STUDY REPORT SYNOPSIS

An epidemiologic study on PD-L1 expression combined with clinical observation of initial treatment pattern and overall survival in the Chinese muscle invasive urothelial bladder carcinoma patients

Study description: This was a multi-center, prospective, epidemiologic study that was planned to enroll 400 consecutive patients with a newly confirmed diagnosis of muscle invasive urothelial bladder carcinoma from approximately 20 hospitals to explore PD-L1 expression status. All the patients were also collected with initial treatment pattern and then followed up for overall survival for up to 2 years.

Milestones:	<<INSERT AS INCLUDED IN THE MILESTONES SECTION OF THIS STUDY REPORT>>
Phase of development:	NA
Sponsor:	AstraZeneca Investment (China) Co., Ltd.

This study was performed in compliance with Good Clinical Practice (GCP) and Good Pharmacoepidemiology Practice (GPP), including the archiving of essential documents.

Background/rationale: Bladder cancer is the ninth most commonly diagnosed cancer each year worldwide. In China, bladder cancer is also one of the 10 predominant malignancies and the most common tumor of the urinary tract (Chen et al 2016). The median survival for patients with metastatic / unresectable / advanced disease is 14–15 months after chemotherapy treatment and a 5-year survival rate of about 5% (National Cancer Institute Surveillance, Epidemiology, and End Results Program. SEER cancer statistics factsheets: Bladder cancer). Two programmed cell death protein 1 (PD-1) / programmed cell death-ligand 1 (PD-L1) inhibitors and 5 PD-1/PD-L1 inhibitors have been approved by United States (US) Food and Drug Administration (FDA) for first line treatment in the cisplatin ineligible patients and second line treatment in urothelial carcinoma, respectively.

Durvalumab, a PD-L1 inhibitor, has been demonstrated its antitumor efficacy and safety in the metastatic urothelial carcinoma patients who progressed after platinum-based chemotherapy in a phase I/II study (study 1108). Based on the results of this study, the patients with high PD-L1 expression seemed to achieve much greater clinical benefit in response to the treatment of durvalumab (Powles et al 2017) than those with low or negative PD-L1 expression. So that PD-L1 expression status is probably a promising predictive biomarker for durvalumab treatment. Currently, a global phase III, registration study (DANUBE study) including 180 Chinese patients with locally advanced or metastatic urothelial carcinoma is being conducted. The PD-L1 expression prevalence will be analyzed. However, the results will be only limited to analyze the patients

at late stage and the samples are mainly just collected from biopsy ≤ 3 years which probably cannot reflect the real-time status of the patients according to the protocol of DANUBE study. Therefor so far there was no relevant data about real-time PD-L1 expression status by Ventana SP263 Immunohistochemistry (IHC) assay in Chinese patients with muscle invasive urothelial bladder carcinoma (MIUBC). In addition, initial treatment pattern in the Chinese patients with MIUBC and the overall survival (OS) status of them were unclear in China. Collecting the related data helped to learn about the unmet medical needs of these patients in China.
This multi-center, prospective, epidemiologic study was proposed to investigate the prevalence of high PD-L1 expression in the Chinese MIUBC patients who also were observed for initial treatment pattern and 2-year OS to aid in making treatment decisions in the future. Meanwhile, the PD-L1 testing outcome of the hospital laboratories was explored to evaluate potential scientific issue such as testing concordance between the central laboratory and the hospital laboratories.

Objectives:
The primary objective of this observational study was:

- To investigate the prevalence of high PD-L1 expression in Chinese patients with MIUBC. High PD-L1 expression was defined as ≥ 25% tumor cell membrane positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control OR ≥ 25% tumor associated immune cell (IC) positivity for PD-L1 at any intensity above background staining as noted on the corresponding negative control.
  Note: PD-L1 High (≥ 25% tumor cell membrane positivity for PD-L1 or 1) IF IC area > 1%: ≥ 25% tumor associated IC positivity for PD-L1; 2) If IC area=1%: 100% tumor associated IC positivity for PD-L1). PD-L1 Low if criteria not met for PD-L1 High.

The secondary objectives of this observational study were:

- To investigate the PD-L1 expression profile in tumor cell (TC) or IC in Chinese patients with MIUBC.
- To assess the concordance of PD-L1 testing results generated from the hospital laboratories with those from the central laboratory.
- To observe the initial treatment pattern for patients with MIUBC in usual clinical practice in China.
- To observe 2-year OS of the Chinese patients with MIUBC.

