Aus der

Universitäts-Hautklinik

Inaugural-Dissertation

Are there tumor or patient specific predictive markers for the development of adverse events under checkpoint blockade in melanoma?

**zur Erlangung des Doktorgrades
der Medizin**

**der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen**

vorgelegt von

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2022

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Tag der Disputation:	09.08.2022

Dedication

For my Family

In Gratitude

Perseverance, secret of all triumphs. – Victor Hugo

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List of Abbreviations

AIRE: Autoimmune regulator

APC: Antigen-presenting cell

cfDNA: Cell-free DNA

CNV: Copy number variation

CTCAE: Common Terminology Criteria for Adverse Events

ctDNA: Circulating tumor DNA

CTLA-4: Cytotoxic T-lymphocyte-associated protein 4

FAN1: FANCD2 and FANCI associated nuclease 1

HLA: Human leukocyte antigen

ICI: Immune Checkpoint Inhibitor

IKZF1: Ikaros family zinc finger 1

IL1RN: Interleukin-1 receptor antagonist

IRAE: Immune-related adverse event

JAK2: Janus kinase 2

LDH: Lactate dehydrogenase

LRRK2: Leucine rich repeat kinase 2

PD: Progressive disease

PDCD1/PD-1: Programmed cell death 1

PD-L1/CD274: Programmed cell death 1 ligand 1/CD274 molecule

PFS: Progression-free survival

PR: Partial response

PRDM1: PR/SET domain 1

SD/MR: Stable disease/mixed response

SH2B3: SH2B adapter protein 3

SLCO1B1: Solute carrier organic anion transporter family member 1B1

SMAD3: SMAD family member 3

TCR: T cell receptor

TERT: Telomerase reverse transcriptase

TMB: Tumor mutational burden

TSHR: Thyroid stimulating hormone receptor

UNG: Uracil DNA glycosylase

VAR: Small variation

1 Introduction

Melanoma of the skin is one of the most common types of cancers, and its incidence has increased significantly in the past few decades (Siegel, Miller et al. 2018). Even though prognosis in early stage melanoma patients is rather favourable, it becomes considerably poorer in advanced stage metastatic melanoma (Wilson, Zhong et al. 2019).

“In the past decade, immune checkpoint inhibitors (ICI) have revolutionized the therapeutic landscape of metastatic melanoma and significantly improved prognosis of metastatic melanoma patients (Leonardi, Candido et al. 2020). ICI activate the endogenous immune response against tumor cells (Leonardi, Candido et al. 2020)” (Wölffer, Battke et al. 2022).

Therefore, this relatively new type of therapy has gained prominence in the clinical routine of dermatological oncology and has been the subject of numerous studies up to this date. Due to successful results in melanoma, the therapeutic use of checkpoint inhibitors has recently also been extended to other cancers notoriously difficult to treat, such as hepatocellular carcinoma (El Dika, Khalil et al. 2019) or non-small cell lung cancer (Herzberg, Campo et al. 2017).

Checkpoint inhibitors are now widely used in various therapeutic approaches for melanoma patients. Anti-PD-1 inhibitors have been approved by the European Medicines Agency for adjuvant therapy after complete resection of metastases (Weber, Mandala et al. 2017). Combined ICI in an adjuvant setting is currently investigated (NCT03068455, CheckMate 915). In a neoadjuvant setting, combined ICI with ipilimumab and nivolumab has been shown to be associated with high response rates and overall survival in patients with advanced stage melanoma (stage III and IV) (Amaria, Reddy et al. 2018). Hence, neoadjuvant ICI of melanoma patients is object of current research.

“However, ICI might be associated with immune-related adverse events (IRAE), up to 59% in case of combined ICI (Weber, Mandala et al. 2017)” (Wölffer, Battke et al. 2022). They seem to be influenced by the therapy regime, can possibly affect every organ system (Choi and Lee 2020) and might be fatal (Wang, Salem et al. 2018).

“Much research is being done in this area, yet up to now there are no genetically based biomarkers associated with IRAE available. Since IRAE can be very limiting in the therapy management, especially in combined ICI, biomarkers associated with IRAE

would be helpful" (Wölffer, Battke et al. 2022). "Furthermore, the knowledge of biomarkers of distinct IRAE might help in the differential diagnosis, for example by distinguishing IRAE from laboratory abnormalities of other cause. Indeed, symptoms of IRAE might be unspecific in some cases, on the other side, early diagnosis is essential and may be life-saving (Choi and Lee 2020).

A few biomarkers have already been identified, such as female sex (Valpione, Pasquali et al. 2018), and pre-existing autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease (Johnson, Sullivan et al. 2016). Blood count parameters at occurrence of IRAE have been hypothesized to predict IRAE, including increased total leukocytes and relative neutrophil count, decreased relative lymphocyte count (Fujisawa, Yoshino et al. 2017) and increased absolute and relative eosinophil counts (Nakamura, Tanaka et al. 2019). However, they have not been systematically examined in larger studies yet and are not sufficient to be used in order to predict the occurrence of IRAE under ICI" (Wölffer, Battke et al. 2022). Cell-free DNA (cfDNA) has been subject to various studies linking it to progress or response under ICI (Váraljai, Elouali et al. 2020), but has not yet been associated with IRAE. "Other markers put forward in literature concern specific genes involved in immune system regulation (Hoefsmit, Rozeman et al. 2019). These include polymorphisms of TSHR, shown to affect the development of central tolerance and associated with autoimmune diseases (Lee, Li et al. 2015, Fujii, Inoue et al. 2017), or polymorphisms of PRDM1 found to influence antigen presentation (Jang, Chen et al. 2017) and associated with systemic lupus erythematodes (Gateva, Sandling et al. 2009)" (Wölffer, Battke et al. 2022). They may have great potential but remain up to this date speculative, as they have not been verified as biomarkers for IRAE yet. "Some authors describe in single case studies correlations between different HLA alleles and occurrence of IRAE (Ishida, Otsuka et al. 2017, Li, Ma et al. 2017)" (Wölffer, Battke et al. 2022), whereas there is a substantial lack of information as for links between HLA homozygosity and IRAE. "Finally, existing literature does not satisfactorily differentiate between specific IRAE. In most of the cases, the authors refer to IRAE in general but not to the specific involved organs. However, markers for organ specific IRAE, especially for those with high fatality rates, would entail a great benefit.

In the following study, we sought to determine whether there are biomarkers associated with the occurrence of IRAE under ICI in melanoma patients. For this purpose, we

evaluated NGS results and clinical data in view of the occurrence of IRAE in melanoma patients. We searched for the occurrence of IRAE in general as well as for specific IRAE and focused on germline genetic alterations known to play a central role in the regulation of the immune system as we hypothesized their influence also on the occurrence of IRAE" (Wölffer, Battke et al. 2022).

2 Material and Methods

2.1 Patients' data

“We analyzed 95 melanoma patients that had been enrolled in a prospective study on the value of liquid biopsy and tumor sequencing between 01/2018 and 06/2018 who subsequently received immune checkpoint inhibitor (ICI) therapy. Details concerning tumor panel analysis and bioinformatics have already been reported (Forschner, Battke et al. 2019). The aim of this evaluation was to investigate a possible association between copy number variations (CNVs), small variations (VARs), human leukocyte antigen (HLA) and the occurrence of IRAE under ICI. After a thorough literature search (pubmed.ncbi.nlm.nih.gov, search terms “immunogenetics ipilimumab” and “genetics adverse events PD-1”), we found 39 genes that have been discussed in the literature” (Wölffer, Battke et al. 2022). These were: SFTPC, AIRE, TERT, MUC5B, CARD9, CARD10, SH2B3, LRRK2, NOD2, HNF4A, IL2RA, RTEL1-TNFRSF6B, IFIH1, IKZF1, GPR35, NKX2-3, SMAD3, JAK2, IL23R, PRDM1, PTPN22, CTLA4, TSHR, MMEL1, LPP, BACH2, FAN1, SLCO1B1, PDCD1, CCL2, NOS3, IL12B, IL1RN, CXCR3, IL6R, CD274, UNG, IFNL4, and IFNW1.

“We compared the list of these 39 genes with the two different tumor panels that our patients had received for NGS by CeGat GmbH, including 711 and 742 genes respectively. We found that 16 of the 39 genes were also included in the tumor panels used for NGS. We focused the evaluation on the following genes: AIRE, TERT, SH2B3, LRRK2, IKZF1, SMAD3, JAK2, PRDM1, CTLA4, TSHR, FAN1, SLCO1B1, PDCD1 (PD1), IL1RN, CD274 (PD-L1), UNG. Except for AIRE, both panels included all the genes mentioned, and AIRE was removed from the analysis due to lack of data. Therefore, we focused our analyses on these 16 genes. Our objective was also to check for possible associations between patient specific parameters such as sex, blood count, pre-existing autoimmune diseases and the occurrence of IRAE” (Wölffer, Battke et al. 2022).

Patients' data was accessed through the patients' organiser of Tübingen University Hospital's SAP programme. Laboratory data was accessed through Lauris programme.

“The patients either received combined immunotherapy or anti-PD-1 monotherapy. If they received both therapies in sequence, for example first anti-PD-1 monotherapy and later combined ICI, we focused on the data of the combined immunotherapy, as IRAE are

more likely to occur here. Subsequently, we documented the time point of occurrence of IRAE, as well as the most common affected organs, in particular: colitis, pneumonitis, hepatitis, encephalitis, myocarditis, myositis, pancreatitis, exanthema, hypophysitis, nephritis, and thyroiditis. Regarding IRAE, we documented the date of first occurrence and the highest grade according to the Common Terminology Criteria of Adverse Events (CTCAE) scale from 1 to 5 (National Institutes of Health 2017), date of last administration of immunotherapy, median time to occurrence of specific IRAE, therapy and number of IRAE. We used the IRAE documentation of the oncologically experienced, treating physicians in the patient`s file. In case of late-occurring IRAE under follow-up therapies, IRAE were considered until 3 months after termination of ICI.
We then documented potential biomarkers for the occurrence of IRAE such as sex, type of ICI, pre-existing autoimmune diseases, and blood count parameter at start of ICI. Continuous variables, in particular blood count parameter, were divided into categories for better assessability" (Wölffer, Battke et al. 2022) (decreased, normal, increased according to the reference values of the Central Laboratory of the University Hospital Tübingen).

Finally, the results of the patients' NGS were analyzed: cell-free DNA (cfDNA), VARs, CNVs, and HLA-class I.
Each date of sample collection of cfDNA along with the reported cfDNA level in ng/ml was documented. Such cfDNA results were available for 70 patients. We focussed on the levels before, during, and after the respective first date of IRAE, as we were expecting an increase and following decrease here. We defined „during" as one day before until one day after the first day of symptoms. CfDNA levels above 1500 ng/ml were defined as elevated. As cfDNA is a value likely to be false positive because it is volatile, we wanted to define a level high enough to ensure it is indeed elevated. The evolution of cfDNA levels of patients with IRAE was visualized by median and quartiles from four measurements before until five measurements after the occurrence of IRAE for absolute and relative values of cfDNA. Considering more than four preceding measurements was not expedient as ICI did not start earlier than this date in most cases. The same procedure was performed for cfDNA levels of patients without IRAE, for up to the last 10 measurements collected. We visualized the evolution of cfDNA around the first IRAE

only. Indeed, second IRAE were rare in our cohort and it would have been clinically difficult to separate an increase in cfDNA due to the first IRAE from one due to the second IRAE, as they were often close in time. We did not distinguish organ specific IRAE for our cfDNA results, as our sample size was too limited for this analysis. Finally, we visualised the evolution of individual cfDNA data for all patients.

"HLA-Class I genes were analysed concerning heterozygosity and homozygosity, discerning HLA-A, HLA-B, and HLA-C homozygosity" (Wölffer, Battke et al. 2022). We distinguished the specific alleles for each HLA-I surface receptor. Forest plots were done with the program Microsoft Excel (Version Excel 2019, Microsoft Corporation, Redmond, WA, USA) in order to gain an overview about confidence intervals and odds ratio for HLA data.

VARs include insertions and deletions (Indels) as well as single-nucleotide variants (SNVs). Synonyms and non-coding exons and introns were excluded from the analysis. We kept VARs leading to one of the following effects: initiator_codon, stop_gained, frameshift, essential_splice_site, stop_lost, inframe, kozak_sequence, splice_region, missense, stop_retained. We proceeded by filtering VARs according to our previously established list of 16 genes and excluded artefacts in germ line VARs by setting an allele frequency of at least 15%. "We examined each selected gene for the effect of VARs. We abstained from further differentiating according to the exact variant location. We thus hypothesised that each polymorphism could lead to the same IRAE" (Wölffer, Battke et al. 2022).

„Softclip calls" were excluded from the analysis of CNVs, leaving only calls presenting a relative difference between coverage and expected value. Each gene was examined for the effect of CNVs. "We discerned deletions and duplications for IRAE in general, as well as colitis, hepatitis, and pancreatitis" (Wölffer, Battke et al. 2022). Considering the resulting subsamples had a very limited size, we did not discern deletions and duplications for less frequent IRAE. "CNVs above a certain frequency in the population are not recorded due to technical reasons, the difference to the comparison group not being large enough for the caller to respond. Consequently, it is possible that high-frequency CNVs are not considered in the analysis" (Wölffer, Battke et al. 2022). Forest plots were prepared with the programme Microsoft Excel (Version Excel 2019, Microsoft Corporation, Redmond, WA, USA) in order to illustrate confidence intervals and odds

ratio for CNVs data.

In summary, the following variables were recorded for this purpose:

- Sex
- Date of birth and age in years
- Date of death
- Type of ICI
- Intention of therapy (neo-adjuvant or adjuvant)
- ICI: first line or not
- Directly preceding and subsequent therapy
- Levels of protein S100, LDH, leukocyte count, total and relative neutrophil count, total and relative lymphocyte count, total and relative monocyte count, total and relative eosinophil count, each at start of ICI
- Number of ICI cycles
- Date and result of first staging
- Date of progressive disease and progression free survival in months
- Number of metastases and location (hepatic, pulmonary, osseous, cerebral, nodal, cutaneous, peritoneal, retroperitoneal)
- Number of liquid biopsy samples
- Radiotherapy during liquid biopsy sampling and duration
- Pre-existing autoimmune diseases
- IRAE: date of last administration of ICI, date of start of IRAE, median time to occurrence of specific IRAE, type of IRAE according to affected organ system, CTCAE grade, therapy of IRAE, number of IRAE
- Histological subtype of melanoma
- Tumor thickness of melanoma
- Level of tumor mutational burden (TMB)
- Germline findings and BRAF mutation
- Stage at inclusion in study
- Alleles on HLA-A1 / 2, HLA-B1 / 2, HLA-C1 / 2
- Homozygosity and heterozygosity on HLA, HLA-A, HLA-B and HLA-C
- All dates of blood sample collection and level of cfDNA in ng/ml, relative and total development of cfDNA around the occurrence of IRAE, relative and total

development of the last 10 measurements of patients without IRAE

- VARs on: AIRE, TERT, SH2B3, LRRK2, IKZF1, SMAD3, JAK2, PRDM1, CTLA4, TSHR, FAN1, SLCO1B1, PDCD1, IL1RN, CD274, UNG
- CNVs on: AIRE, TERT, SH2B3, LRRK2, IKZF1, SMAD3, JAK2, PRDM1, CTLA4, TSHR, FAN1, SLCO1B1, PDCD1, IL1RN, CD274, UNG, with distinction between deletions and duplications

As last date including new data from the patients' records, we fixed December, 17th 2019.

2.2 Statistical analysis

"All patients' data were entered in and statistically analyzed with the statistical program for social sciences SPSS statistics version 25.0. (IBM Corp. 2017), and Microsoft Excel Version 2019. The descriptive data was analyzed by absolute and relative frequency. We retained each variable that was in relative terms more frequently observed in patients with IRAE than in patients of the entire cohort. We proceeded correspondingly with the three most prevalent IRAE for each potential marker. We obtained the potentially significant biomarkers for occurrence of IRAE and proceeded by testing these markers for significance. The exact version of the Chi-Squared-Test was used for statistical significance. The level of significance was set at 0.05 in all analyses. Results of organ specific IRAEs and biomarkers with a prevalence outside the interval of 33% to 66% are purely exploratory. This is due to the limited sample size of our work and the amount of biomarkers to be detected, especially in the VARs and CNVs sections. Within this restriction, differences in proportions of at least 30% could be detected confirmatory with a power of at least 80% (Chi-square test for unequal samples with ratio at most 2:1, n=95 subjects). Our results support the markers previously suggested in the literature but need to be confirmed and further specified in future more comprehensive studies as correction for multiple testing was not feasible due to lack of power" (Wölffer, Battke et al. 2022).

2.3 Ethical statement

This study has been approved by the ethical committee of the Ärztekammer Baden-Württemberg and the ethical committee of the Eberhard-Karls-University Tübingen, approval numbers F-2016-010 from 01/03/2016 and 827/2018BO2 from 27/11/2018. We documented and analyzed all patients' data conforming to the „Best Practice" guideline

for doctoral procedures at Eberhard-Karls-University Tübingen, and to the Helsinki Declaration.

3 Results

The analysis includes 95 patients treated with checkpoint inhibitors. “Our results must be interpreted with caution due to the exploratory approach” (Wölffer, Battke et al. 2022). The following table provides an overview on clinical characteristics of the cohort.

Table 1. Clinical characteristics of the cohort. ICI: Immune checkpoint inhibitors. AJCC: American Joint Committee on Cancer. CNS: Central Nervous System. BRAF: B-Rapidly Accelerated Fibrosarcoma. TMB: Tumor Mutational Burden. IRAE: Immune-Related Adverse Event. LDH: Lactate Dehydrogenase.

Clinical characteristics	Median	1. Quartile	3. Quartile	IQR
Age at beginning of ICI (years)	62.1	51.8	75.1	23.3
Progression free survival under therapy (months)	5.3	2.1	14.9	12.8
Time between last ICI and IRAE (days)				
IRAE 1	12	5	21	16
IRAE 2	20	11	34	24
Tumor thickness (mm)	3.2	1.9	5.2	3.3
	Number of patients			%
Sex				
Male		54		57
Female		41		43
Melanoma type				
Cutaneous		63		66
Acral lentiginous		9		10
Mucosal		3		3
Uveal		6		6
Occult		12		13
Other		2		2
BRAF mutation				
Positive		45		47
Negative		50		53
Germline Mutation				
Yes		15		16
No		80		84
AJCC cancer stage at study inclusion				
3		20		21
4		75		79
TMB values at start ICI				
Low (<3.3 Var/Mb)		29		32
Intermediate (3.3-23.1Var/Mb)		44		48
High (>23.1 Var/Mb)		19		21
Last systemic treatment before ICI				
None		58		61
Targeted		20		21
PD-1 antibody		12		13
CTLA-4 antibody		0		0
Other		5		5

Table 1. *Cont.*

	Number of patients	%
Type of ICI		
Monotherapy	36	38
Combined	59	62
First line		
Yes	58	61
No	37	39
Intention		
Adjuvant	20	21
Palliative	75	79
Cycles		
<4	33	35
4-10	36	38
>10	26	27
Laboratory data at start of ICI		
Leukocytes		
Normal	80	84
Increased	10	11
Decreased	5	5
Neutrophils abs.		
Normal	78	82
Increased	13	14
Decreased	4	4
Neutrophils %		
Normal	85	90
Increased	7	7
Decreased	3	3
Lymphocytes abs.		
Normal	71	75
Increased	2	2
Decreased	22	23
Lymphocytes %		
Normal	53	56
Increased	4	4
Decreased	38	40
Monocytes abs.		
Normal	81	85
Increased	12	13
Decreased	2	2
Monocytes %		
Normal	88	93
Increased	7	7
Decreased	0	0
Eosinophils abs.		
Normal	80	84
Increased	1	1
Decreased	14	15
Eosinophils %		
Normal	77	81
Increased	1	1
Decreased	17	18

Table 1. *Cont.*

	Number of patients	%
Protein S100 at start of ICI		
S100 elevated	42	44
S100 normal	53	56
LDH at start of ICI		
LDH elevated	31	33
LDH normal	64	67
Origin of tissue sequenced (n = 89)		
Lymph node metastasis	31	35
Other metastasis	41	46
Primary melanoma	15	17
CNS metastasis	1	1
Local recurrence	1	1
Metastases at start of ICI		
Hepatic	22	23
Pulmonary	44	46
Osseous	29	31
Cerebral	27	28
Nodal	76	80
Cutaneous	59	62
Peritoneal (incl. intestinal and splenic)	25	26
Retroperitoneal (incl. renal and pancreatic)	8	8
Number of metastases at start of ICI		
1	15	16
2	32	34
3	16	17
4	13	14
5	9	10
6	5	5
7	4	4
8	1	1
Result at first staging		
Partial response	30	32
Stable disease / mixed response	18	19
Progressive disease	37	39
Died before first staging	9	10
Not done yet at time of data collection	1	1
Radiotherapy during ICI		
Yes	39	41
No	56	59
Number of radiotherapies		
One	30	32
Two	7	7
Three	2	2
Pre-existing autoimmune diseases		
Diabetes Mellitus Type 1	2	2
Rheumatoid Arthritis	2	2
Vitiligo	1	1
Crohn’s Disease	1	1
Other	2	2

Table 1. *Cont.*

	Number of patients	%
IRAE under ICI		
Occurrence of IRAE	59	62
Colitis	20	21
Hepatitis	14	15
Pancreatitis	13	14
Hypophysitis	10	11
Pneumonitis	8	8
Thyroiditis	7	7
Exanthema	7	7
Nephritis	3	3
Myositis	3	3
Encephalitis	3	3
Myocarditis	2	2
Treatment of IRAE		
IRAE 1 (n = 52)		
Combined treatment	11	21
Steroids	41	79
IRAE 2 (n = 27)		
Combined treatment	5	19
Steroids	22	82

The flow chart below summarizes the selection process of patients included in our study.

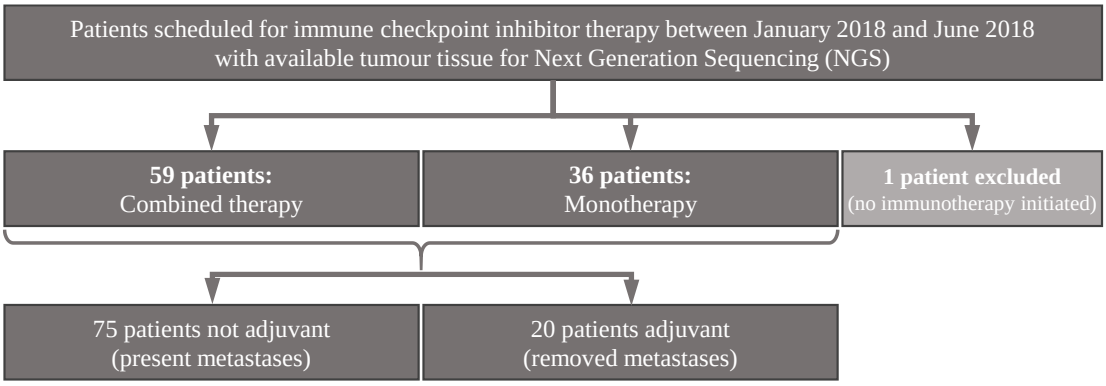


Figure 1. Flow chart of patients' selection.

Some IRAE, as colitis, hepatitis and pancreatitis, were much more prevalent than others. Figure 2 provides an overview of frequency of IRAE under ICI in percent.

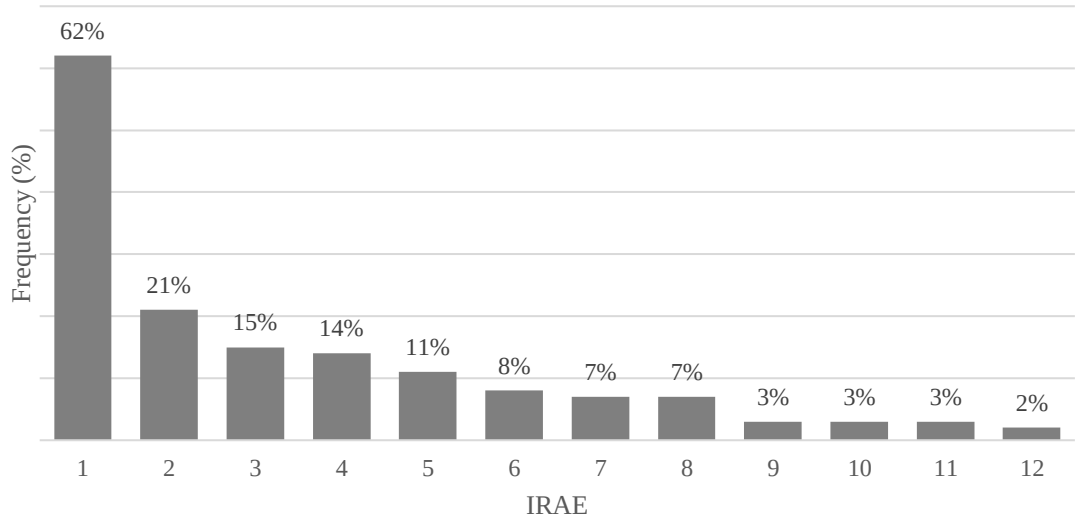


Figure 2. Frequency of IRAE in percent. 1: IRAE. 2: colitis. 3: hepatitis. 4: pancreatitis. 5: hypophysitis. 6: pneumonitis. 7: thyroiditis. 8: exanthema. 9: nephritis. 10: myositis. 11: encephalitis. 12: myocarditis.

The following table outlines the frequency of IRAE and results at first staging. IRAE in general, as well as the three most common IRAE, showed overall a similar frequency between the results "progressive disease" and "partial response". Progressive disease was not observed to correlate with less IRAE.

Table 2. Frequency of IRAE among results at first staging.

IRAE		PR	SD/MR	PD	Died before	Not done yet	Total
IRAE (any)	N	21	8	26	4	0	59
	%	36%	14%	44%	7%	0%	100%
Colitis	N	9	1	9	1	0	20
	%	45%	5%	45%	5%	0%	100%
Hepatitis	N	6	0	8	0	0	14
	%	43%	0%	57%	0%	0%	100%
Pancreatitis	N	6	2	4	1	0	13
	%	46%	15%	31%	8%	0%	100%
Total	N	30	18	37	9	1	95
	%	32%	19%	39%	10%	1%	100%

In table 3, we looked further into a potential link between a lower number of cycles of ICI (<5), the development of IRAE, and the result "progressive disease" at first staging. We did not observe a correlation here. The number of cycles of ICI does not appear to correlate with IRAE.

Table 3. Frequency of IRAE and results at first staging by number of cycles.

IRAE and result at first staging		Cycles			
		≤4	5 to 10	>10	All
	N	61	8	26	95
	%	64%	8%	27%	100%
IRAE (any)	N	41	6	12	59
	%	70%	10%	20%	100%
PR	N	22	2	6	30
	%	73%	7%	20%	100%
SD/MR	N	4	1	13	18
	%	22%	6%	72%	100%
PD	N	25	5	7	37
	%	68%	14%	19%	100%
Died before	N	9	0	0	9
	%	100%	0%	0%	100%
Not done yet	N	1	0	0	1
	%	100%	0%	0%	100%

The following figure shows the frequency of number of IRAE occurred in patients. Among IRAE-developing patients, most had only one IRAE.

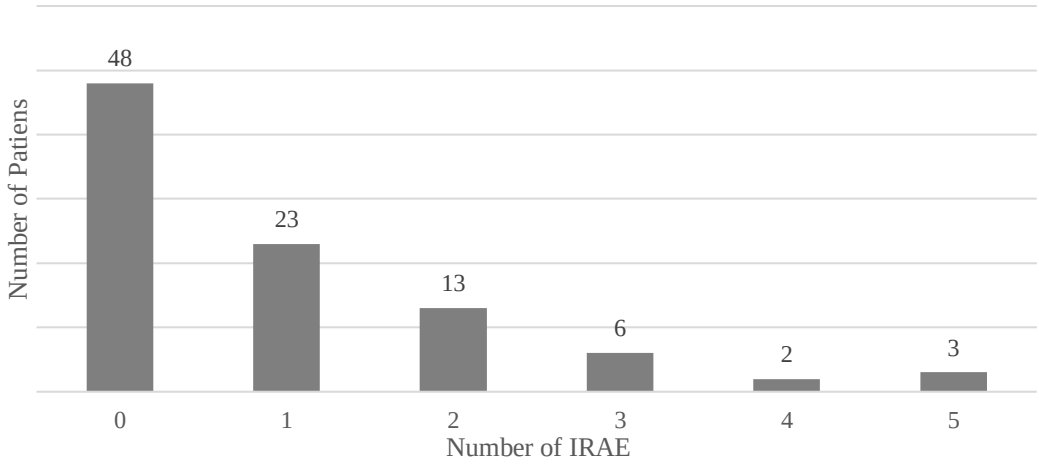


Figure 3. Frequency of occurred IRAE by number of patients.

