REAL-WORLD PERSPECTIVES ON CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITOR THERAPIES

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REAL-WORLD PERSPECTIVES ON CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITOR THERAPIES

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University of Oulu Graduate School; University of Oulu, Faculty of Medicine; Medical Research Center Oulu; Oulu University Hospital

Abstract

Progress in the field of immuno-oncology has changed the treatment landscape of cancer. Typically, with PD-(L)1-inhibitors responses are seen in 20–40% of the patients yet most fail to respond. From a clinical perspective, constantly expanding indications together with the high expenses of immune checkpoint inhibitors (ICIs), are challenging, while the optimal length of PD-(L)1-inhibitors remains undetermined. Therefore, predictive biomarkers are needed to guide patient selection.

Cancer patients suffer from a variety of symptoms, either derived from the malignancy itself, or as side effects of cancer treatments. Worsening of symptoms indicates cancer progression or severe side-effects and is linked to poorer cancer survival. Web-based applications coupled with an urgency algorithm have been developed to monitor cancer patients. Electronic patient-reported outcomes (ePROs) have been shown to improve quality of life (QoL) and increase the number of patients receiving active cancer treatments at disease progression. Furthermore, the use of ePROs in cancer patient monitoring has shown impressive improvements in overall survival compared to standard follow-up.

The toxicity spectrum of ICIs is wide and inadequately characterized. The side effects of ICIs resemble autoimmune disease. These side effects, unlike those of traditional cancer therapies can occur from months to years after therapy initiation or even after therapy discontinuation. Hence, long-term, feasible follow-up of patients is warranted.

This present study aims to evaluate the optimal treatment duration of PD-(L)1-inhibitors, to seek predictive factors for therapy selection, and to investigate the feasibility and clinical relevance of ePRO symptom follow-up on cancer patients receiving immune checkpoint inhibitors in real-life.

Keywords: CRP, immune checkpoint therapy, metastatic cancer, patient reported outcomes, PD-1 therapy, PD-L1, predictive, prognostic, real-world data, survival, symptoms, therapy discontinuation
Iivanainen, Sanna, Kliinisä näkökulmia immuunivasteen tarkastuspisteen estäjää saavien syöpäpotilaiden hoitoon.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala
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Tiivistelmä
Immunologisten syövänhoitojen kehitys on muuttanut erityisesti levinnyttä syöpää sairastavien potilaiden hoitoa. Immuunivasteen tarkastuspisteen estäjä saavistuista syöpäpotilaista vain noin 20–40 prosenttia hyöty hoidosta. On epäselvää, mikä on optimaa linen hoidon kesto ja ketkä hoidosta oletettavia hyötyisivät. Kun käyttöindikaatiot kyseisen ryhmän lääkkeille lisääntyvät kiihtyvällä tahdilla, myös taloudellisesta näkökulmasta näkökulmasta katsottuna hoidon tehoa ennakoivien tekijöiden tutkimus on keskeistä.

Syöpähoitoja saavat potilaat käsivät monenlaisista oireista, joista useiden on osoitettu ennustetavan huonon selviytymistä syövää. On olemassa useita hälytystoiminnollaa varustettuja verkkosovelluksia syövää sairastavien potilaiden voinnin seurannan edistämiseksi, joita on osoitettu parantavan oireiden hallintaa, lisäävän potilaiden ja kliinikoiden välistä kommunikaatiota sekä edistävän potilaiden hyvinvointia ja jopa pidentävän elossaoloa toisaalta.


Tämän väitöskirjatutkimuksen tavoitteena on arvioida immuunivasteen tarkastuspisteen estäjien optimaalista hoidon kestoaa, etsiä hoidon tehoa ennakoivia tekijöitä ja arvioida sähköisen potilastuhon oireseurannan käytettävyyttä ja kliinisä merkitystä immuunivasteen tarkastuspisteen estäjää saavien syöpäpotilaiden seurannassa.

Asiasanat: CRP, elossaolo, ennustetekijät, hoidon tauotus, immuunivasteen tarkastuspisteen estäjät, metastaattinen syöpä, oireet, PD-L1, PRO, tosielämä tieto
‘Success is walking from failure to failure with no loss of enthusiasm’

-Winston Churchill
Preface

For me, the thesis process has been a fascinating journey to the world of clinical research. In the fall 2016, at the first meeting with my thesis supervisor Docent Jussi Koivunen, M.D, Ph.D., he introduced three different research possibilities to me. One of them was a collaboration suggestion from Kaiku Health Oy. They were interested in doing a clinical research on ePRO symptom follow-up of cancer patients receiving immune checkpoint inhibitors based on an ePRO tool developed by them. The idea sounded interesting to me, and I started the work by lending two books handling the basics of Immunology from the library of Medical Faculty since the concept of cancer immunotherapy was albeit familiar to me.

On February 2017, our research protocol for the KISS trial was evaluated at PPSHP ethical board meeting. We started testing the ePRO tool with three cancer patients in the spring, and during our pilot phase in June 2017, at the annual meeting of American Society of Clinical Oncology, Ethan M. Basch, M.D., MSc., presented data from a randomized study showing that web-based symptom monitoring during outpatient chemotherapy prolonged overall survival approximately five months compared to standard of care follow-up. On the 28th of June, we recruited our first study patient for the trial.

This is a clinical study in the field of medical oncology, focusing on cancer patients treated with immune checkpoint inhibitors (ICIs). The pioneer work of Jim Allison, Ph.D., who found that T cells are controlled by a safety mechanism, a negative checkpoint protein called CTLA-4, led to the development of the first immune checkpoint inhibitor, ipilimumab, which blocks the immune checkpoint protein CTLA-4. A second negative immune checkpoint protein, PD-1, was identified by Tasuko Honjo, Ph.D., and his colleagues at Kyoto University which constituted the basis for the development of anti-PD-(L)1 therapies. Since then, the evolution of PD-(L)1 antibodies has truly changed the treatment of many advanced solid cancers.

There is an unmet need for investigator-initiated clinical research aiming to evaluate the efficacy and tolerability of medical treatments outside the clinical trials. Our results of non-inferior survival even after a short anti-PD-(L)1 treatment in responding patients is an important finding, eventhough, due to the small number of patients and retrospective nature of the study, a hypothesis generating. Furthermore, the finding of the negative prognostic value of elevated pretreatment CRP levels befor ICIs is of importance when only ~30% of patients respond to anti-PD-(L)1 therapies and predictive biomarkers for therapy benefit are lacking. For
the latter, I want to thank the collaborators Jarkko Ahvonen, M.D., Tampere University Hospital, Aija Knuuttila, M.D., Ph.D., Helsinki University Hospital, and Satu Tiainen, M.D., Kuopio University Hospital for your invaluable work in the study.

A prospective clinical trial is always an effort, and especially a multicenter one. I sincerely want to thank Tuomo Alanko, M.D., Ph.D., from Docrates Cancer Center and Pia Vihinen, M.D., Ph.D., from Turku University Hospital for your collaboration in the KISS trial. I also want to thank Katriina Peltola, M.D., Ph.D., for the fluent co-work in our first ePRO study. In addition, Teemu Konkala and Jussi Ekström, Ph.D., from Kaiku Health Oy have been a crucial element in the data processing for the ePRO studies. And without the innovative attitude of Lauri Sippola and Henri Virtanen from Kaiku Health Oy, the co-operation would have been less fruitful. The hypothesis of certain symptom correlations with treatment benefit from our retrospective ePRO study was confirmed in the KISS trial, enabling us to further conclude that individual prediction models for treatment benefit of ICIs could be generated based on even some early PROs.

This study was financially supported by The Kaarina and Erkki Piippola Foundation, The Emil Aaltonen Foundation, the University of Oulu Scholarship Foundation and the Northern Cancer Centre. All these sources of financial support are gratefully acknowledged.

I would like to thank my doctoral training follow-up members, Professor Ulla Puistola, M.D., Ph.D., Eeva Rahko, M.D., Ph.D., and Kaisa Lehtiö, M.D., Ph.D. I also want to thank my colleagues for your warmhearted company at work.

I want to express my gratitude to my thesis supervisor Docent Jussi Koivunen for giving me the opportunity and support to reach my dreams. You are an admirable clinical researcher with the ability to inspire others.

I am a mother of two adorable little boys, Antero and Oskari, who sometimes have wondered during my thesis project, do grownups ever have holidays. I want to thank my husband Olli, my parents and my parents-in-law. Without your support this thesis would never have reached a successful conclusion.

With the words of Socrates, I want to thank my pre-examiners Docent Sirkku Jyrkkiö, M.D., Ph.D., and Maarit Bärlund, M.D., Ph.D. for your respectable expertise:” Education is the kindling of a flame, not the filling of a vessel.”

Oulu, August 2019

Sanna Iivanainen
**Abbreviations**

Ab  
Antibody

ADP-ribose  
Adenosine diphosphatase ribose

Ag  
Antigen

AI  
Artificial intelligence

ALK  
Anaplastic lymphoma kinase

CA-125  
cancer antigen 125

CAR T cell therapy  
Chimeric antigen receptor T cell therapy

CDK  
cyclin-dependent kinase

cfDNA  
circulating free DNA

CD  
cluster of designation

CEA  
carcinoembryonic antigen

CIN  
copy number/chromosomal instability

CR  
complete response

CRP  
C-reactive protein

CT  
computed tomography

CTCAE  
Common Terminology Criteria for Adverse Events

ctDNA  
circulating tumor DNA

CTLA4  
Cytotoxic T-lymphocyte associated protein 4

dMMR  
deficient mismatch repair

DNA  
deoxyribonucleic acid

ECOG  
Eastern Cooperative Oncology Group

e.g.  
for example

EGFR  
Epidermal growth factor receptor

EMA  
European Medical Agency

EORTC  
European Organisation for Research and Treatment of Cancer

(e)PRO  
(electronic) patient reported outcome

FDA  
Food and Drug Administration

GCP  
Good Clinical Practice

GI  
Gastrointestinal

GnRh  
Gonadotropin hormone-releasing hormone

GU  
Genito-urinary

HLA  
Human Leukocyte Antigen

H&N  
Head and neck

H(N)SCC  
Head and (neck) squamous cell carcinoma
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
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<tr>
<td>HRQoL</td>
<td>Health-related quality of life</td>
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<td>ICI</td>
<td>Immune checkpoint inhibitor</td>
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<td>iCPD</td>
<td>immune confirmed progressive disease</td>
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<td>IFNγ/β</td>
<td>Interferon gamma/beta</td>
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<td>Ig</td>
<td>Immunoglobulin</td>
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<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<td>Interleukin-2</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
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<tr>
<td>IO</td>
<td>Immuno-oncology</td>
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<td>irAE</td>
<td>immune-related adverse event</td>
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<tr>
<td>iRECIST</td>
<td>immune-response evaluation criteria for solid tumors</td>
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<tr>
<td>iUPD</td>
<td>immune unconfirmed progressive disease</td>
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<tr>
<td>JAK/STAT</td>
<td>Janus kinase/signal transducers and activators of transcription</td>
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<tr>
<td>KRAS</td>
<td>Kirsten RA Sarcoma Virus</td>
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<tr>
<td>LDH</td>
<td>lactate-dehydrogenase</td>
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<td>LKB1</td>
<td>Liver kinase 1</td>
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<td>LOH</td>
<td>Loss of heterozygocity</td>
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<tr>
<td>MDSC</td>
<td>Myeloid-derived suppressor cells</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
<td>dMMR</td>
<td>mismatch-repair deficiency</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MSI</td>
<td>microsatellite instability</td>
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<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
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<td>NK cells</td>
<td>natural killer cells</td>
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<td>NLR</td>
<td>neutrophil-to-lymphocyte ratio</td>
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<td>NSCLC</td>
<td>non-small cell lung cancer</td>
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<td>ORR</td>
<td>objective response rate</td>
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<td>OS</td>
<td>overall survival</td>
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<td>PFS</td>
<td>progression-free survival</td>
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<td>PD</td>
<td>progressive disease</td>
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<td>PD-L1</td>
<td>Programmed death ligand-1</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PR</td>
<td>partial response</td>
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<tr>
<td>QLQ-C30</td>
<td>Quality of Life Questionnaire-Core 30</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>-----------------------------------</td>
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<tr>
<td>RCC</td>
<td>renal cell carcinoma</td>
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<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>ROC curve</td>
<td>receiver operating characteristic curve</td>
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<tr>
<td>SD</td>
<td>stable disease</td>
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<tr>
<td>STK11</td>
<td>Serine/threonine kinase 11</td>
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<tr>
<td>TAMs</td>
<td>Tumor-associated macrophages</td>
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<tr>
<td>TCR</td>
<td>T cell receptor</td>
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<tr>
<td>TIL</td>
<td>Tumor-infiltrating lymphocytes</td>
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<td>TMB</td>
<td>tumor mutation burden</td>
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<td>TNF-(\alpha)</td>
<td>tumor necrosis factor alpha</td>
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<tr>
<td>TNM</td>
<td>TNM Classification of Malignant tumors</td>
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<tr>
<td>TTP</td>
<td>Time to disease progression</td>
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<tr>
<td>UCC</td>
<td>urothelial cell carcinoma</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:


Contributions in the publications: I participated in planning and designing the study, treated the study patients, collected and analysed the data and drafted the manuscript in publications I, II, III and IV.
# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td></td>
</tr>
<tr>
<td>Tiivistelmä</td>
<td></td>
</tr>
<tr>
<td>Preface</td>
<td>9</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>11</td>
</tr>
<tr>
<td>Original publications</td>
<td>15</td>
</tr>
<tr>
<td>Contents</td>
<td>17</td>
</tr>
<tr>
<td>1 Introduction</td>
<td>19</td>
</tr>
<tr>
<td>2 Literature review</td>
<td>23</td>
</tr>
<tr>
<td>2.1 Cancer</td>
<td>23</td>
</tr>
<tr>
<td>2.1.1 Cancer evolution</td>
<td>23</td>
</tr>
<tr>
<td>2.1.2 Hallmarks of cancer</td>
<td>24</td>
</tr>
<tr>
<td>2.1.3 Cancer epidemiology</td>
<td>26</td>
</tr>
<tr>
<td>2.2 Treatment of cancer</td>
<td>28</td>
</tr>
<tr>
<td>2.2.1 Surgery</td>
<td>28</td>
</tr>
<tr>
<td>2.2.2 Radiotherapy</td>
<td>29</td>
</tr>
<tr>
<td>2.2.3 Medical treatment of cancer</td>
<td>30</td>
</tr>
<tr>
<td>2.2.4 Treatment response assessment in solid tumors</td>
<td>31</td>
</tr>
<tr>
<td>2.3 Cancer immunotherapy</td>
<td>32</td>
</tr>
<tr>
<td>2.3.1 Mechanism of action</td>
<td>33</td>
</tr>
<tr>
<td>2.3.2 Immune checkpoint inhibitors</td>
<td>35</td>
</tr>
<tr>
<td>2.3.3 Predictive factors for treatment benefit from immune checkpoint inhibitors</td>
<td>42</td>
</tr>
<tr>
<td>2.3.4 Side effects of immune checkpoint inhibitors</td>
<td>46</td>
</tr>
<tr>
<td>2.4 Symptom burden of cancer</td>
<td>48</td>
</tr>
<tr>
<td>2.4.1 Patient-reported outcomes</td>
<td>48</td>
</tr>
<tr>
<td>2.4.2 (e)PROs in the follow-up of cancer patients</td>
<td>49</td>
</tr>
<tr>
<td>3 Aims of the present study</td>
<td>51</td>
</tr>
<tr>
<td>4 Materials and methods</td>
<td>53</td>
</tr>
<tr>
<td>4.1 Patients receiving PD-1 inhibitors (I)</td>
<td>53</td>
</tr>
<tr>
<td>4.2 Patient cohorts of the study evaluating predictive value of C-reactive protein for PD-1 inhibitors (II)</td>
<td>54</td>
</tr>
<tr>
<td>4.2.1 Discovery cohort</td>
<td>54</td>
</tr>
<tr>
<td>4.2.2 Validation cohort</td>
<td>54</td>
</tr>
</tbody>
</table>
4.3 Patient cohort followed with web-based application for symptoms and Quality of Life (QoL) receiving PD-1 inhibitors (III).......................................................................................................... 54

4.4 Study population in the prospective one-arm multicenter trial for cancer patients receiving immune checkpoint inhibitors (KISS) (IV).......................................................................................................... 55
4.4.1 Inclusion criteria ........................................................................... 55
4.4.2 Exclusion criteria .......................................................................... 55

4.5 Methods................................................................................................... 55
4.5.1 ePRO tool on cancer patients receiving immune checkpoint inhibitors (III, IV) ...................................................... 55
4.5.2 QoL assessment (III)........................................................................ 57
4.5.3 Patient compliance (IV)..................................................................... 57

4.6 Statistics .................................................................................................. 57
4.6.1 Statistical plan (IV)........................................................................ 58

5 Results 59
5.1 Early PD-1 therapy discontinuation in responding metastatic cancer patients ........................................................................................................... 59
5.2 Elevated CRP levels indicate poor progression-free and overall survival in cancer patients treated with PD-1 inhibitors ................................. 60
5.3 ePROs in the follow-up of cancer patients treated with immune checkpoint inhibitors: a retrospective study ..................................................... 61
5.4 Follow-up of Cancer Patients Receiving Immune Checkpoint Inhibitor Therapy by Electronic Patient Reported Outcomes-tool (KISS): a prospective study ................................................................................. 63

6 Discussion 67
6.1 Short-term anti-PD-1 treatment ............................................................... 67
6.2 Prognostic and predictive biomarkers of response to anti-PD-(L)1 therapies ........................................................................................................... 70
6.3 ePRO follow-up of cancer patients ......................................................... 73

References 79
Appendices 109
Original Publications 113
1 Introduction

It has been known for years that the immune system can resist or eradicate malignant tumors (Parish, 2003). However, efficient therapeutic approaches to enhance immune effect on tumors have been missing. In the past five years, there has been a huge development in cancer immunotherapies with the introduction of immune checkpoint therapies such as PD-(L)1 and CTLA-4 antibodies (Pardoll, 2012). Immune checkpoint inhibitor therapies have become the most important medical therapies in many malignancies such as melanoma, non-small cell lung cancer, and urogenital cancers (Bellmunt et al., 2017; Borghaei et al., 2015; Brahmer, J. et al., 2015; Herbst et al., 2016; Motzer et al., 2015; Reck et al., 2016; Rittmeyer et al., 2017; Robert, Long et al., 2015; Robert, Schachter et al., 2015; Wolchok et al., 2017).

