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Loco-Regional Treatment of Cutaneous Melanoma

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Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Jüri Teras

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signature

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Nahamelanoomi lokoregionaalne ravi

JÜRI TERAS

TAL TECH
KIRJASTUS

“I did then what I knew how to do. Now that I know better, I do better”

Maya Angelou

To all my patients

Contents

List of Original Publications

The thesis is based on the following publications, which will be referred to in text by their Roman numerals:

I. Utjes D, Malmstedt J, **Teras J**, Drzewiecki K, Gullestad HP, Ingvar C, Eriksson H, Gillgren P. 2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomised trial. *Lancet* 2019;394:471–477.

II. **Teras J**, Kroon HM, Thompson JF, Teras M, Pata P, Mägi A, Teras RM, Rüütel Boudinot S. First Eastern European experience of isolated limb infusion for intransit metastatic melanoma confined to the limb: Is it still an effective treatment option in the modern era? *Eur J Surg Oncol* 2020;46:272–276.

III. **Teras J**, Kroon HM, Miura JT, Kenyon-Smith T, Beasley GM, Mullen D, Farrow NE, Mosca PJ, Lowe MC, Farley CR, Potdar A, Daou H, Sun J, Carr M, Farma JM, Henderson MA, Speakman D, Serpell J, Delman KA, Smithers BM, Barbour A, Tyler DS, Coventry BJ, Zager JS, Thompson JF. International multicenter experience of isolated limb infusion for in-transit melanoma metastases in octogenarian and nonagenarian patients. *Ann Surg Oncol* 2020;27:1420–29.

IV. **Teras J**, Kroon HM, Zager JS. ASO Author Reflection: Isolated limb infusion for locally advanced melanoma in the extremely old patient is safe and effective. *Ann Surg Oncol* 2020;27:1430–31.

Authors Contribution to the Publications

Publication I:
Jüri Teras collected patient data from Estonia and reviewed the manuscript.
Publication II:
Jüri Teras contributed to study design, patient treatment and data collection, data interpretation, literature search and writing of the manuscript.
Publication III:
Jüri Teras contributed to the literature search, data interpretation, and writing of the manuscript.
Publication IV:
Jüri Teras contributed to the literature search, data interpretation, and writing of the manuscript.

Introduction

With significant improvements in the fight against other diseases, cancer is emerging as the main cause of mortality in many parts of the world. Cancer, being a source of serious medical and socio-economic problems to contemporary mankind, affects us all. As a patient, as a bystander, as a caregiver. In countries, where healthcare systems are least prepared to manage the burden of cancer, large number of patients are denied timely quality diagnosis and treatment.[1] In countries with strong health systems survival rates of many types of cancer are improving, early detection and quality treatment being the key factors.[1]

Skin cancer is one of the most prevalent type of cancers in the world.[2] In the Western world the occurence of skin cancers, mostly basal cell carcinoma, squamous cell carcinoma and melanoma, continues to rise. Of these three, melanoma has the worst prognosis due to its metastatic potential. Therefore, adequate surgical treatment of primary melanoma and its metastases, particularly in-transit metastases, is of utmost importance.

The first study (Publication I) describes ways of radical surgery of primary melanoma. Being part of extended follow-up in an international multicenter randomized controlled trial on surgical excision of localized cutaneous melanoma thicker than 2 mm we were able to provide the answer to a fundamental but simple question of sufficient resection margin for primary surgical removal of cutaneous melanoma.

The second study (Publication II) describes the methods of treatment of a separate entity of metastatic melanoma - the in-transit metastases. The study was conducted to analyze the isolated limb infusion experience in small Estonia, to evaluate its feasibility and its efficacy outside high-volume centers in other countries. This is the first report of this type of treatment in Eastern Europe and one of the few published data from Europe.

The third study (Publication III) describes isolated limb infusion in the subset of a group of patients – the extremely old. The efficacy and safety of the procedure in octogenarian and nonagenarian patients was evaluated in a multicenter trial of USA and Australian centers. In this highly comorbid and fragile population a minimally invasive treatment alternative to systemic therapy with its potential side-effects, is needed.

The fourth study (Publication IV) summarizes the data on isolated limb infusion performed on octogenarian-nonagenarian patients and gives an overview on future perspectives of this treatment modality.

Abbreviations

AJCC	American Joint Committee on Cancer
BCG	Bacille Calmette-Guerin
BOD	Burden of Disease
CLND	Complete lymph node dissection
CM	Cutaneous melanoma
CPK	Creatine Phosphokinase
CR	Complete response
CTLA-4	Cytotoxic T-lymphocyte antigen-4
DFS	Disease-free survival
DPFS	Distant progression-free survival
ECT	Electrochemotherapy
EORTC	European Organisation for the Research and Treatment of Cancer
ESMO	European Society of Medical Oncology
IBW	Ideal body weight
ILP	Isolated limb perfusion
ILI	Isolated limb infusion
IL-2	Interleukin 2
ITM	In-transit metastases
IQR	Interquartile range
IPFS	In-field progression-free survival
L19IL2	Darleukin
LOS	Lenght of hospital stay
MDSCs	Myeloid-derived suppressor cells
MDT	Multidisciplinary Tumor board
MSKCC	Memorial Sloan Kettering Cancer Centre
MSS	Melanoma-specific survival
NCCN	National Comprehensive Cancer Network
NEMC	North Estonia Medical Centre Foundation
NICE	National Institute for Health and Care Excellence
ON	Octogenarians and nonagenarians
OR	Overall response
OS	Overall survival
PDL	Pulse Dye Laser
PFS	Progression-free survival
PR	Partial response
PV-10	Rose Bengal Disodium
RECIST	Response evaluation criteria in solid tumors
RCT	Randomised Controlled Trial
RFS	Recurrence-free survival
TNF	Tumor Necrosis Factor
T-VEC	Talimogene laheparepyec
UV	Ultraviolet
WHO	World Health Organisation

1. Background and review of the literature

Melanoma is the most aggressive skin cancer.[2] It arises from the pigment-producing melanocyte cells, and in over 95% of cases it hits the skin surfaces exposed to sun.[2]

Melanoma is most prevalent in the skin but it can be also detected in other anatomical locations.[3] Still lesions on gastrointestinal or genital mucous membranes, on meninges or in the eye are less prevalent. [2]

History of human medicine provides evidence that melanoma was known to our ancestors already in the 5th century BC, however documentation of melanoma studies dates back to late 18th and early 19th century. John Hunter was recorded as first surgeon successfully removing a melanoma in 1787, in 1804 René Laennec was the first to lecture on melanoma and the first to recognize metastatic melanoma, Sir Robert Carswell introduced the term 'melanoma' in 1838.[2,4]

Understanding and treatment of melanoma of the skin, a disease surrounded by mystery for centuries, has evolved dramatically throughout recent years.[5]

Current work elaborates on the role and future perspectives of loco-regional treatment of cutaneous melanoma in modern healthcare settings.

1.1 Cutaneous melanoma

The incidence of cutaneous melanoma (CM) has increased over the past decades[1]. Although its incidence is still more than a 10-fold lower than that of other, more common skin cancers, its ability to rapidly metastasize to lymph nodes and distant organs makes melanoma the most devastating skin cancer with a significant health and economic burden for the society.[3] Melanoma is generally thought to develop mainly due to direct ultraviolet (UV) radiation in one of following two ways: without any visible precursor or in association with benign melanocytic proliferation. [2]

Several risk-factors like advanced age, male sex, familial melanoma, high number of nevi, history of severe solar erythema and light color of skin and hair have been recognized for melanoma.[7] Due to low skin pigmentation in Caucasians, they have a higher risk of getting melanoma compared to darker-skinned people, whose skin pigment protects them better from the cancerous effects of sunlight.[1]

The World Health Organization (WHO) has listed also the damage of atmospheric ozone layers and increase of solar UV radiation reaching the Earth's surface, as risk-factors for melanoma. Due to the increase in greenhouse gas emissions and their detrimental influence on ozone levels, and the aging of population and the ever popularity of sun bathing, the global incidence of melanoma is expected to increase even further in the next decades.

Furthermore, longer life expectancy, mostly in developed countries, results in decreased immunological performance of the elderly increasing the incidence of melanoma even further. This is due to stem cell exhaustion, altered nutrient and growth factor sensing, reduction in T-cell priming capacity, number of circulating natural killer and B cells, in combination with limited efficacy of the cellular immune response in the setting of melanoma.[8]

Preventative measures for melanoma are the most effective in the fight against this deadly tumor.[9] Thus far, however, primary prevention programs, such as sun protection campaigns have not yet shown to be effective in most western countries.[9] In Australia, however, a country with a high melanoma incidence, prevention campaigns started

already in the 1980s, and have been more successful by showing stabilized melanoma rates.[6]

Also continued surveillance has been beneficial in early melanoma detection and reducing long-term complications of the disease such as distant metastases.

1.2 Treatment of primary melanoma

The British surgeon Samuel Cooper acknowledged already in 1844 that success of melanoma treatment is highly dependent on the early removal of the disease.[5] This statement has guided melanoma treatment for more than a century and is still accepted today. If melanoma is diagnosed in the early stages and confined to the primary location only, cure rates are high.[10]

The management of CM is guided by the tumor (Breslow) thickness and highlighted by the initial surgical excision.[11] The aim of surgery is to assure oncologically safe resection margins, reducing the risk of tumor recurrence, but also with a low risk of surgical complications.[12]

Historically a wide excision margin has been recommended for treatment of a primary CM.[11] Until the 1980's a resection margin of 5cm was common practice for all locally advanced CM, resulting in high rates of consecutive skin grafting and associated morbidity to this extensive surgery.[11] Later in that decade, results of the first studies showed that smaller resection margins of 1cm for ≤2mm thick CM may be adequate in terms of disease-free survival (DFS) and overall survival (OS). Also, no difference in the occurrence of nodal or distant metastases was observed compared to a 3cm excision margin in ≤2mm thick CM.[13,14] During the next decades, several additional randomized controlled trials were conducted showing no difference between narrow excision (1-2cm) and wide excisions (3-5cm) in terms of OS and recurrence-free survival (RFS).[15] The Cochrane review, however, concluded that there was still not sufficient evidence to determine the optimal resection margin for primary CM.[11] To this day, various national guidelines give different guidance for the excision margins of primary CM, and none addresses the depth of the excision. In head and neck melanomas the resection margins can vary from standard recommendations due to anatomical and functional facial structures hindering wide resections.[11]

Table 1: Current guidelines for surgical resection margins of cutaneous melanoma.

	NCCN[1]	European[2]	Australia[3]	UK[4]
In situ	0.5-1.0cm	0.5cm	0.5-1.0cm	0.5cm
<1.0mm	1.0cm	1cm	1cm	1cm
>1.0-2mm	1-2cm	1cm	1-2cm	1cm
>2.0-4mm	2.0cm	2cm	1-2cm	2cm
>4mm	2.0cm	2cm	2cm	2cm

[1] National Comprehensive Cancer Network (NCCN) Guideline Version 1.2020[16]
[2] European Society of Medical Oncology (ESMO) Guideline 2019[17]
[3] Cancer Council Australia Clinical Guidelines 2020[18]
[4] UK National Institute for Health and Care Excellence (NICE) Guidelines 2015[19]

1.3 Melanoma metastases

Melanoma cure rates are high when the disease is controlled and limited to its primary location, however metastases still occur quite frequently.[20]

Metastatic spread of CM may arise from a small tumor load.[21] The biologic behavior of CM is often unpredictable. Metastases can develop via three pathways: satellite or in-transit metastases, regional lymph node metastases and distant metastases.[21] There are no clear predictors for the kind of metastatic behavior.[21] In organs where melanoma metastasation occurs extravasation of melanoma cells is regulated by adhesion molecules, matrix metalloproteases, chemokines and growth factors.[22] The metastatic spreading of melanoma can occur through vessel lumina or via extracellular lattices by angiotropism.[22,23] It is believed that besides other risk factors of metastasation like patient's age, sex, primary tumor location on the body, histological profile, Breslow thickness and level of invasion predicts were metastasis would develop.[22] Primary tumors located in the extremities and trunk have the strongest tendency to develop satellite or in-transit metastases.[22,24] Approximately one-third of melanoma patients develop metastasis in distant sites, two-thirds of patients present with locoregional disease.[24]

1.4 In-transit metastases

In-transit metastases (ITMs) are cutaneous or subcutaneous nodulous metastases occurring between the primary tumor and its regional lymphatic basin.[25,26] Examples of ITM are demonstrated on photos 1 and 2. The incidence of ITMs varies between 5% in patients without nodal disease involvement, to 20% in those with.[26]

ITMs develop mostly at an average of 18 months after local excision of the primary melanoma.[10] ITMs confined to a limb and without distant metastatic disease are classified according to the American Joint Committee on Cancer (AJCC) staging system as stage IIIB or IIIC disease.[27] Interestingly, up to 50% of patients with ITMs of the extremity won't develop distant metastases (Stage IV) in the first 2 years after onset of the disease.[28]

Prognostic factors of ITMs are age, thickness, ulceration of the primary tumor and location of primary tumor on extremities.[21, 29] Increased age as the time dependent accumulation of cellular damage and genomic instability has been shown to be a relevant independent prognostic factor for development of ITMs in several studies.[29,30] Sentinel-node positivity and higher Breslow thickness[31] contribute to higher stage of the primary disease conferring a worse prognosis for developing ITM.[32]

Symptoms of ITMs can be pain, ulceration, bleeding and functional disability all resulting in a reduced quality of life and psychological distress.[28,33]

Asymptomatic cutaneous ITMs can be detected clinically, and high-frequency ultrasound is also considered to be a good modality to detect and diagnose subcutaneous and some cutaneous ITMs.[34] ^{18}F-FDG PET/CT sensitivity for detecting smaller lesions has shown to be inferior compared to high-frequency ultrasound, but has better specificity in case it does detect ITMs, and is less operator dependent.[34] Also, benefit of education of melanoma patients has been demonstrated for detection of skin abnormalities that might be ITMs.[35] Female patients have been shown to be more likely to develop ITM than male.[24] Tumor mutation status seems to play a role in the metastasis occurrence site. BRAF/NRAS wild-type tumors compared to BRAF mutant tumors have an increased risk of developing satellite/in-transit metastasis as first metastasis rather than regional lymph node metastasis.[24]

1.5 Treatment of in-transit metastases of cutaneous melanoma

Treatment of ITM can pose a challenge, especially if the disease is multiple in number, or bulky. Various treatment options are available for melanoma ITMs. The US NCCN guidelines list surgical excision, ILI, ILP, intra-lesional therapies, local ablation therapies, radiotherapy and systemic treatments all as appropriate treatment options for these patients.[16]

1.5.1 Surgical excision

In most cases, true curation of ITMs cannot be achieved as in most patients the tumor does reoccur. However, temporarily curation by local procedures can be achieved, also leading to symptom relief.

Surgical excision of ITMs has been the state-of-the-art approach.[26] If possible, surgical resection of metastases is the most effective therapy, but it can only be performed in case the lesions are limited in number and size.

When the metastases are bulky in size or they are numerous, simple surgical resection is often no longer possible and treatment alternatives have to be considered. In these cases, other treatments are put in place. [16] Example of recurrent ITM after surgical resection is demonstrated on photo 4.

1.5.2 Other treatments for in-transit metastases

NCCN provides among other treatments guidance on use of intra-lesional agents, such as Bacille Calmette-Guerin (BCG), interleukin 2 (IL-2)[28,36], talimogene laherparepvec (T-VEC)[37,38], Darleukin (L19IL2), Daromun, PV-10 (rose bengal)[39], laser therapy (pulsed dye or carbon dioxide), electrochemotherapy (ECT) and topical imiquimod[40] as potential effective treatment options. Also, regional chemotherapy by either isolated limb infusion (ILI) or isolated limb perfusion (ILP) and in some cases - systemic treatment with interferon-α or interleukin-2 (IL-2) can be effective.

The goal of treatment is to eliminate the local and systemic dissemination without unreasonable toxicities or deformities and with a benefit of enhanced life expectancy and quality of life.[41]

These abovementioned treatment modalities act in two ways: firstly, they reduce tumor load and potentially decrease the selection of resistant clones, but also potentially induce immune checkpoints and change the natural history of a tumor by turning cold tumors into hot ones and this way make them detectable by immune system.[42]

A proportion of patients also develop a bystander reaction in adjacent or distant lesions resulting in reduction of tumor burden also in lesions not directly treated.[43] This effect is much similar to the abscopal effect reported in radiotherapy treatment of melanoma metastases.[44,45]

1.5.3 Topical and intra-lesional treatment

Radiotherapy is frequently used to treat microscopic ITMs, but macroscopic lesions are more difficult to treat with this modality.[20]

Clinical trials with topical application of either imiquimod or diphencyprone have shown promising results in treating superficial cutaneous and subcutaneous metastases, however, the patients included in these trials were limited making it difficult to draw firm conclusions.[46-48]

Topical treatment of ITMs with imiquimod and tretinoin 0.1% cream in combination with intralesional IL-2 has also been described in case reports or case series.[40,49-51] It is believed that the response is the combined effect of IL-2 inducing antitumoral response

by activating natural killer cells, CD8 and CD4 T-cells, and imiquimod inducing antitumoral cytokines such as TNF, interferon-α and interleukin-12, while topical retinoids increase epidermal penetration aiding to enhance the inflammatory response.[40] Due to the limited number of patients in these series, firm conclusions about the efficacy of this strategy are difficult to draw although response rates of these series were promising.

T-VEC is the first oncolytic immunotherapy approved for intralesional therapy. It has demonstrated therapeutic efficacy in (sub)cutaneous melanoma metastases.[37] However, these studies reported on a small number of patients only and there is no experience in case of bulky or numerous lesions. Furthermore, T-VEC is only used in a small number of countries due to its significant costs. Therefore, T-VEC is currently used only in clinical trials for ITMs, or in anatomical regions where loco-regional chemotherapy is not feasible. Immunomodulation in intra-lesional injections with Darleukin (L19IL2), Daromun or PV-10 have also shown promising preliminary results with a sustained decrease of circulating immunosuppressive myeloid-derived suppressor cells (MDSCs) however these therapies have only been used in clinical trials so far.[39,52]

1.5.4 Laser ablation

CO_2 laser or V-beam pulsed dye laser (PDL) ablation can be performed in outpatient settings also under local anesthesia. Studies have shown that these modalities can be effective in the treatment of ITMs.[10,53] Mechanism of action of PDL is photocoagulation of tumors' vasculature and induces an inflammatory process, whereas the CO_2 laser uses evaporation with 5-10W to eradicate lesions.[10,54] These laser treatments can be applied repeatedly and can be applied as a first-line treatment modality for patients with numerous ITM.[55] Although laser therapy is relatively cheap, simple and patient friendly[56], it is less effective in larger lesions due to deeper skin infiltration and a higher rate of local recurrences and wound morbidity.[10,54]

1.5.5 Cryotherapy

Cryotherapy, using liquid nitrogen, applies extremely cold temperatures that modify the immunological response, leukocytes mobilization and cytokines levels.[57] Historically, cryosurgery in dermato-oncology has been limited to treating certain non-melanoma skin cancers and pre-cancerous lesions.[58] However, recently it has also been used to treat melanoma ITMs.[58,59] Data on effectiveness and outcome of this modality in treating ITMs is limited, hindering wide application.

1.5.6 Electro-chemotherapy

Some centers have reported their experience with electrochemotherapy, electroporation in combination with intravenous administration of antineoplastic drugs such as bleomycin, to treat ITMs.[26] Electrochemotherapy induces a non-thermal tumor ablation, using the cytotoxic effect of antineoplastic drugs directly invading the DNA in the cell nucleus after the integrity of the cell membrane has been broken by electric current.[26] The symbiosis of electroporation and bleomycin has shown a potent tumoricidal effect, not observed when used separately. Although some studies have shown CR rates of 44.8% at 3 months[26] the use of this modality is limited to dedicated centers due to its associated costs.

1.5.7 Isolated limb perfusion (ILP)

Since the 1950s locoregional high-dose chemotherapy treatments have been used for patients with melanoma ITMs.[60] When these ITMs are confined to a limb and deemed unresectable and unable to be treated with other locoregional measures as discussed above, locoregional treatments such as ILP or ILI can provide good results.[20]

Both treatments administer to the isolated limb intravascularly much higher dose of chemotherapy than would be tolerated if administered systemically.[60] Both techniques are based on the principle that under vascular isolation of the limb, the dose of the locally administered chemotherapy, normally melphalan, can be up to 10-fold higher than what would be tolerated systemically without causing damage to vital organs.

