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Cardiotoxicity during checkpoint inhibitor therapy in patients with advanced skin cancer

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Dedicated to my sons Noah Mateo and Leo Emiliano

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List of abbreviations

AJCC ..... American Joint Comittee on Cancer

CK-MB ..... Creatine kinase myocardial band

CT ..... Computed tomography

CTCAE ..... Common Terminology Criteria for Adverse Events

CTLA-4 ..... Cytotoxic T-Lymphocyte Antigen

DNA ..... Deoxyribonucleic acid

ECG ..... Electrocardiography

G-CSF ..... Granulocyte colony-stimulating factor

hsTnT ..... High sensitivity Troponin T

IHD ..... Idiopathic heart disease

irAEs ..... Immune related adverse events

irM ..... Immune related myocarditis

LAD ..... Left anterior descending artery

MRI ..... Magnetic resonance imaging

nSCLC ..... Non-small cell lung cancer

NT-proBNP ..... N-terminal prohormone of brain natriuretic peptide

OS ..... Overall survival

PD-1 ..... Programmed Death 1

PD-L1 ..... Programmed Death Ligand 1

PET ..... Positron emission tomography

SCC ..... Squamous cell carcinoma

UVR ..... Ultraviolet radiation

Introduction

Immunotherapy

Immunotherapy now plays a central role in the management of a range of cancers, particularly in the context of metastatic melanoma and non-melanoma skin cancer. (Postow et al. 2018). By targeting specific immune checkpoints, including the cytotoxic T-lymphocyte antigen 4 (CTLA-4), the programmed cell death 1 (PD-1), and the programmed cell death ligand 1 (PD-L1), immune checkpoint inhibition has revolutionized the management of malignant disease and now forms an integral part of the treatment of an ever-increasing list of cancers, both in the palliative and adjuvant setting (Postow et al. 2018). Moreover, the combination of immune checkpoint inhibitors, specifically anti-PD-1 and anti-CTLA-4, first licensed for the treatment of metastatic melanoma, is also now employed in the treatment of other tumour entities, including renal cell carcinoma and non-small cell lung cancer (table 1).

Table 1: Clinical applications of immune checkpoint-based immunotherapy

Drug	Target	Indication
Ipilimumab	CTLA-4	Melanoma, Renal carcinoma, Non-small-cell lung cancer
Nivolumab	PD-1	Melanoma, Non-small-cell lung cancer, Renal carcinoma, Hepatocellular carcinoma Hodgkin's lymphoma, Squamous cell carcinoma Urothelial carcinoma, Colorectal carcinoma
Pembrolizumab	PD-1	Melanoma, Non-small-cell lung cancer, Renal carcinoma, Hepatocellular carcinoma Hodgkin lymphoma, Squamous cell carcinoma Urothelial carcinoma, Colorectal carcinoma
Cemiplimab	PD-1	Squamous cell carcinoma
Atezolizumab	PD-L1	Non-small-cell carcinoma, Urothelial carcinoma
Avelumab	PD-L1	Merkel cell carcinoma, Urothelial carcinoma
Durvalumab	PD-L1	Urothelial carcinoma

Indeed, in the case of renal cell carcinoma and non-small cell lung cancer (nSCLC), combined immunotherapy (ipilimumab plus nivolumab) is licensed as a first-line treatment, albeit at a dosage different to that used in metastatic melanoma and, in the case of nSCLC, in combination with platinum-based chemotherapy. The immune checkpoint inhibitors currently licensed in the field of dermato-oncology all target the PD-1- Programmed cell death ligand 1 (PD-L1) axis and/or the expression of the cytotoxic T-lymphocyte antigen 4 (CTLA-4).

The principles of immune checkpoint inhibition in advanced skin cancer

CTLA-4 and PD-1 play an important physiological role in the regulation of the human immune system. Checkpoint inhibitors affect both the priming and the effector phase of T cell interactions. Tumour antigens are presented by dendritic cells to CD4+ T cells in the lymph node during the priming phase. In this phase, a co-stimulatory signal, for example, B7-1 (or CD80) and B7-2 (or CD86) molecules binding to the CD28 receptor on T cells, is necessary for the activation and expansion of antigen-specific T cells (Ribas 2012b). A co-inhibitory signal, namely the binding of CD80 or CD86 on dendritic cells to the CTLA-4 receptor on T cells, prevents the development of self-reactive T cell clones following up-regulation of CTLA-4. Checkpoint inhibitors specifically block the CD80-CTLA-4 interaction, resulting in T cell activation and expansion (figure 1). In the effector phase, which takes place in the tumour itself, activated T cells bind to MHC Class II molecules on the tumour cell via their T cell receptor. The binding of PD-1 (on the T cell) to PD-L1 (on the tumour cell) prevents further T cell activation negatively regulates T-cells (figure 1) (Pardoll 2012). Again, checkpoint inhibitors targeting PD-1 or the PD-L1 ligand remove the inhibitory signal and facilitate activation of cytotoxic T cells targeting the tumour. Given that PD-1 predominantly regulates the effector phase of the tumour response, combined with its specificity for selective promoting tumour-specific T cell activation, its use is associated with fewer side effects than CTLA-4 (Ribas 2012b) or combined anti-CTLA-4 and anti-PD-1 therapy.

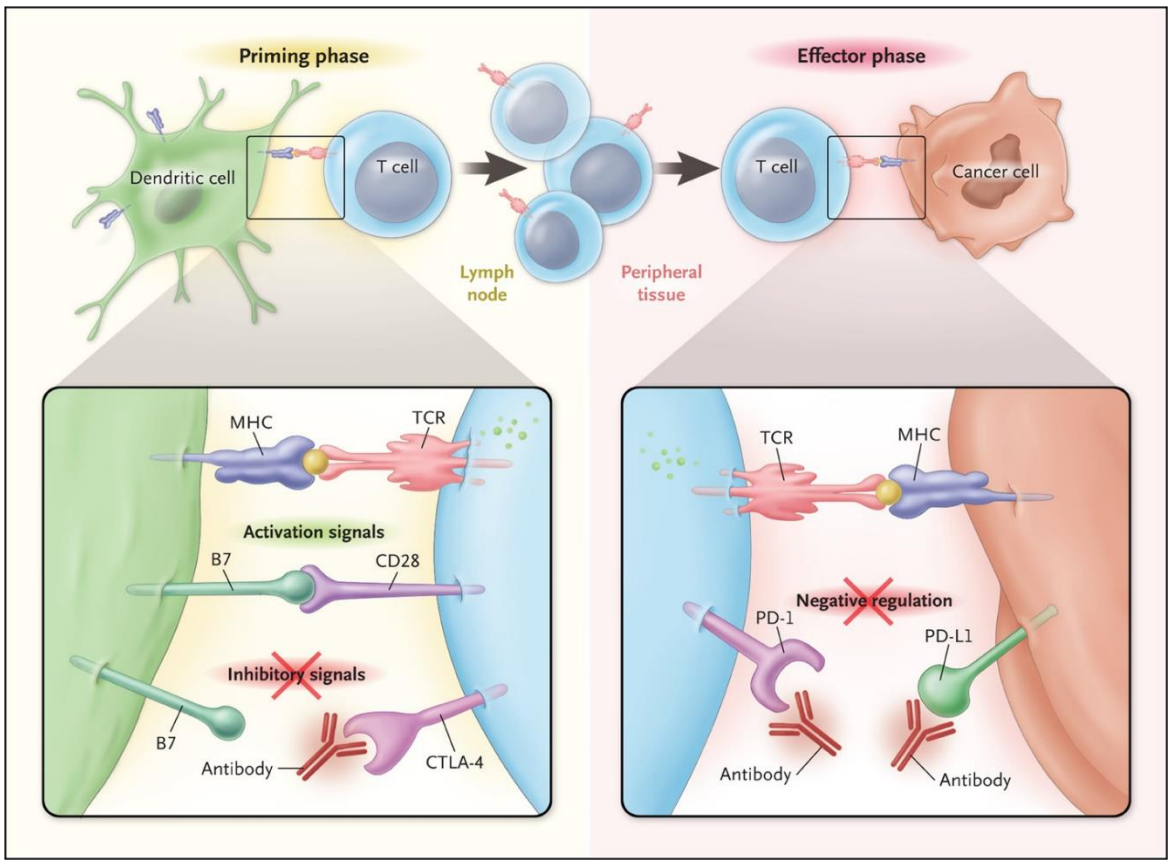

Figure 1: Mechanism of checkpoint-inhibition

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The history and use of immune checkpoint inhibition in advanced skin cancer

Ipilimumab (anti-CTLA-4 monoclonal antibody) was the first-in-class immune checkpoint inhibitor to show promise in the treatment of metastatic melanoma. Hodie et. al demonstrated for patients suffering from stage 4 melanoma, that ipilimumab alone demonstrated a prolonged median overall survival of 10.1 months, in comparison to the cohort receiving ipilimumab plus gp 100 peptide, a synthetic peptide cancer vaccine (median overall survival: 10 months) and gp100 peptide (median overall survival: 6.4 months) (Hodi et al. 2010). Almost half a decade later, in June 2015, both nivolumab and pembrolizumab were approved for the treatment of non-resectable metastatic melanoma. The approval of nivolumab was based on the checkMate-066 and -037 studies. In these studies, patients with unresectable or metastatic melanoma, stage 3 or stage 4, received

either nivolumab 3mg/kg body weight every three weeks or dacarbazine 1000 mg/m² every two weeks. Nivolumab demonstrated a prolonged one-year overall survival of 72.9 % compared to 42.1 percent in the dacarbazine group (Franklin et al. 2017; Weber et al. 2015b). Pembrolizumab was licensed for the treatment of unresectable Stage III or metastatic melanoma based on data from the phase 3 keynote -006 clinical trial. Pembrolizumab (10 mg/kg) was compared to ipilimumab (3 mg/kg). The co-primary and the primary end points in this study were progression free survival and overall survival. The observation time was initially planned as two years. In the monotherapy, pembrolizumab demonstrated a prolonged progression free survival with 47.3 percent, compared to 26.5 percent in the ipilimumab cohort over the first 6 months after commencing treatment (Robert et al. 2015). The one year overall survival for the pembrolizumab group was 74 %, in comparison to just 58 % in the ipilimumab group (Franklin et al. 2017). The proof of the combination therapy was demonstrated In the checkmate 067 trial combined immunotherapy with nivolumab (dose: 1 mg per kilogram of body weight) plus ipilimumab (3 mg per kilogram) every 3 weeks for four doses, followed by nivolumab monotherapy (3 mg per kilogram every 2 weeks). This treatment resulted in a significantly improved progression free survival (11.5 months) in comparison to nivolumab (6.9 months) or ipilimumab monotherapy (2.9 months) (Franklin et al. 2017; Larkin et al. 2015)

In fact, the success of immune checkpoint inhibitors in the palliative (non-operable/metastatic) setting led to an investigation of the use of anti-PD-1 antibodies in the so-called “adjuvant” setting. In this context, immune checkpoint inhibitors were administered to patients with stage III (lymph node metastases) and IV disease (visceral metastases) who were clinically and radiologically tumour free following surgery.

For the adjuvant treatment of melanoma, both the checkmate-238 study and the keynote-54 study investigated the adjuvant use of nivolumab and pembrolizumab respectively (Weber et al. 2017). The most recent data from the checkmate – 238 study showed that nivolumab was associated with a four-year recurrence-free survival rate of 51.7%, significantly greater than the equivalent rate (41.2%) in the ipilimumab group. Regarding

overall survival, nivolumab demonstrated a better overall survival (77.9 percent), in contrast to ipilimumab (76.6 percent) (Ascierto et al. 2020). In addition to that, the keynote-54 trial demonstrated, that pembrolizumab was associated with a significant prolonged one-year recurrence free-survival (75.4 %) versus the placebo group (61 %) (Eggermont et al. 2018).

However, the successful application of immune checkpoint inhibition in dermato-oncology is not restricted to the management of metastatic melanoma. The PD-1 inhibitor cemiplimab is now licensed for the treatment of locally advanced and/or metastatic squamous cell carcinoma, where it is associated with a response rate of up to 47 % (Migden et al. 2018). This is impressive given the co-morbidities encountered in this typically elderly patient population.

Finally, manipulation of the PD-1 – PD-L1 axis has also been successfully employed in Merkel cell carcinoma, a neuroendocrine tumour frequently associated with the Merkel cell polyoma virus. Merkel cell carcinoma is perhaps the most lethal of all metastatic skin tumours, typically affecting elderly patients where it develops on ultraviolet radiation (UVR) exposed skin sites. Prior to the era of immune checkpoint inhibition, the prognosis of metastatic Merkel cell carcinoma was extremely poor, with a 5-year overall survival of 13.5 percent (Grabbe et al. 2018). However, targeting the PD-L1 ligand, the immune checkpoint inhibitor avelumab has resulted in impressive results. The JAVELIN Merkel 200 trial illustrated a partial response for 33 percent of the patients and a complete response of 11.4 of the patients who received avelumab in the second-line setting, i.e. following treatment progression after with chemotherapy (Kaufman et al. 2018; Terheyden et al. 2019) Furthermore, patients who received avelumab as first-line therapy achieved a partial response of 56.4 % and a complete response of 24 % (Kaufman et al. 2018; Terheyden et al. 2019).

Despite the dramatic improvements in progression free- and overall survival for patients with a range of locally advanced and/or metastatic skin cancers, the major limitation of immune checkpoint inhibition is the development of immune-related adverse events (irAEs), including cardiotoxicity, which can be irreversible and potentially life-threatening.

Melanoma

Cutaneous melanoma is a highly aggressive skin cancer arising from melanocytes; melanin producing neural-crest derived cells (figure 2).

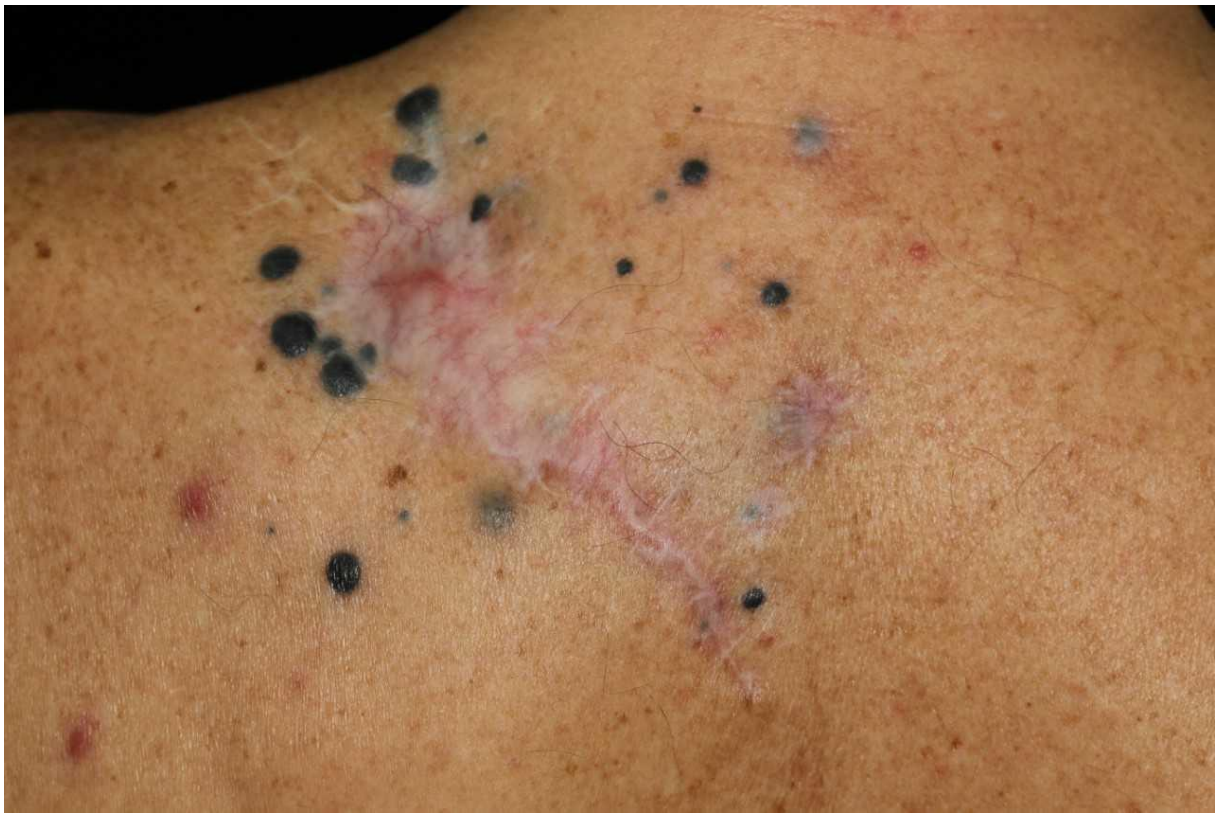

Figure 2: Multiple satellite metastases following excision of the primary melanoma in a stage III melanoma patient.

Melanoma is more common in people with Fitzpatrick skin types I and II, Cutaneous melanoma is rare in African or Asian populations (Garbe and Leiter 2009). Patients with a family or personal history of melanoma are at significantly increased risk of developing melanoma. The incidence of melanoma in Europe is geographically dependent, with an incidence of 12-35/100000 in Nordic countries but only to 3-5/100000 in Mediterranean countries (Hollestein et al. 2012). Although melanoma can occur at any age, its incidence peaks around 65 years of age. The incidence of melanoma has dramatically increased over the last few decades (Hollestein et al. 2012). The relationship between geographical

location and the risk of developing melanoma is thought to be largely mediated by levels of exposure to ultraviolet radiation (UVR), damaging DNA and causing the formation of thymine dimers (Alexandrov et al. 2013). Consistent with this observation, the incidence rate of melanoma in Queensland, Australia is 60/100.000; the highest incidence worldwide (Leitlinienprogramm Onkologie: Melanom 2021.000Z).

