Published in final edited form as:

Lancet Oncol. 2012 July ; 13(7): e301–e310. doi:10.1016/S1470-2045(12)70126-2.

Immunotherapies for non-small-cell lung cancer and mesothelioma

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Abstract

Non-small-cell lung cancer and mesothelioma are thoracic malignancies, which in their advanced stages are incurable and have poor prognosis. Advances in our understanding of immune responses to tumours, tumour immunosuppression mechanisms, and tumour-specific shared antigens enabled successful early clinical trials of several specific and non-specific immuno therapies. For non-small-cell lung cancer, phase 3 clinical trial results of Toll-like receptor agonists show little promise. However, ongoing phase 3 trials are assessing melanoma-associated antigen A3 vaccine, liposomal BLP25, belagenpumatucel-L, and talactoferrin. In mesothelioma, immunotherapies being investigated include dendritic cell-based and *Listeria*-based vaccines, and allogeneic tumour cell and WT1 analogue peptide vaccines. Selection of appropriate patients and disease stages for immunotherapy, measurement of tumour-specific immune responses, and understanding the association between immune and clinical responses are some of the major challenges for the development of immunotherapies for these malignancies.

Introduction

Lung cancer is the most common cause of cancer-related death worldwide.^{1,2} Non-small-cell lung cancer (NSCLC) constitutes about 85% of lung cancers and 40% of patients with newly diagnosed NSCLC have advanced disease.¹ Malignant mesothelioma is an aggressive disease. Although rare, mesothelioma incidence is increasing worldwide and is associated with substantial economic burden.^{3,4} For both advanced NSCLC and mesothelioma, standard chemotherapy only marginally improves overall survival while causing substantial morbidity. Despite the addition of new therapies, median overall survival of patients in advanced stages of both malignancies is roughly 1 year. Only 3-5% of advanced NSCLC

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AT and RH searched the published work, and wrote and had final approval of the report.

Conflicts of interest

We declare that we have no conflicts of interest.

patients and less than 10% of advanced mesothelioma patients survive 5 years after diagnosis.^{1,5–7} New treatment options are clearly warranted for both these malignancies.

Although rarely curative, immunotherapy offers the promise of targeted therapy, which could prolong survival with few toxic effects. However, despite a strong preclinical rationale, clinical trials of immunotherapy agents for solid tumours have had little success.^{7,8} Improvements in overall survival in large, randomised phase 3 clinical trials, and the approvals of ipilimumab for melanoma and sipuleucel-T for prostate cancer have led to a renewed interest in immunotherapy of solid tumours.^{9,10}

Unlike melanoma, mesothelioma and NSCLC are non-immunogenic tumours. Tumour antigen-specific B-cell responses in patients with mesothelioma and spontaneous tumour-specific T lymphocytes in some cases of NSCLC suggest that both tumours are subject to immunosurveillance.^{11–16} Figure 1 summarises the putative steps involved in the generation of a cytotoxic T-lymphocyte response to tumour antigens and peptides. Evidence also suggests that microenvironments in these tumours might have immunosuppressive mechanisms to help evade immunosurveillance.^{17–22} Solid tumours, pleural effusions, and peripheral blood from patients with mesothelioma and NSCLC have increased functionally immunosuppressive T regulatory cells and cytokines, which could suppress an efficient host immune response.^{23–26} In this report, we review immunotherapies that are being investigated for NSCLC and mesothelioma.

Non-small cell lung cancer

Liposomal BLP25

Liposomal BLP25 is a peptide vaccine that targets the exposed core peptide of MUC-1, a membrane-associated glycoprotein overexpressed and aberrantly glycosylated in cancer cells.^{27,28} MUC-1 is associated with cell transformation, migration, immunosuppression, resistance to apoptosis induced by oxidative stress, and resistance to some genotoxic chemotherapeutic agents.^{29–33} Liposomal-BLP25 vaccine incorporates synthetic MUC-1 lipopeptide and monophosphoryl lipid A—an immunoadjuvant—in a liposomal delivery system.²⁸ In mice, liposomal BLP25 induces a cellular immune response characterised by antigen-specific T-cell proliferation and production of interferon- γ , indicating a T helper type 1 response.³⁴ In phase 1 studies, two different formulations of liposomal BLP25 were tolerated and elicited T-cell responses.^{35–37}

