

Rationale: This project aims to develop a classifier using bulk RNA sequencing data to stratify low-grade, stage Ta non-muscle-invasive bladder cancer patients by recurrence risk. It attempts to build on the European Association of Urology (EAU) risk classification system¹ by subdividing the intermediate risk group into low and high genomic risk subsets. A binary classification model predicting recurrence within two years of diagnosis and resection was used, offering greater interpretability and clinical actionability compared with continuous risk scores produced by Cox proportional hazards models.

Data Description: Data from the Knowles² and UROMOL³ cohorts were used in this project. The UROMOL cohort, which is annotated with EAU risk scores, was used for model training and internal validation. Out-of-fold (OOF) predictions from the classifier were used to subdivide patients labeled as intermediate risk by EAU into two groups, enabling downstream analyses. The Knowles cohort lacks EAU risk labels and the demographic information required to derive them; therefore, it was used exclusively for external validation of the binary classifier.

Preprocessing: To ensure comparability between cohorts, only the 19,087 genes common to the expression datasets of the UROMOL and Knowles cohorts were retained for model training and evaluation. To accelerate training, the 1,000 genes with the highest variance in the UROMOL training set were selected. Patients with unknown recurrence or less than two years of follow-up without observed recurrence were excluded from both cohorts, as recurrence status at two years could not be reliably determined for these individuals. Three missing ages were imputed using median values. Prior to model training, all features were centered and scaled.

Classifier Development: Model training was performed using 5-fold cross-validation within the UROMOL cohort. RNA expression data, along with selected demographic and clinical variables (age, sex, concomitant carcinoma in situ, and receipt of BCG treatment), were used as input. A random forest method was used based on superior performance in preliminary testing against elastic net and gradient boosting. Model performance was evaluated using the area under the receiver operating characteristic curve (AUC).

Results: Classifier performance was modest, with AUCs around 0.6 (Figure 1). The Knowles cohort showed slightly higher AUCs than the UROMOL cohort, but this improvement did not correspond to clearer separation of the Kaplan-Meier (KM) survival curves (Figure 2). By contrast, OOF predictions within the UROMOL cohort produced KM curves with good separation and statistical significance (Figure 2). EAU risk groups on their own showed clear and statistically significant KM separation, highlighting the strength of the established clinical risk stratification (Figure 3). Notably, the UROMOL OOF prediction labels effectively subdivided the intermediate EAU risk group into two distinct genetic risk subgroups (Figure 3).

Discussion: Overall, these results are encouraging, as the classifier was able to further stratify EAU risk groups, potentially supporting more informed clinical decision-making. Intermediate risk patients classified as genetically high risk may benefit from closer monitoring or more aggressive therapy than those classified as low risk. The binary classification approach provides a clear distinction between high- and low-genomic-risk groups, facilitating interpretation. However, the moderate performance on the Knowles cohort indicates a lack of generalizability that would need to be addressed before this model could be applied clinically.

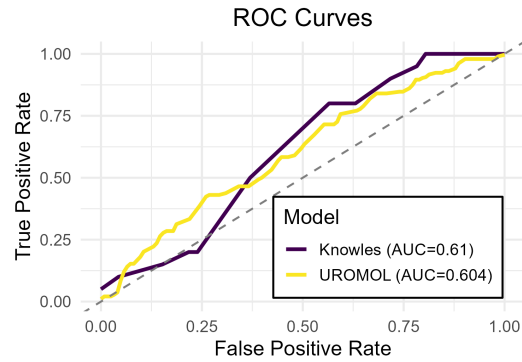


Figure 1: Receiver operating characteristic curves for the binary classifier model using UROMOL out-of-fold predictions and external validation in the Knowles cohort.

