The potential of combined immunotherapy and antiangiogenesis for the synergistic treatment of advanced NSCLC.

Manegold C , Dingemans AC, Gray JE, Nakagawa K, Nicolson M, Peters S, Reck M, Wu YL, Brustugun OT, Crino L, Felip E, Fennell D, Garrido P, Huber RM, Marabelle A, Moniuszko M, Mornex F17, Novello S, Papotti M, Pérol M, Smit EF, Syrigos K, van Meerbeeck JP, van Zandwijk N, Chih-Hsin Yang J, Zhou C, V8okes E.

The potential of combined immunotherapy and antiangiogenesis for the synergistic treatment of advanced NSCLC.

Over the past few years, there have been considerable advances in the treatments available to patients with metastatic or locally advanced NSCLC, particularly those who have progressed during first-line treatment. Some of the treatment options available to patients are discussed here, with a focus on checkpoint inhibitor immunotherapies (nivolumab and pembrolizumab) and antiangiogenic agents (bevacizumab, ramucirumab, and nintedanib). It is hypothesized that combining immunotherapy with antiangiogenic treatment may have a synergistic effect and enhance the efficacy of both treatments. In this review, we explore the theory and potential of this novel treatment option for patients with advanced NSCLC. We discuss the growing body of evidence that proangiogenic factors can modulate the immune response (both by reducing T-cell infiltration into the tumor microenvironment and through systemic effects on immune-regulatory cell function), and we examine the preclinical evidence for combining these treatments. Potential challenges are also considered, and we review the preliminary evidence of clinical efficacy and safety with this novel combination in a variety of solid tumor types.

Keywords

- Antiangiogenesis;
- Combination therapy;
- Immunotherapy;
- NSCLC

Introduction

Over the past decade, there have been considerable advances in the treatment of metastatic or locally advanced NSCLC, particularly in the treatment of patients experiencing progression during or after first-line treatment. First-line treatment for patients with advanced NSCLC without targetable tumor-specific mutations involves the administration of platinum-based chemotherapy doublets with or without bevacizumab or necitumumab. Several targeted therapies are also approved for use by the European Medicines Agency (EMA) and/or the U.S. Food and Drug Administration (FDA) for the first-line treatment of patients with advanced NSCLC with known oncogene addiction. Gefitinib, erlotinib, and afatinib, which are EGFR-specific tyrosine kinase inhibitors (TKIs), are FDA and EMA approved in patients with EGFR activating mutations. Crizotinib, which targets the anaplastic lymphoma kinase (ALK) receptor tyrosine kinase, is also FDA and EMA approved for ALK-positive patients and approved by the FDA for use in patients with *ROS1* gene alterations.

Despite improvements in first-line therapy, all patients with advanced disease will eventually require further treatment. Recently, a number of agents developed for use in patients without oncogene addiction who progress during or after chemotherapy have been approved by the EMA and/or the FDA. Largely, these fall into one of two categories: antiangiogenic agents and checkpoint inhibitor immunotherapies. Antiangiogenic agents include nintedanib (EMA approved), which is a triple angiokinase inhibitor for use in patients with NSCLC of the adenocarcinoma histologic type, and ramucirumab (FDA and EMA approved), which is a humanized monoclonal antibody targeting vascular endothelial growth factor receptor-2 (VEGFR2). Checkpoint inhibitor immunotherapies that are

available for the treatment of NSCLC include nivolumab and pembrolizumab, which are monoclonal antibodies that inhibit the programmed cell death protein 1 (PD-1) receptor. Nivolumab is EMA and FDA approved, whereas pembrolizumab is approved by the FDA when 50% or more of the tumor cells express programmed death ligand 1 (PD-L1) membrane staining of any intensity, as assessed by immunohistochemistry (IHC). Further immunotherapies are also in late-stage development and are discussed later in this review.[7] In addition to antiangiogenic agents and checkpoint inhibitor immunotherapies, afatinib has also been approved by the FDA and has received a positive Committee for Medicinal Products for Human Use opinion for the treatment of squamous NSCLC progressing after platinum-based chemotherapy. Moreover, several targeted therapies are also available for patients with progressive disease and known oncogene addiction. These include alectinib (FDA approved) and ceritinib (FDA and EMA approved), both approved for use in ALK-positive patients who have progressed while receiving crizotinib or are intolerant to it. Osimertinib, an EGFR inhibitor, is EMA and FDA approved in T790M mutation–positive patients irrespective of prior treatment, although data in the first-line setting are limited.

However, despite advances, many patients do not benefit from these therapies, and resistance will develop even in those who initially respond. Thus, there is a clear unmet need to improve the overall survival (OS) in most previously treated patients, while maintaining quality of life and improving tolerability of treatment. This need is particularly urgent in patients who do not have targetable oncogenic mutations or who fail to respond to targeted treatment. Emerging evidence that proangiogenesis factors have immunosuppressive activity has led investigators to evaluate the potentially synergistic combination of antiangiogenic agents and immunotherapy in the treatment of several cancers, including advanced NSCLC.These investigations are of particular interest in patients who are not expected to benefit from monotherapy with PD-1/PD-L1 inhibitors, such as those with PD-L1–negative tumors or, potentially, patients who are refractory to first-line therapy.

In this narrative review, we provide an overview of treatment options for patients with advanced NSCLC, including the use of antiangiogenic or immunotherapeutic agents. We discuss the preclinical and clinical data exploring the potential and the challenges of combining antiangiogenesis with immunotherapy as a novel treatment option.

Angiogenesis Inhibitors

Angiogenesis is essential for primary tumor growth, and tumors are characterized by poorly organized and abnormal vessels with altered permeability. VEGF is the principal regulator of angiogenesis, stimulating proangiogenic signaling pathways by binding to its receptor, VEGFR2. Other key growth factors implicated in the regulation of angiogenesis include the platelet-derived growth factor and fibroblast growth factor families.

The inhibition of angiogenesis signaling pathways is a well-established treatment modality in oncology. Angiogenesis inhibitors used to treat NSCLC fall into two categories: monoclonal antibodies targeting VEGF or VEGFR and small molecule TKIs.