We analyzed the timeline of IRAE by means of Kaplan-Meier-Curves, showing cumulative frequency of patients by time since the start of ICI in weeks, as well as the median time of IRAE occurrence after start of ICI in months (see Figure 4 – 15 and Table 4). In most cases, the IRAE appeared during the first 12 weeks after start of ICI. Two cases of pancreatitis appeared 34 and 41 weeks respectively after start of ICI.

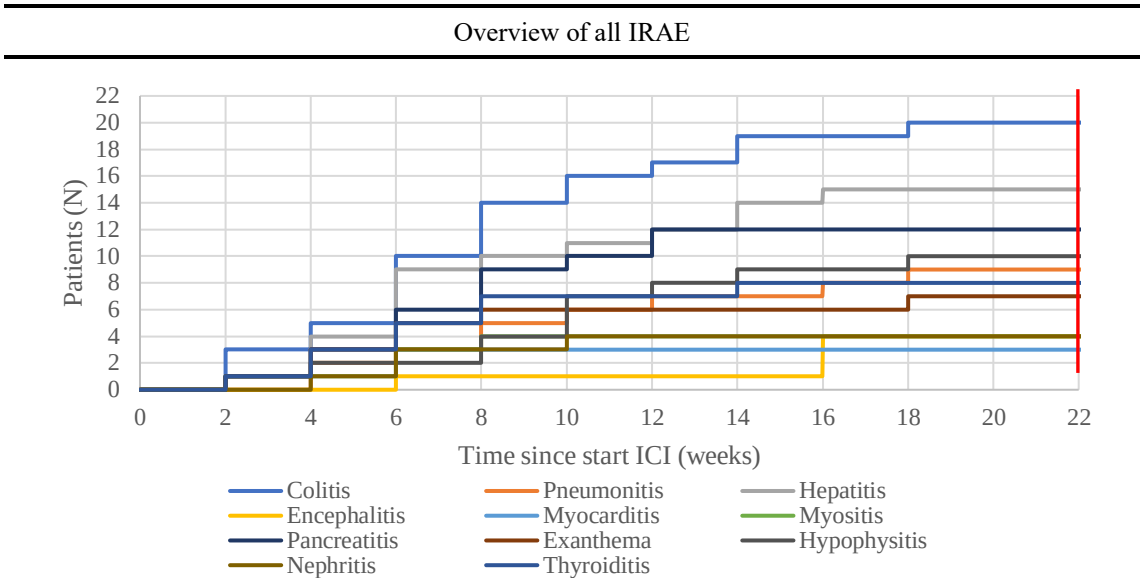


Figure 4. Cumulative Kaplan-Meier-Curve for all IRAE. Y: Patients (N), x: time since start ICI (weeks). Red line: IRAE appearing after week 22, see Figures Pancreatitis, Hypophysitis and Exanthema.

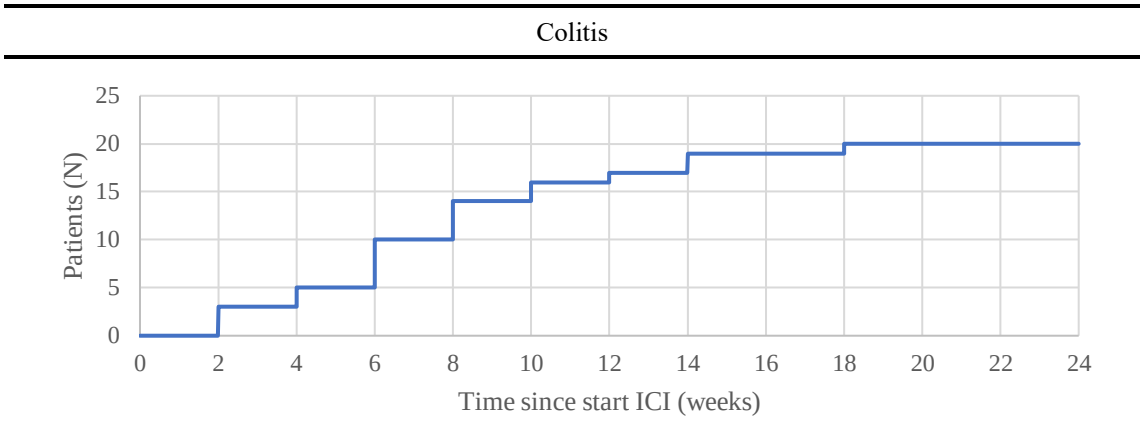


Figure 5. Cumulative Kaplan-Meier-Curve for Colitis. Y: Patients (N), x: time since start ICI (weeks).

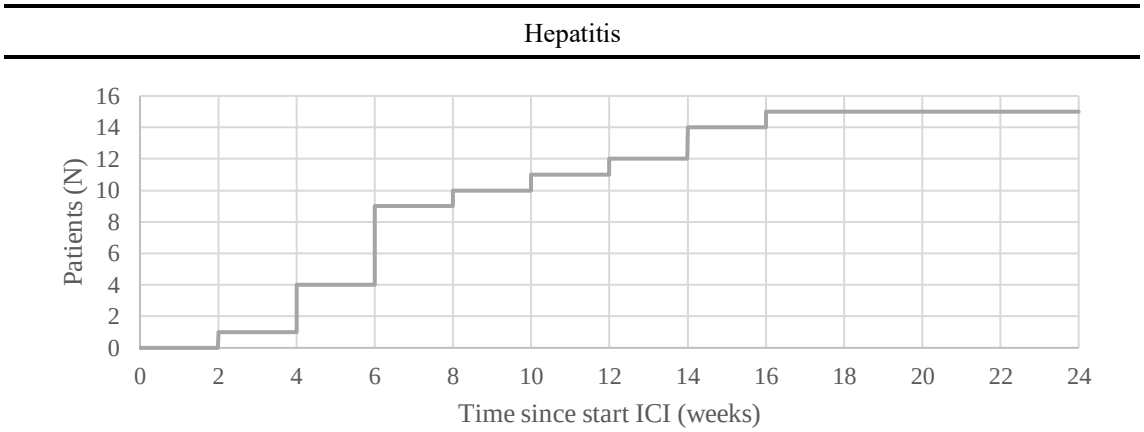


Figure 6. Cumulative Kaplan-Meier-Curve for Hepatitis. Y: Patients (N), x: time since start ICI (weeks).

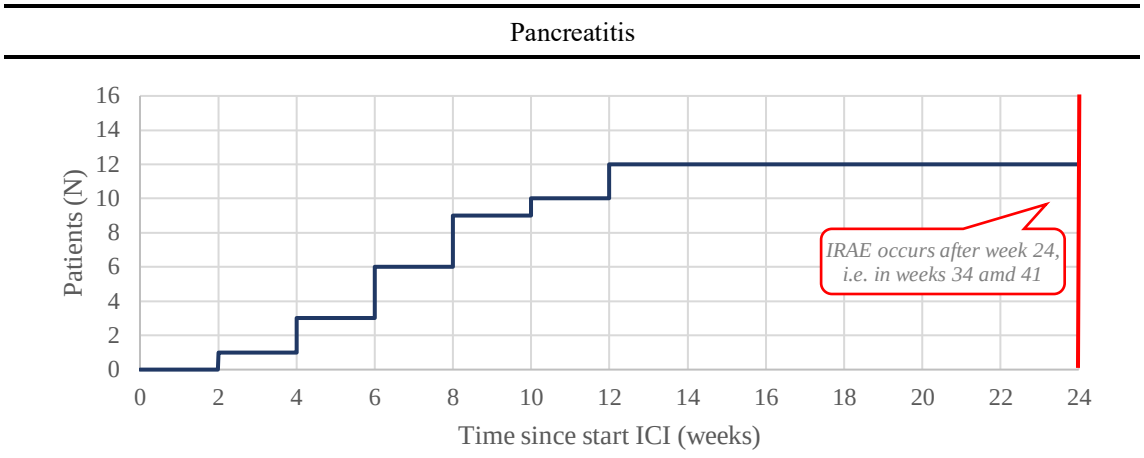


Figure 7. Cumulative Kaplan-Meier-Curve for Pancreatitis. Y: Patients (N), x: time since start ICI (weeks). Red line: IRAE occur after week 24, i.e. in weeks 34 and 41.

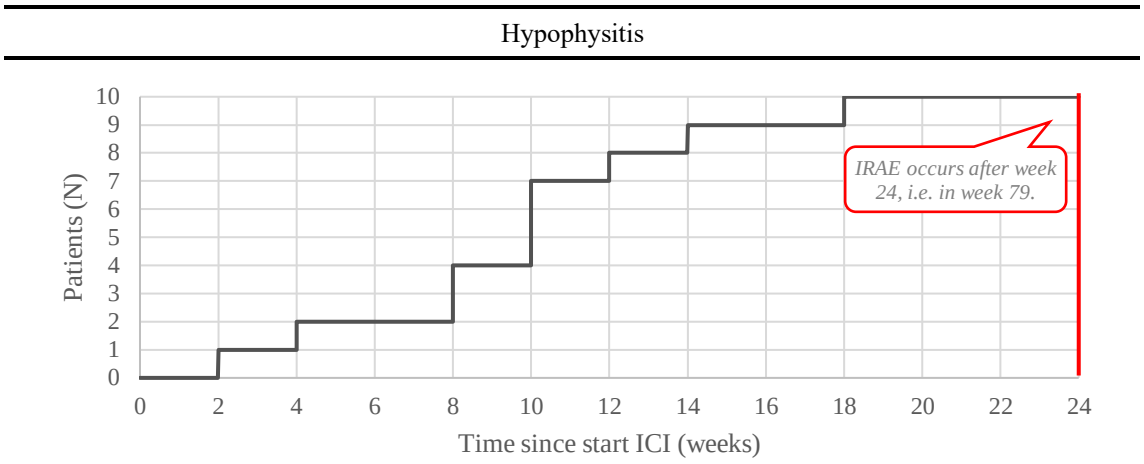


Figure 8. Cumulative Kaplan-Meier-Curve for Hypophysitis. Y: Patients (N), x: time since start ICI (weeks). Red line: IRAE occurs after week 24, i.e. in week 79.

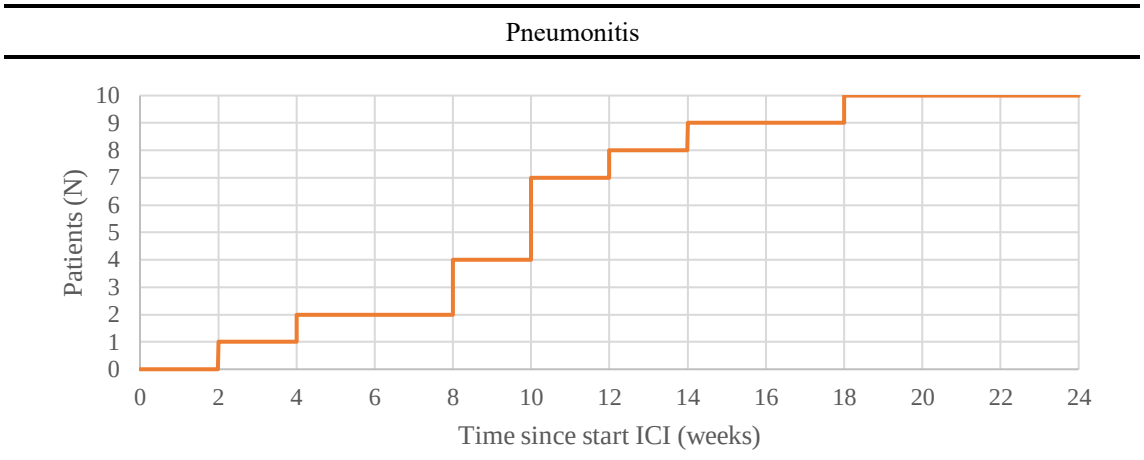


Figure 9. Cumulative Kaplan-Meier-Curve for Pneumonitis. Y: Patients (N), x: time since start ICI (weeks).

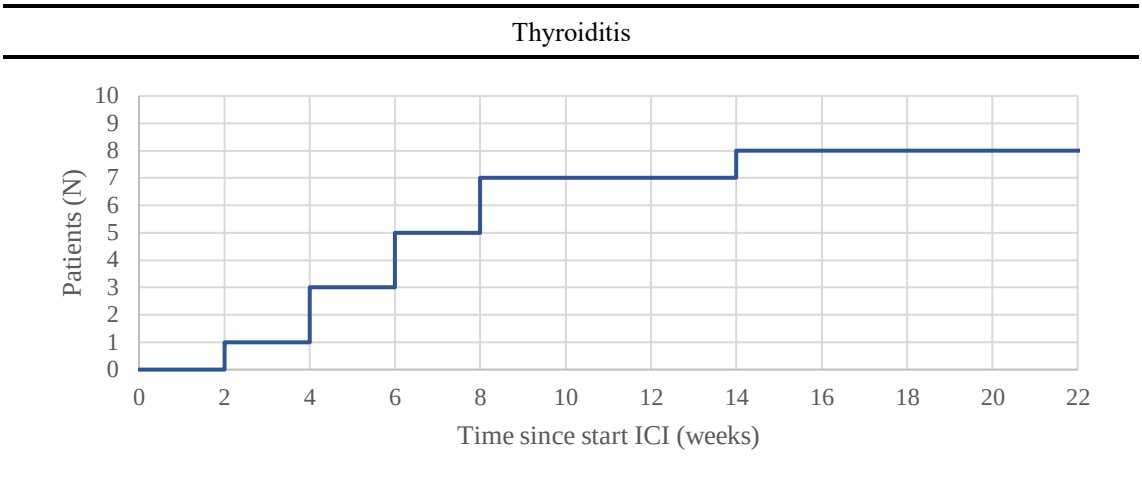


Figure 10. Cumulative Kaplan-Meier-Curve for Thyroiditis. Y: Patients (N), x: time since start ICI (weeks).

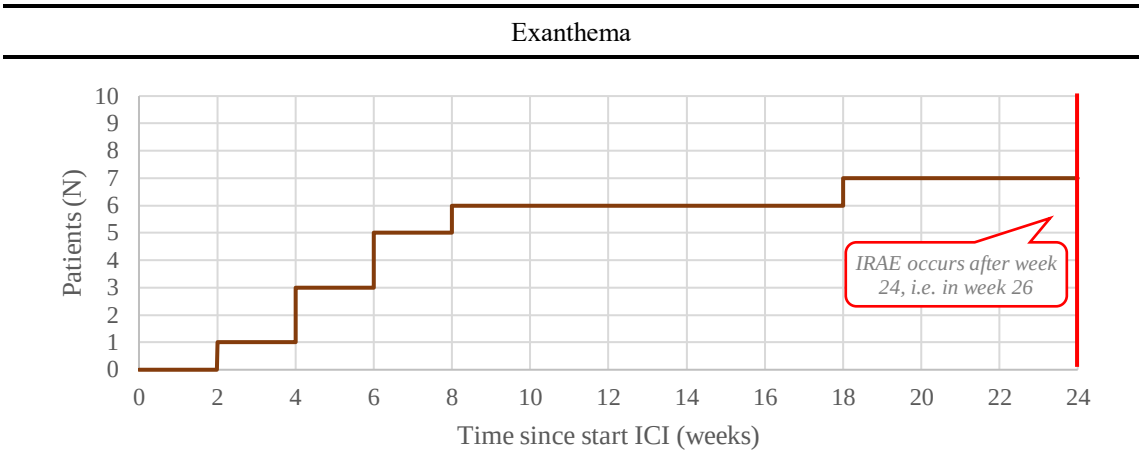


Figure 11. Cumulative Kaplan-Meier-Curve for Exanthema.Y: Patients (N), x: time since start ICI (weeks). Red line: IRAE occurs after week 24, i.e. in week 26.

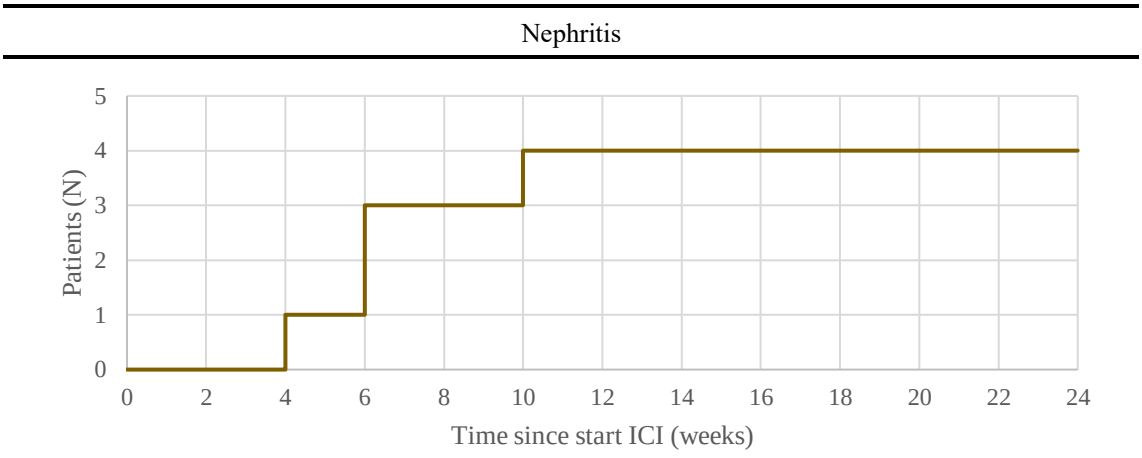


Figure 12. Cumulative Kaplan-Meier-Curve for Nephritis. Y: Patients (N), x: time since start ICI (weeks).

Myositis

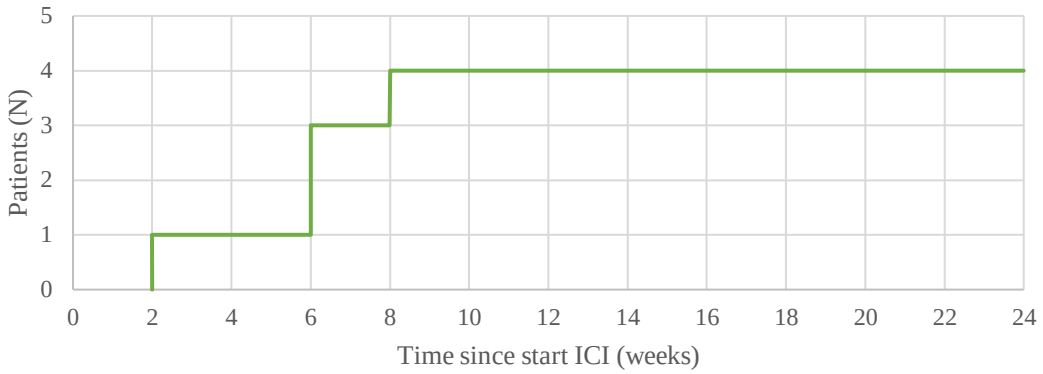


Figure 13. Cumulative Kaplan-Meier-Curve for Myositis. Y: Patients (N), x: time since start ICI (weeks).

Encephalitis

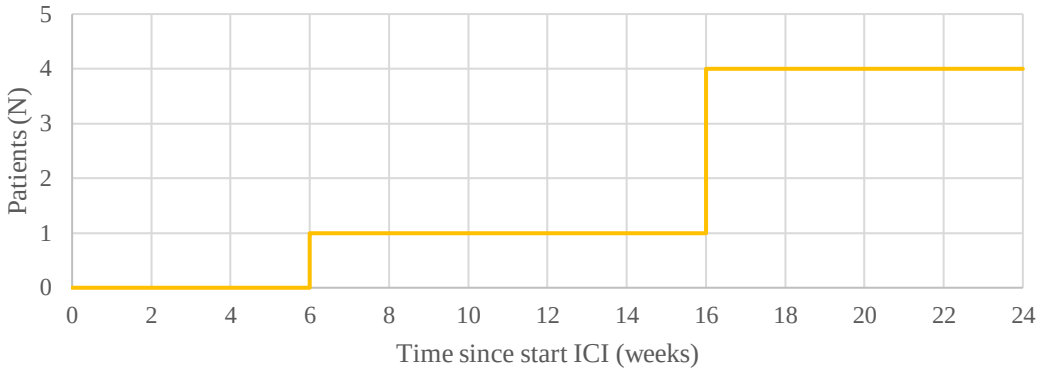


Figure 14. Cumulative Kaplan-Meier-Curve for Encephalitis. Y: Patients (N), x: time since start ICI (weeks).

Myocarditis

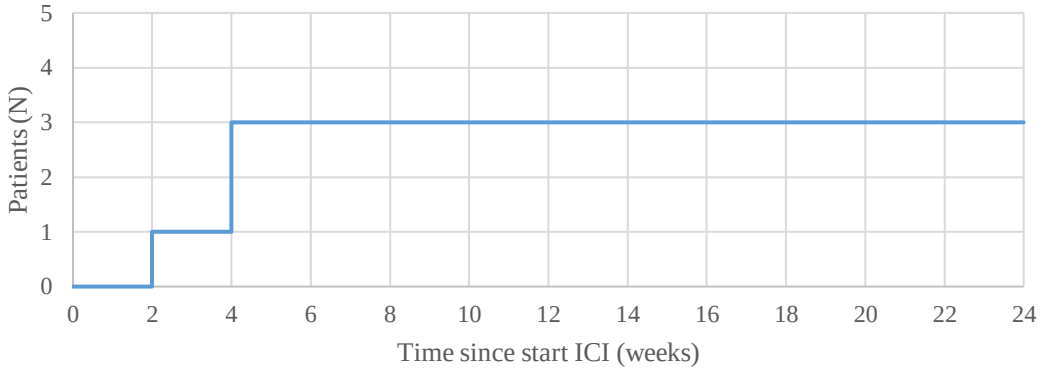


Figure 15. Cumulative Kaplan-Meier-Curve for Myocarditis. Y: Patients (N), x: time since start ICI (weeks).

Table 4. Median time of IRAE occurrence after start of ICI in months.

IRAE	Median time of IRAE occurrence after start of ICI in months
Colitis	1.4
Hepatitis	1.3
Pancreatitis	1.4
Hypophysitis	2.1
Pneumonitis	1.8
Thyroiditis	1.2
Exanthema/skin rash	1.2
Nephritis	1.3
Myositis	1.3
Encephalitis	3.4
Myocarditis	0.5

Table 5 provides an overview of the patients' genetic data.

Table 5. Genetic characteristics of the cohort. HLA: Human Leukocyte Antigen. (Wölffer, Battke et al. 2022).

Genetic characteristics	No. patients	%
HLA		
Homozygosity	23	24
Heterozygosity	72	76
HLA-A		
Homozygosity	13	14
Heterozygosity	82	86
HLA-B		
Homozygosity	8	8
Heterozygosity	87	92
HLA-C		
Homozygosity	8	8
Heterozygosity	87	92
Small variations (VARs)		
AIRE	1	1
TERT	7	7
SH2B3	66	70
LRRK2	95	100
IKZF1	0	0
SMAD3	12	13
JAK2	3	3
PRDM1	45	47
CTLA4	59	62
TSHR	95	100
FAN1	62	65
SLCO1B1	57	60
PDCD1	3	3
IL1RN	48	50
CD274	4	4
UNG	2	2

Table 5. *Cont.*

Genetic characteristics	No. patients	%
Copy number variations (CNVs)		
AIRE	1	1
Duplications	0	0
Deletions	1	1
Both	0	0
TERT	45	47
Duplications	37	39
Deletions	7	7
Both	1	1
SH2B3	27	28
Duplications	26	27
Deletions	1	0
Both	0	0
LRRK2	38	40
Duplications	27	28
Deletions	10	11
Both	1	1
IKZF1	40	42
Duplications	34	36
Deletions	4	4
Both	2	2
SMAD3	36	38
Duplications	36	38
Deletions	0	0
Both	0	0
JAK2	43	45
Duplications	20	21
Deletions	23	24
Both	0	0
PRDM1	32	34
Duplications	10	11
Deletions	22	23
Both	0	0
CTLA4	14	15
Duplications	9	9
Deletions	4	4
Both	1	1
TSHR	20	21
Duplications	7	7
Deletions	10	11
Both	3	3
FAN1	40	42
Duplications	25	26
Deletions	11	12
Both	4	4
SLCO1B1	59	62
Duplications	41	43
Deletions	11	12
Both	7	7

Table 5. *Cont.*

Genetic characteristics	No. patients	%
Copy number variations (CNVs)		
PDCD1	28	29
Duplications	24	25
Deletions	4	4
Both	0	0
IL1RN	15	16
Duplications	9	9
Deletions	5	5
Both	1	1
CD274	18	19
Duplications	3	3
Deletions	15	16
Both	0	0
UNG	31	33
Duplications	27	28
Deletions	3	3
Both	1	1

Some genes were often affected by VARs, such as, in order of descending frequency, LRRK2, TSHR, SH2B3, FAN1, CTLA4, SLCO1B1. 100 % of our patients had VARs on LRRK2 and TSHR. 69.5% had VARs on SH2B3, 65.3% on FAN1, 62.1% on CTLA4, and 60% on SLCO1B1.

IKZF1 was not affected by VARs.

Some genes were more often altered by CNVs than others. SLCO1B1, TERT and JAK2 were the three most common genes here, whereas AIRE, CTLA4, and IL1RN were rarely affected by CNVs.

Overall, duplications affected genes far more frequently than deletions. Only for AIRE, JAK2, PRDM1, TSHR, and CD274, more deletions than duplications were observed.

Sex

64.8% of male patients developed IRAE, compared to 58.5% of female patients. Some IRAE were sex-associated. “24.4% of female patients developed hepatitis ($p = 0.021$), compared to only 7.4% of male patients, whereas 18.5% of male patients developed pancreatitis, opposed to only 7.3% of female patients” (Wölffer, Battke et al. 2022).

There was no significant difference in sex as for colitis.

Type of ICI

“Combined immunotherapy led to more IRAE than monotherapy. 67.8% of patients with combined therapy developed IRAE, compared to 52.8% of patients with monotherapy. 30.5 % of patients with combined therapy compared to 5.6 % with monotherapy (p = 0.004) developed colitis. 18.6% of patients with combined therapy had hepatitis, compared to 8.3% of patients with monotherapy. Finally, 16.9% of patients with combined therapy had pancreatitis, compared to 8.3% of patients with monotherapy” (Wölffer, Battke et al. 2022).

This was also verified for hypophysitis and pneumonitis. 2.8% of patients receiving monotherapy developed hypophysitis, against 15.3% of patients receiving combined therapy. 5.6% of patients receiving monotherapy developed pneumonitis, against 10.2% of patients receiving combined therapy.

Laboratory data

Contrary to our expectation, “[e]levated levels of protein S100 or lactate dehydrogenase (LDH) at therapy initiation were not associated with IRAE.

In the differential blood cell count at therapy initiation, increased leukocytes were associated with colitis, hepatitis or pancreatitis. Increased total neutrophils were associated with colitis or pancreatitis. Decreased relative lymphocytes were associated with pancreatitis. Increased total and relative monocytes were also associated with IRAE. Increased total monocytes were associated with colitis, hepatitis, or pancreatitis (p < 0.0005), whereas increased relative monocytes were only associated with colitis or pancreatitis (p = 0.001). The single patient having had high levels of eosinophils at start of immunotherapy developed colitis, pancreatitis and nephritis during immunotherapy. Table [6] shows laboratory results and frequency of IRAE, colitis, hepatitis or pancreatitis” (Wölffer, Battke et al. 2022).

Table 6. Distribution of laboratory results in relation to development of IRAE in percent (Wölffer, Battke et al. 2022).

