Carbonic Anhydrase IX Expression Is Associated with Improved Outcome of High-dose Interleukin-2 Therapy for Metastatic Renal Cell Carcinoma

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Abstract. *Aim: The objectives of this study were to evaluate treatment responses to high-dose interleukin-2 (HD IL-2) in patients with metastatic renal cell carcinoma (mRCC) and assess correlation between responses and prognostic factors, such as histology, site of metastatic disease, prior treatment, prior nephrectomy, and carbonic anhydrase IX (CAIX) expression. Patients and Methods: A retrospective analysis was performed on all mRCC patients treated with HD IL-2 between 1996 and 2006 at the University of Minnesota Medical Center in Minneapolis, Minnesota, USA. A cycle of HD IL-2 consisted of 600,000 U/kg given once every 8 hours for 14 doses. Cycles were repeated until disease progression or intolerable toxicities developed. CAIX expression and staining intensity were evaluated on available primary tumor tissue. Results: Forty-seven patients with mRCC were identified. Of the 107 cycles of therapy that were given, 97.1% of patients received only two cycles of therapy. Complete response and partial response were seen in 3 (6%) and 15 (32%) patients, respectively. The overall disease control rate was 42.6%. The longest durable CR was 72 months and the shortest was 45 months. The median time to disease progression in patients with a CR or PR was 12 months. Patients with a Memorial Sloan-Kettering Cancer Center prognostic score of '1' were two times more likely to progress after two cycles than patients with a score of '0'. No response was observed in patients whose tumors were negative for CAIX by immunoperoxidase staining. Conclusion: HD IL-2 is a reasonable option for first-line therapy for selected patients with mRCC. Patients with tumors negative for CAIX may not benefit from HD IL-2 therapy. Further research is necessary to define patients with a higher likelihood of disease response to this therapy.*

High-dose interleukin (IL)-2 was approved by the Food and Drug Administration for the treatment of metastatic renal cell carcinoma (mRCC) in 1992. The potential life-threatening complications often associated with HD IL-2 have limited its availability.

The rationale for immunotherapy with IL-2 is based on documented cases of spontaneous remission of mRCC (1, 2). It is thought that these remissions were the result of RCC stimulating the host's immune system. IL-2 is a glycoprotein which regulates lymphocyte function and growth. IL-2 does not act directly on tumor cells; rather, it mediates its antitumor effects by activating and expanding the host's lymphocyte populations (3, 4). Currently, IL-2 is the only treatment modality that offers the possibility of curing patients with mRCC (5-7). In the phase II clinical trials supporting the FDA's approval of HD IL-2 for mRCC, there were 255 individuals from seven different clinical sites. Of these, 17 patients (7%) were in complete response (CR) and 20 patients (8%) were in partial response (PR) almost 11 years after treatment (8). A randomized phase III trial comparing HD IL-2 to subcutaneous IL-2 plus interferon (IFN) showed superior

Abbreviations: CAIX: carbonic anhydrase IX; CR: complete response; FDA: Food and Drug Administration; HD: high-dose; IFN: interferon; IL-2: interleukin-2; mRCC: metastatic renal cell carcinoma; MSKCC: Memorial Sloan-Kettering Cancer Center; PR: partial response; RCC: renal cell carcinoma; TTDP: time to disease progression.

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efficacy for HD IL-2, with response rates of 23.2% and 9.9% for HD IL-2 and subcutaneous IL-2 plus IFN, respectively. A three-year follow-up revealed ten patients receiving HD IL-2 were still progression free, compared to three patients receiving subcutaneous IL-2 plus IFN (p=0.082) (9). Carbonic anhydrase IX (CAIX) expression has been associated with better outcome of patients with RCC (10), although it may not be an independent predictor of better survival (11). The role of CAIX expression as a prognosticator of benefit from IL-2 therapy is under evaluation based on an initial report by Atkins *et al*. (12).

In this single-center, retrospective analysis, we reviewed patient charts to analyze the efficacy of HD IL-2 for the treatment of mRCC and correlate benefit with CAIX expression.

