

Diplomarbeit

**Endocrine adverse events of Immune
Checkpoint Inhibitors: Case Report and
Literature Research**

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Graz, am 09.01.2019

Affidavit

I hereby declare that the present diploma thesis was originated and composed entirely by myself and without any assistance from third parties. Furthermore, I confirm that no other sources than those indicated in the text have been used in the preparation of the diploma thesis.

Graz, am 09.01.2019

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List of Abbreviations

ACTH.....	adrenocorticotropic hormone	L.....	liter
ADL.....	activities of daily living	LH.....	luteinising hormone
AE.....	adverse event	mcg.....	microgram
AJCC.....	American Joint Committee on Cancer	mg.....	milligram
ALT.....	alanine transaminase	MHC.....	major histocompatibility complex
APC.....	antigen-presenting-cell	mL.....	milliliter
AST.....	aspartate transaminase	MRI.....	magnetic resonance imaging
BMI.....	body mass index	mRNA.....	messenger ribonucleic acid
CD.....	cluster of differentiation	MSI-H.....	microsatellite instability-high
cm.....	centimeter	ng.....	nanogram
CTCAE.....	Common Terminology Criteria for Adverse Events	NK cell.....	natural killer cell
CTLA-4.....	cytotoxic T lymphocyte antigen-4	NSAID.....	Non-steroidal anti-inflammatory drug
dL.....	deciliter	PAI.....	primary adrenal insufficiency
dMMR.....	mismatch repair-deficient	PCR.....	polymerase chain reaction
et al.....	et alii (and others)	PD-1.....	programmed death-1
FDA.....	Food and Drug Administration	PD-L.....	programmed death-ligand
FDG.....	fluorodeoxyglucose	PET.....	positron emission tomography
FSH.....	follicle stimulating hormone	pmol.....	picomol
GH.....	growth hormone	RR.....	reference range
GnRH.....	Gonadotropin-releasing hormone	ft3.....	free triiodothyronine
HGH.....	human growth hormone	ft4.....	free thyroxine
HLA.....	human leucocyte-associated antigen	TCR.....	T cell receptor
i.v.....	intravenous	TGF.....	tumor growth factor
ICI.....	immune checkpoint inhibitor	TLR.....	Toll-like-receptor
IF.....	interferon	TNF.....	tumor necrosis factor
Ig.....	immunoglobulin	Treg.....	regulatory T cell
IGF-1.....	insulin-like growth factor-1	TRH.....	thyroliberine
IH.....	immune-related hypophysitis	TSH.....	thyrotropin stimulating hormone
IL.....	interleukin	U.....	units
irAE.....	immune-related adverse event	WHO.....	World Health Organization
kg.....	kilogram	μ.....	microgram
		μU.....	micro units

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Zusammenfassung

Immun-Checkpoint-Inhibitoren (ICI) sind relativ neue und effektive Medikamente in der Krebs-Therapie, die zur Zeit vorwiegend bei Patienten mit metastasierten, nicht-respektablen Tumoren eingesetzt werden, bei denen die konventionellen Therapieregime ausgeschöpft sind. Abgesehen von ihrer ausgezeichneten Wirksamkeit mit Ansprechraten von ca. 20-40% der behandelten Patienten/innen können sie jedoch auch zu sogenannten „immun-medierten Nebenwirkungen“ führen. Die zugrundeliegenden pharmakologischen Mechanismen der Wirkweise der Checkpoint-Inhibitoren sind hochkomplex und noch nicht in Bezug auf jede potentielle Nebenwirkung, die sie auslösen können, verstanden. Die folgende Arbeit soll einen Überblick über die immunologischen Grundlagen, im Besonderen der T-Zell-Funktionen und der Regulationsmöglichkeiten des Immunsystems, geben. Weiters wird der aktuelle Stand der Forschung bezüglich der zugrundeliegenden Prozesse für die Entwicklung von Nebenwirkungen präsentiert. Außerdem werden Wirksamkeitsraten der Checkpoint-Inhibitoren und mögliche Gründe für etwaiges Therapieversagen aufgelistet. Darüber hinaus wird die aktuelle Literatur bezüglich der immun-medierten Nebenwirkungen in Zusammenhang mit den verschiedenen Wirkstoffen, deren Manifestationen und Häufigkeitsverteilungen gesichtet. Dabei wird spezielles Augenmerk auf die Nebenwirkungen der endokrinen Organe gelegt, deren häufigste und zugleich gefährlichste die Hypophysitis ist. Weitere typische endokrinologische Nebenwirkungen sind Schilddrüsen-Dysfunktionen, über deren Inzidenz allerdings kein eindeutiger Konsensus in der Literatur besteht. Hypothyroidismus jedoch wird einhellig als die häufigste Dysfunktion beschrieben. Außerdem werden die Grundzüge seltenerer endokrinologischer Nebenwirkungen, wie primäre Nebennierenrindeninsuffizienz und insulinpflichtiger, autoimmunbedingter Diabetes mellitus, dargestellt. Zusätzlich werden anhand der aktuellen Literatur die derzeit gültigen Empfehlungen für die Therapie von endokrinen Nebenwirkungen, sowie auch im Vergleich zur Therapie von nicht-endokrinen Nebenwirkungen, präsentiert. Zur Abrundung und praktischen Veranschaulichung des Themas werden drei Fallberichte aus der endokrinologischen Abteilung des Universitätsklinikums Graz aus dem Zeitraum von November 2015 bis Jänner 2018 präsentiert.

Abstract

Immune checkpoint inhibitors (ICI) are relatively new and highly efficient drugs in cancer therapy. Their range of indication is currently mainly limited to patients with progressive cancer after chemotherapy but the indication spectrum is expanding these days. Responding rates vary from about 20-40%. Apart from their excellent efficacy, it should be mentioned that they can also cause so-called “immune-related adverse events” (irAEs). The pathophysiological mechanisms of their beneficial actions and side effects are complex and not yet fully understood. Therefore, the aim of this thesis is to give an overview of immunological actions of immune checkpoint inhibitors with a focus on T cell function and modulating mechanisms of the immune system. Furthermore, the current literature is reviewed regarding the efficacy of these agents and potential reasons for unresponsiveness. Furthermore, an overview of the incidence and characteristics of immune-related adverse events is provided. In this context, the differences of the available checkpoint inhibitors with regard to effects and adverse events are outlined and discussed. With reference to the immune-related side effects, a special focus is laid on adverse events affecting the endocrine organs, in particular hypophysitis, as this disease potentially can be life-threatening. Other common endocrine adverse events are thyroid disorders which are described with different incidences across the literature. However, hypothyroidism is widely presented as the most frequent condition. Rare endocrine adverse events like primary adrenal insufficiency and insulin dependent diabetes mellitus are also discussed. Additionally, current guidelines regarding management of endocrine side effects as well as a comparison with management of non-endocrine side effects are provided. Lastly and to give some insights into clinical routine, three case reports from patients experiencing immune-related adverse events are presented.

1. Introduction

1.1. The Immune System

The purpose of the immune system is the defence of exogenous substances. It consists of the innate (unspecific) and adaptive (specific) immune system. Their common functions are recognition, opsonisation and mortification of infected or pathological altered cells and foreign microorganisms, such as bacteria, parasites and worms. Both the innate and the adaptive systems are divided into a cellular and humoral part (1).

1.1.1. Innate Immune System

With exception of lymphocytes all leukocytes belong to the cellular part of the innate immune system. These are the neutrophilic, eosinophilic and basophilic granulocytes, mast cells, monocytes and macrophages, as well as natural killer cells. The humoral part consists of the complement system, lysozyme, acute phase proteins and cytokines. All of those components act to defend the body against unspecific exogenous material. The majority of these cells are located in the interstitium and the minority circulates within the bloodstream. Granulocytes, monocytes and macrophages are so-called phagocytes which means they are able to recognise an opsonised foreign cell with their "Toll-like-receptors" (TLR) and ingest it through pseudopodial evagination and subsequently phagocyte it by the aid of lysosomes which contain several enzymes and oxygen radicals (1).

In case of an inflammatory reaction – which is simply a response to harmful stimulus – leukocytes, which are present at the location of the inflammation, secrete cytokines which attract additional leukocytes from the bloodstream. For that procedure the most important cytokines are tumor necrosis factor (TNF)-alpha, interleukin (IL) -1 and IL-8 which support cells to migrate through the endothelium into the tissue. (Monocytes which have migrated into the tissue are referred to as macrophages.) Thus, the unspecific immune response starts immediately (1).

The complement system is a complex cascade of self-activation after a stimulus which can be either contact with an antigen-antibody-complex or a microbe surface polysaccharide. The central part of that system is the complement factor C3b which acts to proteolytically splitting factor C5 into C5a and C5b. The latter initiates the

formation of the so-called "membrane attack complex" which consists of the mentioned factor C5b, as well as C6, C7, C8 and 10 – 16 molecules of C9. This complex is able to cause pores in the membrane of target cells, which leads to lysis of these cells. Additionally, some factors have an effect of enhancing the vascular permeability and secrete histamine as well as chemotactic substances. Furthermore, C3b can opsonise pathogen microorganisms to enable easier recognition for phagocytes (1).

The macrophages generate a link between the innate and adaptive immune system due to their further function as antigen-presenting cells (APCs) to helper T cells (1) (see next chapter 1.1.2.).

1.1.2. Adaptive Immune System

The adaptive immune system reacts delayed yet with high specificity to foreign stimulus. Its cellular part consists of T lymphocytes and B lymphocytes, whereas its humoral part is represented by B lymphocytes' secreted antibodies (1). The components of the innate and adaptive immune system are depicted in Figure 1 below.

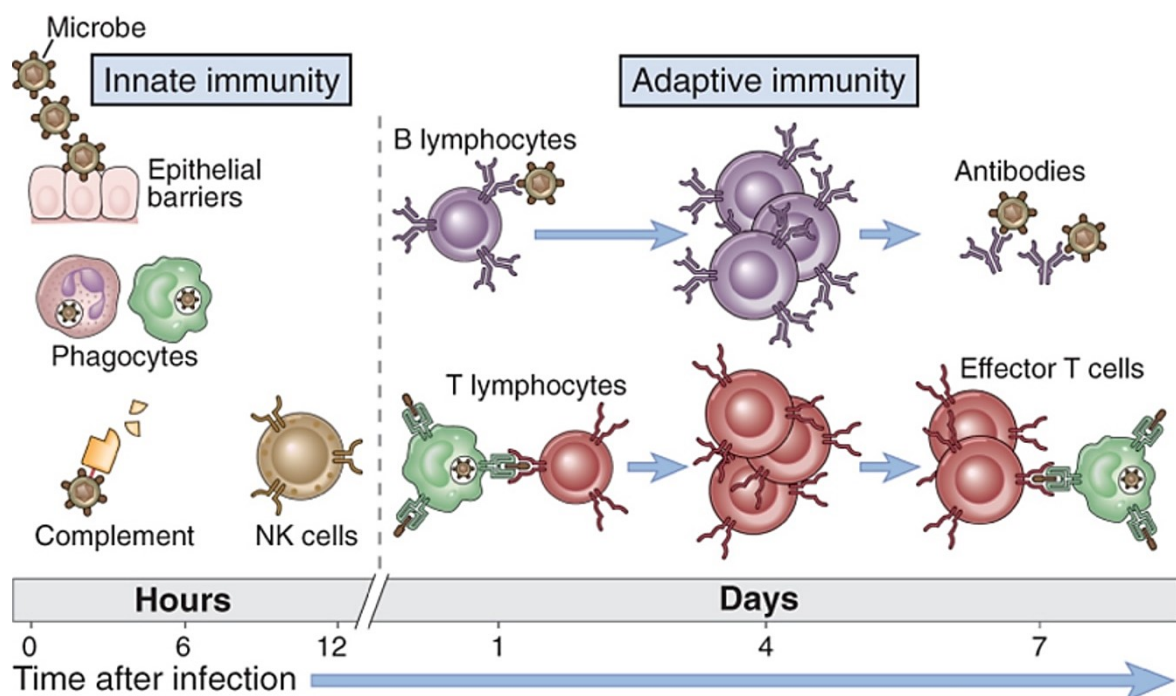


Figure 1: Components of innate and adaptive immune system (2).

1.1.2.1. MHC-molecules

The MHC- (major histocompatibility complex) or synonymously called HLA- (human leucocyte-associated antigens) molecules play a central role in the immune system. These are transmembrane multi-protein-complexes in which antigens are presented and exist as type I and type II. These two types differ in their molecular structure, localisation and their target cells (1).

MHC-I exist on nearly all nucleated cells and thrombocytes but hardly on erythrocytes and neurons. In MHC-I molecules intracellular antigens such as viral components or mutated proteins from cancer cells are presented to CD8 T cells (1). In contrast, MHC-II exists only on APC's which are dendritic cells, monocytes, macrophages and B lymphocytes. They present extracellular antigens which they have absorbed by endocytosis (especially bacterial proteins) to CD4 T cells. These molecules are essential for the engagement between lymphocytes and antigens (1).

1.1.2.1. T lymphocytes

Mature T cells carry an antigen-specific receptor (TCR), which is associated with the CD3-receptor and one of the co-receptors CD4 or CD8. They evolve from bone marrow as T progenitor cells without any receptors ("double negative") and then migrate into the thymus, where they proceed with the second part of their maturation. In the subcapsular area they become "double positive", which means they receive their T cell-CD3-receptor-complex and both co-receptors CD4 and CD8. Afterwards they migrate into the cortex where the positive and negative selection takes place. First, the T cells are tested whether they are able to recognise endogenous MHC-proteins and then, as part of the so-called "positive selection", they lose one of their co-receptors and are left with the second one which is either CD4 or CD8. This is followed by the "negative selection" or "deletion" (1,3) which examines the ability to distinguish between own and foreign antigens. If the T cells are able to recognise self-antigens, apoptosis is triggered. After completing both selections eligible cells leave the thymus as naive T cells and migrate into the secondary lymphoid organs, where they get in loose contact with several in MHC-molecules presented antigens by APCs. If an antigen fits into a specific T cell receptor, engagement of the TCR and the antigen-MHC-complex is triggered. This establishes the "first signal" for T cell activation. However, full activation requires

additional bonding with a co-stimulation factor, like the engagement between CD28-receptor on T lymphocytes and B7-ligand on APCs, that establishes the critical “second signal”. Absence of that co-stimulation factor causes inactivation of the T cells (1). In Figure 2, antigen recognition with and without the co-stimulatory factor CD28:B7 are depicted (2).

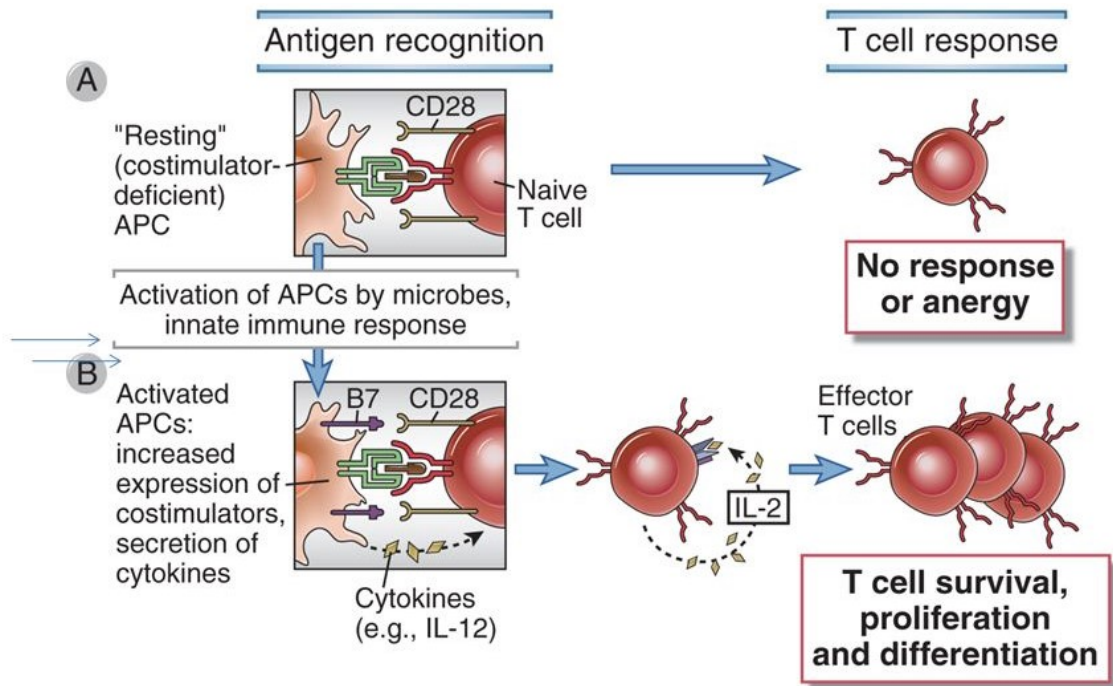


Figure 2: Antigen recognition with and without co-stimulation (4).

