Melanoma surgery in the era of modern immunotherapy

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Till Maja.

"I send greetings on behalf of the people of our planet. We step out of our Solar System into the Universe, seeking only peace and friendship, **to teach if we are called upon, to be taught if we are fortunate**."

- *The Secretary General of the United Nations (1977), as etched on gold metal plates stored onboard the Voyager space probes. The plates contain a greeting with the pictures, sounds and sciences of planet Earth. Voyager 1 will pass the star Gliese-445 in 40,000 years and Voyager 2 will pass the star Sirius in 296,000 years, after which both probes will continue onward into the cosmos.*

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ABSTRACT

Recent advances in immuno-oncology have led to a paradigm shift in the treatment of advanced cutaneous melanoma. The aim of this thesis is to investigate the role of surgery in the management of melanoma in this modern era of effective systemic treatments. This is done using a comprehensive approach of both retrospective and prospective study designs. In *Paper I*, we gathered international data from a large number of melanoma centers to examine the efficacy of immunotherapy on melanoma in-transit metastases and found immunotherapy to be effective in this patient group. In *Paper II*, we investigated the effect of isolated limb perfusion in patients that had received previous systemic immunotherapy and found it remains an effective treatment option. In *Paper III*, we used data from the Swedish national melanoma registry to show the prognostic value of sentinel lymph node status also in thick (>4.0 mm) melanomas, and our study supports a recommendation for sentinel lymph node biopsy also for thick tumors. In *Paper IV*, we performed a randomized controlled trial investigating the safety and preliminary efficacy of a single dose of the PD-1 inhibitor nivolumab prior to isolated limb perfusion for in-transit metastases, and found it to be safe and with promising efficacy.

This thesis shows a strong continued role for surgery and locoregional treatments for patients with advanced melanoma, and underscores the evolving and dynamic nature of melanoma management, where a multidimensional approach holds the key to optimizing patient outcomes.

Keywords: melanoma, surgery, immunotherapy, isolated limb perfusion

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SAMMANFATTNING PÅ SVENSKA

Malignt melanom är en cancertumör som utgår från hudens melanocyter. Sverige har ett av världens högsta insjuknandetal av melanom, med siffror som fortsatt stiger. I de fall där tumören sprider sig med metastaser är sjukdomen aggressiv och utmanande att behandla. Historiskt har detta inneburit kort överlevnad för patienten. Under senaste decenniet har dock nya systemiska immunologiska cancerbehandlingar utvecklats, något som helt revolutionerat fältet. Med dessa moderna immunterapier mobiliseras kroppens egna immunförsvar mot cancern och låter patientens egna T-celler attackera tumörerna. Det har varit oklart hur äldre kirurgiska behandlingstekniker ska användas i denna moderna era, och om det går att uppnå ännu bättre behandlingsresultat genom att kombinera beprövade och nya tekniker. Syftet med denna avhandling var således att undersöka vilken roll kirurgiska behandlingsmetoder har inom ramen för modern immunterapi.

I delarbete I undersöktes behandlingseffekten av immunterapi på s.k. in-transitmetastaser, en bitvis aggressiv och kirurgiskt svårbehandlad form av spridda melanommetastaser. Genom att retrospektivt samla in data från ett stort antal sjukhus från hela världen har vi kunnat utvärdera behandlingseffekt hos specifikt denna patientgrupp, kunskap som tidigare saknats. Vi kan med denna studie styrka att immunterapi har effekt på just in-transitmetastaser.

I delarbete II undersöktes effekten av behandlingen *isolated limb perfusion* (ILP) hos patienter med in-transitmetastaser som haft otillräckligt behandlingssvar efter immunterapi. ILP är en teknik där en tumördrabbad extremitet stängs av från övriga blodcirkulationen, kopplas till hjärt-lungmaskin och sköljs med mycket höga doser upphettade cellgifter. Vår studie bekräftar att ILP är ett bra behandlingsalternativ även hos patienter som tidigare erhållit immunterapi och att metoden har kvar sin nischade roll.

I delarbete III användes data från det nationella Svenska Melanomregistret (SweMR) för att undersöka det prognostiska värdet av kirurgisk biopsering av portvaktskörtlen, d.v.s. den första dränerande lymfkörteln från tumörområdet, hos patienter med specifikt tjocka (>4.0 mm) melanom. Värdet av denna biopsi är tidigare väl fastställt för tunnare melanom, men rekommenderas idag inte i riktlinjer för tjockare tumörer. Med vår studie kunde vi nu bekräfta att resultatet av portvaktskörtelbiopsi är prognostiskt värdefullt även vid tjocka melanom, och vi rekommenderar att rådande internationella riktlinjer uppdateras.

I delarbete IV undersöktes en ny behandlingsstrategi där en enstaka dos av immunterapi ges dagen före ILP med förhoppningen att detta ytterligare ska mobilisera immunförsvaret och öka behandlingseffekten. Studien är en internationell randomiserad kontrollerad studie (NivoILP-studien) där patienter slumpades till antingen placebo eller en dos immunterapi innan ingreppet. I en första preliminär analys kan vi konstatera att detta är en säker behandlingsstrategi som visar lovande behandlingseffekt.

Denna avhandling visar på den fortsatt viktiga rollen för kirurgi i behandlingen av avancerat malignt melanom. Våra studier befäster vikten av en multimodal behandlingsstrategi för patienter med spridd sjukdom, i synnerhet för patienter med in-transitmetastaser. Vi belyser även utmaningarna, men också möjligheterna, i att reorientera beprövade behandlingar inom ramen för det paradigmskifte som införandet av effektiv systemisk immunterapi inneburit.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Holmberg CJ, Ny L, Hieken TJ, Block MS, Carr MJ, Sondak VK, Örtenwall C, Katsarelias D, Dimitriou F, Menzies AM, Saw RP, Rogiers A, Straker RJ 3rd, Karakousis G, Applewaite R, Pallan L, Han D, Vetto JT, Gyorki DE, Tie EN, Vitale MG, Ascierto PA, Dummer R, Cohen J, Hui JY, Schachter J, Asher N, Helgadottir H, Chai H, Kroon H, Coventry B, Rothermel LD, Sun J, Carlino MS, Duncan Z, Broman K, Weber J, Lee AY, Berman RS, Teras J, Ollila DW, Long GV, Zager JS, van Akkooi A, Olofsson Bagge R.

   The efficacy of immune checkpoint blockade for melanoma in-transit with or without nodal metastases – A multicenter cohort study.

   Eur J Cancer. 2022 Jul; 169:210-222.

II. Holmberg CJ, Mattsson J, Olofsson Bagge R.

   Effects of the introduction of modern immunotherapy on the outcome of isolated limb perfusion for melanoma in-transit metastases.

   Cancers (Basel). 2023 Jan; 15(2):472.

III. Holmberg CJ, Mikiver R, Isaksson K, Ingvar C, Moncrieff M, Nielsen K, Ny L, Lyth J, Olofsson Bagge R.

   Prognostic significance of sentinel lymph node status in thick primary melanomas (> 4 mm).

   Ann Surg Oncol. 2023 Aug; 30(13):8026–8033.

IV. Holmberg CJ, Zijlker L, Katsarelias D, Huibers A, Wouters WJM, Schrage Y, Reijers S, van Thienen H, Grünhagen DJ, Martner A, Nilsson J A, van Akkooi ACJ, Ny L, van Houdt W J, Olofsson Bagge R.

   The effect of a single dose of nivolumab prior to isolated limb perfusion for patients with in-transit melanoma metastases: an interim analysis of a phase Ib/II randomized double-blind placebo-controlled trial (NivoILP trial)

   Submitted manuscript.

RELEVANT PUBLISHED WORK NOT INCLUDED IN THE THESIS

Holmberg CJ, Katsarelias D, Jespersen H, Carneiro A, Elander NO, Helgadottir H, Isaksson K, Jansson M, Wirén S, Ullenhag GJ, Ny L & Olofsson Bagge R. **Surgery of metastatic melanoma after systemic therapy – the SUMMIST trial: study protocol for a randomized controlled trial.** Acta Oncologica. 2020 Nov; 60:1, 52-55.

Lindqvist Bagge A-S, Wesslau H, Cizek R, Holmberg CJ, Moncrief M, Katsarelias D, Carlander A, Olofsson Bagge R. **Health-related quality of life using the FACT-M questionnaire in patients with malignant melanoma: A systematic review.** Eur J Surg Oncol. 2021 Sep; 48(2):312-319.