Melanoma Stages

The American Joint Committee on Cancer (AJCC) staging system is used to classify the extent of the tumour burden and is based on the depth of invasion of the primary tumour (Breslow measurement) and the presence or absence of ulceration (Stages I and II). Stage III indicates the presence of regional lymph node involvement and Stage IV disease is characterized by the presence of visceral and/or distant metastases (table 2, table 3, table 4, table 5). This classification follows the internationally accepted T (tumour), N (lymph nodes), M (metastases) staging for cancer. A recent mutli-centre analysis of survival data of 46000 patients with melanoma formed the basis of the 8th version of the melanoma staging system (Gershenwald et al. 2017). The key prognostic factor remains the Breslow measurement of the primary tumour (Gershenwald et al. 2017).

Table 2: T - category (AJCC TNM eight edition staging system of melanoma)

Source: (Keung and Gershenwald 2018)

T category	**Thickness**	**Ulceration status**
Tx Primary tumour thickness cannot be assessed (e.g. diagnosis by curettage	Not applicable	Not applicable
T0 No evidence of primary tumour (e.g. unknown primary or completely regressed melanoma)	Not applicable	Not applicable
Tis (melanoma in situ)	Not applicable	Not applicable
T1	≤ 1,0 mm	Unknown or unspecified
T1a	< 0.8mm	Without ulceration
T1b	< 0.8mm 0.8 mm – 1.0 mm	With ulceration With or without ulceration
T2	>1.0–2.0 mm	Unknown or unspecified
T2a	>1.0–2.0 mm	Without ulceration
T2b	>1.0–2.0 mm	With ulceration
T3	>2.0-4.0 mm	Unknown or unspecified
T3a	>2.0-4.0 mm	Without ulceration
T3b	>2.0-4.0 mm	With ulceration
T4	>4.0 mm	Unknown or unspecified
T4a	>4.0 mm	Without ulceration
T4b	>4.0 mm	With ulceration

Table 3: N – category

Source: (Keung and Gershenwald 2018)

N category	**Number of tumour-involved regional lymph node**	**Presence of in-transit, satellite, and/or microsatellite metastases**
N0	No regional metastases detected	No
N1	One tumour-involved node or in-transit, satellite, and/or microsatellite metastases with no tumour- involved nodes	
N1a	One clinical occult (i.e. detected by SLN biopsy)	No
N1b	One clinical detected	No
N1c	No regional lymph node disease	Yes
N2	Two or three tumour- involved nodes or in-transit, satellite, and/or microsatellite metastases with one tumour involved node	
N2a	Two or three clinical occult (i.e. detected by	No
N2b	Two or three, at least one of which was clinically detected	No
N2c	One clinical occult or clinically detected	Yes
N3	Four or more tumour-involved nodes or in-transit, satellite, and/or microsatellite metastases with two or more tumour-involved nodes, or any number of matted nodes, or any number of matted nodes without or with in-transit, satellite, and/or microsatellite metastases	
N3a	Four or more clinically occult (i.e. detected by SLN biopsy)	No
N3b	Four or more, at least one of which was clinically detected, or presence of any number of matted nodes	No
N3c	Two or more clinical occult or clinically detected and or presence of any number of matted nodes	yes

Table 4: M – category

Source: (Keung and Gershenwald 2018)

M category	**Anatomic site**	**LDH level**
M0	No evidence of distant metastasis	Not applicable
M1	Evidence of distant metastasis	See below
M1a M1a (0) M1a (1)	Distant metastasis to skin, soft tissue including muscle and/or nonregional lymph node	Not recorded or unspecified Normal Elevated
M1b M1b (0) M1b (1)	Distant metastasis to lung with or without M1a sites of disease	Not recorded or unspecified Normal Elevated
M1c M1c (0) M1c (1)	Distant metastasis to non-CNS visceral sites with or without M1a or M1b sites of disease	Not recorded or unspecified Normal Elevated
M1d M1d (0) M1d (1)	Distant metastasis to CNS with or without M1a, M1b or M1c sites of disease	Not recorded or unspecified Normal Elevated

Table 5: Melanoma Stages

Source: (Keung and Gershenwald 2018)

T	N	M	Pathological stage group
Tis	N0	M0	0
T1a	N0	M0	IA
T1b	N0	M0	IB
T2a	N0	M0	
T2b	N0	M0	IIA
T3a	N0	M0	
T3b	N0	M0	IIB
T4a	N0	M0	
T4b	N0	M0	IIC
T0	N1b, N1c	M0	IIIB
T0	N2b, N2c, N3b oder N3c	M0	IIIC
T1a/b–T2a	N1a oder N2a	M0	IIIA
T1a/b–T2a	N1b/c oder N2b	M0	IIIB
T2b/T3a	N1a–N2b	M0	
T1a–T3a	N2c oder N3a/b/c	M0	IIIC
T3b/T4a	Any N ≥N1	M0	
T4b	N1a–N2c	M0	
T4b	N3a/b/c	M0	IIID
Any T, Tis	Any N	M1	IV

Therapy and prognosis

Although the treatment of melanoma is heavily stage-dependent, histological confirmation of the primary tumour, following excision with a 2 mm safety margin, forms the initial standard of care (Tronnier et al. 1997). The safety margin of 2 mm allows complete removal of the tumour for histological analysis whilst simultaneously minimizing damage to the local lymphatics to allow subsequent sentinel lymph node biopsy if indicated (Tran et al. 2008). Standard re-excision, with a 1 or 2 cm safety margin, dependent on the Breslow thickness, reduces the risk of local recurrence. At present, re-excision with a 1 cm margin when the Breslow measurement is < 2 mm and 2 cm when the Breslow measurement is > 2 mm is recommended, although the evidence base for this practice is limited (Wheatley et al. 2016).

Sentinel lymph node biopsy with the re-excision of the scar is recommended for melanoma from stage pT1b (AJCC eighth edition), i.e. primary melanomas with a Breslow thickness of > 0.8 mm without ulceration or < 0,8 mm with ulceration (Han et al. 2013). In the case of tumours> 4 mm with ulceration, or disease at Stage IIC and above, a radiological assessment of the tumour burden should be considered. Several modalities are currently available including computed tomography (CT), positron emission (PET), and magnetic resonance imaging (MRI) (Dummer et al. 2011).

The current guidelines for the management of locally advanced and/or metastatic melanoma recommend treatment with targeted therapy, in the case of patients with a BRAF mutation, or immunotherapy, either combined anti-PD-1 and anti-CTLA-4 or anti-PD-1 monotherapy (Michielin et al. 2019). The current data, in terms of progression free survival, clearly favors combined immunotherapy over anti-PD-1 monotherapy, albeit with a significant increase in the incidence and severity of irAEs. The prognosis is stage-dependent. Patients with stage one disease have a 5-year survival of more than 90 percent, patients with stage two melanoma have a survival rate between 45-77 percent and those with stage 3 melanoma have a 5-year survival rate ranging between 27-70 percent. Patients with untreated stage 4 metastatic melanoma have an extremely poor prognosis with a five-year survival rate of less than 20 percent (Gershenwald et al. 2017). In the adjuvant setting, only anti-PD-1 immunotherapy is currently licensed. Decisions regarding the treatment of metastatic melanoma are made in multidisciplinary tumour boards, tailored to the individual patient and taking into account the tumour burden, tumour activity, and co-morbidities.

Squamous Cell Carcinoma

Squamous cell carcinoma (SCC) is another skin cancer, whose incidence is also closely related to the patient's Fitzpatrick skin time and chronic UVR exposure. In fact, since 2015, SCC is recognized as a work-related cancer in patients whose occupations involved working outdoors (figure 3).

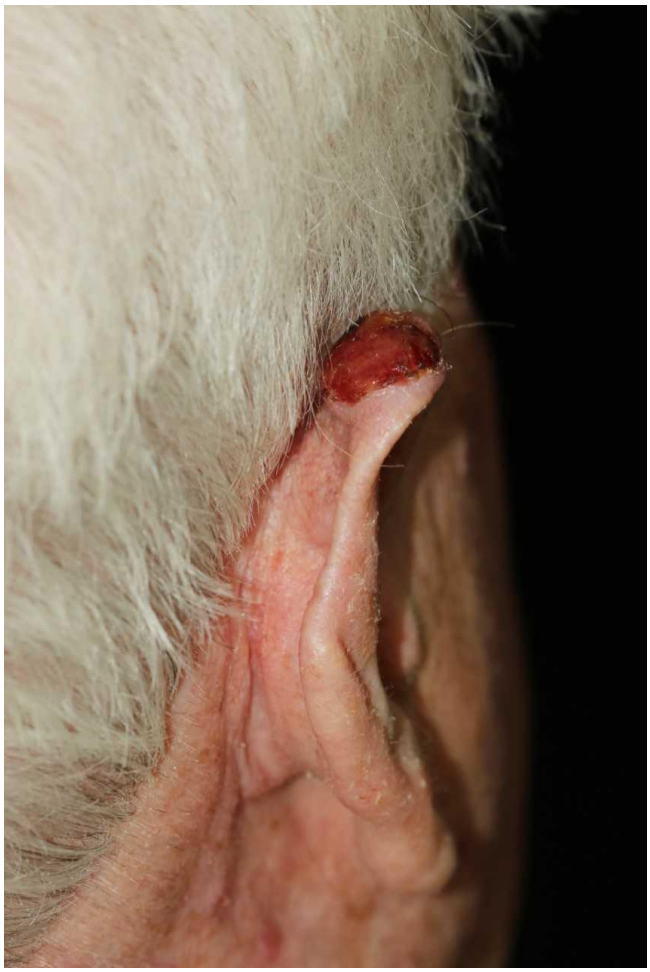

Figure 3: Squamous cell carcinoma in a typical affected localization

Similar to the situation with melanoma, there has been a significant increase in the incidence of SCC over the past few decades (Alam et al. 2018). In fact, the incidence of cutaneous SCC has increased by up to 200 percent over the last 30 years and now accounts for 20 % of all non-melanoma skin cancers (Leiter et al. 2017). In addition to chronic and cumulative UVR exposure, immunosuppression, particularly following solid organ transplantation, is a major risk factor for the development of the disease (Kim et al. 2016). Given the relationship

to exposure to UVR, it is unsurprising that sun-exposed areas (head, neck, arms) are most commonly affected.

The gold standard for the treatment of SCC is complete surgical excision where possible, with an appropriate safety margin (Alam et al. 2018; Leiter et al. 2020) . Nevertheless, SCC is associated with a risk of 4% of both loco-regional and distant metastasis, depending on the tumour stage. This risk is increased two to three-fold in patients who are immunosuppressed or immunocompromised (Cooper and Brown 2006). In contrast to the diagnostic workup in melanoma, the utility of sentinel lymph node biopsy is unclear (Alam et al. 2018). When the primary tumour cannot be fully resected, termed R1 status, or there is evidence of perineural spread, adjuvant radiotherapy should be administered.

Until recently, the treatment of metastatic SCC was based heavily on the use of platinum-based chemotherapy, achieving response rates over 50%, without a solid evidence-base. However, the typically short duration of response, the development of serious toxicity and a lack of prospective randomized controlled clinical trials underpinned the need for the development of new treatment options for locally advanced and/or metastatic disease.

In 2019 the PD-1 immune checkpoint inhibitor cemiplimab was licensed for the treatment for unresectable or metastatic squamous cell carcinoma (Migden et al. 2018). Cemiplimab also achieved treatment response rates of approximately 50%; securing disease control in almost two-thirds of patients (Ahmed, SR et al. 2019). Immune-related adverse events, such as diarrhoea, fatigue or nausea occurred in about 15 percent of the cases (Migden et al. 2018). To date, one case of irM has been reported in the literature (Jeyakumar et al. 2020).

It is worth bearing in mind that up to 80 percent of relapses occur during the first two years after diagnosis and that 30-50 percent of second squamous cell carcinomas occur during the first 4 years after diagnosis (Brantsch et al. 2008). Therefore, regular follow-up of these patients is required to detect disease recurrence and/or progression.

Merkel-Cell Carcinoma

Merkel cell carcinoma is a rare, but highly aggressive tumour of neuroendocrine origin (van Keymeulen et al. 2009). The incidence in Europe is about 2-4 per million inhabitants and the increasing incidence has also been largely attributed to increased levels of UVR exposition. The disease more frequently affects males (61.5 percent) and the median age of diagnosis is 76 years (Girschik et al. 2011; Reichgelt and Visser 2011). In addition to the aetiopatholigcal role of UVR exposure (Agelli and Clegg 2003), both immunosupression (Lanoy et al. 2010) and the Merkel cell polyomavirus (MCPyV) play pivotal roles in the development of the disease (Kassem et al. 2009). Clinically, Merkel cell carcinoma presents as a rapidly growing, erythematous nodule or tumour which may be ulcerated (Heath et al. 2008). Photo-exposed sites are most typically affected, for example, the head and neck area (53 %) and extremities (34-35%). Mucosal sites and the trunk are rarely affected (Lebbe et al. 2015). Histologically, Merkel cell carcinoma is characterized by a high rates of mitosis and occasional necrosis (Lebbe et al. 2015) but the diagnosis relies on demonstration of neuroendocrine markers, for example CK20 expression. Histological examination is mandatory given that the clinical presentation is indistinguishable from that of other tumour entities.

The treatment of choice is complete surgical excision and, depending on the tumour characteristics, post-surgical radiotherapy to reduce the risk of loco-regional recurrence. A safety margin of about 1-2 cm is recommended and the suggested dose of radiotherapy is 50 Gy (Veness et al. 2005). The staging system of the Merkel cell carcinoma is based on the standard TNM classification, T1 is characterized by a tumour size < 2 cm, T2 by 2 – 5 cm and T3 by > 5 cm, whereas T 4 involves deeper layers, such as bones, muscles, fascia and cartilage (Lemos et al. 2010). Similar to other cancer types, the disease stage is closely associated with prognosis (table 6)

Table 6 Stages of Merkel cell carcinoma

Source: (Lemos et al. 2010)

Stage	T	N	M	5 years survival (%)
0	TIS	N0	M0	
IA	T1	pN0	M0	79
IB	T1	cN0	M0	60
IIA	T2/T3	pN0	M0	58
IIB	T2/T3	cN0	M0	49
IIC	T4	N0	M0	47
IIIA	Any T	N1a	M0	42
IIIB		N1b/N2	M0	26
IV		Any N	M1	18

Sentinel lymph node biopsy should be considered in the absence of lymph node or distant organ metastases. A complete lymph node dissection is highly recommended should micrometastases be discovered in the sentinel lymph node, followed by radiotherapy to the tumour bed and the lymphatic drainage areas. Given the highly aggressive nature of Merkel cell carcinoma and the high rate of mortality associated with metastatic disease, follow-up should be conducted on a 3 monthly basis and include complete skin examination, lymph node sonography, and CT/MR Imaging every 3 to 12 months depending on sentinel lymph node status (Becker et al. 2019; Lebbe et al. 2015).

As was the case with the management of squamous cell carcinoma, the treatment of locally advanced and/or metastatic disease was heavily reliant on toxic polychemotherapy regimes, with short durations of response and high rates of relapse. In 2018 the PD-L1 antibody avelumab was licensed for the treatment of metastatic Merkel cell carcinoma based on the JAVELIN Merkel 200 trial. Partial response rates of 33 % and complete response rates of 11.4 % were achieved in patients who received avelumab after the prior treatment with chemotherapy (Kaufman et al. 2018; Terheyden et al. 2019). Moreover, patients who were treated in the first-line therapy setting achieved partial response rates of 56.4 percent and 24% of patients obtained a complete response (Kaufman et al. 2018; Terheyden et al. 2019). The irAE associated with avelumab are similar to those recognized during treatment with anti-PD-1 and anti-CTLA-4 immune checkpoint inhibitors. In addition

to cutaneous irAEs, pneumonitis, hepatitis, colitis, endocrinopathies, and infusion-related reactions have all been reported (Kelly et al. 2020).

In summary, the use of immune checkpoint inhibitor-based immunotherapy has revolutionized the management of a range of highly aggressive and, when untreated, fatal skin cancers. However, their use is associated with the risk of the development of severe, even life-threatening irAE, including irM. To date, the incidence of irM in the “real life” use of immune checkpoint inhibitors is unclear and there is currently no way to identify patients at risk of potentially fatal irAEs, particularly irM.

Immune-related adverse events (irAEs)

Given that immune checkpoint inhibitors essentially stimulate the cellular immune response, by removing the physiological mechanisms which protect the body from excess inflammation, it is unsurprising that their use is associated with the development of organ-specific inflammation which can be severe, life-threatening and irreversible. Termed immune-related adverse events (irAEs), these side effects are a class effect of all immune checkpoint inhibitors and can affect any organ in the body (figure 4).