CD4-cells are activated by binding to an antigen presented in an MHC-II-molecule which causes the differentiation into helper T cells. Whereas, CD8-cells bind to MHC-I-antigen-complexes and consequently differentiate to cytotoxic T cells (= T killer cells). Helper T cells and cytotoxic T cells are so-called T effector cells, hence they migrate into the peripheral tissues to accomplish the desired immune reaction. The activation of T cells additionally initiates cytokine synthesis, especially for IL-2 and its receptor, which they express on their surface and thus leads to auto-stimulation with clonal expansion of T cells (see below) (1).

At second contact of cytotoxic cells with their in an MHC-I-protein presented specific antigen within peripheral tissues, they secrete perforins and granzymes. Perforins, like serine protease, cause perforation on membranes of target cells which is then followed by lysis through granzymes. Furthermore, cytotoxic T cells own a second mechanism to mortify infected or mutated cells. They express the CD95-ligand (=

fas-ligand) and by binding to a CD95-receptor positive cell they are able to initiate apoptosis of this target cell (1).

The purpose of helper T cells is to support the activation of B cells. There exist three common types of helper T cells – Th0, Th1 and Th2 cells. Th0 cells are activated cells yet to be differentiated. Whether those cells primarily develop into Th1 or Th2 cells depends on the predominantly present kind of interleukins. IL-12 leads to the development of Th1 cells while IL-4 will produce Th2 cells. These cell types also differ in the cytokines they produce. Th1 cells primarily produce IL-2 and IF (interferon)-gamma (IF-gamma activates macrophages), while Th2 cells produce especially IL4, -5 and -10 (1).

B lymphocytes recognise their specific antigen by their B cell receptor (BCR). That same antigen was earlier absorbed by APCs and then led to the differentiation of a CD4 T cell into a helper T cell. There also exists a B cell with a BCR specific for that antigen-epitope and if they encounter, the B cell absorbs the antigen, processes it and presents antigen structures in an MHC-II-molecule to its cognate helper T cell. The interaction of T and B cells starts by concurrent co-stimulation. The Th1 cell releases IL-2 which leads to proliferation and differentiation of the B cell to a plasma cell with ensuing antibody-production. IL-4 secreted by Th2 cells causes the so-called "isotype switching" of the antibody-synthesis, which induces an increased affinity by producing optimised immunoglobulin G (Ig) and E instead of IgM (1).

1.1.2.2. B lymphocytes

The B cells represent the humoral part of the adaptive immune system. They completely mature in the bone marrow – originating from lymphoid stem cells and differentiating to pre-B cells and further B cells with their B cell receptor. The BCR consists of either an IgM or an IgD. B cells have to pass a negative selection and are sent to apoptosis if they are able to recognise body-own antigens. As mature naive B cells, they migrate into secondary lymphatic tissues where they get in contact with countless antigens. Bonding with their specific antigen leads to antibody-secretion (1).

Therefore, two types of B-cell activation exist:

1.) T cell-independent (thymus-independent) B cell-activation:

Through binding with the antigen, the B cell is directly activated to proliferation and differentiation to plasma cells with following antibody-secretion. However, this kind of immune response is of moderate quality.

2.) T cell-dependent (thymus-dependent) B cell-activation:

This kind of immune response is already explained under the helper T cell function (see p. 14). Due to the cytokine production of helper T cells and the following additional stimulus for proliferation, differentiation and the synthesis of optimised antibodies (“isotype switching”), the T cell-dependent B cell-activation is much more effective and high-specified (1).

1.1.2.3. Immunological memory

Some of the T effector cells and plasma cells become memory cells. These can survive in the body for years, even without any antigen-stimulus. The consequence therefore is an immediate adaptive immune response, also with antibodies, to a repeated antigen contact. As a result, the antigens get eliminated so fast that the disease rarely ever breaks out at all and in that case, symptoms are moderate. That condition is called immunity (1).

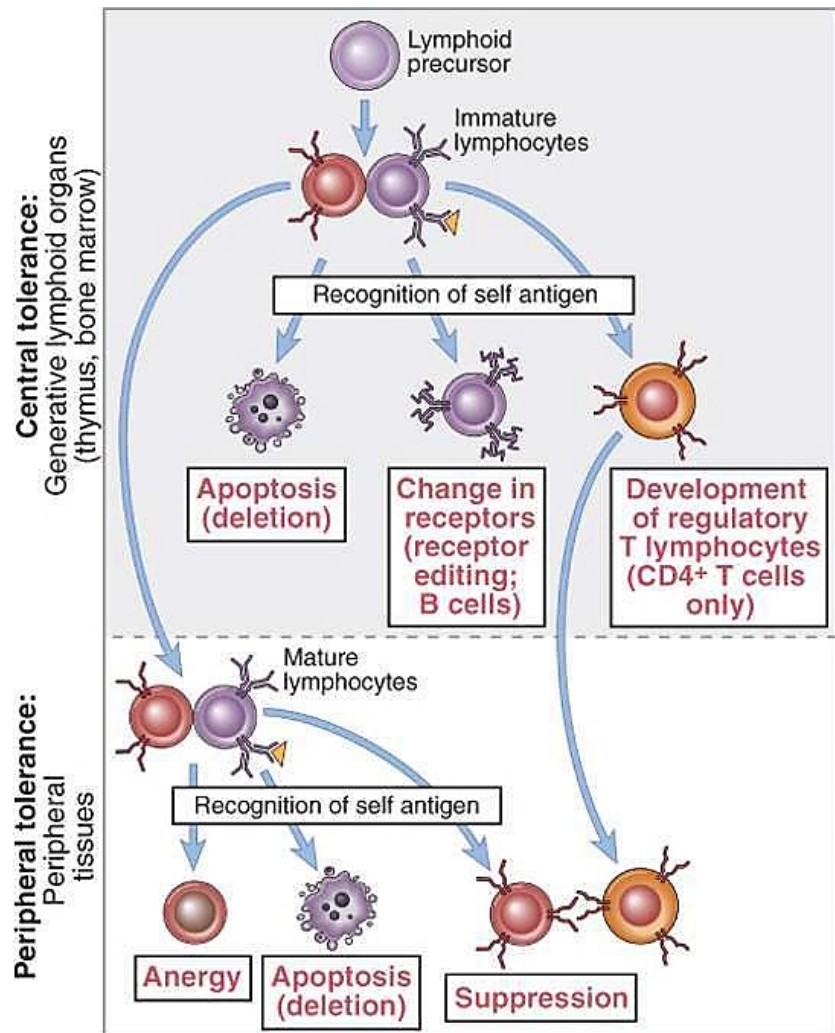
1.2. Immunological tolerance

A balance between activating and inhibiting signals is essential for maintaining the so-called self-tolerance of the immune system. It is defined as unresponsiveness to specific antigens with repetitive encounters to the immune system, which are primarily self-antigens. Dysfunction in this ability leads to autoimmunity with subsequent autoimmune diseases (3).

Central tolerance needs to be distinguished from peripheral tolerance, both are shown in Figure 3. Central tolerance occurs in the generative lymphoid organs, thymus and bone marrow and is ensured by the previously described negative selection of T cells with deletion of T cells that have high-affinity receptors for self-antigens. In the thymus, countless self-antigens containing cell-associated and in the bloodstream circulating proteins as well as peripheral tissue antigens are presented to T cells, the latter by a special mechanism of medullary thymic epithelial

cells (3). Nonetheless, central tolerance is not errorless and furthermore there exist self-antigens, which are exclusively present in peripheral tissues or simply emerge in adult life. Therefore, a mechanism exists to permit tolerance of lymphocytes in peripheral tissues. This peripheral tolerance consists of three main mechanisms: anergy/unresponsiveness, suppression by regulatory T cells and deletion/apoptosis (3). These terms are described in the following chapter 1.2.1.

Figure 3: Central and peripheral tolerance (6).



1.2.1. Regulatory T cells

“Natural” or thymic regulatory T cells (tTregs) develop as a subset from naive CD4 cells in the thymus. Some T cells which recognise self-antigens but are not destroyed during the negative selection instead differentiate into regulatory T cells and are thus specific for a distinct self-antigen in the periphery. Likewise exist

peripheral regulatory T cells (pTregs) which may be specific for self and foreign antigens. They equally develop from naive CD4 cells in secondary lymphoid organs through antigen recognition in the absence of strong innate immunity signals or at inflammatory sites. Their function is suppressing immune reactions through several mechanisms. Firstly, they secrete tumor growth factor (TGF)-beta, which inhibits the proliferation and effector functions of T cells and activation of macrophages. Secondly and very typical for Tregs is a high expression of CTLA-4 (inhibitory receptor, see next chapter). If a regulatory T cell binds through CTLA-4 to B7 on an APC, the availability of B7 as a costimulatory factor for naive T cells is reduced and hence leads to anergy of the T cell which is simultaneously engaged to the APC. Moreover, Tregs express high levels of IL-2 receptors, which may cause a lack of IL-2 as a growth factor for T cells (and B cells, see above), which further leads to enhanced proliferation of Tregs, but diminished proliferation of conventional T cells (3).

Anergy is the inability of activation of naive T cells and is caused, as mentioned, by lack of a costimulatory factor, such as B7:CD28 interaction, or absence of innate immunity (3). To limit immune responses in anergic cells, there exist three different mechanisms (see Figure 4):

- The signal of the TCR is blocked.
- The recognition of self-antigens may activate ubiquitin ligases, which mark specific proteins from the signalling cascade for degradation.
- The recognition of self-antigens may lead to the expression of inhibitory receptors on T cells. Several inhibitory receptors are already discovered, yet the best-known are CTLA-4 and PD-1 and will be described in the following section (3).

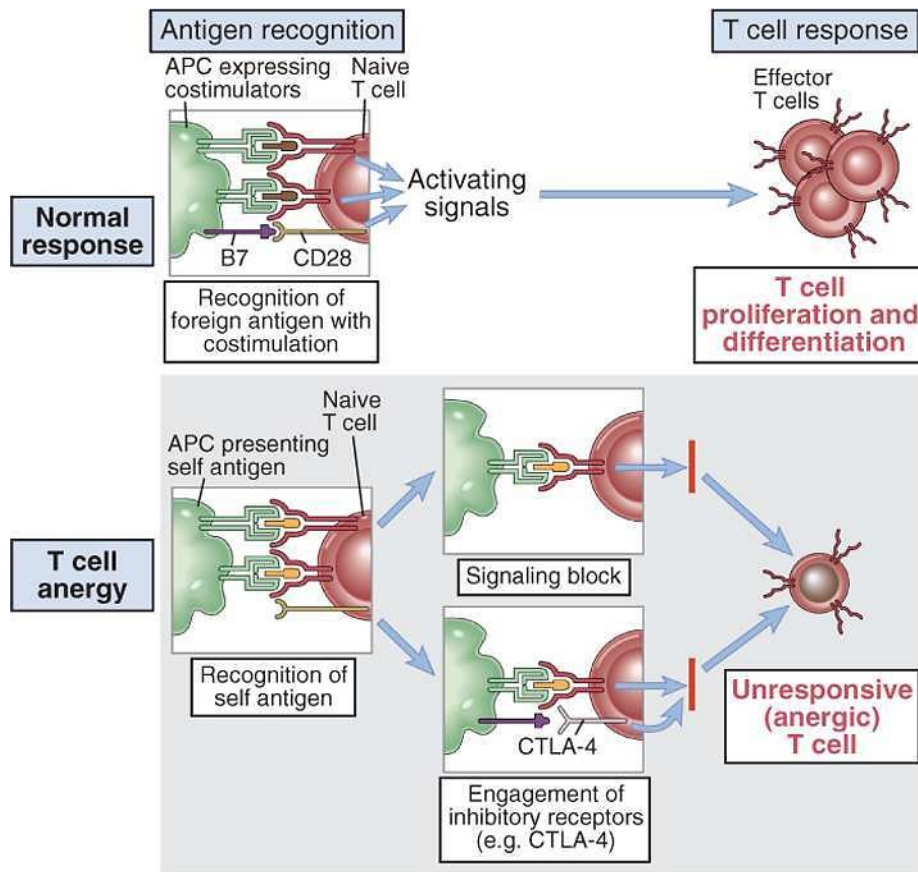


Figure 4: Normal response to foreign antigen presentation with costimulatory signal, compared to mechanisms of energy due to self-antigen recognition: 1.) TCR signalling gets blocked without costimulatory signal. 2.) Engagement of the costimulator (B7) with inhibitory receptors (here: CTLA-4) on T cells, with subsequent unresponsiveness (4).

1.2.2. Modulation of T cell responses by inhibitory receptors

CTLA-4 and PD-1 are both receptors of the CD28 family. They are expressed after T cell activation at their surface to self-limit immune responses (3).

CD28 is a surface receptor on T cells with an extracellular Ig domain. It is expressed on over 90% of CD4 T cells and 50% of CD8 T cells. Its ligand is the B7 protein on APCs, which acts as a co-stimulator for T cell activation. As previously mentioned, the engagement of these two proteins establishes the critical “second signal” for full T cell activation. Resting or inactivated APCs express low levels of B7, whereas microbial products and cytokines induce an increase of B7 levels during innate immune reactions. This mechanism ensures full T cell activation when immune reactions are necessary. The CD28 signal is integrated and works in cooperation with TCR signals. It is important for survival, proliferation and differentiation of the antigen-specific T cell clones through enhanced expression of anti-apoptotic proteins. However, several CD28 receptor- and B7 ligand homologues were detected, which cannot only amplify but also inhibit downstream T cell signalling (3).

1.2.2.1. CTLA-4 (cytotoxic T lymphocyte antigen-4)

CTLA-4 is expressed on naive and regulatory T cells and binds to B7 proteins with 10 to 20 times higher affinity than CD28 does. As a result, CTLA-4 acts as a competitive inhibitor of the CD28 receptor (3). According to Abul K. Abbas, "CTLA-4 inhibits T cell activation in two different ways. In the cell-intrinsic mechanism, upon activation, the responding T cells begin to express CTLA-4, and it shuts off further activation, thus terminating the response. In a cell-extrinsic pathway, Tregs express high levels of CTLA-4 and use it to prevent the activation of responding cells" (3). By engagement with a B7 protein, a clathrin-mediated endocytosis of the receptor-ligand complex into the activated T cell or the regulatory T cell is elicited, with the consequence of limited availability of B7 on APCs to function as a costimulatory signal for T cell activation. Physiologically, this mechanism is important to maintain tolerance to self-antigens. However, during innate immune responses, when B7 levels increase, relatively more low-affinity CD28 binds to B7 ligands, which enables an appropriate immune reaction. To sum up, CTLA-4 inhibits the initial T cell activation directly in secondary lymphoid organs (3).

1.2.2.2. PD-1 (programmed death-1)

PD-1 is an inhibiting T cell receptor expressed on activated T cells and others like B and NK cells (5,6). Its ligands, PD-L1 and PD-L2 are expressed on APCs and additionally on other tissue cells, as well as on cancer cells (7). PD-1 works by activating phosphatases, which dephosphorylate and thus inactivate signalling enzymes of the TCR-CD3-coreceptor-complex and CD28 receptors. Therefore, compared to CTLA-4's inhibition of activation of naive T cells in secondary lymphoid tissues, PD-1 functions to inactivate effector T cells in peripheral tissues, especially cytotoxic T cells. Physiologically, PD-1 plays an important role in controlling immune reactions to prolonged antigen exposure, for example self-antigens, tumour antigens and antigens of chronic infections (3). Besides, it is essential for protecting the surrounding tissue of a centre of inflammation from damage (5). The physiological functions of CTLA-4 and PD-1 are depicted in Figure 5.

