

Study Title	
Study Title:	<p>PURE-01 - Window pre-operative study of aPD-1 MK-3475 (Pembrolizumab) in urothelial bladder cancer patients who are candidates for surgery.</p> <p>An investigator initiated study endorsed by the EAU Research Foundation.</p>
Principal Investigator and Institution Contact Information	
Name:	Andrea Necchi
Name:	Fondazione IRCCS Istituto Nazionale dei Tumori
Address 1	Via G. Venezian 1
City, ST, Zip	Milano, Italy, 20133
website	www.istitutotumori.mi.it
Study Information	
Indication	Urothelial bladder cancer
Phase:	2
Number of Subjects:	90 patients in 2 centres in Fondazione IRCCS Istituto Nazionale dei Tumori and University Hospital Vienna (Prof. Shahrokh Shariat).
Background and Rationale	
<p>Urothelial bladder cancer (UBC) is the fourth most common cancer in the United States with over 60,000 new cases each year. It can usually be treated initially by cystectomy or trans-urethral resection of bladder tumor (TURBT). Unfortunately over 40% of all patients will develop a cancer recurrence in less than two years and all patients remain at increased risk of recurrence for the remainder of their lives.</p> <p>Patients with T2-T4a N0M0 TCC bladder routinely wait for up to 4-8 weeks to have surgery. This is due to the complex nature of the procedure. This results in an opportunity to give a period on MK-3475 prior to cystectomy. Sequential tissue will be available for biomarker analysis.</p> <p>MK-3475 (pembrolizumab) is a humanized IgG4, high-affinity, anti-PD-1 antibody that has been recently approved in the United States for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAFV600 mutation-positive, a BRAF inhibitor. MK-3475 has demonstrated significant activity in UC of the bladder and it appears biomarkers can predict activity.(1) PDL-1 expression alone from archived tissue, measured by immunohistochemistry (IHC) may not be predictive of response to PD-1/PDL-1 therapy in UBC. A major challenge is that we have not had real time tissue for analysis. Moreover, dynamic changes to immunological markers may be crucial in understanding mechanisms of sensitively.</p> <p><i>Bibliography:</i></p> <ol style="list-style-type: none"> 1. Plimack ER, Gupta S, Bellmunt J, et al. LBA23: A phase 1b study of pembrolizumab (Pembro; MK-3475) in patients with advanced urothelial cancer. ESMO 2014 Madrid, Spain, September 2014. 2. Necchi A, Anichini A, Raggi D, Briganti A et al. Pembrolizumab as Neoadjuvant Therapy Before Radical Cystectomy in Patients With Muscle-Invasive Urothelial Bladder Carcinoma (PURE-01): An Open-Label, Single-Arm, Phase II Study. <i>J Clin Oncol</i>. 2018 Oct 20;JCO1801148. doi: https://doi.org/10.1200/JCO.18.01148 https://www.ncbi.nlm.nih.gov/pubmed/30343614 . 	
Objectives	

FACTS AND FIGURES:

To assess whether MK-3475 results in pathological complete response rates (pCR) in T2-T4a N0M0 UBC of the bladder according to the assumptions provided below.
 To evaluate radiological response on those patients with measurable disease (at baseline). Response (CR and PR) after 3 cycles of treatment with the study drug.
 Safety of MK-3475.
 Disease free survival and overall survival at 2 years.

Endpoints:

- Pathologic complete response (pCR) is the primary endpoint.
- Secondary endpoints will include downstaging to non-muscle invasive disease, PFS, OS, and toxicity.
- Translational outcomes

Hypothesis

Pembrolizumab can downstage bladder cancer tumors and reduce recurrence.
 Also sequential tissue will allow to further explore dynamic biomarkers. This tissue will be unique.

Study Design/Clinical Plan

A transurethral resection of the bladder (TURB) for biopsy, histological characterization, and local staging will be executed first.
 MK-3475 will be administered for a total of 3 cycles prior to radical cystectomy. Surgery will be planned at the time of study inclusion to be done within 3 weeks of the last dose of study drug (accounting for a total window time of 9 weeks).