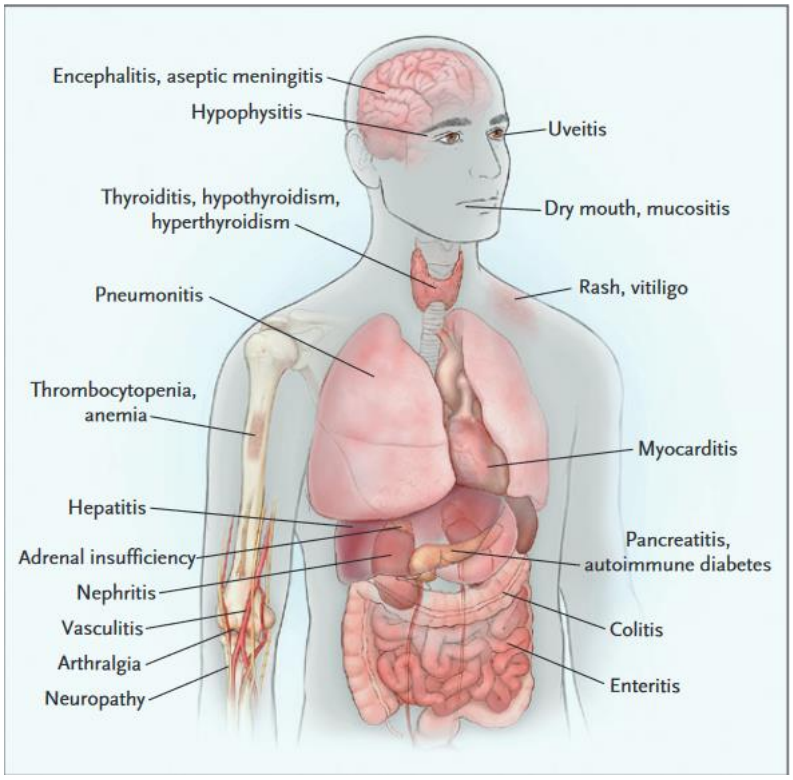

Figure 4: Possible immunotherapy related adverse events

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To date, a comprehensive understanding of the pathophysiology of irAEs remains elusive. CTLA-4 mediated irAEs often occur early in treatment (Krummel and Allison 1996), whereas those related to PD-1 inhibitors occur later (Boussiotis 2016; Dong et al. 2002). Animal studies, either in a CTLA-4-deficient mouse model (Tivol et al. 1995), or a PD-1-

deficient mouse model (Nishimura et al. 1999), have shed light on the role of immune checkpoints in the development of cancer. For example, mice lacking the CTLA-4 receptor die from lymphoproliferation (Tivol et al. 1995) and mice lacking from PD-1 develop autoimmune diseases, including rheumatoid arthritis and immune-mediated cardiomyopathy (Nishimura et al. 1999).

Interestingly, there is some evidence that baseline serum interleukin 17 (IL-17) and G-CSF concentrations may correlate with the development of specific irAEs; namely, patients with increased serum baseline IL-17 concentrations are associated with an increased risk of pneumonitis and lower G-CSF levels at baseline are correlated with the risk of developing colitis (Harbour et al. 2015; Tyan et al. 2021).

To effectively manage irAE, it is important to record their incidence and grade their severity. The “Common criteria of adverse events” (CTCAE) classification is widely used in routine clinical practice and in clinical trials for this purpose. The current version (Version 5) was published in 2017 and adverse events are graded as mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4), or lethal (grade 5.) (Common Terminology Criteria for Adverse Events (CTCAE) | Protocol Development | CTEP 2021; Wang et al. 2019). The treatment of irAE is based on this classification as follows: grade 1 – symptomatic treatment only and continuation of the immune checkpoint therapy, grade 2 – treatment with oral corticosteroids and interruption of the immune checkpoint therapy, grade 3 - treatment with intravenous corticosteroids and interruption of the immune checkpoint therapy and grade 4 – treatment with intravenous corticosteroids and additional immunosuppressive agents and permanent withdrawal of immune checkpoint therapy (Brahmer et al. 2018; Friedman et al. 2016; Haanen et al. 2017; Weber et al. 2015a).

Immune-related adverse events, in general, are a relatively frequent effect of immune checkpoint inhibition and occur in up to 90 percent receiving treatment with anti CTLA-4 (Hodi et al. 2010), and in up to 70 percent in the treatment with anti-PD-1 (Topalian et al. 2012) albeit of varying severity. The risk of developing irAE is also dependent on whether anti-PD-1 monotherapy or combined anti-PD-1 and anti-CTLA-4 therapy are administered.

Moreover, in terms of anti-CTLA-4 therapy, there is evidence that irAEs are dose-dependent. (Wolchok et al. 2010). Reassuringly, there is no evidence of cumulative toxiticty with anti-PD-1 treatment (Michot et al. 2016). Patients treated with anti CTLA-4 therapy demonstrate a different range of organ-specific adverse events when compared to irAEs occurring during anti-PD-1 therapy (Michot et al. 2016). For example, anti-CTLA-4 therapy is more frequently associated with immune-mediated colitis. Whilst the most common irAEs, non-specific inflammatory dermatoses, are mild and can be managed with corticosteroid-based topical therapy. In fact, the development of vitiligo may even correlate with treatment (Hua et al. 2016). But still, whether the development of irAEs correlates with the therapeutic response is controversial. Nonetheless, irAEs, such as cardiotoxicity, are rare and associated with significant mortality despite early immunosuppressive treatment.

The most common adverse events are inflammatory dermatoses. Gastrointestinal adverse events are the second most common irAE (>25 percent for anti-CTLA-4, approximately 20 percent for anti-PD-1/PD-L1), followed by pulmonary adverse events, arthralgia, endocrinopathies, hepatic adverse events, neurological and cardiac adverse events (Abdel-Rahman et al. 2016; Morganstein et al. 2017; Naidoo et al. 2017). Moreover, adverse events due to anti-CTLA-4 are generally more severe. Severe adverse events (grade 3-4), particularly gastrointestinal and endocrine irAE are more common during treatment with ipilimumab (Larkin et al. 2015; Schachter et al. 2017). Indeed, several irAEs can occur concurrently and/or the following cessation of treatment and their incidence may be increased in patients with pre-existing autoimmune diseases. Previous studies have shown that about 30 percent of patients with a pre-existing autoimmune disease may experience an exacerbation during immune checkpoint inhibitor therapy. Therefore, patients with pre-existing inflammatory bowel disease should be kept under close surveillance to facilitate early detection and treatment of immune-mediated colitis (Kähler et al. 2018).

The use of additional immunosuppressive agents is reserved for organ-specific severe (grade 4) or treatment-refractory irAE and includes TNF alpha inhibition (colitis), mycophenolate mofetil (pneumonitis and hepatitis), cyclophosphamide (pneumonitis), and

intravenous immunoglobulins (pneumonitis and encephalitis) followed by accurate tapering of systemic corticosteroids (Haanen et al. 2017).

Cardiotoxicity

Cardiotoxicity is one of the most feared irAE given the high rate of mortality. The term cardiotoxicity, in the context of immune checkpoint therapy, is an umbrella term that encompasses myocarditis, pericarditis, myocardial fibrosis, and pericardial effusions (Varricchi et al. 2017). The clinical presentation of immune-mediated myocarditis (irM) can vary dramatically, ranging from non-specific symptoms of malaise and fatigue to dyspnoea and congestive heart failure, and ultimately resulting in cardiac arrhythmias and fulminant cardiogenic shock (Frigeri et al. 2018; Liu and Wu 2020). Indeed, initial there may be no clinical symptoms at all. This lack of specific symptoms, especially in the initial stages, often leads to a delay in diagnosis (Atallah-Yunes et al. 2019).

Fortunately, cardiac irAEs are rare but taken together with neurological irAE they are responsible for 50 percent of all fatal events due to immune checkpoint inhibitor therapy (Wang et al. 2018). However, it should be borne in mind that although the incidence rate of irM is often reported as being less and 1%, this is likely to be an underestimate given it often presents in a non-specific and rapidly fatal manner. The non-specific presentation often means that crucial diagnostic confirmation is frequently not obtained and death may be attributed to other causes. Nevertheless, from the data which are available to date, the mortality of irM is almost 50 percent (Mahmood et al. 2018; Wang et al. 2019)

One key feature of irM is its rapid onset after the initiation of immune checkpoint inhibitor therapy. According to Moleshi et al., over two-thirds of patients with irM who develop symptoms do so after the first cycle of immunotherapy. Most of those patients received anti-PD-1 checkpoint inhibition (57 %), a smaller amount of number was treated with combination checkpoint inhibitor therapy (27 %)(Moslehi et al. 2018). Similarly, Liu et. al

(Liu and Wu 2020) reported a mean onset time of 34 days after the first treatment cycle. The timing of treatment cycles is dependent on which immune checkpoint inhibitors are used; every two weeks to monthly for nivolumab (anti-PD-1 antibody), every three weeks for cemiplimab, every 3 to 6 weeks for pembrolizumab (anti-PD-1 antibodies), and every three weeks for combined anti-PD-1 and anti-CTLA-4 immunotherapy. There have been reports that some irAEs may be more common when immune checkpoint therapy is administered at an increased dose to enable a longer interval between cycles. For example, Kähler et al reported that longer dosing intervals are associated with the development of additional adverse events, such as diabetes mellitus type 1 (Kähler et al. 2020). To date, there is no evidence that the interval between cycles affects the risk of developing irM. Nevertheless, late presentations have also been reported after 30 treatment cycles (Escudier et al. 2017), therefore clinicians should remain vigilant and have a high index of suspicion.

In terms of pathology, irM is characterized by the presence of a myocardial CD4+ T cell dominant inflammatory infiltrate (Cooper 2009). Whilst the pathogenesis of irM is incompletely understood, evidence from murine models has shown the importance of immune checkpoints, specifically CTLA-4 and PD-1 expression, in preventing and/or limiting immune-mediated cardiac inflammation. For example, anti-CTLA-4 antibody therapy leads to profound inflammation in myocardial cells in $CD28^{-/-}$ mice (Ying et al. 2010). In addition to that, antagonism of the PD-1 signaling pathway, in a PD-1 deficient mouse model, results in a lymphocytic myocardial infiltrate, predominantly involving $CD4^{+}$ and $CD8^{+}$ T cells (Wang et al. 2010).

The troponin complex is responsible for the contraction of the striated muscles and is based on three sub-units: Troponin C, a calcium-binding protein; Troponin T, the binding protein for tropomyosin and troponin I, which is responsible for the binding to actin and the decrease of the troponin c affinity to calcium (figure 5) (Xu et al. 2013)

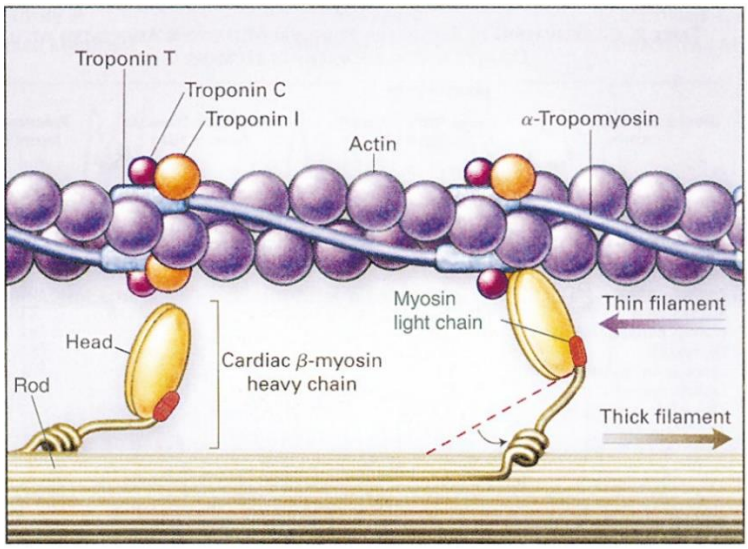


Figure 5: Structure of a sarcomere complex in humans

Source: (Seidman and Seidman 2001)

Damage to cardiomyocytes, whether due to inflammation following the administration of immune checkpoint inhibitors or following ischemia in the context of myocardial infarction, results in the release of Troponin subunits into the circulation, which can be measured by high sensitivity assays (Eggers and Lindahl 2017; Xu et al. 2013). Therefore, serum high sensitivity Troponin measurements, for example, high sensitivity Troponin T (hsTnT), are used in the assessment of irM.

Although irM is reportedly rare, several risk factors have been identified based in the published literature, albeit relying heavily on retrospective analyses and case series. For example, Lyon et. al highlighted the following risk factors (i) the use of combined anti-PD-1 and anti-CTLA-4 immune checkpoint inhibitors, (ii) the presence of pre-existing ischaemic heart disease, and (iii) a history of autoimmune disease (Lyon et al. 2018a). In contrast, Moslehi et al. failed to confirm the presence of pre-existing ischaemic heart disease as a risk factor for irM, but did confirm the increasing incidence of irM over the last few years (Moslehi et al. 2018). Other studies have reported that male patients are more predisposed to developing irM following immune checkpoint inhibitor therapy and that diabetes may represent another important risk factor (Escudier et al. 2017; Mahmood et al. 2018).

Given the frequently non-specific presentation of irM, there is a pressing need for a specific and sensitive diagnostic test. At present, the laboratory and diagnostic work-up consist of an electrocardiogram, echocardiography, serum high sensitivity troponin T (hsTnT) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) measurement, cardiac imaging (magnetic resonance imaging), and ultimately an endomyocardial biopsy. According to Mahmood et. almost all cases of myocarditis are associated with an increased hsTnT level (94 %), abnormal ECG findings (89%), but only 51 percent have an abnormal echocardiogram (Mahmood et al. 2018). The Lake Louise Criteria have become widely accepted for the evaluation of cardiac MRT imaging (Lagan et al. 2018; Luetkens et al. 2014), but the extent to which these criteria can be applied for irM is unclear (Neilan et al. 2018).

The gold standard for the detection of irM remains the endomyocardial biopsy (Atallah-Yunes et al. 2019). Both the detection of myocyte necrosis and lymphocytic infiltration are among the key diagnostic features (Gürdoğan and Yalta 2020). However, even endomyocardial biopsy is not 100% sensitive due to the patchy nature of the lymphocytic infiltrate. Therefore a high degree of expertise is required on the part of the clinician to ensure that a representative area of the endomyocardium is sampled (Kindermann et al. 2012).

Ultimately, given that the clinical presentation of irM is often non-specific and that diagnostic tests have limited sensitivity and specificity, establishing the diagnosis of irM requires a high index of suspicion and a comprehensive evaluation of the clinical, laboratory, and radiological findings (Neilan et al. 2018). Moreover, early recognition and rapid initiation of immunosuppressive therapy is necessary to prevent fulminant cardiac failure and death.

Prompt assessment and appropriate treatment require close collaboration between oncologists and cardiologists. Given the lack of clinical trial data evaluating the treatment of irM, as a consequence of its incidence and frequently fatal clinical course, the management is largely based on the consensus guidelines developed by both the American Society of Clinical Oncology (Brahmer et al. 2018) and the Society for Immunotherapy of Cancer

Toxicity Management Working Group (Weber et al. 2015b). In keeping with the management of other organ-specific irAEs, systemic immunosuppression with corticosteroids plays a central role. Treatment with high-dose intravenous methylprednisolone (1-2 mg/kg or 500-1000mg once daily) is recommended and the patient should be monitored in a coronary care unit to detect and treat potentially fatal arrhythmias. In addition to methylprednisolone, the administration of mycophenolate, infliximab, or anti-thymocyte globulin may be useful (Brahmer et al. 2018; Zhou et al. 2019), particularly in severe and/or treatment-refractory cases.

Therefore, although treatment algorithms are well-established and relatively effective for the majority of irAE, the diagnosis and successful management of cardiotoxicity, particularly immune-mediated myocarditis (irM), still poses a significant challenge. Not only is there a lack of sensitive and specific biomarkers more irM, but there is no reliable way to predict which patients are at most risk of developing irM.

Aims of this thesis

There are currently no established biomarkers that can be used to predict which patients are likely to develop checkpoint inhibitor-mediated cardiotoxicity. The established markers of ischaemic heart disease, namely troponin, creatine kinase (including CK-MB), and NT-proBNP, are neither specific nor sensitive enough to reliably diagnose irM. Nevertheless, immune checkpoint inhibitor therapy guidelines recommend that troponin testing should at least be considered before the initiation of immune checkpoint therapy (Brahmer et al. 2018).

Therefore, the aims of this thesis were fourfold:

1. To determine the incidence of cardiotoxicity in patients with locally advanced and/or metastatic skin cancer who were treated with anti-PD-1, anti-PD-L1, or combined anti-PD-1/anti-CTLA-4 checkpoint inhibitors
2. To assess the extent to which pre-therapeutic serum hsTnT was measured in patients undergoing immune checkpoint therapy for advanced skin cancer.
3. To elucidate whether increased high sensitivity troponin T (hsTnT) values at baseline were associated with an increased risk of cardiotoxicity and identify which factors were associated with increased serum baseline troponin concentrations.
4. Finally, to examine whether increased pre-treatment hsTnT levels correlated with treatment response as measured by overall survival.

Material and methods

Ethical approval for the project was obtained from the Ethics Committee of the University of Luebeck (AZ 20-216, see attachment). The electronic case notes of all patients (121) in whom treatment with immunotherapy was initiated for locally advanced and/or metastatic melanoma and non-melanoma skin cancer between 2018 and 2019 were retrospectively analyzed. All data were collated, anonymized and analyzed after institutional ethical approval was obtained and according to the Declaration of Helsinki principles. In 2019 the routine measurement of baseline pre-immunotherapy treatment hsTnT serum levels was introduced into our departmental standard operating procedure. Data on sex and pre-existing cardiac disease were specifically recorded given that these may be potential risk factors for the development of irM (Escudier et al. 2017; Mahmood et al. 2018) . In addition, age, cancer type, treatment type (anti-PD-1, anti-PD-L1, combined anti-CTLA-4/anti-PD-1) and treatment setting (adjuvant versus palliative) were documented. Baseline echocardiography findings were also recorded. Overall survival (OS) was also calculated and compared between patients with normal versus elevated serum hsTnT levels, calculated from the day of the first applied immunotherapy until the day of exitus of the patient. Finally, the treatment of cardiotoxicity was noted.