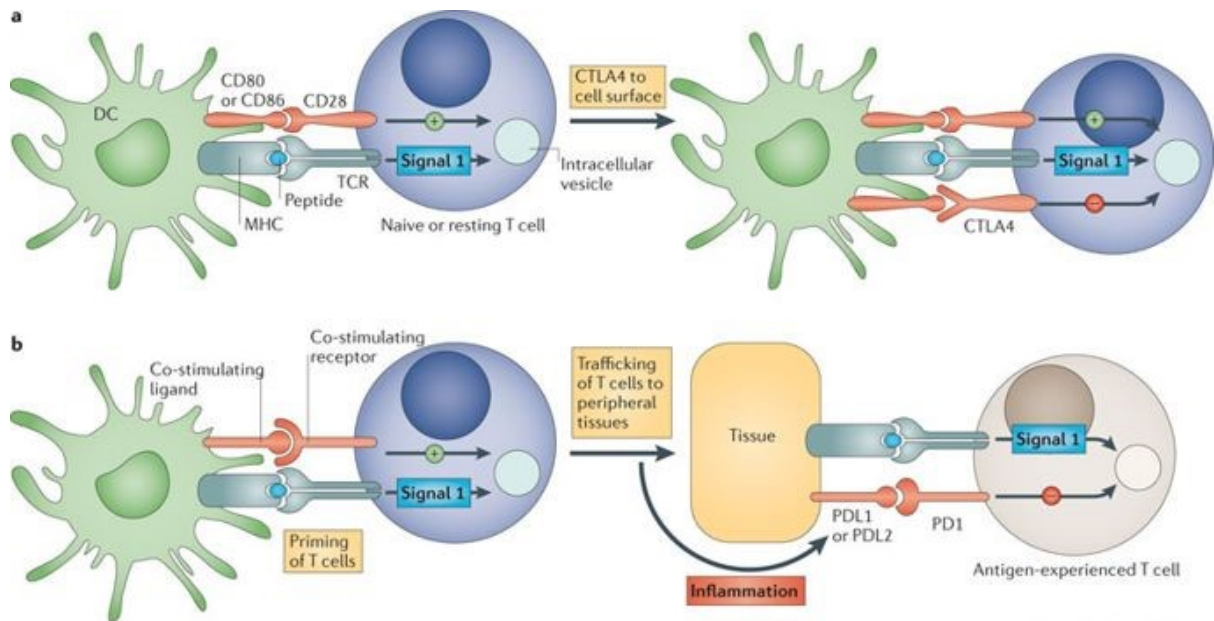


Figure 5: The Physiological function of the inhibitory receptors a) CTLA-4 and b) PD-1. a) Activation of naive or resting T cells in secondary lymphoid organs by an APC (here: dendritic cell) with the TCR-specific antigen. After the activation CTLA-4 is expressed on the T cell's surface with the consequence of attenuating its activity, to avoid excessive immune responses. b) Activated T cells migrate into peripheral tissues to accomplish the immune response. In order to avoid an exaggerated reaction and to protect the surrounding tissue of an inflammation, they express PD-1 and bind the PD-1 ligand 1 or 2, which is expressed on tissue and cancer cells (5).

1.3. Immune Checkpoint Inhibitors

Due to CTLA-4's and PD-1's function to control and regulate immune responses, they are considered as so-called "checkpoints" in immune reactions. Some cancers exploit those natural inhibiting mechanisms to evade the immune system's reactions. One possible option of evading is that tumour antigens are presented in absence of innate immune signals to T cells, what further enables the engagement of CTLA-4 to B7 and thus inhibits T cell activation. On one hand missing, many cancers express PD-L1 whereby anti-tumour T cells become directly unresponsive/anegetic at the site of the cancer. Therefore, drugs and more specifically monoclonal antibodies from the IgG type that inhibit the inhibition have been developed – so-called "immune checkpoint inhibitors" (ICI) – to trigger a checkpoint blockade. The working points of checkpoint inhibitors are shown in Figure 6 below. The anti-tumour T cells involved in these processes and responding to ICI therapy are mainly CD8 T cells (3).

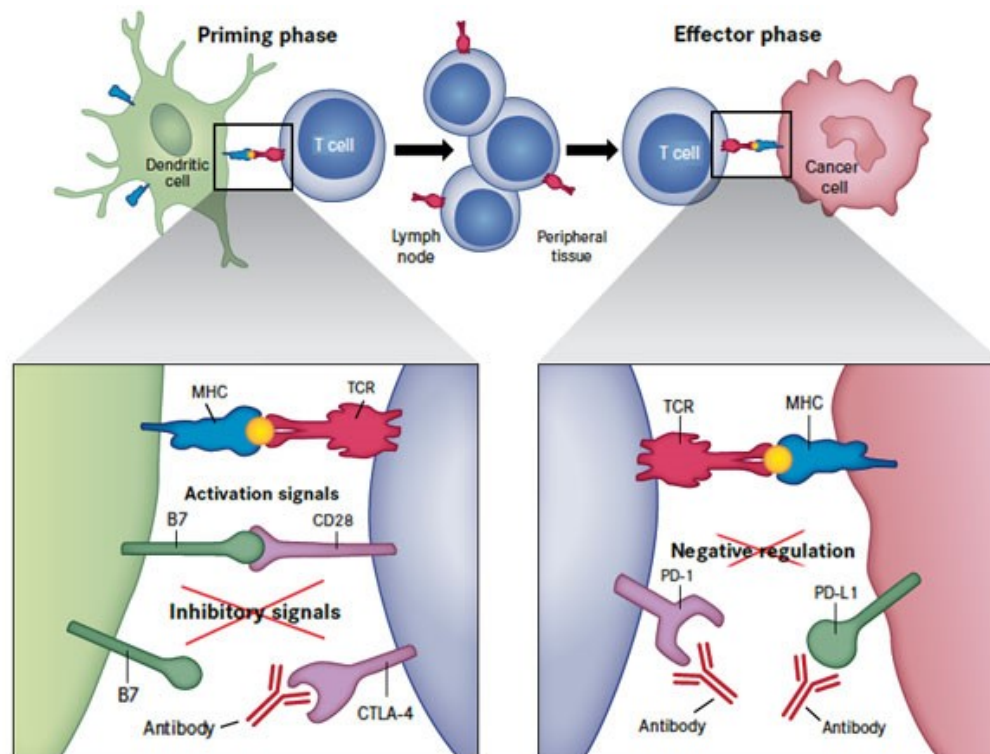


Figure 6: Working points of anti-CTLA-4, anti-PD-1 and PD-L1 antibodies are depicted. The first picture shows the effect of CTLA-4 inhibitors during the priming phase of naive T cells in lymph nodes. Due to the blocking of CTLA-4, enhanced engagement of B7 with the activating receptor CD28 is enabled. The picture on the right shows the effect of PD-1 or PD-L1 inhibitors in peripheral tissues, during the effector phase of activated T cells. Because of blocking of PD-1/L1, the cancer is unable to suppress the immune system's reaction (8).

1.3.1. History and Approval

The first ICIs approved by the U.S. Food and Drug Administration (FDA) were monoclonal CTLA-4 antibodies back in 2011, followed by anti-PD-1 antibodies in 2014 and the latest anti-PD-L1 antibodies in 2016. The first approved cancer type for that kind of therapy was the malignant melanoma (3), which is still the only approved one for CTLA-4 blockade by the FDA (9). Nowadays, anti-PD-1 treatment is also used against several other cancer genes like “non-small cell lung cancer, renal cell cancer, bladder cancer, head and neck cancer, Hodgkin lymphoma, hepatocellular carcinoma, gastric cancer, Merkel cell carcinoma, and unresectable or metastatic, microsatellite instability-high (MSI-H), or mismatch repair-deficient (dMMR) solid tumours that have progressed after prior treatment” (10). Immune checkpoint inhibitors are currently approved as first-line or second-line therapy for melanoma, non-small-cell lung cancer and renal cell cancer (11).

FDA approved immune checkpoint inhibitors are the following:

- CTLA-4 antibody: Ipilimumab
- PD-1 antibodies: Nivolumab, Pembrolizumab
- PD-L1 antibodies: Atezolizumab, Avelumab, Durvalumab (9)

Administration recommendation by the FDA:

- Ipilimumab: 3 mg/kg i.v. every 3 weeks for a total of four doses (12).
- Nivolumab: 240 mg i.v. every 2 weeks until disease progression or unacceptable toxicity, for a maximum of 1 year (13).
- Pembrolizumab: 200 mg for adults, 2 mg/kg for children, every 3 weeks until disease progression, unacceptable toxicity or up to 24 months in patients without disease progression (14).

1.3.2. Efficacy

Michael A. Postow suggested in a recent article in the New England Journal of Medicine that: "... immune checkpoint blockade has shown remarkable benefit in the treatment of a range of cancer types" with a better safety profile than ordinary chemotherapy (9). However, according to data analysis of several studies by Postow and Wolchok (15), an approximate objective response range is specified from 20-40%. As an example, a clinical trial by Topalian et al. (16) regarding advanced melanoma patients who were treated with the PD-1 inhibitor nivolumab showed an objective response with 31% of patients and further "7% of patients ... experienced stable disease lasting for 24 weeks or more" (16). Especially the long-lasting therapeutic effects of PD-1 inhibitors are worth pointing out. The median response duration lasted 2 years and 71% of patients maintained their response for at least 16 weeks after the last drug administration (16). Furthermore, the extent of the anti-tumour effect may correspond with the occurrence of immune-related adverse events (irAE), which suggests that patients might have an overall benefit from irAEs in terms of better drug efficacy through enhanced T cell activity with hence improved response rates and tumour regression (17). Especially regarding the treatment of cancers where similar tumour antigens are also expressed on normal tissues, as it applies to melanoma and normal melanocytes, the development of vitiligo appears to indicate significant increase of response rates

(9,17,18). Hua et al. (19) stated that “An objective (complete or partial) response to pembrolizumab treatment in metastatic melanoma patients was associated with a higher occurrence of vitiligo”. In this study, 71% of patients who developed vitiligo had an objective response, whereas just 28% of melanoma patients without vitiligo experienced an objective response, which is shown in Table 1. Besides, vitiligo correlated with lower progressive disease rates (19).

Patient Group	Response, No. (%) of Patients ^a		Disease, No. (%) of Patients	
	Complete	Partial	Stable	Progressive
Vitiligo (n = 17)	3 (18)	9 (53)	3 (18)	2 (12)
No vitiligo (n = 50)	4 (8)	10 (20)	1 (2)	35 (70)
All (N = 67)	7 (10)	19 (28)	4 (6)	37 (55)

Table 1: Comparison of response rates between patients with and without vitiligo (19).

However, no significant benefit in overall survival compared to those without vitiligo could be detected (19). In contrary, Freeman-Keller et al. (20) reported in his retrospective analysis statistically significant associations between rash and vitiligo with overall survival of patients suffering from metastatic unresectable melanoma treated with anti-PD-1 antibodies.

Moreover, the repression of the PD-1 pathway appears to be more effective with better overall survival and less severe adverse events (3,9,21). Besides, Wolchok et al. (21) stated an increased overall survival rate of advanced melanoma patients when a treatment combination of CTLA-4 and PD-1 inhibitors is used. In this trial, the overall 3-year survival rate with combined therapy amounted 58%, but single anti-PD-1 treatment (nivolumab) showed a similar good outcome with 52%, compared to 34% by only using anti-CTLA-4 antibodies. This data is related to the overall objective response rates, which were also 58% in the combination treatment group and further 44% in the nivolumab and 19% in the ipilimumab group. However, complete response rates were lower, with the highest being 19% resulting from combined treatment and the lowest of just 5% from ipilimumab monotherapy. The median progression-free survival period counted 11.5 months for combined therapy, 6.9 months with nivolumab and 2.9 months with ipilimumab treatment. Progression-free survival at 3 years followed this trend as well. The rates in the combination therapy group and the nivolumab group were resembling with 39% and 32%, while just 10% of patients survived without progression for at least 3 years in the ipilimumab group (21). To sum up, “the two nivolumab-containing groups had

significantly longer survival than the ipilimumab group” (21). In Table 2, complete and partial response rates, as well as stable and progressive disease rates regarding nivolumab and ipilimumab combination and single therapy are presented (21).

Variable	Nivolumab plus Ipilimumab (N=314)	Nivolumab (N=316)	Ipilimumab (N=315)
Best overall response — no. (%) [†]			
Complete response	61 (19)	52 (16)	16 (5)
Partial response	122 (39)	88 (28)	43 (14)
Stable disease	38 (12)	31 (10)	69 (22)
Progressive disease	74 (24)	121 (38)	159 (50)
Unable to determine	19 (6)	24 (8)	28 (9)

Table 2: Comparison of response rates between combined and monotherapy of nivolumab and ipilimumab (21).

1.3.3. Unresponsiveness

According to Abul K. Abbas, more than 50% of patients treated with immune checkpoint inhibitors are nonresponsive or become resistant after an initial response (3). To understand possible reasons for unresponsiveness, it is primarily important to be aware of the mechanisms and effects of checkpoint inhibitors. Due to ICI's intervention in the interaction of T cells and tumor cells, the cancer must feature many somatic mutations for neoantigens, with the consequence of many clones of tumor-specific T cells, to guarantee a sufficient working point for the drugs. Furthermore, the cancer's environment must be infiltrated by plenty of these effector T cells. The cancer obviously must utilise the CTLA-4 or PD-1 pathway to evade the immune system. Last but not least, after an efficient first treatment, the cancer may develop resistance by proliferation of adapted tumor cells, which express other inhibitory receptors than CTLA-4 or PD-1 (3).

1.4. Adverse Events of Immune Checkpoint Inhibitors

As immune checkpoint inhibitors show a disinhibiting effect in immune responses, they may also systematically cause enhanced immune activity with the result of disseminated inflammation and autoimmune diseases (3). Therefore, the provoked

side effects are referred to as “immune-related adverse events” (irAEs) (7,11). They appear in a wide range of various symptoms and from endocrine glands to gastrointestinal and respiratory tract, over skin, joints, cardiovascular and neurologic system nearly all organ systems may be affected (9,22). These irAEs can occur either alone or in combination (22,23). According to Hodi et al. (24), approximately 60% of patients treated with CTLA-4 inhibitors may develop adverse events of all types. Wolchok et al. (21) even stated that 86% of patients treated with either anti-CTLA-4 or anti-PD-1 therapy showed immune-related adverse events. Thus, within the group of patients treated with ICI-therapy, autoimmune effects are significantly increased compared to the general population (< 0,005% to > 0,5%) (25). The majority of side effects cease under immunosuppressive therapy (22), but endocrine adverse events frequently require (additional) hormone replacement therapy (7).

1.4.1. Reasons for the various Range of Adverse Events

The incidence rates of adverse events during anti-CTLA-4 and anti-PD-1 treatment vary (22). For example, colitis, hypophysitis and rash seem to be more common with anti-CTLA-4 antibodies, whereas pneumonitis, hypothyroidism, arthralgia and vitiligo appear to occur more often on anti-PD-1 therapy (9,22). Besides, checkpoint inhibitor-associated adverse events differ from “common” autoimmune diseases. Overall, the prevalence of autoimmune effects caused by checkpoint blockade is usually rare compared to spontaneously developed autoimmune diseases in the general population (3). An underlying mechanism could be the expression of one of the receptors on ordinary tissues, like the ectopic expression of CTLA-4 on normal pituitary cells that may lead to lymphocyte-induced hypophysitis (9,26). Furthermore, owing to PD-1’s function to maintain self-tolerance – especially regarding antigens with permanent existence – its blockade may trigger vulnerability against these antigens, which may exemplary be predominant anti-thyroid antibodies (9,27). In that case, checkpoint inhibitor treatment could cause enhanced activity of those antibodies with risk of developing thyroid disorders (9,27). Moreover, it is assumed that adverse events may also underlie a cross reaction between tumour antigens and self-antigens (9,18). As an early example, in 1995 Cui and Bystryn (28) discovered a correlation between melanoma and vitiligo through the detection of emerged antibodies with attraction to similar antigens that

are expressed on melanocytes and melanoma cells in their patients. By repression of immune checkpoints, anti-tumour T cells may attack antigens on normal melanocytes with the consequence of depigmentation (18). Lastly, there exists a thesis that increased levels of cytokines like IL-17 may contribute to the development of ICI-associated adverse events, specified by Callahan regarding colitis (9,29). Besides, Khoja et al. (22) pointed out associations between the type of cancer and adverse events in a systematic review. For that comparison merely cases treated with PD-1 inhibitors were included. For instance, pneumonitis is more likely to occur in non-small lung cancer patients compared to melanoma, unlike colitis, pruritus, diarrhoea and rash which are more likely to emerge in melanoma patients. Furthermore, pneumonitis is more likely to occur in patients with renal cell cancer than in melanoma patients, while arthralgia, hypothyroidism, rash, pruritus and diarrhoea are more likely to appear in melanoma patients than in those with renal cell cancer. However, the mechanisms of toxicity are not specifically known yet and continue to be an object of research (22).

