Dear **Editor**,

We are thankful for the opportunity of major revision and we are thankful to the Reviewer for taking time to evaluate our work and for the valuable comments that have significantly improved the quality of the article.
Below please find the point-by-point response to reviewer's comments and the changes applied to the manuscript accordingly.

REVIEWER 1

From this manuscript, the authors found that there are differences in TAMA between the two groups of kidney cancer patients with and without collateral vessels. And age was not an influencing factor in the two groups of patients, and it was concluded that " TAMA driven by a reduction in patients with peritumoral collateral vessels". Generally, this is a manuscript of good quality and certain value, but I have the following questions and hope to get appropriate responses.

・Since body mass index is an important indicator of systemic state, although the author uses TAMA as the main research index, did the authors consider the influence of body mass index on TAMA? (Maybe the body mass index of the two groups of patients is different, and TAMA may also be different? That is, does body mass index affect TAMA? These are not reflected in the baseline data and analysis).

RESPONSE:

Thank you for the comment. Body mass index (BMI) not give information about the body composition and about the quantity and distribution of different tissues. CT allows you to specifically evaluate both the quantity and the distribution of different body tissues. Given the retrospective nature of the study, unfortunately we do not have the BMI data, however we have shown in another published study that ccRCC patients with peritumoral collateral vessels show a significant reduction of subcutaneous adipose tissue (Greco F, Quarta LG, Carnevale A, Giganti M, Grasso RF, Beomonte Zobel B, Mallio CA. Subcutaneous Adipose Tissue Reduction in Patients with Clear Cell Renal Cell Carcinoma and Peritumoral Collateral Vessels: A Retrospective Observational Study. *Applied Sciences*. 2021; 11(13):6076. https://doi.org/10.3390/app11136076) which, coherently with the significant decrease in skeletal muscle mass demonstrated in this study suggests a BMI reduction.

CHANGE:

“Body mass index (BMI) is an indicator used for obesity classification that does not give any information about the body composition and it does not provide details about the quantity and distribution of different tissues (such as SM and abdominal adipose tissue compartments).”

“The results of this study are further reinforced and confirmed by recent evidence showing significant reduction of subcutaneous adipose tissue in ccRCC patients with peritumoral collateral vessels[17].“

・In the early stage of the research design, did the authors consider using other indicators to assist in judging the changes in TAMA? For example, abdominal circumference, body mass index, occupation, etc. (for example, engaged in heavy physical labor and athletes' muscle content is significantly higher than that of normal people)?

RESPONSE:

In view of the retrospective nature of the study, we do not have anamnestic data such as BMI and occupation. Further studies will need to evaluate these data, especially occupation, so as not to have confounding factors about SMM. abdominal circumference was not evaluated as we published another study evaluating the distribution and quantification of abdominal adipose tissue compartments. Since this data is more precise than the abdominal circumference, we decided not to include it (Fang H, Berg E, Cheng X, Shen W. How to best assess abdominal obesity. Curr Opin Clin Nutr Metab Care. 2018 Sep;21(5):360-365).

CHANGE:

The limitations of this study are due to the retrospective nature that did not allow us to have detailed clinical and anamnestic data including patients’ occupation, BMI, hormonal blood levels, disease-free survival (DFS), timing of CT imaging, performing status, therapies, and CT follow-up after therapies. For instance, testosterone deficiency is known to be associated with an increase of proinflammatory cytokines. The presence of hormonal data, such

as testosterone, could help better understand the cytokine cascade associated with pathogenesis and changes in body composition[34,35].
Similarly, CT follow-up after therapies (surgery, chemotherapy / targeted immunotherapy) would have been important to understand the changes of sarcopenia index and the relationship with peritumoral collateral vessels after therapies.
The vendor, model, and acquisition parameters (such as slices thickness) of the CT imaging used in this study were not systematically available. Images of the open-source TCIA were often acquired heterogeneously at multiple center as part of clinical routine.
Indeed, it would be helpful to give strengths to our findings to perform multivariate analysis with a larger sample size to verify if collateral vessels are an independent predictor of sarcopenia and the potential impact of other variables such as staging[36-38].
Further studies will evaluate the changes of sarcopenia index after therapies to add robustness to the role of peritumoral collateral vessels as prognostic biomarker in ccRCC patients. Such studied should consider abdominal circumference and patients' occupation, which is a factor that can influence SMM (for example, engaged in heavy physical labor and athletes' muscle content is significantly higher than that normal people)[39].
Finally, it will be estimated the SMM amount in other subtypes of kidney cancer (e.g. chromophobe and papillary) or in other categories of cancer patients, to assess the impact of SMM trophism on the patient's health status and prognosis.

