Clinical Impact of Tumor-Associated Macrophage and T-Cell Contents in Diffuse Large B-Cell Lymphoma

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Background: Tumor infiltrating immune cells can modulate cancer progression and are attractive therapeutic targets. We have previously identified a tumor microenvironment (TME) immune cell signature in diffuse large B-cell lymphoma (DLBCL), which contains genes for cytolytic factors, immune checkpoint molecules, T-cells and macrophages, and separates the patients into immune cell high and low or T-cell inflamed and non-inflamed subgroups (Autio et al., 2020). As macrophages and T-cells are key components of the TME, our aim was to characterize their phenotypes and relationships in the tumor tissue and associate the findings to clinical outcome in patients with DLBCL.

Methods: We used multiplex immunohistochemistry, gene expression data, and unsupervised computational approaches to characterize tumor associated T-cell and macrophage (TAM) phenotypes in 178 samples from DLBCL patients. We correlated the immune cell constitution with clinical data, and validated the findings utilizing gene expression data from three independent DLBCL cohorts (Reddy et al., 2017, Schmitz et al., 2018, Chapuy et al., 2018) and *in silico* immunophenotyping with CIBERSORT.

Results: The proportions of TAMs and infiltrating T-cells varied significantly between the patients. Unsupervised hierarchical clustering divided the samples into T-cell inflamed and non-inflamed subgroups. The T-cell inflamed phenotype was rich in other immune cell subtypes such as TAMs, natural killer (NK) cells, and regulatory T-cells, thus characterizing an immune hot phenotype. Apart from B-symptoms being more common in the patients with T-cell inflamed phenotype, clinical characteristics were equally distributed between the subgroups, and no association with survival was observed. However, when we divided the T-cell inflamed group further into subgroups according to the TAM content, we identified a T-cell high/macrophage low group, which was characterized by clinically less aggressive disease and better survival as compared to the T-cell high/macrophage high or T-cell low groups. Using *in silico* deconvolution analyses, we validated the T-cell high/macrophage low subgroup, and its association with less aggressive disease and survival in an external dataset comprising 496 patients (Reddy et al., 2017). The prognostic impact of T-cell high/macrophage low subgroup on survival was independent of the IPI and molecular subtype. Differential gene expression analyses identified a gene signature corresponding to the T-cell high/macrophage low subgroup. This signature translated to better outcome, and was validated in two additional datasets (Schmitz et al. n=562, Chapuy et al. n=137). Finally, we studied the impact of immune checkpoint expressing TAMs on survival, and whether it differed between the T-cell inflamed and T-cell non-inflamed subgroups. In particular, a high proportion of PD-L1^{+}, TIM3^{+}, and PD-L1^{+}TIM3^{+}CD163^{-} macrophages associated with poor outcome, independent from the IPI and molecular subtype. Furthermore, the impact of these TAM subtypes on survival was evident only in the patients with T-cell inflamed phenotype.

Conclusions: Our data demonstrate that the proportions of different immune cell phenotypes in the DLBCL TME are clinically meaningful, and suggest that the high T-cell/macrophage ratio predicts favorable survival in patients with DLBCL.