SEMMELWEIS EGYETEM

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**Ph.D. értekezések**

**3338.**

# SZÉLES ÁDÁM DÁNIEL

**Urológia**

című program

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# PROGNOSTIC AND PREDICTIVE BIOMARKERS IN UROTHELIAL CANCER

**Ph.D. Thesis**


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2025

***I disapprove of what you say, but I will defend to the death your right to say it***

*Voltaire*

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## 1. LIST OF ABBREVIATIONS

UTUC – upper tract urothelial carcinoma
UBC – urothelial bladder cancer
RC – radical cystectomy
sPD-L1 – soluble programmed death ligand 1
sPD-1 – soluble programmed death 1
NLR – neutrophil-to-lymphocyte ratio
PLR – platelet-to-lymphocyte ratio
CRP – C reactive protein
ICI – immune checkpoint inhibitor
RCC – renal cell carcinoma
(m)UC – (metastatic) urothelial carcinoma
NSCLC – non small cell lung cancer
IHC – immunohistochemistry
HNSCC – head and neck squamous cell cancer
RNU – radical nephroureterectomy
PRISMA – preferred reporting items for systematic reviews and meta-analyses
OS – overall survival
PFS – progression free survival
CTX - chemotherapy
ECOG – eastern cooperative oncology group
ROC – receiver operating characteristic
HR – hazard ratio
CI – confidence interval
ORR – objective response rate
QUIPS – quality in prognosis studies
TUKEB – tudományos és kutatásetikai bizottság - research ethics committee
ELISA – enzyme linked immunosorbent assay
OR – odds ratio
Ki67 – marker of proliferation Kiel 67
CDCA5 – cell division cycle associated 5
PAK1 – p21 activated kinase
ESCC – esophageal squamous cell carcinoma
MMP-7 – matrix metalloproteinase 7

## 2. STUDENT PROFILE

### 2.1. Vision and mission statement, specific goals

My vision is to optimize therapy for ICI-treated urothelial carcinoma patients. As a mission, I am assessing different soluble biomarkers, their role in ICI sensitivity, and their impact on survival outcomes.

### 2.2. Scientometrics

| | |
|---|---|
| **Number of all publications:** | 6 |
| Cumulative IF: | 34.3 |
| Av IF/publication: | 5.72 |
| Ranking (Sci Mago): | D1: 2, Q1: 2, Q2: 5 |
| **Number of publications related to the subject of the thesis:** | 3 |
| Cumulative IF: | 15.2 |
| Av IF/publication: | 5.07 |
| Ranking (Sci Mago): | D1: 1, Q1: 2, Q2: - |
| **Number of citations on Google Scholar:** | 39 |
| **Number of citations on MTMT (independent):** | 11 |
| **H-index:** | 3 |

The detailed bibliography of the student can be found on page 55.

### 2.3. Future plans

My long-term professional objectives are gathered around clinical research and practical patient care.

Urology - especially uro-oncology - is an innovative, rapidly developing field, which demands not only practical skills but an experienced methodological perspective to translate science into everyday patient care. Active participation in clinical environments will enhance my ability to generate research ideas that are directly informed by and applicable to real-world patient needs. Through the integration of empirical research and clinical practice, I seek to develop evidence-based strategies, that improve healthcare delivery and outcomes.

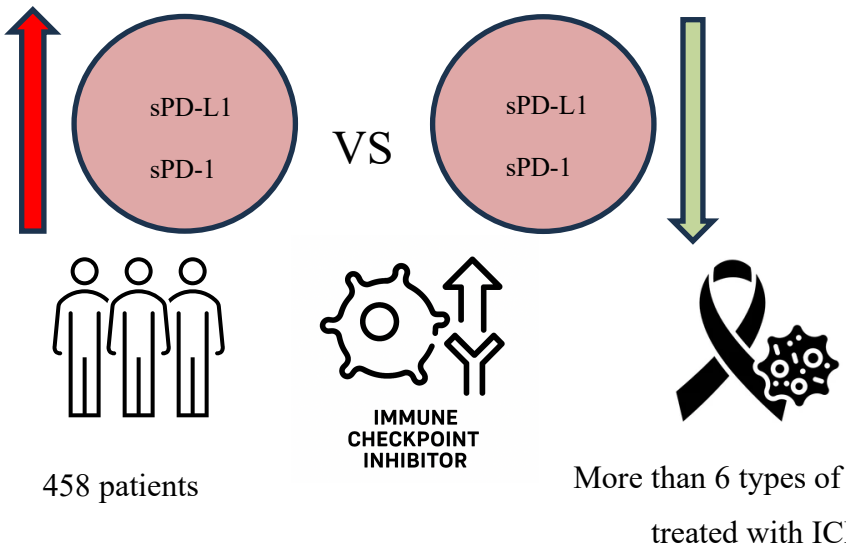
## 3. SUMMARY OF THE PH.D.

Recent development in the field of oncology calls for an exploration of biomarkers of which help us personalize treatment decisions. Immune checkpoint inhibitors (ICI) revolutionized the therapy of urological malignancies like urothelial and renal cell carcinoma. Biomarkers will be a hallmark of survival prognostication, treatment selection and rigorous treatment monitoring. For this reason, we conducted two meta-analyses and an observational post hoc cohort analysis. In these projects, we assessed soluble programmed death ligand 1 (sPD-L1), soluble programmed death 1 (sPD-1) and different laboratory inflammation biomarkers like neutrophil-to lymphocyte ration (NLR), C-reacative protein (CRP) and platelet to lymphocyte ratio (PLR) in the context of human malignancies treated with ICI with a focus of UC. Our findings showed that ICI-treated patients with high sPD-L1, sPD-1 have significantly worse prognosis. This finding is also observed in upper tract urothelial carcinoma patients (UTUC) regardless of treatment modality. We similarly demonstrated that ICI-treated UC patients have inferior survival when presenting with elevated inflammatory biomarkers. In conclusion, sPD-L1 and inflammatory biomarkers are promising biomarkers in ICI-treated human malignancies.

## 4. Graphical abstract

### 4.1. Study I

**Aim: to evaluate the prognostic relevance of sPD-L1 and sPD-1 in patients with different tumor entities who underwent ICI therapy.**

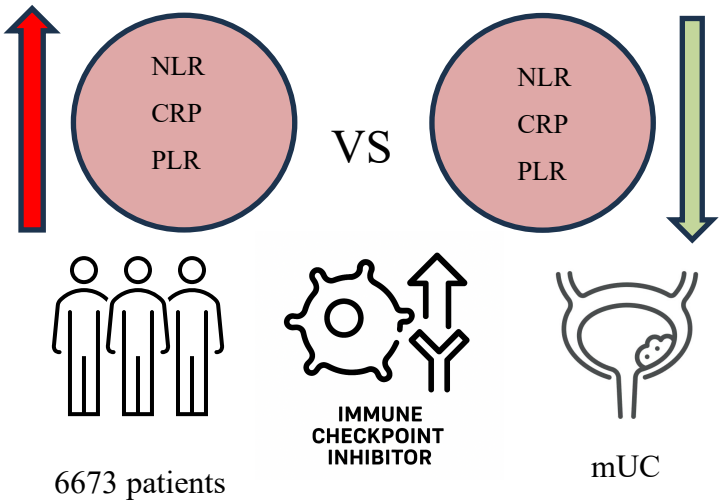



**Results: significantly inferior OS in ICI-treated cancer patients with elevated pre-treatment sPD-L1 levels**

**Conclusion: sPD-L1 is a prognostic biomarker for ICI therapy, which should be interpreted in a tumor type-specific context.**

### 4.2. Study II

**Aim: to analyze the prognostic and predictive significance of liquid biomarkers (NLR, CRP, PLR, and LDH) in mUC patients treated with ICI.**




**Results: elevated pre-treatment NLR, CRP, PLR, and LDH are significantly associated with worse OS and PFS in ICI-treated urothelial carcinoma patients,**

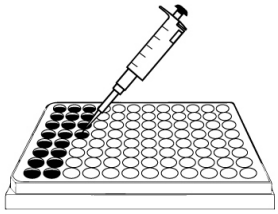
**Conclusion: they have potential prognostic and predictive value in treatment decisions.**

### 4.3. Study III

**Aim: to determine the prognostic value of pretreatment sPD-L1 in UTUC**

65 UTUC

sPD-L1



ELISA

**Results: preoperative sPD-L1 level is a predictor of higher pathological stage and worse survival in UTUC**

**Conclusion: sPD-L1 could be a useful preoperative prognostic biomarker in UTUC.**

# 5. INTRODUCTION

## 5.1. Overview of the topic

### 5.1.1. What is the topic?

Our research focus is on assessing different prognostic and predictive soluble biomarkers in the context of UC and ICI therapy.

### 5.1.2. What is the problem to solve?

ICIs have revolutionized the systemic treatment of UC; however, the majority of patients receiving this therapy do not respond. The main unmet need is to identify those patients who will benefit from ICI therapy.

### 5.1.3. What is the importance of the topic?

UCs are amongst the most prevalent cancers with devastating survival rates, when diagnised in muscle-invasive disease stage. Prognostication and therapy prediction is crucial for personalized cancer therapy. As ICIs have reshaped the treatment landscape of UC, identifying which patients are most likely to benefit remains an unsolved clinical challenge. Investigating potentially predictive biomarkers may help predict treatment response, improve patient stratification, and avoid unnecessary therapy.

### 5.1.4. What would be the impact of our research results?

The impact of our research project lies in the evaluation of the efficacy of novel immunotherapeutic approaches and the identification of reliable predictive biomarkers. Our research aims to guide the selection of patients most likely to benefit from these treatments. This precision approach can lead to improved response rates and mitigate overtreatment**.** Furthermore, the application of soluble blood-based biomarkers could enable disease by tracking therapeutic response, allowing clinicians to make individualized treatment decisions.

## 5.2. Urothelial carcinoma

Urothelial carcinoma (UC) is one of the most prevalent human malignancies worldwide (1). UC develop from the urothelium, presenting as either bladder cancer (UBC) or upper tract urothelial carcinoma (UTUC)

Traditionally, UTUC and urothelial bladder cancer (UBC) were considered the same disease with different localizations as these tumors have a common etiology and show similar therapeutic sensitivities. However, in recent years, a growing body of evidence revealed disparities between UTUC and UBC (2). Therefore, UTUC and UBC should be considered different tumor entities with substantial similarities.

### 5.2.1. Urothelial bladder cancer

Urothelial bladder cancer (UBC) ranks as the top 10$^{th}$ most prevalent cancer worldwide, with the incidence of 573,000 new cases and 213,000 death registered each year. In Hungary, approximately 2,400 new cases are diagnosed with a 1,200 death associated with UBC annually (3). Therefore, UBC considered a substantial health burden with high mortality rates. At the time of the diagnosis, 25% of patients have muscle-invasive bladder cancer (MIBC) (4). At this stage the gold standard therapy is radical cystectomy (RC) with or without perioperative platinum-based chemotherapy. Locally advanced or metastatic UC (mUC) is a clinically challenging, highly aggressive disease characterized by short survival rates and limited treatment options. Platinum-based chemotherapy has been the only therapeutic option for mUC for decades (5). However, only ~50% of patients show radiographic response to this chemotherapy, and only 20% of patients will survive longer than two years, while serious side effects of this agent can deeply affect its administration (6).

In 2016 and 2017, two innovative immune checkpoint inhibitor (ICI) therapies, atezolizumab (from the IMvigor 210 study) and pembrolizumab (from the KEYNOTE-045 study), were introduced for patients with UC that had progressed during or after platinum-based chemotherapy (7, 8). These therapies achieved objective response rates (ORR) of 15-29%, which are substantially higher than the response rates of less than 10% observed with other second-line chemotherapies. Notably, patients who responded to these treatments experienced a durable response lasting longer than 12 months, an unprecedented improvement at this stage of treatment. In 2017, both drugs were also

approved for first-line use in platinum-ineligible patients based on the IMvigor 210 and KEYNOTE-052 studies (8, 9). In addition, maintenance therapy with avelumab became available for patients, who initially responded to platinum chemotherapy [4]. Overall, ICIs represent a promising new therapeutic strategy that offers a lasting therapeutic effect and prolonged survival for a subgroup of patients. Furthermore, other novel targeted therapies and antibody-drug conjugates have become available, such as the FGFR-inhibitor erdafitinib and the Nectin-4 targeting enfortumab vedotin, both used most recently in third-line treatment, and enfortumab vedotin in combination with pembrolizumab in the first-line mUC treatment (10, 11).

5.2.2. Upper tract urothelial carcinoma

Upper tract urothelial carcinoma (UTUC) represents a distinct clinical and biological subset of UC, accounting for approximately 5–10% of all urothelial malignancies (12). Traditionally, UTUC has been managed surgically, with radical nephroureterectomy (RNU) being the gold standard treatment for localized disease. However, the anatomical challenges of the upper urinary tract and limited accuracy of preoperative staging often lead to overtreatment in patients with low-risk tumors (13). Moreover, systemic therapy—particularly platinum-based chemotherapy—is recommended in advanced cases, but its administration is often limited by impaired renal function following RNU(14). For cisplatin-ineligible patients, ICIs offer an important therapeutic alternative (15). Yet, similar to other cancer types, the response to ICI therapy in UTUC is highly variable and currently unpredictable. While PD-L1 overexpression in UTUC has been associated with more aggressive disease and improved ICI responsiveness, its value as a tissue biomarker is constrained by the same limitations observed in other tumor types. The identification of serum-based biomarkers—such as sPD-L1—and their evaluation in UTUC is therefore of high clinical interest but remains underexplored. Likewise, the prognostic significance of routine inflammatory markers in the UTUC subpopulation treated with ICIs has not been thoroughly investigated, leaving a critical gap in the current biomarker landscape.

Given these considerations, there is a compelling need to identify and validate reliable, non-invasive biomarkers that can assist in both the prognostication and therapeutic

stratification of UTUC and broader UC cases undergoing ICI therapy. Blood-based markers such as sPD-L1, sPD-1, and inflammation-related indicators hold promise in addressing this need, offering a practical and scalable alternative to tissue-based approaches. Moreover, their ability to reflect both tumor burden and immune dynamics positions them as ideal candidates for real-time monitoring of treatment response and disease progression.

### 5.3. Immune checkpoint inhibitors

In recent years, ICIs have emerged as a cornerstone in the treatment of several malignancies, marking a paradigm shift in oncology. ICIs primarily target immune checkpoint pathways, such as programmed death protein-1 (PD-1) and its ligand PD-L1, which are critical regulators of immune tolerance and immune evasion in cancer. These therapies function by blocking the interaction between PD-1—primarily expressed on activated T-cells—and PD-L1, which is often overexpressed on tumor cells and various immune cells (16). This blockade reactivates the immune system's ability to recognize and destroy cancer cells and has resulted in significant clinical benefits in a subset of patients across a range of tumors, including non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), and UC (17).