ICIs act through inhibition of T-cell blocking which results in T-cell mediated cancer cell death (Pardoll, 2012). The side effects of ICIs resemble autoimmune disease. The most common ones are rash, endocrine toxicity, gastrointestinal (GI)-toxicity, hepatitis, and respiratory symptoms. Even life-threatening side effects can occur, but they can, in most cases, be managed with early detection, delaying or stopping the immuno-oncological (IO)-therapy, and initiation of immunosuppressive medication, most commonly corticosteroids (Haanen, J. B. A. G. et al., 2017; Puzanov et al., 2017; Spain, Diem, & Larkin, 2016; Wang et al., 2018).

Despite the continuously evolving indications of immune checkpoint inhibitors, clinical knowledge about their efficacy and tolerability in a real-world setting is scarce. Furthermore, the response patterns of ICIs differ from what has been seen with traditional medical treatments, such as chemotherapy, for cancer. Assessment of the change in tumor burden is a central feature in clinical evaluation of cancer therapeutics in metastatic diseases. Tumor shrinkage, or in other words, objective response rate (ORR), time to disease progression (TTP) or progression-free survival (PFS), and overall survival (OS) are key endpoints in clinical trials (El-Maraghi & Eisenhauer, 2008; Paesmans et al., 1997). In addition, the concept of disease-free survival (DFS), the length of time after primary treatment without any signs of symptoms of cancer, is essential in trials assessing cancer therapeutics in adjuvant settings. Nevertheless, all these endpoints are useful only if based on widely accepted and applied standard criteria based on anatomical tumor burden.

Response Evaluation Criteria in Solid Tumors (RECIST), published in 2000 and updated since, was created to standardize and simplify prior response criteria
(Eisenhauer et al., 2009). However, the unique response patterns of ICIs were considered to be better captured with the new response guideline, iRECIST, based on RECIST 1.1. The main differences between iRECIST and RECIST 1.1 are the two distinctive responses: unconfirmed progressive disease (iUPD) and confirmed progressive disease (iCPD). The approach of iRECIST allows typical responses to ICIs, such as delayed responses after pseudoprogession, to be identified (Seymour et al., 2017b).

Typically, with PD-(L)1 inhibitors responses are seen in 20-40% of patients yet most fail to respond. Selective or predictive biomarkers of response to anti-PD-(L)1 are lacking albeit PD-L1 expression in non-small-cell lung cancer tissue, tumor mutational burden (TMB), and in rare cases, microsatellite instability and mismatch-repair deficiency (dMMR). However, the true predictive value of PD-L1, that is, to predict outcomes in the presence of immunotherapy instead of predicting outcomes independent of treatment (as prognostic biomarkers do) is somewhat unclear. In addition, positive and negative predictive values of PD-L1 and TMB are low, and these biomarkers are assessed from tumor biopsies which are time consuming. So far, clinically relevant predictive biomarkers are lacking for evaluating response to ICIs and to guiding patient selection. Furthermore, the optimal length of PD-(L)1 inhibitor therapies, which remains to be elucidated, is not only an economical but also an ethical challenge, as the toxicity spectrum of immune checkpoint inhibitors is wider and more fatal than the first results from clinical studies implied (Le Burel et al., 2017; Pillai et al., 2018; Wang et al., 2018).

Cancer patients suffer from a variety of symptoms derived from the malignancy itself, whereas some arise as side effects of the given treatment. Many symptoms are left unnoticed due to factors such as time discontinuity between prescheduled health care appointments, individual disease history, and inadequate patient coherence (Basch et al., 2009a; Gilbert et al., 2012; Henry et al., 2008; Laugsand et al., 2010; Reilly et al., 2013; Valderas et al., 2008; Velikova et al., 2010). In general, worsening of symptoms indicates cancer progression or severe side effects of the treatment, and is linked to poorer cancer survival (Trajkovic-Vidakovic, de Graeff, Voest, & Teunissen, 2012).

Scheduled electronic patient-reported outcomes (ePROs) enable timely and continuous collection of symptoms in a cost-effective manner (Bennett, Jensen, & Basch, 2012; Cleeland et al., 2011; Holch et al., 2017; Jensen et al., 2014; Kotronoulas et al., 2014; Mullen, Berry, & Zierler, 2004; Pakhomov, Jacobsen, Chute, & Roger, 2008). Furthermore, use of ePROs in cancer patient monitoring
has shown impressive improvements with over five months overall survival benefit compared to standard follow-up (Basch et al., 2017a; Denis, Yossi et al., 2017).

Immune checkpoint inhibitors differ from traditional cancer therapies with potential severe side effects rising from all the organs of the body and late timing of the side effect occurrence. Therefore, comprehensive and long-lasting assessment of symptoms is needed. ePROs provide a unique and cost-effective means to capture broad changes in patients’ symptoms for extended time periods. Since ePRO follow-up has been shown to improve survival and QoL of lung cancer patients (Denis et al., 2017; Denis, Lethrosne et al., 2017) and cancer patients receiving chemotherapy (Basch et al., 2016; Basch et al., 2017), it is feasible to speculate that ePROs could also improve these in cancer patients receiving immune checkpoint inhibitors.

The aim of the present study is to view the use of immune checkpoint inhibitor therapies in a real-world context and to investigate the clinical relevance of ePRO symptom follow-up on cancer patients receiving immune checkpoint inhibitor therapies.
2 Literature review

2.1 Cancer

Cancer is one of the leading causes of death and disease worldwide. Not only does cancer have an enormous effect on the health of patients and survivors but it has a tremendous financial impact on a societal level. In the near future, the number of new cancer cases is expected to go up mostly because of the aging of the population. The evolution of cancer therapies has created multiple new treatment opportunities, improving overall survival among different cancer types, even with metastatic disease. The reverse side of the improved survival among cancer patients treated with multiple therapy lines, increasing the symptom burden of a patient, is the pressure it creates on health care in the form of growing demand for resources. Digitalization, a global megatrend, has attracted growing interest as at least a part of the solution to better utilize scarce reserves. Digital transformation of healthcare aims to deliver the positive impact of technology in many forms e.g. telemedicine, artificial intelligence (AI)-aided medical devices, and vast data pools to create predictive analytics for generating value-based healthcare assets.

2.1.1 Cancer evolution

The present consensus on the origins of cancer stands in the genes. In other words, cancer is a genetic disease (Vogelstein & Kinzler, 2004). A better conception of genetic alterations in cancer initiation and progression has dramatically improved knowledge of this disease in recent years. ‘Cancer genes’ are generally grouped into oncogenes and tumor suppressor genes. An often-used analogy is that oncogenes can be referred to as a car accelerator so that a mutation in an oncogene would be an equivalent of having the accelerator continuously pressed. Conversely, tumor suppressor genes act as brakes, so that when not mutated, they inhibit the carcinogenesis (Kinzler & Vogelstein, 1996). Oncogenes and tumor suppressor genes can be classified by the nature of their somatic mutations found in tumors. That is, mutations in the oncogenes are almost always missense affecting only one allele occurring typically at specific hotspots. In contrast, tumor suppressor genes are typically mutated throughout the gene, and mutations affect both alleles causing loss of heterozygosity (LOH) (Vogelstein & Kinzler, 2004).
Proto-oncogenes are “normal” genes that are involved in cell growth and proliferation thus, onco-proteins produced by oncogenes are typically involved in cell signal transduction or mitogenic signals. In general, mutations occurring during transformation of proto-oncogenes to oncogenes are “gain of function” by nature, leading to up-regulation (Vogelstein & Kinzler, 2004). Examples of oncogene pathways include mutations in the proto-oncogene self or in the promoter (Reddy, Reynolds, Santos, & Barbacid, 1982), changes in the oncoprotein concentrations due, for example, to changes in miRNA stability (Trang, Weidhaas, & Slack, 2008), and chromosomal translocations of which the “Philadelphia” chromosome is an example (Groffen et al., 1984).

Mutations in tumor suppressor genes lead to loss or reduction of function and are considered recessive mutations based on Alfred Knudson’s (1922-2016) classic “two-hit” hypothesis apart from a few exceptions (Cook & McCaw, 2000; Hollstein, Sidransky, Vogelstein, & Harris, 1991; Knudson, 1971). In general, proteins encoded by tumor suppressor genes promote apoptosis and downregulate cell cycle for example through hindering cell cycle promoting genes and recognizing DNA damage and further suspending cell cycle (Kinzler & Vogelstein, 1997; Vogelstein et al., 2013).

2.1.2 Hallmarks of cancer

The idea of certain principal changes needed in cancer genesis is universal, but the concept of Hallmarks of Cancer was invented by Douglas Hanahan and Robert A. Weinberg (Hanahan & Weinberg, 2000), and later redefined (Hanahan & Weinberg, 2011). The eight hallmarks of cancer are constituted of eight functional capabilities thought to be acquired by developing cancers in the multistep carcinogenesis that leads to most forms of human cancer (Fig. 1).
Fig. 1 A replication of Hallmarks of Cancer based on Hanahan and Weinberg (2011).

The abilities to sustain proliferative signalling as well as avoid the negative regulatory programs of cell proliferation are essential for cancer cells to evolve, where the independence from highly regulated yet only poorly understood cell-to-cell control is the central factor (Hanahan & Weinberg, 2011). Also, capabilities of resisting regulated cell death, apoptosis and inducing angiogenesis are distinctive for carcinogenesis alongside changes in the cell energy metabolism favouring aerobic glycolysis, and the defects in immune surveillance, ultimately leading to invasive growth and the formation of metastases (Hanahan & Weinberg, 2011).

Elimination is one of the three steps of the process of "cancer immunoediting" under the concept of immune surveillance, where the immune system eliminates and reshapes malignant disease. According to Dunn et al (Dunn, Bruce, Ikeda, Old, & Schreiber, 2002) the elimination phase includes 1) recognition of tumor cells by innate immunity cells, 2) maturation and migration of antigen-presenting cells, and 3) generation of cytotoxic, tumor-antigen-specific T lymphocytes which home into the tumor bed to eradicate tumor cells. If the host immune system fails to eliminate the malignant cells, it is the beginning of the second phase called equilibrium where dynamic balance between the host immune system and survived tumor cell variants might endure for years. Even though cancer cells go through constant immune selection pressure, some clones emerge and escape immune surveillance due to
insensitivity to immune detection or elimination caused by genetic or epigenetic changes, leading to clinically detectable malignant disease.

According to Hanahan and Weinberg, two enabling characteristics are present at the acquisition of the eight hallmarks of cancer. The most notable is the development of genomic instability in cancer cells that generates random mutations among which are rare genetic changes for example inactivating mutations of tumor suppressor gene TP53 or activation of Ras genes, which can enable individual hallmark capabilities. Also, DNA methylation status is related to genomic integrity as an example of epigenetic mechanism in carcinogenesis (Jones & Laird, 1999). The inflammatory status of neoplastic lesions where the infiltration of certain immune cells of both adaptive and innate immunity can further promote tumor progression through various means is considered as the second enabling attribute (Hanahan & Weinberg, 2011).

2.1.3 Cancer epidemiology

**Non-small-cell lung cancer**

Lung cancer is the most common malignancy and the most common cause of cancer deaths worldwide with a five-year survival rate of under 20%. The incidence of lung cancer among women and the prevalence of the adenocarcinoma subtype are rising (Ferlay et al., 2015). Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer, covering approximately 85% of all lung cancers. The two main subtypes are adenocarcinoma and squamous cell (epidermoid) carcinoma (Travis et al., 2015). The most important risk factor for lung cancer is tobacco smoking. Five-year relative survival rates for NSCLC patients are around 20%, altogether. For stages I-II treated with curative intention, survival rates are ~60% whereas for stage III around one third of patients are alive at the five-year timepoint after diagnosis. Stage IV patients with metastatic disease have a substantially worse prognosis, with only around 5% of patients alive at the same timepoint. (American Cancer Society, National Cancer Institute, 2019, SEER database, NCI).

**Melanoma**

Melanoma is a cancer arising from the malignant transformation of melanocytes and is reported to be the 19th most common cancer in the world. Cutaneous
Melanoma is the most common subtype (~90%), arising from the melanocytes in the epidermis. Two other subtypes are mucosal and uveal melanomas. The incidence of melanoma varies substantially between countries, which is considered a result of variations in racial skin phenotypes and sun exposure around the world (Ferlay et al., 2015).

The incidence of cutaneous melanoma is rising. The median age at diagnosis is 57, lower than with other solid malignancies where the majority of cases are diagnosed at over the age of 65 (Ferlay et al., 2015). There are several risk factors, environmental and genetic, that are considered significant in the development of cutaneous melanoma, ultraviolet (UV) radiation being the most important. Even though a direct causal link between UV radiation and cutaneous melanoma has not been established, epidemiological studies (Elwood & Jopson, 1997; Gilchrest, Eller, Geller, & Yaar, 1999) have demonstrated an association between the pattern and the length of sun exposure.

Melanoma patients with localized disease (stages I-II) have a good overall survival prognosis; the 5-year relative survival rate for cutaneous melanoma is 98% (American Cancer Society, National Cancer Institute, 2019). Stage III patients with regional disease are a heterogenous group; stage IIIA patients have an excellent prognosis with a 5-year survival rate of 93% whereas for stage IIID the prognosis is as low as 32% (8th Edition International melanoma database).

Genitourinary cancers

Genitourinary (GU) cancers include, among others, kidney and bladder cancer. Kidney cancer accounts for 5% and 3% of all adult malignancies in men and women, respectively, representing the seventh most common cancer in men and the tenth most common cancer in women. Renal cell carcinoma (RCC) accounts for ~80% of all kidney cancers. Obesity, tobacco smoking and hypertension are well known risk factors for kidney cancer. In addition, RCC appears to be more common in patients with end-stage renal failure or acquired renal cystic disease. Approximately 2-3% of all RCCs are hereditary (Escudier et al., 2019a). Five-year disease-specific survival for patient with localized disease with low to intermediate risk is ~80-90% compared to high risk patients with survival of around 55%. Patient with metastatic disease have a much lower survival percentage ranging from 20-30% at best (Escudier et al., 2019b).

Bladder cancer was the ninth most common malignancy worldwide with ~400 000 newly diagnosed cases in 2012 (Ferlay et al., 2015). The disease is
prevalent among men, and approximately 70% of patients with bladder cancer are over 65 (So, 2016). The majority (90%) of bladder cancers are of urothelial origin in Western Europe and the United States (Ferlay et al., 2015). The main risk for bladder cancer is tobacco smoking. The five-year relative survival rate for localized bladder cancer is almost 70%, and in the case of in situ alone-findings, the survival rate is ~95%. However, patients with advanced disease have a much poorer prognosis; the 5-year relative survival rate is as low as 5% with stage IVB cancer (American Cancer Society, SEER database, NCI).

**Head and neck cancers**

The annual incidence of head and neck cancers (H&N) worldwide is more than 650,000 cases with around 330,000 deaths each year. The prevalence among men is higher compared to women, and about 90% of all cases are squamous cell carcinomas (Ferlay et al., 2015). Risk factors are among other things of dietary, viral and genetic origin. The incidence rates and prevalence of different subtypes vary substantially geographically.

Survival in head and neck squamous cell carcinoma (HNSCC) is predicted primarily by anatomical site, stage and human-papilloma virus (HPV) status, the latter being a strong positive predictor for survival if positive. In a recent EUROCARE population-based study, five-year relative survival was poorest for hypopharyngeal cancer (25%) and highest for laryngeal cancer (59%). For oral cavity and pharyngeal cancer, around 30% of cases are localized at the time of diagnosis whereas over 50% of laryngeal cancers are localized when diagnosed.

