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# Immune response gene expression in colorectal cancer carries distinct prognostic implications according to tissue, stage and site: a prospective retrospective translational study in the context of a hellenic cooperative oncology group randomised trial.

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## Abstract

### BACKGROUND:

Although host immune response is an emerging prognostic factor for colorectal cancer, there is no consensus on the optimal methodology, surrogate markers or tissue for study.

### PATIENTS AND METHODS:

Tumour blocks were prospectively collected from 344 patients with stage II/III colorectal cancer (CRC) treated with adjuvant chemotherapy. Whole section lymphocytic infiltration was studied along with mRNA expression of CD3Z, CD8, CD4, CXCL9, CXCL13, IGHM, FOXP3, SNAI2 and ESR1 by qRT-qPCR in tissue microarray (TMA) cores from the centre of tumour, invasive margin and adjacent normal mucosa.

### RESULTS:

Lymphocytic infiltration, deficient MMR (10.9%), KRAS (40.7%) and BRAF (4.9%) mutations or single mRNA gene expression were not prognostic. Tumour ESR1 gene expression (Hazard Ratio [HR] for relapse 2.33, 95% CI 1.35-4.02; HR for death 1.74, 95% CI 1.02-2.97) and absence of necrosis (HR for relapse 1.71, 95% CI 1.05-2.71; HR for death 1.98, 95% CI 1.14-3.43) were adverse prognostic features. We used CD3Z and CD8 expression in order to devise the mRNA-based Immune Score (mIS) and proceeded to partitioning analysis in 267 patients, with age, stage, tumour site (Right vs Left CRC), KRAS mutation and tumour mIS as input factors. Only in patients with stage III right-sided colon cancer, a low immune response was associated with inferior disease-free survival (mIS-low, HR for relapse 2.28, 95% CI 1.05-8.02). No prognostic significance was seen for tumour mIS in any other stage or site of CRC, or for a similar mIS score derived from adjacent normal mucosa. Independent adverse prognostic significance was retained in multivariable analysis for absence of necrosis, tumour ESR1 expression in all patients and low tumour mIS in stage III right-sided CRC.

### CONCLUSIONS:

In localised CRC, mRNA-based CD3Z/CD8 profiling of tumour immune response may have stage, site and tissue-specific prognostic significance, along with ESR1 expression.