Despite the impressive outcomes in some cases, the therapeutic success of ICIs is limited by considerable inter-individual variability in treatment response. Only a lower rate of patients experience durable remission, while others show minimal or no response. In metastatic UC (mUC), for example, the response rates to ICIs such as atezolizumab and pembrolizumab remain below 30%, even though these treatments offer the advantage of prolonged response duration and improved survival for those who benefit (7, 8). This variability presents a major clinical challenge, emphasizing the need for predictive biomarkers that can guide patient selection and therapeutic decision-making.

Currently, the most widely used predictive biomarker for ICI therapy is PD-L1 immunohistochemistry (IHC) (18). While tissue-based PD-L1 expression has shown some correlation with treatment response in cancers such as NSCLC and head and neck squamous cell carcinoma, its predictive power is inconsistent across different tumor types (19, 20). In UC, PD-L1 IHC is routinely used to determine eligibility for ICI in cisplatin-ineligible patients, yet its negative predictive value is low, meaning that also some PD-

L1–negative patients may still respond well to treatment (21). Furthermore, the application of tissue-based biomarkers is hindered by technical limitations such as heterogeneous expression within tumors, variability among diagnostic antibodies, and subjective interpretation of results. These issues are compounded by the invasive nature of repeated tumor biopsies, which limits their use for longitudinal treatment monitoring. As a result, there is increasing interest in developing blood-based biomarkers that are minimally invasive, easily repeatable, and more reflective of the patient's dynamic physiological state.

### 5.4. Soluble biomarkers

Of the blood-based biomarkers, soluble forms of immune checkpoint proteins—specifically, soluble PD-L1 (sPD-L1) and soluble PD-1 (sPD-1)—have garnered significant attention. These circulating molecules are detectable in the serum of both healthy individuals and cancer patients. Elevated levels of sPD-L1 and sPD-1 have been associated with more advanced disease and poorer prognosis in various malignancies, suggesting a potential role in cancer progression and immune suppression (22, 23). However, the clinical utility of these soluble biomarkers remains poorly defined, particularly in terms of their predictive value for ICI therapy response. Conflicting evidence from different studies further complicates their interpretation and applicability in routine clinical practice.

In addition to immunological markers, systemic inflammation is increasingly recognized as a key player in UC biology and treatment response (24). Chronic inflammation is known to promote tumor development, immune evasion, and resistance to therapy (25) (26). Therefore, inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and C-reactive protein (CRP) have emerged as potential prognostic indicators in various cancers. These markers are widely available, routinely measured in clinical practice, and provide insight into the systemic immune-inflammatory status of the patient (27, 28). In the context of immunotherapy, these markers may reflect both tumor-associated inflammation and immune system activation or suppression, making them particularly relevant for ICI-treated patients. In mUC—a disease characterized by a high mutational burden and immunogenicity—inflammation

plays a prominent role in disease progression and may significantly impact treatment outcomes (24).

## 6. Objectives

**6.1. Study I.** – In this study, we conducted a systematic review and meta-analysis of published literature data to assess the prognostic significance of circulating sPD-L1 and sPD-1 levels in pre-treatment and on-treatment samples of patients with various cancers who underwent ICI therapy.

**6.2. Study II.** – In this study, we aimed to systematically investigate the prognostic relevance of NLR, CRP, PLR, and LDH in ICI-treated locally advanced and mUC patients.

**6.3. Study III. –** In this study, we aimed to assess the prognostic value of sPD-L1 and its changes in different treatment settings of UTUC. Therefore, in a post hoc pilot study, we determined sPD-L1 levels in prospectively collected pre-treatment and on-treatment serum samples of UTUC patients who underwent either surgical or systemic (platinum-based or ICI) treatment.

## 7. Methods

The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 recommendations, and followed the Cochrane Handbook (29) (30). The study protocols was registered on PROSPERO (Study I: CRD42021283222, Study II: CRD42022291449)

### 7.1. Literature search and eligibility criteria

The electronic databases PubMed, EMBASE, and Cochrane Library were screened for study I. and we also included Scopus for study II. The dates of literature search and the searchkeys are includied in the original articles.

Two independent authors performed the systematic selection process. Disagreements were resolved by a third author. References were screened using Endnote $X^9$ (Clarivate Analytics, Philadelphia, PA, USA) and assessed by title, abstract, and full text.

### 7.2. Study selection, data collection and patient cohort

7.2.1. Study selection and data collection for study I. and study II.

We used the PECO (Population, Exposure, Comparator, Outcome) framework to formulate our research question. We included original English-language studies. For Study I we examined (P) ICI-treated patients with various tumors, and (E and C) compared the hazard of high and low serum or plasma sPD-L1 and/or sPD-1 levels in regard to (O) overall survival (OS) or progression-free survival (PFS). There was no pre-defined cut-off value for the definition of high and low levels of biomarkers. If available, on-treatment sPD-L1 and sPD-1 concentrations (median or mean level, range, or interquartile range) were also considered as additionally assessed parameters. For Study II we invastigated (P) patients with ICI-treated UC and (E and C) compared the hazard of high and low serum or plasma NLR, CRP, LDH, and PLR levels for (O) OS or PFS and ORR. For the assessed biomarkers, we used cut-off values based on the definitions in the original articles. The following exclusion criteria were used: reviews, comments, letters, meta-analyses, systematic reviews, animal experiments, and conference abstracts were excluded.

Data were obtained by reading full-text articles by two independent authors. For Study I we extracted the following data: the first name of the author, year of publication, cancer type, ICI therapy type, country of sample collection, study type, cohort size, patient age, sex, cut-off values for sPD1/ sPD-L1, cut-off definition method (e.g., median, receiver operating characteristic [ROC] curve), assay method, follow-up time, OS and PFS.

For Study II parameters extracted were first name of author, publication year, tumor location (upper *vs.* lower urinary tract), type of ICI therapy, country of sample/data collection, type of study, cohort size, patient age, sex, ECOG performance status, cut-off values for NLR, CRP, LDH and PLR, follow-up time, OS, PFS, and ORR.

For eligible studies, the article provided calculated hazard ratios (HR) with 95% confidence intervals (CI). In addition, when available, ORR, data on sPD-L1 and sPD-1 level changes during ICI treatment were extracted.

7.2.2. Patient cohort for study III

Pre-treatment serum samples were collected from an overall number of 61 UTUC patients (44 males, 17 females), who underwent surgical (RNU cohort; n=37), postoperative platinum (CTX cohort; n=25), or second-line ICI therapy (ICI cohort; n=6) at the Department of Urology, Semmelweis University between 08/2014 and 07/2020. Six patients were included in more than one cohort (three patients both in the RNU and CTX cohorts, two patients in the CTX and ICI cohorts, while one patient in all the three treatment groups). In addition to pre-treatment samples, we collected samples following therapy start at predefined time points. For 14 patients of the RNU cohort, serum samples from the first postoperative day were available. For the CTX cohort, 18 samples from the first day of the second chemotherapy cycle, while for the ICI cohort four samples after three months of therapy were available for analysis.

Blood samples were collected in 9 ml tubes (Vacuette®) and left at room temperature for 30 – 90 minutes, then centrifuged with an Eppendorf 5702R centrifuge at 1500 x g for 10 minutes, and finally aliquoted and kept at -80°C until further analysis. The primary endpoint of this study was OS, which was calculated as the period between initiation of therapy (RNU, CTX, or ICI) and the last follow-up (01/2022) or death. The secondary endpoint was PFS. The study was conducted in accordance with the Declaration of

Helsinki and approved by the institutional ethics committee (TUKEB 256/2014). All patients provided a written informed consent to participate in this study.

### 7.3. Quality assessment and soluble PD-L1 analysis

7.3.1. Quality assasment for study I. and study II.

Risk of bias was assessed by two independent authors using the Quality in Prognostic Studies (QUIPS) tool (31). The study attrition domain was assessed only for prospective studies. The RobVisR tool was used to summarize the results of the evaluations (32). GRADEpro™ program was used to evaluate the evidence (33).

7.3.2. Soluble PD-L1 analysis for study III.

Quantitative sPD-L1 analyses were performed by using the sandwich ELISA method (PD-L1/B7-H1 Quantikine ELISA kit, DB7H10, R&D Systems, Wiesbaden, Germany), according to the manufacturer's instructions. To exclude possible interference between the therapeutic anti-PD-L1 antibody and the used ELISA assay, we also analyzed atezolizumab (anti-PD-L1) and pembrolizumab (anti-PD-1) on our ELISA plates.

### 7.4. Data synthesis and analysis

7.4.1. Statistical analysis for study I and study II

a) All statistical analyses were performed with R (R Core Team 2023, v4.3.2), using the meta (34) package for basic meta-analysis calculations and plots, and dmeta (35) package for additional influental analysis calculations and plots.
b) For time-to-event data, hazard ratio (HR) was used for the effect size measure with 95% confidence interval (CI). To calculate the pooled HR, we calculated the logarithm of HR and its SE from the available data following the methodology of Tierney *et al.*(36).
c) We extracted or calculated the total number of patients and events ("raw data") from available studies. Using these data, we calculated odds ratios (ORs) with 95% confidence intervals (CIs) as the effect size measure. Results are reported as the odds of the event in the experimental group compared to the control group.

d) Pooled OR based on raw data was calculated using the Mantel-Haenszel method (37, 38). The pooled HR was calculated using the inverse variance weighting method (on a logarithmic scale).
e) We used a Hartung-Knapp adjustment (39) for CIs (40). To estimate the heterogeneity variance measure (τ2), for raw data OR calculation, we used the Paule-Mandel method (41) (recommended by Veroniki *et al.*(42) with the Q-profile method for the confidence interval. For HRs, the restricted maximum-likelihood estimator was used with the Q profile method for the confidence interval (42, 43).
f) Results were considered statistically significant if the pooled CI did not contain the null value. We summarized the findings in forest plots. Where applicable, and where the number of studies was sufficiently large and not too heterogeneous, we also reported the prediction intervals (i.e., the expected range of effects of future studies) of results. In addition, between-study heterogeneity was described by the Higgins & Thompson's statistics (44).
g) For Study I subgroup analysis was conducted based on the used ELISA assays and cancer types. For Study II we conducted subgroup analyses by line of therapy (first-line, second-line *vs.* mixed), drug (atezolizumab, pembrolizumab and mixed), study design (prospective *vs.* retrospective), and study site (singlecenter *vs.*multicenter). For subgroup analysis, we used a fixed-effects "plural" model (aka. mixed-effects model). We assumed that all subgroups had a common τ2 value as we did not anticipate differences in the between-study heterogeneity between the subgroups, and the number of studies was relatively small in some subgroups (recommended by Borenstein *et al.* (45)., The "Cochrane Q" test (an omnibus test) was used to assess differences between subgroups (43). The null hypothesis was rejected at the 5% significance level. Biomarker level changes were expressed as fold-changes, and a median fold-change was calculated separately for PD-1 and PD-L1 inhibitors (Table 2.).

7.4.2. Statistical analysis for study III.

The non-parametric two-sided Wilcoxon rank-sum test (Mann-Whitney test) was used for group comparisons. Univariate OS and PFS analyses were performed using the Kaplan-Meier log-rank test and univariate Cox analysis. Low event numbers in each cohort did

not allow the performance of multivariate analyses. Receiver operating characteristics (ROC) curves were applied for RNU and CTX treatment groups to determine PD-L1 cut-off values with the highest sensitivity and specificity for the dichotomized endpoint of death during the follow-up period. Spearman´s rank correlation analysis was used to test for correlation between formerly determined serum MMP-7 and PD-L1 levels (46). P-value of $<0.05$ was considered as significant. All statistical analyses were performed with IBM SPSS Statistics, version 27.0 (IBM Corp., Armonk, N.Y., USA).

## 8. RESULTS

### 8.1. Search and selection, characteristics of the included studies

8.1.1. Study I. – Investigating the prognostic role of sPD-L1 and sPD-1 in human malignancies treated with immune checkpoint inhibitor

We retrieved 458 articles from the accessed databases (Figure 1). After the selection process, 16 articles matched our eligibility criteria. However, the HR and 95% CI estimation in two articles were not possible. Therefore, these two articles were included only in the qualitative synthesis.

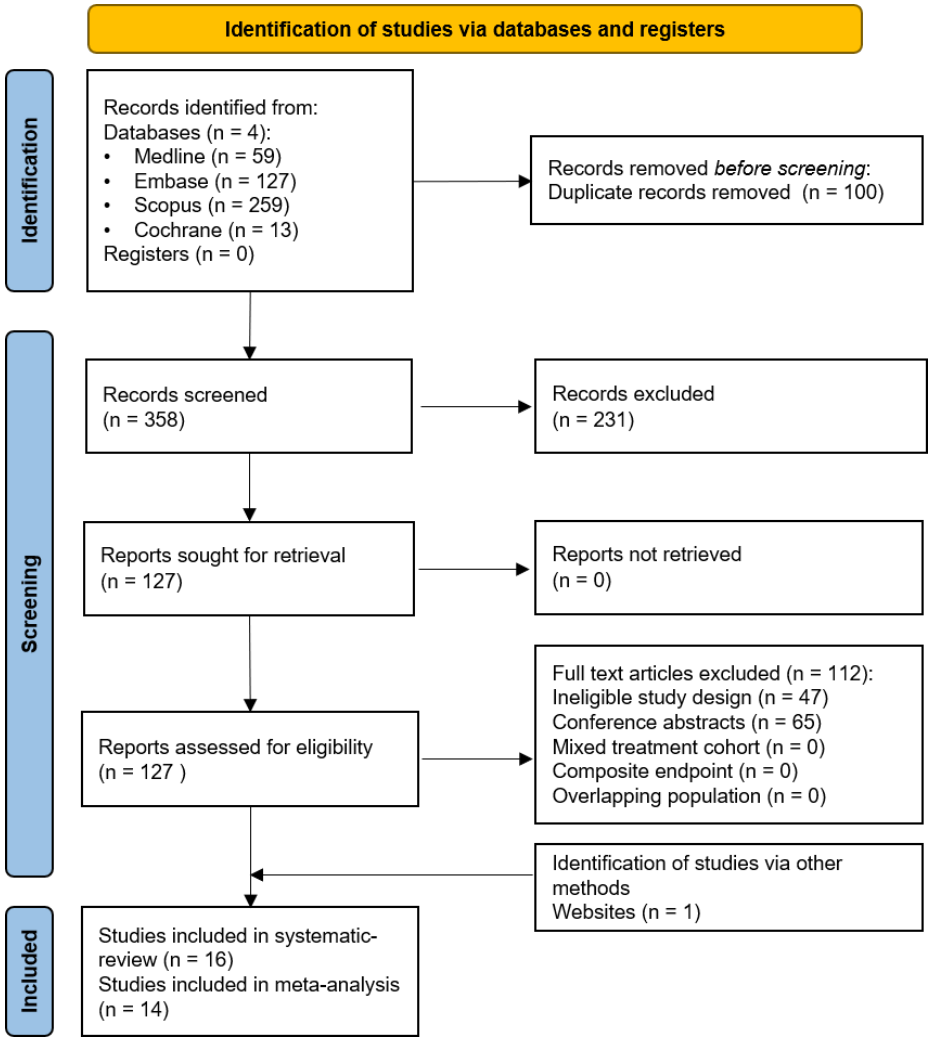



**Figure 1.** (47) PRISMA 2020 flowchart representing the study selection process

**Table 1.** (47) Basic characteristics of included studies
Nivo - ninvolumab, Pembro - pembrolizumab, Termeli - termelimumab, Durva - duvalumab, Atezo - atezolizumab, NSCLC - non-small cell lung cancer, RCC - renal cell carcinoma, ESCC - esophageal squamous cell carcinoma, R - retrospective, P – prospective, ROC – receiver operating curve, CART - Classification and Regression Trees, sd - standard deviation, OS - overall survival, PFS - progression-free survival
*IQR, **mean, † included only in systematic review, # cohort data is supplemented with unpublished data, ## Fold change