1.4.2. Occurrence in Time

The temporal range of onset of irAEs is more variable than of ordinary cancer therapies (22). However, specific adverse events follow the same pattern regarding their occurrence in their therapeutic process (30), as shown in figure 7. They usually appear within the first few weeks or months of therapy (9). Adverse events of skin are typically the earliest to occur with an average onset time of 5 weeks after treatment initiation while renal adverse events seem to be the last to emerge within an average time of 15 weeks (31). However, adverse events may fluctuate and occur at any point of therapy, even after cessation (9).

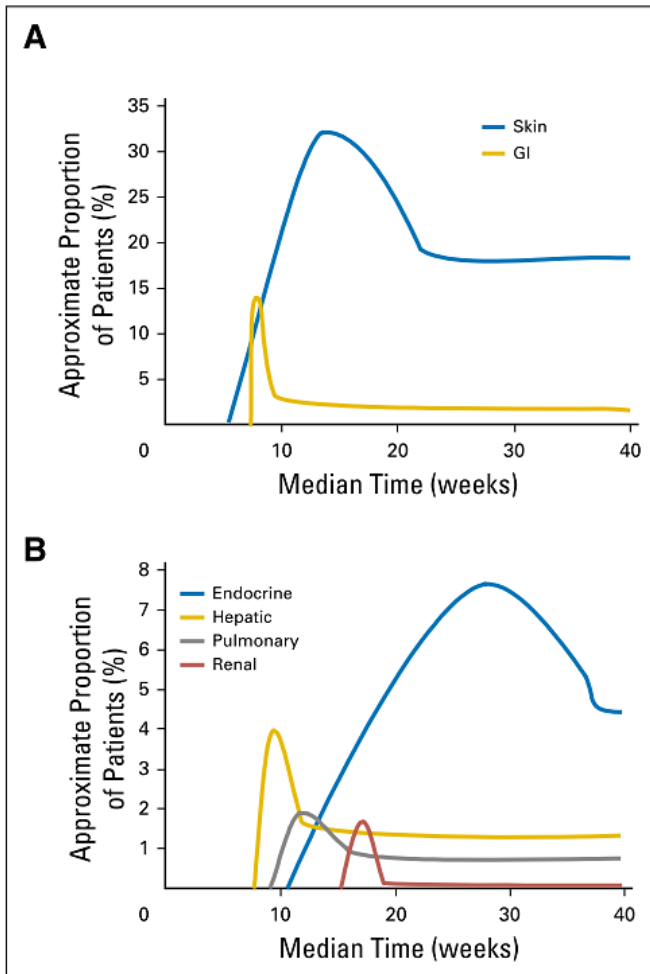


Figure 7: Time of onset of immune-related adverse events (29).

1.4.3. Dose-dependency and Combination Therapy

According to the above mentioned systematic review by Khoja et al. (22), a proportional increase of the frequency of adverse events in relation to an increase of the drug dose may be conceivable. However, pneumonitis caused by nivolumab therapy was the only adverse event which indicated a significant correlation between increased dose level and incidence (22). According to Wolchok et al. (32), there also exists an association between the occurrence of diarrhoea and increased dose level. 17% of advanced melanoma patients treated with low-dose ipilimumab (0,3 mg/kg) developed diarrhoea independent of grades, while this occurred to more than double (40%) the patients who were treated with high-dose ipilimumab (10 mg/kg) (32). Besides, no case of severe diarrhoea was reported in the low-dose group whereas 10 out of 71 patients (14%) in the high-dose group suffered from grade 3 or 4 diarrhoea (32). Furthermore, studies do not show an impact on frequency and severity of side effects regarding long-term treatment (9,16).

However, combined therapy with CTLA-4 and PD-1 inhibitors indicated a cumulative risk for higher incidences of side effects (21). According to several studies, especially pneumonitis, hypophysitis and thyroid disorders occur more frequently with combination therapy (11,33–36). A further clinical trial by Wolchok et al. (21) additionally claimed a higher occurrence of grade 3 and 4 adverse events with combined ipilimumab and nivolumab therapy (59%) compared to 28% irAEs with single ipilimumab and 21% irAEs with single nivolumab treatment. Considering all grades of adverse events, the data displayed the occurrence of adverse events in 96% of patients with combination therapy, compared to 86% each with nivolumab and ipilimumab monotherapy (21).

1.4.4. Classification of Adverse Events

It is advised that immune checkpoint inhibitor-induced adverse events are graded according to the “Common Terminology Criteria for Adverse Events (CTCAE)”, in reference to the National Cancer Institute, U.S. Department of Health and Human Services (37). Adverse events are graded from 1-5 regarding their severity (see Table 3). However, not all grades are appropriate for all adverse events, therefore some AEs are only graded from 1-4 (38).

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local or non-invasive; intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to AE

Table 3: Grades of adverse events according to the CTCAE (version 5.0) (36).

1.4.1. Non-endocrine Adverse Events

Existing data about the frequency distribution of immune-related adverse events differs in the literature. However, skin and gastrointestinal diseases are consistently mentioned as common side effects (15,21,37,39,40).

1.4.1.1. Fatigue

A review of several studies by Naidoo et al. (41) revealed fatigue as one of the most common irAEs. It is reported to occur with rates between 16-37% using PD-1 inhibitors and 12-24% with PD-L1 inhibitors (41). In the majority of the cases, fatigue is of mild or moderate quality and grades 3 or 4 are extremely rare (15,41). The incidences seem to be higher with CTLA-4 inhibitors which induce 40% fatigue of all grades and 7% of grades 3 or 4 in patients with metastatic melanoma, as stated by Hodi et al. (24). An underlying causative mechanism of fatigue owing to ICI therapy is yet unknown (41). However, fatigue often appears as a symptom of various systemic diseases. Regarding endocrinological diseases it should be noted that hypothyroidism as well as hypophysitis (if resulting in secondary hypothyroidism) may also lead to fatigue (40).

1.4.1.2. Skin

Skin-related adverse events are the most common ones (40). According to Brahmer et al. (39), they appear with an overall frequency of 30-50% of all ICI agents and with an incidence of 37-70% on CTLA-4 inhibitor and 17-37% on PD-1 inhibitor therapy. This data resembles data by Wolchok et al. (21), who reported 56% skin-related adverse events with anti-CTLA-4 therapy and 46% with anti-PD-1 treatment. Dermatologic adverse events are led by rash (39). 47-68% of patients treated with the CTLA-4 inhibitor ipilimumab experienced mainly maculopapular rash (30), which is typically manifested on the trunk and extremities (40) and may be accompanied by extreme pruritus (30). Besides, rash is stated to usually be the first manifestation of irAEs to emerge after an average of 4 weeks past treatment initiation (30,40). Another common adverse event of the skin in patients with metastatic melanoma is vitiligo (30,39). Weber, Kähler and Hauschild (30) quoted the occurrence of vitiligo in 11% of ipilimumab-treated patients, whereas Naidoo et al. (41) stated a low rate

of 2% in a more recent review. Vitiligo appears as well-demarcated macules of depigmentation and may emerge months after treatment initiation (39,40). The histological equivalent of dermis and epidermis infiltrated with CD4 and CD8 T cells (30) supports the theory of enhanced T cell reactivity between similar tumour and self-antigens (9,18). Blockade of PD-1 and PD-L1 checkpoints may cause mucosal toxicity. In one trial 6.5% of patients presented symptoms of dry mouth (40).

Data for grades 3 or 4 of cutaneous adverse events range from approximately 1-4% with ipilimumab therapy (31,39). As previously mentioned, dermatologic side effects appear less frequent under PD-1 inhibitor treatment, but grade 3 or 4 irAEs are reported at the same incidence as with CTLA-4 therapy (39,41). Rarely developing, however potentially life-threatening skin-related adverse events are bullous pemphigoid, Stevens-Johnson syndrome and toxic epidermal necrolysis (41). Those can be explained by the cross-reactivity theory of activated T cells (41).

1.4.1.3. Intestine

The most common gastrointestinal side effects of immune checkpoint inhibitors are diarrhoea and colitis (15). They occur in a mean time of 6 weeks after treatment initiation (15). According to the World Health Organization (WHO), diarrhoea is defined as increased stool frequency with loose or liquid stool passage at least three times a day respectively higher than usual (42). Diarrhoea is a symptom owing to osmotic, secretory, or inflammatory processes or due to motility disorders (43). Concerning ICI-treated patients who develop diarrhoea it is important to screen for other causes like infections (15,41). The occurrence of diarrhoea is significantly higher on anti-CTLA-4 therapy than anti-PD-1/-L1 treatment (15,40,41). Patients with metastatic melanoma who were treated with CTLA-4 inhibitors in a clinical trial developed around 30% diarrhoea of all grades with less than 10% severe forms (24,40). A similar trial with nivolumab therapy (anti-PD-1) displayed an occurrence of 18% diarrhoea of all grades (16). Diarrhoea may emerge as a symptom of colitis, but additional symptoms like abdominal pain and histological correlation are required for diagnosing ICI associated colitis (15,41). A histological correlate of neutrophilic and lymphocytic infiltration is proven for anti-CTLA-4 therapy but not yet confirmed for anti-PD-1 agents (15,41). An overview of respective studies showed an incidence of colitis grades 3 or 4 in approximately 5% of CTLA-4 treated patients

and in 1-3% of PD-1/-L1 receiving patients (41). Distinct data is reported by Wolchok et al. (21) who stated gastrointestinal grade 3 or 4 side effects as the most common ones under all grade 3 or 4 irAEs with an incidence of 12% with anti-CTLA-4 and 4% with anti-PD-1 treatment.

1.4.1.4. Hepatotoxicity

According to a review by Naidoo et al. (41), hepatotoxicity is reported in up to 10% of patients with CTLA-4 inhibitor therapy and in 5% or less with PD-1/-L1 inhibitors (15,41). The occurrence of grade 3 or 4 adverse events is rare – around 3% with high-dose ipilimumab (10 mg/kg) therapy (32) and 1-2% with anti-PD-1/-L1 treatment (41). Hepatic toxicity usually manifests as an asymptomatic increase of alanine transaminase (ALT) and aspartate transaminase (AST) levels and sometimes is accompanied by fever or malaise (30,40,41). Findings of diffuse T cell infiltration confirm the picture of immune-related hepatitis (30). In most cases, hepatic side effects emerge at 8-12 weeks into treatment (15,40). A fluctuating appearance may occur with anti-CTLA-4 antibodies (30). Concerning disease management of grade 4 immune-related hepatitis, infliximab should be strictly avoided due to its own potential of hepatotoxicity (30,40,41). Instead, if corticosteroids are not sufficient, the administration of mycophenolate mofetil should be considered (30,40,41).

1.4.1.5. Pneumonitis

Pneumonitis is an uncommon but potentially fatal ICI adverse event (33). It mainly occurs with anti-PD-1 therapy (9). A clinical trial by Naidoo et al. (33) detected a total of 5% of patients who developed pneumonitis in a large pool of checkpoint inhibitor receiving patients (anti-PD-1/-L1 monotherapy or combined with anti-CTLA-4). As already mentioned, the occurrence of pneumonitis seems to be dose-dependent (22). Naidoo et al.'s (33) study stated that the incidence was higher in patients who were treated with combination therapy. The time of onset differed from 9 days to 19 months with a mean onset time of 3 months and pneumonitis generally occurred earlier in patients treated with combination therapy (33). The most common symptoms of pneumonitis were dyspnoea and cough while 33% of patients were asymptomatic at the time of onset (33). Regarding diagnostic features, no

special radiologic signs were found (33). 73% of the cases appeared to be grade 1 or 2 adverse events. Grade 4 and 5 pneumonitis was experienced by 27% of patients, no case of grade 3 pneumonitis was detected (33). 88% of pneumonitis cases were resolved by pausing ICI therapy and/or administering immunosuppressive therapy. 12% of patients worsened under pneumonitis treatment and eventually died. However, only one case of death was explicitly attributable to pneumonitis (33).

1.4.2. Endocrine Adverse Events

Typical endocrine checkpoint inhibitor-associated adverse events are hypophysitis, thyroid disorders, adrenalitis and insulin-deficient diabetes mellitus (10,15,23,44) with the first two being the most common (34,40). Endocrine AEs are usually successfully treatable, therefore early diagnosis and appropriate treatment with hormone replacement therapy are essential. Otherwise they potentially may be life-threatening (23). The difficulty of accurate diagnosis of endocrine adverse events for clinicians consists of the variable and unspecific symptoms like fatigue, headache or malaise, which in most cases are the initial symptoms of endocrine dysfunctions (15,40). Furthermore, the possibility of multi-organ adverse events complicates accurate diagnosis and management (23). Endocrine side effects are difficult to display in numbers because of different methods of assessment, diagnosis and monitoring in clinical trials. However, according to Barroso-Sousa et al. (34), endocrine adverse events seem to affect up to 10% of patients, interestingly with the overall highest rates on anti-PD-1 therapy (39). For easier recognition, regular blood value evaluations before and after drug administration are required (30).

1.4.2.1. Hypophysitis

Hypophysitis is a potentially life threatening endocrine ICI-associated adverse event (7,10,23,36). It occurs nearly solely with anti-CTLA-4 blocking (alone or in combination) (34). According to a recent systematic review by Barroso-Sousa et al. (34), which included 7551 patients from 38 clinical trials, hypophysitis was experienced by 6.4% of patients with combination therapy, 3.2% treated with CTLA-4 inhibitors and just 0.4% receiving PD-1 inhibitors. On treatment with PD-L1

inhibitors hypophysitis hardly existed (< 0.1%). 0.5% of all hypophysitis cases were graded as severe or life-threatening (34). Interestingly, in several studies regarding ICI-induced hypophysitis, tremelimumab which is a yet to be approved CTLA-4 inhibitor is included (10). Compared to ipilimumab it shows lower rates of hypophysitis with only 0.4-2.6% (10). The incidence of ipilimumab-induced hypophysitis is not consistent throughout literature. Barroso-Sousa et al. (34) stated in the above mentioned review a relatively low incidence rate with 3.2% of affected patients. Another clinical review by Joshi et al. (37) presented a wide-ranging rate of 0-17% (respectively 0.4-17% by Girotra et al.(10)) for ipilimumab-associated hypophysitis, however, with the following dose-dependent distribution: Low-dose ipilimumab (<3 mg/kg) caused in 1.8-3.3% of patients hypophysitis, whereas high-dose ipilimumab (3-9 mg/kg) led to 4.9-17% of the hypophysitis cases (7,37). However, dose-dependency is discussed controversially (7,37,45). In a longitudinal analysis by Faje et al. (45) 13 of 17 patients who developed hypophysitis received 3 mg/kg ipilimumab and the remaining 4 were treated with a high-dose of 10 mg/kg ipilimumab. In contrast, data concerning the incidence of PD-1 inhibitors-induced hypophysitis appears to be more consistent throughout literature (10,37). For instance, both Joshi et al. (37) and Girotra et al. (10) stated an occurrence under 1% for each nivolumab and pembrolizumab. Further, Joshi et al. (37) quoted that patients who were pre-treated with brain radio- or chemotherapy were less likely to develop hypophysitis.

The currently accepted hypothesis regarding the pathological mechanisms of the development of ICI-induced hypophysitis was published by Iwama et al. (26) in 2014. Ectopic CTLA-4 antigen expression on pituitary endocrine cells was discovered. The existence of CTLA-4 mRNA substrates was proven through reverse transcription-PCR in the murine model while protein substrates of CTLA-4 were simultaneously proven through Western blotting in humans. In mice CTLA-4 appeared to be primarily present on prolactin- and TSH- but not on GH- and FSH-secreting cells. Similar results were obtained in humans. After further examination, the expression of CTLA-4 was proven on 3% of prolactin- and 1-3% TSH-producing cells (26). Furthermore, after repetitive administration of anti-CTLA-4 antibodies, newly generated antibodies against the anterior pituitary gland were detected. Findings in patients with hypophysitis demonstrated that these antibodies were primarily directed against TSH-secreting cells and less frequently against FSH- and ACTH-

producing cells which was congruent with the symptoms presented by patients (26). Thus, Iwama et al. (26) hypothesized that the administered anti-CTLA-4 antibodies engage with the CTLA-4 antigens on pituitary cells. As a consequence the complement system gets activated through the classical pathway (26). That means that the complement factor C1 binds to the antigen-antibody-complex and thereby gets activated. Several proteolytic cleavages and thereby further bindings of other factors of the cascade follow which ultimately leads to the generation of the membrane attack complex with the result of lysis of cell membranes and consecutive destruction of pituitary cells. Additionally, NK cells get activated and act directly cytotoxic (1). That procedure of opsonisation and lysis by the complement system is consistent with a type 2 hypersensitivity reaction (26). Furthermore, infiltration of lymphocytes and macrophages in the pituitary gland was proven histopathologically through haematoxylin and eosin staining in the murine model, which may also be initiated by the complement system. This cellular mechanism is assumed to play an additional role in the pathomechanism of pituitary gland toxicity after CTLA-4 inhibitor administration (26). More specifically, ipilimumab is a monoclonal antibody of the IgG1 subclass and able to activate the complement system from average quality. On the contrary, tremelimumab is an antibody of the IgG2 subclass and is less likely to activate the complement system (26). These facts are confirmed by Iwama et al.'s (26) findings of higher incidence of hypophysitis with ipilimumab than with tremelimumab therapy.

