

## **Bladimirplus: a precise, multimodal, urine-based tool for non-invasive prediction of immunotherapy response in bladder cancer**

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\*CIEMAT-I+12

### **Abstract**

Introduction:

Non-muscle-invasive bladder cancer (NMIBC) presents challenges due to the varied responses to treatments. The most effective treatment approach is intravesical immunotherapy with BCG (Bacillus Calmette-Guérin), which induces inflammation and an anti-tumor response in patients who respond. However, predicting which patients will benefit is difficult, with around 50% showing inflammation without clinical improvement. Liquid biopsies, especially urine-based methods, offer a promising, non-invasive alternative for personalizing NMIBC treatment. Previously, we demonstrated that miRNAs serve as effective biomarkers for bladder cancer using the BlaDimiR system. We now introduce BlaDimiRplus, an advanced urine-based strategy for predicting response to BCG immunotherapy, either alone or combined with anti-PD-L1 therapy.

Objective:

To create a straightforward method using miRNAs and cytokines to predict the immunotherapy response in NMIBC patients.

Methods:

We identified differentially expressed (DE) miRNAs in primary tumors from responder (R) and non-responder (NR) patients using the nCounter Human v3 miRNA Nanostring panel, and analyzed cytokines in urine samples with LEGENDplex. These findings were validated in a proof-of-concept cohort using RT-qPCR for miRNAs and ELISA for cytokines. We compared samples taken before transurethral resection and before the initial BCG instillation. A multifactorial analysis helped identify the most effective biomarker combination for validation.

Results:

Of the 26 DE miRNAs identified, those with the highest AUC and significant p-values were analyzed in urine samples. We validated individual ratios that distinguished between R and NR patients in pre-treatment samples for BCG alone (ROC AUC = 0.82-0.91) and in combination with anti-PD-L1 (ROC AUC = 0.67). In 57 urine samples, we identified four cytokines that predicted response to BCG, with CXCL10 validated in a second cohort of 74 samples (ROC AUC = 0.74). A multifactorial analysis yielded a ROC AUC = 1 for a combination of six miRNAs and two cytokines.

Conclusions:

BlaDimiRplus is an accurate urine-based tool for predicting immunotherapy response in bladder cancer patients, addressing a critical clinical need. This multimodal approach demonstrates that deregulation of miRNAs and cytokines in urine can differentiate responders from non-responders before treatment.

**Do you have any conflicts of interest?**

No, I do not have a conflict of interest.