ANTICANCER RESEARCH *43*: 2645-2657 (2023)
doi:10.21873/anticanres.16431

Long-term Outcome With Prolonged Use of Interferon-alpha Administered Intermittently for Metastatic Renal Cell Carcinoma: A Phase II Study

MINNA KANKURI-TAMMILEHTO[1,2,3], LAURA PERASTO[4], SEPPO PYRHONEN[1,5] and EEVA SALMINEN[1,5]

[1]*Department of Oncology and Radiotherapy, Turku University Hospital, Turku, Finland;*
[2]*Department of Clinical Genetics, Turku University Hospital, Turku, Finland;*
[3]*Institute of Biomedicine, University of Turku, Turku, Finland;*
[4]*Institute of Clinical Medicine, Department of Biostatistics, University of Turku, Turku, Finland;*
[5]*Institute of Clinical Medicine, Department of Clinical Oncology, University of Turku, Turku, Finland*

Abstract. *Background/Aim: Interferon-alpha (IFN-alpha) has shown survival benefits in metastatic renal cell carcinoma (mRCC), but the knowledge about long-term outcome is sparse. Additional knowledge is beneficial because IFN-alpha usage in combination therapy such as immune checkpoint inhibitor for mRCC is an area of interest. This is the longest follow-up concerning IFN-alpha treatment. Patients and Methods: A total of 117 metastatic renal cell cancer (mRCC) patients without prior chemotherapy were enrolled between 1994-2002 and followed-up until January 2022. The median follow-up was 18 months. After progression to IFN-alpha, the patients were not treated with tyrosine kinase, mTOR inhibitors or bevacizumab as these were not standard therapies at that time or the patients' performance status was too poor. Mean treatment duration was 11 months. Results: Median overall survival was 19.0 months, 5-year survival rate 16.2%, and 10-year survival rate 9.0%. There were statistically significant differences in survival in response to treatment (log-rank test, p<0.001): median overall survival was 52.0 months for objective responses, 25.0 months for stable disease and 5.0 months for progressive disease. Proportion of 5-year survivors was 29% in low, 20% in intermediate, and 7% in high-risk groups, respectively (p=0.001). Conclusion: With prolonged INF-alpha treatment stable and responding patients can obtain late objective responses, long-lasting complete responses, and long-term outcome with acceptable toxicity. IFN-alpha is an alternative therapy when multiple treatment lines are used for mRCC and an interesting option to study for combined therapies such as immune checkpoint inhibitor-based therapies.*

Correspondence to: Minna Kankuri-Tammilehto, Department of Clinical Genetics, Turku University Hospital, Kiinamyllynkatu 4-8, PL 52, 20521 Turku, Finland. E-mail: mikanku@utu.fi

Key Words: Interferon-alpha, renal cell carcinoma.

Renal cell carcinoma (RCC) accounts for 2-3% of all diagnosed malignancies worldwide, and in some Northern/Central Europe countries even 5% (1). The annual increase in the incidence of RCC, since the 1970's, has been in the range of 2% to 4% (2, 3), which has been attributed to the frequent use of imaging techniques, the increasing prevalence of cigarette smoking, and obesity (3, 4). However, a recent plateau in RCC incidence rates has also been reported in some countries (3). Hereditary kidney cancers may account for at least 5-8% of all kidney cancers (5). At diagnosis, 20-30% of RCC patients have metastases. Half of patients diagnosed with localized disease will later have a recurrence of their cancer: of these, two thirds within the first year and the majority within 5 years (6). Although the increase of RCC incidence is due to localized RCC, also metastasized RCC (mRCC) has increased slightly (3, 7). Currently mRCC accounts for approximately 15% of all RCC (7).

Currently, treatments in RCC are evolving. There are several effective therapeutic options for mRCC patients. When selecting the treatment, prognostic factors, other illnesses, and side-affects are taken into consideration. IFN-alpha treatment is an option as first line treatment for mRCC patients with bevacizumab especially for the good and intermediate prognostic risk groups (8, 9). IFN-alpha is also an alternative therapy for patients who have been previously treated with targeted therapies or whose comorbidity or side effect profile prevent other treatments. IFN-alpha usage in combination therapy such as immune checkpoint inhibitor for mRCC is currently an area of interest (10).

Currently, the optimal dosing and duration of IFN-alpha is not known. Previously, the efficacy and feasibility of intermittent and prolonged administration of IFN-alpha in mRCC patients was published in 2001 by Kankuri *et al*. (11), and IFN-alpha was observed to be moderately tolerated. This is a long-term follow-up to that study with 42 more patients; the schema and dose were the same.

Only very few data have been previously published regarding the long-term survival with IFN-alpha and other mRCC therapies. This patient group is homogenous as IFN-alpha has been the routine clinical treatment during 1994-2002 in mRCC patients, and at that time targeted agents or modern immuno-oncological agents were not available at the beginning of treatment. The follow-up was up to January 2022. The present study is an analysis of long-term follow-up of IFN-alpha treatment and of prognostic factors. According to our knowledge this is the longest follow-up published concerning IFN-alpha treatment.

Patients and Methods

Patient eligibility. Between December 1994 and December 2002, 117 consecutive patients (70 males and 47 females) with metastatic RCC gave informed consent to participate in the recombinant IFN-alpha2a (Roferon®) study. Patient characteristics are shown in Table I. A total of 107 (91%) patients had undergone nephrectomy, whereas 10 (9%) were inoperable. Two (2%) patients had partial spontaneous regression, one of them in the lungs and another in the pleura. None of the patients had previously received systemic treatment for mRCC.

Treatment schedule. Inclusion criteria were age under 80 years, performance status of 0-2 (World Health Organization, WHO), a serum creatinine concentration of less than 200 μmol/l and serum concentrations of liver enzymes below twice the upper reference limit. Patients with brain metastases, other concomitant malignancies, serious concomitant disease or with a life expectancy of less than 3 months were excluded. All eligible patients were evaluated for response and toxicity.

IFN-alpha was administered on an outpatient basis subcutaneously three times weekly (tiw) at an escalating dose from 4.5 million units (MU) to the highest tolerable dose of 9, 12, 13.5 or 18 MU. The dose was increased weekly, and the maximum dose was to be reached during the first four weeks. The maintenance dose was balanced based on the patients' tolerance. The treatment cycle consisted of three-weeks' treatment with IFN-alpha followed by a one-week pause. IFN-alpha was planned to be continued until progression or intolerable toxicity, or for up to two years. Together with the injection of IFN-alpha, patients were recommended to use paracetamol or naproxene prophylactics to ameliorate flu-like symptoms. The treatment schedule was described in detail earlier (11), and approved by the joint ethics committee of the University of Turku and Turku University Hospital (11). The study was conducted in accordance with the Declaration of Helsinki and approved by the Turku Clinical Research Center. The patients were treated until May 2006 and followed-up until January 2022 at the Department of Oncology and Radiotherapy of Turku University Hospital. The median duration of follow-up was 18 months [range=1.2-302.4 months (0.1-25.2 years)]. After progression to IFN-alpha, the patients were not treated with tyrosine kinase or mTOR inhibitors or bevacizumab as these were not standard therapies at that time or the patients' performance status was too poor.

Table I. *Patient characteristics*.

Characteristics	No. (%)
Total no. of pts	117 (100)
Sex	
Female	47 (40)
Male	70 (60)
Age, years, median [range]	63 [38-78]
Time to metastases in months, median [range]	0 [0-157]
Prior metastases	64 (55)
Late metastases	53 (45)
WHO performance status	
<2	80 (68)
=2	37 (32)
T-stage*	
T1	20 (20)
T2	19 (19)
T3	51 (50)
T4	12 (12)
Tumor grade#	
G1	38 (37)
G2	39 (38)
G3	25 (25)

*Fifteen patients were not evaluable for T-stage. #Fifteen patients were not evaluable for tumor grade.

Staging and response assessment. The 2002 updated UICC pTNM classification system of renal carcinomas for T-staging was used (12). Staging investigations included a chest x-ray, whole body computed tomography (CT scan) and bone scintigraphy if the patient had bone pain. Histopathologic samples were graded according to the WHO classification (13). The evaluation of the response followed the criteria of the WHO (14). The patients were evaluated weekly during the first four weeks and bimonthly thereafter. At follow-up, all symptoms, signs of the disease, performance status, neurological status, body weight and response to treatment were recorded. Blood counts and serum concentrations of calcium, liver enzymes and creatinine were analyzed. Radiologic evaluations of the tumor spread were performed by chest and abdominal CT scan or chest X-ray and abdominal ultrasound at regular 3-4-month intervals. Treatment was discontinued when obvious disease progression was over 25% in tumor measurements, which means that progressive disease (PD) was observed, or intolerable toxic events were seen. In brief, complete response (CR) was defined as complete disappearance of all tumors and partial response (PR) as at least 50% decrease in tumor area (14). Tumor area was dimensionally measured (multiplication of longest diameter by the greatest perpendicular diameter). Two successive measurements at least 4 weeks apart, confirming response (CR or PR), were required for the patient to qualify as a responder. A PR in bone metastases was defined if clinically stable lesions with decreased uptake in skeletal scintigram and normalization of alkaline phosphatase were observed. The response duration was measured from the observed response.