### 2.2 Treatment of cancer

#### 2.2.1 Surgery

Surgery is the oldest form of therapy for cancer but not until the development of an effective method for anaesthesia and the introduction of antisepsis in the beginning of the nineteenth century was true success met. This laid the basis for modern surgical oncology. By far, surgery has made the greatest contribution to the cure of cancer. Surgical interventions are needed not only to treat cancer, but to diagnose, as a prophylactic measure to prevent cancer, and in some cases, as palliation. As a
part of cancer treatment, surgery may be used along with other treatment modalities such as chemotherapy and radiation therapy.

### 2.2.2 Radiotherapy

The observations of Wilhelm Conrad Roentgen (1845-1923) led to the first published paper about the new form of rays; X-rays. When the Nobel Prize for Physics was awarded for the first time in 1901, Roentgen was recognized. The discovery of natural radioactivity by Henri Poincaré (1854-1912) and the Curies was another milestone in the evolution of radiation for cancer treatment.

Radiotherapy uses high doses of radiation to kill cancer cells and shrink tumors. Radiotherapy is based on the use of two main types of radiation: electromagnetic (X-rays and gamma-rays) and particulate ones, the latter represented by electrons, neutrons and protons (Boone et al., 1977). Ionizing radiation works by damaging the DNA or other important cellular molecules through direct cell death, or through indirect cellular damage which occurs after the production of free radicals, leading to cellular death. Radiation may be delivered externally or internally. During radiation therapy, unfortunately, normal cells may be damaged and killed also; this is controlled by focusing the beam on the tumor, and with dose fractioning (Suit et al., 1982).

Radiotherapy can be given as a curative or palliative treatment in solid cancers, or as consolidating treatments with distinct criteria in hematopoietic malignancies. Stereotactic body radiotherapy (SBRT) or stereotactic ablative radiotherapy (SABR) by another name, is a subset of external radiotherapy aiming for radical outcomes. It can deliver precisely targeted radiation in fewer high-dose treatments than traditional therapy, presumably with less healthy-tissue damage. It may be used to treat primary tumors and (oligo)metastatic lesions. Chemoradiotherapy combines radiotherapy and chemotherapy with radical treatment intention. Chemotherapy can be used to treat local lung cancer, GI-cancers, and head and neck cancers as primary therapy or in an adjuvant setting, prior or after surgery.

Radioisotope treatments are mainly used as an adjuvant therapy after surgery in medium or high-risk cases of thyroid carcinomas (Jonklaas et al., 2010), but also in the treatment of other, rare, neuroendocrine tumors (Spiegel & Libutti, 2010). Radioisotopes can also be used to treat metastatic bone lesions of cancerous origin (Blake et al., 1988), and mainly advanced cases of prostate cancer (Hoskin et al., 2014). The mechanism of action is based on the higher radioisotope-intake of
cancerous cells compared to normal cells, leading to higher doses of radioactivity, and, thus, more lethal effect.

### 2.2.3 Medical treatment of cancer

On December 3, 1947 the first cancer patient was treated with aminopterine, a folic acid antagonist, by Sydney Farber and his team at Children’s Hospital Boston (Miller, 2006), and the concept of chemotherapy was established. Chemotherapy is a treatment of cancer with drugs that can destroy cancer cells by impeding their growth and reproduction. Chemotherapy can be used as a primary treatment to destroy cancer cells both in curative and palliative measures. If chemotherapy is given prior to surgery, it is called neoadjuvant treatment, and if after another cancer treatment, for example breast cancer surgery with curative intention, adjuvant therapy. Chemotherapy can be administered in multiple ways, including orally, intravenously, and by injection. Chemotherapy treatments can be divided as following: alkylating agents (e.g. cisplatin, lomustine); platinum alkaloids (e.g. carboplatin, oxaliplatin); antimicrotubule agents (e.g. docetaxel, vincristine, vinorelbine); antimetabolites (e.g. methotrexate, capecitabine); and topoisomerase interactive agents (e.g. topotecan, irinotecan, etoposide).

Treatment-related toxicity is a major restricting factor in the use of chemotherapy even at the usual therapeutic levels. Around 35-40% of patients treated with chemotherapy face severe side effects, of which some are potentially chronic (Reilly et al., 2013). Common side effects of chemotherapy include among others nausea, fatigue, bone marrow suppression, GI and mucosal irritation, and neuropathy.

Hormonal therapies of cancer are non-tissue damaging treatments with typically mild side effects, used as a treatment for hormonally responsive cancers, such as breast, prostate, or endometrial carcinomas. Hormonal therapies to treat hormone receptor-positive breast cancer both in adjuvant and metastatic settings can be classified as selective estrogen receptor modulators (e.g. tamoxifen, fulvestrant) and aromatase inhibitors (e.g. letrozole, exemestane). Likewise, gonadotropin-releasing hormone (GnRH) analogs (e.g. goserelin) can be used in the treatment of prostate cancer and breast cancer. GnRH analogs and antagonists (e.g. degarelix) can be used as (neo)adjuvant treatment combined with radiotherapy with radical intention as well as in metastatic settings. The role of antiandrogens in the treatment of metastatic prostate cancer has changed with the evolution of novel antiandrogens such as abiraterone acetate and enzalutamide.
The development of targeted therapies has resulted in a wide variety of cancer treatments. Targeted therapy works by targeting cancer’s distinct genes, proteins, or the tissue environment that contributes to cancer growth and survival. There are several types of targeted therapy, including monoclonal antibodies (e.g. cetuximab); small-molecule drugs, like angiogenesis inhibitors (e.g. sunitinib) and tyrosine kinase inhibitors (e.g. erlotinib); poly (ADP-ribose) polymerase inhibitors (e.g. olaparib); and cyclin-dependant kinases (CDK) 4/6 inhibitors (e.g. palbociclib). Furthermore, the effect of epigenetic therapies, which are mainly based on three known epigenetic mechanisms, DNA methylation, histone modifications, or regulatory RNA expression, is under intensive pre-clinical research (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009).

### 2.2.4 Treatment response assessment in solid tumors

The assessment of the efficacy of a given cancer treatment varies depending on the aim of the cancer care. That is, in the adjuvant setting, response evaluation aims to define the disease-free survival (DFS), the length of time after primary treatment during which no detectable signs of cancer are present. Furthermore, assessment of the change in tumor burden is a central feature in clinical evaluation of cancer therapeutics in metastatic diseases. Detection of disease relapse, as well as treatment benefit in metastatic disease, is typically based on imaging, mainly computed tomography (CT) and magnetic resonance imaging (MRI).

In addition, the evaluation of tumor response in the daily clinical practice of oncology might be better captured in terms of clinical improvement instead of objective tumor response. It may, rather, be based on subjective medical judgement resulting from clinical and laboratory data instead of predefined standardized measurement guidelines intended for reporting in clinical trials. However, reliable surrogates for predicting a potential therapeutic benefit for cancer patients in clinical practise are needed, and imaging techniques are an essential part of modern clinical oncology care.

The World Health Organization (WHO) definitions, published originally 1979 and redefined in 1981, aiming to unify response evaluation of tumors to anticancer agents, categorized four response classes: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD), based on the change in the size of measurable and evaluable lesions. Since then, a number of different modifications or clarifications of the WHO criteria have taken place. However, the WHO criteria do not include assessment of other measures of antitumor activity.
beyond tumor shrinkage, such as changes in tumor metabolism or tumor volume
nor the definition of serum tumor marker response of which carcinoembryonic
antigen (CEA) and cancer antigen 125 (CA-125) are examples, which are
nowadays considered part of clinical practice.

Response Evaluation Criteria In Solid Tumors (RECIST), based on the original
WHO guidelines, was developed and published in 2000, and revised in 2009 as
RECIST 1.1. The RECIST criteria are widely adopted, and used in oncology
clinical trials. Endpoints described by the RECIST criteria have been used as either
primary or supportive data for regulatory approval of new therapeutics by both the
Food and Drug Administration (FDA) and European Medicines Agency (EMA).
The RECIST criteria provides a standardized set of rules for response assessment
using tumor shrinkage based upon imaging modalities globally available and
interpretable by most clinicians. However, in recent years, there have been major
changes in the mechanism of action of cancer therapeutics as well as in imaging
technologies. The era of immune checkpoint inhibitors has created the need for
response evaluation guidelines capable of capturing the unique response patterns
of ICIs. A consensus guideline, iRECIST, was developed by the RECIST working
group for the use of modified RECIST 1.1 in cancer immunotherapy trials.
iRECIST differs from conventional RECIST by permitting new lesions, thus,
allowing the capture of pseudoprogression as a beneficial response.

There is growing evidence of the use of circulating tumor DNA (ctDNA) in
detecting microscopic residual disease or early disease progression, aiming to guide
patient selection for adjuvant treatments, curative intended re-interventions or
treatment re-challenge in metastatic context. Furthermore, ctDNA levels in an
individual patient over time correlate well with changes in tumor burden and
treatment. The short half-life of ctDNA in circulation can be advantageous for
measuring real-time tumor burden in response to therapy compared to many
standard serum tumor markers in current clinical use with half-lives of days to
weeks, typically (Dawson et al., 2013).

2.3 Cancer immunotherapy

The pioneer work of William B. Coley (1862-1936) to harness the immune system
in cancer control in the 1890s was initially overlooked due to the lack of
consistency of response. Paul Ehrlich (1854-1915), a German scientist, was the first
to imply the existence of some form of immunosurveillance when stating that the
incidence of cancer would be much higher if the body’s immune system did not
prevent it. However, the term immunosurveillance was coined by Australian virologist, Sir Frank Macfarlane Burnet (1899-1985) when suggesting that lymphocytes maintain a continuous check on tissues, probably through recognition of tumor-related antigens, and then eliminate the transformed cells. At about same time, it was postulated by Lewis Thomas (1913-1993), that rejection of a graft reflects the way the body clears up cancer (Parish, 2003).

The basis for cancer immunotherapy was obtained in the 1940s when evidence for specific tumor recognition by immune system cells was shown in experiments conducted using murine tumors. The observation that mainly CD8+ cytotoxic T cells were responsible for mediating the rejection of tumors led to the identification of genes coding antigens expressed on tumors, and the mechanistic basis of antigen-recognition by human tumor-reactive T cells was discovered (Hellstrom & Hellstrom, 1969).

2.3.1 Mechanism of action

T cell mediated immunity is a cascade of multiple steps from selective antigen recognition to execution of direct effector functions, and to the provision of help to multiple other effector cells of immunity, such as antigen-presenting cells (APCs) and natural-killer (NK) cells. Each step is regulated by counterbalancing stimulatory and inhibitory signals redefining the response.

The ability of T cells to home in to the antigen-expressing tumor bed in all parts of the body, and their feature of continual proliferation in response to immunogenic proteins expressed by tumors until all the tumor cells are extirpated, makes T cell focused cancer immunotherapy mechanistically preferable compared to other forms of cancer therapy. In addition, the generation of immunological memory allows the eradication of reoccurring antigen-bearing tumors (Disis, Bernhard, & Jaffee, 2009). Two main subtypes of T cells are cytotoxic CD8+ T cells and cytokine secreting CD4+ T cells. The roles of T cell subsets in tumor growth are multiple based on specific phenotypes. The CD4+ T cell response can be either immune stimulatory or immune inhibitory by nature, and specific CD4+ T helper (Th) cell phenotypes are essential for the efficient effector function of CD8+ T cells. Th1 CD4+ T cells secrete type I cytokines including IFNγ, leading to activation of antigen-presenting cells (APCs), and enhanced CD8+ effector T cell response (Ossendorp, Mengede, Camps, Filius, & Melief, 1998). On the contrary, Th2 CD4+ T cells can dampen the activity of APCs by secreting type II cytokines like interleucin 4 (IL4) in response to antigen (Ellyard, Simson, & Parish, 2007). A
fairly new subtype of T helper cells has been described: Th17 CD4+ T cells, that among other effector cytokines, secrete IL17 (Dong, C., 2006). Furthermore, there are regulatory T cells (Tregs) counteracting the effector T cell mechanisms. Several subtypes of Tregs have been identified, although their lineage relationships among T cells, and their functions are incompletely understood. Tregs haven been classified as natural and adaptive where the natural Tregs, CD4+FOXP3+, originating from thymoid lineage, were the first well-defined subtype (Sakaguchi, 2000). CD4+FOXP3+ Tregs can inhibit adaptive T cell response via secretion of immunosuppressive cytokines like IL10 as well as by direct inhibition of APCs (Zou, 2006).

The high density of CD8+ effector memory T cells in the tumor parenchyma was shown to be associated with increased survival in over 400 colon cancers (Pages et al., 2005). In another study, based on gene-expression patterns, data in subset of tumors from 75 colon cancer patients suggested that upregulation of genes related to Th1 adaptive immune response was associated with a decreased relapse risk (Galon et al., 2006a). On the contrary, a high number of intratumoral Tregs is associated with a shorter relapse-free period and decreased overall survival compared to individuals with low Treg infiltrate (Bates et al., 2006; Gao, Q. et al., 2007).

Cancer immunotherapies include a wide variety of different approaches, but three main orientations can be defined. The first is non-specific stimulation of immune reactions by stimulating effector cells. The ability of IL-2, a soluble T cell growth factor, to mediate tumor regression by stimulating effector cell functions in solid tumors was shown in the 1980s (Rosenberg et al., 1985), and was later narrowed to melanoma and RCC with higher IL-2-sensitivity in clinical use (Rosenberg et al., 1993). Immune checkpoint blockade in effector cell function is another example of this phenomenon. The second approach is active immunization with cancer vaccines to enhance antitumor reactions. Cancer vaccines are based on the idea of sensitizing a patient to specific cancer antigens, in an attempt to boost cellular immune reactions capable of inhibiting the growth of established cancers (Klebanoff, Acquavella, Yu, & Restifo, 2011). The third path is to passively transfer activated immune cells with antitumor activity, also called adoptive immunotherapy (Rosenberg et al., 1988). Genetic modification of lymphocytes, focusing on T cell receptors (TCRs), for adoptive cell therapy has given promising results in solid tumors although treatment related toxicity has been an issue (Johnson et al., 2009; Robbins et al., 2011).
Chimeric antigen receptor (CAR) T cell therapies are an adaptation of autologous T cell therapy. T cells can be genetically engineered to express either of two types of receptors: CARs or natural TCRs. CARs are artificial fusion proteins that incorporate antigen recognition domains and T cell activation domains. Compared to TCRs, recognition of antigens by CARs is not dependent on MHC molecules (Kochenderfer & Rosenberg, 2013).

Anticancer immunotherapies such as immune checkpoint inhibitors or methods including adoptive cellular transfer, function by overcoming tumor-induced immunosuppression, thus, enabling immune-mediated tumor clearance. Immunoediting, a process of three steps (elimination, equilibrium and escape) is believed to reflect the paradoxical process of the immune system capable of both constraining and promoting tumor development and progression (Schreiber, Old, & Smyth, 2011). If the initial elimination of transformed cells fails, tumor cell subclones can proceed to the equilibrium phase in which net tumor growth is limited, however. The pressure from adaptive immunity to tumor cells with possible other elements of hallmarks of cancer such as genomic instability can lead to selection of subclones with reduced immunogenicity. Immunoediting is a cascade of multiple steps, such as loss of antigen presentation due to epigenetic changes, or changes in the effector cells of the immune system like decreased IFNγ secretion by T cells (Chang, C. C. et al., 2015; Takeda et al., 2017) and can ultimately lead into the escape phase, where the immunoedited tumors become clinically apparent (Mittal, Gubin, Schreiber, & Smyth, 2014; Schreiber et al., 2011). Interestingly, immunoediting is shown to be present not only during the natural progression of tumors but also reoccurring in response to treatment with cancer immunotherapies, as an acquired immunotherapeutic resistance phenomenon (Schreiber et al., 2011).

### 2.3.2 Immune checkpoint inhibitors

Immune checkpoints are cell surface proteins that are responsible for the balance between co-stimulatory and inhibitory signals influencing T cell mediated immune responses (Greenwald, Freeman, & Sharpe, 2005; Zou & Chen, 2008). Immune checkpoints, like cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1) and programmed death ligand-1 (PD-L1) are crucial for the maintenance of self-tolerance under normal physiological conditions. Chronic antigen exposure, for example within tumor microenvironment or during viral infection, can lead to T cell exhaustion. The exhausted state of T cells is characterized, among other features, by their expression of multiple co-
inhibitory receptors, immune checkpoints, which is shown to correlate with their degree of unresponsiveness (Antoine et al., 2012; Wherry, 2011). In contrast, T cell anergy, which also called peripheral tolerance, is thought to develop during initial antigen exposure in the presence of suboptimal TCR stimulation (Schwartz, 2003). However, it seems more likely that these two definitions are intertwined, and T cell exhaustion is a state of T cell anergy developing in response to the presence of chronic antigen exposure aiming to limit extensive pathology (McAfee & Blattman, 2012). The finding that blockade of CTLA-4, as well as PD-1, can restore effector function of exhausted T cells defined immune checkpoints as potential drug therapy targets which lead to the development of immune checkpoint inhibitors.