In 2016, Caturegli et al. (46) were the first to examine the pituitary gland's structure in patients after CTLA-4 therapy post mortem. They analysed 6 autopsy cases, one of them with actual clinical hypophysitis. This patient presented a heterogeneous 30% necrosis and fibrosis with nearly full destruction of the anterior pituitary gland. Remaining endocrine cell areas were intensively infiltrated with haematopoietic mononuclear cells. The endocrine cell residues were primarily GH-producing. Prolactin- and ACTH-secreting cells were rare, FSH/LH- and TSH-secreting cells were completely lost (46). The posterior pituitary gland showed a normal structure in concordance with absence of central diabetes insipidus (46). In magnetic resonance imaging the patient showed an enlarged pituitary gland with hypo-intense areas in the anterior gland, which correlates with necrosis. However, it needs to be mentioned that this patient received the CTLA-4 inhibitor tremelimumab which is yet to be approved by the FDA (46). Another patient showed mild lymphocytic infiltration

and others presented normal pituitary glands although all received anti-CTLA-4 therapy. CTLA-4 antigen expression on pituitary cells was proven in all patient but at different levels (46). Thus, Caturegli et al. (46) concluded that the development of ICI-induced hypophysitis could be associated with the extent of expression of CTLA-4 antigens on pituitary endocrine cells. Further they stated that T cell infiltration, IgG-dependent complement deposition and phagocytosis were also detected in dependence of the level of CTLA-4 expression (46).

Furthermore, male gender and older age seem to be risk factors for the development of ICI-associated hypophysitis (10,37,45). Faje et al. (45) proposed the hypothesis that high oestrogen levels could have a protecting effect, however nothing more is known about that correlation yet.

In general, hypophysitis leads to hypopituitarism by primarily affecting the thyroid and the adrenal axis (10). A lack of central releasing hormones by the pituitary gland leads consequently to a lack of hormones from peripheral glands. Thus, if the TSH axis is impaired, this results in hypothyroidism (10). Reportedly, prolactin levels may be elevated or decreased which is the case in approximately 25% of patients (7). Most male patients suffer from a deficiency of the gonadal axis with the result of hypogonadotropic hypogonadism (7). The growth hormone axis appears to be affected very rarely and would be detected through low serum IGF-1 levels (7). Cases with impairment of the posterior pituitary gland with ensuing diabetes insipidus are extremely rare (7,10,47). One example in the literature was a prostatic cancer patient with CTLA-4 treatment who suffered from diabetes insipidus (47). Initial symptoms of hypophysitis which are independent of the affected axis are usually unspecific like headache (most common according to Faje et al. (45)), fatigue, nausea, vertigo and behavioural change. Moreover, confusion, memory loss, anorexia and temperature intolerance may emerge (7). Symptoms of local compression like cranial nerve paralysis and sometimes headache may occur through inflammatory oedema of the pituitary gland (23). Pressure of the optic chiasm causes visual impairments (7,23). The most important differential diagnosis of these unspecific symptoms are newly emerged brain metastases (30). Additional symptoms, depending on the affected hormone axis, may be for instance loss of libido, erectile dysfunction, hyponatremia (in up to 50% (10)), hyperkalaemia and hypotension (37). These symptoms mostly occur after 6-12 weeks after treatment initiation with a mean onset time of 9-11 weeks – usually after the third ipilimumab

administration (7,37,48). That may suggest a cumulative effect of CTLA-4 inhibitors regarding the development of hypophysitis (7). Endocrine disorders due to hypophysitis are in most cases irreversible (7). Faje et al.'s (45) longitudinal analysis of metastatic melanoma patients receiving ipilimumab presented 11% of patients developing hypophysitis, ensuing persistent hypopituitarism in 76% of the cases. Recovery of the gonadal axis is reported in approximately 57% of men, but varies from 12-57% according to Girotra et al. (7,10,41). Recovery of the thyroid axis is described in 37-50%, but varies from 6-64% according to Girotra et al. (7,10,41). In contrast, recovery of the adrenal axis is extremely unlikely (7,10).

For easier diagnosis, regular laboratory examinations during the time of immune-checkpoint inhibitor therapy are required (30). During the first six months of treatment, monthly evaluations are recommended. If hormone levels remain within the reference ranges the intervals of examinations may be extended to every three months for the next six months and every six to twelve months afterwards (10). Hypopituitarism results in partially or completely decreased levels of mostly anterior pituitary hormones – TSH, ACTH, FSH/LH and prolactin – as well as of effector hormones – free triiodothyronine fT3 and thyroxine fT4, cortisol, oestrogen, testosterone and IGF-1. On suspicion of hypophysitis initial blood examination should be performed with TSH and fT4, as well as 8 a.m. morning ACTH and cortisol levels. If hypophysitis is diagnosed, also gonadotropins – testosterone in men and oestrogen in women respectively – should be measured. In patients with hypogonadotropic hypogonadism, additionally the prolactin level may be examined because hyperprolactinaemia may suppress gonadotropin-releasing hormone (GnRH) secretion. Moreover, serum sodium levels should be evaluated owing to the high prevalence of hyponatraemia. Usually, hyponatraemia is transient and resolves with hormone replacement therapy. Regarding diabetes insipidus, possible polyuria and polydipsia should be assessed. There is no need of biochemical evaluation of growth hormone and IGF-1 levels, since replacement is contradicted in patients with active neoplasia (10). Further, performing brain-imaging through magnetic resonance (MRI) is suggested when hypophysitis is clinically suspected (23) or biochemically established (10). This disease usually causes enlargement with homogenous or heterogeneous appearance of the pituitary gland and occasional thickening of the infundibulum (7,10,30). A MRI should urgently be conducted when symptoms of local compression are present in order to be able to evaluate a need

for administration of methylprednisolone (see below) (23). Pituitary enlargement is sensitive and specific for hypophysitis (45) and was found in respectively 60-100% (7) and 75-100% (10) of patients with ICI-hypophysitis, although several cases without pituitary enlargement were reported (7,36). It is important to note that no distinct radiological patterns to differentiate checkpoint inhibitor-associated hypophysitis and primary hypophysitis could be found (48). According to Corsello et al. (7), ICI-associated hypophysitis overall appears with less intensity of alterations. Radiological signs may occur weeks before biochemical changes (10,45,48).

Regarding management of grade 3 or higher ICI-induced hypophysitis, it has been widely recommended to withhold ICI-therapy and to start with high-dose intravenous glucocorticoid therapy (30,39,40,45). Whether high-dose glucocorticoid therapy should be preferred over physiologic glucocorticoid substitution has, however, been questioned (23). There is no clear proof for the efficacy of high-dose glucocorticoid therapy in terms of recovery from hypophysitis or overall survival (10,23) whereas it is established that high dose glucocorticoid therapy can cause adverse effects. When reviewing the literature it seems that high-dose glucocorticoid therapy, as it is used for other immune related adverse events, like the intestine or the lungs, has been recommended for endocrine adverse events (30,39,40,45) without considering the fundamental differences between different organs and the efficient treatment options for endocrine disorders. In this context, it must be stressed that living without the pituitary gland with adequate hormone replacement therapy is possible but living an ordinary life without lungs is not. Anyway, if the ACTH axis is affected, and high-dose glucocorticoid therapy is initiated it must be performed by subsequent tapering over 4-8 weeks to physiologic replacement levels (30,39,40,45). If both adrenal and thyroid axis are impaired, it is important to administrate corticosteroid substitution (usually hydrocortisone) before thyroid hormone replacement in order to prevent adrenal crisis (10,23,39). Using these above mentioned therapies, the acute symptoms are relieved in most patients within days (7,30). Regarding the long-term course of ICI-associated hypophysitis, the majority of patients require life-long hormone replacement therapy, especially those with insufficiency of the adrenal axis (7,10,30,40). Considering that it is not clear whether high-dose glucocorticoid therapy should be preferred over physiologic replacement therapy (23) and in view of corticosteroid's potential of severe adverse events and adrenal insufficiency risk during the tapering phase, Girotra et al. (10) and Konda, Nabhan and Shah (36)

suggested to reserve high-dose glucocorticoids for severe hypophysitis cases and patients who suffer from symptoms of local compression through pituitary enlargement (11,23,45). Otherwise, in case of cortisol deficiency, administration of physiologic hydrocortisone dosage is recommended (23,36). Methylprednisolone seems to benefit in reducing symptoms of compression (23). The enlarged pituitary gland is reported to diminish after 4-12 weeks (10) or alternatively 2-27 weeks (36). Severe hypophysitis cases with secondary adrenal insufficiency require immediate hospitalisation due to their life-threatening potential of hypo- and hyperkalaemia, dehydration, hypotension and eventually shock (10,30,40). In case of adrenal crisis, substitution of corticosteroids with mineralocorticoid activity like fludrocortisone is essential (23,30). Impairment of the other pituitary-hormone axis is simply treated with lacking effector hormones like thyroxine (T4) and gonadotropins (testosterone or oestrogen). The replacement of growth hormone is contradicted in the case of malignant neoplasia. According to Higham et al. (23), ICI-therapy can be continued as soon as the patient is clinically stable on adequate hormone replacement therapy.

Notably, Faje et al. (45) quoted: "The presence of IH [immune-related hypophysitis] appears to be associated with longer survival and relatively low morbidity in patients who receive appropriate hormonal replacement therapies". The median survival time of patients diagnosed with hypophysitis was 19.4 months, in contrast to 8.8 months for patients without hypophysitis (45).

1.4.2.2. Thyroid disorder

Primary thyroid disorders are also frequent endocrine side effects (7). Within those, hypothyroid metabolic conditions are the most common (10). Thyroid dysfunctions may occur as thyroiditis, primary hypothyroidism, or hyperthyroidism owing to Graves' disease-like conditions associated with ophthalmopathy, with the latter one to emerge rarely and mostly in euthyroid states (7,10,11,41). These disorders have to be distinguished between overt and subclinical conditions (35,36). A few authors reported thyroiditis as the most frequent cause for ICI-induced thyroid disorders (7,10,11,49,50). Typical for thyroiditis is an initial thyrotoxic phase of hyperthyroidism lasting for approximately 2-4 weeks (10), followed by transient or persistent subclinical or overt hypothyroidism (7,23). The hyperthyroid condition

initially occurs through excessive hormone release due to the destructive genesis of thyroiditis (10,36). The majority of cases of thyroid dysfunction are of mild or moderate quality. However, one case of each thyroid storm owing to thyrotoxicosis and myxoedema secondary to hypothyroidism were reported (23).

Regarding Barroso-Sousa et al.'s (34) recent comprehensive systematic review, 472 cases of all grades hypothyroidism and 194 cases of all grades hyperthyroidism were observed among 7551 patients. That led to an estimated overall incidence of hypothyroidism of 6.6% and hyperthyroidism of 2.9%. Within both metabolic conditions, the majority of cases were presented as grade 1 or 2 and severe thyroid disorders were very rare with 0.12% of hypothyroid and 0.10% of hyperthyroid patients (34). Statistically significant differences concerning the incidence of thyroid disorders between the different ICI agents were detected. Patients receiving PD-1 inhibitors or combined therapy (13.2%) were more likely to develop hypothyroidism than those with ipilimumab therapy (3.8%). Hyperthyroidism was more likely to occur on combination therapy (8.0%) than monotherapy. The lowest hyperthyroidism rate of just 0.6% was found in patients with anti-PD-L1 therapy (34). In contrast, Girotra et al. (10) suggested an incidence of hypothyroidism with 8.6% of patients with anti-PD-L1 treatment. However, data from recent publications is inconsistent: According to Illouz et al. (11), hypothyroidism occurs in 10% of patients with monotherapy and up to 25% for combined CTLA-4 and PD-1 or PD-L1 inhibitor treatment. Hyperthyroidism is claimed to occur in a range of 5% to 10% on combination therapy (11). In another recent review, PD-1 inhibition is stated to elicit hypothyroidism in 5-8% of patients and hyperthyroidism in 3% (10). Ipilimumab is quoted to generate approximately 5-6% of hypothyroidism cases (10). Konda et al. (36) stated that hypothyroidism occurs in 5.6% with ipilimumab, 5.9% with PD-1 inhibitors, 4.3% with PD-L1 inhibitors and 13.9% on combination therapy. Hyperthyroidism is claimed to occur in 3.3% of patients treated with PD-1 and 8% with combination treatment. Further, Konda et al. (36) specified the incidence of thyroiditis with 3.2% on ipilimumab therapy. Morganstein et al. (35) differentiated between subclinical and overt conditions in his systemic analysis of ICI-treated melanoma patients. He stated that 23% of CTLA-4-treated patients experienced various conditions of thyroid disorder with most of them developing subclinical hyperthyroidism (16%) and second most subclinical hypothyroidism (6%). Just one out of 126 patients suffered from overt hypothyroidism. Regarding PD-1 inhibitors, 13% developed overt

hypothyroidism (however two patients were already handicapped), further 13% developed subclinical hypothyroidism and another 13% demonstrated signs of subclinical hyperthyroidism. Two of the patients with subclinical hypothyroidism suffered from preceding hyperthyroid episodes. That results in a total of 39% PD-1 inhibitor treated patients who developed a kind of thyroid disorder. With nivolumab and ipilimumab combination therapy 50% of patients generated a thyroid dysfunction. 22.2% developed overt hypothyroidism, with three of them showing initial periods of overt or subclinical hyperthyroidism. Further 22.2% presented subclinical hyperthyroidism without subsequent hypothyroidism. Only one patient (5.6%) developed primary subclinical hypothyroidism (35).

The onset of thyroid dysfunctions is similar to that of ICI-induced hypophysitis. With anti-CTLA-4 therapy, abnormalities usually occur after the second to fourth infusion which means after 6-12 weeks (7,37,41). However, late onset after a few months to several years also has been reported (10,37).

Like hypophysitis, thyroid dysfunction initially presents itself with unspecific symptoms (36). On the one hand, symptoms of hypothyroidism may be fatigue, weakness, loss of concentration, depressive mood, obstipation, cold intolerance and gain of weight. On the other hand, hyperthyroidism may cause restlessness, agitation, trembling, insomnia, voracious appetite, hyperhidrosis, tachycardia, increased stool frequency, loss of weight and muscle weakness. Additionally and typical for Graves' disease is exophthalmia (51).

Underlying pathophysiological mechanisms are not fully clear yet. However, the existence of auto-antibodies could be verified in several affected patients. Regarding thyroiditis, the generated antibodies may be anti-thyroglobulin and anti-thyroid peroxidase antibodies. However, in the rare case of Graves' disease anti-TSH-receptor antibodies are responsible for excessive hormone synthesis (7,41). The existence of autoantibodies is reported at different rates. A clinical trial by Osorio et al. (27) regarding pembrolizumab treatment of non-small lung cancer patients revealed the existence of anti-thyroid antibodies in 8 of 10 patients who developed thyroid disorders in comparison to 3 of 38 who did not. The onset of antibody development was consistent with the beginning of transient hyperthyroidism and predicted subsequent hypothyroidism (27). In comparison, another clinical trial by de Filette et al. (49) with pembrolizumab treatment of melanoma patients showed elevated anti-thyroid antibodies in just 4 out of 10 cases.

Interestingly, Orlov et al. (50) reported 6 out of 10 patients suffering from thyroiditis with an initial thyrotoxic episode during anti-PD-1 therapy without antibody emergence, whereas the other 4 patients developed primary hypothyroidism with thyroid antibody positivity. Since 11% of healthy individuals carry thyroid autoantibodies, it is discussed if immune system enhancing therapy causes unmasking of latent autoantibodies (27). In contrast, de Moel et al. (52) detected an association of newly emerged anti-thyroid antibodies after ipilimumab therapy and significantly more thyroid disorders with subsequent anti-PD-1 therapy. Furthermore, the authors stated an increased treatment response as well as survival rate in patients who developed autoantibodies (52).

Another discussed reason may be genetic polymorphisms in the CTLA-4 gen locus on chromosome 2 (11,37). Patients with autoimmune diseases (Hashimoto's thyroiditis and Addison's disease) exhibit amino acid exchanges at codon 17 compared to the control group, which leads to speculation about associations of that polymorphism and the development of thyroiditis (53).

