UiT Norges arktiske universitet

Faculty of Health Sciences

On indications for cancer immunotherapy:

A review of current practice for immunology-based indications

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1 Preface

1.1 Purpose

The purpose of the study is first of all to clearly summarize how immunotherapy is used and if it's used based on the knowledge of immunological mechanisms. While it is an interesting study to summarize knowledge of immunotherapy use and research, it is most of all a semi-structured/narrative review for personal purposes, so as to gain more knowledge on a complex, modern and interesting subject.

1.2 Basis of the project

The project is highly based on a personal interest in immunotherapy in cancer treatment. It has throughout medical studies and practice become clear that while immunotherapy is an exceptional new modality in the treatment of cancer, it is most often used in late-stage cancer. At some point during my studies, I became aware that immunotherapy for cancer usually has indications for metastatic, locally advanced, refractory or recurrent cancer. I found it odd that a field that is considered to be so exceptionally specific and precise were most often used for what seemed like last-line therapy. And while immunotherapy involves risk of long-lasting and serious adverse effects, why is it being used without knowing if one is treating based on actual mechanisms used by tumor cells? Presenting these thoughts for my supervisor, he suggested that a review of therapies to investigate how common this practice is would be an interesting study, and after some reflection on this theme, the idea of this project was established.

I would like to thank my supervisor, Tor B. Stuge, for always entertaining ideas and thoughts, no matter how high-flying or relevant they are, and for good help in reviewing this study, even on very short notice on days in which it cannot be expected.

Date: 23.08.21, Tromsø

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2 List of abbreviations

- PD-1/PD-L1 – Programmed cell death protein 1 /Programmed cell death ligand 1
- ICI – Immune checkpoint inhibitor
- CTLA-4 – Cytotoxic T-lymphocyte-associated protein 4
- MHC – Major histocompatibility complex
- RCT – Randomized controlled trial
- NSCLC – Non-small cell lung cancer
    - SQ-NSCLC – Squamous-NSCLC
    - Non-SQ-NSCLC – Non-squamous NSCLC
- OS – Overall survival
- PFS – Progression-free survival
- ORR – Objective response rate
- RECIST – Response evaluation criteria in solid tumors
- CR – Complete response
- PR – Partial response
- HR – Hazard ratio
- UC – Urothelial carcinoma/Bladder cancer
- ESMO - European Society of Medical Oncology
- ASCO - American Society of Clinical Oncology
- AIOM - Italian Society of Medical Oncology
- NCCN - National Comprehensive Cancer Network
- IHC – Immunohistochemistry
- TMB – Tumor mutational burden
- TIL – Tumor infiltrating lymphocytes
- MMR – Mismatch repair
- MSI – Microsatellite instability

3 Abstract

3.1 Background

Knowledge of tumor immunology and immune escape mechanisms was the basis for developing immune checkpoint inhibitors (ICI) for cancer therapy. These target and block checkpoint molecules commonly implicated in tumor immune escape by overexpression, such as PD-1, its ligand, PD-L1 or CTLA-4. Use of ICIs has been approved mostly for treatment of advanced disease, some without any requirement for checkpoint overexpression on tumors. The objectives of this review is to investigate how common lack of immunological biomarker requirement is, and for therapies with such a requirement, the reason for and the importance of overexpression, as well as reviewing what challenges clinicians face in this field.

3.2 Methods

Performing two related semi-systematic reviews, all currently approved cancer antibody therapies categorized by The Norwegian Medicines Manual for Health personnel were reviewed to investigate indications for ICI therapy, and a literature review on current practice in their use was performed to investigate reasons for the discrepant requirements in indications. Approved therapies were also screened for antibodies with similar specificity and indications, where one was immunology based and the other was not, to investigate whether there were discrepancies in efficacy based on PD-L1 expression for similar antibodies.

3.3 Results

Five of 16 indications for using ICIs require PD-L1 positive tumors. All had significantly better outcomes with higher expression in clinical trials. Select agents without such requirement also has increased effect with overexpression, but also saw effect in PD-L1 negative tumors. The literature review found 14 articles on current practice of ICI therapy, all focused on different challenges in clinical practice. Key issues are patient selection, lack of predictive biomarkers, no standardization of expression assays and therapy timing.

3.4 Conclusion

It cannot be said that it is common for therapies to have immunology-based indications, as less than one third of all indications require checkpoint overexpression. All of these have higher efficacy with high PD-L1 expression, but also in patients without overexpression. The mechanisms behind this are not well understood, but is highly likely explained by emerging biomarkers that are under investigation. Major challenges in the day-to-day practice include patient selection, lack of biomarkers and therapy timing.

4 Introduction

4.1 Theory

4.1.1 Hallmarks of cancer

Cancer is a leading cause of premature death worldwide, premature death being defined as occurring before the age of 70. In 2019, cancer was estimated to be the first or second leading cause of premature death in 112 out of 183 countries. How highly ranked cancer is on this list is, in part, dependent on how well developed a country is, and considering the continued development of most countries, it is to be expected that the number of countries where cancer is the most significant barrier to increased life expectancy will increase (1).

In 2011, Hanahan and Weinberg published their follow-up to the renowned "Hallmarks of cancer" (2), wherein the most common and defining features of cancer are described. Known and common properties, such as the evasion of apoptosis, growth and cell division without proper signaling and angiogenesis, among others, are described. In the follow-up, emerging hallmarks not included in the original are presented and discussed. Notably, the evasion of immune destruction is one of these new hallmarks. It had become clear that mechanisms for evading, suppressing or modifying immune responses towards cancer cells, so called "tumor immune escape", is vital for the development and persistence of malignant disease. This knowledge is important for a more thorough understanding of all cancers, but perhaps more importantly, it suggests reversal of immune mechanisms utilized by cancer cells as a promising approach in the therapy of cancer (3). While this knowledge was not new, the inclusion of immunotherapy as a promising modality in the treatment of cancer was at this time more widely acknowledged, and later it has gained massive traction, made very clear by the naming of cancer immunotherapy as breakthrough of the year in Science in 2013 (4) and later, the Nobel prize in physiology or medicine in 2018 to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation." (5).

4.1.2 Tumor immunology and tumor immune escape

The significance of tumor immune evasion and escape cannot be sufficiently appreciated without the understanding of how thoroughly involved the immune system is thought to be in the development of cancer. The immune system has several indirect tumor-protective mechanisms, such as elimination of oncogenic viruses and prevention of chronic inflammation as a driver of tumorigenesis (6). The processes of tumor immune surveillance

and cancer immunoediting, however, are the more direct ways of the interaction of the immune system and cancer. The process of immune surveillance involves direct recognition of malignant or potentially malignant cells, and is protective by establishing an immune response specific for tumor antigens and eliminating the cells before malignant disease can develop. This mode of establishing an immune response can happen due to the mutation of normal proteins to such a degree that they are no longer recognized as the host by immune cells, thus called “tumor antigens”. While this process is assumed to be necessary for the immune-mediated elimination of tumors, it does also exert a selection pressure on tumor cells. This is the conceptual idea in the hypothesis of cancer immunoediting. In this process, the elimination of tumor cells is suspected to potentially select for tumor cell variants with increased capacity to evade immune elimination. Ultimately, the result of such a selection can be tumor immune escape, in which the immune system is no longer capable of suppressing or eliminating tumor development and growth, resulting in the development of malignant disease without any physiological means of elimination (7).

The immunological mechanisms involved in suppressing tumor immunity consists of a wide variety of highly complex properties of cancer cells or their modification of the tumor microenvironment, even further complicated by being influenced by the immune competence of the host (8). Very broadly defined, these can be grouped in two. Mechanisms where tumor cells “hide” themselves from being recognized by immune cells, or evasion by disabling or eliminating immune cells. In the first category, some of the most common mechanisms are the down-regulation of antigen processing machinery components, co-stimulatory molecules, antigen-presenting molecules (MHC/HLA-complexes) or tumor-associated antigens. In this way, tumors evade protective immune responses by hiding in plain sight, but without the necessary components to interact properly with immune cells. Most of these are commonly found in malignant disease (9, 10). In the second category, evasion by disabling or eliminating immune cells, tumors directly interact with the host immune system in some way or other. Herein, the manipulation of T-cells toward an anergic, exhausted state is central (8). The best known mechanisms are through interactions between programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), both expressed on the surface of lymphocytes, and their ligands, PD-L1/PD-L2 and B7, respectively. These receptors are involved in negative regulation of antigen receptor signaling, so-called “immune checkpoints” that are involved in deciding whether interaction between T-cell receptors and antigen-presenting molecules on cells result in an activation of T-cells or not. These checkpoints are

so critical in the regulation of immune system activation and immunological tolerance that PD-1 and/or CTLA-4 deficiency is highly associated with a number of autoimmune diseases as well as having a role in feto-maternal tolerance, chronic viral infections, transplantation immunity and cancer (11).

4.1.3 Immunotherapy and checkpoint inhibitors

The development of immunotherapy for cancer is based on the understanding of these basic immunological mechanisms, the interplay between the immune system and cancer cells in the development and persistence of malignant disease, and knowledge of the many tumor escape mechanisms. In addition, they are in part made possible by modern biotechnological methods, many of which are highly complex. The development process is as such, exceptionally time-consuming, and the development of this kind of therapy usually spans over decades, wherein the process of clinical trials is only the tip of the iceberg, on top of the detailed elucidation of basic immunological mechanisms (12).

As tumor immune escape via negative regulation of antigen receptor signaling is considered to be a major and common mechanism, there has been a massive focus on development of therapies directed at disrupting or manipulating these mechanisms in recent years, with approval of a number of therapies directed at a wide variety of cancer types in the last decades. Monoclonal antibody-based therapy, so called “immune checkpoint inhibitors” (ICIs) has proven to be particularly promising for many cancer types in all phases of treatment, as first-line in combination with already approved therapy, as monotherapy or as last-line therapy, giving hope to many patients that have advanced or metastatic disease with few or no treatment options(13). Some of these have proven to be so promising to be granted both accelerated approval process as well as giving rise to the first drug approval on a molecular instead of histopathological basis as indication for use, granted to pembrolizumab, for unresectable or metastatic, microsatellite instable- high or mismatch repair-deficient solid tumors with no other satisfactory treatment options (14).

Checkpoint inhibitors aim to block the interaction between cancer cells overexpressing negative antigen receptor signaling (Fig. 1). For full T-cell function, multiple signals are required. The interaction between T-cell receptors and peptides presented on major histocompatibility complex (MHC) molecules is considered the first signal, in which T-cells recognize and bind specifically to pathogen-derived peptides, or peptides that are host-derived, but mutated to a degree in which these are recognized as non-self (7, 15). In addition

to this, cytokine stimulation is necessary, as well as costimulatory signals, such as B7-binding to CD28 or CTLA-4, or PD-L1 binding to PD-1. In this interaction, interaction of B7 and CTLA-4, or PD-L1 and PD-1, are negative regulators that can dampen T-cell responses and eliminate lymphocyte-mediated cytotoxic reactions on cancer cells (16). Checkpoint inhibitors are monoclonal antibodies specific for these negative regulators or their ligands. In this interplay between cancer cells and T-cells, checkpoint inhibitors binds to and blocks interaction, inhibiting activation of negative regulatory pathways in T-cells and thus reviving the cytotoxic activity against cancer cells (13).

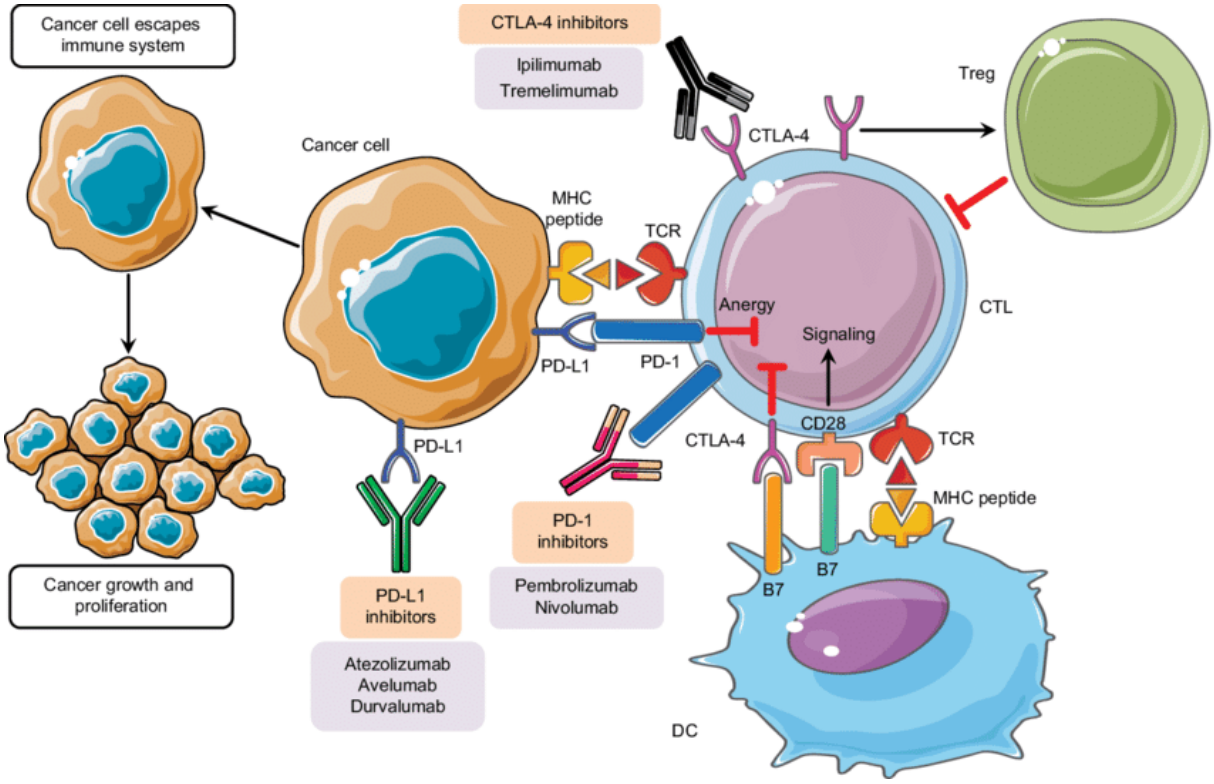

Figure 1: "Immune checkpoint inhibitors in cancer treatment": Cytotoxic T-lymphocytes (CTL) bind to cancer cells expressing cancer antigens presented on MHC-molecules. For full activation of T-cells, costimulatory signals are required, which can be stimulatory (B7-CD28) or inhibitory (PD-L1-PD-1/B7-CTLA-4). Cancer cells overexpressing inhibitory ligands can dampen CTL activity, causing tumor immune escape and further growth and proliferation of cancer cells. Monoclonal antibodies specific for inhibitory receptors (PD-1/CTLA-4) or their ligands (PD-L1) are used to reactivate anti-tumor activity of CTLs *(17).*

4.1.4 Tumor immunogenicity and checkpoint inhibitor efficacy

For therapy with checkpoint inhibitors to work, there has to be an immune response present to begin with, as all ICIs do is to block tumor cells' negative regulation of T-cells that recognize the cells (13). This is not as straight forward as it sounds, considering that tumor cells stem from the hosts own cells. The mutated nature of tumor cells is as previously mentioned a basis for immunological elimination of tumor cells. Highly mutated tumor will become more unlike the host cells, and this makes them more recognizable for immune cells. The measure of how

readily a tumor will have an immune response mounted against it is referred to as the immunogenicity of the tumor. This is affected by how mutated it is, but it is also necessary to present antigen on the surface of tumor cells and to have immune cells present so as to recognize them (18). So considering all this, there are three main factors proposed to affect the efficacy of ICIs: How mutated tumor cells are, due to a large number of mutations causing a larger number of potentially recognizable targets to mount an immune response against; the expression of negative regulators, as in PD-L1 overexpression; and the number of T-cells in the microenvironment of the tumor, necessary for recognition and lymphocyte-mediated cell death (19).

4.1.5 Clinical trials

The process of clinical trials, most often through randomized controlled trials, is the benchmark process for developing and comparing new therapies. The process aims to assess effectiveness and safety of interventions used in prevention and treatment of health conditions in an ethical, rigorous and scientifically sound process in which one strives to minimize all forms of bias. In clinical trials, investigators must design studies with a strict process of selection and exclusion of the study population as well as a control population with similar characteristics but to whom the therapy is not given. It is essential to design the population to whom the therapy is intended and the conditions for exclusion, either for clinical or practical reasons. This is a necessity for the development of evidence-based medicine, as therapies must have specifically defined criteria for on whom and when to use them – the indication to use a procedure or medication. In addition, trials must have defined endpoints, or outcome measures, in which an expected effect is measured in some meaningful way. Endpoints can be highly diverse, some being strictly objective measures and others highly subjective. Clinical trials often have primary and secondary endpoints as well, to measure the main intended effect as well as some directly or indirectly related measure, such as measuring survival time with a drug intervention as a primary endpoint, but in addition measuring the quality of life in the same period as a secondary endpoint. In essence, and very simplified, one can describe clinical trials by these core principles only: “how is (endpoint) affected by this drug when given to (population within selection criteria) in x dose over y time, and is also (secondary endpoint) changed in this period?” (20).