Antiangiogenic monoclonal antibodies that are currently approved for use in the treatment of NSCLC are bevacizumab and ramucirumab, targeting VEGF and VEGFR2, respectively. Bevacizumab, in addition to platinum-based chemotherapy, is EMA and FDA approved for the first-line treatment of advanced, metastatic, or recurrent NSCLC, excluding predominantly squamous cell histologic features. In a meta-analysis of randomized phase II/III trials in treatment-naive patients with advanced NSCLC, bevacizumab plus platinum-based chemotherapy significantly increased the OS versus chemotherapy alone (hazard ratio [HR] = 0.90, 95% confidence interval [CI]: 0.81–0.99, p = 0.03). There was a significantly increased risk for several grade 3 or higher

adverse events (AEs), although no unexpected patterns of toxicity emerged. For patients who have disease progression while or after receiving platinum-based chemotherapy, there are now options for antiangiogenic agents in combination with docetaxel.
Ramucirumab in combination with docetaxel is EMA and FDA approved for the treatment of metastatic NSCLC. The phase III REVEL trial compared docetaxel plus ramucirumab with docetaxel plus placebo in patients with advanced NSCLC (any histologic type) who progressed after platinum doublet chemotherapy.[11] Median OS in the docetaxel plus ramucirumab arm was 10.5 months versus 9.1 months in the docetaxel plus placebo arm (HR = 0.86, 95% CI: 0.75–0.98, $p = 0.023$). Overall, no significant increase in grade 3 or higher hemorrhage was observed in patients receiving ramucirumab, and most toxicities were manageable with appropriate dose reductions and supportive care.
Until recently, the use of antiangiogenic TKI therapy in the treatment of NSCLC has been a decidedly unsuccessful strategy; numerous agents have failed to demonstrate meaningful improvements in OS, while also showing generally unfavorable toxicity profiles. However, nintedanib in combination with docetaxel was recently approved by the EMA for treatment of advanced, metastatic, or locally recurrent NSCLC of the adenocarcinoma tumor histologic type after first-line chemotherapy. Nintedanib is a novel triple angiokinase inhibitor that inhibits the VEGF, platelet-derived growth factor, and fibroblast growth factor signaling pathways In a phase III trial (LUME-Lung 1), nintedanib in combination with docetaxel demonstrated clinically meaningful benefits in patients who progressed after first-line treatment with chemotherapy. The primary end point was progression-free survival (PFS), with OS being the key secondary end point, in predefined subgroups in a stepwise order. In the first predefined population of patients with the adenocarcinoma histologic type who progressed within 9 months after the start of first-line treatment, the median OS was significantly improved in the docetaxel plus nintedanib group (10.9 months versus 7.9 months; HR = 0.75, 95% CI: 0.60–0.92, $p = 0.0073$). Similarly, significant improvements in median OS were obtained in the second predefined population of all patients with the adenocarcinoma histologic type (12.6 months in the docetaxel plus nintedanib group versus 10.3 months; HR = 0.83, 95% CI: 0.70–0.99, $p = 0.0359$). An exploratory analysis was also carried out in patients with adenocarcinoma who were refractory to first-line treatment (progressive disease as best response achieved). The median OS was longer with docetaxel plus nintedanib than with docetaxel plus placebo (9.8 months versus 6.3 months; HR = 0.62, 95% CI: 0.41–0.94, $p = 0.0246$). Although these efficacy results represent clinically meaningful gains for patients, it is worth noting that mutational analysis of the adenocarcinoma histologic type was lacking in this clinical trial. At the time of initiation of the LUME-Lung 1 study, *EGFR* biomarker testing was not standard clinical practice but analysis of the cohort for *EGFR* mutations was available for 19.4% of the population of patients with adenocarcinoma (n = 128); of these patients, only 16 (12.5%) were *EGFR* mutation–positive, with five and 11 *EGFR* mutation–positive tumors in the nintedanib and placebo arms, respectively (Boehringer Ingelheim, unpublished data, 2014).
The LUME-Lung 2 study investigated nintedanib in combination with pemetrexed versus placebo-pemetrexed for the treatment of patients with advanced or recurrent nonsquamous NSCLC who had relapsed or failed one prior line of chemotherapy. Pemetrexed is widely used as a second-line agent for the treatment of nonsquamous NSCLC, and the results showed a significant improvement in independent centrally reviewed PFS with nintedanib/pemetrexed over placebo/pemetrexed (median 4.4 months versus 3.6 months; HR = 0.83, 95% CI: 0.70–0.99, $p = 0.0435$) at the time of the final analysis. On the basis of a preplanned investigator-assessed futility analysis, LUME-Lung 2 recruitment was stopped prematurely, but combining nintedanib with pemetrexed

significantly prolonged PFS in patients with advanced nonsquamous NSCLC after first-line chemotherapy, with a manageable safety profile.
The general safety profile of nintedanib in combination with docetaxel was as expected. Gastrointestinal AEs and increased liver enzyme levels were the most common AEs, and there was a low incidence of AEs typically associated with antiangiogenic agents. These were manageable by application of standard oncological principles of judicious dose reductions and symptomatic treatment. Efficacy results from key phase III trials of angiogenesis inhibitors in NSCLC are reviewed in Table 1.

Table 1.
Efficacy Results from Phase III Trials of Angiogenesis Inhibitors in NSCLC

