The ARON PROJECT

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

ARON-2 Study Protocol

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Summary

List of Abbreviations

BMI = Body Mass Index

CI = Confidence Interval

CR = Complete Response

GCP = Good Clinical Practice

mUC = Metastatic Urothelial Cancer

ORR = Overall Response Rate

OS = Overall Survival

PD = Progressive Disease

PFS = Progression-Free Survival

PR = Partial Response

SD = Stable Disease

UC = Urothelial Cancer

1. Background and Rational

Urothelial cancer (UC) represents the fourth most frequently diagnosed tumor worldwide, accounting for nearly half a million of new cases annually [1,2]. Despite approximately the 70% of patients present with non-muscle invasive UC, more than one fourth of cases are diagnosed with advanced or metastatic disease, and systemic treatments remains the only therapeutic option [3,4]. Cisplatin-based chemotherapy is the standard first-line treatment in metastatic UC (mUC), with these regimens reporting a median survival of approximately 15 months and a 5-year survival rate of 15%, according to landmark registration trials [5,6]. Nonetheless, since the majority of patients with metastatic disease are over 65 years old, a substantial proportion is not deemed eligible for platinum-based chemotherapy, due to concomitant comorbidities [7,8]. In addition, disease progression inevitably occurs following the front-line treatment, and the prognosis of these patients remains poor [9,10].

The advent of modern immunotherapy and immune checkpoint inhibitors (ICIs) has recently revolutionized the treatment landscape of cancer patients in recent years [11,12]. ICIs targeting programmed cell death protein-1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4) are able to enhance T-cell mediated anti-tumor responses, resulting in improved clinical outcomes in several hematological and solid tumors [13,14]. Phase III clinical trials evaluating the role of immunotherapy in mUC patients have reported

important results, as also witnessed by the approval of five ICIs by the United States Food and Drug Administration (FDA) in the last few years: the PD-1 inhibitors, pembrolizumab and nivolumab, and the PD-L1 inhibitors atezolizumab, avelumab, and durvalumab [15,16]. However, only a small subset of advanced-stage UC patients may benefit from immunotherapy reaching a durable response. Antibodies–drug conjugates such as enfortumab vedotin, directed against nectin-4, and sacituzumab govitecan, directed against Trop-2, have been compared with standard chemotherapy in two randomized clinical trials including patients with locally advanced or mUC who had previously received platinum-based treatment and subsequently PD-1 or PD-L1 inhibitor. Both drugs shown to significantly improve survival compared to chemotherapy [17,18]. This led to the FDA approval of enfortumab vedotin for advanced-stage UC patients who had previously received PD-1 or PD-L1 inhibitor and platinum-containing chemotherapy, or who are ineligible for cisplatin-containing chemotherapy and had previously received one or more lines of systemic therapy [17,19].

With the emerging therapeutic options available, there is an urgent need for the identification of predictive biomarkers of response to ICIs, as well as a deeper understanding the role of immunotherapeutic agents in mUC [20]. Recently, several emerging studies aimed to evaluate biomarkers and predictors of response in this setting, including protein markers, immune-related cells, genetic markers, and host-related factors [21−22]. Regarding the latter, only a few studies

have investigated the putative predictive value of routinely assessed clinico-pathological data, including age, gender, liver metastases, and histological features.

The ARON-2 Study has been designed to share and analyze the global real world experiences with the use of pembrolizumab as first and second line and enfortumab vedotin for mUC aimed to analyze the efficacy and safety of these therapeutic approaches in specific subpopulations and to investigate for the presence of prognostic factors in this context.

2. Study Design and Endpoints

The ARON-2 Study is designed as an International Multicentric Retrospective Study to collect global experiences with the use of pembrolizumab and enfortumab vedotin used in different settings in patients with metastatic UC (mUC).

2.1 Primary End-Point

- To analyze real-world data on the efficacy and safety of pembrolizumab and enfortumab vedotin in patients with mUC.

