
# NOVEL ASPECTS OF CLINICAL AND PATHOLOGICAL PROGNOSTIC AND PREDICTIVE MARKERS OF URINARY BLADDER AND KIDNEY CANCERS

Ph.D. Thesis

**János Révész, M.D., Pharm.D.**

Supervisor:

Anikó Maráz, M.D., Ph.D., habil

University of Szeged, Albert Szent-Györgyi Medical School

Doctoral School of Clinical Medicine

Department of Oncotherapy

Szeged, Hungary

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**List of full papers that served as the basis of the Ph.D. thesis**

I.

**Révész J** and Pósfai B, Pajor L, Papdán T, Varga L, Paczona VR, Varga Z, Sükösd F and Maráz A. Correlation between fibroblast growth factor receptor mutation, programmed death ligand-1 expression and survival in urinary bladder cancer based on real-world data

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II.

Maráz A, Cserháti A, Uhercsák G, Szilágyi É, Varga Z, **Révész J,** Kószó R, Varga L, Kahán Z. Dose escalation can maximize therapeutic potential of sunitinib in patients with metastatic renal cell carcinoma. BMC Cancer. 2018 Mar 15;18(1):296. doi: 10.1186/s12885-018-4209-9. PMID: 29544452; PMCID: PMC5856318.

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III.

Maráz A, Csejtei A, Kocsis J, Szűcs M, Kahán Z, Bodoky G, Dank M, Mangel L, **Révész J**, Varga Z, Géczi L. Assessment of the Role of Everolimus Therapy in Patients with Renal Cell Carcinoma Based on Daily Routine and Recent Research Results. Pathol Oncol Res. 2019 Jan;25(1):149-156. doi: 10.1007/s12253-017-0317-0. Epub 2017 Oct 13. PMID: 29027615.

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**Related Articles**

I.Maráz A, Bodoky G, Dank M, Géczi L, Kahán Z, Mangel L, **Révész J**, Szűcs M. Áttétes vesedaganatos betegek everolimusterápiájával szerzett hazai tapasztalatok [Experience with everolimus therapy for patients with metastatic renal cancer in Hungary]. Magy Onkol. 2014 Mar;58(1):4-9. Hungarian. Epub 2014 Mar 4. PMID: 24712001. IF:-

II. Pósfai B, Kuthi L, Varga L, Laczó I, **Révész J**, Kránicz R, Maráz A. The Colorful Palette of Neuroendocrine Neoplasms in the Genitourinary Tract. Anticancer Res. 2018;38(6):3243-3254. doi: 10.21873/anticanres.12589. Review. IF: 1.935

III. Varga L, Bajory Z, Pajor L, **Révész J**, Sükösd F, Maráz A. Edifications and modern strategies of localized prostate cancers' definitive therapy. Orv Hetil. 2018 Aug;159(32):1317-1325. doi: 10.1556/650.2018.31105. Hungarian. IF: 0.564

IV. Maraz A, Takacs P, Lawson J, Santiago-Walker A, Pajor L, Sukosd F, and **Revesz J** Correlation between FGFR mutation and PD-L1 expression of urinary bladder cancers: A real-world based biomarker study. Journal of Clinical Oncology 2019 37:15_suppl, e16030-e16030

V. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. N Engl J Med. 2019;380:1116-1127. doi: 10.1056/NEJMoa1816714.

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# 1.Introduction

Treatment for advanced urothelial bladder- and renal cell cancers have significantly developed in recent years, in addition to modern targeted therapies immunotherapies have opened a new area. Optimal indications of the therapies are primarily based on the results of prospective clinical trials and the approved indications, but it is also useful to analyze the real-life results to predict the possible ineffectiveness of individual treatments or to reach the maximal efficiency of the therapeutic lines.