Study	ClinicalTrials.gov Identifier	Patients	Compounds (Dose)	Median OS, mo (95% CI)	Median PFS, mo (95% CI)	ORR
ECOG4599	NCT00021060	Stage IIIB/IV nonsquamous NSCLC (N = 878)	Paclitaxel + carboplatin vs. paclitaxel + carboplatin + bevacizumab	10.3 (NR) vs. 12.3 (NR) HR = 0.79 (0.67–0.92), *p* = 0.003	4.5 (NR) vs. 6.2 (NR) HR = 0.66 (0.57–0.77), *p* < 0.001	15% vs. 35%, *p* < 0.001
BEYOND	NCT01364012	Chinese patients with stage IIIB/IV nonsquamous NSCLC (N = 276)	Paclitaxel + carboplatin vs. paclitaxel + carboplatin + bevacizumab	17.7 (NR) vs. 24.3 (NR) HR = 0.68 (0.50–0.93), *p* = 0.0154	6.5 (NR) vs. 9.2 (NR) HR = 0.40 (0.29–0.54), *p* < 0.01	25% vs. 54%, *p* < 0.001
AVAiL	NCT00806923	Stage IIIB/IV nonsquamous NSCLC (N = 1043)	Cisplatin + gemcitabine + bevacizumab (7.5 mg/kg or 15 mg/kg) vs. placebo	13.6 (11.8–15.8) and 13.4 (11.1–15.1) vs. 13.1 (11.8–15.2) HR = 0.93 (0.78–1.11), *p* = 0.42; and HR = 1.03 (0.86–1.23), *p* = 0.761	6.7 (NR) and 6.5 (NR) vs. 6.1 (NR) HR = 0.75 (0.64–0.87), *p* = 0.0003; and HR = 0.85 (0.73–1.00), *p* = 0.0456	38% and 35% vs. 22%, *p* ≤ 0.0001 and *p* = 0.0002
REVEL	NCT01168973	Stage IV squamous or nonsquamous NSCLC	Docetaxel + ramucirumab vs. docetaxel +	10.5 (IQR 5.1–21.2) vs.	4.5 (IQR 2.3–8.3) vs. 3.0 (IQR	23% vs. 14%, *p* < 0.0001

Study	ClinicalTrials.gov Identifier	Patients	Compounds (Dose)	Median OS, mo (95% CI)	Median PFS, mo (95% CI)	ORR
		progressed after first-line chemotherapy (N = 1253)	placebo	9.1 (IQR 4.2–18.0) HR = 0.86 (0.75–0.98), *p* = 0.023	1.4–6.9) HR = 0.76 (0.68–0.86), *p* < 0.0001	
LUME-Lung 1	NCT00805194	Stage IIIB/IV NSCLC progressed after first-line chemotherapy (patients with adenocarcinoma histologic type who progressed within 9 mo after start of first-line treatment [n = 405]; all patients with adenocarcinoma histologic type [N = 658])	Docetaxel + nintedanib vs. docetaxel + placebo (patients with adenocarcinoma histologic type who progressed within 9 mo after start of first-line treatment/all patients with adenocarcinoma histologic type)	10.9 (8.5–12.6) vs. 7.9 (6.7–9.1) HR = 0.75 (0.60–0.92), *p* = 0.0073/ 12.6 (10.6–15.1) vs. 10.3 (8.6–12.2) HR = 0.83 (0.70–0.99), *p* = 0.0359	3.6 (2.8–4.3) vs. 1.5 (1.4–2.6) HR = 0.63 (0.48–0.83), *p* = 0.0008/NR HR = 0.77 (0.62–0.96), *p* = 0.0193	4.9% vs. 1.5%, *p* = 0.0393/ 4.7% vs. 3.6%, *p* = 0.4770
LUME-Lung 2	NCT00806819	Stage IIIB/IV nonsquamous NSCLC that progressed after first-line chemotherapy (N = 713)	Nintedanib + pemetrexed vs. placebo + pemetrexed	12.2 vs. 12.7 HR = 1.03 (0.85–1.24), *p* = 0.7921	4.4 vs. 3.6 HR = 0.83 (0.70–0.99), *p* = 0.0435	9.1% vs. 8.3%, NR

OS, overall survival; CI, confidence interval; PFS, progression-free survival; ORR, objective response rate; NR, not reported; HR, hazard ratio, IQR, interquartile range.

a
Limited study data are currently available as a result of termination of the study on the basis of a preplanned futility analysis of investigator-reviewed PFS. Retrospective analysis showed that if the futility analysis had been conducted at a different time point or used centrally reviewed data, termination might not have been recommended.

Immune Checkpoint Inhibitors

In recent years, immunotherapies targeting the T-cell immune checkpoint receptor PD-1, or its ligand PD-L1 and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), have brought significant improvements in some cancer outcomes. The rationale for the utility of immune checkpoint receptor therapy can be summarized as follows: the PD-1 pathway is a T-cell–inhibitory pathway that is induced by binding of the PD-1 receptor on the T-cell

plasma membrane to PD-L1 on the tumor. Tumor cells have hijacked this pathway by upregulating PD-L1 expression, thereby preventing T-cell–mediated destruction. Thus, a PD-1/PD-L1 antagonistic antibody would allow antitumor T cells to be fully activated and generate tumor cell killing. Likewise, the binding of CTLA-4 on activated T cells to the B7 ligand on antigen-presenting dendritic cells results in reduction of the duration and magnitude of functional T-cell activation. Antagonistic anti–CTLA-4 antibodies blocking the CTLA-4–B7 interaction can relieve this inhibitory signal and enhance T-cell activity.

In a rapidly evolving area, two PD-1 inhibitors are currently approved for the treatment of NSCLC: nivolumab and pembrolizumab (September 2016). Nivolumab is a fully human immunoglobulin G4 (IgG4) anti–PD-1 monoclonal antibody that is EMA and FDA approved for use in patients with metastatic NSCLC and progression during or after platinum-based chemotherapy. In two phase III trials, one in patients with the squamous histologic type (Checkmate 017) and the other with the nonsquamous histologic type (Checkmate 057), a reduction in the risk for death was demonstrated with nivolumab versus with docetaxel in previously treated patients. In Checkmate 017, the median OS was significantly longer with nivolumab than with docetaxel (9.2 months versus 6.0 months; HR = 0.59, 95% CI: 0.44–0.79, $p < 0.001$). In Checkmate 057, the median OS was 12.2 months with nivolumab compared with 9.4 months with docetaxel (HR = 0.73, 95% CI: 0.59–0.89, $p = 0.002$). [9] In Checkmate 017, there was also significant improvement in PFS with nivolumab versus with docetaxel (3.5 months versus 2.8 months; HR = 0.62, 95% CI: 0.47–0.81, $p < 0.001$). PD-L1 expression was not predictive or prognostic in patients with the squamous histologic type. In contrast, in Checkmate 057 (nonsquamous histologic type), there was no PFS benefit with nivolumab versus with docetaxel (median PFS 2.3 months versus 4.2 months; HR = 0.92, 95% CI: 0.77–1.11, $p = 0.39$). However, at the interim analysis, there appeared to be a strong predictive association between increasing extent of PD-L1 expression and improved clinical outcome, for all efficacy outcomes, with nivolumab versus with docetaxel. In both trials, fewer grade 3 or higher treatment-related AEs (TRAEs) were reported in the nivolumab group than in the docetaxel group (Checkmate 017, 7% versus 55%; Checkmate 057, 10% versus 54%, respectively).