Holmberg CJ, Alwan G, Ny L, Olofsson Bagge R, Katsarelias D. **Surgery for gastrointestinal metastases of malignant melanoma – a retrospective exploratory study.** World J Surg Oncol. 2019 Jul 12;17(1):123.

CONTENTS

ABBREVIATIONS

AE	Adverse event
AJCC	American Joint Committee on Cancer
BAP1	BRCA1 associated protein-1
BRAF	V-raf murine sarcoma viral oncogene homolog B
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CLND	Completion lymph node dissection
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4
DSMB	Data safety monitoring board
ECT	Electrochemotherapy
FDA	Food and Drug Administration
HRQoL	Health-related quality of life
ICI	Immune checkpoint inhibitor
ILI	Isolated limb infusion
ILP	Isolated limb perfusion
LDH	Lactate dehydrogenase
MEK	MAPK/ERK Kinase
MI-ILP	Minimally invasive isolated limb perfusion
MILND	Minimally invasive inguinal lymphadenectomy
PD-1	Programmed cell death protein 1

RECIST	Response Evaluation Criteria in Solid Tumours
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SweMR	Swedish Melanoma Register
TVEC	Talimogene laherparepvec
UV	Ultraviolet light

DEFINITIONS IN SHORT

Complete response (CR)	Disappearance of all lesions according to RECIST 1.1 criteria.
Duration of response (DOR)	Time from CR or PR to disease progression or death from any cause.
Melanoma-specific survival (MSS)	Time to death from melanoma.
Overall response rate (ORR)	Proportion of patients with CR or PR.
Overall survival (OS)	Time to death from any cause.
Partial response (PR)	Decrease of more than 30% of the total tumor burden, measured as number of lesions or shrinkage in largest tumor diameter, according to RECIST 1.1 criteria or by caliper measurement for cutaneous lesions not visible on radiology.
Progression-free survival (PFS)	Time to disease progression or death from any cause.
Progressive disease (PD)	Increase of more than 20% in existing lesions, or the appearance of new lesions.
Stable disease (SD)	Criteria for CR, PR or PD not met.
Time to local progression (TTLP)	Time to disease progression locally.
Time to nodal progression (TTNP)	Time to disease progression in lymph nodes.
Time to systemic progression (TTSP)	Time to disease progression with distant metastases.

1 INTRODUCTION

Descriptions of melanoma can be found as early as in the writings of Hippocrates (460-375 BC). The first surgical resection of a melanoma is generally credited to John Hunter, a Scottish surgeon working at St George's Hospital in London, who in 1787 removed a recurring black tumor on the face of a 35-year old man [1]. By the 1850's, the disease had been named "melanoma" and its malignant nature understood [2]. The tendency of this cancer to wildly disseminate throughout the body was noted already then, along with recommendations of radical resections to prevent spread (which at this time meant as wide an excision as possible). Interestingly, most modern-day publications on melanoma start with noting the very same things. Treating patients with melanoma thus pose the same basic challenges today as it did then. The tools at our disposal to overcome those challenges have, however, improved significantly.

1.1 EPIDEMIOLOGY

Cutaneous melanoma is currently the 18th most common cancer globally with over 324,000 new cases and 57,000 deaths reported globally in 2020 [3].

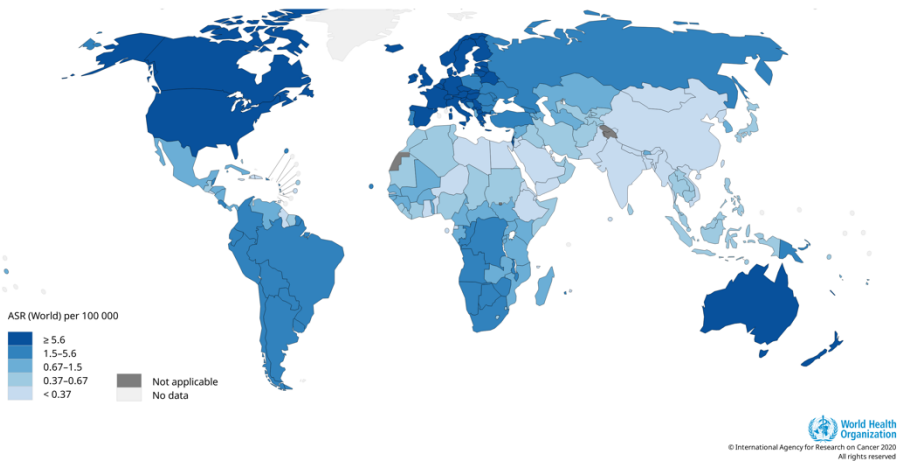

Figure 1. Estimated age-standardized incidence rates of melanoma in 2020. (Data source: GLOBOCAN 2020 Map production: IARC (http://gco.iarc.fr/today), World Health Organization. With permission.)

Incidence rates vary from its highest at 37 per 100,000 people in at-risk fair-skinned populations in Australia, to approximately 17-18 per 100,000 people in North America and Western Europe, to its lowest of less than 1 per 100,000 people in dark-skinned populations in most African countries (Figure 1) [4]. Global average incidence is estimated to be 3.8 and 3.4 per 100,000 people for men and women respectively and higher for men in most regions of the world.

In Sweden, clinicopathological data on all melanoma patients are registered in the Swedish Melanoma Registry (SweMR), a national registry with very high (99%) coverage that allows for population-level data aggregation. Excerpts from this registry show that incidence rates are increasing also in Sweden, currently with as much as 5% per year [5]. The number of reported new patients with invasive melanoma in 2022 were over 5500, a number that has more than quadrupled since the early 90's. The proportion of in-situ (i.e. non-invasive) melanomas have also been increasing, now equaling the invasive group in size. In contrast to continually rising incidence rates, however, Swedish mortality rates have been largely constant over time (Figure 2).

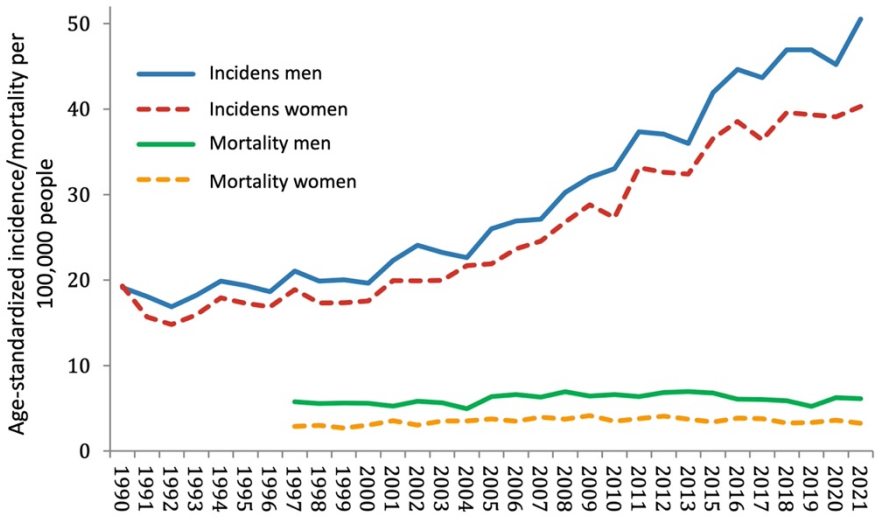

Figure 2. Age-standardized incidence and mortality of invasive melanomas per 100,000 people (Adapted from SweMR).

This mirrors a global trend and is believed to due to a combination of better diagnostics, better treatments and that the main increase comes from tinner melanomas with better prognosis. For instance, thin melanomas (≤1.0 mm), a group of tumors with excellent prognosis, now constitute 60% of all patients in Sweden. In 2022, 535 deaths from melanoma were reported in Sweden, with mortality remaining roughly twice as high in men as in women.

1.2 PATHOPHYSIOLOGY

Cutaneous malignant melanoma is a cancer arising from melanocytes in the superficial layers of the skin. Melanocytes form the pigment melanin that normally functions as an absorbing barrier of protection against harmful light exposure for underlying skin cells. The main cause of these melanocytes malignifying into melanoma cells is believed to be the mutagenic effect of UV radiation on DNA in exposed cells [6-8]. UVA radiation penetrates deep into the skin and causes indirect DNA damage via the formation of free radicals. UVB radiation, even more mutagenic, penetrates the superficial skin layers and causes a type of direct DNA-damage where cytosine and thymine nucleobases form faulty dimers. UVC rays are the most mutagenic but are filtered by the atmosphere and generally does not reach the earth surface. Melanocytes respond protectively to these types of radiation by producing more melanin, leading to the darkening of the skin commonly known as a “suntan”.