Pre-existing thyroid disorders are discussed to worsen through immune checkpoint inhibitor therapy (37). Narita et al. (54) reported two cases that led to serological aggravation of pre-existing clinical and (probably) subclinical Hashimoto's disease with nivolumab therapy, however, correlations are not clear yet (37,54).

Thyroid dysfunction may potentially be dangerous if not treated appropriately in time (10). Therefore, regular laboratory evaluations every four to six weeks as part of routine clinical monitoring are recommended (39). TSH and free T4 levels are essential for basic biochemical assessment (10,11,36,39). On one hand, increased TSH with normal or decreased free T4 indicates primary hypothyroidism. On the other hand, low TSH and normal to raised free T4 levels refer to primary hyperthyroidism (10,36,39). Importantly, hypophysitis has to be ruled out based on blood value levels (36). Hypopituitarism would lead to secondary hypothyroidism with low or normal TSH levels and low fT4 levels (10,36). However, simultaneous occurrence of primary and secondary thyroid disorders is possible. Further, thyroid sonography should be performed. As already noted, thyrotoxicosis is in most cases a transient condition within the frame of thyroiditis (7,10,11) or, less common, a prolonged state consistent with Graves' disease (10,36,39). These conditions may be revealed by diffuse enhanced uptake of the thyroid gland of 18-FDG in PET scans (10,49) and distinguished by iodine uptake scans (10,23). Thyroiditis would

present low iodine uptake compared to Graves' hyperthyroidism with high uptake (10,23). However, routine iodine uptake scans are not indicated (44).

In reference to Girotra et al. (7), 50% of thyroid disorder cases are reported to be transient with recovery of the euthyroid condition, although hypothyroidism due to PD-1 inhibitor therapy appears to stay rather permanent. During the mostly limited periods of thyrotoxicosis owing to thyroiditis, anti-thyroid therapy is usually not indicated because it is not effective in the case of destructive thyroiditis. Thiamazole inhibits synthesis of thyroid hormones, however, cannot inhibit the secretion of already synthesised triiodothyronine and thyroxine (43). If necessary, symptomatic therapy with beta-blocker is sufficient in most cases (10,11,23,39,41). Glucocorticoid therapy is also not indicated but may be taken into account for patients with cardiac diseases (10). Exceptionally, in severe cases of thyrotoxicosis with thyroid storm, administration of anti-thyroid agents may be considered alongside high-dose glucocorticoid therapy (39,44). However, Graves' disease definitely requires anti-thyroid medication like methimazole or propylthiouracil (10,39,41). For hypothyroid conditions with TSH levels $> 10 \mu\text{U/mL}$ (11,39) it is suggested to start with substitution of low-dose levothyroxine (25-50 $\mu\text{g/day}$) with subsequent titrating until normal thyroid hormone levels are reached (10,11,39,41,44). Patients with only subclinical disorders can stay under surveillance without specific treatment (10,41). Because most cases are of mild or moderate quality and thyroid disorders are successfully treatable, pausing or discontinuation of ICI-therapy is usually not required (10,41,44). Furthermore, Osorio et al. (27) stated an increased overall survival rate of patients who developed thyroid dysfunctions on pembrolizumab with a mean survival time of 40 months, compared to 14 months of those who did not develop thyroid adverse events.

1.4.2.3. Primary adrenal insufficiency

Cases of primary adrenal insufficiency (PAI) due to ICI-therapy are quite rare (34). Therefore, not much data regarding its incidence is available. Barroso-Sousa et al. (34) detected among 5831 patients 43 cases (0.7%) of adrenal insufficiency of any grade with 14 of them suffering from grade 3 or higher (0.2%). However, in the cohort of combination therapy PAI occurred in 4.2% of patients (34). Other reports vary from 0.3-1.5% of PAI incidence (7). Primary adrenal insufficiency biochemically

presents low cortisol and high ACTH levels (39). Possibly subclinical conditions of adrenal insufficiency with radiological enlargement of the adrenal gland without clinical symptoms have been reported (37). According to Girotra et al. (10), two studies each conducted on one patient stated adrenal gland enlargement occurring 3-4 months after ipilimumab therapy which resolved over the course of 6 weeks to 4 months. One patient was reported to first have presented low ACTH levels combined with pituitary enlargement, consistent with secondary adrenal insufficiency due to ipilimumab-induced hypophysitis. Therefore, replacement therapy with hydrocortisone was initiated. Additionally, imaging of the adrenal glands during ipilimumab therapy showed bilateral enlargement compared to the baseline scans. The patient's cortisol and aldosterone levels did not respond to a cosyntropin stimulation test which led to the diagnosis of primary adrenal insufficiency (55). Thus, Min and Ibrahim (55) concluded that the dynamic size change of adrenal glands under ICI-therapy is an indicator for potential ICI-induced adrenalitis. The second case presented symmetrically and smoothly enlarged adrenal glands with hypermetabolic 18-FDG uptake in PET scans. Due to this appearance of a smooth enlargement without any signs of nodularity or mass, metastatic modifications could be excluded. Interestingly, the cortisol level of the second patient was elevated once but lacking clinical symptoms. Four months later, the adrenal gland diminished back to normal size (56). Bacanovic (56) concluded that smooth and hypermetabolic adrenal gland enlargement after ipilimumab therapy should raise the suspicion of ICI-associated adrenalitis. However, ICI-induced primary adrenal insufficiency should be an exclusive diagnosis, since several other factors may lead to abnormalities in hormone levels or cause adrenal gland enlargement. For instance, adrenal metastasis and glucocorticoid therapy, which influences the pituitary-adrenal axis, must be ruled out (11). Moreover, if adrenalitis is diagnosed, other causes like preceding infection should be considered (39). For further diagnosis, a cosyntropin stimulation test may be conducted (11). Management of PAI should be performed with glucocorticoid (usually hydrocortisone) replacement and additionally mineralocorticoid (fludrocortisone) substitution may be required (23).

1.4.2.4. Pancreas – Insulin-dependent diabetes mellitus

In consideration of Barroso-Sousa et al.'s (34) systematic review, the occurrence of insulin-dependent diabetes is extremely rare. Only 0.2% of the 5831 patients were diagnosed with insulin-deficiency, however, half of them were graded 3 or higher. Notably, all those cases except one were associated with PD-1 inhibitors (34). According to Girotra et al. (7) and Konda, Nabhan and Shah (39), cases of insulin deficient-diabetes also occurred with anti-PD-L1 therapy but not with anti-CTLA-4 treatment. The median onset averaged 8.5 weeks with an average glucose level of 530 mg/dL and low C-peptide levels (10,36). Hughes et al. (57) reported 5 cases of new onset insulin-dependent diabetes in patients treated with PD-1 inhibitors. They presented severe hyperglycaemia or diabetic ketoacidosis with elevated HbA1c ranging from 1 week to 5 months after initiation of ICI therapy. All patients required prolonged insulin therapy. However, one patient already suffered from pre-existing diabetes mellitus type 2 and newly developed insulin-dependency after ICI-administration. Three out of five patients presented positive autoantibodies for diabetes-associated structures (pancreatic beta-cells and insulin), which is usually the diagnostic criteria for diabetes mellitus type 1. In the other two patients, T cell enhancement against diabetes antigens was proved. These mechanisms indicated an autoimmune genesis which is normally untypical for older patients (> 55 years) (57). Despite of the low prevalence of ICI-induced diabetes, serum glucose should be measured in all patients that are feeling unwell. Furthermore, it must be considered that glucocorticoid therapy used for other irAEs may cause hyperglycaemia (23).

In Table 4 below, the frequency distribution over the various kinds of adverse events is shown.

Event	Nivolumab plus Ipilimumab (N=313)		Nivolumab (N=313)		Ipilimumab (N=311)	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
	<i>number of patients with event (percent)</i>					
Any treatment-related adverse event	300 (96)	184 (59)	270 (86)	67 (21)	268 (86)	86 (28)
Rash	93 (30)	10 (3)	72 (23)	1 (<1)	68 (22)	5 (2)
Pruritus	112 (35)	6 (2)	67 (21)	1 (<1)	113 (36)	1 (<1)
Vitiligo	28 (9)	0	29 (9)	1 (<1)	16 (5)	0
Maculopapular rash	38 (12)	6 (2)	15 (5)	2 (1)	38 (12)	1 (<1)
Fatigue	119 (38)	13 (4)	114 (36)	3 (1)	89 (29)	3 (1)
Asthenia	30 (10)	1 (<1)	25 (8)	1 (<1)	17 (5)	2 (1)
Pyrexia	60 (19)	2 (1)	21 (7)	0	21 (7)	1 (<1)
Diarrhea	142 (45)	29 (9)	67 (21)	9 (3)	105 (34)	18 (6)
Nausea	88 (28)	7 (2)	41 (13)	0	51 (16)	2 (1)
Vomiting	48 (15)	7 (2)	22 (7)	1 (<1)	24 (8)	1 (<1)
Abdominal pain	26 (8)	1 (<1)	18 (6)	0	28 (9)	2 (1)
Colitis	40 (13)	26 (8)	7 (2)	3 (1)	35 (11)	24 (8)
Headache	35 (11)	2 (1)	24 (8)	0	25 (8)	1 (<1)
Arthralgia	43 (14)	2 (1)	31 (10)	1 (<1)	22 (7)	0
Increased lipase level	44 (14)	34 (11)	27 (9)	14 (4)	18 (6)	12 (4)
Increased amylase level	26 (8)	9 (3)	20 (6)	6 (2)	15 (5)	4 (1)
Increased aspartate aminotransferase level	51 (16)	19 (6)	14 (4)	3 (1)	12 (4)	2 (1)
Increased alanine aminotransferase level	60 (19)	27 (9)	13 (4)	4 (1)	12 (4)	5 (2)
Decreased weight	19 (6)	0	10 (3)	0	4 (1)	1 (<1)
Hypothyroidism	53 (17)	1 (<1)	33 (11)	0	14 (5)	0
Hyperthyroidism	35 (11)	3 (1)	14 (4)	0	3 (1)	0
Hypophysitis	23 (7)	5 (2)	2 (1)	1 (<1)	12 (4)	5 (2)
Decreased appetite	60 (19)	4 (1)	36 (12)	0	41 (13)	1 (<1)
Cough	25 (8)	0	19 (6)	2 (1)	15 (5)	0
Dyspnea	36 (12)	3 (1)	19 (6)	1 (<1)	12 (4)	0
Pneumonitis	22 (7)	3 (1)	5 (2)	1 (<1)	5 (2)	1 (<1)
Treatment-related adverse event leading to discontinuation	123 (39)	95 (30)	37 (12)	24 (8)	49 (16)	43 (14)

Table 4: Frequency distribution of adverse events referred to different therapy regimes (21).

1.4.3. Management of adverse events

Grade	Cancer treatment	Management	Example
1	Continue	Supportive	e.g. Loperamide for diarrhoea; emollients and hydrocortisone cream for rash
2	Consider withholding	Targeted to reverse side effect	Oral prednisone 0,5-1 mg/kg/day, weaning to zero slowly over 4 weeks
3	At least withhold or discontinue	Inpatient	i.v. methylprednisolone 1-2 mg/kg/day or oral prednisone 1-2 mg/kg/day until symptoms attenuate till grade 1 or less, then oral prednisone as above, tapering over at least 4-6 weeks
4	Discontinue	Intensive	Infliximab i.v. or oral mycophenolate mofetil, if corticosteroids are insufficient

Table 5: Management of immune-related adverse events. Adapted from (39,58).

Table 5 above presents the general approach of management of ICI-induced adverse events which is based on immunosuppressive therapy with glucocorticoid treatment. However, endocrine adverse events require an adapted approach (11,23,39). First, hormone replacement therapy is the most important part of management of endocrine adverse events. Corticosteroids are strongly recommended to be reserved for severe life-threatening endocrine side effects or symptoms through local compression since regardless of immunosuppressive therapy, there is no evidence of improvement in resolving hypophysitis and most patients stay dependent on a life-long hormone substitution (7,10,11,23,30,39,40). Furthermore, corticosteroids may potentially induce adrenal insufficiency when their dose is reduced to fast, or they may cause other severe adverse events like hyperglycaemia, increased susceptibility to infections, increased coagulation with thrombotic risk, hypertension and gastric or duodenal ulcer (11,51). Second, non-endocrine adverse events of grade 4 require permanent discontinuation of checkpoint inhibitor therapy, but ICI treatment of patients suffering from endocrine adverse events grade 4 may be continued after appropriate hormone replacement therapy (23,39). Currently, management of endocrine side effects by clinicians should be performed according to the “Endocrine Emergency Guidance: Acute management of the endocrine complications of checkpoint inhibitor therapy” by

Higham et al. (23). ICI-treated patients feeling acute severely unwell should be assumed to suffer from acute cortisol deficiency (primary or secondary) and therefore immediately treated with glucocorticoids until proven otherwise. The appropriate glucocorticoid for acute management is hydrocortisone. If the patient's condition does not improve within the first 24 hours of replacement therapy, further diagnosis should be performed. Asymptomatic or mildly unwell feeling patients with cortisol deficiency also should get hydrocortisone replacement therapy. Tapering of glucocorticoid dosage can start after clinical recovery (usually 48-72 hours). Glucocorticoid therapy is maintained by using hydrocortisone or prednisolone (23).

Regarding management of non-endocrine adverse events, immunosuppressive therapy leads to the question whether it causes attenuated effects of the checkpoint blocking therapy which relies on the mechanism of enhanced immune activity. According to Postow et al. (9) and Konda, Nahban and Shah (36), immunosuppressive therapy of irAEs does not impair clinical responses to ICI treatment and the overall outcome compared to patients who did not receive immunosuppressive agents did not worsen. However, prospective studies with these points as primary outcome are missing. Restarting immune checkpoint inhibitor therapy may lead to repeated occurrence of the same or new adverse events, however, recurrent side effects usually seem to be less severe. Absolute contraindication for resuming ICI therapy are life-threatening side effects (9,36).

1.4.4. Death by Immune Checkpoint Inhibitor Therapy

Deaths due to ICI therapy are extremely rare but can occur for instance in consequence of myocarditis, pneumonitis, colitis or neurological damage (9,22). As stated in Khoja's (22) review, mortality during PD-1 therapy with nivolumab occurs in 0.3% of cases and with pembrolizumab just in 0.1% - as sequelae of pneumonitis representing the most frequent cause. However, gastrointestinal causes with consequences of colitis, diarrhoea or bowel perforation are the mainly potential life-threatening side effects with CTLA-4 therapy (22). As a comparison, in the already quoted clinical trial by Wolchok et al. (21) out of 945 patients who participated in the trial, 4 deaths occurred (0.4%). There was one death each in the single nivolumab and single ipilimumab treatment group and additional two deaths in the combined

treatment group (21). Reasons were neutropenia (nivolumab), colon perforation (ipilimumab), autoimmune myocarditis with cardiac insufficiency (patient was priorly cardiac handicapped) and liver necrosis, the last two with combined therapy (21).

2. Material and Methods

Literature research was performed considering relevant books of the immune system and especially its inhibitory receptors as well as the hormone system. Furthermore, PubMed and UpToDate was used to search for respective papers with “Immune Checkpoint Inhibitors”, “Adverse events of Immune Checkpoint Inhibitors” and “Endocrine adverse events of Immune Checkpoint Inhibitors” as keywords. Further literature was generated through the search of primary literature according to the references in papers. In order to examine reasons for the emergence of anti-thyroid antibodies in some patients during ICI-therapy, “Thyroid antibodies in Immune Checkpoint Inhibitor therapy” was used as keyword for literature research in the mentioned online databases. Furthermore, the official homepage of the U.S. FDA was attended to verify the currently approved ICI-agents and browse administration recommendations. The current classification of adverse events was granted through the recent publication of CTCAE of the National Cancer Institute, U.S. Department of Health and Human Services.

Data for the case reports was acquired from an ongoing register study which was already approved by the local ethics committee with the registration number 29-277 ex 16/17 “Endokrinologie Register”. Three patients with malignant melanoma double-blindly treated with ICI therapy – ipilimumab and nivolumab as single or combination therapy – at the dermatologic department at the Medical University of Graz who developed irAEs were selected. Their clinical reports and laboratory values were collected using the hospital information system “MEDOCS” and relevant parameters and events were summarised to case reports.