Clinical trials must be based on pre-clinical investigations. These are often based on basic studies key physiological mechanisms as well as the drugs suspected or confirmed role in this,

its properties, production process and animal studies with the substance to explore safety *in vivo*, pharmacodynamics and pharmacokinetics. After this process, phase I trials can begin. These are the first studies of the therapy used in humans and are characterized by dose-escalation and studies of the pharmacology of a drug in a low number of human subjects. In this process, determination of toxicity and safety is the primary concern, not therapeutic potential. In phase II trials, the therapeutic potential is investigated in a small number of patients fulfilling selection criteria. In this phase, safety and pharmacology is still a primary concern, but this is often in addition to studying therapeutic potential for different doses, frequency and administration routes as well as investigating potential endpoints for further studies. In phase III trials, safety and therapeutic potential is investigated further, often based on results from phase I and II studies. Usually, this involves a larger number of patients, often hundreds to thousands, to establish with enough statistical power that there is therapeutic benefit and to investigate less common adverse reactions. In phase III studies, comparative efficacy is often investigated by comparing the intervention group to a placebo-control group or to some other approved therapy for the condition the drug targets to treat. In this phase, trials are often blinded, meaning that the patient and/or the investigator does not know which group the patient is in, eliminating the potential for analyzing based on knowing which group the patient is in (assessment bias). In contrast, studies can be open label, in which there is no blinding, but these are more often based on objective endpoints that are less prone to assessment bias. Lastly, there are phase IV trials, which are observational studies after drug approval. In this phase, adverse effects and cost-effectiveness is evaluated (20).

4.2 Objectives

One would assume that the detailed knowledge of mechanisms in immune checkpoint regulation and whether they are utilized by cancer cells were of vital importance when choosing checkpoint inhibitors. In reality, this is not commonly involved as a deciding factor in therapy choice. The use of this type of therapy has increased survival for a number of cancer types, including malignant melanoma, lymphoma, lung cancer, renal cancer, among others (21). As described in the previous chapter, this form of immunotherapy is based on relatively new knowledge about the mechanisms of immune checkpoint molecules and their role in cancer development. As these mechanisms are inherently similar across multiple cancer types, one could assume that use of therapy directed at these mechanisms would be based on analysis of tumor expression of ligands involved in immune checkpoints. This does, however, not seem to be the case (22). The approval of pembrolizumab for all unresectable or

metastatic cancers, with high microsatellite instability or mismatch-repair deficiency as late as 2017 is the first approval of a cancer drug with an indication solely based on a molecular marker rather than its site of origin (14), and it seems reasonable that more would follow and base their indications on knowledge of mechanisms utilized by cancer cells to survive and escape immune elimination. There must be sound reasoning and good evidence behind approvals of therapies, but still some very similar agents have different indicational requirements for use. To investigate why this is, a set of questions were designed as the primary objectives to explore the relatively new clinical landscape of immunotherapy and checkpoint inhibitors.

Primary objectives:

The objective of this study is to review the current practice when using antibodies targeting immune checkpoint molecules, the largest group of immunology-based therapy, when used in cancer treatment to target tumor immune escape mechanisms. The questions I wish to answer are these:

- Are the mechanisms of tumor immune escape, the knowledge of which was the basis of developing therapies like checkpoint inhibitors, used as indications for immunotherapy of cancer? If so, is the efficacy correlated to these mechanisms?
- Knowing that there are some differences in indications for similar checkpoint inhibitors in similar cancer types, why is this?
- What are the challenges facing clinicians when considering to use checkpoint inhibitors in cancer therapy?

This study was split into two main tasks for answering these questions:

- Perform a semi-systematic review of approved monoclonal antibodies used in cancer therapy
    - To review their indications for use and the publications associated with the clinical trials that their approval is based on
    - To investigate how common it is to require investigation of tumor immune escape mechanisms before using such treatments and the trial results that this requirement is based on
    - To investigate if there are antibody-therapies with very similar mechanisms that are approved with and without indication requirements to analyze tumor

immune escape ligands, and to review publications associated with their clinical trials to investigate reasoning behind their indications.

- To perform a semi-systematic review for investigating the current practice when using checkpoint inhibitors and the challenges this relatively new field is facing.

To limit the scope of this study, only therapies approved in Norway were selected.

The primary objective questions and tasks for answering them has been quite extensively changed since the first version, per evaluation feedback and supervisor feedback. A summary of these changes are presented in a changelog in the appendix.

5 Methods

5.1 Selection of therapies included in this study

5.1.1 Therapy selection strategy

The selection of therapies has been limited by selecting medicines that are approved for use in Norway, and that are described with properties and indications in The Norwegian Medicines Manual for Health Personnel. Approved monoclonal antibodies in treatment of cancer were systematically reviewed in the manual and in the summary of product characteristics (SPC) for each drug. The SPC is official documentation that is approved and published for all approved therapies and details the characteristics of therapeutic agents as well as the basis for their approval. All monoclonal antibodies used in cancer therapy were screened and evaluated based on their specific target.

5.1.2 Inclusion criteria

Antibodies approved as therapy based on their specificity for ligands or receptors involved in tumor immune escape (PD-1/PD-L1 or CTLA-4) were included for screening and listed by their antigen specificity and approved indications. Among those selected for screening, their approved indications were reviewed and evaluated, and therapies that specifically require expression-analysis of immune checkpoint molecules on tumor cells were selected for review.

In addition. those that had indications for the same cancer type, but with different criteria for treatment, in which one had indications that were based on expression status of immunological checkpoints and the other had not, were selected for review and comparison.

5.1.3 Exclusion criteria

Monoclonal antibodies with targets not directly implicated in tumor immune escape mechanisms were excluded. Presented in table 1, these involve antibodies targeting growth factors and cell surface molecules for targeting of specific cell types or their growth-stimulating mechanisms.

5.2 Selection of publications to review for selected therapies

5.2.1 Publication selection strategy

In the SPC of selected therapies, clinical trials that were the basis for approval is summarized for every indication for the agent. The studies referenced as grounds for approval are listed with title and trial short name, as well as summaries of primary and secondary outcome measures in the studies. In the manual, clinical trials referenced as basis for their indication in the SPC were reviewed on ClinicalTrials.gov, and trial results published in association with the registered trial were reviewed. For trials that have yet to be completed due to patients still being treated and/or examined, and therefore lacking an official publication of results after trial completion, publications indexed to the clinical studies via their NCT number were reviewed as they represent interim-analyses and follow-up studies involved in approval.

5.2.2 Inclusion criteria

Publications of trial results and follow-up studies reporting primary outcome measures and studies involving immune escape mechanisms were selected for review. The list of publications indexed to all clinical trials were continually reviewed throughout the study to be as up-to-date as possible, with the last review occurring at the 31st of May 2021.

5.2.3 Exclusion criteria

Publications that did not present data concerning outcome measures, studies of tumor immunology or tumor immune escape were excluded. These involve publications on safety- and dosing studies, cost-effectiveness, patient feedback and patient quality-of-life studies.

5.3 Review of selected therapies and their clinical trials

Clinical trials selected were systematically reviewed, focusing on type of agent and their specificity, indication, exclusion criteria, study type, status of the study as well as primary and secondary outcome measures. Where applicable, results grouped by immune escape mechanisms like PD-L1/PD-L1 or CTLA-4 expression is to be reviewed and summarized for every clinical trial of an approved therapy with such expression stated in its indication.

5.4 Semi-systematic literature review of current practice in cancer therapy with checkpoint inhibitors

5.4.1 Search strategy

As the primary objectives of this review is not to provide evidence-based guidelines, but rather to explore the present status and issues when using checkpoint inhibitors, a semi-systematic approach was considered appropriate. The structure of the search strategy and review selection is based on a methodology for systematic review of reviews by Smith and colleagues (23). This methodology is developed specifically for systematic reviewing of healthcare interventions when there are many publications and summaries of reviews are productive. While this study is not a systematic review, the iterative methodology described by Smith et. al. was chosen due to it being developed for articles summarizing reviews on healthcare interventions. Humans as subjects were required, as pre-clinical studies on other species are not relevant to the research questions. As the field of immunotherapy is rapidly developing, and many therapies are still in large-scale trials or have been approved in recent years, a time-frame of the 5 last years was chosen.

The literature search was based on three primary terms, “checkpoint inhibitors”, “current practice / clinical practice”, “off-label” and “cancer”. The literature search was conducted in PubMed and EMBASE. For development of a productive and broad search strategy, these three primary terms describing the focus of the study were used as a basis for discovering valid MeSH (Medical subject headings) and EMTREE (EMBASEs MeSH equivalent) terms (collectively referred to as “terms” hereafter). For this purpose, two strategies were used: PubMeds automatic MeSH term mapping and EMBASE with its advanced search and “explode” and “focus” mechanics. In PubMeds engine, it will interpret the search keyword and automatically include associated terms. EMBASE keyword search leads to a guided mapping where one can choose from a list of associated terms to include in the search. In this process, primary searches in each database were performed with the primary terms and all suggested associated terms from both search engines were reviewed. For “cancer”, “current practice”, “clinical practice” and “off-label”, all suggested terms from both search engines were considered to be relevant for the search. For “checkpoint inhibitors”, both engines resulted in many irrelevant terms due to both “checkpoint” and “inhibitors/inhibition” being frequently used terms in articles typically concerned with cancer, cell cycle checkpoints and enzyme inhibitors. To eliminate too many irrelevant results, the term “checkpoint inhibitors” and associated terms were reviewed in both engines and manually mapped through inclusion

of the most relevant terms combined by the “OR” function. By exploring similar terms that were both broad and narrow enough, the search strategy aimed to find many relevant publications without too many irrelevant results, as per the methodology developed by Smith et.al. (23). Detailed search strings are presented in the appendix.

5.4.2 Inclusion criteria

Articles fulfilling these criteria were selected for title and abstract screening: reviews, systematic reviews and meta-analyses published in the last 5 years with humans as subjects. Articles must be fully published and must be primarily focused on the use of checkpoint inhibitors in cancer therapy in a clinical setting.

5.4.3 Exclusion criteria

Publications of clinical trials such as RCTs, pilot trials, safety- and dosing studies were excluded. RCTs were excluded due to the massive amount of ongoing and finished trial series of all phases, and also as secondary sources like systematic reviews of RCTs are more informative on guidelines following trials. Studies on toxicology, adverse events and their management of adverse events were excluded. Any publication not focused on malignant disease were excluded.

6 Results

6.1 Selection of therapies

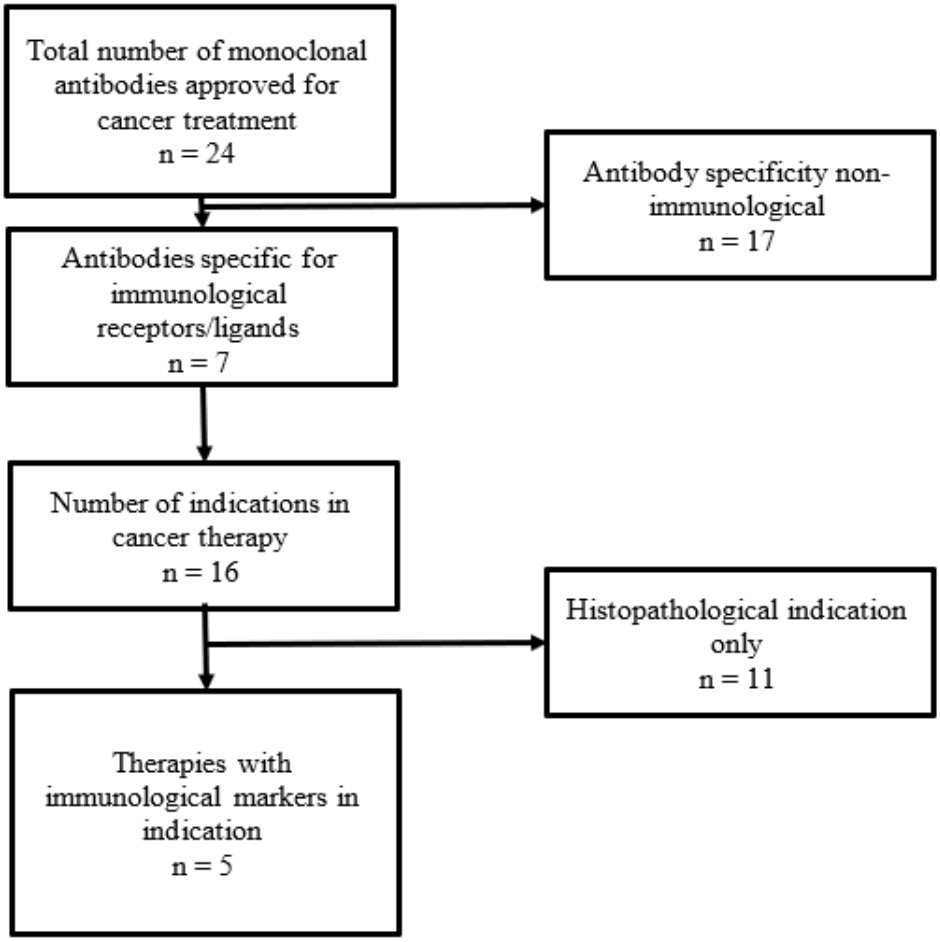

Figure 2: Flowchart of the systematic review of approved antibody-therapies in cancer treatment

As of June 2021, 24 monoclonal antibody-therapies are approved for use in cancer therapy, many approved therapies are specific for different targets often over-expressed on tumors, such as growth factors or different cell surface molecules for specific cell type targeting. As these are not specifically involved in immune checkpoints, immune modulation or tumor immune escape mechanisms, 17 agents were excluded on this basis. 7 antibodies were specific for receptors or ligands suspected to be involved in tumor immune escape, approved with a total of 16 different indications in cancer therapy. 11 indications were purely histopathological, being indicated for use on advanced, recurrent, refractory, metastatic or after failure of first- and/or second-line therapy. 5 indications, from 3 different antibodies are approved with an indication that includes criteria for analysis of immunological markers (Table 1). Overview of selected therapies, their complete indication and overview of screened

monoclonal antibodies used in cancer therapy as well are presented in tables 1-2 in appendix 1.

Table 1: Summary of selected agents approved with checkpoint expression level required in indication. Clinical trials referenced as basis for the indication in agents' SPC are described by trial short name and ClinicalTrials.gov unique NCT-identifier

Cancer type	**Agent**	**Clinical trial short name (NCT-identifier)**	**Checkpoint expression requirement**
Non-small cell lung cancer	Pembrolizumab (Anti-PD-1)	KEYNOTE-010 (NCT01905657)	PD-L1 ≥1%
	Pembrolizumab (Anti-PD-1)	KEYNOTE-024 (NCT02142738)	PD-L1 ≥ 50%
	Durvalumab (Anti-PD-L1)	PACIFIC (NCT02125461)	PD-L1 ≥ 1%
Urothelial carcinoma	Pembrolizumab (Anti-PD-1)	KEYNOTE-052 (NCT02335424)	PD-L1 ≥10%
	Atezolizumab (Anti-PD-L1)	IMvigor210* 1: (NCT02951767) 2: (NCT02108652)	PD-L1 ≥5%

*IMvigor210 trials enrolled patients in 2 cohorts, with different selection criteria

Ipilimumab, the only approved anti-CTLA-4 therapy at the time of this study. While being the only representative of the other class of checkpoint inhibitors, was excluded as there were no criteria for overexpression of CTLA-4 ligands for any indication.

6.2 Publication selection and review of clinical trials

6.2.1 KEYNOTE-010: Pembrolizumab in treatment of locally advanced or metastatic NSCLC after chemotherapy, and expression of PD-L1 with ≥1% TPS (24)

This study has one official publication of results for the trial and one follow-up on long-term outcomes that were included (25, 26). Five indexed publications were screened but excluded for not reporting outcome measures. These were *post hoc* simulation comparisons, studies on adverse effects, dosing and quality of life-studies. The trial is a randomized, open-label, international phase 2/3 intervention study of pembrolizumab, a humanized IgG4 PD-1 specific antibody (27) compared to docetaxel in previously chemotherapy treated NSCLC with at least PD-L1 expression on 1% of tumor cells. Patients previously treated with PD-1 checkpoint inhibitors or docetaxel, with known active brain metastases, carcinomatous meningitis, active autoimmune disease and interstitial lung disease were excluded. The study is completed, meaning the study has completed normally and there are no patients still being treated and/or being examined. Primary outcome measures were overall survival (OS), defined as time in months from randomization to date of death from any cause and progression-free survival (PFS), defined as time from randomization to radiologically confirmed progressive disease or death due to any cause (24).