Of the two treatments, ILP has been performed for almost seven decades. In brief, ILP is performed as follows: circulation of the disease bearing limb is isolated from the systemic circulation by surgical cannulation of the artery and vein at the iliac, femoral, popliteal, axillary or brachial level, and connected to an extracorporeal circuit. Further isolation of the limb is achieved by applying a tourniquet placing it proximally from the affected area to avoid leakage through collateral vessels.

The cytotoxic agents, normally melphalan, are then circulated through the affected limb through an oxygenator and roller pump. The mild limb hyperthermia causes vasodilation of subdermal and dermal tissues improving so local drug delivery but also increasing drug uptake and cell death.[61-63]

Melphalan, or L-phenylalanine mustard, is an alkylating nitrogen mustard used as an antineoplastic drug.[64,65] It has been the standard drug for ILP since its introduction.[28,63]

Phenylalanine plays a major role in the synthesis of melanine and melanocyte metabolism, making melphalan especially effective when administered in the high dosages tolerated during ILP. Cytotoxic alkylating radical attached to phenylalanine produces selective toxicity to melanoma cells.[66] Warming of the infusate helps to prolong the effect of melphalan.[28,61,63,66] Therefore, during ILP, the limb is heated, internally with warmed infusate and externally by a warm air blanket and an overhead radiant heater, with the aim to achieve limb temperatures of 38-40°C.

The first ILP series reported by Creech et al. showed an overall response (CR+PR) rate of 68%.[61] More recent studies have shown that melphalan-based ILP provides OR rates of 75-80% with CR rates of 40-50%.[62]

In many centers, tumor necrosis factor (TNF) is added to melphalan during ILP.[63,66,67] Complete response rates (CR) after ILP with TNF has been reported to be 7-91% (median 46) and partial response (PR) 0-44% (median 34).[68] The RFS has been in median 14 months, after CR 23 months.[68] OS after ILP has been reported 24 months, in the subgroup of complete responders 44 months (IQR 22-120).[68]

Despite the good results of ILP with TNF[62,63,69] the wider usage of TNF based ILP is hindered by the availability and high costs of the therapy.[70]

Several other drug regimens have been explored for ILP such as cisplatin, dacarbazine and dactinomycin.[62,66] However, none of these combinations have shown results superior to melphalan (with/without TNF) and have resulted in greater limb toxicity.[66]

Although effective and resulting in good response rates, this is a technically complex and invasive procedure and can result in high morbidity rates.[28] This technique requires open surgical dissection and cannulation of the iliac artery and vein and cardiopulmonary bypass machinery with a dedicated perfusion team.[28]

Currently, due to its complexity, costs and other limiting factors described above, ILP is performed in few tertiary referral centers in Europe and the USA, hindering wider application of this efficient treatment modality.[28,68]

1.5.8 Isolated limb infusion

In view of the complexity of ILP, a simplified approach to treat locoregional limb melanoma was developed by Thompson et al. in the 1990s and called isolated limb infusion (ILI).[68,71] Basically ILI is a minimally-invasive low-flow ILP without oxygenation performed through percutaneously placed catheters, omitting the need for surgical cannulation of the limb vessels. Therefore, in ILI no invasive surgical approach is required for insertion of catheters, but percutaneous catheters are placed under radiological guidance in local anesthesia using the Seldinger technique either from ipsilateral or contralateral groin. The tips of the catheters are placed at the level of elbow or knee joint.[68] Similarly to ILP, a tourniquet is placed around the root of the affected limb. Tissues proximal to the catheter tips, but distal from the tourniquet are perfused via collateral vessels in a retrograde fashion.[68] Under general anesthesia, full systemic heparinisation is achieved with a heparin dose of 3mg/kg.[68] The catheters are connected with an extracorporeal circuit running through a warming coil and the cytotoxic drugs, mostly melphalan with or without actinomycin-D, are rapidly infused in the limb via the arterial line using a pressure-pump.[72-74] Both agents have a short half-life of only 15 to 20 minutes[68], so the circulation time of 30 minutes during ILI is sufficient for efficient tissue absorption.[68,71-75] For 30 minutes the infusate is circulated through the limb and the extracorporeal circuit by aspiration and reinfusion. Differently from ILP, ILI is a low-flow procedure and the infusate is not oxygenated, resulting in a hypoxic and acidotic environment[68]. Acidosis enhances the antitumor effect of melphalan[76] by potentiating the action of melphan 1.5 times, together with hypoxic environment potentiation it up to three times.[77]

Warming of the limb is essential to improve the efficacy of the cytotoxic drugs. Similarly to ILP, this is achieved by warming the limb internally by warming the infusate, and by applying a hot-air blanket around and a radiant heater placed over the limb.[72-74]

The cytotoxic drug is typically circulated 60 to 90 minutes, after that the limb is flushed with 1 liter of Hartmann`s solution to eliminate the remaining drug in the isolated limb and the effluent is then discarded as cytotoxic waste.[68,76] Systemic heparinisation effect is reversed with protamine, limb tourniquet is deflated and normal limb circulation is restored.[76] The venous and arterial catheters are removed, Angioseal™ device (St. Jude Medical) can be used to reduce the risk of postoperative bleeding and false aneurysm formation offering a considerable advantage over the traditional manual compression.[76,78]

Postoperatively, daily measurement of creatine phosphokinase (CPK) are performed to detect muscle and tissue damage and clinical assessment is carried out to evaluate local and systemic toxicity, as well as tumor response.[68] Muscle and tumor damage typically peaks on post-procedural day three to four, detectable by CPK or lactate dehydrogenase level increase.[77]

Immediate tumor response is assessed by noting the degree of regression, growth and appearance of melanoma deposits.[77] Treatment response is usually determined according to the RECIST 1.1 guidelines for solid tumors[79] three months after ILI or using the WHO criteria for reporting results of cancer treatment, capturing the best response at two observations more than 4 weeks apart[80] according to country-specific traditions.

Reported outcomes of ILI are comparable to those reported after ILP.[67,72-75]

In the early studies of ILI[81,82] overall response rates described as complete response and partial response together has been reported from 44 to 55%. In more recent studies from US and Australian centers overall response rates have been reported 64 to 73%.[77,83] The data of a systemic review show that the results of ILI are relatively similar to those reported after ILP.[77] However, patients in most ILI studies are in a considerably poorer general condition and comorbid status; sometimes ILI is performed also in palliative settings for stage IV disease.[77]

Limb toxicity after ILI is usually described using Wieberdink toxicity grade.[84] In most series reported toxicity stays usually in grade I-III and no grade V limb toxicity necessitating amputation is present.[77,83,85,86] The most common symptomatic side-effects of ILI are limb edema, numbness and stiffness, no long-term residual functional impairment is usually reported.[87]

1.5.9 Immunotherapy and checkpoint inhibitor therapy

In recent years, immunotherapy and checkpoint inhibitor therapy have dramatically changed the treatment landscape for metastatic melanoma. Their role in patients with melanoma ITMs, however, remains unclear. Most clinical trials of these therapies have included only small number of patients with ITMs or none at all and neither EORTC 18071, EORTC 1325, Checkmate 238 nor COMBI–AD trials, among others, have involved patients with in-transit metastases as inclusion criteria.[88-91] Therefore, to date none of these new agents has been shown to be as effective for ITMs as ILI, especially in patients with bulky disease or when numerous lesions are present.[92-94] Furthermore, the systemic therapies can sometimes result in severe systemic side-effects, something not experienced by patients after ILI. Significant toxicities, severe dose-limiting autoimmunity and even death due to checkpoint inhibitor therapy has been reported.[83,95] Finally, around the world, there are large discrepancies in access to these new and costly drugs, largely due to economic differences between countries and available healthcare funding.[96]

2. Aims of the thesis

Current doctoral thesis seeks to improve the loco-regional treatment of cutaneous melanoma and its in-transit metastases by evaluating surgical techniques, assessing and implementing isolated limb infusion technology.

The thesis has three aims:

1. To evaluate the optimal surgical treatment of primary cutaneous melanoma thicker than 2 mm.
2. To evaluate isolated limb infusion as an option to treat in-transit metastases of melanoma in East European healthcare settings.
3. To evaluate the safety and efficacy of isolated limb infusion for in-transit metastases of melanoma in octo- and nonagenarians, a specific vulnerable group of patients.

3. Patients and methods

Separate retrospective studies were conducted to address the questions of appropriate resection margins for primary melanoma, feasibility of ILI in Eastern European settings outside high-volume centers of the USA and Australia, and safety of ILI in octogenarian-nonagenarian patient groups.

Ethics approvals were obtained for all studies from each institutional or regional ethics review boards. Patient data were retrieved from national cancer registries, melanoma registries or hospital information systems according to availability.

To address the issue of the appropriate surgical excision margin without compromising oncological outcome, an international open-label multi-center randomized controlled trial (RCT) was conducted in Swedish, Danish, Norwegian and Estonian melanoma groups for patients with a primary CM on the trunk, and upper or lower extremities (see Photo 5). Patients with a histo-pathologically verified thick (>2mm) CM were randomized to receive either a 2 or a 4cm surgical excision in parallel allocations.[12] The 936 patients with primary CM aged 75 years or younger were recruited from 53 centers in the 4 countries from 1992 to 2004. Inclusion of patients was performed after diagnosis confirmation with a close margin diagnostic excisional biopsy or with an immediate 2-cm excision margin if melanoma was strongly suspected. Definitive surgery was performed within 8 weeks after diagnosis. Surgery was extended to, or included the deep muscle fascia, although removal of the fascia is generally no longer recommended. In Estonian center fascia was excised in all patients.

Short-term follow-up of patients was conducted in accordance with standard clinical routine in the participating centers, long-term follow-up was performed utilizing the resources of individual medical records, national health board data, local cancer registries and national death or cause-of-death registries according to availability in different participating countries **(Publication I).** All 936 patients were included in the extended follow-up analysis, with only two patients of the total cohort lost to follow-up, both due to emigration. All analyses were done on an intention-to treat basis.

The primary objectives of this study were overall survival (OS) and melanoma-specific survival (MSS). MSS was estimated with Kaplan-Meier method from randomization until death due to disease, and patients were censored at time of death if they died from non-melanoma causes or if they were still alive at the date of last follow-up. OS was estimated from the date of randomization until death from any cause. Statistical analyses were done with SPSS version 25 and R version 3.4.3.

The aim of the study "Evaluation of isolated limb infusion as an option to treat in-transit metastases of melanoma in East European health care settings" **(Publication II)** was to evaluate the intraoperative factors and outcomes of the ILI procedures and to compare those to high-volume centers of USA and Australia performing ILI with the aim to improve treatment quality in Estonia. The North Estonia Medical Centre Foundation (NEMC) is treating approximately 80% of all primary CMs in Estonia, from a population of 1.3 million. All patients treated at the NEMC were discussed by a dedicated multidisciplinary tumor-board (MDT) after initial evaluation, biopsy and appropriate imaging have been performed.

Although Estonia has no dedicated melanoma registry, NEMC carefully archives data of all melanoma patients diagnosed and treated in the institution and these data could easily form the content of national population based registries, as described by other teams.[97] The decision to perform an ILI was met by MDT consensus based on each

individual disease presentation, history and general condition. The ILI protocol used was based on and resembled the ones as described by Brady et al. in the USA[28] and Kroon et al. in Australia.[67] A schematic overview of the procedure is shown in Figure 1.

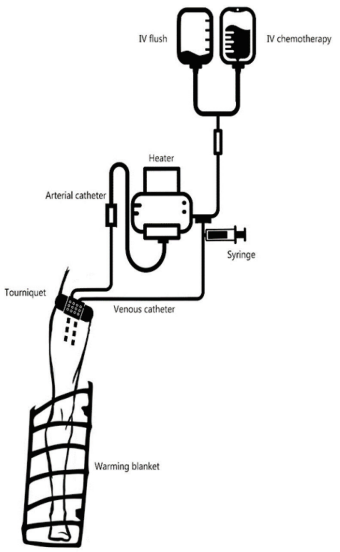

Figure 1: Schematic diagram of the ILI circuit. Chemotherapy is rapidly infused using a pressurized circuit incorporating a blood-warmer with bubble excluder. Venous blood is manually extracted from the limb using a 60ml syringe and reinjected into the isolated circuit.
Source: Teras J, Kroon HM, Thompson JF et al. First Eastern European experience of isolated limb infusion for intransit metastatic melanoma confined to the limb: is it still and effective treatment option in the modern era? Eur J Surg Oncol.2020;46:272-6

High-flow 6F arterial and 7F venous catheters (Bernstein Occlusion Catheter, Boston Scientific®, MA, USA) were placed via the femoral or axillary artery and vein under fluoroscopic guidance the day before the procedure in 4 (19%) patients and in 17 (81%) patients the day of the procedure. Early placement of the catheters was used towards the beginning of the program and abandoned later with growth of experience. Systemic heparinisation during the ILI procedure was achieved immediately before tourniquet placement with a 300IU/kg body weight dosage of unfractionated heparin. This was reversed at completion of the procedure before tourniquet deflation with its antidote protamine 3mg/kg body weight.

Patients were treated with melphalan and actinomycin, agents used also in other centers.[28,68]

Melphalan and actinomycin dosages were corrected for ideal body weight (IBW) as is common practice in US[28,98], but not in Australian centers.[68,85] Several studies have addressed IBW correction suggesting that melphalan dose correction does not decrease the toxicity associated with ILI.[99] Dose adjustment according to IBW reduces grade III toxicity, but at the expense of a lower partial response rate.[77] Limb volume calculation for dosage adjustment was performed by taking circumferential limb measurements of the limb from distal to the proximal at 1.5cm intervals and using a software calculation program provided by dr. Tyler, Duke University Medical Centre, NC, USA.

Limb warming was started in the operating-theater using warm blankets and the forced–air patient warming 3M™ Bair Hugger™ system. Once subcutaneous temperatures

reached 37.0 °C, the chemotherapeutic drugs were infused into the isolated limb over 5-10 minutes via the arterial catheter through a heating coil. The infusate was then circulated manually for 20-25 minutes using a 60mL syringe and a 3-way stopcock. Needle probes were used to monitor the subcutaneous temperatures continuously, aiming for 38.5 ^{0}C by the end of the procedure.

Monitoring of acid base balance with the Astrup method[100] was performed during the procedure to follow patients´ general condition. Likewise, perfusate blood gasses were performed to monitor the extent of hypoxia in the isolated limb.

Upon completion of drug circulation, the limb was flushed with one liter of Ringer´s solution via the arterial catheter, while the effluent was extracted from the limb via the venous catheter using suction attached to it. The effluent was then discarded as cytotoxic waste.

Response to ILI was evaluated at 6 months according to the RECIST 1.1 guidelines for solid tumors.[79] All patients underwent 18F-FDG PET/CT imaging twice during the first year after ILI, and once yearly thereafter.

Data were analysed using descriptive statistics and the Mann-Whitney *U* test. Kaplan-Meier curves were used to display overall survival. Data analysis was performed using JMP 10.0 (SAS®) and Microsoft Excel software (Microsoft®).

In order to evaluate ILI in ON patients, an international multi-center study was conducted, including 687 patients who were treated for melanoma ITMs between 1992 and 2018 at nine US and Australian institutions **(Publication III).** Outcomes of 160 ON patients were compared with the younger cohort of 527 patients. All patients were treated using the same ILI protocol and all centers had demonstrated proficiency with the technical aspects of ILI. The cytotoxic drug combination of melphalan (7.5 mg/L for lower extremities and 10 mg/L for upper extremities) and actinomycin-D (75 mg/L for lower extremities and 100 mg/L for upper extremities) was used. Melphalan dose correction for ideal body weight was performed in the US centers but not in the Australian centers. In case disease progression to the groin or axillary lymph nodes was present, a complete lymph node dissection (CLND) was performed during same anesthesia after completion of the ILI procedure. Following the procedure, patients were monitored to assess limb toxicity according to Wieberdink grading scale.[84]

Treatment response was determined according to the RECIST 1.1 guidelines for solid tumors[79] 3 months after ILI at the US centers or using the WHO criteria for reporting results of cancer treatment, capturing the best response at two observations more than four weeks apart[80], at the Australian centers.

The clinicopathological data that were collected were: age, gender, stage of disease, involved extremity (upper/lower), burden of disease (BOD), and Breslow thickness of primary melanoma. Perioperative data collected were limb volume, melphalan and actinomycin-D doses, tourniquet time, limb temperatures, pH of the perfusate, postoperative serum CK levels, Wieberdink limb toxicity grade, and length of hospital stay (LOS). Outcomes of interest were response to treatment, in-field progression-free survival (PFS; defined as recurrent or progressive melanoma in the affected limb or related nodal basin), distant PFS (defined as melanoma spread beyond the nodal basin of the affected limb), OS and MSS (defined as death due to melanoma), all calculated from time of ILI. Kaplan-Meier curves with log-rank tests were used for displaying and comparing the survival between groups. Statistical analyses were performed using GrapPad Prism version 8.02 software (GraphPad Software Inc., San Diego, CA) and SPSS software version 25.0.0 (SPSS Inc., Chicago, IL).

4. Results and discussion

4.1 Evaluation of optimal surgical treatment of primary cutaneous melanoma thicker than 2 mm (Publication I)

936 clinically staged patients recruited in the study between Jan 22, 1992 and May 19, 2004 were randomly assigned to a 4-cm excision margin (n=471) and a 2-cm excision margin (n=465). Patients were followed-up for a median 19.6 years (IQR 16.7-21.7, maximum 25.6), with a total of 10,039 patient-years of follow-up. 2 patients were lost to follow-up due to emigration after 5.7 years and 8.7 years, respectively. Length of follow-up did not vary between the treatment groups.

Of the study cohort, 621 patients (66.3%) died during the follow-up period. Of those, 397 (64%) died due to CM.

Main finding of this study demonstrated that the incidence of melanoma-related mortality and all-cause mortality did not differ between the groups of 4-cm and 2-cm. There was likewise no difference in overall survival or in melanoma specific survival between the two groups (Figures 2 and 3).

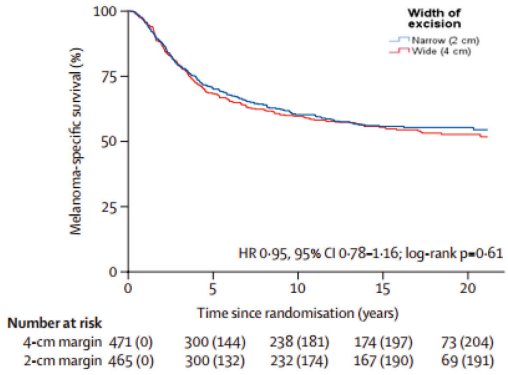

Figure 2: Kaplan-Meier curves of melanoma-specific survival.

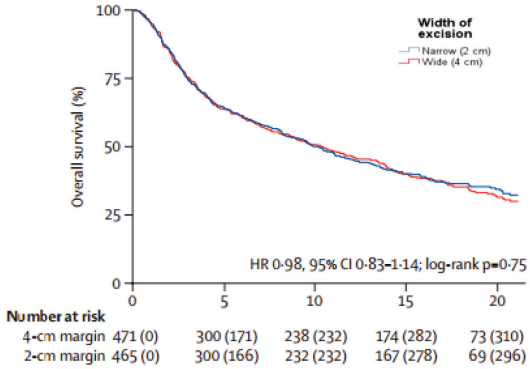

Figure 3: Kaplan-Meier curves of overall survival.
Source: Utjes D, Malmstedt J, Teras J et al. 2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomised trial. Lancet 2019;394:471-477.

Additional analysis stratified by prognostic factors such as age, sex, anatomical site, thickness of the tumor and presence of ulceration did not reveal a difference in OS between narrow (2cm) versus wide (4cm) excision margins in these subgroups. The established prognostic factors, male sex and presence of ulceration, were independent predictors of a shorter MSS.

The overall death rate during the follow-up was the highest during the first 5 years (cumulative 5-year risk for 2-cm group 35%, 95% Cl 31-40 and for 4-cm group 36%, 32-41). The following 5-year periods the risk varied between 5 and 15% in the 2-cm group, and between 7 and 13% in the 4-cm group.