To address the aims of this thesis, the following parameters were collated (table 7):

Table 7: Collected parameters

Development of immune related myocarditis	Yes/no
Sex	Male/female
Age	Age in years
Type and stage of skin cancer	Melanoma, Squamous cell cancer or Merkel cell carcinoma and TNM Stage
Immunotherapy setting	Adjuvant/palliative
Baseline echocardiography	Not performed, performed but unremarkable, performed and the findings were pathological
Baseline hsTnT	Not tested, tested without elevation (<14ng/l), tested and elevated (>14 ng/l)
Overall survival	Months (final date of follow up: December 31, 2019)
Diabetes mellitus	Pre-existing diabetes mellitus, no previous diabetes mellitus
BRAF mutations status	BRAF V 600 E/K mutation positive, BRAF V 600 E/K mutation negative

Statistical analysis

Data were collected and integrated into Microsoft Excel (version 2019). All statistical analyses were performed using Microsoft Excel (version 2019) and survival analyses were calculated using GraphPad Prism (version 8). Statistical advise was obtained from the institute of biomedical statistics in Lübeck (“Institut für Medizinische Biometrie und Statistik“). P values < 0.05 were considered statistically significant. For the descriptive analyses bar and pie charts as well as frequency tables were used. Moreover, Kaplan-Meier curves were used to demonstrate the influence on patients´ overall survival. The following test methods were used to determine statistical significance depending on the distribution of the data and whether it was nominal or ordinal.

- Log-rank (Mantel-Cox) test
- Chi-square test
- Mann Whitney test
- Fisher´s exact test

Results

Demographics

Between the 1st of January 2018 and the 31st of December 2019, a total of 121 patients received immune checkpoint therapy in different therapy settings for locally advanced or metastatic melanoma and non-melanoma skin cancer. All patients attended the immunotherapy out-patient unit of the Department of Dermatology and underwent regular clinical examination and laboratory investigation. 81 of those patients were men, and 40 patients were female (figure 6).

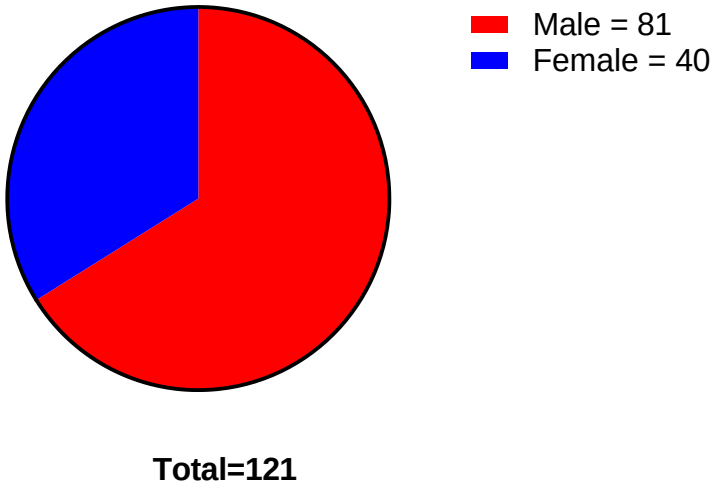


Figure 6: Ratio of men/women

The mean age of the treated patients was 74 years (+/- 15.31 years) (figure 7).

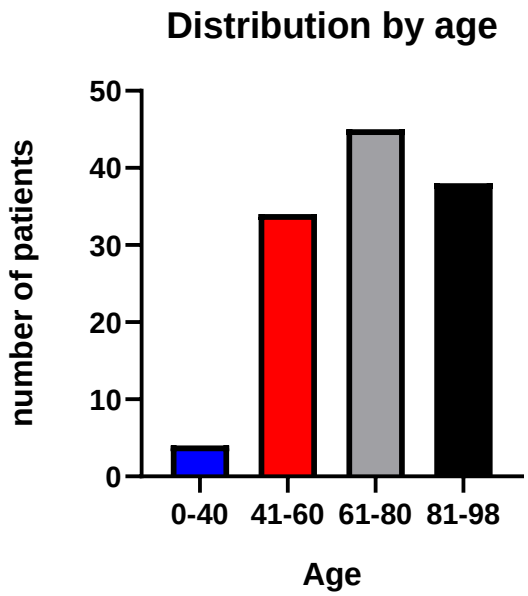


Figure 7: Age distribution

Types of cancer and treatment

Almost all patients were treated for metastatic melanoma (n=116), only two patients received treatment for advanced squamous cell carcinoma and three patients for Merkel cell carcinoma (figure 8).

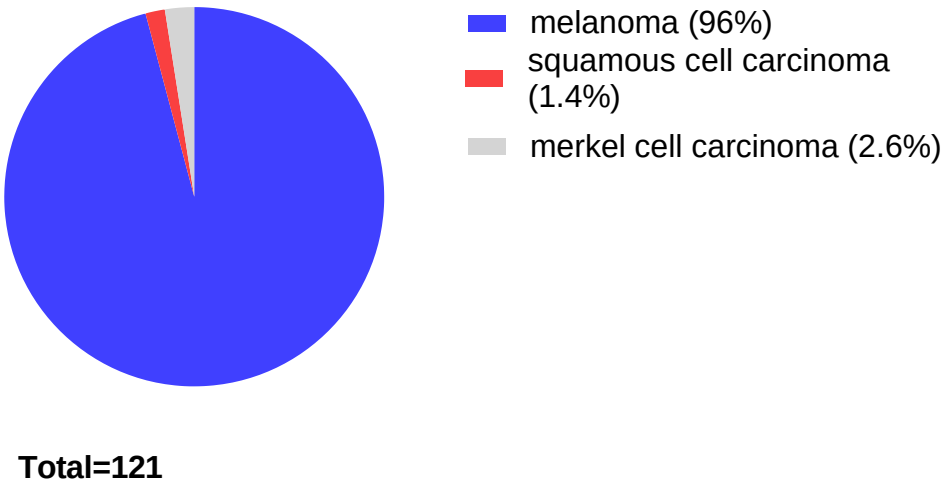


Figure 8: Cancer types

Of the 116 patients suffering from metastatic melanoma, 77 received immune checkpoint therapy in the palliative setting, and 39 received the treatment in the adjuvant setting (figure 9).

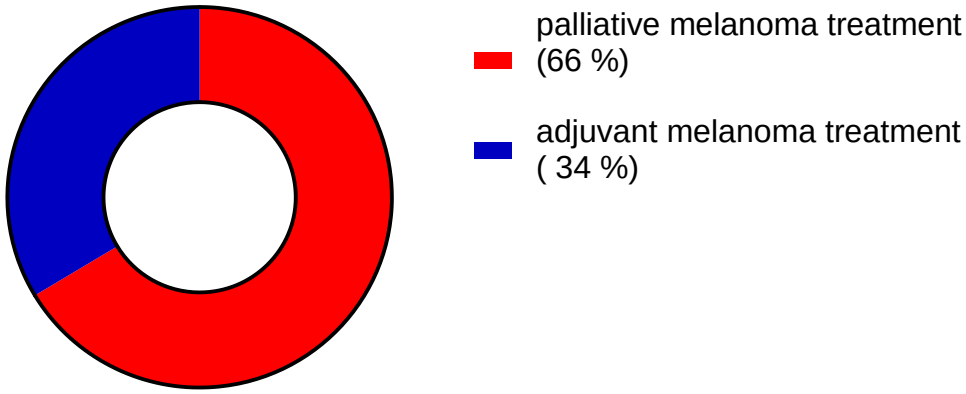


Figure 9: Palliative vs. adjuvant melanoma treatment

From 77 patients receiving palliative treatment for metastatic melanoma, 47 (61 %) received combined anti-CTLA-4 and anti-PD-1 therapy, with the remaining patients receiving monotherapy with pembrolizumab (9) or nivolumab (21) (figure 10). 5 patients with non-melanoma skin cancer were treated with immune checkpoint inhibitors, 2 with locally advanced squamous cell carcinoma (cemiplimab, anti-PD-1) and 3 based on metastatic Merkel cell carcinoma (avelumab, anti-PD-L1).

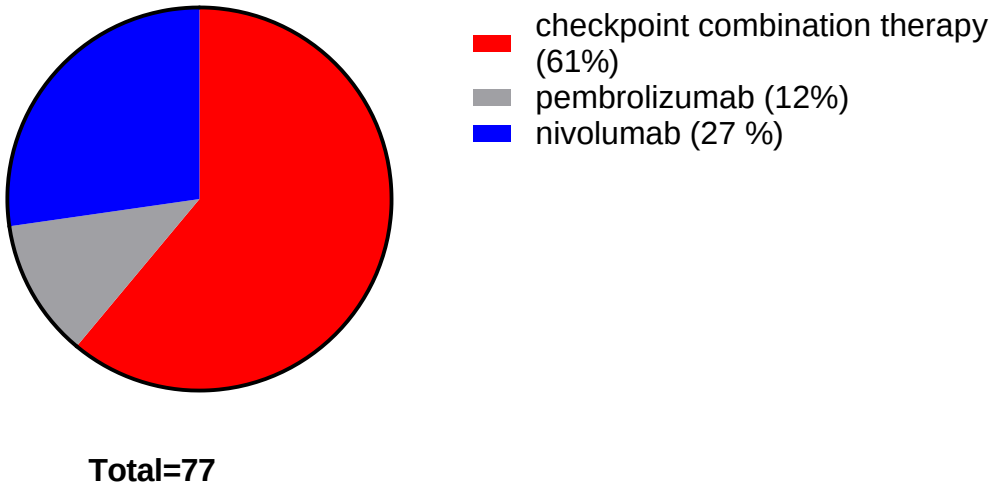


Figure 10: Palliative melanoma treatment

Aim number one

To determine the incidence of cardiotoxicity in patients with locally advanced and/or metastatic skin cancer who were treated with anti-PD-1, anti-PD-L1 or combined anti-PD-1/anti-CTLA-4 checkpoint inhibitors"

As expected, the overall incidence of cardiotoxicity in general and irM in particular within our patient cohort was very low; consistent with the published literature (Mahmood et al. 2018). Only one patient developed cardiotoxicity, namely severe irM, resulting in an overall incidence of 0.8% and 2 % of those in whom pre-treatment hsTnT was measured. This patient underwent treatment with cemiplimab (dose: 350 mg) due to a locally advanced squamous cell carcinoma (figure 11 and figure 12). Inoperability had been established in the multi-disciplinary tumour board given that complete removal of the tumour with adequate safety margins was technically not feasible. Curative radiotherapy was not possible due to the site of the tumor. Therefore, the multi-disciplinary tumour board recommended treatment with the PD-1 inhibitor cemiplimab.

Figure 11: 3 x 3 cm solitary hyperkeratotic tumour with central ulceration

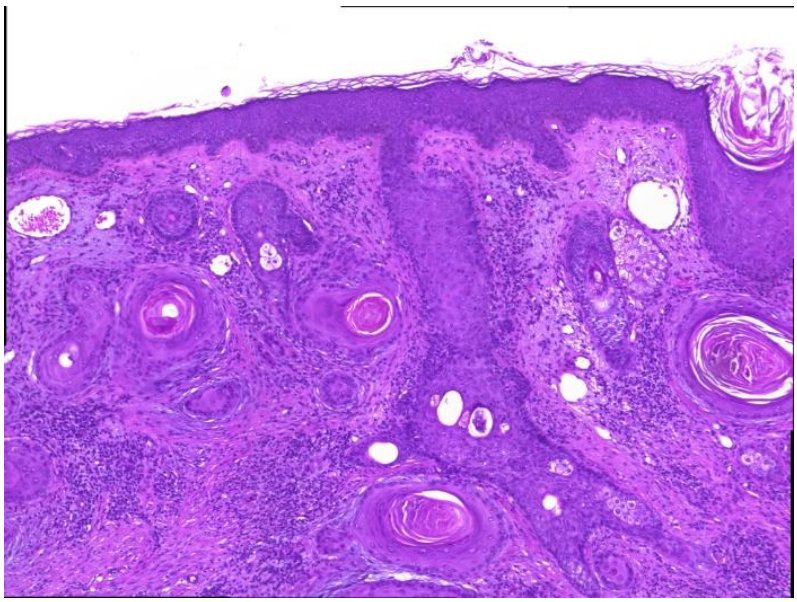


Figure 12: Moderately differentiated squamous cell carcinoma (H&E staining, 200x)

16 days after the first dose of cemiplimab the patient presented to the out-patient department with fatigue, malaise, and generalized myalgia. Routine blood investigations revealed a significantly elevated serum hsTnT with 457 ng/l (normal range: <14ng/l). Prior

to treatment the baseline hsTnT was 6.9 ng/l. As part of the initial cardiological work-up, an ECG was performed which was essentially unremarkable (figure 13).

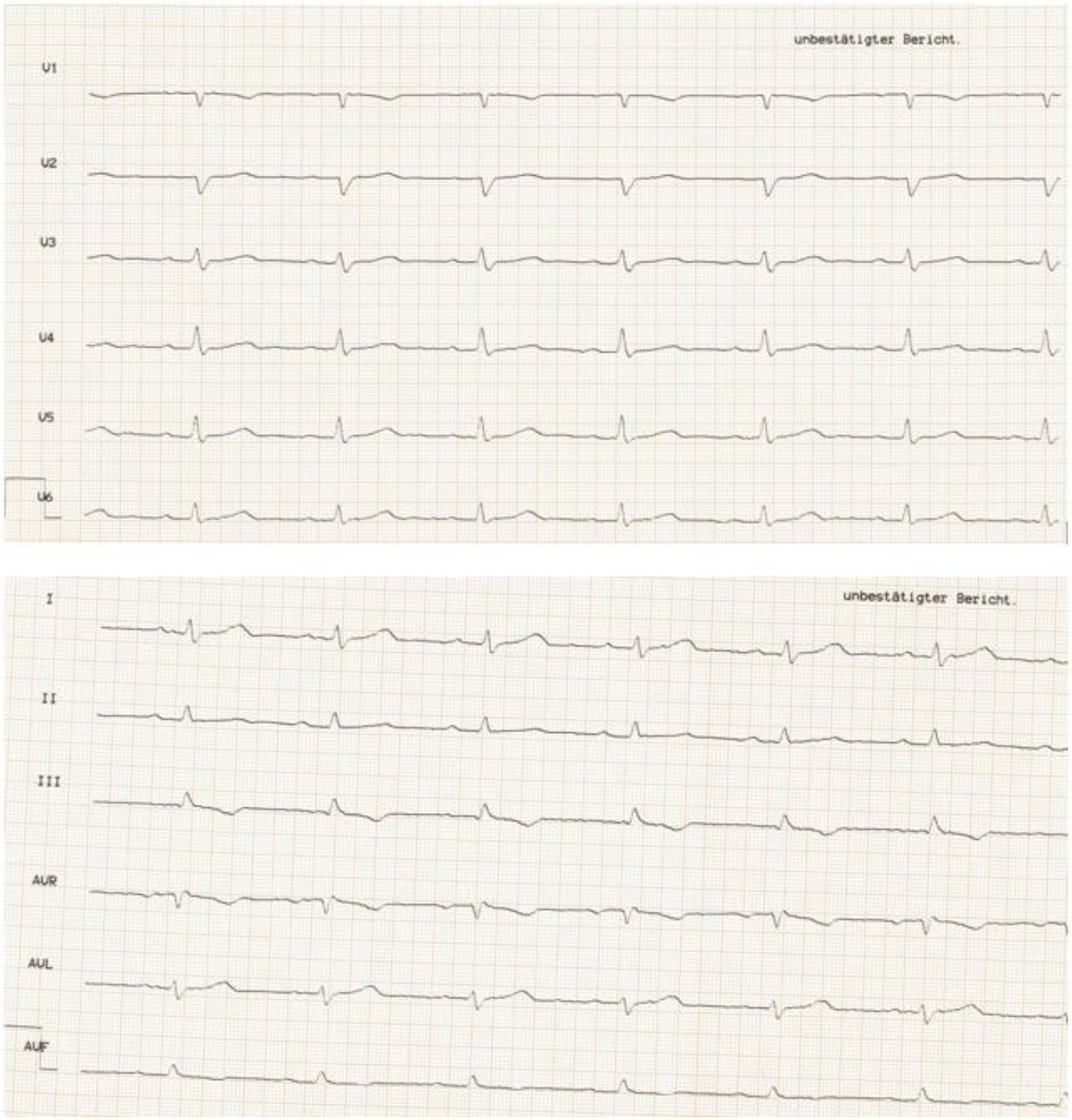


Figure 13: The initial ECG was within normal limits.

