SYNOPSIS

Name of Sponsor/Company: Astra Zeneca KK	**Individual Study Table Referring to Module 5 of the Dossier** **Volume** **Page**	**(For National Authority Use Only)**
Name of Finished Product: Not Applicable		
Name of Active Ingredient: Not Applicable		

Title of Study: Real-world evidence of PD-L1, TMB prevalence and efficacy of 1st line chemotherapy in these high or low population for Stage IV urothelial cancer (YODO)

Investigator sites: 21 Investigator sites in Japan.

Objectives:

Primary Objectives

1. To evaluate prevalence of programmed death ligand 1 (PD-L1) expression on tumor cell and immune cell in Stage IV urothelial cancer patients in real-world setting.

Secondary Objectives

1. To evaluate prevalence of tumor mutation burden (TMB) in tissue tumor or immune cell in Stage IV urothelial cancer patients in real world setting.
2. To assess overall survival (OS), progression-free survival (PFS) from start of first-line treatment for Stage IV urothelial cancer.
3. To investigate treatment pattern in Stage IV urothelial cancer patients.

Exploratory Objectives

1. To evaluate cancer-immune phenotype of Stage IV urothelial cancer patients in real-world setting.
2. To evaluate OS and PFS from start of first-line treatment for Stage IV urothelial cancer patients in subpopulations:
   - PD-L1 high or low/negative population.
   - PD-L1 percentage expression on tumor tissue or immune cell: 0, 10, 25, 50, 75, 100%.
   - TMB high (>10 mut/Mb) or low (≤10 mut/Mb) population.
   - Cancer-immune phenotype: immune-desert, immune-excluded, inflamed, others.
3. To evaluate the presence of gene mutations as prognosis marker in primary tumor tissues of the Stage IV urothelial cancer patients

Methodology:

This study was a multicenter, noninterventional study to evaluate the prevalence of PD-L1, cancer immunity and TMB in Stage IV urothelial cancer patients who had been treated in a real-world setting in Japan.

Patients' background, treatment pattern, treatment outcome, and efficacy were collected from medical records of Stage IV urothelial cancer patients diagnosed from 01 January 2017 to 31 December 2018 who had received at least 1 cycle of chemotherapy. Archived patients' FFPE primary tumor samples were collected from each site and PD-L1 assay, classification of histology and cancer-immune phenotype and next generation sequencer (NGS) assay for TMB were performed. Based on these data, prevalence of PD-L1 expression, TMB, OS, and PFS from start of first-line treatment for Stage IV urothelial cancer were assessed.

Patients had to provide written informed consent for their data and tumor samples to be used in the study. After signing the informed consent form (ICF), patients were screened and if they fulfilled the study inclusion and

exclusion criteria they were enrolled in the study. Patient background at enrollment, sample information and other data were collected from medical records and reported on the case report form (CRF). Personal information, such as patient name and medical record ID, were not collected in the CRF, and patients were each given a specific study identification number.

When it was difficult or not possible to contact a patient, eg, the patient had died or moved, optout was available after recording enrollment in the medical record. At the site, this study information was released to allow opportunity to reject participation to the study. Optout procedure followed ethics committee procedures for each site.

The FFPE samples retained at the site were sent to the central laboratory according to the sample handling procedure, to assay PD-L1 expression, histological classification, cancer-immune phenotype and NGS. All reports from the central laboratory including central pathological examination were not sent to the patients or Investigators.

Number of patients (planned and analyzed):

152 patients screened and enrolled in study.

143 patients were included in eligible population and completed study.

Diagnosis and main criteria for inclusion:

Japanese men and women aged >20 years, diagnosed as Stage IV (American Joint Committee on Cancer [AJCC], 7th edition) urothelial cancer between 01 January 2017 and 31 December 2018, who had started at least 1 cycle of chemotherapy, had a FFPE primary tumor sample collected after 01 January 2017 (before first-line treatment in Stage IV urothelial cancer; preferably the sample was taken before neoadjuvant therapy [NAC], even if it was taken before 01 January 2017), and who had provided informed consent for participation (in dead or moving out cases, optout was applicable according to Institutional Review Board (IRB)/Ethics Committee (EC) rules).

Test product, dose and mode of administration, batch number: Not applicable.

Reference therapy, dose and mode of administration, batch number: Not applicable.

Duration of treatment: Not applicable.

Criteria for evaluation:

Outcomes

The OS rates at 12, 18 and 24 months after start of first-line treatment for Stage IV were estimated using Kaplan-Meier method. Patients enrolled on and before 31 December 2019 and alive at enrollment were followed up until 31 December 2019. Patients enrolled after 01 January 2020 and alive at enrollment were followed at the date of enrollment.

