)	3 (9.7)	
Spitzoid	7 (100.0)	14 (77.8)	16 (51.6)	
BRAF V600E expression, n (%)				
Positive	0 (0.0)	8 (44.4)	18 (58.1)	0.034
Negative	6 (85.7)	9 (50.0)	13 (41.9)	
Not available	1 (14.3)	1 (5.6)	0 (0.0)	
ALK expression, n (%)				0.662
Positive	1 (14.3)	2 (83.3)	2 (6.5)	
Negative	5 (71.4)	15 (11.1)	29 (93.5)	
Not available	1 (14.3)	1 (5.6)	0 (0.0)	
PD-L1 expression, n (%)				1.000
Positive	0 (0.0)	0 (0.0)	1 (3.2)	
Negative	6 (85.7)	17 (94.4)	30 (96.8)	
Not available	1 (14.3)	1 (5.6)	0 (0.0)	
Tumour stage, n (%)				
0	0 (0)	0 (0.0)	3 (9.7)	0.008
I	1 (14.3)	6 (33.3)	19 (61.3)	
II	2 (28.6)	2 (11.1)	7 (22.6)	
III	4 (57.1)	9 (50.0)	2 (6.5)	
IV	0 (0.0)	1 (5.6)	0 (0.0)	
Sentinel lymph node biopsy, n (%)				
Positive	3 (42.9)	8 (44.4)	1 (3.2)	0.004
Negative	2 (28.6)	5 (27.8)	8 (25.8)	
Not performed	2 (28.6)	5 (27.8)	21 (67.7)	
Not available	0 (0.0)	0 (0.0)	1 (3.2)	

5.3 Study III: Increasing Incidence of Melanoma in Children and Adolescents

Analysis of the 122 patients aged 0–19 years recorded in the FCR in 1990–2014 described in the original publication III revealed a statistically significant increasing trend with annual percent change of 5.6% (95% CI 2.7 to 8.6) in the age-adjusted incidence of cutaneous melanoma. The incidence started to increase around 2009, and the increase in the incidence concerned the adolescent melanoma patients aged 11–19 years, and similar increase was not seen in children under 11 years old. The incidence of Spitzoid melanomas appeared to increase in the most recent years, but not in other melanomas.

The percentage of the confirmed 56 melanomas among the 122 cases of the FCR did not differ statistically between the years, the proportion of confirmed melanomas being 67–90% in 5-year periods (p=0.835). Increase in the incidence concerned both melanomas and non-melanoma tumours. However, reliable statistical analysis of non-melanoma tumours was impossible because of the low number of cases per year. The misclassification-corrected mean annual incidence estimates for melanoma in children and adolescents in Finland increased from 1.4/1 000 000 in 1990–1994 to 5.8/1 000 000 in 2010–2014. Incidence trends are visualised in Figure 13 and in Figure 1 of the original publication III.

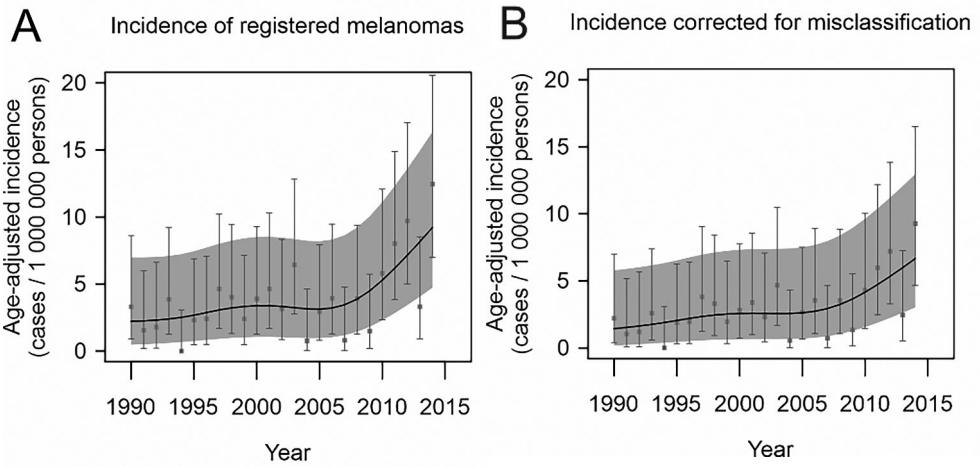

Figure 13. Incidence of melanomas in children and adolescents based on the Finnish Cancer Registry data (**A**), and after its correction for misclassification using re-evaluated melanoma cohort (**B**). (Modified from original publication III)

When comparing the primary tumour characteristics between cases diagnosed in 1990–2002 and 2003–2014, no differences in Clark level, Breslow thickness, ulceration, or mitotic rate between the two time periods were observed. The deaths

due to metastasised melanoma were a bit more frequent in the first half of the study, but this might be a consequence of the longer follow-up time of the patients diagnosed earlier since no difference in the hazard ratios for death to melanoma were observed between the time periods. The proportions between anatomical locations of melanoma were similar in the two time periods, neither there was difference in the age groups or in the sex distribution between the two periods. Significant difference in the proportions of melanomas located in the presumably sun-exposed (head and neck, upper extremities) and non-sun-exposed (trunk, lower extremities) locations was not observed. The proportion of Spitzoid melanomas out of all the re-evaluated melanomas did not differ between sub-periods. Prognostic factors of melanomas diagnosed in the two sub-periods are shown in Table 6, and in table 1 of the original publication III.