Table 2: Overview of KEYNOTE-010 results at latest follow-up (26)

	PD-L1 50% ≥ 1% (n=591)		**PD-L1 ≥50% (n=442)**	
	Docetaxel (n=343)	Pembrolizumab (n=691)	Docetaxel (n=152)	Pembrolizumab (n=290)
Median PFS (months) (95% CI)	4.1 (3.8-4.5)	4.0 (3.1-4.1)	4.2 (3.8-4.7)	5.3 (4.2-6.7)
HR (95% CI)	0.83 (0.72-0.96), P<0.005		0.57 (0.45-0.71), P<0.00001	
Median OS (months) (95% CI)	8.4 (7.6-9.5)	11.8 (7.6-9.5)	8.2 (6.4-9.8)	16.9 (12.3-21.4)
HR (95% CI)	0.78 (0.65-0.94)		0.53 (0.42-0.66)	

1034 patients were randomized, 345 to pembrolizumab 2 mg/kg, 346 to 10 mg/kg and 343 to docetaxel. In the four-year follow-up, all pembrolizumab-treated patients were pooled, as the primary analysis showed no difference in efficacy due between doses. Patients were subgrouped by PD-L1 expression of ≥50% and 1-49%. Summarized in table 1, the trial has resulted in increased overall survival-time for all patients with expression of PD-L1 over the cutoff at 1%, further increased in patients with more than 50% expression. Favorable hazard ratio for PFS when compared to docetaxel was also observed in both subgroups, also with a more favorable outcome for patients with ≥50% PD-L1 expression (25, 26).

In summary, when comparing docetaxel to pembrolizumab, pembrolizumab was found to have better outcomes for overall survival and progression-free survival across all subgroups, and higher efficacy was seen in patients with higher PD-L1 expression (25, 26).

6.2.2 KEYNOTE-024: Pembrolizumab in treatment of metastatic NSCLC (28)

This study has an official publication of results and a five-year follow-up, both were reviewed (29, 30) for this trial. Five indexed publications were screened and all excluded, two for presenting country-specific outcome measures, one dosing study, a quality-of-life study and a study on adverse effects and immunogenicity of the agent. This is a randomized, open-label, international phase 3 intervention study of pembrolizumab compared to platinum-based chemotherapy in patients with previously untreated advanced NSCLC with tumor cell PD-L1 expression on ≥50% of tumor cells and no mutations of EGFR or ALK. Patients receiving systemic glucocorticoids, immunosuppressive treatment, untreated brain metastases, active autoimmune disease, active interstitial lung disease or a history of glucocorticoid-treated pneumonitis were excluded. This study is as of late May 2021 still active as patients are receiving treatment and/or being examined but is not recruiting new patients. The primary outcome measure is progression free survival (PFS), defined as the time from randomization to documented disease progression measured by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1), an often-used guideline for clinical assessment of change in tumor burden in clinical trials (31), or death from any cause. Secondary outcome measures are OS, defined as time from randomization to death due to any cause and objective response rates (ORR), the percentage of participants in the population who experienced complete

response (CR, disappearance of all lesions) or partial response (PR, in which there is a 30% reduction in diameter of lesions), assessed using RECIST 1.1 (28, 30).

Table 3: Overview of KEYNOTE-024 results at latest follow-up (29, 30)

	Pembrolizumab (n=154)	**Platinum based chemotherapy (n=151)**
Median PFS (months)	7.7 (6.1-10.2)	5.5 (4.2-6.2)
HR (95% CI)	0.50 (0.39-0.65)	
Median OS (months) (95% CI)	26.3 (18.3-40.4)	13.4 (9.4-18.3)
HR (95% CI)	0.62 (0.48-0.81)	
Objective response rate (%)	46.1 (38.1-54.3)	31.1 (23.8-39.2)

305 patients were randomized, 154 in the pembrolizumab group, and 151 patients in the chemotherapy group. Median PFS for the pembrolizumab group was 7.7 (95% CI 6,1-10.2) and 5.5 months (4.2-6.2) in the chemotherapy group, significantly longer for the pembrolizumab group (HR 0.50, 95% CI 0.39-0.65, p<0.001). OS was significantly higher in the pembrolizumab group compared to the chemotherapy group (HR 0.62, 95% CI 0.48-0.81, p=0.005) after five years. ORR was 46.1 (38.1-54.3) for pembrolizumab and 31.1 (23.8-39.2)in the chemotherapy group. Overview of results are presented in table 2 (29, 30).

In summary, pembrolizumab significantly increases OS, PFS and ORR compared to platinum-based chemotherapy in patients with ≥50% PD-L1 expression on tumor cells (29, 30).

6.2.3 KEYNOTE-052: Pembrolizumab in treatment of advanced urothelial cancer (32)

This study has 2 official publications of results, one interim analysis, and one long-term outcome publication (33, 34). One publication was excluded as it described adverse effects/immunogenicity of the agent. The study is active, not recruiting. The study is an open-label, non-randomized, international phase 2 interventional study of pembrolizumab in treatment of patients with advanced and unresectable or metastatic urothelial carcinoma who were not candidates for cisplatin-based therapy and not previously treated with chemotherapy. Patients with CNS metastases, carcinomatous meningitis, autoimmune disease, interstitial lung disease, active systemic treated infection, hepatitis B or C, or HIV, were excluded. Primary outcome measures were ORR, defined as complete or partial responses by RECIST 1.1. Secondary outcome measures were determination of duration of response per RECIST 1.1, OS, PFS (also RECIST 1.1), safety and tolerability (33).

Table 4: Overview of KEYNOTE-052 trial results at latest follow-up (33, 34)

	All patients (n=370)	<10% PD-L1 expression (n=251)	≥10% PD-L1 expression (n=110)
Median PFS (months) (95% CI)	2.2 (2.1-3.4)	Not reported	Not reported
Median OS (months) (95% CI)	11.3 (9.7-13.1)	9.7 (7.6-11.5)	18.5 (12.2-28.5)
Objective response rate (%) (95% CI)	28.6 (24.1-33.5)	20.3 (15.5-25.8)	47.3 (37.7-57.0)

370 patients were treated with at least one dose of pembrolizumab. 24% (95% CI 20-29) of patients had ORR, with 5% (95% CI 3-7) of patients achieving complete response and 19% (95% CI 16-24) had a partial response. Median PFS was 2 months (95% CI 2-3), OS after 6 months was 67% (95% CI 62-73). Analysis of PD-L1 expression in patients in the study was used to assess cutoff-values for PD-L1 expression with highest positive predictive value. ORR was 39% (95% CI 28-50) for combined positive score ≥10% (CPS; in which the number

of tumor cells, lymphocytes and macrophages in a tumor sample expresses PD-L1 (35)), 20% (95% CI 14-28) for CPS 1% - 10% and 11% (95% CI 4-24) with CPS lower than 1% (33).

In the follow-up study of long-term outcomes, ORR was 28.6% (95% CI 24.1-33.5) and median OS 11.3 months (95% CI 9.7-13.1) in the total population, and for patients with CPS ≥10% ORR was 49.0% (95% CI 34.8%-63.4%) and median OS 27.0 months (95% CI 12.4-not reached), both higher than the total population (34).

In summary, this study confirms the anti-tumor capacity of pembrolizumab for untreated patients with advanced urothelial cancer. Analyses of checkpoint expression levels indicate higher ORR and OS with high PD-L1 expression on tumor cells (34).

6.2.4 PACIFIC: Durvalumab in treatment of patients with unresectable NSCLC (36)

The study has no official publication of results, only publications indexed by NCT-number. As of May 31st 2021, 9 publications were indexed to the study, of which the first publication of study results, follow-up four year OS analysis and results by PD-L1 status were selected for review (37-39). Interim analysis previous to four-year OS analysis, patient reports, studies on chemoradiation impact were excluded. The study is active, not recruiting. It is a randomized, double-blinded, international phase 3 study of durvalumab, a human IgG1 monoclonal antibody specific for PD-L1 (37) compared to placebo in treatment of locally advanced and unresectable NSCLC without progression after platinum-based chemoradiation therapy. Patients previously treated with anti-PD-1/PD-L1 therapy, with active or prior autoimmune disease or history of immunodeficiency, severe or uncontrolled systemic disease or unresolved high-grade toxicity from previous chemoradiation therapy were excluded. Primary outcome measures were PFS based on RECIST 1.1 and OS (36).

Table 5: Overview of PACIFIC trial results (37-39)

	All patients (n=709)		**≥25% PD-L1 expression**		**≥1% PD-L1 expression**		**<1% PD-L1 expression**	
	Durvalumab	Placebo	Durvalumab	Placebo	Durvalumab	Placebo	Durvalumab	Placebo
Median PFS (months)	17.2	5.6	19.3	3.7	23.9	5.6	10.7	5.6
HR (95% CI)	0.55 (0.44-0.67)		0.42 (0.27-0.65)		0.49 (0.36-0.66)		0.79 (0.53-1.19)	
Median OS (months)	47.5	29.1	Not reached*	21.1	57.4	29.6	33.9	43.0
HR (95% CI)	0.71 (0.57-0.88)		0.53 (0.33-0.85)		0.60 (0.43-0.84)		1.05 (0.69-1.62)	

*At four-year follow-up

709 patients were randomized to treatment groups, 473 to durvalumab and 236 to placebo. In the first published results from 2017, in a planned interim analysis, median PFS was 16.8 months (95% CI 13.0-18.1) for the durvalumab group, compared to 5.6 months (95% CI 4.6-7.8) with placebo. Overall response rates were higher for patients receiving durvalumab compared to placebo 28.4% and 16.0% ($p<0.001$), respectively. Median OS was 23.2 months (95% CI 23.2-not reached) with durvalumab and 14.6 months (95% CI 10.6-18.6) with placebo. With a <25% PD-L1 expression defined as not PD-L1 expression dependent, this interim analysis resulted in no increase in effect from high PD-L1 expression (37).

In a follow-up study, published in 2020, analysis so PD-L1 status and its effect on treatment effect in the original study. 451 patients had PD-L1 assessable samples. Herein, samples were categorized by PD-L1 expression of ≥25%, ≥1%, <1% and 1-24%. When comparing durvalumab to placebo, PFS was increased for the ≥25% group to 17.8 months vs. 3.7 months (HR 0.41, 95% CI 0.26-0.65), the ≥1% group 17.8 months vs. 5.6 months (HR 0.46, 95% CI

0.33-0.64), the 1%-24% group not reached vs. 9.0 months (HR 0.49, 95% CI 0.30-0.80) and in the <1% group 10.7 vs. 5.6 months (HR 0.73, 95% CI 0.48-1.11). OS was increased for all groups except the <1% PD-L1 group (39).

In the four-year follow-up, median PFS was 17.2 months for the durvalumab group, and 5.6 months for the placebo-group (HR 0.55, 95% CI 0.44-0.67), similar to findings in the original publication. Median OS was 47.5 months for durvalumab, and 29.1 months for placebo (HR 0.71, 95% CI 0.57-0.88), both higher than in the original publication. Following the analyses of new subgroups of PD-L1 expression (≥25%, ≥1%, <1% and 1-24% compared to >25% and <25% in the original publication), the result of PD-L1 increasing PFS in all subgroups, and all but the <1% PD-L1 group for OS (HR 1.05, 95% CI 0.69-1.62), was consistent with results from the 2020 analyses (38, 39).

In summary, the original publication and follow-ups at three and four years after randomization and treatment initiation shows promising and consistent results. Findings indicate significantly increased progression-free survival time and overall survival time for patients treated with Durvalumab compared to placebo. Analyses of subgroups by PD-L1 expression indicates increased PFS and OS with high PD-L1 expression across all groups except OS in patients with less than 1% PD-L1 expression on tumor cells (37-39).

6.2.5 IMvigor210: Atezolizumab in treatment of locally advanced of metastatic urothelial bladder cancer

6.2.5.1 Cohort 1: Treatment naïve patients ineligible for cisplatin-based chemotherapy (40)

The study has no official publication of study results, only publications of study results indexed to the study by its NCT-number. As of May 31st, 2021, one publication has been indexed to this study and reviewed. The study is active, not recruiting. The study is an open-label, non-randomized, international phase 2 study of atezolizumab, a humanized IgG1 antibody specific for PD-L1 (41) in treatment of locally advanced or metastatic urothelial bladder cancer. Primary outcome measures were number of patients achieving complete response or partial response based on RECIST 1.1. A number of secondary outcome measures were included, among them only PFS per RECIST 1.1 and OS were, as they were the common endpoints included in other studies reviewed (40).

Table 6: Overview of IMvigor210 cohort 1 trial results (42)

	All patients (n=119)	PD-L1 <1% (ICo) (n=39)	PD-L1 ≥1% < 5% (IC1) (n=48)	PD-L1 ≥5% (IC2/3) (n=32)	PD-L1 ≥1% (IC1/2/3) (n=80)
ORR (%) (95% CI)	23 (16-31)	21 (9-36)	21 (10-35)	28 (14-47)	24 (15-35)
Median PFS (months) (95% CI)	2.7 (2.1-4.2)	2.6 (2.1-5.7)	2.1 (2.1-5.4)	4.1 (2.3-11.8)	Not reported
Median OS (months) (95% CI)	15.9 (10.4-not reached)	19.1 (9.8-not reached)		12.3 (6.0-not reached)	Not reported

In the publication of results for cohort 1, 119 patients were treated with atezolizumab and later assessed for primary outcome measures. Patients were grouped by PD-L1 expression levels, in the article defined as PD-L1 positive immune cells "ICo": <1%, "IC1": ≥1% but <5% and "IC2/3": ≥5%, as well as grouping all positive (>1%) as "IC1/2/3". In the IC2/3 group, ORR was 28% (95% CI 14-47), for patients in the IC1/2/3 group 24% (95% CI 15-35), 21% (95% CI 9-36) in the IC1 group and 21% (95% CI 9-36) in the ICo group. Median PFS was 4.1 months (95% CI 2.3-11.8) in the IC2/3 group, 2.1 months (95% CI 2.1-5.4) in IC1 and 2.6 months (95% CI 2.1-5.7) in ICo. Median OS was 12.3 months (95% CI 6.0-not reached) in IC2/3 and 19.1 months (95% CI 9.8-not reached) in IC0/1 (43).

In summary, all groups had objective responses, and PD-L1 was not a good predictor of efficacy (43).

6.2.5.2 Cohort 2: Patients with progression during or after platinum-based chemotherapy (44)

This cohort of the study has no official publication of results, but 7 publications indexed through its NCT-number. Of these, the first publication of trial results was included. Studies for cohort 1, analyses of systemic and somatic factors other than PD-L1 in clinical response, post-progression studies, studies of outcomes based on previous treatment and

pharmacokinetic studies were excluded. The trial is active, not recruiting. The study is an open-label, non-randomized, international phase 2 study of atezolizumab in treatment of patients that have had progression of disease during or after platinum-based chemotherapy. Primary outcome measures were number of patients with ORR (CR or PR) based on RECIST 1.1 (44). As in cohort 1, several secondary outcome measures were registered in the trial, out of which only PFS and OS was reviewed in addition to the primary endpoint.

Table 7: Overview of latest IMvigor210 coohort 2 trial results (42).

	All patients (n=311)	**PD-L1 <1% (ICo) (n=103)**	**PD-L1 ≥1% < 5% (IC1) (n=108=**	**PD-L1 ≥5% (IC2/3) (n=100)**	**PD-L1 ≥1% (IC1/2/3) (n=208)**
ORR (%) (95% CI)	15 (11-19)	8 (3-15)	10 (5-18)	26 (18-36)	18 (13-24)
Median OS (months) (95% CI)	7.9 (6.6-9.3)	6.5 (4.4-8.3)	6.7 (5.1-8.8)	11.4 (9.0-not reached)	8.8 (7.1-10.6)

310 patients received treatment with atezolizumab. Subgroups were categorized by PD-L1 expression and named as in cohort 1 (ICo": <1%, "IC1": ≥1% but <5% and "IC2/3": ≥5%, as well as grouping all positive (>1%) as "IC1/2/3). For the IC2/3 group, ORR was 26% (95% CI 18-36), 18% (95% CI 13-24) in IC1/2/3, and 15% (95% CI 11-19) in the total population. Median OS was 11.4 months (95% CI 9.0-not reached) in IC2/3, 8.8 months (95% CI 7.1-10.6) in IC1/2/3, 6.5 months (4.4-8.3) in ICo, 6.7 months (5.1-8.8) in IC1 and 7.0 months (95% CI 6.6-9.3) in the total population (42).