Pembrolizumab is a humanized IgG4 anti–PD-1 monoclonal antibody that has been approved by the FDA and EMA for the treatment of patients with metastatic NSCLC whose tumors express PD-L1 and who have disease progression during or after platinum-based chemotherapy. In a phase II/III trial (KEYNOTE-010), patients with previously treated NSCLC with PD-L1 expression in 1% or more of tumor cells (centrally assessed with the Dako IHC assay [Dako, Carpenteria, CA] using murine 22C3 antihuman PD-L1 antibody [Merck, Kenilworth, NJ]) were randomly assigned to receive pembrolizumab, 2 mg/kg, pembrolizumab, 10 mg/kg, or docetaxel, 75 mg/m^2. OS was significantly longer for pembrolizumab, 2 mg/kg, (10.4 months) versus for docetaxel (8.5 months; HR = 0.71, 95% CI: 0.58–0.88, $p = 0.0008$) and for pembrolizumab, 10 mg/kg, (12.7 months) versus docetaxel (HR = 0.61, 95% CI: 0.49–0.75, $p < 0.0001$), whereas PFS benefits were only significantly improved with pembrolizumab in patients with 50% or more of tumor cells expressing PD-L1. Further approvals in this second-line indication may occur in patients expressing PD-L1 at levels in 1% or more of tumor cells, and it is also being considered for first-line treatment in patients with higher levels of PD-L1 expression. Grade 3 to 5 TRAEs were less common with pembrolizumab than with docetaxel (13% in patients given pembrolizumab, 2 mg/kg, 16% in those given pembrolizumab, 10 mg/kg, and 35% in those given docetaxel), and the frequency of immune-related AEs was manageable (1%–5%).

Monoclonal antibodies targeting PD-L1 are also in advanced clinical development. Atezolizumab, a humanized IgG engineered for low FcγR affinity, has been granted breakthrough therapy designation by the FDA on the basis of preliminary phase I efficacy results in previously treated patients with advanced NSCLC. Further support for this

therapy stems from the results of the phase II POPLAR trial, also in patients with NSCLC who had progressed while receiving postplatinum chemotherapy. OS in the intention-to-treat population, the primary end point, was 12.6 months with atezolizumab arm versus 9.7 months with docetaxel (HR = 0.73, 95% CI: 0.53–0.99, $p = 0.04$). There was an association between increasing improvements in OS and increasing PD-L1 expression; PD-L1 expression was assessed by IHC both on tumor cells (percentage of total tumor cells grouped as less than 1%, 1% to <5%, ≥5% to <50%, or ≥50%) and on tumor-infiltrating immune cells (percentage of tumor area grouped as <1%, ≥1% to <5%, ≥5% to <10%, or ≥10%). Atezolizumab was well tolerated, with 11% of patients experiencing grade 3 to 4 TRAEs versus 39% with docetaxel. Several phase III studies with atezolizumab are ongoing in patients with nonsquamous NSCLC, and the treatment looks likely to gain regulatory approval in NSCLC, pending favorable phase III data. Further investigational checkpoint inhibitors undergoing phase III trials in NSCLC include the human PD-L1 monoclonal antibodies, durvalumab (IgG4) and avelumab (IgG1). Efficacy results from phase II/III trials of PD-1/PD-L1 inhibitors in NSCLC are summarized in Table 2. With anti–PD-1 and anti–PD-L1 therapies, immune-related AEs, including pneumonitis, are infrequent and of low severity, but grade 3 to 4 drug-related immune toxicities that require early diagnosis and rapid medical management can develop in a small percentage of patients.

Table 2.
Efficacy Results from Phase II/III Trials of Immune Checkpoint Inhibitors in NSCLC

Study	ClinicalTrials.gov identifier	Patients	Compounds (Dose)	Median OS, mo (95% CI)	Median PFS, mo (95% CI)	ORR (95% CI)
CheckMate-017	NCT01642004	Stage IIIB/IV, previously treated squamous NSCLC (n = 272)	Nivolumab (3 mg/kg) vs. docetaxel (75 mg/m^2)	9.2 (7.3–13.3) vs. 6.0 (5.1–7.3) HR = 0.59 (0.44–0.79), $p < 0.001$	3.5 (2.1–4.9) vs. 2.8 (2.1–3.5) HR = 0.62 (0.47–0.81), $p < 0.001$	20% (14–28) vs. 9% (5–15), $p = 0.008$
CheckMate-057	NCT01673867	Stage IIIB/IV, previously treated nonsquamous NSCLC (n = 582)	Nivolumab (3 mg/kg) vs. docetaxel (75 mg/m^2)	12.2 (9.7–15.0) vs. 9.4 (8.1–10.7) HR = 0.73 (0.59–0.89), $p = 0.002$	2.3 (2.2–3.3) vs. 4.2 (3.5–4.9) HR = 0.92 (0.77–1.11), $p = 0.39$	19% (15–24) vs. 12% (9–17), $p = 0.02$
KEYNOTE-010	NCT01905657	Stage IIIB/IV, previously treated nonsquamous or squamous NSCLC (n = 1034)[a]	Pembrolizumab (2 mg/kg and 10 mg/kg) vs. docetaxel (75 mg/m^2)	10.4 (9.4–11.9) and 12.7 (10.0–17.3) vs. 8.5 (7.5–9.8) HR = 0.71 (0.58–0.88), $p = 0.008$ and HR = 0.61 (0.49–0.75), $p < 0.0001$[b]	3.9 (3.1–4.1) and 4.0 (2.7–4.3) vs. 4.0 (3.1–4.2) HR = 0.88 (0.74–1.05), $p = 0.07$ and HR = 0.79 (0.66–0.94), $p = 0.004$[b]	18% (NR) and 18% (NR) vs. 9% (NR), $p = 0.0005$ and $p = 0.0002$[b]
POPLAR	NCT01903993	Stage IIIB/IV, previously	Atezolizumab (1200 mg fixed dose)	12.6 (9.7–16.4) vs. 9.7 (8.6–12.0)[d]	7.8 (NR) vs. 3.9 (NR) HR = 0.60	17% (11–23.8)

