New strategies in immunotherapy for non-small cell lung cancer

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Introduction

Lung cancer is the leading cause of cancer related death worldwide (1). Cytotoxic chemotherapy can improve survival, but responses are often short-lived (2). Targeted therapies result in high response rates for patients with certain alterations, such as EGFR mutations or EML4-ALK translocations, but these are found only in a minority of patients and resistance inevitably develops (3,4). Clearly, novel therapeutic approaches are urgently needed.

Utilizing the immune system to fight cancer is an attractive goal, promising the possibility of long term disease control without some of the toxicities of more traditional therapies. In order to grow, cancer must evade immune detection. In lung cancer and many other tumors, T-cell tolerance to tumor antigens is a major barrier to effective immunotherapy, and initial studies of non-specific immune stimulation with interleukin-2 and interferon-alpha showed minimal activity (5,6). Therapeutic checkpoint inhibition or activation of co-stimulatory molecules has the potential to combat this tolerance and allow anti-tumor immune responses. Recent trials with these agents have shown promising results and have renewed excitement in the field of immune therapy for lung cancer. In this article, we will review the results of recently reported clinical trials of immunotherapy agents, and we will discuss ongoing and upcoming research in this field.

Vaccine therapy

Cancers often express antigens not found on normal cells. Many clinical trials have studied the efficacy of vaccines against these antigens. Two large trials of vaccine therapy in non-small cell lung cancer (NSCLC), the Stimulating Targeted Antigenic Response to (START) NSCLC trial and the MAGRIT, a double-blind, randomized, placebo-controlled Phase III study to assess the efficacy of the recMAGE-A3 + AS15 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3-positive non-small cell lung cancer (MAGRIT) trial, have recently been reported.

The START trial enrolled 1,513 patients with stage III NSCLC who had completed chemoradiation. Patients without progressive disease were then randomized to receive either tecemotide (L-BLP25), a vaccine against...
MUC1, or placebo, and the primary endpoint of the study was overall survival (OS). There was no significant difference in survival between the two arms (median OS 25.6 months with tecemotide vs. 22.3 months with placebo), though there was a benefit for the vaccine in patients who received concurrent rather than sequential chemotherapy (30.8 vs. 20.6 months, P=0.0175) (7). Unfortunately, these promising results were not confirmed in other studies, and development of this compound has been halted.

In the MAGRIT trial, patients with surgically resected stage IB-IIIA NSCLC were randomized to receive placebo or a vaccine to MAGE-A3. Eligible patients were required to have MAGE-A3 positive tumors. A total of 2,272 patients were randomized and treated. Treatment was well tolerated, but results were disappointing as the trial showed no significant improvement in disease-free survival (60.5 vs. 57.9 months, P=0.0175) (7). Unfortunately, these promising results were not confirmed in other studies, and development of this compound has been halted.

With negative results from these two large trials, enthusiasm for vaccine-based therapy for NSCLC has waned, and focus has shifted to treatment with checkpoint inhibitors and other immunotherapy agents.

**Checkpoint inhibition**

A complex interaction of both inhibition and stimulation of the immune system exists to allow the appropriate destruction of pathogens and abnormal cells while preventing overstimulation that could lead to destruction of healthy cells (autoimmunity). This network of inhibitory and stimulatory signals offers multiple potential targets. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are co-inhibitory factors (“checkpoints”) that have been the focus of much research (Table 1).

**CTXLA-4 inhibitors**

CTLA-4 is expressed on the surface of T-cells. It inhibits T-cell activation and decreases immune response (14,15). Inhibition of this protein with therapeutic antibodies leads to increased activation of T-cells and anti-tumor responses in some patients. Ipilimumab is an anti-CTLA-4 antibody approved for the treatment of metastatic melanoma (16). Its activity is under investigation for NSCLC.