39. Fang H, Berg E, Cheng X, Shen W. How to best assess abdominal obesity. Curr Opin Clin Nutr Metab Care. 2018 21:360-365.

REVIEWER 2

Summary Federico et al. performed a quantitative analysis of cross-sectional muscle area in make patients with clear renal cell carcinoma with/without peritumoral collateral vessels. Although the authors showed decreased cross-sectional muscle area was observed in clear cell renal cell carcinoma with peritumoral collateral vessels, I cannot recommend this article for publication because it has extensive problems.

・Introduction; The authors hypothesized decreased cross-sectional total abdominal muscle area is present in patients with ccRCC and peritumoral collateral vessels as a metabolic systemic consequence related to a locally advanced disease. It seems that this is the main point to perform the study later described in this manuscript. However, there is no rationale whether presence of decreased cross-sectional total abdominal muscle area is related to locally advanced disease nor metabolic systemic consequence.

RESPONSE:

Thank you for the comment. The rationale for this study is based on the fact that the presence of collateral vessels has been shown to correlate with locally advanced and even more aggressive disease (Greco F, Quarta LG, Bernetti C, Grasso RF, van Berge Henegouwen MI, Beomonte Zobel B, Mallio CA. Composition of Perinephric Fat and Fuhrman Grade in Clear Cell Renal Cell Carcinoma: The Role of Peritumoral Collateral Vessels. Applied Sciences. 2021; 11 (9): 394). Cancer cachexia is linked to a decrease in prognosis in patients with advanced or metastatic RCC. In this study we evaluated muscle area in patients with RCC and peritumoral collateral vessels (and therefore with locally advanced disease) so as to actually evaluate whether peritumoral collateral vessels can be considered a sign of muscle mass reduction.

CHANGE:

The presence of collateral vessels adjacent to RCC is a sign of locally advanced disease (i.e. pT stage > T3a) and high Fuhrman grade (e.g., III and IV)[19,20].

20. **Greco F,** Quarta LG, Bernetti C, Grasso RF, van Berge Henegouwen MI, Beomonte Zobel B, Mallio CA. Composition of Perinephric Fat and Fuhrman Grade in Clear Cell Renal Cell Carcinoma: The Role of Peritumoral Collateral Vessels. *Appl Sci* 2021 11: 3941.

・Materials and methods; The authors excluded female patients and non-Caucasian ethnicity patients. However, there is no reasonable evidence that these patients should be excluded from skeletal muscle quantification studies with certain reasons (Campi et al. Minerva Urol Nephrol. 2021 Oct 29. Online ahead of print). Please clarify why patients having these characters were excluded from this study.

RESPONSE:

Thanks for the comment. It has been shown that men have a greater amount of muscle mass and a different distribution than women. It has also been shown that different ethnic groups have a different distribution of muscle mass. To make the sample reliable, robust and avoid such bias, it was decided to conduct the study on Caucasian male patients.

CHANGE

Only caucasian male patients were included in the present study as it has been shown that there are differences of SMM according to gender and ethnicity [24,25].

24. **Janssen I,** Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol (1985)* 2000 89: 81-8.
25. **Silva AM,** Shen W, Heo M, Gallagher D, Wang Z, Sardinha LB, Heymsfield SB. Ethnicity-related skeletal muscle differences across the lifespan. *Am J Hum Biol* 2010 22: 76-82.

・Materials and methods; The timing of CT imaging used for this study was not described. The timing of CT imaging is highly important for this study as the skeletal muscle quantity is assumed to be decreased as the disease progresses (Gu et al. Sci Rep. 2017 Aug 8;7(1):7587.). Usually, in retrospective studies, the timing of CT imaging used was the images taken within 28-day before the initial therapy (Choi et al. PLoS One. 2015 Oct 5;10(10):e0139749. eCollection 2015; Sato et al. Pancreatology. 2021 Aug;21(5):892-902. Epub 2021 Mar 6.)

