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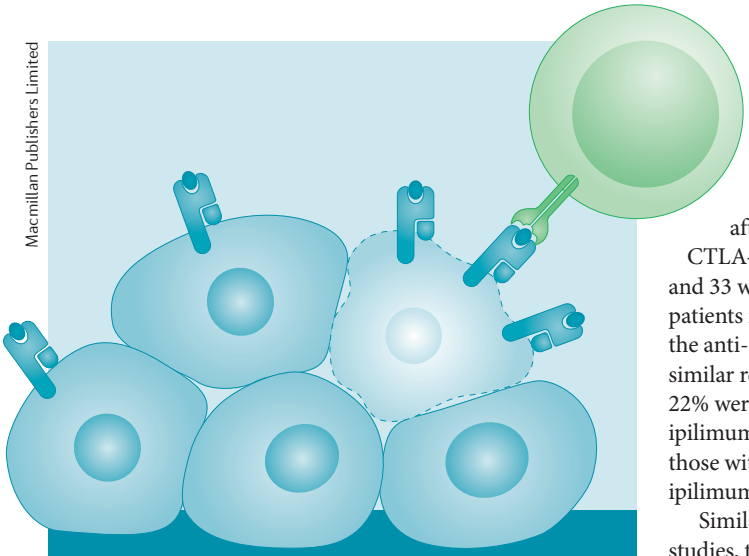

IMMUNOTHERAPY

Nivolumab-induced alterations revealed

Abundant evidence exists that both tumour development, and responses to treatment are dynamic processes. Despite this solid evidence, knowledge of tumour evolution in response to immune-checkpoint inhibition is currently limited. Now, data from a clinical trial involving patients with advanced-stage melanoma that combined analysis of patient outcomes with in-depth investigations of tumour characteristics provides insight into the response to immune-checkpoint inhibition, and thus, the mechanism of action of immune-checkpoint inhibitors.

Co-lead author Nils Weinhold explains: "This project is a formal clinical trial with a robust biomarker component to address what is happening during anti-PD-1 treatment." explains: adding that: "we used genomic sequencing techniques to elucidate several aspects of patients' tumours as well as their immune system, which were believed to be involved in determining a response to this treatment." The investigators enrolled a total of 68 patients with advanced-stage melanoma, of whom 35 had disease progression after treatment with the anti-CTLA-4 antibody ipilimumab and 33 were ipilimumab-naive. All patients received treatment with the anti-PD-1 antibody nivolumab; similar response rates of 21% and 22% were observed in those with ipilimumab-naive disease and in those with disease progression on ipilimumab, respectively.

Similar to the findings of previous studies, the investigators observed a positive correlation of both total and clonal mutational load with overall survival outcomes. Changes in mutational load as a result of anti-PD-1 therapy were then investigated in a subset of paired pretreatment and on-treatment biopsy samples ($n = 41$). Examinations of the clonal compositions of these paired samples revealed a decrease in the frequencies of many single-nucleotide variants (SNVs), termed 'mutational contraction', in nivolumab-responsive tumours. Tumours from patients with stable disease had significantly greater levels of mutational contraction relative to those with progressive disease ($P < 0.01$) and a reduced frequency of novel emergent SNVs on treatment ($P < 0.02$) relative to tumours from patients with progressive disease.

Transcriptomic analyses revealed considerable levels of heterogeneity in gene expression. Notably, samples from patients who responded to nivolumab did have greater expression of a wide range of immune-related genes, reflecting both activation of immune cells and changes in the immune-cell population, such as increased numbers of $CD8^+$ T cells and NK cells, and decreased numbers of M1-like macrophages.

Investigations of the on-treatment T-cell repertoire revealed that the median fold change in number of unique CDR3 sequences (indicating T-cell diversity) was associated with clinical benefit in patients not previously exposed to ipilimumab ($P = 0.016$), but not in those previously treated with ipilimumab ($P = 0.49$). Furthermore, among responders, a linear relationship was observed between the number of expanded T-cell clones and the number of neoantigens, the numbers of which decreased to the point of becoming undetectable during therapy. Computational analyses confirmed that the expected number of neoantigens on therapy was lower than the expected number in all patients with either a complete or partial response to treatment, indicating that nivolumab-induced activation of antitumour immunity results in the elimination of tumour cells expressing specific antigens.

Co-lead author Tim Chan summarizes: "all aspects of the tumour environment and immune system that were profiled in this study are consistent with a mechanism of action involving an immune response induced by the genomic characteristics of tumours in responding patients." He adds that, "because this was a prospective study that was done rigorously, we were able to validate a number of biomarkers: we confirmed that mutational load and clonal mutational load can predict response. We were also able to identify other potential biomarkers that were not further validated, such as IFN gene loss."

These findings provide insight into the mechanism of action of anti-PD-1 antibodies, and might help to guide both the design of future trials and the selection of patients to receive immune-checkpoint inhibition.

Peter Sidaway

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