In recent years, checkpoint inhibitor immunotherapy has revolutionised the treatment of advanced urothelial bladder cancer**.** Based on several previous analyses, it can be assumed that patients with tumor cells showing programmed cell death-ligand 1 (PD-L1) positivity have a better respond to anti-PD-1/PD-L1 monotherapy**.** Despite of these facts there are numerous nonresponding patients at different stages, and their resistance mechanisms are still unclear. The effect of PD-L1 expression in tumor cells as well as in immune cells infiltrating the tumor can be significant, and the combined positive score (CPS) can be determined based on the number of these cells together. The predictive effect of high PD-L1 expression on pembrolizumab immunotherapy has been confirmed in the first-line treatment of metastatic patients unfit for cisplatin and the high CPS of ≥10% was associated with a prolonged median overall survival (OS)**.** The activating point mutation of fibroblast growth factor receptor 3 (FGFR3) is the most frequently occurring one, mainly present in low grade, early stage non muscle invasive bladder cancer (NMIBC)**.** The FGFR pathway is an appealing targeted treatment option, and in the case of its alteration, phase 2 results of the multiple receptor inhibitor erdafitinib therapy are already available. Retrospective analyses of patients with mutation of FGFR3 urothelial cancer enrolled in phase I/II trials of FGFR3 inhibitors have indicated infrequent responses to prior immunotherapies. Lower response rates and shortened OS following anti–PD-L1 therapy was also observed in patients with FGFR alterations.

Sunitinib malate, an oral multi-targeted tyrosine kinase inhibitor (TKI) - its main effect is the inhibition of the vascular endothelial growth factor receptor (VEGFR) - was considered to be one of the standard first-line therapeutic options in metastatic renal cell cancer (mRCC). Sunitinib has been approved by the regulatory authorities after it had been demonstrated to improve progression-free survival (PFS), overall survival, objective response rate (ORR), and quality of life compared with interferon-alpha in previously untreated mRCC patients. According to the international guidelines, sunitinib was used as first-line treatment in patients with advanced or metastatic dominantly clear cell histological type RCC whose condition has good or intermediate prognosis. The standard treatment schedule of sunitinib is

50 mg for 28 days with a 14-day break. The dose can be adjusted according to the patient's response to the treatment, but it should be kept within the range of 25 to 75 mg. At higher sunitinib doses, the direct anti-cancer effect of the drug may be predominant.

Everolimus is a preclinically and clinically tested, oral mTOR (mammalian target of rapamycin) inhibitor. According to the European Society for Medical Oncology (ESMO) guideline, everolimus was recommended for patients with metastatic, clear cell renal carcinoma in second- and third-line after the ineffectiveness of previously administered anti-VEGFR therapy. Efficacy and safety of everolimus monotherapy had been analyzed in a phase III (RECORD-1) placebo-controlled study on patients with metastatic renal cell carcinoma, who previously received sunitinib and/or sorafenib therapy. PFS was significantly longer among patients receiving everolimus therapy (4.9 months vs. 1.9 months (in the placebo arm). According to subgroup analysis one line anti-VEGFR TKI therapy was significantly associated with a longer PFS in the everolimus arm, than in the control arm (5.4 versus 1.9 months).

## 2.Aims

The primary aim of the dissertation was to analyze novel aspects of the clinical and pathological prognostic and predictive markers of urinary bladder and kidney cancers to potentially improve the effectiveness of treatments and maximize the therapeutic effect.

2.1.To demonstrate the frequency of FGFR mutation in different tumor stages of cystectomy samples, and to reveal a possible relationship between the FGFR status, PD-L1 status, CPS score, tumour-stages and the survival of patients.

2.2.To analyze the maximum efficacy and side effects of increased dose first line sunitinib in metastatic RCC in daily practice, and to evaluate the correlation of prognostic factors

2.3.To investigate retrospectively the efficacy and tolerability of everolimus therapy in patients with metastatic renal carcinoma who previously received and progressed on one line of VEGFR-TKI therapy based on the experiences of nine Hungarian institutes, and to search for prognostic clinical factors during treatment to predict outcome.

