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DNA-methylation derived counts of regulatory T cells and the risk of cancer and cardiovascular disease

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Results from laboratory based mechanistic studies and prognosis studies among patients suggest that alterations of immune tolerance may predispose to major chronic diseases. With regard to cancer, increased immune tolerance, i.e. an impaired immune reactivity against malignant cells, may facilitate carcinogenesis. In contrast, reduced immune tolerance has been implicated in cardiovascular disease development through its contribution to excessive immune responses towards atherosclerotic plaque antigens. Despite the general concept of dysregulated immune tolerance constituting a risk for both cancer and cardiovascular diseases, there is a lack of prospective studies, in which the relationship between immune tolerance in the pre-diagnostic situation and disease risk has been evaluated. Thus, associations between pre-diagnostic blood levels of tolerance-inducing T regulatory cells (Tregs) and the risks of common cancers as well as major cardiovascular diseases were evaluated for the first time in a prospective human study, the EPIC– Heidelberg cohort.

As immune cell profiling by flow cytometry is not feasible in large-scale epidemiological studies due to the lack of fresh blood samples, a novel epigenetic assay, developed by Epiontis Inc. (Berlin, Germany), was used to measure Treg cells in stored buffy coat samples. Tregs were quantified by quantitative real-time PCR-assisted cell counting making use of the Treg-specific and highly-preserved differential methylation of the Forkhead-Box-Protein P3 (FOXP3) gene. Similarly, total T cells were quantified based on differential methylation of the cluster of differentiation 3 (CD3) gene. The ratio of Treg to $CD3^+$ T cells ("ImmunoCRIT") was used as a marker of immune tolerance, with higher values indicating greater tolerance.

Multivariable Cox regression analyses of data from a case-cohort subset of the EPIC-Heidelberg Study showed that higher ImmunoCRIT values were associated with significantly increased risks of colorectal cancer (hazard ratio between the highest and the lowest tertile [95% confidence interval]: 1.59 [0.99, 2.54], $p_{trend} = 0.007$) and lung cancer (1.98 [1.06, 3.69],

p_{trend} = 0.026). The ImmunoCRIT was not significantly associated with the overall risks of breast and prostate cancer. However, there was significant heterogeneity in the association between ImmunoCRIT and breast cancer risk by estrogen receptor (ER) status of the tumors and subgroup analyses revealed a significant direct association with the risk of ER-negative tumors (HR_{T1-T3}[95% CI]:3.34 [1.52, 7.35, p_{trend} = 6 0.001), while the association with the risk of ER-positive tumors was not significant. With respect to cardiovascular disease risk, no significant associations between the ImmunoCRIT and the risks of myocardial infarction and ischemic stroke were observed. Notably, the ImmunoCRIT was not associated with most known risk factors such as BMI, C-reactive protein levels, blood lipids, physical activity, reproductive factors, diet, or alcohol intake. By contrast, the present analyses did point to increased immune tolerance among smokers, which is in line with previous experimental and clinical data. While associations between ImmunoCRIT and risks of colorectal, lung and ER-negative breast cancer remained statically significant after adjustment for smoking, future studies are needed to identify further determinants of immune tolerance such as infections, composition of the intestinal microbiome, or genetic factors.

Overall, the present findings suggest that increased peripheral immune tolerance may indeed predispose to certain malignancies, but not to myocardial infarction and ischemic stroke. At the same time, epigenetic cell counting was demonstrated to be an alternative method to evaluate immune cell composition in relation to chronic disease risks in large-scale epidemiological studies. Thus, two additional analyses were carried out, to prepare future extensions of the present thesis project. First, tumor infiltration of ER-positive breast tumors with both Treg and total T cells was shown to be significantly lower compared to ER-negtive tumors in a pilot study using DNA from paraffin-embedded formalin fixed tumor tissue indicating the potential of epigenetic-based immune cell counting for molecular pathology applications in the EPIC- Heidelberg Study and beyond. Second, a study on the biological reproducibility of a wider set of epigenetically determined immune markers in buffy coat samples over one year ($CD4^+$ T cells, $CD8^+$ T cells, monocytes, neutrophils, B-lymyphocytes, natural killer cells, and myeloid-derived suppressor cells in addition to Treg and total T cells) was conducted and revealed adequate temporal stability of the analyzed markers for use in large-scale epidemiological studies. Based on these promising results and the fact that Tregs modulate a broad spectrum of effector cell responses, a follow-up project, for which an expanded set of immune markers is analyzed in the EPIC-Heidelberg Study, was launched to investigate associations between more refined immune cell profiles and cancer risks.