KLINIKAI- ÉS PATOLÓGIAI PROGNOSZTIKUS MARKEREK ÚJSZERŰ ASPEKTUSAI HÚGYHÓLYAG ÉS VESEDAGANATOKBAN

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

PhD Thesis

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I.

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II.

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IV. 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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V. Petrylak DP, de Wit R, Chi KN, at el. Ramucirumab plus docetaxel versus placebo plus docetaxel in patients with locally advanced or metastatic urothelial carcinoma after platinum-based therapy (RANGE): a randomised, double-blind, phase 3 trial. Lancet. 2017;390:2266-2277. doi: 10.1016/S0140-6736(17)32365-6.

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VI.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:-

VII. 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

VIII. 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

IX. 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

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List of abbreviation

ATP	Adenosine Triphosphate
AE	adverse event
bFGF	b-Fibroblast Growth Factor
ccRCC	clear cell Renal Cell Carcinoma
CD8+	cluster of differentiation 8
CI	confidence interval
CPS	combined positive score
CT	Computed Tomography
CTCAE	common terminology criteria for adverse events
DE	Dose Escalation
DR	Dose Reduction
DSM	Dose Scheme Modification
EAU	European Association of Urology
ECOG	Eastern Cooperative Oncology Group
ESMO	European Society for Medical Oncology
EVE	Everolimus
FGFR	Fibroblast Growth Factor Receptor
G	Grade
HR	Hazard Ratio
IFN	Interferon-α
IHC	Immune-Histochemical
IL	Illinois
IL-8	Interleukin-8
LDH	Lactate Dehydrogenase
LL	Lower Limit
LVEF	Left Ventricular Ejection Fraction
MIBC	Muscle-Invasive Bladder Cancer
mOS	median Overall Survival
mPFS	median Progression-Free Survival
mRCC	metastatic Renal Cell Carcinoma
MRI	Magnetic Resonance Imaging
MSKCC	Memorial Sloan Kettering Cancer Center
mTOR	mammalian Target of Rapamycin

NA	Not Applicable
NCCN	National Comprehensive Cancer Network
NCI CTCAE	v.National Cancer Institute Common Terminology Criteria for Adverse Events Version
NGS	Next Generation Sequencing
NMIBC	Non-Muscle-Invasive Bladder Cancer
NWT	Non-Wild Type
ORR	Overall Response Rate
OS	Overall Survival
PA	Pazopanib
PDGFRA	Platelet-Derived Growth Factor Receptor Alpha
PDGFRB	Platelet-Derived Growth Factor Receptor Beta
PD-L1	Programmed Cell Death Ligand 1
PFS	Progression-Free Survival
pts	Patients
QOL	Quality of Life
RCC	Renal Cell Carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RTK	Receptor Tyrosine Kinase
SD	Standard Deviation
SE	Standard Error
SO	Sorafenib
SP	Slight Progression
SPSS	Statistical Product and Service Solutions
SU	Sunitinib
TCGA	The Cancer Genom Atlas
TKI	Tyrosine Kinase Inhibitor
TNM	Tumor, Node and Metastasis
TPS	Tumor Positive Score
TUR	Transurethral Resection
UL	Upper Limit
USA	United States of America
VEGFR	Vascular Endothelial Growth Factor Receptor
WT	Wild Type

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.

Bladder cancer is the tenth most common cancer worldwide with approximately 550,000 new cases annually [1]. The depth of tumor invasion is the most important distinguishing factor from a clinical standpoint and is divided into non-muscle-invasive bladder cancer (NMIBC) and the prognostically less favorable muscle-invasive cancer (MIBC) types. The rate of occurrence of MIBC capable of forming distant metastases is 25-42%, while that of the disseminated stage is 4-15% [1–3]. Localized MIBCs become disseminated in almost 50% of the course of the disease despite the radical cystectomy or locoregional trimodal therapy [3]. In the treatment of advanced disease, for decades only combined chemotherapy was available, with relatively low efficacy and significant toxicity – moreover, molecular possibilities did not exist for predicting treatment ineffectiveness. In recent years, checkpoint inhibitor immunotherapy has revolutionised the treatment of advanced urothelial bladder cancer [3]. However, the role of potential biomarkers predicting the effectiveness of immunotherapy remains incompletely understood, and the prognostic value of programmed cell death ligand-1 (PD-L1) in urothelial cancer remains also controversial [4]. Based on several previous analyses, it can be assumed that patients with tumor cells showing PD-L1 positivity have a better respond to anti-PD-1/PD-L1 monotherapy [5]. Despite of these facts there are numerous nonresponding patients at different stages, and their resistance mechanisms are still unclear [6]. 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 [7,8] and the high CPS of ≥10% was associated with a prolonged median overall survival (OS) [8].

In recent years, due to the emergence of FGFR inhibitor therapy, the clinical significance of FGFR mutation has come into view. Fibroblast growth factor receptor 3 (FGFR3) is a member of protein tyrosine kinase family. and the alteration of the receptor induces an oncogenic signaling pathway [9]**.** Amongst these aberrations, the activating point mutation is the most frequently occurring one, mainly present in low grade, early stage NMIBC [10]**.** 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 [11]**.**

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 [12]**.** An analysis of the IMVigor 211 phase 3 study previously revealed a relatively low response rate with atezolizumab immunotherapy in patients with mutated FGFR3 tumors [13]**.** Lower response rates and shortened OS following anti–PD-L1 therapy was also observed in patients with FGFR alterations [14]. Based on the published data, the ratio of PD-L1 expression, CPS score, and FGFR expression in each tumor stage is not clear, nor is the prognostic or predictive effect of their relation to each other.

Sunitinib malate, an oral multi-targeted tyrosine kinase inhibitor (TKI) was considered to be one of the standard first-line therapeutic options in metastatic renal cell cancer (mRCC) [15]. It is a small molecule [16] selective tyrosine kinase receptor inhibitor, which mechanism is important in RCC. Sunitinib has direct anti-tumor effects via binding the unactivated conformation of KIT and via platelet-derived growth factor receptor alpha polypeptide (PDGFRA) inhibition. The dual inhibitor activity against vascular endothelial growth factor receptors 1 and 3 (VEGFR 1 and 3), and platelet-derived growth factor receptor beta polypeptide (PDGFRB) on endothelial membranes enhances anti-angiogenesis [17].

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 metastatic RCC patients [18–20]. According to the international guidelines (e.g., NCCN, ESMO, EAU), 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 [21–23]. Sunitinib had become the gold standard first-line therapy of mRCC in the past decade,

and it has been used worldwide in this patient population in wider indications as well [21–27].

The standard treatment schedule of sunitinib is 50 mg for 28 days with a 14-day break [24–26]. Alternate scheduling (2 weeks on/1 week off) can also be used to manage toxicity, but currently no robust data are available supporting it [27]. 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.

Drug resistance is associated with a transient increase in tumor vasculature and epigenetic changes in histone proteins in the chromatin, which contribute to tumor angiogenesis by inactivating the anti-angiogenic factors [28]. However, the drug-induced resistance can be overcome by sunitinib dose escalation [28]. If patients tolerate the standard regimen, the increased sunitinib exposure is associated with longer PFS, OS, and a higher response rate [29].

Everolimus is a preclinically and clinically tested, oral mTOR (mammalian target of rapamycin) inhibitor [30]. In case of renal carcinomas according to the decision of European Medicines Agency everolimus can be used for patients with advanced renal cell carcinoma, who progressed on or after anti-VEGFR therapy, e.g sunitinib [30]. According to the 2012 ESMO guideline everolimus was recommended with level 2A of evidence for patients with metastatic, clear cell renal carcinoma in second- and third-line after the ineffectiveness of previously administered anti-VEGF therapy [31]. 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. Patients who progressed in the placebo arm could switch to everolimus therapy. PFS was significantly longer among patients receiving everolimus therapy (4.9 months vs. 1.9 months (in the placebo arm)) [32]. OS was 14.8 months and 14.4 months in case of everolimus and placebo therapy, respectively [33]. 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) [34].

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

All the clinical studies had been approved by the Research Ethics Committee (number of ethical approval: 1011/16, 2017/EKU and 20090/2016 EKU, 3482/2014 and 3483/2014). In the two prospective analyses all the enrolled patients gave their written informed consent before being registered as participating in the study.

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. Patients were included after partial or radical cystectomy, without known metastatic disease. The indication for the majority of cystectomies was primarily to diagnose muscle invasive transitional cell bladder cancer. In a smaller proportion of cases, extensive, multiple recurrent, non muscle-invasive tumors were also indications for surgery, based on the guidelines. Neoadjuvant chemotherapy was allowed. The pT0 cases based on cystectomy specimens were called $pT0_{cyst}$. In these cases the biomarker analysis was performed from the initial sampling tissues, but the stage was not redefined based on the less accurate result of the baseline transurethral resection (TUR) samples. Patients were excluded from the current analysis in the following cases: sequenced samples without clinical information or patients with clinical informations without sequencing results; uncertain sequencing outcomes (due to technical reasons); neuroendocrine histology; immunotherapy or anti-FGFR therapy after progression (to avoid a potential influence on survival data).

The main clinical and demographic data included gender, age, stage and previous therapies. The surgical specimen was graded according to WHO classification and staged by the TNM criteria. 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 (NGS) 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.

Only the FGFR3 mutation status (wild type -WT, non wild type – NWT) was recorded, the exact type of mutation (point mutation, deletion, insertion, etc.) was not analyzed. 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). Nowadays, a more relevant CPS score in clinical application 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 ≥ 10) [8].

Formation of analyzed groups

In our study 392 surgical samples were collected, of which 82 patients were excluded on the basis of insufficient information. 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 in which all results were available **(Figure 1).**

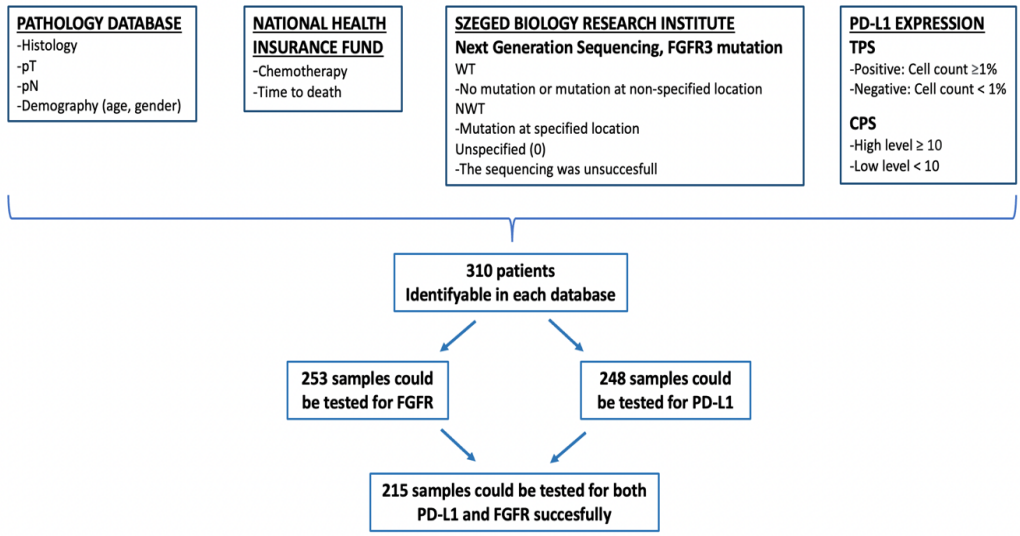

Figure 1. Method of data collection (CPS – combined positive score, FGFR - Fibroblast Growth Factor Receptor, NWT – non wild type, PD-L1 - programmed cell death ligand 1, pN – pathologic lymph node stage, pT – pathologic tumor stage, TPS – tumor positive score, WT – wild type)

Statistical Analysis

Demographic data were characterized using gender, median age, TNM stage and different biomarkers. The independence between the stratifying variables was analyzed using chi-square test for independence. P values < 0.05 were considered significant.

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. Cox proportional hazard models were used to estimate the effect of certain covariates on the overal survival from cystectomy (gender, age at the time of cystectomy, TNM stage, FGFR mutation (WT/NWT), PDL1 expression (positive/negative), chemotherapy).

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).

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 (1 or 2 from the following 5 unfavorable factors: 1. time from diagnosis to systemic treatment ‹1 year; 2. hemoglobin ‹ lower limit of normal level; 3. calcium › 10 mg/dL or 2.5mmol/L; 4. LDH › 1.5 x upper limit of normal; 5. Karnofsky performance status ‹ 80%) were treated with sunitinib between January 2010 and December 2016. The study was performed in accordance with the Hungarian and the EU drug law and relevant medical and financial guidelines of the Hungarian health authorities.

The patients received first-line sunitinib after having undergone nephrectomy or kidney biopsy and embolization if nephrectomy was not feasible. Histological and staging examinations, such as abdominal and chest CT (and bone scintigraphy and skull CT if clinically indicated), were performed before initiating the therapy.

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. Physical and laboratory examinations were performed 2 to 4 weeks after the initiation of sunitinib therapy, and once every six weeks thereafter, while imaging examination, cardiac and thyroid gland function follow-ups were performed every 12 weeks. 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), or therapeutic delay occurred due to the following reasons: grade 3/4 thrombocytopenia, neutropenia, hand–foot syndrome affecting walking, stomatitis or diarrhea of grade 3/4, which significantly influenced the nutrition or resulted in >10% weight loss, hypertension of grade 3/4 developing despite being on combined antihypertensive therapy. 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) [35]. The general condition of the patients was assessed according to the Karnofsky scale [36]. PFS and OS were defined from the onset of the medical treatment to the date of progression based on RECIST 1.1 or death, respectively. The evaluation of tumor response was performed every 12 weeks according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. Sunitinib therapy was discontinued in case of progression per the RECIST criteria in all cases (compared to best response). If the CT indicated slight progression (SP) but still corresponded to stable disease according to the RECIST 1.1 criteria [37] 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. [Figure 2]

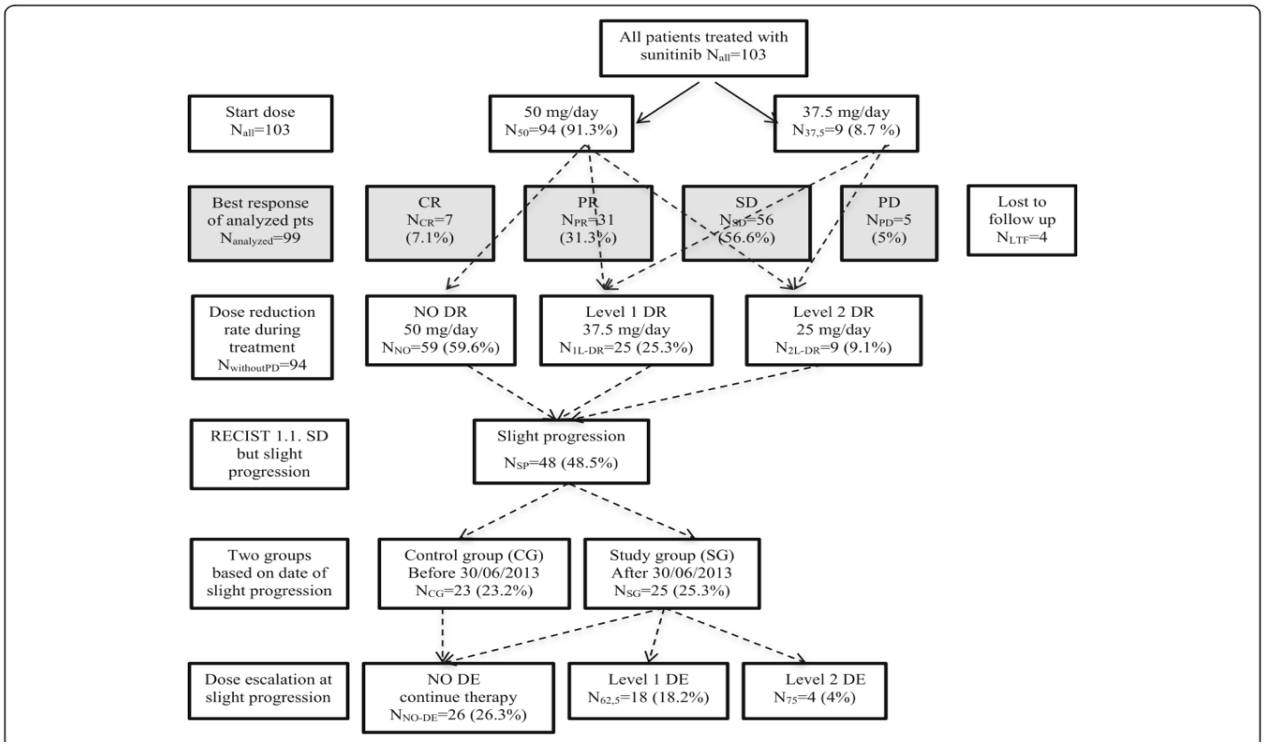

Figure 2 - Flowchart of sunitinib dose modifications (CG – control group, CR – complete remission, DE – dose escalation, DR – dose reduction, LTF – lost to follow-up, N – number of analyzed patients, PD – progressive disease, PR – partial remission, RECIST – Response Evaluation Criteria In Solid Tumors, SD – stable disease, SG – study 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. The outcome was analyzed according to the characteristics of the patients of the two groups as well as the side effects and other factors that could influence the escalation of the dose.

Statistical analysis. 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 (dose escalation, dose reduction, therapeutic lines after sunitinib, nephrectomy, and treatment group), and patient-related factors (gender, MSKCC score) on PFS and OS was analyzed with Kaplan–Meier analysis. To compare the median follow up times between control and study groups, the Mann-Whitney U Test was used. To determine the differences between the control and study groups, independent sample t-test and chi-square test were used for the continuous and categorical variables, respectively. To detect the independent role of nephrectomy and DE 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).

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 sorafenib, sunitinib or pazopanib therapy between January 2010 and July 2013, in nine Hungarian oncological institutes. Histological and staging examinations, such as abdominal-, chest CT, bone scintigraphy and skull CT 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. Minimum 4-weeks washout period followed the previously administered anti-VEGFR therapy. Treatment was started when patients' general condition was good, did not suffer from side-effects of the previous therapies and after stabilization of symptoms caused by new metastases (e.g cerebral metastasectomy, brain- or bone irradiation, anemia control, etc). Dose reduction or delay was performed according to the Summary of Product Characteristics. Physical examination and laboratory test were performed every 4-8 weeks. The imaging examinations were performed first 8 weeks after the initiation of everolimus therapy, thereafter once every twelve weeks, prescribed by the National Health Insurance. Tumor response was evaluated according to RECIST 1.0 every 12 weeks (96% in case of 140 patients) [38]. 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). Patients' general condition was registered according to ECOG scale. After progression on everolimus, treatment in clinical studies, therapy with interferon, progesterone derivatives and best supportive care were available and allowed therapeutic options. Data were collected retrospectively.

Statistical analysis. Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Association between PFS, OS and age was analysed using COX regression. Influence of other therapy-related factors (duration of TKI therapy and the time that elapsed between the stop of TKI therapy and the initiation of everolimus), and patient-related factors (gender, type of previous therapy, ECOG status, anemia) on PFS and OS was analyzed with Kaplan-Meier analysis.

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

Baseline characteristics, TNM stage, FGFR and PD-L1 results

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 (age 61.5 years) than men (median age 63.1 years) **(Table 1).**

	All patients (%) [valid %]	Both FGFR and PD-L1 available (%)
Number	310 (100)	215 (69.4 of all pts)
Gender		
Male	236 (76.1)	171 (79.5)
Female	74 (23.9)	44 (20.5)
Age		
Median age (months)	62.8	62.9
Patients over 65 years (%)	112 (36.1)	80 (37.2)
Stage (%)		
$pT0_{cyst}$ pN0	20 (6.5)	19 (8.8)
St.0-I (pTa, pTis, pT1 pN0)	54 (17.4)	43 (20.0)
St.II (pT2a, pT2b pN0)	60 (19.3)	41 (19.1)
St.III-IV (pT3a, pT3b, pT4 / pN+)	176 (56.8)	112 (52.1)
Any chemotherapy performed (%)	87 (28.1)	59 (27.4)
Neoadjuvant (NA) chemotherapy (%)	18 (5,8)	12 (5,6)
Any chemotherapy except NA (%)	69 (22.3)	47 (21.8)
FGFR		
Missing or unsuccesful	95 (30.7)	NA
Non-wilde type (NWT)	36 (11.6) [16.7]	36 (16.7)
Wilde type (WT)	179 (57.7) [83.3]	179 (83.3)
PD-L1 (TPS)		
Missing or unsuccesful	62 (20.0)	NA
PD-L1 negative (< 1%)	146 (47.1) [58.9]	129 (60.0)
PD-L1 positive (≥ 1%)	102 (32.9) [41.1]	86 (40.0)
PD-L1 (CPS)		
Missing or unsuccesful	62 (20.0)	NA
CPS < 10	169 (54.5) [68.1]	146 (67.9)
CPS ≥ 10	79 (25.5) [31.9]	69 (32.1)

Table 1. The baseline characteristics (CPS – combined positive score, FGFR - fibroblast growth factor receptor, NA – not applicable, NWT - non-wilde type, PD-L1 - programmed cell death ligand 1, pts – patients, St – stage, TPS – tumor positive score, WT - wilde type)

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. The characteristics of the patients were similar in the entire population, in the PD-L1 and FGFR subgroups, as well as in the further analyzed subgroup in which both biomarkers could be evaluated. (Figure 1) (Table 1) Results of FGFR alteration testing were categorized into subgroups based on the non-wild type or wild type, PD-L1 immunostaining data as TPS negative or positive, and CPS < 10 or CPS ≥ 10, respectively (Table 1).