Study	ClinicalTrials.gov identifier	Patients	Compounds (Dose)	Median OS, mo (95% CI)	Median PFS, mo (95% CI)	ORR (95% CI)
		treated nonsquamous or squamous NSCLC (n = 287)[c]	vs. docetaxel (75 mg/m^2)	HR = 0.73 (0.53–0.99), p = 0.04	(0.31–1.16)	vs. 15% (9.3–21.4)

OS, overall survival; CI, confidence interval; PFS, progression-free survival; ORR, objective response rate; HR, hazard ratio; NR, not reported; PD-L1, programmed death ligand 1.

a
This was a randomized, open-label phase II/III study.

b
Results are shown for the total analysis population (patients with PD-L1 expression of at least 1%). For the subgroup of patients with PD-L1 expression on at least 50% of tumor cells, please see main text.

c
Randomized, open-label phase II study.

d
PFS was reported only in the subgroup showing the highest expression of PD-L1 (≥50%).

Synergistic Combination of Antiangiogenesis and Immunotherapy

Current Hypotheses and Preclinical Rationale

There is a growing body of evidence describing a complex relationship between angiogenesis and the immune system. It is also becoming apparent that antiangiogenic agents can stimulate the immune system, and it has been suggested that immunotherapies can also be antiangiogenic. This suggests that when combined, these two types of therapies could operate synergistically to target tumors (Fig. 1). Moreover, evidence indicates that the potential of such a combination regimen may be realized without a substantial increase in AEs. The rationale for the approach and the current preclinical evidence are discussed in more detail in the following paragraphs.

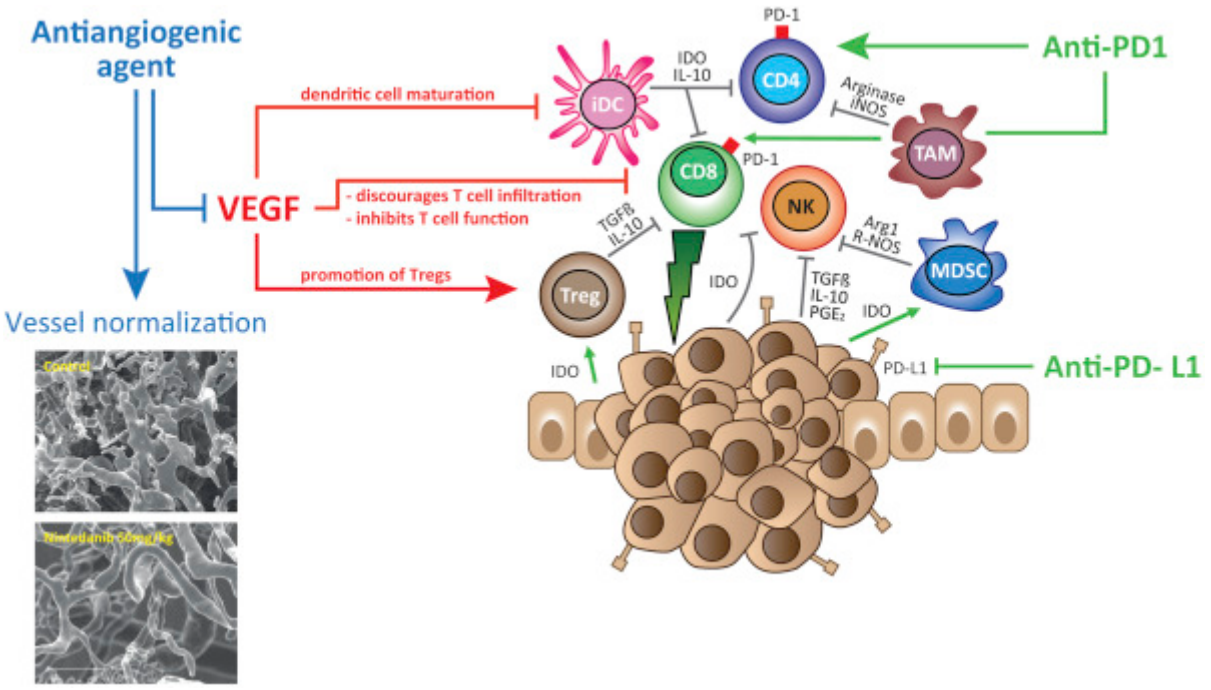

Figure 1.
Combined inhibition of tumor angiogenesis and the immune checkpoint, programmed cell death protein 1 (PD-1). Abbreviations: Arg1, arginase 1; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; iDC, immature dendritic cell; IDO, indoleamine 2, 3-dioxygenase; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD-L1, programmed death ligand 1; PGE_2, prostaglandin E_2; TAM, Tyro3 Axl and Mer; R-NOS, reactive nitrogen oxide species; TGFβ, transforming growth factor β; Treg, T-regulatory cell; VEGF, vascular endothelial growth factor.
Adapted with permission from Metpally RPR, et al.

Over the past 20 years, evidence has accumulated that VEGF not only promotes angiogenesis but also acts as a key mediator of the immunosuppressive microenvironment that enables tumor cells to evade immunosurveillance. VEGF signaling has been shown to attenuate the antitumor response through multiple mechanisms that can be divided into two modes of action. First, VEGF influences lymphocyte trafficking across endothelia to the tumor by inhibiting lymphocyte adhesion to activated endothelial cells and through an association with defects in endothelial intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 clustering at the endothelial cell surface, thereby blocking T-cell infiltration into tumors, and by prevention of T-cell mobilization and trafficking into the tumor through its effect on the Fas ligand. Second, VEGF has a systemic effect on immune-regulatory cell function through multiple mechanisms, including the following: induction and proliferation of inhibitory immune cell subsets, such as T-regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs); suppression of dendritic cell maturation; and inhibition of T-cell development from hematopoietic progenitor cells. Given the immunosuppressive role of VEGF and angiogenesis within tumors, it is not surprising that there is evidence that antiangiogenic agents stimulate the immune response and enhance the efficacy of immunotherapies. The multitarget TKI sunitinib decreased PD-1 expression in tumor-infiltrating T cells in advanced tumor–bearing mice and significantly increased the infiltration of CD8-positive and CD4-positive T cells into the tumor. Moreover, it reduced the number of Tregs and MDSCs in addition to impairing the suppressive function of MDSCs. In patients with renal cell carcinoma (RCC), sunitinib also decreased Treg and MDSC accumulation. Likewise, cabozantinib (a TKI inhibiting VEGFR2 among other targets), alone or in combination with MVA/rF-CEA/TRICOM (an

anticancer vaccine with immune-stimulatory properties), reduced the number of Tregs and MDSCs in mice although significantly increasing the CD4-positive and CD8-positive T-cell infiltration. One reason for the increase seen in immune cell infiltration into tumors is thought to be the effect of antiangiogenic agents on tumor perfusion. Antiangiogenic agents can reduce tumor compactness, which is thought to relieve pressure on existing blood vessels and despite reduced tumor vasculature, result in both improved perfusion and oxygenation of the tumor microenvironment.

