Official Study Title

Biomarker Studies to Predict Treatment Outcomes of Enfortumab Vedotin in Advanced Urothelial Carcinoma

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1. Official Study Title

Biomarker Studies to Predict Treatment Outcomes of Enfortumab Vedotin in Advanced Urothelial Carcinoma

2. Background and Rationale

Advanced urothelial carcinoma (aUC) remains a highly fatal malignancy, with limited durable responses to first-line platinum-based chemotherapy. Enfortumab vedotin (EV), a nectin-4-targeting antibody-drug conjugate, has shown promising efficacy in aUC patients, both in the first-line setting when combined with pembrolizumab and in later-line setting when used as monotherapy (EV/EVP). However, despite its clinical success, the occurrence of significant toxicities and high treatment costs highlight the need for predictive biomarkers to optimize treatment outcomes and mitigate adverse effects. Nectin-4, as the target of EV, along with ADAM10/17, enzymes responsible for nectin-4 shedding, and soluble nectin-4 (sNectin-4), have emerged as potential biomarkers for predicting EV treatment outcomes.

3. Objectives

3-1. Primary Objective

(1) To investigate the correlation between membranous nectin-4 (mNectin-4) expression and treatment outcomes in patients with aUC receiving EV/EV-P.

3-2. Secondary Objectives

(1) To evaluate the association between ADAM10/17 expression and treatment outcomes of EV/EVP in aUC.

(2) To examine the relationship between sNectin-4 levels and treatment efficacy of EV/EVP in aUC patients.

3-3. Exploratory Objective

(1) To assess whether mNectin-4, ADAM10/17, and sNectin-4 can serve as predictive biomarkers for EV/EVP in aUC patients.

4. Study Design

4-1. **Study Type**: Observational

4-2. **Study Design**: Prospective cohort study

4-3. **Study Center**: Single-center (National Taiwan University Hospital, NTUH); may expand to multi-center in the future

4-4. **Enrollment Method**: Consecutive enrollment of eligible patients

4-5. **Study Period**: April 1, 2025 to December 31, 2029

4-6. **Estimated Sample Size**: 100 patients receiving EV/EVP, plus 100 patients treated with platinum-based chemotherapy as a comparator cohort

5. Study Population

5-1. Inclusion Criteria

(1) Histologically confirmed diagnosis of advanced aUC, including bladder cancer or upper tract urothelial carcinoma

(2) Age ≥ 20 years

(3) Received EV/EVP or first-line platinum-based chemotherapy at NTUH

(4) Availability of archival formalin-fixed paraffin-embedded (FFPE) tumor tissue for biomarker analysis

(5) Willingness to provide serum samples at predefined time points

(6) Ability to provide written informed consent

5-2. Exclusion Criteria

(1) Patients who have received prior investigational agents targeting nectin-4

(2) Inadequate tissue samples or insufficient serum collection

(3) Concurrent participation in another interventional clinical trial

(4) Any condition that, in the investigator's judgment, would compromise the patient's safety or compliance with study procedures

5-3. Sampling Method

(1) Consecutive enrollment of eligible patients meeting the inclusion criteria

5-4. Estimated Sample Size

(1) 100 patients treated with EV/EVP

(2) 100 patients treated with first-line platinum-based chemotherapy (for comparative biomarker analysis)

6. Study Groups/Cohorts

6-1. Group 1: EV Therapy Cohort

(1) Population: Patients with aUC receiving EV/EVP at NTUH

(2) Treatment: Standard EV-based regimen (monotherapy or in combination with pembrolizumab)

(3) Biospecimen Collection:

- Archival tumor tissue for immunohistochemistry (IHC) analysis of mNectin-4 and ADAM10/17 expression
- Serum samples collected at predefined time points for sNectin-4 quantification via ELISA

(4) Clinical Data Collection: Demographics, disease characteristics, treatment details, response assessments (RECIST), progression-free survival, overall survival, and adverse events

6-2. Group 2: Chemotherapy Cohort (Comparator Cohort)