A phase 2B randomised trial included 171 stage IIIB or IV NSCLC patients with stable disease or an objective clinical response after first-line chemotherapy or chemoradiation. Patients were assigned to receive either liposomal BLP25 or best supportive care.³⁸ Patients received a single dose of cyclophosphamide (300 mg/m² to a maximum dose of 600 mg/m²) 3 days before the first dose of vaccine, followed by eight weekly vaccinations (1000 μ g subcutaneously). Maintenance vaccinations every 6 weeks starting from week 13 were administered at the investigator's discretion until disease progression. Although not significant, median overall survival (the primary objective) was increased by 4.2 months for patients who received liposomal BLP25 (n=88; 17.2 vs 13.0 months, hazard ratio [HR] 0.745, 95% CI 0.533–1.042). The greatest difference in survival occurred in the stage IIIB

locoregional subgroup (n=35, 40% of patients in the vaccine group), which had a 17.3 month improvement in median overall survival (30.6 vs 13.3 months, 0.548, 0.301–0.999) after median follow-up of 53 months.³⁹ Only 16 of the 78 assessable patients in the liposomal-BLP25 group developed a MUC-1-specific T-cell proliferative response. Common adverse events in the vaccine group were flu-like symptoms, minor injection site reactions, and nausea related to cyclophosphamide. In 16 patients who received prolonged courses of vaccine (2.0 to 7.7 years), adverse events decreased with increasing treatment duration and no long-term safety issues were identified.⁴⁰

Two similarly designed ongoing phase 3 trials (Stimulating Targeted Antigenic Responses To NSCLC [START], registered at [ClinicalTrials.gov](https://clinicaltrials.gov), number NCT01015443, and Stimuvax trial In Asian NSCLC Patients: Stimulating Immune Response [INSPIRE], [NCT00409188]) are assessing overall survival (primary endpoint) with liposomal BLP25 in patients with unresectable stage III NSCLC who have responded to or have stable disease after primary chemoradiotherapy (table 1). With a combined accrual goal of more than 1800 patients, the trials randomly assigned participants (2:1) to receive either liposomal BLP25 or placebo. Intravenous cyclophosphamide 300 mg/m² is administered 3 days before the first vaccination. Weekly subcutaneous vaccinations (930 µg) are administered for 8 consecutive weeks followed by maintenance vaccinations at intervals of 6 weeks, commencing at week 13, until disease progression (table 1).

Belagenpumatucel-L

Belagenpumatucel-L is an allogeneic tumour-cell vaccine cocktail, which incorporates four irradiated NSCLC cell lines (H460, H520, SKLU-1, and RH2) that have been modified with transforming growth factor β2 (TGF-β2) antisense plasmid. TGF-β is a member of a family of multifunctional proteins, which, through complex cell signalling pathways, regulate cell proliferation, differentiation, and angiogenesis.⁴¹ TGF-β helps some tumours escape host immunosurveillance by several mechanisms, including inhibition of cytotoxic T-cell activation and conversion of naïve T cells to T regulatory cells.⁴² In NSCLC, a strong inverse correlation exists between increased TGF-β concentration and prognosis.⁴³

The use of a cell vaccine cocktail is based on reports that NSCLC tumour cell lines share immunogenic epitopes with primary tumours. MHC class I restricted cytotoxic T lymphocytes generated against human lung adenocarcinoma cell lines have cytotoxicity against other lung cancer cell lines. Belagenpumatucel-L vaccine uses four cell lines to increase the number of tumour antigens in the vaccine. Additionally, the cell lines have low TGF-β expression because of transfection with a TGF-β2 antisense plasmid, which is thought to remove a major source of immune suppression at the site of vaccine injection. In preclinical studies, antisense inhibition of TGF-β2 led to inhibition of cellular TGF-β2 expression and increased immunogenicity of cancer cells. The enhanced local immune recognition and effector cell activation that results is purported to induce a systemic immune response capable of targeting cancers.^{44–46}

In a randomised, dose-variable, phase 2 trial, 75 NSCLC patients with stages II, IIIA, IIIB, and IV disease were randomly assigned to receive one of three doses (1.25×10⁷, 2.5×10⁷, or 5.0×10⁷ cells per injection intra dermally) of belagenpumatucel-L on a monthly or alternate

month schedule to a maximum of 16 doses.⁴⁶ Patients were required to have an estimated total tumour burden volume of 125 mL or less, excluding nodal or bone disease. No restrictions existed for the number of previous therapies. Less than 20% of patients had early stage NSCLC (n=14). Although median overall survival did not differ between dose cohorts, patients who received high doses (2.5×10^7 cells per injection) had significantly improved overall survival compared with those who received low doses (1.25×10^7 cells per injection; $p=0.0069$). Compared with patients with progressive disease, clinical responders had increased production of cytokines (interferon- γ and interleukin-6) and increased antibody-mediated response to vaccine human leucocyte antigens. Belagenpumatucel-L was well tolerated. Despite small numbers of patients with low volume disease and the absence of a control group, this trial showed a dose-related improvement in survival and response after belagenpumatucel-L vaccination. A smaller phase 2 study of 20 patients with advanced NSCLC confirmed the responses and safety profile.⁴⁷

With an accrual goal of 506 patients, a phase 3 randomised, double-blind trial (Survival, Tumor-free, Overall, and Progression-free [STOP], NCT00676507) is assessing whether belagenpumatucel-L can prolong overall survival of patients with stages IIIA (T3, N2 only), IIIB, and IV NSCLC by at least 3 months (92 days). Patients are eligible if they have responded to or have stable disease after one first-line platinum-based chemotherapy regimen. Previous adjuvant chemotherapy and concomitant radiation therapy are permitted. Patients in the treatment group will receive 18 monthly intradermal injections of belagenpumatucel-L (2.5×10^7 cells per injection) followed by two quarterly injections (table 1).