Laboratory results		**N**	**IRAE**	**Colitis**	**Hepatitis**	**Pancreatitis**
		(95)	%	%	%	%
Leukocytes	Normal	80	61	18	13	11
	Increased	10	60	40	30	30
	Decreased	5	80	40	20	20

Table 6. *Cont.*

Laboratory results			N	IRAE	Colitis	Hepatitis	Pancreatitis
			(95)	%	%	%	%
Neutrophils	Absolute	Normal	78	62	18	14	10
		Increased	13	62	39	15	31
		Decreased	4	75	25	25	25
	%	Normal	85	66	21	15	14
		Increased	7	29	29	14	14
		Decreased	3	33	0	0	0
Lymphocytes	Absolute	Normal	71	66	24	17	14
		Increased	2	50	0	0	0
		Decreased	22	50	14	9	14
	%	Normal	53	70	25	21	11
		Increased	4	25	0	0	0
		Decreased	38	55	18	8	18
Monocytes	Absolute	Normal	81	62	20	14	9
		Increased	12	75	33	25	50 (p<0.0005)
		Decreased	2	0	0	0	0
	%	Normal	88	61	19	16	10
		Increased	7	71	43	0	57 (p=0.001)
		Decreased	0	-	-	-	-
Eosinophils	Absolute	Normal	80	68	21	16	14
		Increased	1	100	100	0	100
		Decreased	14	29	14	7	7
	%	Normal	77	68	21	17	14
		Increased	1	100	100	0	100
		Decreased	17	35	18	6	6

Result at first staging

64% of patients having had the result “progress” at first staging developed IRAE, against 59% of patients having had the result “no progress”. This was not systematically found with each IRAE.

Metastases

The number of metastases was not linked to IRAE. Indeed, occurrence of one metastasis was associated with IRAE (67% of this cohort developed IRAE). This association was however not progressing with a higher number of metastases, and even diminishing.

Furthermore, the three main IRAE showed ambiguous association to the number of metastases. Colitis and hepatitis were associated with one or at least three organic systems

affected by metastases, whereas the highest association in pancreatitis was found for two systems affected (18.8% of these patients developed pancreatitis).

Radiotherapy

Radiotherapy was not linked to IRAE. 64% of the patients having passed radiotherapy developed IRAE, against 61% of those who did not. The most considerable difference was observed in patients with pancreatitis: 17.9% of patients with radiotherapy developed pancreatitis, versus 10.7% without.

Pre-existing autoimmune diseases

“Pre-existing autoimmune diseases were associated with the occurrence of IRAE, 6 out of 8 patients with pre-existing autoimmune diseases developed IRAE. 100 % of a total of 4 patients suffering from rheumatoid arthritis, vitiligo, or Crohn’s Disease developed IRAE.

The occurrence of colitis was associated with rheumatoid arthritis (50 %), Crohn’s Disease (100%), or Heparin-induced thrombopenia type II (HIT-II; 100%). The development of hepatitis was associated with diabetes mellitus Type 1 (50%), vitiligo (100%), or HIT-II (100%). The development of pancreatitis was associated with rheumatoid arthritis (50%), vitiligo (100%), or HIT-II (100%). Pancreatitis was linked significantly to pre-existing autoimmune diseases ($p = 0.041$)” (Wölffer, Battke et al. 2022).

Table 7. IRAE occurrence in patients with pre-existing autoimmune disease (Wölffer, Battke et al. 2022).

Pre-existing autoimmune disease	N (% occurred IRAE)	IRAE occurred
Diabetes mellitus type I	2 (50%)	Hepatitis, pneumonitis
Rheumatoid arthritis	2 (100%)	Pancreatitis, colitis
Vitiligo	1 (100%)	Exanthema, hepatitis, pancreatitis
Crohn’s disease	1 (100%	Colitis
HIT type II	1 (100%)	Colitis, thyroiditis, hepatitis, pancreatitis, nephritis
AIHA	1 (0%)	-

BRAF mutation

Mutated BRAF seemed to be a protective marker for the occurrence of IRAE. Only 49% of patients with mutated BRAF developed IRAE, against 74% of patients without mutated BRAF ($p = 0.012$). This was also confirmed for colitis and hepatitis. Interestingly, it was reversed for pancreatitis: 20% of patients with mutated BRAF had pancreatitis, compared to only 8% without.

Histological subtype of melanoma

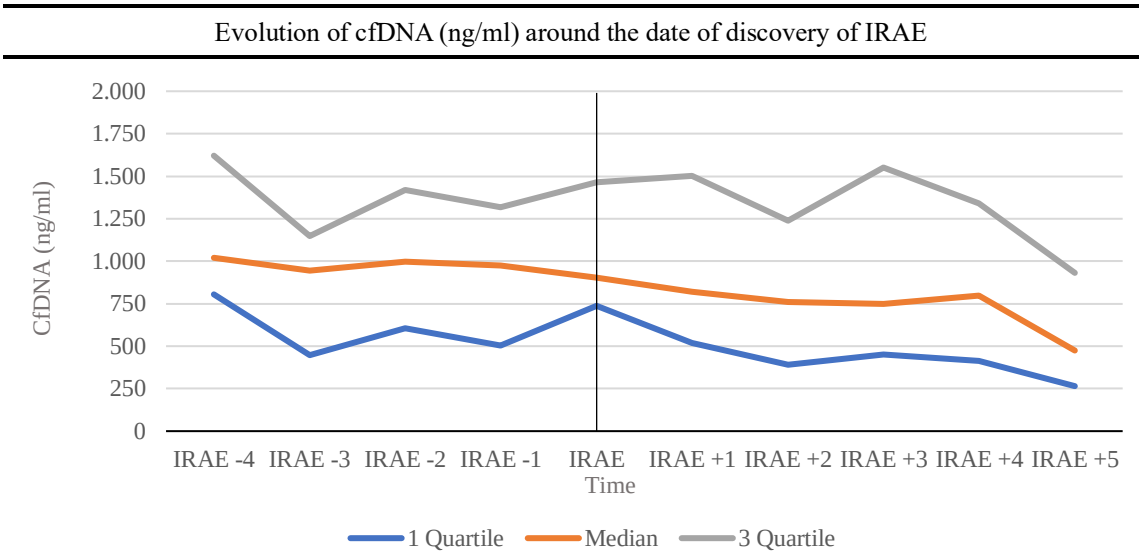
Table 8 shows histological subtype of melanoma and frequency of associated IRAE.
Acral lentiginous and occult melanoma were linked to IRAE. Mucosal and occult melanoma were linked to colitis. Acral lentiginous, mucosal, uveal and occult melanoma were linked to hepatitis. Uveal and occult melanoma were finally linked to pancreatitis.
Cutaneous melanoma seemed to be a protective factor for the occurrence of IRAE.

Table 8. Histological subtype of melanoma and frequency of associated IRAE.

IRAE	Cutaneous	Acral lentiginous	Mucosal	Uveal	Occult	Other
IRAE	52%	89%	67%	67%	83%	100%
Colitis	18%	11%	33%	17%	50%	0%
Hepatitis	10%	22%	33%	33%	25%	0%
Pancreatitis	13%	11%	0%	17%	25%	0%

cfDNA

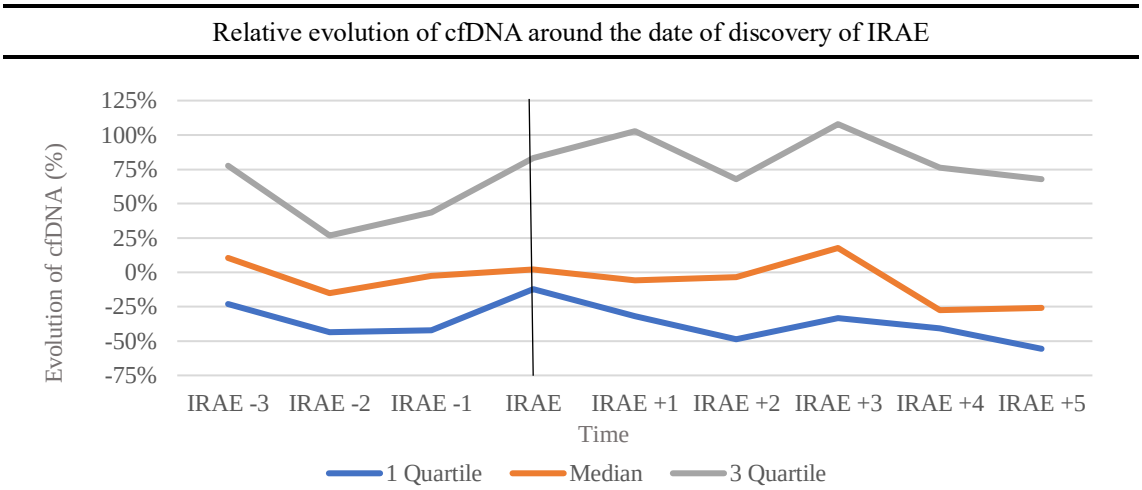
70 patients in our cohort had available cfDNA data, whereof 43 developed IRAE.
We explicitly excluded the mean from our analysis, and resorted to median and quartiles, as very few extreme levels distorted the mean. The highest absolute levels of cfDNA were measured four measurements prior to the occurrence of IRAE for the period of four measurements before until five measurements after the occurrence of IRAE. The median remained on a plateau until one measurement prior to the occurrence of IRAE, then decreased afterwards (see Figure 16).



Measurement	IRAE-4	IRAE-3	IRAE-2	IRAE-1	IRAE	IRAE+1	IRAE+2	IRAE+3	IRAE+4	IRAE+5
Total patients (N)	9	14	29	42	8	42	34	29	25	18
Time to start ICI (days)										
Median	-28	-5	0	20	42	71	118.5	146	175.5	200
1.Quart.	-37	-13.5	-1	0	24.25	46	84.25	113.75	134	163.75
3.Quart.	21	15.75	18	27.25	76.75	109.5	174.75	160.75	194.75	222.25
IQR	58	29.25	19	27.25	52.5	63.5	90.5	47	60.75	58.5

Figure 16. Evolution of cfDNA in ng/ml from four measurements before until five measurements after the occurrence of IRAE. Blue: 1. Quartile. Orange: Mean. Grey: 3. Quartile. Day IRAE: first occurrence of IRAE.

We analyzed also the evolution by percentage, as shown in Figure 17.

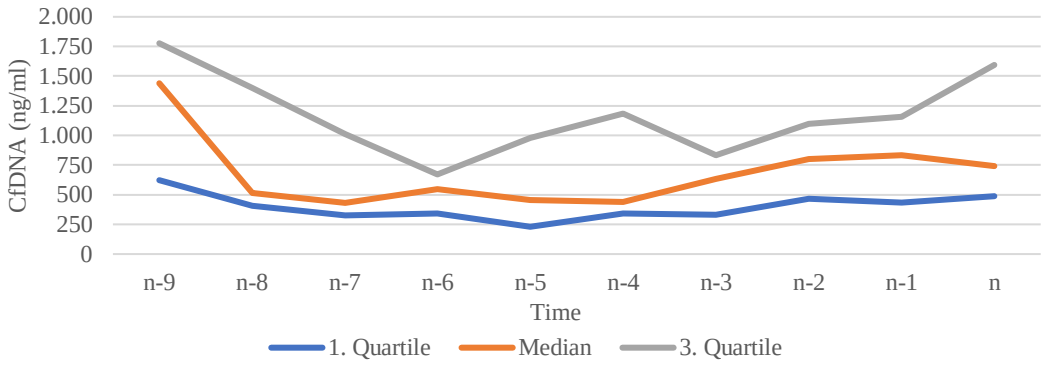


Measurement	IRAE-3	IRAE-2	IRAE-1	IRAE	IRAE+1	IRAE+2	IRAE+3	IRAE+4	IRAE+5
Total patients (N)	14	29	42	8	42	34	29	25	18
Time to start ICI (days)									
Median	5	0	20	42	71	118.5	146	175.5	200
1.Quart.	-13.5	-1	0	24.25	46	84.25	113.75	134	163.75
3.Quart.	15.75	18	27.25	76.75	109.5	174.75	160.75	194.75	222.25
IQR	29.2	19	27.25	52.5	63.5	90.5	47	60.75	58.5

Figure 17. Evolution of cfDNA in ng/ml from four measurements before until five measurements after the occurrence of IRAE. Blue: 1. Quartile. Orange: Mean. Grey: 3. Quartile. Day IRAE: first occurrence of IRAE.

For our control group, composed of 23 patients without IRAE occurrence throughout ICI, we used the last measurements of cfDNA to be compared to cfDNA results of the patients with IRAE above. As before, we analyzed these results in absolute and relative values. Figure 18 and 19 summarize these results. Lower levels of cfDNA over time were observed in patients having no IRAE.

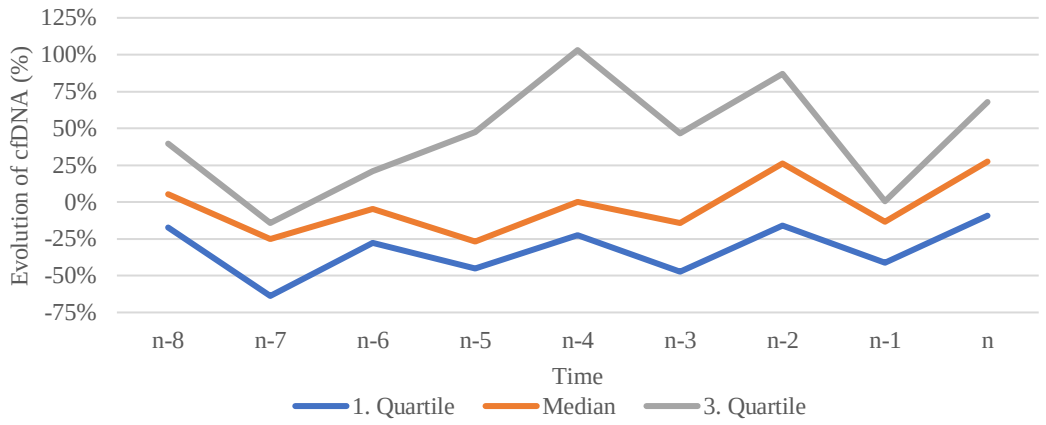
Evolution of cfDNA (ng/ml) in patients without IRAE



	n-9	n-8	n-7	n-6	n-5	n-4	n-3	n-2	n-1	N
N	5	7	7	9	11	12	13	14	20	23

Figure 18. Evolution of cfDNA in ng/ml from nine measurements before last measurement until last measurement in patients without IRAE. Blue: 1. Quartile. Orange: Mean. Grey: 3. Quartile. N: last measurement taken.

Relative evolution of cfDNA in patients without IRAE



	n-9	n-8	n-7	n-6	n-5	n-4	n-3	n-2	n-1	N
N	5	7	7	9	11	12	13	14	20	23

Figure 19. Evolution of cfDNA in percent from nine measurements before last measurement until last measurement in patients without IRAE. Blue: 1. Quartile. Orange: Mean. Grey: 3. Quartile.

As shown in Table 9, we further analyzed the evolution directly around the occurrence of IRAE. The median increased for both IRAE between the last measurement before and the

one during the IRAE. CfDNA was lower directly after the IRAE than before it (-5% and -8%, respectively).

Table 9. Evolution of cfDNA by percentage of median, first and third quartile around IRAE 1 and 2.

	IRAE 1 pre/during	IRAE 1 pre/post	IRAE 2 pre/during	IRAE 2 pre/post
Median	2%	-5%	36%	-8%
1.Quartile	-13%	-31%	-21%	-35%
3.Quartile	121%	44%	123%	87%

As shown in Table 10, the number of patients beyond our critical level of 1500 ng/ml among all patients with IRAE measured was highest during the IRAE (30% for IRAE 1 and 33% for IRAE 2), and rose from the measurement before to the measurement during the IRAE. However, the sample size to support this finding is very limited.

Table 10. Frequency of patients above 1500 ng/ml cfDNA among all patients with IRAE measured, for the three measurements around IRAE 1 and 2, respectively.

	Measurement					
	IRAE 1			**IRAE 2**		
	Before	During	After	Before	During	After
Number patients > 1500 ng/ml	7	3	10	4	1	4
Total number patients measured	42	10	42	17	3	15
%	17%	30%	24%	24%	33%	27%

22% of the measurements taken around the occurrence of IRAE (one measurement prior to IRAE occurrence until one measurement thereafter for both IRAE) were above 1500ng/ml cfDNA, i.e. 29 out of 129 measurements**.** 68 measurements were above 1500ng/ml cfDNA somewhen during the measurements within patients having IRAE. Thereof, only 39 measurements were above 1500ng/ml cfDNA and occurred outside of the IRAE interval, i.e. without a supposed link to IRAE but within patients enduring IRAE at some point. Out of all cfDNA blood samples, only 19%, or 96 out of 507, were above 1500ng/ml cfDNA at some point, including all patients, also those who never developed IRAE.

When looking at patients and not measurements, only 56% (15 out of 27) of patients without IRAE were somewhen above 1500ng/ml cfDNA, as opposed to 70% (30 out of 43) of patients with IRAE at some point. In total, 64% (45 out of 70) were somewhen above 1500ng/ml.

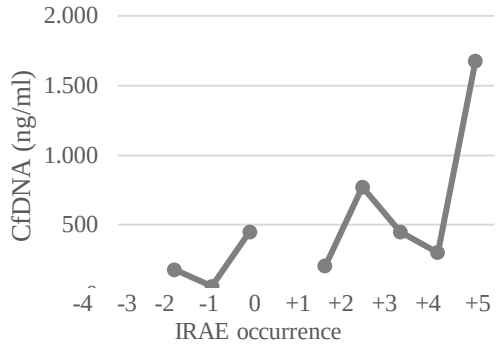
As shown in Table 11, elevated cfDNA was associated with progressive disease at first

staging. 71% of patients with progress at first staging had elevated cfDNA throughout the therapy, whereas only 55% with no progress had elevated cfDNA throughout the therapy.

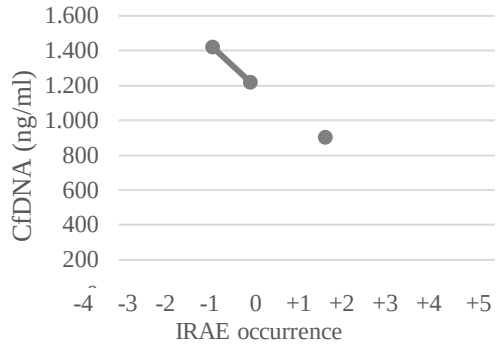
Table 11. Distribution of patients by cfDNA levels and result at first staging examination.

cfDNA / Progress after first staging	No progress	Progress
> 1,500 ng/ml	17	25
< 1,500 ng/ml	14	10

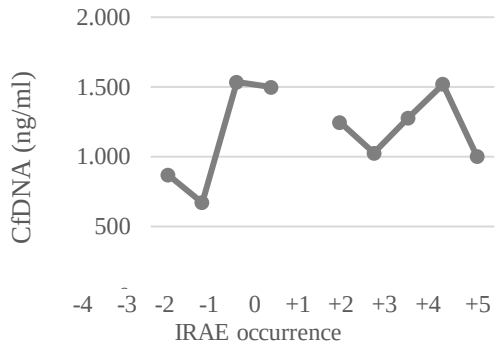
We analyzed the evolution of cfDNA in ng/ml over time for each patient with IRAE whose measurements laid in the interval from four measurements prior to occurrence of IRAE to five measurements after (see Figure 20). We noted for each figure the result at first staging examination. Unfortunately, no result can be postulated here. We did not observe an increase of cfDNA prior to the development of IRAE, or a different evolution of cfDNA as compared to patients without IRAE.



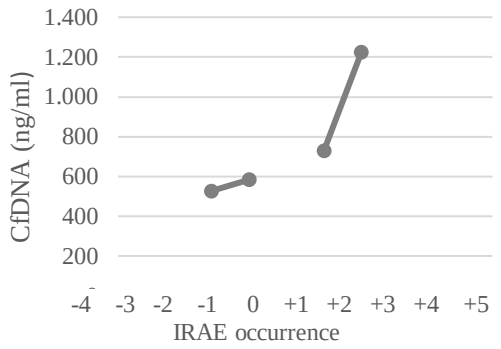
A – Result at first staging: PD.



B – Result at first staging: PD

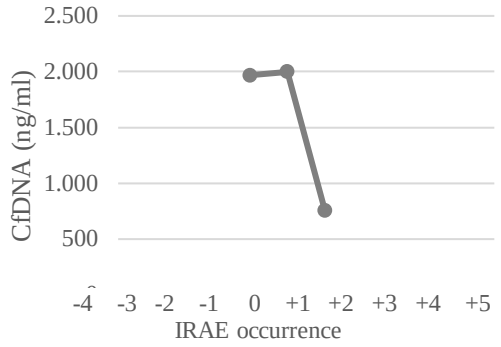


C – Result at first staging: Died before first staging.

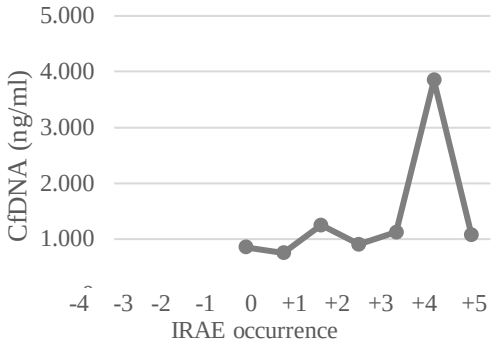


D – Result at first staging: PD.

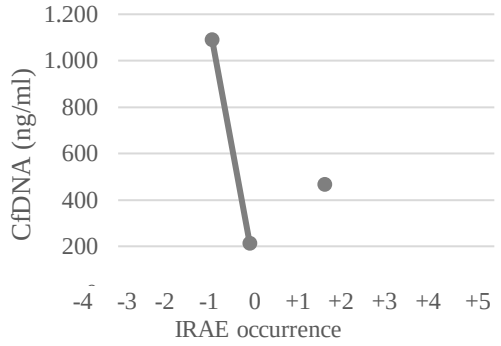
Figure 20. *Cont.*



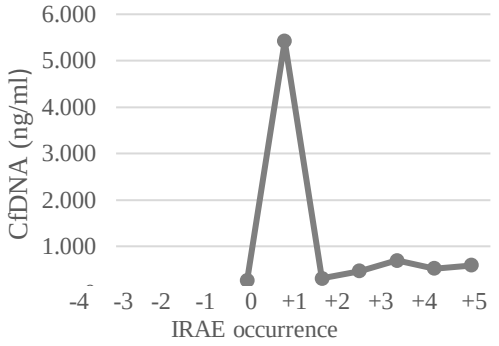
E – Result at first staging: PD.



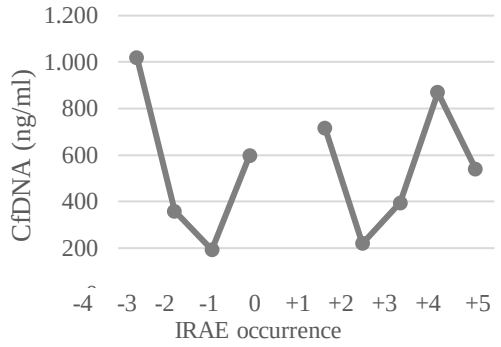
F – Result at first staging: PD.



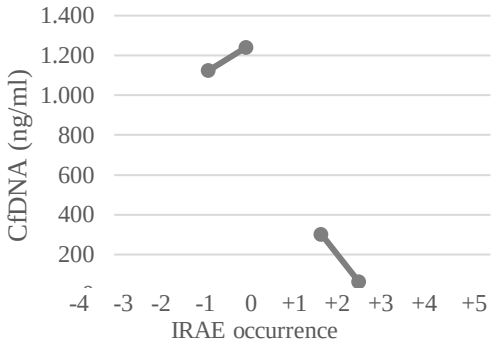
G – Result at first staging: PD.



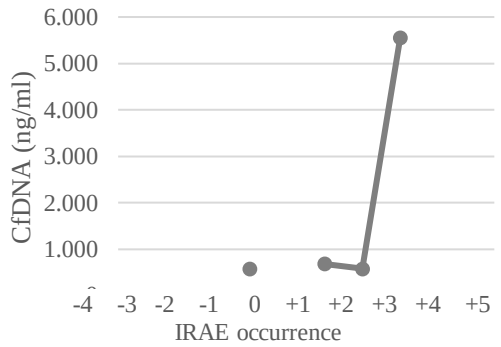
H – Result at first staging: PR.



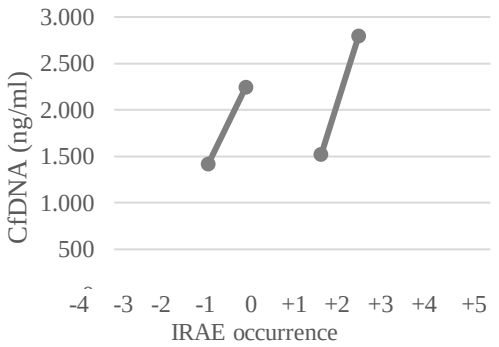
I – Result at first staging: PR.



J – Result at first staging: SD/MR.

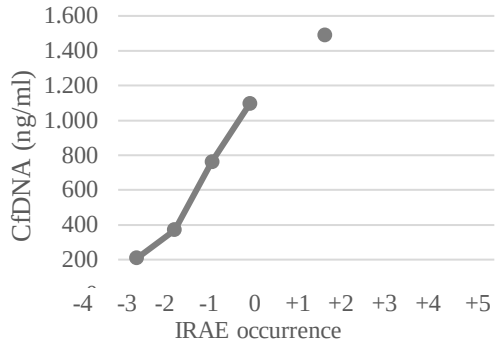


K – Result at first staging: Died before first staging.

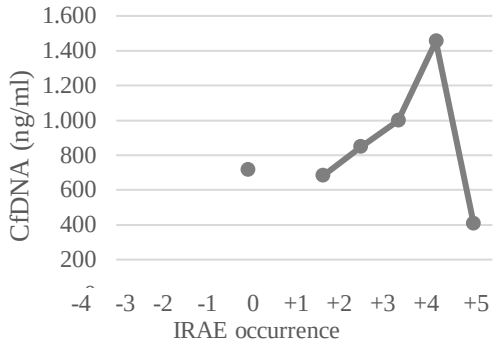


L – Result at first staging: PD.

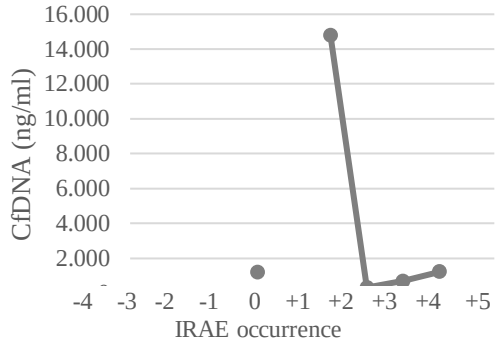
Figure 20. *Cont.*



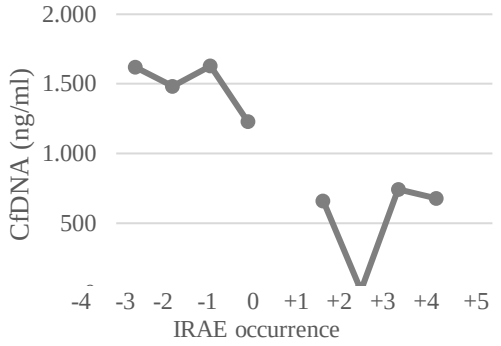
M – Result at first staging: PD.



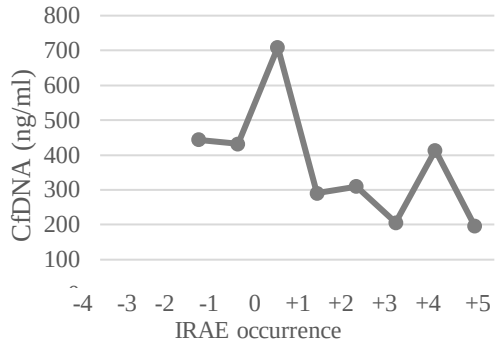
N – Result at first staging: PR.



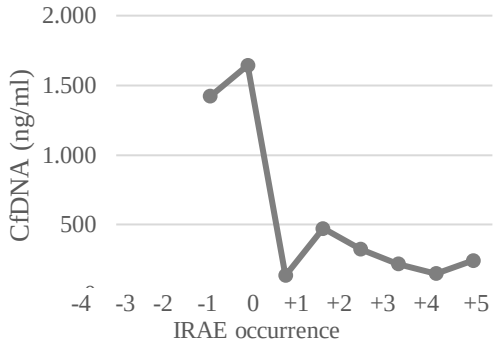
O – Result at first staging: 6.