Table II. *Prognostic values of variables for overall survival and 5-year survival during IFN-alpha in metastatic renal cell carcinoma patients.*

Variable	Overall survival						5-year survival					
	Univariate analysis (*p*-value)	Hazard ratio	95% Confidence interval	Multivariate analysis (*p*-value)	Hazard ratio	95% Confidence interval	Univariate analysis (*p*-value)	Hazard ratio	95% Confidence interval	Multivariate analysis (*p*-value)	Hazard ratio	95% Confidence interval
Performance status (<2 *vs*. 2)	0.001	0.5	0.3-0.8	0.99	1.0	0.4-2.4	0.001	0.5	0.3-0.8	0.990	1.0	0.4-2.7
Hb (normal *vs*. abnormal)	<0.001	0.5	0.3-0.7	0.079	0.6	0.3-1.1	<0.001	0.4	0.3-0.7	0.160	0.6	0.3-1.2
Metastases (prior *vs*. late)	0.220	1.3	0.9-1.8				0.120	1.4	0.9-2.1			
Time to metastases (<24 months *vs*. ≥24 months)	0.330	1.2	0.8-1.9									
Lung metastases (no *vs*. yes)	0.470	0.9	0.6-1.3				0.450	0.8	0.5-1.3			
Liver metastases (no *vs*. yes)	0.280	0.8	0.5-1.3				0.240	0.7	0.4-1.2			
Bone metastases (no *vs*. yes)	0.090	0.7	0.5-1.1				0.066	0.7	0.5-1.0			
No. of disease sites (less *vs*. 4)	0.030			0.012			0.015			0.009		
1 *vs*. 4	0.036	0.4	0.1-0.9	0.001	0.01	0.0-0.1	0.032	0.4	0.1-0.9	0.001	0.01	0.0-0.2
2 *vs*. 4	0.130	0.5	0.2-1.2	0.002	0.01	0.0-0.2	0.120	0.5	0.2-1.2	0.002	0.02	0.0-0.2
3 *vs*. 4	0.580	0.8	0.3-2.0	0.003	0.01	0.0-0.2	0.690	0.8	0.3-2.2	0.007	0.02	0.0-0.3
Response to IFN-alpha (CR+PR *vs*. SD)	0.022			0.034			0.041			0.054		
CR *vs*. SD	0.009	0.3	0.1-0.7	0.009	0.3	0.1-0.7	0.013	0.2	0.1-0.7	0.016	0.2	0.1-0.8
PR *vs*. SD	0.610	1.2	0.6-2.4	0.960	1.0	0.4-2.2	0.880	1.1	0.5-2.2	0.730	0.9	0.4-2.0
Dose of IFN-alpha	0.320						0.180					
1 *vs*. 4	0.610	1.2	0.6-2.8				0.330	1.5	0.7-3.5			
2 *vs*. 4	0.550	0.8	0.4-1.7				0.720	0.9	0.4-1.9			
3 *vs*. 4	0.880	1.1	0.5-2.3				0.660	1.2	0.5-2.7			
Sex (female *vs*. male)	0.140	0.7	0.5-1.1				0.140	0.7	0.5-1.1			
Age at the beginning of IFN-alpha (<65 yrs *vs*. 65 or older)	0.670	1.1	0.7-1.6				0.550	1.1	0.8-1.7			
T-stage (less *vs*. 4)	0.090						0.210					
1 *vs*. 4	0.012	0.4	0.2-0.8				0.037	0.4	0.2-1.0			
2 *vs*. 4	0.093	0.5	0.3-1.1				0.120	0.5	0.2-1.2			
3 *vs*. 4	0.094	0.6	0.3-1.1				0.094	0.6	0.3-1.1			
T-stage (1+2 *vs*. 3+4)[#]	0.084						0.280					
T-size	0.009	1.1	1.0-1.1	0.100	1.1	1.0-1.2	0.025	1.1	1.0-1.1	0.140	1.1	1.0-1.2
Tumor grade (less *vs*. 4)	0.330						0.260					
1 *vs*. 3	0.150	0.7	0.4-1.2				0.110	0.6	0.4-1.1			
2 *vs*. 3	0.230	0.7	0.4-1.2				0.240	0.7	0.4-1.2			
Side of primary kidney tumor (right *vs*. left)	0.210	1.3	0.9-1.9				0.580	1.1	0.7-1.7			

[#]Analyzed by Log-Rank test: The median times are 22 months for (1+2) and 16.5 months for (3+4) in the overall survival and 5-year survival.

The widely used MSKCC risk group scoring from the year 2002 (15) or International Metastatic RCC Database Consortium model (16) could not have been used as lactate dehydrogenase (LDH) and corrected calcium values were not routinely recorded for the patients, as the study included the patients from 1994 onwards. The Cleveland Clinic Foundation scoring system (17) was used as the values of the prognostic factors were easily applied and were available from the patient records.

Statistical analysis. The association of prognostic factors with overall survival time and 5-year survival time was analyzed using survival analysis applying Kaplan–Meier estimation and Cox's regression analysis. The associations between separate prognostic factors (Table II) were analyzed with univariate Cox's regression analysis, *p*-values (two-tailed) less than 0.05 were interpreted as statistically significant. When comparing T-stages 1 and 2 to T-stages 3 and 4 the log rank test was used. All the prognostic factors that were statistically significant in the univariate analysis were used in the multiple Cox's regression. Computing was performed with SAS System version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The mean duration of treatment with IFN-alpha was 11 months (range 2 weeks to 32 months). Younger patients (<65 years) tolerated higher doses than older patients (≥65 years) (p=0.012). For five patients the treatment was continued over two years (range=25-57 months), due to good performance status, response to IFN-α and well-tolerated treatment, two of these patients achieved partial response and three had stabilized disease. Their IFN-alpha dose was between 4.5 and 9 MIU. If disease progressed, patients were not treated with bevacizumab or tyrosine kinase inhibitors. For eight (7%) patients the treatment was discontinued between two and four weeks due to decreased performance status and rapid disease progression. These patients were included in our intention-to-treat analysis.

Response to treatment. Objective response was observed in 20 patients; response rate being 17%. There were 10 (9%) patients with complete and 10 (9%) patients with partial response (Table III). The response duration for patients with CR was 231*, 214+, 126, 118, 50*, 46, 41, 17, 11, and 8 months and for patients with PR 32, 24, 16, 14, 12, 11, 9, 7, 6, and 6 months (* indicates that the response duration was interrupted due to cause of death other than mRCC; + means that the patient was still alive at the end of the follow-up). The median response duration was 15 months. The median time to response was 7 months (range=2-28 months) from the beginning of the treatment. Four of these patients turned to partial response after prolonged disease stabilization after 12 months (range=12-17 months). One of them turned to complete response at 22 months of treatment. One patient with complete response (CR) was treated for an additional period with another IFN-alpha, because the disease progressed slowly. The patient achieved objective response (CR) again after the second treatment. One patient had a partial spontaneous regression in the lung after nephrectomy and the patient achieved CR after beginning of INF-alpha treatment.

Table III. *Response to IFN-alpha*.

Response	No. of patients	Percentage
Complete response	10	9
Partial response	10	9
Stable disease	49	42
Progressive disease	48	41

Forty-nine patients (42%) had stable disease (SD) with median duration of 12 months (ranging from 4 to 290+ months). The patient with SD of more than 290 months had pleural tumor which was operated before INF-alpha treatment, and after INF-alpha treatment for 10 months the lung metastases seemed to slightly regress but criteria for partial response were not met. One SD patient had a partial spontaneous regression in the pleura after nephrectomy, during INF-alpha the disease was stable. Forty-eight patients (41%) progressed during treatment; 25 of them (21%) had early progression, *i.e.*, within three months. Seventeen of 117 (15%) patients developed brain metastases as a sign of progressive disease during or after IFN-alpha therapy. The median detection time of brain metastases was 21 months after start of IFN-alpha therapy and the median overall survival after detection was 2.7 months. Twelve of these patients received palliative radiation therapy.