Test of independence of TNM stage, FGFR and PD-L1 status

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 ($p=0.002$) and a trend based on TPS ($p=0.049$), in more advanced stages the frequency of PD-L1 positivity was higher. (Table 2.a, b, c,)

		FGFR alteration		p-value
		NWT	WT	
		n=36	n=179	
TNM stage	**$pT0_{cyst}$ (%)**	5 (26.3)	14 (73.7)	**<0.001**
	St.I (%)	16 (37.2)	27 (62.8)	
	St.II (%)	7 (17.1)	34 (82.9)	
	St.III-IV (%)	8 (7.1)	104 (92.9)	

Table 2.a. Correlations between the FGFR mutation and the TNM stage

		FGFR alteration		p-value
		NWT	WT	
		n=36 (%)	n=179 (%)	
PD-L1 expression (TPS)	**negative** (< 1%)	29 (80.6)	100 (55.9)	**0.005**
	positive (≥ 1%)	7 (19.4)	79 (44.1)	
PD-L1 expression (CPS)	**< 10**	32 (88.9)	114 (63.7)	**0.003**
	≥ 10	4 (11.1)	65 (36.3)	

Table 2.b. Correlations between the FGFR mutation and the PD-L1 expression (TPS, CPS)

		PD-L1 expression					
		TPS < 1%	TPS ≥ 1%	p-value	CPS < 10	CPS ≥ 10	p-value
		n=129	n=86		n=146	n=69	
TNM stage	**$pT0_{cyst}$**	12 (63.2)	7 (36.8)	**0.07**	14 (73.7)	5 (26.3)	**0.002**
	St.I	33 (76.7)	10 (23.3)		39 (90.7)	4 (9.3)	
	St.II	24 (58.5)	17 (41.5)		23 (56.1)	18 (43.9)	
	St.III-IV	60 (53.6)	52 (46.4)		70 (62.5)	42 (37.5)	

Table 2.c. Correlations between the TNM stage and the PD-L1 expression (TPS, CPS)

(CPS – combined positive score, FGFR - fibroblast growth factor receptor, NWT – non wild type, PD-L1 - programmed cell death ligand 1, TNM - Tumor, Node, Metastasis, TPS – tumor positive score, WT – wild type)

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.

Effect of TNM stage on survival

Stratifying the patients based on the TNM stage at the time of cystectomy showed that the survival at more advanced stages was worse than at earlier cases. 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). Although this is not unexpected, this finding verifies the validity of the model **(Figure 3) (Table 3).**

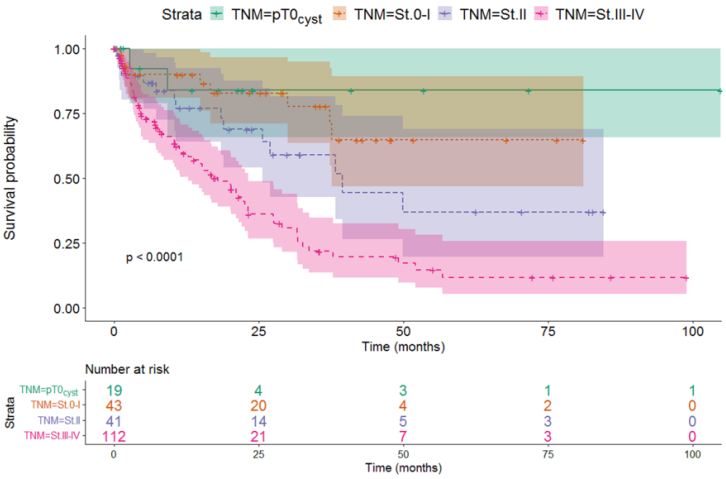

Figure 3. The effect of TNM stages on survival

Based on the pairwise comparison, we found that the survival of the TNM stage III-IV group was significantly worse compared to the other groups, while no difference could be detected between the groups with a better prognosis.

TNM stage	**Median survival (months)**	**95% CI LL**	**95% CI UL**	**p-value**
$pT0_{cyst}$	Not reached	NA	NA	<0.001
St.0-I	Not reached	37.86	NA	
St.II	39.50	20.25	58.75	
St.III-IV	17.97	11.98	23.96	

Table 3. – Connection with TNM stages and median survival (CI – confidence interval, LL – lower limit, St – stage, TNM – Tumor, Node, Metastasis, UL – upper limit)

The effects of analyzed biomarkers on survival

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). **(Figure 4) (Table 4)**

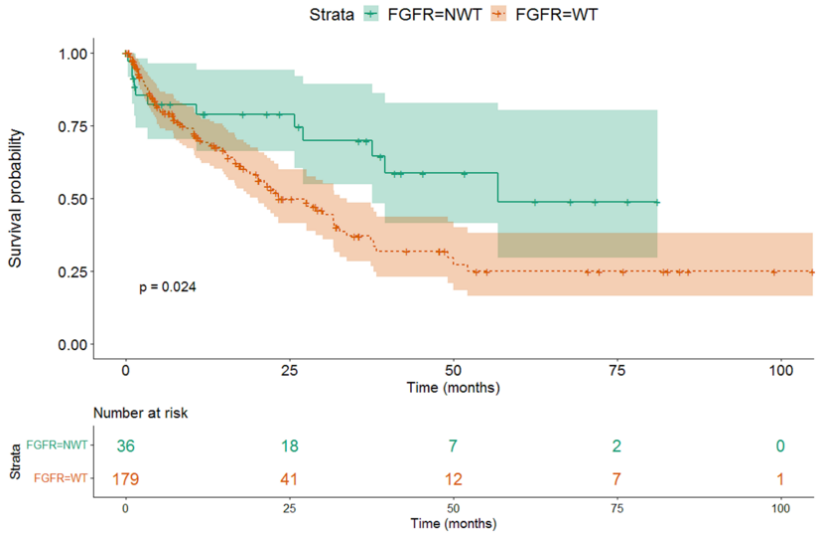

Figure 4. The effect of different FGFR mutations on survival

FGFR	Median survival (months)	95% CI LL	95% CI UL	p-value
NWT	56.73	38.95	NA	0.024
WT	23.23	15.59	30.87	

Table 4. Connection with FGFR mutations and median survival (CI – confidence interval, FGFR – fibroblast growth factor receptor, LL – lower limit, NA – not available, NWT – non wild type, WT – wild type, UL – upper limit)

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). **(Figure 5 a,b).**

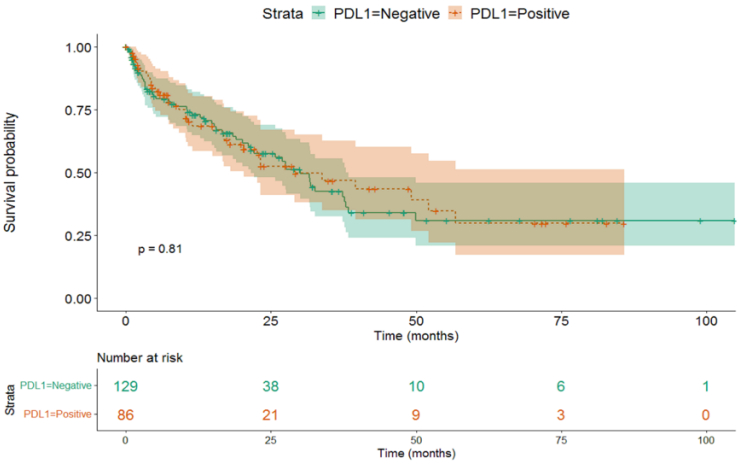

Figure 5a. The effect of different PD-L1 expressions (TPS) on survival

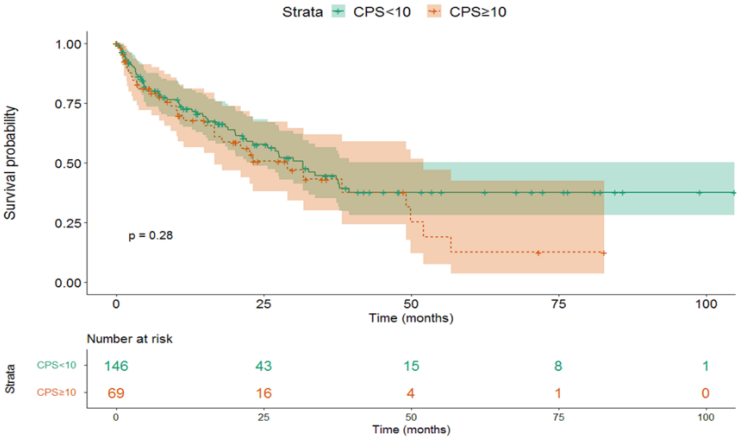

Figure 5b. The effect of different PD-L1 expressions (CPS) on survival

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. The gender (male vs female, HR:1.18, p=0.52), age (older than 65 vs. younger, HR:1.48, p=0.07) of the patients and the chemotherapy use (no versus yes, HR:0.793, p=0.18) did not affect survival.

There was a significant correlation between all variables (TNM, FGFR status, PD-L1 status). All covariates were associated with TNM stage and their impact on survival is through the TNM stage. It is not possible to evaluate the impact of any of the covariates independently from each other, based on multivariate Cox model the only exception is TNM stage.

Hazard ratios for the covariates in univariate and multivariate analysis from Cox model are summed in the Table 5.

Covariate	Univariate				Multivariate			
	HR	**95% CI LL**	**95% CI UL**	**p-value**	**HR**	**95% CI LL**	**95% CI UL**	**p-value**
TNM stage								
$pT0_{cyst}$	reference							
St.0-I	1.612	0.348	7.469	0.541	1.612	0.348	7.469	0.541
St.II	3.156	0.721	13.803	0.127	3.156	0.721	13.803	0.127
St.III-IV	6.812	1.665	27.857	0.008	6.812	1.665	27.857	0.008
FGFR								
NWT	reference							
WT	1.997	1.082	3.685	0.027	NA			0.718
PD-L1								
TPS < 1%	reference							
TPS ≥ 1%	1.052	0.693	1.596	0.813	NA			0.231
CPS < 10	reference							
CPS ≥ 10	1.265	0.825	1.938	0.281	NA			0.776

Table 5. Hazard ratios for the covariates in univariate and multivariate analysis

(CI – confidence interval, CPS – combined positive score, FGFR – fibroblast growth factor receptor, HR – hazard ratio, LL – lower limit, NA – not available, NWT – non wild type, PD-L1 – programmed cell death ligand 1, St- stage, UL – upper limit, WT – wild type)

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

Patient characteristics. Out of the 103 patients who participated in the study, 80 (77.7%) were men and 23 (22.3%) were women [Table 7]. The mean±standard error (±SE) age was 62.27±0.9 (range, 32–80) years, and 84.5% of the patients had undergone nephrectomy. The mean (±SE) MSKCC score was 1.7±0.05, and the mean number of metastatic sites was 2.32±0.11 (range, 1–5). Lungs, bone and distant lymph nodes were the most frequent localizations of metastases [Table 7]. 68% of the patients had a comorbidity that required treatment. Hypertension, other cardiovascular disorders, and diabetes were the most common diseases. Hyperthyroidism and well-managed hypertension at the beginning of the therapy occurred in 5 (4.9%) and 32 (31.1%) patients, respectively. The rate of secondary tumors was relatively high (8.7%) as well as the rate of primary bone metastasis (45.6%). Mean±SE value of baseline LVEF was 61.7±3.2%. The histological type of the tumors was mainly clear cell renal cell cancer (ccRCC) in case of all patients, and in most cases pure ccRCC. No rare variants could be detected, but only sarcomatoid, papillary and chromophobe morphologies, and transformations in the ccRCC were present**.** No genetic analyses were performed to prove the familial origin of the renal cancer. The baseline characteristics of the patients are presented in Table 6.

Sunitinib dose parameters and efficiency. 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). First-level (37.5 mg/day in 4/2 or 2x2/1 scheme) and second-level (25 mg daily dose in 4/2 or 2x2/1 scheme) dose reductions were required during the treatment in 25 (25.3%) and 9 (9.1%) cases, respectively. Sunitinib therapy had to be ultimately ceased within 12 weeks in 5 (5%) patients due to progression of the disease. The follow-up of four patients was incomplete; thus, their data were excluded from the final analyses.

The dosing scheme was modified (DSM) in case of 22 (22.2%) patients. A cycle delay of more than seven days was needed in 15 (15.1%) patients because of an infection, herniotomy, dental intervention, diarrhea, neutropenia, or cardiac decompensation. Mean±SE duration of the delay was 7.8±3.3 days. 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.

Patients		**N_{all}=103**		**N_{SP}=48**	
Mean age, years ± SE		62.27 ± 0.9		61.76 ± 1.62	
Age range, years		32–80			
MSKCC score, mean ± SE		1.7 ± 0.05		1.6 ± 0.1	
Gender	male	80 (77.7 %)		39	81.3 %
	female	23	23 (22.3 %)	9	18.7 %
Number of patients after nephrectomy		87	84.5 %	42	87.5 %
Comorbidities					
Hypertension		32	31.1 %	9	18.8 %
Other cardiovascular disorders		12	11.6 %	5	10.4 %
Diabetes		11	10.7 %	4	8.3 %
Secondary tumors		9	8.7 %	1	2 %
Hyperthyroidism		5	4.9 %	0	0 %
Mean number of metastatic sites (range)		2.32 ± 0.11 (1–5)		1.79 ± 0.1 (1-3)	
Location of metastases					
Lungs		84	81.6 %	39	81.2 %
Bone		47	45.6 %	16	33.3 %
Distant lymph node		36	34.9 %	20	41.7 %
Liver		19	18.4 %	7	14.6 %
Brain		11	10.7 %	0	0 %
Suprarenal gland		9	8.7 %	4	8.3 %
Other (peritoneum, pleura, pancreas, local relapse, contralateral kidney, or thyroid gland)		-	‹8 %	-	‹4 %
Patients with synchronous metastases		94	91.2 %	45	93.8 %
Histopathological types				n	%
Purely clear cell renal cell type (ccRCC)		91	88.3 %	46	95.8 %
ccRCC with sarcomatoid morphology		7	6.8 %	1	2 %

Table 6 – Baseline demographics of all patients and of patients with slight progression (SP) (ccRCC – clear cell renal cell cancer, MSKCC – Memorial Sloan Kettering Cancer Center, n – number of involved patients, N – number of analyzed patients, SE – standard error**)**

In cases of SP, the result of radiological revision 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. Sunitinib therapy is still continued in 10 (10.1%) patients, and 5 patients underwent metastasectomy; their sunitinib therapy was discontinued and rechallenged in 3 (3%) of them. After progression on sunitinib therapy, no further therapy was administered in 30 (30.3%) cases, while in 47 (47.4%) and 5 (5.1%) patients, one and two therapy lines were applied, respectively.

Factors influencing efficacy. PFS and OS were not influenced by the patients' age, gender, the number/type of metastatic organ systems, and dose reduction in the overall population. Patients with nephrectomy and lower MSKCC scores showed more favorable outcomes in the studied population.

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 [Table 7] [Figure 6].

Metastasectomy after an effective sunitinib therapy caused the most favorable overall survival (74.3 months). Median survival of patients with slight progression is longer with dose escalation (58.6 months) than without it (27.9 months), or the outcome of all other patients (17.9 months) ($p<0.001$). The PFS and OS results of patients with SP who underwent radiological revision and showed to have a stable disease (48 patients), did not influence the number of metastatic sites, the MSKCC score, and the dose reduction.

Specifications of analyzed patients N=99		**PFS-HR** (95% CI)	***p***	**OS-HR** (95% CI)	***p***
Age		1.012 (0.987–1.038)	0.351	1.007 (0.981–1.035)	0.590
Number of metastatic organs		1.083 (0.891–1.317)	0.423	1.100 (0.896–1.350)	0.364
		PFS-HR (95% CI)	***p***	**OS-HR** (95% CI)	***p***
Gender	man/ woman	1 / 1.367 (0.807–2.316)	0.245	1 / 1.388 (0.792–2.435)	0.252
MSKCC score	0 / 1 / 2	1 / 3.770 (1.345–28.435) / 6.693 (1.813–49.061)	**0.019**	1 / 2.692 (1.355–20.445) / 5.199 (1.713–37.929)	**0.023**
Dose reduction	Yes / No	1 / 1.492 (0.947–2.506)	0.065	1 / 1.553 (0.963–2.504)	0.071
Nephrectomy	Yes / No	1 / 2.702 (1.508–4.840)	**0.001**	1 / 3.189 (1.741–5.842)	**<0.001**
Dose escalation	Yes / No	1 / 2.665 (1.486–4.780)	**0.001**	1 / 3.157 (1.613–6.179)	**0.001**
Dose scheme modification	Yes / No	1 / 2.569 (1.437–4.595)	**0.001**	1 / 2.444 (1.288–4.636)	**0.006**
Therapeutic lines after sunitinib	2 / 1 / 0	NA	NA	1 / 7.731 (2.318–25.787) / 4.043 (1.228–13.311)	**0.001**

Table 7 – Factors influencing the outcome of sunitinib therapy in all patients (Bold *p*-values are significant ‹0.05) (HR – hazard ratio, MSKCC – Memorial Sloan Kettering Cancer Center, mOS – median overall survival, mPFS – median progression-free survival, NA – not applicable, OS – overall survival, *p* – p-value, PFS – progression-free survival, SE – standard error)

Age and gender of the patients did not influence the OS. PFS was longer in case of younger male patients. PFS and OS were more favorable if patients underwent nephrectomy, in case of DE and DSM [Table 8, 9].

Specifications of all patients with slight progression N=48		**PFS-HR** (95% CI)	***p***	**OS-HR** (95% CI)	***p***
Age		1.047 (1.008–1.089)	**0.019**	1.025 (0.982–1.069)	0.265
		PFS-HR (95% CI)	***p***	**OS-HR** (95% CI)	***p***
MSKCC score	0 / 1 / 2	1 / 3.671 (0.474–28.414) / 5.304 (0.709–39.661)	0.176	1 / 2.965 (0.375–23.430) / 3.841 (0.513–28.786)	0.366
Dose reduction	Yes / No	1 / 0.840 (0.450–1.570)	0.585	1 / 0.724 (0.365–1.436)	0.356
Nephrectomy	Yes / No	1 / 3.397 (1.364–8.461)	**0.009**	1 / 5.583 (2.135–14.601)	**<0.001**
Dose escalation	Yes / No	1 / 2.383 (1.241–4.578)	**0.009**	1 / 2.479 (1.185–5.183)	**0.016**
Dose scheme modification	Yes / No	1 / 2.373 (1.034–5.445)	**0.041**	1 / 2.583 (1.008–6.709)	**0.047**
Therapeutic lines after sunitinib	2 / 1 / 0	NA	NA	1 / 6.163 (1.582–24.016) / 3.873 (1.130–13.280)	**0.032**

Table 8 – Factors influencing the outcome of sunitinib therapy in SP cases (Bold *p*-values are significant ‹0.05) (HR – hazard ratio, MSKCC – Memorial Sloan Kettering Cancer Center, mOS – median overall survival, mPFS – median progression-free survival, NA – not applicable, OS – overall survival, *p* – p-value, PFS – progression-free survival, SE – standard error, SP – slight progression)

Influence of dose escalation on effectivity. There were 23 patients in the control group (they underwent radiological revision before June 30, 2013 and showed slight progression) and 25 patients in the study group (they underwent radiological revision after June 30, 2013).