The potential for increased efficacy by combining an antiangiogenic agent and immunotherapy is supported by preclinical evidence across several tumor types. In two in vivo cancer models, B16F10 melanoma and CT26 colon carcinoma cells, VEGF inhibition was achieved through systemic expression of a chimeric VEGFR that binds VEGF and blocks its function. When VEGF inhibition combined with granulocyte-macrophage colony-stimulating factor–secreting tumor cell immunotherapy, the survival of the animals was significantly increased. Similarly, coadministering cabozantinib with MVA/rF-CEA/TRICOM significantly reduced the growth of MC38-CEA cancer cells in an in vivo model, compared with in the control of MVA/rF-CEA/TRICOM alone, and it resulted in durable regression of the tumors. In three different in vivo NSCLC models, combining immunotherapy (adoptive cellular immunotherapy using cytokine-induced killer [CIK] cell transfer) and inhibition of angiogenesis (with rh-endostatin) significantly inhibited the growth of the tumor, whereas neither treatment alone had a significant effect. Likewise, combined CIK cell therapy and bevacizumab in an in vivo lung adenocarcinoma model synergistically inhibited the growth of the tumors. There was increased infiltration of CIK cells into the tumor versus with other treatments. In a model of colon adenocarcinoma, simultaneous inhibition of PD-1 and VEGFR2 with monoclonal antibodies also inhibited tumor growth significantly compared with monotherapy treatments, but without inducing overt toxicity. This evidence has led to clinical investigations exploring the potential of combined antiangiogenesis and immunotherapy for the treatment of NSCLC. In addition, given the favorable toxicity profiles of both classes of agents, it was anticipated that this novel therapy combination would be well tolerated.

Despite the potential efficacy benefits, combination therapy with an antiangiogenic agent and immunotherapy is not without challenges. One of the most pressing factors to be addressed in the future will be the identification of which patients might gain the most benefit from this novel combination, necessitating robust biomarkers to aid selection of these patients. This combination approach might benefit patients with poor prognosis, such as those not expected to benefit from checkpoint inhibitor monotherapy, including patients who are refractory to first-line therapy or who have PD-L1–negative tumors. In addition to the challenge of patient selection, the optimal sequence and the timing for each combination has to be determined, and for some this will be crucial. The dose of each agent may also be important, as it has been shown that high doses of antiangiogenic agents can have inferior immune-stimulating effects when combined with immunotherapy.

Clinical Data

Multiple trials are currently investigating combinations of angiogenesis inhibitors and immunotherapies in a range of cancers. These trials are supported by preliminary phase I data evaluating the efficacy and safety of these novel therapy combinations in NSCLC, gastric or gastroesophageal junction (G/GEJ) adenocarcinoma, urothelial carcinoma, metastatic colorectal cancer (mCRC), and metastatic RCC (mRCC). Preliminary results are described in the paragraphs that follow, and Table 3 provides information about ongoing phase I trials assessing the combination of bevacizumab, ramucirumab, or nintedanib plus immune checkpoint antibodies in patients with advanced NSCLC.

Table 3.
Phase I Trials Evaluating the Combination of Antiangiogenic Agents and Immune Checkpoint Inhibitors in NSCLC

Relevant Compound(s)	Study Title	ClinicalTrials.gov Identifier	Estimated Enrollment (Total Study)	Primary End Points	Relevant Regimens (Immunotherapy + Angiogenesis Inhibitor in NSCLC)	Relevant Patient Population	Estimated Completion Date
Bevacizumab, nivolumab, ipilimumab	A Multi-arm Phase I Safety Study of Nivolumab in Combination with Gemcitabine/ Cisplatin, Pemetrexed/ Cisplatin, Carboplatin/ Paclitaxel, Bevacizumab Maintenance, Erlotinib, Ipilimumab or as Monotherapy in Subjects with Stage IIIB/IV NSCLC (CheckMate-012)	NCT01454102	412	Safety and tolerability of nivolumab + chemotherapy	Cohort D: nivolumab + bevacizumab maintenance	Newly diagnosed and confirmed stage IIIB/IV NSCLC	November 2017
Bevacizumab, pembrolizumab	A Phase I/II Study of MK-3475 (SCH900475) in Combination with Chemotherapy or Immunotherapy in Patients with Locally Advanced or Metastatic NSCLC	NCT02039674	308	Part I, all cohorts: the recommended phase II dose for pembrolizumab + chemotherapy or immunotherapy	Part I, cohort B: pembrolizumab + paclitaxel + carboplatin + bevacizumab	Stage IIIb/IV NSCLC	June 2019
Bevacizumab atezolizumab	A Phase Ib Study of the Safety and Pharmacology of MPDL3280A Administered	NCT01633970	225	Incidence of AEs; DLTs/MTD	Cohort A: atezolizumab + bevacizumab Cohort B: atezolizum	Locally advanced or metastatic solid tumors (including stage IIIB/IV, or recurrent	September 2017