Melanoma-associated antigen A3 vaccine

Melanoma-associated antigen A3 (MAGE A3), which belongs to the MAGE family, is one of several tumour-specific shared antigens and is encoded by a member of a multigene family located on the X chromosome. Although placental trophoblasts and testicular germ cells are the only normal cells with significant expression of MAGE genes, human tumours—including NSCLC—express MAGE. Several epitopes derived from melanoma-associated antigens are recognised by human leucocyte antigen class I restricted cytotoxic T cells, making them candidates for immunotherapies.⁴⁸ Although the physiologic role of MAGE gene products is unknown, activation of MAGE genes occurs in early carcinogenesis of the lung.⁴⁹ MAGE A3 is detected in about 35–50% of NSCLC, most frequently in squamous cell carcinoma, and is typically associated with higher histological grade and advanced disease. In a retrospective analysis, MAGE A3 expression was inversely correlated with tumour-specific survival in NSCLC patients undergoing surgery.^{50,51} A MAGE A3 protein-based vaccine has been developed, consisting of a recombinant antigen containing protein D—a lipoprotein on the surface of *Haemophilus influenzae* B—MAGE A3 protein, and a polyhistidine tail.⁵²

In a phase 2 trial, 17 stage I or II NSCLC patients with no evidence of disease after resection of primary tumour expressing MAGE A3 received four doses of MAGE A3 fusion protein alone or in combination with an adjuvant, at intervals of 3 weeks.⁵² Of nine patients vaccinated with recombinant MAGE A3, only three had a modest but significant increase in

antibodies against recombinant MAGE A3 protein, as measured by ELISA. By contrast, seven of the eight patients who received recombinant MAGE A3 with adjuvant had a substantial increase in serum anti-MAGE A3 antibodies, suggesting the importance of adjuvant for the development of immunity to MAGE A3. Booster vaccinations of MAGE A3 and adjuvant resulted in stronger antibody responses and a wider spectrum of CD4 and CD8 T cells against MAGE A3 epitopes in patients previously treated with MAGE A3 and adjuvant.⁵³

In a double-blind phase 2 trial,⁵⁴ patients with completely resected stage IB or II NSCLC expressing MAGE A3 (assessed by quantitative reverse transcriptase PCR), were randomly assigned (2:1) to receive postoperative MAGE A3, 300 µg intramuscularly, or placebo. Vaccination was started more than 6 weeks after surgery, with five doses at intervals of 3 weeks (induction), followed by eight doses every 3 months (maintenance). Other adjuvant therapies were not allowed. 363 patients were positive for MAGE-A3 of 1089 screened. For the 182 patients who were enrolled to the treatment groups, after a median follow-up of 28 months, the HR for disease-free interval (the primary endpoint) was 0.74 (95% CI 0.44–1.20; $p=0.107$), for disease-free survival was 0.73 (95% CI 0.45–1.16), and for overall survival was 0.66 (95% CI 0.36–1.20), suggesting a trend, but no statistically significant advantage compared with placebo. A gene signature consisting of immune-related genes associated with the pretherapeutic tumour microenvironment was predictive of a benefit of MAGE A3.⁵⁵ While the reduction in relative risk of cancer recurrence was 25% (95% CI 0.46–1.23) in the overall unselected NSCLC population, it was 43% (0.25–1.34) in the population with a positive gene signature.

An ongoing phase 3 study (MAGE A3 as Adjuvant, NSCLC Immunotherapy [MAGRIT], NCT00480025) is investigating the efficacy of MAGE A3 vaccine in patients with completely resected stage IB, II, or IIIA NSCLC positive for MAGE A3. In this randomised, double-blind, placebo-controlled, four-group, multicentre study including more than 500 institutions, patients will receive MAGE A3 vaccine or placebo (2:1), either immediately after surgery or after adjuvant chemotherapy. Up to four cycles of adjuvant chemotherapy can be administered at the discretion of the investigators. Five doses of vaccine will be administered every 3 weeks, followed by eight doses every 12 weeks. The primary objectives are to evaluate the disease-free survival of MAGE A3 vaccine compared with placebo after complete surgical resection, efficacy in the overall population, and efficacy in the population of patients who did not receive adjuvant chemotherapy (table 1).