# 3.Patients and methods

## 3.1.Correlation between fibroblast growth factor receptor mutation, programmed death ligand-1 expression and survival in urinary bladder cancer based on real-world data

*3.1.1. Patients* Prospective next generation sequencing (NGS) of tumor tissues, and retrospective collections and analyses of clinical data were performed by the collaboration between University of Szeged, and the Szeged Biology Research Institute, with the use of Hungarian National Health Insurance Fund Database. Enrolled patients were previously diagnosed with urothelial bladder cancer and underwent radical operation during a 10-year period (before the immunotherapy era, between 2006-2016) at the University of Szeged, Hungary. The pT0 cases based on cystectomy specimens were called $pT0_{cyst}$. In these cases the biomarker analysis was performed from the initial sampling tissues. The patients' basic pathological (histology, pT, pN, demography, age, gender), clinical, oncological treatment and outcome data were collected from the pathological and medical documents of University of Szeged, and the overall survival data from the National Health Insurance Fund database, respectively. All data of patients from different databases were linked at the patient level then de-identified. Overall survival (OS) was defined from the date of cystectomy to the date of death.

*3.1.2. Methods Tissue sample testing* Two tests were performed on easch tissue sample. The service provider together with University of Szeged performed FGFR next generation sequencing for mutations and PD-L1 stain with DAKO 28-8 tests. This sample collection was supplemented with a retrospectively analyzed anonymized patient's follow up database from the medical reports and funder data. FGFR3 mutation status as wild type -WT and non wild type – NWT was recorded. The expression level of the samples was given in percentages, and the samples were considered positive if the expression level was at least 1% and negative otherwise. PD-L1 positivity was defined if the PD-L1 expressed tumor cell count was at least 1% (tumor positive score - TPS), CPS score has also been defined as the ratio of the number of all PD-L1–expressing cells (tumor cells, lymphocytes, macrophages) to the number of all tumor cells (high level $\geq 10$).

*Formation of analyzed groups* In our study 392 surgical samples were collected, but the data of 310 patients were considered for analysis. Three subgroups were formed based on possible testing for FGFR, PD-L1 and CPS score: in the the first subgroup of patients, FGFR mutation testing of histological samples were performed; in the second subgroup, PD-L1 analysis was available; while in the third subgroup, both tests (PD-L1 and FGFR ) were also performed. The data on the interaction of biomarkers and their role in survival were evaluated in the last subgroup.

## 3.2. Dose Escalation can Maximize Therapeutic Potential of Sunitinib in Patients with Metastatic Renal Cell Carcinoma

### *3.2.1. Patients*

An explorative retrospective analysis of a prospective mRCC register was carried out at the Department of Oncotherapy University of Szeged, Hungary. 103 patients with MSKCC (Memorial Sloan-Kettering Cancer Center) good (0 unfavorable factor) or intermediate risk were treated with sunitinib between January 2010 and December 2016. The patients received first-line sunitinib after histological and staging examinations, such as abdominal and chest CT (and bone scintigraphy and skull CT if clinically indicated).

### *3.2.2. Methods*

*Sunitinib therapy and dose modifications.* Patients received sunitinib monotherapy orally, in six-week cycles, at a dose of 50 mg once a day for 4 weeks, followed by a two-week rest period (4/2 scheme) in 94 (91.3%) cases. In 9 (8.7%) cases with advanced age and concomitant diseases, the therapy was started with a reduced dose of 37.5 mg. Adequate supportive therapy and proactive management of side-effects were applied. Dose reduction (DR), modification of dose scheme (DSM) (2 weeks on/1 week off) were allowed. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (NCI CTCAE v4.0). The evaluation of tumor response was performed every 12 weeks according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. If the CT indicated slight progression (SP) but still corresponded to stable disease according to the RECIST 1.1 criteria in patients enrolled in the study after June 30, 2013 (study group), a dose escalation (DE) strategy was started with careful follow-up if any clinically significant side effect was detected. The dose was elevated first to 62.5 mg, and if a slight progression was still present or occurred again, to a level of 75 mg. Patients showing SP before the date of June 30, 2013 were enrolled in the control group.

*Evaluation of the effect of dose escalation.* The effects of dose escalation was analyzed on PFS and OS of both the entire patient population and the patients showing SP. Two groups of patients with SP were distinguished considering that the SP occurred before or after June 30, 2013; patients before that date were treated with an unchanged standard dose, despite the presence of SP. After that date, in cases without relevant side effects, a DE strategy was applied.