RESPONSE:

Thank you for the comment, It was not possible to perform the timing of CT imaging as this is a retrospective study whose data was taken from the Cancer Imaging Archive. Unfortunately, we do not have enough data to formulate the

timing of CT imaging or to acquire data on CT images taken 28 days prior to initial therapy. This was added to the limitations of the study.

CHANGE

The limitations of this study are due to the retrospective nature that did not allow us to have detailed clinical and anamnestic data including patients' occupation, BMI, hormonal blood levels, disease-free survival (DFS), timing of CT imaging, performing status, therapies, and CT follow-up after therapies. For instance, testosterone deficiency is known to be associated with an increase of proinflammatory cytokines. The presence of hormonal data, such as testosterone, could help better understand the cytokine cascade associated with pathogenesis and changes in body composition[34,35]. Similarly, CT follow-up after therapies (surgery, chemotherapy / targeted immunotherapy) would have been important to understand the changes of sarcopenia index and the relationship with peritumoral collateral vessels after therapies.
The vendor, model, and acquisition parameters (such as slices thickness) of the CT imaging used in this study were not systematically available. Images of the open-source TCIA were often acquired heterogeneously at multiple center as part of clinical routine.
Indeed, it would be helpful to give strengths to our findings to perform multivariate analysis with a larger sample size to verify if collateral vessels are an independent predictor of sarcopenia and the potential impact of other variables such as staging[36-38].
Further studies will evaluate the changes of sarcopenia index after therapies to add robustness to the role of peritumoral collateral vessels as prognostic biomarker in ccRCC patients. Such studied should consider abdominal circumference and patients' occupation, which is a factor that can influence SMM (for example, engaged in heavy physical labor and athletes' muscle content is significantly higher than that normal people)[39].
Finally, it will be estimated the SMM amount in other subtypes of kidney cancer (e.g. chromophobe and papillary) or in other categories of cancer patients, to assess the impact of SMM trophism on the patient's health status and prognosis.

36. Gu W, Wu J, Liu X, Zhang H, Shi G, Zhu Y, Ye D. Early skeletal muscle loss during target therapy is a prognostic biomarker in metastatic renal cell carcinoma patients. Sci Rep 2017 7: 7587.
37. Choi Y, Oh DY, Kim TY, Lee KH, Han SW, Im SA, Kim TY, Bang YJ. Skeletal Muscle Depletion Predicts the Prognosis of Patients with Advanced Pancreatic

Cancer Undergoing Palliative Chemotherapy, Independent of Body Mass Index. PLoS One 2015 10: e0139749.
38. Sato H, Goto T, Hayashi A, Kawabata H, Okada T, Takauji S, Sasajima J, Enomoto K, Fujiya M, Oyama K, Ono Y, Sugitani A, Mizukami Y, Okumura T. Prognostic significance of skeletal muscle decrease in unresectable pancreatic cancer: Survival analysis using the Weibull exponential distribution model. Pancreatology 2021 21: 892-902.
39. Fang H, Berg E, Cheng X, Shen W. How to best assess abdominal obesity. Curr Opin Clin Nutr Metab Care. 2018 21:360-365.

・Materials and methods; The model and parameters used for this study is not clear. Please specify the model (i.e. number of detector rows, manufacturer of the CT scan) and parameters (i.e. thickness of CT scanning) of the CT imaging used in this study.

RESPONSE:

The model (i.e. number of detector rows, manufacturer of the CT scan) and parameters (i.e. thickness of CT scanning) of the CT imaging used in this study were not included. The acquired data comes from TCIA where image data sets are heterogeneous in terms of scanner modalities, manufacturers and acquisition protocols. In most cases the images were acquired as part of routine care and not as part of a controlled research study or clinical trial. However, the data on thickness of CT scanning has no influence on the type of work as the region of interests (ROIs) have been measured on the single slice.

CHANGE

Please see the previous response about limitations.