Accumulated sun exposure over time, exposure in early age and occasional extreme exposures resulting in sunburn are major risk factors in developing melanoma [9]. This includes the heavy UVA exposure from indoor tanning solariums. The damaging effect is potent, with even one hour of UV exposure shown to cause extensive DNA damage requiring cellular repair [10]. The exposure is greatest at high altitudes and around the equator, where sunlight is the most constant and where either a thinner atmosphere or a thinner ozone layer offers less protection [11].

One of the greatest populations at risk are fair-skinned people of northern European ancestry that have migrated to geographically exposed areas, with the perhaps foremost example being white Australians which, as mentioned, have the greatest incidence of melanoma in the world [12]. Interestingly, Australian incidence rates have begun to level off and even drop in the last years. This is likely due to intensive and targeted information campaigns on

the dangers of sun exposure, leading to a reduced risk behavior in the population [13, 14]. A smaller contributing factor has also been changed population demographics with a relative decrease of fair-skinned and high-risk individuals in the population.

Beside direct UV exposure, there are a number of genetically pre-disposing risk factors, e.g. CDKN2A and BAP1 [15, 16]. These lead to familial forms of melanoma and other associated tumor forms, but are relatively rare.

Other than the skin, primary melanoma can also occur on the mucosal membranes of the gastrointestinal tract and in the eye. This wide anatomical diversity is plausibly due to the embryonical origin of melanocytes from the neural crest, cells that during fetal development migrate to a range of functions throughout the body. Is has even been proposed that this very characteristic is what gives melanoma its tendency to metastasize so widely once disseminated [17]. Mucosal melanoma is, due to internal anatomical locations, unlikely to be affected by sun exposure and more likely driven by other primary mutations [18, 19]. Diagnosis is often delayed, and survival is significantly worse than from the cutaneous forms. Uveal melanoma originates from the eye and is biologically distinct from cutaneous melanoma. It is an aggressive malignancy with short patient survival [20]. Uveal melanoma often metastasizes through hematogenous dissemination to the liver and can then be treated locoregionally with isolated hepatic perfusion [21].

1.3 STAGES OF DISEASE

Cutaneous melanoma is staged from stage I to IV (Figure 3). Stages I and II are cutaneous tumors localized to the skin, stage III includes engagement of regional lymph nodes, satellites or in-transit metastasis, and stage IV are distantly metastasized tumors. Staging is performed according to the American Joint Committee on Cancer (AJCC) TNM-classification, currently in its 8th edition [22]. The thickness of the tumor is described by Breslow thickness in millimeters, named after the pathologist Alexander Breslow who in 1970 was the first to report the prognostic significance of melanoma tumor thickness [23].

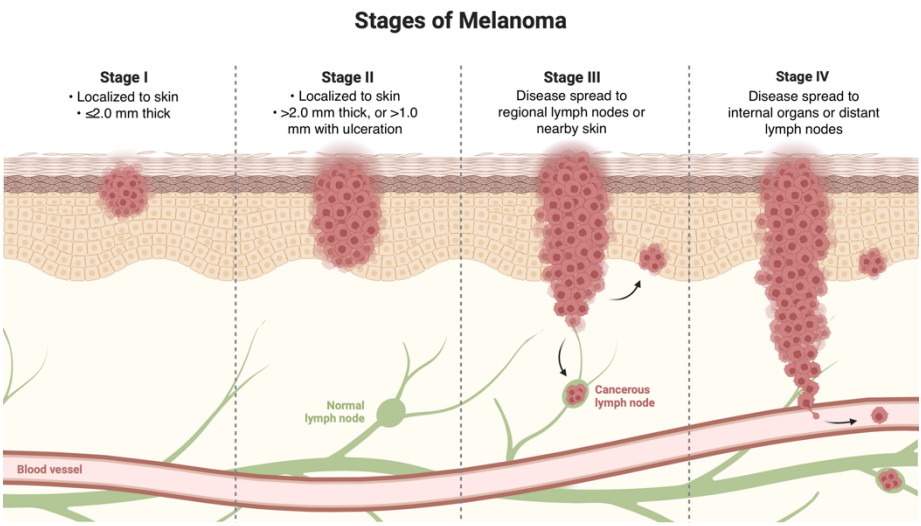

Figure 3. Stages of melanoma. (Created using Biorender.com)

Depending on thickness, T status is defined as either a thin melanoma (T1 ≤1.0 mm), of intermediate thickness (T2 >1.1-2.0 mm, T3 >2.1-4.0 mm) or thick (>4.0 mm). This last group is the focus of Paper III of this thesis. Each level is further subdivided a/b by if histopathological tumor ulceration, a known prognostic factor, is present. T-status also divides stage I and II melanoma, with stage I defined as tumors ≤2.0 mm thick without ulceration, or ≤1.0 mm with ulceration; and stage II defined as tumors >2.0 mm thick, or >1.0 mm with ulceration.

Stage III melanoma encompasses regional lymph nodes and locoregional disease in the skin, as denoted by N status described by number of positive nodes. N1 is defined as one positive node, N2 is defined as two to three positive nodes, and N3 is defined as >3 positive nodes. Each step is further subclassified as a/b/c depending on presence of subclinical nodal micrometastases, clinically detectable nodal macrometastases, or microsatellite/satellite/in-transit metastases, respectively.

In-transit metastases, which are the focuses of Papers I, II and IV, is a distinctive form of metastases that develop in approximately 5-10% of patients, and that appear as tumor nodules between the site of the primary melanoma and the nearest lymph node basin (Figure 4) [24-27]. Importantly, this form of

metastasis is different from local recurrences (which occur at the primary tumor site and have a significantly better prognosis) and from satellite metastases (which occur within 2 cm of the primary tumor site). A hypothesized pathophysiology is that tumor cells get trapped in lymph channels while migrating to the closest lymph nodes. In-transit melanoma is clinically challenging to manage, is associated with significant morbidity and has a high risk of both locoregional and systemic recurrences [22]. Of patients with in-transit metastases, up to approximately 50% will go on to later develop distant metastases [26]. Known prognostic factors for the development of in-transit metastases are positive sentinel lymph node status, Breslow depth, tumor ulceration, age >50 years and primary tumor location on the lower limb.

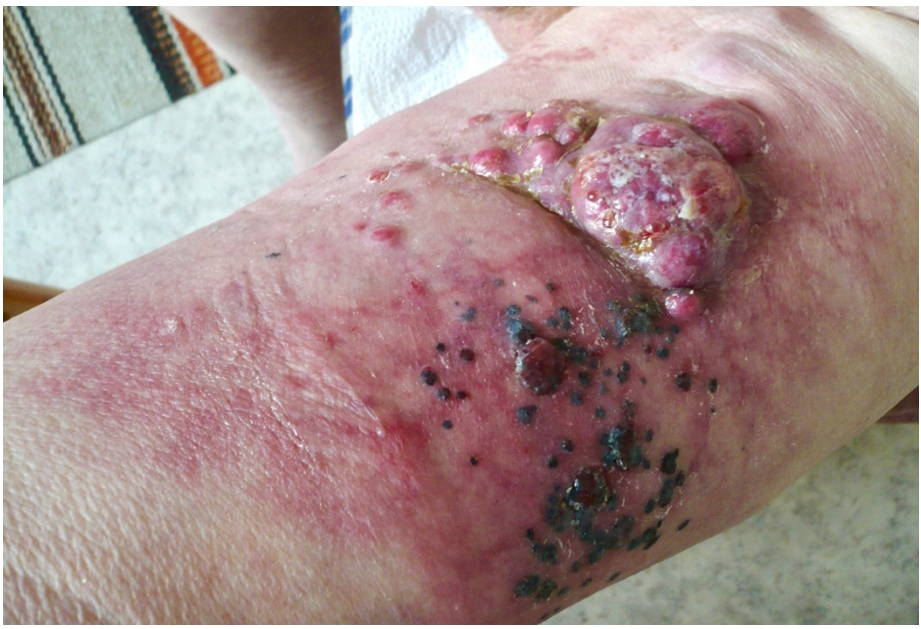

Figure 4. Melanoma in-transit metastases on the upper thigh of the left leg. (Photo by Roger Olofsson Bagge. Patient consent for publication given.)

Stage IV melanoma classifies distant disease. Metastases to distant skin or distant lymph nodes are classified as stage M1a, to the lungs as M1b, to the viscera as M1c, and to the brain as M1d. Subclassification is in this case by presence of elevated lactate dehydrogenase (LDH) levels in serum, and denoted as (0) or (1).