The following factors were similar in the two groups: patients' age, gender, MSKCC score, number of metastatic sites, time elapsed from diagnosis, serum calcium level, LDH, hemoglobin, Karnofsky performance status, DR and DSM. All patients underwent nephrectomy in the study group, whereas it was performed in 17 out of 23 patients in the

control group (p=0.008). Dose escalation was only performed in the study group. It could be performed in case of 18 patients (72.0%), but it could not be carried out in 7 cases (28.0%). 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. The median follow-up time of the cohort with slight progression was 37.3 (11.17-93.83) months.

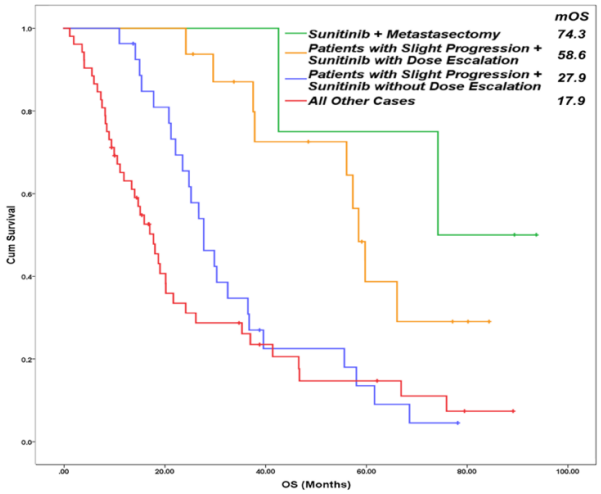

Figure 6 – Overall survival of patients in four subgroups - (Cum – cumulative)

Because of the higher rate of nephrectomy and DE in study group, a multivariate analysis was performed to detect the real effect of these factors. 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..

Specifications of patients with slight progression N_{SP}=48		**Control group Before June 30, 2013 N_{CG}=23**	**Study group After June 30, 2013 N_{SG}=25**	***p***
Nephrectomy	**No**	6 (26.1%)	0 (0.0%)	**0.008**
	Yes	17 (73.9%)	25 (100.0%)	
Dose escalation rate	**No**	23 (100.0%)	7 (28.0%)	**<0.001**
	Level 1 (62.5 mg)	0 (0.0%)	14 (56.0%)	

	Level 2 (75 mg)	0 (0.0%)	4 (16.0%)	
median progression-free survival		14.2±1.3	39.7±5.1	**0.037**
median overall survival		27.9±2.5	57.5±10.7	**0.044**
median follow-up time (range) (months)		30.9 (11.2-89.5)	45.7 (13.9-84.5)	**0.061**

Table 9 –Characteristics and results of patients with slight progression, differences between the control and study groups

The impact of dose escalation on the adverse effects. After dose escalation, the most common adverse effects were the following: worsening or development of fatigue, hypertension, stomatitis, and weight loss (over 10%) [Table 10]. The most upgraded clinical parameters were fatigue and development or worsening of hypertension as a result of the increased sunitinib dose.

New or intensifying adverse effects N_{DE}=22	**Number of patients (percent)**			
	Any grade	**Grade 1**	**Grade 2**	**Grade 3**
All	21 (95.5%)	17 (77.3%)	3 (13.6%)	1 (4.5%)
Fatigue	9 (40.9%)	7 (31.8%)	2 (9.1%)	0
Development / worsening of hypertension	8 (36.4%)	7 (31.8%)	1 (4.5%)	0
Stomatitis	6 (27.3%)	5 (22.7%)	1 (4.5%)	0
Diarrhea	5 (22.7%)	3 (13.6%)	1 (4.5%)	1 (4.5%)
Weight loss 10%≤	4 (18.2%)	4 (18.2%)	0	0
Hand–foot syndrome	4 (18.2%)	4 (18.2%)	0	0
Eyelid edema	2 (9.1%)	2 (9.1%)	0	0
Hypothyroidism	1 (4.5%)	1 (4.5%)	0	0
Elevation in creatinine level	5 (18.2%)	4 (18.2%)	1 (4.5%)	0
Thrombocytopenia	4 (18.2%)	2 (9.1%)	2 (9.1%)	0
Anemia	3 (13.6%)	2 (9.1%)	1 (4.5%)	0
Neutropenia	2 (9.1%)	1 (4.5%)	1 (4.5%)	0

Table 10 – New or intensifying adverse effects in patients after dose escalation

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

Patient characteristics. 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.

	Patients N = 145		
Mean age, years ± SE	62.0 ± 0.9		
Age range, years	28-79		
Gender, n %	male	108	74,5
	female	37	25,5
ECOG, n %	0	45	31
	1	88	60,7
	2	9	6,2
	3	3	2
Comorbidities, n %			
Hypertension		59	40.7
Other cardiovascular disorders		15	10.3
Diabetes		17	11.7
Secondary tumors		11	7.6
Metastases			
Mean number of metastatic sites (range)	2.3 (1-6)		
Location of metastases, n %			
Lung		125	86.2
Bone		59	40.7
Distant lymph node		53	36.5
Liver		27	18.6
Brain		13	9
Suprarenal gland		13	9

Table 11 - Patient characteristics *(N - number of analyzed patients, n – number of involved patients, ECOG* - Eastern Cooperative Oncology Group*, SE – standard error)*

The general condition of the patients was good, ECOG 0 and 1 score were registered in 45 (31%) and 88 (60.7%) cases, respectively. ECOG 2 and 3 was registered in 9 (6.2%) and 3 (2%) cases, respectively. Nephrectomy was performed in 136 (93.8%) cases. Hypertension, other cardiovascular disorders and diabetes occurred in 59 (40.7%), 15 (10.3%) and 17 (11.7%) cases, respectively. Secondary tumor was diagnosed in case of 11 (7.6%) patients. Simultaneous hematological concomitant disease, asthma and psoriasis occurred in 2 (1.4%) cases each. The mean number of metastatic sites was 2.3 (1-6). Pulmonary metastasis was the most common (125 cases, 86.2%)). Bone and distant lymph node metastases occurred in 59 (40.7%) and 53 (36.5%) cases, respectively. Liver metastasis occurred in 27 (18.6%) cases, while controlled brain and suprarenal gland metastases developed in and 13 (9%) cases, each. The prevalence of other rare (peritoneum, pleura, pancreas, local relapse, contralateral kidney, local relapse, thyroid gland) metastases was under 8%. (Table 11).

	Patients N=145	
Previous therapies, n %		
Nephrectomy	136	93.8
Adjuvant IFN	19	13.1
First line IFN before VEGFR-TKI	18	12.4
Sunitinib	128	88.3
Sorafenib	16	11
Pazopanib	1	0.7
First line VEGFR-TKI	123	84.8
Second line VEGFR-TKI after IFN	22	15.2
Duration of previous therapy		
Mean duration of VEGFR-TKI, months (±SE)	11.7 (±0.9)	
Duration of VEGFR-TKI <3 months, n (%)	24	16.6
Mean duration between VEGFR-TKI and EVE, days (±SE)	97.7 (±10.1)	

Table 12 - Previous therapies before everolimus treatment (N - number of analyzed patients, n – number of involved patients, IFN – interferon-α, VEGFR – vascular

endothelial growth factor receptor, TKI - tyrosine kinase inhibitor, EVE - everolimus, SE – standard error)

Previous therapies. After nephrectomy 19 (13.1%) patients received postoperative interferon treatment, while before VEGFR therapy, in first-line 18 (12.4%) patients. 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. TKI treatment was performed after the IFN in 22 (15.2%) cases.

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). (Table 12)

Dose-parameters. 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%). Therapy delay was necessary in 13 (8.9%) cases. Its mean duration was 24 (5-75) days. Reasons for therapy delay longer than 7 days were the following: cardiovascular symptoms, elevation of renal functions that required dialysis (10-10 days), grade 3 diarrhea (9-14 days), cerebral metastasectomy (20 days) and pneumonitis in 2 cases (28 and 30 days).

Efficacy. Currently 38 (26.2%) patients are being treated, 78 (53.8%) patients are alive. Complete regression (CR) 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) (based on the data of 132 patients) was 16.2 months (*95%CI 12.95-19.45*).

Side-effects. The most common side-effects were the following: exanthema (25%), peripheral edema (19%), stomatitis (19%), pneumonitis (13%), nausea, weight loss, fatigue (11% each), diarrhea (10%), dyspnea (10%), mucositis (9%). In the laboratory values anemia (72%), elevation of renal function (45%), liver function (25%), blood sugar

(51%), cholesterol (44%) and lipid level (35%) occurred most commonly. No severe, life threatening side-effect did not occurred.

Factors influencing efficacy. The PFS and OS after everolimus therapy were not influenced by the patients' gender, age, the number of metastatic organ systems, the presence of single lung metastasis, the length and type of previous TKI therapy or the time elapsed between the stop of the TKI treatment and the initiation of everolimus therapy. 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) **(**Table 13). 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)

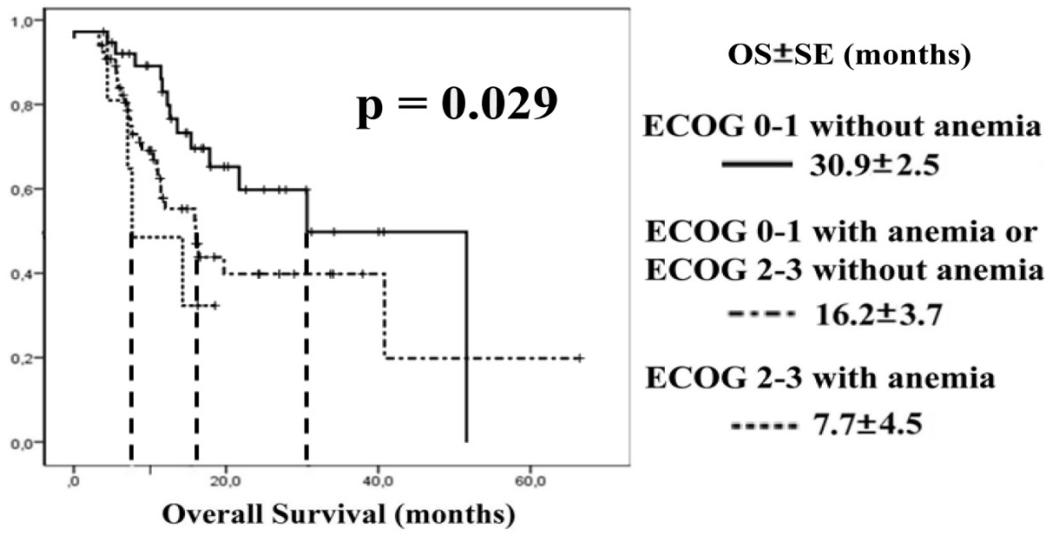

***Figure 7* – Effect of ECOG status and anemia on OS** - Kaplan-Meier analysis of OS was compared in patients with anemia and ECOG 2-3 status *(7.7±4.5 months) vs.* the absence of anemia and ECOG 0-1 status *(30.9±2.5 months) vs.* only one unfavorable prognostic factor is present *(16.2±3.7 months) (p=0.029).*

Specification		**PFS±SE (months)**	***p*-value**	**OS±SE (months)**	***p*-value**
ECOG status	0-1/2-3	6.4±1.1/3.5±0.2	**0.033**	19.9±6.7/7.5±0.6	**0.008**
Duration of TKI therapy (months)	≤3/>3	3.4±0.6/5.9±0.8	0.250	16.0±4.5/19.9±5.9	0.244
	≤6/>6	4.7±0.8/6.4±1.3	0.090	21.9±7.2/16.6±2.4	0,840
	≤9/>9	4.5±0.8/7.2±1.5	**0.019**	16.0±2.8/41.2±18.6	**0.045**
TKI-EVE period (days)	≤30 / >30	6.5±0.9/5.3±0.9	0.774	11.5±5.4/ 30.9±6.8	0.106
	≤60 / >60	5.6±0.6/4.5±1.3	0.601	19.9±4.9/ 16.5±6.8	0.624
Anemia	G0/G1-2-3	4.8±1.2/6.4 ±1.0	0.612	30.9±6.1/16.2±1.4	**0.020**
PFS (months)	<12 / ≥12			15.5±1.8/41.2±9.5	**0.001**

Table 13 – Factors influencing outcome of everolimus therapy (PFS - median progression-free survival, OS – median overall survival, SE – standard error, ECOG – Eastern Cooperative Oncology Group, TKI – tyrosine kinase inhibitor, SU – sunitinib, SO – sorafenib, PA – pazopanib, EVE – everolimus, G – grade)

5. Discussion

5.1. The standard treatment of muscle-invasive, non-metastatic tumors is cystectomy. Surgery may or may not be preceded by neoadjuvant chemotherapy, depending on eligibility to receive cisplatin [1,3]. Neoadjuvant cisplatin-based chemotherapy results in a 5% benefit in 5-year overall survival and a 9% benefit in 5-year disease-free survival based on a meta-analysis (11 trials, 3005 patients) [39]. Most of our analyzed cases were locally advanced, but the use of chemotherapy was still detected at a very low rate. This may be due to the fact that in the period covered, the only treatment option available was the toxic platinum-based chemotherapy combination, which generally yielded little success. As a result, it was used with great caution and viewed with skepticism by both patients and doctors [40]. In our study, the use of chemotherapy did not provide a survival advantage in the entire population. Since the focus of our work was the investigation of biomarkers and the number of patients receiving chemotherapy was small, it was not possible to search for further relevant correlations related to chemotherapy.

The indication for cystectomy is mainly the muscle invasive diagnosis based on TUR. However, it happens that tumor tissue will no longer be detectable in the cystectomy sample (pT0), e.g. due to the ablative effect of TUR or even the effectiveness of neoadjuvant treatment. The life expectency of these patients is more favorable based on literature data [41]. pT0 tumor status occurred in 6.5% of our samples, the survival of these patients was the best amongst all stages, similar to the international multicenter results of Tilki et al [41].

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, regardless of biomarker analysis, but they are also effective and approved as a first choice in case of high PD-L1 (CPS – combined positive score or IC – immune cell score) status [1,3]. In our work, PD-L1 status was determined based on TPS and CPS as well. Their effect on the outcome of the disease had a similar prognostic value.

Among the targeted therapies, FGFR inhibitors can be administered after platinum-based chemotherapy or immune checkpoint inhibitors in the treatment of bladder cancer [1,3].

Approximately 70% of low-grade non-invasive papillary tumors show FGFR3 mutation in literature [42]. In our study, patients with superficial bladder tumors (20%) who underwent

cystectomy were included after a recurrence or if the disease could not be controlled by transurethral resection. Even in this higher-risk superficial group, the proportion of FGFR mutant patients was 37.2%, lower than in published data, but higher than in our analyzed muscle-invasive or locally advanced group [42]. The strongest correlation could be observed between TNM stage and FGFR mutation. Our results represent the high frequency of FGFR3 mutation in earlier stages. Previous studies support our data, and it has been demonstrated that over half of pTa tumors recur, accordingly FGFR alteration is a possible signaling pathway in the development of these tumors [43]. Compared to the literature [43], our findings suggest an oncogenic relationship, as FGFR3 mutation is mostly seen in non-invasive tumors, and less frequently at more advanced stages.

Advanced stage tumors have worse survival than early stage, and stratifying the patients according to TNM stage, we found also significant difference in survival from cystectomy between NWT and WT patients. Our data are similar to other published results that have reported an association between favorable prognosis and FGFR mutation status [44].

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, but the database analysis method and the limited number of elements found in each subgroup did not allow the matching of the individual elements and the adjustment of the data.

Some previous studies verified mutated FGFR3 with increased FGFR3 gene expression and an association with decreased T-cell infiltration, but in this publication was no significant difference in response rate or OS with immunecheckpoint inhibitors in FGFR3 separated groups was found, possibly due to the lower stromal-mediated immune suppression [45].

These findings may propose a negative or immunosuppressive effect of FGFR3 alterations on T-cell gene mechanism. Therefore, the clinical significance of the connection between FGFR3 status and response to immune checkpoint inhibitors was investigated in a large phase II study, ImVigor 210. In the platinum-refractory or cisplatin-ineligible patient group there was no significant difference in ORR and OS with immune checkpoint inhibitors in FGFR3 mutant (NWT) or wild-type (WT) tumor [13]. 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 [14].

Our aim was to investigate whether FGFR mutation is a possible independent prognostic factor of survival. Reflect on many controversial survival and response data in the anti-PD-L1 treated FGFR mutated patient group [11,12,14,46–48], according to other studies we consider a larger investigation of special noninvasive subtypes to be necessary in order to verify its predictive and prognostic value. In addition, we consider it forward-looking to wait for the results of the phase 3 prospective THOR (NCT03390504) study, which compares the effects of erdafitinib and pembrolizumab in patients with advanced mUC, to clarify the real therapeutic significance of FGFR alterations [49].

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 [50]. Another advantage of our work is that we also evaluated the CPS data in relation to PD-L1 expression, used better in the daily practice during first line immunotherapies nowadays, which would provide the opportunity for further potentially predictive conclusions. 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 is one of the most frequently applied first line therapies in patients with metastatic ccRCC with MSKCC good and moderate prognoses. In the investigated cohort, nephrectomy was performed in 84.8% of the cases, and PFS and OS results of these patients were more favorable. Each patient with SP in the Study group (period 2) underwent nephrectomy (which means that the patients were fit enough for this operation). It might have been a potential selectional bias of the compared cohorts. However, the other parameters and the comorbidities of the patients in the two cohorts were not significantly different.

In our study, PFS was longer than in the registration study [19]; however, patients with MSKCC poor prognosis were excluded from our study, but the PFS of our patients was similar to the excellent international data [51,52]. The median OS of patients with

metastatic RCC was no longer than 2 years before the immunotherapeutic era [15], 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 [53]. The recommended starting dose for sunitinib malate is 50 mg daily for 28 days followed by a 14-day break. Although individualized sunitinib therapy improves the outcome, poorer outcomes in patients tolerating the standard schedule treatment without significant toxicity may be the result of underdosing [28]. Several authors [54,55] 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 [56]. According to the meta-analysis of Houk et al. [28], escalated sunitinib exposure (area under the curve) 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 after the summer of 2013 in cases with slight progression, when RECIST 1.1 results confirmed a stable disease if any clinically relevant side effects occurred. 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 trials of sunitinib [19], which might reflect an outstanding benefit from sunitinib mainly in patients with low tumor volume in our studied cohort. After an initial favor tumor response evolving slight progression can be stopped or be reversible with dose escalation and adequate titration has been hypothesized. Drug toxicity and efficacy may depend on the interindividual differences in pharmacokinetics, pharmacodynamics, and pharmacogenetics [57,58]; however, Motzer et al. [25] have not found correlation between sunitinib pharmacokinetic values and the toxicity profile. Adelaiye et al. [28] have detected an increase in sunitinib plasma concentration in animals treated with escalated dose TKI in the drug resistant group, and also a trend for decreased plasma concentration after prolonged sunitinib exposure. Gotink et al. [59] have found 1.7 to 2.5-fold increase in sunitinib concentration in resistant tumor cells due to the increased lysosomal drug sequestration, which was reversible after the

removal of sunitinib from the cell culture. 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 [60,61]. In the retrospective analysis of Bjarnason et al. [62], an individualized treatment strategy and shorter treatment break (14 days on and 7 days off) have resulted in improved PFS and OS as compared to the standard sunitinib schedule, and the PFS detected in patients with ccRCC has been one of the best reported for any TKI. Modified sunitinib schedule is well tolerated and induces optimal drug exposure [63].

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. As the two patient populations are not the same, their effects can be considered independent. Dose escalation can be performed in case of patients with good general condition, who do not have any relevant adverse effects. In case of these patients, based on the prognostic values, the survival rate is potentially better. Therefore, we compared the two (almost similar) groups regarding dose escalation, so selection of patients with better prognosis could not have queried the results. The effect of dose escalation on PFS and OS was confirmed during the comparison of the two groups. No significant difference was found among the number of the subsequent therapies and mOS after sunitinib was equal in two groups as well, which may be because in our country the availability of more active new regimens was very limited during our study period.

The rate of adverse events (AE) in our real world dose escalated patients is lower in the selected cohort than the AE rate in patients administered the standard dose in the pivotal trials [19,20]. 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.

In metastatic RCC patients on standard schedule sunitinib with early disease progression, Adelaiye et al. [28] could increase sunitinib dose from 50 to 62.5 and 75 mg daily, with a 14-day on and 7-day off treatment scheme to some type of grade 2 toxicity, and they observed clinical benefit in the majority of the patients. According to Gotink et al. [59] and Zama et al. [64], sunitinib rechallenging in previously resistant patients also has a therapeutic value.