・Materials and methods; The authors stated that the Shapiro-Wilk test was used for analysis of data distribution. However, the Shapiro-Wilk test is the statistical test whether a variable is normally distributed in a population. There is no result about this test shown in the result section. Also, it is not clear how to affect additional statistical analysis if the population is not distributed. Usually, the Student's t-test performed when the population is normally distributed, however, in recent article, the t-test is robust in non-normality distributed in certain conditions (Posten et al. Robustness of the Two-Sample T-Test. Robustness of Statistical Methods and Nonparametric Statistics. 92-99.)

RESPONSE:

Thank you for the comment. The population showed a Gaussian type of distribution, for this reason the t test was used.

CHANGE:

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・Materials and methods; The authors did not perform multivariate analysis. In terms of large heterogeneity observed in this study, the authors should consider performing multivariate analysis to show that the decreased skeletal muscle is the independent factor. In the manuscript, the authors performed age-correction to exclude age-related effects. However, there is no method was shown in the main text.

RESPONSE:

Thank you for the comment. Multivariate analysis was not performed and is a limitation of this study. Further studies will need to perform multivariate analysis to show that the decreased skeletal muscle is the independent factor. Indeed, the meaning of our paper was also to stimulate scientific curiosity and futher research on the topic, as confirmed by the question mark on the title.

CHANGE:

Please see the previous response about limitations.

・Results; The staging can be a confounder of the results. The authors should perform statistical analysis to show that there is no statistical difference between ccRCCA and ccRCCp group, in terms of staging, T, N and M factor.

RESPONSE:

Thank you for the comment. A statistical analysis to show that there is no statistical difference between ccRCCA and ccRCCp group, in terms of staging, T, N and M factor was not performed. Further studies will need to perform this statistical analysis.

CHANGE:

Please see the previous response about limitations.

▪ Discussion; In the section, the authors did not discuss the clinical significance of peritumoral vessels and cross-sectional muscle area. It is not clear whether the decreased cross-sectional muscle area affects patients' outcome. Minor points 1) Author contributions; The authors stated that "all the authors solely contributed to this paper". However, in this section, the corresponding author should specify each author's role played in this research (i.e. who analyzed and quantified the cross-sectional muscle area, who performed statistical analysis).

RESPONSE:

Thanks for the comment, we agree. To discuss the clinical significance of peritumoral vessels and cross-sectional muscle area and evaluate affects patients' outcome we added the survival data and drew the Kaplan-Meier curve.

CHANGE:

MATERIALS AND METHODS

Statistical analysis

"Finally, Kaplan-Meier curves were included to check survival between ccRCCa and ccRCCp groups."

RESULTS

"No significant differences of survival between the two groups (available data of 54 ccRCCa patients out of 54 and 69 ccRCCp patients out of 70) were found with the Kaplan-Meier method (log-rank test; z = 1.88, p = 0.06; 95% confidence interval) (Figure 4)."

DISCUSSION

"Indeed, patients with ccRCC who were dead, demonstrated a statistically significant reduction of TAMA with respect to those alive, suggesting a link

between sarcopenia and survival in our sample. However, Kaplan-Maier curves showed a difference just above the statistical threshold between ccRCCa and ccRCCp patients."