In summary, Atezolizumab was found to have promising anti-tumor effects with objective response rates across all treatment subgroups. High expression of PD-L1 in this study was associated with higher response rates as well as increased OS time, but was only considered to be only partially indicative of treatment effect. The authors also found that genomic subtypes with high CD8+, indicative of high effector T-cell presence in addition to high PD-L1 expression were associated with significantly better responses (42).

6.3 Comparison of selected therapies

For anti-PD-1, Nivolumab and Pembrolizumab were included for further study. Both are IgG4 antibodies, but pembrolizumab is a humanized variant, while nivolumab is fully human. They are approved with several different indications, most of which were based on cancer type and stage. These had overlap in their indication for treatment of non-small cell lung cancer (NSCLC) and urothelial carcinoma (UC), wherein Nivolumab had no immunological criteria, but Pembrolizumab did. Use of Nivolumab is indicated for locally advanced or metastatic NSCLC (*CheckMate 017*) and locally advanced, inoperable or metastatic UC, where platina-based therapy has failed (CheckMate 275). Pembrolizumab is approved for use in locally advanced or metastatic NSCLC with tumor expression of PD-L1 ≥ 1% tumor proportion score (TPS) *(KEYNOTE-010)* and locally advanced or inoperable UC in adults who cannot be treated with cisplatin-based chemotherapy, with expression of PD-L1 with "combined positive score" (CPS) ≥ 10% (KEYNOTE-052). These methods (TPS and CPS) are different ways to evaluate PD-L1 expression. TPS uses total count of PD-L1 expressing tumor cells in a sample, while CPS, in addition to tumor cells, includes lymphocytes and macrophages in the count. While these produce somewhat different results, they are considered to be of equal value when predicting response of anti-PD-1/PD-L1 therapy (35). These two indications for using either Nivolumab or Pembrolizumab, which are both anti-PD-1 drugs, were considered so similar, only differing in the inclusion of TPS/CPS, which in itself is very similar, that they were selected for further study into whether the inclusion of immunological markers produce different treatment outcomes. Cancer type, generic and trade name for drugs, their indication and associated clinical trial short name and unique national clinical trial identifier (NCT) are summarized in table 2.

Table 8: Selected drugs and their indications after SPC/Clinical trial registry review

Cancer type	Drug	Indication	Trial short name/NCT identifier[a]
NSCLC	Nivolumab (*Opdivo*)	Locally advanced or metastatic after chemotherapy	CheckMate 017 / NCT01642004
	Pembrolizumab (*Keytruda*)	Locally advanced or metastatic after chemotherapy, and expression of PD-L1 with ≥1% TPS	KEYNOTE-010 / NCT01905657
UC	Nivolumab (*Opdivo*)	Locally advanced, inoperable or metastatic where platina-based therapy has failed	CheckMate 275 / NCT02387996
	Pembrolizumab (*Keytruda*)	Locally advanced or inoperable in adults who cannot be treated with cisplatin-based chemotherapy, and with expression of PD-L1 with "Combined positive score" (CPS) ≥ 10%	KEYNOTE-052 / NCT02335424

[a] ClinicalTrials.gov identifier

For Anti-PD-L1 therapy, Durvalumab and Atezolizumab were considered due to both being indicated for use in treatment of NSCLC. Durvalumab is indicated for disease without progression after chemotherapy, while Atezolizumab had no criteria for progression. Because of this difference, they were considered too difficult to compare directly, as the criteria for non-progression is indicative of better outcomes.

6.3.1 Comparison of clinical trial results

6.3.1.1 Clinical trials on treatment of non-small cell lung cancer

6.3.1.1.1 Checkmate 017: Nivolumab in treatment of locally advanced or metastatic NSCLC after chemotherapy (45)

The trial has no official publication with results, but 5 publications are indexed to the NCT. The first publication with results and a publication with two-year outcomes were selected for

review. 3 articles were excluded, one focusing on outcome in patients with liver metastasis, one dosing- and safety-study and one study of efficacy-prediction. The study is a randomized, open-label, international phase 3 safety- and efficacy-study of Nivolumab, a fully human IgG4 PD-1 specific antibody (46), compared to Docetaxel in previously treated advanced or metastatic (stage IIIB/IV) squamous NSCLC (SQ-NSCLC) in patients 18 or older with an Eastern Cooperative Oncology Group (ECOG) performance-status of 0 or 1 – indicating low disability and mild symptoms. Patients with autoimmune disease, symptomatic interstitial lung disease, systemic immune suppression, prior T-cell therapy, prior therapy with checkpoint-targeted therapy or prior docetaxel therapy were excluded. This study is as of late May 2021 still active as patients are receiving treatment and/or being examined but is not recruiting. The primary end point of the study was overall survival (OS) time, defined as time from randomization and date of death from any cause (45).

272 patients were randomly assigned to receive nivolumab or docetaxel, and 260 were ultimately randomized and treated, 131 with nivolumab and 129 with docetaxel. All randomized patients had previously been treated with platinum-based therapy. PD-L1 expression in tumor-biopsy samples retrospectively and characterized by expression levels of 1%, 5% or 10% to evaluate prognostic and predictive roles of expression. Median survival was 9.2 months (95% CI 7,3-13.3) in the nivolumab group, and 6.0 months (95% CI 5.1-7.3) for docetaxel, with a 41% reduction in risk of death (HR 0.59, 95% CI 0.44-0.79, $p<0.001$). Median PFS in the nivolumab group was 3.5 months, and 2.8 months with docetaxel. No significant difference in OS, PFS or ORR was observed with higher expression levels of PD-L1 (47).

Both agents are used in the treatment of locally advanced or metastatic NSCLC after chemotherapy with similar selection criteria, exclusion criteria and end points, but they differ in patient group. In Checkmate 017, only patients with squamous NSCLC were studied, while for KEYNOTE-052, both patients with squamous and non-squamous NSCLC (non-SQ-NSCLC) were included (25, 47).

6.3.1.2 Clinical trials on urothelial cancer

6.3.1.2.1 CheckMate 275: Nivolumab in treating metastatic or unresectable bladder cancer (48)

This study has no official publication with results after completed study. One article reporting interim data is indexed to its NCT-number, this was selected for review. The study is active,

not recruiting. The study is an open-label, non-randomized, phase 2 study of nivolumab in treating patients with metastatic or unresectable urothelial cancer that have had progression or recurrence after treatment with platinum-based therapy. Patients with central nervous system metastases, known or active autoimmune disease, conditions requiring corticosteroid treatment or other immunosuppressive medications, or previous treatment with drugs targeting T-cell co-stimulation or immune checkpoints were excluded. Primary outcome measures were ORR via RECIST 1.1. and ORR per PD-L1 expression. Secondary outcome measures included PFS with RECIST 1.1 and OS, as well as both PFS and OS per PD-L1 expression levels. PD-L1 levels were grouped into ≥1% and ≥5% expression (48)

270 patients were treated with nivolumab, 265 of which were included in analyses. OR occurred in 19.6% (95% CI 15.0-24.9) of patients in the total population. ORR was 28.4% (95% CI 18.9-39.5) for patients with PD-L1 ≥5%, 23.8% (95% CI 16.5-32.3) for those with PD-L1 ≥1% and 16.1% (95% CI 10.5-23.1) in the PD-L1 <1% group. Median OS was 8.74 months (95% CI 6.05-not reached) in the total population, 11.30 months (95% CI 8.74-not reached) for the PD-L1 ≥1% and 5.95 months (95% CI 4.30-8.08) in the PD-L1 <1% group. Median PFS was 2.0 months (95% CI 1.87-2.63) in the total population, and was not presented by PD-L1 expression. The objective responses were better than OR with systemic chemotherapy in all subgroups, having set the lower 95% CI threshold at 10% ORR based on result studies for second-line single agent chemotherapy. While there are higher ORR and OS with higher PD-L1 expression, the authors conclude that there is an improved effect over current second-line single-agent chemotherapy for all PD-L1 expression levels, concurrent with the approved indication. The authors briefly discuss the differences in efficacy by PD-L1 expression in the publication compared to other trials, questioning if differences in analysis, cutoff limits or unknown mechanisms are involved, without concluding on anything specific (49).

6.4 Literature review on current practice and clinical challenges

6.4.1 Literature search results

Using the described search strategy with the search strings presented in the appendix, 1495 publications were identified, 621 results in PubMed and 874 in EMBASE. After pooling all results, removing duplicates and title and abstract review, 50 publications were selected for full text review. 14 articles were found to fulfill selection criteria (inclusion-/exclusion

criteria), 13 systematic reviews (50-62) and one retrospective descriptive study (63). Search strategy results are presented in fig. 3.

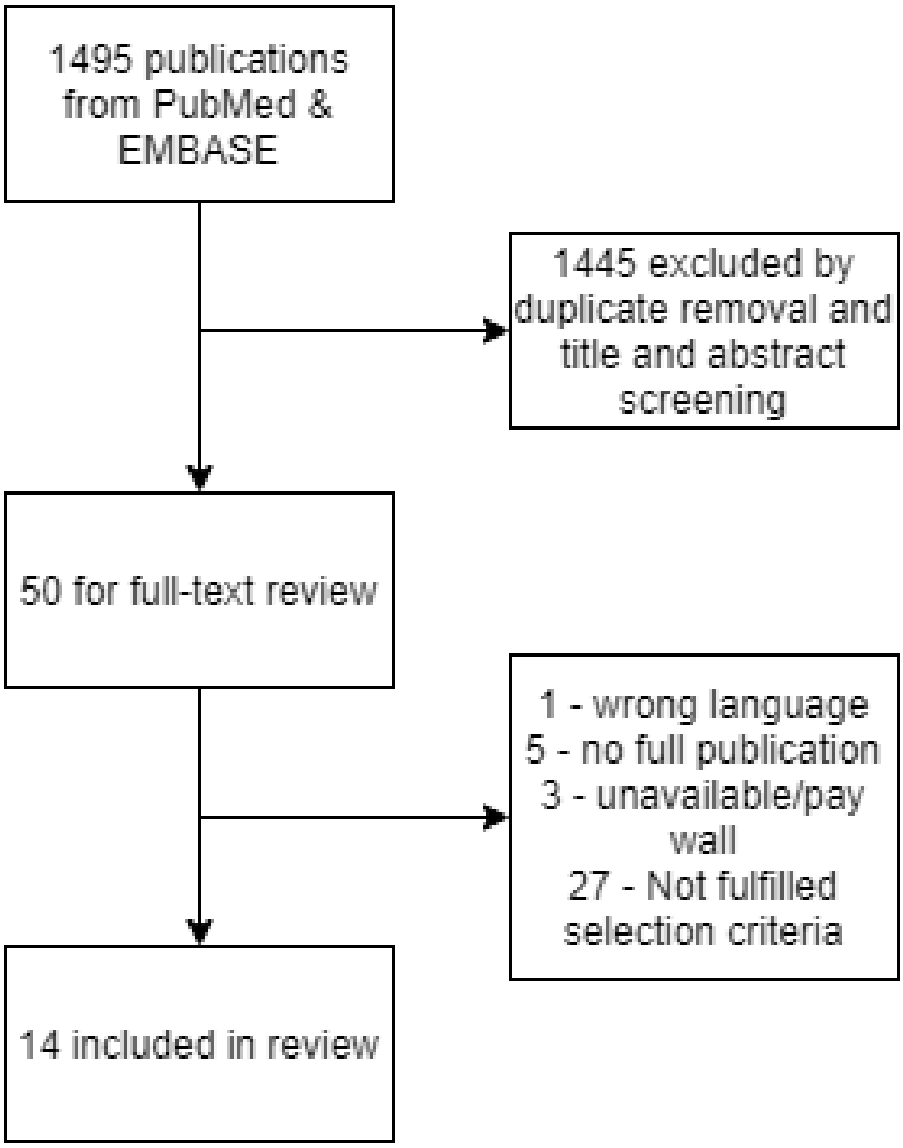

Figure 3: Flow-chart of literature search publication selection

As per the second primary objective of this study, the literature review was focused on investigating the cause for the differences in biomarker requirements for use of similar ICIs and the challenges facing clinicians when using these.

After reviewing all literature search results and the literature review itself, it became obvious that there is limited published data on post-approval clinical experience and practice (A lack that is also presented as an issue by Chen et al.(55) in their 2020 review of checkpoint

inhibitors in clinical practice). Publications selected as the best fit for review were articles reviewing clinical trial data, experiences from clinical practice as well as publications proposing or demonstrating guidelines in clinical practice. In the use of ICIs in clinical practice, the differences seen in requirements for biomarker validation, as well as the approval mostly for late-stage, is a complicated and multifaceted problem. As could be expected with such a relatively new and dynamic therapy paradigm, all reviews found in the literature search has key issues with ICI therapy in clinical practice as a major element (50-63).

6.4.2 Clinical practice guidelines

In reviewing RCTs and guidelines for checkpoint inhibition therapy globally, Bironzo and Di Maio (53) presented some very central thoughts on clinical guidelines that shed some light upon the approval process of ICIs and the rapid pace of their development. They present guidelines from European, Italian and two American scientific societies focused on developing recommendations and guidelines in cancer therapy (European Society of Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO), Italian Society of Medical Oncology (AIOM) and National Comprehensive Cancer Network (NCCN)). All of these have different practices when releasing guidelines. Comparing these, they find that because the field is so rapidly changing, various organs involved in recommendations and guidelines encounter a challenging dilemma: As they await solid and high-level evidence for approving therapies and/or releasing or improving guidelines, therapies vitally important for many patients are in limbo, or are used without clear guidelines. Being quick at releasing new guidelines, however, come at the cost of less rigid evidence. They do not attempt to come up with a specific solution to this, but make it clear that the methodology in developing recommendations is becoming too slow for the fast pace in therapy development (53).

Reviewing the intricacies of clinical guidelines was not part of the planned study. This insight into the challenges of such a rapidly evolving field was still considered appropriate. Considering the findings of the next chapters, the thoughts presented by Bironzo and Di Maio (53) is fitting, as they describe that the need for rapid approval with guidelines that in hindsight might be considered less-than-ideal, but justified.

6.4.3 Clinical practice

Many of the reviewed studies report correlation where high PD-L1 expression increases ICI efficacy in classic Hodgkin lymphoma (51), esophageal- and gastric cancers (56), non-small cell and small cell lung cancer (50, 52, 53, 61, 64), metastatic urothelial carcinoma (59),

metastatic melanoma (63), breast cancer (57, 58) and others. And in practice, ICI therapy is increasingly becoming part of standard cancer care (60), exemplified by Pembrolizumabs approval as the first ICI in first-line monotherapy for SCLC with >50% expression of PD-L1 and for NSCLC with >1% (53). More will likely follow, as many other ICIs are also being explored in trials as first-line therapy, such as for classic Hodgkin lymphoma (51), esophageal cancer (56), lung cancers (61, 64) and urothelial carcinoma (59), mostly in combination with chemotherapy. As becomes clear, the issues facing clinicians is not the lack of efficiency in patients with response, but multiple problems closely associated to the immunological mechanisms involved.

6.4.3.1 Patient selection and biomarkers

The potential of checkpoint inhibition as part of a new paradigm in cancer therapy is obvious, and it has become evident in the first half of this study that ICIs are beneficial for many patients that have increased survival time because of them. However, the objective response rates in the trials presented in the first half of this study are all below 50% (25, 27, 30, 33-35, 37-39, 42). As the increase in other outcome measures are so good, but response rates relatively low, there is clearly an issue with patient selection rather than the therapies efficacy. The issue with patient selection is central issue many of the reviewed studies discuss or try to solve (56, 58-60, 62, 64, 65). One major cause of this problem is a lack of biomarkers that can be reliably used in real-life-practice in an efficient way (60). Presently, while there are candidates, there seems to be no real consensus on how to properly select patients that are likely to have a good response to ICI therapy, or what mechanisms are most important to explore for mapping such markers (60). The obvious candidate, PD-L1 expression, is already presented herein as a viable biomarker for selecting ICI therapy, but it does not consistently predict good responders (55, 56, 62).

6.4.3.1.1 The challenges of PD-L1 as a biomarker

While focused on guidelines for breast cancer, many general issues with PD-L1 analysis are extensively reviewed by Gonzalez-Ericsson et al. (58). In their review, they raise concerns about multiple issues with the use of PD-L1 as a standard biomarker. First, PD-L1 is criticized for being inherently difficult to use due to it being highly variable both over time and in samples taken simultaneously. In addition to this, multiple assays, scoring methods and cut-offs are used for different cancer types and for different agents. All these result in reduced reproducibility and represents a large obstacle for efficient use in practice outside of the controlled setting of a clinical trial. Different practices globally are also described, where

agents approved with required expression analysis accepts any validated assay in the EU, but specific assays in other countries. These concerns are also raised by multiple other reviews (50, 55, 57, 60-62, 64).