5.3. The antitumor effect of everolimus is induced by the inhibition of the mTOR complex. The mTOR plays a central role in the signal transmission pathway. It is activated by stimuli (for example growth factors) that affect the cell [65]. The activation of the mTOR complex can lead to increased production of HIF-1α (hypoxia-inducible factor-1 alpha) and indirectly that of the VEGF, which promote cell migration and proliferation. Blocking of these processes leads to inhibition of the growth and proliferation of tumor cells, fibroblasts, endothelial cells and pericytes are blocked [66].

In our analysis everolimus monotherapy was associated with favorable PFS and OS in case of patients with metastatic, clear-cell renal carcinoma 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 [32] and similar to the subgroup of patients treated after one line of TKI therapy [34]. Median overall survival of patient in our study was 16.2 months. In the phase III study the median overall survival in the everolimus arm and in the placebo arm was 14.8 and 14.4 months, respectively [33]. In case of progression patients in the placebo arm received everolimus therapy. In our study the mean duration between the stop of VEGFR-TKI treatment and the beginning of everolimus therapy was unfortunately 97.7 days. The reasons for delaying the start of the administration of everolimus were manifold: side-effects of previous therapy had to be cured at least to grade 1 severity; symptoms caused by new metastasis had to be stabilized (if necessary cerebral metastasectomy, brain- or bone irradiation); patient flow between the institutes; organization of radiological examinations; and also the availability of drugs were the most important factors. The length of this period between TKI and mTOR inhibitor therapies was similar to the period from the stop of TKI to the beginning of active drug following progression on placebo in the placebo arm of registration study. Besides delaying the start of administration of everolimus therapy, our PFS results were quietly favorable.

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, the type of previous TKI therapy or its duration 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 favorable results can be reached with mTOR inhibitor agents. Although no statistically significant difference could be demonstrated, PFS and OS tended to be less

favorable in case of primary TKI resistance. Similar results can be found in international studies [67]**.** Similarly to our results, Bergmann et al. have not found correlation between the type, the shorter or longer than 3- or 6-month of previous TKI therapy and the outcome [68]. In the Hungarian population those patients whose VEGFR-TKI therapy was longer than 9 months had significantly more favorable PFS and OS. The patients with unfavorable MSKCC prognosis tended to have shorter progression-free survival [68]. 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 [69]. These were created on the basis of subgroup analysis of the phase III study. In our population we could also unambiguously prove that poor general condition negatively influenced the survival. 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 everolimus therapy and PFS we concluded that ECOG is one of the most important factors. The OS was remarkably better in case of everolimus therapy lasting more than 12 months. This underlines the importance of appropriate patient selection. After longer everolimus therapy the number of third-line therapies decreased without influencing the survival, so the properly selected, effective second-line therapy determined the patients' life expectancy.

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. Appearance of pneumonitis and dyspnea were new symptoms during everolimus therapy. 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 are similar to the RECORD-1 study. Development of hypercholesterolemia, hypertrigliceridemia and hyperglycemia are connected to the mode of action of everolimus, 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 [70].

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.

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Appendix

I.

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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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Background: Programmed cell death (PD)-1/PD-ligand 1 (PD-L1) inhibitors have made a breakthrough in the therapy of advanced urothelial bladder cancer (UBC). The impact of Fibroblast Growth Factor Receptor 3 (FGFR3) mutation on the effectiveness of PD-L1 treatment remains still unclear. Objective: Our study aimed to investigate the frequency of FGFR mutations at different tumor stages, and their relation to PD-L1 status and survival.

Methods: 310 patients with urothelial bladder cancer and subsequent radical cystectomy were included in a retrospective study over a 10-year study period at the University of Szeged, Hungary. FGFR3 mutations from the most infiltrative areas of the tumor were analyzed by targeted next-generation sequencing and PD-L1 (28-8 DAKO) tests (tumor positive score -TPS and combined positives score–CPS). In T0 cases FGFR3 mutations were analyzed from the earlier resection samples. Survival and oncological treatment data were collected from the National Health Insurance Fund (NHIF). Neoadjuvant, adjuvant and palliative conventional chemotherapies were allowed; immunotherapies were not. The relationship between the covariates was tested using chi-square tests, and survival analysis was performed using the Kaplan-Meier model and Cox proportional hazards regression.

Abbreviations: CD8, cluster of differentiation 8; CPS, combined positive score; FFPE, formalin-fixed paraffin-embedded; FGFR, fibroblast growth factor receptor; HR, hazard ratio; IHC, immunohistochemistry; LCL, lower control limit; MIBC, muscle-invasive; NGS, next-generation sequencing; NMIBC, non-invasive; NWT, no wild type; OS, overall survival; PCR, polymerase chain reaction; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; St, stage; TCGA, the cancer genom atlas; TNM, tumour, node, metastasis; TPS, tumor positive score; TUR, transurethral resection; UBC, urothelial bladder cancer; UCL, upper control limit; WT, wild type.

Results: PD-L1 and FGFR could be tested successfully in 215 of the 310 UBC samples [$pT0_{cyst}$ 19 (8.8%); St.0-I 43 (20%); St.II 41 (19%); St.III-IV 112 (52%)]. Significant pairwise dependency was found between tumor stage, FGFR3 mutation status and PD-L1 expression ($p < 0.01$). Samples with FGFR mutation were more common in less advanced stages and were also less likely to demonstrate PD-L1 expression. The effect of all investigated factors on survival was found to correlate with tumor stage.

Conclusion: FGFR alteration frequency varied between the different stages of cancer. Higher positivity rates were observed at early stages, but lower levels of PD-L1 expression were detected in patients with FGFR mutations across at all stages of the disease.

KEYWORDS

urinary bladder cancer, fibroblast growth factor receptor, FGFR mutation, programmed death-ligand 1 expression, combined positive score

Introduction

Bladder cancer is the tenth most common cancer worldwide with approximately 550,000 new cases annually (1). The depth of tumor invasion is the most important prognostic factor from a clinical standpoint and is divided into non-muscle-invasive bladder cancer (NMIBC) and the prognostically less favorable muscle-invasive cancer (MIBC) types (2). The rate of occurrence of MIBC capable of forming distant metastases is 25%–42%, while that of the disseminated stage is 4%–15% (1, 3). Localized MIBCs become disseminated in almost 50% over the course of the disease despite the radical cystectomy or locoregional trimodal therapy (3). In the treatment of advanced disease, for decades only combined chemotherapy was available, with relatively low efficacy and significant toxicity—moreover, molecular markers did not exist for predicting treatment ineffectiveness.

In recent years, checkpoint inhibitor immunotherapy has revolutionised the treatment of advanced urothelial bladder cancer (3). However, the role of potential biomarkers predicting the effectiveness of immunotherapy remains incompletely understood, and many factors that assume an immunogenic mechanism are currently under investigations.

In some studies, the presence of tumor infiltrating lymphocytes such as $CD8^+$ (cluster of differentiation 8) T cells, as well as interferons and chemokines has been found to result in improved response to immunotherapies (4); however the prognostic value of programmed cell death ligand-1 (PD-L1) in urothelial cancer remains controversial (5). Based on several previous analyses, it can be assumed that patients with tumor cells showing PD-L1 positivity have a better response to anti-PD-1/PD-L1 monotherapy (6). 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 (7, 8), as high combined positive score (CPS) of ≥10% was associated with a prolonged median overall survival (OS) (8).

In addition to immune mechanisms, the mutations responsible for bladder tumor progression are also the focus of genetic analyses. The mutation rate of urothelial carcinomas was published in The Cancer Genom Atlas (TCGA), however the possibilities and actual effectiveness of targeted drug treatments against mutations remain low (9). MIBC is a molecularly diverse disease with heterogeneous clinical outcomes (10, 11). Several reports have highlighted the clinical significance of molecular stratification of MIBC. A Consensus Molecular Classification of MIBC identified six different molecular classes with the occurrence of the following possible mutations: luminal papillary (24%)—FGFR3, KDM6A, STAG2; luminal non-specified (8%)—ELF3; luminal unstable (15%)—TP53, ERCC2, TMB+, APCBEC+; stroma-rich (15%), basal/squamous (35%)—EGFR+, TP53+, RB1+; and neuroendocrine-like (3%)—TP53-, RB1, by suggesting that responses to immunotherapy and chemotherapy may be enriched in specific subtypes (10). Because of the molecular heterogeneity of bladder cancer, molecular characterization is a very dynamically developing area.

In recent years, due to the emergence of FGFR inhibitor therapy, the clinical significance of FGFR mutation has come into view. Fibroblast growth factor receptor 3 (FGFR3) is a member of protein tyrosine kinase family, which consists of four transmembrane receptors, (FGFR1–4), and the alteration of the receptors induces an oncogenic signaling pathway (12). The aberrations in FGFR1–4—are detected in 5%–10% of all human cancers, although some types, such as urothelial cancer and intrahepatic cholangiocarcinoma display an increased (10%–30%) frequency of FGFR aberrations. Amongst these aberrations, the FGFR3 activating point mutation is the most frequently occurring one (10%–60%), mainly present in low grade, early stage NMIBC, while FGFR3 fusion and FGFR1 amplification can also occur in 6% and 7%, respectively (13).

However point mutation is rarely associated with MIBC, as nearly the half of advanced stage tumors bear wild-type FGFR3 gene (14). 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 (15).

Sweis et al categorised bladder cancer into two subgroups using immune gene profiling; T-cell-inflamed tumors and non-T-cell-inflamed tumors. In the non-T-cell-inflamed subgroup, which is mostly associated with luminal-papillary subtype (or cluster I subtype), they identified some exclusively typical somatic mutation, where FGFR3 was the most common molecular alteration (16, 17). Lower response rates and shortened OS following anti–PD-L1 therapy was observed in patients with FGFR alterations (18).

Based on the published data, the ratio of PD-L1 expression, CPS score, and FGFR expression in each tumor stage is not clear, nor is the prognostic or predictive effect of their relation to each other.

The aim of our study was to demonstrate the frequency of FGFR3 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.

Material and methods

Patients and demographic characterization

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 and 2016) at the University of Szeged, Hungary. Patients were included after partial or radical cystectomy, without known metastatic disease. The indication for the majority of cystectomies was primarily diagnosed muscle invasive transitional cell bladder cancer. In a smaller proportion of cases, extensive, multiple recurrent, non-muscle-invasive tumors were also indications for surgery, based on the guidelines. Neoadjuvant chemotherapy was allowed. The pT0 cases based on cystectomy specimens were called $pT0_{cyst}$. In these cases the biomarker analysis was performed from the initial sampling tissues, but the stage was not redefined based on the less accurate result of the baseline transurethral resection (TUR) samples. Patients were excluded from the current analysis in the following cases: sequenced samples without clinical information or patients with clinical informations without sequencing results; uncertain sequencing outcomes (due to technical reasons); neuroendocrine histology; immunotherapy or anti-FGFR therapy after progression (to avoid a potential influence on survival data).

The main clinical and demographic data included gender, age, stage and previous therapies. The surgical specimen was graded according to WHO classification and staged by the 7th TNM criteria. 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.

Tissue sample testing

Two tests were performed on easch tissue sample. The service provider together with University of Szeged performed FGFR next-generation sequencing (NGS) 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.

Only the FGFR3 mutation status (wild type -WT, non-wild type—NWT) was recorded, the exact type of mutation (point mutation, deletion, insertion, etc.) was not analyzed. The PD-L1 (IHC) 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). Nowadays, a more relevant CPS score in clinical application 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 ≥10) (9).

A detailed description of histological and molecular analyses can be found in the Supplementary Material.

Formation of analyzed groups

In our study 392 surgical samples were collected, of which 82 patients were excluded on the basis of insufficient information. 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 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 in which all results were available (Figure 1).

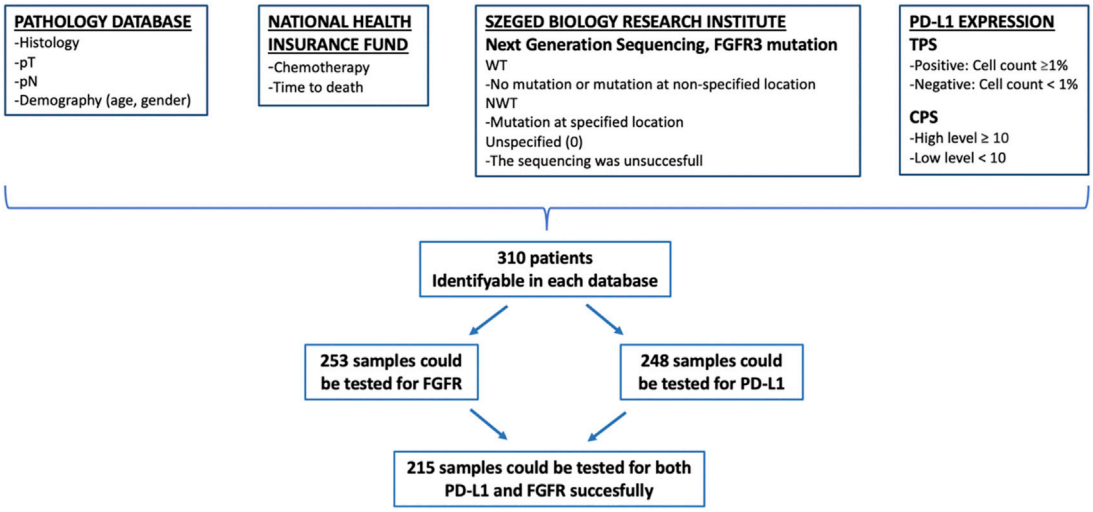

FIGURE 1
Method of data collection. CPS, combined positive score; FGFR, fibroblast growth factor receptor; NWT, non wild type; PD-L1, programmed cell death ligand 1; pN, pathologic lymph node stage; pT, pathologic tumor stage; TPS, tumor positive score; WT, wild type.

Statistical analysis

Demographic data were characterized using gender, median age, TNM stage and different biomarkers. The independence between the stratifying variables was analyzed using chi-square test for independence. Fischer's Exact test was used to examine the relationship between binary variables. P values <0.05 were considered significant.

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 overall survival (OS) was evaluated independently using univariate stratification of the Kaplan-Meier estimation. During survival analyses, Bonferroni correction was used for pairwise comparisons in the case of variables with more than 2 groups. Univariate and multivariate (with forward likelihood ratio method) Cox proportional hazard models were used to estimate the effect of certain covariates on the overal survival from cystectomy. The following variables were used in the Cox models as predictors: gender, age of the patient at the time of cystectomy (dichotomized as under 65 years vs. at least 65 years), TNM stage, FGFR mutation (WT/NWT), PD-L1 expression (positive/negative), chemotherapy (yes/no). Model reference values were the following: gender–male, age–lower than 65 years, chemotherapy–no, TNM stage–$pT0_{cyst}$ pN0, FGFR–NWT, PD-L1—negative.

SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, United States) 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).

Results

Baseline characteristics, TNM stage, FGFR and PD-L1 results

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) (Table 1). 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. The characteristics of the patients were similar in the entire population, as in the further analyzed subgroup in which both biomarkers could be evaluated (Figure 1 and Table 1).

Results of FGFR alteration testing were categorized into subgroups based on the non-wild type or wild type, PD-L1 immunostaining data as TPS negative or positive, and CPS <10 or CPS ≥10, respectively (Table 1).

Test of independence of TNM stage, FGFR and PD-L1 status

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 ($p = 0.003$). Significant relationship was also found between stage and PD-L1 expression based on TPS ($p = 0.070$) or CPS ($p = 0.002$), in

TABLE 1 The baseline characteristics.

	All patients (%) [valid %]	Both FGFR and PD-L1 available (%)
Number	310 (100)	215 (69.4 of all pts)
Gender		
Male	236 (76.1)	171 (79.5)
Female	74 (23.9)	44 (20.5)
Age		
Median age (months)	62.8	62.9
Patients over 65 years (%)	112 (36.1)	80 (37.2)
Stage (%)		
$pT0_{cyst}$ pN0	20 (6.5)	19 (8.8)
St.0-I (pTa, pTis, pT1 pN0)	54 (17.4)	43 (20.0)
St.II (pT2a, pT2b pN0)	60 (19.3)	41 (19.1)
St.III-IV (pT3a, pT3b, pT4/pN+)	176 (56.8)	112 (52.1)
Any chemotherapy performed (%)	87 (28.1)	59 (27.4)
Neoadjuvant (NA) chemotherapy (%)	18 (5.8)	12 (5.6)
Any chemotherapy except NA (%)	69 (22.3)	47 (21.8)
FGFR		
Missing or unsuccesful	95 (30.7)	NA
Non-wilde type (NWT)	36 (11.6) [16.7]	36 (16.7)
Wilde type (WT)	179 (57.7) [83.3]	179 (83.3)
PD-L1 (TPS)		
Missing or unsuccesful	62 (20.0)	NA
PD-L1 negative (<1%)	146 (47.1) [58.9]	129 (60.0)
PD-L1 positive (≥1%)	102 (32.9) [41.1]	86 (40.0)
PD-L1 (CPS)		
Missing or unsuccesful	62 (20.0)	NA
CPS < 10	169 (54.5) [68.1]	146 (67.9)
CPS ≥ 10	79 (25.5) [31.9]	69 (32.1)

CPS, combined positive score; FGFR, fibroblast growth factor receptor; NA, not applicable; NWT, non-wilde type; PD-L1, programmed cell death ligand 1; pts, patients; St, stage; TPS, tumor positive score; WT, wilde type.

TABLE 2 Correlations between the FGFR mutation and the TNM stage.

		FGFR alteration		*p*-value
		NWT	WT	
		***n* = 36**	***n* = 179**	
TNM stage	$pT0_{cyst}$ (%)	5 (26.3)	14 (73.7)	**<0.001**
	St.I (%)	16 (37.2)	27 (62.8)	
	St.II (%)	7 (17.1)	34 (82.9)	
	St.III-IV (%)	8 (7.1)	104 (92.9)	

more advanced stages the frequency of PD-L1 positivity was higher (Tables 2–4).

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.

Effect of TNM stage on survival

Stratifying the patients based on the TNM stage at the time of cystectomy showed that the survival at more advanced stages was worse than at earlier cases. 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, $p < 0.001$). Although this is not unexpected, this finding verifies the validity of the model (Figure 2 and Table 5).

Based on the pairwise comparison, we found that the survival of the TNM stage III-IV group was significantly worse compared to the other groups, while no difference could be detected between the groups with a better prognosis.

TABLE 3 Correlations between the FGFR mutation and the PD-L1 expression (TPS, CPS).

		FGFR alteration		*p*-value
		NWT	WT	
		n = 36 (%)	*n* = 179 (%)	
PD-L1 expression (TPS)	Negative (<1%)	29 (80.6)	100 (55.9)	**0.005**
	Positive (≥1%)	7 (19.4)	79 (44.1)	
PD-L1 expression (CPS)	<10	32 (88.9)	114 (63.7)	**0.003**
	≥10	4 (11.1)	65 (36.3)	

TABLE 4 Correlations between the TNM stage and the PD-L1 expression (TPS, CPS).

		PD-L1 expression					
		TPS < 1%	TPS ≥ 1%	*p*-value	CPS < 10	CPS ≥ 10	*p*-value
		n = 129	*n* = 86		*n* = 146	*n* = 69	
TNM stage	$pT0_{cyst}$	12 (63.2)	7 (36.8)	**0.07**	14 (73.7)	5 (26.3)	**0.002**
	St.I	33 (76.7)	10 (23.3)		39 (90.7)	4 (9.3)	
	St.II	24 (58.5)	17 (41.5)		23 (56.1)	18 (43.9)	
	St.III-IV	60 (53.6)	52 (46.4)		70 (62.5)	42 (37.5)	

The effects of analyzed biomarkers on survival

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 ($p = 0.024$) (Figure 3 and Table 6).

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, $p = 0.28$) (Figures 4A, B).

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.

The gender (male vs. female, HR: 1.18, $p = 0.52$), age (older than 65 vs. younger, HR: 1.48, $p = 0.07$) of the patients and the chemotherapy use (no versus yes, HR: 0.793, $p = 0.18$) did not affect survival.

There was a significant correlation between all variables (TNM, FGFR status, PD-L1 status). All covariates were associated with TNM stage and their impact on survival is through the TNM stage. It is not possible to evaluate the impact of any of the covariates independently from each other, based on multivariate Cox model the only exception is TNM stage.

Hazard ratios for the covariates in univariate and multivariate analysis from Cox model are summed in Table 7.

Discussion

It is well-known that urothelial tumors of the bladder predominantly develop in older adults, owing to the influence of environmental factors. However, such tumors have begun to appear more and more often in younger age groups (19). In our analysis, the average age of patients was 63 years; 36% were over 65 years old, and women were slightly younger than men.

