Ki67 and FOXC1-based complementary diagnostic for neoadjuvant cisplatin-based chemotherapy predicts response and improved survival in muscle-invasive bladder cancer

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**Introduction & Objectives:** Neoadjuvant Cisplatin-based chemotherapy (NACCT) regimens are widely used in the treatment of muscle-invasive bladder cancer (MIBC). However, suitable complementary diagnostics to help guide and tailor treatment recommendations are still lacking. Ki67 is a well accepted and routinely used marker that tracks proliferation and has been shown to predict efficacy of neoadjuvant chemotherapy. Forkhead Box C1 (FOXC1), a transcriptional driver of cell plasticity/partial EMT/metastasis/immune evasion has proven prognostic value, but remains of uncertain predictive value. We sought to evaluate the potential of a Ki67 and FOXC1-based response predictor as a possible complementary diagnostic for NACCT regimens in patients diagnosed with primary MIBC.

**Materials & Methods:** 149 Pre-treatment tumor biopsy MKI67 and FOXC1 mRNA expression values were retrospectively obtained from MIBC patients who had received NACCT prior to surgical resection of their tumors, and correlated with the rate of observed partial/complete pathologic response (PR), or persistent residual disease (RD). The area under the curve (AUC) of each model was calculated and used to determine suitable cutoff values to maximize Negative Predictive Value (NPV) and Sensitivity for PR prediction. Differences in recurrence-free survival (RFS), cause-specific survival (CSS) and overall survival (OS) outcomes were also assessed between the predicted PR and predicted RD groups.

**Results:** The observed PR rate across all 149 patients was 41.22%. A cutpoint-based combined MKI67+FOXC1 predictive biomarker strategy displayed superior response discrimination between the predicted PR group and the predicted RD group (61.9% vs 14.1%, $p<0.0001$). The predictive strategy had an AUC of 0.85, an NPV of 85.9% and sensitivity of 85.2%, with an Odds Ratio of 9.931 (4.33-22.80, 95%CI), $p<0.0001$. The predicted PR group displayed statistically significant improvements in RFS ($p=0.008$), CSS ($p=0.001$) and OS ($p=0.0004$) compared to the predicted RD group. Multiple logistic regression PR-predictive models may further improve predictive accuracy.

**Conclusions:** Complementary diagnostic role of pre-NACCT MKI67 + FOXC1 expression merits further validation in MIBC patients treated with NACCT regimens and is currently underway. Adoption of such a complementary diagnostic approach in the clinic may help to not only optimize achieved PR rates but also significantly extend RFS, CSS and OS in patients diagnosed with MIBC.