Immune checkpoint inhibitors

Inhibitory co-receptors and pathways (immune checkpoint inhibitors) that restrain T-cell functions can impede antitumour immunity. Antibodies that bind to these co-receptors can block inhibitory signals, thus, augmenting T-cell activation and proliferation. Two fully human monoclonal antibodies—ipilimumab and tremelimumab—have been assessed in NSCLC against the most extensively studied inhibitory T cell co-receptor, cytotoxic T-lymphocyte-associated antigen (CTLA)-4.⁵⁶ A double-blind phase 2 trial in 203 chemotherapy-naïve patients with advanced NSCLC assessed the combination of ipilimumab (either concurrently or sequentially) with chemotherapy (carboplatin or

paclitaxel) compared with chemotherapy alone.⁵⁷ Immune-related progression-free survival was improved in the concurrent (median 5.52 months, 95% CI 4.17–6.74; $p=0.094$) and sequential (5.68 months, 4.76–7.79; $p=0.026$) treatment groups compared with chemotherapy only (4.63 months, 4.14–5.52; using a predefined significant p value of 0.1). Ipilimumab was associated with increased toxic effects; 41 patients (58%) in the concurrent group, 35 (52%) in the sequential group, and 18 (42%) in the chemotherapy only group had grade 3 or 4 adverse events.⁵⁷ A phase 3 study will evaluate overall survival with this combination in more than 900 squamous cell lung cancer patients (NCT01285609; table 1). In a phase 2 randomised, open-label trial, tremelimumab maintenance after first-line chemotherapy in NSCLC did not improve progression-free survival, the primary endpoint.⁵⁸

Programmed death-1 (PD-1), an inhibitory co-receptor expressed on activated T and B cells, is homologous to CTLA-4 but provides distinct immune inhibitory signals.⁵⁹ In a dose-escalation study, MDX-1106, a fully human monoclonal antibody against PD-1, was well tolerated to a maximum planned dose of 10 mg/kg in 39 patients with refractory metastatic solid tumours.⁵⁹ Of the six patients with NSCLC, one patient's lesions regressed significantly but did not meet the criteria for objective response. CTLA-4 and PD-1 blockage are both associated with immune-related adverse events from uncontrolled T-cell lymphoproliferation, although these toxic effects are less frequent and milder for PD-1 inhibition than for CTLA-4 inhibition.

Toll-like receptors agonists

Toll-like receptors (TLRs) are type I membrane glycoproteins and belong to a family of pattern recognition receptors that recognise broad classes of molecular structures common to groups of microorganisms.⁶⁰ TLRs have a crucial role in both innate and adaptive immune responses. Ligand binding to TLR results in a localised response, which leads to activation, maturation, and induction of proinflammatory cytokines and other antimicrobial compounds. Immature dendritic cells resident in peripheral tissues also recognise these invading pathogens via numerous TLRs. This activation leads to the activation, maturation, and trafficking of dendritic cells to local lymph nodes, and presentation of microbial antigens to naive T cells, leading to the induction of adaptive immunity against the invading pathogen (figure 2).

TLR9 is the TLR with the narrowest tissue distribution. In humans, its constitutive expression is confined to B cells and plasmacytoid dendritic cells. Cellular activation by highly immunogenic CpG motifs induces TLR9 expression in additional cell types, including monocytes, neutrophils, and CD4 cells.⁶¹ Functionally active TLR9 is overexpressed in lung cancer tissue compared with normal lung.⁶² PF-3512676 (formerly known as CpG 7909) is a synthetic TLR9-activating oligodeoxynucleotide that mimics the natural ligand of TLR9 (unmethylated CpG motifs), thereby inducing a cascade of immune reactions, potentially promoting an antitumour immune response.⁶³ In mouse lung cancer models, PF-3512676 has single-agent activity, and a combination of PF-3512676 and paclitaxel improves survival compared with either treatment alone, without additive toxic effects.⁶⁴ Proposed mechanisms of the synergistic antitumour activity of paclitaxel combined

with PF-3512676 include paclitaxel-induced recruitment of antitumour CD8 cells in the tumour and regulatory T-cell depletion and suppression.⁶⁵

In a phase 2 randomised controlled study, 111 chemotherapy-naïve patients who received a combination of taxane and PF-3512676 had higher response rates (38% vs 19%; $p=0.043$) and a trend towards improved overall survival (12.3 vs 6.8 months; $p=0.188$), compared with patients who received chemotherapy alone.⁶⁶ However, these results did not translate into clinical benefit in two large similarly designed phase 3 trials that assessed PF-3512676 in combination with up to six cycles of gemcitabine and cisplatin ($n=839$) or paclitaxel and carboplatin ($n=828$).^{67,68} In the first study, median overall survival was 11.0 months (95% CI 9.6–12.6) in the intervention group and 10.7 months (9.2–12.4) in the control group ($p=0.98$).⁶⁷ In the second study, median overall survival was 10.0 months (8.9–11.1) in the intervention group and 9.8 months (8.8–11.3) in the control group ($p=0.56$).⁶⁸ In both studies, severe haematological adverse events, transfusions, infections, granulocyte colony-stimulating factor use, chemotherapy dose delays, and dose reductions were more frequent in the intervention group than in the control group. Administration of PF-3512676 was stopped at the first interim analysis in both trials because the predefined futility boundary for overall survival had been reached and because of increased toxic effects. Subgroup analyses did not identify a subpopulation that benefited from the addition of PF-3512676 to standard chemotherapy. Discrepancy in efficacy and toxic effects between randomised phase 2 and the subsequent phase 3 studies might be because of differences in study design, patient populations, and treatment regimens.