| Author (year) | Type of cancer - treatment | Study site/type | No. of patients (female %) | Age (year) | Biomarker/Cut-off(pg/mL)/type of cut-off | Follow-up period (months) | Type of ELISA | Outcome |
|---|---|---|---|---|---|---|---|---|
| Ando *et al.* 2019 † (48) | NSCLC - Nivo/ Pembro<br>Gastric - Nivo/ Pembro<br>Bladder - Pembro | Japan/R | 21 (29) | NA | sPD-L1/347/median | 6.0 (1.0-27.0) | R&D | OS |
| Castello *et al*. 2020 † (49) | NSCLC - mixed | Italy/P | 20 (35) | 77 (51- 86) | sPD-L1/27.2/median | 10.3 (2.0-29.0) | R&D | OS/PFS |
| Chiarucci *et al*. 2020 (50) | Mesothelioma - Durva/Termeli | Italy/P | 40 (34) | 66 (42- 83) | sPD-L1/70/median | 19.2 (13.8 – 20.5) * | R&D | OS |
| Costantini *et al*. 2018 (51) | NSCLC - Nivo | France/R | 43 (33) | 68 (62-72) | sPD-L1/34/ROC | 16.3 (11.7-21.1) * | Abcam | OS/PFS |
| Incorvaia *et al.* 2020 (52) | RCC - Nivo | Italy/P | 21 (10) | 61 (36–70) | sPD-L1/660/ROC<br>sPD-1/2110/ROC | NA | DYNABIO | PFS |
| Ji *et al*. 2020 (53) | ESCC - anti PD-1<br>Mixed – mixed | China/R | 21 (5)<br>61 (39) | 57 (46-70)<br>43 (21-64) | sPD-L1/NA/ROC | NA | Multiplex immunoassay kit | OS/PFS |
| Krafft *et al*. 2021# (54) | Urothelial - Atezo/Pembro | Hungary/P | 19 (26) | 66 (43 – 77) | sPD-L1/76/median | 17.0 (6.0–31.0) | R&D | OS/PFS |
| Mahoney *et al*. 2021 (55) | RCC - Nivo<br>Melanoma - Nivo | USA/P | 91 (33)<br>78 (44) | NA | sPD-L1/NA/NA | NA | SIMOA | OS/PFS |
| Mazzaschi *et al.* 2020 (56) | NSCLC - Nivo/Pembro/Atezo | Italy/P | 109 (33) | 72 (41-85) | sPD-L1/113/CART tree | 17.3 | R&D | OS/PFS |
| Meyo *et al*. 2020 (57) | NSCLC – Nivo | France/P | 51 (43) | 66 (60 – 69) | sPD-L1/160/median<br>sPD-1/70/median | 26.4 (18.1 – 36.5) | Cloud-Clone | OS/PFS |
| Murakami *et al.* 2020 (58) | NSCLC - Pembro/Nivo | Japan/R | 233 (35) | 63 (30-84) | sPD-L1/90/mean+2sd | NA | R&D | OS/PFS |
| Okuma *et al*. 2018 (59) | NSCLC – Nivo | Japan/P | 39 (26) | 69 (50-88) | sPD-L1/3357/ROC | NA | Cloud-Clone | OS |
| Oh *et al*. 2021 (60) | Mixed – mixed | Korea/R | 128 (31) | 62 (21–82) | sPD-L1/11/ROC | NA | Invitrogen | OS/PFS |
| Ugurel *et al.* 2019 (61) | Melanoma - anti PD-1 | Germany/R | 85 (41) | 62** | sPD-L1/10/ROC<br>sPD-1/500/ROC | 12.1 | R&D | OS/PFS |
| Yang *et al*. 2021 (62) | NSCLC – mixed | China/P | 21 (NA) | NA | sPD-L1/0.95## | NA | R&D | OS/PFS |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Zhou *et al*. 2017 (63) | Melanoma - Pembro | USA/NA | 35 (NA) | NA | sPD-L1/1400/NA | NA | R&D | OS |

8.1.2. Study II. – Investigating the prognostic role of blood-based inflammatory biomarkers in urothelial cancer treated with immune checkpoint inhibitor

Using the specified search key, we obtained a total of 6,673 articles from the databases accessed (Figure 2). After the selection process, 31 articles met our eligibility criteria.

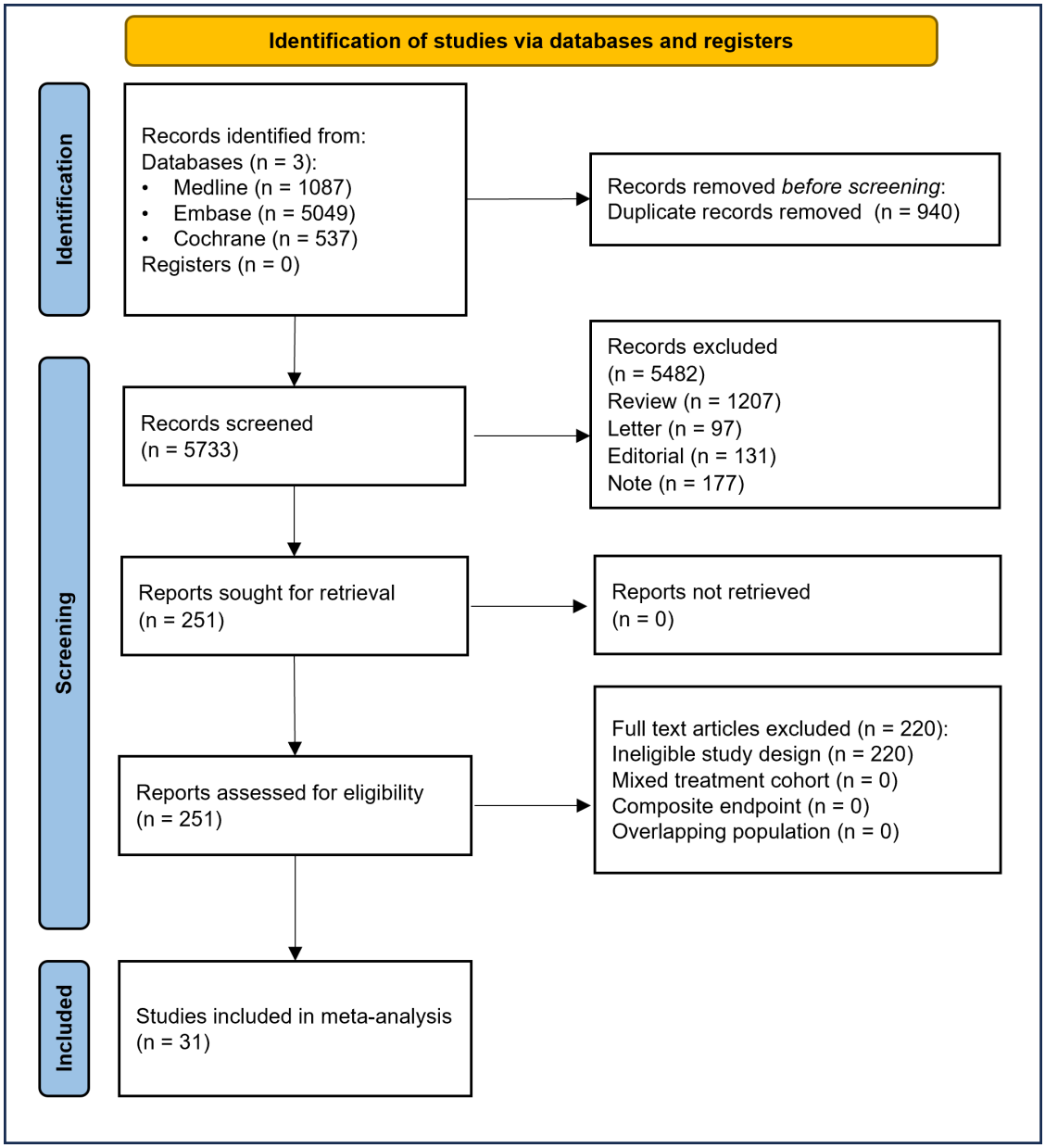



**Figure 2.** (64) PRISMA 2020 flowchart representing the study selection process

**Table 2.** (64) Basic characteristics of articles included

Pembro - pembrolizumab, Atezo - atezolizumab, Single – Singlecenter, Multi – Multicenter, R - retrospective, P – prospective, OS - overall survival, PFS - progression-free survival *IQR, **mean, UTUC – upper tract urothelial carcinoma, ECOG – Eastern Cooperative Oncology Group

| Author (year) | Study site/ center/ type | Nr. of pts. (female %) | Type of treatment | Age (median, range) | UTUC % | Follow-up (mo) (median, range) | ECOG 2-4 (%) | Line of therapy | Biomarker - Outcome |
|---|---|---|---|---|---|---|---|---|---|
| Bamias (2023) | Italy/Multi/P | 936 (22) | Atezo | 68 (61 - 74) | 23 | 12.6 | N/A | 2L | NLR - OS |
| Brown (2021) | USA/Single/R | 53 (15) | Mixed | 70 (32 - 86) | N/A | 27.1 | 11 | Mixed | CRP - OS |
| Kouchkovsky (2021) | USA/Single/R | 119 (35) | Mixed | 71 (65 - 77) | 24 | 6.3 | 21 | N/A | NLR - OS, PFS |
| Fornarini (2021) | Italy/Multi/P | 267 (17) | Atezo | 69 (62 – 74)* | 20 | 9.5 | 5 | 2L | NLR - OS, PFS |
| Fujiwara (2021) | Japan/Single/R | 74 (26) | Pembro | 69 (61 - 73)* | 51 | 8.5 (3.5 – 15.7)* | 10 | 2L | NLR, CRP, LDH - OS |
| Fukushima (2020) | Japan/Single/R | 28 (32) | Pembro | 74 (70 - 82)* | 32 | 6 (3 – 18)* | 11 | 2L | CRP - OS, PFS |
| Furubayashi (2021) | Japan/Multi/R | 105 (29) | Pembro | 72 (67 - 77)* | 39 | 8.4 (4.1 – 15.7)* | 10 | 2L | LDH - OS |
| Isobe (2021) | Japan/Single/R | 94 (18) | Pembro | 72 (47 - 85) | N/A | 13.6 | 1 | 2L | NLR, CRP - OS |
| Ito (2021) | Japan/Multi/R | 755 (25) | Pembro | 72 (63 - 77) | N/A | 7.2 | 20 | 2L | NLR - OS |
| Khaki (2021) | USA-EU/Multi/R | 357 (27) | Mixed | 71 (32 - 93) | 13 | 22 | 29 | 1L | NLR - OS |
| Klümper (2021) | Germany/Multi/R | 154 (26) | Mixed | 68 (43 - 88 | 19 | N/A | 14 | Mixed | CRP - OS, PFS |
| Kobayashi (2021) | Japan/Multi/R | 463 (23) | Pembro | 71 (31 - 88) | 39 | 10.2 | 19 | Mixed | NLR - OS |
| Kurushina (2022) | Japan/Single/R | 54 (32) | Pembro | 70** (51 - 81) | N/A | N/A | N/A | 2L | NLR - OS, PLR - OS, PFS |
| Miyama (2022) | Japan/Single/R | 50 (38) | Pembro | 72 (70 -77) | 46 | N/A | N/A | 2L | NLR - OS, PFS |
| Ogihara (2020) | Japan/Single/R | 78 (31) | Pembro | 72** (46 -89) | 45 | 7.42 (0.9 – 7.9) | N/A | 2L | NLR - PFS |
| Park (2022) | Korea/Multi/R | 224 (28) | Mixed | 68 (32 - 90) | 42 | 10.5 (5.1 – 17.4)* | 10 | 2L | NLR - OS |
| Pond (2021) | Mixed/Multi/R | 79 (N/A) | Mixed | 74 (45 - 93) | N/A | N/A | 28 | 1L | NLR - OS |
| Rijnders (2022) | Netherlands/Single/P | 71 (28) | Pembro | 70 (29 - 85) | 30 | N/A | 33 | Mixed | NLR - OS, PFS |
| Shabto (2020) | USA/Single/R | 67 (21) | Mixed | 69 (32 - 93) | N/A | N/A | 12 | Mixed | NLR, PLR - OS, PFS |
| Shimizu (2020) | Japan/Single/R | 27 (15) | Pembro | 73 (52 - 82) | 44 | 7 (1 – 20) | 44 | 2L | NLR, CRP, PLR - OS, PFS |
| Sonpavde (2020) | USA/Multi/P | 405 (23) | Atezo | 66 (32 - 89) | N/A | 22.8 (19.2 – 30) | N/A | 2L | NLR, LDH - OS |
| Taguchi (2021) | Japan/Multi/R | 150 (26) | Pembro | 71 (66–76) | 45 | 7.5 (4 – 14)* | 12 | Mixed | NLR, CRP - OS, PFS |
| Tamura (2019) | Japan/Single/R | 41 (30) | Pembro | 70 (47 - 82) | 54 | 6.2** (0.3 – 18) | 15 | 2L | NLR, CRP - OS |
| Tomioka-Inagawa (2022) | Japan/Multi/R | 160 (25) | Pembro | 72 (69 - 78)* | 31 | 10 (5 – 19)* | 16 | 2L | NLR, CRP - OS |
| Tural (2021) | Turkey/Multi/R | 113 (13) | Atezo | 65 (37 – 86) | 13 | 23.5 | N/A | Mixed | NLR - OS |
| Uchimoto (2021) | Japan/Multi/R | 212 (29) | Pembro | 72 (66 - 78)* | 39 | 11.7 | N/A | 2L | NLR - OS |
| Une (2022) | Japan/Single/R | 200 (31) | Pembro | 71 (38 - 94) | 45 | 13.3 (0 – 183) | 11 | Mixed | CRP, LDH - OS |
| Váradi (2023) | Hungary/Multi/R | 210 (31) | Mixed | 70 (29- 89) | 12 | 10.2 (0 -68.7) | 12 | Mixed | NLR, CRP, LDH - OS, PFS |
| Yamamoto (2021) | Japan/Multi/R | 121 (28) | Pembro | 74 (50 - 86) | 46 | 7.9 (0.8 – 55.9) | N/A | 2L | NLR, CRP - OS |
| Yasuoka (2019) | Japan/Single/R | 40 (20) | Pembro | 69 (44 - 83) | 48 | 5.3 (1.4 -12.3) | 14 | 2L | NLR, CRP, LDH - OS |
| Yoshida (2022) | Japan/Multi/R | 755 (25) | Pembro | 72 (66 - 77) | 56 | N/A | 20 | 2L | NA |

8.1.3. Study III. – Investigating the prognostic role of sPD-L1 in upper tract urothelial carcinoma

The median age in the RNU, CTX and ICI cohorts were 69, 72 and 65 years and the median follow-up times were 24, 18 and 20 months, respectively. In three patients, histological evaluation after RNU revealed a pT0 stage. Further patients' characteristics and baseline PD-L1 levels are given in Table 3.

