Review Article

Anti-tumor immune response in early stage non small cell lung cancer (NSCLC): implications for adjuvant therapy

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Abstract: The demonstration that systemic chemotherapy improves survival in patients who have had resection of early stage non-small cell lung cancer (NSCLC) represents a significant advance. The absolute benefit of adjuvant chemotherapy in this setting is small with an overall survival improvement of 5%. In addition, there are many patients who have contraindications to cisplatin-based adjuvant therapy. Adjuvant chemotherapy is intended to target systemic micrometastases that remain after primary resection. The observation that cancers can relapse months or years after initial surgery implies that the residual micrometastases exist in a latent or dormant state. The concept of tumor dormancy offers therapeutic potential through induction or maintenance of the dormant state in disseminated tumor cells or through eradication of these dormant cells.

Cancer dormancy is a complex process with multiple potential mechanisms. This review will focus on some of the evidence for immune related tumor dormancy and the potential for immune therapies to improve outcomes in the adjuvant setting in NSCLC.

Key Words: Non-small cell lung cancer (NSCLC); anti-tumor immune response; immunotherapy; checkpoint inhibitors; gene signature (GS); immune dormancy

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Introduction

The demonstration that systemic chemotherapy improves survival in patients who have had resection of early stage non-small cell lung cancer (NSCLC) represents a significant advance in the treatment of this disease (1). The absolute benefit of adjuvant chemotherapy in this setting is small with an overall survival improvement of 5%. In addition, there are many patients who have contraindications to cisplatin-based adjuvant therapy. Hence, a non-cytotoxic therapy that could be combined with chemotherapy to improve survival or could be applied in patients not eligible for adjuvant chemotherapy would be in a welcomed advance.

Adjuvant chemotherapy is intended to target systemic micrometastases that remain after primary resection. The observation that cancers can relapse months or years after initial surgery implies that the residual micrometastases exist in a latent or dormant state. In addition, experimental models of dormancy have been developed that allow for investigation into different mechanisms of tumor dormancy (2). The concept of tumor dormancy offers therapeutic potential through induction or maintenance of the dormant state in disseminated tumor cells or through eradication of these dormant cells.

Cancer dormancy is a complex process with multiple potential mechanisms. More broadly, these can be categorized into cellular dormancy, resulting from tumor growth arrest of disseminated tumor cells, or tumor mass dormancy related to limitations in vascular supply or to an active host immune response (3).

This review will focus on the evidence for immune related tumor dormancy and the potential for immune

therapies to improve outcomes in the adjuvant setting in NSCLC.

Immune dormancy

It has been more than half a century since Burnet and Thomas proposed a formal hypothesis of cancer immune-surveillance wherein one of the primary functions of the immune system is to protect against cancers. Dunn *et al.* (4) expanded this into a broader proposal of immunoediting, which envisages not only elimination of cancer cells (immune-surveillance), but also a dynamic interaction between cancer cells and immune system, which leads to an equilibrium phase. This process selects for less immunogenic cancer cells until the selection eventually leads to cancer cell escape from immune control. The equilibrium phase could account for the period of immune dormancy while escape would signal clinical relapse. The extensive experimental data from those models and correlated clinical studies in human patients that support the concept of immune dormancy have been reviewed elsewhere (5-9).

One line of clinical evidence supporting an interaction between host immune system and human cancer cells are the numerous studies documenting infiltration of human malignancies by immune cells. While many reports focus on tumor infiltrating lymphocytes (TILs), effector cells of the innate immune system (macrophages, mast cells, dendritic cells and natural killer cells) have also been shown to be major constituents of tumor infiltrates (10).

Colorectal cancer is one of the most studied human cancers linking TILs and prognosis. Initial reports linking TILs to better prognosis date back more than thirty years (11). More recently, Galon *et al*. (12) found that, in colorectal cancer, the type, density, and location of TILs within the tumor were strongly linked to survival independent of tumor stage.

