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Role of carbonic anhydrase 9 as diagnostic and prognostic marker in clear cell renal cell carcinoma: A single centre study

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Abstract
Background: Carbonic anhydrase 9 (CA-9) is currently being evaluated as prognostic and predictive biomarker for clear cell renal cell carcinomas (ccRCC) and also aids in diagnosis, monitoring tumour progression and clinical outcome.
Methods: Descriptive study wherein 100 cases of primary ccRCC and 50 controls tissues were evaluated for CA-9 by immunohistochemistry. Immunoreactivity scores and pattern of staining were correlated with Furhman grading, clinical staging and risk stratification.
Results: 88% cases of ccRCC exhibited CA-9 immunopositivity wherein 20% nonclear cell category were positive exhibiting strong statistical correlation between CA-9 immnoexpression and ccRCC. Low grade tumours, lower stage grouping and low risk tumours (UCLA integrated clinical staging) exhibited strong to moderate positivity while high grade, high stage and high risk tumours were either negative or exhibited weak patchy positivity and association was statistically significant.
Conclusion: CA-9 is a valuable diagnostic marker for ccRCC and its expression correlates with low grade low risk tumours with favourable prognosis.

Keywords: Carbonic anhydrase, furhman grade, immunohistochemistry, renal cell carcinoma

1. Introduction
Clear cell renal cell carcinoma (ccRCC), are closely associated with *VHL* gene mutations which are seen in approximately 80% of the ccRCCs. Currently, tumour node metastasis (TNM) stage and grade are used for prognostication with advanced TNM stage resulting in poorer survival [1-2]. Identification of tissue-based RCC biomarkers that provide further prognostic information is vital for monitoring disease progression and response to therapy [3]. Carbonic anhydrase 9 (CA-9) - a protein maintaining intracellular and extracellular pH also influences the regulation of cell proliferation, oncogenesis, and tumor progression. Its expression is von Hippel-Lindau-hypoxia inducible factor pathway dependent [3]. Recent data delineated an association of low CA-9 levels and poor survival of patients and lower response rates to tyrosine kinase inhibitor (TKI) treatment. Hence CA-9 characterisation may help in prognostication and also may aid in selection of patients who might benefit from IL-2 or CA-9 targeted therapies [4]. Hence this study was undertaken to correlate the expression of CA-9 in our cases of ccRCC with reference to grading, staging and risk stratification.

2. Materials and Methods
2.1 Study design: Cross sectional observational study conducted at department of laboratory sciences along with department of urology of a tertiary care multispecialty hospital in New Delhi. Clearance from institutional ethical committee was obtained.

2.2 Selection of cases: 100 cases of ccRCC who underwent surgery as a part of management protocol were included in the study. All cases where morphology was other than that of ccRCC including the variants with clear cell morphology (clear cell papillary renal cell carcinoma and translocation carcinoma) were excluded from the study group. 40 cases of renal tumours other than ccRCC and 10 cases of normal renal autopsy tissue were taken as controls. Informed consent was obtained from all patients.

Demographic and clinical data was accrued from the data register in the urology operation theatre, urology OPD, ward and requisition forms.

2.3 Histopathological Studies

2.3.1 Histopathology: 100 specimen of radical and partial nephrectomy were processed as per standard guidelines issued in college of American pathologist (CAP) protocol. 5µm thick hematoxylin and eosin (H&E) stained sections were examined. Paraffin blocks and H&E stained sections of retrospective cases were retrieved from database and were re-assessed for morphology. In both retrospective and prospective cases morphological diagnosis was made and tumour graded as per conventional Fuhrman grading system.

2.3.2 Immunohistochemistry (IHC): IHC was carried out on formalin fixed paraffin embedded tissues for CA-9 (Sigma Aldrich clone EP161, catalogue no 379R-1, concentrated, dilution 1:100, IgG rabbit monoclonal). Secondary detection system used was single step polymer based detection system (Envision detection system, peroxidise / DAB, rabbit/mouse). Immunohistochemical staining was evaluated by light microscopy using 10X and 20–40X objective lens for confirmation. Membranous staining for CA9 was considered positive. The extent of immunostaining was categorized into 4 groups according to the percentage of positive immunostained neoplastic cells

- Score 0: Absent
- Score 1: ≤25%
- Score 2: 25%–50%
- Score 3: 50%–75%
- Score 4: ≥75%

The intensity of positive immunostaining of tumor cells was categorized into:

- Score 0: Absent
- Score 1: Weak (interrupted membranous staining)
- Score 2: Moderate (box like staining but weaker than score 3)
- Score 3: Intense (complete circumferential box like membranous staining)

A combined immunoreactivity score was calculated by multiplying the score for extent by the score for intensity and cases were grouped as:

- Absent (0): 0
- Mild staining (1): 1 - 4
- Moderate (2) : 5 - 8
- Intense (3): 9 - 12

2.4 Risk Stratification

Risk stratification was carried out using UCLA (University of California and Los Angles) Integrated Staging System. Using the system, patients were placed in low, intermediate and high risk group. These variables were then correlated with CA-9 marker expression [5] (Table 1).

Table 1: UISS (UCLA Integrated Staging System). Escudier B *et al*. [5]

	Prognostic group				
Patient group		**T stage**	**Furhman grade**	**ECOG status**	**5 year disease specific survival**
Localised disease (N0,M0)	Low risk	1	1-2	0	91.1
	Intermediate risk	1	1-2	1 or more	80.4
		1	3-4	Any	
		2	Any	Any	
		3	1	Any	
		3	2-4	Any	
	High risk	3	2-4	1 or more	54.7
		4	Any	Any	
Metastatic disease	Low risk	N1M0	Any	Any	32
		N2M0/M1	1-2	0	
	Intermediate risk	N2M0/M1	1-2	0, 1 or more	19.5
			3	0, 1 or more	
			4	0	
	High risk	N2M0/M1	4	1 or more	0

Legend to Table: ECOG - Eastern Cooperative Oncology group

2.5 Statistical analysis

The clinicopathological and immunohistochemical features were tested for their association with the histological subtype using Student's t-test for continuous variables and the chi-square test for qualitative variables. All statistical analyses were performed using the SPSS 21.0 software. Statistical significance was considered when P value ≤ 0.05.

3. Results

3.1 Demographic and Clinical data

Age of patients (n=100) ranged between 42 years and 75 years with a mean of 57.28 years (SD-10.994) and median of 59 years. Men outnumbered women, with males being 31/50(62%) and females 19/50 (38%). In control group (n=50) age ranged from 45 years to 50 years with mean and median age of 48 years and 47 years respectively. Majority of cases underwent radical nephrectomy (88%) while rest were managed by partial nephrectomy (12%). The tumour stage (T Stage) based on the maximum dimension of the tumour is depicted in Table 2. 24/100 cases were positive for metastasis to bone (8/24), lung (12/24) and liver (5/24). Positive lymph node status was noted in 12/100 cases.

3.2 Histopathological examination

Furhman grading of 100 ccRCC cases were grade 1; 22 (22%), grade II; 36(36%), grade III; 30 (30%) and grade IV; 12(12%). 42 cases (42%) showed tumour necrosis while 04 cases (4%) revealed focal sarcomatous differentiation.

Lymphovascular invasion was observed in 6/100 cases. Majority of tumours were confined within renal capsule (92%) while rest (8%) revealed infiltration of capsule, renal pelvis or both. Based on the tumour stage, nodal status and metastasis (TNM), 62 % cases were stage I,16% were stage II, 2% were stage III and 20% Stage IV. The control group was composed of Type 1 papillary RCC (12/50). Type 2 papillary RCC (7/50), chromophobe RCC (6/50), oncocytoma (3/50), urothelial carcinoma (6/50), clear cell papillary RCC (2/50) angiomyolipoma (4/50) and normal renal autopsy cases (10/50)

3.3 Risk stratification

Based on UCLA integrated staging system, patients were categorised as follows: Low risk: 38%, Intermediate risk: 48% and high risk: 14%.

3.4 Immunohistochemistry

All 100 cases of ccRCC and 50 control cases under study were stained for CA9 and results with immunoreactivity score is depicted in Table 3&4. In ccRCC group 88/100 (88%) cases exhibited positivity for CA9 while rest 12/100 (12%) cases were negative. In nonclear cell category 10/50 cases exhibited CA-9 immunostaining exhibiting strong statistical correlation between CA-9 immunostaining and ccRCC (Pearson $x^2 = 65.08$ p value < 0.05). Table 2 All autopsy tissues composed of normal renal parenchyma were negative for CA9. All cases of ccRCC exhibited membranous staining. In two cases of clear cell papillary RCC, staining was basal and cup like whereas in six cases of urothelial carcinoma immunostaining was membranous and cytoplasmic. Papillary RCC and chromophobe RCC one case each exhibited weak patchy immunostaining. Sarcomatous component in 04 cases of ccRCC did not exhibit CA9 immunostaining or exhibited weak negligible expression