The dynamic expression of PD-L1 is of course an issue, but changing the nature of ligand expression is not really an option in clinical practice, so the improvement of PD-L1 as a biomarker rests on the assays for measuring expression, scoring method, cut-off values and diagnostic guidelines. One complicating factor in standardization of PD-L1 measurement assays is the development process for ICIs. For their clinical trials, developers have all developed their own immunohistochemistry (IHC) assay for PD-L1 expression, and in the approval process, therapies that require or recommend PD-L1 measurement were required to use the assay associated with each agent (64). To assess the different assays, the Blueprint PD-L1 IHC assay Comparison Project, a joint effort from academia and industry, found that the comparability of the different assays was lower than acceptable, and thus it cannot be said that they are interchangeable (50). Considering that many of the reviewed trials in the first half of this study operates with lower cut-off limits at 1%, these differences in assays can have considerable effect in the selection of therapy for patients. The standardization of assays, scoring and cutoffs is a work in progress (58). At the time of publication for the latest article discussing these issues in this review, published in October 2020, this issue is still not resolved (62).

6.4.3.1.2 Emerging biomarkers in clinical practice

Citing Pagni et al. on the issue of patient selection: “Multiple lines of evidence suggests that in this setting, the vision of a single biomarker is somewhat naïve and imprecise, given that immunotherapy does not follow the rules that we have experiences in the past for targeted therapy.” (60, p.1). This statement highlights the main issue on PD-L1 as a biomarker for prediction of ICI effect: The critique from Pagni, Gonzalez-Ericsson and others are not meant to propose the replacement of PD-L1 as a biomarker, but to present the pressing need for multiple biomarkers in combination with PD-L1 for improved patient selection and to increase response rates (57, 58, 60, 62).

Besides PD-L1, tumor mutational burden (TMB) is the most frequently discussed biomarker, reviewed in varying degrees in 10 of the 14 reviewed articles (52, 55-62, 64). TMB is a biomarker that describes the number of somatic mutations in tumor DNA. It is hypothesized that a high mutational burden is associated with an increase in the number of tumor antigens,

leading to a wider and more robust immune response towards tumor cells, a tendency that is further reinforced when treated with ICIs (60). High TMB is associated with increased objective response rates, longer duration of response as well as longer PFS, when using ICIs in cancer therapy (55), and have shown promise across 27 tumor types (62). There are some issues, however. The cancer types where TMB is a good biomarker are often types that typically are highly immunogenic, such as NSCLC and UC (52, 60). In breast cancer on the other hand, which typically is not as immunogenic, TMB is found to have substantially less predictive effect, while the cause for this is not completely clear (57). In breast cancer, however, PD-L1 is a validated and good biomarker for efficacy (58). This is not unique for breast cancer. In some cancer types, TMB is a better predictor for treatment outcome, while in others PD-L1 is more appropriate. Because of this, the testing of PD-L1 and TMB is suggested as a standard when considering to use ICI therapy (50, 55, 60). TMB is still mostly used in investigational settings, somewhat due to it being time-consuming and expensive to perform as whole-genome sequencing, but more efficient methods are in development (50, 64).

Tumor-infiltrating lymphocytes is another biomarker that is widely discussed, being a topic in 10 of 14 reviews (51, 52, 55-58, 60-62, 64). The amount of TILs in the tumor microenvironment is known to be related to survival in a number of cancer types as well as being predictive of both ICI and chemotherapy effect (58, 60), but its role in ICI therapy is not fully understood (57). It is suspected that the effect of ICI depends in part upon the presence of lymphocytes in the tumor microenvironment, as lymphocytes are important for immune mediated cell death. Following this suspicion, TIL measurements are suggested to be highly predictive not only of efficacy, but also for prediction of PD-L1 expression, as the mechanism of tumor evasion by checkpoint inhibition would not be necessary were there no lymphocytes in the environment (56, 58, 60). Because of this, Gonzalez-Ericsson et al. (58), suggests in their review the introduction of TIL analyses in addition to PD-L1 in all diagnostic testing for breast cancer when considering ICI therapy. This combination is also being investigated for head- and neck cancers (62).

In addition to these three major biomarkers being explored as predictors of ICI therapy effect, a number of others are also being investigated. Most notably are mismatch repair-defects in tumors (MMR-deficient) and microsatellite instability (MSI). Both of these have been associated to greater benefit from ICI therapy (56, 62) due to the increase in mutation burden resulting from defects in DNA repair (64). MMR-deficient tumors are in fact so responsive to

Pembrolizumab treatment that in 2017, it was given the first cancer therapy approval without any specific site/tissue requirement (64).

6.4.3.2 Therapy timing

The previous paragraphs on reviews of biomarkers and clinical practice underline the important role of highly immunogenic tumors due to high numbers of tumor antigens as well as lymphocytes in the tumor microenvironment. The next challenge that is reviewed consistently is the issue of timing of ICI therapy. As mentioned previously in this study, many ICIs have been approved for late-line therapy. One issue with this is that the process of tumor immunoediting, where selection of more immune-resistant variants is selected, continues after tumor immune escape. Thus, late-stage therapy is suspected of potentially being less ideal than early therapy. This is reviewed by six of the 14 studies (50, 55, 56, 59, 60, 65).

Tay et al. (61) discuss the potential of using ICIs early in therapy in combination with chemotherapy, and at stages where chemotherapy is still efficient. They describe that in some trials, the increased tumor-antigen exposure that results from highly cytotoxic therapy can have a synergistic effect when combined with ICIs. As checkpoint inhibition benefits from a large number of tumor antigens, the utilization of the highly cytotoxic effects of early-line chemotherapy for releasing large amounts of tumor antigen can be beneficial. This follows from the increased efficacy of checkpoint inhibition where analyses show a high degree of mutation as well as a high degree of neoantigens. As more antigens are released due to widespread tumor cell death, the effects are assumed to be similar to having high mutational burden in that many potential antigens are present at the time of therapy initiation. In addition to discussing this as part of a chemotherapy regime, the authors also discuss this mechanism and its potential for use in combination with radiotherapy, a strategy already utilized in ongoing trials on lung cancers (61, 65).

7 Discussion

7.1 Results of primary objectives

The objective of this study was to review the use of checkpoint inhibitors in cancer therapy. To this end, two primary objectives has been explored:

- To investigate how common it is to require analysis of potential tumor escape mechanisms before treating with therapies developed based on the knowledge of such mechanisms, focusing on checkpoint inhibitors.

    - To review clinical trials for these therapies to assess the effect these mechanisms have on treatment.
    - To investigate whether similar agents were approved with similar indications, but with differences in these requirements.
- It was also a primary objective to perform a literature study on the current practice for using checkpoint inhibitors in clinical practice, to further investigate the reasons for differences in requirements in the first objective, and to review central challenges in checkpoint inhibitor therapy.

To summarize the findings of this study: I found that all therapies aimed at suspected tumor immune escape mechanisms are checkpoint inhibitors, and that it is not that common for their different indications to require analysis of tumor overexpression of checkpoint ligands on tumor cells. Three out of seven checkpoint inhibitors were approved with partly immunology-based indications, and of 16 indications in total for all checkpoint inhibitors, only five require overexpression of PD-L1 on tumor cells. A few select therapies without such requirement show increased efficacy with overexpression, but these also have effect for patients without overexpression (47, 49).

In the literature search, all selected articles reviewed central issues when using checkpoint inhibitors in clinical practice (50-63). As for the differences in clinical trial results leading to differing requirements in the use of ICIs, no conclusive explanation was found in the literature review. Findings in the first half of this study combined with the literature review does, however, reveal parts of the explanation as well as pointing us in the right direction. PD-L1 overexpression is associated with significantly increased treatment effect, as seen in the review of clinical trials, but are plagued by low response rates, clearly indicating a lack of good patient selection (25, 27, 30, 33-35, 37-39, 42). Seeing this in combination with all the issues that were found in the literature review, like lack of standardization of PD-L1 assays and scoring (54, 55, 61), dynamic expression both in time and in localization in samples (50, 58, 62) as well as guidelines being practiced differently in various parts of the world (58), it seems highly likely that PD-L1 alone cannot predict which patients will have effect across all cancer types, especially with so many issues affecting reproducibility. The explanation for the differing trial results might lie in simple differences in measurement of PD-L1. However, if also including the many other biomarkers that are emerging from clinical trials, like tumor mutational burden and amount of tumor-infiltrating lymphocytes (55, 58, 60-62), it seems probable that further trials where more biomarkers are included are not only required to

improve patient selection, but also to fully understand the mechanisms involved in checkpoint inhibitor efficacy.

Answering this primary objective question also reveals many of the challenges of checkpoint inhibitors in clinical practice, as per the third primary objective. In addition to patient selection, the most central challenges in current practice doesn't seem to be the potential and efficacy, but rather therapy timing (50, 55, 56, 59, 60, 65). It is interesting that Tay et. Al. (61) reviews trials where introduction of ICIs early-line in therapy results in better outcomes. Considering the mechanisms of immune surveillance and immunoediting after development of a primary immune escape mechanism (7). One could speculate that these mechanisms not only are involved in the selection process in this development, but that so long as the immune system has any sort of anti-tumor activity it will continue to select for variants that are more resistant to immune elimination, a concept briefly discussed by Pagni et al. (60), stating that not only is this process involved in the sculpting of a budding tumor into immune escape, but also in further progression and relapse. This could be an argument for introducing checkpoint inhibitors earlier in therapy selection algorithms to utilize the immunogenic effect of first-line chemotherapy or radiotherapy, or to utilize the less sculpted anti-immune response mechanisms of cancer. In clinical practice, some agents are approved for first-line (53), and more are in trials as first-line (51, 54, 57, 59, 61). It will be very interesting to see if these gain the suspected synergistic effect in combination with current first-line therapy.

In the following sections I will discuss specific findings of interest, as well as some weaknesses and strengths of methods and literature used in this study.

7.2 On methods

In the selection of therapies, the use of The Norwegian Medicines Manual for Health Personnel is used mainly to reduce the scope of the subject. The manual is intended for general practitioners and for use by medical professionals for information on therapies in fields that are not their specialty. As such, it is likely that the list of included therapies and their indications are not completely up to date, especially considering the rapid development in the field, and therapies will be highly likely be used on different indications than those presented here. As the field of cancer immunotherapy is already massive and complex, a thorough and detailed comparison of every type of therapy for all approved indications as well as practice when used at specialist's discretion would be far beyond the scope of this study. This process of selection clearly represents a weakness in this thesis, as it cannot be

said to be exhaustive and will most likely be too simplified to realistically represent practice of specialists in fields where frequent use of cancer immunotherapy occurs. The addition of a literature study on clinical practice will to some extent make up for this simplification. The goals of this study were to review the approved use of checkpoint inhibitors, the clinical challenges of their use, their use for immunological indications, and to investigate whether these were based on immunological mechanisms or not. The resulting list of therapies, reviews and clinical trials for review was considered to be adequate for its purpose.

The review of trial results through publications indexed via the trials NCT-number and/or trial short name assumes that all publications of trial results and associated studies were indexed. It is highly likely that publications of interest for this study was missed due to faulty indexing and/or lack of registration of NCT-number/trial short name in publications. All trials selected for review had publications presenting results consistent with every therapies SPC as well as results published on ClinicalTrials.gov, and the weakness that potential faulty indexing represented was considered to be significantly reduced by comparing publications to SPC/NCT in this manner.

In selecting trials to review based on indication similarities, the selection process was somewhat superficial. This became obvious when reviewing the study populations, where nivolumab in Checkmate017, only treated patients with SQ-NSCLC (47) and pembrolizumab was used in treating both SQ-NSCLC and non-SQ-NSCLC in KEYNOTE-010 (25). This could be a more thorough review if results for patients with SQ-NSCLC for pembrolizumab was isolated and compared. As results were similar across both groups, however, this was considered precise enough. If such a review were performed, it could reduce the population size and potentially give different results. The objective of the review was to study results for studies within selection criteria, and the result was considered to be precise enough.

In the literature review, the use of PubMed and EMBASE was considered to cover enough databases to find as many relevant publications as was necessary. The difference between their search mechanisms, however, causes some issues. As PubMed uses automatic mapping of search terms to MeSH-terms, one can perform a search and review all mappings so as to include specific terms, but not others. This makes it simpler to directly include every term assessed to be relevant. EMBASEs guided searching is simple to use and be guided through relevant subject categories and using explode and focus mechanisms. While both are simple to use, the different mechanisms for searching can be difficult to get identical. Both resulted

in valid results, but due to the different search mechanisms, there is a possibility of gaps between these searches in which there would be relevant publications. The resulting collection of publications did cover many of the issues central to this field, so no further search strategy was considered necessary to investigate the objectives in this study.

7.3 On results

While it was not planned that lung cancer and urothelial carcinoma would dominate this study as much as it did, they ended up being the most reviewed cancer types. There are probably multiple reasons for this. Lung cancer is one of the most common cancer types in the world, only surpassed by female breast cancer worldwide, but still the most common in many countries, and is the leading cause of cancer death worldwide (1). As such, it is to be expected that it is the focus of companies doing research and drug development. In addition lung cancer is commonly seen amongst smokers, attributing for about two-thirds of lung cancers (1). The large increase in lung cancer risk is due to the chronic irritation and exposure to carcinogens (66). The mutagenic pressure that comes from this exposure to carcinogens often results in tumors with high mutagenic burden and therefore high immunogenicity (52, 60, 64). This increased immunogenicity can cause an increased number of neoantigens, and therefore a more robust immune response. Releasing these responses through checkpoint inhibition is therefore suspected to be more efficient, but not confirmed (64). While not confirmed, the fact that lung cancers dominate this review in the way that it does is potentially due to its highly immunogenic nature in addition to its incidence rate.

Much of the same can be said for urothelial carcinoma. It is the tenth most commonly diagnosed cancer worldwide, and is usually associated with good prognosis (1). Advanced bladder cancer, however, typically has PFS and OS of less than 14 months (59). It is also often highly immunogenic, and therapy effect with Durvalumab is strongly associated with PD-L1 expression as well as TMB (60). As with lung cancer, the high mutational load and subsequent high efficacy from ICI therapy could likely be an explanation as to why it became so prevalent in this study.

The KEYNOTE-010 trials (24-26) on pembrolizumab in NSCLC, while being a well-controlled RCT with a high level of evidence and quite significant results, a major flaw is the lack of a subgroup with no checkpoint overexpression. The subgroups of 1-49% and over 50% PD-L1 expression does not exclude the possibility of similar outcomes for patients with lower than 1% PD-L1 expression (26). Studies on pembrolizumab in combination with

chemotherapy has confirmed greater benefit with increased expression, but also promising results in those with ≤1% expression when compared to placebo and chemotherapy. Whether these results extend to pembrolizumab monotherapy has yet to be determined and will require further trials on this patient group (67).

In the IMvigor210 cohort 1 trials (40), no predictive effect of PD-L1 expression was found. The trial is, however, a phase 2 trial case series with no control arm as well as a limited population of 119 patients (43), all indicative of a low level of evidence (see appendix I for grading overview). As such, further trials with a larger population, more robust study design with control groups and masking is necessary for more conclusive evidence. Cohort 2 (44), also a case series and thus with a low level of evidence, in contrast found PD-L1 expression to be associated with better results across all subgroups (42). With this discrepancy as well as the level of evidence of these trials, findings therein were weighted lightly the conclusion of this study.

For all 6 reviewed clinical trials (5 trials, wherein IMvigor210 had 2 cohorts) (24, 28, 32, 36, 40, 44), the most common outcome measures were objective response rate by RECIST 1.1, overall survival in months from randomization to death of any cause and progression-free survival in time to progression per RECIST 1.1 (25, 30, 33, 42, 47, 68). As these were the common outcome measures for all studies, they were the focus when reviewing all studies. This could be considered a weakness in the study, as which outcome measures that are sensible can vary widely based on cancer type, staging and previous treatment. Focusing on few, but very common outcome measures, was still considered optimal as the measures that were chosen represents important aspects of cancer treatment development in general: increasing survival, treatment responsivity and increasing progression-free survival time.