As a form of adaptive immune resistance, tumors can influence the expression of immune checkpoint proteins. Adaptive immune resistance is a process where a cancer phenotype changes in response to cytotoxic or pro-inflammatory immune response. The process is triggered by the specific recognition of cancer cells by T cells which leads to the production of immune-activating cytokines and to a cascade of other immunological responses (Pardoll, 2012; Ribas, 2015). By contrast, innate immune resistance is characterized as overall lack of response to immunotherapy due to immunological ignorance in patients with an immunosuppressive state, tumors expressing antigens albeit on a small scale, or the immunosuppressive mechanisms in the immunoediting process (Sharma, Hu-Lieskovan, Wargo, & Ribas, 2017). Unlike to most former antibodies approved for cancer therapy, antibodies that block immune checkpoints, do not aim to directly influence tumor cells but instead target lymphocyte receptors or their ligands to enhance intrinsic antitumor activity (Topalian et al., 2012).

**Cytotoxic T-lymphocyte-associated antigen 4 antibodies**

CTLA-4 antibodies were the first immune checkpoint inhibitor therapies to be accepted for the treatment of cancer. CTLA-4 is an inhibitory checkpoint molecule that counteracts CD28, which is constitutively expressed on the cell surface of naïve CD4+ and CD8+ T cells. CTLA-4 inhibits proliferation and IL-2 secretion of T cells (Krummel & Allison, 1995). CD28 takes part in the formation of the immunological synapse where the activation of TCR by antigen recognition is amplified by CD28 signalling, leading to T cell activation. The proposed mechanism of CTLA-4 is the dampening of T cell activation by competing binding with CD28 to CD80 (also known as B7.1) and CD86 (also known as B7.2) both expressed on APCs alongside direct inhibitory signals to T cells (Egen & Allison,
2002; Parry et al., 2005; Riley et al., 2002; Schneider et al., 2006). Under normal physiological conditions the role of CTLA4 is to downmodulate the helper T cell activity and to enhance regulatory T cell immunosuppressive activity (Wing et al., 2008).

The work of Allison and colleagues (Leach, Krummel, & Allison, 1996) led to the development of two fully humanized CTLA-4 antibodies, ipilimumab and tremelimumab. Both antibodies produced tumor responses in ~10% of melanoma patients whereas immune-related adverse events were reported in ~25-30% of patients, colitis being the most common (Beck et al., 2006; Hodi et al., 2003; Phan et al., 2003; Ribas et al., 2005). Ipilimumab fared better and based on survival benefit on patients with metastatic melanoma (Hodi et al., 2010) it was approved by the FDA for the treatment of metastatic melanoma in 2010. More impressing was the effect of ipilimumab on long-term survival, for that, 18% of ipilimumab-treated patients survived over two years. Ongoing responses and survival long after even a short course of therapy discontinuation were a new findings, and as has been shown later, a response specific to yet not prevalent in immune checkpoint inhibitors.

**PD-(L)1 antibodies**

The mechanistic basis of responses to PD-1 or PD-L1 blocking is in inhibiting interferon-induced adaptive immune resistance. Interferon production is increased after the formation of the immunological synapse where antigen presentation activates TCR, thus leading to activation of effector cells of both innate and adaptive immunity alongside upregulation of PD-L1 and indoleamine-pyrrole 2,3-dioxygenase (IDO) on intratumoral cells (Ribas, 2015). In addition to interferon-inducible expression of PD-L1, which is more common in most cancer types, PD-L1 can be constitutively expressed through activation of oncogenic pathways related to the activation of signal transducers and activators of transcription (STAT) proteins or other interferon-receptor downstream modulators, reflecting innate immune resistance (Akbay et al., 2013; Atefi et al., 2014; Loke & Allison, 2003; Parsa et al., 2007).

The expression of PD-1 is induced when T cells become activated (Ishida, Agata, Shibahara, & Honjo, 1992). In the PD-L1-PD-1 pathway, binding of PD-L1 ligand, expressed on tumor cells and APCs to the PD-1 receptor on T cells, halts or limits T cell response by downregulating T cell proliferation, effector function and cytokine production (Topalian et al., 2012). After the PD-L1-PD-1-interaction, PD-
1 can bind SH2-domain containing tyrosine phosphatase 1 (SHP-1) and 2, thus leading to dephosphorylation of key signalling intermediates including kinases like Akt and PI3K, which might terminate early TCR signalling (Chemnitz, Parry, Nichols, June, & Riley, 2004; Freeman et al., 2000), and influence the duration of T cell-target cell contact (Fife et al., 2009). Disruption of PD-1-PD-L1 interaction leads to diminished T cell movement, thus providing the possibility for extended antigen-specific T cell and antigen-bearing APC interaction, which is critical for full T cell activation (Hurez et al., 2003; Scholer, Hugues, Boissonnas, Fetler, & Amigorena, 2008). In contrast to CTLA4, which contributes to T cell activation centrally, PD-1 regulates effector T cell activity in peripheral tissues and tumors. Furthermore, PD-1 is more broadly expressed than CTLA-4 on other non-T lymphocyte subsets of immune cells (Fanoni et al., 2011; Terme et al., 2011).

It has been shown that tumor-infiltrating lymphocytes (TILs) from many types of cancers express PD-1 (Ahmadzadeh et al., 2009; Bremnes et al., 2016; Galon et al., 2006b; Sfanos et al., 2009; Stanton, Adams, & Disis, 2016), as has the upregulation of expression of PD-L1 on tumor cells (Dong, H. et al., 2002; Freeman et al., 2000; Zou & Chen, 2008). In addition, it has been shown that the enhanced PD-1 expression of TILs reflects the large proportion of intratumoral CD4+ regulatory T cells, as well as the anergic state of CD8+ T cells.

The first phase I clinical trial with a fully human IgG4 PD1 antibody took place in 2010 (Brahmer, J. R. et al., 2010) where tumor regressions were observed in four of the five histologies studied: colon, renal and lung cancer, and melanoma. In addition, compared to CTLA4 antibody therapies, the rate of immune-related side effects of anti-PD1 treatment was lower.

In September 2014, pembrolizumab was approved by the FDA as the first PD-1 inhibitor for treatment of patients with unresectable or metastatic melanoma, and in December that year, nivolumab was approved for the same indication as the second PD-1 inhibitor. In April 2016, the first PD-L1 inhibitor, atezolizumab, was granted fast track status for previously treated, advanced or metastatic NSCLC by the FDA. Since then, the efficacy of PD-(L)1 inhibitors has been studied in clinical trials for multiple cancer types, both in monotherapy and in combination with other cancer treatments.
Fig. 2. Mechanism of immune checkpoint blockade through CTLA-4 and PD-L1/PD-1 pathways. Replication of Tray et al (2018). APC, antigen presenting cell; Ab, antibody; MHC, major histocompatibility complex; Ag, antigen

Although the suppressive role of PD-1-PD-L1 interaction in T cell regulation is well established, less is known about the influence of PD-L1 signaling in other immune effector cells (Loke & Allison, 2003; Noman et al., 2014). Tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) are key regulators of cancer-related inflammation and resistance to immunotherapy (Gajewski, Schreiber, & Fu, 2013; Mantovani, Marchesi, Malesci, Laghi, & Allavena, 2017). It has been shown that PD-L1 expression on macrophages is related to a tumor-promoting state, resulting in immunosuppressive cell phenotype, also called the M2-like tumorigenic phenotype (Vesely, Kershaw, Schreiber, & Smyth, 2011), and that in vivo, treatment with PD-L1 antibody reversed the phenotype and triggered macrophage-mediated antitumor activity (Hartley, Chow, Ammons, Wheat, & Dow, 2018). Therapeutic approaches combining PD-(L)1 antibodies and TAM-focused therapies to foster the antitumor effect of cancer immunotherapy are being investigated (Viitala et al., 2019).

Indications

Immune checkpoint inhibitors are indicated for cancer treatment both in adjuvant and metastatic settings in multiple cancer types, and in first and later lines of
treatment. Current (August 9, 2019) indications approved by the EMA for advanced solid cancers are presented in Table 1 and for adjuvant treatment in Table 2.

**Table 1. European Medical Agency approved indications for immune checkpoint inhibitors in advanced solid cancers**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab</td>
<td>Melanoma</td>
<td>1st as single therapy and in combination with nivolumab ≥2nd</td>
</tr>
<tr>
<td></td>
<td>RCC</td>
<td>1st in combination with nivolumab ≥2nd</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Melanoma</td>
<td>1st and ≥2nd as single therapy</td>
</tr>
<tr>
<td></td>
<td>HSCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>NSCLC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>RCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>UCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Melanoma</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>NSCLC</td>
<td>1st ∧ and ≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>HSCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>UCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td></td>
<td>RCC</td>
<td>1st in combination with pemetrexed and platinum chemotherapy ¹</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>NSCLC</td>
<td>1st in combination with bevacizumab and chemotherapy ≥2nd</td>
</tr>
<tr>
<td></td>
<td>UCC</td>
<td>≥2nd ∧</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>NSCLC</td>
<td>as consolidation therapy after chemoradiotherapy ³</td>
</tr>
<tr>
<td>Avelumab</td>
<td>Merkel carcinoma</td>
<td>≥2nd ∧</td>
</tr>
</tbody>
</table>

¹ in EGFR and ALK wild type patients; ² in patients with PD-L1≥50%; ³ in patients with PD-L1≥1%; RCC, renal cell carcinoma; HSCC, head squamous cell carcinoma; NSCLC, non-small cell lung cancer; UCC, urothelial cell cancer.
Table 2. European Medical Agency approved indications for immune checkpoint inhibitors for adjuvant treatment in solid cancers

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Detailed indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>Melanoma</td>
<td>completely resected stage III-IV (^1)</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Melanoma</td>
<td>completely resected stage III (^1)</td>
</tr>
</tbody>
</table>

\(^1\) according to the 7th edition of American Joint Committee on Cancer.

Five year results of CheckMate 067 for patient with advanced/metastatic melanoma showed a mOS of >60 months (not reached; NR) in the nivolumab/ipilimumab, 36.9 months in the nivolumab, and 19.9 months in the ipilimumab arm. Five-year overall survival rates were 52%, 44%, and 26%, respectively. For pembrolizumab in the same indication (Keynote 006) with median follow-up of 57.7 months, the mOS was 32.7 months in the pembrolizumab group versus 15.9 months in ipilimumab group. For advanced RCC (CheckMate 214), the combination treatment of nivolumab plus ipilimumab improved overall survival in patients with intermediate or poor-risk (NR vs 37.9 months/sunitinib) at a median follow-up of 32.4 months whereas single-nivolumab (CheckMate 025) showed a median OS benefit of 25.8 months compared to 19.7 months with everolimus treated patients at the three-year follow-up. In advanced/metastatic UCC (Keynote 045), pembrolizumab is the only therapy that has shown overall survival improvement compared to chemotherapy with a median OS of 10.1 months versus 7.3 months with chemotherapy after a median follow-up of 27.7 months. With single anti-PD-1 therapy in the treatment of HSCC in second line, the median overall survival of nivolumab (CheckMate 141) and pembrolizumab (Keynote 055) was ~ eight months compared to five months with chemotherapy with minor variations based on prior cetuximab exposure.

ICI therapies have moved from the second-line to the first-line treatment in NSCLC. For advanced/metastatic NSCLC, pembrolizumab monotherapy (Keynote 042) is approved as a first line therapy for patients with PD-L1 \(\geq 50\%\) with three-years follow-up of mOS of 26.4 months in the pembrolizumab arm versus 14.2 months in the chemotherapy arm. Pembrolizumab plus chemotherapy has been approved as a first line therapy (Keynote 189) for non-squamous cell lung carcinoma with mOS of 22.0 months (pembro+chemo) versus 10.7 months in chemotherapy arm with median follow-up of 18.7 months and in squamous cell lung cancer (Keynote 407) with mOS of 17.1 (pembro+chemo) months versus 11.6 months (chemo) with median follow-up of 14.3 months. A regimen of atezolizumab+chemotherapy combined with bevacizumab (IMpower150) is
approved in the first line treatment of non-squamous NSCLC with median overall survival of 19.2 months in the atezolizumab four-drug group versus 14.7 months in the control group after a median 13.5 months of follow-up.

Currently, ICI monotherapies in adjuvant treatment of high-risk melanoma have been approved based on phase III results with unmatured overall survival data (3-year RFS rates 58%/nivolumab versus 45%/ipilimumab, Checkmate 238; mRFS NR/pembrolizumab versus 20.4mo/placebo, Keynote 054). Durvalumab as a consolidation therapy after stage III NSCLC chemoradiation (Pacific trial) significantly prolonged progression-free survival compared with placebo, with median durations of 16.8 months and 5.6 months, respectively.

2.3.3 Predictive factors for treatment benefit from immune checkpoint inhibitors

PD-(L)1 inhibitors have become a standard of care in treatment of metastatic lung, renal, urothelial cancers, and melanoma, and are approved for adjuvant treatment for melanoma, and also for consolidation therapy for NSCLC in specific settings. Ipilimumab monotherapy is approved for the treatment of advanced melanoma, and as combination therapy with nivolumab to treat advanced melanoma and RCC in the first-line. Unlike with other cancer treatments, such as chemotherapy or targeted therapies, very prolonged or permanent responses are seen with a subset of patients with treatment-refractory metastatic cancers although the majority of patients fail to respond. This emphasizes the need to identify predictive biomarkers for outcome to help guide the patient selection.

Tumor biomarkers

Despite the pervasive research in the biomarker field, only a few biomarkers have proven to be clinically relevant such as PD-L1 expression. Previous studies have shown that pretreatment PD-L1 expression on tumor cells (Taube et al., 2014; Topalian et al., 2012) and on immune cells (Herbst et al., 2014) predicted clinical outcome on multiple tumor types. Based on a landmark trial KEYNOTE-001, the correlation between PD-L1 expression and improved response rates, progression-free survival (PFS) and overall survival (OS) was comprehensively shown (Daud et al., 2016). The data also implied that preexisting CD8+ TILs localized in the invasive tumor margin were predictive of melanoma response to PD-1 inhibitor therapy (Tumeh et al., 2014). The positive predictive role of CD8+ TILs in
melanoma patients treated with ipilimumab has also been proven (Chen et al., 2016; Hamid et al., 2011). Another example of the predictive role of PD-L1 expression on tumor cells was shown in a study indicating that PD-L1+ melanocytes located next to TILs led to the secretion of interferon-gamma as a form of adaptive immune resistance (Taube et al., 2012).

Even though baseline PD-L1 expression is widely used as a biomarker in clinical practice, its expression is dynamic and may change over the course of clinical treatment (Vilain et al., 2017), raising questions over the rationale of the sole use of baseline expression. Furthermore, patients with PD-L1 negative tumors can benefit from PD-(L)1 inhibitor therapy also. In addition, PD-L1 expression as a biomarker is valid for patient selection only in particular cancers such as NSCLC and urothelial cancers.

Two predictive classification schemas on the interactions between tumor and host immunity in the tumor microenvironment have been proposed. The first one is based on four different types of TME reflecting the PD-L1 status and the presence or absence of TILs (Teng, Ngiow, Ribas, & Smyth, 2015), type I with TIL+/PD-L1+ being the most likely to respond to PD-(L)1 immune checkpoint blockade. The second framework classifies cancers into T cell-inflamed (also called “hot”) tumors versus non-inflamed (or “cold”) tumors (Gajewski, 2015), where the T cell-inflamed tumor-type contributes to a higher probability of response to ICIs.

Another clinically relevant, although rarely usable biomarker used to predict outcome and guide patient selection for ICI therapy is microsatellite instability (MSI) which is a condition of genetic hypermutability that results from impaired DNA mismatch repair (MMR). Approximately 5% of solid tumors are characterized by either the presence of MSI or by the absence of one or more MMR proteins, implying dMMR status. MSI/dMMR has been shown to possess positive predictive value for treatment benefit from the PD-1 blockade (Le et al., 2015; Le et al., 2017). MSI is the first biomarker used to select patients for PD-1 inhibitor therapy irrelevant of tissue/tumor type with FDA approval of pembrolizumab after first-line therapy for metastatic, MSI-high tumors.

Tumor mutation burden (TMB) and neoantigen load are biomarkers similar in concept to MSI, though they have not been validated independently to predict response. TMB is a measurement of mutations carried by tumor cells (Alexandrov et al., 2013). There is data demonstrating the association of higher TMB to improved survival in patients receiving ICIs (Samstein et al., 2019). In clinical practice, the usability of TMB as a predictive biomarker is questionable due to the
somewhat variable definitions of high TMB, and along with the price and technical requirements of its’ determination.

As a part of mutational evolution on tumors, new DNA sequences are generated which may encode neoantigens with immunogenic peptides that are recognized by T cells fostering the sensitivity to immune checkpoint blockade. The concept of mutational landscapes with distinct immune-activation gene-expression signatures predicting responsiveness to PD-(L)1 blockade (Ayers et al., 2017; Hugo et al., 2016; McGranahan et al., 2016; Rizvi et al., 2015), and CTLA-4 antibody therapy has been established (Shukla et al., 2018; Snyder et al., 2014; Van Allen et al., 2015).