Table 2: Distribution of cases with respect to CA-9 staining. Clear cell RCC n=100, Nonclear cell tumours n=40 and normal renal tissue n=10

S No	Tumour	Total No	Positive for CA-9	Negative for CA-9	Percentage positive CA-9
1	Clear cell RCC	100	100	0	100
2	Non Clear cell RCC	50	10	40	20
2a	Type 1 Papillary RCC	12	0	12	
2b	Type 2 Papillary RCC	7	1	6	
2c	Chromophobe RCC	6	1	5	
2d	Clear Cell Papillary RCC	2	2	0	
2e	Oncocytoma	3	0	3	
2f	Angiomyolipoma	4	0	4	
2g	Urothelial Carcinoma	6	6	0	
2h	Normal Renal Autopsy tissue	10	0	10	0

Legend to Table: RCC – Renal cell carcinoma

3.5 Correlation of CA-9 staining with grading and staging

3.5.1 Fuhrman nuclear grade: Distribution of ccRCC cases with respect to CA-9 positivity and immunoreactivity score revealed inverse correlation with Furhman grade of the tumour. It was observed that low grade tumours (Grade I/II) exhibited strong to moderate positivity while high grade tumours (Grade III/IV) were either negative or exhibited weak patchy positivity which was statistically significant. (Pearson $x^2 = 90.833^a$, p value = 0.000). (Figures 1-4)

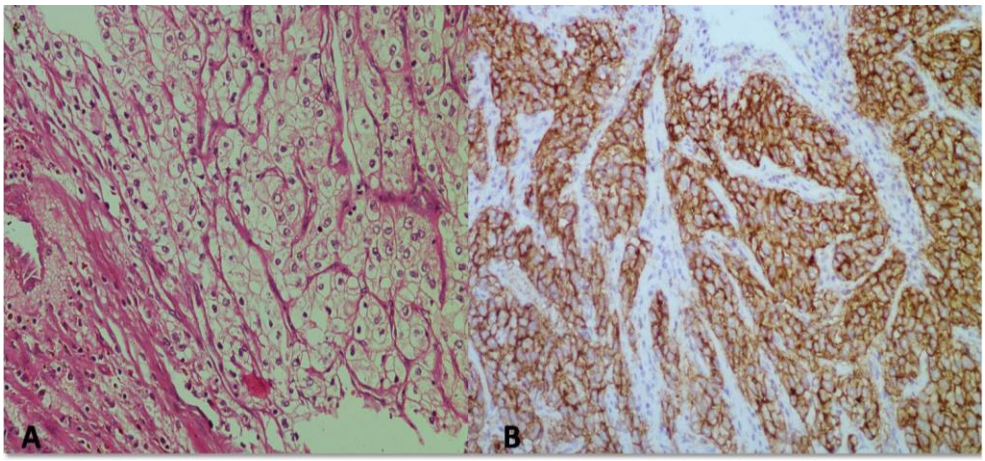

Fig 1: (A) Clear cell renal cell carcinoma, Furhman grade 1, haematoxylin and eosin, magnification 200 X. (B) CA9 immunostaining showing diffuse strong membranous pattern, immunoreactivity score 3. Immunoperoxidase x diaminobenzaldehyde staining; magnification 100 X.