- Patients with T2-T4a N0 M0 UBC will receive 3 cycles of MK-3475 at 200mg 3 weekly prior to surgery.
- Surgery will take place within 3 weeks after the last dose of the study drug.
- After surgery, patients with the evidence of pT3-4 and/or pN+ disease will be managed according to local guidelines (adjuvant chemotherapy will be allowed).
- Further Anti PD-1 therapy will not be given post-operatively.
- PDL-1 status will be assessed for all patients enrolled on the study.
- The study may be extended to include addition PD-L1 positive patients.
- PDL-1 status will be assessed on all samples of patients enrolled on the study. The study may be extended to include additional PD-L1 positive patients.

Treatment

- List the clinical dosage/dosage form, route, and dose regimen:

Pembrolizumab (MK-3475) 200 mg will be administered as a 30 minute IV infusion every 3 weeks (treatment cycle intervals may be increased due to toxicity as it will be described in the full protocol). Investigators will make every effort to target infusion timing to be as close to 30 minutes as possible. However a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Collateral Research

The original tissue (paraffin embedded) need to be located and collected by the Sponsor (Fondazione IRCCS Istituto Nazionale dei Tumori, Milano). This does not need to occur before the patient enters into the study. All previous bladder cancer samples taken from patients will also be collected for future research.

Proposals for Collateral Research:

- a. Correlation of expression and /or localisation of PD-1/PD-L1, CD8+ cells and expression of T cell markers and

FACTS AND FIGURES:

- response/outcome measures.
- b. Status of PD-L1, immune- and disease-related and other exploratory biomarkers in freshly obtained tumor tissues and blood collected before, during, or after treatment with MK-3475.
 - c. Status of tumor-infiltrating immune cells and exploratory biomarkers in biopsy specimens and blood collected at different time points.

More specifically these may include:

- 1) Assessment of the tumor microenvironment by immunohistochemistry (IHC) and RTqPCR.
- 2) Phenotypic analysis of PBMCs and activation status of NK and NK T cells.
- 3) Quantification of T-cell activation and tumor-specific T cell responses.
- 4) Characterization of Myeloid-derived suppressor cells (MDSC) in blood and tumor.
- 5) Serum measurements of relevant cytokines (e.g., IL-6, IL-1, TNF α , IL-12) and chemokines (e.g., MCP-1, MIP-1 β) in peripheral blood before and after therapy.

Furthermore, we will plan a targeted exon sequencing of available samples from each patients through the Ion Ampliseq™ Comprehensive Cancer Panel. The latter targets the exons of 409 tumor suppressor genes and oncogenes frequently cited and mutated in urothelial cancer. It is strategically designed to interrogate coding DNA sequences and splice variants across multiple gene families simultaneously.

Statistical Plans

All patients enrolled who receive at least 1 cycle of study drug will be included in the ITT analysis.

The assumption is to assess whether pre-operative treatment with pembrolizumab results in pCR of 20% or more in T2-T4a N0M0 TCC of the bladder. A two-stage design will be used to estimate the number of patients required. Pembrolizumab is active in UBC and results in CR in the metastatic setting. It is hoped that immune check point inhibitors will be more active in untreated patients. A pCR of $\geq 20\%$ would support further investigation in randomised III clinical trials. pCR should not be 10% or less, and it would only be worth considering in further study if the true rate were 20% or more.

The overall sample size will consist in 90 patients overall, with the first stage of 49 patients, with 80% power and a two-sided test of significance at the 10% level.

The above described assumptions will result in the following decision rules:

If the number of pathologic complete responses is 6 or more in the first stage, the study will go through the second stage. If, out of the total 90 stage 1+2 patients, 13 patients at least will achieve a pCR, the drug will be considered active and warranting further investigation.

Based on the numbers available from the metastatic setting, it is expected that 30% of patients (i.e. approximately 30 patients in this study) will have a 2+ or 3+ IHC staining for PDL-1, and a greater activity of the study drug is hypothesized in these patients.

Such numbers will allow us to run sufficiently powered analyses to test a difference of pCR rate from 10% (H0) to either 25% or 30% (H1) (power of 80% and 92%, respectively) in these patients only.

An expansion of the PDL-1 2+/3+ cohort will be discussed in case the total number of patients will be lower than 30 at the end of planned accrual.