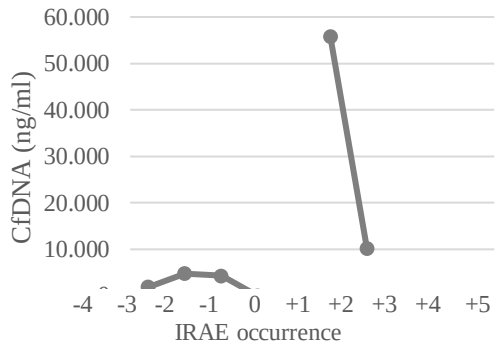
P – Result at first staging: PR.



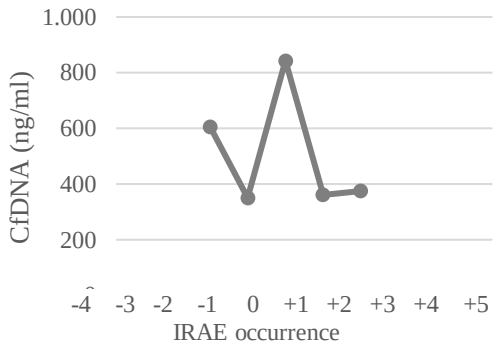
Q – Result at first staging: PR.



R – Result at first staging: PD.

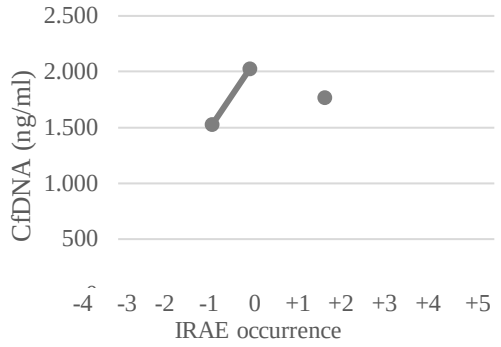


S – Result at first staging: PR.

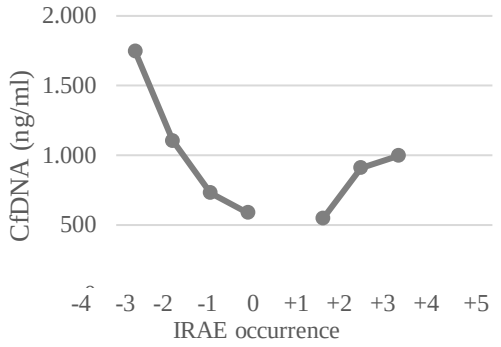


T – Result at first staging: Died before first staging.

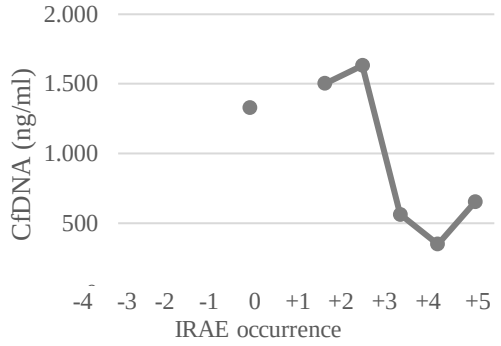
Figure 20. *Cont.*



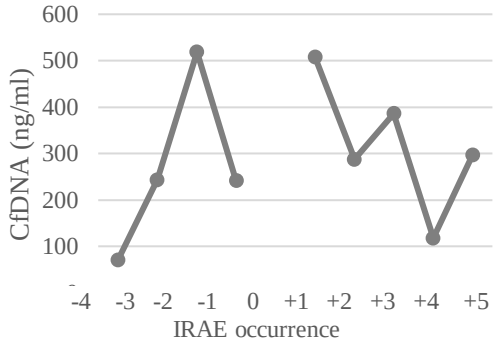
U – Result at first staging: PD.



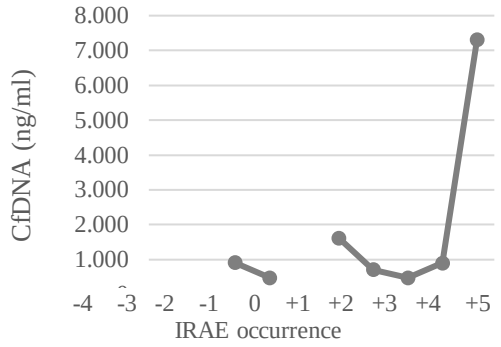
V – Result at first staging: PD.



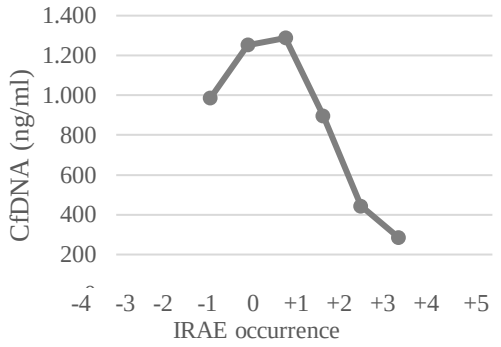
W – Result at first staging: PD.



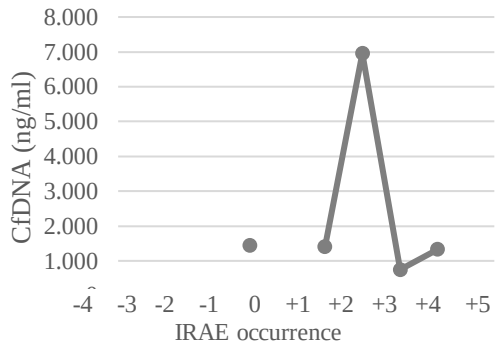
X – Result at first staging: PD.



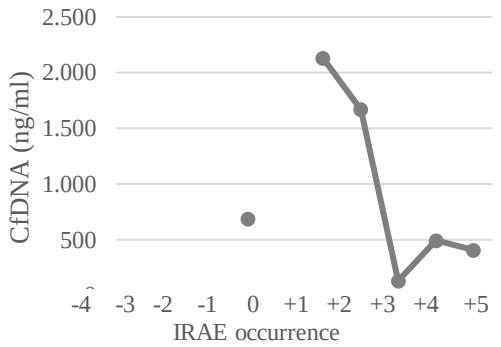
Y – Result at first staging: PR.



Z – Result at first staging: Died before first staging.

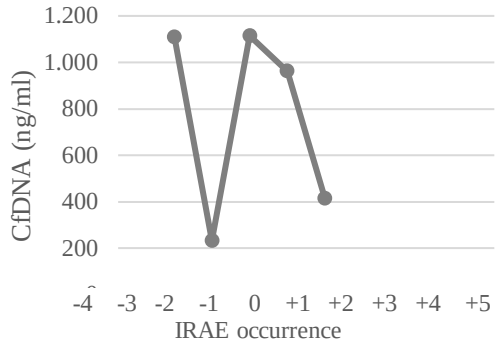


AA – Result at first staging: PD.

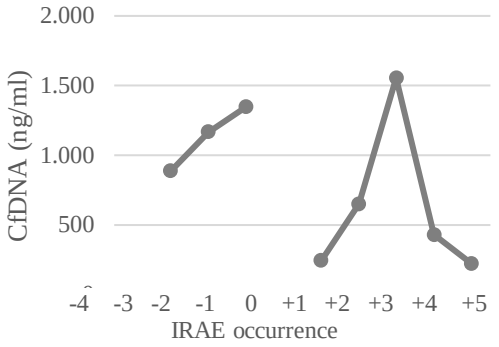


AB – Result at first staging: Died before first staging.

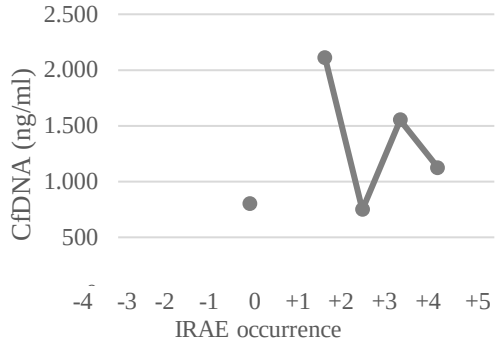
Figure 20. *Cont.*



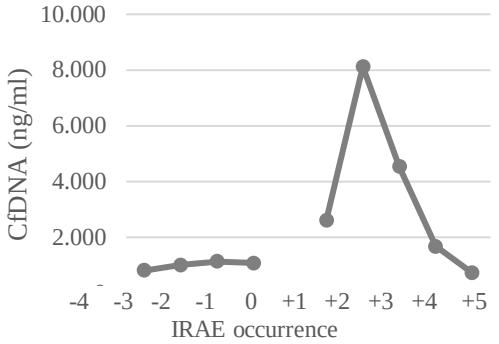
AC – Result at first staging: PR.



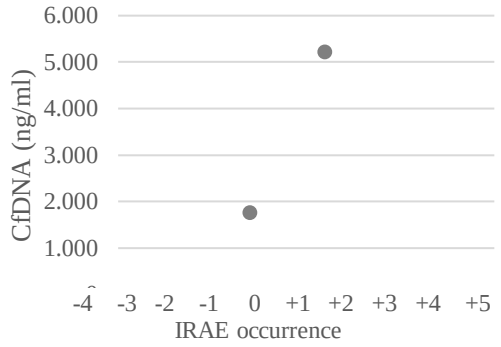
AD – Result at first staging: PD.



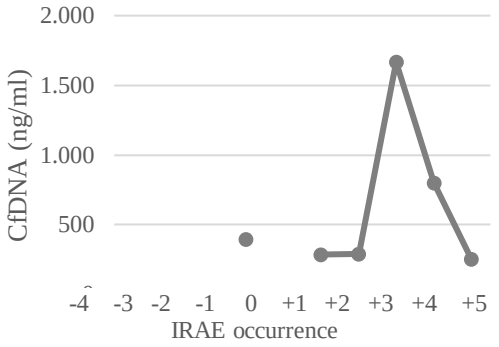
AE – Result at first staging: SD/MR.



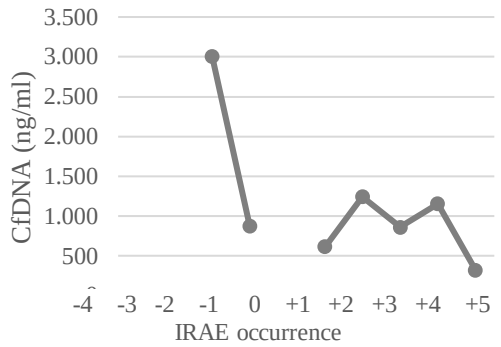
AF – Result at first staging: PR.



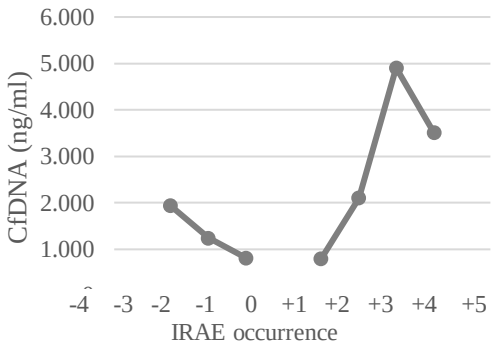
AG – Result at first staging: PD.



AH – Result at first staging: PR.

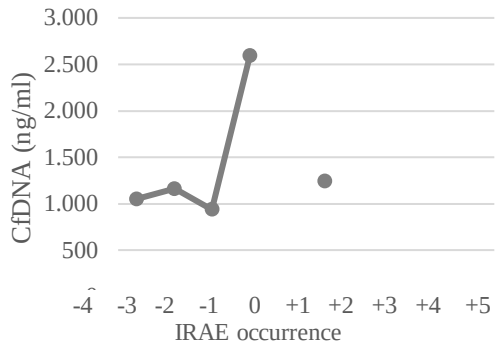


AI – Result at first staging: PD.

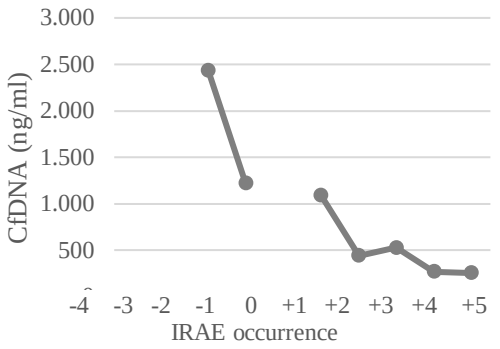


AJ – Result at first staging: PR.

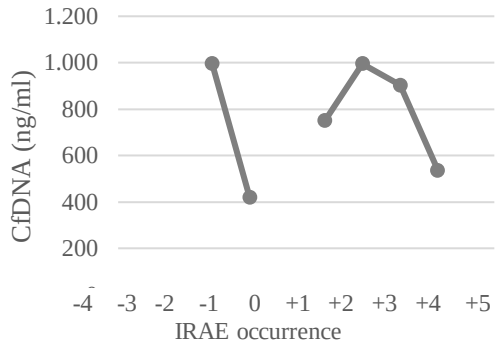
Figure 20. *Cont.*



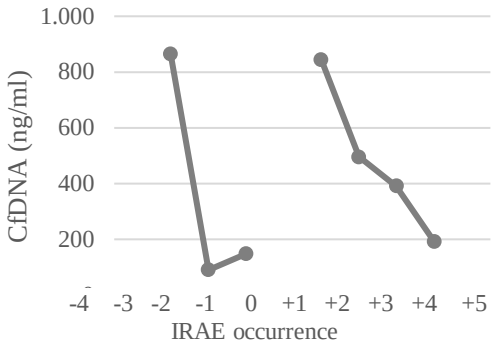
AK – Result at first staging: PR.



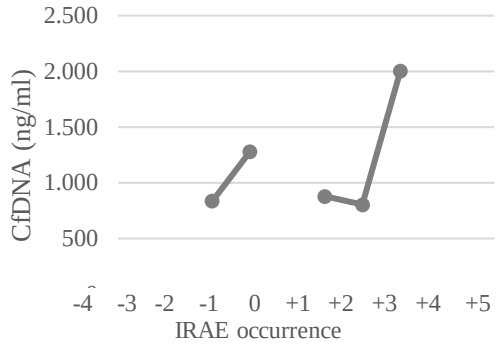
AL – Result at first staging: PR.



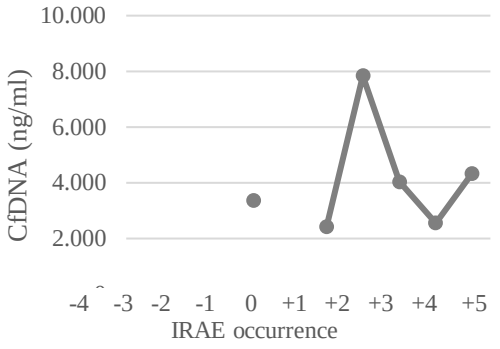
AM – Result at first staging: SD/MR.



AN – Result at first staging: PR.



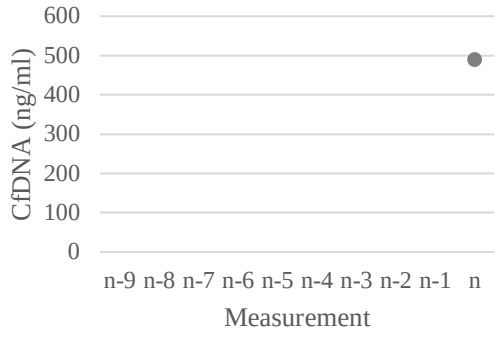
AO – Result at first staging: PR.



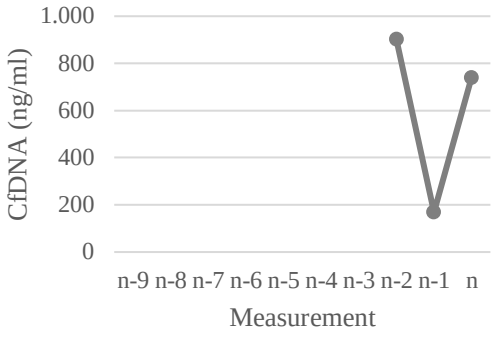
AP – Result at first staging: PD.

Figure 20. Evolution of cfDNA in ng/ml over time for each patient with IRAE with result at first staging.

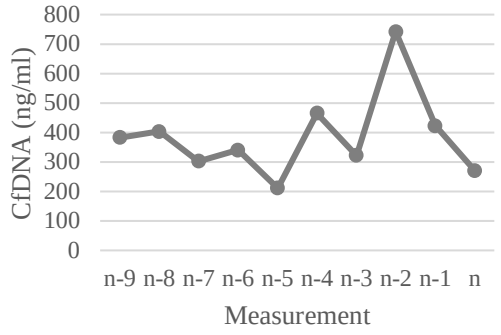
We also analyzed the development of cfDNA over time for each patient without IRAE in ng/ml (see Figure 21). We noted for each figure the result at first staging examination.



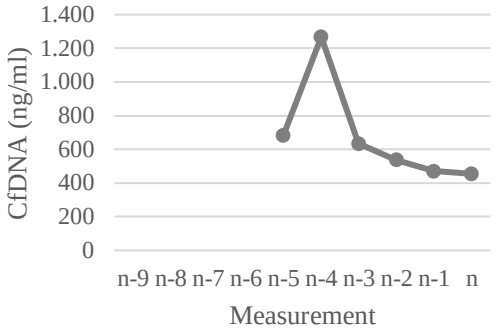
A – Result at first staging: PR.



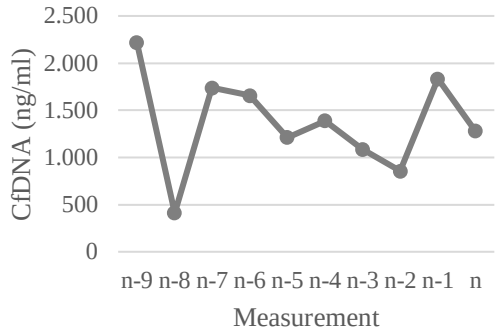
B – Result at first staging: SD/MR.



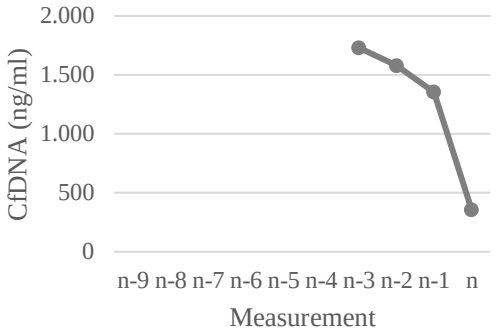
C – Result at first staging: PR.



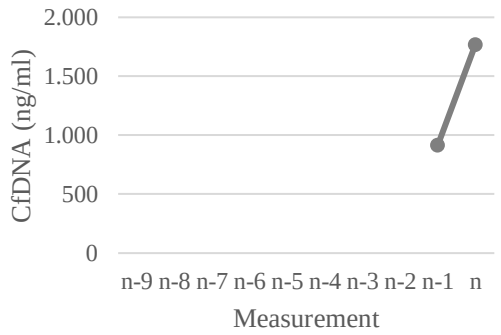
D – Result at first staging: Died before first staging.



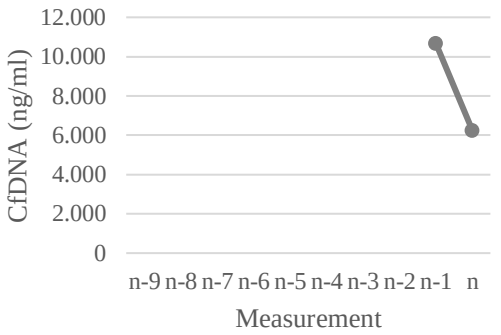
E – Result at first staging: PR.



F – Result at first staging: PD.

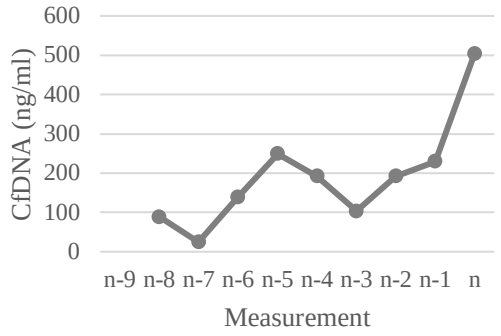


G –Result at first staging: Died before first staging.

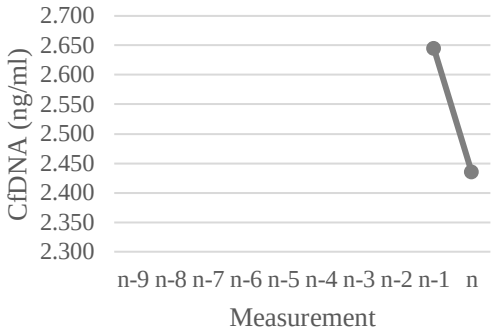


H – Result at first staging: PD.

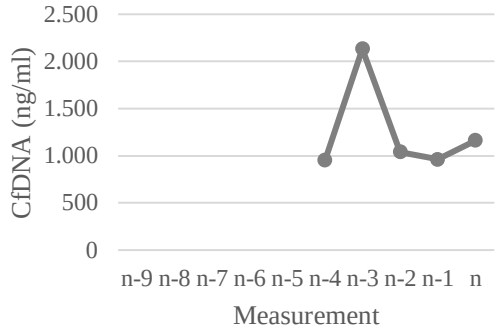
Figure 21. *Cont.*



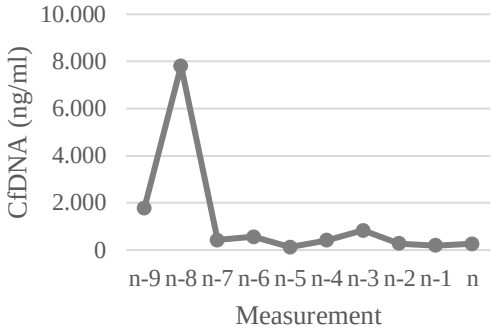
I – Result at first staging: SD/MR.



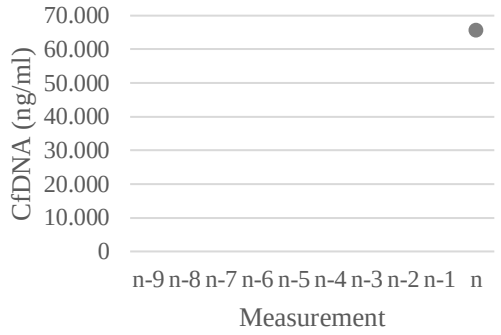
J – Result at first staging: PD.



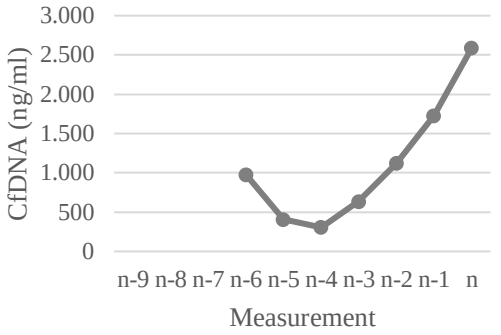
K – Result at first staging: SD/MR.



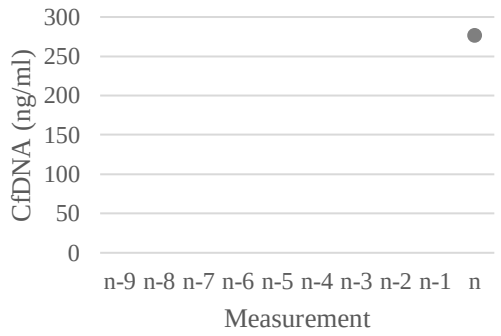
L – Result at first staging: PR.



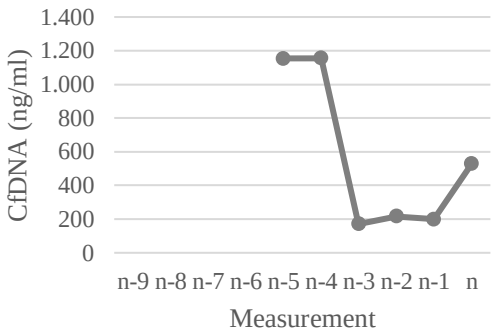
M – Result at first staging: Died before first staging.



N – Result at first staging: PR.

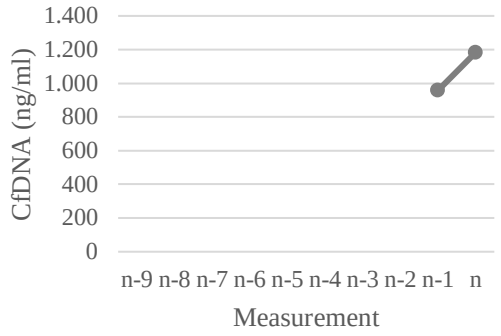


O – Result at first staging: PR.

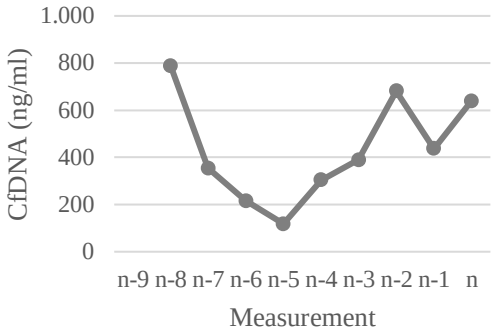


P – Result at first staging: PD.

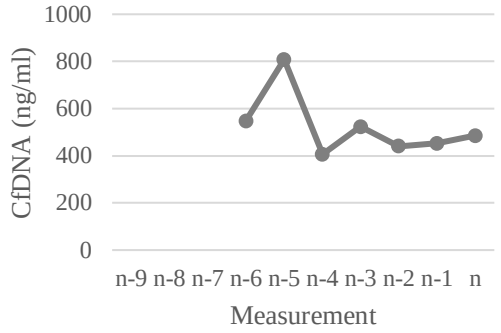
Figure 21. *Cont.*



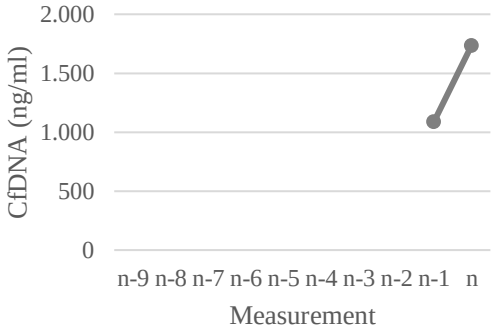
Q – Result at first staging: SD/MR.



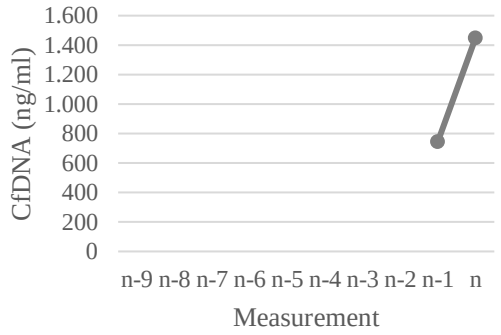
R – Result at first staging: PR.



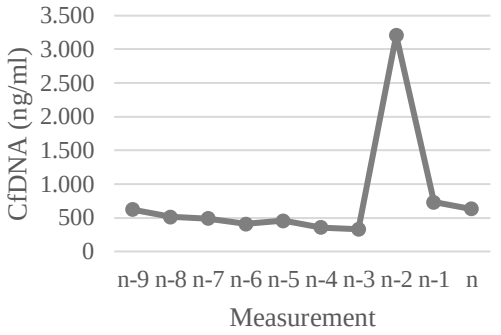
S –Result at first staging: PD.



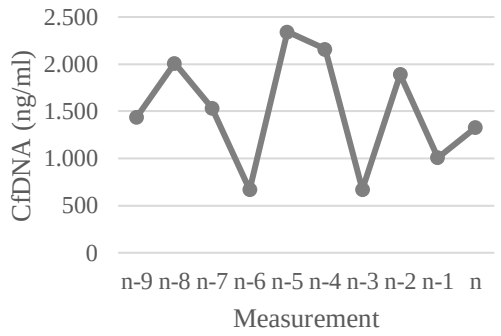
T – Result at first staging: PD.



U – Result at first staging: PR.



V – Result at first staging: SD/MR.



W – Result at first staging: PR.

Figure 21. Evolution of cfDNA in ng/ml over time for each patient without IRAE with result at first staging.

HLA

"74% of HLA homozygous patients developed IRAE, compared to 58% of HLA heterozygous patients. This difference was not statistically significant. We differentiated further between HLA-A, -B, and -C homozygosity. 77% of HLA-A homozygous patients developed IRAE, compared to only 60% of HLA-A heterozygous patients. Notably, only 50% of HLA-B and 63% of HLA-C homozygous patients developed IRAE, compared to 63% and 62% of HLA-B and HLA-C heterozygous patients, respectively. HLA-A homozygosity thus seems to be the determining factor in HLA homozygosity-associated IRAE.