Patients and Methods

Study design, patient eligibility and treatment. The medical record database was retrospectively searched for mRCC patients treated with HD IL-2 between January 1996 through December 2006 at the University of Minnesota Medical Center, Fairview in Minneapolis, Minnesota, USA. The purpose of this analysis was to determine whether the University of Minnesota's CR and PR were comparable to previously published data. This study included adult patients (18 years or older) with histologically documented RCC. Patients eligible for HD IL-2 therapy were required to have an Eastern Cooperative Oncology Group performance score between 0-2 due to the cardiopulmonary toxicity of IL-2. This retrospective chart review was approved by the University of Minnesota's Institutional Review Board.

HD IL-2 was given intravenously in a non-intensive care unit hospital ward. Every eight hours, a bolus of HD IL-2 at a dose of 600,000 IU/kg was administered over 15 minutes. Patients were monitored for side-effects related to capillary leak syndrome, including hypotension and pulmonary edema. These bolus infusions were repeated at eight-hour intervals as tolerated by the patient, with a maximum of 14 doses per cycle. Patients were discharged when hemodynamically stable, and brought back to the hospital usually 5-10 days later to repeat the course of treatment. Treatment was continued until either CR, or the disease no longer responded, or the patient could no longer tolerate the side-effects.

Evaluating response. Computed tomography imaging of the chest, abdomen, and pelvis was performed to assess treatment response (using Response Evaluation Criteria in Solid Tumors (RECIST)) (13) after every two cycles. In brief, CR was defined as disease that had disappeared and stayed in remission for at least one month. PR was defined as disease that had regressed by greater than 30% in tumor size. Stable disease (SD) was classified as disease that had regressed in size by less than 30% or grown by no more than 20%. Disease progression was indicated when the tumor had increased by more than 20% in size. Mixed response was defined as having at least one site of tumor progression and one site of tumor response.

Data collection. The data collected from the patients' medical records included: gender, pathological diagnosis, history of nephrectomy, sites of metastasis, Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic scores (14), history of prior systemic treatment, response to therapy, number of doses per cycle, number of cycles of treatment received and time to disease progression.

Tumor tissue staining for carbonic anhydrase IX. Four-micrometer sections of formalin-fixed, paraffin-embedded tissue from primary kidney tumor were cut and placed on charged glass slides. Tissue sections were deparaffinized and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide. CAIX was identified using rabbit polyclonal antihuman antibody (ab15086; Abcam Inc. Cambridge, MA, USA) at a concentration of 1:1000. Heat antigen retrieval was carried out in 10 mM citrate buffer (pH 6.0). The immunogen for the polyclonal antibody was a synthetic peptide made to a sequence within residues 359-459 of human CAIX. Staining was performed in an automated immunostainer (Ventana Benchmark XT; Ventana, Tucson, AZ, USA). An indirect biotin-streptavidin system was used to detect the primary antibody (New DAB detection kit; Ventana). One representative block per tumor was selected and stained.

CAIX expression and staining intensity were evaluated by a pathologist (J.C.M.) without knowledge of patient outcome. CAIX expression was recorded as the percentage of tumor cells that stained positively for CAIX. Intensity was scored as absent (0), weak (+1), moderate (+2) or strong (+3) for the area of the section demonstrating maximal staining (Figure 1). CAIX tumor expression was analyzed using the cut-off point for low (85%) and high (>85%) expression previously identified by Bui *et al*. (10)

Statistical methods. For the purposes of these analyses, patients were grouped based on clinical benefit of therapy. Patients were considered to have benefited from therapy if they had a PR, mixed or CR. Patients with no response or SD were considered not to have benefited from therapy. Patients with MSKCC prognostic scores of '2' or '3' were grouped together for the analyses due to the small number of patients.

Fisher's exact test was used to determine if histology, metastatic site, prior treatment and prior nephrectomy were related to clinical benefit of therapy. The Spearman correlation coefficient was used to estimate correlation between the response type and MSKCC prognostic score. Both were assumed ordinal, with the total score ranging from 0-3 and the response ranging from no response to CR.