### 3.3. Experiences with everolimus therapy for patients with metastatic renal cell cancer in Hungary

*3.3.1. Patients* Everolimus therapy was administered in 145 cases for patients with metastatic clear cell renal carcinoma, after progression on VEGFR-TKI therapy between January 2010 and July 2013, in nine Hungarian oncological institutes. Histological and staging examinations were performed prior to initiation of the therapy. 61% of the patients had comorbidity that needed to be treated.

*3.3.2. Methods Everolimus therapy.* Everolimus monotherapy was administered orally in a daily dose of 10 mg. The medication was taken continuously in 28-day cycles. The imaging examinations were performed first 8 weeks after the initiation of everolimus therapy, thereafter once every twelve weeks, Severity of adverse events was evaluated based on the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0 (86% in case of 125 patients). Data were collected retrospectively.

*Statistical analyses* Survival analysis was performed to analyze overall survival, Kaplan-Meier estimators were used to characterize the survival function. The effect of TNM, FGFR mutation, and PD-L1 expression on OS was evaluated independently using univariate stratification of the Kaplan-Meier estimation and Cox proportional hazard models SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Survival analyses (Kaplan-Meier plots) were carried out using the statistical software R 4.2.2. (R Core Team 2021). The association between PFS, OS and age, and the number of metastatic organs was analyzed using COX regression. The influence of the therapy-related factors and patient-related factors on PFS and OS was analyzed with Kaplan–Meier analysis. To detect the independent role of covariants on the outcome, multivariate COX regression was used. All statistical analyses were performed by using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

## 4.RESULTS

### 4.1.Correlation between fibroblast growth factor receptor mutation, programmed death ligand-1 expression and survival in urinary bladder cancer based on real-world data

The data of 310 patients were considered for analysis, of 236 (76.1%) were male and 74 (23.9%) female. The median age of the entire patient population was 62.8 years, women were slightly younger (median age 61.5 years) than men (median age 63.1 years) 253 samples could be tested for FGFR mutation, 248 samples for PD-L1 and CPS score, and 215 samples for both PD-L1 and FGFR succesfully.

There was a strong correlation between TNM stage and FGFR mutation ($p<0.001$), i.e. higher stage had a lower NWT ratio. The positive PD-L1 rate was significantly ($p=0.005$) lower in the NWT group (19.4% vs. 44.1%) than in the WT, similar to the CPS ≥ 10 rate. Significant relationship was also found between stage and PD-L1 expression based on CPS ($p=0.002$), in more advanced stages the frequency of PD-L1 positivity was higher.

We focused primarily on the correlation between FGFR and PD-L1 (TPS and CPS) status, where we found that the more likely the samples were FGFR mutated, the less likely they were PD-L1 positive. Our results show that TNM stage has a strong significant effect on FGFR mutation and PD-L1 expression.

Survival of locally advanced patients with TNM stage III-IV at the time of cystectomy was significantly the most unfavorable factor (median: 17.97 months, $p<0.001$).

We found that the survival was longer in FGFR positive, mutant (NWT - median OS 56.7 months, 95%CI 38.9-NA), than in FGFR wild type (WT - median OS 23.2 months, 95%CI 15.6-30.9) patients ($p=0.024$). There was no difference detected in median overall survival between patients with PD-L1 positive or negative (30.07 vs 29.03, $p=0.81$) based on TPS, and high or low level of CPS (31.63 vs 29.03, $p=0.28$). Based on our data, FGFR NWT vs. WT was a factor affecting patient survival, while PD-L1 negativity vs positivity or CPS low vs high level was not found significant. Our data showed that the stage proved to be a significant independent factor for survival, the close connection with FGFR had no independent effect. As in case of TNM, the independency of these variables were rejected with high probability here too.

### 4.2. Dose Escalation can Maximize Therapeutic Potential of Sunitinib in Patients with Metastatic Renal Cell Carcinoma

Out of the 103 patients who participated in the study, 80 (77.7%) were men and 23 (22.3%) were women. The mean age was 62.27 (range, 32–80) years, and 84.5% of the patients had undergone nephrectomy.