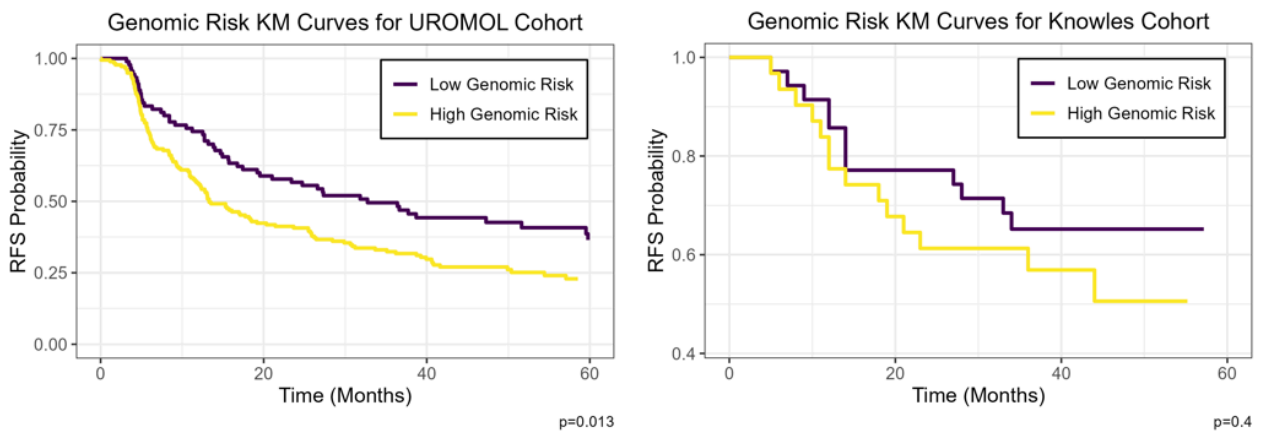


Figure 2: Kaplan–Meier recurrence-free survival curves by genomic risk for UROMOL out-of-fold predictions (left) and Knowles validation (right). High genomic risk means recurrence is predicted within 2 years. P values calculated with the log-rank test.

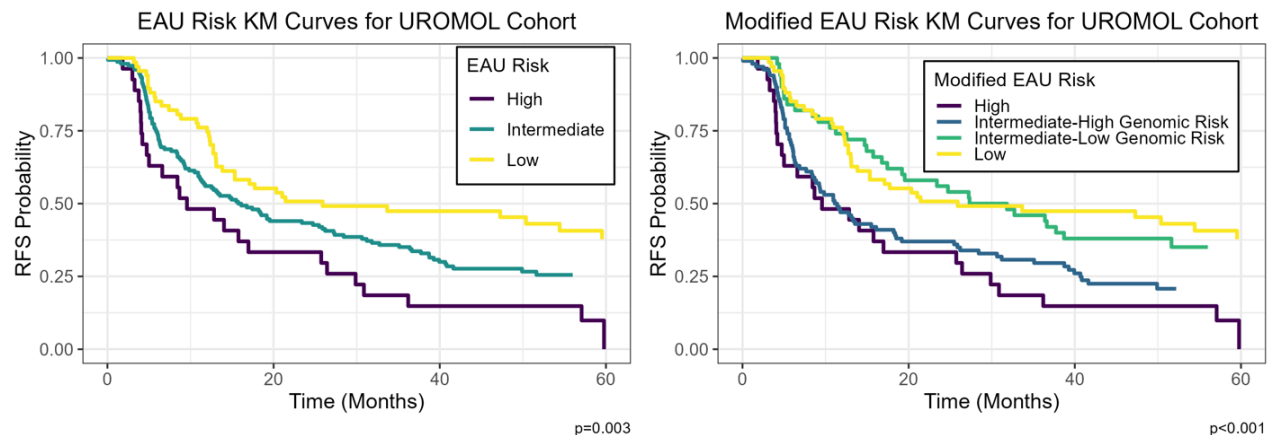


Figure 3: Kaplan–Meier recurrence-free survival curves by EAU Risk (left) and combined EAU risk and out-of-fold genomic risk predictions (right) for the UROMOL cohort. High genomic risk means recurrence is predicted within 2 years. P values calculated with the log-rank test.

References

1. Babjuk, M. *et al.* European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) - 2019 Update. *Eur Urol* **76**, 639–657 (2019).
2. Hurst, C. D. *et al.* Stage-stratified molecular profiling of non-muscle-invasive bladder cancer enhances biological, clinical, and therapeutic insight. *Cell Rep Med* **2**, 100472 (2021).
3. Lindskrog, S. V. *et al.* An integrated multi-omics analysis identifies prognostic molecular subtypes of non-muscle-invasive bladder cancer. *Nat Commun* **12**, 2301 (2021).