Tumor infiltrating immune cells and prognosis in NSCLC

A number of studies have investigated the link between tumor-associated immune response in the primary tumor and patient outcome after surgical resection in early stage NSCLC. While these studies have a number of limitations including small patient numbers, specificity of the markers of immune cells used, lack of correlation with micro-anatomical location and failure to assess functional status of immune cells, they do suggest a potential prognostic role for assessing tumor infiltrating immune cells after resection of early stage non small cell lung cancer.

In an early report of over 700 resected NSCLC specimens, Johnston *et al*. (13) found that non-specific evaluation of tumor infiltrating immune cells was not correlated with prognosis. However, the presence of T cells infiltrating among the cancer cells was associated with favorable prognosis. Wakabayashi (14) also pointed out the importance of assessing immune cell infiltration in various tumor compartments: within the tumor cell nests (epithelial), within the central stroma (stromal), and along the invasive margins.

Tumor associated macrophages (TAMs) constitute a major component of tumor infiltrating immune cells (10) and have the potential to promote tumor progression or support an immune response (15). A number of groups have reported that the number of tumor infiltrating macrophages could be associated with favorable survival in early stage NSCLC (16,17). In contrast, the largest reported trial (18) found no association between TAMs in either stromal or tumor epithelial compartments and survival in 335 resected NSCLCs. These contradictory results might possibly be due to failure to distinguish M2 macrophages, which are tumor angiogenesis promoting, and M1 macrophages which may exert a cytotoxic effect on cancer cells (15). Studies by Ohri (19) and Ma (20) found that macrophages expressing markers for M1 phenotype were associated with better prognosis in early stage NSCLC.

Dendritic cells are the most efficient antigen presenting cells for inducing an immune response to cancer. Al-Shibli *et al*. (18) measured immature dendritic cells in the tumor epithelial and stromal compartments from resected NCLC. Higher stromal DCs were associated with absence of nodal involvement and significantly better disease specific survival. Similar results were reported by Inoshima (21), who assessed 132 resected NSCLC specimens for dendritic cell infiltration in tumor nests. Higher DC counts were more common in stage I patients and an independent prognostic factor for overall survival. This study also looked at the correlation between VEGF expression and DC infiltration. In addition to its role in angiogenesis, VEGF has been shown to inhibit both the maturation and function of DCs (22). Tumors expressing high levels of VEGF had less DC infiltration and the combination of high VEGF expression and low DC infiltration resulted in a significantly lower 5-year survival than patients with low VEGF and high DC infiltration (14.5% versus 43.4%). These results

suggest a potential clinically important interaction between VEGF and an anti-tumor immune response and a role for anti-VEGF therapy in restoring DC function.

A favorable effect on prognosis in early stage NSCLC was seen in studies investigating infiltration of primary NSCLC by $CD8^+$ cytotoxic T lymphocytes (16,23,24). In a report of 335 consecutive stage I-IIIA NSCLC specimens, Al-Shibli *et al*. evaluated tissue microarrays for epithelial and stromal $CD4^+$, $CD8^+$ and $CD20^+$ lymphocytes. High density of both $CD4^+$ and $CD8^+$ lymphocytes in the stromal but not the epithelial compartment were associated independent predictors of disease specific survival.

Malignant tumors are composed not solely of the malignant cells but also stromal, endothelial, and immune/inflammatory cells that interact in complex ways. These interactions may lead to presence of immune cells that are immature or anergic. As a marker of a functional anti-tumor immune response Gottlin (25) looked at the relationship between presence of organized lymphoid structures, germinal centers (GC) and survival in early stage NSCLC. The GCs are organized loci containing mature dendritic cells and T cells adjacent to B cells and are an adaptive immune response. They assessed 91 early stage NSCLC specimens for the presence of GCs and found 32 (35%) to have GCs at tumor margins or tumor center. The presence of intratumoral but not marginal GCs was associated with earlier stage (Stage I) and in stage I patients, presence of intratumoral GCs was associated with better survival than no GCs. A separate study from France (26), looked at the presence of tertiary lymphoid structures, which they designated tumor-induced bronchus associated lymphoid tissues (Ti-BALT) in 74 patients with resected early stage NSCLC. The Ti-BALT is composed of mature dendritic cell/T-cell clusters adjacent to B-cell follicles. The used the density of mature DCs as a marker of Ti-BALT. The density of mature Dcs was significant predictor of overall, disease specific and disease free survival. They concluded that their data suggested that infiltration of tumor cells by mature DCs resulted in organization and proliferation of T and B-cells in Ti-BALT. They proposed a potential role of mature DC density as a prognostic factor for relapse in early stage NSCLC.