Preliminary signs of efficacy in NSCLC were seen in a phase II study of ipilimumab in combination with chemotherapy. Patients with previously untreated metastatic NSCLC were randomly assigned to one of three groups: carboplatin/paclitaxel, carboplatin/paclitaxel with concurrent ipilimumab, or carboplatin/paclitaxel with sequential ipilimumab (9). Results were very promising with a statistically significant improvement in progression-free survival (PFS) in the sequential arm (carboplatin and paclitaxel for two cycles followed by addition of ipilimumab with chemotherapy for four more cycles) compared to the chemotherapy alone arm (median PFS 5.1 vs. 4.2 months,

<table>
<thead>
<tr>
<th>Immunotherapy</th>
<th>Target</th>
<th>Setting</th>
<th>Patient number</th>
<th>Results</th>
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<tbody>
<tr>
<td>Ipilimumab</td>
<td>CTLA-4</td>
<td>Phase II</td>
<td>204</td>
<td>ir-PFS 5.7 months for phased ipilimumab + chemotherapy vs. 4.6 months for placebo + chemotherapy (HR 0.72; P=0.05); ir-PFS 5.5 months for concurrent ipilimumab + chemotherapy vs. placebo + chemotherapy (HR 0.81; P=0.13) (9)</td>
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<tr>
<td>Nivolumab</td>
<td>PD-1</td>
<td>Phase III</td>
<td>272</td>
<td>OS 9.2 months for nivolumab vs. 6 months for docetaxel (P&lt;0.001) (10)</td>
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<tr>
<td>Pembrolizumab</td>
<td>PD-1</td>
<td>Phase I;</td>
<td>495; (Training-182);</td>
<td>ORR 19.4%; MDR 12.5 months; MDOS 12 months (11)</td>
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<td>KEYNOTE-001</td>
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<tr>
<td>MPDL3280A</td>
<td>PD-L1</td>
<td>Phase I</td>
<td>53; (37 evaluable)</td>
<td>ORR 24%; 24-week PFS 48% (12)</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>PD-L1</td>
<td>Phase I</td>
<td>13</td>
<td>3 PR with two additional responses not meeting PR per irRC (13)</td>
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<td>(durvalumab)</td>
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NSCLC, non-small cell lung cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HR, hazard ratio; ir-PFS, immune-related progression-free survival; irRC, immune-related response criteria; MDOS, median duration of overall survival; MDR, median duration of response; ORR, overall response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand of protein 1; PFS, progression-free survival; PR, partial response.

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P = 0.02). Subset analysis showed a greater benefit in patients with squamous histology and thus, a phase III study of ipilimumab in combination with carboplatin and paclitaxel for squamous cell carcinoma of the lung is ongoing (NCT02279732). Ipilimumab is also being studied in NSCLC in combination with radiation (NCT02239900, NCT02221739) and with other immunotherapy agents (NCT02039674, NCT02174172).

**PD-1 inhibitors**

PD-1 is expressed on the surface of activated T-cells and has an inhibitory effect on immune response, and multiple therapeutic antibodies against PD-1 are in development. PD-1 inhibitors that have been most extensively studied for lung cancer are nivolumab and pembrolizumab. NSCLC responses to PD-1 inhibition with nivolumab were first reported in a phase I study, with responses seen in 18% of heavily pre-treated NSCLC patients (17). Some of these responses were durable. These results led to two large, phase III trials of nivolumab vs. docetaxel in previously treated squamous NSCLC (CheckMate 017, NCT01642004) and non-squamous NSCLC (CheckMate 057, NCT01673867). Results of the CheckMate 017 study were recently reported; patients treated with nivolumab had a statistically significant improvement in OS compared to patients receiving docetaxel (9.2 vs. 6.0 months, P < 0.001) (10). Nivolumab was FDA approved for previously treated squamous cell carcinoma of the lung based on these data. CheckMate 057 has completed accrual and results are eagerly awaited. A phase III study comparing nivolumab to platinum-doublets for previously untreated NSCLC is currently enrolling patients (NCT02041533).

