

## **Scientific memorandum**

### **AIM OF THE STUDY**

Despite the advancements in the diagnosis and treatment over the past two decades, clear cell renal cell carcinoma remains a highly lethal malignancy of the urinary system. Chloride intracellular channel 1 (CLIC1) first described in 1997 it is a protein that belongs to the family of ion channels of chlorine and has been identified to have a role in various fundamental biological processes, including maintenance of cell volume, ion homeostasis, trans-epithelial transport, and pH regulation but also, regulates the cell cycle, as well as cell proliferation, apoptosis, and differentiation. CLIC1 is involved in tumor angiogenesis, possibly by regulating the expression of integrins on endothelial cell surfaces. CLIC1 is expressed in the normal kidney, but also in cc RCC tumor cells and may act as sensor or effector of cellular proliferation, invasion and metastasis. Furthermore, it was found to be expressed, not only in cc RCC tumor cells, but also in tumor blood vessel endothelial cells, suggesting that it could be used as a new potential endothelial marker for tumor angiogenesis assessment. Its expression in both tumor and endothelial cells may be a sign for the development of future anti-CLIC1 therapy, able to target both tumor and its associated vessels.

TLS, formed by mature dendritic cells in a T-cell zone, together with a B-cell follicle including a germinal center, are present in most solid cancers, as an ectopic lymphoid tissue surrounding the tumor. It is known that the higher density of TLS into the tumor, the better the patient's prognosis. Cc RCC shows less frequently and also dysfunctional TLS than other cancers.

One of the objectives was to characterize of CLIC1 positive vascular network in tumor and stromal compartments by using NFA (Network Formation Assay) digital tool with special focus on the cc RCC vessel's ability to form vascular loops and branching points, but even more, to assess the tumor-stroma interplay by applying CLIC1 immunohistochemical expression in both tumor and stromal components.

## LIST OF SCIENTIFIC PUBLISHED ARTICLES

1. **Andrei-Alexandru Cosma**, Mihaela Pasca Fenesan, Alexandru Nesiu, Eugen MelnicAdela, Maria Ferician, Ovidiu Catalin Ferician, Emil Ceban, Simona Sarb, Anca Maria Cimpean. Exploring vasculogenesis in the normal human kidney and clear cell renal cell carcinoma: insights from development to tumor progression and biomarkers for therapy response. *Front. Oncol.*, 30 April 2024, Sec. Molecular and Cellular Oncology. Impact factor 4.7
2. **Andrei-Alexandru Cosma**, Alina Gabriela Negru, Ovidiu Catalin Ferician, Mihaela Maria Pasca Fenesan, Vlad Vornicu, Adela Maria Ferician, Eugen Melnic and Anca Maria Cimpean. Predictive Factors for clear cell-Renal Cell Carcinoma (cc-RCC) Multiple Me-tastases Location. *ANTICANCER RESEARCH* 45: 2365-2375 (2025), 15(4), 00, doi: 10.21873/anticanres.17609. Impact factor 1.6
3. **Andrei-Alexandru Cosma**, Alina Gabriela Negru, Ovidiu Catalin Ferician, Mihaela Maria Pasca Fenesan, Vlad Vornicu, Adela Maria Ferician, Eugen Melnic and Anca Maria Cimpean. Tissue Prognostic Markers for Clear Cell Renal Cell Carcinoma Tumor-Stroma Interaction: Impact on TNM Staging Parameters. *ANTICANCER RESEARCH* 45: 2401-2416 (2025), 15(4), 00, doi: 10.21873/anticanres.17612. Impact factor 1.6

## DATA REGARDING THE PROCESS OF THE RESEARCH

Our research team extensively studied before microscopic and molecular aspects of cc RCC malignances in the west part of Romania. Our previous work was focused on tumor compartment mainly, followed by tumor angiogenesis and stimulatory and inhibitory angiogenic factors expression. Based on the results of the present thesis, we will continue the study of cc RCC by extending our research area through an interaction in between tumor and stromal components.

Our previous studies reported CLIC1 expression in tumor cells, but these data were initially correlated to TNM staging parameters. We found heterogenous CLIC1 expression in tumor cells given by the ability of CLIC1 to translocate through all three compartments of the cell: nucleus, cytoplasm and membrane. Based on this ability of translocation in between cellular compartments, it was reported that CLIC1 has a



heterogenous impact on prognosis and OS of ccRCC patients. We also reported that local and metastatic potential differed related to this heterogenous CLIC1 expression in tumour cells. Cosnita et al. used anti-CLIC1 antibodies to illustrate CLIC1-expressing tumor cell necrosis and the blockage of tumor blood vessels in chick embryo chorioallantoic membrane ccRCC tumor model xenografts. This prior investigation was exclusively performed on human tumor specimens sourced from patients with ccRCC malignancy, marking the inaugural report of CLIC1 expression in human endothelial cells. We validated prior in vitro observations about CLIC1 expression at the endothelium level in these cases. Several studies have indicated that CLIC1-positive tumor cells interact with endothelial cells via secreting CLIC1 extracellular vesicles that bind to several endothelial cell receptors. Our team reported variability in CLIC1 expression within the blood vessel endothelium during inflammation-induced angiogenesis in non-malignant conditions, such as rheumatoid and psoriatic arthritis, where vascular CLIC1 expression was significantly affected by therapy and interactions with other stromal components. Substantial correlations between CLIC1-MVD and TNM staging parameters, along with the microscopic identification of CLIC1-positive tumor emboli within blood vessels lined by CLIC1-positive endothelium, substantiate the angiogenic function of CLIC1 secreted by tumor cells, while also affirming its role as a facilitator of cancer progression and metastasis.