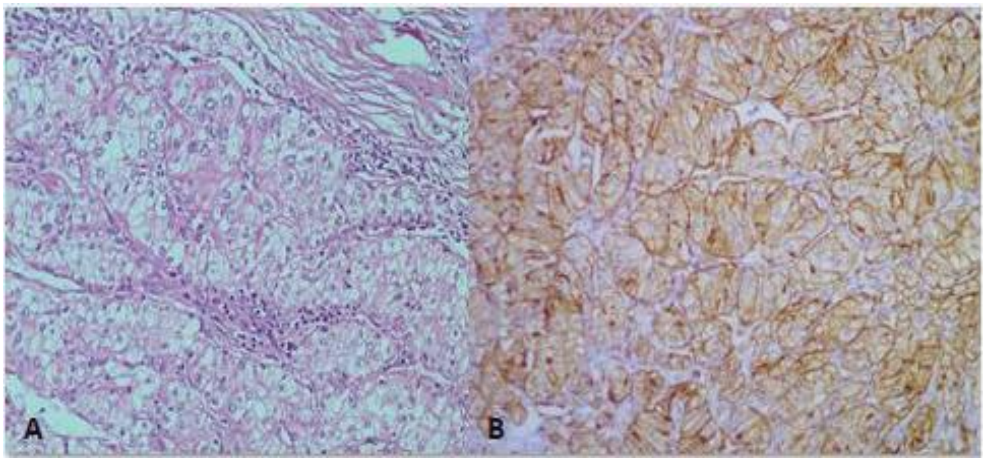

Fig 2: (A) Clear cell renal cell carcinoma, Furhman grade 2, haematoxylin and eosin, magnification 200 X. (B) CA9 immunostaining showing diffuse membranous pattern, immunoreactivity score 2. Immunoperoxidase x diaminobenzaldehyde staining; magnification 200X

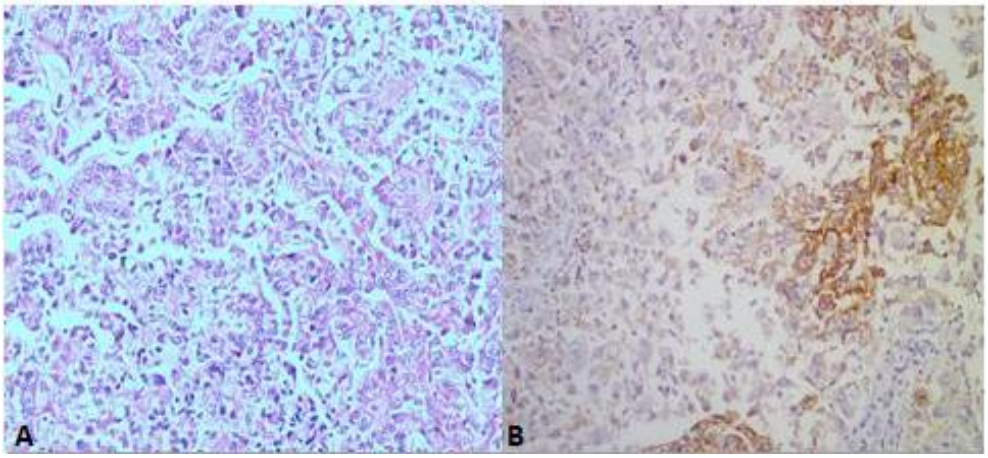

Fig 3: (A) Clear cell renal cell carcinoma, Furhman grade 3, haematoxylin and eosin, magnification 200 X. (B) CA9 expression showing patchy weak immunostaining pattern, immunoreactivity score 1. Immunoperoxidase x diaminobenzaldehyde staining; magnification 200X

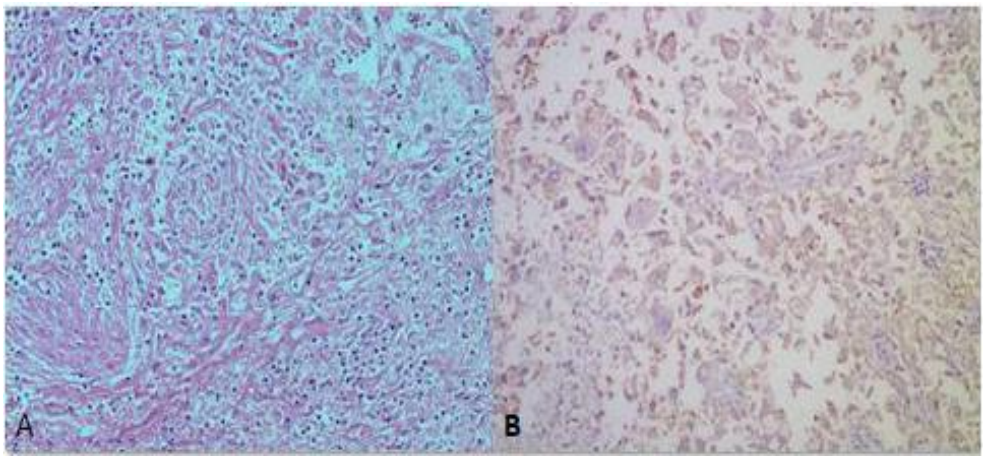

Fig 4: (A) Clear cell renal cell carcinoma, Furhman grade 4, haematoxylin and eosin, magnification 200 X. (B) CA9 expression showing very weak negligible immunostaining. Immunoperoxidase x diaminobenzaldehyde staining; magnification 200 X