The studies cited provide substantial evidence for the existence an antitumor response in NSCLC. These studies suggest a potential role for correlating host immune response with survival in early stage NSCLC and a role in potentially selecting patients for adjuvant therapy. However, there is no convincing evidence that assessment of any component of the innate or adaptive immune response can reliably predict outcomes after surgical resection of NSCLC. The large tumor banks from randomized trials of adjuvant chemotherapy provide the potential to assess the prognostic ability of host immune response in early NSCLC in a large sample size taken in a multi-center setting and whether or not a local immune response to the primary tumor might predict for benefit of adjuvant chemotherapy.

Gene signature (GS) predicting response to immunotherapy

It is believed that a cancer phenotype associated with immune response does occur and may identify patients more likely to respond to immunotherapy. Ulloa-Montoya *et al*. (27) used tissue microarrays from patients with advanced melanoma treated with MAGE-A3 antigen specific cancer immunotherapy (ASCI) to identify a pre-treatment gene expression signature associated with clinical benefit. Clinical benefit was defined as objective response, stable disease for more than 4 months or mixed response with unequivocal tumor shrinkage. Genes selected from the microarray data were corroborated using quantitative polymerase chain reaction (qRT-PCR) and these. The GS identified not only identified patients with clinical benefit but also was predictive of better overall survival (29 versus 16.2 months) with MAGE-A3 ASCI treatment in patients whose tumors were GS positive versus those who were GS negative.

This GS was applied to resection specimens from patients enrolled in an independent randomized phase II trial of MAGE-A3 ASCI as adjuvant therapy in stage IB or II NSCLC. The GS was assessed from 157 of the 162 randomized patients. There were 61 patients who were GS positive. A positive GS was associated with a better disease free interval (DFI), the primary end-point of the trial (HR 0.42; 95% CI: 0.17-1.03; P=0.06). Although no benefit in terms of overall survival was seen for MAGE-A3 ASCI in the overall study population, in patients with GS positive tumors a strong trend in favor of benefit from MAGE-A3 ASCI was seen (HR 0.63; 95% CI: 0.22-1.78; P=0.38).

Analysis of the genes included in the GS positive tumors showed an over-representation of immune related genes. Genes involved included MHC class I and II, T cell markers regulated by interferon gamma (IFN-G), genes involved in antigen presentation and chemokines. The authors concluded that a specific tumor microenvironment favors the presence of immune effector cells in responding

patients. Of interest, the GS did not appear to be prognostic in placebo arm. This might reflect the small sample size or the selection of patients for the trial based on MAGE-A3 expression.

Prospective evaluation of this GS in randomized phase III trials in melanoma and NSCLC are planned. Confirmation of the predictive value of a GS for immune response would be valuable in patient selection for on-going trials and potential could be valuable in selecting patients likely to benefit from MAGE-A3 immunotherapy.

Checkpoint inhibitors

The demonstration of agents targeting immune check-points can result in tumor response in human solid tumors (28) and improve survival (29) has renewed interest in cancer immunotherapy. The fact that these agents have activity when used alone is support for an endogenous host immune response to cancer cells.

Under normal circumstances, immune checkpoint inhibitors are integral to maintaining cell tolerance and protecting normal tissue from damage during immune response (30). Counter-balancing stimulatory and inhibitory signals regulate T cell activation. The two most relevant immune checkpoint inhibitors are cytotoxic T-lymphocyte associated antigen 4 (CTLA4) and programmed cell death protein 1 (PD1).