The major histopathological subtype in children and adolescents, Spitzoid melanoma occurred most often (49%) in the lower limbs. The proportion of the melanomas located in the lower limbs was lower in other melanoma subtypes (26%). Disease stage and Clark level were higher in Spitzoid melanomas compared to other melanomas. There was no significant difference between these two groups regarding Breslow thickness, ulceration, mitotic rate, or TILs. The 5-year-OS of Spitzoid melanoma was 94.5%, and the relative risk of death was not significantly different between Spitzoid and other melanoma subtypes (HR 0.52, 95% CI 0.10 to 2.58).

Spitzoid melanomas were less frequently BRAFV600E -positive than the other melanoma types (35.1% vs. 68.4%). Among the SLNB -positive patients, 55% had Spitzoid melanomas and 14% of the patients had other subtypes, but the difference was not statistically significant. Differences between Spitzoid and other melanomas are shown in Table 7 and in Table 2 and Figure 2 of the original publication III.

Table 5. Distribution of prognostic factors between the two sub-periods. (Modified from original publication III)

	1990–2002	2003–2014	*P*
Cases (n)	18	38	
Sex, n (%)			
Males	9 (50.0)	18 (47.4)	1.000
Females	9 (50.0)	20 (52.6)	
Age at diagnosis, mean (SD) (years)	16.7 (3.2)	14.9 (3.8)	0.089
Metastasis during follow-up, n (%)	3 (16.7)	4 (10.5)	0.669
Melanoma-related deaths during follow-up, n (%)	3 (16.7)	3 (7.9)	0.374
Melanoma location, n (%)			
Head and neck	2 (11.1)	10 (26.3)	0.464
Trunk	4 (22.2)	10 (26.3)	
Upper extremity	2 (11.1)	4 (10.5)	
Lower extremity	10 (55.6)	13 (34.2)	
Not available	0 (0.0)	1 (2.6)	
Clark level, n (%)			
1	1 (5.6)	2 (5.3)	0.207
2	4 (22.2)	2 (5.3)	
3	6 (33.3)	15 (39.5)	
4	7 (38.9)	17 (44.7)	
5	0 (0.0)	2 (5.3)	
Not available	0 (0.0)	0 (0.0)	
Breslow thickness			
Median (range) (mm)	1.1 (0.2 to 5.0)	1.6 (0.6 to 15.3)	0.237
Not available, n (%)	1 (5.6)	2 (5.3)	
Ulceration, n (%)			
Yes	2 (11.1)	7 (18.4)	0.703
No	16 (88.9)	31 (81.6)	
Not available	0 (0.0)	0 (0.0)	
Mitoses / mm^2			
Median (range)	0 (0 to 18)	1 (0 to 12)	0.541
<6 mitoses / mm^2, n (%)	14 (77.8)	31 (81.6)	0.670
≥6 mitoses / mm^2, n (%)	3 (16.7)	4 (10.5)	
Not available, n (%)	1 (5.6)	3 (7.9)	
BRAFV600E expression, n (%)			
Positive	9 (50)	17 (44.7)	0.771
Negative	8 (44.4)	20 (52.6)	
Not available	1 (5.6)	1 (2.6)	
Melanoma type, n (%)			
Superficial spreading	5 (27.8)	7 (18.4)	0.525
Nodular	0 (0.0)	4 (10.5)	
Spitzoid	12 (66.7)	25 (65.8)	
In situ	1 (5.6)	2 (5.3)	
Not available	0 (0.0)	0 (0.0)	
Tumour stage, n (%)			
0	1 (5.6)	2 (5.3)	0.100
I	10 (55.6)	16 (42.1)	
II	6 (33.3)	5 (13.2)	
III	1 (5.6)	14 (36.8)	
IV	0 (0.0)	1 (2.6)	
Not available	0 (0.0)	0 (0.0)	

Table 6. Differences between Spitzoid and other melanomas in children and adolescents. (Modified from original publication III)