1.3.1 PROGNOSTIC FACTORS

The most important prognostic factors in primary melanoma are Breslow tumor thickness, tumor ulceration, mitotic rate, sentinel lymph node status, histopathological subtype, age, sex and anatomical tumor localisation [5, 22, 28, 29]. Of these, Breslow thickness has the strongest prognostic value for survival, with significant differences in long-term survival (Figure 5).

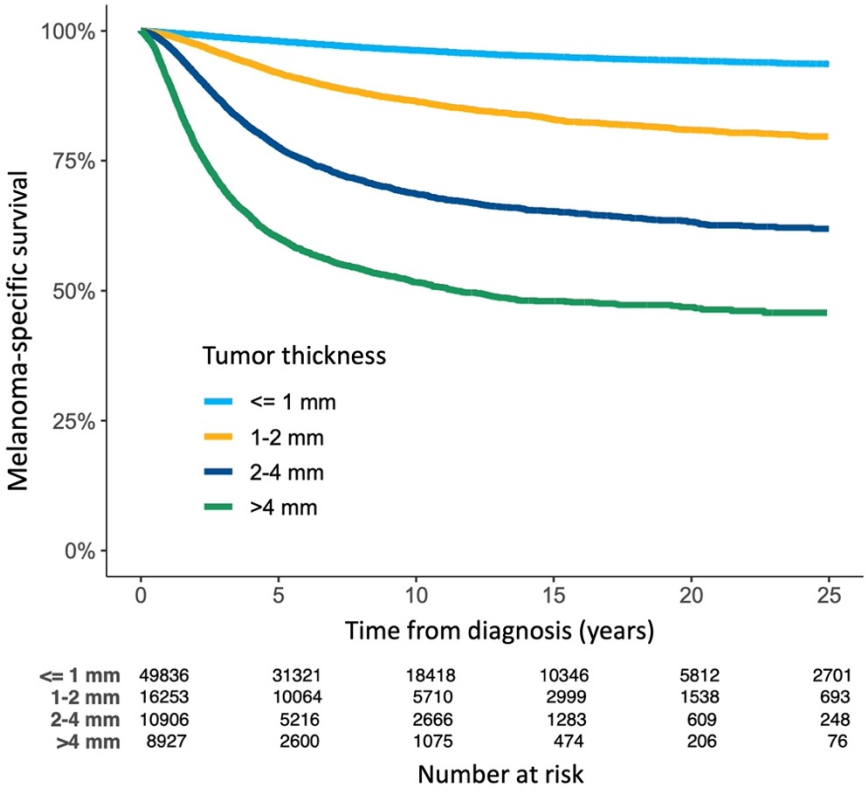

Figure 5. Melanoma-specific survival by tumor thickness, 1992-2022. (Adapted from SweMR)

1.4 TREATMENT

Melanoma is an aggressive disease with a pattern of dissemination infamously difficult to predict. It may present as stubbornly reoccurring and indolent

locoregional metastases in some patients, and as a hostile multifocal disease virtually exploding throughout many separate organs in others. Surgical management of this spectrum poses a clinical challenge. If there are genetic tumor characteristics underlying the difference in aggressiveness, they are yet largely unknown [30-32].

1.4.1 DIAGNOSTIC AND WIDE LOCAL EXCISION

A suspected primary melanoma is first removed by diagnostic excision with 2 mm margins and histopathologically analyzed. If the diagnosis can be confirmed, the melanoma is then treated by a secondary wide local excision with either 1 or 2 cm resection margin, depending on Breslow thickness. In a majority of patients, this ends up being the definitive treatment. The wide local excision is made down to the next underlying anatomical layer, e.g. muscle fascia or periosteum. The optimal skin excision margin has been the subject of a number of studies and clinical trials [27, 33-43]. Current Swedish guideline recommendations vary with the thickness of the tumor, with a 5 mm margin for melanoma in-situ, 10 mm margin for invasive tumors of Breslow thickness ≤2.0 mm and 20 mm margin for invasive tumors of Breslow thickness >2.0 mm. Currently, 10 vs 20 mm excision margins for tumors of >1.0 mm thickness is being investigated in the MelMartT-II randomized controlled trial (clinicaltrials.gov ID NCT02385214) aimed at improving surgical results and quality of life for patients.

1.4.2 SENTINEL LYMPH NODE BIOPSY

The technique of sampling the first draining lymph node of the tumor, the "sentinel" lymph node (SLN), to evaluate subclinical disease was introduced in 1992 by Morton *et al* [44]. The lymph node of interest is identified by injection of a tracer substance around the tumor and observation of which specific node in the regional lymph node basin absorbs the tracer. This node is removed for histopathological analysis. Both a blue color dye (detected visually) and a radioactive tracer substance (detected by scintigraphy and a gamma probe) are used in unison to reduce error. It is a relatively simple and low-morbidity procedure that adds important prognostic information [45]. As illustrated by the MSLT-I trial, removal of the SLN has been shown to in itself reduce regional recurrences but not to improve survival.

Current Swedish guidelines stipulate that sentinel lymph node biopsy (SLNB) be offered to patients with tumors of Breslow thickness >1.0 mm, and the prognostic value of this is well established for melanomas of up to 4.0 mm

thickness. It has previously been less clear if SLNB has value also for melanomas of thickness >4.0 mm. According to current guidelines (American Society of Clinical Oncology (ASCO), Society of Surgical Oncology (SSO), and National Comprehensive Cancer Network (NCCN)), SLNB is "recommended" for melanomas of thickness 1-4 mm, but only labeled as "may be recommended" for patients with thick melanoma [46, 47]. This topic is investigated in Paper III of this thesis.

1.4.3 LYMPH NODE DISSECTION

Based on several landmark clinical trials, management of patients with a positive SLNB has shifted in the last decade [45, 48, 49]. For patients with a positive SLNB, the treatment has historically been a completion lymph node dissection (CLND) in which the affected lymph node basin is surgically cleared. The procedure is not without morbidity, with 41% of patients suffering from lymphedema and 69% of patients afflicted with postoperative seromas or infections in the year after surgery [50]. Recent technical development of the procedure now allows for a laparoscopic approach to the inguinal lymph node basin [51-53]. Referred to as minimally invasive inguinal lymphadenectomy (MILND), it has been shown to reduce postoperative complications compared to open surgery, but without jeopardizing oncological outcome.

No survival benefit has been shown for CLND, as illustrated by the MSLT-II and DeCOG-SLT trials [48, 49]. Instead, current best practice is follow-up by nodal observation and lymph node dissection only in patients presenting with manifest clinical nodal disease.

1.4.4 LOCOREGIONAL TREATMENTS

Locoregional disease recurrence is primarily treated by surgical resection. For patients with primary nodal metastases or recurrences in the draining nodal basin, the current standard treatment is lymph node dissection. However, with bulky or very rapidly recurring in-transit metastases, resection is often not possible. For these patients there is a wide array of locoregional treatments available, including e.g. talimogene laherparepvec (TVEC) and electrochemotherapy (ECT). TVEC is a modified oncolytic form of the herpes simplex virus that is injected locally into the tumor nodules, resulting in increased response rates and moderately prolonged survival [54]. ECT is a procedure in which chemotherapeutic drugs are injected either systemically or at the tumor site with an electrical current then administrated via electrodes, causing a transient increase in cell membrane permeability and increased local

uptake of the cytotoxic drugs [55]. ORR rate is approximately 75%, but application of the technique is limited to smaller metastases.

One of the most effective locoregional therapies is isolated limb perfusion (ILP), used specifically to treat tumors located in the limbs [56-58]. For this procedure, the extremity artery and vein are surgically dissected and cannulated with large bore infusion catheters. The limb is then isolated by a tourniquet, connected to a heart-lung-machine for oxygenation, and perfused with heated high-concentration chemotherapy (melphalan) for up to an hour (Figure 6).