The exploratory objectives of this observational study were:

- To explore the relationship between the demographic characteristics and expression of PD-L1.
- To explore the relationship between OS and the demographic characteristics as well as the expression of biomarkers.
  To explore the relationship between PD-L1 and tumor mutation burden (TMB), PD-L1 and CD8 + T cell and PD-L1 positive IC with CD8+T cell respectively.

Study design: This was a multi-center, prospective, epidemiologic study that was planned to enroll 400 consecutive patients with a newly confirmed diagnosis of MIUBC from approximately 20 hospitals to explore PD-L1 expression status. All the patients were also collected with initial treatment pattern and then followed up for OS for up to 2 years.
Data collection included enrolled period and follow-up period.
During the enrolled period of the study, after informed consent was obtained, the following procedures were performed: review of eligibility criteria, collection of tumor tissue samples obtained from cystectomy, transurethral resection or biopsy within 60 days before the enrollment for testing the expression of PD-L1 and other biomarkers including IC subset CD8+ T cells, TMB, and collection of required patients' data through chart review. The study collected retrospective data relevant to the MIUBC diagnosis, including medical history and treatment history. Because all the enrolled patients were required to be the patient population who had not received any systemic treatment yet, after enrollment, the initial treatment pattern such as surgery and chemotherapy (including regimen, timing of administration, etc.), was documented when determined. All the patients were treated according to usual clinical practice on the study sites during the study, which might include surgery and/or systemic drug treatment such as chemotherapy and radiotherapy. No experimental drug was provided to the patients during this study.
All the 400 patients were tested for the expression of PD-L1 and other biomarkers including IC subset CD8+ T cells, TMB at baseline in the central laboratory. Two hundred out of 400 patients were also planned to be tested for assessing PD-L1 testing concordance within approximately 10 hospital laboratories. PD-L1 testing was conducted by Ventana SP263 IHC in TC and IC. PD-L1 testing results were categorized into high

expression and low/negative expression. The testing concordance between the central laboratory and all 10 hospital laboratories was calculated based on categorization results. CD8+ T cells were marked by IHC with commercial primary antibody. DNA-based sequencing was used to explore TMB in MIUBC tumor samples. Ten qualified formalin fixed paraffin embedded (FFPE) tissue sections were collected for the testing of PD-L1 and other biomarkers in the central laboratory for each patient. If there were less than 10 sections from one sample in case the sample was too small, the priority to collect sections for certain testing was as following: PD-L1 (3 sections) > CD8 (2 sections) > TMB (5 sections). The minimal required sections number was 3, specially for PD-L1 testing. Additional 3 tissue sections were collected for the concordance of PD-L1 IHC testing and stained in the hospital laboratories.
During the follow-up period of the study, all the patients was planned to be followed up by the doctors through on-site visit or phone call every 6 months for OS for up to 2 years.

Data source: Data were collected after enrollment in the study and entered in the electronic case report form (eCRF). All data were collected through the patients' medical records, patient interview, and other methods not limited to contacting a family member, friend, or primary care physician. The site investigator was responsible for ensuring that all the required data were collected and recorded in the eCRF.

Study population: Patients with a new diagnosis of MIUBC.

Inclusion criteria:
The patient population that was observed in the study fulfilled all the following criteria:
- Age ≥18 years at the time of screening.
- Be able and willing to sign the informed consent form (ICF).
- Patients with histologically or cytologically documented, muscle invasive urothelial carcinoma (ie, T2 toT4, any N, any M) of bladder (see National Comprehensive Cancer Network [NCCN] Bladder Cancer Guidelines), who had not been previously treated with any systemic chemotherapy, radiotherapy, investigational product, or biologic therapy for cancer treatment.
- For PD-L1 testing by IHC assay, all patients were able to provide a newly acquired tumor sample within 60 days before enrollment by cystectomy, transurethral resection or biopsy. Samples with limited tumor content and fine needle aspirate specimens were not acceptable. Specimens from metastatic bone lesions were typically unacceptable unless there was a significant soft tissue component. The tumor specimen submitted to establish PD-L1 status should be of sufficient quantity to allow for PD-L1 IHC analyses and was preferred in FFPE blocks.