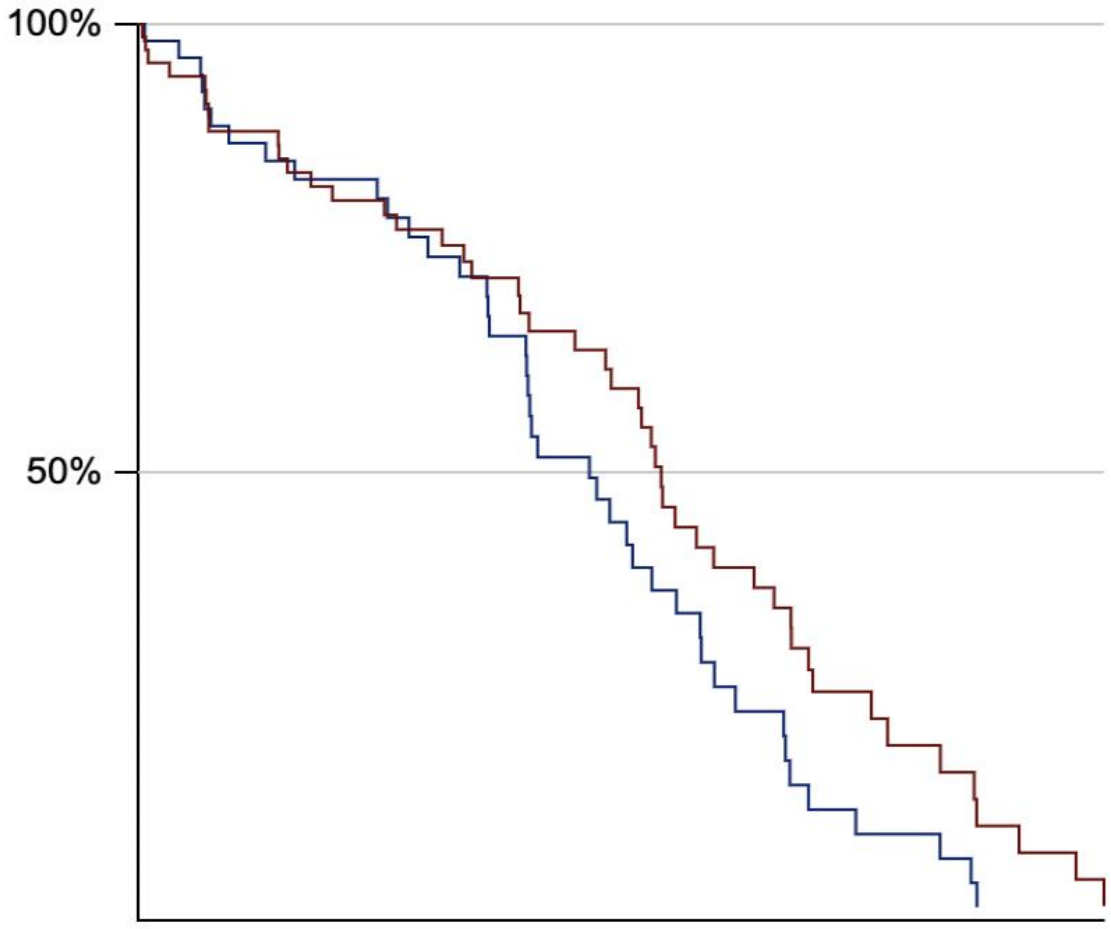


Figure 4 Kaplan-Meier curves showing no statistically significant difference of survival between the two groups (ccRCCa group is depicted as blue curve and ccRCCp group is depicted as red curve).

・Minor points 1) Author contributions; The authors stated that "all the authors solely contributed to this paper". However, in this section, the corresponding author should specify each author's role played in this research (i.e. who analyzed and quantified the cross-sectional muscle area, who performed statistical analysis).

RESPONSE:

Author contributions has been added.

CHANGE:

Author Contributions: Conceptualization, F.G. and C.A.M.; methodology, F.G. and C.A.M.; software, F.G. and C.A.M.; validation, F.G. B.B.Z. and C.A.M.; formal analysis, F.G. and C.A.M.; investigation, F.G. and C.A.M.; resources, F.G. and C.A.M.; data curation, F.G. and C.A.M.; writing—original draft preparation, F.G. and C.A.M.; writing—review and editing, F.G. and C.A.M.; visualization, F.G. and C.A.M.; supervision, F.G., B.B.Z. and C.A.M.; project administration, F.G. and C.A.M. All authors have read and agreed to the published version of the manuscript.

REVIEWER 3

Dear Author, I am sincerely pleased to review your manuscript. I read with interest the paper that the presence of collateral blood vessels in ccRCCs is associated with a decrease in SMM. As stated in the limitation, clinical data could not be obtained, so the results are from univariate analysis only and the evidence level is low, but I think your point of view is very important and interesting.

・Is the formula for calculating TAMA_C correct as follows?: TAMA_C/age. Please describe in the Methods how you corrected for age.

RESPONSE:

thank you for the comment, we agree. The groups were corrected for age by dividing TAMA by the age of the respective ccRCC patient: TAMA_C/age .