4.2 Evaluation of isolated limb infusion as an option to treat in-transit metastases of melanoma in East European health care settings (Publication II)

Results of the retrospective study conducted to evaluate the data of patients treated by ILI between January 2012 and May 2018 at the NEMC is reviewed.[101]

In total 21 patients treated by ILI for melanoma ITMs confined to a limb at the NEMC were identified. Most patients (67%) undergoing ILI had a high burden of disease (defined as having >10 lesions or any one lesion >2 cm on an affected limb). Limb toxicity evaluation and short-term follow-up of patients was performed in the surgical wards with a mean hospital stay of 15.8 days, ranging from 9 to 24 days.

Local toxicity assessed by the Wieberdink toxicity scale[84] was mild to moderate in 19 (90%) patients. Photos 2 and 3 show a limb before and after ILI. Photo 6 demonstrates grade IV limb toxicity according to Wieberdink toxicity scale. Two patients (10%) experienced grade IV limb toxicity: one patient underwent a fasciotomy and recovered fully. The second patient developed muscle necrosis of the superficial posterior tibial compartment, which was surgically debrided at the time of a fasciotomy. No patient experienced grade V toxicity – severe tissue damage necessitating amputation.

Sixteen patients (76%) experienced an OR (complete + partial response), with a complete response (CR) in eight patients (38%). Four of them (19%) developed new ITMs after a median of 18 months (range 6-30 months). A partial response (PR) was achieved in eight patients (38%) and was sustained in one patient (5%), however this patient developed distant metastases and died 12 months after ILI. Five patients (24%) had either stable disease or progressive disease following ILI. Long-term limb salvage was achieved in 19 patients (90%). In two patients an amputation had to be carried out: In one patient a lower limb amputation was performed due to uncontrollable local recurrence causing pain 13 months after ILI. In the other patient, amputation of the lower limb was carried out 24 months after ILI due to limb ischemia. Median follow-up was 31.0 months (range 1-70 months). In total, four patients (19%) developed distant metastases, two of whom were still alive at the time of data analysis.

Five-year overall survival was 57% (Figure 4). Thirteen patients (62%) were still alive at the time of analysis, 11 of them without evidence of active disease in the treated limb.

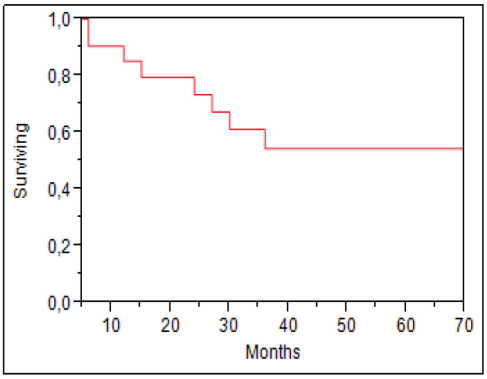

Figure 4: Overall survival (months, red line) following isolated limb infusion for the complete cohort. Source: Teras J, Kroon HM, Thompson JF et al. First Eastern European experience of isolated limb infusion for intransit metastatic melanoma confined to the limb: is it still and effective treatment option in the modern era? Eur J Surg Oncol.2020;46:272-6

4.3 Evaluation of safety and efficacy of isolated limb infusion for in-transit metastases of melanoma in onto- and nonagenarian groups of patients (Publication III)

Of the 687 patients undergoing a first ILI 160 were octogenarians and nonagenarians (ON).

Median age of the 160 patients in the ON group was 84 years, which was 67 years for the younger group. The ON cohort included more female patients than the younger group (70.0% vs. 56.9%; *p*=0.003), and more upper limb procedures were performed in these patients (16.9% vs. 9.5%; *p*=0.009). More ON patients were treated in Australia (71.9% vs. 28.1% in the USA; p=0.0004). Limb volumes were significantly lower in ON patients (median 5.7 vs. 7.3 L; *p*<0.0001). Toxicity was similar in both groups, with the majority experiencing Wieberdink grade I/II limb toxicity, but with younger patients experiencing more grade IV limb toxicity (1.9% vs 4.6%, *p*=0.45). No grade V limb toxicity necessitating an amputation or severe systemic side-effects were experienced in either group.

The OR rate (CR+PR) was similar in both groups: 67.3% in ON patients and 64.6% in the younger patients (*p*=0.53, table 2). CR rate was higher in younger patients (30.5% vs 26.3%) while PR was experienced more by older patients (41.0% vs 34.1%, *p*=0.047).

Table 2: Clinical outcomes.

Characteristic	**<80 years** (n=527)	**≥80 years** (n=160)	**p-value**
Treatment Response, n (%)			
Complete response	159 (30.5)	41 (26.3)	**0.047**
Partial response	178 (34.1)	64 (41.0)	
Stable disease	71 (13.6)	29 (18.6)	
Progressive disease	114 (21.8)	22 (14.1)	
Overall response	*337 (64.6)*	*105 (67.3)*	*0.53*
Resection of residual disease, n (%)	106 (21.2)	30 (18.7)	0.71
Time to resection of residual disease in months, median (range)	5 (0 - 38)	6 (2 - 22)	0.21

Source: Teras J, Kroon HM, Miura JT et al. International multicenter experience of isolated limb infusion for in-transit melanoma metastases in octogenarian and nonagenarian patients. Ann Surg Oncol 2020;27:1420-29

Median follow-up was 92 months for ON patients and 78 months for the younger group (p=0.68). The in-field PFS was 9 months for both groups (p = 0.88), and distant PFS was 36 and 23 months in the ON and younger patients respectively (p=0.16). OS for the ON patients was 29 versus 40 months for the younger patients (p<0.0001), and MSS was 46 versus 78 months, respectively (p=0.0007).

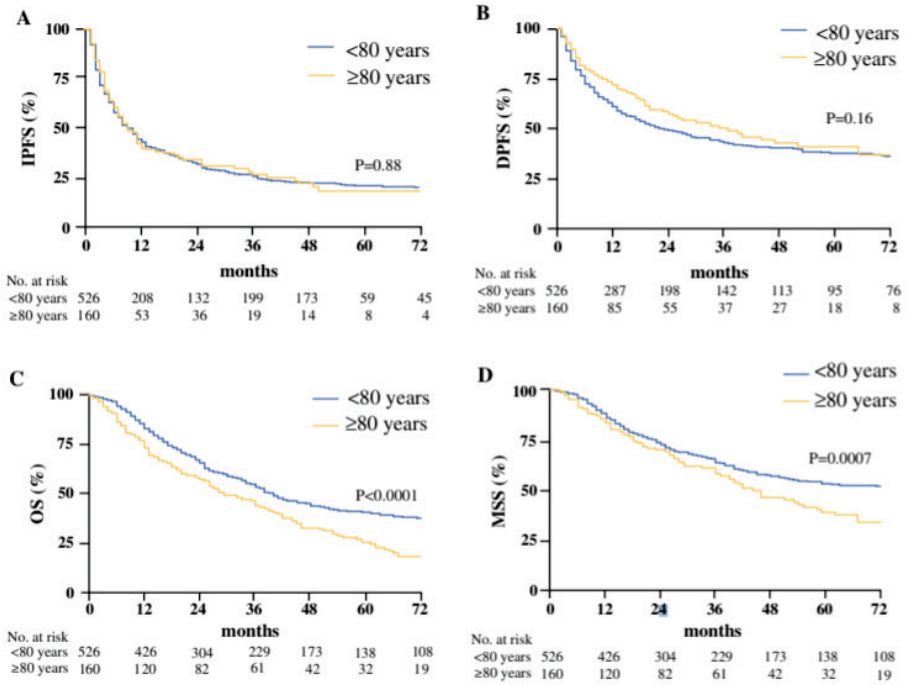

Figure 5 - (a) In-field progression-free survival (IPFS, p=0.88), (b) distant progression-free survival (DPFS, p=0.16), (c) overall survival (OS, p<0.0001), and (d) melanoma-specific survival (MSS, p=0.0007) for octogenarian-nonagenarian patients (≥80 years; dotted line) compared to younger patients (<80 years; solid line).
Source: Teras J, Kroon HM, Miura JT et al. International multicenter experience of isolated limb infusion for in-transit melanoma metastases in octogenarian and nonagenarian patients. Ann Surg Oncol 2020;27:1420-29

Disease-free survival (DFS) was increased for ON patients with lower stage of disease and after CR.

4.4 Discussion

Historically a wide excision margin has been a preferred treatment option for primary CM.[11] In the 1980's a resection margin of 5cm was common practice for all locally advanced CM mandating often wound closure with extensive skin grafting resulting in increased morbidity.[11] Later in early 1990's, results of the first studies showed that smaller resection margins of 1cm for ≤2mm thick CM may be adequate in terms of DFS and OS. Also, no difference in the occurrence of nodal or distant metastases was observed compared to a 3cm excision margin.[13,14]

Despite the fact that surgery is used for loco-regional treatment of cutaneous melanoma for more than two hundred years, modern medicine has raised the need for further development of optimal surgical treatment. Currently, the aim is not only to fully remove the malignant tissue, but also to assure highest possible level of safety and comfort for the patient.

Although melanoma can present with a devastating clinical picture, most melanoma patients in Europe are diagnosed at an early stage of disease.[102] In these patients, definitive treatment can usually be achieved by simple surgical excision. Although much more international research is focused on finding complex genomic and proteomic treatment options for metastatic melanoma[103], guidelines and recommendations for adequate surgical excision of primary melanoma vary around the world.[15] Randomized controlled trials have been conducted in an effort to determine the appropriate resection margin for local control of CM, but all conducted trials have concluded that further research is required.[11,104]

Present study with an extended follow-up period comparing outcomes of 2-cm surgical excision margin with 4-cm margin for treating primary CM with more than 2 mm thickness have shown data in favour of treating thick CM with a 2-cm excision margin.

No difference was found in melanoma specific or overall survival in the long-term follow-up of median 19.6 years between a 2-cm or a 4-cm resection margin for patients with localized CM of >2mm Breslow thickness. In the light of the present study, the conclusion can be drawn that a resection margin wider than 2 cm for melanoma with a Breslow thickness ≥2mm does not provide any survival benefit for the patient, but could potentially result in higher morbidity and increases healthcare expenditure. Whether a resection margin less than 2 cm is also feasible, is for the future studies to establish. Male gender and the presence of ulceration were independent predictors for a shorter MSS, a finding also described by others.[105] Female superiority in survival has been described in previous studies and is thought to be related to the higher stage of disease at presentation in males.[106] It has also been suggested that female do better in melanoma survival due an increase in the female immune response triggered by hormone estrogen.[107,108]

It is commonly believed that melanoma with thickness between 0.75 mm and 1.5 mm tend to develop more often distant metastases, while tumors with thickness <0.76 mm and >1.5 mm have increased rates of in-transit metastases.[24] Still two-thirds of patients developing metastases present initially with locoregional disease – regional lymphnode, ITM or satellite metastases.[24] The incidence of CM and correspondingly loco-regional metastatic disease in the form of ITMs have increased significantly over the past decade.[1] This increase in incidence is even larger in the elderly population, which can be caused by the longer life expectancy, more sun exposure and improved treatments for primary CM resulting in longer survival and later presentation of metastatic disease. Over the last decades, the incidence of CM has shown a significant increase also in Estonia[109], consequencing also in more cases of ITM.

When melanoma ITM develop also distant metastases occurrence is more likely to happen resulting in a reduced 5-year survival. The incidence of ITMs varies between 5% in patients without nodal disease involvement, to 20% in those with.[26]

Sentinel-node positivity and higher Breslow thickness[31] contribute to higher stage of the primary disease conferring a worse prognosis for developing ITM[32]. In the NEMC study the high mean Breslow thickness (7.5mm, range 2.10-14.0mm), rate of ulceration (42%), high mitotic rate (67%) and high BOD (67%) is consistent with this and comparable to previous ILI series.[60,74] Patients with tumors located on upper trunk or head-neck regions have increased risk of developing ITM rather than lymph node metastases compared to patients with other primary regions.[24]

In advanced cases with numerous malignant foci or bulky disease loco-regional surgical resection cannot assure sufficient treatment effect of CM. Despite huge progress

in the development of novel drugs, systemic therapy may lack efficiency in such cases and serious systemic side-effects may discourage patients from these treatments. In these situations isolated limb infusion may be a sound therapeutic option.

ILI program in the NEMC was started in 2012 with the adoption of the isolated limb infusion protocol from the Memorial Sloan Kettering Cancer Centre (MSKCC), USA. To date, the NEMC is the only institution in the Baltic region to perform ILI for melanoma ITMs. Patients are referred for the procedure to the NEMC from all over Estonia.

Symptoms of ITMs can be pain, ulceration, bleeding and functional disability all resulting in a reduced quality of life and psychological distress.[28,33] Therefore a prompt treatment of ITM-s, even if only for symptomatic relief is of vital importance. In the NEMC series majority of patients (80%) presented with complaints on pain and bleeding from the ITMs.

As summed up in **Publication IV** most ITMs can be treated by surgical excision or intralesional injections with for instance PV-10 or T-VEC, electro-chemotherapy, laser therapy or cryotherapy, but when ITMs are multiple, large and bulky, treatment can be challenging. Surgical excision is reported to give local recurrence in 30% of patients[110], in the NEMC study cohort repeated surgical resection of the ITMs was attempted in 24%. In the last decade effective systemic therapy options have been approved and made available in many countries worldwide, but still little is known about the efficacy of these treatments in ITM-s. For selected patients ILI has an advantage also in the era of effective targeted and immune therapies.

This first Eastern European ILI series for melanoma ITMs showed that ILI can be safely and effectively implemented also outside high-volume centres in the USA and Australia, resulting in comparable toxicity and response rates. Our CR rate of 38% and OR rate of 76% are comparable to those reported by larger series.[70,74] This relatively cheap and simple treatment modality may be particular important in Eastern European healthcare settings where budgetary limitations might hinder the availability of other treatments.

Prognostic factors of ITMs are age, thickness, ulceration of the primary tumor and location of primary tumor on extremities.[21,29] Increased age as the time dependent accumulation of cellular damage and genomic instability has been shown to be an important independent prognostic factor for development of ITMs in several studies.[29,30]

Elderly patients, especially the extreme ON, suffer from more comorbidities and are, due to their fragile general state, at increased risk for adverse events when treated for metastatic melanoma with the full armamentarium of modern medicine such as systemic therapies.[86] The introduction of these effective treatment options in the recent years has changed the therapeutic landscape for metastatic melanoma substantially. Immunotherapy and targeted therapies can provide high response rates, however, they can also result in severe and sometimes even fatal side-effects, making clinicians reluctant to administer these agents to ON patients. Most clinical trials of these therapies have included only small numbers of patients with ITMs or none at all.[88-91] Furthermore, it is currently unknown if these systemic therapies are as effective when administered to treat ITMs. Therefore, treatment alternatives with lower chances of side-effects but with a high potential response rate are of special interest in this patient category.

Octogenarian and nonagenarian patients with ITMs, a specifically vulnerable patient group with possible comorbidities and fragility, may not be offered systemic therapies for fear of intolerable side effects. Due to its locoregional and minimally invasive nature ILI is a perfect treatment option also for such patients.

Based on this first ILI study with a specific focus on ON patients, it was concluded that in these extreme old patients, ILI is safe and effective, resulting in similar response and regional control rates compared to younger patients. However, overall and melanoma-specific survival in the ON cohort are shorter. In selected ON patients, ILI is the preferred treatment option for melanoma ITMs.

In the future, the treatment of melanoma ITMs is definitely continuing its evolution, new effective loco-regional treatment alternatives, new systemic immunotherapy and targeted therapy agents are likely to be introduced. It is unlikely that intra-lesional therapy alone provides a sustainable tumor control both locally and distanly.[111]

In recent years interesting new approaches have been introduced combining ILI with different other treatment modalities. Combinational treatment of ILI with intra-lesional agents like T-VEC or PV-10 have been described to be promising.[112] Also novel strategies of intra-lesional delivery of oncolytic viruses and immunocytokines in combination with immunotherapy are currently being assessed.[83]

Studies reporting results of combining ILI with systemic immunotherapy have shown promising results.[106] Evidence that local chemotherapy in combination with CTLA-4 blockade provides a rapid response in treating melanoma metastases suggests that local chemotherapy can be an additional tool to increase the immune response against cancer.[106]

The future aim is to identify those patients who might benefit the most from regional therapy by examining tumor microenvironent[83], using tumor gene expression profiling or other precision methods.

The current work has shown that ILI can be efficiently performed in low-volume dedicated medical centers and on patients in their extreme age, but patient response rates and long-term survival are being far from ideal. Therefore effort should be put into finding treatment modalities being able to really cure patients with locally advanced CM.

5. Conclusions

Current work allows to draw following conclusions:

1. It is safe to conclude that a surgical resection margin wider than 2 cm is not required for thick melanomas (Breslow thickness >2 mm). This data clearly shows that major surgical procedure for treating primary melanoma with very wide resection, sometimes mandating plastic surgery for defect closure, is not necessary, reducing thereby morbidity and saving health care costs.
   Based on current data, a 2-cm resection margin is stated as standard treatment in excision of thick cutaneous melanoma.
2. Isolated limb infusion is a suitable treatment modality for in-transit metastasis of cutaneous melanoma, especially in numerous foci and bulky disease. In this first series of ILI for melanoma ITM in an Eastern European country, we have shown that ILI can be safely and effectively implemented outside of high-volume centers in the USA and Australia. In the Estonian healthcare system, ILI offers the advantage of being a relatively cheap and simple method with low morbidity.
3. Isolated limb infusion for melanoma ITMs in the vulnerable group of octogenarian patients is safe and effective, with comparable responses and disease control rates to younger patients. A favorable response after ILI in ON patients is associated with improved long-term oncological outcomes, and thus widens their treatment options. In current era of ongoing developments in immunotherapy for melanoma, ILI remains an important treatment option for appropriately selected patients with ITMs in view of its low locoregional toxicity and long-term efficacy, especially in octogenarians and nonagenarians.

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Abstract

Loco-regional treatment of cutaneous melanoma

Skin cancer, being one of the most common cancers in Caucasian population, is a global healthcare and socioeconomic burden. The incidence of melanoma, the most devastating skin cancer, continues to rise worldwide. Adequate surgical treatment of primary melanoma and its metastases, particularly in-transit metastases, is of utmost importance.

Most melanoma cases are cured by simple surgical excision. The expansion of surgical treatment must be sufficient to avoid local recurrence while minimizing accompanying morbidity. Historically a wide excision margin has been recommended for treatment of a primary CM. Until the 1980's a resection margin of 5cm was common practice for all locally advanced CM, resulting in high rates of consecutive skin grafting and associated morbidity to this extensive surgery. Later in that decade, results of the first studies showed that smaller resection margins of 1cm for ≤2mm thick CM may be adequate in terms of disease-free survival (DFS) and overall survival (OS). Also, no difference in the occurrence of nodal or distant metastases was observed compared to a 3cm excision margin in ≤2mm thick CM. During the next decades, several additional randomized controlled trials were conducted showing no difference between narrow excision (1-2cm) and wide excisions (3-5cm) in terms of OS and recurrence-free survival (RFS). To this day, various national guidelines give different guidance for the excision margins of primary CM,

The **first goal of this dissertation** was to evaluate the optimal surgical treatment of primary cutaneous melanoma thicker than 2 mm.

A sound compromise between efficient surgical resection margin with reasonable morbidity and melanoma-specific survival has been found to be 2 cm for melanomas greater than 2mm in Breslow thickness. This concept was proven in a multicenter international randomized trial with a median follow-up period of 19.6 years. It is safe to conclude that a surgical resection margin wider than 2 cm is not required for thick melanomas (Breslow thickness >2 mm). Our data clearly shows that major surgical procedure for treating primary melanoma with very wide resection, sometimes mandating plastic surgery for defect closure, is not necessary, reducing thereby morbidity and saving health care costs.

Based on current data, a 2-cm resection margin is stated as standard treatment in excision of thick cutaneous melanoma.

Although efforts are made to surgically treat melanoma in due time and efficiency, distant metastases still occur, in 5-20% of patients in the form of in-transit metastases (ITM). ITMs usually can be treated with simple surgical excision, intralesional injections with several different substances, or isolated limb infusion (ILI) if located in a limb. Treating large and bulky ITMs poses a challenge even in the era of efficacious systemic treatment, especially in the subgroup of octogenarian and nonagenarian patients, whom may be not offered systemic therapies for fear of intolerable systemic effects. ILI on the other hand can be safely performed also in these usually comorbid and fragile patients with overall response in 67.3 % cases.