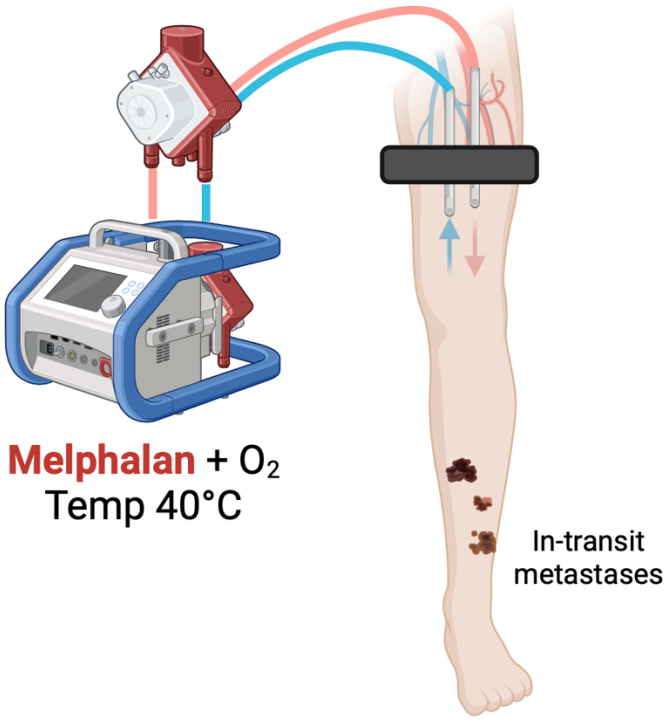

Figure 6. Isolated limb perfusion of the lower limb. (Created using Biorender.com)

Such treatment conditions would be lethal if applied systemically but are well tolerated when applied isolated to only the extremity. Postoperative local

toxicity reactions are not uncommon, but more serious reactions are rare. ILP response rates are excellent and among the highest of the locoregional treatments, with ORR of 65-100% and CR rates of up to 65%. The procedure can be repeated in patients with recurrences, making it a versatile and pragmatic tool. Recent technical advances now allow for percutaneous cannulation of the vessels instead of open dissection, which has been dubbed minimally-invasive isolated limb perfusion (MI-ILP) [59].

Another variation of ILP is isolated limb infusion (ILI), a technical alternative in which the extremity vessels are also cannulated percutaneously but perfused only using a low-flow syringe. This technique lacks the benefit of increased temperature and oxygenation added by a heart-lung machine [60-63]. The resulting circuit has both a lower flow rate and a lower response rate than what can be achieved with ILP, but can be an appropriate technical choice for select patients.

The toolbox of available locoregional therapies is thus varied. It is currently unknown if an optimal combination and sequence of these (and other) therapies exist. It is also not yet fully understood what role these treatments have in relation to effective systemic immunotherapies.

1.4.5 SYSTEMIC TREATMENT

Historically, the only available systemic treatment for metastatic melanoma was chemotherapy. The toxicity was significant and treatment effect limited with survival times not significantly better than the natural course of the disease [64-66]. The field was propelled into the current modern era with several major laboratory findings. First, several key driver mutations in melanoma were identified, leading to the development of drugs targeting the MAPK pathway. These “targeted therapies” act on the BRAF and MEK proteins in the MAPK signal pathway to limit cancerous cell proliferation [67-71]. BRAF mutations occur in approximately 50% of melanomas [72, 73].

The next breakthrough was the discovery of inhibitory regulating pathways on cytotoxic T-cells and the development of immune checkpoint inhibitors (ICI), which is now the core of modern immunotherapy. The first available ICI was ipilimumab, a monoclonal antibody that targets the cytotoxic T-lymphocyte associated protein 4 (CTLA-4) receptor and inhibits the immunosuppressive interaction between antigen-presenting cells and cytotoxic T-cells (Figure 7) [74]. The result is an activated T-cell that can identify and, with the help of co-stimulatory signals, attack melanoma cells.

Next came monoclonal antibodies that target the programmed cell death-1 (PD-1) receptor. Interaction between PD-1 and its ligand results in a deactivation of T-lymphocytes. Cancer cells, including melanoma cells, can overexpress the surface PD-1 ligand and thereby suppress the T-cell attack. Inhibition of the receptor-ligand interaction removes this suppression and makes recognition and an attack by T-cells possible.

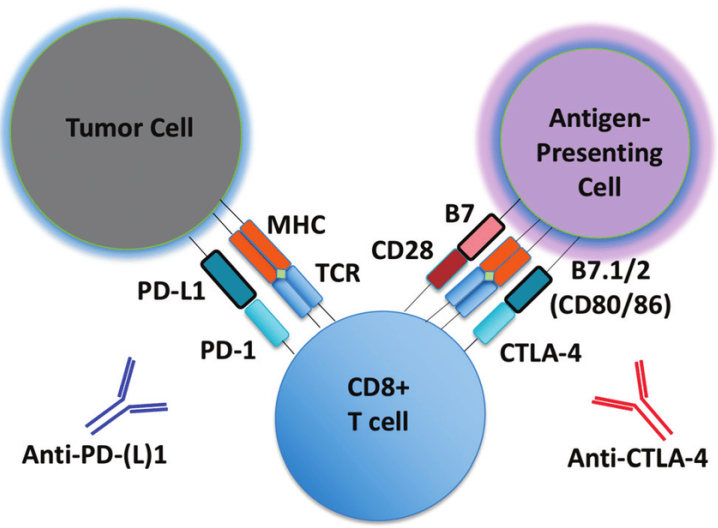

Figure 7. PD-1 and CTLA-4 inhibitor mechanism of effect. (Creative commons license CC BY-NC 3.0).

CTLA-4 and PD-1 inhibitors thus have similar mechanisms of effect, but the latter acts more directly on the interaction between T-cell and cancer cell. PD-1 inhibitors such as nivolumab and pembrolizumab are currently the first-line treatment for stage IV melanoma [75, 76]. The work underlying the development of CTLA-4 and PD-1 inhibitors was performed by James P. Allison and Tasuku Honjo respectively, for which the pair was jointly awarded the 2018 Nobel Prize in Medicine.

These effective systemic immunotherapies have revolutionized the treatment of melanoma and has led to a paradigm shift in the field. Historically dismal 5-year survival rates of 5% have now increased to over 50%, with up to 22%

of patients having CR and 38% having SD/PR [75, 77]. The highest response rates are currently achieved with a combination treatment of both CTLA-4 and PD-1 inhibitors (ipilimumab + nivolumab), but at the expense of higher toxicity rates than seen with monotherapy.

1.4.5.1 ADJUVANT AND NEOADJUVANT TREATMENT

Following use in metastatic disease, ICIs have also been introduced as adjuvant therapies in patients lacking macroscopically visible disease. For patients with resected stage III disease, adjuvant treatments with both PD-1 and BRAF/MEK inhibitors have shown robust effects on recurrence-free survival [78-82]. Recently published trial data also shows such an effect for adjuvant PD-1 inhibition in stage IIB/C disease, a patient group that paradoxically has a worse survival prognosis than stage IIIA/B patients [22, 80]. Importantly, this improvement is only on recurrence-free survival and no trial using PD-1 inhibitors has yet been able to show an effect on overall survival for these patients compared to control [83]. Recent phase II trial data also supports neoadjuvant treatment, which has now quickly become standard clinical practice in Sweden [33, 84].

1.4.6 SURGERY FOR METASTATIC MELANOMA

There is currently limited evidence to guide the use of metastasectomy in metastatic melanoma. Historically, metastatic surgery was used as a complement to chemotherapy in select patients. Encouraging long-term survival rates of up to 20-40% could be shown [29, 85-90]. However, these studies were generally conducted on carefully selected patients and likely subject to significant selection-bias, making them poor guidance for the treatment of the stage IV patient group overall. As such, what was first identified as predictive factors aimed at selecting the right patient for metastasectomy was more likely prognostic biomarkers for long-term survival.

The dawn of effective systemic treatments over the metastatic melanoma landscape has opened new questions on the role of surgical metastasectomy. Using PD-1 inhibitors, 20% of stage IV patients see CR and another 48% see PR or SD [75, 76]. This means half of all patients will have clear treatment benefit, but also live with remaining tumors (Figure 8). That these patients would benefit from complementary metastasectomy after immunotherapy is an attractive premise. Reduction of tumor mass and removal of lesions with less than complete response could hypothetically shift the immunological fight in favor of the patients T-cells [91]. This is an idea not without support from

recent trial data where the effect of ICI greatest in patients with low-volume disease burden [92-95].

