ORIGINAL ARTICLE

Bevacizumab Reduces S100A9-Positive MDSCs Linked to Intracranial Control in Patients with *EGFR*-Mutant Lung Adenocarcinoma

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ABSTRACT

Introduction: In vitro models have demonstrated immune-modulating effects of bevacizumab (BEV). Combinations of an EGFR tyrosine kinase inhibitor (TKI) with BEV improve progression-free survival (PFS) in patients with *EGFR*-mutated lung adenocarcinoma. How BEV confers this clinical effect and the underlying mechanisms of its effect are not clear.

Methods: A total of 55 patients with stage 4 *EGFR*-mutated lung adenocarcinoma were enrolled. Myeloid-derived suppressor cells (MDSCs), type 1 and type 2 helper T cells, and cytotoxic T lymphocytes were analyzed by flow cytometry. Clinical data were collected for analysis.

Result: In all, 25 patients received EGFR TKI and BEV combination therapy (the BEV/TKI group) and 30 patients received EGFR TKI monotherapy (the TKI-only group). The BEV/TKI group had longer PFS (23.0 versus 8.6 months [$p = 0.001$]) and, in particular, better intracranial control rates (80.0% versus 43.0% [$p = 0.03$]), a longer time to intracranial progression (49.1 versus 12.9 months [$p = 0.002$]), and fewer new brain metastases (38.0% versus 71.0% [$p = 0.03$]) than the TKI-only group did. The BEV/TKI group had a lower percentage of circulating MDSCs (20.4% ± 6.5% before treatment versus 12.8% ± 6.6% after treatment, respectively [$p = 0.02$]), and higher percentages of type 1 helper T cells (22.9% ± 15.3% versus 33.2% ± 15.6% [$p < 0.01$]) and cytotoxic T lymphocytes (15.5% ± 7.2% versus 21.2% ± 5.6% [$p < 0.01$]) after treatment, changes that were not seen in the TKI-only group. Pretreatment percentage of MDSCs was correlated with PFS, with this correlation attenuated after BEV/TKI treatment. Percentage of MDSCs was also associated with shorter time to intracranial progression.

Conclusion: Combining a EGFR TKI with BEV extended PFS and protected against brain metastasis. Those effects were probably due to the reduction of circulating S100A9-positive

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MDSCs by BEV, which leads to restoration of effective antitumor immunity. Our data also support the rationale for a BEV–immune checkpoint inhibitor combination.

Keywords: EGFR; Angiogenesis; Myeloid-derived suppressor cells; Lung cancer

Introduction

EGFR tyrosine kinase inhibitors (TKIs) have great efficacy and improve quality of life in patients with lung adenocarcinoma harboring activating *EGFR* mutations.[1,2] However, the disease eventually progresses, primarily or distantly, by developing acquired resistance. In the brain, disease control might also be dampened by low central nervous system (CNS) concentrations of EGFR TKIs (hemodynamic failure). Brain metastasis is common in patients with advanced lung cancer (with the incidence being about 20% to 40%) and is a poor prognostic factor for survival.[3] For asymptomatic brain metastasis, EGFR TKIs had an intracranial control rate of 60% to 70%.[4,5] Although the combination of whole brain radiotherapy (WBRT) and an EGFR TKI improved intracranial response rate and prolonged progression-free survival (PFS), it did not prolong overall survival (OS).[6] To reduce unwanted neurotoxic side effects, WBRT deferred until intracranial progression might be sufficient in closely monitored patients with asymptomatic brain metastasis treated with EGFR TKIs.[7] Although EGFR TKIs have shown much better CNS efficacy than conventional chemotherapy has, the brain is still a frequent site of disease recurrence.[8]

Bevacizumab (BEV), which is a humanized immunoglobulin G1 monoclonal antibody to the vascular endothelial growth factor (VEGF), has been demonstrated to provide a survival benefit when used in combination with platinum-based doublet chemotherapy in patients with nonsquamous NSCLC.[9] Adding BEV to chemotherapy robustly increases both extracranial and intracranial efficacy in *EGFR* wild-type nonsquamous NSCLC and probably delays the onset of brain metastasis.[10–12] In *EGFR*-mutant lung adenocarcinoma, combination of BEV with EGFR TKI, albeit similarly in OS comparing to EGFR-TKI alone, did improve response rate and PFS.[13] However, neither the clinical details of the combination of BEV and an EGFR TKI nor its underlying mechanism have been clearly demonstrated in a clinical setting. Myeloid-derived suppressor cells (MDSCs) are known to play important roles in tumor immune evasion and to contribute to tumor metastasis. Tumor-infiltrating monocytic MDSCs facilitate tumor cell dissemination by inducing the epithelial-mesenchymal transition phenotype.[14] MDSCs expressed both VEGF receptor (VEGFR) 1 and VEGFR 2. In a tumor-bearing mouse model, infusion of VEGF could result in accumulation of granulocyte-differentiation antigen-1–positive MDSCs and inhibition of dendritic cell maturation.[15,16] In patients with ovarian cancers, VEGF was demonstrated to induce MDSCs, as confirmed by a mouse model.[17] We thus hypothesized that anti-VEGF monoclonal antibody might be able to reduce MDSC level.

We have previously reported that the level of circulating monocytic S100A9-positive MDSCs correlated with treatment response and PFS with first-line chemotherapy in patients with *EGFR* wild-type lung adenocarcinoma.[18] Our unpublished observation showed a similar correlation in *EGFR*-mutant EGFR TKI–treated lung adenocarcinoma. A growing body of preclinical evidence has supported an immune-modulating role of BEV, including a reduction in MDSC level; however, evidence in humans and clinical relevance have not been well elucidated.

In this study, through recruiting patients with *EGFR*-mutated lung adenocarcinoma treated with an EGFR TKI alone or in combination with BEV, we were able to conduct a sophisticated analysis of clinical outcomes and immune profiles before and after treatment and thereby evaluate the immune-modulating effects of BEV, focusing on its clinical relevance.

Subjects and Methods

Subjects

This study was approved by both the Taipei Medical University Joint Institutional Review Board (TMU-JIRB No. 201402046) and the Chang Gung Medical Foundation Institutional Review Board (IRB 102-3377B), and informed consent was obtained from all subjects. All subjects had stage IV lung adenocarcinoma harboring an activating *EGFR* mutation. Thirteen patients were recruited from Chang Gung Medical Foundation–Linko Branch, and 42 patients were recruited from Shuang Ho Hospital. In all, 25 patients received combination therapy consisting of an EGFR TKI and BEV, and 30 patients received first-line EGFR TKI treatment only. EGFR TKI treatment included erlotinib, 150 mg, or gefitinib, 250 mg, once daily. BEV was given in a dose of 7.5 to 15 mg/kg at the physician's decision every 3 weeks. Tumor response was evaluated by computed tomography according to the Response Evaluation Criteria in Solid Tumors, version 1.1, criteria.[19] The site of image-confirmed first tumor progression and new brain metastasis or progress of known brain metastasis were both documented. PFS was defined as the interval from the start of first-line treatment until image-documented progress of the lesion or death, and OS was defined as the interval from the start of first-line of