Acquired resistance mechanisms to immunotherapy

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Harnessing the immune system to act against malignant tumors has long been investigated. Recently, checkpoint blockade mechanisms were identified as possible targets to be used in the immunotherapy field.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) can be found in CD4+ and CD8+ T cells cell surface. It acts by connecting to co-stimulatory receptors B7-1 and B7-2, present on antigen presenting cells (APCs). CTLA-4 expression is upregulated by different mechanisms, namely interleukin 2 (IL-2), interferon (IFN) γ and T cell receptor activation. This leads to a physiologic negative feedback on CD4+ and CD8+ T cells activated by APCs (1).

Programmed cell death 1 (PD-1) is a transmembrane protein that can be expressed in NK cells, B cells and T cells. When connect to PD-ligand 1 (PD-L1) or PD-L2 it acts as an inhibitory molecule. PD-L1 is expressed in different tissues and cells, namely tumor and hematopoietic cells whereas PD-L2 is mainly expressed in hematopoietic cells. PD-1-PD-L1/2 interaction inhibits apoptosis, induces T cell conversion to regulatory T cells and T cell exhaustion (2,3). IL-2 and INF-γ can physiologically upregulate PD-1 and PD-L1/2, emphasizing and also explaining the inhibitory effect on T cells cytotoxic function.

Ipilimumab is an immune checkpoint inhibitor that targets CTLA-4, blocking the negative feedback on activated T cells. It was the first agent to be associated with an improvement in overall survival (OS) and long-term survival in a phase III clinical trial, which enrolled patients with metastatic melanoma (MM) (4,5).

Nivolumab and pembrolizumab are PD-1 antibodies, approved for the first-line treatment of patients with MM (mutant or wild-type BRAF tumors), or after pre-treatment with a BRAF inhibitor (mutant BRAF tumors). Nivolumab has been shown to be much more effective in comparison to conventional chemotherapies (6) and pembrolizumab proofed significantly higher activity as compared to ipilimumab (7). Consequently, PD-1 antibodies are currently the main therapeutic approach in first-line therapy of MM.

Combination immunotherapy with CTLA-4 and PD-1 inhibitors has also shown benefit in advanced melanoma patients. Data from phase II (8) and phase III (9) clinical trials, show that patients treated with ipilimumab plus nivolumab have higher objective response rates and prolonged progression-free survival as compared to the single substances.

Resistance mechanisms to both chemotherapy and targeted therapy have been described (10,11). Although different response patterns can be found in patients treated with immune checkpoint inhibitors—non-response, early or late response and relapse—the exact resistance mechanisms to immunotherapy are not yet known (12). The authors described the first known mechanism associated with late acquired resistance to PD-1 blockade in MM patients.

Patients included in this analysis were patients with MM that fulfilled three inclusion criteria, as follows: (I) patients were included in a clinical trial at the University of California, Los Angeles, were treated with pembrolizumab

monotherapy and had an objective tumor response while participating in the clinical trial; (II) had a late recurrence defined as "*in situ* recurrence or new lesion development, despite continuous dosing, after more than 6 months of tumor response"; (III) had available tumor biopsies before therapy and at the time of progression.

From the initial 78 patients that were treated with pembrolizumab, 42 had an objective tumor response, 15 had progressive disease and only 4 met all the previous mentioned inclusion criteria. The authors evaluated the tumor samples from the 4 enrolled patients and the genetic and transcriptional profiling analyses were done using whole-exome sequencing.

For 3 patients (patients 2, 3 and 4), tumor samples before therapy with pembrolizumab were available, whereas for one patient (patient 1) the available tumor biopsy was performed before therapy with BRAF inhibitor vemurafenib.

All patients had an initial objective response with late relapse. The mean time to relapse was almost two years (624 days).

Immunohistochemical staining and multiplexed immunofluorescence analysis was used to further characterize tumor cells and immune infiltrates. This analysis was performed before treatment, at the time of response and at the time of disease progression.

Before pembrolizumab treatment, samples from patients 1, 2 and 3 showed CD8+ T-cells infiltration at the invasive margin. The CD8+ T cells distribution overlapped with PD-L1 expression on surrounding macrophages and melanoma cells. At the time of response, an increase in intratumoral CD8+ T-cells infiltrates was seen in tumor samples from patients 2, 3 and 4. At time of progression, all samples were available and the analyses showed that, again, CD8+ T-cell infiltration and PD-L1 expression were stronger in the tumor margins.