CTLA4 is expressed exclusively on T cells and primarily acts to regulate the amplitude of early T cell activation (30). It is not expressed on the naive or memory T cells and expression is triggered only after antigen binds to T cell receptor (TCR). Hence, the clinical activity of the CTLA blocking antibodies implies that a host T cell response to tumor antigens exists but is suppressed by factors in the tumor microenvironment.

The major role for PD1 is to limit activity of T cells in peripheral tissue and to limit autoimmunity (30). The expression of PD1 is induced after T cell activation. PD1 must bind to one of its ligands, PD1 Ligand1 (PD L1 or B7-H1) or PD1 Ligand2 (PD L2 or B7-DC) in order to inhibit T cell activation signals. Unlike CTLA4, the expression of PDL1 is not limited to T cells. It is also expressed on B cells and natural killer cells.

The ligands for PD1 are commonly upregulated on tumor cells including NSCLC (31) and they have potential as biomarkers for response to PD1 ligand blockade. The expression of PD L1 on tumors may be a form of adoptive immune resistance and is further evidence of an endogenous host immune response and a potential mechanism of immune escape. Zhang *et al*. (32) compared PD1 expression on peripheral blood CTLs from healthy controls to those obtained from peripheral blood of 21 NSCLC patients undergoing surgical resection and to PD1 expression on TILs from resected specimens from 16 of these patients. The expression of PD1 was higher in peripheral blood CTLs from NSCLC patients than healthy controls and highest in the TILs from the surgical resection specimens. The PD1 expressing TILs showed less differentiated phenotype and were less capable of production of IFN G and IL-2 and of proliferation. Blocking antibodies to PDL1 but not PDL2 lead to increased cytokine production and T cell proliferation.

Immunosuppressive regulatory T cells (Treg) also highly express PD1 and early expression of PD1 can shift T cells from an activated state to one of anergy. Assessing TILs for expression of PD1 or its ligands may be important in studies evaluating TILs and the association with prognosis in NSCLC. Schneider *et al*. (33) assessed tumors from 12 patients undergoing potentially curative resection for early stage NSCLC for the expression of B7-H3, a member of the PDL1 ligand family, on DCs from tumor and normal lung distant from tumor. Expression of B7-H3 was significantly upregulated on DCs from tumor compared to healthy lung and these DCs were inferior at stimulating T cell proliferation. The ability to stimulate T cell proliferation could be restored by blocking antibodies for B7-H3.

The above evidence, although taken from small, single institution studies, suggests that the PD1/PDL1 pathway may play a role in immune escape in human NSCLC, even at an early stage. The potential to reverse the immunosuppression with blockade of PD1/PDL1 pathway provides rationale for studies of PD1/PDL1 blocking agents in the adjuvant setting.

Immunotherapy as adjuvant therapy in NSCLC

The concept of immunotherapy as an adjuvant treatment after resection of early NSCLC is not a new one. The Ludwig Lung Cancer Study Group investigated immune-stimulation with intrapleural Bacillus Calmette-Guérin (BCG) versus placebo as adjuvant therapy in early stage NSCLC more than 30 years ago (34). This non-specific immunotherapy which was administered once in early post-operative setting, resulted in an increased complications, mainly empyema, and inferior disease-free survival in the BCG group. Since that time, our understanding of cancer

immunology has increased tremendously (35) and has led to the development of more specific immunotherapies. Recent successes with specific immunotherapy strategies in castrate resistant prostate cancer (36) and melanoma (29) have renewed excitement in the potential immunotherapy to modify the clinical course of solid malignancies.

There are few cancer immunotherapies that have been assessed in the adjuvant setting in NSCLC. The two agents that have completed phase III clinical trials are both antigen specific (vaccine) strategies: tecemotide, previously known as L-BLP25, and MAGE-A3 ASCI.