Interestingly, only pembrolizumab and nivolumab fulfilled selection criteria for review based on similar indications. Both are IgG4 anti-PD-1 antibodies, only differing in nivolumab being fully human and pembrolizumab being humanized (25, 47). They are approved for many of the same cancer types and the same staging, while nivolumab has no indicational criteria for status of PD-1/PD-L1 for any cancer type, the majority of those Pembrolizumabs indications were based on expression of PD-L1 (69, 70). In CheckMate275, an increased effect of treatment with Nivolumab was seen for groups with higher PD-L1 expression, but due to the design of the study and primary outcome measures, this has not been included in approval. In the study, a lower 95% CI threshold of 10% was considered improvement over comparison

chemotherapy, and because all groups achieved this, it has been approved without such requirement (49). This is a very interesting approach to result analysis, but also interesting for understanding the mechanisms behind checkpoint inhibitors. As patients with high PD-L1 expression likely will have tumors where this expression plays a role in tumor immune escape, and this will have effect from checkpoint inhibitors, any patient could in theory be effectively treated with checkpoint inhibitors as it can act as a general "brake-releasing" therapy for the immune system – causing increased immune elimination of tumor cells and potentially overcoming whatever immune escape mechanism is utilized (71). Further studies are needed to clearly illustrate if this is the case, or if there are other mechanisms involved.

8 Conclusion

Less than half of all approved checkpoint inhibitors have immunology-based indications, and less than one third of all indications for checkpoint inhibitors require expression analysis of checkpoint ligands. All reviewed trials of antibodies with immunology-based indications had significant improvements in their primary outcome measures, either in comparison to described chemotherapy regimens, placebo or objective response for non-comparative studies. When assessing outcome measures by PD-L1 expression, all studies found significant increase of primary outcome measures with PD-L1 positive tumor cells or immune cells defined as PD-L1 expression ≥1%. In addition, a majority of the studies found increased OS and PFS with increasing PD-L1 expression.

The findings in this study suggest that immunology-based indications for use of checkpoint inhibitors are not common. In all clinical trials of agents with an immunology-based indication, significant improvement of overall survival, progression-free survival and objective response rates are affected by PD-L1 expression, but was not exclusive to patients with high PD-L1 expression. In addition, similar antibodies indicated for the same cancer type and stage are found to be more effective with increased PD-L1 expression in some studies, but not in others, indicating that there could be mechanisms involved in deciding therapy efficacy that are currently not included in indications.

The mechanisms involved in the clinically significant effects on patients with PD-L1 negative tumors is not fully understood, so the differences in trials and ultimately indications cannot be thoroughly explained yet. It seems highly likely that these differences can be explained by further investigating emerging biomarkers that complement PD-L1, such as tumor mutational

burden and measures of tumor-infiltrating lymphocytes and their predictive value in patient selection.

There are multiple central challenges clinicians face when using checkpoint inhibitors in clinical practice today. First, the assays used in testing PD-L1 expression are usually developed alongside the therapeutic agent, and these are not necessarily interchangeable between agents. In addition, there is a lack of standardization of scoring as well as cut-off limits for defining tumors as PD-L1 positive or negative. Second, patient selection is further complicated due to the lack of other biomarkers, some emerging ones are as mentioned being investigated, but so far only in the setting of clinical trials. Timing of therapy is also an issue, and it is suspected that it might be beneficial to use checkpoint inhibitors in a first-line setting. This remains to be clearly demonstrated.

The findings of this study could form the basis for designing a meta-analysis and/or a systematic review of checkpoint inhibitors in cancer treatment and biomarker expression to further elucidate the mechanisms involved in checkpoint inhibitor efficacy and proper patient selection.

9 References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
4. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. Science. 2013;342(6165):1432-3.
5. The Nobel Prize in Physiology or Medicine 2018. NobelPrize.org. Nobel Media AB 2021. Sat. 1 May 2021. [Available from: https://www.nobelprize.org/prizes/medicine/2018/summary/.
6. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest. 2007;117(5):1137-46.
7. Bruce AT, Ikeda H, Old LJ, Dunn GP, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11):991-8.
8. Ferro S, Huber V, Rivoltini L. Mechanisms of tumor immunotherapy, with a focus on thoracic cancers. J Thorac Dis. 2018;10(7):4619-31.
9. Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer. Immunologic Research. 2005;33(2):113-33.
10. Whiteside TL. Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention. Seminars in Cancer Biology. 2006;16(1):3-15.
11. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. International Immunology. 2007;19(7):813-24.
12. Pardoll DM. Immunology beats cancer: a blueprint for successful translation. Nat Immunol. 2012;13(12):1129-32.
13. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. Int Immunopharmacol. 2018;62:29-39.
14. Prasad V, Kaestner V, Mailankody S. Cancer Drugs Approved Based on Biomarkers and Not Tumor Type-FDA Approval of Pembrolizumab for Mismatch Repair-Deficient Solid Cancers. JAMA Oncol. 2018;4(2):157-8.
15. Joffre O, Nolte MA, Sporri R, Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol Rev. 2009;227(1):234-47.
16. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13(4):227-42.
17. Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: recent advances and combination therapeutic approaches. Breast Cancer (Dove Med Press). 2019/01/31 ed2019. p. 53-69 License: CC BY-NC 3.0.
18. Wang S, He Z, Wang X, Li H, Liu X-S. Antigen presentation and tumor immunogenicity in cancer immunotherapy response prediction. Elife. 2019;8:e49020.
19. Zappasodi R, Merghoub T, Wolchok JD. Emerging Concepts for Immune Checkpoint Blockade-Based Combination Therapies. Cancer Cell. 2018;33(4):581-98.
20. Umscheid CA, Margolis DJ, Grossman CE. Key Concepts of Clinical Trials: A Narrative Review. Postgraduate Medicine. 2011;123(5):194-204.
21. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019;19(3):133-50.

22. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. J Immunother Cancer. 2018;6(1):8.
23. Smith V, Devane D, Begley CM, Clarke M. Methodology in conducting a systematic review of systematic reviews of healthcare interventions. BMC Medical Research Methodology. 2011;11(1):15.
24. Merck Sharp & Dohme Corp. Study of Two Doses of Pembrolizumab (MK-3475) Versus Docetaxel in Previously Treated Participants With Non-Small Cell Lung Cancer (MK-3475-010/KEYNOTE-010) [Available from: https://ClinicalTrials.gov/show/NCT01905657.
25. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540-50.
26. Herbst RS, Garon EB, Kim D-W, Cho BC, Perez-Gracia JL, Han J-Y, et al. Long-Term Outcomes and Retreatment Among Patients With Previously Treated, Programmed Death-Ligand 1–Positive, Advanced Non–Small-Cell Lung Cancer in the KEYNOTE-010 Study. Journal of Clinical Oncology. 2020;38(14):1580-90.
27. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;372(21):2018-28.
28. Merck Sharp & Dohme Corp. Study of Pembrolizumab (MK-3475) Compared to Platinum-Based Chemotherapies in Participants With Metastatic Non-Small Cell Lung Cancer (MK-3475-024/KEYNOTE-024) [Available from: https://ClinicalTrials.gov/show/NCT02142738.
29. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Five-Year Outcomes With Pembrolizumab Versus Chemotherapy for Metastatic Non–Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score ≥ 50%. Journal of Clinical Oncology. 2021;39(21):2339-49.
30. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2016;375(19):1823-33.
31. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.
32. Merck Sharp & Dohme Corp. Study of Pembrolizumab (MK-3475) in Participants With Advanced Urothelial Cancer (MK-3475-052/KEYNOTE-052) [Available from: https://ClinicalTrials.gov/show/NCT02335424.
33. Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18(11):1483-92.
34. Vuky J, Balar AV, Castellano D, O'Donnell PH, Grivas P, Bellmunt J, et al. Long-Term Outcomes in KEYNOTE-052: Phase II Study Investigating First-Line Pembrolizumab in Cisplatin-Ineligible Patients With Locally Advanced or Metastatic Urothelial Cancer. J Clin Oncol. 2020;38(23):2658-66.
35. De Marchi P, Leal LF, Duval da Silva V, da Silva ECA, Cordeiro de Lima VC, Reis RM. PD-L1 expression by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) are similar in non-small cell lung cancer (NSCLC). Journal of Clinical Pathology. 2021:jclinpath-2020-206832.

36. AstraZeneca. A Global Study to Assess the Effects of MEDI4736 Following Concurrent Chemoradiation in Patients With Stage III Unresectable Non-Small Cell Lung Cancer [Available from: https://ClinicalTrials.gov/show/NCT02125461.
37. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med. 2017;377(20):1919-29.
38. Faivre-Finn C, Vicente D, Kurata T, Planchard D, Paz-Ares L, Vansteenkiste JF, et al. Four-Year Survival With Durvalumab After Chemoradiotherapy in Stage III NSCLC-an Update From the PACIFIC Trial. J Thorac Oncol. 2021;16(5):860-7.
39. Paz-Ares L, Spira A, Raben D, Planchard D, Cho BC, Ozguroglu M, et al. Outcomes with durvalumab by tumour PD-L1 expression in unresectable, stage III non-small-cell lung cancer in the PACIFIC trial. Ann Oncol. 2020;31(6):798-806.
40. Hoffmann-La Roche. A Study of Atezolizumab in Participants With Locally Advanced or Metastatic Urothelial Bladder Cancer (Cohort 1) [Available from: https://ClinicalTrials.gov/show/NCT02951767.
41. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563-7.
42. Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909-20.
43. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017;389(10064):67-76.
44. Hoffmann-La Roche. A Study of Atezolizumab in Participants With Locally Advanced or Metastatic Urothelial Bladder Cancer (Cohort 2) [Available from: https://ClinicalTrials.gov/show/NCT02108652.
45. Bristol-Myers Squibb. Study of BMS-936558 (Nivolumab) Compared to Docetaxel in Previously Treated Advanced or Metastatic Squamous Cell Non-small Cell Lung Cancer (NSCLC) (CheckMate 017) [Available from: https://ClinicalTrials.gov/show/NCT01642004.
46. Wang C, Thudium KB, Han M, Wang XT, Huang H, Feingersh D, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014;2(9):846-56.
47. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;373(2):123-35.
48. Bristol-Myers Squibb. A Study of Nivolumab in Participants With Metastatic or Unresectable Bladder Cancer [Available from: https://ClinicalTrials.gov/show/NCT02387996.
49. Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. The Lancet Oncology. 2017;18(3):312-22.
50. Abdel Karim N, Kelly K. Role of Targeted Therapy and Immune Checkpoint Blockers in Advanced Non-Small Cell Lung Cancer: A Review. Oncologist. 2019;24(9):1270-84.
51. Bair SM, Mato A, Svoboda J. Immunotherapy for the Treatment of Hodgkin Lymphoma: An Evolving Paradigm. Clin Lymphoma Myeloma Leuk. 2018;18(6):380-91.

52. Barnet MB, Cooper WA, Boyer MJ, Kao S. Immunotherapy in non-small cell lung cancer: Shifting prognostic paradigms. Journal of Clinical Medicine. 2018;7(6).
53. Bironzo P, Di Maio M. A review of guidelines for lung cancer. Journal of Thoracic Disease. 2018;10(Supplement13):S1556-S63.
54. Califano R, Lal R, Lewanski C, Nicolson MC, Ottensmeier CH, Popat S, et al. Patient selection for anti-PD-1/PD-L1 therapy in advanced non-small-cell lung cancer: Implications for clinical practice. Future Oncology. 2018;14(23):2415-31.
55. Chen Y, Pei Y, Luo J, Huang Z, Yu J, Meng X. Looking for the Optimal PD-1/PD-L1 Inhibitor in Cancer Treatment: A Comparison in Basic Structure, Function, and Clinical Practice. Front Immunol. 2020;11:1088.
56. De Mello RA, Lordick F, Muro K, Janjigian YY. Current and Future Aspects of Immunotherapy for Esophageal and Gastric Malignancies. Am Soc Clin Oncol Educ Book. 2019;39:237-47.
57. de Melo Gagliato D, Buzaid AC, Perez-Garcia J, Cortes J. Immunotherapy in Breast Cancer: Current Practice and Clinical Challenges. BioDrugs. 2020;34(5):611-23.
58. Gonzalez-Ericsson PI, Stovgaard ES, Sua LF, Reisenbichler E, Kos Z, Carter JM, et al. The path to a better biomarker: application of a risk management framework for the implementation of PD-L1 and TILs as immuno-oncology biomarkers in breast cancer clinical trials and daily practice. Journal of Pathology. 2020;250(5):667-84.
59. Grivas P, Agarwal N, Pal S, Kalebasty AR, Sridhar SS, Smith J, et al. Avelumab first-line maintenance in locally advanced or metastatic urothelial carcinoma: Applying clinical trial findings to clinical practice. Cancer Treat Rev. 2021;97:102187.
60. Pagni F, Guerini-Rocco E, Schultheis AM, Grazia G, Rijavec E, Ghidini M, et al. Targeting immune-related biological processes in solid tumors: We do need biomarkers. International Journal of Molecular Sciences. 2019;20(21).
61. Tay RY, Heigener D, Reck M, Califano R. Immune checkpoint blockade in small cell lung cancer. Lung Cancer. 2019;137:31-7.
62. Wang HC, Yeh TJ, Chan LP, Hsu CM, Cho SF. Exploration of feasible immune biomarkers for immune checkpoint inhibitors in head and neck squamous cell carcinoma treatment in real world clinical practice. International Journal of Molecular Sciences. 2020;21(20):1-22.
63. Arheden A, Skalenius J, Bjursten S, Stierner U, Ny L, Levin M, et al. Real-world data on PD-1 inhibitor therapy in metastatic melanoma. Acta Oncologica. 2019;58(7):962-6.
64. Califano R, Lal R, Lewanski C, Nicolson MC, Ottensmeier CH, Popat S, et al. Patient selection for anti-PD-1/PD-L1 therapy in advanced non-small-cell lung cancer: implications for clinical practice. Future Oncol. 2018;14(23):2415-31.
65. Tay R, Prelaj A, Califano R. Immune checkpoint blockade for advanced non-small cell lung cancer: Challenging clinical scenarios. Journal of Thoracic Disease. 2018;10(Supplement13):S1494-S502.
66. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health. 2017;32(8):1018-36.
67. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2018;378(22):2078-92.
68. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. The Lancet. 2017;389(10064):67-76.

69. Norsk legemiddelhåndbok for helsepersonell 2021 [cited 31.05.21]. Available from: https://www.legemiddelhandboka.no/L2.3.7.2/Pembrolizumab.
70. Norsk legemiddelhåndbok for helsepersonell 2018 [cited 31.05.21]. Available from: https://www.legemiddelhandboka.no/L2.3.7.1/Nivolumab.
71. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018;50(12):1-11.

APPENDIX 1:

1 Tables

Table 1: Overview of monoclonal antibodies used in cancer treatment

Antibody generic name	Specificity	Indications	Imm. Indications
Cetuximab	EGFR	2	0
Panitumumab	EGFR	1	0
Trastuzumab	HER2	2	0
Trastuzumab-emtansin	HER2	1	0
Pertuzumab	HER2	2	0
Aflibercept	VEGF	1	0
Bevacizumab	VEGF	7	0
Ramucirumab	VEGF	2	0
Dinutiksimab beta	GD2	1	0
Ofatumumab	CD20	1	0
Rituximab	CD20	3	0
Obinutuzumab	CD20	2	0
Inotozumab	CD22	2	0
Brentuksimab vedotin	CD30	3	0
Gemtuzumab	CD33	1	0
Daratumumab	CD38	1	0
Polatuzumab vedotin	CD79b	1	0
Ipilimumab	CTLA4	1	0
Nivolumab	PD-1	6	0
Pembrolizumab	PD-1	4	3
Cemiplimab	PD-1	2	0
Atezolizumab	PD-L1	1	1
Avelumab	PD-L1	1	0
Durvalumab	PD-L1	1	1

Table 2: Overview of checkpoint inhibitors. Checkpoint inhibitors used in cancer therapy are listed with generic name and categorized by their main specificity (Anti-CTLA-4, Anti-PD-1 or Anti-PD-L1) and description of indications listed in their SPC. Drugs/indications selected for review listed in cursive. Drug indications selected for comparison listed in bold.