Pembrolizumab is another PD-1 inhibitor with promising activity in NSCLC. In the recently reported phase I KEYNOTE-001 study, patients with NSCLC were treated with pembrolizumab in different doses and schedules. Most patients (81%) had received prior systemic therapy. Tumor samples from all patients were assessed for programmed cell death ligand of protein 1 (PD-L1) expression prior to treatment. The overall response rate (ORR) in all tumor types was 19.4% with a median duration of response (MDR) of 12.5 months. Median OS was 12 months (11). A phase III trial comparing pembrolizumab to docetaxel for previously untreated NSCLC is currently enrolling patients (NCT02041533).

**PD-L1 inhibitors**

An alternate to inhibiting PD-1 is blocking interactions at the ligand level (PD-L1). The two anti-PD-L1 antibodies that are furthest in development for lung cancer are MPDL3280A and MEDI4736. In a phase I study of MPDL3280A, NSCLC patients had a 24% response rate. Some of these responses were long-lasting; a number of patients continued to maintain a response even after the antibody was stopped (12). Larger studies are ongoing: the single arm, phase II BIRCH trial is enrolling patients with PD-L1 positive NSCLC (NCT02031458); the phase III OAK trial randomizes patients with previously treated NSCLC to either MPDL3280A or docetaxel (NCT02008227).

MEDI4736 also has shown promising activity in a phase I study (7), and larger studies are ongoing. The phase III PACIFIC trial is enrolling patients with stage III NSCLC who have completed radiation and randomizing them to either MEDI4736 or placebo (NCT02125461); this is the largest ongoing immunotherapy effort for locally advanced disease. In the phase III ARTIC trial, NSCLC patients who have received multiple prior therapies will be randomized to MEDI4736 vs. standard chemotherapy if PD-L1 positive or MEDI4736 in combination with anti-CTLA4 antibody tremelimumab if PD-L1 negative (NCT0235948).

**T-cell co-stimulation**

“Taking the brakes off” the immune system with checkpoint inhibition has been the major focus of immunotherapy research in NSCLC so far; however, agents that augment co-stimulatory signals are also under study. Ongoing studies are focused on 4-1BB (CD-137), OX40 (CD134), and CD-27 agonists that augment T-cell response, often in combination with checkpoint inhibitors. These three receptors are members of the tumor necrosis factor receptor family and are primarily expressed on T-cells. When activated by interactions with their ligands or with antibodies, these receptors lead to T-cell proliferation and survival, as well as cytokine production (8). Therapeutic antibodies against these receptors are in clinical development.

**4-1BB agonists**

4-1BB, also known as CD137, is a potent co-stimulatory molecule expressed on activated T-cells, and is not seen
in resting T-cells (13). Interactions between 4-1BB and its ligand lead to T-cell proliferation and activation (18). In pre-clinical models, administration of 4-1BB agonists inhibits tumor growth (19). Urelumab is a fully human anti-4-1BB antibody with agonistic activity. In an initial phase I study, activity was observed in melanoma; no patients with NSCLC enrolled on this trial (20). There is an ongoing phase I/II trial studying the combination of urelumab with nivolumab; this study will enroll patients with NSCLC in an expansion cohort (NCT02253992).

**OX40 agonists**

Like 4-1BB, OX40 is expressed primarily on activated T-cells and its ligand is expressed on antigen presenting cells (21). When OX40 is activated by either its ligand or therapeutic antibodies, the result is survival and proliferation of T-cells (22). Multiple compounds are in development targeting OX40. MEDI6469 is an anti-OX40 antibody with agonist activity; it is being studied in a phase Ib/II trial as a single agent or in combination with checkpoint inhibition (NCT02205333). An expansion cohort for NSCLC is planned. MEDI6383 is another OX40 agonist currently in phase I studies (NCT02221960).