A positive correlation between IRAE and homozygosity was found for HLA-A and HLA-C homozygosity for colitis, and HLA-A, HLA-B, and HLA-C homozygosity for hepatitis. HLA homozygosity was significantly linked to the development of hepatitis (p = 0.015). HLA-B homozygosity is seemingly a protective factor in general IRAE, colitis or pancreatitis. Table [12] illustrates the results on HLA homo- and heterozygosity" (Wölffer, Battke et al. 2022).

Table 12. Distribution of HLA zygosity in our cohort, subset by HLA class I locus in relation to development of IRAE in absolute values and percent (Wölffer, Battke et al. 2022).

HLA class I locus	Patients total		IRAE		Colitis		Hepatitis		Pancreatitis	
	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)
Total	95	100	59	62	20	21	14	15	13	14
HLA										
Homozygosity	23	24	17	74	8	35	7	30 (p=0.015)	3	13
Heterozygosity	72	76	42	58	12	17	7	10	10	14
HLA-A										
Homozygosity	13	14	10	77	5	39	4	31	2	15
Heterozygosity	82	86	49	60	15	18	10	12	11	13
HLA-B										
Homozygosity	8	8	4	50	1	13	2	25	1	13
Heterozygosity	87	92	55	63	19	22	12	14	12	14
HLA-C										
Homozygosity	8	8	5	63	3	38	2	25	1	13
Heterozygosity	87	92	54	62	17	20	12	14	12	14

We collected HLA alleles of patients developing IRAE in our cohort (see Table 13).

Table 13. HLA alleles of patients developing IRAE in our cohort.

HLA-A	HLA-B	HLA-C
0101	0801	0202
0201	1402	0303
2301	1501	0304
2402	1801	0401
2501	3501	0501
2601	3502	0602
2902	3503	0701
3001	3701	0702
3101	3801	0704
3201	3906	0802
6601	4001	1202
6802	4101	1203
6824	4402	1402
	4403	1502
	4701	1602
	4901	1701
	5101	
	5108	
	5201	
	5701	
	5801	

"We analyzed confidence intervals and odds ratio for HLA homo- and heterozygosity for IRAE, colitis, hepatitis and pancreatitis (see Figure [22])" (Wölffer, Battke et al. 2022).

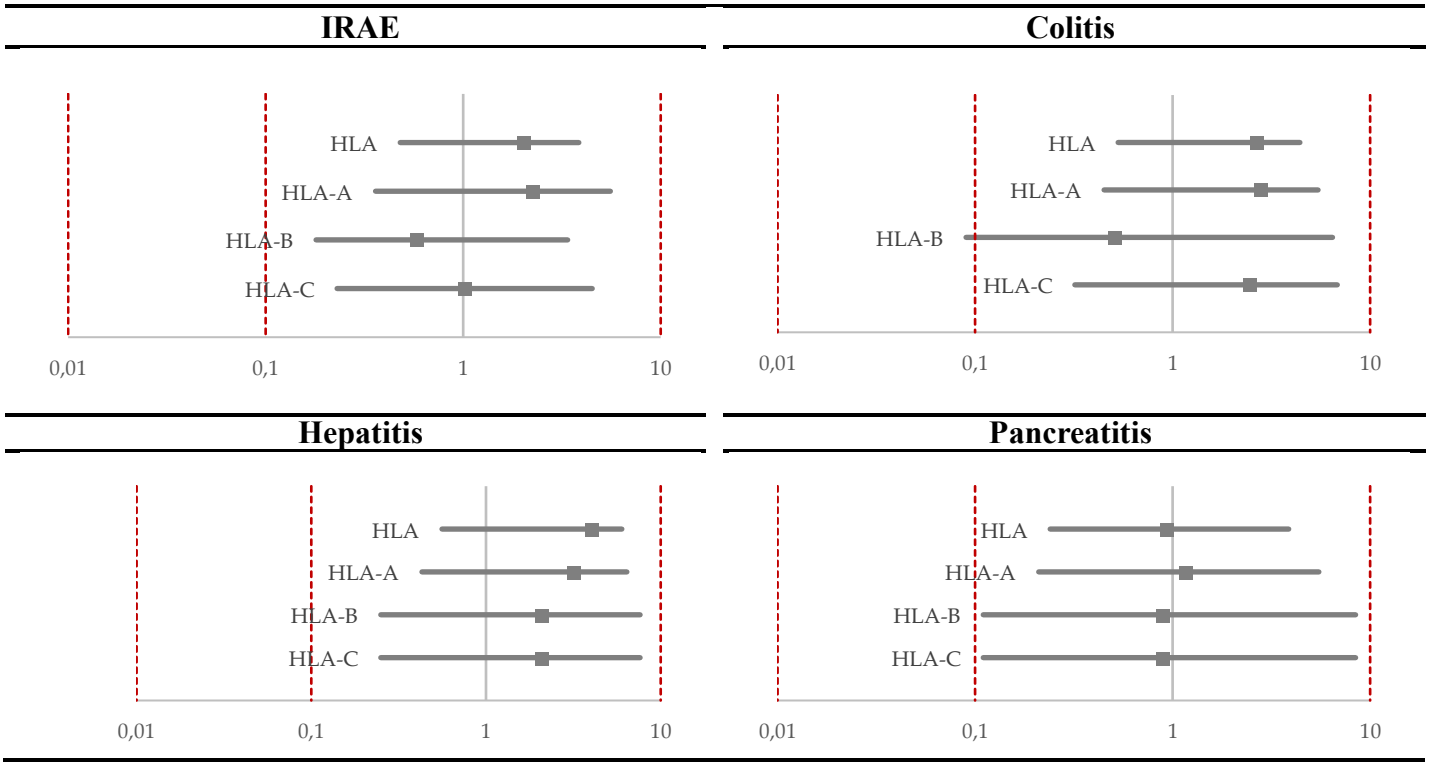


Figure 22. Forest plots HLA homo- and heterozygosity depicting the confidence intervals and odds ratios on a logarithmic scale. Note that all confidence intervals include 1, meaning that we cannot conclude that there is a statistically significant difference (Wölffer, Battke et al. 2022).

Small Variations (VARs)

"VARs on SMAD3 were significantly linked to the development of pancreatitis (p = 0.034). This result must be read and interpreted with caution due to the exploratory approach" (Wölffer, Battke et al. 2022). We analyzed genes affected by VARs and associated with the development of IRAE, for IRAE in general and in particular (see Table 14).

Table 14. Genes affected by VARs associated with IRAE and organ specific IRAE. Note that we only list genes with observed incidence of IRAE above the respective cohort average and concerning at least 2 patients (Wölffer, Battke et al. 2022).

IRAE	Genes affected by VARs	N of patients with VARs and IRAE	% patients with VARs having IRAE
IRAE in general	SMAD3	8	67%
	PRDM1	29	64%
	PDCD1	3	100%
	IL1RN	31	65%
	CD274	3	75%
Colitis	TERT	2	29%
	SMAD3	3	25%
	CTLA4	16	27%
	IL1RN	11	23%
Pneumonitis	PRDM1	4	9%
Hepatitis	PRDM1	7	16%
	CTLA4	10	17%
Myocarditis	SLCO1B1	2	4%
Myositis	IL1RN	2	4%
Pancreatitis	TERT	2	29%
	SH2B3	10	15%
	SMAD3	4	33% (p = 0.034)
	FAN1	9	15%
	IL1RN	9	19%
Exanthema	PRDM1	4	9%
	CTLA4	5	9%
Hypophysitis	SMAD3	3	25%
	PRDM1	6	13%
	IL1RN	7	15%
Nephritis	FAN1	3	5%
	IL1RN	3	6%
Thyroiditis	CTLA4	5	9%
	FAN1	5	8%

Copy Number Variations (CNVs)

"IKZF1, FAN1 and IL1RN were linked to IRAE in general, as well as all three main IRAE in particular. Deletions were found to be more frequently associated with IRAE than duplications. CNVs on IL1RN (p = 0.033) and deletions on PRDM1 (p = 0.026) were significantly linked to IRAE. Duplications on CD274 (p = 0.043) and CNVs on SLCO1B1 (p = 0.010) were significantly linked to hepatitis. These results must be interpreted with caution due to the exploratory approach. We distinguished deletions and duplications for each CNV for IRAE in general, as well as colitis, hepatitis and pancreatitis (see Table [15])" (Wölffer, Battke et al. 2022).

Table 15. Genes affected by CNVs (deletions or duplications) in relation to observed IRAE. Note that we only list genes with observed incidence of IRAE above the respective cohort average and concerning at least 2 patients (Wölffer, Battke et al. 2022).

IRAE	**Gene affected**	**Patients with CNV and IRAE**		**Deletions with IRAE**		**Duplications with IRAE**	
		N	%	N	%	N	%
IRAE	TERT	31	69%	-	-	27	73%
	LRRK2	24	63%	9	90%	-	-
	IKZF1	27	68%	3	75%	23	68%
	SMAD3	25	69%	-	-	25	69%
	JAK2	28	65%	18	78%	-	-
	PRDM1	24	75%	19	86% (p=0.026)	-	-
	CTLA4	10	71%	3	75%	6	67%
	TSHR	16	80%	9	90%	-	-
	FAN1	29	73%	9	82%	-	-
	SLCO1B1	39	66%	7	64%	27	66%
	PDCD1	19	68%	-	-	17	71%
	IL1RN	13	87% (p=0.033)	5	100%	7	78%
	CD274	14	78%	12	80%	2	67%
	UNG	22	71%	2	67%	19	70%
Colitis	TERT	10	22%	2	29%	-	-
	IKZF1	11	28%	-	-	11	32%
	SMAD3	8	22%	-	-	8	22%
	FAN1	8	20%	3	27%	-	-
	IL1RN	4	27%	-	-	3	33%

Table 15. *Cont.*

IRAE	Gene affected	Patients with CNV and IRAE		Deletions with IRAE		Duplications with IRAE	
		N	%	N	%	N	%
Hepatitis	SH2B3	5	19%	-	-	5	19%
	LRRK2	8	21%	3	30%	5	19%
	IKZF1	7	18%	-	-	7	21%
	SMAD3	8	22%	-	-	8	22%
	PRDM1	5	16%	-	-	2	20%
	CTLA4	3	21%	-	-	3	33%
	TSHR	5	25%	2	20%	2	29%
	FAN1	8	20%	-	-	7	28%
	SLCO1B1	13	22% (p=0.010)	2	18%	9	22%
	IL1RN	4	27%	-	-	4	44%
	CD274	4	22%	-	-	2	67% (p=0.043)
	UNG	5	16%	-	-	5	19%
Pancreatitis	TERT	5	11%	2	29%	-	-
	IKZF1	5	13%	-	-	5	15%
	JAK2	4	9%	-	-	3	15%
	FAN1	4	10%	2	18%	-	-
	SLCO1B1	7	12%	-	-	6	15%
	IL1RN	2	13%	-	-	-	-
	UNG	3	10%	-	-	-	-

“CNVs on PRDM1 and CD274 were significantly linked to encephalitis (p = 0.014 and p = 0.032) and myositis (p = 0.014 and p = 0.032). CNVs on TSHR and FAN1 were significantly linked to myositis (p = 0.049 and p = 0.039). These results must be interpreted with caution due to the exploratory approach. Since a distinction between duplications and deletions would have made the sample size too small for statistical statements for less common specific IRAE, this has been omitted here (see Table [16])” (Wölffer, Battke et al. 2022).

Table 16. Genes affected by CNVs in general associated with IRAE. Note that we only list genes with observed incidence of IRAE above the respective cohort average and concerning at least 2 patients (Wölffer, Battke et al. 2022).

IRAE	Gene affected by CNV	N of patients with CNV and IRAE	% of patients with CNV and IRAE
Pneumonitis	PRDM1	3	9%
	CD274	2	11%
Encephalitis	TERT	3	7%
	IKZF1	2	5%
	JAK2	3	7%
	PRDM1	3	9% (p = 0.014)
	CD274	2	11% (p = 0.032)

Table 16. *Cont.*

IRAE	Gene affected by CNV	N of patients with CNV and IRAE	% of patients with CNV and IRAE
Myocarditis	SLCO1B1	2	3%
Myositis	TERT	2	4%
	LRRK2	2	5%
	IKZF1	2	5%
	SMAD3	2	6%
	JAK2	2	5%
	PRDM1	3	9% (p = 0.014)
	TSHR	2	10% (p = 0.049)
	FAN1	3	8% (p = 0.039)
	CD274	2	11% (p = 0.032)
	UNG	2	6%
Exanthema	TERT	4	9%
	SH2B3	3	11%
	SMAD3	3	8%
	JAK2	4	9%
	PRDM1	4	13%
	CTLA4	2	14%
	TSHR	2	10%
	SLCO1B1	6	10%
	IL1RN	2	13%
	CD274	3	17%
Hypophysitis	TERT	5	11%
	SMAD3	4	11%
	JAK2	5	12%
	PRDM1	5	16%
	FAN1	7	18%
	CD274	3	17%
	UNG	4	13%
Nephritis	IKZF1	2	5%
Thyroiditis	LRRK2	4	11%
	SMAD3	3	8%
	PRDM1	3	9%
	TSHR	2	10%
	SLCO1B1	6	10%
	PDCD1	3	11%
	CD274	2	11%
	UNG	3	10%

We did not find a significant correlation between CNVs on a specific gene and histological subtype of melanoma (see Table 17).

Table 17. Frequency of CNVs by histological subtype of melanoma.

Genes affected by CNVs		Melanoma subtype						
		Cutaneous	Acral lentiginous	Mucosal	Uveal	Occult	Other	Total
	N	63	9	3	6	12	2	95
	%	66%	10%	3%	6%	13%	2%	100%
AIRE	N	0	0	0	0	1	0	1
	%	0%	0%	0%	0%	100%	0%	100%
TERT	N	32	3	2	3	4	1	45
	%	71%	7%	4%	7%	9%	2%	100%
SH2B3	N	20	1	1	2	2	1	27
	%	74%	4%	4%	7%	7%	4%	100%
LRRK2	N	21	5	2	3	5	2	38
	%	55%	13%	5%	8%	13%	5%	100%
IKZF1	N	28	2	2	1	6	1	40
	%	70%	5%	5%	3%	15%	3%	100%
SMAD3	N	21	3	2	3	6	1	36
	%	58%	8%	6%	8%	17%	3%	100%
JAK2	N	26	5	3	2	6	1	43
	%	61%	12%	7%	5%	14%	2%	100%
PRDM1	N	18	4	2	2	4	2	32
	%	56%	13%	6%	6%	13%	6%	100%
CTLA4	N	10	1	0	2	1	0	14
	%	71%	7%	0%	14%	7%	0%	100%
TSHR	N	12	1	2	1	3	1	20
	%	60%	5%	10%	5%	15%	5%	100%
FAN1	N	25	4	2	3	5	1	40
	%	63%	10%	5%	8%	13%	3%	100%
SLCO1B1	N	36	6	3	5	7	2	59
	%	61%	10%	5%	9%	12%	3%	100%
PDCD1	N	19	3	1	3	1	1	28
	%	68%	11%	4%	11%	4%	4%	100%
IL1RN	N	9	1	0	2	2	1	15
	%	60%	7%	0%	13%	13%	7%	100%
CD274	N	13	1	1	0	2	1	18
	%	72%	6%	6%	0%	11%	6%	100%
UNG	N	17	4	1	3	4	2	31
	%	55%	13%	3%	10%	13%	7%	100%

“We analyzed confidence intervals and odds ratio for CNVs for IRAE, colitis, hepatitis and pancreatitis (see Figure [23]). [CNVs on AIRE were eliminated from all forest plots

due to lack of data.] CNVs on CTLA-4 were eliminated from the forest plot for pancreatitis due to lack of data" (Wölffer, Battke et al. 2022).

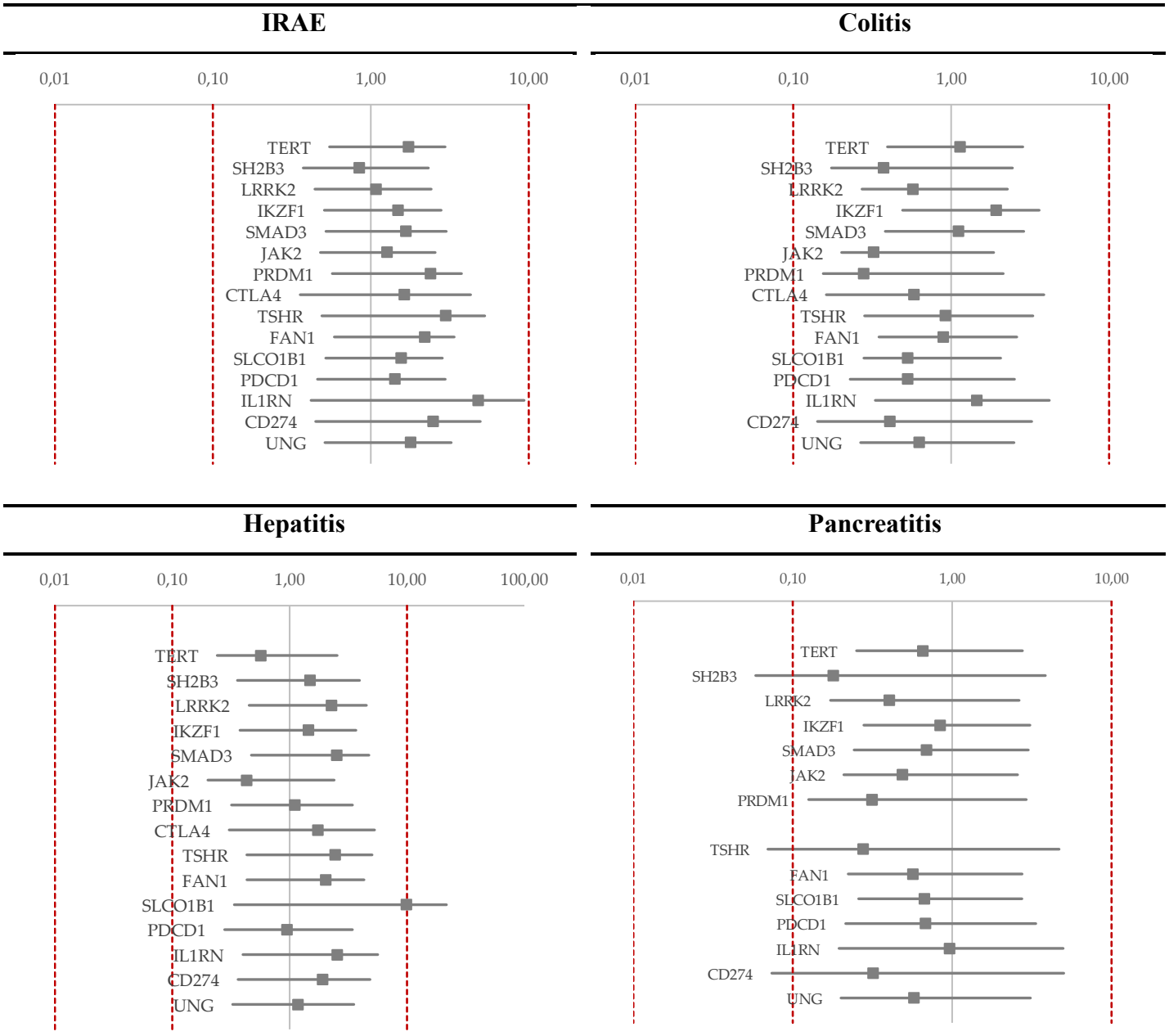


Figure 23. Forest plots CNVs depicting confidence intervals and odds ratios on a logarithmic scale. Note that all confidence intervals include 1, meaning that we cannot conclude that there is a statistically significant difference (Wölffer, Battke et al. 2022).

4 Discussion

Summary of results

The three most common IRAE in our cohort were colitis, hepatitis and pancreatitis. Early-onset IRAE included exanthema, thyroiditis, nephritis, myocarditis and myositis, whereas late-onset IRAE included hypophysitis, pneumonitis and encephalitis. “We found a significant link between female sex and hepatitis, and a correlation between male sex and pancreatitis. Combined immunotherapy was associated with a higher incidence of IRAE and significantly linked to colitis. We found an association between increased leucocytes at start of immunotherapy and occurrence of colitis, hepatitis or pancreatitis. Increased absolute neutrophils at start of immunotherapy were associated with colitis or pancreatitis, whereas decreased relative lymphocytes at start of immunotherapy were associated with pancreatitis. Increased total and relative monocytes at start of immunotherapy were associated with IRAE or colitis. Increased absolute and relative monocytes at start of immunotherapy were significantly linked to the occurrence of pancreatitis. We found furthermore a significant link between pre-existing autoimmune diseases and pancreatitis” (Wölffer, Battke et al. 2022). Concerning genetic data, we were not able to find a correlation between rising levels of cfDNA and the occurrence of IRAE. However, we found an association between high levels of cfDNA and progress of disease, as well as overall elevated levels of cfDNA and occurrence of IRAE. “HLA homozygosity was linked to IRAE in general or colitis. HLA homozygosity was significantly associated with hepatitis. HLA-A homozygosity was strikingly linked to the occurrence of IRAE in general, colitis or hepatitis. VARs on SMAD3 were significantly linked to pancreatitis. CNVs on IKZF1, FAN1 and IL1RN were linked to IRAE, colitis, hepatitis or pancreatitis. CNVs on IL1RN and deletions on PRDM1 were significantly linked to IRAE, whereas duplications on CD274 and CNVs on SLCO1B1 were significantly linked to hepatitis. Finally, CNVs on PRDM1 and CD274 were significantly linked to encephalitis, and CNVs on PRDM1, CD274, TSHR and FAN1 were significantly linked to myositis” (Wölffer, Battke et al. 2022).

Pathophysiology of checkpoint inhibitors and IRAE

The two main targets of immune checkpoint inhibitor therapy are CTLA-4 and PD-1, two

T cell receptors (TCR) responsible for T cell activation and proliferation.

After antigen recognition by TCR, T cell activation necessitates a supplementary binding of CD80 or CD86 of dendritic cells to CD28 TCR (Ramos-Casals, Brahmer et al. 2020). CTLA-4 also binds CD80 and CD86, inhibiting T cell activation (Ramos-Casals, Brahmer et al. 2020). Under physiological conditions, this is supposed to prevent autoimmune reactions. Indeed, genetic deletion of CTLA-4 in mice has been shown to induce autoimmunity and expansion of forkhead box (Fox) P3+ regulatory T cells (Klocke, Sakaguchi et al. 2016), confirming the role of CTLA-4 as an antagonist of CD28. CTLA-4 inhibitors prevent the connection of CTLA-4 and CD80 or CD86, supporting T cell activation (Ramos-Casals, Brahmer et al. 2020).

PD-1 is another receptor on T cells supporting T cell apoptosis and inhibiting apoptosis of regulatory T cells and T cell activation if bound by PD-L1 (Ramos-Casals, Brahmer et al. 2020). Under physiological conditions, this prevents autoimmune reactions through activation of regulatory T cells (Gianchecchi and Fierabracci 2018). However, tumor cells express PD-L1 in order to escape the immune system. Indeed, a plethora of factors lead to an upregulation of PD-L1 expression on tumor cells. Interferon-γ originating from cytotoxic T cells attacking the tumor, Interleukin-6 (IL-6) and IL-10 stimulate PD-L1 expression through the intermediary of STAT1 transcription factor (Frydenlund and Mahalingam 2017). Secondly, oncogenic abnormalities in the MAPK and EGFR signalling pathways, and increased expression of STAT3 and HIF-1α transcription factors also lead to increased PD-L1 expression on tumor cells (Frydenlund and Mahalingam 2017). This so called adaptive immune resistance is targeted by PD-1 and PD-L1 inhibitors which prevent the connection between PD-1 and PD-L1 and thereby support T cell activation (Ramos-Casals, Brahmer et al. 2020).

CTLA-4 inhibition results thus in T cell activation and proliferation, inhibition of regulatory T cells and B-cell mediated autoantibody production, while PD-1 inhibition results in inhibition of regulatory T cells and increase of cytokine production such as IL-17, CXCL10, and tumor necrosis factor (TNF) (Ramos-Casals, Brahmer et al. 2020). This not only leads to a stimulation of the immune system with increased anti-tumor activity, but also to autoimmune reactions. Indeed, polymorphisms on CTLA-4 have been associated with autoimmune disorders such as type 1 diabetes (Jin, Xiang et al. 2015).

The following two figures illustrate the mechanisms of immune checkpoints and

checkpoint inhibitors.

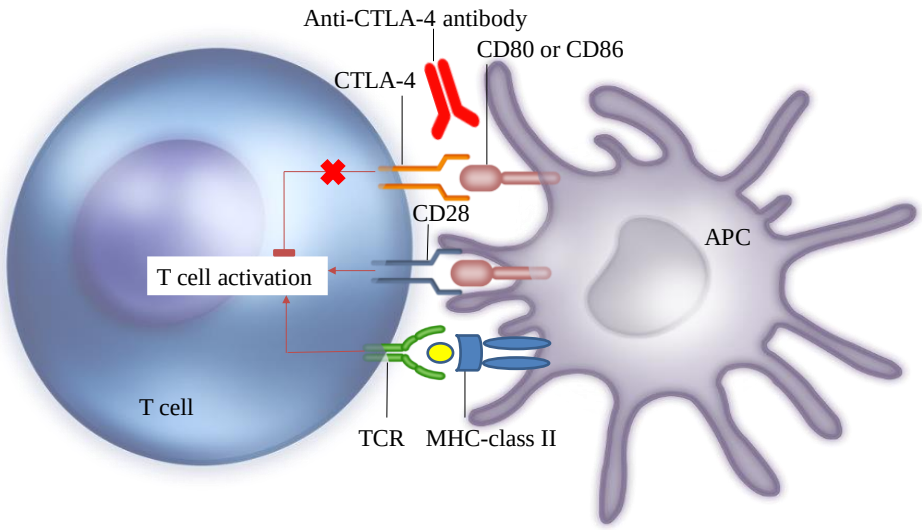


Figure 24. Mechanism of Anti-CTLA-4 antibodies. TCR: T cell receptor. APC: antigen-presenting cell.

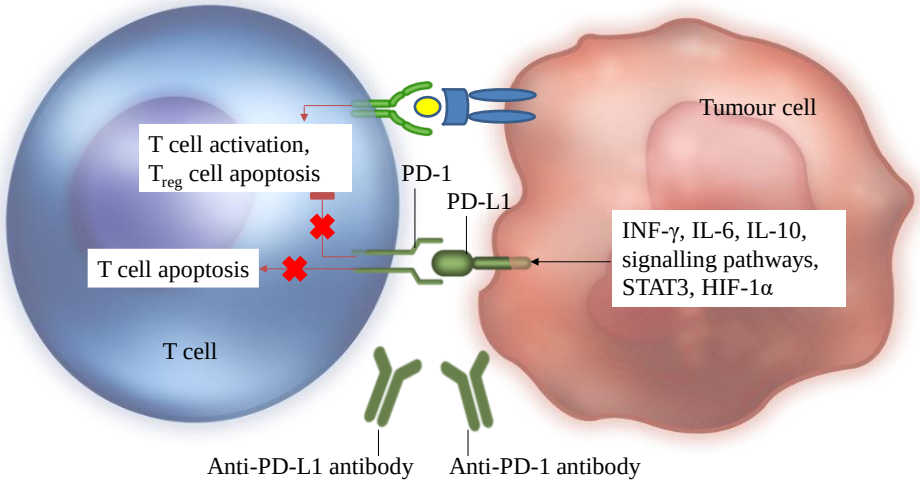


Figure 25. Mechanism of Anti-PD-1 and Anti-PD-L1 antibodies.

The pathophysiology of IRAE themselves is not yet fully understood. Authors hypothesised that CD8+ cytotoxic T cells hyperactivated through the mechanisms mentioned above attack tumor tissue, releasing tumor antigens and auto-antigens, and diversifying thus the T cell repertoire, which may lead to interactions between some T cell clones and self-tissue (Passat, Touchefeu et al. 2018). Inhibition of regulatory T cells contributes additionally to the reduction of immune tolerance (Passat, Touchefeu et al.