The group of complete responders (n=10) included three patients with lung metastases, three patients with lung and lymph node metastases, one patient with lung and renal metastases, one patient with liver and lymph node metastases, one patient with bone, spleen, and lymph node metastases, and one patient with bone metastases. In four (20%) of 20 responders the INF-alpha dose was under 9 MU 3 tiw. The dose for the remaining 16 (80%) responders was between 9 to 18 MU 3 tiw.

Progression-free and overall survival. Median progression-free survival was 8 months. Median overall survival for the entire study population was 19.0 months (1.58 years). The 5-year, 10-year, 15-year, 20-year, and 25-year overall survival rates were 16,2%, 9,0%, 5,8%, 4,5%, 4,5%, respectively. Two patients were alive at the end of follow-up. Five patients died of causes other than mRCC during follow-up. There were statistically significant differences in survival in response to treatment (log-rank test, p<0.001, Figure 1). Median overall survival was 52.0 months (4.33 years) in the group of CR + PR, 25.0 months (2.08 years) in the group of SD, and 5.0 months (0.42 years) in the group

of PD (Figure 1). Patients with a response had better survival than patients with stable disease up to 10-years, but after that there was no significant differences in outcomes between response and stable disease patients (Figure 1); of these five patients with CR + PR two died of other causes at the age of 95 and 80 years, two were still alive and 1 died of mRCC.

Prognostic factors. Prognostic factors for overall and progression-free survival were identified by Cox analysis and the occurrence of brain metastasis was identified by Log-Rank test. In multivariate analysis, significant prognostic factors for improved overall survival were low number of disease sites (p=0.012), and response to IFN-alpha (p=0.034), and for 5-year survival low number of disease sites (p=0.009) (Table II). Univariate results are shown in Table II.

Treatment results in different risks groups. The patients were classified according to the Cleveland Clinic Foundation scoring system into three risk groups (17). Poor prognostic factors for short-term survival were ECOG performance status more than zero, number of involved metastatic sites more than two, baseline levels of hemoglobin below the lower limit of normal, and primary RCC in the left kidney (17). The median overall survival and 5-year survival proportions as well as the number of objective responses (CR+PR) are shown in Figure 2 and Table IV.

Discussion

Overall survival rates. Our long-term results of prolonged and intermittently administered IFN-alpha therapy in mRCC (intention-to-treat analysis) shows that approximately 1 in 6 patients with mRCC who had prolonged IFN-alpha therapy reach 5-year survival, and 1 in 11 mRCC patients reach 10-year survival. Previously reported 5-year long-term survival of patients with IFN-alpha-based therapy has been approximately between 9% and 16% (18, 19), and in all of these studies additional agents have been used, e.g., IL-2 or vinblastine (18, 19). Our result on IFN-alpha therapy alone is aligned with the upper range of these results.

Only few reports about long-term outcome with IFN-alpha therapy alone in mRCC have been published (18, 20). Our extended follow-up showed better long-term survival than that previously reported by Minasian *et al*. (1993), where the 5-year survival rate was 3% with IFN-alpha therapy alone (20). In the study of Minasian *et al*., the planned treatment duration was 3 months in one trial and until progression of disease, the median treatment duration was not reported (20).

Median overall survival of 1.6 years (=19.1 months) in our study was longer compared to the weighted average median survival of 1.0 years (=11.4 months) in a meta-analysis of 644 mRCC patients treated with IFN-alpha (21). Our median overall survival result was similar to a randomized controlled trial, in which patients were treated with IFN-alpha alone (1.6 years, 18.8 months) or with combination of IFN-alpha, IL-2 and fluorouracil (1.6 years, 18.6 months) (22). In that study the median duration of treatment with continuous IFN-alpha was 4.2 months. In the CALGB study the median overall survival did not reach over 18.3 months in IFN-alpha or in IFN-alpha plus bevacizumab group (23). The results of the present study confirm our previous results (11). This study shows that despite the treatment cycle pause the overall survival was similar or even longer compared to other reports with continuous IFN-alpha with or without additional agents, such as IL-2 or vinblastine (19, 22, 24, 25).

The patient material was homogenous; after progression, no patients were treated with bevacizumab or tyrosine kinase inhibitors or mTOR inhibitors. Two of the patients had high-dose (HD)-IL-2 therapy after the progression to IFN-alpha without success. In addition, two patients were treated with capecitabine without response.

Prognostic factors. For overall survival, response to treatment was a significant prognostic factor in multivariate analysis (Table II). Objective response rate of 17% in the current study is comparable to that of other studies with IFN-alpha alone (24, 25), or IFN-alpha with IL-2 (25), and IFN-alpha with vinblastine (19). Our patients with stable disease (42%) evidently benefitted from the prolonged treatment. The median overall survival of responding patients was four years and twice as long compared to those with stable disease, and ten times longer compared to those with progressive disease. The complete responders had metastases mainly in the lungs (7 out of 10 complete responders); in addition, one of complete responders had bone metastases, and one had liver metastases. Based on our results, for patients with objective response, a median response duration of four years, and a response duration of even more than 20 years is possible to achieve (Figure 1). The observation of late responses during prolonged IFN-alpha has been reported in our previous study (11). Four patients (3%) achieved partial response after 12 months of treatment, longest time to response was 17 months after the initiation of therapy. One of them achieved later a complete response at 22 months after initiation of therapy. It has been noted that with novel immunotherapy, such as immune checkpoint inhibitors (ICI) therapy, some patients have a long survival even if the disease seems to have progressed during treatment (26). In our study no one in the PD group survived past 5 years. However, the long-term survival was reached also by a portion of the stable disease group as 11 SD patients from 49 (22%) achieved 5-year survival and 5 SD patients (10%) more than 11 years (Figure 1). Long-term and durable responses can thus be reached with interferon-based treatments. In this single-arm IFN-alpha study patient material was homogeneous as targeted agents or modern

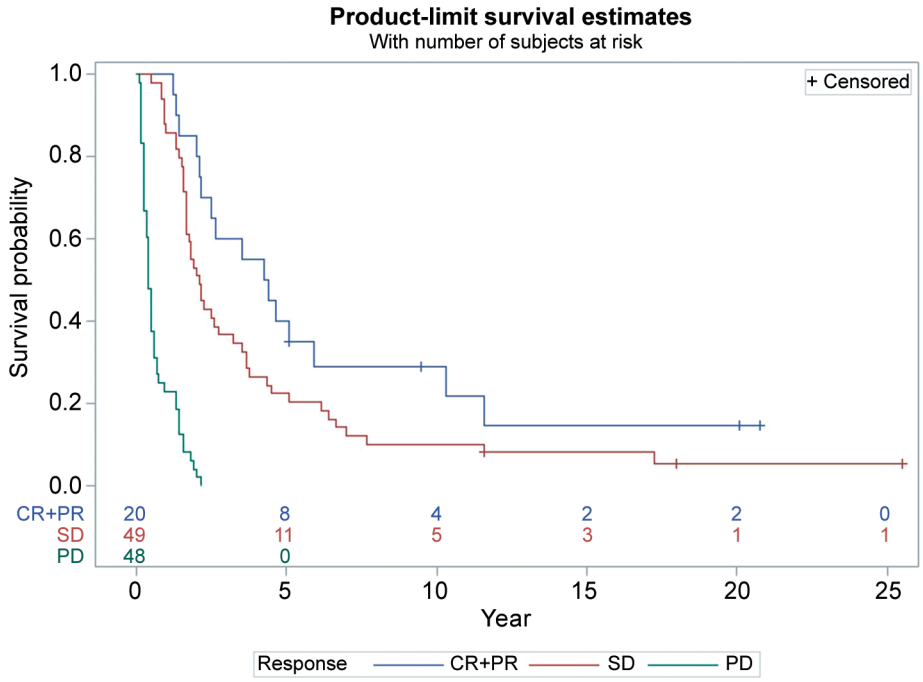

Figure 1. *Kaplan–Meier survival curves for responding patients, patients with stable and progressive disease (log-rank p<0.001). Two complete response (CR)+ partial response (PR) patients died of a cause other than metastatic renal cell carcinoma. SD: Stable disease; PD: progressive disease.*