The second and third objectives of this dissertation were to evaluate isolated limb infusion as an option to treat in-transit metastases of melanoma in East European healthcare settings and in octo- and nonagenarians (ON), a specific vulnerable group of patients.

Isolated limb infusion is a suitable treatment modality for in-transit metastasis of cutaneous melanoma, especially in numerous foci and bulky disease. In this first series of ILI for melanoma ITM in an Eastern European country, we have shown that ILI can be safely and effectively implemented outside of high-volume centers in the USA and Australia. In the Estonian healthcare system, ILI offers the advantage of being a relatively cheap and simple method with low morbidity. Due to the minimal invasive and locoregional nature of ILI, systemic side effects are insignificant and complications rare. We conclude that ILI can be efficiently performed in both high-volume cancer centers and smaller dedicated cancer treatment institutions with reasonable response and survival rates.

Based on this first ILI study with a specific focus on ON patients, it was concluded that ILI for melanoma ITMs in the vulnerable group of octogenarian patients is safe and effective, with comparable responses and disease control rates to younger patients. A favorable response after ILI in ON patients is associated with improved long-term oncological outcomes, and thus widens their treatment options. However, overall and melanoma-specific survival in the ON cohort are shorter. In selected ON patients, ILI is the preferred treatment option for melanoma ITMs.

In current era of ongoing developments in immunotherapy for melanoma, ILI remains an important treatment option for appropriately selected patients with ITMs in view of its low locoregional toxicity and long-term efficacy, especially in octogenarians and nonagenarians.

With new immune-chemotherapy concepts evolving, utilizing ILI as an inducer of inflammatory tumor microenvironment with subsequent immune- or targeted therapy for mobilizing cytotoxic effectors, can still be an effective additional tool to increase the immune response against cancer.

Lühikokkuvõte

Nahamelanoomi lokoregionaalne ravi

Nahakasvajate esinemine europiidse rassi seas on kasvajalistest haigustest kõige sagedasem, olles ühiskondlikus plaanis suureks meditsiiniliseks ja majanduslikuks koormaks. Kõige laastavama nahakasvaja, melanoomi, esinemisagedus maailmas on jätkuvas tõusutrendis. Siiski on enamus naha melanoome ravitavad lihtsa kirurgilise ekstsisiooniga, mille ulatus peab olema piisav tagamaks retsidiivivaba elulemust ilma protseduuri järgsete tüsistuste suurenemiseta. Ajalooliselt on melanoomi esmaseks kirurgiliseks raviks soovitatud laia marginaaliga ekstsisiooni. Kuni 1980-ndate aastateni soovitati resektsioonipiiriks kuni 5-cm, mis enamasti tõi kaasa vajaduse hilisemaks nahaplastikaks koos oma võimalike tüsistustega. Hiljem on mitmed uuringud näidanud on õhukeste melanoomide puhul Breslow paksusega ≤2mm on piisavaks resektsioonipiiriks 1cm tagamaks hea haigusvaba- ja üldise elulemuse. Tänapäeval on rahvusvaheliste ravijuhiste soovitused melanoomi kirurgilise eemaldamise osas siiski varieeruvad.

Käesoleva doktoritöö **esimene eesmärk** oli hinnata Breslow >2 mm paksusega melanoomide optimaalset kirurgilist ravi.

On leitud, et efektiivne kirurgiline resektsioon eelnimetatud melanoomide raviks adekvaatse ravitulemuse ning tagasihoidliku operatsioonijärgse morbiidsusega on 2 cm. Selle kontseptsiooni paikapidavust kontrolliti rahvusvahelise keskmise 19.6 aastase jälgimisperioodiga mitmekeskuselises randomiseeritud uuringus, mille tulemustele tuginedes võib väita, et 2 cm resektsioonipiir nahamelanoomide puhul, mille kasvajapaksus Breslow järgi on üle 2 mm, on piisav, tagamaks minimaalsete operatsioonijärgsete tüsistustega hea melanoomispetsiifilise elulemus. Meie uuringu tulemused näitavad selgelt, et suuremahulisem ja seega ka kallim kirurgiline operatsioon laiema resektsioonijoone, hilisema võimaliku nahaplastika ja tüsistustega, ei ole vajalik.
Hetketeadmiste valguses saab kinnitada, et paksu nahamelanoomi esmase kirurgiline ravi standardmeetodiks on resektsioon mitte laiema, kui 2-cm marginaaliga.

Kuigi primaarse kirurgilise ravi ajastatus ja efektiivsus on oluliselt paranenud, toimub siiski melanoomi metastasteerumine, 5-20% patsientidest esineb see in-transit metastaasidena (ITM). Enamasti on ITM-id lihtsalt kirurgiliselt eemaldatavad või neid saab ravida koldesiseste süstidega. Kui ITM-id paiknevad jäsemel, saab neid ravida ka isoleeritud jäseme kemoinfusiooniga (ILI). Kuigi tänapäeval on järjest enam laienemas efektsiivse süsteemravi võimalused, siis suurte ja vohavate ITM-ide puhul on pakutava süsteemravi võimalused ahtamad, eriti võttes arvesse ka sellise raviga kaasneda võivaid süsteemseid kõrvalmõjusid. Samas ILI saab kasutada ka eakatel ja kaasuvate haiguste tõttu riskialtimatel patsientidel üldise ravivastusega 43-84% juhtudest

Käesoleva doktoritöö **teine ja kolmas eesmärk** oli uurida ILI ravimetoodika kohaldatavust Eestis ning eakatel patsientidel. Esimese Ida-Euroopa keskusena kirjeldasime ILI metoodika kasutamist väljaspool suuri USA ja Austraalia vähikeskusi ning näitasime oma ravitulemuste ja –tüsistuste võrdluses, et antud ravimeetodit on edukalt võimalik implementeerida ka väiksematest keskustes.

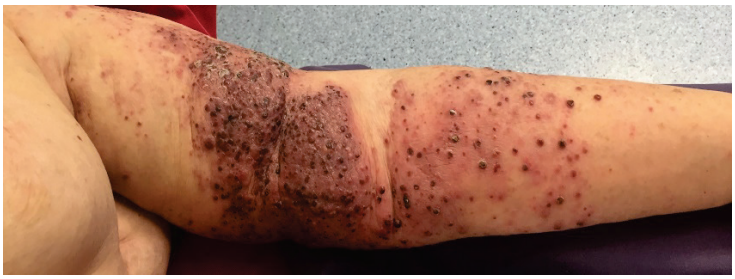
ILI-il on minimaal-invasiivse lokoregionaalravi meetodina harva kõrvatoimeid või ravitüsistusi. ILI võimaldab saavutada ka väiksemates spetsialiseeritud vähiravikeskustes, nagu Põhja-Eesti Regionaalhaigla, häid ravi kaugtulemusi, mis on võrreldavad nimekate suurkeskuste ravitulemustega.

Tuginedes rahvusvahelisel Austraalia ja USA eakatel kaheksa-ja üheksakümnendates eluaastates patsientidel läbiviidud uuringul saame väita, et ILI on edukalt teostatav ka selles vanusegrupis patsientidel, kelle ravivõimalused jäseme ITM-ide puhul ei pruugi olla väga laiad ning seda eeskätt kaasneda võivate muude haiguste tõttu. Tihti ei pakuta antud vanusegrupi patsientidele ka kõrvaltoimete kartuses süsteemravi või märklaud/immuunravi võimalusi. Seevastu ILI hea efektiivsus koos paranenud üldise onkoloogilise tulemusega avardab selle eagrupi patsientide ravivõimalusi.

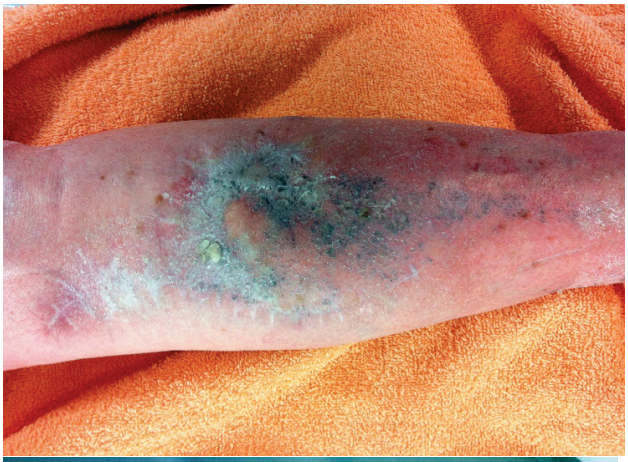
Tänapäevase immuun-kemoteraapia võidukäigu ajastul on ILI säilitanud oma rolli, kui kasvaja mikrokeskkonna mõjutaja. ILI ravile võiks järgneda immuun- või märklaudravi tsütotoksiliste effektorite mobiliseerimiseks ja organismi immuunvastuse parandamiseks.

Appendix 1: Photos

Photo 1 In-transit metastasis on upper limb (personal observation of J.Teras)



Photos 2,3 In-transit metastasis on lower limb pre ILI (photo 2, above) and four weeks later (photo 3, below). Pre-ILI photo demonstrates massive in-transit metastases (blue color). Post-ILI photo demonstrates complete response to ILI with disappearance of ITM-s. (personal observation of J.Teras)



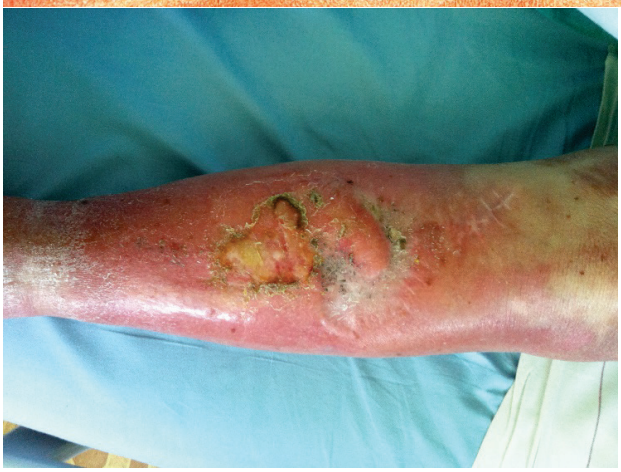


Photo 4 Recurrent in-transit metastasis of thorax 6 months after surgical resection (personal observation of J.Teras)

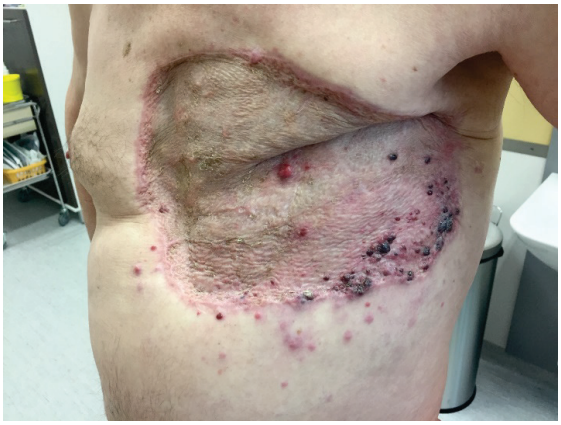


Photo 5 Bulky primary melanoma (Breslow >2cm) with adjacent in-transit metastasis on the skin of thorax (personal observation of J.Teras)

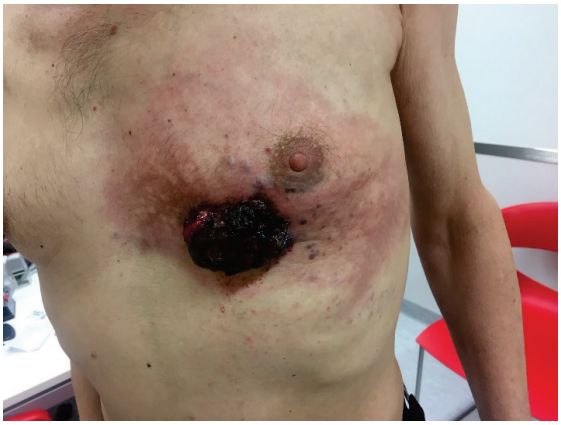
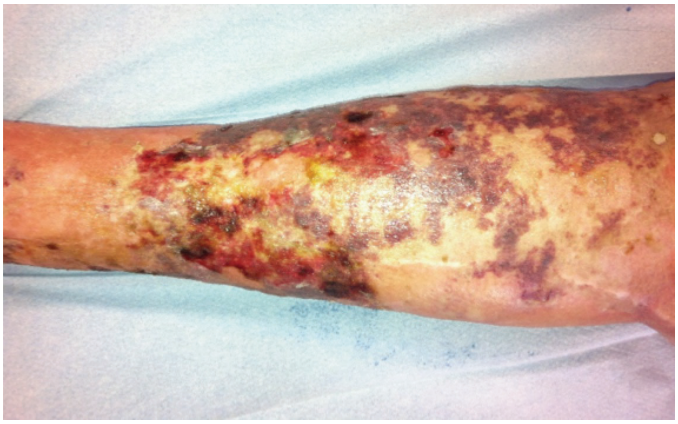


Photo 6 Local toxicity of ILI: epidermiolysis (Wieberdink grade IV) of lower limb 3 weeks after procedure (personal observation of J.Teras)



Appendix 2: Publications

Publication I

Debora Utjes, Jonas Malmstedt, **Jüri Teras**, Krzysztof Drzewiecki, Hans Peter Gullestad, Christian Ingvar, Hanna Eriksson, Peter Gillgren. 2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomised trial. *Lancet* 2019;394:471–477.

2-cm versus 4-cm surgical excision margins for primary cutaneous melanoma thicker than 2 mm: long-term follow-up of a multicentre, randomised trial

Deborah Utjés, Jonas Malmstedt*, Jüri Teras, Krzysztof Drzewiecki, Hans Petter Gullestad, Christian Ingvar, Hanna Eriksson†, Peter Gillgren†*

Summary

Background The optimal surgical excision margins are uncertain for patients with thick (>2 mm) localised cutaneous melanomas. In our previous report of this multicentre, randomised controlled trial, with a median follow-up of 6·7 years, we showed that a narrow excision margin (2 cm *vs* 4 cm) did not affect melanoma-specific nor overall survival. Here, we present extended follow-up of this cohort.

Methods In this open-label, multicentre randomised controlled trial, we recruited patients from 53 hospitals in Sweden, Denmark, Estonia, and Norway. We enrolled clinically staged patients aged 75 years or younger diagnosed with localised cutaneous melanoma thicker than 2 mm, and with primary site on the trunk or upper or lower extremities. Patients were randomly allocated (1:1) to treatment either with a 2-cm or a 4-cm excision margin. A physician enrolled the patients after histological confirmation of a cutaneous melanoma thicker than 2 mm. Some patients were enrolled by a physician acting as responsible for clinical care and as a trial investigator (follow-up, data collection, and manuscript writing). In other cases physicians not involved in running the trial enrolled patients. Randomisation was done by telephone call to a randomisation office, by sealed envelope, or by computer generated lists using permuted blocks. Patients were stratified according to geographical region. No part of the trial was masked. The primary outcome in this extended follow-up study was overall survival and the co-primary outcome was melanoma-specific survival. All analyses were done on an intention-to-treat basis. The study is registered with ClinicalTrials.gov, number NCT03638492.

Findings Between Jan 22, 1992, and May 19, 2004, 936 clinically staged patients were recruited and randomly assigned to a 4-cm excision margin (n=465) or a 2-cm excision margin (n=471). At a median overall follow-up of 19·6 years (235 months, IQR 200–260), 621 deaths were reported—304 (49%) in the 2-cm group and 317 (51%) in the 4-cm group (unadjusted HR 0·98, 95% CI 0·83–1·14; p=0·75). 397 deaths were attributed to cutaneous melanoma—192 (48%) in the 2-cm excision margin group and 205 (52%) in the 4-cm excision margin group (unadjusted HR 0·95, 95% CI 0·78–1·16, p=0·61).

Interpretation A 2-cm excision margin was safe for patients with thick (>2 mm) localised cutaneous melanoma at a follow-up of median 19·6 years. These findings support the use of 2-cm excision margins in current clinical practice.

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Introduction

The incidence of invasive cutaneous melanoma has increased worldwide, especially in white populations.[1,2] The initial surgical excision of the primary melanoma, guided by tumour thickness according to Breslow's depth, is critical for the management of melanoma.[3–6] The ultimate aim of surgical excision is to improve disease outcome and reduce the risk of complications by use of smaller surgical margins.[7] Therefore, the width of the resection margins is of high importance in primary melanoma.

The risk of recurrence with a narrow margin must thus be balanced against the excess morbidity from larger skin defects after wider excision. Over time, and in light of the findings of several randomised studies,[8–10] less extensive surgery for primary melanoma with tumour thickness greater than 2 mm has become more established, although evidence to support this approach has been challenged.[6,11] Most recent guidelines advocate a 2-cm margin for tumours thicker than 2 mm, including the American National Comprehensive Cancer Network and American Academy of Dermatology guidelines.[12,13] The British National Institute for Health and Care Excellence guideline[14] for melanoma changed recommendations from a 3-cm margin to a 2-cm margin for all tumours thicker than 2 mm in 2015, whereas Australian guidelines suggest a less extensive 1-cm margin for melanomas with thickness 4 mm or less and a margin of 2-cm for thicker melanomas (in this case >4 mm).[4,6] Several randomised controlled trials have addressed the issue of appropriate margins for localised thick melanoma.[8–11,15,16] Hayes and colleagues[11] questioned whether a

Research in context

Evidence before this study

Guidelines concerning local excision of primary cutaneous melanoma vary worldwide. Randomised controlled trials have addressed appropriate margins for localised melanoma thicker than 2 mm and meta-analyses have concluded that more evidence is needed on the minimum safe width of the excision margin (ie, the smallest excision margin that can be used without jeopardising survival outcome). We searched PubMed, the Cochrane Library, and book reference lists from Jan 1, 1960, until Nov 30, 2018, for randomised controlled trials published in English, with the search terms "surgical excision margins", "melanoma", "tumour thickness ≥2 mm", "tumour thickness >2–4 mm", and "tumour thickness >4 mm".

Added value of this study

The optimal surgical excision margins are unclear for patients with thick localised cutaneous melanomas. In the first report of this multicentre randomised controlled trial (follow-up 6·7 years), we showed that a narrow excision margin (2 cm *vs* 4 cm) did not affect the melanoma-specific survival nor overall survival. In 2004, Thomas and colleagues published a similar trial (1-cm *vs* 3-cm excision margins); neither this study nor our study used sentinel node biopsy for pathological staging. Thomas and colleagues published their long-term results (median follow-up 8·8 years) in 2016, and showed a significantly longer survival with the wider surgical excision margin. The present study found that a 2-cm excision margin is safe for patients with thick melanomas (>2 mm) at a follow-up of 19·6 years. To our knowledge, this is the longest follow-up of outcome following surgery for thicker melanoma (>2 mm).

Implications of all the available evidence

Our long-term results show that thicker melanomas (>2 mm) can safely be excised with a 2-cm margin.

1-cm margin is safe for melanomas with thickness greater than 2 mm in a long-term follow-up (median 8·8 years) of melanoma-specific survival in a randomised trial comparing 1-cm surgical excision margins with 3-cm surgical excision margins. Therefore, uncertainty remains over the use of 1-cm margins versus 3-cm margins, and a recent meta-analysis highlighted the need for more evidence.[6]

We present long-term follow-up data of patients with localised melanoma thicker than 2 mm, who were randomly assigned to 2-cm surgical excision margins versus 4-cm surgical excision margins.[9] We aimed to prove that a 2-cm margin is non-inferior to a 4-cm margin with regard to melanoma-specific and overall survival, and to strengthen the evidence base for a 2-cm policy by providing results from an extended follow-up.

Methods

Study design and patients

The study design, patient eligibility criteria, trial protocol, procedures, follow-up, and endpoints have previously been described in detail.[9] Briefly, our original, open-label, multicentre randomised controlled trial was an international study from the Swedish and Danish Melanoma groups, with planned follow-up until at least 2016 (appendix p 2). Patients were recruited from 53 hospitals in Sweden, Denmark, Estonia, and Norway.

