The ARON PROJECT

Global experiences, perspectives and access to cure in patients with genitourinary tumors

ARON-1 Study Protocol

Version 5.0, 28st October 2023

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Summary

List of Abbreviations

BMI = Body Mass Index

CI = Confidence Interval

CR = Complete Response

FDA = Food and Drug Administration

GCP = Good Clinical Practice

IMDC = International mRCC Database Consortium

mRCC = metastatic Renal Cell Carcinoma

ORR = Overall Response Rate

OS = Overall Survival

PD = Progressive Disease

PFS = Progression-Free Survival

PR = Partial Response

RCC = Renal Cell Carcinoma

SD = Stable Disease

1. Background and Rational

The advent of immunotherapy has revolutionized the treatment landscape of several hematological and solid tumors [1,2], and the number of patients eligible for immune-based cancer therapies continues to rise as these treatments are currently part of the first- or later-line standards for many malignancies [3–5]. Over the past decade, an impressive number of trials have been conducted on the role of immune checkpoint inhibitors, with the results of these studies paving the way towards the Immunotherapy Era in cancer care [6,7].

The first immune checkpoint inhibitor approved by the United States Food and Drug Administration (FDA) was ipilimumab, approved in 2011 for the treatment of advanced melanoma, following the results of the pivotal phase III trial conducted by Hodi and colleagues [8,9]. More than ten years after the publication of this study, there is a plethora of ongoing trials aiming to evaluate the efficacy and safety of immune checkpoint inhibitors, as a monotherapy or in combination with other anticancer agents [10,11]; thus, the number of cancer patients receiving immunotherapy is expected to further increase in the near future, especially considering that a wide range of combination therapies is being explored, with the aim of producing a synergistic effect [12,13].

The treatment scenario of metastatic Renal Cell Carcinoma (mRCC) has recently undergone a revolution following the results of landmark phase III clinical trials on the combination of immune checkpoint and tyrosine kinase

inhibitors [14,15], such as pembrolizumab plus axitinib, nivolumab plus cabozantinib, pembrolizumab plus lenvatinib [16,17], as well as dual checkpoint bloackade with nivolumab plus ipilimumab in intermediate–poor-risk patients [18,19]. These treatments currently represent novel standards of treatment, as is recommended by the most up-to-date international guidelines [20,21].

The ARON-1 Study has been designed to share and analyze the global real world experiences with the use of immuno-combination therapies for advanced RCC, aimed to analyze the efficacy of this therapeutic approach in specific subpopulations and to investigate for the presence of prognostic factors in this context.

2. Study Design and Endpoints

The ARON-1 Study is designed as an International Multicentric Retrospective Study to collect global experiences with the use of immuno-combinations in patients with metastatic RCC.

2.1 Primary End-Point

- To assess and compare real-world data on first-line immuno-combinations in patients with metastatic RCC in terms of Progression-Free Survival (PFS), Overall Survival (OS) and Overall Response Rate (ORR).

2.2 Secondary End-Points

- To apply artificial intelligence technology to the study of real-world data for cluster and Artificial Neural Network analyses
- To explore the role of immuno-combination in the elderly populaton
- To compare the efficacy of immuno-combinations in patients with different metastatic sites (i.e. bone and brain metastases)
- To assess the prognostic role of smoking attitude and obesity in RCC patients treated with different immuno-combinations
- To investigate real-world data on the use of immuno-combinations in patients with good risk RCC
- To observe real-world data on the role of nephrectomy in RCC patients treated with different immuno-combinations
- To compare different therapeutic sequences in RCC patients
- To explore the efficacy of different immuno-combinations in patients with non-clear RCC

2.3 Evaluation of the Endpoints

- Overall Survival (OS) was defined as the time from the start of immuno-combination to death from any cause
- Progression-Free Survival (PFS) was defined as the time from the start of

treatment to progression or death from any cause. Patients without a tumor progression to following line of treatment or death or lost at follow-up at the time of analysis were censored at their last follow-up date

- Tumor radiological assessment was led according to the RECIST 1.1 criteria [22] and data on tumor response (complete (CR) or partial responses (PR), stable (SD) or progressive disease (PD)) were collected and analyzed
- The Overall Response Rate (ORR) was defined as the rate of CR + PR according to the RECIST 1.1 criteria [22]

3. Study Population

The ARON-1 study retrospectively analyze patients treated with first-line immuno-combinations for metastatic RCC according the standard clinical practice in the different Countries involved in this project.