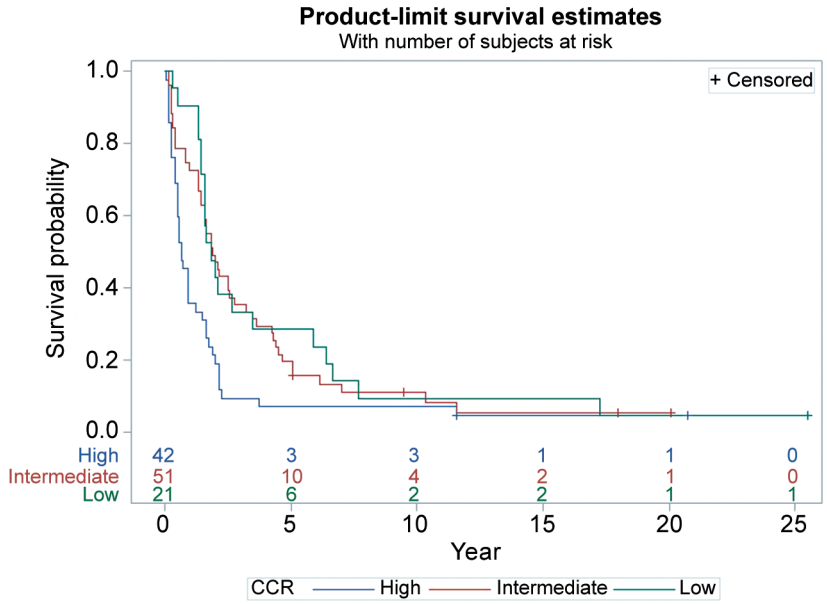

Figure 2. *Kaplan–Meier survival curves for risk groups according to the Cleveland Clinic Foundation scoring system (log-rank p=0.0067).*

immuno-oncological agents were not available at the beginning of treatment. Treatment efficacy is supported by the fact that response to IFN-alpha was a significant prognostic factor in 5-year and overall survival.

Many scoring systems for mRCC patients have been created, and this study pointed that Cleveland Clinic Foundation can stratify prognostic subgroups with only a few variables, *i.e.*, poor performance status, decreased level of Hb

Table IV. *Survival for risk groups according to Cleveland Clinic Foundation scoring system.*

Risk group	Number of poor prognostic factors	No. of patients[#]	No. of objective responses (CR+PR)	5-year survival rate (%)*	Median overall survival (years) [range]
Low	<2	21 (18%)	4 (3.5%)	29	1.8 [0.33-25.50]
Intermediate	=2	51 (45%)	12 (10.5%)	20	1.9 [0.17-20.08]
High	>2	42 (37%)	4 (3.5%)	7	0.66 [0.08-20.75]

*5-year survival rate *p*-value=0.0011. [#]For 3 patients, score could not be evaluated due to missing data and were excluded.

baseline, number of disease sites and side of primary kidney tumor (Table IV). In our study, all the Cleveland Clinic Foundation variables were independent prognostic factors except the side of primary kidney tumor (Table II). In our study, a considerably high number of the responding patients in the intermediate risk group may indicate that they would also benefit from IFN-alpha therapy. In the previous study of Négrier *et al*., the survival benefit was not observed in the intermediate risk group patients (25), but Pyrhönen *et al*. (1999) observed (IFN-alpha plus vinblastine *versus* vinblastine) that the survival benefit of IFN-alpha may be greater in those patients with adverse prognostic factors, such as poor performance status, age over 60 years and male sex (19).

In metastatic RCC, also the following independent prognostic factors have been reported: other metastatic site than the lung and T-size (27, 28). Similarly in the present study, they were significant predictors of survival in Cox regression analysis. For 5-year survival and overall survival, the presence of bone metastases showed a trend in univariate analysis (Table II). This observation is in line with previous observations in which bone metastases have been associated with poor prognosis (29). In our study tumor size was a significant prognostic factor in univariate analyze. This is in accordance with earlier studies where patients with tumor less than 3 cm were more likely long-term survivors compared with those with tumors greater than 3 cm (30).

Sex-specific survival advantage was observed at 5-year overall survival but not after 10 years in patients treated with subcutaneous IL-2 and IFN-alpha (31). In our study we did not observe sex differences.

Dose and duration. In most previous studies the duration of the treatment with IFN-alpha has been 1.5 to 6 months (18, 24), and most responses have been observed within 2 or 6 months of treatment start (24). Since host immune mechanisms are apparently important in regulating tumor growth and in patients responding to IFN-alpha (32), we used a prolonged schedule of IFN-alpha administration. The treatment with IFN-alpha was planned to be continued for 24 months or as long as progression or severe side-effects were encountered. In some responding patients with good performance status, IFN-alpha therapy was longer, even 2.6 years. In addition, restarting INF-alpha after a pause may result in a complete response. Our previous data showed that prolonged and intermittently administered IFN-alpha is well tolerated; the one-week pause every four-weeks allows most patients to continue prolonged treatment with the highest tolerable dose (11). Previous studies pointed that doses of 5 to 18 MU of IFN-alpha three times a week seem to be effective and tolerable. This is supported by our result; only one responding patient had a lower dose of 3 MU; 80% of responses were achieved with a dose of at least 9 MU. As responses were achieved also with doses lower than 9 MU, this may imply that particularly the immunological effects of IFN-alpha are pivotal compared to cytostatic effects in mRCC. It is assumed that population aging in the Nordic countries will lead to an increasing incidence and prevalence of renal cancer (33). Treatment of cancer among the elderly possess challenges because of co-morbidities. ICIs and tyrosine kinase inhibitors (TKIs) treatment-related adverse events lead to discontinuation in approximately 20% or 10% of the patients, respectively (34), and this area is under current investigation with recent publications on predictors and specific patient groups (35, 36). Mortality of 4% has been observed with ICI-based and TKI therapy due to cardiac side effects (37).

Progression-free survival (PFS). In the current analysis the 8 months median PFS of patients with mRCC treated with IFN-alpha was better compared to previous studies with a different IFN-alpha treatment modality or IFN-alpha-based combination therapy that included sunitinib, bevacizumab, IL-2, vinblastine or fluorouracil (22, 23, 38-40), where treatment duration with IFN-alpha alone was less or equal to 5.4 months. However, due to lack of comparative data no conclusions can be made between these different studies. In the present study, the 1-week pause every 4-weeks allowed the patients to continue IFN-alpha longer with the highest tolerable dose. This might be the reason for better PFS.

The incidence of primary or late brain metastases due to RCC has been reported to be as high as 11% in a large autopsy series (41). In the present study, no patients with brain metastases were included in the study because the presence of brain metastases was an exclusion criterion. The incidence

of brain metastases during IFN-alpha or after discontinuation of IFN-alpha was 15% (17 of 117 patients): five out of 17 patients developed brain metastases during IFN-alpha and the remaining patients after discontinuation of the therapy. RCC patients with pulmonary metastases were more prone to develop brain metastases. This is in accordance with previous reports, where the lungs are the most common metastatic location in RCC patients with brain metastases (42). We suggest that this is due to the fact that patients with lung metastases survive longer and consequently are more prone to have brain metastases. The median overall survival after detection of brain metastases was only 2.7 months, which is comparable to other studies (43). The median detection time of brain metastases was 21 months after the start of IFN-alpha therapy. Men were more likely to suffer from brain metastases than women. Brain metastases are a late manifestation of the disease and are usually associated with progression also in the extracranial sites.

Immunomodulatory effects. IFN-alpha enhances immune responses by multiple mechanisms. It can promote the differentiation and activity of host immune cells. It activates natural killer cells, enhances macrophage activity, cytotoxic T-cells and activates and induces differentiation of dendritic cells to induce antitumor activity (44). IFN-alpha has also antiproliferative, antiangiogenic activities and induces apoptosis of tumor cells (44). However, the mechanism of antitumoral activity still is unclear. Recent findings highlight the importance of the interactions of IFN-alpha with the immune system for the generation of a durable antitumor response (44-46).

RCC is an "immunogenic" tumor where regression, including metastases, has been proposed to occur in up to 1% of patients (7). In our study the amount of spontaneous regression was 1.7%. Of interest is the theory that bevacizumab acts predominantly as an immune modulator rather than VEGF treatment in mRCC patients (47). ICIs target programmed cell death 1 (PD-1), receptors present on T cells, and programmed cell death ligand 1 (PD-L1) on tumor cells. When PD-1 is engaged to its ligand PD-L1 immune responses are down-regulated. Tumor cells often express PD-L1 to mediate this blockage. Thus, inhibition of PD-1 or PD-L1 aims to prevent this down-regulation of antitumor immunity of T cells (45). Also, interferons have been shown to be able to enhance and regulate PD-L1 expression not only on tumor but also on several non-tumor cell types such as dendrite cells in microenvironment (46). This mechanism to modulate PD-L1 expression in dendrite cells can be used in IFN-alpha based therapeutic strategies (46). The studies on PD1 ligand (PD-L1) treatments have shown that microenvironment is linked to the efficacy of the treatment (45, 46). Stimulation of dendrite cells by vaccinations has boosted cancer specific immune response (45, 46). An effective treatment on a tumor requires knowledge of the cancer's microenvironment (48, 49).