See Online for appendix

We enrolled clinically staged patients aged 75 years or younger diagnosed with localised cutaneous melanoma thicker than 2 mm, and with primary site on the trunk or upper or lower extremities. We defined clinical stage according to the 7th edition of the American Joint Committee on Cancer melanoma staging and classification.[17] We excluded patients with melanoma of the hands, feet, head and neck, and anogenital region, and those with a history of melanoma, squamous cell carcinoma, or other known malignant disease (other than basal cell carcinoma and in-situ cancer of the cervix uteri). Histological diagnosis was by tumour thickness according to Breslow,[18] histogenetic type,[19] and ulceration of the primary tumour.

The study was approved by the ethics review board of the Karolinska Institutet (Stockholm, Sweden). Participating centres not covered by this review board obtained approval from regional ethics review boards. Patients provided verbal informed consent. The study protocol is available in the appendix (pp 7–14).

Randomisation and masking

Patients were randomly assigned to have either a 2-cm or a 4-cm surgical excision margin in a 1:1, parallel allocation. A physician enrolled the patients after histological confirmation of a cutaneous melanoma thicker than 2 mm. Some patients were enrolled by a physician acting as responsible for clinical care and as a trial investigator (follow-up, data collection, and manuscript writing). In other cases physicians not involved in running the trial enrolled patients. Randomisation was done by telephone call to a randomisation office, by sealed envelope, or by computer generated lists using permuted blocks. Patients were stratified according to geographical region. No part of the trial was masked.[9]

Procedures

The primary excision of the tumour was done either by an excisional biopsy (margin of 1–3 mm) or with an immediate 2-cm excision margin if melanoma was strongly suspected. Patients could then be allocated to receive further surgery with a margin of up to either 2 cm or 4 cm. Patients with an initial 2-cm excision margin based on melanoma suspicion (as was done in some instances) received either no further surgery (those

randomised to the 2-cm group) or an additional wide local excision with a margin up to 4 cm. Definitive surgery, if not achieved initially, was done less than 8 weeks after the date of diagnosis. Surgery extended to, or included, the deep muscle fascia, although removal of the fascia is generally no longer recommended. The pathological excision margin was not recorded.

Patients were followed-up with standard clinical routines within the participating centres and data on recurrence and survival analyses were recorded. In the present study, information on date and cause of death was collected from individual medical records, local cancer registries, and national cause-of-death registries. In Sweden, the Swedish Cause of Death register is annually maintained by the National Board of Health and Welfare and includes date of death, cause of death, and underlying cause(s) of death for all deceased Swedish citizens. In Sweden and Denmark, we obtained outcome data from the National Board of Health, which compiles a register covering all deaths among deceased citizens.[20,21] In Norway, we collected date and cause of death through patient records. In Estonia, we obtained information on date and cause of death through national electronic health records, which are linked to national death registries.

Swedish and Danish patients were followed-up until the most recent updates in the Swedish and Danish national registries at the time of the study, which were Dec 31, 2017, and June 15, 2016, respectively. In Norway, patients were followed-up until April 25, 2016. In Estonia, patients were followed-up until June 29, 2016 (appendix p 2). Two patients were lost to follow-up due to emigration (data on country of residence were updated from national registries), one in each allocation arm, and thus censored at that time. For detailed follow-up data see appendix (p 2).

Outcomes

The primary outcome in this extended follow-up study was overall survival and the co-primary outcome was melanoma-specific survival. Melanoma-specific survival was measured from randomisation until death due to disease, and patients were censored at time of death if they died of non-melanoma causes or at the date of last follow-up if still alive. For calculation of overall survival, the time from randomisation until death from any cause was used. In the original study, recurrence-free survival and number of local recurrences were secondary endpoints, but these endpoints were not assessed in the present long-term follow-up.

Classification of cause(s) of death was done in accordance with WHO rules[22] and the current version of the International Statistical Classification of Diseases and Related Health Problems in the national registers.[20,21]

Statistical analysis

The initial plan was to recruit 1000 patients for an interim analysis and continue and add another 1000 patients to do an equivalency study containing 2000 patients in total. Towards the end of the enrolment period, clinical practice started to change at many centres (tumours close to 2-mm in thickness were routinely excised with small surgical margins) and the inclusion rate abated, therefore enrolment was stopped in 2004 before reaching the initial goal of 1000 patients. The interim analysis was based on the assumption that 500 patients in each treatment group would enable detection of a reduction of survival to 50% with acceptable statistical power (α=0·05, β=10, power=90%). The actual number of patients recruited provided a power of 87% to detect the differences in survival projected in the original power calculation.[9]

All analyses were done on an intention-to-treat basis. No per-protocol analyses were done as all patients received the allocated treatment. Each patient contributed risk time from day of randomisation until death, and patients who remained alive were censored at the end of follow-up. Censoring was considered non-informative (the reason[s] for patients ending follow-up without an event should not be related to the condition under study). We calculated crude incidence rates with 95% CIs for melanoma-specific and overall survival in both groups with the mid-p exact test using Miettinen's modification.[23]

We estimated overall survival with the Kaplan-Meier method and used the Mantel-Haenszel log-rank test to compare overall survival between the study groups. We calculated pointwise 95% CIs for the Kaplan-Meier estimates at each year of follow-up with the Kalbfleisch and Prentice method.[24] We used competing risk analysis with cumulative incidence to estimate melanoma-specific survival and compared survival between the groups with Gray's test.[25]

We calculated median follow-up duration with reversed Kaplan-Meier estimates according to Schemper and Smith.[26] We used standard Cox proportional hazards models for estimation of hazard ratios (HRs) and 95% CIs for overall survival. We used Fine and Gray sub-distribution hazard models[25] to estimate the HR for melanoma-specific survival with death from other causes

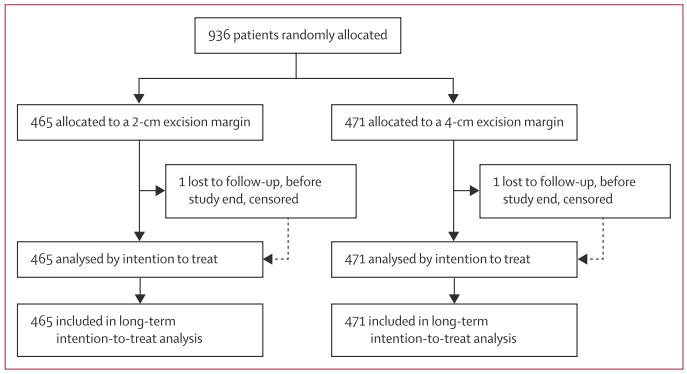

Figure 1: **Trial profile**

as a competing event. We used the 4-cm margin as a reference group for all HR estimates.

We did post-hoc subgroup analyses and used 99% CIs to compensate for multiple testing. Additionally, we did an analysis with adjustment for sex, age, anatomical site of tumour, tumour thickness, and ulceration to enhance comparability with other studies. Furthermore, inspection of log–log plots and a global test based on Schoenfeld residuals indicated that the proportional hazards assumption was not violated. All tests were two-sided and statistical significance was set at p<0·05. Continuous data are reported as median and IQR.

Statistical analyses were done with SPSS version 25 and R version 3.4.3. The study is registered with ClinicalTrials.gov, number NCT03638492.

	2-cm margin group (n=465)	4-cm margin group (n=471)
Age (years)	59 (49–68)	60 (50–68)
Sex		
Men	289 (62%)	311 (66%)
Women	176 (38%)	160 (34%)
Site		
Neck	2 (<1%)	0
Trunk	273 (59%)	292 (62%)
Upper extremity	69 (15%)	74 (16%)
Lower extremity	119 (26%)	104 (22%)
Sole of foot	2 (<1%)	1 (<1%)
Tumour thickness (mm)	3·1 (2·5–4·4)	3·1 (2·5–4·4)
Tumour thickness (mm)		
≤3	230 (49%)	230 (49%)
>3	233 (50%)	241 (51%)
Data unavailable	2 (<1%)	0
Histogenetic type of melanoma		
Superficial spreading melanoma	176 (38%)	169 (36%)
Lentigo maligna melanoma	5 (1%)	4 (1%)
Nodular melanoma	247 (53%)	251 (53%)
Acral lentiginous melanoma	1 (<1%)	1 (<1%)
Unclassifiable	29 (6%)	37 (8%)
Data unavailable	7 (2%)	9 (2%)
Clark level of invasion		
II	6 (1%)	9 (2%)
III	107 (23%)	121 (26%)
IV	294 (63%)	282 (60%)
V	34 (7%)	37 (8%)
Data unavailable	24 (5%)	22 (5%)
Ulceration		
Present	210 (45%)	224 (48%)
Absent	194 (42%)	188 (40%)
Unclassifiable	2 (<1%)	1 (<1%)
Data unavailable	59 (13%)	58 (12%)

Data are median (IQR) or n (%) unless otherwise stated.

Table 1: **Baseline patient and surgical characteristics**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 22, 1992, and May 19, 2004, 936 clinically staged patients were recruited and randomly assigned to a 4-cm excision margin (n=471) or a 2-cm excision margin (n=465; figure 1). Patients were followed-up for a median of 19·6 years (235 months, IQR 200–260; maximum 307 months) after randomisation, with a total of 10 039 patient-years of follow-up. Two patients emigrated and were therefore lost to follow-up after 5·7 years and 8·7 years, respectively. Length of follow-up did not differ between the treatment groups (appendix p 2). All patients from the original trial were included in this extended followed-up analysis and baseline characteristics were similar between the two groups (table 1), as previously described.[9]

Protocol deviations occurred in 145 (15%) of 936 patients, as described previously.[9] 621 patients died during follow-up—317 (51%) in the 4-cm margin group and 304 (49%) in the 2-cm margin group (unadjusted HR 0·98, 95% CI 0·83–1·14; p=0·75; table 2; figure 2). 205 (52%) of 397 deaths attributed to melanoma occurred in the 4-cm excision margin group and 192 (48%) of such deaths occurred in the 2-cm excision margin group (HR 0·95, 95% CI 0·78–1·16; p=0·61; table 2; figure 2). The incidence of melanoma-related mortality

	2-cm margin group (n=465)		4-cm margin group (n=471)		Crude HR* (95% CI)	p value	Adjusted HR for age and sex (95% CI)	p value
	n (%)	Absolute rate per 100 person-years (95% CI)	n (%)	Absolute rate per 100 person-years (95% CI)				
Death	304 (65%)	6·1 (5·5–6·8)	317 (67%)	6·3 (5·6–7·0)	0·98 (0·83–1·14)	0·75	1·02 (0·87–1·19)	0·83
Death due to melanoma	192 (41%)	3·9 (3·3–4·4)	205 (435%)	4·1 (3·5–4·6)	0·95 (0·78–1·16)	0·61	0·99 (0·81–1·20)	0·89

HR=hazard ratio. *4-cm margin group is reference.

Table 2: **Number of events, absolute rates per 100 person-years, and HRs for overall survival and melanoma-specific survival**

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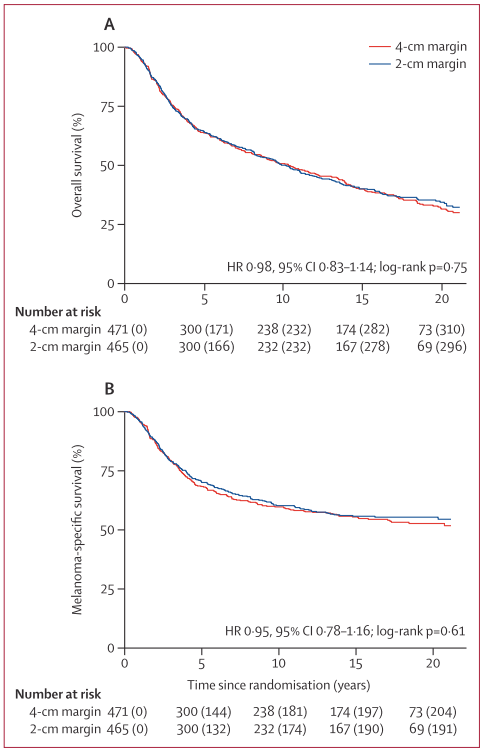

Figure 2: **Overall survival (A) and melanoma-specific survival (B)**
HR=hazard ratio.

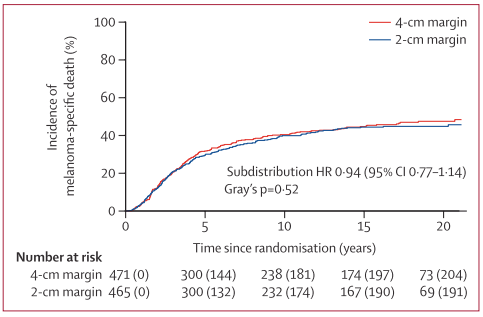

Figure 3: **Cumulative incidence of death due to melanoma**
HR=hazard ratio.

	2-cm margin group	4-cm margin group
5 years	0·65 (0·60–0·69)	0·64 (0·59–0·68)
10 years	0·50 (0·45–0·55)	0·51 (0·46–0·55)
15 years	0·40 (0·36–0·44)	0·40 (0·36–0·44)
20 years	0·35 (0·30–0·39)	0·33 (0·28–0·37)
25 years	0·25 (0·18–0·33)	0·26 (0·20–0·31)

Data are survival probability (95% CI).

Table 3: **Long-term overall survival probabilities in the 2-cm *vs* 4-cm excision margin groups**

and all-cause mortality did not differ between the 4-cm and 2-cm group (table 2).

Results of the competing risk analysis using the Fine and Gray model for melanoma-specific deaths were similar to the Cox model for melanoma-specific survival (HR 0·94, 95% CI 0·77–1·14; p=0·52; figure 3; appendix p 4).

The overall death rate was highest during the first 5 years of follow-up (cumulative 5-year risk for 4-cm group 36%, 95% CI 32–41 and for 2-cm group 35%, 31–40), whereas the risk varied between 7% and 13% in the 4-cm group, and between 5% and 15% in the 2-cm group over the following 5-year periods (table 3; figure 2).

We included 929 patients in the multivariate analysis (seven patients had missing data for at least one variable). Adjustment for prognostic factors did not change the HRs for the 4-cm excision margin group versus the 2-cm excision margin group. The combination of male sex, age older than 60 years, an ulcerated melanoma, with thickness greater than 3 mm, and located on the trunk conferred the highest risk of death due to melanoma (table 4). Furthermore, subgroup analysis of prognostic factors (age, sex, anatomical site, thickness, and ulceration) did not reveal any difference in the effect caused by margin of excision (appendix pp 5–6).

Discussion

We found no difference in melanoma-specific survival or overall survival in this long-term follow-up of a randomised controlled trial comparing 2-cm with 4-cm surgical excision margins for melanomas thicker than 2 mm. Additionally, analysis stratified by prognostic factors did not reveal any association between survival and narrow versus wide excision margins in these specific subgroups. As expected, we found that established prognostic factors, such as male sex and presence of ulceration, were independent predictors of a shorter melanoma-specific survival.

Previous randomised controlled trials comparing a 3–5-cm margin with a 1–2-cm margin have found no difference in overall survival.[8–10] However, a long-term follow-up[11] of a study by Thomas and colleagues[10] concerning melanoma thicker than 2 mm implied a less favourable melanoma-specific survival for clinically staged patients randomly assigned to a narrow (1-cm) versus wide (3-cm) margin. Median follow-up duration was 8·8 years and death certificates were not obtained for 110 (12%) of 900 patients.[11] We were able to follow up patients for a median of 19·6 years and had complete data on cause of death, and only two patients were lost to follow-up. Our present results are in line with our previous report,[9] in which we did not find any difference in melanoma-specific

	n	Overall survival		Melanoma-specific survival	
		HR (95% CI)	p value	HR (95% CI)	p value
Margin of excision*					
4-cm	470	1 (ref)	..	1 (ref)	..
2-cm	459	1·04 (0·89–1·22)	0·61	0·99 (0·82–1·21)	0·96
Sex					
Women	335	1 (ref)	..	1	..
Men	594	1·39 (1·16–1·67)	0·0004	1·51 (1·19–1·90)	0·0006
Age (years)					
<60	473	1 (ref)	..	1 (ref)	..
≥60	456	2·11 (1·79–2·48)	<0·0001	1·34 (1·09–1·63)	0·0046
Site					
Lower extremity	226	1 (ref)	..	1 (ref)	..
Trunk	560	1·20 (0·98–1·48)	0·078	1·29 (1·00–1·68)	0·051
Upper extremity	143	0·97 (0·74–1·27)	0·84	0·92 (0·65–1·31)	0·64
Thickness (mm)					
≤3	459	1 (ref)	..	1 (ref)	..
>3	470	1·45 (1·23–1·70)	<0·0001	1·74 (1·42–2·14)	<0·0001
Ulceration					
Absent	379	1 (ref)	..	1 (ref)	..
Present	433	1·39 (1·17–1·65)	0·0002	1·68 (1·35–2·10)	<0·0001
Data unavailable†	117	1·02 (0·78–1·33)	0·90	1·22 (0·87–1·70)	0·26

The 2-cm *vs* 4-cm HR is adjusted for all other factors in the table. HR=hazard ratio. *One patient in the 4-cm group and six patients in the 2-cm group were excluded (two melanoma located in head and neck region, two no data for tumour thickness, and three unclassifiable ulceration). †Analyses without these cases did not affect the results.

Table 4: **Multivariate analysis of overall survival and melanoma-specific survival**

survival or overall survival between patients in the 2-cm margin group and the 4-cm margin group.

The patient population in our study was similar to the population in Thomas and colleagues' trial[10] in terms of age and tumour thickness, but our study had a higher proportion of men and tumour ulceration. Male sex and tumour ulceration are associated with decreased melanoma-specific survival.[27,28] Both sex and tumour ulceration were adjusted for in the multivariate analyses in our trial and the long-term follow-up of this previous trial,[11] and are therefore not a plausible explanation for the contradictory findings.

The original study by Thomas and colleagues[10] found no differences in overall survival, but reported a higher rate of locoregional recurrence in the narrow (1-cm margin) group, which contrasts with our initial report[9] of the present trial in which we found no significant difference in locoregional events or risk of death. Neither the long-term follow-up of our trial nor the long-term follow up of the trial by Thomas and colleagues[11] have data on locoregional recurrence.

There is one main difference in outcome between our long-term results and the long term follow-up of Thomas and colleagues' study.[10,11] The latter study reported a lower long-term melanoma-specific survival (with both Cox and competing risk models) in the 1-cm margin group, whereas, using the same models, we found similar melanoma-specific survival between the groups.[11]

Hence, these main differences might indicate that a 1-cm excision margin is too narrow, whereas a 2-cm margin is safe for patients with melanomas thicker than 2 mm, although further study is needed. Furthermore, the potential impact on relapse-free and overall survival with more effective systemic adjuvant therapies in high-risk cutaneous melanoma might one day obviate the need for wider margins.[29] This strategy requires potential molecular testing or other techniques to determine how aggressive cutaneous melanomas should be treated surgically and systemically.

The limitations of our study are as follows: our original trial did not achieve the intended target inclusion (2000 patients), but the interim analysis indicated an actual power of 87%;[9] nodal staging was not done during the study period, with a possible, but unlikely—given our good balance for other characteristics—consequence of imbalance for nodal stage between the two treatment arms; and pathological excision margins were not recorded, meaning that the results of this trial address only the surgical margin in clinically localised melanoma.

In conclusion, we present an extended long-term follow-up of median 19·6 years of a previously published multicentre trial,[9] which provides robust data that a 2-cm resection margin is safe for melanoma thicker than 2 mm. Results of the ongoing Australia and New Zealand MelMarT trial[30] (NCT02385214), which directly compares 1-cm margins with 2-cm margins for melanoma with thickness 1 mm or greater might provide further evidence of the safety and appropriateness of narrower surgical margins for excision of cutaneous melanoma.

Contributors

DU contributed to the literature search, data collection, data interpretation, and writing of the manuscript. JM was responsible for statistical analysis, figures, and data interpretation, and contributed to the literature search, data collection from Sweden, and writing of the manuscript. JT, KD, and HPG reviewed the manuscript and collected data from Estonia, Denmark, and Norway, respectively. CI contributed to data interpretation and writing of the manuscript. HE contributed to the literature search, data collection, data interpretation, and writing of the manuscript. PG contributed to the study design, literature search, data collection from Sweden, data interpretation, and writing of the manuscript. All authors have seen and approved the final text.

Declaration of interests

We declare no competing interests.

