BRIEF REPORT

A Phase I Trial of Atezolizumab and Varlilumab in Combination With Radiation in Patients With Metastatic NSCLC

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ABSTRACT

Introduction: Anti-programmed cell death 1 (PD-1) immunotherapy is the standard of care for metastatic NSCLC but many tumors develop resistance. We hypothesized that combining a T-cell agonist such as varlilumab (anti-CD27 antibody) with checkpoint inhibition may be synergistic and this synergy may be potentiated further by using targeted radiation (RT).

Methods: We conducted an open-label, single-center, phase I trial (NCT04081688) to determine the safety and clinical benefit of the atezolizumab and varlilumab in combination with palliative RT in patients with advanced or metastatic NSCLC with progression on prior programmed cell death ligand 1therapy. On day 1 of each 21-day cycle, patients received varlilumab followed by atezolizumab on day 2. RT to a lung lesion was administered between cycle 1 and cycle 2.

Results: A total of 15 patients were enrolled (one patient did not start treatment). The median age was 64 years; 10 patients were female. Eight patients (57%) had at least one treatment-related adverse event (AE) and 7 (50%) had at least one grade III or worse treatment-related AE. There was only one grade III immune-related AE requiring steroids (1 diarrhea and colitis); there were no treatment-related deaths. Of the 12 patients evaluable for efficacy, three patients had stable disease (2 with stable disease $>$ 4 mo) and the clinical benefit rate was 25%. The median progression-free survival was two months and the median overall survival was 6.4 months.

Conclusions: Varlilumab in combination with atezolizumab and RT was safe and well tolerated; no additional signal was identified for toxicity. Clinical activity for the combination was modest with 25% of patients with stable disease as the best response.

Keywords: Immunotherapy; anti-CD27; radiation; NSCLC

Introduction

Anti-programmed cell death 1 (PD-1) immunotherapy is the standard of care for the treatment of metastatic NSCLC.[1] However, many tumors have primary immunotherapy resistance or develop resistance on therapy. There is a need to improve response rates further and increase the potential for long-term clinical benefit with PD-1/PD-L1 inhibitors. The costimulatory molecule CD27 is a member of the tumor necrosis factor receptor superfamily and is constitutively expressed on mature T cells, memory B cells, and a portion of natural killer cells.[2–5] Varlilumab (CDX-1127) is a first-in-class

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fully human IgG1 agonist anti-CD27 monoclonal antibody that activates CD27 expressing T cells in the context of T cell receptor stimulation.[6] Varlilumab mimics CD70 to enhance the CD27-mediated T-cell costimulatory pathway when combined with T-cell receptor activation. Preclinical studies demonstrate the synergistic antitumor activity of PD-1 blockade and varlilumab by increasing CD8+ T-cell expansion and effector function, Fc receptor-mediated crosslinking, and Fc-dependent effector function and CD27-mediated regulatory T cell depletion.[7]

Based on the available supporting data, we conducted a phase I trial of the PD-L1 inhibitor Atezolizumab and anti-CD27 antibody, Varlilumab in combination with palliative radiation in patients with pretreated stage IV NSCLC who have progressed on prior PD-L1 therapy (NCT04081688). Combining RT and immunotherapy has a synergistic effect on immune-mediated tumor regression even in sites outside of radiation fields.[8,9] Tumor-antigen release achieved through RT promotes specific tumor targeting by the adaptive immune system that is further augmented by systemic immunotherapy.[8] Also, in our trial, atezolizumab and varlilumab were administered sequentially (varlilumab on day 1 and atezolizumab on day 2). Prior research in mouse models has demonstrated that a sequential combination of T cell agonists followed by anti-PD-1/PD-L1 antibody results in an increase in therapeutic efficacy as sequential administration does not result in T-cell exhaustion.[10]

Methods

Study Design

In this single-center, phase I trial, patients with advanced or metastatic NSCLC who had progressed on prior anti–PD-L1 immunotherapy were enrolled. Eligible patients were at least 18 years or older, progressed on prior platinum-based chemotherapy and anti-PD1 or anti–PD-L1 agents, had adequate hepatic, renal, and marrow functions, and measurable disease per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). Participants with symptomatic, untreated central nervous system metastases or active autoimmune diseases requiring systemic immunosuppression were excluded. Informed consent for the trial was approved by the Institutional Review Board of Rutgers University and conformed to the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from all patients before enrollment. All patients were enrolled at the Rutgers Cancer Institute in New Jersey.