The standard treatment of muscle-invasive, non-metastatic tumors is cystectomy. Surgery may or may not be preceded by neoadjuvant chemotherapy, depending on eligibility to receive cisplatin (1, 3). Neoadjuvant cisplatin-based chemotherapy results in a 5% benefit in 5-year overall survival and a 9% benefit in 5-year disease-free survival based on a meta-analysis (11 trials, 3,005 patients) (20). Most of our analyzed cases were locally advanced, but the use of chemotherapy was still detected

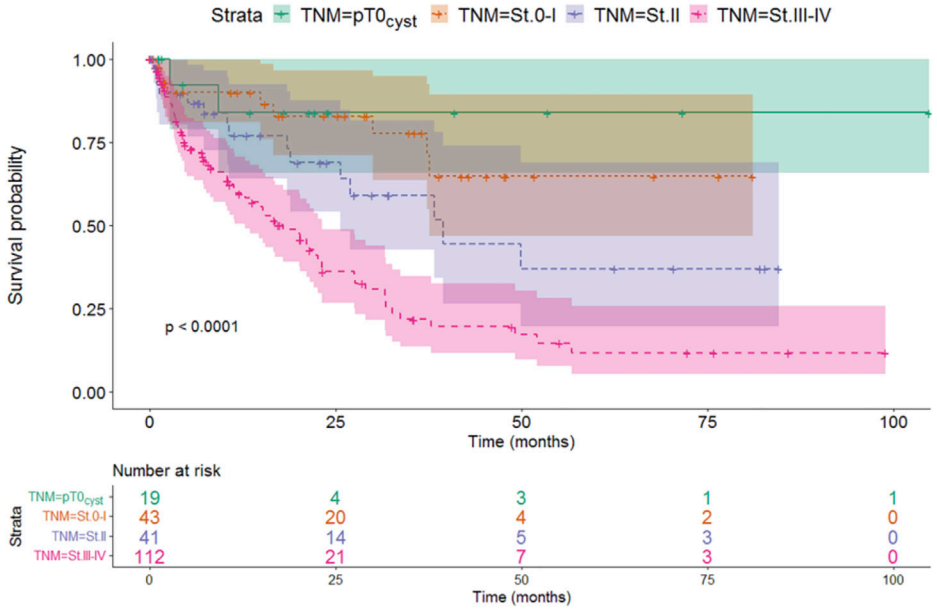

FIGURE 2
The effect of TNM stages on survival.

TABLE 5 Connection with TNM stages and median survival.

TNM stage	Median survival (months)	95% CI LL	95% CI UL	*p*-value
$pT0_{cyst}$	Not reached	NA	NA	<0.001
St.0-I	Not reached	37.86	NA	
St.II	39.50	20.25	58.75	
St.III-IV	17.97	11.98	23.96	

CI, confidence interval; LL, lower limit; St, stage; TNM, Tumor, Node, Metastasis; UL, upper limit.

at a very low rate. This may be due to the fact that in the period covered, the only treatment option available was the toxic platinum-based chemotherapy combination, which generally yielded little success. As a result, it was used with great caution and viewed with skepticism by both patients and urologists (21). In our study, the use of chemotherapy did not provide a survival advantage in the entire population. Since the focus of our work was the investigation of biomarkers and the number of patients receiving chemotherapy was small, it was not possible to search for further relevant correlations related to chemotherapy.

In recent years, the appearance of immunotherapy has been a breakthrough in the treatment of UBC, nowadays clinical trials are also taking place with the use of neoadjuvant indications (22). Radical surgery can also be recommended in the case of multiple recurring, non-muscle invasive tumors, as was the case in 18.7% of our results, if the patient's general condition allowed it (1, 3).

The indication for cystectomy is mainly the muscle invasive diagnosis based on TUR. However, it is possible that tumor tissue is no longer detectable in the cystectomy sample (pT0), e.g., due to the ablative effect of TUR or even the effectiveness of neoadjuvant treatment. The life expectancy of these patients is more favorable based on literature data (23). pT0 tumor status occurred in 6.5% of our samples, the survival of these patients was the best amongst all stages, similar to the international multicenter results of Tilki et al (23).

PD-L1 expression and FGFR alterations 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 are mostly used in advanced urothelial cancer after platinum-based chemotherapy, regardless of biomarker analysis, but they are also effective and approved as a first choice in case of high PD-L1 (CPS–combined positive score or IC–immune cell score)

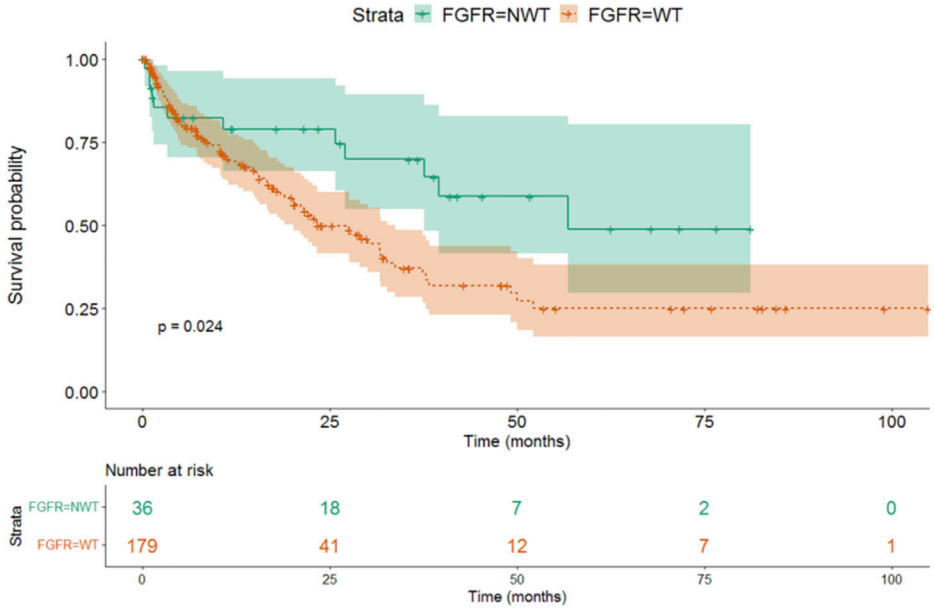

FIGURE 3
The effect of different FGFR mutations on survival.

TABLE 6 Connection with FGFR mutations and median survival.

FGFR	Median survival (months)	95% CI LL	95% CI UL	*p*-value
NWT	56.73	38.95	NA	0.024
WT	23.23	15.59	30.87	

CI, confidence interval; FGFR, fibroblast growth factor receptor; LL, lower limit; NA, not available; NWT, non-wild type; WT, wild type; UL, upper limit.

status (1, 3). In our work, PD-L1 status was determined based on TPS and CPS as well. Their effect on the outcome of the disease had a similar prognostic value. PD-L1 expression on urothelial tumor cells was associated with muscle-invasive disease and with worse overall survival (24).

TCGA project supports the high molecular heterogenity of MIBC such as in non-small cell lung cancer and in melanoma (25). The most common form of bladder cancer is NMIBC at diagnosis. Histologically these tumors are papillary tumors, they recur in more than the half of the cases, but have rare progression tendency (26). Approximately 70% of low-grade non-invasive papillary tumors show FGFR3 mutation in literature (27). In our study, patients with superficial bladder tumors (20%) who underwent cystectomy were included after a recurrence or if the disease could not be controlled by transurethral resection. Even in this higher-risk superficial group, the proportion of FGFR mutant patients was 37.2%, lower than in published data, but higher than in our analyzed muscle-invasive or locally advanced group (30). The strongest correlation could be observed between TNM stage and FGFR mutation. Our results represent the high frequency of FGFR3 mutation in earlier stages. Previous studies support our data, and it has been demonstrated that over half of pTa tumors recur, accordingly FGFR alteration is a possible signaling pathway in the development of these tumors (27). Compared to the literature (26), our findings suggest an oncogenic relationship, as FGFR3 mutation is mostly seen in non-invasive tumors, and less frequently at more advanced stages. Fernandez et al. found FGFR genomic alterations as an independent factor associated with the survival and as a relevant biomarker of mUC that may influence response to systemic therapy (28).

Advanced stage tumors have worse survival than early stage, and stratifying the patients according to TNM stage, we found also significant difference in survival from cystectomy between NWT and WT patients. Our data is similar to other published results that have reported an association between favorable prognosis and FGFR mutation status (29). The real prognostic effect of FGFR mutation is questionable because of the strong

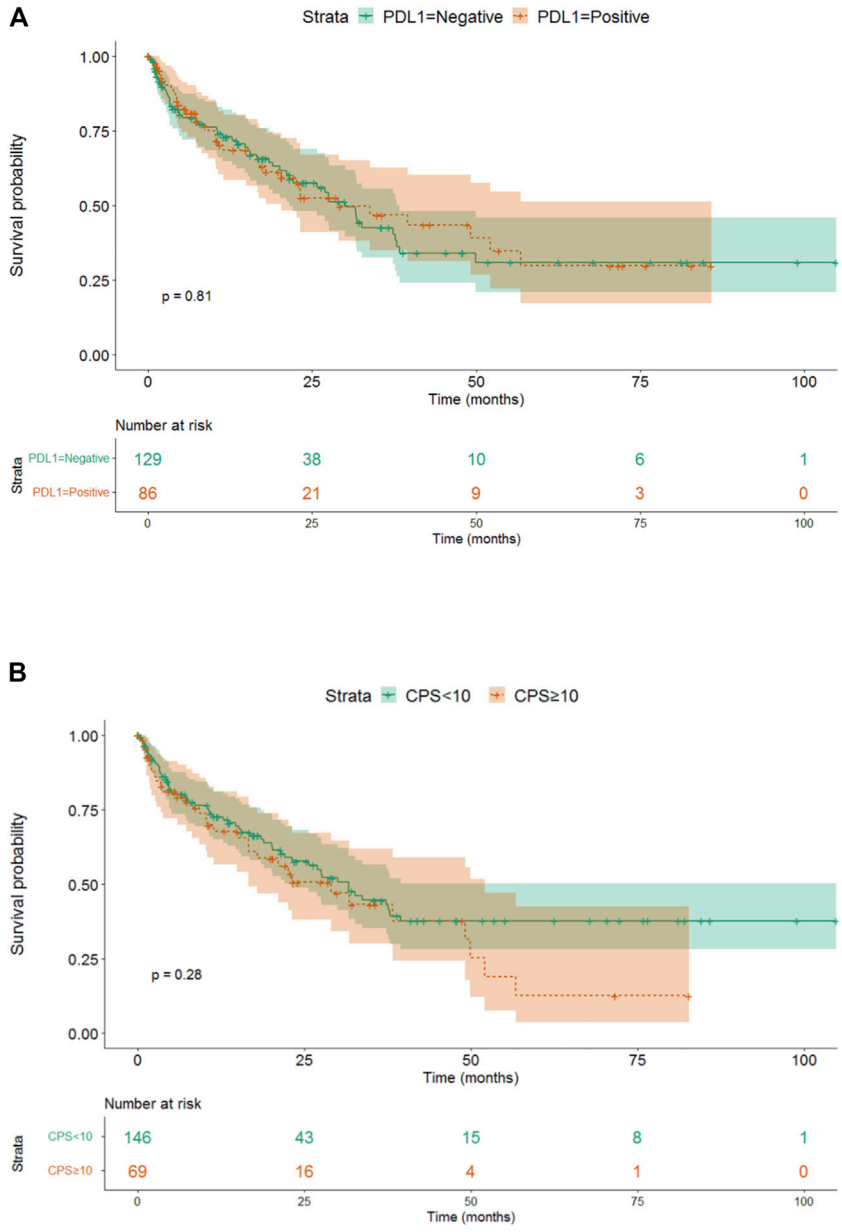

FIGURE 4
(A) The effect of different 256 PD-L1 expressions (TPS) on survival. **(B)** The effect of different PD-L1 expressions (CPS) on survival.

correlation with low stage tumors. Based on our results, FGFR alteration is not an independent prognostic parameter for survival, but occurs more often at lower stages, which is why it affects the overall survival of patients through the stage.

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, but the database analysis method and the limited number of elements found in each subgroup did not allow the matching of the individual elements and the adjustment of the data.

Even if there is an assumable connection between these results, there is no clear evidence that FGFR3 alteration would enhance a resistance mechanism against immune checkpoint inhibitors. Some previous studies verified mutated FGFR3 with increased FGFR3 gene expression and an association with decreased T-cell infiltration, but in this publication there was no significant difference in response rate or OS with

TABLE 7 Hazard ratios for the covariates in univariate and multivariate analysis.

Covariate	Univariate				Multivariate			
	HR	95% CI LL	95% CI UL	*p*-value	HR	95% CI LL	95% CI UL	*p*-value
TNM stage								
$pT0_{cyst}$	References							
St.0-I	1.612	0.348	7.469	0.541	1.612	0.348	7.469	0.541
St.II	3.156	0.721	13.803	0.127	3.156	0.721	13.803	0.127
St.III-IV	6.812	1.665	27.857	0.008	6.812	1.665	27.857	0.008
FGFR								
NWT	References							
WT	1.997	1.082	3.685	0.027	NA			0.718
PD-L1								
TPS < 1%	References							
TPS ≥ 1%	1.052	0.693	1.596	0.813	NA			0.231
CPS < 10	References							
CPS ≥ 10	1.265	0.825	1.938	0.281	NA			0.776

CI, confidence interval; CPS, combined positive score; FGFR, fibroblast growth factor receptor; HR, hazard ratio; LL, lower limit; NA, not available; NWT, non-wild type; PD-L1, programmed cell death ligand 1; St, stage; UL, upper limit; WT, wild type.

immunecheckpoint inhibitors in FGFR3 separated groups, possibly due to the lower stromal-mediated immune suppression (17). The controversial manifestation of FGFR3 and PD-L1 in various stages of the examined cystectomic samples in our study suggest a deeper stratification in molecular and immunological status in urothelial carcinomas.

The T-cell based subtyping of bladder cancers shows that tumors with high FGFR3 expression are associated with lower T-cell infiltration based on the count of the $CD8^+$ T-cells (16). These findings may propose a negative or immunosuppressive effect of FGFR3 alterations on T-cell gene mechanism. 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 (18).

Our aim was to investigate whether FGFR mutation is a possible independent prognostic factor of survival. Reflect on many controversial survival and response data in the anti-PD-L1 treated FGFR mutated patient group (15, 18, 30–33), according to other studies we consider a larger investigation of special non-invasive subtypes to be necessary in order to verify its predictive and prognostic value. In addition, we consider it forward-looking waiting for the results of the phase 3 prospective THOR (NCT03390504) study, which compares the effects of erdafitinib and pembrolizumab in patients with advanced mUC, to clarify the real therapeutic significance of FGFR alterations (34).

Limitations of our study include that we could not obtain retrospective relevant clinical data in almost 1/3 of the cystectomized patients, and molecular analysis was unsuccessful in 15% of the samples suitable for FGFR analysis. Another limitation is that although a strong correlation was detected between the individual investigated parameters, due to the limited number of elements of each subgroup, and the type of database analysis method, matching the individual elements and the adjustment of the data was not feasible.

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 (35). Another advantage of our work is that we also evaluated the CPS data in relation to PD-L1 expression, used better in the daily practice during first line immunotherapies nowadays, which would provide the opportunity for further potentially predictive conclusions. 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.

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, based on all of our results, the role of tumor stage can be highlighted as the strongest survival factor in this group of patients.

While molecular subtyping of urothelial cancers has yet to find its exact place in managing the disease, more and more data are being collected on the molecular profile of each subtype. The goal of newer clinical trials is to combine immunotherapy with modern, antigen-drug conjugates, and to find a place for targeted therapies against individual genetic abnormalities. Although immunotherapy is now the standard treatment for UBC, the frequency of FGFR3 alterations in NMIBC underscores the importance of a new molecular classification for the future of targeted therapy. FGFR inhibitors may represent an additional solution in the treatment of urothelial cancer, perhaps in a possible combination of immune and molecularly targeted therapies, or in halting the progression of early-stage FGFR-mutant tumors.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

"The genetic classification of urinary bladder cancer and research on molecular diagnostic and prognostic markers" study had the permission of the Hungarian Medical Research Council under (1011/16, 2017/EKU and 20090/2016 EKU) numbers. The samples were anonymized according to the Bio Bank Regulation (250/C-2/2017K.K) of the University of Szeged.

Author contributions

Study concept: AM, FS, LP, and JR; study design: AM, FS, and LP; data acquisition: JR, BP, TP, LV, AM, and FS; quality control of data and algorithms: AM and ZV; data analysis and interpretation: ZV, AM, and FS; statistical analysis: ZV; manuscript preparation: JR, BP, TP, LV, VP, ZV, FS, and AM; editing: JR, BP, ZV, and AM; manuscript review: JR, BP, LP, ZV, FS, and AM.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.por-journal.com/articles/10.3389/pore.2023.1611077/full#supplementary-material

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Dose escalation can maximize therapeutic potential of sunitinib in patients with metastatic renal cell carcinoma

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Abstract

Background: In patients with metastatic renal cell cancer, based on limited evidence, increased sunitinib exposure is associated with better outcome. The survival and toxicity data of patients receiving individualized dose escalated sunitinib therapy as compared to standard management were analyzed in this study.

Methods: From July 2013, the data of metastatic renal cell cancer patients with slight progression but still a stable disease according to RECIST 1.1 criteria treated with an escalated dose of sunitinib (first level: 62.5 mg/day in 4/2 or 2 × 2/1 scheme, second level: 75 mg/day in 4/2 or 2 × 2/1 scheme) were collected prospectively. Regarding characteristics, outcome, and toxicity data, an explorative retrospective analysis of the register was carried out, comparing treatments after and before July 1, 2013 in the study (selected patients for escalated dose) and control (standard dose) groups, respectively.

Results: The study involved 103 patients receiving sunitinib therapy with a median overall and progression free survival of 25.36 ± 2.62 and 14.2 ± 3.22 months, respectively. Slight progression was detected in 48.5% of them. First and second-level dose escalation were indicated in 18.2% and 4.1% of patients, respectively. The dosing scheme was modified in 22.2%. The median progression free survival (39.7 ± 5.1 vs 14.2 ± 1.3 months ($p = 0.037$)) and the overall survival (57.5 ± 10.7 vs 27.9 ± 2.5 months ($p = 0.044$)) were significantly better in the study group (with dose escalation) than in the control group. Patients with nephrectomy and lower Memorial Sloan Kettering Cancer Center (MSKCC) scores showed more favorable outcomes. After dose escalation, the most common adverse events were worsening or development of fatigue, hypertension, stomatitis, and weight loss of over 10%.

Conclusions: Escalation of sunitinib dosing in selected patients with metastatic renal cell cancer, especially in case of slight progression, based on tolerable toxicity is safe and improves outcome. Dose escalation in 12.5 mg steps may be recommended for properly educated patients.

Keywords: Metastatic renal cell cancer, Sunitinib, Dose escalation, Improved outcome, Toxicity

Background

Sunitinib malate, an oral multi-targeted tyrosine kinase inhibitor (TKI) is considered to be one of the standard first-line therapeutic options in metastatic renal cell cancer (mRCC) [1]. It is a small molecule indolinone [2] which binds directly to the kinase domain of receptor tyrosine kinases (RTKs) within an adenosine triphosphate (ATP) binding pocket between two lobes of the KIT kinase domain, preventing phosphorylation and activation [3–5]. It selectively targets RTKs, which are important in RCC. Sunitinib has direct anti-tumor effects via binding the unactivated conformation of KIT and via platelet-derived growth factor receptor alpha polypeptide (PDGFRA) inhibition. The dual inhibitor activity against vascular endothelial growth factor receptors 1 and 3 (VEGFR 1 and VEGFR3), and platelet-derived growth factor receptor beta polypeptide (PDGFRB) on endothelial and pericyte membranes enhances anti-angiogenesis [6].

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Sunitinib has been approved by the regulatory authorities after it had been demonstrated to improve progression-free survival (PFS), overall survival (OS), objective response rate (ORR), and quality of life compared with interferon-alpha in previously untreated metastatic RCC patients [1, 7–9]. According to the international guidelines (e.g., NCCN, ESMO, EAU), sunitinib can be used as first-line treatment in patients with advanced or metastatic dominantly clear cell histological type RCC whose condition has good or intermediate prognosis [10–12]. Sunitinib has become the gold standard first-line therapy of mRCC in the past decade, and it has been used worldwide in this patient population in wider indications as well [10–16].

The therapeutic administration of sunitinib and the dedicated patient population for this drug would be changing and would be refined in the near future. The preliminary results of the presented Checkmate-214 phase 3 trial with respect to mRCC, in which sunitinib was the comparator of the investigated drugs [17], the survival rates were more favorable in case of the immune checkpoint inhibitor nivolumab and ipilimumab combination compared to sunitinib administered alone, in poor and intermediate risk groups.