Anti-CTLA4 (=CD152)
Ipilimumab
Monotherapy*:* Treatment of inoperable or metastatic melanoma
Combination therapy*:* Treatment of inoperable or metastatic melanoma, in combination with nivolumab
Anti-PD1
Nivolumab (Anti PD-1)
Malignant melanoma*:* Inoperable or metastatic
Non-small cell lung cancer: Locally advanced or metastatic after chemotherapy
Renal cell carcinoma: Advanced after first line treatment
Hodgkins lymphoma: Recurrent or refractory after high-dose therapy and autologous stem-cell transplantation (HDT-ASCT) and brentuksimab-vedotin
Squamous cell carcinoma of head and neck: Progressed during or after platina-based therapy
Urothelial carcinoma: Locally advanced, inoperable or metastatic where platina-based therapy has failed
Pembrolizumab (Anti PD-1)
Malignant melanoma: Inoperable or metastatic
Non-small cell lung cancer: Metastatic and with tumor expression of PD-L1 ≥ 50% "tumour proportion score" (TPS) without EGFR- og ALK-positive mutations
Non-small cell lung cancer: Locally advanced or metastatic and expression of PD-L1 with ≥1% TPS
Classic Hodgkins lymphoma: Failure to treat after HDT-ASCT and brentuksimab vedotin, or not candidates for HDT-ASCT
Urothelial carcinoma: Locally advanced or inoperable in adults who cannot be treated with cisplatin-based chemotherapy, and with expression of PD-L1 with "Combined positive score" (CPS) ≥ 10%
Cemiplimab (Anti PD-1)
Cutaneous squamous cell carcinoma: Metastatic or locally advanced and not candidate for curative surgery or radiation
Anti PD-L1
Durvalumab (Anti PD-L1)
Non-small cell lung cancer: Locally advanced, inoperable and with tumor expression of PD-L1 ≥ 1% of tumor cells and without progression after platina-based chemoradiation
Avelumab (Anti PD-L1)
Merkel cell carcinoma: Metastatic
Atezolizumab (Anti PD-L1)
Urothelial carcinoma: Metastatic or locally advanced after platina-based chemotherapy or not candidates for platina-based therapy, and with tumour expression of PD-L1 ≥5%
Non-small cell lung cancer: Locally advanced or metastatic after chemotherapy.

2 Literature review search strings

2.1 PubMed MeSH-term search string

(immune checkpoint inhibitor OR (immune checkpoint AND therapy) OR ("immunotherapy" AND "checkpoint") OR "checkpoint inhibitors" OR "immune checkpoint blockade" OR "checkpoint inhibitor" OR "checkpoint inhibition" OR antibodies OR antibody OR "programmed cell-death protein 1" OR programmed cell death ligand OR "PD-L1 inhibition" OR "PD-L1 inhibitor" OR "PD-1 inhibition" OR "PD-1 inhibitor" OR "CTLA-4 inhibition" OR "CTLA-4 inhibitor") AND ("current practice" OR "clinical practice" OR "off-label" OR "off label") AND (cancer OR malignancy OR "malignancies" OR "tumor" OR carcinoma OR neoplasm)

2.2 EMBASE search description

1. Exp clinical practice/
2. Exp malignant neoplasm/dt, th [Drug Therapy, Therapy]
3. Exp antineoplastic agent/dt, th [Drug Therapy, Therapy]
4. (immune checkpoint inhibitor or (immune checkpoint and therapy) or ("immunotherapy" and "checkpoint") or "checkpoint inhibitors" or "immune checkpoint blockade" or "checkpoint inhibitor" or "checkpoint inhibition" or antibodies or antibody or "programmed cell-death protein*" or programmed cell death ligand or "PD-L1 inhibition" or "PD-L1 inhibitor" or "PD-1 inhibition" or "PD-1 inhibitor" or "CTLA-4 inhibition" or "CTLA-4 inhibitor").mp
5. 1 and 2 and 3 and 4
6. from 5 keep 6,12-13,20,30,48-49,52,54,88-89,107-108,112,116,121,124-125,130,132,136,154-156,160,171,189,196,200,203,205,222,226,231,235,237,241,244,248,256-257,261,265,269,272,277,280,289-290,297,304-305,321,324,326,329-330,335-336,338,355,358,362,371-373,378,380,382,393,396-397,402,405-406,414,417,421,426,432,442,446,450,458,464,467,497,500,503,507-508,524,532,536-537
7. from 6 keep 4,6,12,16,17,18,19,23,25,27,34,36,37,39,40,41,42,44,47,48,51,55,58,63,64,65,66,67,84,86
8. from 7 keep 6-7,9,16,18,20,22,25-26

3 Changelog from previous version and comments on feedback

Evaluation feedback: Too limited for a master thesis

- Lack of tables and figures
    - Presented results in tables and added short text summaries to all clinical trial reviews.
- Theme too far removed from clinical practice
    - Theming closer to clinical practice by review of clinical practice and challenges faced by clinicians outside of a clinical trial setting
- More extensive immunological background and further explanation for effects of checkpoint inhibitors in PD-1/PD-L1-negative patients
    - More immunological basis in introduction as well as more extensive review of checkpoint inhibitor mechanisms through literature review
- Large focus on approval trials, and interesting to review literature beside clinical trials
    - Suggestion used as inspiration for a more extensive study by including literature review of current practice, not only widening the objectives but also giving more insight into findings of therapy- and trial review. Attempted to design literature review so as to cover both two previous feedback points as well.

Evaluation feedback: Lack of references in discussion, repeating introduction and results

- Attempted to clearly demonstrate sources both in contents of text as well as including references to reference list for all parts of discussion.
- Attempted to limit repeating results and introduction statements as much as possible without losing comprehensiveness of discussed subjects

Other feedback:

- Lack of more theory on cancer forms included
    - Most common cancer types in the study now more extensively presented in literature review and discussion
- Little need for extensive methodology and lack of presentation of selection criteria etc.
    - Expanded method section of first half of the study
    - More literature and guidelines used in development of search strategy and methodology in literature review
- Lacking names in references for clinical trial overviews
    - Added appropriate names for text and information responsible sponsor/company for all clinical trials

4 GRADE-evaluation

All 8 official/first publications from clinical trials on selected and reviewed has been evaluated. 4 were case series, both IMvigor210, CheckMate275, and KEYNOTE-052. 4 were randomized controlled trials, KEYNOTE-010, KEYNOTE-024, CheckMate017 and PACIFIC. All fulfilled major criteria for their study-type, and most had significant results for primary outcome measures as well as secondary outcome measures. GRADE-charts are presented in the following section.

Referanse: Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540-50.

Studiedesign: RCT

Grade - kvalitet 1b

Purpose

Assessment of efficacy of pembrolizumab compared to docetaxel in treatment of previously treeated, PD-L1 positive advancec non-small cell lung cancer

Conclusion

Pembrolizumab increases overall survival for patients in this group.

Countries

Argentina, Australia, Belgium, Brazil, Canada, Chile, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Japan, Lithuania, Netherlands, Portugal, Russia, South Africa, South Korea, Spain, Taiwan, UK, and USA

Years data collection

August 28, 2013 – feb. 27, 2015.

Materials and methods

Study population recruitment

2699 patients screened, 2222 w/PD-L1 expression, 1034 matching all eligibility criteria and 991 treated with at least first dose

Inclusion-/exclusion criteria

Inclusion: Written consent, over 18 years, life expectancy of at least 3 months, histologically or cytologically confirmed non-small cell lung cancer and at least one measurable lesion by RECIST 1.1, radiographically confirmed progression per RECIST 1.1 after at least two cycles of platinum-containing chemotherapy, ECOG 0 or 1, adequate organ function, provided PD-L1 biomarker tissue for analysis, PD-L1 positive tumor, none or low-grade toxic effects after chemotherapy, negative pregnancy test for female participants

Exclusion: Prior docetaxel therapy for NSCLC, participating or have participated in trials of an agent or device within last 30 days, receiving systemic steroid therapy or any form of immunosuppressive within 3 days prior to first dose, expecting to require systemic or localized antineoplastic therapy, prior systemic cytotoxic chemotherapy, antineoplastic og biological therapy or major surgery within 3 weeks prior to first dose, thoracic radiation therapy >30 Gy within 6 months prior to first dose, prior tyrosine kinase inhibitor therapy or palliative radiotherapy within 7 days of first dose, prior therapy with anti-PD-1/anti-PD-L1/L2, anti-CD137 or anti CTLA-4, known history of prior malignancy except when without evidence of recurrence for 5 years, no CNS metastases and/or carcinomatous meningitis, active autoimmune disease

Data

Study population, grouped by dose regime and PD-L1 expression

Outcome validation

Increased progression-free survival (PFS)time and overall survival (OS) time measured by objective criteria in RECIST 1.1.

Exposure variables (validated/non-validated)

Pembrolizumab treatment/docetaxel treatment

Important confounding factors

Unknown mechanisms affecting outcomes in addition to PD-L1 expression: tumor infiltrating cells, mutational load, other differences in tumor microenvironment

Statistical methods

Kaplan-Meier estimation of overall survival, progression-free survival and duration of response.
Log-rank test to asess differences in PFS and OS.
Cox proportional hazard models for calculations of HR and 95% confidence intervals

Resultater

Total pop:

2 mg/kg group: (HR vs. Docetaxel)
- OS: 10.4 months
  - HR 0.71, 95% CI 0.58-0.88, p=0.0008
- PFS: 3.9 months
  - HR 0.88, 0.74-1.05, p=0.07

10mg/kg group: (HR vs. Docetaxel)
- OS: 12.7 months
  - HR 0.61, 95%CI 0.49-0.75, p<0.0001
- PFS: 4.0 months
  - HR 0.79, 0.66-0.94, p=0.004

Docetaxel group:
- OS: 8.5 months
- 4.0 months

Pop. w/PD-L1 >50%

2 mg/kg group: (HR vs. Docetaxel)
- OS: 14.9 months
  - HR 0.54, 0.38-0.77, p=0.0002
- PFS: 5.0 months
  - HR 0.59, 0.44-0.78, p=0.0001

10mg/kg group: (HR vs. Docetaxel)
- OS: 17.3 months
  - HR 0.50, 0.36-0.70, p<0.0001
- PFS: 5.2 months
  - HR 0.59, 0.45-0.78, p<0.0001

Docetaxel group:
- OS: 8.2 months
- 4.1 months

Secondary findings

Grade 3-5 adverse effects were less common with pembrolizumab compared to docetaxel, 13%, 16% and 35% for 2 mg/kg, 10 mg/kg and docetaxel, respectively.

Diskusjon/kommentarer/sjekkliste

Sjekkliste:

- **Er formålet klart formulert?**
- **Yes**
- **Hvem er inkludert/ekskludert? (seleksjon/generaliserbarhet)**
- Clear selection and exclusion criteria
- **Var gruppene like ved starten? (selesksjon?, har randomiseringen fungert?)**
- Yes
- **Randomiseringsprosedyre?**
- Randomized by interactive voice-response systems into 3 groups 1:1:1
- **Ble deltakere/studiepersonell blindet mht gruppetilhørighet?**
- No, only the study statistician were masked, and remained so until completion of final analysis
- **Ble gruppene behandlet likt utover «intervensjonen»?**
- No, corticosteroid premedication were allowed for the docetaxel group
- **Primary endpoints validated?**
- Yes.
- **Ble deltakernne gjort rede for på slutten av studien? (attrition/follow-up bias)**
- Yes
- **Hva er resultatene? Presisjon?**
- Increase in OS, increasing with higher PD-L1
- Improved PFS, not significant
- **Kan resultatene overføres til praksis?**
- Yes
- **Ble alle utfallsmål vurdert?**
- Yes
- **Er fordelene verdt ulemper/kostnader?**
- Yes
- **Annen litteratur som styrker resultatene?**
- The authors did a small review of findings from trials with all similar in-use antibodies for comparison and to gather evidence from similar therapies.

Hva diskuterer forfatterne som:

- Strengths: Well documented efficacy seen in previous trials, reviewing previous trials on PD-1 inhibition for safety as well as the use of PD-L1 as a biomarker.

- Weaknesses: Lack of knowledge on mechanisms that can influence effect of checkpoint inhibition, such as the dynamic tumor immune microenvironment, tumor infiltrating cells, among others.

Har resultatene plausible forklaringer?

Yes. Inhibition of immune checkpoints are proven to be an efficient strategy in the treatment of cancer, though many mechanisms are currently not well understood.

Referanse: **Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2016;375(19):1823-33**

Studiedesign: **RCT**

Grade - kvalitet 1b

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste

Phase 3 trial for comparing pembrolizumab to platinum-based chemotherapy in treatment of PD-L1 positive, advanced non-small cell lung cancer (NSCLC)
Conclusion
Patients with at least 50% expression of PD-L1 on tumor cells have significantly longer progression-free survival (PFS) and overall survival (OS), as well as fewer adverse events, when treated with pembrolizumab
Countries
Australia, Austria, Belgium, Canada, France, Germany, Hungary, Ireland, Israel, Italy, Japan, Netherlands, New Zealand, Spain, United Kingdom, United States
Years data collection
Sept. 19, 2014 – May 9, 2016
	Study population recruitment
	1934 patients screened for enrollment, from 142 sites in 16 countries. 1653 could have PD-L1 evaluated. 500 with PD-L1 >50%. 305 patients at 102 sites met inclusion criteria and were randomized, 154 to pembrolizumab and 151 to chemotherapy
	Inclusion-/exclusion criteria
	Inclusion: 18 or older, histologically or cytologically confirmed stage 4 NSCLC, ECOG 0 or 1, at least one measurable lesion per RECIST 1.1., >3 months life expectancy, PD-L1 tumor-proportion score >50%
	Exclusion: Sensitizing EGFR or ALK mutations, previous systemic treatment for metastatic disease, systemic glucocorticoid use, immunosuppressive treatment, untreated brain metastases, active autoimmune disease with syustemic treatment, active interstitial lung disease, history of glucocorticoid-treated pneumonitis
	Data
	Study population receiving 1 dose of either pembrolizumab or chemotherapy (154 and 150, respectively)
	Outcome validation
	PFS – time from randomization to disease progression/death
	Objective response rate (ORR), measured by RECIST 1.1.
	Overall survival – time from randomization to death from any cause
	Exposure variables (validated/non-validated)
	Pembrolizumab treated/chemotherapy treated
	Important confounding factors
	Potential for crossover from the chemotherapy group
	Statistical methods
	Kaplan-Meier method for estimation of PFS and OS.
	Stratified log-rank test: Differences between groups in PFS and OS
	Hazard ratios and 95% CI with Cox proportional-hazard model
		Main findings
		Total pop:
		Pembrolizumab (HR vs. Chemotherapy)
		- Median PFS: 10.3 months (95% CI 6.7-not reached)
		  - HR: 0.50, 95% CI 0.37-0.68, p<0.001
		- OS at 6 months: 80.2%
		  - HR 0.60, 0.41-0.89, p=0.005
		- Response rate: 44.8% (95% CI 36.8-53.0)
		Chemotherapy
		- Median PFS: 6.0 months (95% CI 4.2-6.2)
		- OS at 6 months: 72.4%
		- Response rate: 27.8% (95% CI 20.8-35.7)
		
		Secondary findings
		Treatment-related adverse events
		- 73.4% in pembrolizumab group
		  - Discontinuation of therapy due to adverse events: 7.1%
		- 90.0% in chemotherapy group
		  - Discontinuation of therapy due to adverse events: 10.7%
			Sjekkliste:
			• **Purpose clearly formulated?**
			• Yes
			• **Inclusion criteria? (selection/generalizeability)**
			• Yes
			• **Were the groups equal at the start of the study?**
			• Yes
			• **Randomizing procedure?**
			• Not described
			• **Masking/blinding?**
			• Open-label to patients and investigators. RECIST criteria for PFS and ORR evaluated by blinded and independent radiological review
			• **Similar treatment of groups besides «intervention»?**
			• Yes
			• **Primary endpoints validated?**
			• Yes
			• **Participants accounted for at end of study? (attrition/follow-up bias)**
			• Yes
			• **Results? Precision?**
			• Significant results on all primary outcome measures.
			• **Practical applications?**
			• Yes
			• **All selected outcomes evaluated?**
			• Yes
			• **Pros worth cons/costs?**
			• Yes
			• **Other litterature supporting results?**
			• Other studies in KEYNOTE series with confirmation of safety, dosing regimens and efficacy for high PD-L1 expressing tumors.
			
			Authors discussion of:
			- **Strengths:**
			- **Weaknesses:** The PD-L1 cutoff of 50% is potentially too high, studies ongoing at the time of were investigating the potential benefit of a cutoff at 1%.
			- **Strength/weakness:** Due to the significantly longer OS in the pembrolizumab group, the trial was stopped by the monitoring committee and it was decided that all patients be offered pembrolizumab. The authors discuss the potential confounding effect of crossover from the pembrolizumab group to the chemotherapy group
			
			Plausible explanations for findings?
			Yes.