	Spitzoid melanoma	Other melanoma types	*P*
Cases (n)	37	19	
Sex, n (%)			
Males	18 (48.6)	9 (47.4)	1.000
Females	19 (51.4)	10 (52.6)	
Age at diagnosis, mean (SD) (years)	14.5 (4.0)	17.4 (2.0)	0.006
Melanoma location, n (%)			
Head and neck	7 (18.9)	5 (26.3)	0.530
Trunk	8 (21.6)	6 (31.6)	
Upper extremity	4 (10.8)	2 (10.5)	
Lower extremity	18 (48.6)	5 (26.3)	
Not available	0 (0.0)	1 (5.3)	
Clark level, n (%)			
1	0 (0)	3 (15.8)	0.030
2	4 (10.8)	2 (10.5)	
3	12 (32.4)	9 (47.4)	
4	20 (54.1)	4 (21.1)	
5	1 (2.7)	1 (5.3)	
Not available	0 (0.0)	0 (0.0)	
Breslow thickness			
Median (range) (mm)	1.7 (0.2 to 6.0)	1.2 (0.2 to 15.3)	0.455
Not available, n (%)	0 (0.0)	3 (15.8)	
Ulceration, n (%)			
Yes	6 (16.2)	3 (15.8)	1.000
No	31 (83.8)	16 (84.2)	
Not available	0 (0.0)	0 (0.0)	
BRAFV600E expression, n (%)			
Positive	13 (35.1)	13 (68.4)	0.020
Negative	23 (62.2)	5 (26.3)	
Not available	1 (2.7)	1 (5.3)	
Tumour stage, n (%)			
0	0 (0)	3 (15.8)	0.025
I	16 (43.2)	10 (52.6)	
II	8 (21.6)	3 (15.8)	
III	12 (32.4)	3 (15.8)	
IV	1 (2.7)	0 (0.0)	
Not available	0 (0.0)	0 (0.0)	
Sentinel lymph node biopsy, n (%)			
Positive	11 (29.7)	1 (5.3)	0.143
Negative	9 (24.3)	6 (31.6)	
Not performed	17 (45.9)	11 (57.9)	
Not available	0 (0.0)	1 (5.3)	

5.4 Study IV: Co-morbidities and Risk Factors in Adolescent and Young Adult Melanoma Patients

From 241 AYA patients diagnosed in 1981–2011 and 1990–2014 included into the original publication IV, 102 (42%) were male and 139 (58%) were female. The mean age of the patients was 30.3 years. Most melanomas in AYA occurred in the trunk (38%) and in the lower limbs (37%). Male patients had more often melanomas of the

trunk and the head and neck, whereas females had more often melanomas located in the lower limbs (p=0.00028).

The mean Breslow thickness was higher in male patients compared to female patients (1.9 mm vs. 1.4 mm). The highest mean Breslow thickness was seen in head and neck melanomas (2.7 mm), but this did not differ significantly from the melanomas in other anatomical locations, apart from the melanomas in the upper limbs (1.1 mm, P=0.021). Breslow thickness was similar between the age groups when divided by 5-year intervals. Ulcerated melanomas had higher Breslow thickness compared to non-ulcerated melanomas (mean 3.0 mm vs. 1.4 mm). Patients with ulcerated tumours had also worse prognosis (OS 76% vs. 91%). Statistically significant patient and primary tumour characteristics are shown in Table 8 and in Table 1 of the original publication IV.

Melanoma-specific OS and DFS of all patients were 89%, and only two patients who had melanoma recurrence were alive during the follow up. The male patients had worse prognosis compared to the female patients (OS and DFS 81% vs. 94%, P=0.002 for OS and P=0.001 for DFS). The prognosis was worst in patients with melanomas of the trunk and head and neck. Interestingly, the prognosis of female patients with melanomas of the trunk was better than in their male counterparts (OS 93% vs. 74%). Within the age groups, melanoma prognosis was best in the age group of 25–29 with 97% OS, but the differences between in the survival between the age groups were not statistically significant. (IV: Figs. 1 and S1)

From 103 cases with information on whether the melanoma had developed into pre-existing nevus, 77 (75%) melanomas had developed into precursor lesion and 26 (25%) melanomas had appeared *de novo*. In females, melanoma had developed *de novo* in 31% vs. in 18% of male patients, but this was not statistically significant. No significant differences were observed in Breslow thickness, patient age, or in survival was observed between tumours developed into precursor lesion or *de novo*.

Table 7. Statistically significant patient and primary tumour characteristics of adolescents and young adults between sexes. (Modified from original publication IV)

Characteristic	**All patients (*n*=241)**	**Males (*n*=102)**	**Females (*n=139*)**	***P*-value**
Mean age in years (range)	30.3 (15.3–39.9)	30.8 (15.5–39.9)	29.9 (15.3–39.9)	0.384
Primary tumour location				
Trunk	92 (38.2%)	50 (49.0%)	42 (30.2%)	0.00028
Upper extremity	39 (16.2%)	15 (14.7%)	24 (17.3%)	
Lower extremity	89 (36.9%)	24 (23.5%)	65 (46.8%)	
Head and neck	20 (8.3%)	13 (12.7%)	7 (5.0%)	
Unknown	1 (0.4%)	0 (0%)	1 (0.7%)	
Breslow thickness (Mean, range)	1.6 mm (0.2–15.3 mm)	1.9 mm (0.3–15.0 mm)	1.4 mm (0.2–15.3 mm)	0.0013
Ulceration				
Yes	38 (15.8%)	22 (21.6%)	16 (11.5%)	0.048
No or unknown	203 (84.2%)	80 (78.4%)	123 (88.5%)	

Co-morbidities encountered in AYA are shown in Table 9 and in Table 2 of the original publication IV. Autoimmune disease was present in 23 (10%) of the 164 AYA patients with the electronical treatment records available. These included systemic lupus erythematosus (SLE), various types of arthritis, psoriasis, sarcoidosis, inflammatory bowel diseases, celiac diseases, type 1 diabetes, hyperthyroidism with Basedow's disease and Addison's disease.