The standard treatment schedule of sunitinib is 50 mg for 28 days with a 14-day break [13–15]. Alternate scheduling (2 weeks on/1 week off) can also be used to manage toxicity, but currently no robust data are available supporting it [16]. 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 [18]. At higher sunitinib doses, the direct anticancer effect of the drug may be predominant.

Despite the efficacy of sunitinib therapy, the condition of initially responding patients may progress due to the acquired resistance. The underlying mechanisms for that may be the continuous VEGF axis activation via upstream or downstream effectors [19–22], b-fibroblast growth factor (bFGF), c-met, interleukin-8 (IL-8), and angiogenic cytokine pathways [23], altered pharmacokinetics, drug sequestration [24], and epithelial to mesenchymal transition [25]. Drug resistance is associated with a transient increase in tumor vasculature and epigenetic changes in histone proteins in the chromatin, which contribute to tumor angiogenesis by inactivating the anti-angiogenic factors [26]. However, the drug-induced resistance can be overcome by sunitinib dose escalation [26]. If patients tolerate the standard regimen, the increased sunitinib exposure is associated with longer PFS, OS, and a higher response rate [27, 28].

The aim of our study was to analyze the maximal efficiency and the side-effects of escalated dose sunitinib for metastatic RCC in the everyday practice.

Methods

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 (1 or 2 from the following 5 unfavorable factors: 1. time from diagnosis to systemic treatment < 1 year; 2. hemoglobin < lower limit of normal level; 3. calcium > 10 mg/dL or 2.5 mmol/L; 4. LDH > 1.5 x upper limit of normal; 5. Karnofsky performance status < 80%) [1, 18] were treated with sunitinib between January 2010 and December 2016. The study was performed in accordance with the Hungarian and the EU drug law and relevant medical and financial guidelines of the Hungarian health authorities. The study was approved by the regional ethics committee (registration number WHO 3482/2014).

The patients received first-line sunitinib after having undergone nephrectomy or kidney biopsy and embolization if nephrectomy was not feasible. Histological and staging examinations, such as abdominal and chest CT (and bone scintigraphy and skull CT if clinically indicated), were performed before initiating the therapy.

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. Physical and laboratory examinations were performed 2 to 4 weeks after the initiation of sunitinib therapy, and once every 6 weeks thereafter, while imaging examination, cardiac and thyroid gland function follow-ups were performed every 12 weeks. 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), or therapeutic delay occurred due to the following reasons: grade 3/4 thrombocytopenia, neutropenia, hand–foot syndrome affecting walking, stomatitis or diarrhea of grade 3/4, which significantly influenced the nutrition or resulted in > 10% weight loss, hypertension of grade 3/4 developing despite being on combined antihypertensive therapy. 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) [29]. The general condition of the patients was assessed according to the Karnofsky scale [30]. PFS and OS were defined from the onset of the medical treatment to the date of progression based on RECIST 1.1 or death, respectively. The evaluation of

tumor response was performed every 12 weeks according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. Sunitinib therapy was discontinued in case of progression per the RECIST criteria in all cases (compared to best response). If the CT indicated slight progression (SP) but still corresponded to stable disease according to the RECIST 1.1 criteria [31] 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 (Fig. 1).

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. The outcome was analyzed according to the characteristics of the patients of the two groups as well as the side effects and other factors that could influence the escalation of the dose.

Statistical analysis

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 (dose escalation, dose reduction, therapeutic lines after sunitinib, nephrectomy, and treatment group), and patient-related factors (gender, MSKCC score) on PFS and OS was analyzed with Kaplan–Meier analysis. To compare the median follow up times between control and study groups, the Mann-Whitney U Test was used. To determine the differences between the control and study groups, independent sample t-test and chi-square test were used for the continuous and categorical variables, respectively. To detect the independent role of nephrectomy and DE 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).

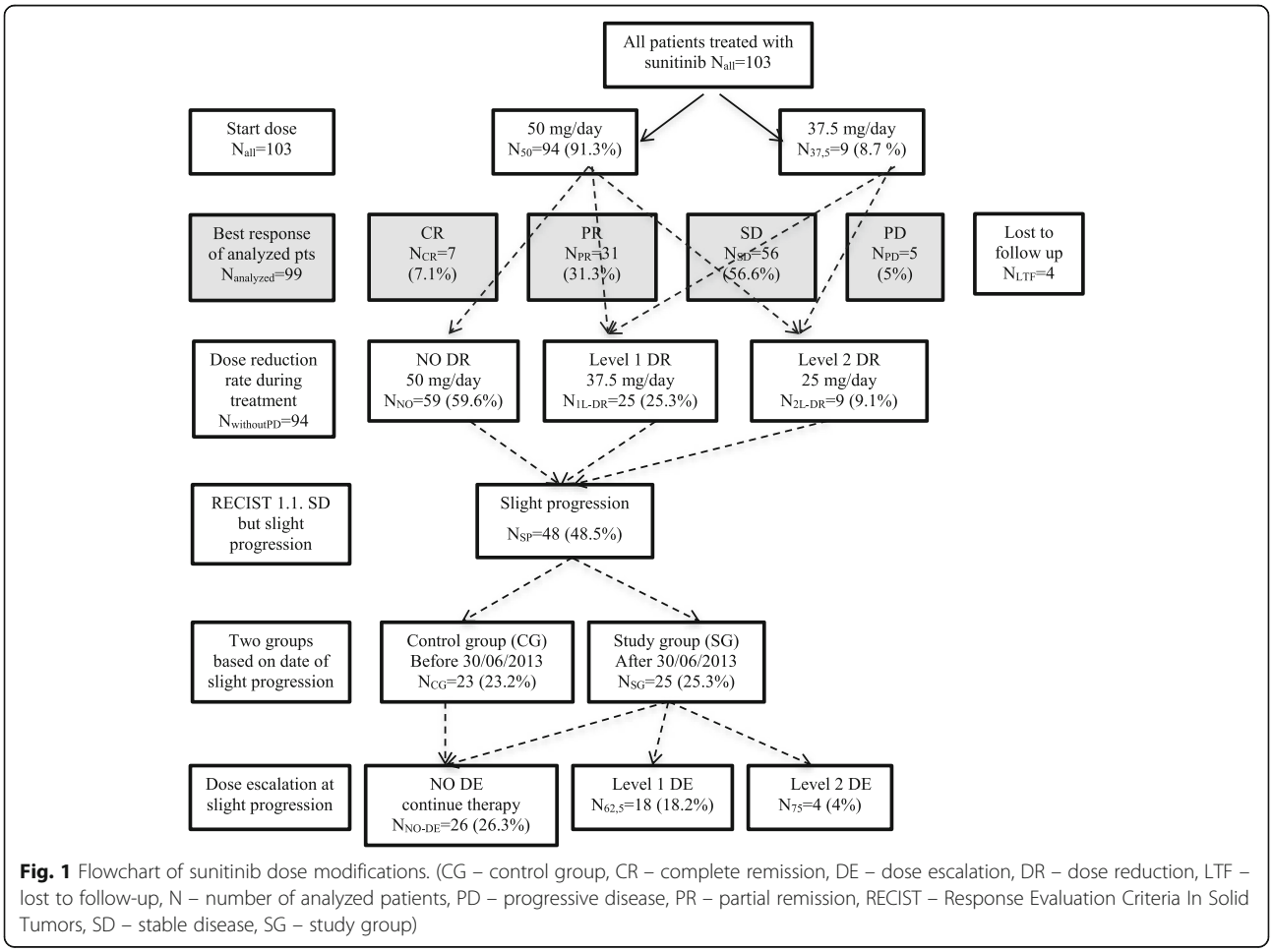

Fig. 1 Flowchart of sunitinib dose modifications. (CG – control group, CR – complete remission, DE – dose escalation, DR – dose reduction, LTF – lost to follow-up, N – number of analyzed patients, PD – progressive disease, PR – partial remission, RECIST – Response Evaluation Criteria In Solid Tumors, SD – stable disease, SG – study group)

Results

Patient characteristics

Out of the 103 patients who participated in the study, 80 (77.7%) were men and 23 (22.3%) were women (Table 1). The mean ± standard error (±SE) age was 62.27 ± 0.9 (range, 32–80) years, and 84.5% of the patients had undergone nephrectomy. The mean (±SE) MSKCC score was 1.7 ± 0.05, and the mean number of metastatic sites was 2.32 ± 0.11 (range, 1–5). Lungs, bone and distant lymph nodes were the most frequent localizations of metastases (Table 1). 68% of the patients had a comorbidity that required treatment. Hypertension, other cardiovascular disorders, and diabetes were the most common diseases. Hyperthyroidism and well-managed hypertension at the beginning of the therapy occurred in 5 (4.9%) and 32 (31.1%) patients, respectively. The rate of secondary tumors was relatively high (8.7%) as well as the rate of primary bone metastasis (45.6%). Mean ± SE value of baseline LVEF was 61.7 ± 3.2%. The histological type of the tumors was mainly clear cell renal cell cancer (ccRCC) in case of all patients, and in most cases pure ccRCC. No rare variants could be detected, but only sarcomatoid, papillary and chromophobe morphologies, and transformations in the ccRCC were present. No genetic analyses were performed to prove the familial origin of the renal cancer. The baseline characteristics of the patients are presented in Table 1.

Table 1 Baseline demographics of all patients and of patients with slight progression

Patients	$N_{all} = 103$		$N_{SP} = 48$	
Mean age, years ± SE	62.27 ± 0.9		61.76 ± 1.62	
Age range, years	32–80			
MSKCC score, mean ± SE	1.7 ± 0.05		1.6 ± 0.1	
Gender				
Male	80 (77.7%)		39	81.3%
Female	23	23 (22.3%)	9	18.7%
Number of patients after nephrectomy	87	84.5%	42	87.5%
Comorbidities				
Hypertension	32	31.1%	9	18.8%
Other cardiovascular disorders	12	11.6%	5	10.4%
Diabetes	11	10.7%	4	8.3%
Secondary tumors	9	8.7%	1	2%
Hyperthyroidism	5	4.9%	0	0%
Hematological disease	3	2.9%	0	0%
Psoriasis	2	1.9%	0	0%
Metastases				
Mean number of metastatic sites (range)	2.32 ± 0.11 (1–5)		1.79 ± 0.1 (1–3)	
Location of metastases				
Lungs	84	81.6%	39	81.2%
Bone	47	45.6%	16	33.3%
Distant lymph node	36	34.9%	20	41.7%
Liver	19	18.4%	7	14.6%
Brain	11	10.7%	0	0%
Suprarenal gland	9	8.7%	4	8.3%
Other (peritoneum, pleura, pancreas, local relapse, contralateral kidney, or thyroid gland)	–	‹8%	–	‹4%
Patients with synchronous metastases	94	91.2%	45	93.8%
Histopathological types			n	%
Purely clear cell renal cell type (ccRCC)	91	88.3%	46	95.8%
ccRCC with sarcomatoid morphology	7	6.8%	1	2%
ccRCC with papillary–/chromophobe–/ both	3 / 2 / 1	2.9 / 1.9 / 1.0%	1/0/0	2/0/0%

ccRCC clear cell renal cell cancer, *MSKCC* Memorial Sloan Kettering Cancer Center, *n* number of involved patients, *N* number of analyzed patients, *SE* standard error

Sunitinib dose parameters and efficiency

No dose reduction (DR) had to be applied in 59 (59.6%) patients (50 mg/day in 4/2 or 2 × 2/1 scheme or 37.5 mg daily dose administered continuously in 2 cases). First-level (37.5 mg/day in 4/2 or 2 × 2/1 scheme) and second-level (25 mg daily dose in 4/2 or 2 × 2/1 scheme) dose reductions were required during the treatment in 25 (25.3%) and 9 (9.1%) cases, respectively. Sunitinib therapy had to be ultimately ceased within 12 weeks in 5 (5%) patients due to progression of the disease. The follow-up of four patients was incomplete; thus, their data were excluded from the final analyses.

The dosing scheme was modified (DSM) in case of 22 (22.2%) patients. A cycle delay of more than 7 days was needed in 15 (15.1%) patients because of an infection, herniotomy, dental intervention, diarrhea, neutropenia, or cardiac decompensation. Mean ± SE duration of the delay was 7.8 ± 3.3 days. 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, the result of radiological revision according to RECIST 1.1 was stable disease in 48 (48.5%) cases. First-level (62.5 mg/day in 4/2 or 2 × 2/1 scheme) and second-level (75 mg daily dose in 4/2 or 2 × 2/1 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. Sunitinib therapy is still continued in 10 (10.1%) patients, and 5 patients underwent metastasectomy; their sunitinib therapy was discontinued and rechallenged in 3 (3%) of them. After progression on sunitinib therapy, no further therapy was administered in 30 (30.3%) cases, while in 47 (47.4%) and 5 (5.1%) patients, one and two therapy lines were applied, respectively.

Factors influencing efficacy

PFS and OS were not influenced by the patients' age, gender, the number/type of metastatic organ systems, and dose reduction in the overall population. Patients with nephrectomy and lower MSKCC scores showed more favorable outcomes in the studied population (Table 2).

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

Table 2 Factors influencing the outcome of sunitinib therapy in all patients

Specifications of analyzed patients $N = 99$	PFS-HR (95% CI)	*p*	OS-HR (95% CI)	***p***
Age	1.012 (0.987–1.038)	0.351	1.007 (0.981–1.035)	0.590
Number of metastatic organs	1.083 (0.891–1.317)	0.423	1.100 (0.896–1.350)	0.364
	PFS-HR (95% CI)	*p*	OS-HR (95% CI)	***p***
Gender				
man/ woman	1 / 1.367 (0.807–2.316)	0.245	1 / 1.388 (0.792–2.435)	0.252
MSKCC score				
0 / 1 / 2	1 / 3.770 (1.345–28.435) / 6.693 (1.813–49.061)	**0.019**	1 / 2.692 (1.355–20.445) / 5.199 (1.713–37.929)	**0.023**
Dose reduction				
Yes / No	1 / 1.492 (0.947–2.506)	0.065	1 / 1.553 (0.963–2.504)	0.071
Nephrectomy				
Yes / No	1 / 2.702 (1.508–4.840)	**0.001**	1 / 3.189 (1.741–5.842)	**< 0.001**
Dose escalation				
Yes / No	1 / 2.665 (1.486–4.780)	**0.001**	1 / 3.157 (1.613–6.179)	**0.001**
Dose scheme modification				
Yes / No	1 / 2.569 (1.437–4.595)	**0.001**	1 / 2.444 (1.288–4.636)	**0.006**
Therapeutic lines after sunitinib				
2 / 1 / 0	NA	NA	1 / 7.731 (2.318–25.787) / 4.043 (1.228–13.311)	**0.001**

Bold *p*-values are significant ‹0.05, *HR* hazard ratio, *MSKCC* Memorial Sloan Kettering Cancer Center, *mOS* median overall survival, *mPFS* median progression-free survival, *NA* not applicable, *OS* overall survival, *p* *p*-value, *PFS* progression-free survival, *SE* standard error

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 (Table 2) (Fig. 2).

The PFS and OS results of patients with SP who underwent radiological revision and showed to have a stable disease (48 patients), did not influence the number of metastatic sites, the MSKCC score, and the dose reduction. Age and gender of the patients did not influence the OS. PFS was longer in case of younger male patients. PFS and OS were more favorable if patients underwent nephrectomy, in case of DE and DSM (Table 3).

Influence of dose escalation on effectivity

There were 23 patients in the control group (they underwent radiological revision before June 30, 2013 and showed slight progression) and 25 patients in the study group (they underwent radiological revision after June 30, 2013). The following factors were similar in the two groups: patients' age, gender, MSKCC score, number of metastatic sites, time elapsed from diagnosis, serum calcium level, LDH, hemoglobin, Karnofsky performance status, DR and DSM. All patients underwent nephrectomy in the study group, whereas it was performed in 17 out of 23 patients in the control group ($p = 0.008$). Dose escalation was only performed in the study group. It could be performed in case of 18 patients (72.0%), but it could not be carried out in 7 cases (28.0%). 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 (Table 4). The median follow-up time of the cohort with slight progression was 37.3 (11.17–93.83) months.

Because of the higher rate of nephrectomy and DE in study group, a multivariate analysis was performed to detect the real effect of these factors. 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 ($p_{DE} = 0.083$).

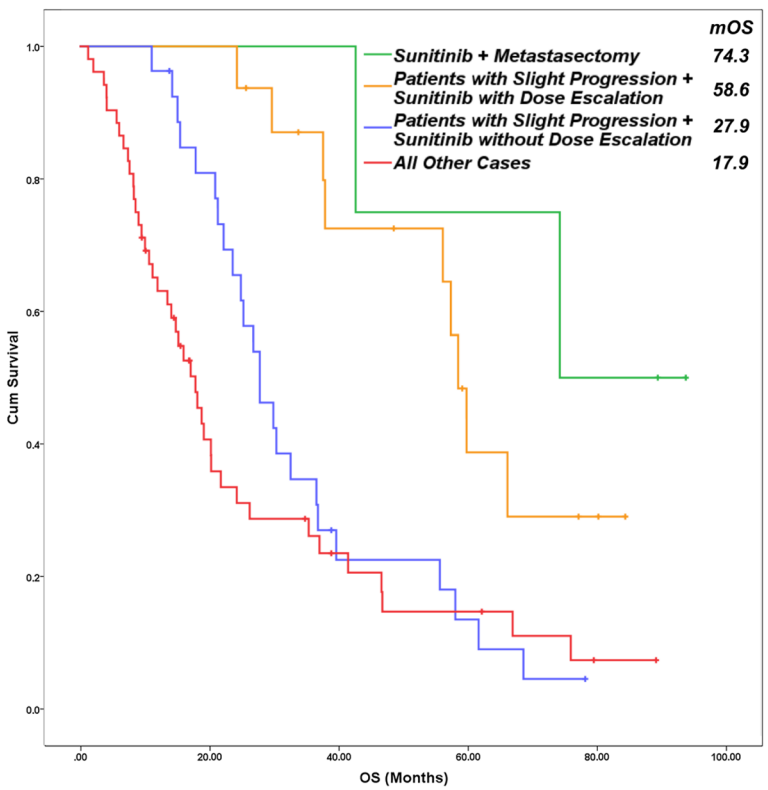

Fig. 2 Overall survival of patients in four subgroups. Metastasectomy after an effective sunitinib therapy caused the most favorable overall survival (74.3 months). Median survival of patients with slight progression is longer with dose escalation (58.6 months) than without it (27.9 months), or the outcome of all other patients (17.9 months) (p<0.001). (Cum – cumulative, OS – overall survival)

Table 3 Factors influencing the outcome of sunitinib therapy in SP cases

Specifications of all patients with slight progression $N = 48$	PFS-HR (95% CI)	*p*	OS-HR (95% CI)	***p***
Age	1.047 (1.008–1.089)	**0.019**	1.025 (0.982–1.069)	0.265
Number of metastatic organs	1.159 (0.873–1.538)	0.307	1.107 (0.820–1.494)	0.508
	PFS-HR (95% CI)	*p*	OS-HR (95% CI)	***p***
Gender				
man/woman	3.202 (1.473–6.962)	**0.003**	2.077 (0.891–4.846)	0.091
MSKCC score				
0 / 1 / 2	1 / 3.671 (0.474–28.414) / 5.304 (0.709–39.661)	0.176	1 / 2.965 (0.375–23.430) / 3.841 (0.513–28.786)	0.366
Dose reduction				
Yes / No	1 / 0.840 (0.450–1.570)	0.585	1 / 0.724 (0.365–1.436)	0.356
Nephrectomy				
Yes / No	1 / 3.397 (1.364–8.461)	**0.009**	1 / 5.583 (2.135–14.601)	**< 0.001**
Dose escalation				
Yes / No	1 / 2.383 (1.241–4.578)	**0.009**	1 / 2.479 (1.185–5.183)	**0.016**
Dose scheme modification				
Yes / No	1 / 2.373 (1.034–5.445)	**0.041**	1 / 2.583 (1.008–6.709)	**0.047**
Therapeutic lines after sunitinib				
2 / 1 / 0	NA	NA	1 / 6.163 (1.582–24.016) / 3.873 (1.130–13.280)	**0.032**

Bold *p*-values are significant ‹0.05, *HR* hazard ratio, *MSKCC* Memorial Sloan Kettering Cancer Center, *mOS* median overall survival, *mPFS* median progression-free survival, *NA* not applicable, *OS* overall survival, *p* p-value, *PFS* progression-free survival, *SE* standard error, *SP* slight progression

The impact of dose escalation on the adverse effects

After dose escalation, the most common adverse effects were the following: worsening or development of fatigue, hypertension, stomatitis, and weight loss (over 10%) (Table 5). The most upgraded clinical parameters were fatigue and development or worsening of hypertension as a result of the increased sunitinib dose.