Referanse: **Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18(11):1483-92**

Studiedesign: **Case series**

Grade - kvalitet 4

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
To assess the activity and safety of pembrolizumab as first-line therapy in cisplatin-ineligible patients with locally advanced, unresectable or metastatic urothelial carcinoma **Conclusion** Pembrolizumab used as first-line therapy has antitumor activity for patients enrolled in this study **Countries** 20 countries, not clearly listed **Years data collection** Feb. 24, 2015 – Aug. 8, 2016	**Study population recruitment** 541 patients were screened, and 374 met eligibility criteria. 370 patients received at least one dose of pembrolizumab. **Inclusion-/exclusion criteria** **Inclusion:** 18 years or older, histologically or cytologically confirmed locally advanced and unresectable or metastatic urothelial cancer of the renal pelvis, ureter, bladder or uretra, ineligible for cisplatin-based therapy, ECOG 2, creatinine clearance 30-60 mL/min, grade >2 audiometric hearing loss, grade >2 peripheral neuropathy, NYHA classification III heart failure, adequate haemotoligical status, life expectancy > 3 months **Exclusion:** Not received systemic chemotherapy for advanced disease, centrally confirmed and measurable disease per RECIST 1.1, ECOG 0-2, active CNS metastases, carcinomatous meningitis, autoimmune disease, interstitial lung disease, systemically treated infection, hepatitis B or C, HIV, previous therapy with drugs targeting T-cell costiumulation or checkpoint pathways within 4 weeks of trial start, chemotherapy or targeted small-molecule treatment <2 weeks before start, systemic therapy for locally advanced and unresectable or metastatic urothelial cancer was not allowed **Data** 370 patients whom received at least one dose of pembrolizumab **Outcome validation** Objective response (OR) – proportion of patients who achieved complete or partial response (CR or PR), measured by RECIST 1.1 **Exposure variables (validated/non-validated)** Pembrolizumab treatment **Important confounding factors** **Statistical methods** Kaplan-Meier method for estimating duration of response, median progression-free survival and overall survival.	**Main findings** Objective response rate: 24% (95% CI 20-29) - 19% with partial response - 5% with complete response **Secondary findings** Median time to response: 2 months (2.0-2.1) Disease control rate 47% (42-52) Median duration of response not reached Median progression-free survival: 2 months (2-3) 6 month overall survival: 67% (62-73)	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • No control group. • **Randomizing procedure?** • No randomization • **Masking/blinding?** • Open-label to patients and investigators. Responses by RECIST 1.1 assessed by independent radiology review • **Similar treatment of groups besides «intervention»?** • Not relevant • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes. Large number of discontinued treatments (63% discontinuation at data cutoff point) • **Results? Precision?** • Low precision due to large number of discontinuation • **Practical applications?** • Yes • **All selected outcomes evaluated?** • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** - **Weaknesses:** No control arm is a limiting factor. Short follow-up. Exclusion of patients with autoimmune disease prevents generalizability for a large patient group. **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.

Referanse: Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med. 2017;377(20):1919-29

Studiedesign: **RCT**

Grade - kvalitet 1b

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
Comparing durvalumab to placebo in patients with stage III non-small cell lung cancer in patients without progression after two or more cycles of platnum-based chemoradiotherapy **Conclusion** Progression-free survival is significantly longer with durvalumab compared to palcebo. Response rates and survival time was increased **Countries** Australia, Belgium, Canada, Chile, France, Germany, Greece, Hungary, Israel, Italy, Japan, Korea, Mexico, The Netherlands, Peru, Poland, Singapoe, South Africa, Slovakia, Spain, Taiwan, Thailand, Turkey, United Kingom, United States, Vietnam **Years data collection** May 2014 – April 2016	**Study population recruitment** 713 patients were screened and eligible, and 709 underwent randomization and received at least one dose **Inclusion-/exclusion criteria** **Inclusion:** Cytologically or histologically confirmed stage 3, locally advanced, unresectable non-small cell lung cancer, received two or more cycles of chemoradiotherapy, no disease progression after chemoradiotherapy, 18 or older, WHO performance status 0 or 1, estimated life expectancy >3 months, completed last radiation within 1-14 days before randomization **Exclusion:** Previous therapy with anti-PD-1/PD-L1 antibodies, treatment with immunotherapy or an investigational agent within 4 weeks before treatment, active or history of autoimmune disease in past 2 years, history of immunodeficiency, uncontrolledillness or ongoing or active infections, unresolved toxic effects after chemoradiotherapy, grade 2 or higher pneumonitis from chemoradiotherapy **Data in study population** 473 patients receiving durvalumab, 236 received placebo **Outcome validation** Progression-free survival (PFS) time by RECIST 1.1 Overall survival (OS) in time from randomization to death from any cause **Exposure variables (validated/non-validated)** Treatment with durvalumab or placebo **Important confounding factors** Not discussed **Statistical methods** Kaplan-Meier method for estimation of progression-free survival times and overall survival times Log-rank test for assessment of differences between groups	**Main findings** Durvalumab group (HR vs. Placebo) Median PFS: 16.8 months (95% CI 13.0-18.1) - HR 0.52 (0.42-0.65, p<0.001) 12 month survival rate: 55.9% (51.0-60.4) - HR 0.61 (0.50-0.76, p<0.001) 18 month PFS rate: 44.2% (37.7-50.5) Placebo group Median PFS: 5.6 months (4.6-7.8) 12 month survival rate: 35.3% (29.0-41.7) 18 month PFS rate: 27.0 (19.9-34.5) **Secondary findings** PFS-benefit irrespective of PD-L1 expression. Objective response rate by RECIST 1.1: 28.4% vs. 16.0% in the placebo group (p<0.001) Adverse events ocurred in 96.8% of patients receiving durvalumab, and 94.9% of patients receiving placebo	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • Yes • **Randomizing procedure?** • Not clearly stated • **Masking/blinding?** • RECIST assessment by blinded independent review, • **Similar treatment of groups besides «intervention»?** • Yes • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes • **Results? Precision?** • Significant • **Practical applications?** • Yes • **All selected outcomes evaluated?** • Yes • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** - **Weaknesses:** Immature data on overall survival due to short follow-up time, uncertainty of mechanisms in the interaction between chemoradiotherapy and immunotherapy **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.

Referanse: Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017;389(10064):67-76

Studiedesign: **Case series**

Grade - kvalitet 4

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
Assess atezolizumab in treatment of metastatic urothelial cancer in cisplatin-ineligible patients **Conclusion** Atezolizumab has durable responses and tolerable safety in patients with metastatic urothelial cancer for cisplatin-ineligible patients **Countries** Germany, United States, Spain, Italy, Canada, United Kingom, France, Netherlands **Years data collection** June 9, 2014 – March 30, 2015	**Study population recruitment** 167 patients were screened and 123 were enrolled. **Inclusion-/exclusion criteria** **Inclusion:** Inoperable, locally advanced or metastatic urothelial cancer in the renal pelvis, ureters, bladder or urethra, RECIST 1.1. verified disease, ECOG of 2 or less, tumor sample available for analysis, chemotherapy or radiation in last 12 months, cisplatin ineligible due to GFR >30 mL/min and <60 mL/min, grade 2 or higher hearing loss, peripheral neuropathy or ECOG 2 **Exclusion:** Any anti-cancer 3 weeks before study start, treatment with other investigational drug, CNS metastases, leptomeningeal disease, uncontrolled tumor pain, uncontrolled pleural effusion, uncontrolled hyercalcemia, other malignancies within last 5 years, pregnancy, sever allergy, autoimmune disease, pulmonary fibrosis, active pneumonitis, HIV positive, active hepatitis B or C, active tuberculosis, NYHA II or greated heart failure, major surgery in last 28 days, previous allogeneic stem cell or organ transplant, previous treatment with checkpoint inhibitors and/or CD137 agonists, previous treatment with systemic immunostimulatory agents, systemic corticosteroid treatment **Data** 119 patients that received one or more doses atezolizumab. 102 discontinued treatment. **Outcome validation** Objective response rate per RECIST 1.1 Duration of response Progression-free survival Overall survival **Exposure variables (validated/non-validated)** Atezolizumab therapy **Important confounding factors** Not discussed **Statistical methods** Clopper-Pearson method for calculating 95% CI and objective response rate	**Main findings** Objective response rate: 23% (95% CI 16-31) - Complete response: 9% - Median response duration not reached **Secondary findings** Responses occured in all PD-L1 groups Median progression-free survival: 2.7 months (2.1-4.2) Median overall survival: 15.9 months (10.4-not reached)	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • Not applicable • **Randomizing procedure?** • Not applicable • **Masking/blinding?** • Independent review of RECIST criteria • **Similar treatment of groups besides «intervention»?** • Not applicable • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes • **Results? Precision?** • Low precision due to having no control group and high discontinuation numbers • **Practical applications?** • Yes • **All selected outcomes evaluated?** • Yes • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** Not discussed - **Weaknesses:** Single arm study **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.

Referanse: Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909-20

Studiedesign: **Case series**

Grade - kvalitet 4

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
Investigate use on atezolizumab in treating patients with metastatic urothelial carcinoma after failure of platinum-based treatment **Conclusion** Atezolizumab has activity with high durability and is tolerated well. PD-L1 expression on immune cells is associated with better response **Countries** «Global» otherwise not described **Years data collection** May 2014 – november 2014	**Study population recruitment** 486 patients were screened and 315 were eligible and enrolled **Inclusion-/exclusion criteria** **Inclusion:** Histologically or cytologically documented locally advanced or metastatic urothelial carcinoma in the renal pelvis, ureter, urethra or urinary bladder, ECOG 0 or 1, disease measurable and confirmed with RECIST 1.1, adequate hematologic and end-organ function, autoimmune disease, active infections **Exclusion:** **Data** 310 patients treated with atezolizumab **Outcome validation** Objective response rate by RECIST 1.1 **Exposure variables (validated/non-validated)** Atezolizumab treatment **Important confounding factors** Not discussed **Statistical methods** Exact binomial test for assessing objective response rate Kaplan-Meier method for estimating times for duration or response, PFS and OS Broodmeyer and Crowley method for 95% CI calculated for median duration of response, PFS and OS	**Main findings** All groups had significantly improved ORR compared to historical control OS. All patients: ORR: 15% (95% CI 11-20, p=0.0058) PFS: 2.7 months (2.1-3.9) OS: 7.9 months (6.6-9.3) IC2/3 PD-L1 >5% ORR: 27% (19-37) PFS: 4.0 months (2.6-5.9) OS: 11.4 months (9.0-not reached) IC1/2/3: PD-L1 >1% ORR: 27% (95% CI 19-37, p<0.0001) PFS: 2.9 months (2.1-4.1) OS: 8.8 months (7.1-10.6) **Secondary findings** Duration of treatment: 12 weeks Any grade adverse events registered in 97% of all patients.	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • Not applicable • **Randomizing procedure?** • Not applicable • **Masking/blinding?** • Independent review of RECIST analysis • **Similar treatment of groups besides «intervention»?** • Not applicable • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes. • **Results? Precision?** • Yes, high. • **Practical applications?** • Yes • **All selected outcomes evaluated?** • Yes • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** Not discussed - **Weaknesses:** Short follow-up time **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.

Referanse: Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2015;373(2):123-35

Studiedesign: **RCT**

Grade - kvalitet 1b

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
Investigate efficacy and safety of nivolumab in treatment of advanced squamous non-small cell lung cancer, compared to docetaxel **Conclusion** Nivolumab is significantly better than docetaxel on overall survival, response rate and progression-free survival, regardless of PD-L1 expression **Countries** United States, Canada, Argentina, Australia, Chile, Mexico, Peru, The Netherlands, Italy, Germany, Russia, Poland, Spain, Czech Republic **Years data collection** October 2012 - December 2013	**Study population recruitment** 272 patients were randomized, 260 of these received treatment **Inclusion-/exclusion criteria** **Inclusion:** 18 years or older, ECOG 0 or 1, submitted pretreatment tumor-tissue **Exclusion:** Autoimmune disease, symptomatic interstitial lung disease, systemic immunosuppression, prior T-cell costimulating therapy or checkpoint inhibitor therapy, prior docetaxel therapy **Data** 131 patients receiving nivolumab and 129 patients receiving docetaxel followed up over minimum 11 months **Outcome validation** Overall survival – time from randomization to death from any cause Objective response rate by RECIST 1.1 Progression-free survival by RECIST 1.1 Efficacy by PD-L1 expression **Exposure variables (validated/non-validated)** Nivolumab treatment or docetaxel treatment **Important confounding factors** **Statistical methods** OS and PFS analysis with two-sided log-rank test and Kaplan-Meier method HR and CI estimated with stratified Cox proportional-hazards model	**Main findings** Nivolumab: (HR vs. Docetaxel) Median overall survival: 9.2 months (95% CI 7.3-13.3) 1 year OS rate: 42% (34-50) - HR 0.59 (0.44-0.79, p<0.001) Response rate: 20% Median PFS: 3.5 months - HR 0.62 (0.47-0.81, p<0.001) Docetaxel: Median overall survival: 6.0 months (5.1-7.3) 1 year OS rate: 24% (17-31) Response rate: 9% Median PFS 2.8 months **Secondary findings** PD-L1 expression not prognostic nor predictive of benefit Treatment related adverse events graded 3 or 4 occured in 55% of patients in the docetaxel group and 7% in the nivolumab group	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • Yes • **Randomizing procedure?** • Yes • **Masking/blinding?** • Open-label • **Similar treatment of groups besides «intervention»?** • Yes • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes • **Results? Precision?** • Significant improvement on all endpoints. • **Practical applications?** • Yes • **All selected outcomes evaluated?** • Yes • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** Significant improvement on all reported primary and secondary outcome measures - **Weaknesses:** Comparison with historical data, **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.

Referanse: Sharma P, Retz M, Siefker-Radtke A, Baron A, Necchi A, Bedke J, et al. Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): a multicentre, single-arm, phase 2 trial. The Lancet Oncology. 2017;18(3):312-22

Studiedesign: **Case series**

Grade – kvalitet 4

Purpose	Materials and methods	Resultater	Diskusjon/kommentarer/sjekkliste
Assessment of nivolumab in the treatment of patients with metastatic urothelial carcinoma after platinum-based therapy **Conclusion** Nivolumab used as monotherapy has clinical effect regardless of PD-L1 expression levels for these patients **Countries** Australia, Belgium, Czech Republic, Finland, Germany, Italy, Japan, Poland, Spain, Sweden and the USA. **Years data collection** March 9, 2015 – Oct. 16, 2015	**Study population recruitment** 270 patients enrolled, 5 with insufficient follow-up **Inclusion-/exclusion criteria** **Inclusion:** 18 years or older, histologically confirmed metastatic and unresectable locally advanced urothelial carcinoma, radiologically measurable disease by RECIST 1.1 criteria, progression or recurrence after at least one platinum-based therapy, ECOG 0 or 1, submittal of tumor samle **Exclusion:** Active brain metastases, malignancy within last 3 years, serious or uncontrolled conditions, autoimmune disease, immunosuppressive treatment within 14 days of randomization, previouos treatment with checkpoint inhibitors, persisting toxicity from previous cancer therapy **Data** 265 patients followed for minimum 6 months **Outcome validation** Objective response, time to response, progression-free survival, overall survival **Exposure variables (validated/non-validated)** Treatment with nivolumab **Important confounding factors** Not discussed **Statistical methods** Based on historical data, a 10% objective response rate was set as the lower threshold for confirmed improvement over chemotherapy monotherapy. Clopper-Pearson method for assessment of objective response Kaplan-Meier method was used for estimation of PFS, OS and duration of response	**Main findings** Confirmed objective response rate: Total population: 19.6% (95% CI 15.0-24.9) PD-L1 >5%: 28.4% (18.9-39.5) PD-L1 >1%: 23.8% (16.5-32.3) PD-L1 <1%: 16.1 (10.5-23.1) Median progression-free survival: 2.00 months (95% CI 1.87-2.63) Median OS: Total population: 8.74 months (6.05-not reache) PD-L1 >1%: 11.30 months (8.74-not reached) PD-L1 <1%: 5.95 months (4.30-8.08) **Secondary findings** Treatment-related adverse events of grade 3-4: 18% of patients No optimal PD-L1 cutoff was found Treatment-related adverse events: 64%, grade 3 or 4 in 18%	**Sjekkliste:** • **Purpose clearly formulated?** • Yes • **Inclusion criteria? (selection/generalizeability)** • Yes • **Were the groups equal at the start of the study?** • Not applicable • **Randomizing procedure?** • Not applicable • **Masking/blinding?** • Open-label. PFS determined by independent review • **Similar treatment of groups besides «intervention»?** • Not applicable • **Primary endpoints validated?** • Yes • **Participants accounted for at end of study? (attrition/follow-up bias)** • Yes • **Results? Precision?** • Significant improvement on all endpoints. • **Practical applications?** • Yes • **All selected outcomes evaluated?** • Yes • **Pros worth cons/costs?** • Yes • **Other litterature supporting results?** • Yes. **Authors discussion of:** - **Strengths:** Objective response rate higher than historical date for other second-line chemotherapies - **Weaknesses:** Comparison with historical data, case series and no control group, short follow-up **Plausible explanations for findings?** Yes. Checkpoint inhibitors show antitumor capabilities against a range of cancer types.