We kept such classification of CLIC1 expression in tumor cells, and based on this, we extended our study through the assesment of CLIC1 not in the tumor exclusively, but also in the stromal compartment components with special focus on vascular endothelium and tertiary lymphoid structures.

Even more, our team reported in one previous study that human cc RCC tumor implants were verified to be CLIC1 positive before their application as grafts on CAM, establishing this as the inaugural experimental model of CLIC1-positive human-derived cc RCC implants utilized for testing anti-CLIC1 antibodies as a prospective treatment approach. The efficacy of this experimental model was enhanced by integrating stereomicroscopic and immunohistochemical techniques with an ultrasonographic method (with and without the Echo-Doppler module) to assess blood vessel acquisition by the implants, demonstrate implant perfusion, and, most importantly, to emphasize the inhibitory effects of anti-CLIC1 antibodies on intratumor vasculature in vivo. Utilizing the same ultrasonographic approach, we assessed the reduction in tumor size in treated compared to non-treated specimens. Ultrasonographic techniques offer a more precise assessment of tumor xenografts implanted on the chorioallantoic membrane, focusing on tumor dimensions and vascularization. Incorporating ultrasonographic techniques into the evaluation of CAM-implanted tumor xenografts will enhance the efficacy of drug testing studies on CAM, which is recognized as one of the most commonly utilized in vivo platforms for drug assessment.

Some of the data regarding vasculogenesis in renal cancer have been presented in the oral section of Romanian Society of Medical Oncology Conferences. The Tissue Prognostic Markers for Clear Cell Renal Cell Carcinoma Tumor stroma Interaction will be exposed as a poster at European Pathology Congress in September 2025.

## APPRECIATION OF THE SCIENTIFIC COORDINATOR

The initial findings from this research indicate that CLIC1's function as an angiogenic factor in human cancers warrants further exploration across various tumor types and its correlation with additional prognostic and survival indicators.

Presence of tertiary lymphoid structures in cc RCC was previously described in few papers related to cc RCC, but none of them reported CLIC1 expression and pattern in such local lymphoid structures.

We consider that this could be of high importance, due to the accurate personalized therapies, such as anti PD1, anti PD-L1.

Predictive factors in cc RCC are incompletely assessed and understood, most of clinico-pathological parameters being neglected. First part of our study related to predictive factors in cc RCC highlighted the importance of patients stratification related to age and blood cell counts in the prediction of multiple metastases locations at the time of diagnosis. We found that cc RCC metastatic potential is highly heterogenous among different age groups. Brain, bone and liver metastases are predominant in the young patients group. For patients between age 35-50, brain and bone metastases are significant correlated to peripheral blood neutrophils count. In the same age subgroup, liver metastases are most commonly in women. Our preliminary data suggested an interplay in between lymph node and skin metastases, but due to the low limit of cases, this preliminary data must be validated by further studies. Skin metastases were totally absent in the age group ranged 51-65 years old. 51-65 years old group was governed by pleural metastases with a high prevalent in males, partially correlated to peripheral neutrophil count. A high neutrophil count in the peripheral blood seems to have a protective role against brain metastases developed in this age of group. The oldest patient group had a unique feature of the highest percentage of multiple metastatic sites at the time of diagnosis. Also, significant correlation has been found between neutrophils and platelets, suggested a high thrombotic risk in elderly patients.

Moreover, we proved that tumor-stroma interaction in cc RCC is depended by CLIC1 expression which seems to act as a mediator for communication in between tumor cells and tumor stroma. By comparatively assessment of TLS positive and negative

cases, we found significant differences related to nodal and distal metastases, which were influenced by CLIC1 expression in tumor cells. CLIC1 MVD for peritumoral blood vessels was highly correlated to CLIC1 expression pattern in tumor, which also influenced the size and local invasion of the tumor. This highly depended by CLIC1 pattern especially when CLIC1 was expressed at the level of the tumor cells membrane. Lack of TLS in the stroma strongly favored nodal and distant metastases.

Even more, this research paper shows first time description of aspects of TLS in the cc RCC by mentioning them into the normal adjacent renal tissue, inside peritumoral adipos tissue and stromal compartment, but also, first time description of CLIC1 expression in the germinal center of TLS in cc RCC.

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