Study Treatment

Patients received the study treatment in 21-day cycles with varlilumab administered on day 1 (10 mg/kg cycle 1; 3 mg/kg cycle 2 onwards, intravenous) followed by atezolizumab (1200 mg, intravenous) on day 2 (Fig. 1). Palliative RT to a lung lesion was administered between cycle 1 and cycle 2 (40 to 50 Gy in 4 to 10 fractions). A site was selected for potential radiation in the lung or in the mediastinum and prioritized based on the following criteria: (1) Lesion progressing on prior PD-1/PD-L1 targeted therapy, or (2) the largest feasible lesion that may provide palliative benefit. Imaging scans to assess tumor response were performed every nine weeks and adverse events (AEs) were defined by the National Cancer Institute Common Terminology Criteria for Adverse Events version 5 (CTCAE v.5).

Statistical Considerations and Analyses

The goal of the trial was to recruit a maximum of 15 patients and continuously monitor the serious AEs. The stopping rule was to suspend enrolment if there was an 85% probability that the serious AE was greater than 50%. The primary endpoint was the safety and tolerability of atezolizumab and varlilumab in combination with radiation. Secondary end points were objective response rate, clinical benefit rate (CBR), and median progression-free survival (PFS). Primary resistance was defined as disease progression after receiving at least six weeks of PD-1/PD-L1 inhibitor (~ two complete cycles of therapy), but no more than six months of therapy.[11] For correlative analysis, pre- and on-treatment peripheral blood mononuclear cells samples were collected for immunophenotyping by multiparameter flow cytometry.

The incidence of AEs was summarized by type of AE, grade, and attribution, with the most severe grade per patient being reported. The objective response rate was defined as the proportion of patients with a confirmed complete response (CR) or partial response (PR) per Response Evaluation Criteria in Solid Tumors version 1.1. The CBR was defined as the proportion of patients with a CR, PR, or stable disease (SD). PFS was defined as the time from treatment initiation to the date of first documentation of disease progression or death due to any cause. Overall survival (OS) was defined as the time from treatment initiation to death due to any cause. Patients still alive were censored at the last date known to be alive. Survival probabilities were estimated and plotted using the Kaplan-Meier method. Estimates along with 95% pointwise confidence intervals were reported.

Results

Between September 2019 and April 2021, 15 patients were enrolled (one patient did not receive treatment due to prior AE, and 14 evaluable for safety). Participant's baseline characteristics are described in Supplementary Table 1-2 and Supplementary Figure 1.