Relevant Compound(s)	Study Title	ClinicalTrials.gov Identifier	Estimated Enrollment (Total Study)	Primary End Points	Relevant Regimens (Immunotherapy + Angiogenesis Inhibitor in NSCLC)	Relevant Patient Population	Estimated Completion Date
	with Bevacizumab and/or with Chemotherapy in Patients with Advanced Solid Tumors				ab + bevacizumab + FOLFOX	NSCLC)	
Ramucirumab, pembrolizumab	Study of Ramucirumab Plus Pembrolizumab in Participants with Gastric or GEJ Adenocarcinoma, NSCLC or Transitional Cell Carcinoma of the Urothelium	NCT02443324	92	DLTs	Cohort 3: ramucirumab + pembrolizumab in NSCLC patients	Locally advanced and unresectable or metastatic gastric or GEJ adenocarcinoma, NSCLC, or transitional cell carcinoma of the urothelium	February 2017
Nintedanib, pembrolizumab	A Phase Ib Trial of Pembrolizumab and Nintedanib (PEMBIB) in advanced NSCLC	NCT02856425	258	Part I: MTD of nintedanib + pembrolizumab Part Ib: safety and tolerability of nintedanib + pembrolizumab and to evaluate the first efficacy signals	Nintedanib + pembrolizumab	Part I: patients with any advanced solid tumors Part Ib, cohort 1: locally advanced, metastatic or locally recurrent NSCLC (adenocarcinoma histologic type) Part Ib, cohort 2: locally advanced, metastatic or locally recurrent NSCLC (SCC histologic type)	July 2021

Relevant Compound(s)	Study Title	ClinicalTrials.gov Identifier	Estimated Enrollment (Total Study)	Primary End Points	Relevant Regimens (Immunotherapy + Angiogenesis Inhibitor in NSCLC)	Relevant Patient Population	Estimated Completion Date
Nintedanib, nivolumab	A Phase Ib Trial Combining Nivolumab and Nintedanib in Second-Line NSCLC With An Expansion Cohort	TBD (Boehringer Ingelheim, unpublished data, 2016)	50	Part I: MTD of nintedanib + nivolumab Part Ib: safety and tolerability of nintedanib + nivolumab and to evaluate first signs of efficacy in second-line NSCLC (adenocarcinoma histologic type)	Nintedanib + nivolumab	Patients with advanced NSCLC (adenocarcinoma histologic type)	TBD

AE, adverse event; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; GEJ, gastroesophageal junction; FOLFOX, fluorouracil, folinic acid (leucovorin), and oxaliplatin; TBD, to be determined; SCC, squamous cell carcinoma.

In patients with advanced NSCLC who did not progress while receiving first-line platinum-based chemotherapy, the safety and efficacy of switching to nivolumab maintenance therapy, as monotherapy or combined with bevacizumab, was evaluated (NCT01454102). The nivolumab plus bevacizumab arm included only patients with the nonsquamous histologic type, whereas the nivolumab monotherapy arm included patients with both the squamous and nonsquamous histologic types. Median OS was not reached in either arm. In the nivolumab plus bevacizumab arm, median PFS was 37.1 weeks, whereas the median PFS was 16 weeks in patients with squamous cell carcinoma and 21.4 weeks in patients with nonsquamous cell carcinoma in the nivolumab monotherapy arm. The objective response rate (ORR) was similar between the nivolumab plus bevacizumab (8%) and nivolumab-alone (10%) arms. In addition, nivolumab plus bevacizumab demonstrated a tolerable safety profile, with a low frequency of grade 3 or higher TRAEs. In conclusion, switching to nivolumab plus bevacizumab demonstrated a PFS similar to that seen with agents approved for maintenance therapy after platinum-based chemotherapy in advanced NSCLC, with an acceptable side effect profile.

The combination of ramucirumab and pembrolizumab is being evaluated in patients with advanced NSCLC, G/GEJ adenocarcinoma, or urothelial carcinoma who had progressed during prior systemic therapy (NCT02443324). Preliminary results from the dose-limiting

toxicity (DLT) portion of the study show no unexpected safety concerns. No DLTs were reported in patients with NSCLC, and one was reported in a patient with G/GEJ adenocarcinoma.

In patients with mCRC, the safety and efficacy of combined treatment with bevacizumab and the PD-L1 inhibitor atezolizumab was assessed as a combination in refractory patients, or in oxaliplatin-naïve patients in conjunction with FOLFOX (fluorouracil, folinic acid [leucovorin], and oxaliplatin), which is a standard-of-care chemotherapy regimen for mCRC. Preliminary results in refractory patients who were treated with atezolizumab and bevacizumab and had at least one tumor assessment demonstrated an unconfirmed ORR of 8%. In oxaliplatin-naive patients treated with atezolizumab plus bevacizumab and FOLFOX, the unconfirmed ORR in patients with at least one tumor assessment was 36%, and in first-line patients it was 44%. In patients treated with atezolizumab and bevacizumab, 7% of grade 3 or higher AEs were considered to be related to atezolizumab, compared with 20% in patients also treated with FOLFOX. Both combination treatments were well tolerated, and no unexpected AEs emerged.

Several trials are also currently ongoing with antiangiogenic agents and checkpoint inhibitor immunotherapies in patients with mRCC, with preliminary results being available for three phase I trials. In patients with mRCC and at least one tumor assessment, combined treatment with atezolizumab and bevacizumab in the first-line achieved a preliminary ORR of 40% (three of four responses confirmed at the time of data cutoff) and was well tolerated, with no grade 3 or higher AEs being attributed to treatment with atezolizumab (NCT01633970). A dose escalation study that evaluated bevacizumab plus pembrolizumab in patients with mRCC who had progressed while receiving at least one systemic therapy reported no DLTs or serious AEs related to the study drugs at the doses investigated (NCT02348008). Lastly, the combination of nivolumab and either pazopanib or sunitinib (both antiangiogenic agents approved for use in mRCC) is being evaluated in patients with mRCC who have received at least one prior systemic therapy (NCT01472081). An ORR of 45% was demonstrated in the nivolumab plus pazopanib arm, compared with 52% in the nivolumab plus sunitinib arm (expanded to include first-line patients). Grade 3 or higher related AEs occurred in 73% and 60% of patients, respectively, and the safety profile was considered to be manageable. Several trials investigating other combinations of antiangiogenic agents and immunotherapies in RCC are currently
ongoing: NCT02231749, NCT02210117, NCT02014636, NCT02133742, NCT01984242, NCT02420821, and NCT02493751. In addition, trials combining nintedanib with nivolumab and pembrolizumab, respectively, are in preparation. The results of these, and ongoing trials in other tumor types, are eagerly awaited to better understand the potential of combining antiangiogenic agents and immunotherapies.