Altogether 31 (19%) patients were diagnosed with another cancer besides the primary melanoma. Another skin cancer was diagnosed in 14 (9%) patients. Nine of them had more than one primary melanoma. From the keratinocyte carcinomas, one patient had squamous cell carcinoma and basal cell carcinoma was diagnosed in 9 patients (6%). Another cancer was associated with sun sensitive skin (P=0.007) and the history of sunburns (P=0.011). Multiple melanomas were diagnosed more often in patients with basal cell carcinomas (22% vs. 5%), but this difference did not reach statistical significance. Another extracutaneous cancer was present in 17 (10%) patients, from which breast cancer (n=9) was the most common. Patients were also diagnosed with prostate cancer, lung cancer, glioblastoma, (chronic) lymphatic leukaemia, diffuse large B-cell lymphoma and Hodgkin's disease.

Dermatological disease was present in 23 (10%) patients. Psoriasis and atopic dermatitis were most common dermatological diseases, followed by different types of dermatitis, cutaneous sarcoidosis, cutaneous SLE, SLE panniculitis, skin sensitivities, acne, and rosacea. Allergies, such as contact dermatitis, atopic dermatitis, asthma, allergic rhinitis, or food allergies were reported in 22 (13%) patients. It was not always clear, whether these allergies were diagnosed by an allergologist or a general practitioner.

Neurological disease or a benign tumour of the nervous system including the pituitary gland was reported in eight patients (5%). These diseases were amyotrophic lateral sclerosis (ALS), meningiomas, two benign peripheral neural tumours in same patient, macroadenoma of the hypophysis, hydrocephalus in childhood and epilepsy related to mental disability.

Thirty-one patients (19%) had psychiatric illness, and 14 (45%) of them were present before the diagnosis of melanoma. After the melanoma diagnosis, 17 (55%) developed psychiatric morbidity. Conditions diagnosed prior melanoma diagnosis were depression, anxiety, panic attacks, neurotic behaviour, bipolar disorder, paranoid schizophrenia, obsessive compulsive disease (ODC) and personality disorder. Morbidities diagnosed after the first primary melanoma were most often depression, followed by anxiety, panic attacks, binge eating, OCD, chronic insomnia, and impulsivity.

Immunoglobulin deficiencies were diagnosed in two patients, one had IgA-deficiency and the other had IgG subtype deficiency. One patient had stroke and another patient experienced dissection of the aorta while under 40 years old.

Germline mutation of CDKN2A was reported in one patient, and one patient had Bannaya-Riley-Ruvalcaba syndrome. Genetic testing or counselling was rarely offered for AYA melanoma patients.

Known risk factor for melanoma, history of sunburns, was reported in 12 (7%) patients. Sun sensitive skin type was mentioned in 17 (10%) patient's records. Seven patients (4%) had had history of irradiation, from which radiation therapy was given for two patients on the body site into which melanoma developed. Three patients reported a history of solarium use, and two psoriatic patients were treated with UV311/UVB-treatments.

5.4.1 Pregnancy and Melanoma in Young Adults

From the 68 AYA females with reported reproductive history available, 10 (15%) patients developed melanoma during pregnancy or in the first year after the delivery (pregnancy group). Eight of these melanomas (12%) had developed during pregnancy. Two (3%) patients were diagnosed with cutaneous melanoma during pregnancy. Fourteen patients (21%) were diagnosed with melanoma within two years postpartum.

Patients in the pregnancy group demonstrated with OS of 100%, and their OS did not differ from the OS of 97% of the remaining 58 female patients (control group). Breslow thickness was higher in the pregnancy group (mean 1,8 mm) compared to the control group (mean 1.0 mm). Statistical difference in Breslow thickness or in ulceration was not observed between the groups, or in the development on melanoma into precursor lesion or *de novo*. (IV: Table 3)

After excluding the basal cell carcinomas from other cancers, patients in the pregnancy group were diagnosed more often with another cancer than the patients in the control group (40% vs. 10%, P=0.034). There were four cases of another cancer in the pregnancy group, of which two were multiple primary melanomas and two were breast cancer. History with sunburns was reported more often in the pregnancy group than in the control group (30% vs. 10%, P=0.009).

Dermatological diseases were more common in the control group than in the pregnancy group (19% vs. 10%), out of which none of the patients in the pregnancy group had psoriasis. One patient in the pregnancy group developed depression after melanoma diagnosis, and none of the pregnancy group patients had a psychiatric diagnosis prior melanoma diagnosis. Co-morbidities observed in all patients and in the patients in the pregnancy group are shown in Table 9. Schematic illustration on risk-factors and co-morbidities in AYA melanoma patients in seen in Figure 2. Of the original publication IV.