Tecemotide is an antigen specific immunotherapy targeting the MUC1 glyco-peptide. A randomized phase IIB (37) trial with tecemotide versus observation showed a potential survival advantage in patients with stage III NSCLC. This led to the design of a global randomized placebo controlled phase III trial of tecemotide versus placebo in patients with stage III NSCLC after primary therapy with chemo-radiation (START). Hence, the tecemotide was given as adjuvant to the primary chemo-radiation. Patients with stage III NSCLC who achieved partial response or stable disease to the primary chemo-radiation were randomized to tecemotide or placebo until disease progression. Patients were stratified based on stage at initial presentation, response to primary chemo-radiation (stable disease versus partial response), region of the world, and mode of delivery of chemo-radiation (sequential versus concurrent). Despite challenges related to a clinical hold imposed on this START trial, more than 1,500 patients were randomized and 1,239 patients were included in the primary analysis (38). Tecemotide was well tolerated even when administered for prolonged periods. However, the primary end point of improvement in overall survival in the primary analysis population was not met. A preplanned subgroup analysis based on stratification variables did show, in the largest sub-group of patients treated with concurrent chemo-radiation (n=806), tecemotide adjuvant therapy resulted in a 10.2 months improvement in median survival (HR, 0.78; 95% CI: 0.64-0.95; P=0.016). Although this could not be considered a statistically significant result, the clinically significant difference in survival seen with tecemotide in a large sub-group of patients with stage III disease suggests a strong signal of efficacy.

MAGE-A3 gene is expressed in a number of cancers, including melanoma and NCLC. It is not expressed on normal tissues with the exception of testis and placenta and is considered a tumor specific antigen and ideal candidate for active immunotherapy. MAGE-A3 ASCI targets the MAGE-A3 tumor specific antigen. In a randomized phase II trial (39) in 182 patients with resected stage IB or II NSCLC, MAGE-A3 ASCI showed a strong trend for improved disease-free interval compared to observation. This trial was conducted prior to wide-spread of the use of adjuvant chemotherapy. An updated survival analysis of this phase II trial was recently published (40). Further follow up to 70 months continues to show a strong trend in favor of MAGE A3 ASCI in terms of DFI (HR 0.78; 95% CI: 0.49-1.24; P=0.248) although no difference was seen in overall survival (HR 0.99). A very large global double-blind placebo controlled phase III trial was initiated to test MAGE-A3 ASCI as adjuvant therapy in NSCLC. The MAGE-A3 as Adjuvant Non-Small Cell LunG CanceR Immuno Therapy (MAGRIT) trial is perhaps the largest adjuvant trial in NSCLC. More than 9,300 patients with stage I-IIIA NSCLC who had undergone surgical resection were screened for MAGE-A3 expression. Patients with tumors expressing MAGE-A3 were stratified based on whether they received adjuvant chemotherapy or not and then randomized to receive MAGE-A3 ASCI or placebo. The MAGRIT trial completed its target accrual of 2,270 patients in late 2011. The results of this trial are eagerly awaited.

Future directions

The limited success of adjuvant chemotherapy in early stage NSCLC is not surprising given the multiple mechanisms involved in tumor dormancy. Cytotoxic chemotherapy is likely to target those micro-metastases that are actively proliferating during the relatively brief time adjuvant therapy is administered. Although elimination of micro-metastases may be the optimal goal, maintaining them in a dormant state may prove an equally valuable strategy. The recent demonstration that very prolonged (10 years) adjuvant anti-estrogen therapy with tamoxifen is superior to shorter durations (5 years) would suggest that maintaining dormancy could be an effective adjuvant strategy (41). Future improvements in adjuvant therapy for NSLC will likely involve combination strategies that target different aspects of dormancy and can build on the gains made with adjuvant chemotherapy or be effective in patients for whom cytotoxic therapy is not an option.

The wide range of immunotherapies currently under investigation makes it difficult to generalize, but there are a number of features that that make immunotherapy particularly attractive in the adjuvant setting. Vaccine or

active specific immunotherapies are easy to administer, have a favorable toxicity profile and can thus be administered for prolonged periods. Immunotherapy has the potential to induce T cell memory and hence, the effect may persist long after the treatment is completed. Finally, contrary to what is popularly believed, immunotherapy may be synergistic when combined with chemotherapy (42) and targeted therapies (43).