Data sharing

Individual participant data underlying the results reported in this Article will be made available after de-identification, alongside a data dictionary, study protocol, and informed consent form. The data will be available at Article publication and for 10 years subsequently. Data will be shared for individual participant data meta-analysis with other members of the research community who have an affiliation to a recognised medical university. Data will only be shared with investigator support and after approval of a proposal, and with a signed data access agreement. Additional restrictions apply according to Swedish law. Proposals should be directed to peter.gillgren@sll.se.

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Publication II

Jüri Teras, Hidde M. Kroon, John F. Thompson, Marina Teras, Pille Pata, Andrus Mägi, Roland M. Teras, Sirje Rüütel Boudinot. First Eastern European experience of isolated limb infusion for intransit metastatic melanoma confined to the limb: Is it still an effective treatment option in the modern era? *Eur J Surg Oncol* 2020;46:272–276.

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First Eastern European experience of isolated limb infusion for in-transit metastatic melanoma confined to the limb: Is it still an effective treatment option in the modern era?

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ABSTRACT

Background: Isolated limb infusion (ILI) with cytotoxic agents is a simple and effective treatment option for patients with melanoma in-transit metastases (ITMs) confined to an extremity. Data for ILIs performed in Europe are sparse and to date no Eastern European ILI experience has been reported. The aim of the current study was to evaluate the efficacy of ILI in Estonia.

Patients and methods: Data for twenty-one patients were collected and analysed. All patients had melanoma ITMs and underwent an ILI between January 2012 and May 2018. The cytotoxic drug combination of melphalan and actinomycin-D was used. Drug circulation times were 20–30 min under mildly hyperthermic conditions (38–39 °C). Primary outcome measures were treatment response and overall survival.

Results: Nineteen lower limb and two upper limb ILIs were performed. The female to male ratio was 18:3. The overall response rate (complete + partial response) was 76% (n = 16), with a complete response in 38% (n = 8). The overall long-term limb salvage rate was 90% (n = 19). During follow-up, eight patients (38%) died, two due to metastatic melanoma. Five-year overall survival was 57%.

Conclusion: This first Eastern European report of ILI for melanoma ITMs shows results comparable to those from other parts of the world. In this era of effective targeted and immune therapies, ILI remains a useful treatment option, with a high overall response rate and durable responses in patients with melanoma ITMs confined to a limb.

Introduction

The incidence of skin cancers, mostly basal cell carcinoma, squamous cell carcinoma and melanoma, continues to rise in the Western world [1]. Of these three, melanoma has the worst prognosis due to its metastatic potential. After treatment of the primary melanoma, it has been reported that 4.3–6.6% of patients develop in-transit metastases (ITMs), defined as metastatic lesions between the primary tumour site and the nearest lymph node basin with reported rates of ITMs exceeding 20% in patients with more advanced stage primary melanomas [2,3]. Without treatment, the quality of life of these patients is often poor, due to ulcerated, bleeding and sometimes painful lesions as well as limited limb functionality [4]. Furthermore, achieving a favourable response following treatment of ITMs is associated with improved overall survival [5,6].

In recent years, new immune-modulating and targeted

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therapies have dramatically changed the treatment of patients with metastatic melanoma. However, there are large variations in the availability of these new and costly therapies around the world, and according to a recent study from Europe a large proportion of melanoma patients have restricted access to them [7]. In these circumstances, clinicians mostly continue to use less costly loco-regional treatment options.

Traditionally, ITMs confined to a limb have often been treated by hyperthermic isolated limb perfusion (ILP). However, due to its technical complexity, the procedure has not been widely used, despite complete response rates of 25–89% and favourable five-year overall survival rates of 32–50% [6]. Isolated limb infusion (ILI) was introduced in the early 1990's by Thompson et al. at the Sydney Melanoma Unit in Australia (now Melanoma Institute Australia) as a minimally-invasive alternative to ILP [8]. Since then, ILI has proved to be an attractive treatment modality to achieve regional disease control and limb preservation in melanoma patients with ITMs confined to a limb [9]. However, there are few ILI reports available from centres outside Australia and North America, with only two publications originating from Europe and none from Eastern Europe [10,11]. Therefore, the present study was conducted to analyse the Eastern European ILI experience in Estonia, to evaluate its feasibility and its efficacy outside of high-volume centres elsewhere in the world.

Patients and Methods

The data for all patients with melanoma ITMs treated by ILI between January 2012 and May 2018 at the Centre for Surgical Oncology and General Surgery in the North Estonia Medical Centre Foundation, Tallinn were collected retrospectively. All patients provided informed consent for data collection and usage for clinical research.

The North Estonia Medical Centre Foundation is the only institution in the region with an ILI program and functions as a tertiary referral centre for melanoma. The decision for either radical surgical resection or an ILI if the ITMs were deemed unresectable was made during a melanoma multidisciplinary team meeting in which all patients were discussed and evaluated by an expert group. Burden of disease (BOD) of the affected limb was defined as low (≤10 lesions with no lesion >2 cm) or high (>10 lesions or any one lesion >2 cm) [9].

All patients underwent ^{18F}-FDG PET/CT imaging preoperatively to identify the presence and extent of active disease in the affected limb and elsewhere.

A schematic overview of the ILI procedure is shown in Fig. 1. The ILI procedure was performed in accordance to the Memorial Sloan Kettering Cancer Center (MSKCC) protocol [12,13]. In brief: high-flow 6F or 7F arterial and venous catheters (Bernstein Occlusion Catheter, Boston Scientific®, MA, USA) were inserted via the femoral or axillary artery and vein under fluoroscopic guidance with their tips placed at the level of the knee or elbow joint. Catheters were placed on the day of the procedure or the day before in case of an early ILI. No systemic heparin was administered during catheter placement. In eight patients, the catheters were inserted via the contra-lateral femoral vessels and in 13 patients they were inserted via the ipsilateral femoral or axillary vessels. After insertion of the catheters, low-dose heparin infusion through them was initiated and continued until full systemic heparinization immediately before the ILI procedure.

Limb volume was determined by taking circumferential limb measurements at 1.5 cm intervals as described by Beasley et al. [14]. A standard dose of 7.5 mg/L of melphalan and 75μg/L of actinomycin-D was used for both lower limb and upper limb ILIs, with dosage modification made according to the patient's ideal

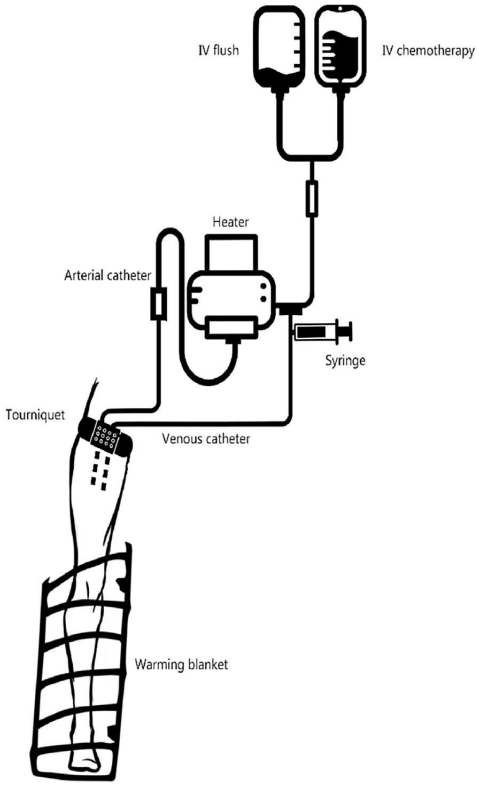

Fig. 1. Schematic diagram of the ILI circuit. Chemotherapy is rapidly infused using a pressurised circuit incorporating a blood-warmer with bubble excluder. Venous blood is manually extracted from the limb using a 60 ml syringe and reinjected into the isolated circuit.

body weight (IBW) [15,16]. The cytotoxic agents were admixed with 400 mL of heparinized normal saline. A proximal pneumatic tourniquet was applied once subcutaneous (SC) temperatures reached 37.0 °C, after which the chemotherapeutic drugs were infused into the limb over 5–10 min via the arterial catheter through a heating coil. Heating of the limb started before chemotherapeutic drug infusion and continued throughout the study with patient-warming systems. The infusate was then circulated manually for 20–25 min using a 60 mL syringe and 3-way stopcock. Needle probes were used to monitor the SC temperatures continuously, aiming for 38.5 °C by the end of the procedure. Upon completion of drug circulation, the limb was flushed with 1 L of Ringer's solution via the arterial catheter, while as much venous blood as possible was extracted from the limb via the venous catheter using suction attached to it, with the effluent discarded as cytotoxic waste. The tourniquet was then deflated and removed and heparin fully reversed with protamine 3 mg/kg.

Postoperatively, patients were monitored in the recovery unit for 2–3 h and subsequently transferred to the surgical ward. Limb toxicity was assessed by physical examination daily during the hospital stay and after 3 months using the scale proposed by Wieberdink et al. [17]. As a prophylactic measure, for the duration of their hospital stay, all patients received low-molecular-weight heparin (LMWH), 4000IU enoxaparin sodium SC, starting directly after the procedure until discharge. Post-procedure limb pain was

assessed using the visual analogue scale (VAS); myoglobin levels and limb circumference changes were monitored, to assess limb muscle toxicity and limb swelling.

Follow-up consisted of an outpatient clinic visit every 3 months, with assessment of response using the RECIST 1.1 guidelines for solid tumors [18]. The response to ILI was evaluated at 6 months. All patients underwent ^{18F}-FDG PET/CT imaging twice during the first year after ILI, and once yearly thereafter.

Data were analysed using descriptive statistics and the Mann Whitney *U* test. Kaplan–Meier curves were used to display overall survival. Data analysis was performed using JMP 10.0 (SAS®) and Microsoft Excel software (Microsoft ®).

Results

Patient details, tumour characteristics and perioperative parameters are listed in Table 1. A total of 21 patients underwent an ILI procedure, 18 of whom were female. The median age was 72 years (range 30–88 years). There were 19 lower limb ILIs performed and 2 upper limb ILIs. BOD was low in 7 patients (33%), and high in 14 patients (67%). The mean Breslow thickness of the primary melanoma was 7.5 mm (range 2.01–14.0 mm) and ulceration of the primary melanoma was present in 8 patients (42%) and 14 patients (67%) had a high mitotic rate.

In all but one patient a single ILI procedure was performed, while one female patient underwent a repeat ILI for recurrent upper limb ITMs two years after an initial ILI. On average, removal of the primary melanoma was performed 24 months (range 4–60 months) prior to the ILI procedure. In all patients, at least one attempt at surgical removal of one or more ITMs had been carried out before ILI was considered. Three patients (14%) underwent multiple surgical resections of ITMs with a median time to recurrence of 5 months (range 2–8 months).

Median limb volume for the lower limb was 10.7 L (range 5.7–12.9 L) and for the upper limb 2.6 L (range 2.5–2.7 L). The median melphalan dose for lower limb procedures was 46.6 mg (range 13.6–66 mg), and 16.3 mg (range 13.6–19.1 mg) for upper limb ILIs. For actinomycin-D the median dose was 490.1 μg (range 427.5–500 μg) and 237 μg (range 195–280 μg), respectively. Median hospital stay was 16 days (range 9–24 days). Limb toxicity was mild (Wieberdink grade II) in 13 patients (60%) and moderate (grade III) in six patients (30%). Two patients (10%) experienced grade IV limb toxicity: One patient underwent a fasciotomy and recovered fully. The second patient developed muscle necrosis of the superficial posterior tibial compartment, which was surgically debrided at the time of a fasciotomy.

Limb swelling, assessed by the change in circumference, reached its maximum on postoperative day 4. The median post-procedure peak myoglobin level was 1366 μg/L (range 67–6937 μg/L; normal range 19–72 μg/L). Patients with Wieberdink grade II toxicity had a significantly lower peak myoglobin (319 μg/L, range 67–2193 μg/L) compared to patients with who had Wieberdink grade III/IV toxicity (2202 μg/L, range 1430–6937 μg/L; $p = 0.001$). Myoglobin levels rose on post-procedure day 3 and peaked on day 5. Thirty-day mortality was 5%: one patient died on day 20 post-ILI due to pulmonary embolism.

Sixteen patients (76%) experienced an overall response (complete + partial response). A complete response was achieved in 8 patients (38%). Four of these patients (19%) developed new ITMs after a median of 18 months (range 6–30 months). A partial response was achieved in 8 patients (38%) and was sustained in one patient (5%), however this patient developed distant metastases and died 12 months after the ILI procedure. Five patients (24%) had either stable disease or progressive disease following ILI. Long-term limb salvage was achieved in 19 patients (90%). In two patients an amputation was carried out: In one a lower limb amputation was

Table 1
Patient and tumor characteristics and perioperative parameters.

Characteristic	Value		
Gender, n (%)			
Male	3 (14)		
Female	18 (86)		
Age in years, median (range)	72 (30–88)		
Involved limb, n (%)			
Lower	19 (90)		
Upper	2 (10)		
Limb volume in litres, median (range)	8.8 (2.6–13.0)		
Burden of disease, n (%)			
Low (<10 lesions and all < 2 cm)	7 (33)		
High (≥10 lesions or any ≥ 2 cm)	14 (67)		
Melphalan dose in mg, median (range)	46.6 (13.6–66)		
Actinomycin-D dose in μg, median (range)	450 (195–500)		
Drug circulation time in minutes, median (range)	28.5 (20–30)		
Tourniquet time in minutes, median (range)	58.4 (50–65)		
Length of hospital stay in days, median (range)	15.8 (9–24)		
Postoperative myoglobin peak value in μg/L, median (range)	1366 (67–6937)		
Myoglobin peak postoperative day, median (range)	4 (2–6)		
Perfusate blood gas, after 30 min of ischaemia	Overall (n = 21)	Upper Limb (n = 2)	Lower Limb (n = 19)
pH, median (range)	7.12 (7.03–7.32)	7.22 (7.12–7.32)	7.18 (7.03–7.32)
Base excess, median (range)	−10.39 (−5,3–20.60)	−6,4 (−4,4–8.4)	−10.77 (−5,3–20.60)
PaO_2 in mmHg, median (range)	19.69 (7.7–41.3)	18.0 (16–20)	19.76 (7.7–41.3)
Lactate mmol/L, median (range)	3.28 (1.88–8.29)	2.65 (2.56–2.73)	3.35 (1.88–8.29)
Wieberdink toxicity grade, n (%)			
I no visible effect	0		
II slight erythema/oedema	13 (60)		
III considerable erythema/oedema with blistering	6 (30)		
IV extensive epidermolysis/obvious damage to deep tissues with threatened or actual compartment syndrome	2 (10)		
V severe tissue damage necessitating amputation	0		

performed due to uncontrollable local recurrence causing pain 13 months after ILI, and in the other an amputation of the lower limb was carried out 24 months after ILI due to limb ischaemia. Median follow-up was 31.0 months (range 1–70 months). In total, four patients (19%) developed distant metastases during follow-up, two of whom were still alive at the time of data analysis. Five-year overall survival was 57% (Fig. 2). Thirteen patients (62%) were still alive at the time of analysis, 11 of them without evidence of active disease in the treated limb.

Discussion

This study demonstrates that ILI for melanoma ITMs can safely and effectively be performed in a tertiary referral centre in Eastern Europe. The results achieved were comparable to those reported by high-volume centres in Australia and the USA, with a long-term limb salvage rate of 90% and acceptable locoregional toxicity [5,19].

Multiple treatment options are available for melanoma ITMs. The US National Comprehensive Cancer Network guidelines list surgical excision, ILI, ILP, intra-lesional therapies, local ablation therapies, radiotherapy and systemic treatments all as appropriate treatment options for these patients [20]. Surgical excision is a reasonable procedure, and can be performed repeatedly when lesions are relatively small and limited in number. If surgical excision can provide satisfactory disease control, often no further treatment is necessary. However, it has been reported that over 30% of patients have another local recurrence and 23% develop distant metastases [21]. Options for local treatment include intra-lesional injection of Bacille Calmette-Guerin (BCG), interleukin 2 (IL-2), talimogene laherparepvec (T-VEC), Darleukin (L19IL2), Daromun, PV-10 (rose bengal), and electrochemotherapy (ECT) [22,23].

Patient selection is important when starting a ILI program. In previous studies, for instance, it has been shown that on average ILI is applied to older patients than ILP [24,25]. In this regard, the median age of 72 years in our series is comparable to that reported in previous ILI studies, reflecting the fact that we selected patients similarly to high-volume centres in Australia and the USA [9]. Furthermore, our patients had a Breslow thickness of the primary melanoma (7.5 mm), ulceration (42%), high mitotic rate (67%), and high BOD (67%) also comparable to previous ILI series [5,9,26]. Since the abovementioned melanoma features are prognostic factors for a worse outcome following ILI, our complete response rate of 38% and overall response rate of 76% are comparable to those reported by larger ILI series, as they relate to similar patients [5,9].

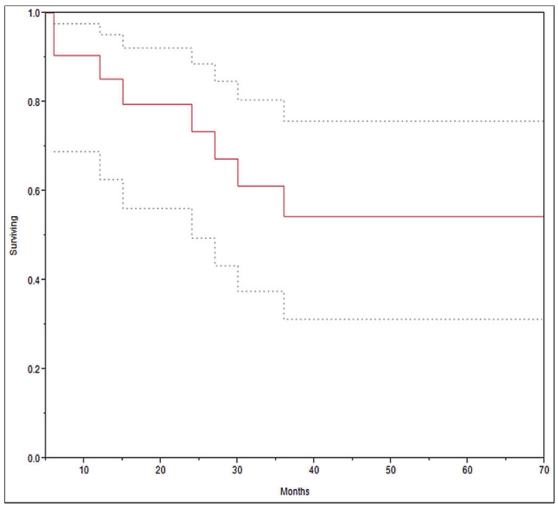

Fig. 2. Overall survival (months) following isolated limb infusion for the complete cohort.

According to the MSKCC ILI protocol, we used the same dosages of melphalan (7.5 mg/L) and actinomycin-D (75μg/L) for upper and lower limb procedures, and corrected both for IBW. Although compared to Australian ILI series this resulted in substantially reduced cytotoxic drug doses in the upper limb procedures, a complete response was achieved in both patients in our series [9,27]. However, we will need more experience in order to draw any firm conclusions.

In our experience, the ILI procedure was well-tolerated, with mild to moderate limb toxicity in most patients (grade II and III). However, two patients suffered grade IV limb toxicity, both requiring a fasciotomy, one of whom also requiring surgical debridement of necrotic muscle in the superficial posterior tibial compartment. As in previous reports from high-volume ILI centres, no patient experienced limb toxicity necessitating amputation, however, one patient died following ILI due to a pulmonary embolism despite the use of LMWH prophylaxis [9,28]. In comparison, following ILP, toxicity-induced amputations have been reported, and systemic side-effects are regularly observed due to leakage into the systemic circulation because of the high vascular pressure in the isolated limb circuit [6,29].

In recent years, immunotherapy and checkpoint inhibitor therapy have dramatically changed the treatment landscape for metastatic melanoma. Their role in patients with melanoma ITMs, however, remains unclear. Most trials of these therapies have included only small numbers of patients with ITMs or none at all [30–33]. Therefore, to date none of these new agents has been shown to be as effective for ITMs as ILI, especially in patients with bulky disease or when numerous lesions are present [26,34,35]. Furthermore, the systemic therapies can sometimes result in severe systemic side-effects, something not experienced by patients after ILI. Finally, around the world, there are large discrepancies in access to these new and costly drugs, largely due to economic differences between countries and available healthcare funds [7]. In contrast, the drugs for ILI, melphalan and actinomycin-D, are readily available and not unduly expensive. Therefore, there is still a place for ILI in the treatment of ITMs in this modern era.

Some limitations of this single-centre retrospective analysis have to be addressed. The small number of patients does not allow us to make conclusions on response rates and survival outcomes. Also, we followed the MSKCC ILI protocol, resulting in lower melphalan and actinomycin-D dosage and shorter drug circulation times compared to the Australian centres [36]. This could potentially have negatively impacted response to ILI. In previous ILI studies, serum creatine phosphokinase levels have been measured to indicate the degree of limb toxicity [9,14]. For practical reasons, we used serum myoglobin levels in the current study for the same purpose and found, in contrast to a recent ILP publication, a significant correlation between a high post-ILI serum myoglobin and increased Wieberdink toxicity grades [37,38]. Lastly, the current series includes the learning curve of mastering the ILI procedure at our institution. Despite these limitations, however, we feel that the current study does provide a realistic overview of the possibility of ILI treatment in Eastern Europe.