IFN-alpha in mRCC patients. Immunomodulators, such as IFN-alpha and interleukin-2 (IL-2) came into the clinical use for the treatment of metastatic renal cell carcinoma (mRCC) (50, 51) and melanoma in the early 80's (52), and randomized trials have indicated that IFN-alpha increases the survival of patients with RCC (19, 24). In the end of 2000, first line therapy choices for mRCC widened with tyrosine kinase inhibitors (TKIs) against VEGF receptor, such as sunitinib, sorafenib, and the mammalian target of rapamycin (mTOR) inhibitor, temsirolimus, and an antiangiogenic monoclonal antibody/vascular endothelial growth factor (VEGF)–targeted therapy, bevacizumab (38, 53-55). Favorable outcome results from phase III trials on bevacizumab + IFN-alpha treatment were published in 2010 (23, 56). Recently, immune checkpoint blockers (ICBs) have been associated with improved outcomes in cancer patients (57, 58). The most promising treatments in mRCC are combinations with programmed cell death 1 (PD-1)/PD-L1–targeted therapy, ICIs, such as nivolumab or pembrolizumab, and there are ongoing trials without or with TKIs. A Belgian retrospective study observed overall survival improvement in patients with metastatic ccRCC since the introduction of VEGFR-TKIs and ICIs (59). In the Belgian study the median overall survival with IFN-alpha as first line therapy was 13 months, which is lower than the 19 months in our studies with IFN-alpha alone (59). In the same study VEGFR-TKI as first line therapy resulted in median overall survival of 19 months (59). Manageable toxicity with high response rates with ICIs in first line therapy have been observed, but long-term results have not been published yet. Many trials have investigated ICIs alone or in combination with other ICI therapy or with antiangiogenic TKIs and have found to be superior to the prior treatments in mRCC with high rate of responses. Long-term outcome of ICIs, such as nivolumab and iplimumab has been published in the CheckMate 214 trial and of pembrolizumab with VEGFR-TKI axitinib in the KEYNOTE-426 trial. In these trials, better objective response rates (42%, 59%, respectively) (34, 60) and better median PFS (11.6 months, 15.4 months, respectively) (34, 37) were achieved compared to previous treatments. An improvement of treatment free survival compared to TKI was observed in intermediate and poor risk patient groups in which it was 6.9 months with the combination of two ICIs (61). Interest in immunotherapy for mRCC is now strong. Usage of IFN-alpha in the treatment of mRCC has become a topic that is subject to re-evaluation (10).

Understanding the significance of immunological response in RCC has increased further over time. McKay, Bossé and Choueiri (2018) proposed a systemic treatment algorithm for metastatic ccRCC, and they pointed that bevacizumab +

IFN-alpha treatment is a choice for mRCC patients, especially for the good prognostic risk group (8).

Future prospects. National Comprehensive Cancer Network (NCCN) (62), European Society for Medical Oncology (ESMO) (9), and European Association of Urology (EAU) (63) have issued guidelines for the treatment of RCC. IFN-alpha based therapy has been defined as an optional therapy (62, 63), and bevacizumab plus interferon are currently considered the drug of choice, especially for the good risk group (39). The results of our study support that with immunotherapy very long survival is possible and that it is of interest to study combined INF-alpha and ICI therapies in the treatment of mRCC. Side effect profile differs between these medications. In addition, the results of this study show that prolonged and intermittently used IFN-alpha is a treatment for mRCC when the patient is given multiple-step treatment lines. Currently, there are many choices for the therapy of RCC patients. Treatment selection is affected by the patient's previous treatments, side-effects profile and concomitant diseases. Prolonged IFN-alpha therapy may also be considered as an additional choice for therapy as it is well tolerated in patients whose concomitant disease or medication does not allow other modalities. Combinations of IFN-alpha and targeted therapies are being evaluated. ICI is a golden standard especially for intermediate and high-risk patients, although their long-term outcome is yet to be defined.

However, the optimal timing of IFN-alpha is not yet known. Our initial results show that the schema used in this study is well tolerated and can achieve long term outcome. The timing of different immunotherapy modalities and targeted therapy should be explored in future studies. The current questions concerning the use of IFN-alpha, is not only the timing (38), but also the optimal dose in combination with other therapy modalities. Also, the optimal dose, schedule, and duration of IFN-alpha alone in the treatment of mRCC has not yet been determined. In a meta-analysis of 644 RCC patients IFN-alpha was associated with survival benefits compared to hormonal therapy, chemotherapy, and other biologic response modifiers (21). Additionally, a low dose of intravenous or subcutaneous IL-2 or other agents have failed to improve survival compared to IFN-alpha alone. HD-IL-2 may improve the number of complete responses, especially in patients with the worst prognosis, e.g., with either liver or bone metastases (64), but (very) good performance status and cardiac function are required (51). Pegylated-IFN-alpha has not been a superior treatment in mRCC (65). The median overall survival and long-term survival analyses of sunitinib and bevacizumab with IFN-alpha are still ongoing.

Prognostic factors, such as PD-L1 expression, for indicating those patients who could respond to IFN-alpha therapy and other immunotherapies in renal RCC should be studied (30, 66-69). Recently, GWAS studies have pointed some single nucleotide polymorphisms (SNPs) and certain chromosomal gains and losses to be associated with the outcome of RCC (70, 71). In the future sub-groups of patients with mRCC who can be benefited from long-term INF-alpha treatment should be investigated.

Conclusion

According to our knowledge this is the longest follow-up published concerning IFN-alpha treatment. Long-lasting complete responses and long-term survival is possible with prolonged and intermittently used IFN-alpha therapy in patients with mRCC. Approximately one in six patients were alive at 5-years and one in 11 patients at 10-years. Patients with few disease sites and a small primary tumor are most likely to achieve long-term survival. Long-term prognosis was more likely, if the patient responded to the treatment, but the same prognosis was reached also by a portion of patients with response of stable disease. Also, patients in the intermediate risk group may benefit from IFN-alpha therapy. IFN-alpha is still an interesting option to study for combined therapies such as immune checkpoint inhibitor-based therapies. In addition, IFN-alpha is an alternative therapy when the patient is given multiple treatment lines for mRCC.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization, M.K-T., E.S. and S.P.; methodology, M.K-T, E.S. and S.P; validation, M.K-T, L.P.,E.S. and S.P; formal analysis, M.K-T, E.S. and S.P; data curation, M.K-T., L.P..; writing – original draft preparation, M.K-T.; writing – review and editing, M.K-T, E.S. and S.P.; supervision, E.S. and S.P.. All Authors have read and agreed to the published version of the manuscript.

References

1 Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, Rawla P and Barsouk A: Epidemiology of renal cell carcinoma. World J Oncol *11(3)*: 79-87, 2020. PMID: 32494314. DOI: 10.14740/wjon1279

2 Rossi SH, Klatte T, Usher-Smith J and Stewart GD: Epidemiology and screening for renal cancer. World J Urol *36(9)*: 1341-1353, 2018. PMID: 29610964. DOI: 10.1007/s00345-018-2286-7

3 Saad AM, Gad MM, Al-Husseini MJ, Ruhban IA, Sonbol MB and Ho TH: Trends in renal-cell carcinoma incidence and mortality in the United States in the last 2 decades: a SEER-based study. Clin Genitourin Cancer *17(1)*: 46-57.e5, 2019. PMID: 30391138. DOI: 10.1016/j.clgc.2018.10.002

4 Chow WH, Gridley G, Fraumeni JF Jr and Järvholm B: Obesity, hypertension, and the risk of kidney cancer in men. N Engl J Med *343(18)*: 1305-1311, 2000. PMID: 11058675. DOI: 10.1056/NEJM200011023431804

5 Kankuri-Tammilehto M: Genetic Susceptibility to Kidney Cancer. Review. In: Kommu SS and Gill IS (ed.). Evolving Trends in Kidney Cancer. IntechOpen, pp 3-18, 2020.