The evidence currently available on the prognostic implications of tumor infiltrating immune cells is insufficient to support routine use. The tumor banks available for the large randomized adjuvant trials present an ideal opportunity to explore not only their prognostic value but also to help better understand the complex interactions between cancer and the immune system in early stage NSCLC. Such studies should focus not only on TILs and their location, but also the potential role of PD1/PDL1 in early stage NSCLC. This could help define whether a population suitable for a potential adjuvant trial with agents targeting this pathway might be identified. This is particularly relevant given the demonstration of clinical activity of checkpoint inhibitors such as anti-CTLA4 and anti-PD1/PDL1 anti-bodies. However, these are but a fraction of the receptors and ligands the have been identified as modulators of an anti-cancer immune response (30). It will be important to define which, if any, of these are relevant in a particular cancer.

The ultimate value of immunotherapies in the adjuvant setting will await demonstration of improved clinical outcomes in randomized trials. The recently completed START trial, while not meeting its primary end-point, shows a strong signal for improved survival with tecemotide as adjuvant therapy after concurrent chemo-radiation in stage III NSCLC. A confirmatory trial, focusing on patients completing concurrent chemo-radiation is being planned. The MAGRIT trial of MAGE-A3 ASCI in the adjuvant setting will define whether this immunotherapy can improve outcomes when given after adjuvant cytotoxic chemotherapy and/or in patients not suitable for adjuvant chemotherapy. This trial will also help define whether a GS might be used to predict patients likely to benefit from the MAGE-A3 ASCI.

Conclusions

The multitude of strategies used by tumors to circumvent immune recognition means that immunotherapy strategies aimed at enhancing one aspect of the immune response or overcoming one aspect of immune resistance are likely to meet with limited success. The availability of clinically active immunotherapies targeting different aspects of the immune response allow for the exploration of combinations of immune therapies. As an example, the activity seen with the checkpoint inhibitors is likely to be limited to patients in which a pre-existing anti-tumor immune response has occurred. By combining checkpoint inhibitors with other strategies such as ASCI, cytotoxic chemotherapy or certain targeted therapies that stimulate an anti-tumor immune response, their clinical activity might be enhanced. Although developing combination therapies presents many challenges, the opportunity to improve clinical outcomes is greatest with such strategies.

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References

1. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J Clin Oncol 2008;26:3552-9.
2. Akslen LA, Naumov GN. Tumor dormancy--from basic mechanisms to clinical practice. APMIS 2008;116:545-7.
3. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. Nat Rev Cancer 2007;7:834-46.
4. Dunn GP, Bruce AT, Ikeda H, et al. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991-8.
5. Teng MW, Swann JB, Koebel CM, et al. Immune-mediated dormancy: an equilibrium with cancer. J Leukoc Biol 2008;84:988-93.
6. Zitvogel L, Apetoh L, Ghiringhelli F, et al. The anticancer immune response: indispensable for therapeutic success? J Clin Invest 2008;118:1991-2001.
7. Bhardwaj N. Harnessing the immune system to treat cancer. J Clin Invest 2007;117:1130-6.
8. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest 2007;117:1137-46.
9. Quesnel B. Tumor dormancy and immunoescape. APMIS 2008;116:685-94.
10. Talmadge JE, Donkor M, Scholar E. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev 2007;26:373-400.
11. House AK, Watt AG. Survival and the immune response

