AZIENDA SANITARIA LOCALE RIETI
Via del Terminillo, 42 – 02100 RIETI - Tel. 0746.2781 – PEC: asl.rieti@pec.it
www.asl.rieti.it C.F. e P.I. 00821180577

Molecular biomarker analysis and immUnotherapy in patients with clear cell RenAl Cell CarciNoma: an Observational, retrospective/prospective, multicenter study. URANO trial

Study Principal Investigator (PI)	Dr.ssa Anna Ceribelli *UOC Oncologia* *Ospedale San Camillo de Lellis-ASL RIETI* Via del Terminillo, 42 – 02100 RIETI a.ceribelli@asl.rieti.it
TRIAL STEERING COMMITTEE	Dr.ssa Anna Ceribelli *UOC Oncologia* *Ospedale San Camillo de Lellis-ASL RIETI* Dr.ssa Maria Bassanelli *UOC Oncologia Medica 1* *IRCCS IFO-IRE Istituto Nazionale Tumori "Regina Elena"*
STATISTIC	Dott.ssa Diana Giannarelli Facility Epidemiology and Biostatistics, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy

BACKGROUND

Clear cell renal cell carcinoma (ccRCC) is the most frequent histologic subtype (70-85%) [1] . ccRCC is characterized by inactivation of the von Hippel-Lindau gene (VHL) in 44-90% of cases. An early event during the evolution of ccRCC is loss of function mutation of the von Hippel-Lindau (VHL) gene [2]. Direct VHL sequencing experiments from subjects with sporadic ccRCC show that up to 75% of these subjects have biallelic loss of function mutation of VHL genes, and up to 19% exhibit expression inactivation by hypermethylation [3]. The normal function of the HIF complex (a heterodimer composed of a and β subunits) is to regulate expression of several genes in response to hypoxia[4] Many of these proteins are involved in cell growth and survival, motility, invasion, metastatization, angiogenesis, hydrogen ion (pH) regulation, and glucose metabolism. Phenotypically, RCC is a highly vascular tumor, with increased VEGF levels, and its growth can be stimulated by factors produced through the HIF-1 pathway. Following VHL, the most prevalent mutations are: PBRM1 (Polybromo 1) (32–41%), BAP1 (BRCA-associated protein-1) (6–15%), SET domain containing 2 (SETD2) (3-11%), TP53 (5%), KDM5C (3–5%), PIK3CA (3%), ATM (3%), TSC1 (3%), ARID1A (2%), CDKN2A (2%) [5]. VHL, PBRM1, SEDT2 and BAP1 genes are located on chromosome 3p [6]. PBRM1, SETD2, BAP1, KDM5C (JARID1C) and KMD6A (UTX) genes encode the chromatin regulatory proteins and mutation in these gene could be alter the chromatin landscape and transcriptional program[7] [8] [9]. Many studies indicated the basis of kidney cancer in a metabolic disease due to activation of gene involved in metabolic pathway as VHL, MET, FLCN, TSC1, TSC2, TFE3, TFEB, MITF, fumarate hydratase (FH), succinate dehydrogenase B (SDHB), succinate dehydrogenase D (SDHD) and PTEN [10] [11] [12] . The TCGA data showed the correlation between disease aggressiveness and metabolic shift that involved increased dependence on pentose phosphate shunt, downregulation of AMP-activated protein kinase (AMPK) and the Krebs cycle, increased glutamine transport and fatty acid production [13]. BAP1 is a tumor suppressor gene which encodes a nuclear deubiquitinase and is located on chromosome region 3p21[14] .Peña-Llopis S. et colleagues studied histological features of tumors with loss of BAP1 showing a correlation with high tumor grade and mTORC1 activation [15]. Kapur et al. described the prognostic role of BAP1 and PBRM1 mutations in patients underwent to surgical resection of a clear cell renal cell carcinoma comparing findings from the University of Texas Southwestern Medical Center (UTSW) with a cohort from The Cancer Genome Atlas (TCGA). Presence of BAP1 mutation was characterized by higher Fuhrman grade, sarcomatoid and rhabdoid histology, tumor necrosis and mTORC1 activation ($p < 0.05$). BAP1 mutant tumors were associated with the expression of gene involved in growth factor signaling (NGF, prolactin ErbB, PTEN, IGF-1; insulin receptor, neuregulin, IL8) [16]. Hakimi AA et al. showed the impact of BAP1 and SEDT2 mutation on cancer specific survival (CSS) in TOGA and Memorial Sloan-Kettering Cancer Center (MSKCC) cohorts.[17]. Kapur P et colleagues studied the association of BAP1 immunohistochemical (IHC) expression with survival in patients with non metastatic ccRCC treated with nephrectomy. BAP1 loss correlated significantly with pathological features as higher Fuhrman grade (p