Liver kinase 1 (LKB1), also known as serini/threonine 11 (STK11), is a tumor suppressor gene that is inactivated for example in NSCLC and melanoma (Guldberg et al., 1999; Sanchez-Cespedes et al., 2002). In NSCLC, mutations in LKB1/STK11 have been shown to be related to a lack of response to immunotherapy. Studies have hypothesized that this might relate to a specific immune environment linked to LKB1/STK11 tumors (Koyama et al., 2016; Mansuet-Lupo et al., 2016; Pecuchet et al., 2017). In a study investigating prognostic factors for treatment benefit from combination immunotherapy among NSCLC patients by Hellman et al, the researchers showed that none of the patients with STK11 mutations responded to therapy (Hellmann et al., 2018). Researchers stated that STK11 was exclusively associated with resistance which aligns with previous studies showing association of these variants to specific T cell excluded phenotypes (Skoulidis, F. et al., 2015). Furthermore, there is data showing that NSCLC patients with STK11 mutations neither benefit from combination therapy of chemo and anti-PD-1 nor from single-chemotherapy (Skoulidis, Ferdinandos et al., 2019) which emphasized the negative prognostic value of the mutated STK11. In addition, concurrent mutations among NSCLC patient in STK11 and KEAP1 are associated with inferior treatment responses to ICIs despite other favorable molecular features such as high TMB (Arbour et al., 2018). The KRAS mutation is the most common oncogenic aberration in NSCLC with up to 30% incidence in patients with adenocarcinoma in Western countries (Murray et al., 2008), and the vast majority of the KRAS mutations in lung adenocarcinoma happen in the codons 12 and 13 with the most common subtypes including G12C, G12V and G12V (Villaruz et al., 2013). A phase I trial assessing the safety and efficiency of AMG 510 as a second-line treatment in KRASG12C mutation positive NSCLC patients, has shown promising results also in combination with ICIs (Fakih et al., 2019).

Pre-clinical findings suggest negative predictive value of deletion of IFNy receptors (Ifngr1 and Ifngr2) and JAK/STAT pathway components (Jak1, Jak2,
Stat1) that resulted in resistance to the PD-1 blockade. According to the study, IFNγ-pathway-deficient tumor cells had significant growth advantage over wild-type tumor cells when exposed to interferon-γ (IFNγ) or interferon-β (IFNβ). Furthermore, tumor cells deficient in Stat1, Jak1 or Ifngr1 failed to upregulate MHC-I presentation molecules after IFNγ stimulation. The results suggested decreased sensitivity to the effects of cytotoxic T cells of Stat1-null tumor cells. In addition, it was shown that deletion of Ptpn2 which encodes a protein tyrosine phosphatase that regulates a range of intracellular processes including IFNγ signaling, markedly increases the response of tumors to immunotherapy (Manguso et al., 2017). The tyrosine phosphatase encoded by Ptpn2 can inhibit the IFNγ signaling by dephosphorylating STAT1 and JAK1 (Kleppe et al., 2010; Kleppe et al., 2011; Pike & Tremblay, 2016).

Blood-based biomarkers

Blood-based biomarkers identified so far for treatment benefit of immune checkpoint blockade are mainly markers of systemic inflammation. C-reactive protein (CRP) has been used to make prognostic and predictive determinations of clinical outcome in cancer patients treated with ICIs (Hopkins et al., 2017; Naqash et al., 2018; Simeone et al., 2014). Other widely acknowledged markers for systemic inflammation with prognostic value include neutrophil-to-lymphocyte ratio (NLR) and lactate-dehydrogenase (LDH). NLR is a marker for the general immune response to various stress stimuli, and it is shown to predict outcome among NSCLC and melanoma patients treated with PD-1 inhibitors (Bagley et al., 2017; Diem et al., 2016; Gibney, Weiner, & Atkins, 2016; Hopkins et al., 2017; Soyano et al., 2018), and CTLA-4 antibodies (Diem et al., 2015; Gibney et al., 2016; Hopkins et al., 2017; Zaragoza et al., 2016). Elevated LDH level is a classic inflammatory marker in patients with cancer. High baseline levels of LDH are linked to poor survival and to inferior response to ICIs on melanoma and NSCLC patients (Martens et al., 2016; Mezquita et al., 2018).

Other potential biomarkers include TCR diversity and clonality (Postow et al., 2015; Snyder et al., 2017), circulating immune cell subsets (Huang et al., 2017; Kitano et al., 2014; Weber, J. et al., 2016), serum protein signatures associated with acute phase, complement, and wound-healing pathways (Weber, J. S. et al., 2018), and soluble PD-L1 (sPD-L1) (Zhou et al., 2017).
Microbiome and host genetics

There is evolving evidence that the gut microbiome has both prognostic and predictive value to treatment benefit from PD-(L)1 blockade (Gopalakrishnan et al., 2018; Matson et al., 2018; Routy et al., 2018; Sivan et al., 2015), and in melanoma patients treated with ipilimumab (Chaput et al., 2017). The role of human leukocyte antigen (HLA) class I genotype variations (Chowell et al., 2018), and germline single-nucleotide polymorphism (SNP) of the PD-L1 gene as biomarkers is unconfirmed (Nomizo et al., 2017).

2.3.4 Side effects of immune checkpoint inhibitors

The toxicity spectrum of immune checkpoint inhibitor therapies is wide and inadequately characterized (Le Burel et al., 2017; Pillai et al., 2018). Immune checkpoint inhibitors act through inhibition of T-cell blocking which results in T-cell mediated cancer cell death. Approximately 15% of patients receiving immune checkpoint inhibitor monotherapies have been reported to have severe Grade 3-4 side effects, and about 30% face lower grade adverse events. NCI’s Common Terminology Criteria for Adverse Events (CTCAE) is a standardized set of criteria for adverse events of the drugs used in cancer therapy, where Grade refers to the severity of the adverse event. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each adverse event based on following the general guideline where Grade 1 is a mild symptom and clinical or diagnostic interventions are not indicated and Grade 4 is a life-threatening condition requiring urgent interventions. The scale goes from zero to five (Grade 5, death), and not all grades are appropriate for all adverse events.

The side effects of immune checkpoint inhibitors resemble autoimmune disease due to the mechanism of action with activated T-cells attacking healthy tissues. Timing of side effects differs from traditional cancer medical therapy and they can occur from months to even years after therapy initiation or after discontinuation of therapy (Li et al., 2017; McDermott et al., 2015; Weber, J. S. et al., 2017). At present, diagnostic measurements aim to exclude other possible causes for symptoms suspected to be related to ICIs because there are no feasible means to confirm immune-related side effects, besides by the response to given immunosuppressive medication. Laboratory test and other possible diagnostic interventions based on the organ of suspicion as origin of symptoms are to be scheduled with low threshold. Neither are there any clinically relevant factors to
predict side effects. Tissue biopsies are to be considered with higher Grade 3-4 toxicities if there is diagnostic doubt and management would be altered by the outcome (Haanen, J. B. A. G. et al., 2018).

**Treatment of side effects**

Common side effects of immune checkpoint inhibitors are skin rash, fatigue, endocrine toxicity, GI-toxicity, and hepatitis. Immune-related adverse events (irAEs) associated with immune checkpoint inhibitors are consistent across tumor types, in general. Some of the side effects can be life-threatening such as cardiovascular toxicities (Ball et al., 2019). However, in most cases early detection, delaying or stopping immune checkpoint therapy, and initiation of immunosuppressive medication, typically corticoids, can resolve or prevent further worsening of side effect (Haanen, J. B. A. G. et al., 2017; Puzanov et al., 2017; Spain et al., 2016; Wang et al., 2018).

As a general rule, in the occurrence of NCI-CTCAE Grade 2 side effects immune checkpoint inhibitor therapy is delayed until they resolve, with Grade 3-4 side effects therapy is discontinued and medical treatment interventions are initiated. Though, with endocrine toxicities, treatment discontinuation is not considered necessary even with symptomatic patients but instead hormone replacement therapies are warranted. Other possible medications in the treatment schemas are chosen based on the affected organ; for example, in the case of suspected central neurological toxicity, empiric antiviral and bacterial treatments are recommended (Haanen, J. B. A. G. et al., 2017).

**Correlation of side effects to treatment response**

There is growing evidence that patients treated with immune checkpoint inhibitors developing side effects due to the given treatment are more likely to benefit from the therapy (Fujii et al., 2018; Liew et al., 2019; Martini et al., 2018; Sznol et al., 2017). However, there are many open questions concerning the strength of that correlation, as well as the incidence of symptoms with respect to the duration of the therapy which might cause bias.
2.4 Symptom burden of cancer

Symptoms such as fatigue, pain, weakness, dyspnea, nausea, and insomnia are commonly observed among cancer patients with advanced disease (Barbera et al., 2010; Walsh, Donnelly, & Rybicki, 2000). Such symptoms have been shown to cause poor quality of life and psychological stress (Chang, V. T., Hwang, Feuerman, & Kasimis, 2000; Cooley, Short, & Moriarty, 2003). However, many physical and psychological symptoms of patients with cancer are often under-recognized by their clinicians based on large data from three randomized trials (n= 1,090) where toxicity rates reported by physicians were always lower than those reported by patients. For patients who reported toxicity of any severity, under-reporting by physicians ranged up to 74.4% (Di Maio et al., 2015). Furthermore, studies show that clinicians often fail to reliably detect their patients’ symptoms and frequently underestimate the severity of the symptoms (Basch et al., 2009b; Basch, Barbera, Kerrigan, & Velikova, 2018).

Patients’ symptom burden also correlates to their use of health care services (Brooks et al., 2014; Prieto et al., 2002). In addition, cancer patients experiencing symptoms related to pain, fatigue and nausea, often require hospitalization (Numico et al., 2015). There is evidence that patients with a higher symptom burden have a longer duration of hospitalization and higher risk for hospital readmissions (Brooks et al., 2014; Nipp et al., 2017; Prieto et al., 2002). It is fair to say that the symptom control of a cancer patient should be improved not only to foster cancer care but also from the perspective of healthcare resource utilization.

2.4.1 Patient-reported outcomes

Patient-reported outcomes (PROs) consist of health-related questionnaires related to health care or treatment directly reported by the patient without interpretation of the patient’s response by a clinician. PROs can be general or condition-specific, and the data can be used for example in evaluation of health outcome measures (www.ichom.org) or as a part of drug approval processes (Kluetz, O’Connor, & Soltyš, 2018). PROs can be captured by traditional paper questionnaires or by web-based approaches. Web-based reporting of PROs has many advantages compared to paper questionnaires such as reducing limitations of time and location. These advantages make web-based PRO capturing more likely to better follow changes in symptoms or quality of life (QoL). Furthermore, web-based PROs, or electronic PROs (ePROs) in all, can be coupled to an urgency algorithm which sends an alert
to the care unit on severe or altering symptoms of a patient. This enables rapid reaction to and treatment of important medical events. A systematic PRO collection for audit and benchmarking purposes is another attempt to assess patient-centred health gain enabling a wider perspective for health outcomes measuring in real-world settings (Calvert, O'Connor, & Basch, 2019).

2.4.2 (e)PROs in the follow-up of cancer patients

In the context of a patient-centered care approach, health-related quality of life (HRQoL) has gained increasing importance (Aaronson et al., 1993; Cella, Chang, Lai, & Webster, 2002; Mauer, Bottomley, Coens, & Gotay, 2008; Priestman & Baum, 1976; Quinten et al., 2009; Quinten et al., 2014; Spitzer et al., 1981; Zikos et al., 2015). Patient-reported outcomes provide clinicians data that can play an essential role in identifying given treatment toxicities and improving symptom management in routine clinical practice, and as a prognostic factor for survival in clinical trials (Atkinson et al., 2016; Basch et al., 2005; Basch, Rogak, & Dueck, 2016; Basch et al., 2017b; Greenhalgh et al., 2018; Howell et al., 2015; Maillet, Gan, Blay, You, & Peron, 2016; Patel et al., 2018; Sivendran et al., 2014). The implementation of PROs combined with patient-centered interventions has already been shown to be associated with better HRQoL, fewer hospitalizations and even increased survival compared to standard care in the follow-up of lung cancer patients and patients receiving chemotherapy for advanced cancers (Basch, Dueck et al., 2017; Jordan et al., 2018).

In recent years, ePROs have been studied in oncological care, mainly in outpatient settings (Basch et al., 2016; Berry et al., 2014; Strasser et al., 2016) but a pilot randomized trial of an electronic symptom monitoring intervention for hospitalized patient with cancer has just been reported (Nipp et al., 2019). Web-based applications coupled with an urgency algorithm have been developed to monitor cancer patients and currently, the most convincing data exists on patients receiving chemotherapy or undergoing follow-up for lung cancer (Basch et al., 2016; Denis et al., 2017). ePROs have been shown to improve quality of life, decrease emergency clinic visits, and improve ECOG (Eastern Cooperative Oncology Group) performance status and the number of patients receiving active cancer treatments at disease progression (Denis et al., 2017) (Basch et al., 2016; Denis et al., 2017; Velikova et al., 2004). Furthermore, use of ePROs in patient monitoring has shown impressive improvements in overall survival compared to standard follow-up (Basch et al., 2017; Denis et al., 2017). Increasing use of
smartphones and apps in the general population supports the idea of collection of individual health data based on such communication channels (Benze et al. 2017).
3 **Aims of the present study**

1. To study whether early discontinuation of even a short PD-1 inhibitor treatment period in responding metastatic cancer patients can lead to long term tumor responses. (I)
2. To evaluate the correlation of pretreatment CRP values and NLR ratio to survival outcomes among cancer patients receiving PD-1 inhibitors. (II)
3. To investigate whether symptoms collected by the ePRO tool on cancer patients receiving immune checkpoint inhibitors follows to symptoms reported in clinical trials aiming to validate the questionnaire and whether coupling of specific symptoms occur. (III)
4. Spectrum of patient-reported symptoms and their severity (IV)
5. Etiology of urgency algorithm alerts triggered by immunological treatment side effects questionnaire (IV)
6. Correlation of PROs to treatment benefit (IV)
7. Correlation between PROs (IV)
8. Patient compliance with ePRO surveillance during treatment period according to response rates of Patient experience questionnaire and immunological treatment side effects questionnaire (IV)
4 Materials and methods

All data collection was carried out according to national legislation and under permit from the medical director of each research center (I: Oulu University Hospital (299/2016); II: Oulu University Hospital (299/2016), Helsinki University Hospital (HUS/395/2018, Kuopio University Hospital (112/2018, 192/2018), Tampere University Hospital (R18612); III: permits from Kaiku Health, Docrates Cancer Center and Oulu University Hospital Ethics committee (9/2017); IV: Oulu University Hospital Ethics Committee (9/2017), Valvira n:o 361). Anonymization was performed before data analysis. Individual informed consents were not sought due to the register nature of the studies I, II, and III. Study IV was registered at the Clinical Trials Register (http://clinicaltrials.gov) with identifier code NCT03928938.

4.1 Patients receiving PD-1 inhibitors (I)

We retrospectively collected all patients who had received at least one dose of intravenous PD-1 inhibitor therapy at Oulu University Hospital (2014-2018) from pharmacy records. Demographic and disease specific information was also included in the data collection from the electronic patient records. Patients whose PD-1 treatment was discontinued in at least at stable disease (SD) response because of adverse events, or maximum institutional recommended treatment length (six months), and patients with complete response were subjects to IO-therapy-free survival. Progression-free survival (PFS) and overall survival (OS) were calculated from the first date of PD-1 inhibitor treatment to documented tumor progression, death or end of follow-up (PFS), or to death or end of follow-up (OS). Tumor progression and/or death were counted as events. IO-therapy-free survival was calculated from the last dose of PD-1 inhibitor therapy to next treatment regimen, death or end of follow-up, first two counted as events. Tumor responses were retrospectively analyzed from electronic health care records by two independent investigators (SI and JPK) with 100% concordance according to RECIST criteria.
4.2 Patient cohorts of the study evaluating predictive value of C-reactive protein for PD-1 inhibitors (II)

4.2.1 Discovery cohort

All patients who had received at least one dose of intravenous PD-1 inhibitor treatment at Oulu University Hospital (Finland) 8/2014-9/2018 were retrospectively collected together with clinical variables (age, cancer type, TNM staging, ECOG, and blood sample results). Laboratory values four weeks prior and two weeks post from the first PD-1 inhibitor infusion were included. In case of multiple values, the closest laboratory value to the first PD-1 inhibitor therapy was selected. If blood samples were taken during an acute infection, another pre- or post-infection value was chosen which aligned in time the closest to the first PD-1 infusion. All the laboratories responsible for analysing CRP values used Finnish Accreditation Service accredited (SFS-EN ISO 15189) immunoturbidometric test. Progression-free survival and overall survival were calculated, tumor progression and/or death counted as events.

4.2.2 Validation cohort

The validation cohort consisted of cancer patients treated with PD-1 inhibitors during 3/2015-9/2018 in three Finnish university hospitals: Tampere, Helsinki, and Kuopio. All the subjects included had received at least one dose of PD-1 inhibitor treatment and had CRP values available as for the discovery cohort. All the laboratories responsible for analysing CRP values used Finnish Accreditation Service accredited (SFS-EN ISO 15189) immunoturbidometric test.

