More than 85% of lung cancer cases are classified as non-small-cell lung cancer (NSCLC), with predicted 5-year survival of 16% (1). However, after many years of dismal prognosis we are now experiencing a revolution in lung cancer treatment. Apart from targeted therapies, the potential of immunotherapy has created great excitement in the oncology community and has ushered in a new era of optimism. Cancer immunotherapy encompasses different approaches designed either to boost or restore immune functions. Therapeutic cancer vaccines are designed to stimulate the immune system of a cancer patient to act against tumor antigens (2). Sipuleucel-T, a vaccine designed to stimulate an immune response to prostatic acid phosphatase (PAP), was the first cancer vaccine approved for treatment of metastatic prostate cancer (3). Tālimogene laherparepvec (T-VEC) is the first oncolytic virus therapy approved for inoperable metastatic melanoma. Unfortunately, other therapeutic cancer vaccines such as MAGE-A3 or tecemotide have not proved successful in the treatment of NSCLC (4,5).

Checkpoint blockade therapy releases the ‘brakes’ of the immune system and enhances the anti-tumor T-cell response (6) and in 2015 two such therapies, nivolumab (7,8) and pembrolizumab (9) were approved for the treatment of NSCLC.

The TIME trial is a phase 2b/3 randomized, double-blind, placebo-controlled study evaluating TG4010 in combination with chemotherapy as first-line treatment of advanced NSCLC patients. Two hundred and twenty two patients from 45 centers in France, Belgium, the UK, Italy, Spain, Hungary, Poland, Israel and the USA were included. All patients had MUC1 expression by
immunohistochemistry in at least 50% of the tumoral cells. The primary objective of the phase 2b part of the study was to analyze progression-free survival (PFS) and Quoix and colleagues demonstrated that adding TG4010 to first line chemotherapy improves PFS in comparison to placebo plus chemotherapy in advanced NSCLC patients (24). Overall, the TIME study demonstrated a significant benefit in PFS with the addition of TG4010 to first line chemotherapy. However, although the results were statistically significant, they were not notable, with an increase in PFS of only 5.9 months for patients in the TG4010 plus chemotherapy arm, compared to 5.1 months for the placebo plus chemotherapy arm. Interestingly, almost 50% of patients had unknown status of EGFR mutations at baseline. TG4010 was well tolerated and the trial is set to continue to phase 3.

Low baseline values of triple positive lymphocytes (TrPAL) for CD16, CD56 and CD69, corresponding to a phenotype of activated natural killer cells, were found to be predictive of TG4010 activity in combination with chemotherapy. In fact, the TrPAL test, developed as a companion diagnostic for TG4010, would be validated in the TIME trial. Validation was planned for if patients with baseline TrPAL values less or equal to the upper limit of normal (ULN) had a more than 95% probability of hazard ratio (HR) for PFS being less than 1. Also, and if patients with baseline TrPAL values greater than ULN had a more than 80% probability of HR for PFS of more than 1. The probability of HR being greater than 1 was 31.3%, and the primary endpoint was not met. From the clinical point of view, the most interesting results were in the 127 patients with non squamous histology and TrPAL values less than the third quartile (Q3). These patients had the highest benefit in terms of PFS and overall survival (OS) with the addition of TG4010 to chemotherapy (HR, 0.59, 95%CI 0.40–0.87; P=0.0033 and HR, 0.59 95%CI, 0.39–0.91; P=0.0072, respectively).

The pretreatment normal levels of activated natural killer cells (TrPAL) defined a subgroup of patients who derived significant benefit in several parameters including OS (20). But is there a reliable way to define the heterogeneous and plastic natural killer cells repertoire? There are two primary phenotypically defined subsets of natural killer cells. The majority has low-density expression of CD56 (CD56$^{\text{dim}}$) and high levels of Fc$\gamma$ receptor III (FcγRIII, CD16) (CD56$^{\text{dim}}$CD16$^+$), while others are CD56$^{\text{bright}}$CD16$^{\text{dim}}$ or CD56$^{\text{bright}}$CD16$^-$. CD56$^{\text{dim}}$ natural killer cells predominate in the blood and are more cytotoxic than the CD56$^{\text{bright}}$, which produce abundant cytokines following activation of monocytes but have low cytotoxicity (25). Therefore, natural killer cells should be treated as distinct CD56$^{\text{bright}}$ and CD56$^{\text{dim}}$ subsets, rather than as a homogenous population.

In a post-hoc exploratory analysis, programmed cell death 1 ligand 1 (PD-L1) expression was assessed by immunohistochemistry in pretreatment tumor specimens;
both in tumoral cells and the immune infiltrate (24). Quoix and colleagues did not find improvement in PFS in the TG4010 group compared with placebo according to PD-L1 expression in tumoral cells. However, there was significant improvement in PFS in the TG4010 group compared with placebo according to PD-L1 expression in the immune infiltrate. Those patients with low PD-L1 expression in their immune infiltrate derived greater benefit from TG4010 (24) but whether these results are relevant is a matter of debate (26,27).

Immunotherapy trials targeting tumor antigens have yet to meet expectations. To better define patients who can benefit from immunotherapy, other factors such as tumor-infiltrating immune cells and gene signatures are now being evaluated. Hong et al. described a transcriptional signature related to innate anti-PD-1 resistance (28). CD8+ T cells play a significant role in antitumor immunity in many types of cancers (29) and it would be reasonable to assume that good prognosis is associated with CD8+ T cell infiltrate into the tumor, while accumulations of regulatory T cells (Treg) or myeloid-derived suppressor cells (MDSC) correlate with worse outcome. Signal transducer and activator of transcription 3 (STAT3) has been found to promote an immunosuppressive tumor microenvironment (30) (Figure 1). The S100A8 peptide belongs to the transcriptional network of STAT3 and decreases tumor infiltration of CD8+ T-cells, promoting a tumor growth-enhancing immune microenvironment through activation of the toll like receptor 4 (TLR4)/MD2 pathway (31) (Figure 1). Inhibition of acetyl-CoA acetyltransferase (ACAT1), a cholesterol esterification enzyme, significantly enhances the function and proliferation of CD8+ T-cells (32). The immunoproteosome (formed by proteasome subunits B; PSMB) generates peptides that bind to human leukocyte antigen (HLA) molecules, facilitating CD8+ T cell response. Reduced immunoproteosome expression has been linked to NSCLC with mesenchymal phenotype and reduced repertoire of HLA-bound peptides (33). STAT3 acts as an antagonist for STAT1, a key regulator of immunoproteosome and antigen presenting machinery (Figure 1) (33). STAT3 activates DNA methyltransferase 1 (DNMT1) which methylates the promoter region of interferon regulatory factor 1 (IRF1), PSMB8 and PSMB9 and HLA molecules that are crucial components of the antigen-presenting mechanism (33) (Figure 1).

A major impediment to cancer vaccines is that tumors with inherent resistance to T cell-mediated immunotherapy may never respond to therapies that target tumor antigens. Therefore, new strategies combining a MUC1 vaccine with antagonists of tumor-induced immune suppression are warranted.

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Footnote
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