<0.0001), advanced pT stage (p = 0.0021), tumor necrosis (p <0.0001) and sarcomatoid dedifferentiation (p = 0.0001)[18]. Joseph RW et al. showed that patients with loss of BAP1 protein expression had an increased risk to die from ccRCC (HR: 3.6; 95% CI, 2.28-4.10, p= 6.77 x 10^{-14}). Jones J et al identified a gene signature detectable in metastatic setting and then related to progression [19].Brannon AR et colleagues stratified ccRCC in two molecular prognostic groups designed by two different gene expression profiling: clear cell type A (ccA) and clear cell type B (ccB). Afterwards, it was developed a molecular model comprising 34-gene expression signature (ClearCode34) using NanoString platform to identified these subtype of tumor which confirmed the same prognostic trend: ccB group showed a higher recurrence risk compared to ccA group (HR: 2.3; 95% CI, 1.6-3.3; p= 0.0000043) [20]. Rini B, et al. developed a 16-gene signature to predict recurrence risk in patients with stage I-III clear cell RCC who underwent nephrectomy [21] .

Checkpoint blockade immunotherapy has rapidly demonstrated unprecedented efficacy becoming the new standard of care for several cancer. The approval of immune checkpoint inhibitors targeting programmed death 1 (PD-1), nivolumab[22], and the combination therapy with ipilimumab, an anti–cytotoxic T-lymphocyte–associated antigen 4 antibody[23], has significantly changed the treatment landscape of renal cell carcinoma . Despite the encouraging success of immune checkpoint inhibitors, only a small subset of patients respond to this treatment. Bassanelli M et al[24] identified a 17-gene expression signature (unfavorable genomic signature [UGS]) to predict a poor prognosis (recurrence-free survival <1 years) in patients with stage I-III ccRCC treated with nephrectomy (cytoreductive, partial or radical nephrectomy). Currently, several biomarker analysis are identified molecular subsets associated with differential response to immune checkpoint inhibitor or tyrosine kinase inhibitor targeting vascular endothelial growth factor receptors (VEGFR) 1, 2 and 3 [25] [26]

Hypothesis/ PURPOSE:

The objective of the current study is to identify molecular biomarkers to associate with different outcome checkpoint inhibitor (nivolumab or ipilimumab + nivolumab) that could lead to different therapeutic approaches in patients with mRCC

STUDY DESIGN:

Observational, retrospective/prospective, multicenter trial to investigate the correlation between molecular biomarkers and histological features with outcome of patients with clear cell renal cell carcinoma, treated with nivolumab or ipilimumab + nivolumab

POPULATIONS:

Adult patients (age ≥ 18 years) with advanced/metastatic clear cell renal cell carcinoma, who received nivolumab or ipilimumab plus nivolumab, as clinical indication

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria

- Age ≥ 18 years
- Histological diagnosis of clear cell renal cell carcinoma
- Advanced or metastatic disease
- At least one cycle of nivolumab or nivolumab plus ipilimumab, as clinically indicated
- Written informed consent

Exclusion criteria

Non clear cell renal cell carcinoma

EFFICACY ASSESSMENT

- Tumour response to treatment will be defined according to RECIST criteria [27]
- Timing of radiological assessment will be based on local practice patterns.

STATISTICAL METHODS

As a general approach quantitative variables distributions will be tested for normality assumption through the Shapiro-Wilks test, items not normally distributed will be reported as medians and interquartile ranges (IQR= 1st and 3rd quartile) and analysed using the nonparametric Mann-Whitney U test for unmatched group. Variables respecting normality assumptions will be reported as mean ± standard deviation and compared among subgroups using Student't test. Categorical variables will be expressed as absolute frequency and percentage and proportions will be compared by Chi-square test or Fisher's exact test, as appropriate according to the expected frequencies in each cells. Survival times will be estimated with the Kaplan-Meier method and differences in survival curves will be assessed with the log-rank test. This analysis is explorative and 'hypotheses generating' in nature, a 2-tailed p value <0.10 will be considered as suggestive of statistical significance without any adjustment for multiple testing. All analyses will be performed using SPSS v. 21.0 (SPSS, Chicago, IL, USA).

IMPLICATIONS IN CLINICAL PRACTICE: To personalize the therapeutic approach in patients ccRCC

REFERENCES

[1] Patard JJ, Leray E, Rioux-Leclercq N, et al. Prognostic value of histologic subtypes in renal cell carcinomas: a multicenter experience. J Clin Oncol 2005; 23: 2763-2771

[2] Latif F, Tory K, Gnarra J, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. May 28 1993;260(5112):1317-1320.