4.3 Patient cohort followed with web-based application for symptoms and Quality of Life (QoL) receiving PD-1 inhibitors (III)

All the patients included in the study had cancer and were treated with immune checkpoint inhibitor therapy at Docrates Cancer Center (Helsinki, Finland) and Oulu University Hospital (Oulu, Finland) in an outpatient setting 4/2017-9/2018, and they were followed with the ePRO module. Included patients filled out at least one symptom questionnaire during the follow-up period. Of the clinical variables, the registry included only age and sex of a patient.
4.4 Study population in the prospective one-arm multicenter trial for cancer patients receiving immune checkpoint inhibitors (KISS) (IV)

All patients signed a written informed consent prior to participating in the study.

4.4.1 Inclusion criteria

1. Signed informed consent
2. Advanced cancers
3. Immune checkpoint inhibitor therapy initiated within +/- 2 weeks
4. Age ≥18y
5. ECOG 0-3
6. Patient compliant with the study procedures

4.4.2 Exclusion criteria

1. Immune checkpoint inhibitor therapy initiated > 2 weeks ago
2. General vulnerability affecting the participation in the trial
3. No internet access

4.5 Methods

4.5.1 ePRO tool on cancer patients receiving immune checkpoint inhibitors (III, IV)

Symptom Questionnaire (III, IV)

The current ePRO tool is a web-based solution scaled to be used in smartphones and home computers. The IO-module developed by Kaiku Health consists of 18 questions. The symptoms selected for the symptom tracking tool for cancer immunotherapy are based on the most common adverse events that have occurred during clinical trials of anti-PD-1, anti-PD-L1 and anti-CTLA-4 antibody monotherapies. The symptoms tracked by the instrument are potential signs and symptoms of immune-related adverse events. The symptom selection is based on the reported publications of the following clinical trials: CheckMate 017,
CheckMate 026, CheckMate 057, CheckMate 066, CheckMate 067, KEYNOTE-010, and OAK. FDA labels for nivolumab, pembrolizumab and atezolizumab were also used in the symptom selection for the instrument. The questions for each symptom in the instrument were developed based on NCI-CTCAE v4.03 register by converting the description of the grades into a patient-friendly language e.g. using terms like pain in joints instead of arthralgia. The readability of the symptom questionnaire was assessed by cancer patients receiving ICIs in Oulu University Hospital outside the trials (III, IV).

Questions assess presence of blood in stool, blood in urine, blurred vision, chest pain, cough, loss of appetite, diarrhea, dizziness, fatigue, fever, headache, itching, nausea, other symptoms, pain in joints, rash, shortness of breath, stomach pain, and vomiting. Besides recording the presence of a symptom, the application has a severity algorithm that grades the symptom according to NCI-CTAE v. 4.03 protocol.

Patients were requested (via e-mail) to fill in online symptom questionnaires at 3-7 days intervals aiming to evaluate the optimal frequency during the pilot study (III). In the KISS trial, patients were supposed to answer the 18 question immunological treatment side effects questionnaire before initiation of the treatment or within 2 weeks, and thereafter weekly or symptom-based up to disease progression or 24 weeks. Email reminders were sent on a weekly basis (IV).

**Urgency algorithm (IV)**

Besides recording the presence of a symptom, the application has a severity algorithm that grades the symptom according to NCI-CTCAE v.4.03 protocol. If algorithm analysis suggests the presence of a Grade 3 or higher symptom, or if the patient has repeatedly reported a Grade 2 symptom, demanding an evaluation as to whether the ICI therapy should be discontinued, an alert will be sent to the care unit after which the care unit contacts the patient, and if necessary, further investigation and/or assessments will be ordered. The patients were informed that the care unit reacts to alerts promptly but no later than three days after an alert. The patient can be contacted by messaging through the software or phone call. Furthermore, it was stated that ePRO follow-up was intended for non-urgent matters.
4.5.2 QoL assessment (III)

QLQ-30

The European Organization of Research and Treatment of Cancer (EORTC) assembled a Quality of Life Group in 1980 to enhance the assessment of QoL in clinical trials, which orchestrated the development of a core questionnaire, QLQ-C30, supplemented with disease-specific questionnaires (Fayers, Bottomley, EORTC Quality of Life Group, & Quality of Life Unit, 2002). QLQ-C30 is a validated instrument for measuring cancer patients’ symptoms and QoL in clinical trials as well as in palliative care (Aaronson et al., 1993). The core QLQ-C30 questionnaire comprises five functional scales (physical, role, cognitive, emotional, social), one item on health-related QoL, five single items (constipation, diarrhoea, sleep, dyspnoea, financial problems) and three symptom scales (fatigue, pain, nausea/vomiting). Patients were requested to fill in electronic QLQ-C30 questionnaires with 1-2 months interval (III).

4.5.3 Patient compliance (IV)

Patient compliance with ePRO surveillance during the treatment period is evaluated based on response rates to the Patient experience questionnaire, and immunological treatment side effects questionnaire. The Patient experience surveys consists of six questions concerning the usability and feasibility of the ePRO tool and was developed for the trial. Patients were asked to answer every four weeks up to disease progression or 24 weeks, and email reminders were sent to the patients to fill in the survey.

4.6 Statistics

IBM SPSS Statistics 24.0.0.0 for Windows was applied for statistical analysis. Survival was analyzed by using the Kaplan–Meier method with the log-rank test. Probability values below 0.05 were considered significant. ROC curves were calculated for CRP and NLR to define the optimal cut-off-point. Multivariate analysis was performed using Cox regression analysis (II). Correlations of different patient-reported symptoms were analyzed using heat maps, and the same method was also applied to correlation analysis between QoL scales (QLQ-C30) and patient-reported symptoms (III). In the heat map analysis, he intensity of the color
signifies the level of correlation; red negative, blue positive correlation A strong correlation ratio is defined as > 0.5 or <-0.5; intermediate 0.5-0.3 or -0.5- -0.3; weak 0.3-0.1 or -0.3- -0.1; very weak 0.1- -0.1 (III, IV).

4.6.1 Statistical plan (IV)

Power assessment

Due to the study nature (one-arm study) statistical plans are not calculated. Approximately 15% of patients receiving immune checkpoint inhibitor therapies have been reported to have severe grade 3-4 side effects, and about 30% face lower-grade adverse events. In a patient cohort of 40, three to six patients will experience a severe iAE. It is estimated that the expected study population is sufficient to evaluate the feasibility of the symptom questionnaire in detecting severe adverse events and directing patients to further investigations. Questionnaires from several timepoints are estimated to be collected from 90% of the study population (~35 patients) which will enable a more comprehensive assessment of feasibility, patient experience and correlation of ePRO changes to treatment response and survival.

Data analysis

Data analysis will be carried out when the length of follow-up time of the last recruited patient is at least 12 weeks. For the final analysis statistical methods will be redefined based on actual distributions.
5 Results

5.1 Early PD-1 therapy discontinuation in responding metastatic cancer patients

This current retrospective study investigated PD-1 treated patients whose therapy had been discontinued in response in Oulu University Hospital 2014-2018. All patients who had received at least one dose of intravenous anti-PD-1 were retrospectively identified from the pharmacy records. In all, 59 patients were subjects in this study. The cohort included patients with melanoma (n=23), lung cancer (n=22) and GU cancers (n=14). Median age of patients was 64 years and the majority of the patients were male, most of them (n=57) ECOG 0-1 performance status. Anti-PD-1 therapy was given as a first-line therapy for three lung cancer patients (13.6%) and for nine melanoma patients (39.1%) while all the GU cancer patients received treatment in the second or later line.

Median duration of IO therapy was 3.0 months (CI 2.2-3.8). Four patients (6.8%) had CR as a best response, 11 (18.6%) had PR, and 14 (23.7%) SD. 29 patients (49.2%) had progressive disease. Median PFS was 4.0 months (CI 2.4-5.6) and median OS 17.0 months (CI 11.8-22-2). Median OS for different tumor types were 27.0 months (CI 12.6-41.4) in melanoma, 11.0 months (CI 9.0-15.0) in lung cancer and not reached for GU cancers. Grade 3 or higher treatment related adverse events occurred in eight patients (13.6%) which included anaphylaxis (n=2), hepatitis (n=2), type 1 diabetes (n=2), colitis (n=1) and pneumonitis (n=1).

17 patients had PD-1 therapy discontinued in response, most of them because of reaching the maximum, institutional recommended PD-1 therapy length (n=12, 70.6%). Other reasons were adverse events (n=4) or complete response (n=1). Patients whose therapy was discontinued in response experienced a long IO-free survival of median 12 moths and only six patients (35.3%) required therapy re-initiation during the follow-up. Of the six patients whose therapy was continued after IO therapy-free period in progressive status, three were re-exposed to PD-1 agents and none responded, and three had radiotherapy as a next line of treatment.

We also investigated alternative response evaluation criteria which outperformed conventional RECIST 1.1 in predicting IO-free survival. These modified response assessment criteria, however, were not better than RECIST 1.1 or iRECIST in prediction of OS. Our modified response criteria, however, were superior compared to RECIST 1.1 or iRECIST in predicting IO-therapy-free time.
5.2 Elevated CRP levels indicate poor progression-free and overall survival in cancer patients treated with PD-1 inhibitors

PD-1 inhibitors are standard of care treatments in various cancers but predictive factors for therapy selection are limited. In the current study, we hypothesized that markers of systemic inflammation would predict adverse outcomes in multiple cancers treated with PD-1 inhibitors.

A total of 73 patients treated with single agent anti-PD-1 therapy for advanced cancers in 2014-2018 at Oulu University Hospital Oncology Department were assessed for eligibility, and 56 of them were included in the final analysis. Median age was 66.0 and the majority of the patients were male (73.2%). The discovery cohort included patients with melanoma (n=23), GU cancers (n=17) and NSCLC (n=16). A ROC curve was calculated to define the optimal cut-off point of CRP in the cohort, and the value 9.50 mg/l was rounded up to 10 which is an validated upper limit normal (ULN) rank for CRP in most laboratories.

In the discovery cohort the median PFS was 4.0 months (CI 2.4-5.6) and there was a statistical difference (p=0.05) between patients with CRP≤10 (7.0 months, CI 2.9-11.1) and CRP>10 (2.0 months, CI 1.6-2.4). Median OS for the whole discovery cohort was 17.0 months and there was a statistical difference (p=0.000004) between patients with CRP≤10 (not reached) and CRP>10 (10.0 months, CI 6.8-13.2). In subgroup analysis per tumor type, there was a statistically significant difference in PFS (p=0.03) and OS (p=0.0001) according to CRP in melanoma but not in GU cancers or NSCLC. However, a similar tendency for improved survival was seen, respectively.

We also analyzed survival according to neutrophil-to-lymphocyte ratio (NLR) in the discovery cohort, and a ROC curve was calculated to define the optimal cut-off point of 2.65. Median PFS for the whole cohort was 4.0 months (CI 1.8-6.3) and there was a statistical difference (p=0.02) between patients with NLR≤2.65 (7.0 months, CI 4.4-9.6) and NLR>2.65 (2.0 months, CI 1.5-2.5). Median OS for the whole cohort was 19.0 months (CI 9.3-28.7) and there was a statistical difference (p=0.009) between patients with NLR≤2.65 (19.0 months, CI 15.0-23.0) and NLR>2.65 (7.0, CI 0.0-15.1). We also calculated PFS and OS with NLR 5.0 defined as an optimal cut-off point in previous studies and the results showed statistically non-significant PFS and OS. The Cox regression model was used to evaluate the dependence between CRP and NLR ratio. In multivariate analysis, these inflammatory biomarkers were non-independent.
We also investigated other blood-based markers suggestive of systemic inflammation and previously linked to poor survival and benefit from anti-PD-(L)1 agents. We analyzed the correlation of LDH, total leucocytes, and total lymphocytes to PFS and OS but none of these markers predicted survival in the cohort.

The validation cohort consisted of 107 cancer patients treated with single anti-PD-1 therapy in three other Finnish university hospitals between March 2015 and November 2018. The cohort consisted of patients with melanoma (n=44, 41.4%), NSCLC (n=42, 39.3%), RCC (n=13, 12.1%), bladder cancer (n=4, 3.7%), and other cancers not specified (n=4, 3.7%). Median PFS for the whole cohort was 7.0 months (CI 4.1-9.9) and there was a statistical difference (p=0.0000008) between patients with CRP ≤ 10 (17.0 months, CI 10.2-23.8) and CRP > 10 (3.0 months, CI 1.9-4.1). Median OS for the whole cohort was 19.0 months and there was a statistical difference (p=0.000006) between patients with CRP ≤ 10 (not reached) and CRP > 10 (12.0 months, CI 7.6-16.4). In subgroup analysis for melanoma, GU cancers (RCC and bladder) and NSCLC, there was a statistically significant difference in PFS (p=0.000008) and OS (p=0.002) according to CRP in melanoma and NSCLC PFS (p=0.03) and OS (p=0.006) but not in GU cancer, though, a similar tendency for improved survival was seen in GU cancers.

5.3 ePROs in the follow-up of cancer patients treated with immune checkpoint inhibitors: a retrospective study

Prior to our study, there were no published works investigating ePRO follow-up approach on cancer patients treated with ICIs. The current study investigated symptoms collected by the ePRO tool on cancer patients receiving ICIs and their correlation to data presented in clinical trials and coupling of the reported symptoms. The study hypothesizes were that ePRO collection of symptoms would be similar or higher compared to clinical trials and certain symptoms would co-occur.

A total of 37 patients with median age of 61 were included in the study, the majority of them male (64.9%). The ePRO tool was used to follow these 37 patients treated with ICIs in Docrates Cancer Center and Oulu University Hospital who had filled in altogether 559 symptom questionnaires focusing on known immunologically related adverse events. During the follow-up, 133 QoL questionnaires were filled in. There was good compliance to ePRO surveillance up to 25 weeks from baseline. The answering rate for symptom questionnaires was
highest at 3-4 weeks with 73 filled questionnaires, 11-14 weeks (n=62) and at baseline (n=51). The median number of filled symptom questionnaires was 11 per patient (CI 1-47; SD 12.3). All the patients had QoL questionnaires filled at baseline, but the answering rate was much lower at later timepoints analyzed. Median number of filled QoL questionnaires was two (CI 1-18; SD 3.47) and the answering rate was highest at 11-14 weeks.

At the time of analysis, the majority of the patients (n=28) had over 12 weeks from the first filled symptom questionnaire and 19 of those 28 had continued symptom reporting for over 12 weeks suggesting a good adherence to ePRO follow-up. One patient out of 28 (3.6%) had the highest reported symptoms at severity Grade 0, seven (25%) at Grade 2, and 20 patients (71.4%) at Grade 3 or over. According to reported symptom severity, patients with Grade 0 had an average 0.1 questionnaires filled per week, Grade 2 patients 0.65 questionnaires per week, and Grade 3 patients 0.66 questionnaires per week.

Patients were asked via email to fill in symptom questionnaires at the baseline and thereafter at 3-7 days frequency. Reported symptoms were categorized by a severity algorithm of the application (Grade 0-4) and grouped timely to baseline, 12 and 24 weeks. The most common reported Grade 1-2 symptoms were fatigue (47%), shortness of breath (31%) and cough (29%). Of the Grade 3-4 symptoms, loss of appetite (5%), other symptoms (5%) and chest pain (3%) were the most frequent. Email reminders to patient about QoL questionnaires to be filled were sent out initially and thereafter, at 1-2 months frequency. In general, there was a tendency for improvement in all the scales from baseline to 12 and 24 weeks.

Heatmap analysis was used to analyze correlations of different patient reported symptoms. The strongest positive correlations were seen between itching and rash, and between nausea and vomiting. In addition, positive correlations were seen between nausea, loss of appetite and stomach pain; and cough and shortness of breath. Analyses did not show a high level of negative correlations between individual symptoms. Interestingly, negative correlations were seen between certain groups of symptoms. Rash, itching, joint pain and diarrhea negatively correlated with cough, shortness of breath and chest pain. With the same method, we also analyzed correlations between QoL scales (QLQ-C30) and patient reported symptoms. According to the results, lower QoL scale and lower global health status had the strongest correlation with fatigue, loss-of-appetite, nausea and dizziness.
5.4 Follow-up of Cancer Patients Receiving Immune Checkpoint Inhibitor Therapy by Electronic Patient Reported Outcomes-tool (KiSS): a prospective study

The addition of electronic patient reported outcome (ePRO) to standard follow-up has been shown to improve survival and QoL of cancer patients receiving chemotherapy. In this prospective one-arm multi-institutional study, we investigated whether ePRO follow-up of cancer patients treated with ICIs is feasible. The study analyzed the variety of patient-reported symptoms, etiology of the alerts generated by the urgency algorithm of the ePRO tool, symptom correlations, and patient compliance.