Mycobacterial adjuvant-based agents

Cell wall components of *Mycobacterium* spp—including lipoglycans, which bind to TLR2—induce non-specific immune stimulation, activate antigen-presenting cells and natural killer cells, and enhance T helper type 1 response.⁶⁹

In a randomised phase 2 study of 28 previously untreated patients with NSCLC and mesothelioma, administration of SRL172—a suspension of heat-killed *Mycobacterium vaccae*—combined with chemotherapy did not significantly affect response rates (54% vs 33%, $p=0.3$) and median survival (9.7 months vs 7.5 months, $p=0.235$) compared with chemotherapy alone.⁷⁰ Results of an open-label, randomised phase 3 study of 419 NSCLC patients showed no difference between the treatment groups for overall survival, progression-free survival, or objective response rate.⁷¹ Patients were randomly assigned to receive up to six cycles of mitomycin, vinblastine, and cisplatin or carboplatin with or without monthly SRL172 (0.1 mL, intradermally). Because of a high dropout rate, after the initial treatment phase of 15 weeks, only 30 patients (14%) received one or more SRL172 injection and 132 (63%) did not receive any injections.⁷¹ In a post-hoc analysis, the adenocarcinoma subgroup had significantly improved survival.⁷²

Mycobacterium indicus pranii had synergistic effects with chemo therapeutic agents in preclinical studies.⁷³ In a randomised phase 2 study of 221 chemotherapy-naïve NSCLC patients, addition of Cadi-05 to paclitaxel and cisplatin improved overall survival (9.8 vs 7.8 months, HR 0.55, 95% CI 0.37–0.82; $p=0.0034$) and progression-free survival (8.4 vs 5.2 months, 0.43, 0.25–0.73; $p=0.0446$). However, these results were not significant in the

intention-to-treat population. Adverse events were not more frequent in vaccinated patients.
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Talactoferrin

Lactoferrin is an iron-binding glycoprotein first identified in breast milk, which is known to have several immunomodulatory functions.⁷⁵ Talactoferrin alfa, or recombinant human lactoferrin, is an orally active recombinant lactoferrin purified from *Aspergillus niger* var *awamori* and is structurally and functionally similar to human lactoferrin. Orally administered talactoferrin binds gut epithelium, is transported into the gut-associated lymphoid tissue, recruits circulating immature dendritic cells bearing tumour antigens, and induces dendritic cell maturation. Dendritic cell activation in the presence of tumour antigens and lymphoid effector cells induces a strong systemic innate and adaptive immune response, which might underlie the anti-neoplastic properties of talactoferrin. Talactoferrin is not systemically absorbed and does not increase serum lactoferrin concentrations or talactoferrin-specific antibodies.⁷⁶ In phase 1 trials, oral talactoferrin was very well tolerated and showed activity in patients with NSCLC.⁷⁷ Results of two phase 2 studies of talactoferrin in NSCLC show improved overall survival with monotherapy in patients who failed first-line or second-line chemotherapy and improved response rates in combination with first-line chemotherapy.^{78,79}

In the first phase 2 placebo-controlled trial, 110 patients with advanced NSCLC were randomly assigned to receive carboplatin and paclitaxel once every 3 weeks for up to six cycles with either talactoferrin or placebo.⁷⁸ Talactoferrin (1.5 g twice per day) or placebo was administered orally for up to three 6-week cycles (35 consecutive days followed by 1 week off), starting from the day after chemotherapy administration in the first, third, and fifth chemotherapy cycles. Addition of talactoferrin improved the response rates (the primary endpoint) compared with placebo in the intention-to-treat population (27% vs 42%) and in the 100 assessable patients (29% vs 47%; $p=0.05$). Median overall survival in the talactoferrin group was 1.9 months higher (10.4 vs 8.5 months, HR 0.87; $p=0.26$) in the intention-to-treat population and 2.8 months higher (11.3 vs 8.5 months, 0.75; $p=0.11$) in the assessable population compared with placebo.

In a second phase 2 placebo-controlled study, 100 patients with stage IIIB or IV NSCLC with progressive disease after one or two lines of chemotherapy, including a platinum-based chemotherapy, were randomly assigned to receive talactoferrin for up to three cycles of 14 weeks (12 weeks on, 2 weeks off; 1.5 g orally, twice per day) or placebo, until disease progression.⁷⁹ Median overall survival was 3.7 months in the talactoferrin group, compared with 6.1 months in the control group (HR 0.68, 90% CI 0.47–0.98; one-tailed $p=0.0404$). Talactoferrin had a consistent effect on overall survival across patient and histological subsets.