Table 8. Co-morbidities observed in adolescents and young adults in all patients and in the pregnancy group patients. (Modified from original publication IV)

Co-morbidity	**All patients *(n=164)***	**Patients in the pregnancy group (*n=10*)**
Autoimmune diseases	**23**	**1**
SLE	3	
(Juvenile) rheumatoid arthritis	3	
Spinal arthritis	1	
Sacroiliitis	1	
Mixed connective tissue disease	1	
Psoriasis	7	
Sarcoidosis	3	
Ulcerative colitis	4	
Crohn's disease	1	
Celiac disease	3	
Type 1 diabetes	1	
Hyperthyroidism + Basedow	2	1
Addison's disease	1	
Another cancer	**31**	**4**
Another skin cancer	***14***	***2***
Multiple melanomas	9	2
Basal cell carcinoma	9	
Squamous cell carcinoma	1	
Another extracutaneous cancer	***17***	***2***
Breast cancer	9	2
Prostate cancer	1	
Lung cancer	1	
Glioblastoma	2	
(Chronic) lymphatic leukaemia	2	
Diffuse large B-cell lymphoma	1	
Hodgkin's disease	1	
Dermatological diseases	**23**	**1**
Psoriasis	7	
Atopic dermatitis	7	
Allergic dermatitis	4	
Hand eczema	1	
Unspecific bullous dermatitis	1	
Cutaneous sarcoidosis	1	
Cutaneous SLE	1	
SLE panniculitis	1	
Highly immunoreactive skin	1	1
Skin photosensitivity	1	
Rosacea	1	
Acne	1	
Neurological disease	**4**	**1**
ALS	2	1
Hydrocephalus	1	
Epilepsy	1	
Benign nervous system tumour	**5**	
Meningioma	3	
Peripheral nerve tumour	1	
Macroadenoma of hypophysis	1	

Co-morbidity	**All patients *(n=164)***	**Patients in the pregnancy group (*n=10*)**
Psychiatric diseases	**31**	**1**
Before melanoma diagnosis	***14***	
Depression	7	
Anxiety	2	
Panic attacks	1	
Neurotic behaviour	2	
Bipolar disorder	2	
Paranoid schizophrenia	2	
OCD	1	
Personality disorder	1	
After melanoma diagnosis	***17***	***1***
Depression	13	
Anxiety	5	1
Panic attacks	2	
Binge eating	1	
OCD	1	
Chronic insomnia	1	
impulsivity	1	

6 Discussion

This thesis indicates that melanomas in children and adolescents often show clinically atypical features. Especially the melanomas in younger, prepubertal children are more frequently amelanotic, and the diagnosis of malignant melanoma is often unexpected for both the patient and the health care professional. The diagnosis of melanoma, particularly of Spitzoid subtype, challenges even the experts.

Although rare, the incidence of melanomas in the young, especially in adolescents and in Spitzoid melanomas, has increased in Finland. A substantial increase from 1.4/1 000 000 to 5.8/1 000 000 was observed in the incidence of melanomas in children and adolescents in 1990–2014. The latest incidence rates of melanoma in children and adolescents seen in Finland are similar to the rates reported in the USA and in the Netherlands.[1,2,34,37]

This increase could either have been a result of a true change of melanoma incidence or changes in diagnostic criteria and by this, in register coverage. Increased UV-exposure may be a contributing factor for increased, true melanoma incidence observed in this study. Since the domestic sun-exposure in Finland is relatively low, the increased recreational travel among Finns may have contributed to the UV exposure.[188] On the other hand, possible increase in the register coverage might have also contributed to the increased incidence. Significant improvements to the classification of Spitzoid melanomas have been made during the recent years.[19,189] As a result, Spitzoid tumours previously not diagnosed as melanomas, and therefore not registered in the FCR, could have been classified as Spitzoid melanomas when the knowledge of these rare tumours has increased. Hypothetically, this may have resulted in enhanced register coverage causing an apparent increase in the incidence of melanoma in children and adolescents without a true change in incidence. However, the results of this study did not support this hypothesis, and the estimations performed with the re-evaluated tumour material suggest that the increase in the incidence is in fact true.

The histopathological diagnosis of Spitzoid and other atypical melanocytic tumours is known to be challenging even for experienced pathologists.[33,77,80] This could be seen also in our studies II and III, as the Cohen's kappa for inter-rater agreement of 0.50 indicated a moderate agreement between the two experienced

dermatopathologists. The tumours with discordant diagnoses were typically associated with favourable prognostic factors, such as low mitosis count, low Breslow thickness and lack of ulceration. Majority of these tumours demonstrated with Spitzoid histology. None of these tumours was fatal or metastasised to distant organs, but without applicable biomarkers inclusive for malignant melanoma, these tumours had been treated as malignant melanomas in the clinical setting. Based on these findings and the diagnostic challenges reported in the literature, it is recommended that the diagnosis of melanoma in children and adolescents in made by at least two experienced dermatopathologists.[33,80]

The youngest patients in our study cohort did not seem to have any particular risk factors described for cutaneous melanoma.[51] If all the information on these patients, such as family history or history of UV-exposure or skin phototype would have been in our use for each case, possible risk factors might have been identified. For this purpose, a prospective study would be more suitable as a study design. However, the pre-disposing patient phenotypes observed in AYA patients were of two main subtypes, those with immune related dysfunctions and those with possible cancer disposing genotype. Also, the possible effect of pregnancy in melanoma development might have connections with the development of melanomas in adolescents as well, hypothetically due to changes in hormonal and immunological factors during these periods of life. These factors would be interesting to study also in children, if a larger number of patients with information on co-morbidities would be available. UV-exposure and sun sensitive skin type were also applicable risk factors in AYA, but their role in children is not as clear.