In conclusion: In this first series of ILI for melanoma ITMs from an Eastern European country, we have shown that ILI can be safely and effectively implemented outside of high-volume centres in the USA and Australia. In the Estonian healthcare system, ILI offers the advantage of being a relatively cheap and simple method with low morbidity.

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Declaration of competing interest

None.

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Publication III

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ORIGINAL ARTICLE – MELANOMA

International Multicenter Experience of Isolated Limb Infusion for In-Transit Melanoma Metastases in Octogenarian and Nonagenarian Patients

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ABSTRACT

Background. Isolated limb infusion (ILI) is used to treat in-transit melanoma metastases confined to an extremity. However, little is known about its safety and efficacy in octogenarians and nonagenarians (ON).

Patients and Methods. ON patients (≥ 80 years) who underwent a first ILI for American Joint Committee on Cancer seventh edition stage IIIB/IIIC melanoma between 1992 and 2018 at nine international centers were included and compared with younger patients (< 80 years). A cytotoxic drug combination of melphalan and actinomycin-D was used.

Results. Of the 687 patients undergoing a first ILI, 160 were ON patients (median age 84 years; range 80–100 years). Compared with the younger cohort (n = 527; median age 67 years; range 29–79 years), ON patients were more frequently female (70.0% vs. 56.9%; p = 0.003), had more stage IIIB disease (63.8 vs. 53.3%;

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$p = 0.02$), and underwent more upper limb ILIs (16.9% vs. 9.5%; $p = 0.009$). ON patients experienced similar Wieberdink limb toxicity grades III/IV (25.0% vs. 29.2%; $p = 0.45$). No toxicity-related limb amputations were performed. Overall response for ON patients was 67.3%, versus 64.6% for younger patients ($p = 0.53$). Median in-field progression-free survival was 9 months for both groups ($p = 0.88$). Median distant progression-free survival was 36 versus 23 months ($p = 0.16$), overall survival was 29 versus 40 months ($p < 0.0001$), and melanoma-specific survival was 46 versus 78 months ($p = 0.0007$) for ON patients compared with younger patients, respectively.

Conclusions. ILI in ON patients is safe and effective with similar response and regional control rates compared with younger patients. However, overall and melanoma-specific survival are shorter.

For several decades, the incidence of melanoma around the world has been increasing steadily.[1] This increase has been proportionally greater in the elderly and is expected to continue to rise in this age group.[2,3] Melanoma can be effectively treated in most patients, however, when in-transit metastases (ITMs) develop, distant metastases are more likely to occur, with an associated decrease in 5-year survival.[4] Moreover, ITMs are often associated with pain, ulceration and bleeding, restricted mobility, and reduced quality of life.[5]

With the recent introduction of effective immunotherapy, the therapeutic landscape for advanced melanoma has changed dramatically. However, elderly octogenarian and nonagenarian (ON; $\geq$ 80 years old) patients present a treatment challenge. This is particularly the case when ITMs develop in a limb and are too numerous or bulky for surgical excision, since systemic therapy is normally not considered in ON patients due to a larger incidence of serious medical comorbidities and potential side-effects that may be poorly tolerated in older patients.[6] Alternative treatment options are therefore of importance for this growing patient group and should preferably be noninvasive or minimally invasive in nature, provide durable responses, cause limited locoregional toxicity, and avoid systemic side-effects.

In view of this, isolated limb infusion (ILI) is a potentially suitable treatment for ON patients as it provides durable results and causes limited locoregional toxicity. However, little is known about its efficacy and safety in ON patients.[7,8] To evaluate this, the present multicenter study of ILI in ON patients was conducted.

PATIENTS AND METHODS

The outcomes of ILI in ON patients ($\geq$ 80 years of age) performed at the five Australian and four US participating centers were compared with those in younger patients ($<$ 80 years). All patients who underwent a first-time ILI between 1992 and 2018 at one of the nine centers were identified. Patients who had melanoma ITMs confined to a limb without distant metastatic disease were included, i.e., American Joint Committee on Cancer (AJCC) seventh edition stage IIIB or IIIC disease.[4] Previous treatments [isolated limb perfusion (ILP), intralesional therapy, surgical resection or systemic therapy] prior to the first ILI were not considered as exclusion criteria. Ethics approval was obtained from each institutional ethics research committee.

All participating centers had demonstrated proficiency with the technical aspects of ILI, and the procedure was performed as previously described.[7,9] The two patient groups, viz. ON patients and the younger group, were treated using the same ILI protocol. A cytotoxic drug combination of melphalan (7.5 mg/L for lower extremities and 10 mg/L for upper extremities) and actinomycin-D (75 μg/L for lower extremities and 100 μg/L for upper extremities) was used. The maximum melphalan dose was 100 mg for lower extremities and 50 mg for upper extremities. The US centers routinely corrected the melphalan dose for ideal body weight (IBW).[10] If inguinal or axillary node metastases were present at the time of ILI, a lymph node dissection was performed under the same general anesthetic. Postprocedure, patients were monitored by physical examination and daily measurement of serum creatine phosphokinase (CK) levels. The Wieberdink scale was used to assess limb toxicity.[11]

At the US centers, treatment response was determined 3 months after ILI, according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.[12] The Australian centers determined response using the World Health Organization (WHO) criteria for reporting results of cancer treatment, capturing the best response at two observations more than 4 weeks apart.[13] Additional locoregional therapy (repeat ILI, surgery, or intralesional injections) or systemic therapy (chemotherapy, immunotherapy, or targeted therapy) was given at the discretion of the treating multidisciplinary team if disease recurrence or progression occurred.

Clinicopathological data collected were age, gender, stage of disease, involved extremity (upper/lower), burden of disease (BOD), and Breslow thickness of primary melanoma. BOD was considered low if there were $<$ 10 lesions with all $<$ 2 cm in diameter. BOD was considered high if there were $\geq$ 10 lesions or if any lesion was $\geq$ 2 cm in diameter.

TABLE 1 Patient and tumor characteristics

Characteristic	< 80 years (n = 527)	≥ 80 years (n = 160)	p value
Gender, n (%)			
Male	227 (43.1)	48 (30.0)	**0.003**
Female	300 (56.9)	112 (70.0)	
Age in years, median (range)	67 (29–79)	84 (80–100)	N/A
Involved limb, n (%)			
Upper limb	50 (9.5)	27 (16.9)	**0.009**
Lower limb	477 (90.5)	133 (83.1)	
Stage of disease at time of ILI, n (%)[4]			
IIIB	281 (53.3)	102 (63.8)	**0.02**
IIIC	246 (46.7)	58 (36.2)	
Burden of disease, n (%)			
High	235 (44.8)	78 (49.1)	0.34
Low	290 (55.2)[a]	81 (50.9)[b]	
Breslow thickness of primary melanoma in mm, median (range)	2.6 (0.3–18.0)[c]	3.0 (0.2–12.0)[d]	0.15
Country of treatment, n (%)			
USA	231 (43.8)	45 (28.1)	**0.0004**
AUS	296 (56.2)	115 (71.9)	
Treatment period, n (%)			
1992–2009	111 (69)	335 (33)	0.19
2010–2018	49 (31)	192 (36)	

Statistically significant p values are given in bold

ILI isolated limb infusion, *N/A* not applicable

[a]2 patients missing

[b]1 patient missing

[c]76 patients missing

[d]31 patients missing

Perioperative data collected were limb volume, melphalan and actinomycin-D doses, tourniquet time, limb temperatures, pH of the perfusate, postoperative serum CK levels, Wieberdink limb toxicity grade, and length of hospital stay (LOS). Outcomes of interest were response to treatment, in-field progression-free survival (PFS; defined as recurrent or progressive melanoma in the affected limb or related nodal basin), distant PFS (defined as melanoma spread beyond the nodal basin of the affected limb), overall survival (OS), and melanoma-specific survival (MSS; defined as death due to melanoma), all calculated from time of ILI.

Continuous variables are presented as medians (with range) and categorical variables as frequencies. The Chi squared test was used to compare frequency distributions, and the Mann–Whitney *U*-test for nonparametric variables. Kaplan–Meier curves with log-rank tests were used to display and compare survival between groups. Multinominal logistic regression and Cox proportional hazard models with stepwise backward methods were used for multivariate analyses of response and survival. The variables entered in the multivariate analyses models were gender, extremity, limb volume, Breslow thickness, burden of disease, melphalan dose in mg, melphalan dose in mg/L, circulation time in minutes, ischemia time, country, Wieberdink toxicity, disease stage (IIIB/IIC), and response to ILI [complete response (CR), partial response (PR), stable disease (SD) versus progressive disease (PD)]. Within the multivariate model, response was defined as a multilevel measure. Alpha was set at 0.05. Statistical analyses were performed using GraphPad Prism version 8.0.2 software (GraphPad Software Inc., San Diego, CA) and SPSS software version 25.0.0 (SPSS Inc., Chicago, IL).

RESULTS

In total, 687 patients underwent a first ILI at one of the nine institutions between 1992 and 2018. The ON group included 160 patients (23.3%) and the younger group 527 patients (76.7%). Median age was 84 years for the ON patients and 67 years for the younger patients. There were more women in the ON group (70.0% vs. 56.9% in the younger group; p = 0.003), and more upper limb ILIs were

TABLE 2 Intra- and postoperative data

Characteristic	< 80 years (n = 527)	≥ 80 years (n = 160)	p-value
Limb volume in liters, median (range)	7.0 (0.5–17.7)[a]	5.4 (1.4–11.0)[b]	< **0.0001**
Lower limb	7.3 (1.9–15.5)	5.7 (2.8–11.0)	< **0.0001**
Upper limb	2.5 (0.5–6.45)	2.4 (1.4–7.7)	0.52
Melphalan dose in mg, median (range)	45.0 (12.0–100.0)[c]	36.5 (8.5–100.0)[d]	< **0.0001**
Lower limb	46.9 (25.0–100.0)	40.0 (22.5–100.0)	< **0.0001**
Upper limb	20.0 (12.0–50)	20.5 (8.5–50.0)	0.40
Melphalan dose in mg/L, median (range)	6.7 (2.2–27.8)	7.5 (2.7–25.0)	< **0.0001**
Lower limb	6.7 (2.2–27.8)	7.5 (4.1–20.0)	**0.001**
Upper limb	8.2 (4.2–27.8)	9.2 (2.7–25.0)	0.39
Actinomycin-D dose in µg, median (range)	500 (125–900)[e]	395 (140–800)[f]	< **0.0001**
Lower limb	500 (200–900)	400 (225–800)	< **0.0001**
Upper limb	220 (125–450)	235 (140–500)	0.36
Actinomycin-D dose in µg/L, median (range)	75 (22–167)	78 (27–250)	**0.007**
Lower limb	75 (22–167)	75 (41–143)	0.44
Upper limb	98 (48–160)	100 (27–250)	0.40
Drug circulation time in minutes, median (range)	30 (3–42)	30 (12–43)	0.87
Tourniquet time in minutes, median (range)	54 (20–120)[g]	54 (25–101)[h]	0.74
Limb temperature initial in °C, median (range)	37.0 (32.4–41.0)[i]	37.2 (28.5–40.2)[j]	0.38
Limb temperature final in °C, median (range)	38.4 (30.5–41.5)[k]	38.4 (26.9–41.1)[l]	0.80
pH at 30 min of ILI, median (range)	7.17 (6.88–7.38)[m]	7.14 (6.86–7.72)[n]	**0.004**
Postoperative CK peak in IU/L, median (range)	675 (21–56,540)[o]	315 (20–19,417)[p]	**0.001**
CK peak postoperative day, median (range)	4 (0–8)[q]	4 (1–9)[r]	0.52
Wieberdink toxicity score, n (%)			
I No visible effect	57 (11.0)	19 (11.9)	0.45
II Slight erythema and/or edema	311 (59.8)	100 (62.5)	
III Considerable erythema and/or edema with blistering	128 (24.6)	37 (23.1)	
IV Extensive epidermolysis and/or obvious damage to deep tissue with a threatened or actual compartment syndrome	24 (4.6)	3 (1.9)	
V Severe tissue damage necessitating amputation	0[s]	0[t]	
Length of hospital stay in days, median (range)	6.5 (2–49)[u]	7.5 (3–54)[v]	**0.007**

Statistically significant p values are given in bold

ILI isolated limb infusion, *CK* creatine phosphokinase

[a]34 patients missing

[b]8 patients missing

[c]3 patients missing

[d]1 patient missing

[e]86 patients missing

[f]30 patients missing

[g]7 patients missing

[h]1 patient missing

[i]149 patients missing

[j]32 patients missing

[k]38 patients missing

[l]18 patients missing

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[n]59 patients missing

[o]56 patients missing

[p]20 patients missing

[q]68 patients missing

[r]25 patients missing

[s]7 patients missing

[t]1 patient missing

[u]10 patients missing

[v]6 patients missing

TABLE 3 Clinical outcomes

Characteristic	< 80 years (n = 527)	≥ 80 years (n = 160)	p-value
Treatment response, n (%)			
Complete response	159 (30.5)	41 (26.3)	**0.047**
Partial response	178 (34.1)	64 (41.0)	
Stable disease	71 (13.6)	29 (18.6)	
Progressive disease	114 (21.8)[a]	22 (14.1)[b]	
Overall response (combined complete and partial response)	337 (64.6)	105 (67.3)	0.53
Resection of residual disease after ILI, n (%)	106 (21.2)[c]	30 (18.7)	0.71
Time to resection of residual disease in months, median (range)	5 (0–38)	6 (2–22)	0.21

Statistically significant p value is given in bold

[a]5 patients missing

[b]4 patients missing

[c]21 patients missing

performed (16.9% vs. 9.5%; $p = 0.009$). ON patients had less stage IIIC disease (36.2% vs. 46.7% for younger patients; $p = 0.020$). More ON patients were treated in Australia (71.9% vs. 28.1% for patients treated in the USA; $p = 0.0004$; Table 1). There was no difference in the proportion of ON patients compared with the younger patients treated in the earlier treatment period (1992–2009) and the more recent treatment period during which systemic therapies became available (2010–2018; $p = 0.19$).

ON patients had significantly lower limb volumes compared with younger patients (median 5.7 vs. 7.3 L; $p < 0.0001$; Table 2), but upper limb volumes were similar (median 2.4 vs. 2.5 L; $p = 0.52$). ON patients received a lower total melphalan dose (median 36.5 vs. 45.0 mg; $p < 0.0001$) but a higher melphalan dose per liter of infused tissue compared with younger patients (median 7.5 vs. 6.7 mg/L; $p = 0.001$). Similarly, a lower total actinomycin-D dose was administered to ON patients (median 395 vs. 500 μg; $p < 0.0001$), but they received a higher actinomycin-D dose per liter infused tissue (median 78 vs. 75 μg/L; $p = 0.007$). ON patients had a significantly lower pH in limb blood compared with younger patients after 30 min of ILI (median 7.14 vs. 7.17; $p = 0.004$). Other intraoperative measurements were similar between both groups.

Postoperative peak CK was significantly lower for ON patients compared with younger patients (median 315 vs. 675 IU/L; $p = 0.001$); this occurred at a median of 4 days postoperatively in both groups. Wieberdink toxicity grades were similar, with the majority of patients experiencing grade I/II toxicity (74.4% for ON patients vs. 70.8% for younger patients), while more grade IV limb toxicity was experienced by younger patients (1.9% vs. 4.6%, respectively), although this difference was not significant ($p = 0.45$). No grade V limb toxicity or severe systemic side-effects were experienced in either group. Finally, LOS was longer for ON patients (median 7.5 vs. 6.5 days; $p = 0.007$).

The overall response rate (OR: CR + PR) was 67.3% in ON patients, which was similar to the 64.6% of the younger group ($p = 0.53$; Table 3). Fewer ON patients achieved CR (26.3% vs. 30.5% for the younger patients) while more experienced PR (41.0% vs. 34.1%, respectively; $p = 0.047$). Resection of remaining lesions after ILI was performed with equal frequency in the two groups ($p = 0.71$).

Median follow-up was 92 versus 78 months for ON patients and the younger group, respectively ($p = 0.68$). Both groups had a median in-field PFS of 9 months ($p = 0.88$; Fig. 1a). In the younger group, 89 (16.8%) underwent a repeat ILI for in-field recurrence or progressive disease, while none of the ON patients underwent a repeat ILI. Median distant PFS for ON patients was 37 months, and 23 months for younger patients ($p = 0.16$; Fig. 1b). ON patients showed a median OS of 29 months compared with 40 months in the younger group ($p < 0.0001$; Fig. 1c), and MSS rates were 46 and 78 months, respectively ($p = 0.0007$; Fig. 1d). For ON patients specifically, median in-field PFS was 35 months after a CR, and 11 months after a PR ($p < 0.0001$; Fig. 2a). Median distant PFS was also significantly longer after a CR (not reached vs. 41 months after a PR; $p = 0.0004$; Fig. 2b). Median OS after a CR was 41 months, which was not significantly different to the 31 months after a PR ($p = 0.38$; Fig. 2c), while median MSS following a CR was significantly longer compared with a PR (74 vs. 44 months, respectively; $p = 0.01$; Fig. 2d).

Table 4 lists the summarized results of the multivariate analyses for ON patients. Lower Breslow thickness and female gender remained significant predictors for CR,

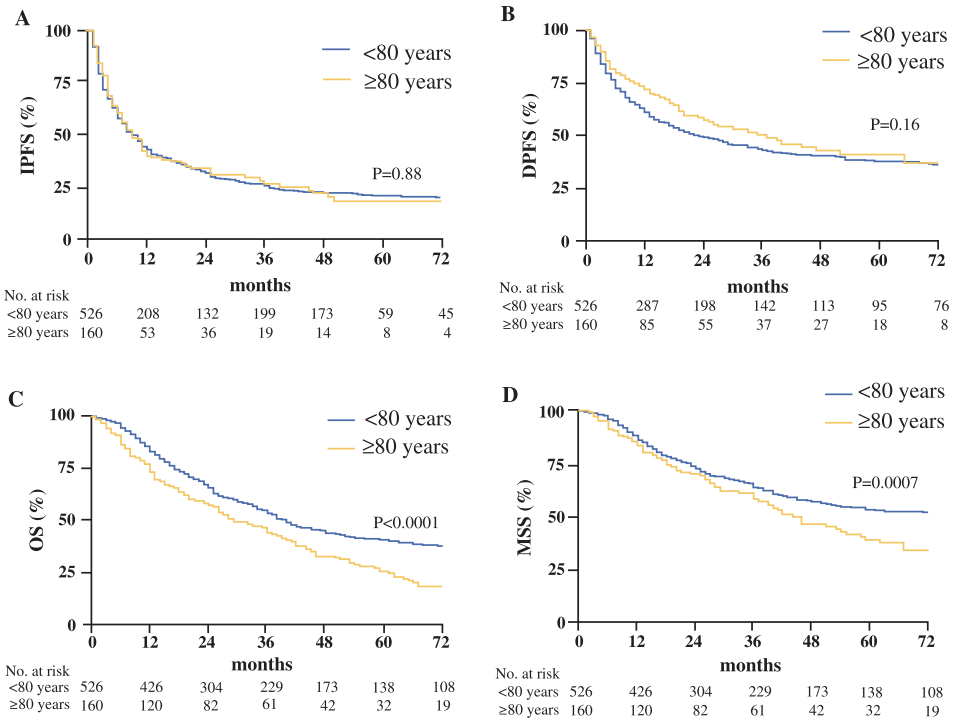

FIG. 1 **a** In-field progression-free survival (IPFS, $p = 0.88$), **b** distant progression-free survival (DPFS, $p = 0.16$), **c** overall survival (OS, $p < 0.0001$), and **d** melanoma-specific survival (MSS, $p = 0.0007$) for octogenarian–nonagenarian patients (≥ 80 years; yellow line) compared with younger patients (< 80 years; blue line)

while lower BOD remained a predictor for OR. CR and PR to ILI were predictors for increased in-field PFS, and lower stage of disease and female gender for increased distant PFS. Disease-free survival (DFS) was increased for ON patients with lower stage of disease and after a CR. Increased OS was observed for patients treated in the USA, those with lower stages of disease, and after CR or PR to ILI. MSS was significantly improved after a CR or PR to ILI. The summarized results of the multivariate analysis of the younger patient group are listed in Table 5. The complete results of the multivariate analyses are listed in Appendices A and B.