**Table 3.** (65) Patients' characteristics. For RNU, CTX and ICI treatment

*Non-malignant – in three cases of RNU histological examination resulted in a pT0 finding, RNU – radical nephroureterectomy, CTX – chemotherapy, ICI – immun checkpoint inhibitor therapy, ECOG PS—Eastern Cooperative Oncology Group performance status, R+ — positive surgical margin, N+ —lymph node metastasis, M+ – distant metastasis, n. a. – not available

| | | RNU | | | CTX | | | ICI | |
|---|---|---|---|---|---|---|---|---|---|
| **General data** | | **n** | **median (range)** | **p** | **n** | **median (range)** | **p** | **n** | **median (range)** |
| Age at baseline, median (range) | | 34 | 68.9 (46.0 - 90.0) | - | 25 | 72.0 (46.0 - 84.0) | - | 6 | 64.5 (50.0 - 76.0) |
| Follow-up in months, median (range) | | 34 | 24.2 (1.1 - 81.9) | - | 25 | 17.6 (1.1 - 67.7) | - | 6 | 20.4 (2.6 - 28.3) |
| Number of patients died | | 11 | - | - | 13 | - | - | 2 | - |
| **Parameters / sPD-L1 conc.** | | **n** | **sPD-L1 cc.** | **p** | **n** | **sPD-L1 cc.** | **p** | **n** | **sPD-L1 cc.** |
| Total No. of patients, median (range) | | 34 | 84.0 (49.9 - 172.3) | 0.347 | 25 | 96.1 (53.1 - 152.9) | - | 6 | 78.3 (42.17 - 192.1) |
| Non-malignant* | | 3 | 68.4 (65.6 - 83.2) | | | | | | |
| Age ≤ 65 | | 10 | 77.3 (49.9 – 162.4) | 0.183 | 5 | 78.6 (53.1 – 139.5) | 0.408 | 3 | 94.8 (61.9 – 122.9) |
| Age > 65 | | 24 | 91.4 (59.3 – 172.3) | | 20 | 99.4 (65.0 – 152.9) | | 3 | 57.2 (42.2 – 192.1) |
| Sex | male | 21 | 93.7 (49.9 – 172.3) | 0.600 | 21 | 102.7 (53.1 – 152.9) | 0.452 | 5 | 94.8 (57.2 – 192.1) |
| | female | 13 | 80.7 (57.9 – 166.1) | | 4 | 93.9 (65.0 – 106.8) | | 1 | 42.2 |
| ECOG PS | 0 | 19 | 80.6 (50.1 – 166.1) | - | 11 | 89.0 (53.1 – 128.8) | - | 5 | 61.9 (42.2 – 192.1) |
| | 1 | 10 | 89.8 (49.9 – 162.4) | - | 10 | 103.9 (65.0 – 139.5) | - | 0 | - |
| | 2 | 4 | 98.4 (73.1 - 172.3) | - | 4 | 107.7 (105.6 – 152.9) | - | 0 | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3 | 1 | 119.6 | - | 0 | - | - | 1 | 122.9 |
| ECOG PS | 0-1 | 29 | 80.7 (49.9 – 166.1) | 0.149 | 21 | 91.8 (53.1 – 139.5) | 0.132 | 5 | 61.9 (42.2 – 192.1) |
| ECOG PS | 2-3 | 5 | 106.7 (73.1 - 172.3) | | 4 | 107.7 (105.6 – 152.9) | | 1 | 122.9 |
| **Nephroureterectomy data** | | | | | | | | | |
| pT0 | | 3 | 68.4 (65.6 - 83.2) | - | - | - | - | - | - |
| pTa | | 7 | 70.2 (50.1 - 111.7) | - | 0 | - | - | - | - |
| CIS | | 1 | 57.9 | - | 0 | - | - | 1 | 57.2 |
| pT1 | | 9 | 68.9 (49.9 - 113.3) | - | 1 | 135.3 | - | 1 | 122.9 |
| pT2 | | 2 | 110.0 (64.1 - 155.3) | - | 6 | 80.0 (68.3 - 128.8) | - | 1 | 94.78 |
| pT3 | | 14 | 102.0 (72.7 - 172.3) | - | 14 | 99.4 (53.1 - 152.9) | - | 3 | 61.9 (42.2 - 192.1) |
| pT4 | | 1 | 126.8 | - | 2 | 92.5 (89.02 - 96.1) | - | 0 | - |
| n.a. | | 0 | | | 2 | | | 0 | |
| pTa-pT1-CIS (non-invasive) | | 17 | 69.4 (49.9 - 113.3) | **<0.001** | 1 | 135.3 | - | 2 | 90 (57.2 - 122.9) |
| pT2-pT4 (invasive) | | 17 | 106.7 (64.6 - 172.3) | | 22 | 93.9 (53.1 - 152.9) | | 4 | 78.3 (42.2 - 192.1) |
| n.a. | | 0 | | | 4 | | | 1 | |
| G1-G2 | | 19 | 80.6 (49.9 - 117.3) | **0.019** | | - | - | 3 | 94.8 (61.9 - 122.9) |
| G3 | | 15 | 97.4 (57.9 - 172.3) | | | - | - | 2 | 124.6 (57.2 - 192.1) |
| Metastatic status at RNU | | | | | | | | | |
| N0/M0 | | 25 | 76.8 (49.9 - 155.3) | **0.002** | 14 | 86.0 (53.1 - 134.5) | 0.096 | 2 | 76.0 (57.2 - 94.8) |
| N+ or M+ | | 9 | 119.6 (73.1 - 172.3) | | 9 | 102.7 (78.6 - 152.9) | | 3 | 61.9 (42.2 -192.1) |
| n.a. | | 0 | | | 2 | | | 1 | |
| Metastatic status at CTX baseline | | | | | | | | | |
| M0 | | - | - | - | 10 | 76.2 (53.1 - 134.5) | **<0.001** | - | - |
| M+ | | - | - | - | 14 | 110.2 (78.6 - 152.9) | | - | - |
| n.a. | | - | - | - | 1 | - | | - | - |
| Chemotherapy regimen | | | | | | | | | |
| Gem/Cis | | - | - | - | 14 | 89.9 (53.1 - 125.9) | **0.013** | - | - |
| Gem/Carbo | | - | - | - | 11 | 111.8 (78.6 - 152.9) | | - | - |

## 8.2. Results of the quantitative analysis

8.2.1. Study I. – Investigating the prognostic role of sPD-L1 and sPD-1 in human malignancies treated with immune checkpoint inhibitor

*8.2.1.1. Elevated pre-treatment sPD-L1 predicts OS in NSCLC and melanoma*

Thirteen articles reported univariate OS as a primary outcome. The pooled overall estimate showed that patients with high sPD-L1 levels had worse OS (HR:1.67; CI:1.26-2.23, $I^2$=79%, p<0.001; Figure 3). As for publication bias, the funnel plot seems asymmetric; however, Egger's test shows no publication bias (p=0.177).

Four of the included articles reported a multivariate Cox proportional hazard model. The pooled multivariate analysis confirmed that patients with high sPD-L1 levels had shorter OS (HR:1.62; CI:1.00-2.62, $I^2$=84%, p=0.05.

A subgroup analysis was performed according to cancer type. Based on six studies with NSCLC patients, high sPD-L1 levels were consequently associated with poor OS (HR:2.93; CI:2.52-3.40, $I^2$=0%, p<0.001). According to three publications, poor OS were found for malignant melanoma patients with high sPD-L1 (HR:1.73; CI:1.01-2.97, $I^2$=19% p=0.047). No difference was found between high and low sPD-L1 levels in OS in the subgroup of mixed tumor types (HR:1.22; CI:0.86-1.72, $I^2$=0%, p=0.263), but in this case various studies showed rather heterogeneous results (Figure 3).

*8.2.1.2. Elevated pre-treatment sPD-L1 predicts poor PFS in NSCLC*

Eleven articles reported univariate PFS as the primary outcome. The pooled overall estimate found no PFS difference between high and low sPD-L1 groups (HR:1.20; CI:0.85-1.70, $I^2$=78%, p=0.305; Figure 4). The visual presentation of the Funnel plot and Egger's test suggested publication bias (p=0.007)

Four of the included articles reported a multivariate Cox proportional hazard model. The pooled multivariate analysis showed that patients with high sPD-L1 levels tended to have inferior PFS (HR:1.71; CI:1.00-2.94, $I^2$=82%, p=0.051).

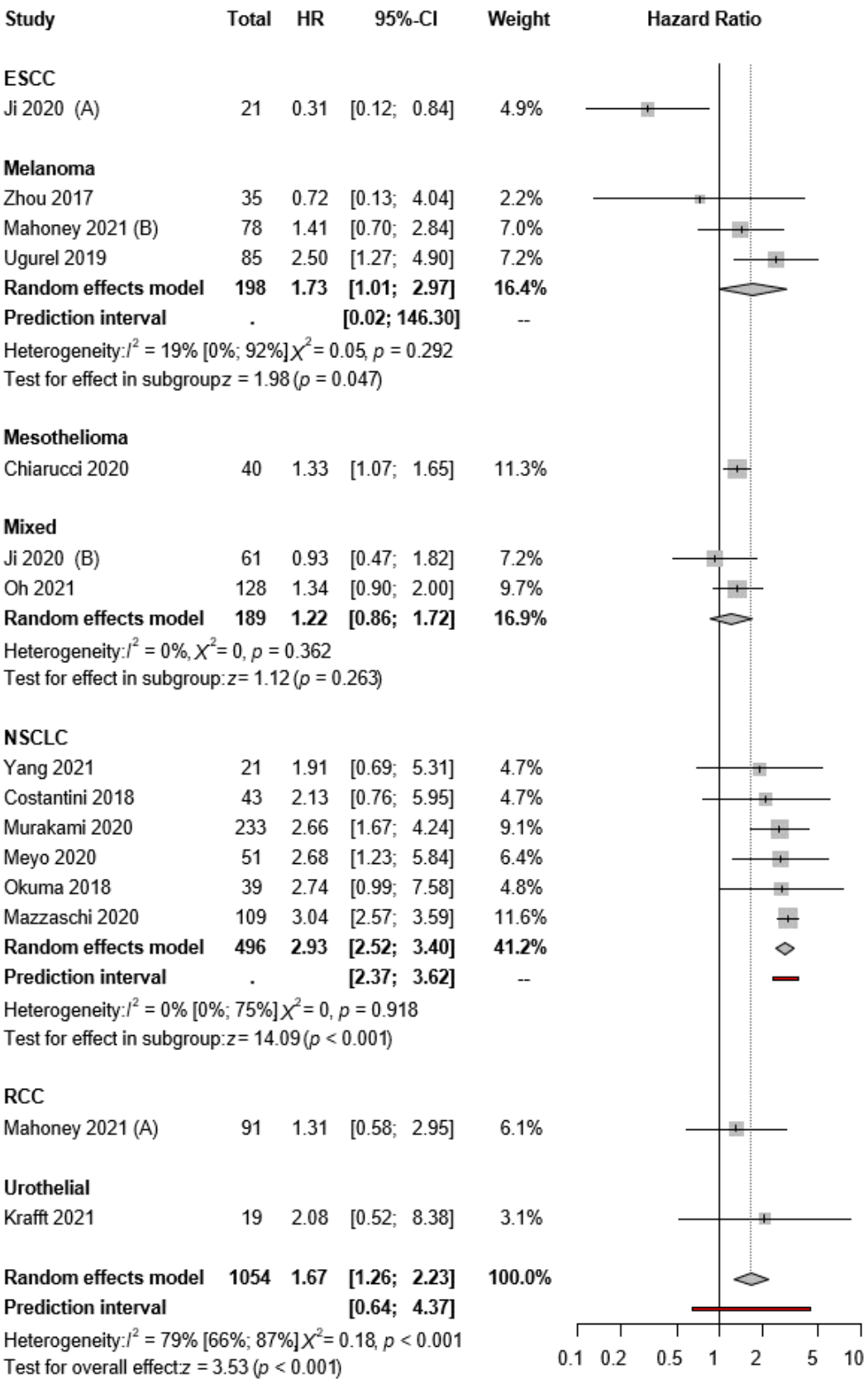

Study
Total
HR
95%-CI
Weight
Hazard Ratio
ESCC
Ji 2020 (A) 21 0.31 [0.12; 0.84] 4.9%
Melanoma
Zhou 2017 35 0.72 [0.13; 4.04] 2.2%
Mahoney 2021 (B) 78 1.41 [0.70; 2.84] 7.0%
Ugurel 2019 85 2.50 [1.27; 4.90] 7.2%
Random effects model 198 1.73 [1.01; 2.97] 16.4%
Prediction interval . [0.02; 146.30] --
Heterogeneity: $I^2$ = 19% [0%; 92%], $\chi^2$ = 0.05, $p$ = 0.292
Test for effect in subgroup: $z$ = 1.98 ($p$ = 0.047)
Mesothelioma
Chiarucci 2020 40 1.33 [1.07; 1.65] 11.3%
Mixed
Ji 2020 (B) 61 0.93 [0.47; 1.82] 7.2%
Oh 2021 128 1.34 [0.90; 2.00] 9.7%
Random effects model 189 1.22 [0.86; 1.72] 16.9%
Heterogeneity: $I^2$ = 0%, $\chi^2$ = 0, $p$ = 0.362
Test for effect in subgroup: $z$ = 1.12 ($p$ = 0.263)
NSCLC
Yang 2021 21 1.91 [0.69; 5.31] 4.7%
Costantini 2018 43 2.13 [0.76; 5.95] 4.7%
Murakami 2020 233 2.66 [1.67; 4.24] 9.1%
Meyo 2020 51 2.68 [1.23; 5.84] 6.4%
Okuma 2018 39 2.74 [0.99; 7.58] 4.8%
Mazzaschi 2020 109 3.04 [2.57; 3.59] 11.6%
Random effects model 496 2.93 [2.52; 3.40] 41.2%
Prediction interval . [2.37; 3.62] --
Heterogeneity: $I^2$ = 0% [0%; 75%], $\chi^2$ = 0, $p$ = 0.918
Test for effect in subgroup: $z$ = 14.09 ($p$ < 0.001)
RCC
Mahoney 2021 (A) 91 1.31 [0.58; 2.95] 6.1%
Urothelial
Krafft 2021 19 2.08 [0.52; 8.38] 3.1%
Random effects model 1054 1.67 [1.26; 2.23] 100.0%
Prediction interval [0.64; 4.37]
Heterogeneity: $I^2$ = 79% [66%; 87%], $\chi^2$ = 0.18, $p$ < 0.001
Test for overall effect: $z$ = 3.53 ($p$ < 0.001)
0.1
0.2
0.5
1
2
5
10

**Figure 3.** (47) Forest plots representing hazard ratios of OS for sPD-L1 in different tumor types, RCC – renal cell carcinoma, NSCLC – non-small cell lung cancer, ESCC-esophageal squamous cell carcinoma

The subgroup analysis of cancer types revealed high pre-treatment sPD-L1 as a strong risk-factor in the NSCLC subgroup (HR:2.08; CI:1.81-2.38, $I^2$=0% p<0.001), whereas rather heterogeneous results were observed in RCC (HR:0.67; CI:0.12-3.86, $I^2$=88% p=0.653), melanoma (HR:1.18; CI: 0.56-2.50, $I^2$=74%, p=0.668) and mixed cohorts (HR:0.96; CI:0.47-1.96, $I^2$=74%, p=0.903) (Figure 4).