Creatine kinase and NT-proBNP were also elevated at 4596 U/L (normal range: <170 U/L) and 901 pg/ml (normal range: <125 pg/ml) respectively. A coronary angiography within 48

hours of admission and revealed a chronic 50% stenosis of the left anterior descending artery (LAD); there was no evidence for acute coronary ischemia (figure 14)

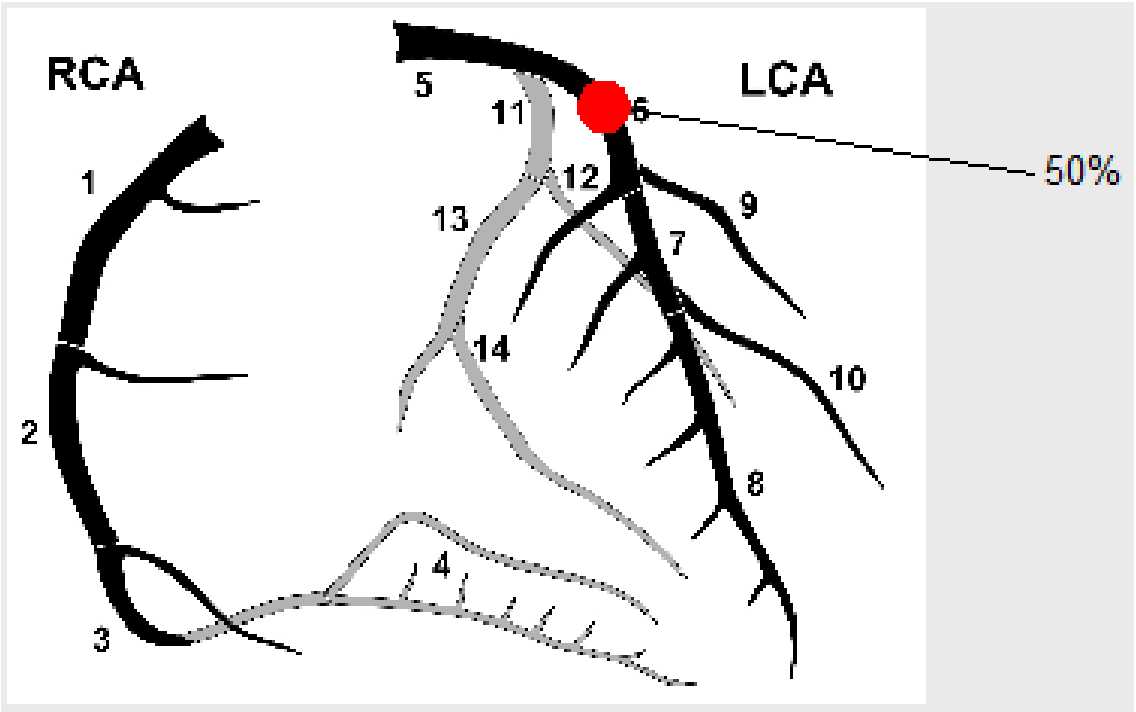


Figure 14: Coronary angiography revealed a moderate chronic proximal stenosis in the LAD which did not account for the elevated serum hsTnT concentrations

The patient's hsTnT and NT-proBNP levels peaked at 2238 ng/l (normal limit <14) and 1366 pg/ml (normal limit <486) at 32 and 44 days after the first dose of cemiplimab. Based on the clinical suspicion of irM the patient was admitted to the coronary care unit for monitoring and high-dose immunosuppression (predisolone 2 mg/kg). The patient declined to undergo an endomyocardial biopsy, but cardiac MR imaging demonstrated focal transmural, almost

global subendocardial myocardial oedema and an epi- to mid-myocardial enhancement with apical pericardial involvement laterally, consistent with irM (figure 15).

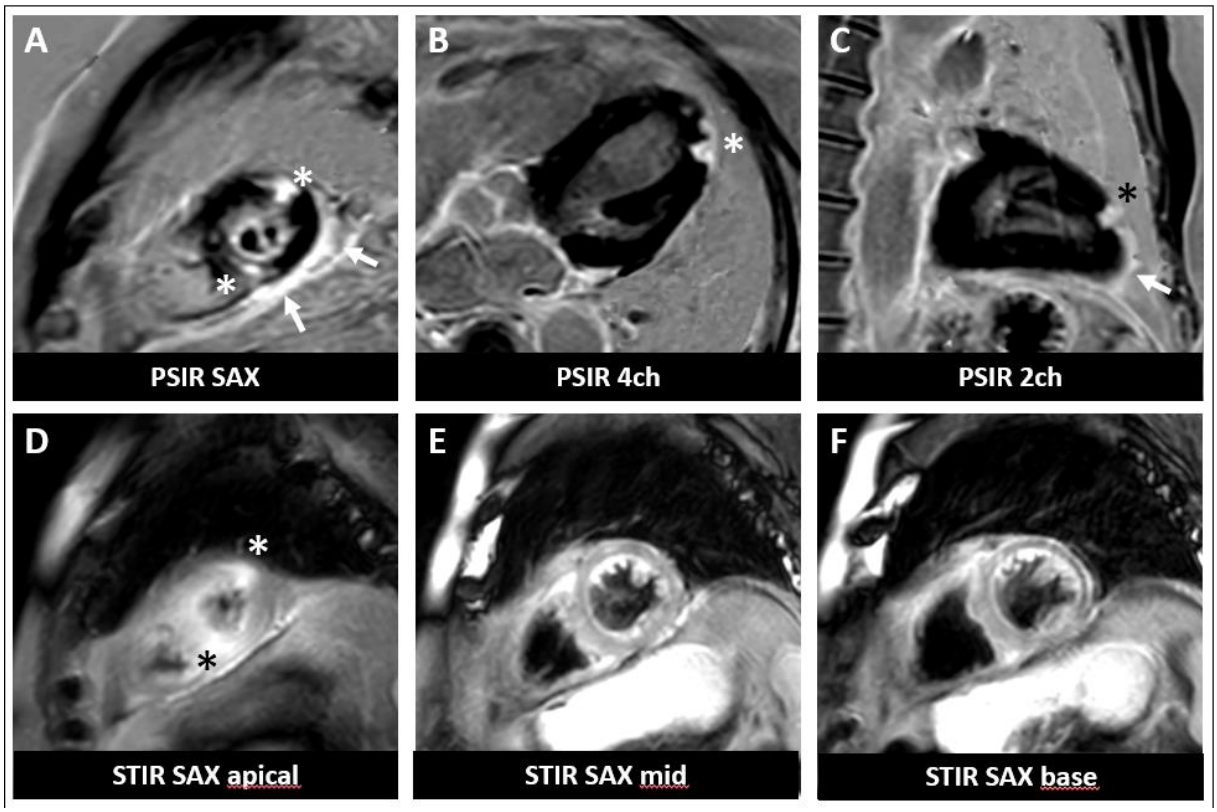


Figure 15: Cardiac MR revealed focal subepicardial to midmyocardial delayed gadolinium enhancement (A-C) associated with edema (D-F) at the lateral and inferoseptal apex (asterisks) involving the pericardium (arrows) in a delayed gadolinium enhancement

Despite an initial improvement with high-dose i.v. prednisolone the patient's condition deteriorated. Immunosuppressive therapy was intensified with the addition of mycophenolate mofetil (3g/d). Repeat electrocardiography 6 weeks after admission revealed persistent abnormalities as a consequence of irM, namely mitral value regurgitation and apical hypokinesia. Pre-treatment echocardiography was unremarkable. The patient also developed ECG abnormalities (figure 16).

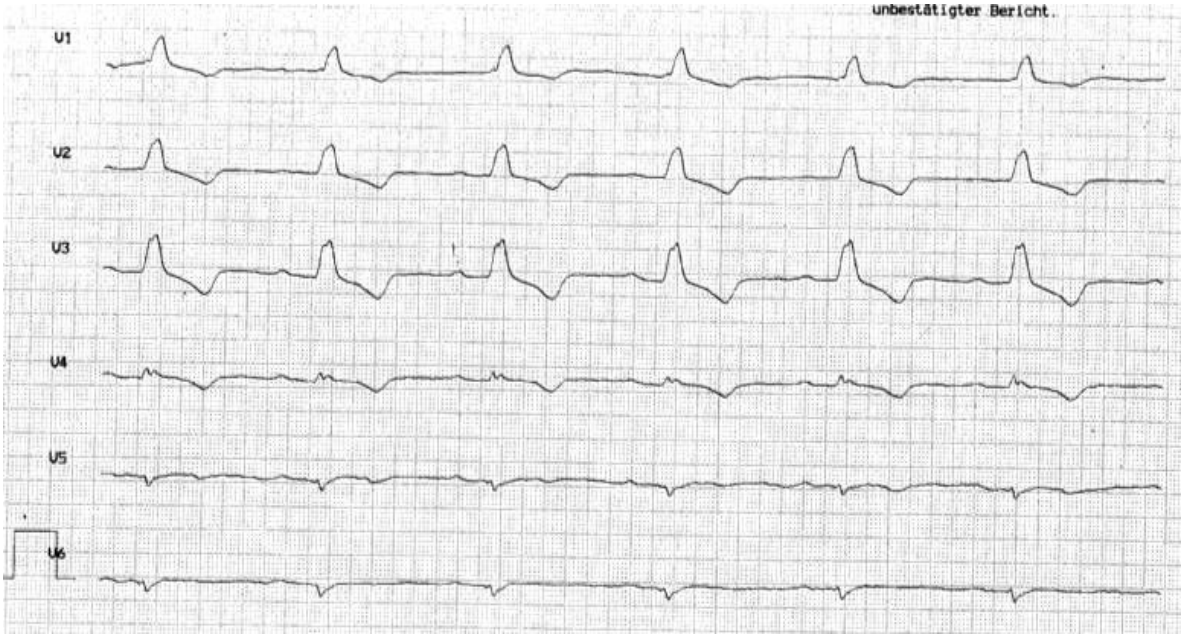


Figure 16: ECG during the 6th week in the hospital with several abnormalities (right bundle branch block)

Despite a complicated clinical course, marked by staphylococcal sepsis followed by reactivation of cytomegalovirus infection due to immunosuppression, the patient's condition stabilized to such an extent that he could be discharged for further in-patient rehabilitation therapy after a hospital stay of 61 days. Unfortunately, he died 129 days after the first dose of cemiplimab (Figure 17). Importantly, the patient had no history of renal impairment, diabetes, or cardiac disease, and pre-treatment ECG, echocardiogram, serum hsTnT, and CK concentrations were all within normal limits. The patient's only likely risk factor for irM was being male.

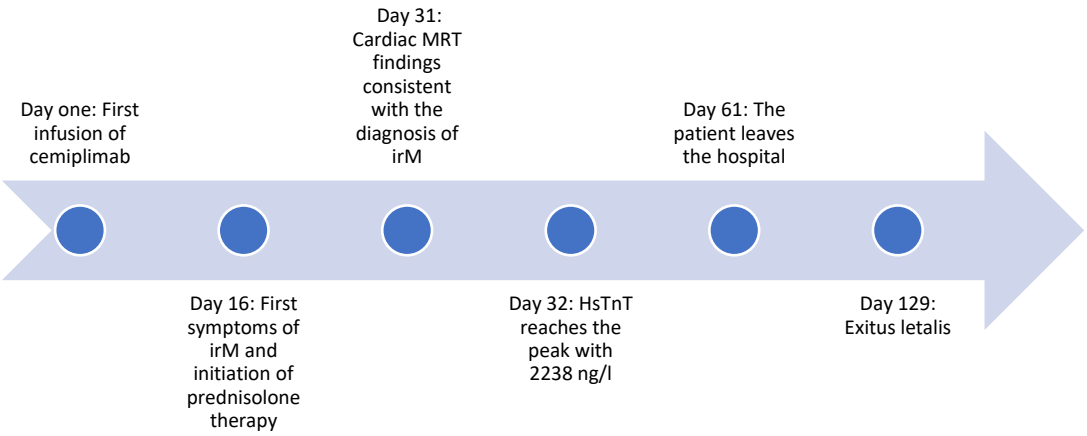


Figure 17: Timeline of the patient suffering from irM

Overall, 56 out of 121 patients had pre-existing cardiac comorbidities prior to the initiation of immune checkpoint inhibitor immunotherapy (figure 18), including atrial fibrillation, coronary heart disease, structural heart disease, or heart failure (figure 19).

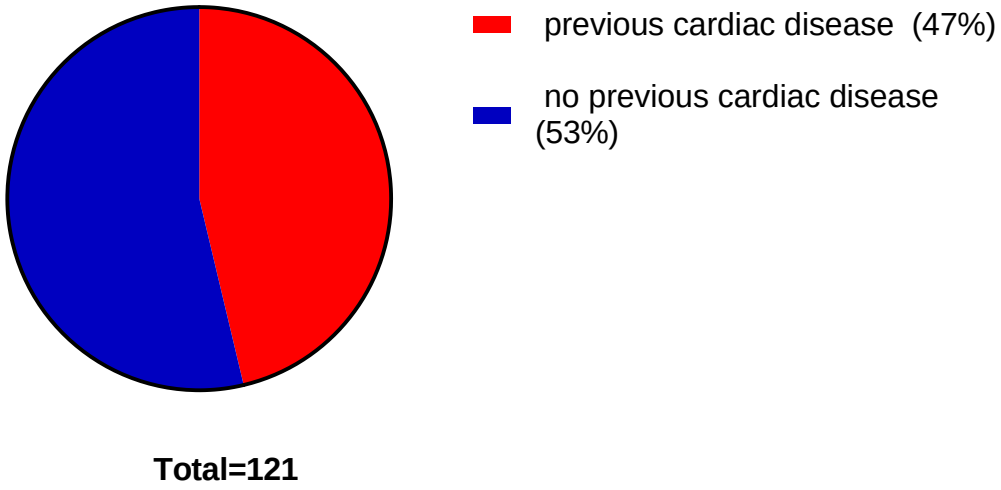


Figure 18: Ratio of previous cardiac disease/no previous cardiac disease

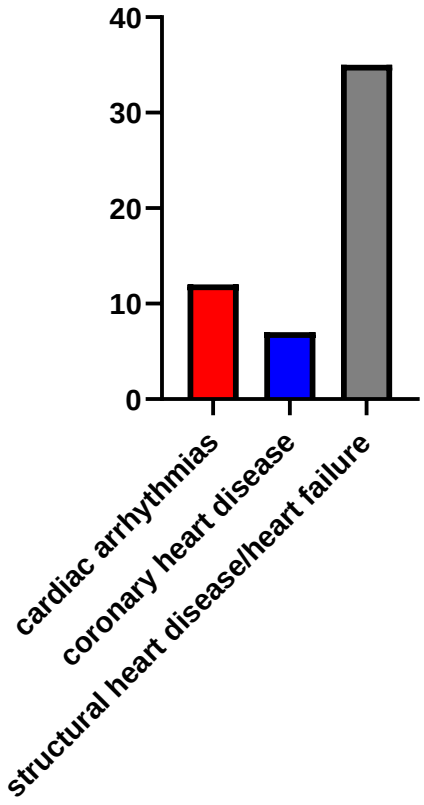


Figure 19: Pre-existing cardiac disease

All in all, baseline echocardiography was performed for 59 patients. Abnormalities were detected in 33 patients (figure 20). Among those were the common abnormalities such as heart failures, valvular stenosis, and arrhythmias.

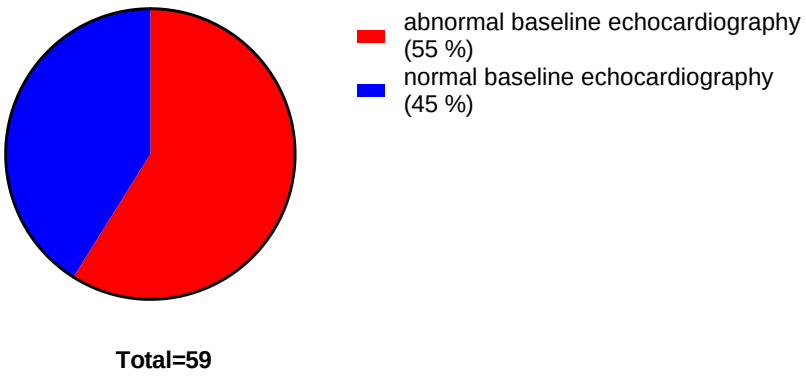


Figure 20: Ratio of normal/abnormal baseline echocardiography

Aim number two

"*To assess the extent to which pre-therapeutic hsTnT was measured in patients undergoing immune checkpoint therapy for advanced skin cancer."*

Measurement of pre-treatment serum hsTnT concentration was introduced in 2019. Results were available for 47 patients (table 8). HsTnT was measured using the Elecsys Assay (Roche) according to the manufacturer's instructions. Elevated serum hsTnT levels were detected in 28 % of the patients (n=13) (table 8). The mean serum concentration was 25 ng/l +/- 10.6 (range: 14.0 – 50.7) All patients had asymptomatic hsTnT elevations. The mean age of the patients in whom hsTnT was measured was 78 years +/-8.8 (range: 53 – 88). All of the patients with elevated serum hsTnT concentrations had metastatic melanoma. 9 were treated in the palliative setting, 4 patients were treated in the adjuvant setting. 6 patients received combined checkpoint therapy, 6 received nivolumab monotherapy and one patient received pembrolizumab monotherapy. Only two patients had a BRAF mutation. The mean baseline creatinine-score was 105. 8 umol/l +/- 30.24 (69 – 180 umol/l). 10 out of those 13 patients had a previous cardiac disease. 7 of those patients had abnormalities in the baseline echocardiograph which were mostly complementary for the pre-existing cardiac heart disease, such as aortic stenosis, heart failure, and arrhythmias. Moreover, 4 had coexisting/previous diabetes mellitus type 2 disease.