Two ongoing phase 3 trials are assessing talactoferrin in patients with advanced NSCLC (table 1). FORTIS-M (NCT00707304) is a double-blind, placebo-controlled, multicentre study that randomly assigned about 720 patients in a 2 to 1 ratio to compare overall survival between patients who receive talactoferrin and placebo. The study is designed to have roughly 85% power to detect a 30% improvement in median overall survival in patients in

the intention-to-treat population, from 4.6 months in the placebo group to 6.0 months in the intervention group. Eligible patients have received at least two previous courses of treatment, including one platinum-containing regimen for stage IIIB or IV NSCLC. Talactoferrin (1.5 g, orally twice per daily) or placebo is administered for 12 weeks followed by 2 weeks off study drug up to a maximum of five cycles of 14 weeks, until progressive disease. FORTIS-C (NCT00706862) is a randomised, double-blind placebo-controlled trial assessing overall survival and progression-free survival for addition of talactoferrin (1.5 g, orally twice per day) to first-line chemotherapy with carboplatin or paclitaxel in 1100 newly diagnosed patients with stage IIIB or IV NSCLC.

Mesothelioma

WT1 analogue peptide vaccine

The Wilms' tumour suppressor gene, WT1, is a transcription factor commonly overexpressed in leukaemias and some solid tumours, including mesothelioma.⁸⁰ In normal adult tissues, WT1 expression is restricted to low levels in nuclei of normal CD34 haemopoietic stem cells, myoepithelial progenitor cells, renal podocytes, and some cells in the testes and ovary. In human mesothelioma cell lines, peptide epitopes from WT1 induce CD4 and cytotoxic CD8 WT1-specific T-cell responses.⁸¹ In a pilot trial in nine patients, six doses of a vaccine of four WT1 analogue peptides were administered subcutaneously over 12 weeks followed by six additional monthly injections for responding patients. Injection sites were pre-stimulated with granulocyte macrophage colony-stimulating factor. The vaccine was safe and induced an immune response: six of nine patients tested had CD4 T-cell proliferation to WT1-specific peptides, and five of the six HLA-A0201 patients tested mounted a CD8 T-cell response.^{82,83} An ongoing randomised phase 2 trial (NCT01265433) is assessing adjuvant WT1 analogue peptide vaccine after completion of combined modality therapy for malignant pleural mesothelioma. Table 2 shows the other ongoing vaccine studies in mesothelioma.

Dendritic cell vaccine

Dendritic cells are potent antigen-presenting cells present in peripheral tissues, where they capture, process, and transport antigens to naive T cells in secondary lymphoid organs. Tissue inflammation results in dendritic cell maturation, migration to draining lymph nodes, and expression of peptide–MHC complexes and co-stimulatory molecules, which allows priming of CD4 T helper cells and CD8 cytotoxic T lymphocytes, activation of B cells, and initiation of an adaptive immune response. However, immature dendritic cells present antigens to T cells in the lymph nodes without co-stimulation, leading to either the deletion of T cells or the generation of inducible regulatory T cells. Owing to their capacity to regulate T-cell immunity, dendritic cells are increasingly used as vaccine adjuvants.^{84,85}

Dendritic cells that have been matured with a standard cytokine cocktail were pulsed with autologous tumour cell lysate, an antigen source for immunotherapy. In a mouse model, dendritic cells pulsed with tumour lysate induced strong tumour-specific cytotoxic T-lymphocyte responses, prolonging survival. The efficacy of immunotherapy was dependent on tumour load, with the most beneficial effects at early stages of tumour development.⁸⁶

The safety of, and ability to induce immunological responses with, dendritic cell vaccination in mesothelioma has been tested in a phase 1 study of 10 newly diagnosed malignant pleural mesothelioma patients with partial response or stable disease after previous combination chemotherapy. Patients underwent leukapheresis 10 weeks after their last dose of chemotherapy. 2 weeks later and at subsequent intervals of 2 weeks for a total of three doses, dendritic cells pulsed with autologous tumour lysate were administered intradermally (a third of the dose) and intravenously (two thirds of the dose). Mild-to-severe flu-like symptoms and local skin rash were the common toxic effects. Three patients had partial responses, one had stable disease, and six had no response after vaccinations. Post-vaccination serum samples from all patients showed a significant humoral response to keyhole limpet haemocyanin, a xenogeneic immunogenic antigen used as a marker of immune response. Nine patients had increased CD3 and CD8 T cells expressing granzyme B after vaccination, suggesting lymphocyte activation by dendritic cell immunotherapy. However, the measured humoral or cellular immunological responses and clinical responses were not correlated.⁸⁷ Addition of cyclophosphamide to dendritic cell vaccination induces beneficial immunomodulatory effects, increasing overall survival in mouse models of mesothelioma.⁸⁸ A phase 1 study (NCT012416812) is assessing this combination in mesothelioma patients (table 2).

