# Differential Pathway Analyses of BCG Treated Bladder Cancer Using Philips OncoSignal: A Pilot Study

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## BACKGROUND

- Intravesical Bacillus Calmette-Guérin (BCG) is the recommended treatment option in high-risk non-muscle invasive bladder cancer patients (NMIBC). In ~40% of patients treatment fails, leading to an increased risk of disease progression and death. In treatment failures, surgical removal of the bladder with urinary diversion is advised.
- Based on transcriptomic data, we previously identified three molecular subtypes with divergent response to BCG treatment (Erasmus BCG subtypes). The BCG3 subtype had a high risk of progression and was characterized by EMT/basal-like and immune-suppressive features (such as MAPK and JAK-STAT enrichment).
- Because molecular subtyping using RNA-seq is difficult to implement in the clinic, we used Philips OncoSignal to quantify the activity of eight signal-transduction pathways (including MAPK and JAK-STAT), using whole-transcriptome data as input.
- Our aim was to determine if OncoSignal predicted the BCG3 subtype. Eventually, this might be used for early stratification of high risk NMIBC patients.

## METHODS

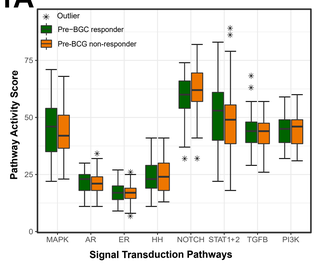
- Philips OncoSignal analysis was performed on whole-transcriptome data from N=64 pre-BCG T1HG NMIBC patients with an ongoing complete (BCG-responders) vs N=68 tumors from pre-BCG T1HG NMIBC patients, with paired N=44 post-BCG recurrences that failed BCG (BCG non-responders).
- The Erasmus BCG subtypes were previously identified. Clinical and molecular subtyping results were combined with Philips OncoSignal pathway activity score to determine differences between clinical outcome (BCG responders and BCG non-responders) and between molecular subtypes.

## HIGHLIGHTS

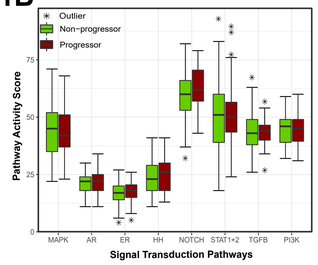
- Oncosignal was successfully executed in 104/132 pre-BCG samples (79%) and 31/44 (71%) of the post-BCG samples. Main reason for test failure was a too low read count.
- OncoSignal data analysis showed no differential activity between BCG responders and BCG non-responders, nor between patients with or without disease progression (1A-B).
- OncoSignal data analysis showed that the poor prognosis BCG3 subtype was associated with the highest MAPK and STAT1/2 activity (2A-C)
- MAPK and STAT1/2 pathways activity are also increased in matched post-BCG tumors vs pre-BCG tumors from BCG failures (3A-B).

## RESULTS

1A



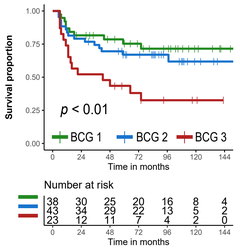

1B



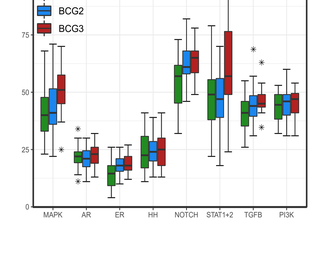

**(1)** Boxplots depicting Oncosignal signal transduction pathway activity between BCG responders and BCG non-responders **(A)** and between patients with and without disease progression **(B).**

Median follow-up for BCG responders was 95 months (IQR 66-119); median follow-up for BCG non-responders was 58 months (IQR 25-77). Within the non-responders to BCG, 41/68 patients had progressive disease, with a median time to progession of 13 months (IQR 8-39).