Time to disease progression (TTDP) was calculated from study entry date to the date of progression or was censored at the date of last contact for patients who remained disease free. TTDP was summarized using Kaplan-Meier methods (15). Cox proportional hazards models and log-rank statistics were used to evaluate the relationship between clinical benefit of therapy measured by TTDP and MSKCC prognostic score. The log-rank test was used to compare the survival curves by CAIX stain. All analyses were performed using SAS version 9.1. (SAS Institute Inc., Cary, NC, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Patient characteristics. Forty-seven patients were identified through a retrospective review of medical records (Table I). By histological analysis, 85.1% of tumors had been classified as conventional and 14.9% as sarcomatoid RCC. All pathology specimens available at the University of Minnesota were re-reviewed; however, in some cases slides had already

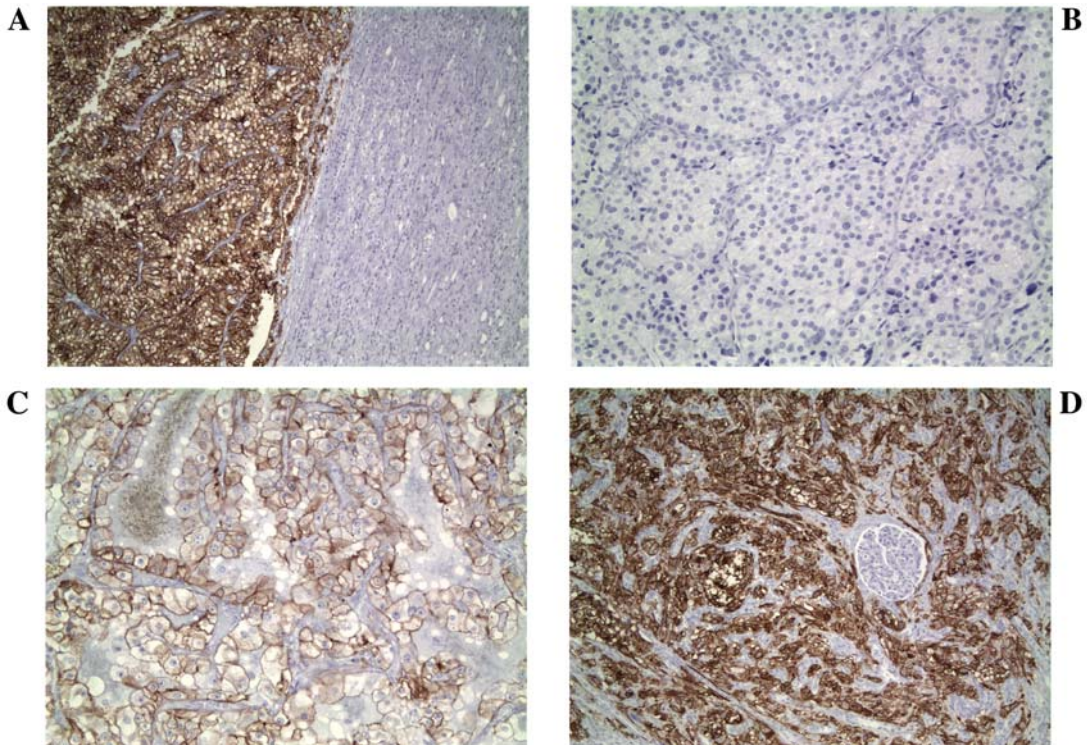

Figure 1. *Representative photomicrographs of carbonic anhydrase IX (CAIX) immunostaining. A: Tumor strongly positive for CAIX (left) in contrast to normal kidney negative (right) (magnification: ×40). B: Tumor negative for CAIX expression (magnification: ×200). C: Tumor weakly positive for CAIX (magnification: ×200). D: Tumor strongly reactive for CAIX expression surrounds normal glomerulus negative for this marker (magnification: ×40).*