3. Results

3.1. Case 1

A 57-year old male patient was admitted to the endocrine department at the Medical University of Graz in October 2017. The patient suffered from malignant melanoma

with progression from stage IB (pT2a N0(i+) M0 AJCC 2009) at the initial diagnosis in September 2015 to stage IIIb (pT2a N2 M0 AJCC 2017). Since end of August 2017 he received immune checkpoint blockade therapy. One and a half months after the beginning of the treatment, he presented tremor which lasted for 14 days. His weight slightly reduced and he suffered from mild fatigue syndrome and increased heart rates. He has already been treated with levothyroxine 25 µg 1-0-0 for one and a half years. Moreover, he has been taking ramipril 2.5 mg 1-0-0 and amlodipine 5 mg 1-0-0.

Thyroid sonography showed an orthotopic located thyroid with the measures of 2.3 x 2.6 x 5.0 cm (right lobe) and 1.8 x 2.1 x 4.9 cm (left lobe). The overall thyroid parenchyma was distinctly hypoechoic and inhomogeneous with faveolated structure and low perfusion. No obvious thyroid nodules could be found. The biochemical assessment revealed abnormalities in thyroid and gonadal hormone levels. TSH was extremely low with 0.01 µU/mL [reference range (RR) 0.10-4.00], also fT4 was decreased at 8.6 pmol/L [RR 9.5-24.0] but fT3 was within the reference range (4.3 pmol/L [RR 3.0-6.3]). Furthermore, thyroid peroxidase antibodies were enormously above the reference range (664 U/mL, RR 0-60) but thyroglobulin antibodies were normal (61 U/mL [RR 0-60]). Regarding gonads, the total amount of testosterone was decreased (1.70 ng/mL [RR 2.41-8.30]), probably as part of the therapy and underlying disease. All neuroendocrinological parameters (ACTH, HGH, prolactin and IGF-1) were within the reference ranges.

The patient's symptoms in synopsis with altered thyroid blood levels indicated a destructive thyroiditis associated with immune checkpoint inhibitor therapy. He presented a common initial hyperthyroid phase followed by peripheral hypothyroid blood levels, however, still with a suppressed TSH and hyperthyroid symptoms (tremor), which was probably related to the preceding extant thyroid hormone excess. It was recommended to continue with thyroid hormone replacement of levothyroxine 50 µg for 3 days, ensued by a raised dose of 75 µg 1-0-0 daily. Additionally, propranolol 20 mg 1-1-1 was prescribed as symptomatic therapy if required. 3 and ½ months later, at the end of January 2018, the patient had his second examination. In the meantime, during a dermatologic check-up, elevated TSH levels with the maximum of 66.87 µU/mL [RR 0.10-4.00] had been diagnosed. The levothyroxine dose was therefore increased to 150 µg in mid-December. Since then, the patient reported feeling subjectively better except for a sometimes-

emerging sensation of cold. Thyroid blood values showed still an increased basal TSH level with 17.54 $\mu\text{U}/\text{mL}$ [RR 0.10-4.00]. Free T4 was within the reference range (12.9 pmol/L [RR 9.5-24.0]), as well as fT3 (4.4 pmol/L [RR 3.0-6.3]), in accordance with subclinical hypothyroidism. The total testosterone level had normalised. In conclusion, the patient showed a peripheral euthyroid metabolism within context of ICI-associated hypothyroidism with distinctly decreasing TSH levels. It was recommended to continue the levothyroxine treatment with 150 μg per day. Re-assessment of endocrine blood values was suggested.

3.2. Case 2

A 32 year old male patient was admitted to the endocrine department at the Medical University of Graz in September 2017 due to elevated thyroid hormone levels. He suffered from a malignant melanoma of the choroid stage IV (pTx N0 M1c AJCC 2009) with hepatic metastasis (increased liver values). The patient received his first ICI dose in August 2017. Exactly 4 weeks later, he was admitted to the endocrine department and reported to suffer from weight loss of 8 kg within the last 3 weeks as well as a limited capability and dyspnoea under exercise. Moreover, hand tremor and an enhanced tendency to hyperhidrosis were reported. The second ICI dose was withheld owing to his mentioned condition and increased thyroid hormone levels.

Thyroid sonography showed an orthotopic located gland with measures of 1.6 x 1.3 x 3.8 cm (right lobe) and 1.6 x 1.5 x 4.7 cm (left lobe). Echogenicity was ubiquitously highly attenuated consistent with an autoimmune thyroid disease. The biochemical assessment showed abnormalities concerning the thyroid values while the other endocrine parameters were within the reference ranges. Basal TSH was completely suppressed (0.00 $\mu\text{U}/\text{mL}$ [RR 0.10-4.00]), fT4 was drastically elevated at 55.0 pmol/L [RR 9.5-24.0] same as fT3 which was increased at 8.6 pmol/L [RR 3.0-6.3]. This condition was consistent with overt hyperthyroidism. Furthermore, the patient presented positive thyroid peroxidase antibodies with 356 U/mL [RR 0-60] and thyroglobulin antibodies with 484 U/mL [RR 0-60]. The patient was prescribed thiamazole and a beta-blocker (propranolol 10 mg 1-1-1). Three days later, he felt subjectively well. He was additionally taking glucocorticoid medication (prednisolone 75 mg 1-0-0). His biochemical assessment (a second blood sample was taken 9

days after the first one) did not present any changes regarding the TSH level (0.00 $\mu\text{U/mL}$ [RR 0.10-4.00]), however, fT4 (17.5 pmol/L [RR 9.5-24.0]) and fT3 (3.1 pmol/L [RR 3.0-6.3]) were within their reference range. Additionally, anti-TSH receptor antibodies were examined which also were within their reference range (3.1 U/L [RR 0.0-15.0]) and therefore excluded Graves' disease. Basal cortisol was enormously heightened at 471.3 ng/mL [RR 43.0-220.0] and the basal ACTH level was low with 5.1 pg/mL [RR 10.0-51.0]. In conclusion, thyroid hormone levels had normalised and just TSH was still suppressed. The high cortisol and low ACTH levels were interpreted in frame of prednisolone therapy. It was recommended to continue thiamazole and propranolol therapy as usual. Two weeks later, the patient reported to be free of symptoms. His laboratory values were consistent with primary hypothyroidism, presented as following: TSH basal had increased to 8.60 $\mu\text{U/mL}$ [0.10-4.00], fT4 and fT3 decreased to 7.9 pmol/L [9.5-24.0] and 1.5 pmol/L [3.0-6.3] respectively. Due to these values it was recommended to halve the thiamazole dose and taper the beta-blocker over a few days. After another 2 weeks, his blood values presented an enormously increased basal TSH to 81.24 $\mu\text{U/mL}$ [RR 0.10-4.00], while fT4 with 5.1 pmol/L [RR 9.5-24.0] and fT3 with 1.2 pmol/L [RR 3.0-6.3] had further decreased compared to previous values. Additionally, the total testosterone level had reduced to 1.03 ng/mL [RR 2.41-8.30]. Due to the opposite altered thyroid hormone levels compared to the first evaluation, thiamazole was discontinued and thyroid hormone replacement therapy was initiated with levothyroxine 50 μg daily. Shortly afterwards, the patient did not show any symptoms, gained weight again and reached with 24.9 a body mass index (BMI) in the upper reference area. It was recommended to increase the levothyroxine dose up to 100 μg . In summary of these findings, a destructive thyroiditis associated with ICI-therapy and an initial episode of hyperthyroidism with subsequent hypothyroidism was diagnosed. Furthermore, the existence of hypogonadism and hypophysitis remained questionable due to the recently enhanced cortisol level (cortisol basal 471.3 ng/mL [RR 43.0-220.0]). After another two weeks, the patient's endocrine biochemical assessment was comparable to the last one: TSH basal was still extremely high with 86.44 $\mu\text{U/mL}$ [RR 0.10-4.00]. Free T4 now was in the lower reference area with 9.6 pmol/L [RR 9.5-24.0] and fT3 remained reduced at 1.7 pmol/L [RR 3.0-6.3]. The basal cortisol level increased (232.4 ng/mL [RR 43.0-220.0]). Total testosterone (1.08 ng/mL [RR 2.41-8.30]) and free testosterone (4.35 pg/mL [RR 6.69-54.69]) were decreased.

The basal ACTH level was within its reference range and in synopsis with a nearly normal cortisol level, therefore, a hypophysitis could be ruled out. It was suggested to further increase the levothyroxine dose to 150 µg per day. One month later, at the patient's last visit, he described intensive fatigue and a recent loss of muscles. Moreover, he presented a discrete ascites. His laboratory values were still pathologically altered. TSH basal had slightly decreased to 66.02 µU/mL [RR 0.10-4.00] compared to the last examinations, fT4 was at 14.7 pmol/L [RR 9.5-24.0] within the reference range and fT3 had further decreased to 1.3 pmol/L [RR 3.0-6.3] – in accordance with a low T3 syndrome. Basal cortisol had again increased to 348.4 ng/mL [RR 43.0-220.0], accompanied by a slightly elevated basal ACTH level with 60.4 pg/mL [RR 10.0-51.0]. Additionally, HGH basal (9.94 ng/mL [RR 0.00-3.00]) and prolactin basal (24.3 ng/mL [RR 2.1-17.7]) were increased. LH and FSH were within the reference ranges but total testosterone (0.42 ng/mL [RR 2.41-8.30]) and free testosterone (2.76 pg/mL [RR 6.69-54.69]) had strongly reduced consistent with hypogonadotropic hypogonadism. Also, IGF-1 was decreased with 50.7 ng/mL [RR 100.0-300.0]. It was recommended to continue the thyroid hormone replacement therapy with 150 µg and to initiate additional testosterone substitution with testogel.

3.3. Case 3

A 33-year old male patient suffering from a metastatic melanoma stage IIIc (pT3b N3 M0 AJCC 2009) was admitted to the endocrine department at the Medical University of Graz in November 2015. The patient showed elevated peripheral thyroid hormone levels. He reported having noticed a neck swelling in the area of the thyroid gland for two days and feeling a mild globus sensation and numbness during swallowing although breathing difficulties were negated. Furthermore, he described having alternating periods of fatigue and agitation as well as sleeping disorders. Additionally, the patient reported a weight loss of 2 kg in one month.

The thyroid sonography showed an orthotopic located, but obviously enlarged gland with inhomogeneous parenchyma and intensified perfusion. The right lobe measured 2.4 x 2.4 x 6.2 cm, the isthmus had a width of over 1 cm and the left lobe measured 3.0 x 2.1 x 7.1 cm. No distinct nodes or cysts could be found. The patient's blood examination presented a basal TSH of 0.00 µU/ml [RR 0.10-4.00] and concordantly increased peripheral thyroid hormone levels (fT4 35.7 pmol/l [RR 9.5-

24.0], fT3 11.3 pmol/l [RR 3.0-6.3]). All thyroid-specific antibodies were negative. Because of his condition of overt hyperthyroidism, therapy with thiamazole 20 mg 1-0-0, propranolol 40 mg ½ - 0 - ½ and methylprednisolone 40 mg 2-0-0 for 5 days was initiated with ensuing tapering of the latter one. After 2 weeks, the symptoms were diminished and respectively his condition improved. The patient claimed his weight to remain constant. The biochemical assessment still presented low TSH basal with 0.01 µU/mL [RR 0.10-4.00] while fT4 (21.3 pmol/L [RR 9.5-24.0]) and fT3 (5.0 pmol/L [RR 3.0-6.3]) had normalised. These blood values were consistent with subclinical hyperthyroidism which was concordant with his sonographic results. Furthermore, thyroglobulin was elevated at 44.88 ng/mL [RR 0.00-30.00] which may indicate a destructive genesis. Due to these findings, it was recommended to reduce his thiamazole dosage to 20 mg ½-0-0 every second day and further continue with tapering of methylprednisolone and discontinue with the propranolol therapy. One month later, the patient was prescribed levothyroxine 100 µg 1-0-0. After another two months, he was still free of symptoms. The laboratory values showed elevated TSH basal at 20.49 µU/mL [RR 0.10-4.00] while fT4 (17.7 pmol/L [RR 9.5-24.0]) and fT3 (4.1 pmol/L [RR 3.0-6.3]) were within the reference range and consistent with subclinical hypothyroidism. It was suggested to continue the hormone replacement therapy with levothyroxine 100 µg. Two months later, the patient required hospitalisation due to newly emerged symptoms along with decreased ACTH and cortisol levels. He suffered from vertigo, diffuse muscle pain, weakness and fatigue. The last ICI administration had been two weeks ago. Therefore, he received additional hydrocortisone replacement therapy with 20 mg 1-½-0 (suspending on the day of blood sampling). The biochemical assessment still presented a hypothyroid metabolism with increased TSH basal at 11.64 µU/mL [RR 0.10-4.00], decreased fT4 to 8.5 pmol/L [RR 9.5-24.0] and fT3 with 4.1 pmol/L [RR 3.0-6.3] within the reference range. Basal cortisol as well as ACTH were below the detection range. Gonadal hormones, prolactin, growth hormone and IGF-1 presented normal levels. These laboratory values indicated an ICI-induced hypophysitis with impairment of the pituitary-adrenal axis. Since the patient felt subjectively well, a continuation of the ICI-therapy was recommended as planned, under regular examination. Furthermore, the dose of levothyroxine was increased to 125 µg. Another four weeks later, the patient was free of symptoms. He still took hydrocortisone as directed but levothyroxine was increased to 150 µg. The

biochemical assessment presented normalised basal TSH levels with 0.32 μ U/mL [RR 0.10-4.00], fT4 had increased to 26.4 pmol/L [RR 9.5-24.0] on replacement therapy and fT3 was within the reference range at 6.0 pmol/L [RR 3.0-6.3]. Moreover, thyroid peroxidase antibodies were slightly positive with 68 U/mL [RR 0-60] while TSH receptor antibodies and thyroglobulin antibodies were negative. Cortisol basal was elevated with 245.1 ng/mL [RR 43.0-220.0], whereas ACTH basal was still below the limit of detection (<5.0 pg/mL [RR 10.0-51.0]). Likewise, basal aldosterone was decreased with <3.7 ng/dL [RR 3.7-43.2] and total testosterone with 2.26 ng/mL [RR 2.41-8.30]. IGF-1 remained within the reference range (237.7 ng/mL [RR 100.0-300.0]). As a consequence of these parameters, it was recommended to continue with glucocorticoid replacement as usual with 1- $\frac{1}{2}$ -0 and to reduce levothyroxine to 125 μ g daily because of the slightly increased fT4. Four months later which equals 11 months after the patient's first introduction at the endocrine department his ICI treatment was completed. The patient was completely free of symptoms. His hydrocortisone therapy was reduced to $\frac{3}{4}$ - $\frac{1}{2}$ -0 which he took on the day of blood sampling. The laboratory values showed nearly the same low basal TSH level as last time (0.33 μ U/mL [RR 0.10-4.00]), fT4 (20.8 pmol/L [RR 9.5-24.0]) and fT3 (5.5 pmol/L [RR 3.0-6.3]) were within the reference ranges which was consistent with an euthyroid state under hormone substitution therapy. However, cortisol basal had further increased to 284.3 ng/mL [RR 43.0-220.0] and ACTH basal had stayed low (<5.0 pg/mL [RR 10.0-51.0]), which is a usual consequence after taking hydrocortisone in the morning before the blood collection. However, every other examined central and peripheral hormone value was normal. It was suggested to continue the thyroid and adrenal replacement therapy as before. In November 2016, a 1-year check-up of the thyroid sonography was conducted. Compared to the dimensions in 2015, the thyroid gland had reduced in size of all measurements. The right lobe measured 1.1 x 1.0 x 4.5 cm and the left one 1.1 x 0.9 x 4.6 cm which therefore correlated with a shrinking range from 1.2-2.5 cm. The parenchyma was presented extremely inhomogeneous with nodal modifications. However, the vascularisation had normalised. The patient negated any symptoms. Regarding the biochemical assessment, all thyroid parameters were within their reference range. Cortisol basal was still elevated at 284.3 ng/mL [RR 43.0-220.0] and the basal ACTH level was low as always (<5.0 pg/mL [RR 10.0-51.0]). The

gonadal and growth hormone axis overall presented normal values. It was recommended to continue with the hormone replacement therapies as before. After further six months, the patient reported to still be free of symptoms – he stated to feel powerful, his weight remained constant and no enhanced susceptibility to infection could be noticed. Under medication every single hormone parameter appeared to be within the reference ranges for the first time, apart from ACTH (<5.0 pg/mL [RR 10.0-51.0]). Due to the efficient substitution, it was suggested to continue with the replacement therapies as before. Eight months later, at the end of December of 2017, the patient reported having suffered from weight loss of a few kilograms over the last months. The laboratory evaluation showed nearly all parameters to still be at normal levels, except for suddenly massively increased TSH basal at 58.08 μ U/mL [RR 0.10-4.00]. ACTH basal was as low as usual (<5.0/ pg/mL [RR 10.0-51.0]). Owing to the elevated TSH level, an increase of the levothyroxine dose to 150 μ g was recommended, glucocorticoid replacement remained as usual. After five months, in May of 2018, the patient was still taking levothyroxine 125 μ g against the doctor's recommendation. In the meantime, hydrocortisone was again increased to 1- $\frac{1}{2}$ -0. The patient reported of overall well-being except for recent weight fluctuations. The laboratory values had worsened compared to the last evaluations. TSH basal had further increased to over 150.00 μ U/mL [RR 0.10-4.00] and the peripheral thyroid hormone levels were much too low with fT4 at 3.0 pmol/L [RR 9.5-24.0] and fT3 at 1.4 pmol/L [RR 3.0-6.3]. Also, cortisol basal was massively above the reference range with 462.3 ng/mL [RR 43.0-220.0]. ACTH basal was low as always (<5.0 pg/mL [RR 10.0-51.0]). It was again suggested to reduce hydrocortisone to $\frac{3}{4}$ - $\frac{1}{2}$ -0 and increase the levothyroxine dose to 160 μ g, whereas a different preparation from euthyrox were tried due to the ongoing fluctuations in TSH that might also be due to non-adherence to levothyroxine treatment..