In AYA, interesting co-morbidities and possible risk-factors for melanoma were observed in the fourth original publication. Particularly interesting topic is the association with 17 (IL-17) and several co-morbidities. Th17 type of inflammation has been known to be crucial in psoriasis, for example.[190–192] In our AYA cohort, the prevalence of psoriasis was 4%, which is higher than a population-wide prevalence reported from Sweden (2%). The risk of cutaneous melanoma is known to be higher in patients with psoriasis regardless of systemic, biologic or UV-therapies.[193–195] Other co-morbidities encountered, such as autoimmune diseases, schizophrenia and ALS are also known to be associated with psoriasis or IL-17.[191,196,197] Also, autoimmune diseases were more common in the AYA patients of this study than reported in the literature.[198] Skin diseases are reported in 30–70% of populations in total, but the data could not be reliably compared with ours due to differences in the reporting.[199]

The role of IL-17 has been recently investigated in melanoma.[192,200–202] *In vitro*, IL-17 has shown to increase the proliferation human and murine cell lines. Decreased disease progression was also seen in IL-17 knockout mouse model and in xenograft melanoma tumours after the silencing of the IL-17 receptor A.[200] Also, the Hmgb1-

IL-23-IL-17-IL-6-Stat3 axis is associated with increased melanoma growth in a murine model form melanoma.[202] In human melanoma IHC samples, increased IL-17 positivity of the tumour mass was seen in malignant melanomas compared to benign melanocytic nevi and Spitz nevi.[201] It has also been shown in mice that depletion of gut microbiota reduced melanoma tumour burden by decreasing IL-17 and other pro-tumorigenic cytokines, which were present in the tumour microenvironment.[203] These findings and the results of the this study raise a question on IL-17 mediated immune disturbances being a possible risk-factor for melanomas in AYA, and their role in melanomas in children would also be interesting to study further.

Cancer predisposing genotype could be another possible risk-factor for melanoma in AYA based to the study IV. Patients with multiple melanomas often had history of sunburns, and the number of basal cell carcinomas was higher in patients with multiple melanomas compared to the other patients. Breast cancer was the most common other cancer with nine cases, compared to only one patient with prostate cancer. A part of these patients may be carriers for cancer predisposing germline mutations, such as CDKN2A and BRCA2.[204] Besides to familial melanomas, mutations in CDKN2A have been linked to rare melanoma and neural system tumour syndrome and Li-Fraumeni -syndrome[204–206]. Even though the former of the rare syndromes has not been described in Finland, it could be hypothesised, since two patients developed glioblastomas, and two other patients had reported their father having a brain tumour, and the patient with four primary melanomas had also two benign peripheral neural sheath tumours.

Supporting the hypothesis of some of the AYA melanoma patients carrying cancer disposing pre-disposing germline mutations, the proportion of the patients with another cancer (19%) was higher in our patient population than observed in the general population. A large population wide study made in the USA reported the overall risk for second malignancy being 8.1% in all cancer survivors, and prostate cancer being most common second cancer of melanoma survivors.[207] Another study made in Sweden showed an increased risk for especially to multiple melanomas and other skin cancers in melanoma survivors, and these patients had also higher prevalence of neuroectodermal tumours and cancers of the immune system. The study in question found no increased risk for breast cancer in melanoma survivors.[208]

Applying the genotypic risk assessment together with phenotypic risk assessment to AYA patients could be helpful in assessing the risk for another cancer in this patient group.[209,210] The number of keratinocyte carcinomas observed in the AYA cohort is relatively low, and favours genotypic risk assessment over phenotypic assessment in this patient population.[211] However, basal cell carcinomas are in most cases treated in the primary health care in Finland, and it is possible that all of these cases were not in our knowledge.

The numbers of patients with pregnancy-associated melanomas are difficult to compare within the studies published for their differences in the inclusion criteria of the patients and in their means of analysis.[57,58,169] In Sweden, 3,3% of melanomas were reported to have been diagnosed during pregnancy, but this did not include the first year postpartum.[57] A Californian study reported the incidence of melanoma during pregnancy and first year postpartum being 8.5 per 100 000 pregnant women.[58] As in the study IV, these two studies did not report differences in the survival rates between pregnant and non-pregnant melanoma patients.[57,58] A Finnish study associated previous pregnancy with improved survival among female melanoma patients.[169] This study at hand did not find associations with other diseases or risk-factors than between another cancer than basal cell carcinoma and sun exposure in the pregnant group patients, which supports the hypothesis of genotypic predisposition in pregnant melanoma patients.

In melanomas of children and adolescents, BRAFV600E-positivity of 48% was at like that in adults.[123] Interestingly, majority of the patients who died of metastatic melanoma the primary tumour and/or the metastases were BRAFV600E-positive. These patients with lethal tumours were all adolescents, and therefore they might have experienced higher level of UV-exposure than smaller children. In this respect, many of the melanomas found in the adolescents may have followed the pathogenesis described in the literature, in which a BRAF mutated precursor lesion develops into malignant melanoma while the mutational burden increases.[74,75] The 10-year-OS of BRAFV600E-positive patients was worse than BRAFV600E negative patients, and this patient population possibly benefits from a similar treatment and surveillance than their adult counterparts.