Mesothelin

Listeria monocytogenes has been investigated as a platform for presentation of tumour-associated antigens because it potently stimulates innate immunity and also initiates an adaptive immune response through recruitment and activation of CD4 and CD8 T cells specific for encoded heterologous antigens.⁸⁹ CRS-207 is a live-attenuated double-deleted *L. monocytogenes* strain (*actA* / *inlB*) that expresses human mesothelin. Mesothelin is an immunogenic glycoprotein that is highly expressed in pancreatic, ovarian, NSCLC, and malignant mesotheliomas. Mesothelin is an attractive candidate for tumour-specific immunotherapy in mesothelioma because of its low expression in normal mesothelial cells and high expression in mesotheliomas.^{90,91} CRS-207 is derived by deleting the entire coding sequences of two virulence determinant genes from the genome of wild-type *L. monocytogenes* and integration of a human mesothelin expression cassette at the *inlB* locus. The deletion of these coding sequences in mice causes a more than 1000-times attenuation of virulence.⁸⁹ However, uptake of CRS-207 by macrophages and phagocytic cells in the liver and spleen is retained and results in a local inflammatory response and activation and recruitment of immune effector cells, such as natural killer and T cells, to the liver.⁸⁹ After uptake and multiplication of CRS-207 by phagocytic cells, mesothelin is expressed and released into the cytosolic compartment. Mesothelin is then processed through the endogenous MHC class I presentation pathway, resulting in activation of anti-mesothelin cell-mediated immunity. In preclinical studies, CRS-207 elicited mesothelin-specific cellular immunity in mice and in non-human primates and was efficacious in tumour-bearing mice.

CRS-207 was assessed in an open-label phase 1, dose-escalation study of 17 patients with advanced treatment-refractory cancers known to express mesothelin.⁹² CRS-207 was well tolerated; the maximum tolerated dose was 1×10^9 colony forming units. *L. monocytogenes* was not shed in either stool or urine. CRS-207 induced both listeriolysin-specific (in six of

seven tested patients) and mesothelin-specific (in six of ten tested patients) T-cell responses. Overall, six of 17 patients, including one of five patients with mesothelioma survived 15 months or more after the first dose. Sequential treatment with CRS-207 was especially effective: all six long-term survivors had previous immunotherapy or subsequent local radiation therapy (all three pancreatic cancer patients received previous treatment with granulocyte macrophage colony-stimulating factor gene-transfected tumour cell vaccine; the sole mesothelioma patient received previous treatment with adenovirus expressing interferon- β ; both NSCLC patients received subsequent local radiation therapy). CRS-207 is being investigated in mesothelioma in combination with first-line chemotherapy.

Conclusion

Approval of immunotherapeutic agents for prostate cancer and melanoma has spurred a renewed interest in such therapies for NSCLC and mesothelioma. Despite being considered non-immunogenic cancers, several lines of evidence suggest that NSCLC and mesothelioma are subject to immune surveillance. In NSCLC, specific immunotherapies targeted against the MAGE A3 and MUC-1 antigens, as well as an allogeneic tumour cell vaccine cocktail modified with antisense inhibition of TGF- β 2, are in phase 3 trials based on modest activity in previous studies. Some of the non-specific immunotherapies assessed for NSCLC include PF-3512676, a TLR9 agonist, agents based on mycobacterial adjuvant, and talactoferrin. Although data from a large randomised phase 2 study of PF-3512676 combined with chemotherapy seemed promising, two phase 3 trials did not detect improvements in survival and were halted because of toxic effects of the combination. Mycobacterial adjuvant-based agents and talactoferrin have shown promise. In mesothelioma, immunotherapies being investigated include vaccines based on dendritic cells and *L. monocytogenes*, and allogeneic tumour cell and WT1 analogue peptide vaccines.

Several important issues need to be addressed to fully harness the therapeutic potential of antitumour immune responses in NSCLC and mesothelioma. A major challenge is the need to improve measurements of tumour-specific immune responses and understand the relation between immune and clinical responses. A first step is harmonisation of immune response assays used in multicentre trials to reduce variability and help establish cellular immune response as a reproducible biomarker.⁹³ The commonly used WHO or RECIST criteria might not effectively capture the clinical activity of immunotherapies because of differences in the dynamics of antitumour activity between immunotherapeutic and cytotoxic agents. The immune-related response criteria adapt the standard response criteria to include the potential for delayed clinical response and early increase of tumour burden because of immunotherapies.⁹⁴ Other important considerations include patient selection for specific interventions, selection of the appropriate stage of disease to test, dosing schedules, and improved immunological adjuvants. Increasingly, immunotherapies are being combined with conventional chemotherapies as a result of preclinical data that suggest synergy between these treatment types.⁹⁵ As such, identification of the correct chemotherapy for the combination is important, as is the timing of administration—simultaneous or sequential—with immunotherapies. In mesothelioma, a rare malignancy, researchers should collaborate to improve patient accrual for immunotherapy trials.

Acknowledgments

Intramural Research Program of the Center for Cancer Research, National Cancer Institute, NIH (USA).

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Search strategy and selection criteria

We identified data for this review by systematic search of Medline with the MeSH terms “Immunotherapy”, “Mesothelioma”, and “Carcinoma, Non-Small-Cell Lung” for peer-reviewed clinical studies and other studies of clinical significance. The search was restricted to reports written in English, published between Jan 1, 1995, and Dec 15, 2011. Bibliographies of identified articles, guidelines, and conference proceedings of professional societies were reviewed for additional references.