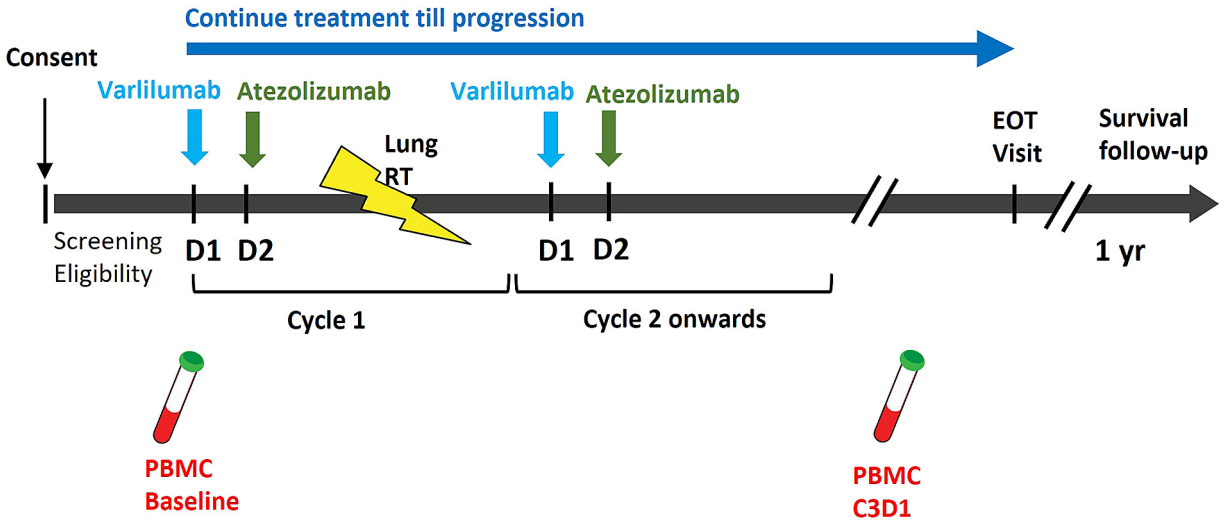

Figure 1. Treatment administration schema on trial. C3D1, cycle 3 day 1; D1, day 1; D2, day 2; EOT, end of treatment; PBMC, peripheral blood mononuclear cells.

Median age was 64 years (range 35 to 73); 10 patients were female; five had received greater than or equal to two prior lines of therapy for metastatic NSCLC. Three patients had primary immunotherapy resistance and 11 patients had acquired immunotherapy resistance. The median number of cycles on study treatment was 3 (range 1–9). The AEs are listed in Table 1. Eight patients (57%) had at least one treatment-related AE (TRAE); seven patients (50%) had at least one grade III or worse TRAE. The most common TRAEs (all grades) was lymphopenia (29%) but this was not associated with neutropenia. There was only one grade III immune-related AE requiring steroids (one diarrhea or colitis) and there were no treatment-related deaths. Two patients discontinued study treatment for AEs (colitis and coronavirus infection).

Of the 12 patients evaluable for efficacy, there were no PR or CR observed. Three patients had SD (2 with SD $>$ 4 mo) and CBR was 25%. In patients with SD, PD-L1 expression was 80% in two patients and 0% in one patient. Median PFS was two months (95% confidence interval: 1.6–3.5) and median OS was 6.4 months (95% confidence interval 2.1–16.9). Peripheral blood mononuclear cells samples were collected pre-treatment and on cycle 3 day 1 and multiplex immunophenotyping on the samples was performed (Fig. 2). The proportion of CD8+ T cells and monocytes increased following study treatment, while the proportion of CD4 T cells decreased in circulating immune cell populations. No differences were observed in other circulating cells such as Treg (CD3+CD4+FoxP3+), NK (CD56+), and B cells (CD19+CD45+). Pairwise comparisons using Mann-Whitney test were not significant likely due to the small sample size.

Discussion

This study was designed to explore the safety and efficacy of the combination of atezolizumab, varlilumab, and palliative RT for the treatment of advanced or metastatic NSCLC pre-treated with platinum-based chemotherapy and PD-L1 inhibitors. While the combination was well-tolerated, efficacy was limited, and the best overall response was stable disease in three patients, with no clinical responses observed. The combination did influence circulating immune phenotypes with an increase appreciated in the proportion of circulating CD8+ T cells and monocytes with the study combination. Improving the outcomes for patients with metastatic NSCLC in the second line setting, or beyond progression on immunotherapy remains a challenge. Current guidelines recommend treatment with chemotherapy, such as docetaxel or gemcitabine, in this setting and outcomes are poor.[1]

A biomarker-based approach incorporating validated markers of T-cell activation as well as tumor microenvironment may be the way forward to explore future immunotherapy-based novel combinations with T cell agonists such as varlilumab. One challenge to the clinical development of T-cell agonists is the potential for immune depletion due to constant T-cell stimulation or T-cell exhaustion.[12] Exploring different doses and schedules for administering T-cell agonists as used in our trial may be a strategy. Ongoing trials are also investigating other approaches to target T-cell agonists such as oncolytic virus therapy (NCT05076760) and T-cell therapy (NCT05681780). Suboptimal receptor crosslinking with varlilumab may also impact efficacy as effective CD27 downstream signaling requires a hexameric ligand format and a hexameric CD27 complex.[13,14]