Exclusion criteria:
Patients meeting the following exclusion criterion were not eligible:
- Prior acquiring tumor tissue samples exposure to immune-mediated therapy (including Bacillus Calmette Guerin), including but not limited to, any anti-CTLA-4, anti-PD-1, anti-PD-L1, or anti-PD-L2 antibodies, therapeutic anticancer vaccines.
- Any concurrent chemotherapy, investigational product, or biologic therapy for cancer treatment. Note: Local treatment of isolated lesions, excluding target lesions, for palliative intent was acceptable (eg, local surgery or radiotherapy).

Statistical methods:
Statistical analysis was descriptive primarily. Continuous variables were summarized by the number of observations, mean (or geometric mean as appropriate), standard deviation, median, interquartile range (Q1, Q3), minimum, and maximum. Categorical variables were summarized by frequency counts and percentages for each category. The 95% confidence interval (CI) was calculated as appropriate. OS was summarized using Kaplan-Meier estimates of the median time to event (death due to any cause) and quartiles together with their 95% CI. PD-L1 expression rate was also summarized descriptively by subgroups according to age (< 70 vs ≥ 70), gender, metastatic disease, prior tobacco use, etc. OS was summarized descriptively by subgroups according to age group (< 70 vs ≥ 70), gender, metastatic disease, and different expression levels of PD-L1 as well as other biomarkers, as appropriate. Cox proportional hazards model was employed to further explore the impact factors of OS.
Spearman's rank correlation analysis was used to explore the association between any two biomarkers.

Results: A total of 248 patients were enrolled from 17 sites, among which 229 (92.3%) patients who met the inclusion/exclusion criteria and had any valid data of PD-L1 expression status were included in the full

analysis set (FAS), while 228 (91.9%) patients who entered the follow-up period were included in the follow-up analysis set (FUAS). Based on the FAS, most patients were male, and all were Asian. The mean (SD) age was 67.9 (10.8) years. The mean (SD) BMI was 23.2 (3.1) kg/m^2. No obvious abnormality of vital signs and physical variables was found at baseline. Most patients had surgical history and therapy, of which, radical cystectomy (72.1%) and transurethral bladder resection (38.9%) by preferred terms were most common experienced. The primary tumor site, regional lymph nodes, and distant metastases were classified by TNM classification, and most patients were diagnosed with T2, N0, M0.

This study was well conducted with no statistical/analytical issues or bias affecting the interpretation of the results.

The primary objective of this observational study was to investigate the prevalence of high PD-L1 expression in Chinese patients with MIUBC. Based on the FAS, the TC for PD-L1 overall evaluation result showed that 59 (25.8%) patients met +TC, and the other 170 (74.2%) patients met -TC. The IC overall evaluation results showed that 5 patients whose IC area = 1% were evaluated as -IC (< 100% or none tumor associated IC positivity for PD-L1). Of other 224 patients whose IC area ≠ 1%, 82 (36.6%) patients met +IC (≥25%) and 142 (63.4%) patients were -IC (<25%). The prevalence of high PD-L1 expression is 52.4% (120/229).

Regarding the results of central PD-L1 expression profile in TC or IC in the FAS, of 120 patients with high PD-L1 expression, +TC was detected in 59 (25.8% of the total) patients, while -TC was detected in 61 (26.6% of the total) patients; and +IC was detected in 82 (35.8% of the total) patients, while -IC was detected in 38 (16.6% of the total) patients.

Of 46 patients with available PD-L1 data from hospital laboratories, the concordance of PD-L1 testing results between the central laboratory and hospital laboratories was 80.4%.

Total 50 (21.8%) patients experienced cancer therapies as the initial treatment for MIUBC. Among the 50 patients, the therapy classes included cytotoxic chemotherapy (64.0%), immunotherapy (26.0%), and other (32.0%). The therapy type included systemic therapy (58.0%) and local therapy (52.0%). The treatment status included intravesical chemotherapy (52.0%), medication therapy alone (not combined with surgery) (28.0%), adjuvant (22.0%), and neo-adjuvant (10.0%). Only 1 patient experienced at least one concomitant chemoradiotherapy, and the number of the chemoradiotherapy was 2-3. Of 37 patients with available treatment cycles data, the mean (SD) number of treatment cycles was 2.9 (2.16). The mean (SD) duration of cancer therapy agent of the 44 patients who had available data was 0.83 (1.044) months. The most common previous and planned surgery in 226 patients was cystectomy or transurethral bladder resection. The surgeries included: radical cystectomy, with/without transurethral bladder resection (81.9%); transurethral bladder resection only, no partial or radical cystectomy (14.6%); partial cystectomy, with/without transurethral bladder resection (2.2%) and partial and radical cystectomy, with/without transurethral bladder resection (1.3%).