2.2 Secondary End-Points

- To apply artificial intelligence technology to the study of real-world data for clustering and Artificial Neural Network analyses

- To assess the prognostic role of smoking attitude, obesity, age, renal insufficiency, primary tumor location, histology and concomitant medication in patients treated with pembrolizumab and/or enfortumab vedotin
- To compare different therapeutic sequences in mUC patients

2.3 Evaluation of the Endpoints

- Overall Survival (OS) will be defined as a time from the start of the therapy until death from any cause
- Progression-Free Survival (PFS) will be defined as a time from the start of the therapy until progression or death from any cause. Patients switched to the following line of treatment without progression or lost at follow-up at the time of analysis were censored at their last follow-up date
- Radiological assessment was performed according to the RECIST 1.1 criteria [23] and the data on objective response (complete regression=CR, partial regression=PR, stable disease=SD or progressive disease=PD) will be collected and analyzed
- The Overall Response Rate (ORR) and Disease Control Rate (DCR) will be defined as the rates of CR + PR and CR + PR + SD, respectively (according to the RECIST 1.1 criteria) [23]
- Treatment related adverse events (*e.g.*, cutaneous toxicity, neurological toxicity and hyperglycemia/mellitus diabetes) will be assessed according to

the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0 [24]. Grade ≥3 adverse events will be analyzed with a special focus

3. Study Population

The ARON-2 study retrospectively analyze patients treated with pembrolizumab, as first-line therapy in platinum-unfit patients or as second-line therapy in patients progressed after previous platinum-based chemotherapy, and/or enfortumab vedotin, as third-line therapy in patients previously treated with platinum-based chemotherapy and subsequently with PD-1 or PD-L1 inhibitor.

3.1 Inclusion Criteria

- Patients aged >18y
- Histologically confirmed diagnosis of UC of upper and/or lower urinary tract
- Histologically or radiologically confirmed metastatic disease
- Treatment with pembrolizumab in patients progressed after previous platinum-based chemotherapy
- Treatment with pembrolizumab as a first-line therapy in the platinum-unfit patients
- Treatment with enfortumab vedotin in patients progressed to previous platinum-based chemotherapy and anti-PD-1/PD-L1 inhibitor.

3.2 Exclusion Criteria

- Patients without histologically confirmed diagnosis of UC
- Patients without histologically or radiologically confirmed metastatic disease

3.3 Estimated Enrollment

In order to determine the minimum sample size for the purpose of the analysis on patients treated by pembrolizumab, we identified the smallest subpopulation among the planned sub-analyses, which was represented by obese patients with advanced UC. According to the literature, the incidence of obesity can be estimated at 30% in patients with UC [21]. In the study that led to the FDA approval of Pembrolizumab in UC patients [15], the median OS in the standard control arm with chemotherapy was 7.4 months, with a hazard ratio of 0.73. In our study, we set a significance level of 0.05 and the power to 80% for a minimal observation time of 12 months [22,25]. Under these conditions, we estimated an enrollment of 110 obese UC patients, in order to expect to observe at least 78 events in this group, which corresponds to an estimated overall study population of 367 patients affected by advanced UC.

In the study that led to the FDA approval of enfortumab vedotin in UC patients [17], the estimated percentage of patients alive at 12 months was 51.5% (95% CI, 44.6 to 58.0) in the enfortumab vedotin group and 39.2% (95% CI, 32.6 to 45.6) in the chemotherapy group with a delta of 12.3 among the two groups. In this study,

we tested the hypothesis that, using the enfortumab vedotin in clinical practice, OS would reach at least 60% after 12 months from the start of therapy. Study plan will include at least 361 patients with an estimated drop out of 10–15%. It was calculated that a minimum of 311 evaluable patients should be enrolled with an α of 0.05 and a β of 0.80 (STATASOFt).

4. Statistical Analysis and Data Collection

PFS and OS will be estimated by Kaplan-Meier method with Rothman's 95% confidence intervals (CI) and compared by using the log-rank test. Univariate and multivariate analyses will be performed by using Cox proportional hazards models. The chi-square test will be used to compare categorical end-points. Significance levels will be set at a 0.05 value and all *p* values will be two-sided. The statistical analysis will be performed using MedCalc version 19.6.4 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium).