CHANGE:

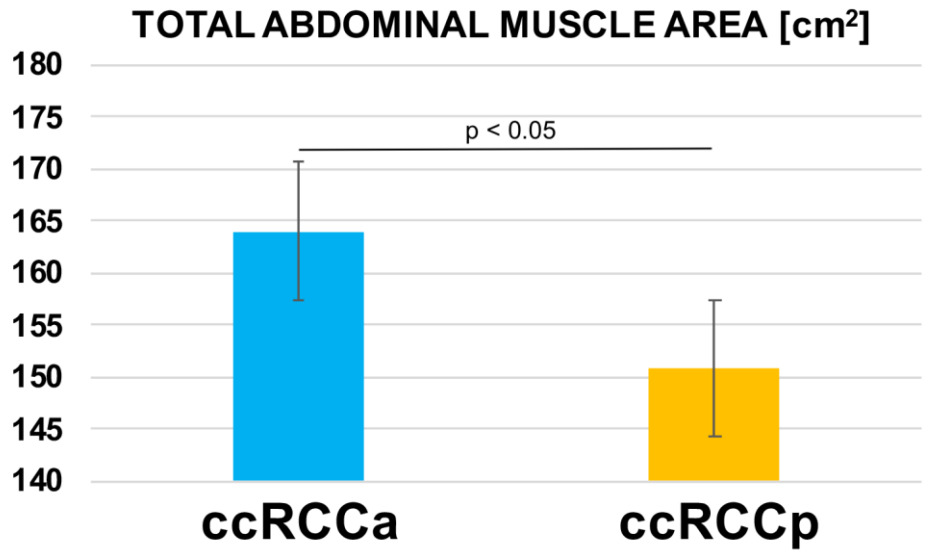
“TAMA measures were also corrected for age in order to rule out age-related effects. Thus, the analysis was repeated between ccRCCa and ccRCCp groups using age-corrected values and Student's t-test. Corrected values of TAMA were obtained dividing individual values of TAMA by the age of each subject: TAMA_C/age.”

・In figure.2, please show that there is a significant difference between the two groups.

RESPONSE:

Thanks for the comment. The significant difference between the two groups was added in the figure and in caption of the figure.

CHANGE



"Figure 2 bar chart with error bars showing a significant difference of mean values of TAMA between ccRCCa and ccRCCp groups."

- Show the Intraclass correlation coefficient for the TAMA measurements.

RESPONSE:

Thanks for the comment. The intraclass correlation coefficient for the TAMA measurements has been added.

CHANGE:

MATERIALS AND METHODS

CT Analysis

"All the ROIs were drawn by 2 radiologists separately (F. G., 5 years of experience; C. A. M., 9 years of experience), blinded to clinical data."

Statistical analysis

"Intraclass correlation coefficient for the TAMA measurements was performed using the Cronbach's alpha (or coefficient alpha), to evaluate the reliability of measures between the two tracers."

RESULTS

"The Cronbach's alpha of the two tracers was α = 0.913, indicating an excellent reliability".

・In this study, was there any difference in the formation of collateral blood vessels between RCC at the level of the lower renal pole and CC at the level of the upper renal pole?

RESPONSE:

Thanks for the comment. Most of the renal tumors with peritumoral collateral vessels in our sample were very large tumors showing extension to both the upper and lower poles. For this reason, we would prefer not to include this parameter to the current analysis. We would like to test this hypothesis in a future paper including only T1 and T2 tumors, that can be easily locate in one of the poles and not take into account collateral vessels since they are associated to higher stages and very large tumoral size.

CHANGE

No changes were made.

・For table.3, why don't you use the Kaplan-meier method to check survival and TAMA? Couldn't you get the survival time? If you can get survival data, you should draw a Kaplan-meier curve.

RESPONSE:

Thanks for the comment, we agree. As you suggested, we added the survival data and drew the Kaplan-Meier curve.

CHANGE:

MATERIALS AND METHODS

Statistical analysis

“Finally, Kaplan-Meier curves were included to check survival between ccRCCa and ccRCCp groups.”