Promising results have also been reported in a phase I advanced melanoma trial combining bevacizumab and the CTLA-4 checkpoint inhibitor ipilimumab. The combination of ipilimumab and bevacizumab yielded an ORR of 19.6% and a median OS of 25.1 months in patients with metastatic melanoma, almost doubling the survival time obtained in previous studies for ipilimumab alone. Tumor biopsy revealed endothelial activation associated with qualitative increases in T-cell and myeloid/monocyte cell infiltration into tumor deposits. Further evidence for immunological changes resulting from the addition of bevacizumab was an increased number of circulating memory T cells in peripheral blood. Importantly, the combination of bevacizumab and ipilimumab was well tolerated, in contrast to the combination of ipilimumab and nivolumab.

Conclusions

Angiogenesis mediated by VEGF is essential for tumor growth and metastasis, and tumor angiogenesis pathways are established therapeutic targets in NSCLC. Angiogenesis inhibition in the treatment of NSCLC has utilized two main strategies: monoclonal antibodies targeting VEGF (bevacizumab) or VEGFR (ramucirumab) or the small molecule TKI that inhibits multiple angiogenic and proliferative pathways (nintedanib). In recent years, a better understanding of the role of the immune system in repressing tumor growth and the mechanisms by which tumors evade immunosurveillance has stimulated the clinical development of immune checkpoint inhibitors for the treatment of NSCLC. The plethora of phase III studies of PD-1 and PD-L1 inhibitors currently under way will surely lead to an expansion of the use of immunotherapy in the clinical management of NSCLC. Recent findings indicating an intertwined regulation of VEGF signaling and immunosuppression in the tumor microenvironment suggest that the combination of anti-VEGF agents and immune checkpoint blockade could have synergistic antitumor activity, along with favorable tolerability. A number of phase I trials evaluating this novel therapy combination in patients with advanced NSCLC, and other solid tumors, are currently in progress. Existing data on such combinations are promising but preliminary, and many challenges remain to be overcome before the full potential of combined immunotherapy and antiangiogenesis treatment can be realized.

Acknowledgments

We thank all the members of the advisory board Second Expert Panel, which was held at the Post-WCLC 2015 Conference, in Dresden, Germany, on 4–5 November 2015, at which these topics were presented and discussed. We would like to acknowledge the contribution of Dr. Suresh Senan (VU University Medical Centre, Amsterdam, the Netherlands) and Dr. Alex Adjei (Roswell Park Cancer Institute, New York, NY). Nicola Davis, PhD, and Aurora O'Brate, PhD, of inVentiv Health (London, United Kingdom), provided medical writing assistance for this manuscript, funded by Boehringer Ingelheim.

All the authors were members of an advisory board held at the Post–World Conference on Lung Cancer 2015 Conference (November 5, 2015; Dresden, Germany), which was funded by Boehringer Ingelheim.

Disclosure: Dr. Manegold has received consultation fees and travel expenses from Boehringer Ingelheim. Dr. Dingemans has received fees for consultation or advisory boards from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novartis, Pfizer, and Roche. Dr. Gray has received a grant from AstraZeneca and fees from AstraZeneca, Boehringer Ingelheim, and Genentech. Dr. Nakagawa has received research funding from EPS Associated, Japan Clinical Research Operations, Oncotherapy Science, and Quintiles. Dr. Nakagawa has also received research funding and personal fees from Astellas, AstraZeneca, Boehringer Ingelheim, Chugai Pharmaceuticals, Daiichi Sankyo, Eisai, Eli Lilly Japan, Merck Sharp and Dohme, Novartis, Ono Pharmaceuticals, Takeda, and Taiho Pharmaceutical. Dr. Nicolson has received research funding, meeting sponsorship, advisory board fees and nonfinancial support from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, Merck Sharp and Dohme, Novartis, and Roche. Dr. Reck has received honoraria/consultancy fees from AstraZeneca, Boehringer Ingelheim, Celgene, Eli Lilly, Bristol-Myers Squibb, Merck Sharp and Dohme, Novartis, Pfizer, Roche, and Samsung. Dr. Wu has received speaker fees from AstraZeneca, Eli Lilly, Pfizer, Roche, and Sanofi. Dr. Brustugun has received a grant from Boehringer Ingelheim. Dr. Crinò has received personal fees from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, and Pfizer. Dr. Felip has received lecture fees from AstraZeneca, Bristol-Myers Squibb, and Novartis and

consulting or advisory fees from Boehringer Ingelheim, Eli Lilly, Merck Sharp and Dohme, Pfizer, and Roche. Dr. Fennell has received personal fees from Bayer, Bristol-Myers Squibb, Clovis Oncology, Eli Lilly, and Roche/Chuagi. Dr. Garrido has received personal fees from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Celgene, Merck Sharp and Dohme, Novartis, Pfizer, and Roche, as well as nonfinancial support from AstraZeneca, Bristol-Myers Squibb, and Roche. Dr. Huber has received personal fees from Ariad, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Eli Lilly, Pfizer, Pierre Fabre, and Roche. Dr. Moniuszko has received a research grant from Berlin-Chemie/Menarini and honoraria from Berlin-Chemie/Menarini, Boehringer Ingelheim, and Takeda. Dr. Novello has received speaker honoraria from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Merck Sharp and Dohme, and Roche, as well as nonfinancial support from Boehringer Ingelheim. Dr. Papotti has received a grant from Novartis and personal fees from AstraZeneca, Boehringer Ingelheim, Clovis Oncology, Eli Lilly, Novartis, Pfizer, and Roche. Dr. Perol has received honoraria from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Eli Lilly, Novartis, Pfizer, and Roche. Dr. Yang has received personal fees from Astellas, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Clovis Oncology, Eli Lilly, Merck Sharp and Dohme, Merck Sorono, Merrimack, Novartis, Ono Pharmaceuticals, Pfizer, and Roche/Genentech/Chugai. Dr. Zandwijk has received personal fees from AstraZeneca, Boehringer Ingelheim, Eli Lilly, and Roche. Dr. Vokes has received personal fees from Amgen, AstraZeneca, Boehringer Ingelheim, Celgene, Eli Lilly, Genentech, Merck Sharp and Dohme, Synta, and VentiRx. The remaining authors declare no conflict of interest.