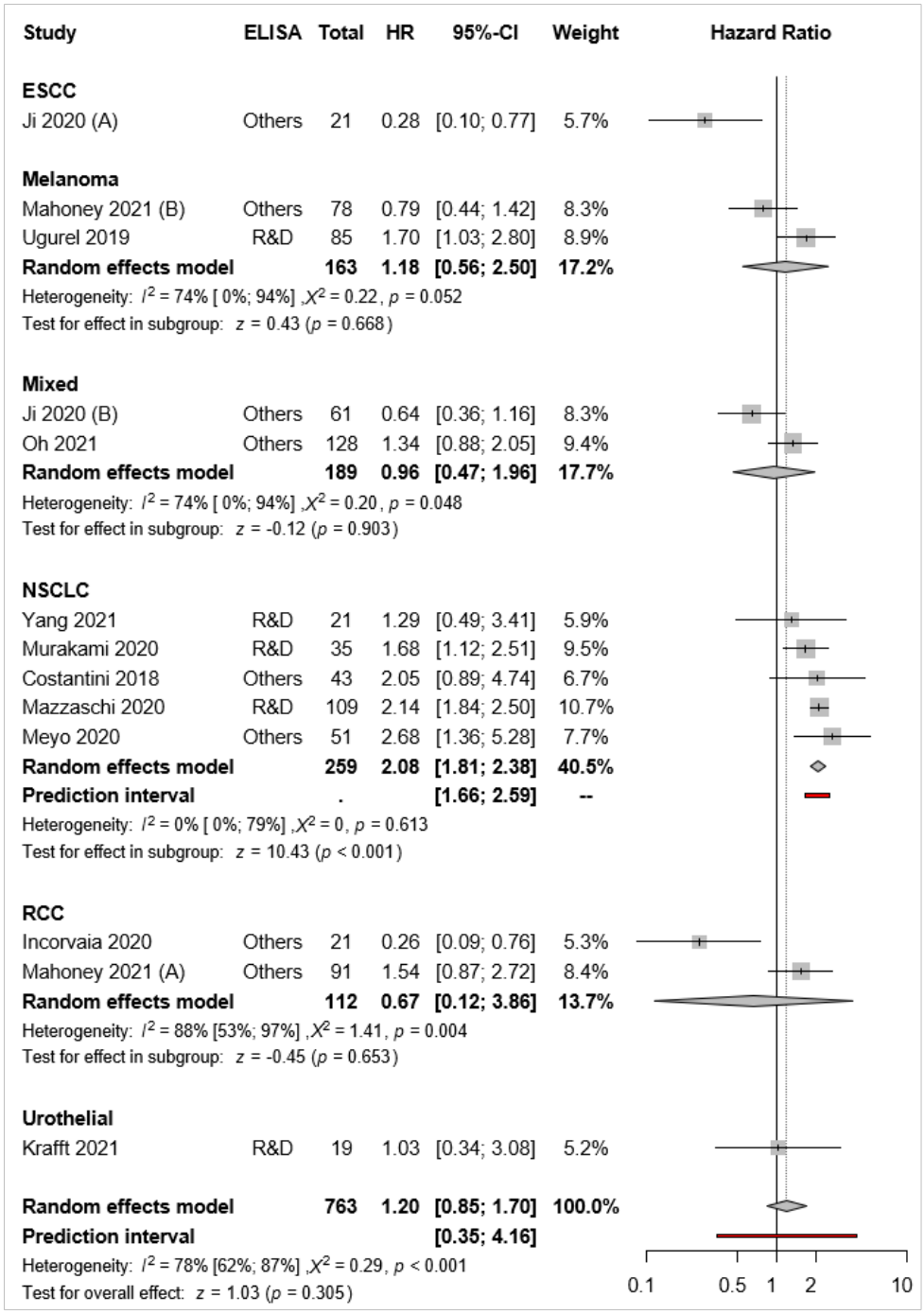



**Figure 4.** (47) Forest plots representing hazard ratios of PFS for sPD-L1 in different tumor types, RCC – renal cell carcinoma, NSCLC – non-small cell lung cancer, ESCC-esophageal squamous cell carcinoma

*8.2.1.3. Pre-treatment sPD-1 and PFS and OS*

Three articles reported PFS for sPD-1 (HR:1.16; CI:0.23-5.75, $I^2$=89%, p=0.858) (Figure 5) with heterogenous results. Meyo *et al*. in NSCLC and Ugurel *et al*. found in melanoma that higher sPD-1 level patients had shorter PFS, whereas Incorvaia *et al*. found the

opposite result in metastatic RCC. Meyo *et al.* (HR:2.28; CI:1.11-4.68; p=0.025) and Ugurel *et al.* (HR:2.70; CI:1.10-6.25; p=0.055) reported sPD-1 and OS.

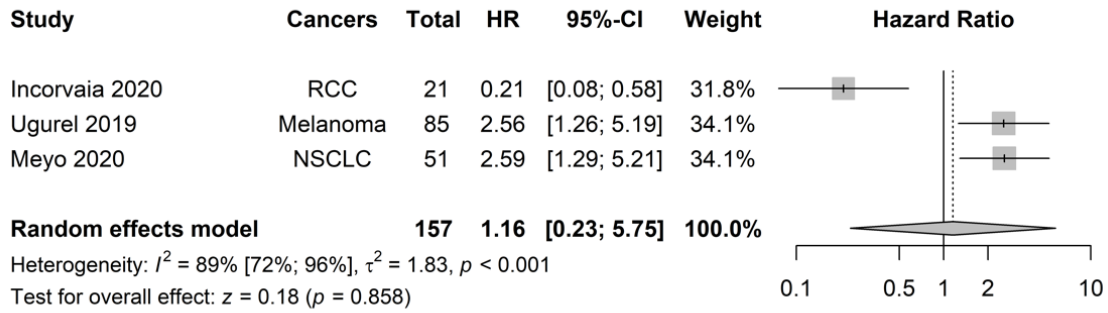



**Figure 5.** (47) Forest plots representing hazard ratios of progression-free survival for sPD-1, RCC – renal cell carcinoma, NSCLC – non-small cell lung cancer

*8.2.1.4. sPD-L1 levels strongly increase during anti-PD-L1 therapy*

Ten articles reported both pre-treatment and on-treatment sPD-L1 levels in 12 tumor entities. Serum sPD-L1 levels remained unchanged under anti-PD-1 therapy, whereas anti-PD-L1 therapy caused a remarkable (27.67-fold) elevation of sPD-L1 levels (Table 4). Two articles reported both pre-treatment and on-treatment sPD-1 levels during anti-PD-1 (nivolumab) therapy (Table 4).

**Table 4.** (47) Dynamic changes of sPD-L1 and sPD-1 levels before and after 1-3 months of immune checkpoint inhibitor therapy

RCC – renal cell carcinoma, NSCLC – non-small cell lung cancer, R – range, IQR – interquartile range

| Author (year) | Type of cancer | Type of treatment | No. of patients | Pre-treatment median sPD-L1 (pg/mL) | No. of patients | On-treatment (1-3 months) median sPD-L1 (pg/mL) | Fold change |
|---|---|---|---|---|---|---|---|
| Incorvaia *et al.* 2020 | RCC | anti PD-1 | 9 | 1090.0 (R 470.0 - 2410.0) | 9 | 730.0 (R 560.0 - 1390.0) | 0.67 |
| Meyo *et al*. 2020 | NSCLC | anti PD-1 | 50 | 160.0 (IQR 30.0 - 440.0) | 50 | 130.0 (IQR 30.0 - 380.0) | 0.81 |
| Mahoney *et al*. 2021 | Melanoma | anti PD-1 | 78 | 2312.0 | 78 | 2247.0 | 0.97 |
| Yang *et al*. 2021 | NSCLC | anti PD-1 | 19 | 37.7 (R 15.6 - 152.0) | 19 | 36.7 (R 15.6 - 109.0) | 0.97 |
| Mahoney *et al.* 2021 (55) | RCC | anti PD-1 | 91 | 1978.0 | 91 | 2179.0 | 1.10 |
| Costantini *et al*. 2018 | NSCLC | anti PD-1 | 43 | 39.8 (IQR 29.8 - 59.2) | 43 | 51.6 (IQR 31.9 - 72.1) | 1.30 |
| Ando *et al*. 2019 | mixed | anti PD-1 | 21 | 347.4 (R 251.9 - 1491.1) | 9 | 468.8 (R 256.5 - 881.3) | 1.35 |
| Oh *et al*. 2021 | Genitourinary | mixed | 10 | 11.8 (R 5.9 - 21.5) | 10 | 17.1 (R 6.0 - 93.5) | 1.46 |
| Castello *et al*. 2020 | NSCLC | anti PD-1 | 20 | 27.2 (R 11.2 - 61.3) | 20 | 43.9 (R 19.6 - 77.8) | 1.61 |
| Oh *et al*. 2021 | NSCLC | mixed | 16 | 15.0 (R 3.8 - 51.9) | 10 | 58.4 (R 8.7 - 139.5) | 3.89 |
| Krafft *et al*. 2021 | Urothelial | anti PD-L1 | 19 | 71.2 (R 42.2 - 192.1) | 8 | 1946.5 (R 1694.0–1993.0) | 27.34 |
| Chiarucci *et al*. 2020 | Mesothelioma | anti PD-L1 | 29 | 70.0 (R 20.0 - 190.0) | 14 | 1960.0 (R 1330.0 - 2750.0) | 28.00 |
| Author (year) | Type of cancer | Type of treatment | No. of patients | Pre-treatment median sPD-1 (pg/mL) | No. of patients | On-treatment (1-3 months) median sPD-1 (pg/mL) | Fold change |
| Incorvaia *et al.* 2020 | RCC | anti PD-1 | 9 | 13250.0 (R 1220.0- 25000.0) | 9 | 1230.0 (R 1060.0 - 1930.0) | 0.09 |
| Meyo *et al*. 2020 | NSCLC | anti PD-1 | 50 | 70.0 (IQR 30.0 - 180.0) | 50 | 70.0 (IQR 30.0 - 200.0) | 1.0 |

*8.2.1.5. The assay method does not influence the correlations between sPD-L1 and OS*

Our subgroup analysis according to the used assay methods suggested that the sPD-L1 assay method had no major influence on the OS (R&D: HR:2.11; CI:1.44-3.08, $I^2$=84%, p=0.003 vs. "others": HR:1.35; CI:0.79-2.30, $I^2$=54%, p=0.224; Figure 6). The same subgroup analysis was further evaluated based on PFS. Our subgroup analysis suggested that the sPD-L1 assay method might influence PFS. (R&D: HR:1.87; CI:1.52-2.32, $I^2$=2%, p=0.025 vs. "others": HR:0.96; CI:0.55-1.66, $I^2$=76%, p=0.873; Figure 5). Because of the low number of studies with sPD-1, no comparison was possible between various assay methods.

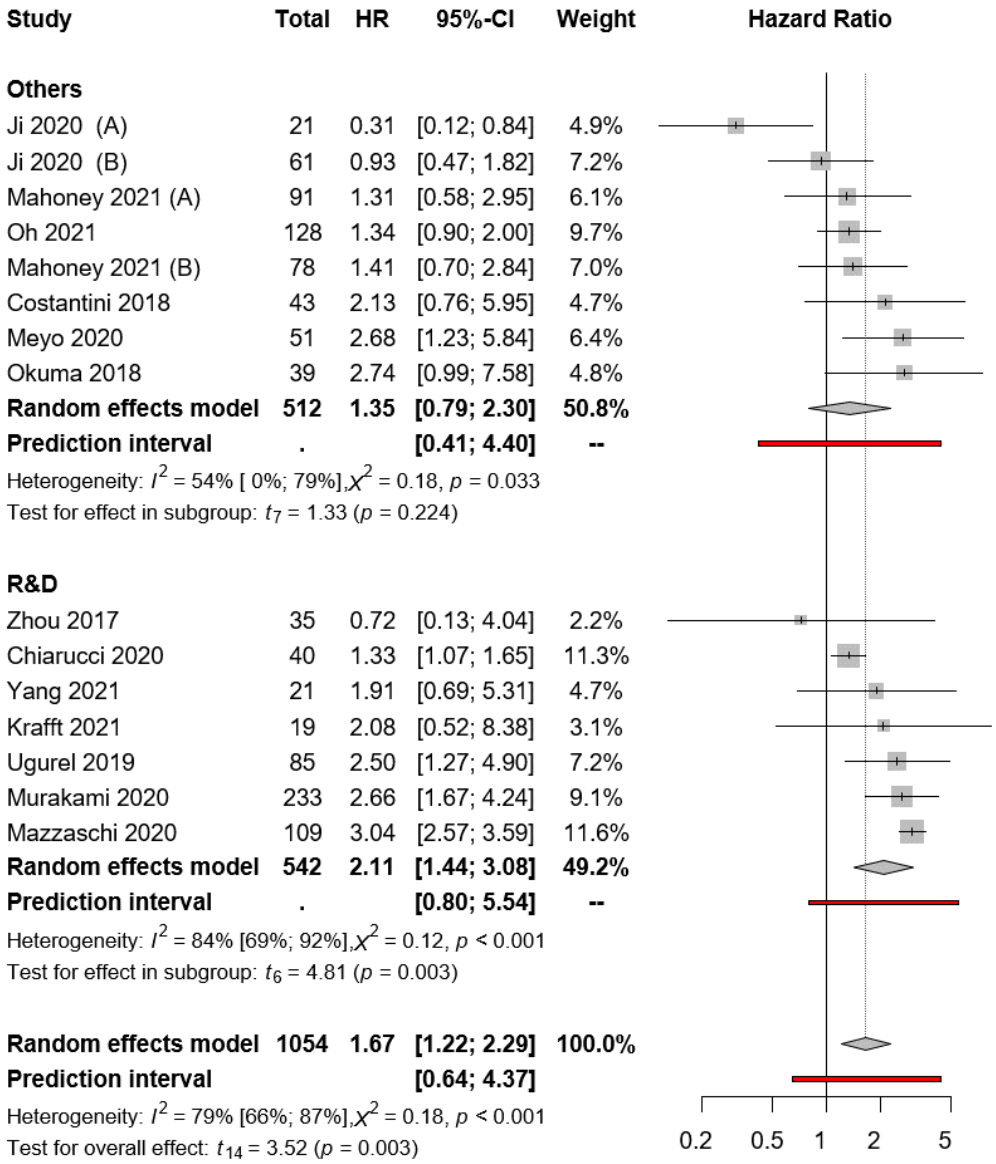


**Figure 6.** (47) Forest plots representing hazard ratios of OS for sPD-L1 for different ELISA kits, HR – hazard ratio

8.2.2. Study II. – Investigating the prognostic role of blood-based inflammatory biomarkers in urothelial cancer treated with immune checkpoint inhibitor therapy

*8.2.2.1. High pre-treatment NLR is associated with inferior OS and PFS.*

Twenty articles provided information on NLR and OS. Pre-treatment high NLR was associated with worse OS both in univariate (HR: 2.19; 95%CI: 1.80-2.68) (Figure 7*)*and multivariate analyses (HR: 1.77; 95%CI: 1.61-1.94).