[3] Herman JG, Latif F, Weng Y, et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci U S A. Oct 11 1994; 91(21): 9700-9704.

[4] Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A. May 1 1993; 90(9): 4304-4308

[5] Randall JM, Millard F, Kurzrock R. Molecular aberrations, targeted therapy, and renal cell carcinoma: current state-of-the-art. Cancer Metastasis Rev 2014; 33:1109–1124.

[6] Peña-Llopis S, Christie A, Xie XJ, et al. Cooperation and antagonism among cancer genes: the renal cancer paradigm. Cancer Res. 2013 Jul 15;73(14):4173-9

[7] Simon JM, Hacker KE, Singh D, et al. Variation in chromatin accessibility in human kidney cancer links H3K36 methyltransferase loss with widespread RNA processing defects. Genome Res. 2014 Feb;24(2):241-50

[8] Dalgliesh GL, Furge K, Greenman C, et al. Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature. 2010; 463(7279): 360–363.

[9] Hakimi AA, Chen YB, Wren J, et al. Clinical and Pathologic Impact of Select Chromatin Modulating Tumor Suppressors in Clear Cell Renal Cell Carcinoma. Eur Urol. 2013; 63(5): 848–854.

[10] Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 2010; 7 (5): 27-285

[11] Linehan WM, Ricketts CJ. The metabolic basis of kidney cancer. Semin Cancer Biol 2013; 23 (1): 46-55

[12] Zaravinos A, Pieri M, Mourmouras N, et al. Altered metabolic pathways in clear cell renal cell carcinoma: A meta-analysis and validation study focused on the deregulated genes and their associated networks. Oncoscience 2014; l (2): 117-131.

[13] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 2013; 499: 43-49

[14] Farley MN, Schmidt LS, Mester JL, et al. A Novel Germline Mutation in BAP1 Predisposes to Familial Clear-Cell Renal Cell Carcinoma. Mol Cancer Res 2013; 11(9); 1061–71.

[15] Peña-Llopis S, Vega-Rubín-de-Celis S, Liao A, et al. BAP1 loss defines a new class of renal cell carcinoma. Nat Genet 2012; 44:751-759

[16] Kapur P, Peña-Llopis S, Christie A, et al. Effects on survival of BAP1 and PBRM1 mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. Lancet Oncol 2013; 14: 159–67

[17] Hakimi AA, Ostrovnaya I, Reva B, et al. Adverse Outcomes in Clear Cell Renal Cell Carcinoma with Mutations of 3p21 Epigenetic Regulators BAP1 and SETD2: A Report by MSKCC and the KIRC TCGA Research Network. Clin Cancer Res 2013; 19 (12): 3259-3267.

[18] Kapur P, Christie A, Raman JD, et al. BAP1 Immunohistochemistry Predicts Outcomes in a Multi-Institutional Cohort with Clear Cell Renal Cell Carcinoma. J Urol. 2014 Mar;191(3):603-10

[19] Jones J, Otu H, Spentzos D, et al. Gene Signatures of Progression and Metastasis in Renal Cell Cancer. Clin Cancer Res 2005;11(16): 5730-5739.

[20] Brooks SA, Brannon AR, Parker JS, et al. ClearCode34: A Prognostic Risk Predictor for Localized Clear Cell Renal Cell Carcinoma. European Urology 2014; 66: 77-84.

[21] Rini B, Goddard A, Knezevic D, et al. A 16-gene assay to predict recurrence after surgery in localised renal cell carcinoma: development and validation studies. Lancet Oncol 2015; 16: 676–85.

[22] Motzer RJ , Escudier B , McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal Cell Carcinoma. N Engl J Med. 2015; 373(19): 1803–1813

[23] Albiges L, Tannir NM, Burotto M, et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 4-year follow-up of the phase III CheckMate 214 trial. ESMO Open 2020;5:e001079

[24] Bassanelli M, Borro M, Roberto M, , et al. A 17-Gene Expression Signature for Early Identification of Poor Prognosis in Clear Cell Renal Cell Carcinoma. Cancers (Basel) 2021 Dec 30;14(1):178

[25] Motzer RB, Robbins PB, Powles T,et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. Nature Medicine 2020 ; 1733–1741

[26] Motzer R J, Banchereau R, Hamidi H, et al. Molecular Subsets in Renal Cancer Determine Outcome to Checkpoint and Angiogenesis Blockade. Cancer Cell 2020; 38, 803–817.

[27] Eisenhauera E.A, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228-247