No dose reduction (DR) had to be applied in 59 (59.6%) patients (50 mg/day in 4/2 or 2x2/1 scheme or 37.5 mg daily dose administered continuously in 2 cases). The dosing scheme was modified (DSM) in case of 22 (22.2%) patients. The median PFS±SE was 14.2±3.22 (95% CI 7.87–20.52) months. Complete remission as the most favorable tumor response was achieved in 7 (7.1%) cases. Partial remission and stable disease were accomplished in 31 (31.3%) and 56 (56.6%) patients, respectively. In cases of SP, according to RECIST 1.1 was stable disease in 48 (48.5%) cases. First-level (62.5 mg/day in 4/2 or 2x2/1 scheme) and second-level (75 mg daily dose in 4/2 or 2x2/1 scheme) dose escalations were indicated in 18 (18.2%) and 4 (4.1%) patients, respectively. The median±SE duration of sunitinib therapy was 19.45±2.01 (95%CI 14.87–22.94) months until definition of slight progression and 7.8±1.55 (95%CI 4.74–10.85) months from date of SP to progression. The median OS was 25.36±2.62 (95% CI 20.23-30.5), and the median follow-up time was 24.37 (1.33-93.83) months, respectively.

DE was performed in 18 (18.2%) cases among the evaluated 99 patients. PFS and OS results were more favorable when the dose was escalated rather than in case of patients without escalation. The dosing scheme was modified in 22 (22.2%) patients. If DSM was performed, the median PFS and OS were longer than without DSM. Dose escalation and DSM were independent parameters. The survival was longer as patients received more therapeutic lines after sunitinib treatment.

There were 23 patients in the control group (showed slight progression) and 25 patients in the study group. Median PFS (39.7±5.1 *vs* 14.2±1.3 months ($p$=0.037)) and mOS (57.5±10.7 *vs* 27.9±2.5 months ($p$=0.044)) results were significantly better in the study group than in the control group.

Based on a multivariate COX analysis, both DE ($HR_{DE}$: 2.12, 95% CI 1.077–4.181; $p_{DE}$=0.030) and nephrectomy ($HR_{nephr.}$: 2.47, 95% CI 1.023–6.315; $p_{nephr.}$=0.049) were independent factors of PFS in patients with SP. In relation to OS, only nephrectomy influenced the results independently ($HR_{nephr.}$: 5.02, 95% CI 1.94–12.98; $p_{nephr.}$=0.001) but DE did not..

After dose escalation, the most upgraded clinical parameters were fatigue and development or worsening of hypertension as a result of the increased sunitinib dose.

### 4.3. Experiences with everolimus therapy for patients with metastatic renal cell cancer in Hungary

The mean age of the patients was 62 (28-79) years. One hundred and eight (74.5%) male and 37 (25.5%) female patients took part in the study. The general condition of the patients was good, ECOG 0 and 1 score were registered in 45 (31%) and 88 (60.7%) cases, respectively. Before everolimus therapy 128 (88.3%), 16 (11%) and 1 (0.7%) patients received sunitinib, sorafenib and pazopanib, respectively. One hundred and twenty-three (84.8%) patients received first-line TKI therapy. Mean (±SE) duration of TKI therapy was 11.7 (±0.9) months. Duration of TKI was shorter than 3 months in 24 (16.6%) cases, they were defined as primary TKI-resistant patients**.** Mean (±SE) duration between the end of TKI therapy and the beginning of everolimus was 97. 7 (±10.1) days (period between TKI-EVE). Dose reduction was necessary in 9 (6.2%) cases due to the following reasons: pneumonitis (6 cases; 4.1%), grade 2 skin problems (2 cases; 1.4%), face and neck edema (1 case; 0.7%).