4. Discussion

As of today, the main target group of immune checkpoint inhibitor therapy are malignant melanoma and non-small lung cancer patients. Therefore, the majority of patients concluded in trials suffer from these malignancies (16,19,33,41,45,49,50,59,20–22,24,26,27,31,32). Thus, parameters like response

rate, overall survival and adverse events are mainly evaluated regarding these patient groups. However, due to the fact that some authors stated an association of the development of adverse events with the type of cancer (22), there are more studies required to also confirm these results for other malignancies.

4.1. Case 1

The first case report represents a typical case of ICI-induced thyroiditis with preceding hyperthyroidism followed by hypothyroidism (7,10). Hypothyroidism is the most frequent condition of ICI-associated thyroid disorders reported in the literature (10) and according to a few studies, thyroiditis seems to be its main cause (7,11,49,50). However, in just one trial it is differentiated between hypo-/hyperthyroidism and thyroiditis regarding their frequency of occurrence (36). Another author distinguished between overt and subclinical conditions (35). Therefore, studies which only present cases of current hypo- or hyperthyroidism lack further examination of possible varied preceding or ensuing metabolic conditions. An increased TSH level combined with normal fT3 and fT4 levels are conform with subclinical hypothyroidism. According to a clinical trial by Morganstein et al. (35), subclinical hypothyroidism is the second most frequent presentation of ICI-induced thyroid dysfunction with ipilimumab therapy and equally frequent as overt hypothyroidism and subclinical hyperthyroidism with anti-PD-1 treatment. With combination therapy, Morganstein et al. (35) described alternating conditions of hyper- to hypothyroidism as the most frequent ones. Autoantibody positivity against thyroid peroxidase and thyroglobulin is reported at different levels in the literature and would be consistent with Hashimoto's disease in an ICI-independent setting (27,49,50). However, the reason for the emergence of these autoantibodies is not clear yet. It could be due to unmasking of pre-existing, latent autoantibodies caused by the immune enhancing therapy or may be newly developed (27,52). According to de Moel et al. (52), the generation of thyroid autoantibodies indicates a better therapy response and improved survival. The onset of the patient's adverse event with estimated 4-6 weeks after treatment initiation is earlier than the median times quoted in literature (6-12 weeks with anti-CTLA-4 antibodies) (7,37,41). The patient's moderate symptoms led to the classification of a grade 2 adverse event, decreasing to grade 1 (asymptomatic, TSH < 10 μ U/mL) over the course of

treatment (39). Regarding therapy, only a beta-blocker without thyreostatic therapy were recommended as suggested in the literature for transient hyperthyroid states as symptomatic therapy (10,39,41). Later, during the subsequent episode of hypothyroidism, the patient required thyroid hormone replacement which ultimately led to a peripheral euthyroid condition. Withholding or even discontinuation of ICI therapy was not indicated at the grade 2 adverse event because the patient became asymptomatic with adequate hormone replacement therapy (39,41). At this point, it was not possible to evaluate if the state of subclinical hypothyroidism would be permanent or transient. Although, it is claimed that 50% of patients regain an euthyroid metabolism but less likely with anti-PD-1 therapy (7). Since hypothyroidism appears more frequently with PD-1 inhibitors or combination therapy (34), I suppose that nivolumab was part of the patient's treatment.

4.2. Case 2

The second patient initially presented typical symptoms for hyperthyroidism (weight loss, limited capability, hyperhidrosis, tremor) which was categorised as a grade 2 adverse event (39). Due to TSH receptor antibody negativity, Grave's disease could be ruled out which anyway would have been a very rare condition with checkpoint inhibitor therapy (7,10,41). The onset of the adverse event was weight loss one week after treatment initiation which is rather early, compared to the literature (7,37,41). This patient received, due to his marked symptoms, thyreostatic drugs and a short-term oral glucocorticoid therapy although corticosteroids are usually not required at grade 2 AEs according to the literature as well as thiamazole is ordinarily only suggested in the case of Graves' disease because thyreostatic drugs do not have an impact on the course of autoimmune thyroiditis. (39). However, those recommendations were just published in 2018 and due to the former simply adoption of management regimes of non-endocrine AEs also to endocrine AEs, this patient got these two questionable drugs prescribed. Beta-blocker were guideline-orientated recommended as symptomatic therapy (10,39,41). Despite the current recommendations the patient became free of symptoms within three days after starting with thiamazole and glucocorticoid therapy and presented normalised FT4 levels nine days afterwards. However, I do not ascribe this improvement to these drugs because thiamazole cannot inhibit the secretion of thyroid hormones nor

inhibit already secreted hormones. I assume these changes were part of the natural disease process, as two weeks later, the patient developed typical subsequent hypothyroidism. Summarising, over the course of two weeks, the patient's thyroid metabolism changed from overt hyperthyroidism through a temporary peripheral euthyroid state with a still completely suppressed TSH level to overt hypothyroidism. This transition is extremely common with ICI-induced thyroiditis (7,10). It is explained owing to the destructive genesis – initially the stored hormones are excessively released, ensued by an inability of the destroyed thyroid cells to synthesis new hormones (10,36) with the consequence of an increase of TSH. Therefore, the patient's subsequently strongly elevated TSH additionally indicated a definite primary cause of hypothyroidism. I suppose that the hyperthyroid condition lasted for 3 weeks and therefore exactly correlates with the information in literature which indicates a duration of initial hyperthyroidism of 2-4 weeks (10). The impaired ACTH-cortisol axis was interpreted to result from glucocorticoid therapy. Despite of halving the thiamazole dose, the patient's thyroid hormone levels worsened and therefore hormone replacement therapy had to be initiated instead of the anti-thyroid therapy which then was discontinued. Additionally, the patient's total testosterone level decreased and with reference to the preceding low ACTH level a hypophysitis had to be ruled out. Apart from TSH; ACTH and FSH/LH, especially in men, are the hormones to most commonly be affected in ICI-induced hypophysitis (7,10). However, further blood sample evaluations presented fluctuating ACTH and cortisol levels, lastly with both values increased, as well as elevated HGH and prolactin which rather led to an exclusion of hypophysitis (although, prolactin levels may be both, elevated or decreased with hypophysitis (7)). Instead, these altered hormone values seemed to be a consequence of a massive stress situation due to the primary disease. The elevated prolactin could also be a result from the hypothyroid condition which elicits over increased TRH secretion, not only high TSH levels but also heightened prolactin release. High prolactin levels suppress FSH/LH secretion and could therefore lead to secondary hypogonadism (51). However, that could be ruled out due to normal FSH/LH levels in this case. An explanation for the low IGF-1 level despite of increased HGH could be that hypothyroidism may cause decreased hepatic protein synthesis and IGF-1 is one of the many proteins synthesised in the liver (51). Besides, the patient's hepatic metastasis is not to take out of consideration. The patient's last two biochemical assessments still showed

increased TSH levels, decreased fT3, but normalised fT4. That led to the suspicion of a low T3 syndrome within the context of the underlying disease. Topical testosterone substitution was recommended despite of pre-existing transaminase elevation under risk-benefit weighting for the patient's quality of life. In conclusion, this patient suffered from immune-related thyroiditis due to immune checkpoint inhibitor therapy and other hormone value abnormalities probably resulted from a massive physical stress response to the metastatic melanoma stage IV.

4.3. Case 3

The third patient presented a fluctuating course of thyroid and adrenal hormone levels. Initially, he showed mild systemic symptoms of hyperthyroidism and a local swelling, what was consistent with the sonographic findings of a distinctly enlarged thyroid gland. The biochemical assessment concordantly demonstrated an overt hyperthyroid condition without antibody positivity. Due to the lower level of knowledge in 2015 also this patient got thiamazole and short-term glucocorticoid therapy prescribed. Within two weeks, he developed an asymptomatic euthyroid state however, resulting in overt hypothyroidism after two months. Due to the low severity, this adverse event was categorised as grade 2 (39). Again, this appearance of thyroid dysfunction is consistent with thyroiditis which is extremely common for ICI-induced thyroid adverse events (7,10). This pathogenesis was additionally confirmed by temporarily increased thyroglobulin values owing to cell destruction. Once the hypothyroid state was reached, thiamazole was discontinued and thyroid hormone replacement therapy started. Six months after the patient's first introduction, he was hospitalised due to dropping of ACTH and cortisol levels below the detection area, accompanied by clinical manifestations. Recent glucocorticoid therapy could be excluded which led to the diagnosis of ICI-induced hypophysitis with impairment of the adrenal axis. The onset of hypophysitis more than 6 months after ICI-treatment initiation is uncommon, compared to 6-12 weeks according to the literature (7,37,48). Impairment of the adrenal axis is the most common alongside impairment of thyroid axis (10). In consideration of his moderate symptoms (vertigo, diffuse muscle pain, weakness, fatigue) and the usual irreversibly damaged ACTH-cortisol axis, high-dose glucocorticoid therapy was refused. Furthermore, according to Faje et al. (45), immune-related hypophysitis indicates improved overall survival

and is associated with relatively low morbidity. For that reason, ICI-therapy was recommended to continue as planned, however, under continuous physiologic glucocorticoid and thyroid hormone replacement therapy and regular surveillance. One month later, in June of 2016, the patient again presented slightly increased fT4 and first-time positive thyroid peroxidase antibodies. This indicated that the patient newly generated antibodies rather than unmasking latent, pre-existing ones. I assume that after a probably failed negative selection in the thymus, a naive T cell with a TCR specific for, in this case, a thyroid self-antigen migrated into the periphery. Ordinarily, peripheral tolerance with its mechanisms of deletion, suppression and anergy functions as backup (3). However, for two of these three mechanisms inhibitory receptors are essential and hence impaired with ICI-therapy. CTLA-blocking attenuates regulatory T cell function (in reference to “suppression”) and facilitates T cell activation in secondary lymphoid organs without innate immune responses taking place. To bring to mind, under physiological conditions, signals of the innate immune system which lead to an increased level of expressed B7 on APCs are required for the engagement of the low-affinity costimulatory receptor CD28 with B7. Otherwise, mainly the high-affinity receptor CTLA-4 binds to B7 on APCs and thus prevents T cell activation (3). However, under blocking therapy T cells are easier activated and if helper T cells subsequently activate B cells, antibody production is elicited. Whereas, PD-1 is responsible for maintaining tolerance to self-antigens and terminating immune reactions directly in peripheral tissues through engagement to PD-L1 or PD-L2, which are expressed on tissue (and cancer) cells and on APCs (3). However, I suppose on inhibition therapy this immune attenuating mechanism is impaired which may cause exaggerated immune reactions at inflammatory sites and intensified T cell activation with, in this case, the result of thyroiditis. Besides, onetime the patient presented decreased total testosterone, however, without clinical symptoms and because of always normal FSH/LH levels, an additional impairment of the pituitary-gonadal axis was excluded. Although usually secondary hypogonadism would be a common further presentation of hypophysitis, especially in men (7). The elevated cortisol is interpreted within the context of glucocorticoid replacement therapy. In August 2016, 3-4 months after the hypophysitis diagnosis and start of hormone replacement therapy, no tumour lesions were detectable, what is consistent with a total response which was maintained at least until July of 2017. Thus, with this clinical course of grade 2 (39)

ICI-induced hypophysitis and ensuing total response, the optimal desirable outcome was reached (45). The next three evaluations over the following 19 months always showed hormone levels within the reference ranges, apart from ACTH and cortisol. In December of 2017 the TSH level had raised again, however, with a peripheral euthyroid condition, wherefore the levothyroxine dose was increased. Because the patient did not follow the doctor's instructions, the thyroid hormone levels worsened until the next visit and due to the again elevated cortisol level, a reduction of the hydrocortisone therapy was suggested. Since hypophysitis nearly solely occurs with anti-CTLA-4 therapy, I suppose ipilimumab was part of the patient's treatment regime, either alone or in combination with nivolumab. (Incidences according to Barroso-Sousa et al. (34): 6.4% of patients with combination therapy, 3.2% with CTLA-4 inhibitors and 0.4% on PD-1 inhibitors.)

Follow-ups of the patient's further clinical courses are missing and not planned to be part of this thesis. Furthermore, it is not yet possible to give a recommendation on what ICI-agent should be used preferred.

5. Conclusion

In conclusion, immune checkpoint inhibitor therapy is in general an effective treatment for cancer patients who suffer from a progressive disease after conventional treatment modalities and are specifically a possible first or second-line therapy for metastatic melanoma, non-small-cell lung cancer and renal cell cancer (10). Nearly 50% of treated patients show signs of response (3,15), concomitant with increased survival (21). The agents work through inhibition of CTLA-4 and PD-1, two inhibitory receptors in the immune system which are primarily expressed on T cells. Physiologically, they function to enable tolerance to self-antigens, avoid exaggerated and limit immune responses. However, some cancers express the ligands of the PD-1 receptor on tumour cells and thus exploit the inhibitory mechanism to escape the immune system's reaction (3). Therefore, nivolumab and pembrolizumab therapy, anti-PD-1 antibodies, are supposed to intervene directly at the location of the cancer. Likewise act anti-PD-L1 antibodies. Whereas ipilimumab, an anti-CTLA-4 antibody, intervenes in the process of activating naive T cells to effector T cells in secondary lymphoid organs (3,5). CTLA-4 physiologically gets

expressed on T cells after their activation as a negative feedback mechanism or on regulatory T cells. These mechanism leads to attenuated further activation of the T cells. Ipilimumab is supposed to inhibit the attenuation of T cell activation with the result of enhanced immune activity (3). However, the agents cannot only affect specific anti-tumour T cells, hence overall intensified immune reactions are elicited, what causes various iatrogenic induced autoimmune diseases – so-called “immune-related adverse events” (3,7,11). In the literature, irAEs are reported from 60% of patients with monotherapy to 96% with combination therapy (21,24). Basically, all organs can be affected (9,22), however, the most frequent adverse events involve skin and intestine (15,21,37,39,40). Endocrine side effects rank with up to 10% in the lower to mid-area of the frequency distribution of irAEs (39). A potentially life threatening endocrine adverse event is hypophysitis which at the same time is the only potentially irreversible one (7,10,23,36). Hypophysitis leads to hypopituitarism with mainly impairing the ACTH and TSH axis (10). Another frequent endocrine adverse event is thyroid dysfunction, with the general consequence of hypothyroidism resulting from thyroiditis (7,10,11,49,50). Whereas, primary adrenal insufficiency and insulin-deficient diabetes are uncommon (34). The incidence of most side effects depends on the used ICI-agent (9,22,34). Furthermore, adverse events are usually more likely to emerge with combination therapy (21). For instance, side effects of the skin, intestine and liver are of higher risk with anti-CTLA-4 treatment (15,39–41). Hypophysitis even occurs nearly exclusively with ipilimumab or combination therapy (34). On the contrary, hypothyroidism is more likely to emerge with anti-PD-1 or combination therapy and hyperthyroidism shows higher incidences with combined treatment (34). Notably, the occurrence of irAEs appears to indicate improved treatment response and survival (17–19,27,45). Endocrine side effects necessarily require hormone replacement therapy which is vital for life, especially in the case of adrenal insufficiency (30,37,39,40,45). According to risk-benefit considerations, additional high-dose glucocorticoid treatment is only suggested for severe endocrine adverse events. Besides, most patients stay dependent on life-long hormone substitution regardless of immunosuppressive therapy (7,10,11,30,39,40). Discontinuation of ICI-therapy is usually not required, even at grade 4 endocrine AEs, if the symptoms cease with hormone substitution (39).

6. References

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