The PFS was defined as the time period from the start of first-line treatment for Stage IV until the first progressive disease (PD) as assessed by the Investigator, or death. Patients enrolled on and before 31 December 2019 and whose PD or death was not confirmed at enrollment were followed up until 31 December 2019. Patients enrolled after 01 January 2020 were followed at the date of enrollment. The proportion of patients without disease progression at 6, 12 and 18 months after the start of first-line treatment for Stage IV were estimated using the Kaplan-Meier method.

FFPE block samples or serial sections were shipped to a central laboratory (Riken Genesis Co, Ltd) for TMB measurement. For block samples, serial sections were prepared at Riken Genesis and the remaining blocks after slicing were returned. If block samples were submitted, one section was stained by HE to confirm tumors. DNA was extracted from the sections. TMB was measured on NextSeq (NGS equipment) using Illumina's TruSight Oncology 500 panel (TSO500). Approximately 500 genes, including 15 HRR genes, were analyzed for mutations.

Another central laboratory (SRL Inc) performed the PD-L1 immunohistochemistry and hematoxylin and eosin (HE) staining procedure using the Ventana PD-L1 (SP263) assay, and then shipped the samples to the Central Pathological Committee.

Using the slide specimens stained by HE as described above, 2 pathologists from the Central Pathological Committee made pathological determinations. These included evaluations of cancer-immune phenotype as immune-desert/immune-excluded/inflamed. They also evaluated PD-L1 expression as 0%, 10%, 25%, 50%, 75%, or 100% using the PD-L1-stained slide specimens.

Safety: Not applicable

Statistical Methods:

Analysis Populations

The eligible population was the main population used in all the analyses, and comprised all patients included in the study who met all inclusion/exclusion criteria and for whom the pathologists at the central laboratory had diagnosed their main FFPE sample as being urothelial carcinoma (transitional cell carcinoma).

Primary Analyses

For the primary objective and endpoint regarding PD-L1 expression on real-world data, descriptive statistics of PD-L1 categories were presented using summary statistics. PD-L1 was described as its original value (0%, 10%, 25%, 50%, 75%, 100%) and its derivation (high, low/negative).

Secondary Analyses

Secondary Analysis 1

The prevalence of TMB was determined according to the frequencies of the categories high (>10 mut/Mb) and low (≤10 mut/Mb). TMB was descriptively analyzed as a continuous variable.

Secondary Analysis 2

Kaplan-Meier plots were presented for OS. Median OS and OS rates, along with their 95% confidence intervals (CIs), were estimated using the Kaplan-Meier method. PFS and second line PFS were similarly analyzed.

Secondary Analysis 3

Treatment used in each line was presented using absolute (n) and relative frequency (%). Frequencies of different treatment patterns from first- to third-line treatment were determined.

Exploratory Analyses

Exploratory Analysis 1

Absolute (n) and relative frequency (%) of cancer-immune phenotype were determined.

Exploratory Analysis 2

The OS and PFS analysis from secondary analysis 2 was repeated for subgroups. For each variable used to define the subgroups, the hazard ratio (HR) between categories and its 95% CI was estimated using a Cox model including only the corresponding variable. The second line PFS was analyzed and presented similarly to OS and PFS.

Exploratory Analysis 3

Absolute (n) and relative frequency (%) of gene mutations present in patients' samples were determined. Additionally, ratios were added based on disease progression assessed during the study for each gene.

Exploratory Analysis 4

PD-L1 expression and change between before and after NAC were described by absolute (n) and relative frequency (%).

Determination of Sample Size

The primary objective in this study was to evaluate prevalence of PD-L1.

The ratio of PD-L1 high patients stained using Ventana PD-L1 (SP263) assay was speculated to be 60%. With 150 patients, the 2 sided 95% CIs were determined as 8.0%, 7.8%, and 7.3%, respectively, when the observed proportion of patients with high PD-L1 was 50%, 60% and 70%. Based on these calculations, 150 patients were considered acceptable for this study.

Results

This was a study of real-world evidence to evaluate the prevalence of PD-L1, TMB and cancer-immune phenotype as well as the correlation between these immunogenic biomarkers and clinical manifestation including prognosis and treatment regimens in 143 eligible patients with Stage IV urothelial cancer and a mean (SD) age of 71.7 (9.84) years. The study involved retrospective analysis of data from medical records, but also de novo assay of the patients' archived FFPE primary tumor samples. By performing validated assay techniques on the FFPE samples, including Ventana PD-L1 (SP263) assay and NGS, PD-L1 expression, histological classification, cancer-immune phenotype, and TMB could be determined. Based on these data, prevalence of the immunogenic biomarkers including PD-L1 expression, TMB and OS, PFS from start of first-line treatment for Stage IV urothelial cancer was assessed.

Since this study has not been made progress as schedule because of COVID19 pandemic, updated study results will be reported by the end of 2021.

Date of Report: 09 February 2021