In adult melanomas, previous studies have reported 2–3% positivity rates for ALK IHC.[83,139] In this study, the higher amount (9%) of ALK-positive melanomas in children and adolescents could result from higher number of Spitzoid melanomas, since approximately 10% rate for ALK-positivity has been described in Spitzoid tumours.[81] In the studies II and III, 13.5% of Spitzoid melanomas showed ALK-positivity, whereas none of the other subtypes were ALK-positive. In our study, only one patient had weak PD-L1-positivity of the primary tumour. In adults, PD-L1-positivity rates have been reported up to 53% while using the 1% threshold.[212] It is not clear, whether this is due to alterations in the host immune responses or in the tumour biology between the patients of different ages.

All prepubertal patients <11 years had Spitzoid melanomas, and they did not stain positive for BRAFV600E, ALK or PD-L1 in five out of six cases. If these melanomas would be further analysed, mutations of NRAS or HRAS could be found from some of the melanomas left IHC negative in our study, and kinase fusions could also be present in some of the Spitzoid melanomas.[19,81] No patient under 11 years old died of melanoma or developed distant metastases. The prognosis of the

prepubertal patients is at times described poor in the literature, but this seems to be associated with melanomas occurring in congenital nevi of young children, or those with neurocutaneous melanosis.[1,19]

The high SLNB-positivity rate of 44% observed in the study II is a notably larger percentage than in described in adults, among whom SLNB-positivity sets between 10–30% in different melanoma subtypes.[213] In this study, positive SLNB result did not indicate for decreased survival, since both SLNB -positive and -negative groups had 100% OS. Similar results have been described also in the literature.[112,113] Children and adolescents had also higher melanoma specific 10-year-OS of 89%, which is similar to the rates of the adult melanoma patients with negative SLNB result.[213] The difference of this level could be explained with the higher proportion of Spitzoid melanomas in children and adolescents, since these tumours could potentially metastasise into regional lymph nodes without worsening the prognosis.[101]

In children and adolescents withs stage II melanoma, the prognosis was better than in adults of the same stage reported in a Swedish study.[214] The 10-year-OS in children and adolescents was higher 100% in stages IIIA and IIIB than reported in their adult counterparts (100%/100% vs. 80%/55%). The survival was also better in children and adolescents of stage IIIC melanomas compared to the literature on adult patients of the same disease stage (78% vs. 43%).[214] In this study, patients in the stage II-group had worse prognosis than those in the stage group III, which is most likely caused by a phenomenon called stage migration, which results from clinically occult lymph node metastases found with SLNB. Also, this stage group is relatively heterogenic in terms of the lymph node involvement, which also influences the differences in the survival of the patients within this group.

The survival of AYA (OS and DFS 89%) is relatively good when compared with in patients aged 55–74 years in Finland in 2017–2019, whose 5-year-OS was 92%.[24] In this study, AYA with melanomas located in head and neck and trunk had the worse prognosis, which is in line with the previous reports.[215] Known prognostic factors, Breslow thickness and ulceration, were applicable in AYA also in this study.[5,6] The worsened survival of AYA males compared to females was also seen in this study, but the survival was not adjusted for prognostic factors such as Breslow thickness like in some of the other publications.[5,216,217] Interestingly, 15–19 years old adolescents had worse survival than young adults aged 20–30 years. Possible explanations for this difference in survival could lay in differences in the tumour biology and pre-disposing conditions, or in the low suspicion for melanoma among adolescents.

The results of this study did not give definitive answers on the role of the UV exposure in the melanomas in children and adolescents. In the latest WHO classification, Spitz melanomas are regarded not having UV-induced pathogenesis.[19]

However, there were also other tumour subtypes included in the studies II and III. Therefore, UV-radiation may have contributed to the increase in the incidence observed in the study III. Based on this study, the role of the UV-exposure is more evident in young adults, since the history of sunburns was associated with multiple melanomas and with basal cell carcinomas. History of sunburns in especially during childhood are well known risk factors for melanoma, but the tumours rarely form in children and adolescents.[1,2,23] It may be expected that the parents of the young children are in most cases careful with their sun protection, but in adolescents, the sun protective behaviour might not be on the same level. Together with possible solarium use before its banning for minors in EU and in the USA, aiming for a trendy sun or artificial tan could partly explain the increased incidence noted in this study. The risk of cutaneous melanoma is shown to increase with the use of solarium also in younger adults.[218] Public health measures, such as health protection campaigns executed in schools or in the social media could increase healthy sun protective habits in the adolescent population.

The risk for a second primary melanoma is reported to be approximately 4% in 5-year-follow up in the Netherlands, and the incidence of the second malignant melanoma has been increasing in Sweden. The Dutch study showed that cumulative sun exposure, such as outdoor working, was associated with higher risk for second primary melanoma, and the authors of the Swedish study suggest increasing UV exposure in forms of e.g., recreational travelling to be a possible cause for the increasing incidence of the second primary melanoma observed since the 1960's. Both studies suggest 10-year follow-up to be planned with yearly nevus screening by a clinician, also after *in situ* melanoma.[219,220] In children, it is not clear how many will develop second primary melanoma, and how long they should be followed. Perhaps, 10-year annual follow up could be advisable in younger patients, and it could be made also in the primary health care depending on the disease stage and primary tumour characteristics. Also, patient education on self-examination of the skin would be advisable, and suspicious lesions in these patients should be excised at a low threshold.