**CD27 agonists**

CD27 is another co-stimulatory receptor related to OX40 and 4-1BB. An agonist anti-CD27 antibody, varililumab, was well tolerated in phase I trials (23). There are ongoing trials enrolling NSCLC patients with varililumab as a single agent (NCT01460134) and in combination with nivolumab (NCT02335918).

**Combination therapy**

Striking responses to immunotherapy have been seen; however, with single agent therapy, only a minority of patients will respond. A number of studies are exploring combination therapy in an effort to increase response rates and duration of disease control.

**Immunotherapy combinations**

As briefly discussed above, ongoing studies are combining multiple immunotherapy agents. Especially promising are combinations of dual checkpoint inhibition with CTLA-4 inhibitors and PD-1 inhibitors. Nivolumab combined with ipilimumab is associated with high response rates in melanoma (24); early phase studies enrolling NSCLC patients are ongoing (NCT01454102). The combination of MEDI4736 and tremilimumab is also under study (NCT02000947). Other studies are ongoing with checkpoint inhibition combined with agents that activate co-stimulatory molecules, such as 4-1BB, CD-27, and OX40.

Immune related toxicity is a concern with these combinations; more than half of all patients treated with a combination of ipilimumab and nivolumab had a grade 3 or 4 treatment-related adverse event (25).

**Combinations with chemotherapy**

Theoretically, administration of cytotoxic chemotherapy may increase antigen release from tumor cells, potentially increasing efficacy of immunotherapy. A phase II study of ipilimumab combined with carboplatin and paclitaxel suggests improved outcomes (immune-related PFS hazard ratio (HR), 0.72; P=0.05 vs. immune-related PFS HR, 0.81; P=0.13) when chemotherapy is administered for several cycles prior to initiation of immunotherapy (9). A number of studies combining checkpoint inhibition with cytotoxic chemotherapy are ongoing (NCT02039674, NCT01454102, and NCT02279732, among others).

**Combinations with targeted therapies**

EGFR inhibitors have revolutionized the treatment of EGFR mutant lung cancer; however, resistance inevitably develops, usually after less than a year (25,26). Studies combining EGFR inhibition with erlotinib or gefitinib with checkpoint inhibitors are ongoing (NCT01454102, NCT0208812). MEK inhibitors such as trametinib and selumetinib have shown some evidence of activity, especially in KRAS mutant NSCLC (27,28). Unfortunately, response rates are low and PFS is short with single agent therapy (28). Trials combining MEK inhibition with immunotherapy in melanoma are ongoing (NCT02224781); trials in NSCLC are likely to follow.

Caution must be exercised when combining targeted agents with immunotherapy. A recent phase I study combining ipilimumab with vemurafenib in metastatic melanoma found the combination to be intolerable, with an unexpectedly high rate of hepatic toxicity (29). This trial shows that unexpected synergistic toxicity may be observed when distinct therapeutics is combined.
**Combinations with radiation**

Like cytotoxic chemotherapy, radiation can cause tumor antigen release, which may increase efficacy of immune therapy. There have been reports of radiation resulting in response in tumors well outside the radiation field; this is known as the abscopal effect (30), and pre-clinical evidence suggests radiation may increase the efficacy of checkpoint inhibition (31). Ongoing studies are combining checkpoint inhibition with radiation (NCT02239900, NCT02221739, NCT02303990). Toxicity is a concern here as well—pneumonitis is a common and potentially severe toxicity of both immunotherapy and radiation. Patients on these trials will be carefully monitored for pulmonary toxicity.

**Biomarker development**

Though immunotherapy agents appear to be very active in a subset of NSCLC, many patients will have no response to therapy. Several strategies are being developed to try to select patients most likely to respond to therapy.