RESULTS

“No significant differences of survival between the two groups (available data of 54 ccRCCa patients out of 54 and 69 ccRCCp patients out of 70) were found with the Kaplan-Meier method (log-rank test; z = 1.88, p = 0.06; 95% confidence interval) (Figure 4).”

DISCUSSION

“Indeed, patients with ccRCC who were dead, demonstrated a statistically significant reduction of TAMA with respect to those alive, suggesting a link between sarcopenia and survival in our sample. However, Kaplan-Maier curves showed a difference just above the statistical threshold between ccRCCa and ccRCCp patients.”

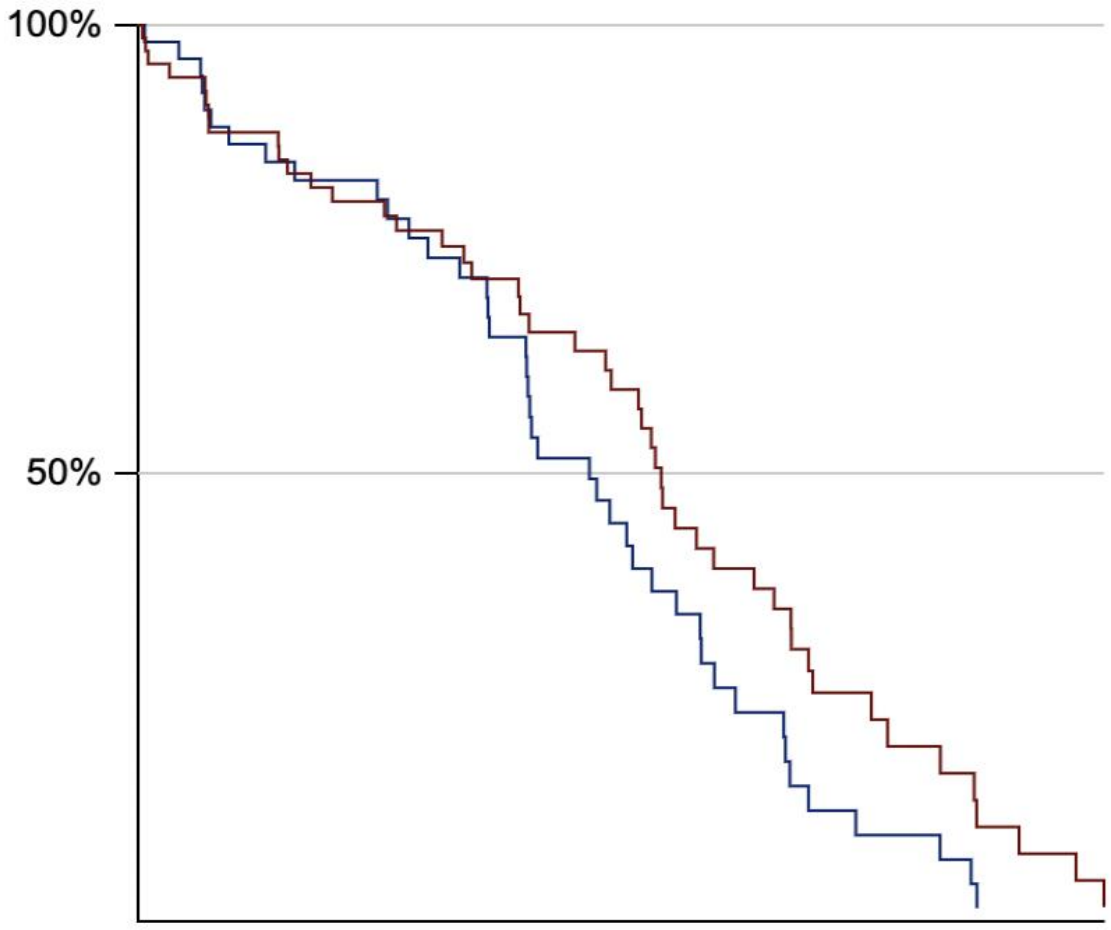


Figure 4 Kaplan-Meier curves showing no statistically significant difference of survival between the two groups (ccRCCa group is depicted as blue curve and ccRCCp group is depicted as red curve).