In the fourth study, we did not find significant associations between melanomas developed in precursor lesions or *de novo* in AYA. A Brazilian study linked *de novo* melanomas to worse prognosis, but this association was not seen in our study or in a study with Turkish and Caucasian patients.[221,222] Possible explanations for these differences could be related to the tumour biology, its initiating epigenetic factors, or in the differences in the genetic background of the studied patients. If histopathological subtypes of the tumours would have been in our knowledge in the fourth study, some differences between development of melanoma subtypes could have been observed.

The findings of the study II encourage the careful consideration on performing SLNB in especially among the youngest patients. Also in healthy individuals, benign melanocytic nevus cells can be seen in the lymph nodes without signs of malignancy, and atypical Spitzoid tumours are shown to metastasise into sentinel lymph nodes without worsening the prognosis like in malignant melanoma.[101,223] One limitation of the study II was the lack of the re-evaluation of the SLNB samples. Nevertheless, SLNB will still bring prognostic information, which may influence to the treatment decisions.[112,113] The decision to perform SLNB in children and adolescents should be carefully considered in an expert meeting consisting of a member from each specialty participating in diagnosing and treating the patient, and not to be left for the surgeon alone. Melanomas in this age group are rare, and before larger meta-analyses from the treatment outcomes are available, it would be advisable to perform the SLNB also in Spitzoid melanomas if the histological features are worrisome. In case the primary tumour resembles more of an atypical Spitz tumour than malignant melanoma, but it is still too atypical to be treated as atypical Spitz nevus, SLNB may be omitted and careful follow-up by ultrasound and clinical examinations planned instead according to the MDT meeting's advice.

However, CLND following a positive SLNB result may not be advisable in young patients and follow up by clinical examination and ultrasound might be preferential.[1,18] In adults, routine CLND after positive SLNB result was abandoned after the release of the MSLTII trial results in 2017.[28] If metastatic nodes would be then found by clinical examination or by ultrasound in children and adolescents, TLND could be performed. In this way, unnecessary morbidity due to surgery and its possible complications could be avoided for majority of the patients. This is especially important in the young patients, who are still in their growth phase, and have hopefully many years still ahead. For the same reasons together with preservation of the lymphatics, it would be advisable to perform the initial surgery by using narrow excisional margins instead of wide local excision or incisional biopsy.[1,18] Since the melanomas in the young can be very challenging to diagnose histopathologically, obtaining the whole lesion for histopathological examination would be of utmost importance. Also, medical oncological therapies need to be considered carefully for their benefits and possible side effects and toxicities in especially in children and adolescents. In addition, a reactive-type psychiatric morbidity increased after melanoma diagnosis in AYA, which emphasises the need for psychiatric support after melanoma diagnosis in the young patients.

The limitations of these studies were its retrospective nature and the low number of patients, especially in the youngest age group, which did not allow us to reach statistical significance in many parameters, such as in the survival rates. Nevertheless, the materials used in this nationwide study comprised all paediatric and adolescent melanoma patients diagnosed in 1990–2014, and relatively high

number of the primary tumour samples were obtained for the re-evaluation. Also, having access to all the patient records of also from the youngest patients for analysing risk factors and co-morbidities would have been beneficial. In AYA the information on primary tumour subtype would have brought new information on the pathogenesis of melanoma in the young. However, interesting findings on melanoma in children and adolescents could be made in our studies, and with those, we hope to aid pathologists and clinicians in diagnosing and treating these rare malignancies.

7 Conclusions

- Melanomas in children were more often clinically amelanotic and raised, and they might not raise the suspicion for melanoma. Therefore, all suspicious lesions in children with evolution should be excised or biopsied.
- Primary cutaneous melanomas in children and adolescents showed different IHC profile for possible therapeutic targets than reported in adults. Even though BRAFV600E-positivity was similar, ALK-positivity was higher than reported in adults. Children and adolescents basically did not have PD-L1-positive tumours. The prognosis of cutaneous melanoma in children and adolescents is better than described in the adult population, despite the fact they have more often SLNB positive lymph nodes. Younger adolescents and patients with BRAV600E-positive tumours seemed to have worse prognosis compared to the others, and their treatment and follow-up should follow adult guidelines. Spitzoid melanoma is most common melanoma subtype in children and adolescents in Finland. These melanomas may have better prognosis than SSMs and NMs, but their diagnosis is more challenging both clinically and histopathology, and the diagnosis should be made at least by two dermatopathologists.
- Melanoma in children and adolescents is rare, but its incidence has increased approximately 4-fold in Finland in 1990–2014. This increase is most prominent in adolescents and in Spitzoid melanoma subtype. The changes in the incidence seem to be true, and not caused by larger register coverage because of e.g., over-diagnostics of benign or atypical Spitzoid tumours as Spitzoid melanomas. Avoiding UV-radiation exposure would be advisable in children and adolescents.
- Evident risk-factors for melanoma were not observed in children, but in adolescents, they may by like those of AYA, such as UV-exposure and sun sensitive skin type. Immune related factors and possible tumour susceptible genotype seemed to be two main patient subtypes in AYA melanoma patients, and suspicious lesions of these patient types should be excised at the early stage.

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