2018). Activation of Th1 and Th17 T cells leads to a massive production of cytokines, especially Interferon-γ and IL-17 (Passat, Touchefeu et al. 2018). Especially IL-17 is associated with the occurrence of severe IRAE and has been associated with the occurrence of colitis during ICI (Tarhini, Zahoor et al. 2015). Additionally, gastrointestinal IRAE in particular have been shown to be caused by a dysregulation of gastrointestinal mucosal immunity (Berman, Parker et al. 2010).

IRAE, temporal occurrence and type of therapy

The CheckMate 067 study (Larkin, Chiarion-Sileni et al. 2015) notes IRAE occurring during Anti-PD-1 monotherapy as well as combined Anti-PD-1 and Anti-CTLA-4 therapy in patients with previously untreated melanoma which we summarise in Table 18.

Table 18. Results of the CheckMate 067 study for frequencies of common IRAE.

IRAE	Frequency under Nivolumab (%)	Frequency under combined therapy (%)
Any select IRAE	62.0	87.9
Gastrointestinal (incl. colitis)	19.5 (1.3)	46.3 (11.8)
Hepatitis	6.4	30.0
Exanthema	21.7	28.4
Hypo- and hyperthyroidism	12.8	24.9
Hypophysitis	0.6	7.7
Pneumonitis	1.3	6.4
Renal	0.9	5.4

We note similarities as to the incidence of IRAE. In our cohort, 62.0% of patients developed IRAE, as well as in the results of the CheckMate 067 study when considering nivolumab monotherapy. However, the frequency of occurrence of IRAE under combined therapy was higher in the cited study (87.9%).

Colitis and hepatitis are the two most common select IRAE under combined therapy in the results of the CheckMate 067 study, which coincides with our results.

We differentiated between frequencies of colitis, hepatitis and pancreatitis under Anti-PD-1 monotherapy and combined therapy. The frequency of colitis mentioned in our results is higher than in the CheckMate 067 study for both therapies (5.6% and 30.5% respectively). However, when comparing the frequency of colitis in our cohort to general

gastrointestinal symptoms in the CheckMate 067 study, the frequency found in our cohort is lower for both therapies. General gastrointestinal symptoms encompass diarrhoea according to the author's information. Cases reported as "colitis" in our cohort include severe cases of diarrhoea necessitating treatment, according to the CTCAE criteria. We can thus assume to have results overall comparable to the CheckMate 067 study. We found a slightly higher frequency for hepatitis under Anti-PD-1 monotherapy (8.3%), and a lower frequency for hepatitis under combined therapy (18.6%).
Frequencies of less common IRAE in our cohort are mostly situated within the interval between frequency under Anti-PD-1 monotherapy and frequency under combined therapy of the CheckMate 067 study. We note a slightly higher frequency for hypophysitis (15.3% under combined therapy versus 7.7% here) and pneumonitis (10.2% under combined therapy versus 6.4% here) in our results. Furthermore, we note a lower frequency for thyroiditis and exanthema in our results (7% each), probably due to the limited sample size of our cohort. Finally, thyroiditis can only be compared to hypo- and hyperthyroidism noted by the CheckMate 067 study, which encompasses more cases.

"Concerning the type of ICI, in the CheckMate 067 study there was a considerably higher incidence of IRAE during combined immunotherapy compared to Anti-PD-1 monotherapy (Larkin, Chiarion-Sileni et al. 2015). This fits very well to the findings of our study. We also noted a comparable impact of type of ICI on frequency of IRAE in our cohort" (Wölffer, Battke et al. 2022).

As the Checkmate 067 study did not provide incidence data of rarer IRAE, we compare our results with different studies (see Table 19).

Table 19. Incidence of less common IRAE according to literature.

IRAE	Frequency under nivolumab monotherapy (%)	Frequency under combined ICI (nivolumab and ipilimumab) (%)
Encephalitis (Larkin, Chmielowski et al. 2017)	0.1	0.24
Myocarditis (Salem, Manouchehri et al. 2018)	0.41	1.3
Myositis (Johnson, Balko et al. 2016)	0.02	0.24
Pancreatitis (George, Bajaj et al. 2019)	1	10.6

The results of George et al. are not melanoma specific. However, they concluded that patients treated for melanoma had an incidence of pancreatitis higher by around 50% compared to non-melanoma patients (George, Bajaj et al. 2019). This would coincide with our results, stating an occurrence of 16.9% under combined therapy and 8.3% under monotherapy for pancreatitis. Notably, the incidences found in our results for myocarditis, myositis and encephalitis are slightly higher than those found in literature. However, these minor discrepancies can be explained by our limited sample size.

On closer evaluation, we also note a similarity of the time to occurrence of IRAE between our results and results found in literature (Martins, Sofiya et al. 2019). We compared the medians of occurrence of IRAE between our results and the cited study (see Table 20).

Table 20. Comparison between our results in median time to onset of IRAE and results of Martins et al. (Martins, Sofiya et al. 2019).*:"neurological".

IRAE	Median of IRAE occurrence after start of ICI in months in sample	Median of IRAE occurrence after start of ICI in months (Martins, Sofiya et al. 2019)	
		Anti-PD-1 monotherapy	Combined therapy
Colitis	1.4	1.5	1.2
Hepatitis	1.3	2.5	2.5
Pancreatitis	1.4	-	-
Endocrinological	-	2.0	1.0
Thyroiditis	1.2	1.3	-
Hypophysitis	2.1	2.5	-
Pneumonitis	1.8	3.0	1.6
Exanthema	1.2	1.2	1.0
Nephritis	1.3	3-12	2.6
Myositis	1.3	-	-
Encephalitis	3.4	1.1*	0.5*
Myocarditis	0.5	-	-

The median time to onset of most IRAE is located within the second month of ICI, which is similar in our cohort. As we did not differentiate between median time of occurrence during monotherapy and combined therapy, the median time of occurrence in our results is generally in between the two medians given in the cited study. Notably, the median of occurrence of hepatitis and nephritis was much lower in our cohort, whereas these IRAE were among the latest occurring IRAE in the cited study. This may be explained by our limited sample size. Combined ICI is associated with a higher IRAE incidence as well as

an earlier occurrence of the same IRAE (Martins, Sofiya et al. 2019).

“Recently, it has also been shown that dosage of ipilimumab and nivolumab in combined immunotherapy seems to be decisive for the occurrence of IRAE. Lebbé *et al.* showed that nivolumab 3mg/kg combined with ipilimumab 1mg/kg as opposed to the established dosage nivolumab 1mg/kg and ipilimumab 3g/kg was associated with a significantly lower occurrence of grade 3 to 5 IRAE while survival outcomes and treatment response were similar between the two dosages (Lebbé, Meyer et al. 2019). This dosage has also been identified as the optimal dosage for combined immunotherapy in a neoadjuvant setting as it presented comparable response rates and lower incidence of IRAE during the opACIN-neo trial (Rozeman, Menzies et al. 2019)” (Wölffer, Battke et al. 2022).

This dosage is nowadays the standard dosage in renal cell carcinoma patients (Motzer, Rini et al. 2019) but has not yet been completely established in treatment of melanoma. A wider use of Nivolumab 3mg/kg and Ipilimumab 1mg/kg could result in less IRAE in the light of new insights mentioned above.

Current studies examine possible combinations of neoadjuvant and adjuvant immunotherapies in stage III melanoma. The neoadjuvant approach has been shown to increase relapse-free survival and pathological complete response rates, facilitate surgical options, and optimize adjuvant treatment after surgical intervention (Garutti, Buriolla et al. 2020). The ongoing PRADO trial complements the opACIN-neo results, examining possible escalations and de-escalations of adjuvant therapy depending on complete pathological response after neoadjuvant therapy (Blank C.U. 2020).

If several factors in the neoadjuvant approach, such as therapy duration, remain up to this date unknown, current studies will provide more information in the near future. These results may change standard therapy of advanced melanoma and thereby have an impact on occurrence and severity of IRAE.

Sex and IRAE

“[F]emale sex is a known risk factor for the development of autoimmune diseases, especially autoimmune hepatic diseases (Schwinge and Schramm 2019), and is linked to the occurrence of IRAE during Anti-CTLA-4 monotherapy (Valpione, Pasquali et al. 2018)” (Wölffer, Battke et al. 2022). “Furthermore, Kitagataya *et al.* (Kitagataya, Suda

et al. 2020) reported an association between female sex and autoimmune hepatitis under immunotherapy. However, the underlying pathomechanism is not fully understood yet. Unlike hepatitis, pancreatitis was more common in the male sex, which also fits very well with the literature. Others also reported an association between male sex and pancreatic injury during immunotherapy (Abu-Sbeih, Tang et al. 2019). Additional information is available on type I autoimmune pancreatitis (IgG4-related pancreatitis), which has also been shown to be linked to male sex (Uchida and Okazaki 2018). In addition, male patients had worse responses to glucocorticoid therapy, more relapses of pancreatitis and higher levels of peripheral eosinophil count (Wang, Zhang et al. 2019), the latter also being linked to IRAE (Nakamura, Tanaka et al. 2019). Recent studies showed a significant correlation between western diet and autoimmune pancreatitis in mice, whereas caloric restriction halved the occurrence (Jaster, Gupta et al. 2020). Furthermore, chronic pancreatitis was detected more frequently in men, and alcohol has been shown to be its most important risk factor (Yadav and Lowenfels 2013)" (Wölffer, Battke et al. 2022). Women were found to be more frequently lifetime abstainers, to drink less and to rarely develop alcohol-induced diseases compared to men (Erol and Karpyak 2015). "Therefore male patients might be more likely to develop pancreatic IRAE because of their diet" (Wölffer, Battke et al. 2022).

Laboratory data

"Furthermore, changes in blood cell counts during immunotherapy had been found to be predictive for the risk for IRAE. These laboratory findings included increased total leucocytes and relative neutrophil count, and decreased relative lymphocyte count (Fujisawa, Yoshino et al. 2017), as well as increased absolute and relative eosinophil counts for endocrinological IRAE (Nakamura, Tanaka et al. 2019). Corresponding to these reports, we also found an association between increased leucocytes at start of immunotherapy and the occurrence of IRAE, such as colitis, hepatitis or pancreatitis. We also found an association between increased absolute number of neutrophils at start of immunotherapy and the occurrence of colitis or pancreatitis. Finally, decreased relative number of lymphocytes at the start of immunotherapy was linked to pancreatitis. However, these results were not statistically significant. It has to be considered, that Fujisawa *et al.* analysed blood counts at start of IRAE (Fujisawa, Yoshino et al. 2017),

whereas we considered blood count data already at start of immunotherapy. Given the parallels in our results, we assume that these markers might be indicative for IRAE. We could not confirm the reported correlation between higher total and relative levels of eosinophils and the occurrence of IRAE (Nakamura, Tanaka et al. 2019), probably due to the limited sample size of our cohort. We had only one single patient in our cohort with high levels of eosinophils at start of immunotherapy. This patient indeed developed colitis, pancreatitis and nephritis during immunotherapy.

We found furthermore an association between increased absolute and relative monocytes at the start of immunotherapy and the occurrence of IRAE or colitis. Increased total and relative monocytes were significantly associated with pancreatitis. Increased monocyte count has been linked to decreased overall survival in melanoma patients (Chasseuil, Saint-Jean et al. 2018). This would suggest a correlation of increased monocyte count with both decreased overall survival and occurrence of IRAE, which contradicts findings in recent literature about a supposed link between response and occurrence of IRAE (Eggermont, Kicinski et al. 2020)" (Wölffer, Battke et al. 2022). Chasseuil et al. also found increased leukocyte-lymphocyte-ratio and neutrophil-lymphocyte-ratio to be associated with a decreased overall survival, confirming the results of Fujisawa et al. (Fujisawa, Yoshino et al. 2017, Chasseuil, Saint-Jean et al. 2018).

Pre-existing autoimmune diseases

Patients with pre-existing autoimmune diseases form a non-inconsiderable group of melanoma patients receiving ICI due to their high risk of malignancy (Boland, Pavlick et al. 2020). Thereby, understanding whether these patients can receive ICI without potentially fatal exacerbation of their disease or occurrence of severe IRAE is crucial. "A history of autoimmune disease has been shown to be associated with a higher incidence of IRAE during immunotherapy of melanoma (Kartolo, Sattar et al. 2018). Several studies analyzed risk of exacerbation and occurrence of IRAE depending on the type of therapy. Johnson et al. noted exacerbations of rheumatoid arthritis and inflammatory bowel disease (IBD), as well as increased occurrence of IRAE, during Anti-CTLA-4 monotherapy (Johnson, Sullivan et al. 2016). Menzies et al. showed that melanoma patients with pre-existing autoimmune diseases had a risk of exacerbation when administered Anti-PD-1 therapy, but no increased risk of developing de novo IRAE

compared to general population (Menzies, Johnson et al. 2017). The inhibition of CTLA-4 or PD-1/PD-L1 pathways in the gastrointestinal tract has been shown to intensify the immune response. Moreover, patients with pre-existing IBD in particular have been shown to be predisposed to an exacerbation of IBD / development of colitis under ICI (Abd El Aziz, Facciorusso et al. 2020). Meserve et al. showed that 40% of IBD patients experienced flares during ICI, often requiring corticosteroids (76%) or biologicals (37%). However, these flares were mostly manageable, and only rarely led to therapy discontinuation (35%) (Meserve, Facciorusso et al. 2021). The same, Abdel-Wahab et al. have shown that IRAE occurring in patients with pre-existing autoimmune diseases only rarely led to termination of therapy. Nevertheless, considering that patients with autoimmune diseases developing exacerbations or IRAE have response rates at least as high as in the general population (Abdel-Wahab, Shah et al. 2018), they should not be excluded from therapy" (Wölffer, Battke et al. 2022).

Limited data on specific pre-existing autoimmune diseases is available up to this date. It would be desirable to further differentiate between autoimmune diseases in future studies, as they may have a different impact on the occurrence of IRAE, exacerbation of pre-existing autoimmune diseases, and treatment response.

Histological subtype of melanoma

Response to ICI is not comparable between histological subtypes of melanoma. Metastatic uveal melanoma patients are known to present less response and progression free survival rates than cutaneous melanoma patients (Algazi, Tsai et al. 2016). Non-resectable mucosal melanoma was however found to present comparable response and progression free survival rates compared to cutaneous melanoma (Moya-Plana, Herrera Gómez et al. 2019). Other studies suggested the same result for acral melanoma (Shoushtari, Munhoz et al. 2016).

Rabbie et al. reviewed the genomic profiles of melanoma subtypes, concluding that mainly mucosal, acral and uveal melanoma were associated with higher numbers of CNVs and lower levels of tumor mutational burden (TMB) compared to cutaneous melanoma (Rabbie, Ferguson et al. 2019). High levels of TMB are found in sun-exposed cutaneous melanoma, whereas melanoma subtypes such as mucosal and acral are associated with low levels of TMB (Hayward, Wilmott et al. 2017). Low levels of TMB

combined with high numbers of CNVs have been associated with unfavourable prognosis in patients with metastatic cancers receiving ICI, whereas high levels of TMB and low numbers of CNVs have been associated with better response (Liu, Bai et al. 2019). Hence, mucosal, acral and uveal melanoma have a lower chance of response under ICI, which contradicts studies mentioned above underlining comparable response rates for acral, mucosal and cutaneous melanoma.

Notably, we found that cutaneous melanoma was a protective marker for the occurrence of IRAE, and that other subtypes were associated with higher incidence of IRAE. This contradicts however the results found by other authors mentioned above. If cutaneous melanoma presents low numbers of CNVs and high levels of TMB, we would suppose better response rates and higher incidence of IRAE as suggested by recent studies (Eggermont, Kicinski et al. 2020) in cutaneous melanoma patients receiving ICI rather than in other histological subtypes. This discrepancy probably may be explained by our limited sample size.

Moreover, the recent CheckMate 172 study analyzed extensively efficacy and safety in melanoma subtypes other than cutaneous, concluding that there was no significant difference in the occurrence of grade ≥3 IRAE between acral, cutaneous, mucosal, ocular, and other melanoma subtypes during therapy with Nivolumab, whereas median overall survival was considerably lower in mucosal and ocular melanoma compared to cutaneous and acral melanoma (Nathan, Ascierto et al. 2019). Histological subtypes of melanoma might be predictive markers for response, but rather no reliable markers for IRAE.

cfDNA

As cfDNA is an established biomarker in several autoimmune rheumatic diseases such as systemic lupus erythematodes and rheumatic arthritis (Duvvuri and Lood 2019), the question arises whether it could also serve as a biomarker for IRAE.

In our results, cfDNA was not confirmed as a predictive marker for the occurrence of IRAE. However, we noted a general association between increased levels of cfDNA and patients enduring IRAE as compared to patients without IRAE, albeit this could not be reliably detected at the same time IRAE occurred. We also observed a correlation between elevated levels of cfDNA and progressive disease.

This has been confirmed by other authors, who found that elevated cfDNA levels were

associated with presence of metastases and progressive disease, leading to decreased overall survival independently of the tumor genotype (Váraljai, Elouali et al. 2020). On the other hand, high levels of TMB, as well as decreasing levels of cfDNA and circulating tumor DNA (ctDNA) shortly after start of combined ICI have been associated with response (Forschner, Battke et al. 2019). These results should be considered with caution, as other authors found that cfDNA does neither positively nor negatively correlate with response to treatment within patients having various cancers, including melanoma under ICI (Jensen, Goodman et al. 2019). Jensen et al. concluded that various factors may influence the precision of cfDNA as marker for treatment response.

As recent studies suggested a link between occurrence of IRAE and progression-free survival (Eggermont, Kicinski et al. 2020), we would expect rather low levels of cfDNA in patients exhibiting IRAE, offering an explanation why elevated levels of cfDNA were not confirmed as predictive marker for occurrence of IRAE in our cohort.

As it is well established that hypermutated ctDNA (the part of cfDNA specifically attributed to apoptotic tumor cells) is a marker for treatment response under ICI (Khagi, Goodman et al. 2017), it would be desirable in future studies to further elucidate the link between ctDNA and IRAE occurrence. This could also contribute to an explanation about the rather paradoxical association of cfDNA to both progressive disease and IRAE in our cohort, allowing to differentiate between DNA from apoptotic cells originating from the tumor and apoptotic cells in general. The isolation of ctDNA however is an expensive task and has not yet been rolled out in clinical routine.

HLA

“Although there is a substantial lack of information in the literature about links between HLA and IRAE, several findings point out that there might be an association between [high levels of HLA-A,] specific HLA-A alleles and HLA homozygosity on one hand, and response to immunotherapy, autoimmune diseases, as well as IRAE occurrence on the other” (Wölffer, Battke et al. 2022).

First, several findings point out an association between high levels of HLA-A and response to ICI. It has been found that a higher mRNA expression of intratumoral HLA-A prior to Anti-PD-1 monotherapy was linked to tumor response (Inoue, Park et al. 2016). On the other hand, authors underlined that lower levels of HLA-A were associated with

lower response rates. Huang et al. have shown that RNA-binding protein MEX3B was responsible for downregulation of HLA-A2 on tumor cells in Anti-PD-1-treated melanoma. This induced resistance to PD-1 blockade, as T cells were unable to identify tumor cells (Huang, Malu et al. 2018).

Secondly, several HLA-A alleles have been associated to treatment response as well as occurrence of IRAE. HLA-A*26 has been found to correlate with response during Anti-PD-1 monotherapy in melanoma patients (Ishida, Otsuka et al. 2017). Notably, HLA-A*26:01 was among the alleles patients exhibiting IRAE expressed in our cohort. “Li et al. noted in a case report that a HLA-A*02:01 homozygous patient with metastatic lung squamous cell cancer treated with nivolumab showed durable remission after occurrence of severe immune-related pneumonitis (Li, Ma et al. 2017). Hayashi et al. found the HLA-A downstream regulatory region to be the decisive factor in pathogenesis of autoimmune vitiligo. Through increase of HLA-A expression and HLA-A*02:01 protein, which presents several vitiligo autoimmune antigens, it facilitates recognition and attack of melanocytes by autoreactive T cells (Hayashi, Jin et al. 2016).

We noted an association between HLA-I homozygosity and the occurrence of IRAE, colitis or hepatitis. HLA-A homozygosity was strikingly associated with IRAE, colitis, or hepatitis, proving to be the determining factor of this association. To our best knowledge, this finding has not yet been identified in literature. Other authors concluded that HLA-class I homozygosity might be linked to inferior outcome and found simultaneous heterozygosity on all HLA-I loci (A, B and C) to be associated with higher survival rates compared to patients homozygous on at least one HLA-I locus (Chowell, Krishna et al. 2019). Inferior outcome was explained by elements impairing T-cells’ recognition of tumor antigens on HLA-B. This coincides with our results about HLA-B homozygosity being a protective factor for the occurrence of IRAE, when considering recent results about an association between IRAE occurrence and treatment response (Eggermont, Kicinski et al. 2020). It seems imperative to differentiate between HLA-A, HLA-B, and HLA-C homozygosity as they seem to have opposite effects on treatment response and IRAE occurrence” (Wölffer, Battke et al. 2022).

Finally, concercing HLA-B and HLA-C alleles expressed by patients exhibiting IRAE in our cohort, we note parallels to results found in the literature. A current study analyzes correlations between distinct HLA alleles and specific IRAE. In their preliminary results,

the authors associated significantly HLA-B*18:01 to colitis, HLA-C*04:01 to hepatitis, and HLA-C*05:01 to neurological IRAE. Furthermore, HLA-B*18:01 was associated to pancreatitis (Y. Angela 2020). All these alleles have been associated with the occurrence of IRAE in our results. Angela et al. also established a significant association between HLA-C*18:01 and colitis, which was not confirmed by our results. Interestingly, these alleles were found to be associated with autoimmune diseases. HLA-B*18:01 and HLA-C*05:01 were linked to autoimmune hepatitis (Littera, Chessa et al. 2016), as well as HLA-C*04:01 (Amarapurkar, Patel et al. 2003). This does indeed suggest an association between these alleles and occurrence of IRAE. Further studies are essential for further investigation and understanding.

VARs

"VARs on SMAD3 were significantly linked to the development of pancreatitis: 33 % of patients with SMAD3 variants suffered from pancreatitis while the incidence of pancreatitis in the complete cohort was only 14 %. To our best knowledge, SMAD3 has not yet been linked to IRAE" (Wölffer, Battke et al. 2022).

SMAD3 is however known to be a key molecule in the TGF-β pathway and thereby to induce renal fibrosis (Meng and Lan 2018). Wu et al. have furthermore shown that SMAD3 and Interferon regulatory factor 7 (IRF7) interact to stimulate TGF-β and induce fibrosis in the pathogenesis of systemic sclerosis (Wu, Skaug et al. 2019). High expression levels of SMAD3 have been identified as negative predictive marker for acute myeloid leukemia in patients under chemotherapy, being associated to a shorter overall survival (Zhang, Zhang et al. 2019).

"[S]tudies have shown the inhibitory effect of SMAD3 and the TGF-β pathway on natural killer cells in the tumor microenvironment: disruption of SMAD3 in natural killer cells was associated to enhanced activity of natural killer cells and cytokine production (Wang, Tang et al. 2018). If SMAD3 plays an immunosuppressive role, as shown by Wang et al., an affection of SMAD3 by VARs could lead to a dysfunction potentially explaining an IRAE. We observed a correlation between SMAD3 affected by VARs and partial response at first staging" (Wölffer, Battke et al. 2022) (5 out of 12 patients with SMAD3 affected by VARs had partial response as result at first staging). This confirms findings in the literature about an association between response and occurrence of IRAE (Eggermont,

Kicinski et al. 2020).

CNVs

CNVs include duplications and deletions of DNA sequence, and play an important role in adaptive evolution as well as cancer (Lauer and Gresham 2019). “[H]igher numbers of CNVs in melanoma tissue have been linked to a higher incidence of metastases, recurrence and death (Alomari, Miedema et al. 2020)” (Wölffer, Battke et al. 2022). Seemingly a negative predictive factor in melanoma patients by numbers, the question arises whether specific CNVs may predict occurrence of IRAE during ICI.

Existing literature (Hoefsmit, Rozeman et al. 2019) describes a hypothetical association of specific genetic loci and IRAE. Among our selected genetic loci (see Material and Methods, 2.1), these genetic loci included:

Table 21. Summary of genetic loci potentially associated with different IRAE under ICI found in literature (Hoefsmit, Rozeman et al. 2019).

IRAE generally	PDCD1 (PD-1)
Colitis	LRRK2, IKZF1, SMAD3, JAK2, PRDM1
Hepatitis	HLA-haplotypes I and II, SH2B3
Pneumonitis	AIRE, TERT
Thyroiditis	CTLA4, TSHR
Nephritis	FAN1
Myositis	SLCO1B1

Furthermore, it has been found that “responsiveness to ICI might be linked to specific polymorphisms (Refae, Gal et al. 2020), including rs419598 on IL1RN. The same, a higher incidence of toxicity under ICI was found being associated with alterations of CD274” (Wölffer, Battke et al. 2022), CTLA-4, and UNG.

“CNVs on IL1RN were significantly linked to IRAE in general and associated with all three main IRAE in particular, i.e., colitis, hepatitis and pancreatitis. If the role of CNVs on IL1RN concerning the occurrence of IRAE during ICI is not yet fully understood, IL1RN is known for its role in the development of autoimmune diseases. Yoshizaki et al. showed an increased risk for the development of autoimmune aortitis in IL1RN-deficient mice, which they explained by stronger signalling of IL-1 (Yoshizaki, Itoh et al. 2019). Polymorphisms on IL1RN have been found to be associated with the occurrence of Hashimoto’s thyroiditis and to predict severity (Zaaber, Mestiri et al. 2014).

CNVs on CD274 (PD-L1) were a recurrent marker for IRAE, being significantly linked to hepatitis, encephalitis and myositis. Once again, the role of CNVs on CD274 in view of the occurrence of IRAE during immunotherapy has not yet been fully understood. Polymorphisms on CD274 are however known to be associated with multiple autoimmune diseases, such as type 1 diabetes (Pizarro, García-Díaz et al. 2014), ankylosing spondylitis (Huang, Wong et al. 2011), and Graves' disease as well as autoimmune Addison's disease (Mitchell, Cordell et al. 2009).

Polymorphisms on PRDM1 have been associated with systemic lupus erythematodes (SLE) (Gateva, Sandling et al. 2009). More precisely, rs548234 has been found to be a risk allele on PRDM1 associated with SLE, decreasing the expression of BLIMP1 (B-lymphocyte-induced maturation proteine-1) in dendritic cells, which is involved in immunological tasks such as antigen presentation (Jang, Chen et al. 2017). Polymorphisms on PRDM1 have also been associated with other autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease (Kim 2015). The role of PRDM1 in preventing autoimmunity has been analyzed in mice by Roberts et al. The authors showed that PRDM1 is expressed by medullary thymic epithelial cells, involved in the deletion of self-reactive T cells and the development of regulatory T cells. Deletion of PRDM1 resulted in autoimmune pathology (Roberts, Adams et al. 2017). Our results confirm these findings: deletions of PRDM1 resulted in IRAE. Xia et al. revealed that homozygous deletions of PRDM1 correlated with decreased plasma cell differentiation and upregulation of genes involved in tumor cell proliferation in activated B-cell-like diffuse large B-cell lymphoma (ABC-DLBCL) patients (Xia, Xu-Monette et al. 2017). Xia et al. also found that patients with PRDM1 deletions were showing upregulation of transcription factors such as STAT3. STAT3 has been identified as main factor in increased PD-L1 expression on tumor cells (Frydenlund and Mahalingam 2017), which is targeted by Anti-PD-1 therapy. PRDM1 deletions may thus be regarded as factor supporting Anti-PD-1 therapy, and thereby inducing IRAE. Of note, the association of CNVs on PRDM1 to encephalitis and myositis has not been reported in the literature yet. It would certainly be interesting if this could be tested in larger cohorts.

Polymorphisms of TSHR in enhancer regions, especially rs4411444 and rs4903961, are known to be associated with autoimmune diseases such as Graves' disease and Hashimoto's disease (Fujii, Inoue et al. 2017). Polymorphisms of TSHR have been shown

to affect the development of central tolerance, thus explaining the occurrence of autoimmune phenomena such as Graves' disease (Lee, Li et al. 2015).

Polymorphisms of SLCO1B1 have not yet been found to be associated with autoimmune diseases or IRAE. SLCO1B1 is a drug transporter and member of the solute carrier family, and polymorphisms of SLCO1B1 have been associated with drug metabolism disorders such as atorvastatin-induced adverse events (Du, Wang et al. 2018) or sorafenib-induced adverse events (Bins, Lenting et al. 2016). Polymorphisms of SLCO1B1 have also been associated with the occurrence of methimazole-induced liver injury in patients with Grave's disease (Jin, Li et al. 2019).