Figure 1: Generation of cytotoxic T-lymphocyte response to tumour antigens and peptides

The TCR recognises antigenic peptides presented in by the MHC. TCR-mediated recognition of a peptide antigen presented by MHC in the presence of a co-stimulatory signal (eg, B7, present on professional antigen-presenting cells, such as dendritic cells, and CD28 present on T cells) triggers the cytotoxic T-lymphocyte response. By contrast, binding of CTLA-4 on T cells to B7 inhibits T-cell activation. Use of a monoclonal antibody to CTLA-4 that interferes with its binding to B7 leads to T-cell activation. TCR=T-cell receptor. CTLA-4=cytotoxic T-lymphocyte antigen 4. CTL=cytotoxic T lymphocyte.

Figure 2: Activation of innate and adaptive immune systems by TLR-mediated dendritic cell activation

Ligand binding to TLR causes a localised response, which leads to induction of pro-inflammatory cytokines, type 1 interferons, and chemokines. This activates adaptive immune responses by polarisation of Th1 CD4 T cells, the development of cytolytic memory T cells, and antibody responses. Activated dendritic cells then migrate to local lymph nodes and present microbial antigens to naive T cells, causing induction of adaptive immunity against the invading pathogen. TLR=Toll-like receptor. PAMP=pathogen-associated molecular pattern. IL=interleukin. NK=natural killer. CTL=cytotoxic T lymphocyte. Th1=T helper type 1.

Table 1:

Ongoing clinical trials of immunotherapies for non-small-cell lung cancer

Intervention	Study design	Estimated enrolment (n)	Stage	Main eligibility requirements	Endpoints
START	Liposomal BLP25 or placebo	1476	Unresectable stage III	Stable disease or objective response after primary chemoradiotherapy. Two or more cycles of platinum-based chemotherapy, 50 Gy radiation chemotherapy, 50 Gy radiation	Primary: OS. Secondary: time to symptom progression, time to disease progression, 1, 2, and 3 year survival, safety
INSPIRE	Liposomal BLP25 or placebo	420	Unresectable stage III	Stable disease or objective response after primary chemoradiotherapy. Two or more cycles of platinum-based chemotherapy, 50 Gy radiation	Primary: OS. Secondary: time to symptom progression, time to disease progression, PFS, time to treatment failure, safety
STOP	Belagenpumatucel-L or placebo	506	Unresectable stage III or IV	Stable disease or objective response after primary platinum-based chemoradiotherapy	Primary: OS. Secondary: PFS, quality of life, time to progression, objective response, response duration, rate of CNS metastases development, safety
MAGRIT	MAGE-A3 vaccine or placebo	2270	Completely resected, stage IB, II, or IIIA	Tumour expresses MAGE-A3 gene.	Primary: disease-free survival. Secondary: lung-cancer-specific survival, OS, anti-MAGE-A3 and anti-protein D seropositivityrate, adverse events
Randomised trial of ipilimumab in squamous cell lung cancer	Ipilimumab, carboplatin, paclitaxel or placebo, carboplatin, and paclitaxel	920	Stage IV or recurrent	Squamous cell histology	Primary: OS. Secondary: PFS, objective response
FORTIS-M	Talactoferrin or placebo	720	Stage IIIB or IV	Progressive disease after two or more previous systemic therapies	Primary: OS. Secondary: PFS, objective response, disease stabilisation rate, safety
FORTIS-C	Talactoferrin, carboplatin, paclitaxel or placebo, carboplatin, paclitaxel	1100	Stage IIIB or IV	No previous systemic anti-cancer therapy for non-small-cell lung cancer	Primary: OS, PFS. Secondary: objective rate, disease stabilisation rate, safety

MAGE=melanoma-associated antigen. PFS=progression-free survival. OS=overall survival.

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Table 2:

Ongoing clinical trials of immunotherapies for mesothelioma

Intervention	Study design	Estimated enrolment (n)	Stage and disease	Main eligibility requirements	Endpoints
Adjuvant WT1 analogue peptide vaccine	Phase 2 randomised, double-blind	78	Mesothelioma	Immunohistochemistry positive for WT1 (>10% of cells). Completed combined modality therapy *	Primary: 1 year PFS. Secondary: immunogenicity
Dendritic cell vaccine, cyclophosphamide	Phase 1	10	Mesothelioma	Stable disease or objective response after chemotherapy. Availability of sufficient tumour material	Primary: immunogenicity. Secondary: safety
Tumour cell vaccine, adjuvant, and celecoxib	Phase 1	120 (30 assessable)	Mesothelioma and other malignancies [†]	Completed surgical resection plus adjuvant chemotherapy or radiation, or both	Primary: safety. Secondary: immunogenicity
Tumour cell vaccine, cyclophosphamide and celecoxib	Phase 1 and 2	25	Thoracic malignancies	No evidence of disease or minimal residual disease after standard multimodality therapy	Primary: safety. Secondary: immunogenicity

PFS=progression-free survival.

* Combined modality therapy: surgical resection plus chemotherapy or radiation, or both.

[†] Lung and oesophageal carcinoma or sarcoma.