Baseline serum hsTnT levels were within the normal range in 34 patients (table 8). The mean age of this patient cohort was 61.4 years +/-13.36 (range: 23 – 85 years). 33 of those patients had metastatic melanoma and one patient had a locally advanced squamous cell carcinoma. The mean hsTnT score was 7.04 ng/l +/-2.3 (range: 5 – 13.4 ng/l). The mean baseline creatinine score was 79.1 umol/l +/- 13.41 (range: 54 – 110 umol/l). 18 patients were treated in the adjuvant therapy setting and 16 patients were treated in the palliative therapy setting. 13 of those patients received the combination checkpoint therapy, seven patients received nivolumab monotherapy, 13 patients were treated with pembrolizumab and one patient had therapy with cemiplimab. Echocardiography was performed prior to

treatment initiation in 16 patients; 3 patients had abnormal ECGs and the ECGs were within normal limits in the remaining patients. The BRAF V-600 (E/K) mutation was present in 13 patients (38 %). Only one patient had diabetes mellitus type 2. One patient in the normal baseline hsTnT cohort developed severe irM.

Table 8 Summary of patients´ baseline characteristics

Patients´ baseline characteristiscs	Troponin elevated	Troponin normal
Sex		
Male	9	22
Female	4	12
Age		
Mean (years)	78	61.4
Range (years)	53 - 88	23 - 85
Tumor type		
Melanoma	13	33
Squamous cell carcinoma	0	1
Baseline hsTnT		
Mean (ng/l)	25.5	7.04
Range (ng/l)	14 - 50.7	5 - 13.4
Baseline Creatinine		
Mean (µmol/l)	105.8	79.1
Range (µmol/l)	69 - 180	54 - 110
Echocardiography		
Abnormal echocardiography	7	3
Normal echocardiography	2	13
Not performed	4	18
Previous cardiac disease		
	10 out of 13	8 out of 34
Immunotherapy		
First combinated therapy, afterwards PD-1 Inhibitor	6	13
Nivolumab monotherapy	6	7
Pembrolizumab monotherapy	1	13
Cemiplimab	0	1
BRAF mutation		
BRAF positive	2	13
BRAF negative	11	21
Diabetes mellitus		
Co-existing Diabetes mellitus Type 2	4	1
No history of Diabetes mellitus Type 2	9	33
Therapeutic setting		
Adjuvant	4	18
Palliative	9	16
immunotherapy related myocarditis (irM)		
Events	0	1

Aim number three

"*To elucidate whether increased high sensitivity troponin T (hsTnT) values at baseline were associated with an increased risk of cardiotoxicity and identify which factors were associated with increased serum baseline troponin concentrations.*"

Importantly none of the patients with an elevated baseline hsTnT developed any cardiac irAEs. As described, one patient with a normal serum baseline hsTnT concentration developed irM despite the absence of pre-existing heart disease or diabetes. All patients with an elevated hsTnT underwent a full cardiological workup, including serial hsTnT measurements, ECG, echocardiography, and assessment by a cardiologist. There was no evidence of acute ischemia in any of these patients and none of the patients in this group required coronary angiography. The resultant cardiological assessment did not delay the initiation of immune checkpoint inhibitor therapy.

Both pre-existing diabetes mellitus (figure 21) and previous cardiac disease (figure 22) were highly associated with an elevated serum hsTnT, p= <0.0001 and 0.0017 respectively, using the Fisher-Test.

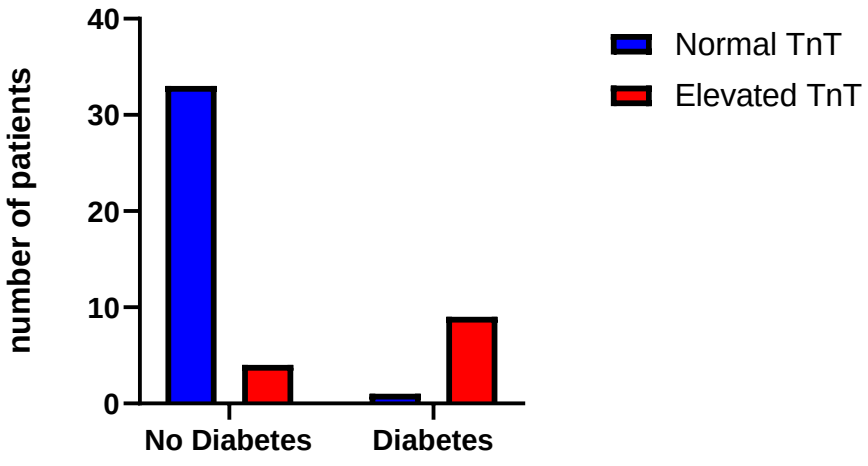


Figure 21: Preexisting diabetes mellitus and hsTnT

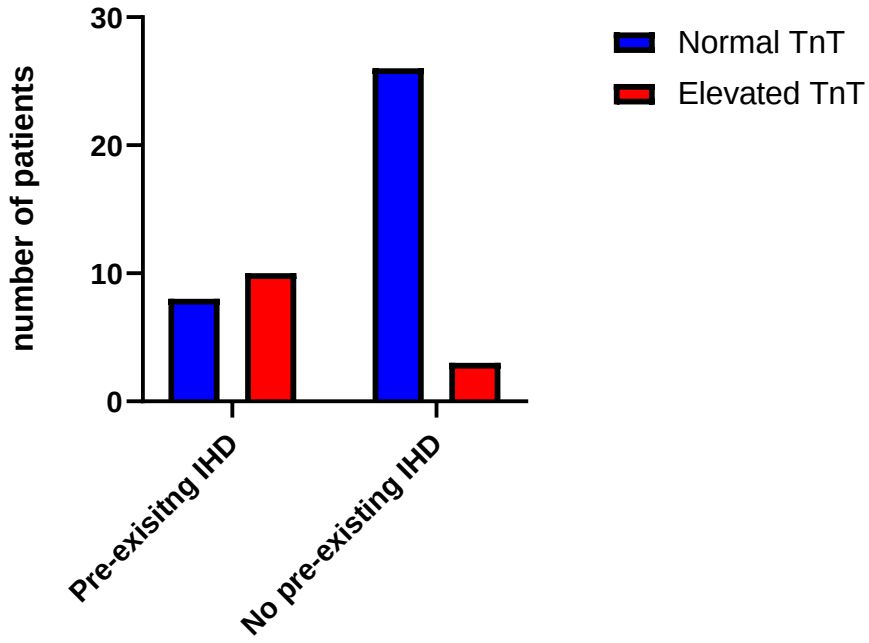


Figure 22: Baseline hsTnT and pre-existing idiopathic heart disease (IHD)

There was no correlation between baseline hsTnT levels and sex (figure 23) or BRAF status (in patients with melanoma) (figure 24), p>0.05 using Fisher Test. 31 of those patients were male and 16 were female.

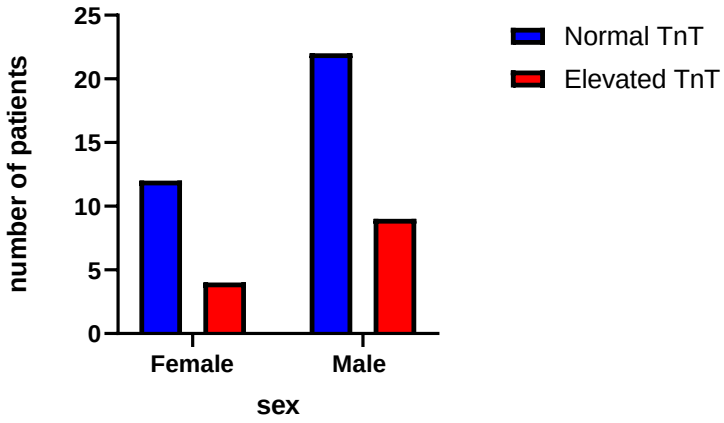


Figure 23: Baseline hsTnT according to sex

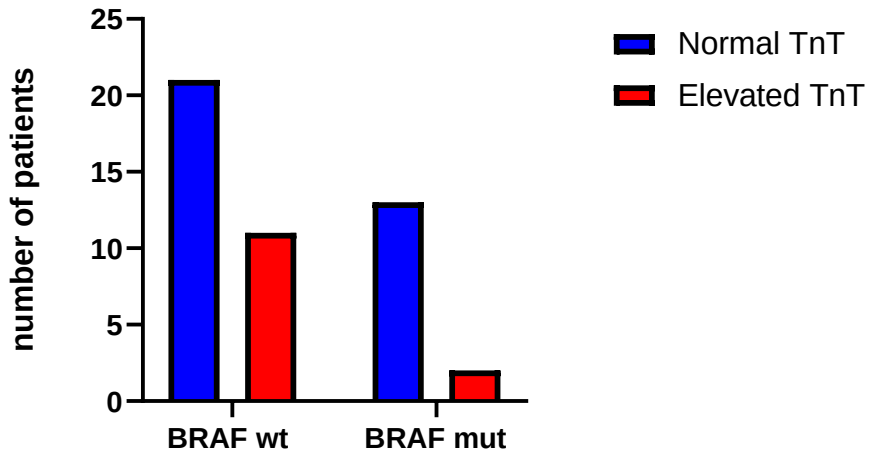


Figure 24: Baseline hsTnT and BRAF-mutation

Patients with an elevated baseline hsTnT concentration were significantly older (figure 25 ,p=0.0001, Mann Whitney Test) in contrast to the patients with a normal baseline hsTnT, and had a significantly higher serum creatinine concentration (p = 0.0007, Mann Whitney Test) (figure 26). Finally, there was (figure 27, figure 28) a significant correlation between both baseline hsTnT and baseline serum creatinine concentration (p=0.0034, r=0.41*)*, and between baseline hsTnT and age (p=0.0011, r=0.4625).

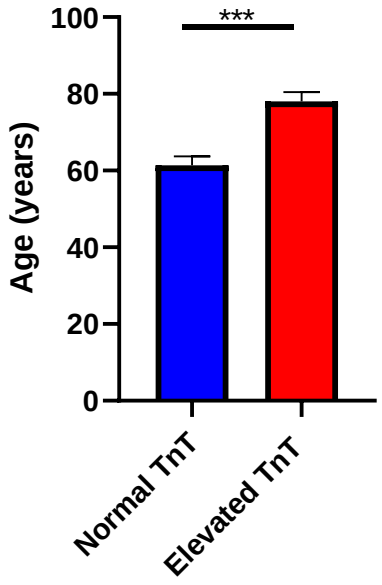


Figure 25: Baseline hsTnT depending on the age (***p<0.001)

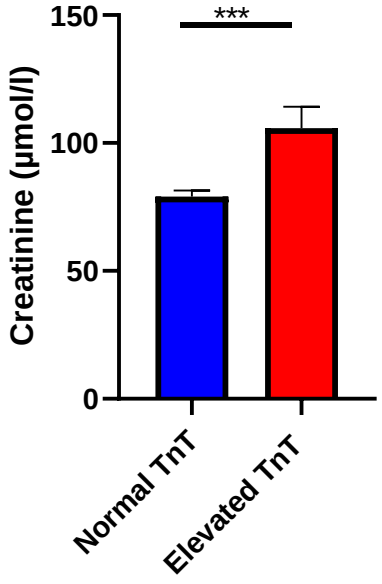


Figure 26: Baseline hsTnT depending on creatinine scores (***p<0.001)

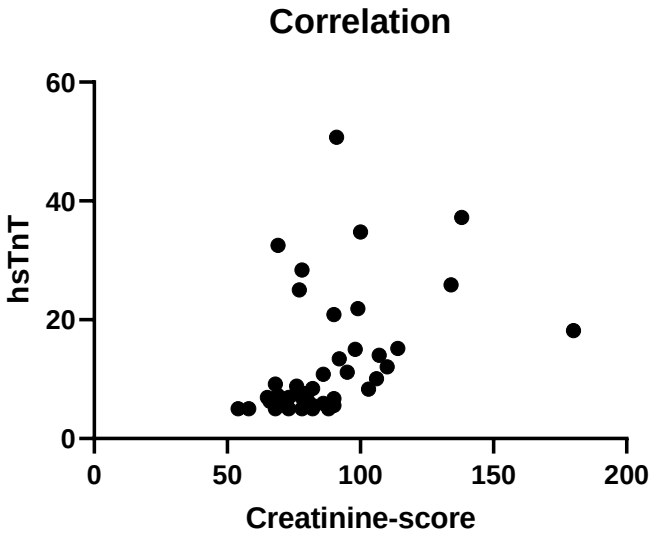


Figure 27: Correlation between baseline hsTnT and baseline creatinine (p=0.0034, r=0.41)

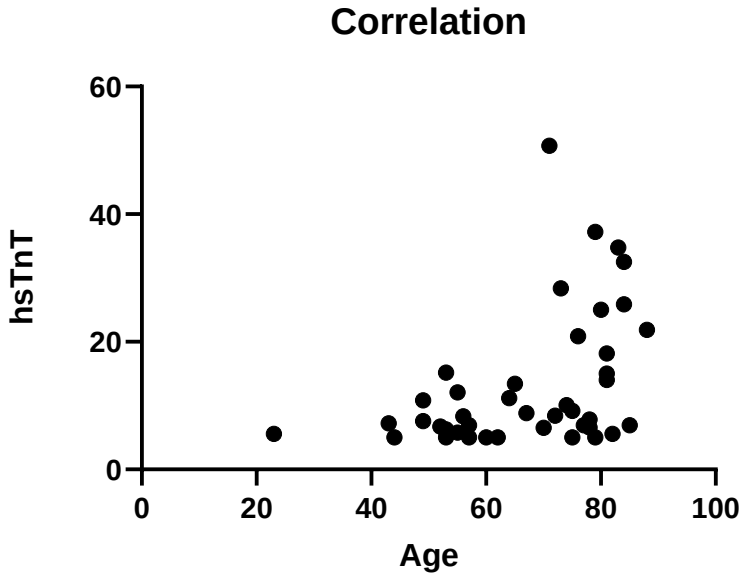


Figure 28: Correlation between baseline hsTnT and age (p=0.0011, r=0.4625)

There was no correlation (figure 29) between treatment setting (adjuvant versus palliative) and elevated baseline serum hsTnT concentrations (p<0.21, Fisher Test).

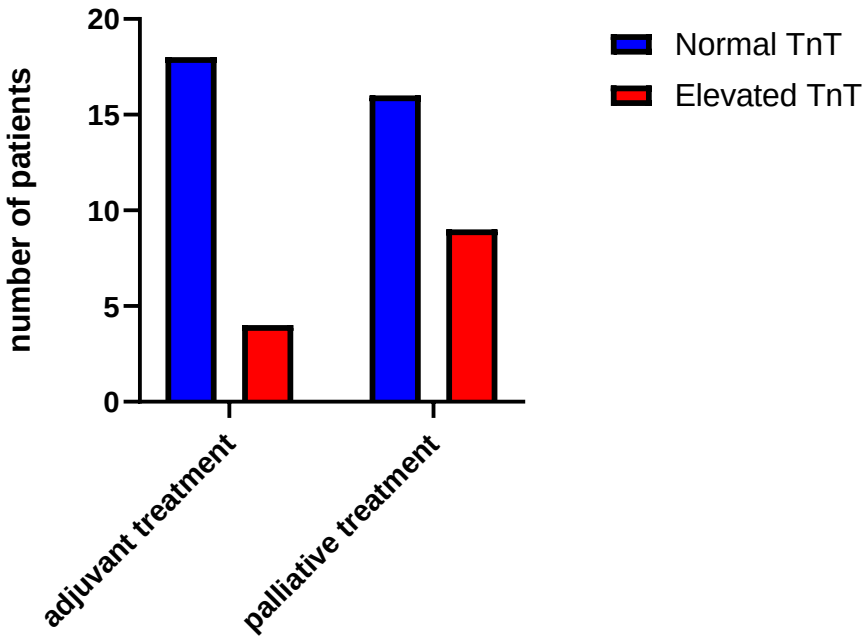


Figure 29: Baseline hsTnT depending on an adjuvant or palliative therapy regime

Patients with an elevated baseline hsTnT were more likely to have abnormal echocardiography findings where these were available. The result was statistically significant, $p<0.001$ (Fisher Test) (figure 30).

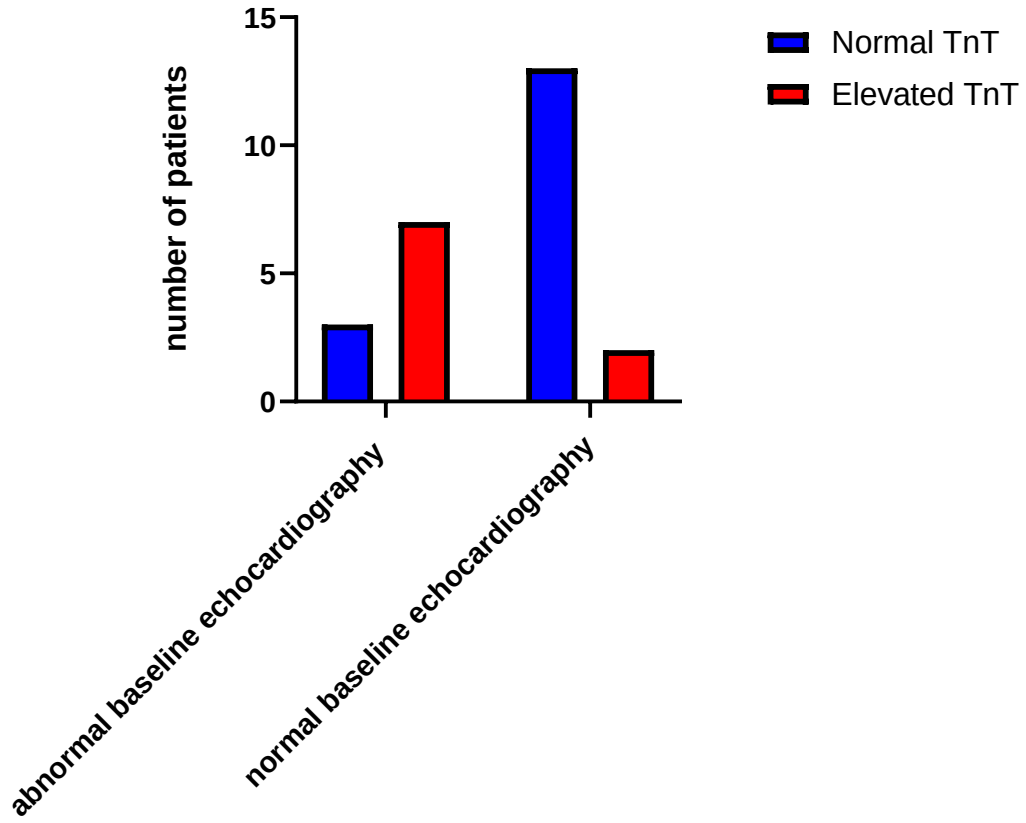


Figure 30: Baseline hsTnT and the ratio of abnormal/normal baseline echocardiography

Aim number four

The next lines refer to aim number four: "*To examine whether increased pre-treatment hsTnT levels correlated with treatment response as measured by overall survival. "*

HsTnT did not show any effect concerning overall survival, even though specific changes in cancer survival were not expected due to the more or less short follow-up period (figure 31).