FAN1 has not yet been associated with autoimmune diseases or IRAE. FAN1 plays an important role in the removal of DNA interstrand crosslinks (Wang, Persky et al. 2014), and has been identified as a protective factor in the occurrence and progression of Huntington's disease (Goold, Flower et al. 2019).

The occurrence of myositis as well as encephalitis was associated with CNVs on CD274 and PRDM1 in our study, possibly explaining the combined occurrence of these IRAE in clinical practice. Sato et al. also reported the frequent combined occurrence of neurological IRAE such as myasthenia gravis, encephalitis and meningitis with myositis during immunotherapy (Sato, Mano et al. 2019). Reynolds et al. confirmed these findings, showing that neurological IRAE often overlap between one another and are associated with myositis (Reynolds and Guidon 2019)" (Wölffer, Battke et al. 2022).

Limitations

"[T]here are several limitations in our study. First, results of organ specific IRAE and biomarkers with a prevalence outside the interval of 33% to 66% are purely exploratory. This is due to the limited sample size of our work and the number of biomarkers to be detected, especially in the VARs and CNVs sections. Our results may well support the biomarkers previously described in the literature but will have to be confirmed in a larger sample size. Second, we did not differentiate between CNVs duplications and deletions for our analysis of less prevalent IRAE, as our sample size was too small to perform further differentiation. Moreover, CNV detection from NGS data on single exon level is less accurate compared to methods like qPCR or MLPA. Thus, confirmation of identified CNV markers by an alternative method in a larger cohort seems advisable to avoid

misinterpretations due to technical limitations. We furthermore did not distinguish between individual VARs and simplified that most of them were similarly located on each gene, and had thereby the same effect.

However, there is also great strength in our study. We have carefully evaluated the patient records and can therefore assume with a high degree of confidence that the clinical data are accurate. We used a comprehensive tumor panel, which increases the chance of identifying biomarkers for IRAE. By specifically differentiating distinct IRAE, HLA-I homozygosity, CNVs deletions and duplications, we have been able to obtain important results" (Wölffer, Battke et al. 2022).

5 Conclusion

Immunotherapy plays an increasing role in the clinical routine of dermatological oncology and has been the subject of numerous studies up to this date. IRAE however remain relatively unexplored, even though they occur regularly during ICI in melanoma patients and are potentially lethal. To be able to estimate the risk of IRAE and to choose the best possible therapy in the sense of personalised medicine, biomarkers associated with the occurrence of IRAE specific to the patient and the tumor are needed.

“Our study can help to define biomarkers associated with the occurrence of IRAE in general and of several specific IRAE. We found a significant association between several genetic markers and the occurrence of IRAE, which were merely hypothesized in the literature” (Wölffer, Battke et al. 2022). We further specified other authors’ findings, as in our results about HLA-A homozygosity. “[T]o estimate the risk of developing IRAE, on the one hand, we have markers such as [sex], blood count parameters, and pre-existing autoimmune diseases, which are easy to obtain; on the other hand, NGS-based results are a more complex and expensive option. However, NGS provides us with additional information and should be considered in risk assessment, especially when multiple therapies are available” (Wölffer, Battke et al. 2022).

Several conclusions can be drawn from our results for clinical practice. “We propose that in future, basic screening for biomarkers associated with the occurrence of IRAE should be carried out before initiation of ICI, in particular in patients for whom a therapeutic alternative, for example with BRAF-/MEK inhibitors is possible, even though ICI is a mainstay of therapy for BRAF-mutated patients as well (Trojaniello, Vitale et al. 2021)” (Wölffer, Battke et al. 2022). Generally, blood samples should be collected after each administration of ICI in order to keep in view the mentioned biomarkers in blood counts. “Where NGS data is already available, a focused query should be made regarding the presence of potentially relevant polymorphisms in genes associated with the development of IRAE. If future studies support our findings by validation or potentially the discovery of additional genetic biomarkers, NGS may become the screening method of choice” (Wölffer, Battke et al. 2022).

The use of a risk assessment of IRAE is however challenged by the fact that ICI represent the best possible treatment in advanced melanoma in terms of response and toxicity and

remain without alternative according to current state of research (Weber, D'Angelo et al. 2015). The currently ongoing SECOMBIT study (NCT02631447) analyzes combinations of targeted therapy and ICI in BRAF-mutated patients; according to the first report on efficacy and toxicity it is recommendable to administer targeted therapy until PD, and to switch thereafter to combined ICI (P.A. Ascierto 2020). Since ICI is a pillar of therapy for BRAF-mutated patients as well, it is questionable to what extent screening for biomarkers in the absence of an effective alternative would have a real benefit.

We showed that IRAE occurred mainly primarily during the first two months of therapy, and that combined therapy was associated with higher IRAE incidence. Patients exhibiting numerous biomarkers for IRAE occurrence could be treated with monotherapy preferably unless combined therapy is necessary for a faster response. If response to monotherapy proves unsatisfactory, introduction of a combined therapy in a second step would be possible.

If unspecific symptoms occur during ICI, IRAE should always be considered for differential diagnosis, especially when occurring in patients presenting biomarkers associated with IRAE. Knowledge about these markers on a multidisciplinary level and cooperation between medical specialists is necessary and could be decisive for patients' outcome.

"Since ICI are used more and more frequently across many different cancer types, further studies on biomarkers associated with the development of IRAE should be performed" (Wölffer, Battke et al. 2022) and put an emphasis on the patients' genetic background including a more differentiated approach and much larger sample size.

6 Summary

The aim of this thesis “was to check for possible associations between clinical parameters or NGS-based genetic alterations and the occurrence of immune-related adverse events (IRAE) in melanoma patients with immune checkpoint inhibitors (ICI). We analyzed 95 melanoma patients” (Wölffer, Battke et al. 2022), focusing on polymorphisms of 16 selected genes hypothesized to be associated with IRAE in the literature. “Our objective was also to check for possible associations between patient specific parameters such as sex, [type of immunotherapy,] blood count [at start of ICI], pre-existing autoimmune diseases and the occurrence of IRAE” (Wölffer, Battke et al. 2022). The analysis of IRAE included organ specific IRAE such as colitis, pneumonitis, hepatitis, encephalitis, myocarditis, myositis, pancreatitis, exanthema, hypophysitis, nephritis, and thyroiditis. The analysis of NGS-based data included cfDNA, HLA-Class I, VARs and CNVs. “We examined each selected gene for the effect of VARs[, t]he same procedure was done for CNVs […]. We discerned deletions and duplications for IRAE in general, as well as colitis, hepatitis, and pancreatitis. […] All patients' data were entered in and statistically analysed with the statistical program for social sciences SPSS statistics version 25.0. (IBM Corp., Armonk, NY, USA), and Microsoft Excel Version 2019. The descriptive data was analyzed by absolute and relative frequency. We retained each variable that was in relative terms more frequently observed in patients with IRAE [and the three most prevalent IRAE] than in patients of the entire cohort. […] We obtained the potentially significant biomarkers for occurrence of IRAE and proceeded by testing these markers for significance. The exact version of the Chi-Squared-Test was used for statistical significance. The level of significance was set at 0.05 in all analyses. Results of organ specific IRAEs and biomarkers with a prevalence outside the interval of 33% to 66% are purely exploratory […] due to the limited sample size of our work” (Wölffer, Battke et al. 2022). “We found a significant link between female sex and hepatitis, and a correlation between male sex and pancreatitis. Combined immunotherapy was associated with a higher incidence of IRAE and significantly linked to colitis. We found an association between increased leucocytes at start of immunotherapy and occurrence of colitis, hepatitis or pancreatitis. Increased absolute neutrophils at start of immunotherapy were associated with colitis or pancreatitis, whereas decreased relative lymphocytes at start of

immunotherapy were associated with pancreatitis. Increased total and relative monocytes at start of immunotherapy were associated with IRAE or colitis. Increased absolute and relative monocytes at start of immunotherapy were significantly linked to the occurrence of pancreatitis. We found furthermore a significant link between pre-existing autoimmune diseases and pancreatitis. HLA homozygosity was linked to IRAE in general or colitis. HLA homozygosity was significantly associated with hepatitis. HLA-A homozygosity was strikingly linked to the occurrence of IRAE in general, colitis or hepatitis. VARs on SMAD3 were significantly linked to pancreatitis. CNVs on IKZF1, FAN1 and IL1RN were linked to IRAE, colitis, hepatitis or pancreatitis. CNVs on IL1RN and deletions on PRDM1 were significantly linked to IRAE, whereas duplications on CD274 and CNVs on SLCO1B1 were significantly linked to hepatitis. Finally, CNVs on PRDM1 and CD274 were significantly linked to encephalitis, and CNVs on PRDM1, CD274, TSHR and FAN1 were significantly linked to myositis" (Wölffer, Battke et al. 2022). These results coincide with results of other authors found in the literature. Most genes affected by CNVs were found to be associated with autoimmune diseases. Concerning SMAD3 and PRDM1, we were able to hypothesize the pathophysiology of an IRAE caused by polymorphisms on these genes. "Myositis and encephalitis, both, were associated with alterations of PRDM1 and CD274, which might explain their joined appearance in clinical practice" (Wölffer, Battke et al. 2022).

"Our study can help to define biomarkers associated with the occurrence of IRAE" (Wölffer, Battke et al. 2022). "[B]asic screening for biomarkers associated with the occurrence of IRAE should be carried out before initiation of ICI, in particular in patients for whom a therapeutic alternative […] is possible, even though ICI is a mainstay of therapy for BRAF-mutated patients as well. Where NGS data is already available, a focused query should be made regarding the presence of potentially relevant polymorphisms in genes associated with the development of IRAE. If future studies support our findings by validation or potentially the discovery of additional genetic biomarkers, NGS may become the screening method of choice. Since ICI are used more and more frequently across many different cancer types, further studies on biomarkers associated with the development of IRAE should be performed" (Wölffer, Battke et al. 2022).

7 German Summary

Ziel dieser Arbeit war es, mögliche Zusammenhänge zwischen klinischen Parametern oder NGS-basierten genetischen Veränderungen und dem Auftreten von immunbezogenen unerwünschten Ereignissen (IRAE) bei Melanompatienten mit Immun-Checkpoint-Inhibitoren (ICI) zu untersuchen. Wir analysierten 95 Melanompatienten und konzentrierten uns auf Polymorphismen von 16 ausgewählten Genen, von denen in der Literatur angenommen wurde, dass sie mit IRAE assoziiert sein könnten. Unser Ziel war es auch, Zusammenhänge zwischen patientenspezifischen Parametern wie Geschlecht, Art der Immuntherapie, Blutbild zu Beginn der ICI, vorbestehenden Autoimmunerkrankungen und dem Auftreten von IRAE zu prüfen.

Die Analyse der IRAE umfasste organspezifische IRAE wie Kolitis, Pneumonitis, Hepatitis, Enzephalitis, Myokarditis, Myositis, Pankreatitis, Exantheme, Hypophysitis, Nephritis und Thyreoiditis. Die Analyse der NGS-basierten Daten umfasste cfDNA, HLA-Klasse I, VARs und CNVs. Wir untersuchten jedes ausgewählte Gen auf die Wirkung von VARs und CNVs. Wir analysierten Deletionen und Duplikationen für IRAE im Allgemeinen sowie für Colitis, Hepatitis und Pankreatitis. Die Daten aller Patienten wurden mit dem Statistikprogramm SPSS Statistics Version 25.0 und Microsoft Excel Version 2019 ausgewertet. Die deskriptiven Daten wurden nach absoluter und relativer Häufigkeit ausgewertet. Wir behielten jede Variable bei, die relativ gesehen bei Patienten mit IRAE und den drei häufigsten IRAE häufiger beobachtet wurde als bei Patienten der gesamten Kohorte. Wir ermittelten die potenziell signifikanten Biomarker für das Auftreten von IRAE und testeten diese Marker mit der exakten Version des Chi-Quadrat-Tests auf ihre Signifikanz. Das Signifikanzniveau wurde in allen Analysen auf 0.05 festgelegt. Die Ergebnisse der organspezifischen IRAE und Biomarker mit einer Prävalenz außerhalb des Intervalls von 33 % bis 66 % sind aufgrund des begrenzten Stichprobenumfangs unserer Arbeit rein explorativ.

Wir fanden einen signifikanten Zusammenhang zwischen weiblichem Geschlecht und Hepatitis und eine Korrelation zwischen männlichem Geschlecht und Pankreatitis. Eine kombinierte Immuntherapie war mit einer höheren Inzidenz von IRAE und signifikant mit Kolitis assoziiert. Wir fanden einen Zusammenhang zwischen erhöhten Leukozyten zu Beginn der Immuntherapie und dem Auftreten von Kolitis, Hepatitis oder Pankreatitis.

Erhöhte absolute Neutrophile zu Beginn der Immuntherapie waren mit Kolitis oder Pankreatitis assoziiert, während verringerte relative Lymphozyten zu Beginn der Immuntherapie mit Pankreatitis assoziiert waren. Erhöhte absolute und relative Monozyten zu Beginn der Immuntherapie waren mit IRAE oder Kolitis assoziiert. Erhöhte absolute und relative Monozyten zu Beginn der Immuntherapie waren signifikant mit dem Auftreten von Pankreatitis verbunden. Darüber hinaus bestand ein signifikanter Zusammenhang zwischen vorbestehenden Autoimmunerkrankungen und Pankreatitis. HLA-Homozygotie war mit IRAE im Allgemeinen oder Kolitis assoziiert. HLA-Homozygotie wurde signifikant mit Hepatitis in Verbindung gebracht. HLA-A-Homozygotie stand in auffälligem Zusammenhang mit dem Auftreten von IRAE im Allgemeinen, Kolitis oder Hepatitis. VARs auf SMAD3 waren signifikant mit Pankreatitis assoziiert. CNVs auf IKZF1, FAN1 und IL1RN wurden mit IRAE, Colitis, Hepatitis oder Pankreatitis in Verbindung gebracht. CNVs auf IL1RN und Deletionen auf PRDM1 waren signifikant mit IRAE assoziiert, während Duplikationen auf CD274 und CNVs auf SLCO1B1 signifikant mit Hepatitis assoziiert waren. Schließlich waren CNVs auf PRDM1 und CD274 signifikant mit Enzephalitis assoziiert, und CNVs auf PRDM1, CD274, TSHR und FAN1 wurden signifikant mit Myositis in Verbindung gebracht.

Diese Ergebnisse stimmen mit den Ergebnissen anderer Autoren überein. Bei den meisten von CNVs betroffenen Genen wurde ein Zusammenhang mit Autoimmunerkrankungen festgestellt. Bei SMAD3 und PRDM1 konnten wir eine Hypothese über die Pathophysiologie der durch Polymorphismen verursachten IRAE aufstellen. Sowohl Myositis als auch Enzephalitis wurden mit Veränderungen von PRDM1 und CD274 assoziiert, was ihr gemeinsames Auftreten in der klinischen Praxis erklären könnte.

Unsere Studie kann dazu beitragen, Biomarker zu definieren, die mit dem Auftreten von IRAE in Verbindung stehen. Ein grundlegendes Screening auf Biomarker sollte vor Beginn der ICI durchgeführt werden, insbesondere bei Patienten, für die eine therapeutische Alternative in Frage kommt, auch wenn die ICI auch bei BRAF-mutierten Patienten ein Therapieansatz ist. Wo bereits NGS-Daten vorliegen, sollte gezielt nach dem Vorhandensein von mit IRAE assoziierten Polymorphismen in Genen gefragt werden. Wenn künftige Studien unsere Ergebnisse validieren oder zusätzliche genetische Biomarker entdecken, könnte NGS die Screening-Methode der Wahl werden.

8 References

Abd El Aziz, M. A., A. Facciorusso, T. Nayfeh, S. Saadi, M. Elnaggar, C. Cotsoglou and R. Sacco (2020). "Immune Checkpoint Inhibitors for Unresectable Hepatocellular Carcinoma." Vaccines (Basel) **8**(4).
Abdel-Wahab, N., M. Shah, M. A. Lopez-Olivo and M. E. Suarez-Almazor (2018). "Use of Immune Checkpoint Inhibitors in the Treatment of Patients With Cancer and Preexisting Autoimmune Disease: A Systematic Review." Ann Intern Med **168**(2): 121-130.
Abu-Sbeih, H., T. Tang, Y. Lu, S. Thirumurthi, M. Altan, A. A. Jazaeri, R. Dadu, E. Coronel and Y. Wang (2019). "Clinical characteristics and outcomes of immune checkpoint inhibitor-induced pancreatic injury." J Immunother Cancer **7**(1): 31.
Algazi, A. P., K. K. Tsai, A. N. Shoushtari, R. R. Munhoz, Z. Eroglu, J. M. Piulats, P. A. Ott, D. B. Johnson, J. Hwang, A. I. Daud, J. A. Sosman, R. D. Carvajal, B. Chmielowski, M. A. Postow, J. S. Weber and R. J. Sullivan (2016). "Clinical outcomes in metastatic uveal melanoma treated with PD-1 and PD-L1 antibodies." Cancer **122**(21): 3344-3353.
Alomari, A. K., J. R. Miedema, M. D. Carter, P. W. Harms, L. Lowe, A. B. Durham, D. R. Fullen, R. M. Patel, A. C. Hristov, M. P. Chan, M. Wang and A. A. Andea (2020). "DNA copy number changes correlate with clinical behavior in melanocytic neoplasms: proposal of an algorithmic approach." Mod Pathol **33**(7): 1307-1317.
Amarapurkar, D. N., N. D. Patel, A. D. Amarapurkar and S. R. Kankonkar (2003). "HLA genotyping in type-I autoimmune hepatitis in Western India." J Assoc Physicians India **51**: 967-969.
Amaria, R. N., S. M. Reddy, H. A. Tawbi, M. A. Davies, M. I. Ross, I. C. Glitza, J. N. Cormier, C. Lewis, W. J. Hwu, E. Hanna, A. Diab, M. K. Wong, R. Royal, N. Gross, R. Weber, S. Y. Lai, R. Ehlers, J. Blando, D. R. Milton, S. Woodman, R. Kageyama, D. K. Wells, P. Hwu, S. P. Patel, A. Lucci, A. Hessel, J. E. Lee, J. Gershenwald, L. Simpson, E. M. Burton, L. Posada, L. Haydu, L. Wang, S. Zhang, A. J. Lazar, C. W. Hudgens, V. Gopalakrishnan, A. Reuben, M. C. Andrews, C. N. Spencer, V. Prieto, P. Sharma, J. Allison, M. T. Tetzlaff and J. A. Wargo (2018). "Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma." Nat Med **24**(11): 1649-1654.
Berman, D., S. M. Parker, J. Siegel, S. D. Chasalow, J. Weber, S. Galbraith, S. R. Targan and H. L. Wang (2010). "Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma." Cancer Immun **10**: 11.
Bins, S., A. Lenting, S. El Bouazzaoui, L. van Doorn, E. Oomen-de Hoop, F. A. Eskens, R. H. van Schaik and R. H. Mathijssen (2016). "Polymorphisms in SLCO1B1 and UGT1A1 are associated with sorafenib-induced toxicity." Pharmacogenomics **17**(14): 1483-1490.
Blank C.U., R. I. L., Pennington T., Versluis J.M., Saw R.P., Rozeman E.A., Kapiteijn E., Van Der Veldt A.A.M., Suijkerbuijk K., Hospers G., et al. (2020). "First safety and efficacy results of PRADO: A phase II study of personalized response-driven surgery and adjuvant therapy after neoadjuvant ipilimumab (IPI) and nivolumab (NIVO) in resectable stage III melanoma." J. Clin. Oncol.
Boland, P., A. C. Pavlick, J. Weber and S. Sandigursky (2020). "Immunotherapy to treat malignancy in patients with pre-existing autoimmunity." J Immunother Cancer **8**(1).

Chasseuil, E., M. Saint-Jean, H. Chasseuil, L. Peuvrel, G. Quéreux, J. M. Nguyen, A. Gaultier, E. Varey, A. Khammari and B. Dréno (2018). "Blood Predictive Biomarkers for Nivolumab in Advanced Melanoma." Acta Derm Venereol **98**(4): 406-410.
Choi, J. and S. Y. Lee (2020). "Clinical Characteristics and Treatment of Immune-Related Adverse Events of Immune Checkpoint Inhibitors." Immune Netw **20**(1): e9.
Chowell, D., C. Krishna, F. Pierini, V. Makarov, N. A. Rizvi, F. Kuo, L. G. T. Morris, N. Riaz, T. L. Lenz and T. A. Chan (2019). "Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy." Nat Med **25**(11): 1715-1720.
Du, Y., S. Wang, Z. Chen, S. Sun, Z. Zhao and X. Li (2018). "Association of SLCO1B1 Polymorphisms and Atorvastatin Safety and Efficacy: A Meta-analysis." Curr Pharm Des **24**(34): 4044-4050.
Duvvuri, B. and C. Lood (2019). "Cell-Free DNA as a Biomarker in Autoimmune Rheumatic Diseases." Front Immunol **10**: 502.
Eggermont, A. M. M., M. Kicinski, C. U. Blank, M. Mandala, G. V. Long, V. Atkinson, S. Dalle, A. Haydon, A. Khattak, M. S. Carlino, S. Sandhu, J. Larkin, S. Puig, P. A. Ascierto, P. Rutkowski, D. Schadendorf, R. Koornstra, L. Hernandez-Aya, A. M. Di Giacomo, A. J. M. van den Eertwegh, J. J. Grob, R. Gutzmer, R. Jamal, P. C. Lorigan, C. Krepler, N. Ibrahim, S. Marreaud, A. van Akkooi, C. Robert and S. Suciu (2020). "Association Between Immune-Related Adverse Events and Recurrence-Free Survival Among Patients With Stage III Melanoma Randomized to Receive Pembrolizumab or Placebo: A Secondary Analysis of a Randomized Clinical Trial." JAMA Oncol **6**(4): 519-527.
El Dika, I., D. N. Khalil and G. K. Abou-Alfa (2019). "Immune checkpoint inhibitors for hepatocellular carcinoma." Cancer **125**(19): 3312-3319.
Erol, A. and V. M. Karpyak (2015). "Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations." Drug Alcohol Depend **156**: 1-13.
Forschner, A., F. Battke, D. Hadaschik, M. Schulze, S. Weißgraeber, C. T. Han, M. Kopp, M. Frick, B. Klumpp, N. Tietze, T. Amaral, P. Martus, T. Sinnberg, T. Eigentler, U. Keim, C. Garbe, D. Döcker and S. Biskup (2019). "Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma - results of a prospective biomarker study." J Immunother Cancer **7**(1): 180.
Frydenlund, N. and M. Mahalingam (2017). "PD-L1 and immune escape: insights from melanoma and other lineage-unrelated malignancies." Hum Pathol **66**: 13-33.
Fujii, A., N. Inoue, M. Watanabe, C. Kawakami, Y. Hidaka, Y. Hayashizaki and Y. Iwatani (2017). "TSHR Gene Polymorphisms in the Enhancer Regions Are Most Strongly Associated with the Development of Graves' Disease, Especially Intractable Disease, and of Hashimoto's Disease." Thyroid **27**(1): 111-119.
Fujisawa, Y., K. Yoshino, A. Otsuka, T. Funakoshi, T. Fujimura, Y. Yamamoto, H. Hata, M. Gosho, R. Tanaka, K. Yamaguchi, Y. Nonomura, I. Hirai, S. Furudate, H. Okuhira, K. Imafuku, M. Aoki and S. Matsushita (2017). "Fluctuations in routine blood count might signal severe immune-related adverse events in melanoma patients treated with nivolumab." J Dermatol Sci **88**(2): 225-231.
Garutti, M., S. Buriolla, E. Bertoli, M. G. Vitale, E. Rossi, G. Schinzari, A. M. Minisini and F. Puglisi (2020). ""To Anticipate": Neoadjuvant Therapy in Melanoma with a Focus on Predictive Biomarkers." Cancers (Basel) **12**(7).
Gateva, V., J. K. Sandling, G. Hom, K. E. Taylor, S. A. Chung, X. Sun, W. Ortmann, R. Kosoy, R. C. Ferreira, G. Nordmark, I. Gunnarsson, E. Svenungsson, L. Padyukov, G.

Sturfelt, A. Jönsen, A. A. Bengtsson, S. Rantapää-Dahlqvist, E. C. Baechler, E. E. Brown, G. S. Alarcón, J. C. Edberg, R. Ramsey-Goldman, G. McGwin, Jr., J. D. Reveille, L. M. Vilá, R. P. Kimberly, S. Manzi, M. A. Petri, A. Lee, P. K. Gregersen, M. F. Seldin, L. Rönnblom, L. A. Criswell, A. C. Syvänen, T. W. Behrens and R. R. Graham (2009). "A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus." Nat Genet **41**(11): 1228-1233.
George, J., D. Bajaj, K. Sankaramangalam, J. W. Yoo, N. S. Joshi, S. Gettinger, C. Price and J. J. Farrell (2019). "Incidence of pancreatitis with the use of immune checkpoint inhibitors (ICI) in advanced cancers: A systematic review and meta-analysis." Pancreatology **19**(4): 587-594.
Gianchecchi, E. and A. Fierabracci (2018). "Inhibitory Receptors and Pathways of Lymphocytes: The Role of PD-1 in Treg Development and Their Involvement in Autoimmunity Onset and Cancer Progression." Front Immunol **9**: 2374.
Goold, R., M. Flower, D. H. Moss, C. Medway, A. Wood-Kaczmar, R. Andre, P. Farshim, G. P. Bates, P. Holmans, L. Jones and S. J. Tabrizi (2019). "FAN1 modifies Huntington's disease progression by stabilizing the expanded HTT CAG repeat." Hum Mol Genet **28**(4): 650-661.
Hayashi, M., Y. Jin, D. Yorgov, S. A. Santorico, J. Hagman, T. M. Ferrara, K. L. Jones, G. Cavalli, C. A. Dinarello and R. A. Spritz (2016). "Autoimmune vitiligo is associated with gain-of-function by a transcriptional regulator that elevates expression of HLA-A*02:01 in vivo." Proc Natl Acad Sci U S A **113**(5): 1357-1362.
Hayward, N. K., J. S. Wilmott, N. Waddell, P. A. Johansson, M. A. Field, K. Nones, A. M. Patch, H. Kakavand, L. B. Alexandrov, H. Burke, V. Jakrot, S. Kazakoff, O. Holmes, C. Leonard, R. Sabarinathan, L. Mularoni, S. Wood, Q. Xu, N. Waddell, V. Tembe, G. M. Pupo, R. De Paoli-Iseppi, R. E. Vilain, P. Shang, L. M. S. Lau, R. A. Dagg, S. J. Schramm, A. Pritchard, K. Dutton-Regester, F. Newell, A. Fitzgerald, C. A. Shang, S. M. Grimmond, H. A. Pickett, J. Y. Yang, J. R. Stretch, A. Behren, R. F. Kefford, P. Hersey, G. V. Long, J. Cebon, M. Shackleton, A. J. Spillane, R. P. M. Saw, N. López-Bigas, J. V. Pearson, J. F. Thompson, R. A. Scolyer and G. J. Mann (2017). "Whole-genome landscapes of major melanoma subtypes." Nature **545**(7653): 175-180.
Herzberg, B., M. J. Campo and J. F. Gainor (2017). "Immune Checkpoint Inhibitors in Non-Small Cell Lung Cancer." Oncologist **22**(1): 81-88.
Hoefsmit, E. P., E. A. Rozeman, J. Haanen and C. U. Blank (2019). "Susceptible loci associated with autoimmune disease as potential biomarkers for checkpoint inhibitor-induced immune-related adverse events." ESMO Open **4**(4): e000472.
Huang, C. H., R. H. Wong, J. C. Wei, M. D. Tsay, W. C. Chen, H. Y. Chen, W. T. Shih, S. P. Chiou, Y. C. Tu and H. S. Lee (2011). "Effects of genetic polymorphisms of programmed cell death 1 and its ligands on the development of ankylosing spondylitis." Rheumatology (Oxford) **50**(10): 1809-1813.
Huang, L., S. Malu, J. A. McKenzie, M. C. Andrews, A. H. Talukder, T. Tieu, T. Karpinets, C. Haymaker, M. A. Forget, L. J. Williams, Z. Wang, R. M. Mbofung, Z. Q. Wang, R. E. Davis, R. S. Lo, J. A. Wargo, M. A. Davies, C. Bernatchez, T. Heffernan, R. N. Amaria, A. Korkut, W. Peng, J. Roszik, G. Lizée, S. E. Woodman and P. Hwu (2018). "The RNA-binding Protein MEX3B Mediates Resistance to Cancer Immunotherapy by Downregulating HLA-A Expression." Clin Cancer Res **24**(14): 3366-3376.
IBM Corp., A., N.Y., USA (2017). "SPSS Statistics version 25.0."
Inoue, H., J. H. Park, K. Kiyotani, M. Zewde, A. Miyashita, M. Jinnin, Y. Kiniwa, R.