**PD-L1 staining**

Multiple studies have assessed whether expression of PD-L1 can predict for response from checkpoint inhibition. In the phase I study of nivolumab in multiple tumor types including NSCLC, patients whose tumors were positive for PD-L1 (defined as >5% of cells with expression) had a response rate of 36% with nivolumab. None of the patients with PD-L1 negative tumors had a response (17). In the KEYNOTE-001 trial, NSCLC patients with staining for PD-L1 in greater than 50% of cells had longer PFS and OS than those with lower rates of expression when treated with pembrolizumab (11).

The early phase studies with nivolumab and pembrolizumab described above used tumor cell staining for PD-L1 as a marker; Herbst et al. examined expression of PD-L1 in both tumor cells and infiltrating lymphocytes in specimens from a phase I study of MPDL3280A (32). Interestingly, they found that expression of PD-L1 on tumor infiltrating immune cells was a significant predictor of response, while PD-L1 expression on tumor cells was not. In patients with NSCLC, the response rate for patients with the highest PD-L1 expression on immune cells (IHC 3) was 83%; the response rate in patients with no staining (IHC 0) was 20%. Of note, a response rate of 20% still compares favorably to response rates seen with cytotoxic chemotherapy for previously treated NSCLC (33).

In summary, studies so far suggest that patients with PD-L1 expression have a higher likelihood of responding to PD-1 or PD-L1 inhibition. These results, however, are all from single-arm trials, and some patients with PD-L1 negative tumors have responded to therapy. The clinical utility of this biomarker has not yet been determined. Ongoing phase III trials randomizing patients to either checkpoint inhibition or standard chemotherapy are incorporating analysis of PD-L1, in addition to other markers. These results should define the role of this marker in selecting patients for therapy. Of note, nivolumab was FDA approved for previously treated squamous cell carcinoma of the lung, without reference to PD-L1 status (10).

**Mutational burden**

Some of the cancers in which immunotherapy seems particularly active (melanoma, NSCLC, and head and neck squamous cell carcinoma) are cancers with high mutational loads. It has been hypothesized that a high mutation burden correlates with creation of neoantigens, which may be targets for immune cells activated by checkpoint inhibition (34). Several studies have examined the correlation between number of somatic mutations detected by whole exome sequencing and response to immunotherapy agents.

Elegant work by Snyder et al. has demonstrated that mutational load in melanoma is associated with clinical benefit to anti-CTLA-4 antibodies tremilimumab and ipilimumab. Specific neoepitopes were identified that predict for benefit—these may resemble epitopes from certain viruses (35).

In the KEYNOTE-001 study in NSCLC, current or former smokers had higher response rates to pembrolizumab than non-smokers (22.5% vs. 10.3%) (11). Smokers tend to have far more mutations in their tumors than never smokers; 10 times more in one study (36). Additional studies on tumor samples from this study showed that higher mutation burden was associated with higher rates of durable clinical benefit [partial response (PR) or stable disease lasting longer than 6 months], higher response rates, and longer PFS (34).

Though these results are intriguing, whole genome sequencing has not yet been incorporated into routine clinical care, and mutational burden does not yet have a role in selecting patients for therapy.

**Management of immunotherapy related toxicity**

The agents described above are generally well tolerated;
however, severe toxicities can occur. Pneumonitis, colitis, and hepatitis have been noted with these agents and can be fatal. Appropriate and timely management of the unique toxicities associated with these agents in critically important; this topic is discussed by Villadolid and Amin in detail in another review in this issue.

Conclusions

It is clear that immunotherapy will play an increasing role in the therapy of lung cancer. Over the next year, the results of several randomized phase III trials comparing checkpoint inhibition with standard chemotherapy will be reported. These studies will help to establish the role of immunotherapy in the treatment of advanced NSCLC. Future research should focus on identification of patients most likely to benefit from therapy, and on rational combination therapy.

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None.

Footnote

Conflicts of Interest: Dr. Carrizosa: Honoraria—Boehringer Ingelheim, Advisory Board—Pfizer. Dr. Gold: Honoraria—Bristol-Myers Squibb, Advisory Board—Pfizer.

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