The current study recruited adult cancer patients whose advanced cancer was treated with single anti PD-(L)1 therapy in outpatient settings. The ePRO tool consisted of a weekly questionnaire of 17 questions evaluating the presence of typical side effects of ICIs with algorithm assessing the severity of the symptom according to NCI-CTCAE v.4.03, and an urgency algorithm with preset limits sending alerts to the care team. During the treatment phase patients received email notification to fill in the electronic symptom questionnaire ≤2 weeks from the first anti-PD-(L)1 infusion and weekly thereafter. They were asked to fill in a monthly electronic patient experience survey. Both questionnaires were supposed to be completed until treatment discontinuation or six months of follow-up.

Patient recruitment took place between June 2017 and March 2019, and the last study patients visit was in June 2019. Anticipated recruitment for the study was 40 patients in 12 months but due to the slow recruiting pace, the period was extended. Altogether 43 patients signed the informed consent to participate. Data analysis was carried out when the last patient included had 12 weeks of follow-up data available. A total of 37 patients who had PD-(L)1 therapy initiated and had at least two symptom questionnaires (baseline and following) answered were included in the final analysis. Median age of the study participants was 62 (range 32-80). The majority of the patients were male (n=27), and five patients had history of an autoimmune disease, hypothyreosis (n=4) being the most common. Tumor types included lung cancer (n=15), melanoma (n=9), genito-urinary cancer (n=9), and head and neck cancer (n=4). 75.7% of patients had stage IV disease.

During the study, 889 filled symptom questionnaires were registered. The range of answered questionnaires was 0.583-1.27 per patient/week with high answering rate throughout the whole follow-up period up to 24 weeks. During the first 12 weeks of ePRO follow-up, the most common Grade 1-2 symptoms were
fatigue (39%), cough (21%), pain in joints (18%), itching (17%), loss of appetite (17%), nausea (17%), and shortness-of-breath (15%). The most common Grade 3-4 symptoms were cough (6%), loss of appetite (4%), and nausea (4%). None of the patients reported blood in stool or hematuria.

Of the 391 symptom questionnaires answered during the first 12 weeks, the ePRO tool triggered 67 (17.1%) alerts. The most common reasons for alerts were loss of appetite, shortness of breath, pain in joints, blurred vision, and cough. The treating physicians were asked to evaluate the etiology of the alerts by grading them to cancer, treatment, or unclear categories. Unclear reasons were the most common cause of alerts (57.1%) followed by treatment (31.4%), and cancer (11.4%).

Patient compliance was analyzed by patient experience survey which was answered through the ePRO tool for the first time four weeks after patient registration and thereafter in a four-weeks cycle. After the first 12 weeks all the patients replied that using the Kaiku software was easy or very easy and only one out of six patients reported needing assistance after initial training. Over 90% of the patients reported that questions were understandable. Also, over 90% of the patients felt that ePRO follow-up improved their cancer care and that they would recommend using it in the follow-up of cancer patients.

The results of the heatmap analysis for the first 12 weeks and for all the symptom questionnaires were very similar. During the first 12 weeks, strong positive correlations were seen between nausea, diarrhea, decreased appetite, and vomiting, stomach pain and decreased appetite, and rash and itching. Only weak negative correlations were detected between cough and vomiting, and between itching and chest pain and fever. 34 of 37 patients were eligible for treatment response benefit analysis. 22 (64.7%) patients had CR, PR, or SD as the best response while 12 (35.3%) patients had PD. Heatmap analysis suggested weak positive correlations between clinical benefit (CR/PR/SD) and itching (0.23/0.25) and intermediate correlations between PD and chest pain (-0.41/-0.47).

We further analyzed the symptom progression for itching and chest pain. During the first 12 weeks, 15-23% of the patients with clinical benefit reported itching while the rate was much lower for the PD patients (0-14.3%). Furthermore, the grade average was much higher for clinical benefit (week 1-12 0.26-0.37, all 1.18) compared to PD patients (week 1-12 0-0.17, all 0.75). For the whole follow-up period, most of the patients with clinical benefit had itching (63.6%) while this was much lower for PD patients (33.3%). The severity of itching for the clinical benefit patients was mainly low-grade (Grade 1 27.3%, Grade 2 18.2%). During the whole follow-up period, chest pain was much more common on PD (58.3%)
compared to clinical benefit patients (18.2%). In the first 12 weeks, patients with PD tended to have gradually increasing chest pain grade average while conversely, a continuing decrease in the grade average was seen for patients with clinical benefit.
6 Discussion

The principal purpose of clinical research is to find effective and better ways to treat patients. According to Good Clinical Practice (GCP), an international quality standard that defines a set of standards for clinical trials involving human subjects, researchers must maximize benefits and minimize harms associated with research, and study-related risks must be reasonable compared to the expected benefits. Cancer is a possibly lethal disease and the toxicity of cancer therapies is justified in that sense. According to studies, patients with advanced cancer may have unrealistic expectations of cure for a given cancer therapy (Weeks et al., 2012). On the other hand, a recent study revealed that metastatic cancer patients treated with drugs aiming to only control disease, no longer consider these drugs worthwhile if they are experiencing severe side effects (Jenkins et al., 2018).

Whether this is the case in wider sense, is still a question, but it can be fairly stated that health-related quality of life is important in the context of a patient-centered care approach. The aim of every physician is most likely to find the best treatment for the patient. Pivotal aspects of treatment interventions in relation to expected benefit-risk ratio should be communicated thoroughly between clinicians and patients when making individual therapy decisions.

6.1 Short-term anti-PD-1 treatment

One of the aims of this study was to investigate whether non-inferior treatment responses and survival benefit can be achieved with markedly limited duration of PD-1 inhibitor therapy compared to clinical trials, in the absence of optimal treatment length for immune checkpoint inhibitors in metastatic cancers. Durable treatment responses of ICIs persisting even after treatment interruption are not totally absent with other cancer therapies (Pons-Tostivint et al., 2019); however, the incidence of such phenomenon is much higher with immune checkpoint blockade. Clinical evidence has suggested that patients can experience long-term progression-free survival if an immune checkpoint inhibitor is discontinued due to side effects in response (Li et al., 2017; McDermott et al., 2015; Robert et al., 2018; Topalian et al., 2014; Wolchok et al., 2017). In addition, a recent neoadjuvant study in the field of NSCLC demonstrated that 45% of patients showed major tumor responses even after a four-weeks PD-1 inhibitor therapy (Forde et al., 2018). Due to the institutionally limited maximal continuous PD-(L)1 inhibitor therapy of six months, our cohort provided a unique opportunity to study effects of a shorter than
until progression PD-1 inhibitor therapy length in the absence of severe immune-mediated side effects. Furthermore, our study investigated multiple tumor types making the findings more generalizable.

In our retrospective study we showed that some patients can experience long-term tumor responses even after a short anti-PD-1 treatment period. Our lung cancer cohort consisted mostly of second- or later-line (86.3%) patients with median OS of 11 months which is similar (9-12 months) to what has been seen in the second line lung cancer trials (Borghaei et al., 2015; Brahmer et al., 2015; Herbst et al., 2016; Rittmeyer et al., 2017). In addition, OS in our melanoma cohort was 27 months landing in the similar range of first-line trials of single-agent anti-PD-1 therapies (33-37 months) despite only 39% of our patients receiving the treatment in the first-line (Robert et al., 2015; Wolchok et al., 2017).

Furthermore, a single prospective study has addressed treatment length of anti-PD-1 treatment in non-small-cell lung cancer. In the trial patient were randomized to either discontinue the anti-PD-1 therapy at one-year time point or continue treatment until progression. The results of the study showed inferior progression-free survival in the discontinuation group but no overall survival benefit (Spigel et al., 2017).

Interestingly, the first phase III clinical trial investigating treatment with the CTLA-4 antibody, ipilimumab, on advanced melanoma patients had a treatment schema of induction therapy of four doses once every three weeks followed by a possible reinduction of the therapy at the 24th week if progressive disease was detected (Hodi et al., 2010). Some earlier phase II trials of melanoma patients treated with ipilimumab had also investigated an ipilimumab maintenance therapy schema with similar results (O'Day et al., 2010; Weber, J. et al., 2009; Wolchok et al., 2010). Hodin et al reported a disease control rate of 28.5% in the ipilimumab-alone group after induction therapy, and that responses continued to improve after 24 weeks. Nine patients (23.1%) from the ipilimumab-alone cohort had reinduction therapy due to disease progression, and eight of those were included in the efficacy analysis. According to Hodin et al, 64.2% of the patients in the ipilimumab-alone group received the total four doses during induction therapy, disease progression being the main reason for therapy discontinuation. These results mirror ours’ where patients treated with a short course of anti-PD-1 therapy discontinued in response had a long-term response.

The first clinical trials for anti-PD-1 therapies, like the phase I CheckMate-001 or the phase II/III Keynote-010, had a study design of continuous anti-PD-1 treatment up to 2 or 3 years in the absence of disease progression or severe,
treatment-related adverse events leading to treatment discontinuation. One could claim that the mechanism of action for immune checkpoint inhibitors is the same, activation of T cell mediated immune reactions. Based on the clinical data from previous CTLA-4 antibody trials, one could have chosen a study design of different cohorts investigating dosing not only in terms of drug concentration but also treatment durations in the spirit of ipilimumab trials with a twelve-week induction phase followed by a re-induction or maintenance treatment possibility for patients with at least SD at the twelve-week treatment response evaluation.

The response patterns observed with ICIs have some unique aspects compared to targeted therapies or chemotherapy (Borcoman et al., 2019). Since RECIST criteria were initially developed for chemotherapy response assessment, it might not capture the unique nature of immuno-oncological responses. Modified RECIST criteria (iRECIST) have been developed, although not widely used (Seymour et al., 2017a), to better assess responses to immuno-oncological therapies allowing the capture of pseudoprogression as a beneficial response. Pseudoprogression is a unique pattern of response where patients experience an objective response after having an initial disease progression, which was firstly observed in patients with advanced melanoma (Di Giacomo et al., 2009). After these initial findings, the possibility to continue immunotherapy after a RECIST-defined progressive disease was rapidly adopted in the majority of clinical trials investigating ICIs and also including tumor types other than melanoma. However, pseudoprogression is a rare phenomenon (George et al., 2016; Robert et al., 2015; Tazdait et al., 2018), meaning that in the majority of cases, radiographic progression at the first response assessment reflects true disease progression. By far, no clear predictors of pseudoprogression exist. However, there are interesting early findings on the meaning of circulating tumor DNA (ctDNA) in distinguishing pseudoprogression from true progression (Cabel et al., 2017; Lee et al., 2017). In one study, the sensitivity of ctDNA for predicting pseudoprogression was 90%, and specificity 100% among metastatic melanoma patients (n=125) treated with either PD-1 inhibitor therapy alone or with combination to ipilimumab (Lee et al., 2018).

Another response pattern unique to ICIs is hyperprogression. Per se, the phenomenon was based on clinical observations of patients whose disease seemed to grow faster after the initiation of immune checkpoint inhibitors in retrospective studies of patients (Champiat et al., 2017; Parseghian et al., 2018). Later the concept was supported by OS data from randomized trials showing survival curves crossing at three months, suggesting that immunotherapy did worse than standard treatment in some subgroups (Bellmunt et al., 2017; Borghaei et al., 2015).
Hyperprogression was shown to correlate with a worse survival in multiple studies (Rodriguez Freixinos et al., 2018; Saada-Bouzid et al., 2017). The data on biological rationale of hyperprogression is scarce. Lo Russo and colleagues (Lo Russo et al., 2019) suggested the potential role of innate immunity in the form of macrophage polarization into M2-like as a mechanism of action. Another interesting observation behind hyperprogression were changes in copy number instability (CIN) (Weiss et al., 2017). In the study, researchers showed that study patients not having a substantial decrease in the CIN score based on chromosomal instability quantification in plasma cfDNA, had an over 90% risk of progression. In addition, the study showed that in five out of six cases, the CIN score could be used to predict hyperprogression earlier than routine imaging processes. However, such methods are at present not routinely available outside clinical trials.

Therefore, we wanted to study whether alternative response criteria would better find patients benefitting from PD-1 inhibitor therapy in routine clinical practice (I). Based on our clinical experience, outcome of patients with partial response (PR) only in single assessment versus patients whose lesions are regressing in multiple assessment substantially differ. For that, we created three-class response criteria: complete response (CR) or PR with lesions regressing in at least two consecutive assessments, other PR or stable disease (SD) and progressive disease (PD) to see if this could better sort out patients for OS or IO-free survival. Our reformed response evaluation criteria could not predict OS better than RECIST or iRECIST but it outperformed both in predicting improved IO-free survival.

Outcomes of the current study (I) closely followed results seen in clinical trials in regards to survival and the side effects of PD-1 inhibitors. Based on these results, it can be stated that similar efficiency and safety results can be achieved even though maximal continuous PD-1 inhibitor therapy was limited to six months. One cannot argue the economic benefit of such a finding. Furthermore, from patients’ perspectives, a shorter treatment course also has many indisputable advantages. However, due to the retrospective nature of our study and the limited number of subjects, results are hypothesis generating and should be investigated further in prospective clinical trials.

6.2 Prognostic and predictive biomarkers of response to anti-PD-(L)1 therapies

For a patient, the result of the given treatment is of a binary nature: either the treatment will work or the cancer will progress. From clinicians’ point of view,
treatment decisions are, of course, based on results from clinical trials, guided by the national decrees, but at the final step, more or less, is educated guesses orchestrated by clinical experience. Even though durable, long-lasting treatment responses have been seen in patient treated with immune checkpoint inhibitors, the majority of the patients fail to respond.

An economical challenge with immune checkpoint inhibitors is the lack of clinically relevant factors predicting treatment response. There is also an ethical aspect to be considered: when we are faced with an inadequately characterized spectrum of immune-related adverse events, of which some are potentially lethal, and at the same time lacking predictive biomarkers, how do we balance the benefit-risk ratio of the therapy? Luckily, the incidence of severe immune-related adverse events with immune checkpoint inhibitors as monotherapies is rather low.

Although the negative impact of systemic inflammation on prognosis of cancer patients receiving chemotherapy and targeted therapies is widely studied (Diakos, Charles, McMillan, & Clarke, 2014; Dolan, Laird, Horgan, & McMillan, 2018), the predictive meaning of systemic inflammatory status in patient receiving ICIs, is inadequately defined. In a recent study (Weber et al., 2018), it was shown that serum protein signatures related to acute phase, complement, and wound-healing pathways, according to a study-related pre-treatment serum test, were predictive for treatment benefit of ICIs among melanoma patients. According to the PSEA (protein set enrichment analysis), an analysis developed for the study, the serum of PD-1 inhibitor therapy-resistant patients was characterized by acute phase, complement, and wound-healing proteins. Based on the results, activation of these pathways seemed to be upregulated in the resistant group compared with the sensitive group.

The role of tumor microenvironment (TME), the area immediately surrounding the tumor, which is typically composed of nonmalignant lymphoid and/or myeloid cells as well as fibroblast, vascular cells and lymphatic vessels, in predicting treatment response for immune checkpoint inhibitors is under fierce investigation. Analysis of genomic and transcriptomic data has led to the discovery of so called metagene signatures, like IPRESS (innate anti-PD-1 resistance), a transcriptomic tumor phenotype (Hugo et al., 2017), and distinctive mutational landscapes, such as genomic defects in the IFNγ pathway in tumors of patients classified as non-responders to ipilimumab (Gao, J. et al., 2016), with prognostic and predictive nature in cancer patients (Bindea et al., 2013; Hugo et al., 2017; Kandoth et al., 2013; Kirilovsky et al., 2016).
Recently, a redefined stratification of TME with respect to immune characteristics has been proposed (O'Donnell, Teng, & Smyth, 2019). The earlier classification which was based on existence of TILs and PD-L1 expression was enriched with data from studies investigating pan-tumor genomic aspects (Cristescu et al., 2018; Ock et al., 2016). The authors state that the presence of TILs and PD-L1 in the TME according to immunohistochemistry (Taube et al., 2012), is likely to reflect the presence or absence of T cell inflammatory gene signature and high TMB. Thus, a stratification of TME based on combination analysis of TMB, neoantigen burden, PD-L1 amplification, and infection of oncogenic virus would give the most comprehensive depiction, in regards to the existence of immunosuppressive pathways, and response to ICIs (O'Donnell et al., 2019). Furthermore, distinct analysis of the immunosuppressive mechanisms utilized by individual tumors could be used to guide the treatment selection, possibly combining different therapy modalities aiming to overcome both innate and adaptive resistance to cancer immunotherapy. However, implementing a DNA or RNA-based gene signature is challenging from a clinical perspective, and more simplified surrogate biomarkers are needed.

Despite the pervasive research in the biomarker field, only a few have proven to be clinically relevant such as PD-L1 expression, TMB, and in rare cases MSI and dMMR. However, positive and negative predictive values of PD-L1 and TMB are low, and they are valid for patient selection only in particular cancers such as NSCLC and urothelial cancers. Furthermore, these biomarkers are assessed from tumor biopsies, which are time consuming and seldom readily available. Blood-based biomarker assays as non-invasive analysis are thus more compelling in various cancers.

