

Characterisation of peripheral blood mononuclear cells (PBMC) in patients with combination ipilimumab and nivolumab therapy-related colitis.

Background: T-cell checkpoint inhibitors (TCI) are revolutionising the treatment of metastatic melanoma and other cancer but come at the cost of immune-related adverse events (irAE). Up to 44% of patients receiving combination ipilimumab and nivolumab therapy develop colitis (IN-C). While it is presumed irAE colitis is the result of activating autoimmune T-cells, the underlying pathophysiology of this entity has not been well characterised. We studied peripheral blood mononuclear cells (PBMC) from patients with metastatic melanoma who developed IN-C and compared these to those who received the same immunotherapy with no autoimmune side effects (IN-NAE), patients with active ulcerative colitis (UC) and healthy volunteers in a retrospective single-centre cross-sectional study. **Materials and Methods:** Cryopreserved PBMC from patients with IN-C (N=9) were studied at 4 timepoints: Baseline, early on-treatment but before colitis, at the time of colitis and post-colitis. IN-NAE (N=11) were studied at baseline and at Week 7-10 of treatment (a comparator to the IN-C “colitis” time-point). Patients with treatment-experienced UC (N=6) and healthy volunteer (N=17) were studied at single time-points. Thawed PBMC were stained with a near infra-red live/dead stain and fluorochrome-conjugated antibodies to CD45RA, CD3, CD19, IgA, CD27, CD38, CD56, CD4, CD8, CD25, CD127 α 4-integrin, β 7-integrin and HLA-DR. Cells were analysed on a five-laser LSRFortessaX20 flow cytometer (BD) and FloJo software. Differences between groups were assessed by non-parametric Mann-Whiney and Chi-square tests for continuous and categorical data, respectively (SPSS Software). **Results:** There was no significant difference between IN-C and IN-NAE groups in terms of age, sex, melanoma stage, presence of visceral metastases or serum lactate dehydrogenase level. IN-C was not associated with a change in the proportion of total, CD4+ or CD8+ T-cells. Treatment with combination ipilimumab and nivolumab was associated with a rise in activated memory gut-homing CD8+ T-cells in both IN-C and IN-NAE groups. IN-C differed from active UC in that it was not associated with a rise in circulating plasmablasts. Compared with healthy volunteers, patients with melanoma had a lower proportion of total T-cells and higher proportion of NK-cells at baseline, but these changes were not predictive of colitis. **Conclusions:** Treatment with combination ipilimumab and nivolumab therapy is associated with a rise in circulating activated memory gut-homing CD8+ T-cells, however this was independent of the development of colitis. Further work is needed to determine if these cells are pathogenic and/or the presence of host protective factors. IN-C was distinct from active UC in that it does not generate a significant plasmablast response, indicating pathogenic B-cells might play less of a role in IN-C. We are currently running a prospective trial on TCI-associated colitis, studying PBMC, gastrointestinal tissue biopsies and microbiome. We aim to confirm if changes seen in PBMC are mirrored in the gastrointestinal tract. Our ultimate goal is to define baseline risk factors and biomarkers that can be used clinically to reduce the morbidity of this condition.