High pre-treatment NLR was associated with poor PFS both in univariate (HR: 1.90; 95%CI: 1.57-2.31) (Figure 2) and multivariate analysis (HR: 1.77; 95%CI: 1.16-2.71).

Subgroup analysis of therapy lines revealed that high pre-treatment NLR was associated with worse OS rates in the second-line (12 articles) (HR:2.21 95%CI: 1.75-2.80) and the mixed-line (5 articles) (HR:3.03 95%CI: 1.67-5.52) ICI settings but no significant association was found in the first-line setting (3 articles) (HR:1.32 95%CI: 0.58-3.00);. Furthermore, subgroup analysis by ICI drug type revealed that NLR was associated with worse OS rates both in the pembrolizumab (12 articles) (HR: 2.09; 95%CI: 1.69-2.60) (Figure 7) and in the atezolizumab (4 articles) (HR: 2.90; 95%CI: 1.30-6.49) (Figure 2) treatment groups. In an additional subgroup analysis, OS rate remained consistently associated with NLR regardless of study design, with an HR of 2.24 (95%CI: 1.67-3.01) (Figure 7) for prospective studies (3 articles) and an HR of 2.15 (95%CI: 1.67-2.78) (Figure 7) for retrospective studies (17 articles). In addition, singlecenter studies had results similar to those of multicenter studies, with singlecenter studies (8 articles) giving an HR of 2.16 (95%CI: 1.50-3.10) (Figure 7) and multicenter studies (12 articles) an HR of 2.23 (95%CI: 1.64-3.02) (Figure 7). Three articles provided information on NLR and ORR, with a pooled ORR of 1.66 (95%CI: 0.47-5.89).

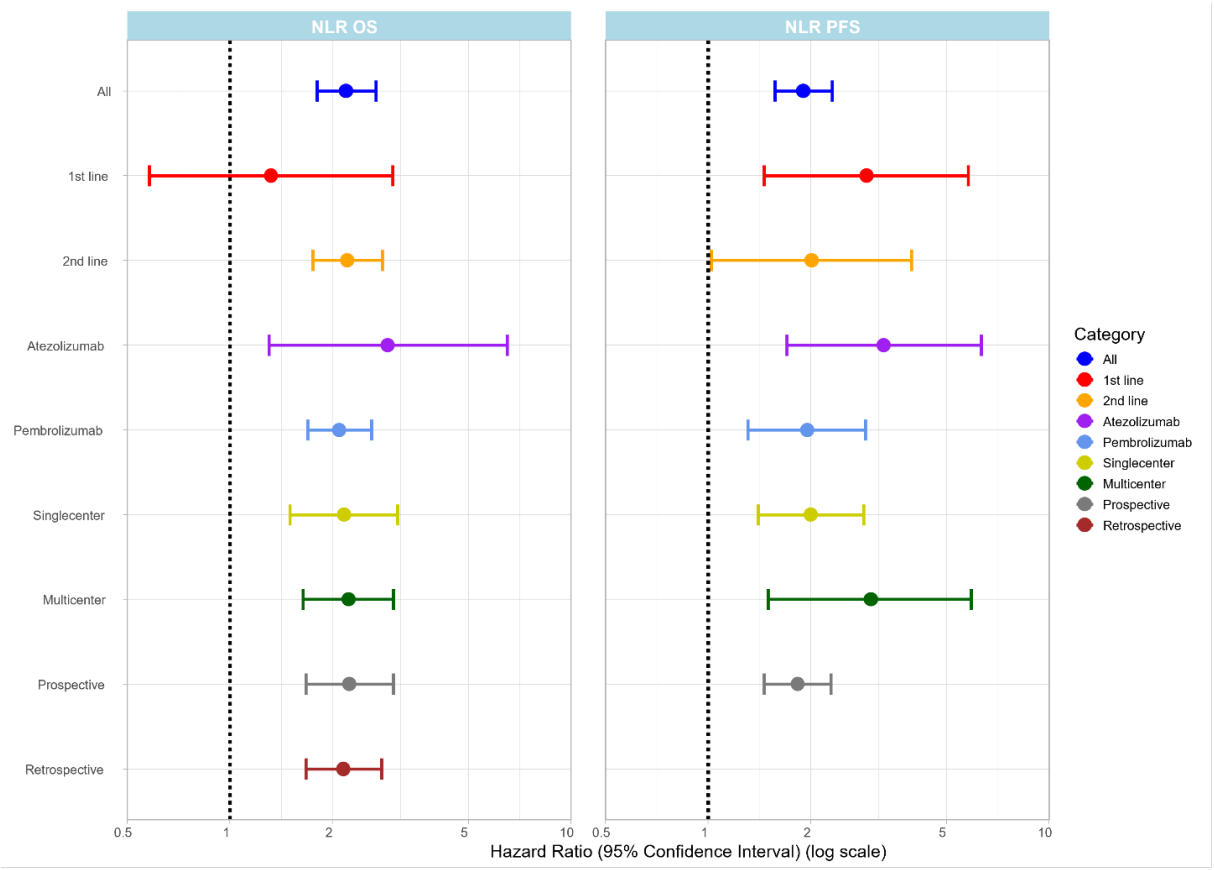



**Figure 7.** (64) Summary plot showing pooled HR values (x-axis) with 95% CI for OS and PFS for NLR in different subgroups (y-axis). NLR – neutrophil-to-lymphocyte ratio, OS – overall survival, PFS – progression free survival

*8.2.2.2. High pre-treatment CRP levels are associated with inferior OS and PFS*

Eleven articles provided information on pre-treatment serum CRP levels. High pre-treatment CRP levels were associated with lower OS rates in both the univariate (HR: 1.75; 95%CI: 1.37-2.24) (Figure 8) and multivariate (HR: 1.66; 95%CI: 1.18-2.33) analyses. Similarly, poor PFS was associated with elevated pre-treatment CRP levels (HR: 1.58; 95%CI: 1.26-1.99) (Figure 8).

Our subgroup analysis revealed that in the second-line ICI setting (7 articles), high pre-treatment CRP was associated with worse OS rates (HR: 1.85; 95% CI: 1.19-2.88) (Figure 8). Furthermore, CRP was also associated with worse OS rates in the pembrolizumab (9 articles) (HR: 1.69; 95%CI: 1.20-2.38) (Figure 8) treatment group, whereas for atezolizumab, the two available studies did not allow a statistical evaluation. In addition,

CRP levels were associated with poor OS in singlecenter (7 articles) (HR: 1.87; 95%CI: 1.23-2.86) (Figure 8), but not in multicenter studies (4 articles).

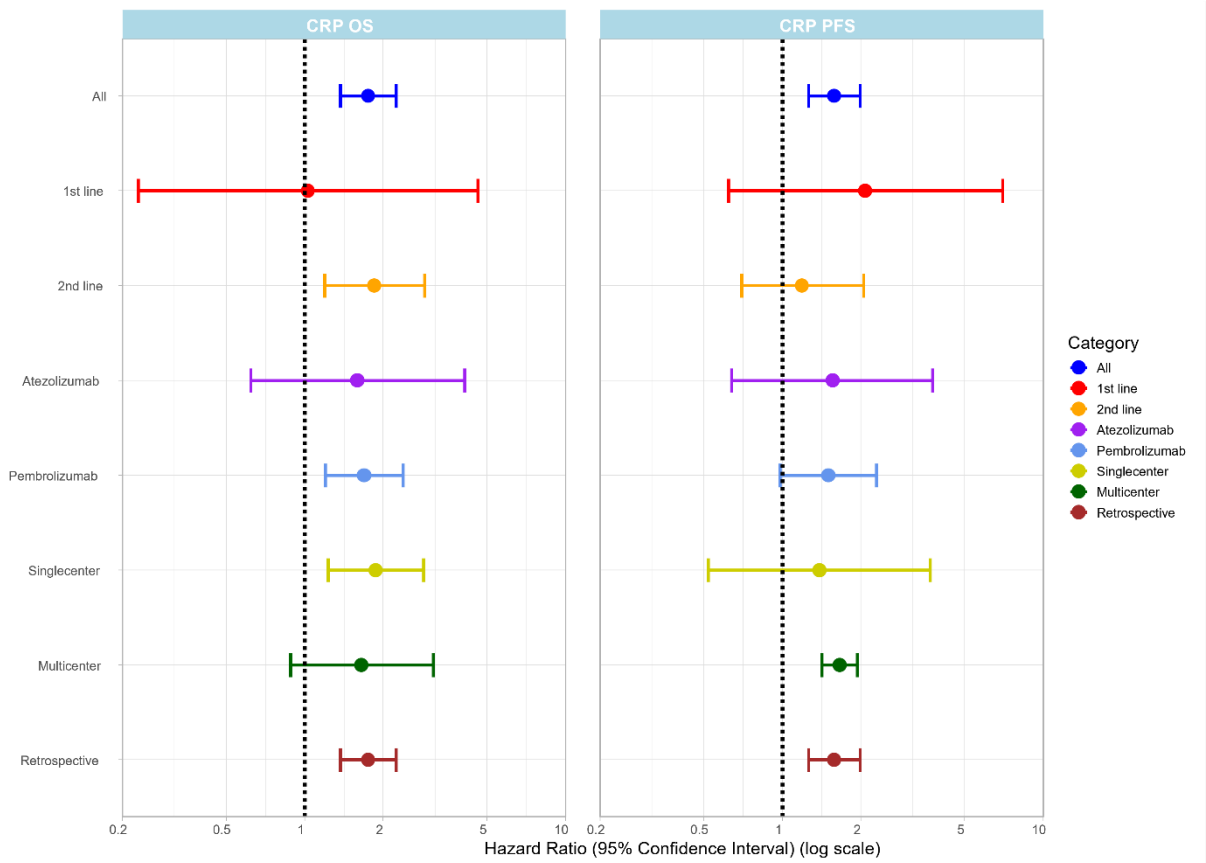



**Figure 8.** (64) Summary plot of pooled HR values (x-axis) with 95% CI for OS and PFS for the CRP based on in different subgroups on the (y-axis). CRP – C reactive protein, OS – overall survival, PFS – progression free survival

*8.2.2.3. High pre-treatment PLR is associated with inferior OS and PFS*

Three articles provided data on PLR and survival endpoints (OS, PFS). In univariate analysis, high pre-treatment PLR was associated with shorter OS (HR: 2.74; 95%CI: 1.74-4.31) (Figure 9) and PFS (HR: 2.25; 95%CI: 1.46-3.47) (Figure 9). Subgroup analyses were not possible due to the low number of available articles.

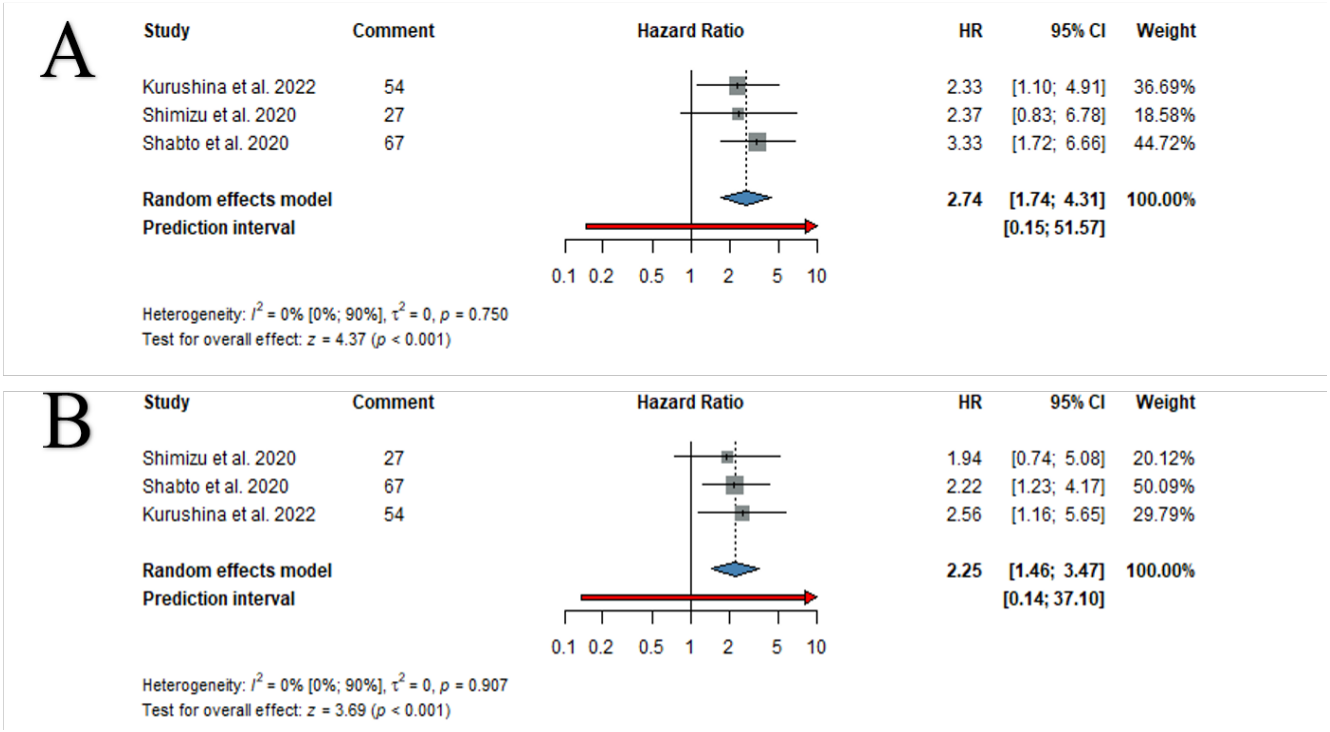



**Figure 9.** (64) Forest plots of pooled univariate HR values and 95% CI for PLR OS (A), PLR PFS (B), PLR – platelet-to-lymphocyte ratio, OS – overall survival, PFS – progression free survival

8.2.3. Study III. – Investigating the prognostic role of sPD-L1 in UTUC

*8.2.3.1. Correlation of sPD-L1 concentrations with clinicopathological parameters*

For the RNU cohort, age, sex and ECOG performance status showed no significant association with preoperative sPD-L1 levels. Higher sPD-L1 levels were found in muscle-invasive, high grade (G3) as well as in lymph node and/or distant metastatic cases ($p<0.001$, $p=0.019$ and $p=0.002$ respectively) (Table 3, Figure 10).

