

## IMMUNE-BASED BIOMARKER ACCURATELY PREDICTS RESPONSE TO IMIQUIMOD IMMUNOTHERAPY IN CERVICAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

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**Background** The complete response rate of cervical high-grade squamous intraepithelial lesion (cHSIL) patients to imiquimod immunotherapy is 60%. Consequently, many patients are exposed to unnecessary adverse effects of imiquimod. On the other hand, conventional surgical large loop excision therapy is associated with increased risk of premature births in subsequent pregnancies. An in-depth analysis of the cHSIL immune microenvironment was performed in order to identify and develop a predictive biomarker for response to imiquimod, to maximize therapy efficacy and avoid adverse effects in patients unlikely to respond.

**Methods** Biopsies of 35 cHSIL patients, before and 10 weeks on imiquimod treatment, were comprehensively analyzed by two multispectral seven-color immunofluorescence panels for T cell and myeloid cell composition in relation to treatment response. Based on these results a simplified immunohistochemical detection protocol was developed. Samples were scanned with the Vectra multispectral imaging system, and cells were automatically identified using machine learning.

**Results** The immune microenvironment of complete responders (CR) prior to imiquimod is characterized by a strong and coordinated infiltration by T helper cells (activated PD1<sup>+</sup>/type 1 Tbet<sup>+</sup>), M1-like macrophages (CD68<sup>+</sup>CD163<sup>-</sup>) and dendritic cells (CD11c<sup>+</sup>). The lesions of non-responders (NR) displayed a high infiltration of CD3<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells. At 10 weeks on imiquimod treatment, a strong influx of intraepithelial and stromal CD4<sup>+</sup> T cells was observed in CR but not NR patients. A steep decrease in macrophages occurred both in CR and NR patients, leveling the pre-existing differences in myeloid cell composition between the two groups. Based on the pre-existing immune composition differences, the sum of intraepithelial CD4 T cell, macrophage and dendritic cell counts was used to develop a quantitative simplified one color immunohistochemical biomarker, the CHSIL Immune Biomarker for Imiquimod (CIBI), which can be automatically and unbiasedly quantified and has an excellent predictive capacity (ROC AUC 0.95, p<0.0001).

**Conclusions** The capacity of cHSIL patients to respond to imiquimod is associated with a pre-existing coordinated local immune process, fostering an imiquimod-mediated increase in local T cell infiltration. The CIBI immunohistochemical biomarker has strong potential to select cHSIL patients with a high likelihood to experience a complete response to imiquimod immunotherapy.

**Ethics Approval** Ethics approval was provided by all the participating hospitals: Maastricht University Medical Center, Erasmus University Medical Center Rotterdam and Catharina Hospital Eindhoven. Patients were included in this study after providing written informed consent. This study was conducted in accordance with the Declaration of Helsinki and in accordance with Dutch law.