2A



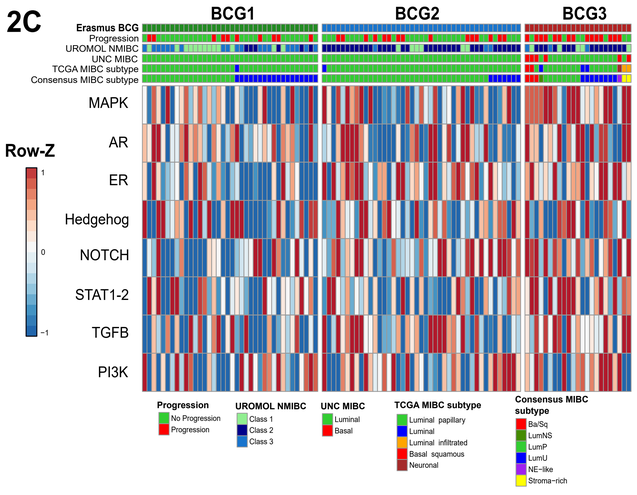

2B




**(2A)** Kaplan-Meier estimates indicating progression-free survival according to the Erasmus BCG subtypes and for whom OncoSignal Pathway analyses are available.

**(2B)** Boxplots depicting differences in OncoSignal pathway activity, grouped according to the Erasmus BCG subtypes. BCG3 tumors had increased pathway activity for MAPK and STAT1-2 as compared to BCG1-2 ($p<0.05$) ER and NOTCH pathway activity was lower in BCG1 patients as compared to BCG2-3 patients ($p<0.05$).

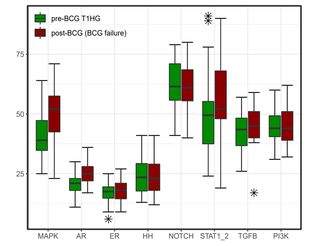
2C




**(2C)** Heatmap of OncoSignal pathway transduction activity grouped according to the Erasmus BCG subtypes. Annotations per sample from top to bottom: Erasmus BCG subtype, UNC MIBC classification, TCGA MIBC classification and Consensus MIBC classification.

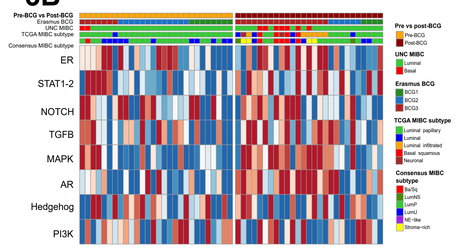
**Abbreviations:** BCG = Bacillus Calmette-Guérin; (N)MIBC = (non-)muscle invasive bladder cancer; TGCA = The Cancer Genome Atlas; UNC = University of North Carolina.

3A



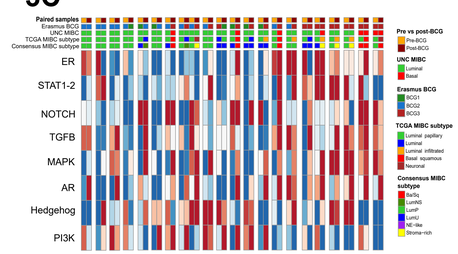

**(3A)** Boxplots depicting Oncosignal signal transduction pathway activity comparing NMIBC patients pre-BCG treatment and post-BCG treatment. All samples are from paired analyses containing only patients with matching pre- vs post-BCG samples.

3B




**(3B)** Heatmap of OncoSignal signal transduction pathway activity pre-BCG treatment and post-BCG treatment. Note the increase in MAPK and STAT1-2 and AR. Furthermore, most pre-BCG tumor samples with the BCG1/BCG2 subtype have switched to the agressive BCG3 subtype post-BCG.

3C




**(3B)** Heatmap of OncoSignal signal transduction pathway activity pre-BCG treatment and post-BCG treatment. Heatmap only contains matching-pair patient samples and can be used to determine between and within patient heterogeniety. In most patients, activation of the MAPK, STAT1-2 and AR pathways is observed, while other signal transduction pathways were less affected.

## CONCLUSION

**Philips OncoSignal confirms increased activity of MAPK and STAT1-2 signaling in pre-treatment BCG3 tumors and in tumor recurrences. As a next step, the OncoSignal qPCR based assay will be used on tumor RNA of the same individuals, instead of performing whole-transcriptome data analysis on archived material. The qPCR based test allows for standard lab routine and turnaround time, thereby facilitating future use in a clinical setting.**

*OncoSignal Pathway activity analyses were provided by Philips*