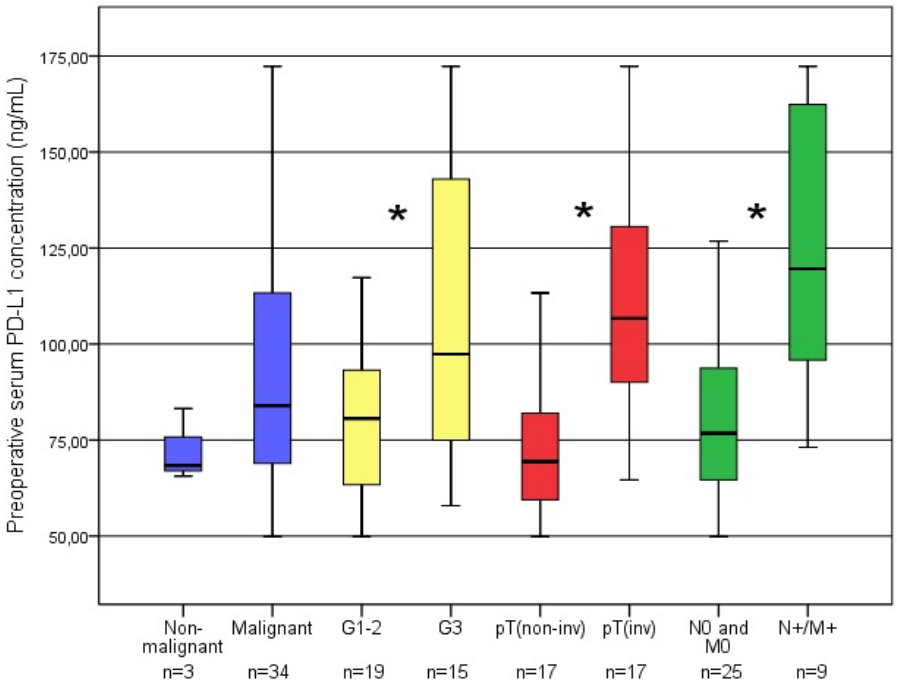



**Figure 10.** (65) Association of preoperative sPD-L1 concentration and clinicopathological parameters in the RNU cohort. * significant difference, pT(non-inv): pTa-pT1, pT(inv): pT2-pT4, G – grade, N0 and M0 – no metastasis, N+/M+ - metastasis

*8.2.3.2. Correlation of pre-treatment sPD-L1 levels with patients' prognosis*

For the RNU cohort, three patients with pT0 histopathological findings were excluded from survival analyses. Muscle-invasive disease (≥pT2) and the presence of lymphatic or distant metastases at RNU were associated with shorter OS (HR: 7.115; 95% CI 1.504 – 33.659; $p=0.013$ and HR: 4.891; 95% CI 1.379 – 17.345; $p=0.014$, respectively). Similarly, shorter PFS was significantly associated with the same factors: ≥pT stage (HR: 10.836; 95% CI 2.865 – 40.978; $p<0.001$), lymph node or distant metastasis (HR: 6.185; 95% CI 2.199 – 17.397; $p=0.001$) (Table 5).

**Table 5.** (65) Correlation of clinicopathological parameters and pretreatment sPD-L1 concentrations with patients' prognosis

* - median cut-off value for RNU is 84.0 ng/ml, median cut-off value for CTX is 96.1 ng/ml; ** ROC cut-off value for RNU is 118.5 ng/ml, ROC cut-off value for CTX is 93.9 ng/ml; RNU – radical nephroureterectomy, CTX – chemotherapy; OS – overall survival; PFS – progression-free survival; ECOG PS – Eastern Cooperative Oncology Group performance status, Gem/Cis – gemcitabine + cisplatin; Gem/Carbo – gemcitabine + carboplatin; bold font represents significant value.

| | | RNU | | | | | | | CTX | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OS | | | PFS | | | | OS | | | PFS | | |
| **General data** | | **n** | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** | **n** | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** |
| Age | ≤ 65 | 10 | ref. | | | ref. | | | 5 | ref. | | | ref. | | |
| | > 65 | 24 | 2 142 | 0.459 - 9.994 | 0.332 | 1.512 | 0.485 - 4.710 | 0.476 | 20 | 1.548 | 0.338– 7.094 | 0.574 | 0.461 | 0.157-1.350 | 0.160 |
| Sex | male | 21 | ref. | | | ref. | | | 21 | ref. | | | ref. | | |
| | female | 13 | 0.281 | 0.060 - 1.301 | 0.104 | 0.376 | 0.120-1.179 | 0.093 | 4 | 0.426 | 0.055 - 3.310 | 0.415 | 0.322 | 0.042-2.450 | 0.274 |
| ECOG PS | 0-1 | 29 | ref. | | | ref. | | | 21 | ref. | | | ref. | | |
| | 2-3 | 5 | 3 451 | 0.707 - 16.832 | 0.126 | 3.153 | 0.868 - 11.451 | 0.081 | 4 | 2.490 | 0.655 – 9.465 | 0.181 | 0.931 | 0.209 - 4.151 | 0.925 |
| Nephroureterectomy data | | | | | | | | | | | | | | | |
| Stage | pTa-pT1 | 17 | ref. | | | ref. | | | | - - | - | - | - | - | - |
| | pT2-pT4 | 17 | 7 115 | 1.504 - 33.659 | **0.013** | 10.836 | 2.865 - 40.978 | **<0.001** | | - | - | - | - | - | - |
| Metastases | N0/M0 | 25 | ref. | | | ref. | | | 14 | ref. | | | ref. | | |
| | N+ or M+ | 9 | 4 891 | 1.379 - 17.345 | **0.014** | 6.185 | 2.199 - 17.397 | **0.001** | 9 | 3.948 | 1.096-14.221 | **0.036** | 3.470 | 1.209 - 9.959 | **0.021** |
| Chemotherapy baseline data | | | | | | | | | | | | | | | |
| Metastases | N0/M0 | | - | - | - | - | - | - | 10 | ref. | | | ref. | | |
| | N+ or M+ | | - | - | - | - | - | - | 14 | 14.737 | 1.810-119.98 | **0.012** | 7.638 | 2.218-26.301 | **0.001** |
| CTX regimen | Gem/Cis | | - | - | - | - | - | - | 14 | ref. | | | ref. | | |
| | Gem/Carb | | - | - | - | - | - | - | 11 | 1.077 | 0.341- 3.401 | 0.899 | 1.738 | 0.623 - 4.846 | 0.291 |
| Pretreatment sPD-L1 | | | | | | | | | | | | | | | |
| median cut-off* | | 17 | ref. | | | ref. | | | 11 | ref. | | | ref. | | |
| median cut-off* | | 17 | 4.023 | 1.060 - 15.269 | **0.041** | 2.793 | 1.011 - 7.716 | **0.048** | 14 | 6.956 | 1.461 - 33.110 | **0.015** | 1.584 | 0.560 - 4.478 | 0.386 |
| ROC cut-off** | | 27 | ref. | | | ref. | | | 11 | ref. | | | ref. | | |
| ROC cut-off** | | 7 | 12.114 | 2.990 - 49.082 | **<0.001** | 6.667 | 2.140 - 20.764 | **0.001** | 14 | 6.956 | 1.461 - 33.110 | **0.015** | 1.584 | 0.560 - 4.478 | 0.386 |

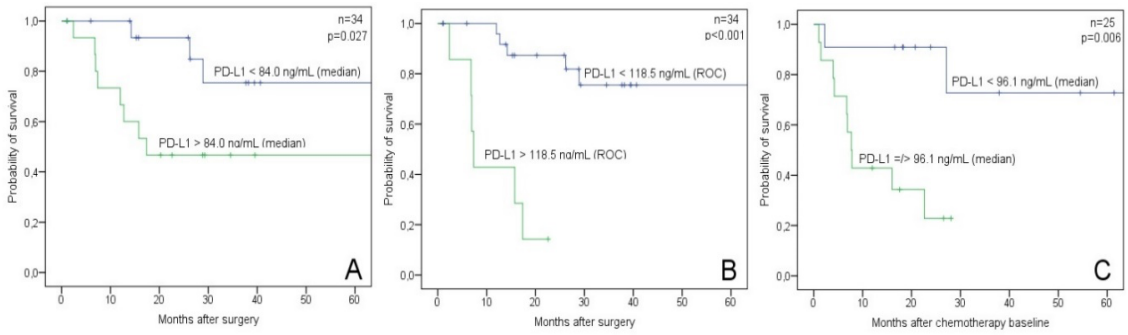



**Figure 11.** (65) Kaplan-Meier OS analyses with log-rank tests (A) for the RNU cohort using the median as the cut-off, (B) for the RNU cohort applying the ROC-based cut-off, (C) for the CTX cohort with the median cut-off (in this cohort, median- and ROC-based cut-off values resulted the same groups) (blue line – low sPD-L1 cc., green line – high sPD-L1 cc., cut-off values are shown on each line). RNU – radical nephroureterectomy; CTX – chemotherapy;

*8.2.3.3. Changes of sPD-L1 levels during and after therapy*

In the RNU cohort, the median preoperative sPD-L1 concentration was 84.0 ng/ml. In 14 cases, postoperative (first day after RNU) sPD-L1 levels were available with a median of 114.5 ng/ml, which was significantly higher than the pre-treatment serum concentrations (p=0.011) (Figure 12 A,D).

In the CTX cohort, the baseline median of sPD-L1 level was 96.1 ng/ml which remained unchanged (99.4 ng/ml, n=18) after the first treatment cycle (Figure 12 B,D).

Interestingly, we observed a remarkable, 25-fold increase of sPD-L1 levels after 3 months of ICI treatment from 78.3 ng/ml to 1955.5 ng/ml (p<0.001) (Figure 12 C,D, which was in accordance with our former observation in ICI-treated UBC patients. In addition, we measured atezolizumab and pembrolizumab directly on our assay plates. These, substances did not result positive signals, excluding an assay incompatibility with the therapeutic antibodies.

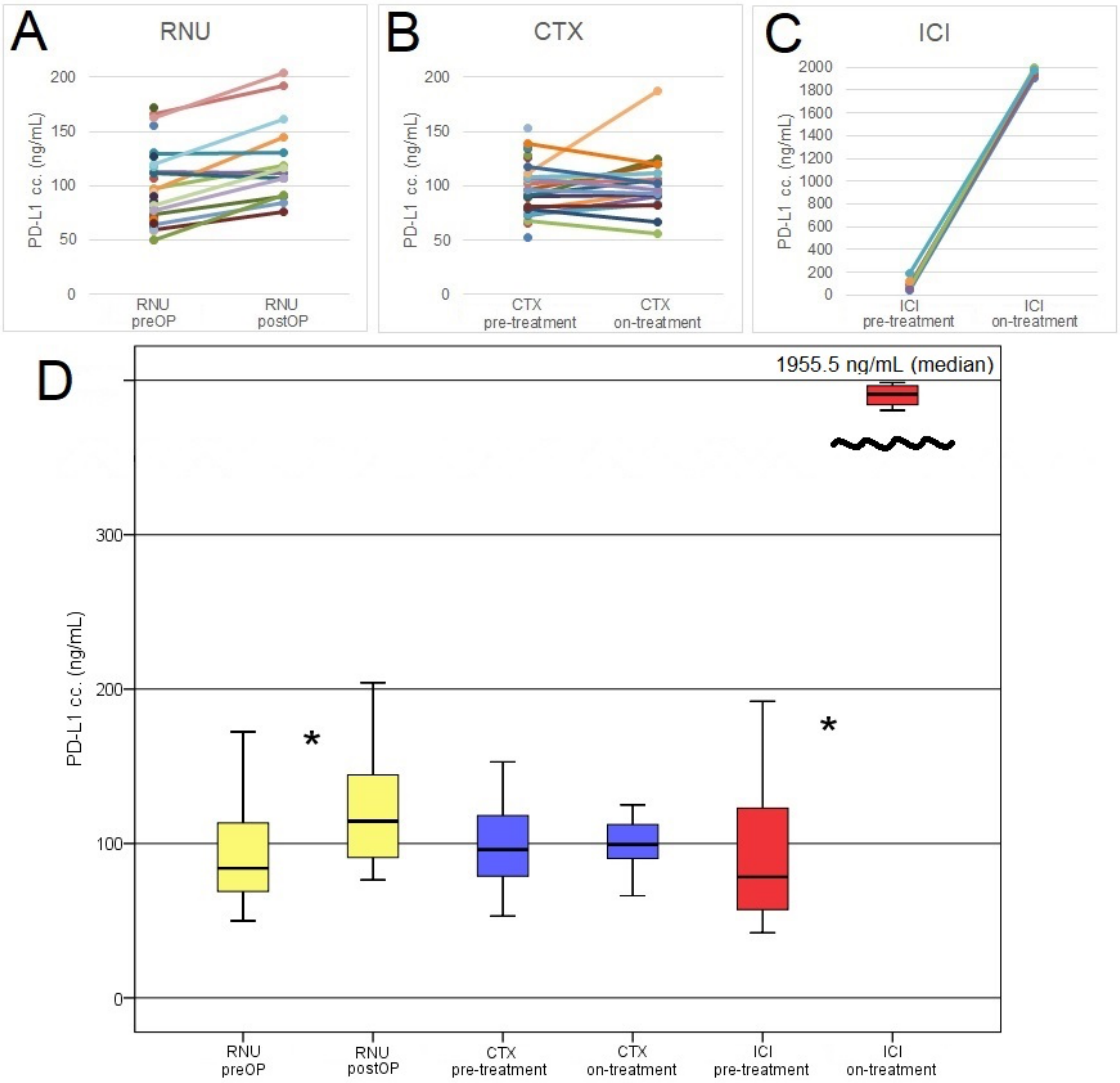



**Figure 12.** (65) Box-plot presentation of changes of sPD-L1 concentrations in the RNU, CTX and ICI cohorts (A) RNU cohort (preop. and postop. values), (B) CTX cohort (at chemotherapy baseline and on the first day of cycle 2), (C) ICI cohort (pre-treatment and on-treatment values at 3 months), (D) (* significant difference); RNU – radical nephroureterectomy; CTX – chemotherapy; ICI – immune checkpoint inhibitor therapy.