Complete regression as the most favorable tumor response did not occur. Partial regression, stable disease and progression occurred in 18 (12.9%), 85 (60.7%) and 37 (26.4%) cases, respectively. Objective tumor response was 18 (12.9%), while clinical benefit was 103 (73.6%). The median PFS at a median follow-up time of 18.0 months (*95%CI 7.05-28.95*) was 5.4 months (*95%CI 3.83-6.97*). The median overall survival time (OS) was 16.2 months (*95%CI 12.95-19.45*).

The median value of PFS and OS in the cases treated with TKI therapy for ≤3 months, *vs.* >3 months were 3.0 *vs.* 5.2 months and 16.0 *vs.* 19.9 months, respectively, however, no statistical significance could be detected ($p$=0.250 and $p$=0.244, respectively). PFS and OS were more favorable after more than 9 month long TKI therapy (PFS $p$=0.019, OS $p$=0.045) and in case of ECOG 0-1 performance status (PFS $p$=0.033, OS $p$=0.008).

The presence of anemia predicted a poorer survival (p=0.020), while a PFS >12 months was a favorable prognostic factor (p=0.762). Only 37 patients (25.5%) received third-line therapy: 26 (17.9%) progesterone derivatives, 6 (4.1%) TKI in clinical studies and 5 (3.5%) interferon therapies were given. OS was not different as compared these patient's data to those who did not received oncological therapy after everolimus (post EVE therapy) (p=0.001). Examining the effect of ECOG status and anemia on survival, the most favorable median OS could be seen in non-anemic patients with ECOG 0-1 (30.9±2.5 months), while it was the most unfavorable in anemic patients with ECOG 2-3 status (7.7±4.5 months) (p=0.029). None of the patient- or therapy-related parameters influenced PFS or OS.

## 5.Discussion

**5.1.** PD-L1 and FGFR are the most frequently investigated biomarkers in connection with the treatment of advanced bladder tumors today, due to the clinical need related to therapeutic options. Immunotherapies can mostly be used in advanced urothelial cancer after platinum-based chemotherapy, but they are also approved as a first choice in case of high PD-L1 status. In our work, PD-L1 status was determined based on TPS and CPS as well. Among the targeted therapies, FGFR inhibitors can be administered after platinum-based chemotherapy or immune checkpoint inhibitors. Approximately 70% of low-grade non-invasive papillary tumors show FGFR3 mutation in literature. In our study, patients with superficial bladder tumors (20%) who underwent cystectomy were included if the disease could not be controlled by transurethral resection. Even in this higher-risk group, the proportion of FGFR mutant patients was 37.2%, lower than in published data, but higher than in our analyzed muscle-invasive group. The strongest correlation could be observed between TNM stage and FGFR mutation. Our results represent the high frequency of FGFR3 mutation in earlier stages. Our data are similar to other results that have reported an association between favorable prognosis and FGFR mutation**.** We detected across TNM stages that tumors with high ratio of FGFR3 mutation are less likely associated with positive PD-L1 expression. Regarding the covariates examined with the cox regression model, we found that their occurrence is not independent of each other. Based on the results obtained, a very strong correlation could be identified between the individual parameters.

Some previous studies verified mutated FGFR3 with increased FGFR3 gene expression and an association with decreased T-cell infiltration. Based on one of the latest retrospective analyses with a relatively high number of patients available in the literature, a lower response rates and shorter OS was observed in patients with FGFR alteratations following anti-PD-L1 immunotherapy. Our aim was to investigate whether FGFR mutation is a possible independent prognostic factor of survival. Reflect on many controversial response data in the anti-PD-L1 treated FGFR mutated patient group, according to other studies we consider a larger investigation to verify its predictive and prognostic value.

The strength of our work is that it processes the real-life results of a relatively large number of bladder tumor patients who have undergone cystectomy. Another advantage is that we also evaluated the CPS data in relation to PD-L1 expression, used better in the daily practice nowadays. It should also be emphasized that it was possible to connect the data available in the clinical and pathological medical systems precisely and individually with the survival results available in the funder's database, thus facilitating the accuracy of our work.