Okuyama, R. Tanaka, Y. Fujisawa, H. Kato, A. Morita, J. Asai, N. Katoh, K. Yokota, M. Akiyama, H. Ihn, S. Fukushima and Y. Nakamura (2016). "Intratumoral expression levels of PD-L1, GZMA, and HLA-A along with oligoclonal T cell expansion associate with response to nivolumab in metastatic melanoma." Oncoimmunology **5**(9): e1204507.
Ishida, Y., A. Otsuka, H. Tanaka, M. P. Levesque, R. Dummer and K. Kabashima (2017). "HLA-A*26 Is Correlated With Response to Nivolumab in Japanese Melanoma Patients." J Invest Dermatol **137**(11): 2443-2444.
Jang, S. H., H. Chen, P. K. Gregersen, B. Diamond and S. J. Kim (2017). "Kruppel-like factor4 regulates PRDM1 expression through binding to an autoimmune risk allele." JCI Insight **2**(1): e89569.
Jaster, R., Y. Gupta, S. Rohde, L. Ehlers, H. Nizze, A. Vorobyev, R. J. Ludwig and S. M. Ibrahim (2020). "Impact of diet and genes on murine autoimmune pancreatitis." J Cell Mol Med **24**(15): 8862-8870.
Jensen, T. J., A. M. Goodman, S. Kato, C. K. Ellison, G. A. Daniels, L. Kim, P. Nakashe, E. McCarthy, A. R. Mazloom, G. McLennan, D. S. Grosu, M. Ehrich and R. Kurzrock (2019). "Genome-Wide Sequencing of Cell-Free DNA Identifies Copy-Number Alterations That Can Be Used for Monitoring Response to Immunotherapy in Cancer Patients." Mol Cancer Ther **18**(2): 448-458.
Jin, P., B. Xiang, G. Huang and Z. Zhou (2015). "The association of cytotoxic T-lymphocyte antigen-4 + 49A/G and CT60 polymorphisms with type 1 diabetes and latent autoimmune diabetes in Chinese adults." J Endocrinol Invest **38**(2): 149-154.
Jin, S., X. Li, Y. Fan, X. Fan, Y. Dai, H. Lin, W. Cai, J. Yang and X. Xiang (2019). "Association between genetic polymorphisms of SLCO1B1 and susceptibility to methimazole-induced liver injury." Basic Clin Pharmacol Toxicol **125**(6): 508-517.
Johnson, D. B., J. M. Balko, M. L. Compton, S. Chalkias, J. Gorham, Y. Xu, M. Hicks, I. Puzanov, M. R. Alexander, T. L. Bloomer, J. R. Becker, D. A. Slosky, E. J. Phillips, M. A. Pilkinton, L. Craig-Owens, N. Kola, G. Plautz, D. S. Reshef, J. S. Deutsch, R. P. Deering, B. A. Olenchock, A. H. Lichtman, D. M. Roden, C. E. Seidman, I. J. Koralnik, J. G. Seidman, R. D. Hoffman, J. M. Taube, L. A. Diaz, Jr., R. A. Anders, J. A. Sosman and J. J. Moslehi (2016). "Fulminant Myocarditis with Combination Immune Checkpoint Blockade." N Engl J Med **375**(18): 1749-1755.
Johnson, D. B., R. J. Sullivan, P. A. Ott, M. S. Carlino, N. I. Khushalani, F. Ye, A. Guminski, I. Puzanov, D. P. Lawrence, E. I. Buchbinder, T. Mudigonda, K. Spencer, C. Bender, J. Lee, H. L. Kaufman, A. M. Menzies, J. C. Hassel, J. M. Mehnert, J. A. Sosman, G. V. Long and J. I. Clark (2016). "Ipilimumab Therapy in Patients With Advanced Melanoma and Preexisting Autoimmune Disorders." JAMA Oncol **2**(2): 234-240.
Kartolo, A., J. Sattar, V. Sahai, T. Baetz and J. M. Lakoff (2018). "Predictors of immunotherapy-induced immune-related adverse events." Curr Oncol **25**(5): e403-e410.
Khagi, Y., A. M. Goodman, G. A. Daniels, S. P. Patel, A. G. Sacco, J. M. Randall, L. A. Bazhenova and R. Kurzrock (2017). "Hypermutated Circulating Tumor DNA: Correlation with Response to Checkpoint Inhibitor-Based Immunotherapy." Clin Cancer Res **23**(19): 5729-5736.
Kim, S. J. (2015). "Immunological function of Blimp-1 in dendritic cells and relevance to autoimmune diseases." Immunol Res **63**(1-3): 113-120.
Kitagataya, T., G. Suda, K. Nagashima, T. Katsurada, K. Yamamoto, M. Kimura, O. Maehara, R. Yamada, T. Shigesawa, K. Suzuki, A. Nakamura, M. Ohara, M. Umemura,

N. Kawagishi, M. Nakai, T. Sho, M. Natsuizaka, K. Morikawa, K. Ogawa, S. Ohnishi, Y. Komatsu, H. Hata, S. Takeuchi, T. Abe, J. Sakakibara-Konishi, T. Teshima, A. Homma and N. Sakamoto (2020). "Prevalence, clinical course, and predictive factors of immune checkpoint inhibitor monotherapy-associated hepatitis in Japan." J Gastroenterol Hepatol.

Klocke, K., S. Sakaguchi, R. Holmdahl and K. Wing (2016). "Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood." Proc Natl Acad Sci U S A **113**(17): E2383-2392.

Larkin, J., V. Chiarion-Sileni, R. Gonzalez, J. J. Grob, C. L. Cowey, C. D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P. F. Ferrucci, A. Hill, J. Wagstaff, M. S. Carlino, J. B. Haanen, M. Maio, I. Marquez-Rodas, G. A. McArthur, P. A. Ascierto, G. V. Long, M. K. Callahan, M. A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L. M. Rollin, C. Horak, F. S. Hodi and J. D. Wolchok (2015). "Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma." N Engl J Med **373**(1): 23-34.

Larkin, J., B. Chmielowski, C. D. Lao, F. S. Hodi, W. Sharfman, J. Weber, K. P. M. Suijkerbuijk, S. Azevedo, H. Li, D. Reshef, A. Avila and D. A. Reardon (2017). "Neurologic Serious Adverse Events Associated with Nivolumab Plus Ipilimumab or Nivolumab Alone in Advanced Melanoma, Including a Case Series of Encephalitis." Oncologist **22**(6): 709-718.

Lauer, S. and D. Gresham (2019). "An evolving view of copy number variants." Curr Genet **65**(6): 1287-1295.

Lebbé, C., N. Meyer, L. Mortier, I. Marquez-Rodas, C. Robert, P. Rutkowski, A. M. Menzies, T. Eigentler, P. A. Ascierto, M. Smylie, D. Schadendorf, M. Ajaz, I. M. Svane, R. Gonzalez, L. Rollin, J. Lord-Bessen, A. Saci, E. Grigoryeva and J. Pigozzo (2019). "Evaluation of Two Dosing Regimens for Nivolumab in Combination With Ipilimumab in Patients With Advanced Melanoma: Results From the Phase IIIb/IV CheckMate 511 Trial." J Clin Oncol **37**(11): 867-875.

Lee, H. J., C. W. Li, S. S. Hammerstad, M. Stefan and Y. Tomer (2015). "Immunogenetics of autoimmune thyroid diseases: A comprehensive review." J Autoimmun **64**: 82-90.

Leonardi, G. C., S. Candido, L. Falzone, D. A. Spandidos and M. Libra (2020). "Cutaneous melanoma and the immunotherapy revolution (Review)." Int J Oncol **57**(3): 609-618.

Li, H., W. Ma, K. Y. Yoneda, E. H. Moore, Y. Zhang, L. L. Pu, G. M. Frampton, M. Molmen, P. J. Stephens and T. Li (2017). "Severe nivolumab-induced pneumonitis preceding durable clinical remission in a patient with refractory, metastatic lung squamous cell cancer: a case report." J Hematol Oncol **10**(1): 64.

Littera, R., L. Chessa, S. Onali, F. Figorilli, S. Lai, L. Secci, G. La Nasa, G. Caocci, M. Arras, M. Melis, S. Cappellini, C. Balestrieri, G. Serra, M. Conti, T. Zolfino, M. Casale, S. Casu, M. C. Pasetto, L. Barca, C. Salustro, L. Matta, R. Scioscia, F. Zamboni, G. Faa, S. Orrù and C. Carcassi (2016). "Exploring the Role of Killer Cell Immunoglobulin-Like Receptors and Their HLA Class I Ligands in Autoimmune Hepatitis." PLoS One **11**(1): e0146086.

Liu, L., X. Bai, J. Wang, X. R. Tang, D. H. Wu, S. S. Du, X. J. Du, Y. W. Zhang, H. B. Zhu, Y. Fang, Z. Q. Guo, Q. Zeng, X. J. Guo, Z. Liu and Z. Y. Dong (2019). "Combination of TMB and CNA Stratifies Prognostic and Predictive Responses to Immunotherapy Across Metastatic Cancer." Clin Cancer Res **25**(24): 7413-7423.

Martins, F., L. Sofiya, G. P. Sykiotis, F. Lamine, M. Maillard, M. Fraga, K. Shabafrouz, C. Ribi, A. Cairoli, Y. Guex-Crosier, T. Kuntzer, O. Michielin, S. Peters, G. Coukos, F. Spertini, J. A. Thompson and M. Obeid (2019). "Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance." Nat Rev Clin Oncol **16**(9): 563-580.
Meng, X. M. and H. Y. Lan (2018). "[Transforming growth factor-β and renal fibrosis]." Sheng Li Xue Bao **70**(6): 612-622.
Menzies, A. M., D. B. Johnson, S. Ramanujam, V. G. Atkinson, A. N. M. Wong, J. J. Park, J. L. McQuade, A. N. Shoushtari, K. K. Tsai, Z. Eroglu, O. Klein, J. C. Hassel, J. A. Sosman, A. Guminski, R. J. Sullivan, A. Ribas, M. S. Carlino, M. A. Davies, S. K. Sandhu and G. V. Long (2017). "Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab." Ann Oncol **28**(2): 368-376.
Meserve, J., A. Facciorusso, A. K. Holmer, V. Annese, W. J. Sandborn and S. Singh (2021). "Systematic review with meta-analysis: safety and tolerability of immune checkpoint inhibitors in patients with pre-existing inflammatory bowel diseases." Aliment Pharmacol Ther **53**(3): 374-382.
Mitchell, A. L., H. J. Cordell, R. Soemedi, K. Owen, B. Skinningsrud, A. B. Wolff, M. Ericksen, D. Undlien, E. Husebye and S. H. Pearce (2009). "Programmed death ligand 1 (PD-L1) gene variants contribute to autoimmune Addison's disease and Graves' disease susceptibility." J Clin Endocrinol Metab **94**(12): 5139-5145.
Motzer, R. J., B. I. Rini, D. F. McDermott, O. Arén Frontera, H. J. Hammers, M. A. Carducci, P. Salman, B. Escudier, B. Beuselinck, A. Amin, C. Porta, S. George, V. Neiman, S. Bracarda, S. S. Tykodi, P. Barthélémy, R. Leibowitz-Amit, E. R. Plimack, S. F. Oosting, B. Redman, B. Melichar, T. Powles, P. Nathan, S. Oudard, D. Pook, T. K. Choueiri, F. Donskov, M. O. Grimm, H. Gurney, D. Y. C. Heng, C. K. Kollmannsberger, M. R. Harrison, Y. Tomita, I. Duran, V. Grünwald, M. B. McHenry, S. Mekan and N. M. Tannir (2019). "Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial." Lancet Oncol **20**(10): 1370-1385.
Moya-Plana, A., R. G. Herrera Gómez, C. Rossoni, L. Dercle, S. Ammari, I. Girault, S. Roy, J. Y. Scoazec, S. Vagner, F. Janot, A. M. M. Eggermont and C. Robert (2019). "Evaluation of the efficacy of immunotherapy for non-resectable mucosal melanoma." Cancer Immunol Immunother **68**(7): 1171-1178.
Nakamura, Y., R. Tanaka, H. Maruyama, Y. Ishitsuka, N. Okiyama, R. Watanabe, M. Fujimoto and Y. Fujisawa (2019). "Correlation between blood cell count and outcome of melanoma patients treated with anti-PD-1 antibodies." Jpn J Clin Oncol **49**(5): 431-437.
Nathan, P., P. A. Ascierto, J. Haanen, E. Espinosa, L. Demidov, C. Garbe, M. Guida, P. Lorigan, V. Chiarion-Sileni, H. Gogas, M. Maio, M. T. Fierro, C. Hoeller, P. Terheyden, R. Gutzmer, T. K. Guren, D. Bafaloukos, P. Rutkowski, R. Plummer, A. Waterston, M. Kaatz, M. Mandala, I. Marquez-Rodas, E. Muñoz-Couselo, R. Dummer, E. Grigoryeva, T. C. Young and D. Schadendorf (2019). "Safety and efficacy of nivolumab in patients with rare melanoma subtypes who progressed on or after ipilimumab treatment: a single-arm, open-label, phase II study (CheckMate 172)." Eur J Cancer **119**: 168-178.
National Institutes of Health, N. C. I., Division of Cancer Treatment & Diagnosis, Cancer Therapy Evaluation Program; Bethesda, MD, USA. (2017). "Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Quick Reference.".
P.A. Ascierto, M. M., P.F. Ferrucci, P. Rutkowski, M. Guidoboni, A.M. Arance

Fernandez, V. Ferraresi, E. Maiello, M. Guida, M. Del Vecchio, M.T. Fierro, P. Queirolo, C. Lebbé, H. Helgadottir, I. Melero, G. Palmieri, D. Giannarelli, A.M. Grimaldi, R. Dummer, V. Chiarion Sileni (2020). "First report of efficacy and safety from the phase II study SECOMBIT (SEquential COMBo Immuno and Targeted therapy study)." Annals of Oncology (2020) 31 (suppl_4): S1142-S1215. 10.1016/annonc/annonc325.

Passat, T., Y. Touchefeu, N. Gervois, A. Jarry, C. Bossard and J. Bennouna (2018). "[Physiopathological mechanisms of immune-related adverse events induced by anti-CTLA-4, anti-PD-1 and anti-PD-L1 antibodies in cancer treatment]." Bull Cancer **105**(11): 1033-1041.

Pizarro, C., D. F. García-Díaz, E. Codner, F. Salas-Pérez, E. Carrasco and F. Pérez-Bravo (2014). "PD-L1 gene polymorphisms and low serum level of PD-L1 protein are associated to type 1 diabetes in Chile." Diabetes Metab Res Rev **30**(8): 761-766.

Rabbie, R., P. Ferguson, C. Molina-Aguilar, D. J. Adams and C. D. Robles-Espinoza (2019). "Melanoma subtypes: genomic profiles, prognostic molecular markers and therapeutic possibilities." J Pathol **247**(5): 539-551.

Ramos-Casals, M., J. R. Brahmer, M. K. Callahan, A. Flores-Chávez, N. Keegan, M. A. Khamashta, O. Lambotte, X. Mariette, A. Prat and M. E. Suárez-Almazor (2020). "Immune-related adverse events of checkpoint inhibitors." Nat Rev Dis Primers **6**(1): 38.

Refae, S., J. Gal, N. Ebran, J. Otto, D. Borchiellini, F. Peyrade, E. Chamorey, P. Brest, G. Milano and E. Saada-Bouzid (2020). "Germinal Immunogenetics predict treatment outcome for PD-1/PD-L1 checkpoint inhibitors." Invest New Drugs **38**(1): 160-171.

Reynolds, K. L. and A. C. Guidon (2019). "Diagnosis and Management of Immune Checkpoint Inhibitor-Associated Neurologic Toxicity: Illustrative Case and Review of the Literature." Oncologist **24**(4): 435-443.

Roberts, N. A., B. D. Adams, N. I. McCarthy, R. M. Tooze, S. M. Parnell, G. Anderson, S. M. Kaech and V. Horsley (2017). "Prdm1 Regulates Thymic Epithelial Function To Prevent Autoimmunity." J Immunol **199**(4): 1250-1260.

Rozeman, E. A., A. M. Menzies, A. C. J. van Akkooi, C. Adhikari, C. Bierman, B. A. van de Wiel, R. A. Scolyer, O. Krijgsman, K. Sikorska, H. Eriksson, A. Broeks, J. V. van Thienen, A. D. Guminski, A. T. Acosta, S. Ter Meulen, A. M. Koenen, L. J. W. Bosch, K. Shannon, L. M. Pronk, M. Gonzalez, S. Ch'ng, L. G. Grijpink-Ongering, J. Stretch, S. Heijmink, H. van Tinteren, J. Haanen, O. E. Nieweg, W. M. C. Klop, C. L. Zuur, R. P. M. Saw, W. J. van Houdt, D. S. Peeper, A. J. Spillane, J. Hansson, T. N. Schumacher, G. V. Long and C. U. Blank (2019). "Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial." Lancet Oncol **20**(7): 948-960.

Salem, J. E., A. Manouchehri, M. Moey, B. Lebrun-Vignes, L. Bastarache, A. Pariente, A. Gobert, J. P. Spano, J. M. Balko, M. P. Bonaca, D. M. Roden, D. B. Johnson and J. J. Moslehi (2018). "Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study." Lancet Oncol **19**(12): 1579-1589.

Sato, K., T. Mano, A. Iwata and T. Toda (2019). "Neurological and related adverse events in immune checkpoint inhibitors: a pharmacovigilance study from the Japanese Adverse Drug Event Report database." J Neurooncol **145**(1): 1-9.

Schwinge, D. and C. Schramm (2019). "Sex-related factors in autoimmune liver

diseases." Semin Immunopathol **41**(2): 165-175.
Shoushtari, A. N., R. R. Munhoz, D. Kuk, P. A. Ott, D. B. Johnson, K. K. Tsai, S. Rapisuwon, Z. Eroglu, R. J. Sullivan, J. J. Luke, T. C. Gangadhar, A. K. Salama, V. Clark, C. Burias, I. Puzanov, M. B. Atkins, A. P. Algazi, A. Ribas, J. D. Wolchok and M. A. Postow (2016). "The efficacy of anti-PD-1 agents in acral and mucosal melanoma." Cancer **122**(21): 3354-3362.
Siegel, R. L., K. D. Miller and A. Jemal (2018). "Cancer statistics, 2018." CA Cancer J Clin **68**(1): 7-30.
Tarhini, A. A., H. Zahoor, Y. Lin, U. Malhotra, C. Sander, L. H. Butterfield and J. M. Kirkwood (2015). "Baseline circulating IL-17 predicts toxicity while TGF-β1 and IL-10 are prognostic of relapse in ipilimumab neoadjuvant therapy of melanoma." J Immunother Cancer **3**: 39.
Trojaniello, C., M. G. Vitale and P. A. Ascierto (2021). "Triplet combination of BRAF, MEK and PD-1/PD-L1 blockade in melanoma: the more the better?" Curr Opin Oncol **33**(2): 133-138.
Uchida, K. and K. Okazaki (2018). "Clinical and pathophysiological aspects of type 1 autoimmune pancreatitis." J Gastroenterol **53**(4): 475-483.
Valpione, S., S. Pasquali, L. G. Campana, L. Piccin, S. Mocellin, J. Pigozzo and V. Chiarion-Sileni (2018). "Sex and interleukin-6 are prognostic factors for autoimmune toxicity following treatment with anti-CTLA4 blockade." J Transl Med **16**(1): 94.
Váraljai, R., S. Elouali, S. S. Lueong, K. Wistuba-Hamprecht, T. Seremet, J. T. Siveke, J. C. Becker, A. Sucker, A. Paschen, P. A. Horn, B. Neyns, B. Weide, D. Schadendorf and A. Roesch (2020). "The predictive and prognostic significance of cell-free DNA concentration in melanoma." J Eur Acad Dermatol Venereol.
Wang, D. Y., J. E. Salem, J. V. Cohen, S. Chandra, C. Menzer, F. Ye, S. Zhao, S. Das, K. E. Beckermann, L. Ha, W. K. Rathmell, K. K. Ancell, J. M. Balko, C. Bowman, E. J. Davis, D. D. Chism, L. Horn, G. V. Long, M. S. Carlino, B. Lebrun-Vignes, Z. Eroglu, J. C. Hassel, A. M. Menzies, J. A. Sosman, R. J. Sullivan, J. J. Moslehi and D. B. Johnson (2018). "Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis." JAMA Oncol **4**(12): 1721-1728.
Wang, L., P. Zhang, X. Zhang, W. Lin, H. Tang, J. Li, M. Wang, X. Liu, Y. Fei, H. Chen, L. Peng, L. Zhang, Y. Lai, X. Zeng, X. Li, H. Xue, Y. Zhao, F. Zhang and W. Zhang (2019). "Sex disparities in clinical characteristics and prognosis of immunoglobulin G4-related disease: a prospective study of 403 patients." Rheumatology (Oxford) **58**(5): 820-830.
Wang, Q. M., P. M. Tang, G. Y. Lian, C. Li, J. Li, X. R. Huang, K. F. To and H. Y. Lan (2018). "Enhanced Cancer Immunotherapy with Smad3-Silenced NK-92 Cells." Cancer Immunol Res **6**(8): 965-977.
Wang, R., N. S. Persky, B. Yoo, O. Ouerfelli, A. Smogorzewska, S. J. Elledge and N. P. Pavletich (2014). "DNA repair. Mechanism of DNA interstrand cross-link processing by repair nuclease FAN1." Science **346**(6213): 1127-1130.
Weber, J., M. Mandala, M. Del Vecchio, H. J. Gogas, A. M. Arance, C. L. Cowey, S. Dalle, M. Schenker, V. Chiarion-Sileni, I. Marquez-Rodas, J. J. Grob, M. O. Butler, M. R. Middleton, M. Maio, V. Atkinson, P. Queirolo, R. Gonzalez, R. R. Kudchadkar, M. Smylie, N. Meyer, L. Mortier, M. B. Atkins, G. V. Long, S. Bhatia, C. Lebbé, P. Rutkowski, K. Yokota, N. Yamazaki, T. M. Kim, V. de Pril, J. Sabater, A. Qureshi, J. Larkin and P. A. Ascierto (2017). "Adjuvant Nivolumab versus Ipilimumab in Resected Stage III or IV Melanoma." N Engl J Med **377**(19): 1824-1835.

Weber, J. S., S. P. D'Angelo, D. Minor, F. S. Hodi, R. Gutzmer, B. Neyns, C. Hoeller, N. I. Khushalani, W. H. Miller, Jr., C. D. Lao, G. P. Linette, L. Thomas, P. Lorigan, K. F. Grossmann, J. C. Hassel, M. Maio, M. Sznol, P. A. Ascierto, P. Mohr, B. Chmielowski, A. Bryce, I. M. Svane, J. J. Grob, A. M. Krackhardt, C. Horak, A. Lambert, A. S. Yang and J. Larkin (2015). "Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial." Lancet Oncol **16**(4): 375-384.
Wilson, M. A., J. Zhong, B. E. Rosenbaum, K. Utter, U. Moran, F. Darvishian, D. Polsky, R. S. Berman, R. L. Shapiro, A. C. Pavlick and I. Osman (2019). "Impact of initial stage on metastatic melanoma survival." Melanoma Res **29**(3): 281-288.
Wölffer, M., F. Battke, M. Schulze, M. Feldhahn, L. Flatz, P. Martus and A. Forschner (2022). "Biomarkers Associated with Immune-Related Adverse Events under Checkpoint Inhibitors in Metastatic Melanoma." Cancers **14**(2): 302.
Wu, M., B. Skaug, X. Bi, T. Mills, G. Salazar, X. Zhou, J. Reveille, S. K. Agarwal, M. R. Blackburn, M. D. Mayes and S. Assassi (2019). "Interferon regulatory factor 7 (IRF7) represents a link between inflammation and fibrosis in the pathogenesis of systemic sclerosis." Ann Rheum Dis **78**(11): 1583-1591.
Xia, Y., Z. Y. Xu-Monette, A. Tzankov, X. Li, G. C. Manyam, V. Murty, G. Bhagat, S. Zhang, L. Pasqualucci, C. Visco, K. Dybkaer, A. Chiu, A. Orazi, Y. Zu, K. L. Richards, E. D. Hsi, W. W. Choi, J. H. van Krieken, J. Huh, M. Ponzoni, A. J. Ferreri, M. B. Møller, B. M. Parsons, J. N. Winter, M. A. Piris, J. Westin, N. Fowler, R. N. Miranda, C. Y. Ok, Y. Li, J. Li, L. J. Medeiros and K. H. Young (2017). "Loss of PRDM1/BLIMP-1 function contributes to poor prognosis of activated B-cell-like diffuse large B-cell lymphoma." Leukemia **31**(3): 625-636.
Y. Angela, M. V., M. Hallensleben, G. Lehner, L. Wulfken, I. Satzger, R. Gutzmer (2020). "Korrelation von HLA-Status und Nebenwirkungen unter medikamentöser Tumortherapie bei onkologischen Erkrankungen." ADO **2020**.
Yadav, D. and A. B. Lowenfels (2013). "The epidemiology of pancreatitis and pancreatic cancer." Gastroenterology **144**(6): 1252-1261.
Yoshizaki, T., S. Itoh, S. Yamaguchi, T. Numata, A. Nambu, N. Kimura, H. Suto, K. Okumura, K. Sudo, A. Yamaguchi and S. Nakae (2019). "IL-25 exacerbates autoimmune aortitis in IL-1 receptor antagonist-deficient mice." Sci Rep **9**(1): 17067.
Zaaber, I., S. Mestiri, H. Marmouch, S. Mahjoub, N. Abid, M. Hassine, B. Bel Hadj Jrad-Tensaout and K. Said (2014). "Polymorphisms in TSHR and IL1RN genes and the risk and prognosis of Hashimoto's thyroiditis." Autoimmunity **47**(2): 113-118.
Zhang, J., L. Zhang, H. Cui, X. Zhang, G. Zhang, X. Yang, S. Yang, Z. Zhang, J. Wang, K. Hu, J. Shi, X. Ke and L. Fu (2019). "High expression levels of SMAD3 and SMAD7 at diagnosis predict poor prognosis in acute myeloid leukemia patients undergoing chemotherapy." Cancer Gene Ther **26**(5-6): 119-127.

9 Declaration of Contributions

The work was carried out in the Universitäts-Hautklinik Tübingen under the supervision of PD Dr. Andrea Forschner. The study was designed by PD Dr. Andrea Forschner (habilitated supervisor) and Marcus Wölffer (medical student submitting this thesis). The statistical analysis was performed by myself after consultation of and checked by Prof. Peter Martus at the Institute for Clinical Epidemiology and Applied Biostatistics (IKEaB) Tübingen. I assure that I have written the manuscript independently and have not used any sources other than those indicated by me.

Tübingen, the 27th January 2022

Marcus Wölffer

10 Reference to the publication

Parts of this dissertation have already been published in the following publication: Wölffer et al., Biomarkers Associated with Immune-Related Adverse Events under Checkpoint Inhibitors in Metastatic Melanoma, *Cancers*, **2022**, *14*(2), 302.

11 Acknowledgements

At this point, I would like to express my great gratitude to all the people who have supported me in the preparation of this dissertation.
My special thanks go to my doctoral supervisor PD Dr. Andrea Forschner for her excellent supervision during the dissertation and her continuous support.
I would also like to thank all the people who made this dissertation possible through their years of support and outstanding educational achievements: Mme. Dorothee Abed. The teachers of the Lycée français de Berlin, in particular retired director M. Bernhard Frank, Mme. Anne Videau, M. Sébastien Illiano, M. Olivier Michel. The teachers of the Lycée Henri IV. Paris, in particular M. Laurent Ferec and M. Marc Midan.
I would also like to express my great gratitude to my friend Stephan Senfle for his numerous thoughts and support during the writing of the thesis. I would like to express my gratitude to my friend Sophie Jasper, who accompanied me on my way with advice and kind words. Finally, I thank my mother and brother for their encouragement during my studies and my work on this dissertation.