3.1 Inclusion Criteria

- Patients aged >18y
- Cytological or Histologically confirmed diagnosis of clear cell or non-clear cell RCC
- Histologically or radiologically confirmed diagnosis of metastatic disease
- First-line treatment with nivolumab plus ipilimumab or nivolumab plus cabozantinib or pembrolizumab plus axitinib or pembrolizumab plus

lenvatinib or avelumab plus axitinib or atezolizumab plus bevacizumab

3.2 Exclusion Criteria

- Patients without histologically confirmed diagnosis of RCC
- Patients without histologically or radiologically confirmed metastatic disease
- Patients treated with immuno-combinations not included in the list reported in the Inclusion Criteria Section

3.3 Estimated Enrollment

We planned to retrospectively analyze a minimum of 1000 patients with advanced RCC treated in Internationally recognized Oncological Centers for genitourinary tumors.

4. Statistical Analysis and Data Collection

PFS and OS were estimated by Kaplan-Meier method with Rothman's 95% confidence intervals (CI) and compared by using the log-rank test. Univariate and multivariate analyses were performed by using Cox proportional hazards models. The chi-square test was used to compare categorical end-points. Significance levels were set at a 0.05 value and all *p* values were two-sided. The statistical analysis was led by using MedCalc version 19.6.4 (MedCalc Software, Broekstraat

52, 9030 Mariakerke, Belgium).

Data will be retrospectively extracted from paper and electronic charts. For each patient, the following data will be collected by the database of each Institution and analyzed: histology, nephrectomy status, International mRCC Database Consortium (IMDC) criteria, sites of metastases, Body Mass Index (BMI), laboratory tests and treatments. Patients without sufficient data on tumor assessment and response to therapy will be excluded from this study. Data will be collected in Excel datasets. A code will be used to identify each patient included in this analysis.

5. End-dates for data collection and publication

Due to the large number of Centers involved from different Countries, we have scheduled a series of deadlines in order to complete step-by-step the planned analyses and prepare the manuscripts for publication:

- The first deadline is July 31th - Analysis of data collection and preparation of the first manuscripts within September 30th, 2023
- The second deadline is September 30th, 2023 - Second analysis of data collection and preparation of four of our planned manuscripts within 1st November, 2023

For each manuscript, one author's name for each Istitution involved in this project will be considered. We will consider, according to Journal policies, two names for

each Oncological Center that has included at least 50 patients in the dataset or which has majorly participated to the different phases of the manuscripts.

Before the submission, a draft of each manuscript will be sent to all the authors from the Institutions involved in order to revise potential errors and propose additional comments and suggestions.

Potential publication fees with be substained by the Center of the Principal Investigator (Macerata Hospital).

6. Supplemetary studies: ARON-1α and ARON-1β

Each Institution involved in the ARON Project could decide to participate also to one or both of the ARON-1α and ARON-1β Supplementary Studies.

6.1 ARON-1α

The ARON-1α Supplementary Study has been designed to investigate for the presence of genomic signatures from tumor samples of patients treated with first-line immuno-combinations for advanced RCC. In particular, we aimed to:

- Correlate between genomic signatures and the outcome in terms of OS, PFS and ORR of mRCC patients treated with first-line immuno-combinations
- Compare the results obtained by our assay among patients treated with different immuno-combinations

- Assess for the presence of intratumor heterogeinity between samples from the primary tumors and metastases from the same patients and investigate for its association with the clinical outcomes

6.1.1 Materials and Methods

Twenty unstained slides (5um-thick, unbaked) or FFPE are required for each primary tumor. Tissue surface area should be up to 15 x 15 mm. Thicker sections may result in lower RNA yields. Total RNA RCC fixed paraffin-embedded slice tissues will be extracted by using "RNeasy® FFPE" kit (Qiagen). Then, mRNA will be converted into cDNA products bythe "RT^{2}PreAMP cDNA Synthesis" (QIAGEN). Ten microliters of the resulting cDNA products will be used as template for TaqMan Array. The TaqMan® Array 96-well Plate, containing specific assays for RCC-associated genes and 3 assays to candidate endogenous control genes, will be designed, customized and purchased (Thermo Fisher, Grand Island, NY, USA) and used to evaluate the effects of the treatments in modulating RCC-related genes.