Further analysis of the tumor tissue at the time of progression was performed. The aim was to identify genetic changes or the presence of mutations that could explain the late relapse in these patients.

Results showed that the baseline samples and the samples at time of progression were genetically very similar in patients 1 and 2. At the time of progression, new homozygous loss-of-function mutations were found in the kinases associated with the IFN-receptor pathway. In tumor sample from patient 1 the authors found a nonsense mutation in the gene encoding Janus kinase 1 (JAK1), whereas in the tumor sample from patient 2 a splice-site mutation in the gene encoding JAK2 was present. Both mutations were absent in the baseline sample.

Based on these results, cell lines were used to establish the functional effects of *JAK* mutations, particularly *JAK2* mutations. Baseline cell lines were able to generate responses to INF α, β and γ. In contrast, relapsing cell lines did not respond, highlighting the fact that INF-γ signaling pathway requires a non-mutated *JAK2*.

The authors further confirmed that in these cells with acquired *JAK2* mutation, INF-γ was no longer able to induce cell growth arrested. This configures a functional advantage to these tumor cells, potentially explaining their tumor progression.

In tumor sample from patient 3, a frame-shift deletion in exon 1 of the beta-2-microglobulin (*B2M*) was described. B2M is a component of the major histocompatibility complex class I (MHC-I). The absence of a functional B2M translates into dysfunctional MHC-I, required for CD8+ T-cell recognition. The authors highlight that loss of functional B2M was already described as an acquired resistance mechanism to immunotherapy.

In tumor samples from patient 4 none of the previous mutations were present. Moreover, although in this sample stroma and T cells expressed PD-L1, tumor cells did not. The authors hypothesized that in this case non-genetic mechanisms leading to altered expression of INF-inducible genes could be responsible for resistance development.

Based on the presented results, the authors suggest that there is homogeneity in tumors resistant to anti-PD-1 therapy, that *JAK* mutations acquisition is an early event on the resistance development process and that the induced resistance to INF-γ antiproliferative effects contributes to immune resistance and relapse.

Taking into account these data, we would like to highlight some aspects.

First: in fact, this is the first known description of a potential mechanism associated with acquired resistance to immune checkpoint blockade.

Second: matched tumor samples were available only for 4 patients, and it should be noted that only in 3 of these 4 patients a potential resistance mechanism was clearly identified. Moreover, *JAK1* mutation was identified in the tumor sample from the patient that was previously treated with a BRAF inhibitor. Although this mutation was not detected in the baseline tumor sample, we think that a possible relation between these results and the previous therapy cannot be excluded at this time, and further investigation is needed.

Third: the INF-γ signaling pathway seems to play an important role in immunotherapy resistance mechanism. In fact, the role of INF-γ and TNF-α in tumor cell growth control and cell senescence induction was already described in a pancreatic cancer mouse model (13). The disruption of INF-γ and TNF-α induced cellular senescence could partially explain late acquired resistance and relapse.

Fourth: the impaired cytotoxic activity of CD8+ T cells was also mentioned as potentially connected to immunotherapy resistance. Recently, CD8+ T cell exhaustion, CD8+ T cell deficiency and loss of antigen presentation were described in samples from melanoma that progressed after MAPK pathway inhibition, possibly leading to cross-resistance to anti-PD1/anti-PDL1 therapy (14). Could a similar mechanism explain the loss of cytotoxic activity of CD8+ T cell in patients treated with first line immunotherapy? The description of a B2M mutation in tumor sample from patient 3 seems to confirm the role of loss of antigen presentation function as a possible immunotherapy resistance mechanism.

Fifth: these potential resistant mechanisms were identified in patients with late recurrence, defined as "in situ recurrence or new lesion development, despite continuous dosing, after more than 6 months of tumor response". The optimal duration of immunotherapy is not yet established. This is particularly true for patients that had a complete response and no significant treatment toxicity. Sixty-one patients included in the KEYNOTE 001 trial stopped pembrolizumab treatment for observation after a complete response (15). With a median time off treatment of 10 months, in 97% of the patients complete response is still ongoing. Should we look for different acquired resistance mechanisms in these patients?

Finally, several groups are currently investigating combination of targeted and immunotherapy (16-19). The potential acquired resistance mechanisms described by Zaretsky *et al.* should be taken in consideration when knew therapeutic combinations are tested, particularly because cross-resistance is possible.

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Footnote

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