3.5.2 TNM status and stage grouping: CA-9 immuno reactivity showed inverse statistical association with higher T status of the tumour wherein intensity of the staining reduced with the increasing T status. (x^2 = 5.027, p value = .046). Similarly low CA-9 expression was seen with lymph nodal metastasis (x^2 = 33.500, p value = .000) and with distant metastasis (x^2 = 5.02, p value = 0.17). (Table 3) The distribution of CA-9 intensity and positivity vis a vis stage grouping also revealed diminished CA-9 expression with higher stage. (x^2 = 20.317, p value =0.016) (Table 4)

Table 3: Distribution of cases as per Tumour stage, lymph node status and metastasis, n=100

Immunoreactivity Scores		Tumour Stage			Total
		1	2	3	
CA9	0	6	2	4	12
	1	14	6	2	22
	2	12	6	0	18
	3	42	4	2	48
Total		74	18	8	100
		Lymph Node Status			Total
		Negative		Positive	
CA9	0	2		10	12
	1	20		2	22
	2	18		0	18
	3	48		0	48
Total		88		12	100
		Metastasis			Total
		Negative		Positive	
0		6		6	12
1		16		16	22
2		16		16	18
3		42		42	48
Total		80		80	100

Table 4: Correlation of CA9 immunoreactivity score with Furhman grade, stage and risk Stratification. N = 100

CA-9 (immuno-reactivity score)	Furhman grade				Total
	1	2	3	4	
0	0	0	0	10	10
1	0	0	22	2	24
2	0	12	6	0	18
3	22	24	2	0	48
Total	22	36	30	12	100
	Stage				
	1	2	3	4	Total
0	2	2	2	6	12
1	10	6	0	6	22
2	10	6	0	2	18
3	40	2	0	6	48
Total	62	16	2	20	100
	Risk stratification				
	Low	Intermediate	High	-	Total
0	0	0	14		14
1	0	22	0		22
2	4	14	0		18
3	34	12	0		46
Total	38	48	14		100

3.5.3 Risk stratification: All 100 cases of ccRCC were stratified into low risk, intermediate risk and high risk cases according to UCLA guidelines. The distribution of cases in each category vis a vis the expression of all four markers is given in Tables 4. Strong CA9 expression was associated with low risk. ($x^2 = 70.32$, p value =0.001)

4. Discussion

CA-9 expression is associated with VHL mutational status and has a significant role in carcinogenesis and tumour progression. ccRCC are genetically heterogenous with varied biological behaviour and clinical disease outcome. There are conventional prognostic factors, which can be used to predict the prognosis of RCC important being Fuhrman nuclear grading system, TNM staging system, presence of tumour necrosis, sarcomatoid component with perirenal fat, renal pelvis and vascular invasion. However adjuncts like molecular profiling and tissue based IHC markers are required to provide precise insight into treatment methods, disease monitoring and response. CA-9 is one such marker characterised by high tissue expression in ccRCC and whose role as a diagnostic and prognostic marker is currently being evaluated in various studies

An overall percentage positivity of 88% and strong statistical association of CA-9 with ccRCC was observed in our patients, the intensity of which varied according to the pathological profile of the tumour. CA-9 positivity was also observed in all cases of urothelial carcinomas though positivity observed in papillary RCC and chromophobe RCC was weak and patchy while that in clear cell papillary RCC was basal. Hence strong statistical association of CA-9 positivity with ccRCC makes it a useful marker for its differentiation from other categories of RCC [6-8]. However in contrast to Wenjuan Yu *et al* none of our cases with sarcamatoid differentiation stained definitively for CA-9.[8] However it is in consonance with several studies that have

also reported CA-9 positivity in urothelial carcinomas and other nonrenal malignancies like endometrium, cervix, breast, gastric lung, and liver tumours. Though the pattern of staining observed was cytoplasmic and diffuse in urothelial carcinomas CA9 may be not useful, as a solitary diagnostic marker in metastatic sites and also in small biopsies [8-10].