**5.2.** Sunitinib was one of the most frequently applied first line therapies in patients with metastatic RCC. In our study, PFS was longer than in the registration study, but the PFS of our patients was similar to the excellent real-world data. The OS of patients was no longer than 2 years before the immunotherapeutic era, as it can be seen in our results as well. One of the most important things in case of a successfully optimized medical therapy is appropriate dosing: the individually titrated, tolerable dose, with the administration of the maximum daily dose. It is important to choose the most suitable dosing scheme after taking comorbidities into consideration. Several authors have reported that both PFS and OS are significantly higher in patients with at least grade 2 hypertension. As on-target side effects determine the drug effect, toxicity profile can be used to optimize dosing and treatment schedules individually. According to the meta-analysis of Houk et al., escalated sunitinib exposure is associated with improved clinical outcomes as well as with an increased risk of adverse effects. The appropriate management of adverse events is necessary for effective sunitinib treatment, which requires the active contribution of the satisfactorily informed patient. Based on the above mentioned data, dose escalation has been applied in cases with slight progression in our work, when RECIST 1.1 results confirmed a stable disease. Our idea was to achieve the optimal titration of sunitinib until the appearance of on target side effects depending on the tolerable off target adverse events. The rate of CR according to RECIST in our studied population was relatively high (7.1%) compared to pivotal phase III trial. After an initial favor tumor response evolving slight progression can be stopped or be reversible with dose escalation and adequate titration has been hypothesized. Blood levels of sunitinib reach a steady state at 10 to 14 days, and a maximum value on day 14, and disease progression usually occurs during treatment interruption. In the retrospective analysis of Bjarnason et al., shorter treatment break (14 days on, 7 off) have resulted in improved PFS and OS as compared to the standard schedule, and the PFS detected in patients with RCC has been one of the best reported for any TKI. Modified sunitinib schedule is well tolerated and induces optimal drug exposure.

Based on our results, PFS and OS results can be improved by sunitinib dose escalation as by dose scheme modification in case of patients poorly tolerating the therapy. Dose escalation can be performed in case of patients with good general condition, who do not have any relevant adverse effects. The effect of DE on PFS and OS was confirmed during the comparison of the two groups.

The rate of adverse events (AE) in our real world dose escalated patients was lower in the selected cohort than the AE rate in patients administered the standard dose in the pivotal trials. It might be partly explained by the favorable VEGFR inhibitor tolerability and the better proactive management of toxicity, which may improve the tolerability of the drug.

**5.3.** In our analysis everolimus monotherapy was associated with favorable PFS and OS in case of patients with mRCC after VEGFR-TKI therapy. PFS of 5.3 months in the population treated in 9 different Hungarian institutes is slightly longer than the result of RECORD-1 registration study and similar to the subgroup of patients treated after one line of TKI therapy. Median OS of patient in our study was 16.2 months. In the phase III study the OS in the everolimus arm, and in the placebo arm was 14.8 and 14.4 months, respectively. In our study the mean duration between the stop of VEGFR-TKI treatment and the beginning of EVE therapy was unfortunately 97.7 days. The reasons for delaying the start of the administration of everolimus were manifold: side-effects of previous therapy; patient flow between the institutes; organization of radiological examinations; and also the availability of drugs were the most important factors.

The patients' unfavorable general condition (ECOG 2-3) was associated with a shorter PFS and OS. The presence of anemia deteriorated the survival. We did not find correlation between patients' other general characteristics and therapeutic outcome. We also analyzed data of patients who showed primary resistance to VEGFR-TKI therapy, because due to the different mode of action, we supposed that slightly favorable results can be reached with EVE, although no statistically significant difference could be demonstrated, Similar results can be found in international studies**.** In the Hungarian population those patients whose VEGFR-TKI therapy was longer than 9 months had significantly more favorable PFS and OS. The prognostic score system published by Motzer for second-line therapy proved unfavorable prognosis in the presence of 3 factors – anemia (beside normal value), poor general condition (under Karnofsky 80) and high value of corrected calcium (>10 mg/dL or >2.4 mmol/L) – instead of 5 factors used in first-line. In our population, if the patients' general condition was good and they did not have anemia, the OS was 30.9 months, but in case of poor condition and anemia this time decreased to 7.7 months. Analyzing the efficacy of EVE therapy and PFS we concluded that ECOG is one of the most important factors. The OS was remarkably better in case of EVE therapy lasting more than 12 months. This underlines the importance of appropriate patient selection.