been returned to the referring institutions and were unavailable for confirmatory review and CAIX staining. Multiple metastases were common, with the most common sites being the lungs (N=34, 72%), bones (N=15, 32%), mediastinum (N=10, 21%), and liver (N=8, 17%). Other metastatic sites included the retroperitoneum, renal bed, adrenal glands, brain and spleen. Prior treatments consisted of nephrectomy (96%), radiation (14.9%), or one of a variety of systemic treatments (27.7%). The male-to-female ratio was 4:1. At presentation, patients had average hemoglobin of 13 g/dl, lactate dehydrogenase of 433.5 IU/l and calcium level of 9.4 mg/dl. Of 47 patients, 43 completed two cycles of therapy. Eight patients completed three cycles, and six of these proceeded to a fourth cycle. Only one patient completed a sixth cycle. The average number of IL-2 injections per cycle was 8.6 (range 2-14). The median patient follow up was 20 months (range: 2-103 months).

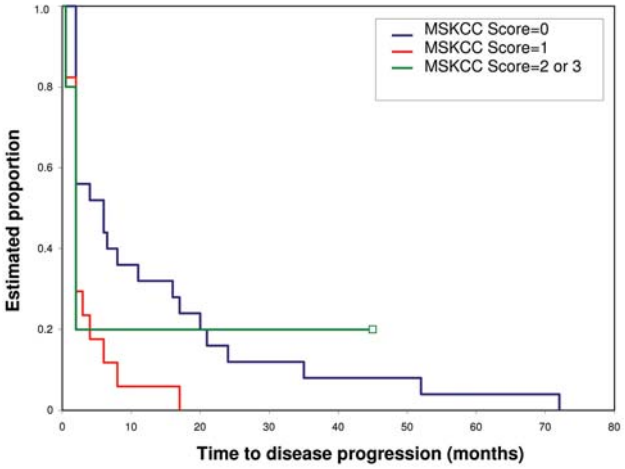

Figure 2. *Time to disease progression differed significantly according to renal cell carcinoma Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic score group (χ^2=7.491, p=0.024).*

Treatment responses. CR, PR and mixed response were achieved in 6.4% (N=3), 31.9% (N=15) and 2.13% (N=1) of patients, respectively. No response to treatment was seen in 57.5% (N=27) of patients. Clinical benefit was seen in 40.4% (N=19) of patients. Patients with CR received on average 7.4 IL-2 injections per cycle, while patients with PR and PD received 8.6 and 8.8 injections per cycle, respectively. Overall median TTDP was 2 months. The median TTDP for PR and CR groups was 8 and 62 months, respectively. The longest and shortest CR was 72 and 45 months, respectively.

When evaluating TTDP, one patient was censored at the last follow-up date due to a lack of disease progression. All other participants progressed during follow-up. TTDP differed significantly according to clinical benefit of therapy (χ^2=35.315, p<0.0001). The hazard ratio for patients without benefit compared to those with benefit was 7.73 (95% confidence interval=3.06-19.52; p<0.001). TTDP also differed significantly by MSKCC prognostic score group (χ^2=7.491, p=0.024) (Figure 2). The hazard ratio for patients with a score of '1' compared to those with a score of '0' was 2.05 (95% CI=1.06-3.92; p=0.033), and the hazard ratio for patients with a score of '2' or '3' compared to those with a score of '0' was 1.16 (95% CI=0.40-3.38; p=0.786).

An analysis of clinical benefit and MSKCC prognostic score group using the Spearman correlation coefficient suggests that a lower score is associated with clinical benefit of therapy (p=0.0916), although this was not significant at the standard 0.05 level. Additionally, when looking at response type (none, SD, PR, mixed, CR) and total score, a lower score was also associated with a better response (p=0.076).

Prognostic factors. No significant association was seen between clinical benefit of therapy or histology (p=1.00), presence of lung metastases (p=0.228), other metastatic site (p=0.394), prior treatment (p=0.635) or prior nephrectomy (p=0.508).