The statistical correlation between high immunoreactivity score and low grade and low stage ccRCC was observed in our study. Strong CA-9 expression also correlated with the low risk tumours as per the UCLA integrated staging system for stratifying patients into modern therapy trials. The association of high CA-9 expression with good prognosis in patients with ccRCC is supported by a various studies [6, 10]. A meta-analysis by Zhihong Zao *et al* found that low CA-9 expression was associated with poor disease free survival, worse overall survival and unfavourable progression free survival [6]. These results indicate the potential of CA-9 as a valuable biological marker to predict prognosis in patients with ccRCC.

Li G *et al* also investigated the association between CA-9 expression and clinicopathological profile of ccRCCs [11]. Low CA-9 expression correlated with high ccRCC grade, existence of lymph node metastases, vascular invasion and distant metastases [12]. Bui *et al*, reported that within the category of metastastic disease strong CA-9 immunostaining was associated with a better prognosis than those patients with weak CA-9 staining [13-14]. Their study demonstrated that CA-9 expression is independently associated with outcome and prognosis in advanced ccRCC. Few studies have demonstrated conflicting results in other organs wherein high CA-9 expression predicts poor prognosis in patients with ovarian, gastric and lung malignancies. The mechanism for this difference is unclear but may be associated with VHL tumour suppressor gene inactivation [6, 15].

Studies have been conducted to demonstrate association of CA9 expression with selection of management protocols and as predictive marker in ccRCC. Patients with ccRCC exhibiting strong CA-9 expression levels have exhibited better treatment response with IL-2 [6, 11]. Patients with metastatic disease who received IL-2, the response rate was higher in patients with the strong CA-9 expression [13-15.] However significant association has not been demonstrated between CA9 expression and clinical outcome in patients treated with sorafenib or temsirolimus [4]. In contrast to Leibovich *et al* and Zang, B.Y. *et al* who in their studies stated that CA-9 expression is not a reliable factor for prognostication or risk stratificationwe in our study found high CA-9 expression with low grade low stage tumours and low risk tumours [16,1 7].

Availability of CA9-specific monoclonal antibody (mAb) cG250 have opened new vistas for ccRCC management. ARISER study, a randomized phase III clinical trial demonstrated that CA-9 immunoreactivity cut off value of score 2 may help in stratifying patients who can benefit from cG250 adjuvant therapy [18-19]. Chamie *et al*, using data from ARISER study also recommended that CA-9 scoring should be carried out for all patients with high-risk disease after nephrectomy for CA9 monotherapy [20]. The methodology of scoring system used in both studies is similar to the one used in our study. Finally CA-9 is not expressed in normal kidney tissue as observed in our study, and hence serum estimation of CA-9 levels as tumour marker can facilitate noninvasive diagnosis and follow up [21-22].

5. Conclusion

Molecular profiling and tissue based immunohistochemical indicators are important modalities for prognostication and to determine treatment methods and predict response. Our study revealed strong CA-9 expression in ccRCCs is associated with low grade and low stage tumours with low risk stratification. However it will be prudent to carry out CA-9 immunostaining with quantification for all cases of clear cell RCC to facilitate identification of patients who might benefit from CA-9 monotherapy. The findings however need validation with increased sample size, embedding of treatment arm in the study and long term follow up of the patients with incorporation of their survival data.

6. References

1. Brugarolas J. Molecular Genetics of Clear-Cell Renal Cell Carcinoma. Journal of Clinical Oncology. 2014; 32(18):1968-76.
2. Linehan WM. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013; 499(7456):43-9.
3. Clark PE. The role of VHL in clear-cell renal cell carcinoma and its relation to targeted therapy. Kidney International. 2009; 76(9):939-45.
4. Choueiri TK, Cheng S, Qu AQ, Pastorek J, Atkins MB, Signoretti S. Carbonic anhydrase IX as a potential biomarker of efficacy in metastatic clear-cell renal cell carcinoma patients receiving sorafenib or placebo: analysis from the treatment approaches in renal cancer global evaluation trial (TARGET). Urol Oncol. 2013; 31:1788-1793.
5. Escudier B, Porta C, Schmidinger M, Rioux-Leclercq N, Bex A, Khoo V *et al*. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2014; 25(Supplement 3):iii49 - iii56, doi:10.1093/annonc/mdu 259.
6. Zhao Z, Liao G, Li Y, Zhou S, Zou H, Fernando S *et al*. Prognostic value of carbonic anhydrase IX immunohistochemical expression in renal cell carcinoma: A meta-analysis of the literature. PLoS One. 2014; 9(11):e114096.
7. Alshenawy HA. Immuno histochemical panel for differentiating renal cell carcinoma with clear and papillary features. Pathol Oncol Res. 2015; 21:893-9
8. Yu W, Wang Y, Jiang Y, Zhan W, Li Y. Distinct immunophenotypes and prognostic factors in renal cell carcinoma with sarcomatoid differentiation: a systematic study of 19 immuno histochemical markers in 42 cases. BMC Cancer. 2017; 17:293-97
9. Driessen A, Landuyt W, Pastorekova S, Moons J, Goethals L, Haustermans K *et al*. Expression of Carbonic Anhydrase IX (CA IX), a Hypoxia-Related Protein, rather than Vascular-Endothelial Growth Factor (VEGF), a pro-angiogenic factor, correlates with an extremely poor prognosis in esophageal and gastric adenocarcinomas. Ann Surg. 2006; 243(3):334-340.
10. Genega EM, Ghebremichael M, Najarian R, Fu Y, Wang Y, Argani P *et al*. Carbonic anhydrase IX