6 Lam JS, Shvarts O, Leppert JT, Pantuck AJ, Figlin RA and Belldegrun AS: Postoperative surveillance protocol for patients with localized and locally advanced renal cell carcinoma based on a validated prognostic nomogram and risk group stratification system. J Urol *174(2)*: 466-72; discussion 472; quiz 801, 2005. PMID: 16006866. DOI: 10.1097/01.ju.0000165572.38887.da

7 Hemminki K, Försti A, Hemminki A, Ljungberg B and Hemminki O: Progress in survival in renal cell carcinoma through 50 years evaluated in Finland and Sweden. PLoS One *16(6)*: e0253236, 2021. PMID: 34157049. DOI: 10.1371/journal.pone.0253236

8 McKay RR, Bossé D and Choueiri TK: Evolving systemic treatment landscape for patients with advanced renal cell carcinoma. J Clin Oncol: JCO2018790253, 2018. PMID: 30372392. DOI: 10.1200/JCO.2018.79.0253

9 Powles T, Albiges L, Bex A, Grünwald V, Porta C, Procopio G, Schmidinger M, Suárez C, de Velasco G and ESMO Guidelines Committee: ESMO Clinical Practice Guideline update on the use of immunotherapy in early stage and advanced renal cell carcinoma. Ann Oncol *32(12)*: 1511-1519, 2021. PMID: 34597799. DOI: 10.1016/j.annonc.2021.09.014

10 Blank CU, Wong DJ, Ho TH, Bauer TM, Lee CB, Bene-Tchaleu F, Zhu J, Zhang X, Cha E and Sznol M: Phase Ib study of atezolizumab plus interferon-α with or without bevacizumab in patients with metastatic renal cell carcinoma and other solid tumors. Curr Oncol *28(6)*: 5466-5479, 2021. PMID: 34940094. DOI: 10.3390/curroncol28060455

11 Kankuri M, Pelliniemi TT, Pyrhönen S, Nikkanen V, Helenius H and Salminen E: Feasibility of prolonged use of interferon-alpha in metastatic kidney carcinoma: a phase II study. Cancer *92(4)*: 761-767, 2001. PMID: 11550145. DOI: 10.1002/1097-0142(20010815)92:4<761::aid-cncr1380>3.0.co;2-#

12 Brierley JD, Gospodarworicz M and Wittekind C (eds.): Union for International Cancer Control. TNM Classification of Malignant Tumors, 8th edition. John Wiley & Sons, 2002.

13 Mostofi FK and Davis CJ in Collaboration with Sobin LH and Pathologists in 6 Countries World Health Organization: Histological Typing of Kidney Tumors. 2nd ed. In: International Histological Classification of Tumors. Berlin, Heidelberg, Germany, Springer, pp. 1-117, 1998.

14 Miller AB, Hoogstraten B, Staquet M and Winkler A: Reporting results of cancer treatment. Cancer *47(1)*: 207-214, 1981. PMID: 7459811. DOI: 10.1002/1097-0142(19810101)47:1<207::aid-cncr2820470134>3.0.co;2-6

15 Motzer RJ, Bacik J, Murphy BA, Russo P and Mazumdar M: Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol *20(1)*: 289-296, 2002. PMID: 11773181. DOI: 10.1200/JCO.2002.20.1.289

16 Heng DY, Xie W, Regan MM, Harshman LC, Bjarnason GA, Vaishampayan UN, Mackenzie M, Wood L, Donskov F, Tan MH, Rha SY, Agarwal N, Kollmannsberger C, Rini BI and Choueiri TK: External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. Lancet Oncol *14(2)*: 141-148, 2013. PMID: 23312463. DOI: 10.1016/S1470-2045(12)70559-4

17 Choueiri TK, Rini B, Garcia JA, Baz RC, Abou-Jawde RM, Thakkar SG, Elson P, Mekhail TM, Zhou M and Bukowski RM: Prognostic factors associated with long-term survival in previously untreated metastatic renal cell carcinoma. Ann Oncol *18(2)*: 249-255, 2007. PMID: 17060490. DOI: 10.1093/annonc/mdl371

18 Atzpodien J, Hoffmann R, Franzke M, Stief C, Wandert T and Reitz M: Thirteen-year, long-term efficacy of interferon 2alpha and interleukin 2-based home therapy in patients with advanced renal cell carcinoma. Cancer *95(5)*: 1045-1050, 2002. PMID: 12209689. DOI: 10.1002/cncr.10783

19 Pyrhönen S, Salminen E, Ruutu M, Lehtonen T, Nurmi M, Tammela T, Juusela H, Rintala E, Hietanen P and Kellokumpu-Lehtinen PL: Prospective randomized trial of interferon alfa-2a plus vinblastine *versus* vinblastine alone in patients with advanced renal cell cancer. J Clin Oncol *17(9)*: 2859-2867, 1999. PMID: 10561363. DOI: 10.1200/JCO.1999.17.9.2859

20 Minasian LM, Motzer RJ, Gluck L, Mazumdar M, Vlamis V and Krown SE: Interferon alfa-2a in advanced renal cell carcinoma: treatment results and survival in 159 patients with long-term follow-up. J Clin Oncol *11(7)*: 1368-1375, 1993. PMID: 8315435. DOI: 10.1200/JCO.1993.11.7.1368

21 Coppin C, Porzsolt F, Kumpf J, Coldman A and Wilt T: Immunotherapy for advanced renal cell cancer. Cochrane Database Syst Rev *(3)*: CD001425, 2000. PMID: 10908496. DOI: 10.1002/14651858.CD001425

22 Gore ME, Griffin CL, Hancock B, Patel PM, Pyle L, Aitchison M, James N, Oliver RT, Mardiak J, Hussain T, Sylvester R, Parmar MK, Royston P and Mulders PF: Interferon alfa-2a *versus* combination therapy with interferon alfa-2a, interleukin-2, and fluorouracil in patients with untreated metastatic renal cell carcinoma (MRC RE04/EORTC GU 30012): an open-label randomised trial. Lancet *375(9715)*: 641-648, 2010. PMID: 20153039. DOI: 10.1016/S0140-6736(09)61921-8

23 Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, Archer L, Atkins JN, Picus J, Czaykowski P, Dutcher J and Small EJ: Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. J Clin Oncol *26(33)*: 5422-5428, 2008. PMID: 18936475. DOI: 10.1200/JCO.2008.16.9847

24 Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Medical Research Council Renal Cancer Collaborators. Lancet *353(9146)*: 14-17, 1999. PMID: 10023944.

25 Negrier S, Perol D, Ravaud A, Chevreau C, Bay JO, Delva R, Sevin E, Caty A, Escudier B and French Immunotherapy Intergroup: Medroxyprogesterone, interferon alfa-2a, interleukin 2, or combination of both cytokines in patients with metastatic renal carcinoma of intermediate prognosis: results of a randomized controlled trial. Cancer *110(11)*: 2468-2477, 2007. PMID: 17932908. DOI: 10.1002/cncr.23056

26 Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, Maio M, Binder M, Bohnsack O, Nichol G, Humphrey R and Hodi FS: Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res *15(23)*: 7412-7420, 2009. PMID: 19934295. DOI: 10.1158/1078-0432.CCR-09-1624

27 Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A and Ferrara J: Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. J Clin Oncol *17(8)*: 2530-2540, 1999. PMID: 10561319. DOI: 10.1200/JCO.1999.17.8.2530

28 Kankuri M, Söderström KO, Pelliniemi TT, Vahlberg T, Pyrhönen S and Salminen E: The association of immunoreactive p53 and Ki-67 with T-stage, grade, occurrence of metastases and survival in renal cell carcinoma. Anticancer Res *26(5B)*: 3825-3833, 2006. PMID: 17094408.

29 Szendroi A, Dinya E, Kardos M, Szász AM, Németh Z, Ats K, Kiss J, Antal I, Romics I and Szendroi M: Prognostic factors and survival of renal clear cell carcinoma patients with bone metastases. Pathol Oncol Res *16(1)*: 29-38, 2010. PMID: 19639393. DOI: 10.1007/s12253-009-9184-7

30 Sunela KL, Lehtinen ET, Kataja MJ, Kujala PM, Soimakallio S and Kellokumpu-Lehtinen PL: Development of renal cell carcinoma (RCC) diagnostics and impact on prognosis. BJU Int *113(2)*: 228-235, 2014. PMID: 23890347. DOI: 10.1111/bju.12242

31 Bonetti E, Jenzer M, Nientiedt C, Kaczorowski A, Geisler C, Zschäbitz S, Jäger D, Hohenfellner M, Duensing S and Reimold P: Interleukin-2 and interferon-α for advanced renal cell carcinoma: Patient outcomes, sexual dimorphism of responses, and multimodal treatment approaches over a 30-year period. Urol Int *106(11)*: 1158-1167, 2022. PMID: 35477131. DOI: 10.1159/000524097

32 Motzer RJ, Bander NH and Nanus DM: Renal-cell carcinoma. N Engl J Med *335*: 865-875, 1996.

33 Næraa SH, Bersang AB, Dahl C, Subhi Y and Azawi N: Burden of renal cancer in Nordic countries. Scand J Urol *53(4)*: 177-184, 2019. PMID: 31174451. DOI: 10.1080/21681805.2019.1624610