Everolimus therapy was well tolerated. Dose reduction and cycle delay were necessary only in 6.2% and 8.9% of the cases, respectively. The longest delay was applied due to pneumonitis. Use of corticosteroids and dose reduction, in severe cases oxygen therapy, may be necessary. Laboratory disorders such as anemia, abnormalities of renal and liver function and increase of blood sugar level were similar to the RECORD-1 study, these are connected to the mode of action of EVE, i.e. the interaction with the mTOR complex and the associated signal transduction ways. Therefore it is essential to monitoring these parameters when administering this group of medicine.

## 6.Summary, conclusions

6.1. **Our results highlight** the high FGFR alteration rate in non-muscle invasive tumors, thereby pointing to a potentially new area for future analysis of the effect of FGFR inhibitors. The higher rate of PD-L1 expression in more advanced stages also confirms the immune mechanism of bladder tumors. Although the survival of FGFR mutant patients was more favorable than wild-type, this effect was established through the tumor stage. **In summary**, the role of tumor stage can be highlighted as the strongest survival factor in this group of patients.

6.2. **As conclusion**, an individual escalated sunitinib therapy optimized by toxicity profile in metastatic RCC patients prolongs PFS and OS, and it is a safe treatment option with a moderate increase in adverse effects. Based on our data, dose escalation in 12.5 mg steps may be recommended for properly educated patients with slight progression, when RECIST 1.1 results confirm a stable disease in case any clinically relevant adverse effects occurred.

6.3. **In summary,** based on all of our results, the mTOR inhibition is an effective way to treat metastatic renal carcinoma after VEGFR-TKI therapy. According to the Hungarian experience, everolimus can be safely used and is well tolerated. Therapeutic results from our everyday practice, PFS and OS are similar to that of the appropriate subgroups of the registration study. Poorer outcome can be expected in case of anemic patient with poor general condition, so their therapy may only be started after the adequate consideration and the improvement of their general condition. Using everolimus as a second-line approach, the progression can be delayed and survival can be improved with the maintenance of good quality of life if the patient is in a good general condition having appropriate hematological parameters.

**New findings of the dissertation**

1. Based on real life data FGFR3 mutations rate is higher in case of early bladder cancers, while PD-L1 positivity (above TPS 1 and CPS 10) is more common in advanced stages. There is a strong correlation between FRGF mutation, PD-L1 expression and TMM stage. Patients' survival depends on FGFR status and TNM stage, while PD-L1 expression is independent.

   The only independent parameter that influence the survival is the TNM stage. The effect of FGFR status on survival can be explained by its strong correlation with TNM.

   Real clinical benefit of the above-mentioned results is that FGFR mutant cases, due to the lower PD-L1 expression and the consecutive poor immunological environment, potentially have worse tumor response for immunotherapies. Therefore, in these cases FGFR inhibitor therapies seem to be potentially optimal choices.

2. In case of moderate progression of metastatic renal cancers treated with sunitinib first line, if there is no relevant toxicity, dose escalation can be performed till the development of on target side-effects. Dose can be elevated daily by 25 mg till the maximum dose of 75 mg. Progression-free and overall survival are also increase due to the dose escalation in comparison to standard dose of the control arm. Dose escalation and modification are independent factors that both influence the progression free survival.

   Clinical benefit of dose escalation is that therapeutic potential of sunitinib can be maximized and it increases the patients' survival.

3. In the analyzed population of metastatic renal cancers survival was more favorable in case of everolimus therapy in second- or multiple lines, than in the registration study. PFS and OS were more favorable if the duration of TKI therapy was longer than 9 months and in case of ECOG 0-1 status. In case of everolimus therapy poorer ECOG status and the presence of anemia were associated with worse therapeutic benefit.

   It has to be mentioned that everolimus therapy is used less frequently nowadays in comparison to the modern therapies, but if we choose this medication, it is worth keeping the above result in mind.