expression in renal neoplasms: Correlation with tumor type and grade. Am J Clin Pathol. 2010; 134:873-879.
11. Li G, Feng G, Gentil-Perret A, Genin C, Tostain J. Serum carbonic anhydrase 9 level is associated with postoperative recurrence of conventional renal cell cancer. J Urol. 2008; 180:510-513.
12. Al-Ahmadie HA, Alden D, Qin LX, Olgac, Olgac S, Fine SW *et al*. Carbonic anhydrase IX expression in clear cell renal cell carcinoma: an immunohistochemical study comparing 2 antibodies. Am J Surg Pathol. 2008; 32(3):377-82
13. Bui M, Visapaa H, Seligson D, Kim H, Han KR, Huang Y *et al*. Prognostic value of carbonic anhydrase ix and ki67 as predictors of survival for renal clear cell carcinoma. J Urol. 2004;171(6):2461-2466
14. Bui MH, Seligson D, Han KR, Pantuck AJ, Dorey FJ, Huang Y *et al*. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res. 2003; 9:802-811
15. Choschzick M, Woelber L, Hess S, Christine E, Schwarz J, Simon R *et al*. Overexpression of carbonic anhydrase IX in vulvar cancer is associated with tumor progression and development of locoregional lymph node metastases. Virchows Arch. 2010; 456(5):483-90
16. Leibovich BC, Sheinin Y, Lohse CM, Thompson RH, Cheville JC, Zavada J *et al*. Carbonic anhydrase IX is not an independent predictor of outcome for patients with clear cell renal cell carcinoma. J Clinical Oncology. 2007; 25:4757-4764.
17. Zhang BY, Thompson RH, Lohse CM, Dronca RS, Cheville JC, Kwon ED *et al*. Carbonic anhydrase IX (CAIX) is not an independent predictor of outcome in patients with clear cell renal cell carcinoma (ccRCC) after long-term follow-up. BJUInt. 2013; 111(7):1046-1053.
18. Pickering LM, Larkin J. Carbonic anhydrase IX in resected clear cell RCC. Nature Reviews Urology. 2015; 12:309-310.
19. Belldegrun AS, Chamie K, Kloepfer P, Fall B, Bevan P, Störkel S *et al*. ARISER: A randomized double blind phase III study to evaluate adjuvant cG250 treatment versus placebo in patients with high-risk ccRCC—Results and implications for adjuvant clinical trials. Journal of Clinical Oncology. 2013; 31(Suppl.):a4507
20. Chamie K, Klopfer P, Bevan P, Chamie K, Klöpfer P, Bevan P *et al*. Carbonic anhydrase-IX score is a novel biomarker that predicts recurrence and survival for high-risk, nonmetastatic renal cell carcinoma: Data from the phase III ARISER clinical trial. Urologic Oncology. 2015; 33(5):204.e25-204.e33
21. Hulick P, Zimmer M, Margulis V, Skates S, Hamel M, Dahl DM *et al*. Blood levels of carbonic anhydrase 9 correlate with clear cell renal cell carcinoma activity. Clin proteom. 2009; 5:37-45
22. Lucarini L, Magnelli L, Schiavone N, Crisci A, Innocenti A, Puccetti L *et al*. Plasmatic carbonic anhydrase IX as a diagnostic marker for clear cell renal cell carcinoma. Journal of Enzyme Inhibition and Medicinal Chemistry. 2018; 33(1):234-240.