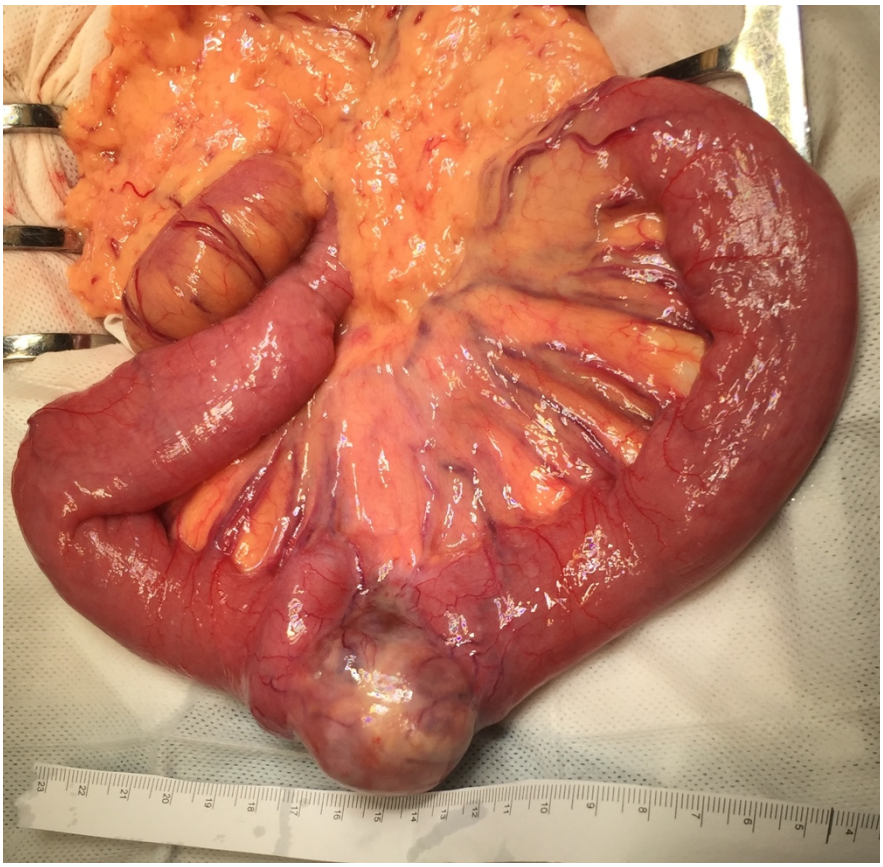

Figure 8. Obstructing small bowel melanoma metastasis (stage M1c), perioperative photo. (Reprinted with permission from Holmberg et al, WJSO, 2019)

2 AIM

The overall objective of this thesis was to evaluate the role of surgery in the treatment of melanoma in the current era of effective systemic treatments. The specific objectives of the individual studies were:

I To evaluate the effect of immune check-point inhibition as first-line treatment on melanoma in-transit metastases.

II To examine any change in population characteristics of melanoma patients receiving isolated limb perfusion treatment before and after the introduction of immune check-point inhibition, as well as evaluate effect of ILP in patients that had failed first-line treatment with immune check-point inhibition.

III To establish the prognostic significance of sentinel lymph node status in patients with thick (>4.0 mm) melanomas.

IV To evaluate the treatment effect of the addition of a single pre-operative dose of the PD-1 inhibitor nivolumab prior to isolated limb perfusion.

3 PATIENTS AND METHODS

3.1 OVERVIEW

***Table 1**. Papers included in the thesis with their respective study designs.*

Paper	Study design	Sample	Outcome measure
I	International multicenter cohort study	In-transit metastases treated with ICI	Response rate
II	Case-control study	ILP in patients treated with or without ICI	Response rate
III	Registry study	Melanoma >4.0 mm	Prognostic significance of SLNB
IV	Randomized controlled trial	In-transit metastases treated with ILP +/- ICI	Safety + Response rate

3.2 PAPER I

3.2.1 STUDY DESIGN

A retrospective multicenter cohort study evaluating the outcome of patients with melanoma in-transit metastases treated with ICI. An international cooperative study group was formed, pooling data from a large number of institutions from around the globe.

3.2.2 PATIENT SELECTION AND INCLUSION

Patients with in-transit metastases of cutaneous melanoma with or without nodal involvement (AJCC8 stage N1c, N2c and N3c) treated with ICI, specifically with PD-1 inhibition (pembrolizumab or nivolumab) and/or

CTLA-4 inhibition (ipilimumab) between 2015 and 2020 were included. Patients with either a history of or current distant metastases (AJCC8 stage M1) were excluded. Previous treatments such as surgery, locoregional therapies or systemic therapies other than ICI, were allowed, however not if given in parallel with current ICI therapy.

Patients were included from 21 institutions in 8 countries, including Europe, USA, the Middle East and Australia. All participating study sites were national or regional referral centers for treatment of melanoma.

3.2.3 DATA COLLECTION

Data was retrospectively collected from patient records. Data sharing agreements were negotiated and established, and the data was transferred to Sahlgrenska University Hospital (Gothenburg, Sweden) as the central coordinating center.

3.2.4 OUTCOME MEASURES

The primary endpoint was rate of complete response (CR). Secondary endpoints were overall survival (OS), melanoma-specific survival (MSS) progression-free survival (PFS), time to local progression (TTLP), time to nodal progression (TTNP) and time to systemic progression (TTSP). Response was calculated according to the RECIST 1.1 criteria modified for cutaneous lesions, allowing for caliper measurements of lesions not visible on radiology [96].

3.3 PAPER II

3.3.1 STUDY DESIGN

An analysis of a prospectively kept database of patients treated with first-time ILP for in-transit metastases at Sahlgrenska University Hospital. Patients were grouped and compared by the time period in which they received treatment: the pre-ICI era (2010-2014) when ILP was considered first-line treatment for metastases of the extremities, versus the ICI era (2017-2021) when immunotherapy was available. The primary aims were to compare response rates in patients treated with ILP that had failed previous ICI with those that were treatment naïve, and to compare patient characteristics before and after the introduction of ICI.

3.3.2 PATIENT SELECTION AND INCLUSION

Patients with cutaneous melanoma in-transit metastases (AJCC8 stages IIIB-D) of the extremities undergoing first-time ILP between 2010 and 2021 were included. Sahlgrenska University Hospital is a national referral center for such treatments, and patients were sourced from throughout Sweden.

3.3.3 DATA COLLECTION

Data on patient and tumor characteristics, treatment response, toxicity, recurrence and survival were prospectively recorded in a locally kept database. Data on therapies given prior to ILP was recorded. Data on any therapies given after ILP was not systematically available due to patients being discharged back to their local hospital for follow-up not specific to the ILP.

3.3.4 OUTCOME MEASURES

The primary analysis was comparisons of response rates, local and systemic progression rates, melanoma-specific survival, toxicity rate and surgical complications rate; as well as comparison of patient characteristics between treatment eras.

3.4 PAPER III

3.4.1 STUDY DESIGN

An analysis of the Swedish Melanoma Registry (SweMR) to evaluate the prognostic significance of positive SLN status in thick (>4.0 mm) melanomas.

3.4.2 PATIENT SELECTION AND INCLUSION

Data on all patients with diagnosis of cutaneous melanoma of Breslow thickness >1.0 mm (AJCC8 stage pT2a-pT4b) between 2007 and 2020 was extracted from the registry. Patient with locoregional or systemic metastases, patients that had not undergone SLNB, and patients that had information missing on SLN status were excluded from the analysis.

3.4.3 DATA COLLECTION

Data was collected from SweMR, a nationwide, population-based registry of Swedish melanoma patients with a high (>99%) level of coverage. To establish

date and cause of death, the registry was cross-linked with the Swedish Cause of Death Registry.

3.4.4 OUTCOME MEASURES

Primary analysis was to compare the prognostic importance of SLN status for MSS over increasing levels of Breslow thickness. Secondary analyses were to verify known predictive factors for SLN status.

3.5 PAPER IV

3.5.1 STUDY DESIGN

A multicenter randomized double-blind placebo-controlled trial evaluating the effect of the addition of a single dose of ICI prior to ILP for melanoma in-transit metastases. Patients were randomized 1:1 to receive either an infusion of the PD-1 inhibitor nivolumab (experimental arm) or placebo (control arm) the day before ILP. The overall trial consists of two stages. First, this current phase 1b trial of 20 patients in which safety and preliminary efficacy is evaluated. If safety can be confirmed, the trial will then proceed to a phase II trial further evaluating efficacy.

3.5.2 PATIENT SELECTION AND INCLUSION

Patients with cutaneous melanoma in-transit metastases (AJCC8 stage N1c, N2c, N3c), with an expected life expectancy of >6 months, and without any autoimmune disease or corticosteroid treatment were included. Prior systemic immunotherapy was allowed if given more than 30 days prior to ILP.