# 9. DISCUSSION

## 9.1. Summary of findings, international comparisons

Recently, advances in biomarker research have significantly influenced the optimization of immunotherapy treatment modalities. Programmed death-ligand 1 (PD-L1) immunohistochemistry has become the standard predictive tool for determining the likelihood of response to ICI therapy in several tumor types. However, soluble biomarkers have yet to be integrated into clinical risk stratification models. Therefore, we performed three distinct yet related analyses: (1) we investigated the clinical relevance of sPD-L1 levels in ICI-treated solid tumors; (2) we examined the role of routinely used inflammatory biomarkers in ICI-treated UC patients; and (3) we evaluated the prognostic and therapy predictive value of sPD-L1 in UTUC, a distinct, less common but significant subset of UC.

In UTUC elevated pretreatment sPD-L1 levels were associated with muscle-invasive disease, higher tumor grade, metastasis, and poor survival, particularly in the surgically and chemotherapy (CTX) treated cohorts. One of the major challenges in UTUC management is the inconsistency of preoperative staging due to inaccurate biopsy and variability in therapy response. Studies have reported significant rates of upstaging and downstaging between pre- and postoperative assessments (66, 67). Grading appears more reliable, with higher consistency between biopsy and definitive findings (68). Although clinical factors such as age, multifocality, and performance status serve as prognostic indicators, they provide limited predictive value (69). Thus, molecular biomarkers reflecting tumor biology are needed to improve clinical decision-making. Tissue-based biomarkers such as Ki67, CDCA5, and PAK1, while informative, are limited by biopsy quality and lack prospective validation (70). Circulating biomarkers like MMP-7, NLR, and CRP have shown promising but require larger validation studies (46, 71, 72). Our serum sample analysis revealed that high sPD-L1 levels correlate with adverse pathological features and shorter survival in UTUC, indicating its potential as a preoperative tool for assessing disease severity and guiding surgical or systemic treatment decisions. Literature data also revealed heterogeneity in UTUC's response to systemic treatments. In the neoadjuvant CTX setting, cisplatin-based chemotherapy showed pathological response rates of 47–52% and 8-10% pathological complete response rates,

while metastatic cases responded at rates of 35–46% (73, 74). However, PD-L1 tissue expression has been linked to improved survival in cisplatin-ineligible patients receiving ICI therapy, though individual responses vary widely (75). In our study, high baseline sPD-L1 predicted worse outcomes in CTX-treated patients, while the small sample size in the ICI cohort prevented statistically robust conclusions.

Conflicting findings in other tumor types further complicate the predictive interpretation of sPD-L1. For instance, in esophageal carcinoma and RCC, high sPD-L1 correlated with better outcomes under ICI therapy, while in NSCLC and melanoma, high levels predicted worse survival (51-53, 61). These results imply that the prognostic or predictive value of sPD-L1 may be tumor-type specific.

To advance the understanding of the therapy predictive relevance of sPD-L1, a meta-analysis of 16 studies across six cancer types evaluated sPD-L1 and sPD-1 as prognostic markers in ICI-treated patients. Overall, high sPD-L1 levels were associated with a 67% increased risk of death and a 20% higher risk of disease progression. Subgroup analyses revealed that this prognostic value is more consistent in NSCLC than in melanoma, suggesting tumor-specific interpretations are essential. For NSCLC, pre-treatment sPD-L1 may serve as a predictive marker, though its overall behavior appears more prognostic than predictive. Notably, various studies applied different assay kits, but ELISA methodology did not significantly influence outcomes. Additionally, multivariate analyses from selected studies identified high sPD-L1 as an independent risk factor for worse outcomes, along with ECOG performance status and PD-L1 tissue expression.

The biological source of sPD-L1 remains uncertain. Current evidence suggests it is not primarily derived from tumor tissue expression, as studies have shown no direct correlation between tissue and serum PD-L1 levels (49, 51, 54). Matrix metalloproteinases (MMPs), particularly MMP-7, have been implicated in the proteolytic release of PD-L1 into circulation (54). In a previous study of the UTUC cohort, a significant correlation between serum MMP-7 and sPD-L1 levels supports the hypothesis that an active proteolytic tumor environment contributes to elevated sPD-L1 (46). Furthermore, the moderate post-surgical increase in sPD-L1 levels hints at non-tumoral sources, possibly related to the inflammatory response induced by surgery. Comparing the pre-and postoperative sPD-L1 levels, we detected a mild but significant increase after RNU, which suggests that tumor cells are not the predominant source of sPD-L1.

Postoperative sPD-L1 levels may be increased as a consequence of an inflammatory response to the surgical procedure itself. In the CTX cohort, no differences were detected between baseline and on-treatment sPD-L1 levels. In contrast, we found strongly, 25-fold elevated sPD-L1 concentrations after three months of ICI treatment. These striking results are in line with our former observation made in UBC showing a similar increase of sPD-L1 levels in PD-L1 inhibitor-treated (atezolizumab) patients after three months of therapy (54). Interestingly, in PD-1 inhibitor-treated (pembrolizumab) bladder cancer patients no sPD-L1 increase could be detected, suggesting that the detected sPD-L1 flare is therapy-specific.

To better understand this phenomenon we investigated on-treatment levels in our meta-analysis (Study 1) with different tumor entities treated with ICI. Similar findings were reported in NSCLC, where only PD-L1 inhibitors (e.g., atezolizumab) led to sPD-L1 elevation (50). The biological relevance of this flare remains unknown but may involve immune complex formation that enhances ELISA signal detection. Interestingly, Music et al. also observed an increase in soluble PD-1 following pembrolizumab treatment, further emphasizing therapy-specific effects (76).

Comparison between baseline and on-treatment sPD-L1 levels during ICI treatment was possible in 12 studies. Based on our previous observation in UC, we hypothesized that anti-PD-L1 therapy leads to an elevation in sPD-L1 levels (54). Accordingly, in the two studies with presenting patients who received anti-PD-L1 therapy a strong (27- and 28-fold) increase in sPD-L1 levels could be observed (50, 54), whereas no such difference was detected in anti-PD-1 treated patients (48, 49, 51, 52, 55, 57, 60, 62). Furthermore, sPD-1 levels did not increase after anti-PD-1 (nivolumab) therapy (52, 57). In contrast, Music *et al*. observed that sPD-1 elevated after the administration of anti-PD-1 pembrolizumab therapy (76). Therefore, it appears that anti-PD-L1 rather than anti-PD-1 therapy induces a significant increase in sPD-L1 levels. However, one possible explanation could be that ICIs – especially atezolizumab – can trigger a strong anti-drug-antibody (ADA) production, which may form antibody complexes that can enhance the measured ELISA signal (77). On the basis of these, the on-treatment flare-up of sPD-L1 seems to be therapy-specific for anti-PD-L1 therapy but the biological and clinical relevance of this elevation needs to be further evaluated.

Inflammatory markers such as NLR and CRP also play critical roles in predicting ICI efficacy, particularly inmUC. Ourcomprehensive meta-analysis involving over 6,000 mUC patients found that high NLR was associated with a 119% increased risk of death and a 90% higher risk of progression. Subgroup analyses of ICI types confirmed these associations across both atezolizumab and pembrolizumab. In contrast, the predictive value of NLR for chemotherapy or enfortumab vedotin therapy was weaker, suggesting its relevance in immunotherapy settings (78, 79).

Similarly, CRP—an acute-phase protein—showed consistent prognostic value across treatment types. Elevated pre-treatment CRP levels were associated with a 75% increased risk of death and a 58% higher risk of progression. Dynamic CRP changes during ICI treatment further refined prognostic assessments. For instance, patients classified as "CRP flare responders," showing a temporary spike followed by normalization, demonstrated favorable ORRs of 69–75%. This indicates that CRP may serve not only as a prognostic marker but also as a potential early indicator of treatment efficacy (80-83).

In summary, sPD-L1, along with inflammation-based biomarkers like NLR and CRP, holds significant promise for stratifying patients undergoing ICI therapy. The prognostic value of sPD-L1 appears consistent across multiple studies and tumor types, particularly in NSCLC and UTUC, though its predictive value islikely tumor-dependent. On-treatment increases in sPD-L1 - particularly in response to PD-L1 inhibitors - further supports its potential role in therapy monitoring, although the underlying mechanisms need further clarification.

### 9.2. Strengths

One of the key strengths of my thesis is the comprehensive investigation of sPD-L1 as both a preoperative prognostic biomarker of UTUC by using own institutional patients' samples as well as a potential therapy predictive marker for ICI treatment. Furthermore, we incorporated routinely available inflammation-related biomarkers such as NLR, CRP and PLR adding a broader immunological perspective. We evaluated more than 45 eligible studies with >7,000 patients using both OS and PFS as endpoints and evaluated on-treatment biomarker level changes. The observed therapy-specific sPD-L1 flare during anti-PD-L1 treatment contributes novel insights to the understanding of biomarker behavior under immunotherapy. Furthermore, the parallel findings from a meta-analysis

strengthen the generalizability of the observed associations, particularly in NSCLC and UTUC. Additionally our meta-anylysis is the first review and meta-analysis for PLR in ICI-treated UC.

### 9.3. Limitations

Both the cohort analysis and the two meta-analyses have limitations inherent from their retrospective observational nature. In our UTUC cohort analyisis, the limited number of patients treated with ICIs prevented us from evaluating the therapy predictive value of sPD-L1. This limitation, however, should be judged in light of the low incidence of this disease. A further limitation of our study is that tumor samples were not available for correlation analysis between tissue and serum sPD-L1 levels, however, literature data based on a large number of cases in various cancers uniformly showed no correlation between serum and tissue PD-L1 levels. Regarding the meta-anylses, we faced some limitations mainly related to the heterogeneity of the included studies regarding their cohort sizes, tumor types, applied ICI drugs, and cut-off values. The limited number of studies on certain biomarkers, such as PLR restricted subgroup analyses and weakened the conclusions on this marker. While most studies had a low risk of bias, several had severe concerns or high risk, which could impact the overall reliability.

## 10. CONCLUSION

We assessed sPD-L1 levels in UTUC for the first time, demonstrating significantly elevated levels in advanced tumor stages. High pre-treatment concentrations shown to be associated with shorter survival in both radical nephroureterectomy (RNU) and chemotherapy-treated patients. These findings, if validated in larger prospective cohorts, may enhance patient stratification and inform therapeutic decision-making in UTUC. In addition, we found that high baseline sPD-L1 levels were associated with significantly worse OS in ICI-treated cancer patients; however, this prognostic association appears to be tumor type-dependent. Thus, sPD-L1 may serve as a valuable pre-treatment prognostic biomarker, but its interpretation should be tailored to the specific tumor context. We also observed a markedly strong increase in sPD-L1 levels during anti-PD-L1 therapy, a phenomenon that appears therapy-specific; its biological basis and clinical implications require further investigation. Notably, elevated pre-treatment inflammatory biomarkers such as NLR, CRP, and PLR hold promise as reliable prognostic indicators in ICI therapy. Therefore, these markers are strong candidates for inclusion in future risk stratification models for mUC.

## 11. IMPLEMENTATION FOR PRACTICE

The above detailed findings may significantly improve clinical practice by helping both urologist and clinical oncologist to identify patients who are most likely to benefit from immunotherapy. If validated, these biomarkers can assist in selecting the appropriate therapy, particularly in the light of the increasing complexity of treatment regimens in UC. Incorporating soluble predictive biomarkers into everyday clinical pre-treatment diagnostics, clinicians my better select treatment modalities. This can help improving survival outcomes, diminishing unnecessary treatment toxicity to ineffective therapies. Furthermore, blood-based biomarkers can aid clinicians in close treatment monitoring, which can facilitate real-time assessment of therapy response.

## 12. IMPLEMENTATION FOR RESEARCH

Precision medicine is unimaginable without understanding the underlying biochemical pathways, which leads to the inefficacy of a certain drug. Especially in immunotherapy, where we can see great survival benefits for a rather small group of patients, but shows complete ineffectiveness for most patients. Future research should aim to validate the identified biomarkers in larger prospectively designed biomarker-based randomized trials. Additionally, future research investigating the underlying biological pathways connected to these markers could help our understanding of failure of immunotherapy. Integrating biomarker data with clinical and genomic profile may also develop patient stratification strategies. This line of research has the potential to shape precision medicine practices and enhance the development of adaptive treatment regimes. Ultimately, broaden the biomarker scope may provide more tailored and effective cancer care.

## 13. IMPLEMENTATION FOR POLICYMAKERS

Our findings may inform policymakers by supporting the incorporation of soluble biomarker testing into everyday standardized diagnostics for UC. Healthcare policymakers should consider allocating reimbursement for the promotion of development and implementation of predictive biomarker assays in order to enhance the cost-effectiveness of ICI treatment. By enabling more precise patient selection health systems can limit nonessential treatment expanses and reduce patient exposure to ineffective therapies. This approach aligns with the goals of precision medicine, which could lead to a personalized medicine and value-based care. Moreover, implementing biomarkers into national cancer guidelines could help standardize care and enhance overall clinical outcomes. Funding this field has the potential to standardize resource utilization while helping access to an equitable and innovative patient centered cancer-care.

## 14. FUTURE PERSPECTIVES

In the future, I am planning to specialize in the field of urooncology, with a particular focus on UC. My goal is to integrate everyday clinical practice with translational research by combining real-life case studies and surgical experience with the latest scientific research. Considering my background in biomarker research within the context of ICI-treated UC, I aim to investigate molecular mechanisms and contribute to enhanced patient management.

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## 16. BIBLIOGRAPHY

### 16.1. Publications related to the thesis

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### 16.2. Publications not related to the thesis

Fazekas T, Széles Á, Teutsch B, Kói T, Vékony B, Hadaschik B, Csizmarik A, Ács N, Hegyi P, Nyirády P, Szarvas T, Poly (ADP-ribose) Polymerase Inhibitors Have Comparable Efficacy with Platinum Chemotherapy in Patients with BRCA-positive Metastatic Castration-resistant Prostate Cancer. A Systematic Review and Meta-analysis
**European Urology Oncology** 7(3):365-375.
**D1, IF: 9.3**

Fazekas T, Széles Á, Teutsch B, Kói T, Vékony B, Váradi A, Lang Zs, Kopa Zs, Grünwald V, Hadaschik B, Csizmarik A, Ács N, Hegyi P, Nyirády P, Szarvas T, Therapeutic sensitivity to standard treatments in BRCA positive metastaic castration-resistant prostate cancer patients - A Systematic Review and Meta-analysis
**Prostate Cancer Prostatic Disease** 26(4):665-672.
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Kovács P T, Mayer T, Riesz P, **Széles Á** , Váradi M, Oláh Cs, Krafft U, Hadaschik B, Tschirdewahn S, Csizmarik A, Nyirády P, Szarvas T,
Elevated Pre-Treatment Serum MMP7 Levels Are Associated with the Presence of Metastasis and Poor Survival in Upper Tract Urothelial Carcinoma
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