Discussion

Sunitinib is one of the most frequently applied first line therapies in patients with metastatic ccRCC with MSKCC good and moderate prognoses.

The role of cytoreductive nephrectomy seems to be equivocal in the era of tyrosine-kinase inhibition. The results of the SURTIME study were presented by Bex et al. last year, in which the overall survival and post surgical complication rates were better with deferred versus immediate cytoreductive nephrectomy, while progression rates at 16 and 28 weeks were not significantly different between both sequences [32]. The ongoing CARMENA study (NCT00930033) may give an answer to this issue in the near future.

According to the recent knowledge, nephrectomy is recommended to be performed in patients in good general condition before the systemic therapy; however, randomized studies analyzing survival data have been performed only in combination with INFα therapy [33–35]. In our study, nephrectomy was performed in 84.8% of the cases, and PFS and OS results of these patients were more favorable. Each patient with SP in the Study group (period 2) underwent nephrectomy (which means that the patients were fit enough for this operation). It might have been a potential selectional bias of the compared cohorts. However, the other parameters and the comorbidities of the patients in the two cohorts were not significantly different.

In our study, PFS was longer than in the registration study [8]; however, patients with MSKCC poor prognosis were excluded from our study, but the PFS of our patients was similar to the excellent international data [36, 37]. Nowadays, the median OS of patients with metastatic RCC is longer than 2 years [1], 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 [38]. The recommended starting dose for sunitinib malate is 50 mg daily for 28 days followed by a 14-day break. Although individualized sunitinib therapy improves the outcome, poorer outcomes in patients tolerating the standard schedule treatment without significant toxicity [1, 14] may be the result of underdosing [27]. Several authors [39, 40] have

Table 4 Characteristics and results of patients with slight progression in the control and study groups

Specifications of patients with slight progression $N_{SP} = 48$	Control group Before June 30, 2013 $N_{CG} = 23$	Study group After June 30, 2013 $N_{SG} = 25$	*p*
Mean age, years ± SE	62.87 ± 1.73	60.74 ± 1.52	0.358
Gender			
male	17 (73.9%)	22 (88.0%)	0.190
female	6 (26.1%)	3 (12.0%)	
MSKCC score, mean ± SE	1.61 ± 0.1	1.60 ± 0.1	0.952
Number of metastatic sites, mean ± SE	2.17 ± 0.24	2.36 ± 0.21	0.559
Location of metastases			
Lungs	19 (82.6%)	20 (80%)	0.556
Bone	7 (30.4%)	9 (36%)	0.460
Distant lymph node	8 (34.8%)	12 (48%)	0.263
Liver	3 (13%)	4 (16%)	0.549
Suprarenal gland	1 (4.3%)	3 (12%)	0.337
Comorbidities			
Hypertension	4 (17.4%)	5 (20%)	0.556
Other cardiovascular disorders	2 (8.7%)	3 (12%)	0.541
Diabetes	2 (8.7%)	2 (8%)	0.663
Secondary tumors	0	1	0.521
Nephrectomy			
No	6 (26.1%)	0 (0.0%)	**0.008**
Yes	17 (73.9%)	25 (100.0%)	
Time from diagnosis to initiation of sunitinib			
< 1 year	11 (47.8%)	15 (60.0%)	0.289
> 1 year	12 (52.2%)	10 (40.0%)	
Hemoglobin level			
< normal range	6 (26.1%)	3 (12.0%)	0.190
> normal range	17 (73.9%)	22 (88.0%)	
Elevated corrected calcium level			
> 2.5 mmol/L	2 (8.7%)	1 (4.0%)	0.468
< 2.5 mmol/L	21 (91.3%)	24 (96.0%)	
Elevated LDH level			
> 1.5× normal level	2 (8.7%)	0 (0.0%)	0.224
< 1.5× normal level	21 (91.3%)	25 (100.0%)	
Elevated corrected calcium level			
> 2.5 mmol/L	2 (8.7%)	1 (4.0%)	0.468
< 2.5 mmol/L	21 (91.3%)	24 (96.0%)	
Karnofsky performance status			
< 80	0 (0.0%)	1 (4.0%)	0.521
≥ 80	23 (100.0%)	24 (96.0%)	
Dose reduction rate			
No	10 (43.5%)	16 (64.0%)	0.226
Level 1 (37.5 mg)	11 (47.8%)	6 (24.0%)	
Level 2 (25 mg)	2 (8.7%)	3 (12.0%)	

Table 4 Characteristics and results of patients with slight progression in the control and study groups *(Continued)*

Specifications of patients with slight progression $N_{SP} = 48$	Control group Before June 30, 2013 $N_{CG} = 23$	Study group After June 30, 2013 $N_{SG} = 25$	*p*
Dose escalation rate			
No	23 (100.0%)	7 (28.0%)	**< 0.001**
Level 1 (62.5 mg)	0 (0.0%)	14 (56.0%)	
Level 2 (75 mg)	0 (0.0%)	4 (16.0%)	
Dosing scheme modification			
No	19 (82.6%)	18 (72.0%)	0.300
Yes	4 (17.4%)	7 (28.0%)	
Therapeutic lines after sunitinib 0 / 1 / 2 (%)	6 (30) / 13 (65) / 1 (5)	3 (15) / 13 (65) / 4 (20)	0.247
mOS after sunitinib therapy	9.33 ± 2.0	9.76 ± 2.5	0.599
mPFS	14.2 ± 1.3	39.7 ± 5.1	**0.037**
mOS	27.9 ± 2.5	57.5 ± 10.7	**0.044**
median follow-up time (range) (months)	30.9 (11.2–89.5)	45.7 (13.9–84.5)	**0.061**

Bold *p*-values are significant ‹0.05, *mOS* median overall survival, *mPFS* median progression-free survival, *MSKCC* Memorial Sloan Kettering Cancer Center, *N* number of analyzed patients, *p* p-value, *SE* standard error, *SP* slight progression

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 [41]. According to the meta-analysis of Houk et al. [28], escalated sunitinib exposure (area under the curve) 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 after the summer of 2013 in cases with slight progression, when RECIST 1.1 results confirmed a stable disease if any clinically relevant side effects occurred. 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 trials of sunitinib [8], which might reflect an outstanding benefit from sunitinib mainly in patients with low tumor volume in our studied cohort. After an initial favor tumor response evolving slight progression can be stopped or be reversible with dose escalation and adequate titration has been hypothesized. Drug toxicity and efficacy may depend on the interindividual differences in pharmacokinetics,

Table 5 New or intensifying adverse effects in patients after dose escalation

New or intensifying adverse effects $N_{DE} = 22$	Number of patients (percent)			
	Any grade	Grade 1	Grade 2	Grade 3
All	21 (95.5%)	17 (77.3%)	3 (13.6%)	1 (4.5%)
Fatigue	9 (40.9%)	7 (31.8%)	2 (9.1%)	0
Development / worsening of hypertension	8 (36.4%)	7 (31.8%)	1 (4.5%)	0
Stomatitis	6 (27.3%)	5 (22.7%)	1 (4.5%)	0
Diarrhea	5 (22.7%)	3 (13.6%)	1 (4.5%)	1 (4.5%)
Weight loss 10%≤	4 (18.2%)	4 (18.2%)	0	0
Hand–foot syndrome	4 (18.2%)	4 (18.2%)	0	0
Eyelid edema	2 (9.1%)	2 (9.1%)	0	0
Hypothyroidism	1 (4.5%)	1 (4.5%)	0	0
Elevation in creatinine level	5 (18.2%)	4 (18.2%)	1 (4.5%)	0
Thrombocytopenia	4 (18.2%)	2 (9.1%)	2 (9.1%)	0
Anemia	3 (13.6%)	2 (9.1%)	1 (4.5%)	0
Neutropenia	2 (9.1%)	1 (4.5%)	1 (4.5%)	0

pharmacodynamics, and pharmacogenetics [42, 43]; however, Motzer et al. [14] have not found correlation between sunitinib pharmacokinetic values and the toxicity profile. Adelaiye et al. [26] have detected an increase in sunitinib plasma concentration in animals treated with escalated dose TKI in the drug resistant group, and also a trend for decreased plasma concentration after prolonged sunitinib exposure. Gotink et al. [24] have found 1.7 to 2.5-fold increase in sunitinib concentration in resistant tumor cells due to the increased lysosomal drug sequestration, which was reversible after the removal of sunitinib from the cell culture. Blood levels of sunitinib reach a steady state at 10 to 14 days, and a maximum value on day 14 [27], and disease progression usually occurs during treatment interruption [44, 45]. In the retrospective analysis of Bjarnason et al. [27], an individualized treatment strategy and shorter treatment break (14 days on and 7 days off) have resulted in improved PFS and OS as compared to the standard sunitinib schedule, and the PFS detected in patients with ccRCC has been one of the best reported for any TKI. Modified sunitinib schedule is well tolerated and induces optimal drug exposure [46].

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. As the two patient populations are not the same, their effects can be considered independent. Dose escalation can be performed in case of patients with good general condition, who do not have any relevant adverse effects. In case of these patients, based on the prognostic values, the survival rate is potentially better. Therefore, we compared the two (almost similar) groups regarding dose escalation, so selection of patients with better prognosis could not have queried the results. The effect of dose escalation on PFS and OS was confirmed during the comparison of the two groups. No significant difference was found among the number of the subsequent therapies and mOS after sunitinib was equal in two groups as well, which may be because in our country the availability of more active new regimens was very limited during our study period.

The rate of adverse events (AE) in our real world dose escalated patients is lower in the selected cohort than the AE rate in patients administered the standard dose in the pivotal trials [8, 9]. 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.

Acquired resistance to sunitinib therapy, driven by several likely mechanisms, is a central issue in the treatment of metastatic RCC patients. However, drug resistance may be reversible, and gradual dose escalation may restore tumor sensitivity to sunitinib, as reported in preclinical and clinical studies as well. Adelaiye et al. [26] have treated mice with patient-derived xenografts 5 days/week with a 40–60-80 mg/kg sunitinib dose increase schedule, and they have found selected intrapatient dose escalation safe, resulting in prolonged PFS due to a greater and longer effect on tumor regression. Although xenografts initially responsive to 40 mg/kg sunitinib developed drug resistance, it could be overcome by incremental dose escalation. In metastatic RCC patients on standard schedule sunitinib with early disease progression, Adelaiye et al. [26] could increase sunitinib dose from 50 to 62.5 and 75 mg daily, with a 14-day on and 7-day off treatment scheme to some type of grade 2 toxicity, and they observed clinical benefit in the majority of the patients. As reported by Mitchell et al. [47], the daily dose of sunitinib can be safely up-titrated to 87.5 mg. According to Gotink et al. [24] and Zama et al. [48], sunitinib rechallenging in previously resistant patients also has a therapeutic value. Drug resistance is also associated with epigenetic changes in histone proteins in the chromatin, which may be reversible upon DE; thus, epigenetic therapies could be successful in ccRCC patients [26].

The limitations of our study are, on the one hand, its retrospective design, that is, an explorative retrospective analysis of a prospective RCC register, and on the other hand, the relatively small number of patients involved.

Conclusion

In 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.

Abbreviations

ATP: Adenosine triphosphate; bFGF: b-fibroblast growth factor; ccRCC: Clear cell renal cell carcinoma; CT: Computed tomography; DE: Dose escalation; DR: Dose reduction; DSM: Dose scheme modification; EAU: European Association of Urology; ESMO: European Society for Medical Oncology; HR: Hazard ratio; IL: Illinois; IL-8: Interleukin-8; LDH: Lactate dehydrogenase; LVEF: Left ventricular ejection fraction; mOS: Median overall survival; mPFS: Median progression-free survival; mRCC: Metastatic renal cell carcinoma; MSKCC: Memorial sloan kettering cancer center; NA: Not Applicable; NCCN: National Comprehensive Cancer Network; NCI CTCAE v4.0: National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0; ORR: Overall response rate; OS: Overall survival; PDGFRA: Platelet-derived growth factor receptor alpha; PDGFRB: Platelet-derived growth factor receptor beta; PFS: Progression-free survival; RCC: Renal cell carcinoma; RECIST: Response evaluation criteria in solid tumors; RTK: Receptor tyrosine kinase; SE: Standard error; SP: Slight progression; TKI: Tyrosine kinase inhibitor; USA: United States of America; VEGFR: Vascular endothelial growth factor receptor

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
AM was responsible for study design, treatment of patients, collection, analysis and interpretation of data, and was a major contributor in writing the manuscript. AC evaluated CT scans according to RECIST 1.1. GU, ES and JR were involved in treating the patients, acquisition and interpretation of data. ZV was responsible for performing statistical analyses and preparing the figures and Tables. RK, JR and LV were involved in drafting the article and revising it critically for important intellectual content. ZK, the GUARANTOR of the article, was responsible for the conception of the study, the interpretation of data, and drafted the article. All authors had participated sufficiently in the work to take public responsibility for appropriate portions of the content, agreed to be accountable for all aspects of the work, read and approved the final manuscript.

Ethics approval and consent to participate
All the procedures performed were in full accordance with the ethical standards of the appropriate national and institutional committees on human experimentation and with the Helsinki Declaration. The study was approved by the Regional Human Biomedical Research Ethics Committee, Albert Szent-Györgyi Health Center, University of Szeged, Hungary (registration number: WHO 3482/2014). The enrolled patients gave their written informed consent before being registered in the study.

Consent for publication
Not applicable.

Competing interests
Anikó Maráz has received honoraria from Bayer, Bristol-Myers Squibb, and has served on advisory boards for Novartis. János Révész has served on advisory boards for Novartis and Pfizer. *Adrienn Cserháti*, *Gabriella Uhercsák*, *Éva Szilágyi*, *Zoltán Varga*, *János Révész*, *Renáta Kószó*, *Linda Varga*, *Zsuzsanna Kahán* declares that they have no competing interests.

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ORIGINAL ARTICLE

Assessment of the Role of Everolimus Therapy in Patients with Renal Cell Carcinoma Based on Daily Routine and Recent Research Results

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Abstract Everolimus is indicated for adults with metastatic renal cell carcinoma (mRCC) after failure of vascular endothelial growth factor receptor-tyrosine kinase inhibitors (TKI). Currently, the therapeutic applicability of EVE has been changing. Multicenter evaluation of efficacy and safety of everolimus in daily routine and definition of patient characteristics with favorable outcome. Data of 165 patients from 9 oncology institutes in Hungary were analyzed retrospectively. Everolimus therapy was used after one TKI in 10 mg starting dose. Physical and laboratory examinations and imaging tests were performed monthly and every 3 months, respectively. Median progression-free survival (PFS) was 5.4 months. Median overall survival (OS) was 16.2 months. PFS and OS results were more favorable in patients with ECOG 0–1 (p_{PFS} = 0.033, p_{OS} = 0.008) and after >9 months of TKI therapy (p_{PFS} = 0.019, p_{OS} = 0.045). Survival was longer in nonanemic patients with ECOG 0–1 than in anemic patients with ECOG 2–3, 30.9 and 7.7 months, respectively (p = 0.029). Dose reduction and treatment delay was required in 6.2% and 8.9% of patients, respectively. Common adverse events were exanthema, edema, stomatitis, anemia, and abnormal kidney functions and glucose levels. Results of this study show that everolimus is safe and efficacious in a real-world setting. Everyday practice showed that nonanemic patients with good performance status receiving TKI therapy for >9 months are favorable candidates for this treatment. Despite the efficiency of novel, registered drugs, everolimus still plays an important role during and after second-line therapy for mRCC when availability of modern remedies is limited.

Keywords Metastatic kidney cancer · mTOR inhibitor · Everolimus · Anemia · ECOG · RCC

Introduction

Everolimus (Afinitor®, Novartis) (EVE), an oral mammalian target of rapamycin (mTOR) inhibitor, has been evaluated in

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preclinical studies and in numerous clinical trials in the past decade [1]. PI3K/AKT/mTOR is an intracellular signaling pathway in which mTOR is a protein kinase involved in the regulation of several cellular functions such as proliferation, growth and survival [2]. This mentioned pathway plays a central role in tumorigenesis of renal cell cancers (RCC) [3]. The anti-tumor effect of EVE had been confirmed in the therapy of advanced or metastatic RCC (mRCC), and then neuroendocrine tumors of pancreatic origin, of other gastrointestinal or lung origin, and hormone receptor-positive advanced breast cancers [4–7].

The first registration study of EVE was a phase 3 placebo-controlled study for the treatment of advanced RCC (RECORD-1), in which the patients' disease has previously progressed on or after sunitinib and/or sorafenib therapy. Progression-free survival (PFS) was significantly longer in patients who received EVE than those who received placebo (4.9 months vs 1.9 months) [4]. The difference between the overall survival (OS) of the two arms was equalized due to crossover after progression (14.8 months with EVE vs 14.4 months with placebo) [8]. The results of the subgroup after failure on one line vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI) therapy demonstrated more favorable PFS (5.4 months) [9]. According to the international guidelines (e.g., NCCN, ESMO, EAU), EVE can be used to treat patients with mRCC whose condition progressed during or after anti-VEGFR TKI [10–12].

Currently, the therapeutic administration of EVE has been changing. The results of one new phase 2 and two phase 3 studies have been published in the past two years with respect to mRCC, in which EVE was the comparator of the investigated drugs. The survival rates were more favorable in the immune checkpoint inhibitor nivolumab, TKI cabozantinib, and also in the tri-specific targeted VEGFR-, RET- and fibroblast growth factor receptor (FGFR) inhibitor lenvatinib combined with the EVE arms compared to EVE administered alone [13–16] [Table 1]. According to the recent guidelines, the role of EVE should be amended in the clinical practice [17].

Besides the therapeutic efficiency of novel remedies, the availability of new therapeutic options also influences the survival of oncologic patients. In some economic regions, the financing of new therapies with high cost is limited, so in the everyday practice, the oncologist has to maximize the efficiency of new therapeutic options with the available resources.

Aim of our study was to retrospectively analyze the maximal efficiency and the side-effects of EVE in the everyday practice of different oncology centers. We wished to define patient characteristics which made the therapy more effective.

Patients and Methods

Patients Everolimus was administered to 165 patients with mRCC between January 2010 and December 2013 in nine Hungarian oncological institutes. The study was performed in accordance with the Hungarian drug law and relevant guidelines of the Hungarian health authorities. The study design was approved by the ethics committee (registration number WHO 3483).

Patients were administered everolimus after they had progressed mostly on sunitinib, and in some cases on sorafenib or pazopanib therapy. Histological and staging examinations, such as abdominal and chest CT (if clinically indicated, bone scintigraphy and skull CT) were performed before initiating the therapy. 71% of the patients had a comorbidity that required treatment.

Everolimus Therapy Everolimus 10 mg daily was administered orally in continuous 28-day cycles. A minimum washout period of 4 weeks followed the previously administered anti-VEGFR therapy. Treatment was started when patients' general condition was good; they did not suffer from side-effects of the previous therapies, and after stabilization of symptoms caused by new metastases (e.g., cerebral metastasectomy, brain or bone irradiation, anemia control, etc.). Dose reduction

Table 1 Second and third line clinical trials with everolimus in clear cell renal cell cancer

Trial, Author	Phase	N	Arms	mPFS (months)	ORR (%)	mOS (months)
RECORD-1	III	416	EVE	4.9	2	14.8
Motzer et al. [4, 8]			PBO	1.9	0	14.4 (crossover)
CheckMate 025	III	821	NIVO	4.6	25	25.0
Motzer et al. [13]			EVE	4.4	5	19.6
METEOR	III	658	CABO	7.4	17	21.4
Choueiri et al. [14, 15]			EVE	3.9	3	16.5
Motzer et al. [16]	II	151	LEN + EVE	14.6	43	25.5
			LEN	7.4	27	18.4
			EVE	5.5	6	17.5

CABO cabozantinib, *EVE* everolimus, *LEN* lenvatinib, *mOS* median overall survival, *mPFS* median progression free survival, *NIVO* nivolumab, *ORR* overall response rate, *PBO* placebo

or delay was performed according to the Summary of Product Characteristics [1]. Physical examination and laboratory tests were performed every 4 to 8 weeks. Imaging examinations were performed 8 weeks after the initiation of everolimus therapy, and once every twelve weeks thereafter, as indicated by the National Health Insurance. Tumor response was evaluated every 12 weeks according to RECIST 1.0 [18]. Severity of AEs was evaluated based on the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0 (88% in case of 145 patients) [19]. The patients' general condition was assessed according to ECOG scale [20]. After progression on everolimus, treatment in clinical studies, therapy with interferon, progesterone derivatives, and best supportive care were available as therapeutic options. Our data were collected retrospectively.