Patients were included from two sites: Sahlgrenska University Hospital (Gothenburg, Sweden) and the Netherlands Cancer Institute (Amsterdam, The Netherlands), both of which are national referral centers for the treatment of advanced melanoma.

3.5.3 DATA COLLECTION

Data was locally collected and centrally aggregated in a digital case report form. Treatment was blinded to both patients and investigators, and only unblinded to the local pharmacy preparing the nivolumab/placebo drug at each site.

3.5.4 OUTCOME MEASURES

Primary endpoints were rate and severity of adverse events (AE), and CR rate at 3 months after ILP. Secondary endpoints were melanoma-specific survival (MSS), overall survival (OS), time to local progression (TTLP) and duration of response (DOR). Adverse events were defined in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Surgical complications were reported according to the Clavien-Dindo classification and toxicity reactions according to the Wieberdink scale [97, 98]. Incidence and severity of adverse events were monitored by an independent data safety monitoring board (DSMB).

3.6 STATISTICAL METHODS

For all four papers, survival was estimated using the Kaplan-Meier method and analyzed using the log-rank test for group comparisons. Statistical hypothesis testing was performed using the Mann-Whitney test for non-parametric continuous variables and Fischer's exact test for categorical variables. For Papers I and III, regression analyses were performed to investigate the strength of relationship between chosen variables. Logistic regression was used for binary categorical outcomes, in Paper I to find predictors of CR and in Paper III to find predictors of SLN status. Cox regression analysis was used for continuous time-to-event outcomes, in Paper I to find predicators of TTLP, PFS and MSS; and in Paper III to find predictors of MSS. Statistical significance set at $p<0.05$. All statistical analysis was done using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA).

3.7 METHODOLOGICAL CONSIDERATIONS

3.7.1 ENDPOINTS

To understand the evolution of endpoints is to understand the evolution of modern clinical cancer research. In the 1970's, treatment effect was often evaluated strictly by tumor response as assessed by radiology or clinical examination [99]. There was, however, a need for an outcome measurement with greater objectivity and with clearer clinical benefit to the patient. This led

to the general adoption of overall survival (OS) as an endpoint, which has now long been considered the golden standard of outcome evaluation in clinical oncology research. In the 1980's, OS was often requested by such regulating and influential bodies as the USA's FDA (Food and Drug Administration) for efficacy evaluations in drug approval. Though it is a rigorous and objective measurement with minimized risk of interpretation bias, evaluation of OS requires a long observation period (de facto all remaining time the patient has). With better cancer treatments came longer patient survival and follow-up, making OS more time consuming and expensive to evaluate.

When the FDA in 1992 adopted a new accelerated drug approval regulation aimed at getting new therapies to the market faster, OS was no longer the most pragmatic choice of endpoint. In addition, with the growth of patient-centered care came the understanding that patients value treatment outcomes other than strict survival time. For example, in one study on the preferences of patients with advanced cancer, 27% of patients preferred health-related quality of life (HRQoL) outcome measurements to survival time, with another 55% assigning the two equal importance [100]. Improvements other than survival time, such as symptom reduction, disease-free time and time to progression, are not captured by a purely survival-based outcome measurement. In response, surrogate endpoints were introduced. This is an alternative measurement that, if properly validated, reflects the true endpoint and allows for conclusions to be made on the true outcome of interest without direct measurement. A major benefit is that an outcome measurement can be made earlier, and examples include progression-free survival (PFS), disease-free survival (DFS) and objective response rate (ORR). In the 1970's and 1980's, 0% of randomized controlled used either PFS or time to progression (TTP) as an endpoint, a figure that climbed to 2% in the 1990's, 7% in the early 2000's and to 26% in 2006 [101]. Today, PFS is a widely accepted surrogate for survival in the metastatic setting and a commonly used basis for regulatory drug approval.

3.7.1.1 RECIST CRITERIA FOR IN-TRANSIT METASTASES

The clinical evaluation of treatment response in melanoma in-transit metastases is central to three (Papers I, II and IV) out of four papers in this thesis. There is, however, currently no agreed upon or standardized methodology for this assessment. Commonly used methods intended for solid-mass tumors, such as the RECIST criteria, are not applicable for cutaneous lesions not detectable on CT [96]. Many previously published studies report their method for evaluating these lesions as RECIST *modified for cutaneous lesions*, but do not specify the modifications further. This makes objective

comparisons, both on patient and cohort level, difficult. For our studies, we specify that all lesions not measurable by CT are measured by calipers instead. This prerequisite condition allowed for the successful pooling of data from a large number of diverse centers in Paper I.

3.7.2 SOURCES OF BIAS

Paper I is, due to its retrospective design, subject to the risk of information bias. Though Papers II and III are retrospectively analyzed, they are both based on prospectively kept databases of high quality and the risk is thus small. The risk of information bias was minimized in Paper IV by a randomized prospective approach, which reduces the amount of information missed at collection. Selection bias is likely present in Papers I and IV but minimized in Papers II and III due to consecutive patient inclusion and due to the data being drawn from a population-based registry respectively.

3.8 ETHICAL CONSIDERATIONS

The ethical clinical study of patients with advanced melanoma comes with a number of technical requirements. The study center must not only be able to offer technically demanding procedures and therapies but must also be capable to fully handle any complications that follow. Sahlgrenska University hospital is a highly specialized such center. We offer patients advanced multidisciplinary treatments in surgical oncology, immuno-oncology and general oncology. This allows us, together with our patients, to pursue state-of-the-art research questions in a safe and ethical way.

The ethical considerations of clinical research are perhaps never made clearer than when sitting down with a patient to discuss potential inclusion in a study. The process of truly informed consent requires that the researcher not only fully understands the science and the clinical procedure, but also the personal priorities of the patient. The dividing line between *information* and *persuasion* can be thin, especially with a patient with whom you have already developed trust as their treating clinician.

When including patients in a randomized controlled trial investigating a novel treatment (such as in Paper IV), one must also consider the consequences to the patient if randomised to the control group. It will mean that they are denied a treatment that we, the researchers, have good reason to believe can help them.

The stress to the patient of not receiving such a treatment, or in the case of a blinded trial not knowing, should not be underestimated. The ethical stress to the researchers of managing disease progression in a control arm can also be significant.

All studies in this thesis were conducted in adherence to the ethical principles of the Declaration of Helsinki and with the approval of either the Regional Ethical Review Board of Gothenburg (Papers I and II) or, after its formation in 2019, the national Swedish Ethical Review Authority (Papers III and IV).

4 RESULTS

4.1 PAPER I

Using our large international multi-institutional patient cohort of real-world data, historically the largest such cohort ever assembled, we demonstrated a higher CR rate for immunotherapy in melanoma in-transit metastases than what has previously been shown, 36% compared to 13-26% [102, 103]. It is also higher than the up to 22% CR rate (for combination therapy PD1 + CTLA-4 inhibitors) reported from the clinical registration trials underlying the use of immunotherapy in unresectable stage III and IV patients [74, 75, 104-109].

As for survival, we can report a 5-year MSS of 72% with a median PFS of 10 months. The 5-year PFS was only 19%, reflecting the long-term morbidity and persistence of in-transit disease.

As previously noted, any comparative analysis of *response rate* is dependent on a standardized and stringently applied method for evaluating clinical *response*. Though such standardization was undertaken for the pooling of data in our cohort and, as a result, we believe internal validity to be high in our study, the same standardization has not been specified in previously published studies. This limits the certainty of any external comparisons.

In addition to presenting data on response and survival, this paper also highlights the current diversity of treatment practices for melanoma in-transit metastases, with great local variance between countries and institutions in the type of locoregional therapies used. Though the paradigm is shifting towards systemic immunotherapy it is important to remember response rates are far from perfect. In this study, we demonstrate a rate of PD as best response of 32%. That is, almost one in three patients see disease progression during systemic treatment and must be considered for complementary locoregional therapy. There exists a possible, and indeed widely hypothesized, synergistic benefit in combining the diverse tool kit of locoregional treatments with the broad effect of immunotherapy. The best timing, sequence, and type of therapies to combine are, however, still unknown. In Papers II and IV, we aim to explore this further by examining the therapeutic overlap between systemic PD1-inihibition and locoregional ILP.

4.2 PAPER II

When comparing the characteristics of patients undergoing ILP before and after the introduction of systemic immunotherapy, we found only minor differences. A statistically significant increase in median age at time of ILP of 5 years was evident; as well as statistically non-significant tendencies towards increased tumor size, increased number of metastases, decreased concurrent lymph node dissection rate and decreased number of patients with stage M1 disease. This may suggest a shift in referral patterns of ILP towards older patients with more advanced disease in the modern era, but our data does not support a more definitive conclusion.