At the time of the data cut-off, the median OS was not reached in the FUAS. The OS rate (95% CI) at 12 and 24 months was 0.84 (0.79, 0.89) and 0.77 (0.70, 0.82), respectively.

As the results of OS subgroup analysis, the median OS in most subgroups was not reached, except that the median (95% CI) OS of MX subgroup (30 patients) by metastatic disease was 19.19 (12.550, -) months and the median OS (95% CI) of N3 regional lymph nodes subgroup (4 patients) was 5.31 (2.370, -) months. The separation of Kaplan-Meier (KM) curves was observed in following subgroups: The KM curves showed separation between the age subgroups, and there appeared to be a better survival curve in < 70-year-old patients than in ≥70-year-old patients; the KM curves showed separation after approximately 8 months between T2 subgroup and other subgroups but showed no separation between the T3 and T4 subgroups up to approximately 13 months; the KM curves showed separation after about 6 months between M0 subgroup and other subgroups but showed no separation between the M2 and MX subgroups; the KM curves showed slight separation after about 12 months between the high and low CD8+ T cells expression subgroups.

Regarding the association analysis between PD-L1 expression and subgroups, the differences within all subgroups were not statistically significant ($P > 0.05$). In the saturated model and final model, the ORs for all characteristics in both models were not statistically significant ($P > 0.05$), indicating the characteristics of subgroups had few influences on patients' PD-L1 expression levels.

Cox proportional hazards models were employed to further explore the impact factors of OS, including age, gender, primary tumor site, metastatic disease, the PD-L1 expression level, and other biomarkers (TMB, CD8+ T cells). The results showed that the HRs for primary tumor site (T3 vs. T2 and T4 vs. T2) and metastatic disease (MX vs. M0) were statistically significant, indicating that the primary tumor site and

metastatic disease may influence patients' OS.

Regarding other biomarkers, of 229 patients in the FAS, 102 (44.5%) patients were observed with high CD8+ T cell and 126 (55.0%) patients were observed with low CD8+ T cell; 124 (54.1%) patients were observed with high TMB and 92 (40.2%) patients were observed with non-high TMB.

As the exploration results of the association between any two biomarkers, for TMB, there was positive weak but statistically significant correlation between the percentage IC present and TMB (P = 0.0324) and the percentage of IC with PD-L1 positivity and TMB (P = 0.0202); for CD8+ T cell, there was statistically significant correlation

in expression level of PD-L1 between the percentage of TC with membrane PD-L1 positivity and CD8+ T cell (P < 0.0001); between the percentage IC present and CD8+ T cell (P < 0.0001); between the percentage of IC with PD-L1 positivity and CD8+ T cell (P < 0.0001).

Conclusion:

- The prevalence of high PD-L1 expression was observed in 52.4% Chinese patients with MIUBC in this study.
- High PD-L1 expressed broadly consistent in patients with +TC and -TC, and expressed more in patients with +IC than patients with -IC.
- The concordance of PD-L1 testing results between the central laboratory and hospital laboratories (n=46) was 80.4% with moderate agreement.
- The most common therapy classes were cytotoxic chemotherapy (64.0%). The most common therapy type was systemic therapy (58.0%). The most common treatment status was intravesical chemotherapy (52.0%). The most common reported previous and planned surgery was radical cystectomy, with/without transurethral bladder resection (81.9%).
- At the time of the data cut-off, a total of 51 patients (22.4%) had died. The median OS was not reached. The OS rate (95% CI) at 12 and 24 months was 0.84 (0.79, 0.89) and 0.77 (0.70, 0.82), respectively. The median OS in most subgroups was not reached.
- The variables of subgroups had few influences on PD-L1 expression levels.
- The HRs for primary tumor site (T3 vs. T2 and T4 vs. T2), and metastatic disease (MX vs. M0) were statistically significant, indicating that the primary tumor site (T3 vs. T2 and T4 vs. T2) and metastatic disease (MX vs. M0) may influence patients' OS.
- Regarding the correlation between the expression level of PD-L1 and other biomarkers, there was statistically significant correlation observed between the percentage IC present and TMB and between the percentage of IC with PD L1 positivity and TMB; between the percentage of TC with membrane PD-L1 positivity and CD8+ T cell; between the percentage IC present and CD8+ T cell; and between the percentage of IC with PD-L1 positivity and CD8+ T cell.

Publications: Not applicable.