(1) Population: Patients with aUC who received first-line platinum-based chemotherapy at NTUH during the same enrollment period

(2) Treatment: Standard first-line cisplatin- or carboplatin-based chemotherapy

(3) Biospecimen Collection:

- Archival tumor tissue for IHC analysis of mNectin-4 and ADAM10/17
- Serum samples for sNectin-4 quantification

(4) Clinical Data Collection: As per EV therapy cohort, for comparative analysis

7. Endpoints / Outcome Measures

7-1. Primary Outcome Measure

(1) Objective Response Rate (ORR): Proportion of patients achieving complete or partial tumor response according to RECIST 1.1 criteria after enfortumab vedotin treatment. Time Frame: Up to 12 months after treatment initiation

7-2. Secondary Outcome Measure

(1) Progression-Free Survival (PFS): Time from treatment start to disease progression or death from any cause. Time Frame: Up to 24 months

(2) Overall Survival (OS): Time from treatment initiation to death from any cause. Time Frame: Up to 36 months

(3) Treatment-Related Adverse Events: Incidence and severity of adverse events graded by CTCAE v5.0

during treatment. Time Frame: Throughout treatment duration

(4) Correlation of Biomarker Expression with Treatment Outcomes: Association between mNectin-4, ADAM10/17 expression, serum sNectin-4 levels, and clinical outcomes. Time Frame: Assessed at baseline and during follow-up

8. Methods / Assessments

8-1. Sample Collection

(1) Tumor Tissue:

- Archival formalin-fixed paraffin-embedded (FFPE) tumor specimens will be retrieved from diagnostic pathology samples.
- Tumor samples will be analyzed for mNectin-4 and ADAM10/17 expression by IHC.

(2) Serum Samples:

- Blood samples will be collected at predefined time points: before the initiation of systemic therapies/at first radiographic response assessment (approximately week 6–8)/t disease progression or end of treatment
- Serum will be isolated and stored at –80°C for subsequent analysis of sNectin-4 by ELISA.

8-2. Biomarker Analysis

(1) IHC:

- FFPE tissue sections will be stained for nectin-4 and ADAM10/17 using validated antibodies.
- Expression will be semi-quantitatively scored based on H-score and/or staining intensity and proportion.

(2) Enzyme-Linked Immunosorbent Assay (ELISA):

- Serum sNectin-4 levels will be quantified using a commercially available ELISA kit following manufacturer’s instructions.

8-3. Statistical Analysis

(1) Descriptive statistics will be used to summarize patient characteristics and biomarker distributions.

(2) Group comparisons:

- Mann–Whitney U test or t-test for continuous variables
- Chi-square or Fisher's exact test for categorical variables

(3) Survival analysis:

- Kaplan–Meier method for estimating PFS and OS
- Log-rank test for between-group comparisons

(4) Multivariable analysis:

- Cox proportional hazards model to assess the independent association of biomarkers with survival outcomes
- Logistic regression for associations with objective response

9. Ethics / Regulatory Compliance

9-1. The study protocol has been reviewed and approved by the Research Ethics Committee E of National Taiwan University Hospital.

IRB Approval Number: 202502065RINE

Approval Date: 2025-03-19

9-2. This study will be conducted in accordance with the principles of the Declaration of Helsinki, ICH Good Clinical Practice (GCP) guidelines, and applicable local regulatory requirements.

9-3. Informed consent will be obtained from all participants before enrollment. The consent process will include information on study purpose, procedures, risks, benefits, data confidentiality, and the

voluntary nature of participation.

9-4. Confidentiality and data protection:

- All personal identifying information will be coded and stored securely in password-protected databases.
- Only authorized study personnel will have access to identifiable data.
- Samples will be labeled with de-identified study codes.
- Data will be reported in aggregate to prevent the identification of individual participants.

10. Study Timeline

10-1. Planned Study Start Date: April 1, 2025

10-2. **Planned Study End Date (Final Data Collection):** December 31, 2029

10-3. **Primary Completion Date:** September 30, 2029

10-4. **Study Completion Date:** December 31, 2029