Moreover, no statistically significant change in response, time to local progression or time to systemic progression for ILP was evident when comparing the pre-ICI and ICI eras, nor was any difference in complications or toxicity rates found. The efficacy of ILP has thus remained high through the historic shift towards systemic immunotherapy. An improvement in MSS was, however, seen. This was expected and reflects the treatment benefits of modern systemic treatment for the melanoma in-transit patient group.

Further, we found no statistically significant difference in response for patients that had received ICI prior to ILP, i.e. the patients that have seen disease progression on immunotherapy and then received ILP as second-line treatment. Failed immunotherapy does not, it appears, influence the effect of ILP. There has been concern over reduced such effects. In addition to the well-known cytotoxic effects of ILP, we have hypothesized immunological mechanisms as also underlying the treatment effect of perfusions [110-112]. This has given rise to concerns that progression on immunotherapy would translate to a selection towards patients with reduced effect to ILP. As no such reduction could be seen, our study further strengthens the high utility role of ILP.

The study highlights the interplay between effective locoregional therapies and modern systemic treatments. This overlap, currently largely unmapped, is further explored in Paper IV.

4.3 PAPER III

Using the Swedish Melanoma Registry, we were able to analyze real-world data for over 10,400 patients with primary melanomas of Breslow thickness >1.0 mm that had undergone SLNB, including 1,943 patients with >4.0 mm (T4) tumors. We found that patients with positive SLN status had a persistently worse prognosis in terms of MSS compared to their negative status counterparts, also for melanomas >4.0 mm. Notably, the hazard ratio for this survival remained unchanged with increasing Breslow thickness. This was not only the case when patients were stratified by Breslow thickness in millimeters, but also when stratified by T-stage.

We could also confirm SLN status, tumor ulceration, Breslow thickness and age > 80 years as independent prognostic factors for MSS, which is consistent with previously published data and the official AJCC classification [22, 113].

In addition, multivariable analysis of specifically the T4 patient subgroup found tumor ulceration, Breslow thickness, tumor site (with increased OR for trunk and palm/subungual localization; and decreased OR for head & neck localization) and histopathological subtype (with increased OR for acral lentiginous melanoma; and decreased OR for nodular melanoma and Other) to be factors predictive of positive SLN status.

These results help in identifying patients with potential benefit from adjuvant systemic immunotherapy and can ultimately guide patients in their treatment choices. The results also support an update of current guidelines to strengthen the role of SLNB in thick melanomas.

4.4 PAPER IV

In the Ib-phase of the Nivo-ILP randomized controlled trial, we found comparable rates of AEs in the interventional and placebo study arms. A total of 20 patients were enrolled in the trial, with 18 patients successfully completing the ILP procedure (two patients were unable to do so due to procedural technical failure), with 8 patients in the nivolumab arm and 10 patients in the placebo arm. As this initial phase of the trial was focused on safety, our current analysis was focused on adverse events. The total AE incidence was 90% in the nivolumab arm and 80% in the placebo arm, with no

grade 4 or 5 AEs observed in either arm. The nivolumab arm had a higher absolute number of AEs (n=45 in the nivolumab arm vs. n=21 in the placebo arm) and a higher rate of reported grade 3 AEs (40%, n=11 in the nivolumab arm vs. 10%, n=3 in the placebo arm). When examining the grade 3 events specifically, they were found to be mainly surgical in nature and most commonly related to wound complications. As PD-1 inhibitors are not known to be associated with an increased risk of surgical complications, there is no cause to suspect nivolumab to be the primary risk factor in these patients. When comparing ILP-related toxicities, no additional risk was seen in the nivolumab arm.

In terms of efficacy, response was evaluated in the 18 patients that successfully underwent ILP. The patients in the nivolumab arm showed tendencies toward improved CR rate at 3 months (75% vs. 60%), ORR rate at 3 months (100% vs. 90%) and 1-year local progression-free rate (86% vs. 67%), with 1-year OS 100 in both study arms. Though encouraging, more definitive conclusions are limited by the small number of patients analyzed in this first part of the trial.

In summary, we found the addition of a single dose of the PD-1 inhibitor nivolumab prior to ILP to be a safe and feasibly treatment. The trial will be continued as a phase II trial to further evaluate efficacy.

5 DISCUSSION

Within the scope of this thesis, we have shown a vital and persistent role for surgery in the treatment of advanced melanoma. Our work further solidifies the effectiveness of systemic immunotherapies for patients with melanoma in-transit metastases. We highlight the intricacies of managing in-transit disease and the importance of a multimodal approach to this disease form, including the use of locoregional treatments. The thesis shows the benefits of sentinel lymph node biopsy in guiding continued treatment, as well as the potential benefits derived from combining locoregional and systemic therapies.

However, our work also illustrates the challenges of integrating novel treatments into established clinical practices and existing bodies of knowledge. The current landscape of cancer treatment is undergoing paradigm shifts, presenting the challenge of determining what aspects to retain and what to discard. For example, while advocating for the benefits of SLNB, we acknowledge that this perspective may evolve with a deeper understanding of the immunological impact of such interventions.

The therapeutic efficacy of PD-1 inhibition may rely on the presence of a certain amount of remaining tumor mass, creating a battlefield on which the drug can mobilize T-cells in the fight against melanoma cells. Removing immunologically vital organs, like lymph nodes, may eliminate a crucial stage for interaction, potentially affecting the outcome of the treatment. Current observations that adjuvant immunotherapy may not improve survival may be a reflection of this [83]. A possible future direction lies in genomic testing, which already today shows promising sensitivity and specificity in predicting SLN status, to get the same prognostic information guiding adjuvant treatment without surgery [114]. Innovative techniques, such as using intracutaneously injected superparamagnetic iron oxide and magnetic resonance imaging as a marker for not only SLN location but also status, holds promise in reducing unnecessary or immunologically disadvantageous surgical biopsies [115].

Our work also underscores the benefits of ILP, emphasizing its continued robust role within the framework of systemic treatments with room for further evolution. The development of the minimally invasive perfusion technique has already increased its utility. There are ongoing efforts to increase the treatment effect and further reduce side effects. If, as we hypothesize, the effect of ILP is not only mediated by the cytotoxicity of chemotherapy but also through immunological mechanisms, the same beneficial effect can perhaps be

achieved at lower doses of chemotherapy and temperatures than what is used today if combined with ICI. Alternative systemic agents, apart from PD-1 inhibitors, that may further amplify the immune-mediated effect warrants exploration. Finally, if lower toxicity rates can be achieved, even at the calculated expense of decreased response rate, ILP could be repeated more often in patients with recurrent disease. The continual translational development of these surgical techniques is imperative for advancing melanoma treatment strategies.

6 CONCLUSION

Based on the studies here presented, it can be concluded that:

- Immune checkpoint inhibition is an effective treatment for melanoma in-transit metastases.

- Isolated limb perfusion (ILP) for melanoma in-transit metastases continues to be a high utility tool in the modern immunotherapy era.

- Sentinel lymph node biopsy adds useful prognostic information also for thick (>4.0 mm) primary melanomas.

- The addition of a single dose of the immunotherapy nivolumab prior to ILP is a safe treatment with promising efficacy, and this treatment combination should be further explored.

7 FUTURE PERSPECTIVES

The future role of the cancer surgeon is yet undecided, presenting both challenges and opportunities. Surgical resection still remains the most important treatment for solid tumors. However, there is little doubt that the way towards better future cancer cures lies primarily in the direction of better antibodies, immunoreceptors, and manufactured molecules; and not in the direction of better scalpels, sutures, or resection techniques. However, one field will not be able to advance without the other. Should surgeons remain as they have, their roles will be relegated to suppliers of biopsies and tumor samples, on which the definitive treatments are tailored by someone else and elsewhere. A decline unfitting a proud tradition stretching back thousands of years, it could be argued.

Instead, we surgeons must choose to embrace the shifting paradigms and integrate new advances into our discipline. We are well placed to define clinically relevant questions in multidisciplinary settings. By principle, our work is patient centered and well suited for the “bedside-to-lab-bench-to-bedside” research methodology.

It is imperative for surgeons to transcend their traditional roles and assume the mantle of surgeon-scientists. We must stride into multidisciplinary research with the same enthusiasm we do into the operating theatre The integration of cutting-edge research into surgical practice is not just an option; it is a necessity for the continued relevance of our discipline.

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To my son, **Henry**.

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