34 Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, Plimack ER, Barthélémy P, Porta C, George S, Powles T, Donskov F, Neiman V, Kollmannsberger CK, Salman P, Gurney H, Hawkins R, Ravaud A, Grimm MO, Bracarda S, Barrios CH, Tomita Y, Castellano D, Rini BI, Chen AC, Mekan S, McHenry MB, Wind-Rotolo M, Doan J, Sharma P, Hammers HJ, Escudier B and CheckMate 214 Investigators: Nivolumab plus ipilimumab *versus* sunitinib in advanced renal-cell carcinoma. N Engl J Med *378(14)*: 1277-1290, 2018. PMID: 29562145. DOI: 10.1056/NEJMoa1712126

35 Kobayashi Y, Arai H and Honda M: Nivolumab and ipilimumab immunotherapy for hemodialysis patients with advanced renal cell carcinoma. Curr Oncol *27(4)*: 225-228, 2020. PMID: 32905370. DOI: 10.3747/co.27.6439

36 Kartolo A, Sattar J, Sahai V, Baetz T and Lakoff JM: Predictors of immunotherapy-induced immune-related adverse events. Curr Oncol *25(5)*: e403-e410, 2018. PMID: 30464691. DOI: 10.3747/co.25.4047

37 Powles T, Plimack ER, Soulières D, Waddell T, Stus V, Gafanov R, Nosov D, Pouliot F, Melichar B, Vynnychenko I, Azevedo SJ, Borchiellini D, McDermott RS, Bedke J, Tamada S, Yin L, Chen M, Molife LR, Atkins MB and Rini BI: Pembrolizumab plus axitinib *versus* sunitinib monotherapy as first-line treatment of advanced renal cell carcinoma (KEYNOTE-426): extended follow-up from a randomised, open-label, phase 3 trial. Lancet Oncol *21(12)*: 1563-1573, 2020. PMID: 33284113. DOI: 10.1016/S1470-2045(20)30436-8

38 Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM and Figlin RA: Sunitinib *versus* interferon alfa in metastatic renal-cell carcinoma. N Engl J Med *356(2)*: 115-124, 2007. PMID: 17215529. DOI: 10.1056/NEJMoa065044

39 Escudier B, Pluzanska A, Koralewski P, Ravaud A, Bracarda S, Szczylik C, Chevreau C, Filipek M, Melichar B, Bajetta E, Gorbunova V, Bay JO, Bodrogi I, Jagiello-Gruszfeld A, Moore N and AVOREN Trial investigators: Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet *370(9605)*: 2103-2111, 2007. PMID: 18156031. DOI: 10.1016/S0140-6736(07)61904-7

40 Bracarda S, Bellmunt J, Melichar B, Négrier S, Bajetta E, Ravaud A, Sneller V and Escudier B: Overall survival in patients with metastatic renal cell carcinoma initially treated with bevacizumab plus interferon-α2a and subsequent therapy with tyrosine kinase inhibitors: a retrospective analysis of the phase III AVOREN trial. BJU Int *107(2)*: 214-219, 2011. PMID: 20942831. DOI: 10.1111/j.1464-410X.2010.09707.x

41 Saitoh H: Distant metastasis of renal adenocarcinoma. Cancer *48(6)*: 1487-1491, 1981. PMID: 7272969. DOI: 10.1002/1097-0142(19810915)48:6<1487::aid-cncr2820480635>3.0.co;2-9

42 Mori Y, Kondziolka D, Flickinger JC, Logan T and Lunsford LD: Stereotactic radiosurgery for brain metastasis from renal cell carcinoma. Cancer *83(2)*: 344-353, 1998. PMID: 9669818. DOI: 10.1002/(sici)1097-0142(19980715)83:2<344::aid-cncr19>3.0.co;2-t

43 Wrónski M, Maor MH, Davis BJ, Sawaya R and Levin VA: External radiation of brain metastases from renal carcinoma: a retrospective study of 119 patients from the M. D. Anderson Cancer Center. Int J Radiat Oncol Biol Phys *37(4)*: 753-759, 1997. PMID: 9128947. DOI: 10.1016/s0360-3016(97)00006-0

44 Ferrantini M, Capone I and Belardelli F: Interferon-alpha and cancer: mechanisms of action and new perspectives of clinical use. Biochimie *89(6-7)*: 884-893, 2007. PMID: 17532550. DOI: 10.1016/j.biochi.2007.04.006

45 Benci JL, Xu B, Qiu Y, Wu TJ, Dada H, Twyman-Saint Victor C, Cucolo L, Lee DSM, Pauken KE, Huang AC, Gangadhar TC, Amaravadi RK, Schuchter LM, Feldman MD, Ishwaran H, Vonderheide RH, Maity A, Wherry EJ and Minn AJ: Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. Cell *167(6)*: 1540-1554.e12, 2016. PMID: 27912061. DOI: 10.1016/j.cell.2016.11.022

46 Bazhin AV, von Ahn K, Fritz J, Werner J and Karakhanova S: Interferon-α up-regulates the expression of PD-L1 molecules on immune cells through STAT3 and p38 signaling. Front Immunol *9*: 2129, 2018. PMID: 30356906. DOI: 10.3389/fimmu.2018.02129

47 McDermott DF, Huseni MA, Atkins MB, Motzer RJ, Rini BI, Escudier B, Fong L, Joseph RW, Pal SK, Reeves JA, Sznol M, Hainsworth J, Rathmell WK, Stadler WM, Hutson T, Gore ME, Ravaud A, Bracarda S, Suárez C, Danielli R, Gruenwald V, Choueiri TK, Nickles D, Jhunjhunwala S, Piault-Louis E, Thobhani A, Qiu J, Chen DS, Hegde PS, Schiff C, Fine GD and Powles T: Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab *versus* sunitinib in renal cell carcinoma. Nat Med *24(6)*: 749-757, 2018. PMID: 29867230. DOI: 10.1038/s41591-018-0053-3

48 Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC, Cogdill AP, Zhao L, Hudgens CW, Hutchinson DS, Manzo T, Petaccia de Macedo M, Cotechini T, Kumar T, Chen WS, Reddy SM, Szczepaniak Sloane R, Galloway-Pena J, Jiang H, Chen PL, Shpall EJ, Rezvani K, Alousi AM, Chemaly RF, Shelburne S, Vence LM, Okhuysen PC, Jensen VB, Swennes AG, McAllister F, Marcelo Riquelme Sanchez E, Zhang Y, Le Chatelier E,

Zitvogel L, Pons N, Austin-Breneman JL, Haydu LE, Burton EM, Gardner JM, Sirmans E, Hu J, Lazar AJ, Tsujikawa T, Diab A, Tawbi H, Glitza IC, Hwu WJ, Patel SP, Woodman SE, Amaria RN, Davies MA, Gershenwald JE, Hwu P, Lee JE, Zhang J, Coussens LM, Cooper ZA, Futreal PA, Daniel CR, Ajami NJ, Petrosino JF, Tetzlaff MT, Sharma P, Allison JP, Jenq RR and Wargo JA: Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science *359(6371)*: 97-103, 2018. PMID: 29097493. DOI: 10.1126/science.aan4236

49 Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF and IMPACT Study Investigators: Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med *363(5)*: 411-422, 2010. PMID: 20818862. DOI: 10.1056/NEJMoa1001294

50 Cantell K, Hirvonen S, Mogensen KE and Pyhälä L: Human leukocyte interferon: production, purification, stability, and animal experiments. In Vitro Monogr *(3)*: 35-38, 1974. PMID: 4620359.