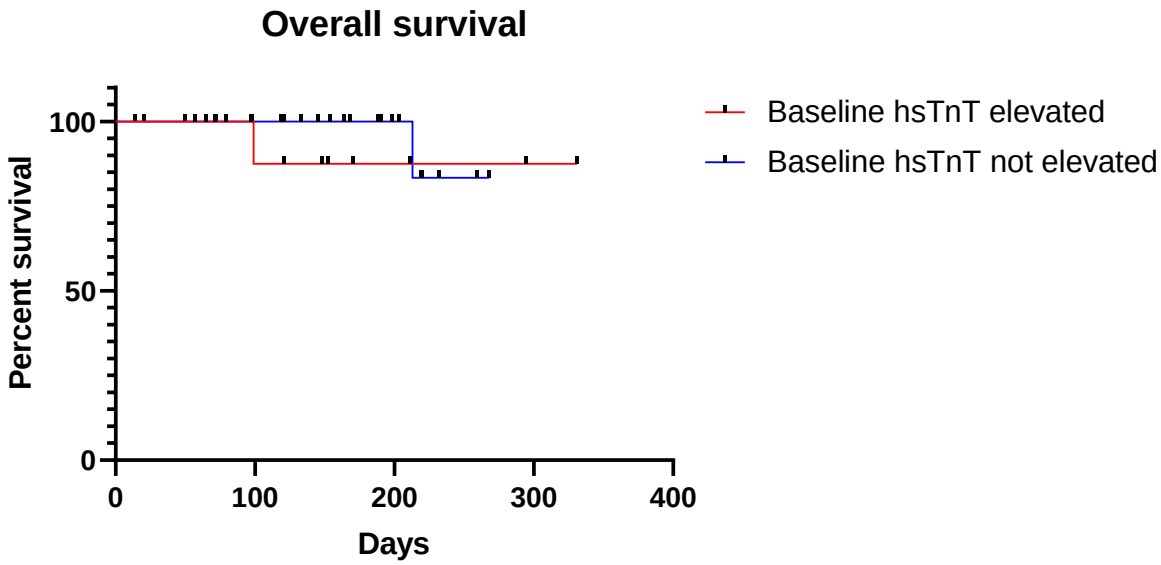


Figure 31: Overall survival was not significantly different between the elevated and normal hsTnT groups.

There was no correlation between overall survival and baseline serum hsTnT concentration.

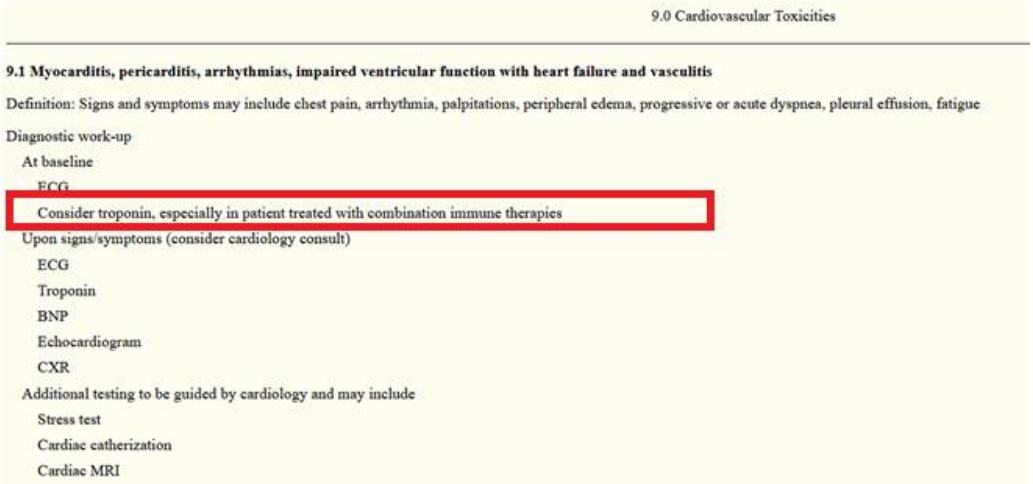
Discussion

Checkpoint inhibitor-based immunotherapy has been shown to produce a significant and enduring anti-tumor response in a range of locally advanced and metastatic skin cancers (Larkin et al. 2015). In addition to the drastic improvements in OS and PFS, the widespread and increasing application of immune checkpoint inhibitor, not only in the palliative setting but also in the adjuvant and the neo-adjuvant setting, will inevitably result in an increase in the number of irAE, some of which will be life-threatening and permanent (Ansari-Gilani et al. 2020). The early detection of such serious irAE, to commence immunosuppressive treatment with minimal delay, remains a key challenge. Early recognition and specific treatment for patients suffering from irM is essential to maximize the patients´ chances of survival (Mahmood et al. 2018).

To this end, given that the measurement of pre-treatment hsTnT should be considered in patients due to undergo immune checkpoint inhibitor therapy, the extent to which this was performed in dermato-oncology patients was reviewed. During 2018 and 2019, out of 121 patients who underwent immune checkpoint inhibitor therapy, pre-treatment serum hsTnT levels were measured in 38 % (n=47). Given that measurement of baseline, hsTnT was incorporated into our standard operating procedure in 2019, the level of compliance reached 75%.

The incidence of cardiotoxicity, specifically irM, was then determined in our cohort of 121 patients with a variety of metastatic and/or locally advanced skin cancers who underwent immune checkpoint inhibitor therapy between January first, 2018, and December 31 in 2019. IrM only occurred in one patient. Baseline hsTnT was measured in 47 patients and was elevated in over 28%, but was normal in the patient who developed fulminant irM. Elevated serum hsTnT levels were associated with chronic renal failure ($p=0.02$) and diabetes mellitus ($p<0.0002$). Given that almost one-third of patients had asymptomatically elevated hsTnT levels, routine measurement resulted in additional hospital visits, frequently without therapeutic consequences.

Although the molecular mechanisms underlying the development of irM are poorly understood, the incidence and time to occurrence in our cohort were in keeping with the published literature. Namely, the incidence was 0.8% and the time to the occurrence was 16 days, after only a single treatment administration. This typical early incidence has led to speculation that irM may reliant upon pre-existing cardiac disease, backed up by reports that its incidence is increased in patients with the classical cardiovascular risk factors (Ganatra and Neilan 2018; Lyon et al. 2018b). Indeed, the American Society of Clinical Oncology (ASCO) has recommended that clinicians consider measuring baseline serum pre-treatment hsTnT levels, especially if combined checkpoint therapy with anti-CTLA-4 and anti-PD-1 is planned (Brahmer et al. 2018) (figure 32).


9.0 Cardiovascular Toxicities

9.1 Myocarditis, pericarditis, arrhythmias, impaired ventricular function with heart failure and vasculitis

Definition: Signs and symptoms may include chest pain, arrhythmia, palpitations, peripheral edema, progressive or acute dyspnea, pleural effusion, fatigue

Diagnostic work-up

At baseline

ECG

Consider troponin, especially in patient treated with combination immune therapies

Upon signs/symptoms (consider cardiology consult)

ECG

Troponin

BNP

Echocardiogram

CXR

Additional testing to be guided by cardiology and may include

Stress test

Cardiac catherization

Cardiac MRI

Figure 32: Suggested pre-therapeutic diagnostic work up, as proposed by the American Society of Clinical Oncology (ASCO)

However, there is a distinct lack of "real-world" data to support this strategy and to determine its utility in identifying patients who will go on to develop irM. In fact, Sarocchi et al. reported that serum TnT elevations in patients undergoing anti-PD-1 treatment for lung cancer were not associated cardiotoxicity in 80% of cases (Sarocchi et al. 2018). In our retrospective analysis of all patients, hsTnT was elevated in 28 % of patients tested and was not associated with the development of irM. As expected, less than 1% of patients developed irM and in this case the baseline hsTnT, ECG and echocardiography were within normal limits. The patient's past medical history was also negative for diabetes mellitus and pre-

existing heart disease. Heinzerling et. al reported an increased risk irM in the presence of cardiac diseases in a retrospective case series. (Heinzerling et al. 2016). The only identifiable potential risk factor for irM in this patient was being male (Escudier et al. 2017; Postow et al. 2018). To date, there is only one other case of cemiplimab associated irM reported in the literature (Jeyakumar et al. 2020).

The majority of the published cases of irM following immunotherapy are in patients with melanoma, specifically, those treated with ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), or pembrolizumab (anti-PD-1) (Mahmood et al. 2018; Moslehi et al. 2018). Both cases of cemiplimab associated irM reported to date occurred in the absence of pre-existing heart disease. This observation raises the question of whether there is a difference in the pathophysiology of irM in patients being either treated with cemiplimab when compared to irM in patients treated with combined immunotherapy and whether the risk of irM is in fact both treatment and tumour-specific. Future prospective studies are needed to address these important clinical questions to counsel patients regarding the risk and to maximize patient safety.

In terms of treatment, the mainstay of therapy for irM is the early initiation of high-dose intravenous corticosteroids. The use of additional immunosuppressive therapies also always bears an additional risk of opportunistic infection, such as aspergillus pneumonia, cytomegalovirus reactivation, resulting in colitis and hepatitis, and pneumocystis jerovcei pneumonia (Arriola et al. 2015; Uslu et al. 2015); Whilst the life-saving benefits of immunosuppression outweigh the risks of potential opportunistic infection, the duration of immunosuppression must be carefully weighed against the risk of the promotion of tumour progression via impaired tumour immune surveillance and antagonism of immune checkpoint inhibition.

In 28 % of our patients who underwent baseline hsTnT measurement, serum concentrations were elevated. This resulted in an extensive cardiological work-up, often on an emergency basis. Part of the definition of acute myocardial infarction relies on the demonstration of an acute elevation in serum hsTnT concentrations (Thygesen et al. 2018). In contrast to acute

myocardial infarction, immunotherapy-related myocarditis is defined as structural heart disease, more precisely a myocardial injury without an acute atherothrombotic event; obviating the need for angiography and reperfusion. In our cases, an elevated serum hsTnT concentration often necessitated presentation in the emergency department for cardiological assessment to exclude acute ischaemia. This was excluded in all of our patients and no patient underwent coronary angiography. All of the patients received their immunotherapy as planned (table 9).

Table 9: Cardiological assessment in patients with elevated hsTnT levels

Patient	Initial hsTnT (ng/l) normal range < 14 ng/l	Follow-up hsTnT (ng/l) normal range < 14 ng/l	NT-proBNP (ng/l) normal range < 486 ng/l	ECG	Echocardiography performed	Creatinine (µmol/l) normal range 59-104µmol/l	Cardiological Evaluation
1	37.2	36	-	No evidence of acute ischaemia	yes	138	hsTnT elevation due to chronic renal impairment
2	15	13.2	-	No evidence of acute ischaemia	yes	98	hsTnT due to pre-existing cardiac disease
3	25.9	18.9	1654	No evidence of acute ischaemia	yes	134	hsTnT due to pre-existing cardiac disease/chronic renal impairment
4	25	23.2	-	No evidence of acute ischaemia	no	77	No evidence of ischaemic heart disease
5	34.8	34.0	4081	No evidence of acute ischaemia	yes	100	hsTnT due to pre-existing chronic cardiac failure
6	32.5	30.5	1395	No evidence of acute ischaemia	yes	69	No evidence of ischaemic heart disease
7	28.4	28.8	-	No evidence of acute ischaemia	yes	78	No evidence of ischaemic heart disease
8	18.2	14.0	-	No evidence of acute ischaemia	no	180	hsTnT elevation due to chronic renal impairment
9	15.2	14.5	-	No evidence of acute ischaemia	no	114	hsTnT elevation due to chronic renal impairment
10	50.7	45.8	700	No evidence of acute ischaemia	yes	91	No evidence of ischaemic heart disease
11	21.9	17.6	-	No evidence of acute ischaemia	no	99	hsTnT elevation due to chronic renal impairment
12	20.9	18.9	1800	No evidence of acute ischaemia	yes	90	hsTnT due to pre-existing chronic cardiac failure
13	14.9	-	-	No evidence of acute ischaemia	yes	107	hsTnT due to pre-existing cardiac disease

However, this may not be the case in all hospital settings, depending upon the availability of cardiological expertise. It is at least conceivable that patients may have to wait for cardiological assessment, potentially delaying the introduction of immune checkpoint therapy with the incumbent risk of disease progression. The cost of extensive cardiological assessment should also not be underestimated, nor the physical and psychological stress associated with emergency hospital visits.

At present, and given the increasing incidence of irM, intense research efforts are focused on identifying “at-risk” patient groups, despite the incidence and pathogenesis of immune-mediated cardiotoxicity being poorly understood (Lyon et al. 2018a). However, at present, there is little “real-world” evidence that this pragmatic approach to identify “at-risk” groups to maximizes early recognition and treatment of irM produces clinically tangible benefits.

Recent studies, both cross-sectional and longitudinal, have highlighted the association between elevated hsTnT levels and diabetes mellitus, consistent with our findings (Fu et al. 2017; Zheng et al. 2012). HsTnT has even been proposed as a predictor of incident diabetes (Whelton et al. 2016). The mechanism underlying this association is currently unclear but may be mediated by concurrent chronic renal impairment or reflect microangiopathy associated structural nerve damage in type II diabetes mellitus (Johann M.E. Jende et al. 2020; Yang et al. 2017). Elevated cardiac biomarkers, including hsTnT, have been reported in cancer patients prior to anti-cancer therapy and are strongly related to all-cause mortality. However, the studies to date have largely included patients with breast, lung, and hematological malignancies (Danese et al. 2013; Lim et al. 2006; Pavo et al. 2015). In one study of over 550 cancer patients, only 2 (0.4%) patients with skin cancer were included (Pavo et al. 2015).

Heart disease in general is common among elderly patients. In our cohort of 121 patients, 56 patients (46%) with a mean age of 74 years had pre-existing cardiac disease. 81 of those patients were male and only 40 were female. Even though 56 patients reported a previous cardiac disease, baseline echocardiography was only carried out in 59 patients, in whom 55% had abnormal findings. Indeed this was 10% higher than the percentage of patients who reported a history of heart disease, suggesting that it may have been largely asymptomatic.

Patients with an elevated baseline hsTnT were significantly older than patients with a normal hsTnT ($p<0.0001$, Man Whitney Test), perhaps reflecting the increased prevalence of heart disease in this age group. (Steenman and Lande 2017). Interestingly, irAEs, in general, do not occur more frequently in older patients compared to younger patients. Betof et. al (Betof et al. 2017) demonstrated no correlation between age and a range of irAEs including hepatitis, pneumonitis, and colitis. Furthermore, age did not impact efficacy as measured by progression-free and/or overall survival. Other groups have reported that pre-existing comorbidities do not correlate with the development of organ-specific irAEs

(Cybulska-Stopa et al. 2019). It is currently clear whether this is the case with irM given its rare incidence.

An association between elevated pre-treatment hsTnT serum concentration and pre-existing diabetes mellitus type 2 was detected, similar to that reported in the literature (Zhong et al. 2016). This may be potentially clinically relevant given recent reports of the development of type 1 diabetes as an irAE in patients receiving 6-weekly cycles of pembrolizumab (Kähler et al. 2020). The combination of pre-existing heart disease and diabetes underpins the importance of a thorough assessment of co-morbidities in patients due to undergo immune checkpoint inhibitor therapy.

In our cohort, there was a significant correlation between elevated baseline hsTnT concentrations and previous cardiac diseases (figure 22), consistent with Sarocchi et. al (Sarocchi et al. 2018). Elevations of hsTnT may be due to several factors including concurrent chronic heart disease (Torre and Jarolim 2015), (ii) the underlying malignancy itself resulting in an oxygen demand and supply mismatch, which may be exacerbated by age (Eggers and Lindahl 2017). This is consistent with our finding that elevated baseline hsTnT concentrations were correlated with age. We could confirm the findings of Spallarossa et. al that elevated hsTnT concentrations are correlated with chronic kidney disease (Figure 26) (Spallarossa et al. 2019). Additionally, we did not see a difference in overall survival in the patients with an elevated baseline hsTnT as illustrated in figure 31. However, this may be due to the short observation period.