Clinical data retrospectively extracted from paper and electronic charts will be collected in Excel datasets. The data will be anonymized and a code will be used for each individual patient included in the study. For each patient, the following data will be collected in the database of each participating institution: histology, surgery, sites of metastases, Body Mass Index (BMI), concomitant medication, radiotherapy, laboratory tests and treatments. Patients without sufficient data on follow-up and response to therapy will be excluded from this study.

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 1st, 2024 - analysis of data collection and preparation of four of our planned manuscripts within 31th August, 2024
- The second deadline is September 1st, 2024 - analysis of data collection and preparation of the other planned manuscripts within October 31th, 2024

For each manuscript, one author's name for each Institution involved in this project will be considered. We will consider, according to Journal policies, two names for each Oncological Center that has enrolled at least 50 patients in the dataset or which has sustantially 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 sustained by the Center of the Principal Investigator (Macerata Hospital).

6. 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 seven (7) years from the completion of the Study.

7. Ethical Consideration

The ARON-2 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.

8. Informed consent procedures

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), informed consent form. Informed consent must be obtained before conducting any study-specific procedures (i.e.,

any of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

9. Fundings and Fees

No fees are required from or provided to the Centers that participate to the ARON-2 project. Potential publication fees will be sustained by the PI of the Study.

10. References

[1] Smith, A.B.; Deal A.M.; Woods, M.E.; Wallen E.M.; Pruthi R.S.; Chen R.C.; Milowsky, M.I.; Nielsen M.E. Muscle-invasive bladder cancer: evaluating treatment and survival in the National Cancer Data Base. *BJU Int.* **2014**, *114*, 719–726.

[2] Antoni, S.; Ferlay, J.; Soerjomataram, I.; Znaor, A.; Jemal, A.; Bray, F. Bladder cancer incidence and mortality: a global overview and recent trends. *Eur. Urol*. **2017**, *71*, 96–108

[3] Mollica, V.; Rizzo, A.; Montironi, R.; Cheng, L.; Giunchi, F.; Schiavina, R.; Santoni, M.; Fiorentino, M.; Lopez-Beltran, A.; Brunocilla, E.;et al. Current Strategies and Novel Therapeutic Approaches for Metastatic Urothelial Carcinoma. *Cancers (Basel)*. **2020**, *12*, 1449.

[4] Godwin, J.L.; Hoffman-Censits, J.; Plimack, E. Recent developments in the treatment of advanced bladder cancer. *Urol. Oncol*. **2018**, 36, 109–114.

[5] Galsky, M.D.; Hahn N.M.; Rosenberg, J.; Sonpavde, G.; Hutson, T.; Oh, W.K.; Dreicer, R.; Vogelzang, N.; Sternberg, C.N.; Bajorin, D.F.; et al. Treatment of patients with metastatic urothelial cancer "unfit" for Cisplatin-based chemotherapy. *J. Clin. Oncol*. **2011**, *29*, 2432–2438.

[6] Hanna, K.S. Updates and novel treatments in urothelial carcinoma.*J. Oncol. Pharm. Pract*. **2019**, 25, 648-656.

[7] Sternberg, C.N.; de Mulder, P.; Schornagel, J.H.; Theodore, C.; Fossa, S.D.; van Oosterom, A.T.; Witjes, J.A.; Spina, M, van Groeningen C.J.; Duclos, B.; et al. Seven years update of an EORTC phase III trial of high-dose intensity M-VAC chemotherapy and G-CSF versus classic M-VAC in advanced urothelial tract tumours. *Eur. J. Cancer*. **2006**, *42*, 50–54.