51 Spanknebel K, Cheung KY, Stoutenburg J, Hurst-Wicker K, Hesdorffer C, Deraffele G and Kaufman HL: Initial clinical response predicts outcome and is associated with dose schedule in metastatic melanoma and renal cell carcinoma patients treated with high-dose interleukin 2. Ann Surg Oncol *12(5)*: 381-390, 2005. PMID: 15915372. DOI: 10.1245/ASO.2005.03.063

52 Mattila K, Raanta P, Lahtela V, Pyrhönen S, Koskivuo I and Vihinen P: Long-term survival of stage IV melanoma patients treated with BOLD combination chemotherapy and intermediate-dose subcutaneous interferon-alpha. Anticancer Res *38(11)*: 6393-6397, 2018. PMID: 30396963. DOI: 10.21873/anticanres.12999

53 Sun M, Larcher A and Karakiewicz PI: Optimal first-line and second-line treatments for metastatic renal cell carcinoma: current evidence. Int J Nephrol Renovasc Dis *7*: 401-407, 2014. PMID: 25378943. DOI: 10.2147/IJNRD.S48496

54 Rodenburg RJ and Eskens F: Tivozanib for the treatment of renal cell carcinoma: patient selection and perspectives. Int J Nephrol Renovasc Dis *12*: 137-141, 2019. PMID: 31190952. DOI: 10.2147/IJNRD.S169056

55 Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, Staroslawska E, Sosman J, McDermott D, Bodrogi I, Kovacevic Z, Lesovoy V, Schmidt-Wolf IG, Barbarash O, Gokmen E, O'Toole T, Lustgarten S, Moore L, Motzer RJ and Global ARCC Trial: Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med *356(22)*: 2271-2281, 2007. PMID: 17538086. DOI: 10.1056/NEJMoa066838

56 Escudier B, Bellmunt J, Négrier S, Bajetta E, Melichar B, Bracarda S, Ravaud A, Golding S, Jethwa S and Sneller V: Phase III trial of bevacizumab plus interferon alfa-2a in patients with metastatic renal cell carcinoma (AVOREN): final analysis of overall survival. J Clin Oncol *28(13)*: 2144-2150, 2010. PMID: 20368553. DOI: 10.1200/JCO.2009.26.7849

57 Choueiri TK, Larkin J, Oya M, Thistlethwaite F, Martignoni M, Nathan P, Powles T, McDermott D, Robbins PB, Chism DD, Cho D, Atkins MB, Gordon MS, Gupta S, Uemura H, Tomita Y, Compagnoni A, Fowst C, di Pietro A and Rini BI: Preliminary results for avelumab plus axitinib as first-line therapy in patients with advanced clear-cell renal-cell carcinoma (JAVELIN Renal 100): an open-label, dose-finding and dose-expansion, phase 1b trial. Lancet Oncol *19(4)*: 451-460, 2018. PMID: 29530667. DOI: 10.1016/S1470-2045(18)30107-4

58 Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, Castellano D, Choueiri TK, Gurney H, Donskov F, Bono P, Wagstaff J, Gauler TC, Ueda T, Tomita Y, Schutz FA, Kollmannsberger C, Larkin J, Ravaud A, Simon JS, Xu LA, Waxman IM, Sharma P and CheckMate 025 Investigators: Nivolumab *versus* everolimus in advanced renal-cell carcinoma. N Engl J Med *373(19)*: 1803-1813, 2015. PMID: 26406148. DOI: 10.1056/NEJMoa1510665

59 Demasure S, Spriet I, Debruyne PR, Laenen A, Wynendaele W, Baldewijns M, Dumez H, Clement PM, Wildiers H, Schöffski P, Roussel E, Kinget L, Albersen M and Beuselinck B: Overall survival improvement in patients with metastatic clear-cell renal cell carcinoma between 2000 and 2020: a retrospective cohort study. Acta Oncol *61(1)*: 22-29, 2022. PMID: 34711121. DOI: 10.1080/0284186X.2021.1989720

60 Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, Pouliot F, Alekseev B, Soulières D, Melichar B, Vynnychenko I, Kryzhanivska A, Bondarenko I, Azevedo SJ, Borchiellini D, Szczylik C, Markus M, McDermott RS, Bedke J, Tartas S, Chang YH, Tamada S, Shou Q, Perini RF, Chen M, Atkins MB, Powles T and KEYNOTE-426 Investigators: Pembrolizumab plus axitinib *versus* sunitinib for advanced renal-cell carcinoma. N Engl J Med *380(12)*: 1116-1127, 2019. PMID: 30779529. DOI: 10.1056/NEJMoa1816714

61 Regan MM, Jegede OA, Mantia CM, Powles T, Werner L, Motzer RJ, Tannir NM, Lee CH, Tomita Y, Voss MH, Plimack ER, Choueiri TK, Rini BI, Hammers HJ, Escudier B, Albiges L, Huo S, Del Tejo V, Stwalley B, Atkins MB and McDermott DF: Treatment-free survival after immune checkpoint inhibitor therapy *versus* targeted therapy for advanced renal cell carcinoma: 42-month results of the CheckMate 214 trial. Clin Cancer Res *27(24)*: 6687-6695, 2021. PMID: 34759043. DOI: 10.1158/1078-0432.CCR-21-2283

62 Motzer RJ, Agarwal N, Beard C, Bolger GB, Boston B, Carducci MA, Choueiri TK, Figlin RA, Fishman M, Hancock SL, Hudes GR, Jonasch E, Kessinger A, Kuzel TM, Lange PH, Levine EG, Margolin KA, Michaelson MD, Olencki T, Pili R, Redman BG, Robertson CN, Schwartz LH, Sheinfeld J and Wang J: NCCN clinical practice guidelines in oncology: kidney cancer. J Natl Compr Canc Netw *7(6)*: 618-630, 2009. PMID: 19555584. DOI: 10.6004/jnccn.2009.0043

63 Ljungberg B, Albiges L, Abu-Ghanem Y, Bensalah K, Dabestani S, Fernández-Pello S, Giles RH, Hofmann F, Hora M, Kuczyk MA, Kuusk T, Lam TB, Marconi L, Merseburger AS, Powles T, Staehler M, Tahbaz R, Volpe A and Bex A: European Association of Urology Guidelines on renal cell carcinoma: the 2019 update. Eur Urol *75(5)*: 799-810, 2019. PMID: 30803729. DOI: 10.1016/j.eururo.2019.02.011

64 McDermott DF, Regan MM, Clark JI, Flaherty LE, Weiss GR, Logan TF, Kirkwood JM, Gordon MS, Sosman JA, Ernstoff MS, Tretter CP, Urba WJ, Smith JW, Margolin KA, Mier JW, Gollob JA, Dutcher JP and Atkins MB: Randomized phase III trial of high-dose interleukin-2 *versus* subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J Clin Oncol *23(1)*: 133-141, 2005. PMID: 15625368. DOI: 10.1200/JCO.2005.03.206

65 Sunela KL, Koskinen S and Kellokumpu-Lehtinen PL: A phase-II study of combination of pegylated interferon alfa-2a and capecitabine in locally advanced or metastatic renal cell cancer.

Cancer Chemother Pharmacol *66(1)*: 59-67, 2010. PMID: 19771431. DOI: 10.1007/s00280-009-1134-2

66 Sayapina MS and Bykovskaia SN: The plasticity of CD4(+)CD25(+)FOXP3(+)CD127(low) T cells in patients with metastatic renal cell carcinoma in the course of interferon-alpha immunotherapy. J Oncol *2018*: 7828735, 2018. PMID: 29853890. DOI: 10.1155/2018/7828735

67 Chehrazi-Raffle A, Meza L, Alcantara M, Dizman N, Bergerot P, Salgia N, Hsu J, Ruel N, Salgia S, Malhotra J, Karczewska E, Kortylewski M and Pal S: Circulating cytokines associated with clinical response to systemic therapy in metastatic renal cell carcinoma. J Immunother Cancer *9(3)*: e002009, 2021. PMID: 33688021. DOI: 10.1136/jitc-2020-002009

68 Lopez-Beltran A, Henriques V, Cimadamore A, Santoni M, Cheng L, Gevaert T, Blanca A, Massari F, Scarpelli M and Montironi R: The identification of immunological biomarkers in kidney cancers. Front Oncol *8*: 456, 2018. PMID: 30450335. DOI: 10.3389/fonc.2018.00456

69 Spisarova M, Melichar B, Juranova J, Zemankova A, Adam T, Matousova K, Javorska L, Krcmova LK, Turonova D and Studentova H: Biomarkers of inflammation and progression during immunotherapy in patients with metastatic renal cell carcinoma. In Vivo *37(1)*: 393-399, 2023. PMID: 36593038. DOI: 10.21873/invivo.13091

70 Köhn L, Svenson U, Ljungberg B and Roos G: Specific genomic aberrations predict survival, but low mutation rate in cancer hot spots, in clear cell renal cell carcinoma. Appl Immunohistochem Mol Morphol *23(5)*: 334-342, 2015. PMID: 24992170. DOI: 10.1097/PAI.0000000000000087

71 Shu X, Gu J, Huang M, Tannir NM, Matin SF, Karam JA, Wood CG, Wu X and Ye Y: Germline genetic variants in somatically significantly mutated genes in tumors are associated with renal cell carcinoma risk and outcome. Carcinogenesis *39(6)*: 752-757, 2018. PMID: 29635281. DOI: 10.1093/carcin/bgy021

Received February 26, 2023
Revised March 29, 2023
Accepted March 30, 2023