We investigated the role of CRP and other markers for systemic inflammation in multiple advanced cancers treated with anti-PD-1 therapy (II). CRP is a known marker for systemic inflammation but its correlation to treatment benefit from anti-PD-(L)1 therapy was scarcely studied, and little was known about the independent prognostic role of CRP level. Interestingly, in our study the optimal cut-off value for CRP, as prognostic marker in anti-PD-1 treated patients, was 10mg/l, which is also the ULN value for CRP in most laboratories. The results suggested a very strong negative prognostic role of elevated pre-therapy CRP in PD-1 inhibitor treated patients. Our conclusion was that pretreatment CRP value could prove to be a cheap and non-invasive prognostic marker and that its’ possible predictive value should be investigated in prospective clinical trials. However, the median PFS and OS for patients with CRP>10 in both discovery (2.0mo; 10mo, respectively) and
validation cohorts (3.0mo; 12mo, respectively) were short, which implies that the natural history of the disease at that point might simply be beyond the reach of anti-PD-(L)1 therapeutic intervention.

Furthermore, a recent study, presented at the annual meeting of the American Society for Clinical Oncology reported similar data on the use of pretreatment CRP and interleukin-6 (IL-6) levels measured before the initiation of ICIs. The data presented was from three randomized melanoma studies, CheckMate-064, CheckMate-066 and CheckMate-067 (Weber, Jeffrey S. et al., 2019). IL-6 is a cytokine with multiple effects on immune cells which also stimulates the liver to produce CRP, so CRP levels reflect IL-6 levels in the blood. The data confirmed our finding that pretreatment CRP level is a strong prognostic marker, and that elevated pre-therapy blood CRP values correlate with poor treatment benefit from ICIs. In addition, in the randomized Checkmate 067 study, serum CRP above the median was associated with shorter survival for ipilimumab, nivolumab or the combination of them, and for nivolumab in the randomized Checkmate 066 study, respectively. Interestingly, they reported that CRP levels above 10mg/l suppressed T cell proliferation and altered T cell signaling, implying that CRP affected the earliest steps in T cell functions. The findings are in line with our prior results and the confirmation of the same cut-off of CRP over ULN alongside the results revealing the direct negative impact of high CRP levels to T cell functions make our findings even more plausible.

Targeting prognostic proinflammatory biomarkers to improve the outcome of ICIs opens a new therapeutic window. According to preclinical data, IL-6 and other proinflammatory cytokines like IL-1β and TNF-α, impact in multiple ways on the tumor microenvironment, favoring myeloid inflammatory response and promoting tumor growth and affecting metastatic potential (Bergmann et al., 2017; Coffelt et al., 2015; Kaplanov et al., 2019). Furthermore, there is preliminary data of synergism in antitumor immunity when combining blockade of these proinflammatory cytokines with ICIs to overcome resistance to ICIs alone (Kaplanov et al., 2019; Mace et al., 2018; Perez-Ruiz et al., 2019).

6.3 ePRO follow-up of cancer patients

The value of PROs combined with patient-centered interventions in cancer care has proven to be substantial. The implementation of ePROs in the treatment of cancer patients with advanced cancers and lung cancer follow-up has been shown to be associated with better HRQoL, fewer hospitalizations, and most remarkably,
increased survival compared to standard care, thus, these data collections and evaluations are considered as an essential part of good cancer care. One could say that by implementing PROs in routine clinical practice, the integration of patient experience and perspective to the cancer care continuum could truly be reached. However, the implementation of PRO measures into everyday clinical practice leaves many open questions.

Firstly, ePROs used for cancer patient treatment-specific follow-up need to be standardized. At present, there is a wide variety of patient-reported outcome measures (PROMs) are used to evaluate physical symptoms, treatment toxicities, psychosocial distress, and HRQoL (Howell et al., 2015). Of no surprise, is the finding that clinicians, patients and decisionmakers value PROMs that can capture the ‘overall’ effect of cancer, and treatment on health outcomes (Howell et al., 2013). Secondly, the comparability of distinct ePRO tools should be validated among different patient cohorts with respect to disease modalities. Another question is the generalizability of ePROs, thus, how to minimize the patient burden of PROs by reducing the questions, and questionnaires used in cancer care, to a reasonable and meaningful minimum yet not losing the possibility to target specific patient groups with tailored patient-centered approaches. The concept of computerized adaptive test (CAT) aims to maximize the individual information of HRQOL by selecting items to be filled in based on previous answers. The same is true also from the clinicians’ point of view. Physicians adherence to PROs in clinical practice is probably enhanced if interpreting and utilizing PRO results is not too time-consuming and use of them is considered to optimize the workflow. If not, PROs are just another hinder between patient and clinician. Thirdly, implementation of ePROs requires structural changes in health care procedures to truly facilitate symptom management strategies (Zhang et al., 2019). That is, based on patients’ input when utilizing different web-based applications or mobile apps in a medical practice, pseudonymization, data privacy and data protection alongside with feedback by a physician are of great value to patients (Kessel et al., 2017), and these aspects should be considered when planning care pathways.

In our retrospective study (III) investigating electronic symptom follow-up, symptom correlation analysis revealed coupling of certain symptoms with positive and negative correlations. Strong positive correlations were seen between typical irAEs like rash and itching, and on the other hand, pulmonary symptoms (cough and shortness of breath). Interestingly, rash, itching and joint pain had negative correlations with cough, shortness of breath and chest pain which often are related to disease progression in the context of lung cancer or lung metastases (Haanen, J.
Our finding of negative correlation between well-known irAEs and disease progression related symptoms supports the hypothesis that incidence of irAEs with ICI therapies is suggestive of potential clinical benefit to the patient. Furthermore, correlation of patient-reported symptoms to QoL assessed by QLQ-C30 (global health status and functioning scales) showed general highly negative correlations excluding joint pain, headache, shortness of breath, chest pain and diarrhea. This could be interpreted that clinical decision-making aiming to improve patients’ wellbeing based on merely QoL reporting is difficult (Field, Holmes, & Newell, 2019). Our findings support the idea that the clinical value of follow-up of cancer patients is increased when individualized symptom analysis is combined with standard measurements (Velikova et al., 2008).

In summary, our results show that ePRO follow-up of cancer patients treated with immune checkpoint inhibitors is feasible. The symptom variety, incidence, and grading collected with the ePRO questionnaire from real-world patients mimics what has been reported in anti-PD-(L)1-trials making our results clinically convincing. The correlation analysis showed a negative correlation between common irAEs and symptoms suggesting disease progression, but due to the retrospective nature of our study, the findings were hypothesis-generating, lacking a confirmation from data linked to clinical outcomes.

Our prospective study, the KISS trial (IV), analyzed the variety of patient-reported symptoms, etiology of alerts, symptom correlations, and patient compliance with the ePRO symptom follow-up. The symptom variety based on patient reporting and grading algorithm performed well and symptom data followed closely what has been reported in clinical trials investigating ICIs. A recent meta-analysis with more than 20 000 patients revealed that fatigue (18%), pruritus (11%) and diarrhea (9%) are the most common adverse events reported on patients treated with anti-PD-(L)1 therapies (Wang et al., 2018). The percentages of AEs in clinical trials are generally bit lower than in our study which might relate to better capture of low-grade symptoms which are often overlooked in physician-based adverse event reporting in clinical trials but come to light using patient reporting. A recent data analysis of PROs from registration trials of anti-PD-(L)1 inhibitors stated that the collection of PRO data was variable and did not consistently assess important symptomatic adverse events. The researchers stated that use of well-defined PRO outcomes combined with relevant symptomatic adverse events alongside clinical data on hospitalizations and supportive care medication could help to estimate the risk-benefit ratio for regulatory purposes (King-Kallimanis et al., 2019).
In the current study (IV), the symptom questionnaire was also coupled to an urgency algorithm which generated alerts in 17.1% of the answered questionnaires during the first 12 weeks. Loss of appetite and fatigue were among the most common symptoms generating alerts. These symptoms rarely alter the cancer treatment, and symptomatic treatments for them are scarce. Furthermore, physicians assessed that most of the alerts were caused for unclear reasons. Fine-tuning of the alerts not only focusing on the symptom grade but also the nature of the symptom could lower the number of alerts and staff workload without sacrificing the performance of ePROs.

Patient adherence to and experience on ePRO follow-up was found to be very good in the study (IV). The patients were requested by email to fill out symptom questionnaires weekly and the number of filled questionnaires was very close to one per patient/week for the first 12 weeks. Based on the Patient experience survey, the system was easy to use, and patients felt that ePRO follow-up improved their cancer care which is in line with prior studies (Basch et al., 2005; Basch et al., 2018; Velikova et al., 1999; Wright et al., 2003).

Our previous (III) retrospective study suggested that some ePRO reported dermatological, gastrointestinal and pulmonary symptoms co-occur. Similarly, we saw strong positive correlations between GI- and dermatological symptoms in the prospective KISS trial (IV). Furthermore, the data from the KISS trial showed weak negative correlations between pulmonary symptoms and some GI-symptoms, and between itching, pulmonary symptoms and fever. In our previous retrospective study, which did not include data on treatment responses, we generated a hypothesis that GI and skin symptoms might relate to immune activation and treatment benefit while pulmonary symptoms could signal tumor progression. Since the current (IV) study also included data on treatment benefit, it enabled us to confirm our hypothesis. The results showed that there was a weak positive correlation between treatment benefit and itching, and PD and chest pain. Similarly, previous studies have linked autoimmune skin toxicity (rash) to anti-PD-1 treatment benefit (Berner et al., 2019; Freeman-Keller et al., 2016; Sanlorenzo et al., 2015). Compared to physician-based adverse event reporting, it is likely that ePROs enable enhanced capturing of low-grade AEs without visible presentation such as itching, and therefore facilitate predicting treatment benefit with higher frequency.

ePROs enable cost-effective capturing of symptoms and their change over long time-periods based on lung cancer follow-up (Lizée et al., 2019). Changes over time might better predict treatment side effects and benefit than just a single presentation of a symptom. Furthermore, data of the current study (IV) showed that
early (during the first 12 weeks) changes in symptoms correlate with treatment benefit as well as symptoms from the whole follow-up period. This highlights the possibility that early changes in symptoms predict outcomes. Large scale symptom data combined with treatment benefit and side effects could be used to build prediction models using artificial intelligence methods. The models could predict risk for an individual patient for symptom development, treatment related side effects, and treatment benefit.

An interesting dimension of ePROs is the possibility to enhance the communication between physicians and patients. Advisable patient education and communication can improve quality of cancer care with multiple subjective and objective enhancements (LeBlanc & Abernethy, 2017). Patient education and communication should include discussing goals of care and prognosis, treatment options and clinical trials, end-of-life care, and the cost of care, and should facilitate family involvement in care (Gilligan et al., 2017). A more comprehensive interaction presumably benefits both parties and could lead to an improved adherence through increased sense of self-efficacy on the part of the patient. An increasing number of cancer survivors in need of follow-up is a challenge, even though a positive one. A recent study (Mayer & Alfano, 2019), introduced the idea of personalized risk-stratified cancer follow-up to better allocate the scarce resources of oncological care. Researchers suggested that survivors should be triaged to different care pathways based on the complexity of their needs and the types of care providers needed. In the development of cost-effective and feasible follow-up measures, ePROs certainly can be part of the solution.

Artificial intelligence (AI) or machine intelligence is intelligence demonstrated by machines. In health care, knowledge representation as part of the clinical decision support system is currently the most used AI approach. There are high hopes that AI could improve health care with early diagnostics and improved care in a more cost-effective manner compared to current approaches. The intriguing aspect of AI is its’ capability to analyze vast amounts of data, and based on that, detect correlations. The possibility of utilizing AI in the ePRO follow-up of treatment toxicities in that it could detect developing severe symptom cascades, and thus, instruct physicians and patients in advance, is extremely interesting. Our findings in the KISS trial (IV) suggest that even early ePROs on symptoms could be used to create individual prediction models for treatment benefit. Whether this could be enhanced by combining the symptom reports of a patient to other eHealth apps sensing for example metabolic or physiologic changes, is another fascinating possibility. However, scientific knowledge on the true value of AI and, web and
mobile applications apart from symptom tracking in cancer care is scarce. But many novel projects are ongoing (Abbasi, 2019), and future data will show, if they are truly adding value to cancer care and improving overall survival.
References


81


87


102


### EORTC QLQ-C30 (version 7)

**Appendices**

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no right or wrong answers. The information that you provide will remain strictly confidential.

Please fill in your details:

<table>
<thead>
<tr>
<th>Your birthday (Day, Month, Year)</th>
<th>05/01/1960</th>
</tr>
</thead>
<tbody>
<tr>
<td>Today’s date (Day, Month, Year)</td>
<td>05/01/2023</td>
</tr>
</tbody>
</table>

#### QLQ-C30 Questions

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you have any trouble doing strenuous activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. Do you have any trouble carrying a heavy shopping bag or a suitcase?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. Do you have any trouble going up or down stairs or steps?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. Do you have any trouble doing a housework outside of the house?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. Do you need help with eating, drinking, or bathing yourself or being in your bed?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

#### During the past week:

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Were you tired in doing either your work or other daily activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. Were you limited in doing your hobbies or other leisure time activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. Were you short of breath?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. Have you had pain?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. Did you need rest?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11. Have you had trouble sleeping?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12. Have you felt hungry?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13. Did you feel depressed?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>14. Have you felt sad, lonely, or hopeless?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15. Have you been worried?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>16. Have you been constipated?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
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</table>

Please go on to the next page.

#### During the last week:

<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all</th>
<th>A little</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>17. Have you had headaches?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>18. Were you tired?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>19. Did you have difficulty with your daily activities?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>20. Did you have difficulty in concentrating on things, like reading a newspaper or watching television?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>21. Did you feel sort?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>22. Did you feel fatigued?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>23. Did you feel depressed?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>24. Did you feel sad, lonely, or hopeless?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>25. Did you have difficulty breathing?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>26. Did you have difficulty walking?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>27. Did you have difficulty in daily physical activities or eating?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>28. Did you have difficulty in daily physical activities due to your medical treatment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

For the following questions please circle the number between 1 and 7 that best applies to you:

<table>
<thead>
<tr>
<th>Question</th>
<th>Very poor</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td>30. How would you rate your overall health during the past week?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>Excellent</td>
</tr>
<tr>
<td>31. How would you rate your overall quality of life during the past week?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>Excellent</td>
</tr>
</tbody>
</table>

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**Elämänlaatukysely**

109
Immunoterapian oirekysely

1. Onko sinulla lännynyt 
   HENESINAKOSTUSTA?
2. Onko onnilla siitä 
   OSEAA?
3. Onko onnilla oman 
   MATSAVARUA?
4. Onko onnilla lännynyt 
   KIVUAA?
5. Onko onnilla lännynyt 
   VATAA?
6. a) Onko UUSITSEESSAG-SIVAA 
   VERTAA?
    b) Onko Virtuehtos uo 
   YRTAA?
7. Onko onnilla lännynyt 
   HOUSKAULUN 
   VÄHENEMISTA?
8. Onko onnilla lännynyt 
   PIINKONTA?
9. Onko onnilla lännynyt 
   DIENITYLAA?
10. Onko onnilla siitä 
    KUNIKETTA?
11. Onko onnilla lännynyt 
    LIIHETYSLAA?
12. Onko onnilla lännynyt 
    LEAARIIKAA?
13. Onko onnilla lännynyt 
    NÄIN SVYNAMEESTA?
14. Onko onnilla lännynyt 
    SÄÄNNÖKSI?
15. Onko onnilla lännynyt 
    NÄSEWPIDUA?
16. Onko onnilla lännynyt 
    HUPPEMARAA?
17. Onko onnilla lännynyt 
    KUTINOAA?

© Ikuun health Oy 2020 kalle
Orinudet pohtimusten.
1. Missä kaukoulevunen käyttö on tärkein?
   • Entistä keholla
   • Kevoilla
   • Vaikealla
   • En ole tällä

2. Oletteko tärkeät toisen henkilön apua kaukoulevyn käytossa? Jos luo ten kaukoulevyn yksikössä erottaa ymmärryksen?
   • Kyllä (jos kyllä, mihin keskustaa, mistä olitte tärkeät toisen henkilön apua)
   • Ei

3. Kaukoulevynen omakseen kyynelkää olevat ymmärtäviä?
   • Työntävän samaa miettä
   • Josain määritin samaa miettä
   • Josain määrän en miettä
   • Ei en neljää

Jos olet en miettä, mitä olet voinut olla seikkaamassa?

4. Usko teko kaukoulevynen käytön parantavan yhdelle teon kotona seurantaa/ymmärtää?
   • Kyllä
   • Ei

5. Onko kaukoulevynen käyttöä silloin siljää hyötä?
   • Kyllä
   • Ei
   • Ei osaa sanne

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Original publications are not included in the electronic version of the dissertation.
1536. Terho, Henri (2019) Electrocardiographic risk markers for cardiac events in middle-aged population
1538. Ylönen, Susanna (2019) Genetic risk factors for movement disorders in Finland
1541. Tiri, Hannu (2019) Comorbidities and mortality of hidradenitis suppurativa in Finland
1542. Hynynen, Johanna (2019) Status epilepticus in mitochondrial diseases and the role of POLG1 variants in the valproic-acid induced hepatotoxicity
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Sanna Iivanainen

REAL-WORLD PERSPECTIVES ON CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITOR THERAPIES