Statistical Analysis Statistical analyses were performed by using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA). The association between PFS, OS and age was analyzed using COX regression. The influence of other therapy-related factors (duration of TKI therapy and the time that elapsed between the cessation of TKI therapy and the initiation of everolimus), and patient-related factors (gender, type of previous therapy, ECOG status, and anemia) on PFS and OS was analyzed with Kaplan-Meier analysis.

Results

Patient Characteristics Out of the 165 patients who participated in the study, 76.4% were men and 23.6% were women [Table 2]. The mean age was 63.2 (range, 28–79) years, and 93.9% of patients had undergone nephrectomy. The general condition of the patients was good with 27.9% and 63.6% of patients having ECOG scores of 0 and 1, respectively; 6.1% and 2.1% of patients had ECOG scores of 2 and 3, respectively. Common comorbidities were hypertension, other cardiovascular disorders, and diabetes.

Table 2 Patient characteristics

		Patients N = 165	
Mean age, years ± SE		63.2 ± 0.9	
Age range, years		28–79	
		n	%
Gender	Male	126	76.4
	Female	39	22.6
ECOG	0	46	27.9
	1	105	63.6
	2	10	6.1
	3	4	2.1
Comorbidities		n	%
Hypertension		66	40.0
Other cardiovascular disorders		16	9.7
Diabetes		18	10.9
Secondary tumors		13	7.9.
Hematological disease		4	2.4
Asthma		4	2.4
Psoriasis		3	1.8
Metastases			
Mean number of metastatic sites (range)		2.4 (1–6)	
Location of metastases		n	%
Lung		142	86.0
Bone		67	40.6
Distant lymph node		60	36.4
Liver		31	18.8
Brain		21	12.7
Suprarenal gland		15	9.1
Other (peritoneum, pleura, pancreas, local relapse, contralateral kidney, thyroid gland)		–	‹8
Histopathological types		n	%
Purely clear cell renal cell type (ccRCC)		146	88.5
ccRCC with sarcomatoid morphology		9	5.45
ccRCC with papillary- / chromophobe- / both morphology		2 / 2 / 2	1.2 / 1.2 / 1.2
ccRCC with sarcomatoid + papillary- / chromophobe- / or both morphology		1 / 1 / 1	0.6 / 0.6 / 0.6
ccRCC with collecting duct component		1	0.6

ECOG Eastern Cooperative Oncology Group, *n* number of involved patients, *N* number of analyzed patients, *ccRCC* clear cell renal cell cancer, *SE* standard error

The mean number of metastatic sites was 2.4 (range, 1–6), and the most common sites of metastasis were the lungs, bones, distant lymph nodes and the liver. The histological type of the tumors was mainly clear cell renal cell cancer (ccRCC) in case of all patients, in most cases pure ccRCC. No rare variants could be detected, only sarcomatoid, papillary, chromophobe or collecting duct morphologies and transformations in the ccRCC were present [Table 2].

No genetic analyses were performed to prove the familial origin of the renal cancer. Renal cancer has developed in 11 (7.27%) and in 3 (1.8%) patients under 50 and 40 years of age, respectively. In these cases, there was no information about any benign tumor, paraganglioma, pheochromocytoma or bilateral tumor. Familial origin and multifocality could be observed in 1 and 2 cases, respectively. Bilateral renal cancer and secondary malignancy (3 rectal cancers, 2 CLLs, 1 breast cancer) could be detected in 5 and 6 cases, respectively.

Previous Therapies After undergoing nephrectomy, 9.1% of the patients received adjuvant INF treatment, and 4.8% of patients received IFN before the administration of VEGFR-targeted therapy. Before receiving everolimus, 93.9%, 4.8%, and 1.2% of patients were given sunitinib, sorafenib, and pazopanib, respectively. The mean (±SE) duration of TKI therapy was 11.7 (±0.9) months. The duration of TKI was <3 months in 15.7% of patients, who were defined as being resistant to primary TKI therapy [21]. The mean (±SE) duration between the end of TKI therapy and the beginning of everolimus was 97. 7 (±10.1) days (period between TKI–EVE) [Table 3].

Dose Parameters Overall, 6.2% of the patients required a dose reduction to manage pneumonitis (4.1%), grade 2 skin problems (1.4%), and face and neck edema (0.7%). Furthermore, 8.9% of the patients required a dose delay with a mean duration of 24 (range, 5–75) days. The reasons for delaying the dose for >7 days were cardiovascular symptoms, elevation of renal functions that required dialysis (10 days each), grade 3 diarrhea (9–14 days), cerebral metastasectomy (20 days), and pneumonitis in 2 cases (28 and 30 days).

Efficacy At the time of the analysis, 26.2% of the patients were being treated, and 53.8% of the patients were alive. Partial regression, stable disease, and progression occurred in 12.9%, 60.7% and 26.4% of the patients, respectively. No patients experienced complete regression (CR). The objective tumor response was 12.9%, and the clinical benefit rate was 73.6% (partial regression + stable disease). The median PFS at a median follow-up time of 21.2 months (*95%CI 7.05–31.45*) was 5.4 months (*95%CI 3.83–6.97*). The median overall survival time (OS) (based on data from 145 patients) was 16.2 months (*95%CI 12.95–19.45*).

AEs The most common AEs were exanthema (25%), peripheral edema (19%), stomatitis (19%), pneumonitis (13%), nausea, weight loss, fatigue (11% each), diarrhea (10%), dyspnea (10%), and mucositis (9%). The most common abnormalities identified in laboratory findings were anemia (72%), and elevation in renal function (45%), liver function (25%), blood glucose (51%), cholesterol (44%) and lipids (35%). AEs compared with data from the phase III study are presented in Table 4. No severe or life threatening AEs occurred.

Table 3 Previous therapies before everolimus treatment

	Patients N = 165	
Previous therapies	n	%
Nephrectomy	155	93.9
Adjuvant IFN	22	13.3
First line IFN before VEGFR-TKI	21	12.7
Sunitinib	155	93.9
Sorafenib	8	4.8
Pazopanib	2	1.2
First line VEGFR-TKI	157	95.1
Second line VEGFR-TKI after IFN	8	4.8
Duration of previous therapy		
Mean duration of VEGFR-TKI, months (±SE)	11.7 (±0.9)	
Duration of VEGFR-TKI <3 months, n (%)	26	15.7
Mean duration between VEGFR-TKI and EVE, days (±SE)	97.7 (±10.1)	

EVE everolimus, *IFN* interferon-α, *n* number of involved patients, *N* number of analyzed patients, *SE* standard error, *TKI* tyrosine kinase inhibitor, *VEGFR* vascular endothelial growth factor receptor

Table 4 Adverse events of patients who received everolimus

Most common adverse events	Hungarian analysis			RECORD-1 Registration study [4]	
	n = 145			*n* = 269	
	All grade %	Grade 2%	Grade 3%	All grade %	Grade 3/4%
Exanthema (rash)	25	5	1	29	1/0
Peripheral edema	20	–	1	25	<1 / 0
Stomatitis	24	2	–	44	4 / <1
Weight loss (asthenia)	17	1	–	33	3 / <1
Fatigue/ Weakness	21	–	–	31	5 / 0
Diarrhea	13	2	–	31	1 / 0
Nausea	15	–	–	26	1 / 0
Mucositis	13	2	–	19	1 / 0
Dyspnea	12	–	–	24	6 / 1
Pneumonitis	11	2	1	14	4 / 0
Decreased hemoglobin	73	21	6	91	9 / <1
Elevated creatinine	43	5	1	46	<1 / 0
Elevated liver transaminases	21	4	1	25–21	0–1
Elevated glucose level	53	6	–	50	12 / 0
Elevated cholesterol	45	3	–	76	3 / 0
Elevated lipid	37	4	–	71	<1 / 0
Hypothyroidism/ hyperthyroidism	<1 / <1	–	–	–	–

n number of analyzed patients

Factors Influencing Efficacy PFS and OS with everolimus were not influenced by the patients' gender, age, the number and type of metastatic organ systems, the presence of the metastasis only in the lungs, the length and type of the previous TKI therapy, or the time between the cessation of TKI treatment and initiating everolimus.

Patients without lung metastasis showed favorable outcome (PFS 5.3 vs 9.1 months $p = 0.042$, OS 10.3 vs 15.9 months $p = 0.006$) [Table 5].

Median PFS and OS of patients treated with TKI therapy ≤3 months, vs > 3 months were 3.0 vs 5.2 months and 16.0 vs 19.9 months, respectively; however, the differences were not

Table 5 Factors influencing the outcome of everolimus therapy

Specifications		PFS ± SE (months)	*p* -value	OS ± SE (months)	*p* -value
Gender	Man/Woman	5.3 ± 0.7/ 6.4 ± 1.7	0.929	19.9 ± 3.5 /18.2 ± 2.7	0.544
Number of metastatic organs	1 / More	5.3 ± 1.5/ 5.5 ± 1.0	0.660	18.0 ± 1.9/16.6 ± 3.2	0.186
Only lung met. / Other met.		4.2 ± 0.5/ 6.4 ± 0.9	0.116	15.5 ± 3.1/21.9 ± 6.6	0.916
Presence / Lack of lung met.		5.3 ± 0.6 / 9.1 ± 2.8	**0.042**	10.3 ± 1.1 / 15.9 ± 4.7	**0.006**
ECOG status	0–1 / 2–3	6.4 ± 1.1/ 3.5 ± 0.2	**0.033**	19.9 ± 6.7/7.5 ± 0.6	**0.008**
Duration of TKI therapy (months)	≤3 / >3	3.4 ± 0.6/ 5.9 ± 0.8	0.250	16.0 ± 4.5/ 19.9 ± 5.9	0.244
	≤6 / >6	4.7 ± 0.8/ 6.4 ± 1.3	0.090	21.9 ± 7.2/ 16.6 ± 2.4	0.840
	≤9 / >9	4.5 ± 0.8/ 7.2 ± 1.5	**0.019**	16.0 ± 2.8/ 41.2 ± 18.6	**0.045**
Type of TKI	SU / SO / PA	5.5 / 6.9 / 2.8	0.140	18 / 19.9 / 30.9	0.690
Period between TKI–EVE (days)	≤30 / >30	6.5 ± 0.9/ 5.3 ± 0.9	0.774	11.5 ± 5.4/ 30.9 ± 6.8	0.106
	≤60 / >60	5.6 ± 0.6/ 4.5 ± 1.3	0.601	19.9 ± 4.9/ 16.5 ± 6.8	0.624
Anemia	G0 / G1–2-3	4.8 ± 1.2/ 6.4 ± 1.0	0.612	30.9 ± 6.1/16.2 ± 1.4	**0.020**
PFS (months)	<12 / ≥12	–	–	15.5 ± 1.8/41.2 ± 9.5	**0.001**

ECOG Eastern Cooperative Oncology Group, *EVE* everolimus, *G* grade, met –metastasis, *OS* median overall survival, *PA* pazopanib, *PFS* median progression-free survival, *SE* standard error, *SO* sorafenib, *SU* sunitinib, *TKI* tyrosine kinase inhibitor

Significant level is: $p < 0.05$

statistically significant ($p = 0.250$ and $p = 0.244$, respectively). PFS and OS were more favorable for patients who received everolimus after receiving TKI therapy for >9 months (PFS $p = 0.019$, OS $p = 0.045$) and for patients with an ECOG performance status of 0 or 1 (PFS $p = 0.033$, OS $p = 0.008$).

The presence of anemia predicted a poorer survival rate ($p = 0.020$), while a PFS >12 months was a favorable prognostic factor ($p = 0.762$) [Table 5]. Only 25.5% of the patients received third-line therapy: progesterone derivatives (17.9%), a TKI in a clinical study (4.1%), and INF therapy (3.5%). OS was not significantly different between patients who received these specific third-line therapies and patients who did not receive oncological therapy after everolimus (post EVE therapy) ($p = 0.001$). Examining the effect of ECOG performance status and anemia on survival, the most favorable median OS was observed for patients without anemia and with an ECOG performance status of 0 or 1 (30.9 ± 2.5 months), whereas it was the most unfavorable median OS observed in patients with anemia and with an ECOG performance status of 2 or 3 (7.7 ± 4.5 months) ($p = 0.029$). None of other patient or therapy related parameters influenced PFS or OS [Fig. 1].

Discussion

Modifying the mTOR signal transduction pathway by blocking the proliferation, migration, growing and survival and by indirectly inhibiting VEGF is an important therapeutic strategy of hypervascular RCCs [3]**.** EVE as an orally administered mTOR serine/threonine kinase inhibitor shows efficiency in second- and third-line therapies of patients with mRCC after failure of at least one VEGFR-TKI. The safety profile of the drug is favorable. No clear predictive biomarkers are known related to efficacy of EVE. The real world data could confirm results of registration studies and help understand the integration of novel drugs into the daily routine practice.

Values of our retrospective post-registration study with EVE are the multicenter data processing, the high case number in comparison to the population, and the homogeneity of the patients regarding previous therapies.

In our analysis, EVE monotherapy was associated with favorable PFS and OS in patients with mRCC refractory to previous VEGFR-TKI therapy. Our reported median PFS of 5.3 months is slightly longer than the median PFS of 4.9 months reported in the RECORD-1 registration study [4], and similar to the median PFS of 5.4 months reported in the subgroup of RECORD-1 patients, who had previously received only one line of TKI therapy [9]. The median OS of patients in our study was 16.2 months. In RECORD-1, the median OS was 14.8 in the everolimus arm [8]. Based on the previous details, results of survival data in our study are comparable to the results of the registration study and even the EVE standard arm in recent clinical studies (Checkmate 025 PFS_{EVE}: 4.4 months, OS_{EVE}: 19.6 months), METEOR (PFS_{EVE}: 3.9 months, OS_{EVE} 16.5 months), LEN-EVE (PFS_{EVE}: 5.5 months, OS_{EVE}: 17.5 months) [13–16] [Table 1].

Regarding PFS, as an indicator of the efficiency of an active agent, results from the everyday practice can be compared with and do not differ significantly from the newly published results. Overall survival data that refer to efficiency of therapeutic sequences based on new results suggest that introducing new therapeutic options positively affect the OS [4, 13, 14].

In the registration studies and retrospective analyses of EVE, and new active agents (carbozantinib and nivolumab), the safety profiles were homogenous [4, 13, 14, 22].

In our study, the mean duration between ceasing VEGFR-TKI treatment and initiating everolimus therapy was 97.7 days. There were several reasons for delaying the start of the administration of everolimus, including resolving AEs associated with VEGFR-TKI therapy to at least to grade 1, stabilizing symptoms caused by new metastases (if necessary cerebral metastasectomy, brain or bone irradiation), patient flow between the institutes, organizing radiological examinations, and drug

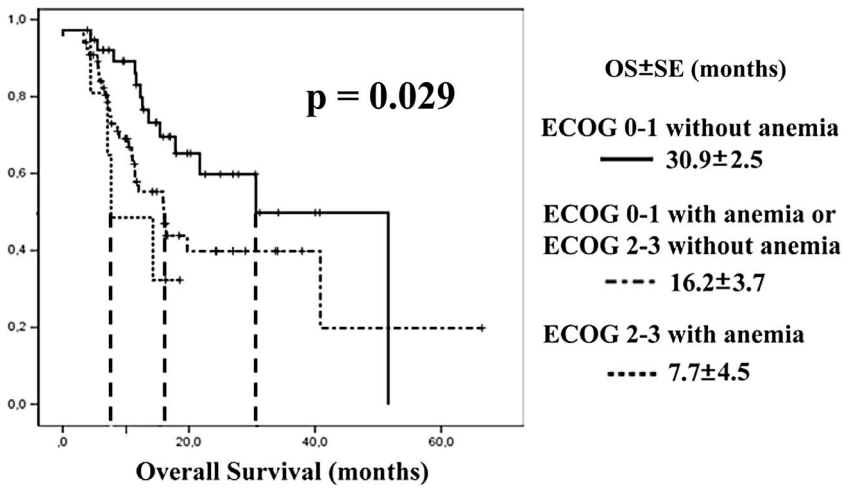

Fig. 1 Effect of ECOG status and anemia on overall survival. Kaplan-Meier analysis of OS was compared in patients with anemia and ECOG 2–3 status *(7.7 ± 4.5 months)* vs the absence of anemia and ECOG 0–1 status *(30.9 ± 2.5 months)* vs only one unfavorable prognostic factor is present *(16.2 ± 3.7 months) (p = 0.029)*. (ECOG – Eastern Cooperative Oncology Group, OS – overall survival, SE – standard error)

availability. The length of time between TKI and mTOR inhibitor therapies was similar to the time between ending TKI therapy and beginning everolimus following progression on placebo in the RECORD-1 study. Surprisingly, despite the length of time between TKI-EVE, we could not have proven any unambiguous, negative effect of it in our population.

We also investigated parameters that could influence the efficacy of everolimus.

Patients' favorable general condition (ECOG 0–1) was associated with a longer PFS and OS. The lack of anemia was associated with longer survival. After the introduction of new, registered therapeutic options, analysis of these prognostic factors might be useful during the evaluation of early experience.

We did not find a correlation between patients' other general characteristics, the type of previous TKI therapy or and its therapeutic outcome. We also evaluated the effect of primary resistance to VEGFR-TKI therapy on subsequent everolimus efficacy. Although differences were not statistically significant, PFS and OS tended to be less favorable in patients who experienced primary TKI resistance. Similar results have been reported in international studies [23]. Similarly to our results, Bergmann et al. found no correlation between the type or duration (< or >3 or 6 months) of previous TKI therapy and the efficacy of everolimus in VEGF-refractory patients with mRCC [24]. In our study, we found that patients whose VEGFR-TKI therapy was >9 months had significantly more favorable PFS and OS [24].

The prognostic score system published by Motzer for second-line therapy demonstrated unfavorable prognosis in the presence of 3 factors: anemia, poor general health (Karnofsky performance status <80), and a high level of corrected calcium (>10 mg/dL or >2.4 mmol/L), instead of the 5 factors used to determine prognosis for first-line therapy [25]. In our population, we demonstrated that poor general health negatively influenced survival. If the patients' general condition was good, and they did not have anemia, the OS was 30.9 months, but if they had poor health and anemia, OS time decreased to 7.7 months. In our analysis, we found that ECOG performance status was one of the most important factors that affect PFS. OS was remarkably better in patients with a duration of everolimus therapy >12 months. This underlines the importance of appropriate patient selection. After longer duration of everolimus therapy, the number of third-line therapies decreased without influencing survival, so the properly selected, effective second-line therapy determined the patients' life expectancy.

Conclusions mTOR inhibition is an effective way to treat patients with VEGFR-TKI refractory mRCC. According to experience in the Hungarian everyday practice, VEGFR-TKI refractory patients in good general health, having adequate hematological values, and >9 months of previous VEGFR-TKI therapy may experience delayed disease progression and improved survival while maintaining good quality of life during the second-line everolimus therapy. Despite the more favorable efficiency of new, registered drugs, EVE therapy still plays role during and after second-line therapy for mRCC in regions where modern remedies are only limitedly available, they have not been introduced yet, or their administration is contraindicated due to medical reasons.

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Compliance with Ethical Standards

Conflict of Interest Anikó Maráz has received honoraria from Bayer, Bristol-Myers Squibb, and has served on advisory boards for Novartis.

András Csejtei has served on advisory boards for Novartis and Pfizer.

Judit Kocsis has received honoraria from Bayer and served as a member of advisory board: Novartis, Bristol-Myers Squibb and Pfizer.

Miklós Szűcs has received honoraria from Bayer, Novartis, Bristol-Myers Squibb and has served on advisory boards for Novartis and Pfizer.

Zsuzsanna Kahán has no actual or potential conflicts of interest to report.

György Bodoky has received honoraria from Bayer, Bristol-Myers Squibb and Pfizer and has served on advisory boards for Novartis and Pfizer.

Magdolna Dank has received honoraria from Bayer, Novartis and Pfizer and has served on advisory boards for Novartis and Pfizer.

László Mangel has received honoraria from Pfizer and has served on advisory boards for Novartis and Pfizer.

János Révész has served on advisory boards for Novartis and Pfizer.

Zoltán Varga has no actual or potential conflicts of interest to report.

Lajos Géczi has received honoraria from Bayer, Novartis, Bristol-Myers Squibb and Pfizer and has served on advisory boards for Bristol-Myers Squibb, Novartis and Pfizer.

Ethical Approval All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study no formal consent is required.

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