[8] Galsky, M.D.; Chen G.J.; Oh W.K.; Bellmunt, J.; Roth, B.J.; Petrioli, R.; Dogliotti, L.; Dreicer, R.; Sonpavde, G. Comparative effectiveness of cisplatin-based and carboplatin-based chemotherapy for treatment of advanced urothelial carcinoma. *Ann. Oncol* **2012**, *23*, 406–410.

[9] Smith, A.B.; Deal, A.M.; Woods, M.E.; Wallen, E.M.; Pruthi, R.S.; Chen, R.C.; Milowsky, M.I.; Nielsen, M.E. Muscle-invasive bladder cancer: evaluating treatment and survival in the National Cancer Data Base. *BJU Int.* **2014**, *114*, 719–726.

[10] Powles, T.; Smith, K.; Stenzl, A.; Bedke, J. Immune checkpoint inhibition in metastatic urothelial cancer. Eur. Urol. **2017**,*72*,477–481.

[11] Motzer, R.J.; Escudier, B.; McDermott, D.F.; George, S.; Hammers, H.J.; Srinivas, S.; Tykodi, S.S.; Sosman, J.A.; Procopio, G.; Castellano, D.; et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N. Engl. J. Med*. **2015**, *373*, 1803e13.

[12] Reck, M.; Rodríguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csőszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S.; et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. *J. Clin. Oncol*. **2019**, *37*, 537-546.

[13] Parry, R.V.; Chemnitz, J.M.; Frauwirth, K.A.; Lanfranco, A.R.; Braunstein, I.; Kobayashi, S.V.; Linsley, P.S.; Thompson, C.B.; Riley, J.L. CTLA- 4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol. Cell. Biol.***2005**, *25*, 9543–9553 (2005).

[14] Nadal, R.; Bellmunt, J. Management of metastatic bladder cancer. *Cancer Treat. Rev.***2019**, *76*, 10-21.

[15] Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, Necchi A, Gerritsen W, Gurney H, Quinn DI, Culine S, Sternberg CN, Mai Y, Poehlein CH, Perini RF, Bajorin DF; KEYNOTE-045 Investigators. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. N Engl J Med **2017,** 376:1015-1026.

[16] Zarrabi, K.; Paroya, A.; Wu, S. Emerging therapeutic agents for genitourinary

cancers. *J Hematol Oncol.***2019**, *12*, 89.

[17] Powles, T.; Rosenberg, J.E.; Sonpavde, G.P.; et al. Enfortumab Vedotin in Previously Treated Advanced Urothelial Carcinoma. *N. Engl. J. Med.* **2021**, 384:1125-1135.

[18] Tagawa, S.T.; Balar A.B.; Petrylak D.P.; et al. TROPHY-U-01: A Phase II Open-Label Study of Sacituzumab Govitecan in Patients With Metastatic Urothelial Carcinoma Progressing After Platinum-Based Chemotherapy and Checkpoint Inhibitors. *J Clin Oncol*. **2021**,1;39(22):2474-2485.

[19] July O (2021) FDA grants regular approval to enfortumab vedotin-ejfv for locally advanced or metastatic urothelial cancer. FdaGov 2021–2023

[20] Al Harthy, M.; Redman, J.; Madan, R.A. Novel immunotherapy combinations for genitourinary cancers.*Expert Opin. Biol*. Ther.**2020**, *27*, 1-10.

[21] Leiter A, Doucette J, Krege S, Lin CC, Hahn N, Ecke T, Sonpavde G, Bamias A, Oh WK, Galsky MD. Obesity and Outcomes in Patients with Metastatic Urothelial Carcinoma. Bladder Cancer. **2016**;2:341-349.

[22] Epstein B, Sobei M. Life testing. J Am Stat Assoc **1953**, 48:486-502.

[23] Schwartz LH, Litière S, de Vries E, et al. RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer **2016**;62:132-7.

[24] NIH/NCI (2017) Common Terminology Criteria for Adverse Events (CTCAE) | Protocol Development | CTEP. Protocol 147.

[25] Narula SC, Li FS. Sample size calculation in exponential life testing.

Technometrics **1975**, 17:229-31.