in patients with carcinoma of the colorectum. Gut 1979;20:868-74.
12. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960-4.
13. Johnson SK, Kerr KM, Chapman AD, et al. Immune cell infiltrates and prognosis in primary carcinoma of the lung. Lung Cancer 2000;27:27-35.
14. Wakabayashi O, Yamazaki K, Oizumi S, et al. CD4+ T cells in cancer stroma, not CD8+ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. Cancer Sci 2003;94:1003-9.
15. Bremnes RM, Al-Shibli K, Donnem T, et al. The role of tumor-infiltrating immune cells and chronic inflammation at the tumor site on cancer development, progression, and prognosis: emphasis on non-small cell lung cancer. J Thorac Oncol 2011;6:824-33.
16. Dai F, Liu L, Che G, et al. The number and microlocalization of tumor-associated immune cells are associated with patient's survival time in non-small cell lung cancer. BMC Cancer 2010;10:220.
17. Welsh TJ, Green RH, Richardson D, et al. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J Clin Oncol 2005;23:8959-67.
18. Al-Shibli K, Al-Saad S, Donnem T, et al. The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma. Histopathology 2009;55:301-12.
19. Ohri CM, Shikotra A, Green RH, et al. Macrophages within NSCLC tumour islets are predominantly of a cytotoxic M1 phenotype associated with extended survival. Eur Respir J 2009;33:118-26.
20. Ma J, Liu L, Che G, et al. The M1 form of tumor-associated macrophages in non-small cell lung cancer is positively associated with survival time. BMC Cancer 2010;10:112.
21. Inoshima N, Nakanishi Y, Minami T, et al. The influence of dendritic cell infiltration and vascular endothelial growth factor expression on the prognosis of non-small cell lung cancer. Clin Cancer Res 2002;8:3480-6.
22. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2:1096-103.
23. Al-Shibli KI, Donnem T, Al-Saad S, et al. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 2008;14:5220-7.
24. Kawai O, Ishii G, Kubota K, et al. Predominant infiltration of macrophages and CD8(+) T Cells in cancer nests is a significant predictor of survival in stage IV nonsmall cell lung cancer. Cancer 2008;113:1387-95.
25. Gottlin EB, Bentley RC, Campa MJ, et al. The Association of Intratumoral Germinal Centers with early-stage non-small cell lung cancer. J Thorac Oncol 2011;6:1687-90.
26. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 2008;26:4410-7.
27. Ulloa-Montoya F, Louahed J, Dizier B, et al. Predictive gene signature in MAGE-A3 antigen-specific cancer immunotherapy. J Clin Oncol 2013;31:2388-95.
28. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.
29. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23.
30. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
31. Konishi J, Yamazaki K, Azuma M, et al. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res 2004;10:5094-100.
32. Zhang Y, Huang S, Gong D, et al. Programmed death-1 upregulation is correlated with dysfunction of tumor-infiltrating CD8+ T lymphocytes in human non-small cell lung cancer. Cell Mol Immunol 2010;7:389-95.
33. Schneider T, Hoffmann H, Dienemann H, et al. Non-small cell lung cancer induces an immunosuppressive phenotype of dendritic cells in tumor microenvironment by upregulating B7-H3. J Thorac Oncol 2011;6:1162-8.
34. Immunostimulation with intrapleural BCG as adjuvant therapy in resected non-small cell lung cancer. The Ludwig Lung Cancer Study Group (LLCSG). Cancer 1986;58:2411-6.
35. Finn OJ. Cancer immunology. N Engl J Med 2008;358:2704-15.
36. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010;363:411-22.
37. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol 2005;23:6674-81.

38. Butts CA, Socinski M, Mitchell P, et al. START: A phase III study of L-BLP25 cancer immunotherapy for unresectable stage III non-small cell lung cancer. J Clin Oncol 2013;31:abstr 7500.
39. Vansteenkiste J, Zielinski M, Linder A, et al. Final results of a multicenter, double-blind, placebo-controlled randomized phase II study to assess MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/II non-small cell lung cancer (NSCLC). J Clin Oncol 2007:25:abstr 7554.
40. Vansteenkiste J, Zielinski M, Linder A, et al. Adjuvant MAGE-A3 immunotherapy in resected non-small-cell lung cancer: phase II randomized study results. J Clin Oncol 2013;31:2396-403.
41. Gray RG, Rea D, Handley K, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. J Clin Oncol 2013;31:abstr 5.
42. Lake RA, Robinson BW. Immunotherapy and chemotherapy--a practical partnership. Nat Rev Cancer 2005;5:397-405.
43. Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237-51.

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