Only 20 patients had tissue available for CAIX staining, all with conventional histology. The staining pattern and intensity was very uniform throughout the slides of each case. In 6 patients, tumors had no staining, and in 14 patients, tumor tissue was strongly positive. All patients with negatively stained tumors had no response to interleukin therapy, except for one who had a mixed response. Eight out of 14 patients with CAIX-overexpressing tumors had at least a PR to therapy. Median TTDP in patients with tumors negatively stained for CAIX was 2 months (range: 0.5-35 months), whereas that for those with positively stained tumors was 4.5 months (range: 2-72 months, p=0.42). Median overall survival of patients with tumors negatively stained for CAIX was 12.5 months (range: 4-not reached), whereas that for those with positively stained tumors was 21.5 months (range: 16-60 months, p=0.58) (Table II).

The median number of cycles before response was detected was 2 (range: 2-6). Out of 36 patients with clear cell carcinoma, 14 had PR and 1 had CR. Of the seven patients with sarcomatoid carcinoma, two had PR and one had CR.

Table I. *Patient demographics*.

	N	(%)
Number of patients	47	
Male/female	37/10	79/21
Response		
None	27	57.5
Stable	1	2.1
Partial	15	31.9
Mixed	1	2.1
Complete	3	6.4
Clinical benefit		
Yes	19	40.4
No	28	59.6
Prognostic score		
0 - Good	25	53.2
1 - Intermediate	17	36.2
2 - Intermediate-poor	4	8.5
3 - Poor	1	2.1
Histology		
Clear cell	36	76.6
Granular	3	6.4
Sarcomatoid	7	14.9
Spindle	1	2.1
Metastasis site		
Bone only	3	6.4
Brain only	2	4.3
Liver only	2	4.3
Lungs only	19	40.4
Viscera only	17	36.2
Multiple sites	4	8.5
Prior treatment		
None	23	48.9
Resection	3	6.4
XRT	7	14.9
Systemic TX	13	27.7
Resection/XRT	1	2.1
Prior nephrectomy		
Yes	45	95.7
No	2	4.3

Discussion

The purpose of our retrospective study was to compare treatment responses of patients treated at the University of Minnesota to previously published data and correlate outcomes with expression of CAIX. Both the CR rate (6.4% N=3) and median TTDP for CR (62 months) in our patient population are similar to those of previous reports (6, 7).

An important limitation of this study is the relatively small patient population. There were few patients with SD or CR and few with MSKCC scores of '2' or '3'. This affects the statistical significance of any evaluation of a potential correlation between treatment response and MSKCC prognostic score. Nevertheless, patients with a MSKCC prognostic score of '1' were approximately twice as likely not to benefit from IL-2 therapy compared to those patients with a score of '0'. These data suggest that the MSKCC prognostic score might be a useful predictor of response to HD IL-2 therapy.

Despite the limitations inherent in a retrospective study and the relatively small patient population, our data show that HD IL-2 has the potential of inducing a durable response.

Table II. *Analysis of high-dose IL-2 therapy and carbonic anhydrase IX positive versus negative staining.*

	N	(%)	No. of events	Median time in months (95% CI)	Log-rank statistic	*p*-Value
Disease progression						
Yes	46	97.9				
No	1	2.1				
CAIX stain available	20	42.6	20	2.00 (2.0, 6.5)		
CAIX stain					0.6520	0.4194
Positive	14	70.0	14	4.50 (2.0, 17.0)		
Negative	6	30.0	6	2.00 (NA)		
Survival						
Alive						
Yes	18	38.3				
No	29	61.7				
CAIX stain available	20		14	20.0 (12.0, 60.0)		
CAIX stain					0.3015	0.5829
Positive	14		10	21.5 (16.0, 60.0)		
Negative	6		4	12.5 (4.0, ∞)		

CI: Confidence interval; NA: not available.

HD IL-2 therapy remains the only treatment modality that offers the possibility of cure. Although the response rate is low, we think that HD IL-2 should continue to be the first-line treatment option for selected patients with no other pre-existing co-morbidities that would preclude them from receiving such a toxic regimen. We believe that tissue markers such as CAIX may be of help in predicting the likelihood of response to HD IL-2 and in selecting patients for this therapy. Chaperon-like function of CAIX has been recently proposed as factor in augmenting immunogenicity of kidney tumors (16). Further research is needed in the form of a larger prospective study looking at mRCC patients and immunotherapy.

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