DISCUSSION

This first study specifically focusing on ILI in elderly patients (≥ 80 years) shows that the procedure is safe and results in similar OR and in-field and distant PFS rates when compared with younger patients in an international multicenter cohort. MSS and OS rates following ILI, however, are lower for ON patients.

In the present study, ON patients treated by ILI presented different baseline characteristics compared with the more frequently reported for younger population. The ON patient group included more female patients, had lower stages of disease, and suffered more frequently from upper limb disease. Some previous ILI and ILP studies have focused on elderly patients (> 70 or > 75 years of age), but no analyses specifically in patients ≥ 80 years of age have been carried out to date.[14–16] Similarly, these previous studies of elderly patients have included more females with lower stages of disease but have not identified a difference between upper and lower limb procedures. This is an important finding, as it has been shown that patients undergoing upper limb ILI are less likely to achieve a favorable response.[17]

Interestingly, more ON patients underwent ILI in Australia compared with the USA. A possible explanation for this could be that the higher average life expectancy in Australia resulted in more fit ON patients being considered for ILI.[18] In contrast, US ON patients showed an increased OS in the multivariate analysis, which could indicate stricter patient selection.

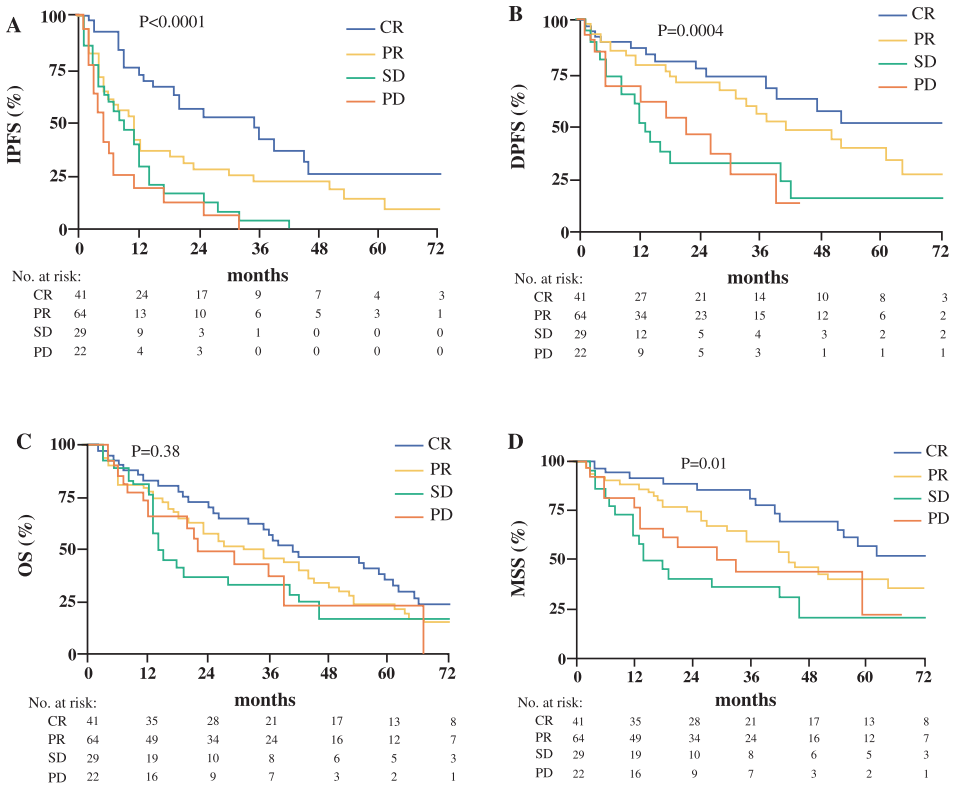

FIG. 2 **a** In-field progression-free survival (IPFS, $p < 0.0001$), **b** distant progression-free survival (DPFS, $p = 0.0004$), **c** overall survival (OS, $p = 0.38$), and **d** melanoma-specific survival (MSS, $p = 0.01$) for octogenarian–nonagenarian patients (≥ 80 years) stratified by treatment response: *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Despite the fact that ON patients had lower CR rates, possibly due to a decline in functional immunity and changes in tumor biology at advanced ages,[19,20] they did show higher PR and OR rates compared with younger patients, showing that successful ILI procedures can be performed in the "extremely" old age group. Furthermore, ILI in ON patients was safe, with only three patients experiencing grade IV toxicity and none developing grade V limb toxicity, despite the higher dosages per liter of infused issue of both melphalan and actinomycin-D in the ON patients.

In contrast to previous ILI and ILP studies in the elderly, we found worse MSS and OS in ON patients, indicating the differences between "elderly" and "extremely old" ON patients.[8,14–16] The worse MSS and OS may be the result of reduced function of the immune system in this age group, as suggested above, but could also be the result of less aggressive treatment in ON patients with recurrent or progressive disease. In immunotherapy studies, for example, ON patients have been less frequently selected to undergo systemic therapy for distant metastatic disease.[6,21] Unfortunately, due to the retrospective nature of this study, we were unable to retrieve accurate information about systemic treatment following ILI in our cohort, but effective therapies only became available in the latter part of the study period. Since the proportion of ON patients remained similar in the earlier treatment period (1992–2009) and the more recent treatment period when systemic therapies became available (2010–2018), we could not identify a shift away from ILI in either cohort.

The US National Comprehensive Cancer Network guidelines list surgical excision, regional chemotherapy (ILI or ILP), and systemic agents as appropriate treatment possibilities for melanoma ITM treatment.[22] Other options include intralesional agents such as Bacille Calmette-Guérin (BCG), interleukin 2 (IL-2), talimogene laherparepvec (T-VEC), Darleukin (L19IL2), Daromun, PV-10 (rose bengal), and electrochemotherapy (ECT).[23–26] However, reports documenting the use of these agents have mostly been of small series, without experience in patients

TABLE 4 Multivariate analysis of octogenarian and nonagenarian (≥ 80 year old) patient group

Endpoint–variable	*p*-value	Odds ratio	95% CI
Complete response			
Breslow thickness	0.049	1.261	1.001–1.589
Gender: female	0.050	2.989	1.002–8.916
Overall response			
Burden of disease	0.037	0.365	0.142–0.939
Endpoint–variable	*p*-value	Hazard ratio	95% CI
In-field progression-free survival			
Complete response	< 0.0001	0.095	0.038–0.238
Partial response	< 0.0001	0.136	0.054–0.343
Distant progression-free survival			
Stage IIIB	0.003	0.315	0.149–0.667
Gender: female	0.019	2.503	1.164–5.379
Disease-free survival			
Stage IIIB	0.008	0.402	0.206–0.787
Complete response	0.005	0.189	0.060–0.597
Overall survival			
Country: USA	0.017	2.706	1.190–6.149
Stage IIIB	0.002	0.414	0.240–0.715
Complete response	0.001	0.221	0.094–0.522
Partial response	0.007	0.302	0.126–0.726
Melanoma-specific survival			
Complete response	< 0.0001	0.142	0.063–0.320
Partial response	< 0.0001	0.193	0.085–0.440

CI confidence interval

TABLE 5 Multivariate analysis of younger (< 80 year old) patient group

Endpoint–variable	*p*-value	Odds ratio	95% CI
Complete response			
Stage IIIB	0.033	0.583	0.355–0.958
Burden of disease	0.012	1.863	1.148–3.025
Melphalan dose	0.009	1.042	1.010–1.075
Overall response			
Country: USA	0.001	0.342	0.177–0.661
Extremity: lower	0.002	0.187	0.660–0.532
Stage IIIB	0.004	0.483	0.292–0.797
Breslow thickness	0.007	1.321	0.813–2.146
Circulation time	0.010	0.932	0.883–0.983
Ischemic time	0.019	1.023	1.004–1.042
Endpoint–variable	*p*-value	Hazard ratio	95% CI
In-field progression-free survival			
Complete response	< 0.0001	0.059	0.038–0.092
Partial response	< 0.0001	0.109	0.071–0.166
Stable disease	< 0.0001	0.282	0.183–0.434
Distant progression-free survival			
Stage IIIB	< 0.0001	0.573	0.424–0.773
Ischemic time	0.023	1.012	1.002–1.022
Complete response	< 0.0001	0.295	0.192–0.454
Partial response	< 0.0001	0.581	0.388–0.870
Disease-free survival			
Stage IIIB	0.002	0.678	0.529–0.869
Complete response	< 0.0001	2.145	1.568–2.934
Partial response	< 0.0001	4.050	2.713–6.046
Stable disease	< 0.0001	12.558	8.318–18.961
Overall survival			
Country: USA	< 0.0001	2.328	1.562–3.468
Stage IIIB	< 0.0001	0.561	0.423–0.744
Burden of disease	0.046	1.130	1.005–1.709
Breslow thickness	0.025	1.057	1.007–1.109
Complete response	< 0.0001	2.142	1.529–3.001
Partial response	< 0.0001	2.940	1.904–4.541
Stable disease	< 0.0001	4.403	2.878–6.735
Melanoma-specific survival			
Country: USA	< 0.0001	3.589	2.195–5.869
Stage IIIB	0.005	0.618	0.442–0.865
Burden of disease	0.004	1.584	1.154–2.174
Complete response	< 0.0001	0.207	0.302–0.790
Partial response	< 0.0001	0.489	0.451–1.311

CI confidence interval

with a high BOD and not evaluating them specifically in ON patients. Furthermore, in ILP studies, the median age is normally lower compared with ILI reports, which may be due to more restrictive patient selection for ILP in view of its invasive nature and the greater risk of postoperative morbidity.[14–16,27,28] Interestingly, however, despite the similar in-field PFS observed in the two age groups in the current study, none of the ON patients underwent a repeated ILI.

Although the present study represents the largest ILI series to date reporting results in ON patients, some limitations must be addressed. Firstly, there was likely a bias in selecting eligible ON patients to undergo ILI, meaning that those who were expected to be able to tolerate the procedure were selected for treatment. The results of the multivariate analysis of the current study may help clinicians to better select patients who may benefit from ILI. The retrospective nature of the study is another limitation. As mentioned above, details of previous and subsequent treatments, systemic therapies in particular, could not be captured accurately. Finally, minor protocol variations between participating institutions and differences in response reporting guidelines used between the USA and Australia existed.

In the future, the treatment of melanoma ITMs is likely to continue to evolve, with the introduction of more effective locoregional treatment alternatives and new systemic immunotherapy agents. An interesting approach for ON patients would be to combine ILI with intralesional injection of ITMs with T-VEC or PV-10, agents unlikely to result in systemic side-effects.[26,29,30] The role of systemic immunotherapy in patients with ITMs remains to be explored as most immunotherapy trials have included only small numbers of patients, making it difficult to compare the results with those achieved by ILI.[6,21,31] Furthermore, systemic immunotherapy can sometimes result in severe, occasionally even fatal, side-effects, making clinicians reluctant to administer these systematic therapies to ON patients, whereas systemic side-effects after ILI are rarely observed.[7,32,33] Combination therapy of ILI with systemic immunotherapy has been reported and may become a useful treatment option, however, despite durable responses observed after combining ILI with systemic CTLA-4 blockade, a large proportion of the patients (38%) experienced grade 3–4 systemic adverse events.[34,35]

In conclusion, this multicenter study shows that ILI for melanoma ITMs in ON patients is safe and effective, with comparable responses and disease control rates to younger patients. Overall and melanoma-specific survival are shorter. A favorable response after ILI in ON patients is associated with improved long-term oncological outcomes. In this era of ongoing developments in immunotherapy for melanoma, ILI remains an important treatment option for appropriately selected patients with ITMs in view of its low locoregional toxicity and long-term efficacy, especially in octogenarians and nonagenarians.

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Publication IV

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ASO AUTHOR REFLECTIONS

ASO Author Reflection: Isolated Limb Infusion for Locally Advanced Melanoma in the Extremely Old Patient is Safe and Effective

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PAST

When melanoma in-transit metastases (ITMs) develop, distant metastases are more likely to occur, with an associated decrease in 5-year survival. If located in a limb, most ITMs can be treated by surgical excision or intralesional injection with, for instance, PV-10 or T-VEC, but when ITMs are large and bulky, treatment can be challenging.[1] In the last decade, much more efficacious systemic treatment options for metastatic melanoma have been approved and made available. However, the efficacy of these therapies specifically for ITMs is unknown. Furthermore, octogenarian and nonagenarian (ON) patients may not be offered systemic therapies for fear of intolerable systemic side effects. Isolated limb infusion (ILI) is an effective treatment for ITMs, providing an overall response in 43 to 84% of cases.[2] Due to its locoregional and minimally invasive nature, systemic side effects are negligible, and complications are rare. However, not much is known about its safety and efficacy for ON patients, a growing group of patients in an aging population and increasing incidence of melanoma.

PRESENT

The current international multi-center study presents the outcomes of ILI for a cohort of 160 ON patients with a median age of 84 years compared with the outcomes for a younger cohort of 527 patients with a median age of 67 years.[3] Limb toxicity was mild to moderate in both groups, with severe limb toxicity (Wieberdink grade 4) observed in three ON patients (1.9%). No toxicity-related limb amputations (grade 5) were performed. The overall response for ON patients was 67.3% compared with 64.6% for younger patients ($p = 0.53$). The in-field progression-free survival was 9 months for both groups ($p = 0.88$), and distant progression-free survival was 36 and 23 months, respectively ($p = 0.16$). Overall survival for the ON patients was 29 versus 40 months for the younger patients ($p < 0.0001$), and melanoma-specific survival was 46 versus 78 months, respectively ($p = 0.0007$). Therefore, ILI for ON patients is safe and effective, with response and regional control rates similar to those of younger patients. However, overall and melanoma-specific survival are shorter.

FUTURE

The results indicate that ILI is suitable for treatment of ON patients with melanoma ITMs. In the future, treatment for melanoma ITMs likely will continue to evolve, with the introduction of more effective locoregional treatments and new systemic immunotherapy agents. A future aim is to identify those ON patients who will respond to regional therapy, for instance, using tumor gene expression

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profiling. This will allow clinicians to direct ILI or other regional methods to those likely to benefit from it while offering other treatment methods such as systemic therapy or combination treatments to others. One interesting approach for ON patients would be to combine ILI with intralesional injection of T-VEC or PV-10, agents unlikely to cause systemic side effects.[1,4] Also, therapy combining ILI with systemic immunotherapy is reported to be highly effective; albeit a grade 3 or 4 systemic adverse event rate of 38% has been reported for ILI combined with anti-CTLA-4.[5] These combination strategies need further investigation.

DISCLOSURE Jonathan S. Zager has a patent for high-flow isolated limb infusion.

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2014	Observership in Memorial Sloan Kettering Cancer Center, Supervisor dr Daniel Coit, NY, USA
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2010–2011	Effective Health Care Resources Management, Stockholm School of Economics Riga Executive Education, Riga, Latvia
2007	Observership Hospital Militar 1 Lomas de Sotelo, Mexico City, Supervisor mjr Luiz Garcia, Mexico
2005	American College of Surgeons (ACS) International Travel Scholarship MD Anderson Cancer Centre Houston, Texas Mount Sinai Hospital New York, University of Medicine and Dentistry of New Jersey

4. Current Occupation:

Chief of General and Oncological Surgical Centre North Estonia Medical Centre, Tallinn, Estonia
European Union of Medical Specialist (UEMS) section surgery – national representative

5. **Humanitarian work:**

2010–2020	Humanitarian missions in Africa and Asia

6. **Scientific work:**

My scientific work has been focused on inflammatory processes of soft tissue and abdominal cavity; soft tissue, abdominal cavity and skin malignant tumors, their treatment options and new diagnostic modalities.

7. **Publications:**

- Kenyon-Smith TJ, Kroon HM, Miura JT, Teras J, Beasley GM, Mullen D, Farrow NE, Mosca PJ, Lowe MC, Farley CR, Potdar A, Daou H, Sun J, Farma JM, Henderson MA, Speakman D, Serpell J, Delman KA, Smithers BM, Barbour A, Coventry BJ, Tyler DS, Zager JS, Thompson JF. Factors predicting toxicity and response following isolated limb infusion for melanoma: An international multi-centre study. Eur J Surg Oncol 2020 (accepted for publication).
- Teras J, Kroon HM, Thompson JF, Teras M, Pata P, Mägi A, Teras RM, Rüütel Boudinot S. First Eastern European experience of isolated limb infusion for intransit metastatic melanoma confined to the limb: Is it still an effective treatment option in the modern era? *Eur J Surg Oncol* 2020;46:272–276.
- Teras J, Kroon HM, Miura JT, Kenyon-Smith T, Beasley GM, Mullen D, Farrow NE, Mosca PJ, Lowe MC, Farley CR, Potdar A, Daou H, Sun J, Carr M, Farma JM, Henderson MA, Speakman D, Serpell J, Delman KA, Smithers BM, Barbour A, Tyler DS, Coventry BJ, Zager JS, Thompson JF. International multicenter experience of isolated limb infusion for in-transit melanoma metastases in octogenarian and nonagenarian patients. *Ann Surg Oncol* 2020;27:1420–29.
- Teras J, Kroon HM, Zager JS. ASO Author Reflection: Isolated limb infusion for locally advanced melanoma in the extremely old patient is safe and effective. *Ann Surg Oncol* 2020;27:1430–31.
- PelvEx Collaborative. Management Strategies for Patients With Advanced Rectal Cancer and Liver Metastases Using Modified Delphi Methodology: Results From the PelvEx Collaborative. *Colorectal Dis* 2020 Feb 11 [Epub ahead of print].
- Kenyon-Smith TJ, Kroon HM, Miura JT, Teras J, Beasley GM, Mullen D, Farrow NE, Mosca PJ, Lowe MC, Farley CR, Potdar A, Daou H, Sun J, Farma JM, Henderson MA, Speakman D, Seprell J, Delman KA, Smithers BM, Barbour A, Coventry BJ, Tyler DS, Zager JS, Thompson JF. Factors predicting toxicity and response following isolated limb infusion for melanoma: An international multi-centre study. *Eur J Surg Oncol* (submitted).
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Elulookirjeldus

1. Isikuandmed:

Nimi:	Jüri Teras
Sünniaeg:	27.05.1968
Sünnikoht:	Tallinn, Eesti
Rahvus:	eestlane

2013–2020	Doktorantuur, Tallinn Tallinna Tehnikaülikool, Tallinn, Eesti
1994–1997	Residentuur Mustamäe Haigla, Tallinn, Eesti
1996	Residentuur Sundsvalli Haigla, Sundsvall, Rootsi
1993–1994	Internatuur Johanniter-Krankenhaus Dohna-Heidenau, Saksamaa
1992–1993	Internatuur Mustamäe Haigla, Tallinn, Eesti
1986–1992	Tartu Ülikooli Arstiteaduskond, Tartu, Eesti
1975–1986	Tallinna 7. Keskkool

2. Diplomijärgne koolitus:

2019	Stažeerimine University Hospital of Adelaide, juhendaja dr Hidde Kroon, Adelaide, Austraalia
2014	Stažeerimine Memorial Sloan Kettering Cancer Center, juhendaja dr Daniel Coit, NY, USA
2013	Stažeerimine Memorial Sloan Kettering Cancer Center, juhendaja dr Daniel Coit, NY, USA
2010–2011	Stažeerimine Memorial Sloan Kettering Cancer Center, juhendaja dr Daniel Coit, NY, USA
2007	Stažeerimine Hospital Militar 1 Lomas de Sotelo, juhendaja mjr Luiz Garcia, Mexico City, Mehhiko
2005	American College of Surgeons (ACS) International Travel Scholarship MD Anderson Cancer Centre Houston, Texas Mount Sinai Hospital New York, University of Medicine and Dentistry of New Jersey

3. Töökoht:

Ülemarst-keskuse juhataja
Üld-ja onkokirurgia keskus, SA PERH
European Union of Medical Specialist (UEMS) section surgery – rahvuslik esindaja

4. Humanitaartöö

2010–2020	Humanitaarmissioonid Aafrikas ja Aasias

5. Teadustöö:

Teadustöö on keskendunud pehmekoe ja kõhukoopa infektsioonidele ning kasvajalistele haigustele, nende diagnostilistele ja ravimodaliteetidele.