Nevertheless, in our experience, pre-therapeutic elevated hsTnT concentrations were not associated with the development of irM. This may provide some reassurance to treating physicians and patients. Given the dramatic increase in the number of cancer patients who are now eligible for treatment with immune checkpoint inhibitors, there will inevitably be more patients who develop irAEs. A key challenge over the next decade will be the identification of biomarkers not only to maximize the benefit of immunotherapy among patients receiving it but also to maximize patient safety and optimize treatment of irAEs, especially those associated with significant morbidity and mortality. To this end, interleukin

6, C-reactive protein, and melanoma inhibitory activities have recently been reported to correlate with the onset of irAEs (Husain et al. 2021). Should these results be confirmed, a role for anti-IL-6R antibodies in the treatment of irAEs may emerge.

However, any decision to administer immune checkpoint inhibitors must include a careful assessment of treatment-associated risks, particularly in the adjuvant setting where there is no evidence of residual tumour. In fact, due to the lack of reliable biomarkers, it is currently not possible to predict which individual patients with fully resected high-risk metastatic melanoma for example will develop disease recurrence.

In summary, we confirm that irM is rare and report that pre-ICI treatment hsTnT concentrations were frequently elevated in patients with advanced skin cancer in the absence of acute ischemia. Following cardiac evaluation, immunotherapy was administered as planned and none of the patients with an elevated hsTnT concentration developed irM, although this was not expected given the sample size. Nevertheless, pre-ICI treatment hsTnT concentrations should be routinely performed before the initiation of immune checkpoint inhibition (Brahmer et al. 2018; Rassaf et al. 2020; Spallarossa et al. 2019) and thoroughly investigated when elevated. Following cardiological assessment and the decision to initiate ICI therapy, pre-and early-treatment serum hsTnT concentrations should be measured and closely monitored especially during the initial treatment cycles, when the risk of irM is greatest, particularly in patients with additional risk factors for irM, including the male sex, diabetes, a history of heart disease, and those undergoing combined anti-PD-1 and anti-CTLA-4 immunotherapy.

Strengths of this thesis

The cohort was large enough to confirm the incidence of irM reported in the literature and, although the study was retrospective, serial hsTnT measurements and full cardiology work-ups of the affected patients could be analyzed. The follow-up period also allowed conclusions to be drawn on the utility measuring pre-treatment hsTnT concentrations in the early detection of irM, given that it typically occurs in the initial treatment cycles. Moreover, in the case of irM the close collaboration with the department of Radiology and the department of Cardiology allowed prompt radiographic imaging to substantiate the diagnosis of irM, given that the patient refused an endomyocardial biopsy. Another strength was the range of treatments and tumour entities which we could investigate.

Limitations of this study

Our study was neither designed nor powered to evaluate the positive or negative predictive value of pretreatment elevated serum hsTnT for the development of irM, which would also require large multicenter studies. Additional limitations of our study include the predominance of a single cancer type (melanoma), the single-center setting, its retrospective nature, and that only one patient developed irM. Moreover, as we only measured pretreatment serum hsTnT concentrations our study does not allow any conclusions to be drawn about the sensitivity or specificity of serum hsTnT concentrations in the diagnosis of irM. Finally, the patient declined an endomyocardial biopsy, which may have shed light on the extent and nature of the immune-cell infiltrate

Conclusion

The results of this study demonstrate that serum hsTnT concentrations are frequently elevated before ICI therapy in patients who do not go on to develop irM. However, hsTnT may still provide useful information regarding the patient´s general health and may identify patients at risk of a poorer overall prognosis, independent of the development of irM. Therefore, as per the ASCO guidelines, hsTnT should be measured prior to immune checkpoint immunotherapy and investigated when abnormal. Close cooperation between cardiologists and oncologists is also crucial to ensure patient safety. Until new biomarkers or imaging techniques become available, patients with elevated pre-treatment hsTnT levels should be carefully monitored during therapy and serial measurements should be considered during the initial treatment cycles in patients with known risk factors for the development of irM.

Summary

Immunotherapy has dramatically improved the therapeutic landscape for the management of a range of locally advanced and/or metastatic skin cancers including melanoma, squamous cell carcinoma and merkel cell carcinoma. Among immune-related adverse events (irAEs), immune-related myocarditis (irM), with mortality of almost 50 percent, is probably the most feared. The regular measurement of pre-treatment baseline hsTnT concentrations has been proposed by the American Society of Clinical Oncology (ASCO), in order to screen patients for the risk of developing irM, although “real-world” data to support this strategy is lacking.

Therefore, to evaluate the utility of this strategy we retrospectively analyzed all patients in the department of Dermatology at the University of Lübeck, who started immunotherapy in the year 2018 and 2019 (n=121), to both determine the incidence of irM in our department and to value the use of baseline hsTnT as a predictable marker for irM.

As expected, the incidence of irM was low (0,82 %). Baseline hsTnT was determined in 47 patients and was elevated in 28 % of the cases. An elevation of baseline hsTnT was strongly correlated with a pre-existing diabetes mellitus, a pre-existing cardiac disease, chronic renal failure, age and abnormal echocardiography. Most importantly, the one patient who developed irM did not present with an elevation of baseline hsTnT, nor did he have a history of a previous cardiac disease. In addition to that, none of the patients with an abnormal baseline hsTnT developed neither irM, nor any other kind of immune-related cardiac events, nor did any of them suffer from acute cardiac ischemia.

In fact, almost 30 percent of the patients in our cohort presented with an elevated baseline hsTnT, in the absence of any specific signs of cardiac ischemia or any clinical symptoms, questioning whether routine measurement of hsTnT is a useful tool. Routine measurement of abnormal hsTnT concentrations often resulted in emergency room visits, frequently without any clinical consequence.

However, given that irM is rare, large multi-center studies are needed in order to identify the sensitivity and specificity of baseline hsTnT measurement as predictors of this serious irAE. Given that elevated hsTnT levels may be associated with a poorer overall prognosis *per se,* we recommend routine measurement, especially for patients with echocardiography and/or ECG abnormalities, diabetes mellitus and/or known ischemic heart disease, and monitoring during the initial treatment cycles when the risk of irM is greatest.

German summary

Einleitung

Aufgrund von Immun-Checkpoint-Inhibitoren haben sich die Therapieoptionen und die Prognose für sowohl lokal fortgeschrittene als auch metastasierte Formen des Hautkrebs, wie beispielsweise dem Melanom, dem Plattenepithelkarzinom oder dem Merkelzellkarzinom, stark verbessert. Die Immuntherapie wird aktuell in Deutschland nicht nur für palliatives Hauttumorleiden im Stadium IV angewendet, sondern hat aufgrund beeindruckender Resultate ebenfalls die Zulassung im adjuvanten Bereich, dem Stadium III, erlangt. Trotz der vielversprechenden Langzeitergebnisse ist die Anwendung für einen Teil der Patienten mit einer Vielzahl von möglichen Nebenwirkungen verbunden. Neben den typischen Immuncheckpoint-Inhibitor-assoziierten Nebenwirkungen, wie beispielsweise exanthematischen Hautveränderungen, der immun-mediierten Colitis oder der Immuntherapie assoziierten Hepatitis, ist die Immuncheckpoint-Inhibitor-assoziierte Myokarditis, mit einer Mortalität von fast 50 Prozent, die am meisten gefürchtetste. Wegen der sehr unspezifischen klinischen Symptomatik und dem fulminanten Verlauf ist ein schneller Therapiebeginn für den betroffenen Patienten meist lebensentscheidend. Aktuell ist noch nicht eindeutig geklärt, welche Patientengruppen prädisponiert für diese Nebenwirkung sind. Aus diesem Grund empfiehlt die „American Society of Clinical Oncology“ (ASCO) die Bestimmung eines Troponin Ausgangswerts vor dem Beginn einer Immunkombinationstherapie, um Patienten mit einem möglichen vorbestehenden Risiko für diese schwere Nebenwirkung vorzeitig zu erkennen. Diesbezüglich ist die Datenlage jedoch sehr uneindeutig.

Ziele der Arbeit

Um den Nutzen dieser Empfehlung zu untersuchen, wurden diejenige Patienten an der Klinik für Dermatologie, Allergologie und Venerologie der Universität Lübeck retrospektiv in den Jahren 2018 und 2019 analysiert (n=121), welche eine Immuntherapie erhielten.

Beabsichtigt war nicht nur die Inzidenz der Immuncheckpoint-Inhibitor-assoziierten Myokarditis innerhalb von unserer Kohorte zu bestimmen, sondern auch um den möglichen Zusammenhang eines Troponin Ausgangswerts als nützlichen prädiktiven Marker für diese fatale Nebenwirkung zu bewerten. Das Ziel der Arbeit lag hier auf 4 Kernpunkten:

- Die Bestimmung der Inzidenz einer kardialen Nebenwirkung bei Patienten mit fortgeschrittenen Hauttumorleiden, welche eine Immuntherapie, oder eine Immunkombinationstherapie erhielten.
- Die Bestimmung der Anzahl der Patienten, bei denen wir einen Ausgangs Troponin Wert vor Einleitung der Immuntherapie erhoben haben.
- Die Beantwortung der Fragestellung, ob einer erhöhter Ausgangs Troponin Wert ein erhöhtes Risiko für Kardiotoxizität darstellt und die Darstellung derjenigen Risikofaktoren, welche mit einem erhöhten Ausgangs Troponin Wert in unserem Patientenkollektiv einhergingen.
- Die Beantwortung der Frage, ob ein erhöhter Ausgangs Troponin Wert ein Auswirken auf das Gesamtüberleben der Patienten in unserem Kollektiv hatte.

Ergebnisse

Von den insgesamt 121 analysierten Patienten bestand bei 116 ein Melanom, bei zwei Patienten ein Plattenepithelkarzinom und bei drei Patienten ein Merkelzellkarzinom. 81 der Patienten waren Männer, 40 der Patienten waren Frauen. Das Durchschnittsalter lag bei 74 Jahren. Von den 116 Melanom Patienten bekamen mehr als zwei Drittel eine Immuntherapie im palliativen Therapiesetting, während das andere Drittel eine Immuntherapie im adjuvanten Therapieregime erhielt. 56 der Patienten berichteten über eine vorbestehende kardiale Nebenerkrankung.

Wie erwartet war die Inzidenz der Immuncheckpoint-Inhibitor-assoziierten Myokarditis gering (0,82%). Ein Patient aus der gesamten Kohorte entwickelte eine Immuncheckpoint-Inhibitor-assoziierte Myokarditis nach nur einer Gabe Cemiplimab aufgrund eines nicht

operablen Plattenepithelkarzinom. Bei diesem Patienten war weder der Troponin Ausgangswert erhöht, noch bestand eine kardiale Grunderkrankung, noch gab es mögliche Risikofaktoren, welche prätherapeutisch auf eine kardiale Nebenwirkung hätten hinweisen können. Der Troponin Wert erreichte bei dem Patienten einen Höchstwert von 2238 ng/l (Norm: <14ng/l) 31 Tage nach der Immuntherapie. Nach vier Wochen intensivster medizinischer Betreuung konnte der Patient das Krankenhaus in die Rehabilitation verlassen, verstarb jedoch 121 Tage nach der Infusion.

Der Troponin Wert wurde insgesamt bei 47 Patienten vor der Einleitung einer Immuntherapie bestimmt und war in 28% (13 von 47) der Fälle erhöht. Eine Erhöhung des Troponin Ausgangswert korrelierte stark mit einem vorbestehenden Diabetes mellitus, einer kardialen Grunderkrankung, einem chronischem Nierenversagen, dem Alter und einer von der Norm abweichenden Echokardiographie. Im Gegensatz dazu konnten wir in unserem Patientenkollektiv keine Korrelation zwischen einem erhöhten Troponin Wert und dem Geschlecht, dem BRAF-Status oder dem Therapieregime (adjuvante oder palliativ) erkennen. Bei keinem der Patienten mit erhöhtem Troponin Wert gab es nach gründlicher kardiologischer Abklärung den Hinweis für eine akute Ischämie und alle Patienten konnten ihre Immuntherapie ohne Zeitverzug erhalten. Ergänzend entwickelte keiner der Patienten mit einem erhöhten Troponin Wert eine kardiale Nebenwirkung. Ebenfalls zeigte sich kein Zusammenhang eines erhöhten Troponin Wert auf das Gesamtüberleben.

Diskussion

Die zunehmende Verwendung von Immun-Checkpoint-Inhibitoren wird neben dem vielversprechenden Nutzen für die Patienten auch eine deutlich ansteigende Zahl an Immuncheckpoint-Inhibitor-assoziierten Nebenwirkungen, teilweise mit hoher Letalität einhergehend, mit sich bringen. Eine schnelle Diagnostik und schnelles therapeutisches Handeln sind besonders für die fatalen Nebenwirkungen, wie der Immuncheckpoint-Inhibitor-assoziierte Myokarditis, lebensentscheidend.

In unserer Kohorte hatten fast 30 Prozent der Patienten vor der Einleitung der Immuntherapie einen asymptomatisch erhöhten Troponin Ausgangswert. Bei allen dieser Patienten wurde ein leitliniengerechter Ausschluss einer kardialen Ischämie durchgeführt, teilweise kurzfristig bei den Kollegen der Kardiologie. Bei keinem der Patienten gab es nach sorgfältiger Diagnostik den Hinweis auf akute kardiale Probleme, welche mit einem plötzlichen Troponin Anstieg assoziiert sind. Es zeigte sich jedoch ein direkter signifikanter Zusammenhang zu einem vorbestehenden Diabetes mellitus ($p<0.0001$), einer kardialen Grunderkrankung ($p= 0.0017$) einem chronischem Nierenversagen ($p=0.0007$), dem Alter ($p<0.0001$) und einer von der Norm abweichenden Echokardiographie ($p<0.0001$). Schlussendlich konnten alle Patienten ihre Immuntherapie ohne Zeitverzug erhalten. Ergänzend hierzu entwickelte keiner der Patienten mit einem erhöhten Troponin Wert eine Immuncheckpoint-Inhibitor-assoziierte kardiale Nebenwirkung oder Myokarditis.

Diese Erkenntnis wirft die Frage aus, ob die Empfehlung der ASCO nützlich ist für die prätherapeutische Erkennung von Risikogruppen, da wir keinen Zusammenhang zwischen einem erhöhten Ausgangs Troponin Wert und der Entwicklung einer Myokarditis zeigen konnten. Zusätzlich sahen wir keine Auswirkung eines erhöhten Troponin Wert auf das Gesamtüberleben, wobei hier berücksichtigt werden muss, dass unsereF Beobachtungsphase verhältnismäßig kurz war.

Da die Immuncheckpoint-Inhibitor-assoziierte Myokarditis selten ist, werden zukünftig große multizentrische Studien erforderlich sein, um weitere nützliche prädiktive Marker für diese gefürchtete Nebenwirkung zu bestimmen. Zusätzlich wird es eine Kernaufgabe der nahen Zukunft für die Wissenschaft sein, Risikogruppen für seltene Nebenwirkungen im Rahmen der Immuntherapie zu bestimmen. Da man in Bezug nehmend auf die Myokarditis nicht ausschließen kann, dass Patienten mit erhöhten Troponin Werten eine schlechtere Gesamtprognose haben, empfehlen wir weiterhin die routinemäßige Messung eines Troponin Werts vor der ersten Gabe der Immuntherapie, insbesondere bei Patienten mit Echokardiographie- und / oder EKG-Anomalien, Diabetes mellitus und / oder bekannten

ischämischen Herzerkrankungen. Zusätzlich raten wir während der ersten Behandlungszyklen zu einer engmaschige Kontrolle der Troponin Werte, da besonders in dieser Zeit das Risiko für eine Myokarditis im Rahmen der Immuntherapie am höchsten ist.

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List of figures

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Presentations and publications arising from this thesis

1: A conference abstract has been submitted to the annual meeting of the "Arbeitsgemeinschaft Dermatologische Onkologie."

Titel: "Prätherapeutisches Troponin ist häufig erhöht vor Immun-Checkpoint Inhibitor Therapie bei fortgeschrittenem Hautkrebs"

Jonas K. Kurzhals, Tobias Graf MD, Katherina Boch MD, Ulrike Grzyska MD, Alex Frydrychowicz, Detlef Zillikens, MD, Patrick Terheyden MD, Ewan A. Langan PhD MB

2: The following article arising from this thesis has been published:

Frontiers in Medicine section Dermatology

DOI: 10.3389/fmed.2021.691618

Title: "Serum Troponin T concentrations are frequently elevated in advanced skin cancer patients prior to immune checkpoint inhibitor therapy: experience from a single tertiary referral center"

Authors: **Jonas K. Kurzhals**, Tobias Graf MD, Katherina Boch MD, Ulrike Grzyska MD, Alex Frydrychowicz, Detlef Zillikens, MD, Patrick Terheyden MD, Ewan A. Langan PhD MB

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Attachments

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Verkürztes Verfahren (retrospektive Anlyse anonymisierter Daten)

Cardiotoxicity during immune checkpoint therapy in patients with advanced skin cancerIhre E-Mail vom 18. Mai 2020

Sehr geehrter Herr Dr. Langan,

Sie informieren uns über das Dissertationsvorhaben von Herrn Kurzhals.

Folgendes Dokument lag vor:

- Studienskizze vom 18.05.2020

Die Ethik-Kommission nimmt das beschriebene Vorhaben zustimmend zur Kenntnis. Eine Behandlung im normalen Antragsverfahren wird nicht für notwendig erachtet.

Mit freundlichen Grüßen

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Curriculum Vitae

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