The list of analyzed genes includes: *AKT1, ARNT, BRAF, CA9, CAV1, CREBBP, CX3CL1, CX3CR1, EGFR, EGLN3, ETS1, FH, FHIT, FLCN, HGF, KANK1, KDR, KIT, KRAS, KRT7, MAPK1, MET, MME, MOK, MUC1, NRAS, PAK1, PBRM1, PDGFB, PDGFRB, PI3KCA, PI3KR1, RAC1, RAF1, RAP1B, SLC2A1, TCEB1, VEGFA, VEGFR1, VEGFR2, VHL, VIM* and *TRP genes.*

Measurement of housekeeping genes expression (*GAPDH*; *GUSB*) on the samples

will be used to normalize mRNA content. Gene Array will be performed in QRT-PCR by using the Multicolor Real-Time PCR Detection System (BioRad, Milano, Italy).

6.1.2 End-dates for sending tumor samples and data publication

Similarly to the collection of clinical data, we have scheduled two deadlines in order to complete step-by-step the planned analyses and prepare the manuscripts for publication:

- The first deadline for sending tumor samples is December 31th 2023 - First analysis of data collection and preparation of the first manuscripts within 28th February 2024
- The second deadline for sending tumor samples is 28th February 2024 - Second analysis of data collection and preparation of the other planned manuscripts within 1st April 2024

6.2 ARON-1β

6.3 The *ARON-1β* Supplementary Study has been designed to charaterize the immune cell populations and assess their relationship with the clinical outcome of mRCC patients treated with first-line immuno-combinations.

6.2.1 Materials and Methods

Twenty unstained slides (5um-thick, unbaked) or FFPE are required for each primary

tumor. An anonymized copy of the original pathology report (identified by the same code reported in the clinical dataset) must be provided, and the tissue collection date must be recorded so the sample age can be derived. Sections should be obtained from the FFPE block of the tumor sample with the highest grade and with at least 70% tumor cells.

Up to 5 slides will be used for DNA/RNA extraction. Exploratory analysis of immune cell populations includes immunostaining for T-cells CD3, CD4, CD8, B lymphocytes CD20, macrophages CD68 and CD163, dendritic cells CD1a, plasma cells CD38, cytotoxic T and NK cells CD3, CD8, CD16, CD56 and granzyme. We also intend to investigate the expression of PD-1/PD-L1 and other immuno-checkpoints on tumor and immune cells.

7. Data confidentiality and management

Information about study subjects will be kept confidential and managed under the applicable laws and regulations. The data collection system for this study uses built-in security features to encrypt all data for transmission in both directions, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of individually assigned user identification codes and passwords, made available only to authorized personnel who have completed prerequisite training. The investigator must ensure anonymity of the patients; patients must not be identified by names in any documents.

The Principal Investigator is responsible for assuring that the data entered are complete and accurate. Essential documents (written and electronic) should be retained for a period of not less than five (5) years from the completion of the Study.

8. Ethical Consideration

The ARON-1 Study will be conducted according to Good Clinical Practice (GCP) and has been designed with the ethical principles laid down in the Declaration of Helsinki on human experimentation. The Study will be approved by the Ethical Commitees of the particpating Institutions according to local guidelines.

9. Fundings and Fees

No fees are required from or provided to the Centers that participate to the ARON-1 project. The costs for sending tissue samples for the Centers participating also to one or both of the Supplementary studies ARON-1α and ARON-1β will be sustained by the PI, as well as the potential publication fees.

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