Table 1. All Adverse Events, Maximum Grade Per Patient

	All AEs		Treatment-related AEs	
Adverse Events	All, n (%)	≥ Grade III, n (%)	All, n (%)	≥ Grade III, n (%)
Lymphopenia	4 (29)	4 (29)	4 (29)	4 (29)
Colitis	1 (7)	1 (7)	1 (7)	1 (7)
Diarrhea	1 (7)	1 (7)	1 (7)	1 (7)
Pain	6 (43)	-	1 (7)	-
Nausea	3 (21)	-	1 (7)	-
Fatigue	1 (7)	1 (7)	1 (7)	1 (7)
WBC decreased	1 (7)		1 (7)	-
Thromboembolic event	2 (14)	2 (14)	-	-
Dyspnea	2 (14)	1 (7)	-	-
Fever	1 (7)	-	1 (7)	-
Headache	1 (7)	-	1 (7)	-
Lipase increased	1 (7)	-	1 (7)	-
Confusion	1 (7)	1 (7)	-	-
Lung infection	2 (14)	1 (7)	-	-
Hypomagnesemia	3 (21)	-	-	-
Acute kidney injury	1 (7)	-	-	-
ALT increased	1 (7)	-	-	-
AST increased	1 (7)	-	-	-
Alkaline phosphatase increased	1 (7)	-	-	-
Constipation	1 (7)	-	-	-
Cough	1 (7)	-	-	-
Dehydration	1 (7)	-	-	-
Depression	1 (7)	-	-	-
Hypophosphatemia	1 (7)	-	-	-
Hypotension	1 (7)	-	-	-
Hypoxia	1 (7)	-	-	-
Mucositis	1 (7)	-	-	-
Numbness	1 (7)	-	-	-
Odynophagia	1 (7)	-	-	-

Note: If a subject experienced more than one adverse event within an AE category, the subject is counted once under that category.
AE, adverse event; ALT, alanine transaminase; AST, aspartate transaminase; WBC, white blood cells.

Combining RT and immunotherapy has a synergistic effect on immune-mediated tumor regression even in sites outside of radiation fields.[9] This abscopal response has been reported specifically in NSCLC also[15] and may contribute to a durable response. In animal models, stereotactic body radiotherapy (SBRT) delivered to melanoma or breast tumors resulted in the development of antigen-specific T cell- and B cell-mediated immune responses. These immune-stimulating effects of SBRT were significantly increased when combined with anti-PD-1 therapy or regulatory T cell (Treg) depletion, resulting in improved local tumor control.[16] In a trial combining SBRT and pembrolizumab reported by Luke et al.,[17] SBRT doses varied from 45 Gy in three fractions for peripheral lung, liver, and abdominal or pelvic; 50 Gy in five fractions for central lung and mediastinal/cervical; 30 Gy in three fractions for osseous and spinal/paraspinal lesions. To assess whether SBRT might result in favorable immunologic changes in the tumor microenvironment, the expression of four preselected IFN-γ–associated genes was analyzed in post-irradiation biopsy specimens. Increased gene expression was significantly correlated with responses in nonirradiated tumors ($p = 0.023$). The NRG Oncology Group also used 50 Gy (in 5 fractions) for central lung lesions and mediastinal lymph nodes or 45 Gy (in three fractions) for peripheral lung lesions in a phase I trial of SBRT for the treatment of multiple metastases.[18] Based on these prior studies, we used SBRT at a dose of 40 to 50 Gy (in four or five fractions) in our trial. In our trial, we observed control and stabilization of disease even in non-radiated sites in three patients. Moreover, the treatment was well-tolerated with no cases of pneumonitis observed even when the site of radiation was in the lung for all patients. It is unclear what number of lesions should be radiated to elicit a systemic immune response. In our study, only one lung lesion received RT which may have limited the clinical benefit from the combination.

In conclusion, varlilumab in combination with atezolizumab and RT was safe and well tolerated; no

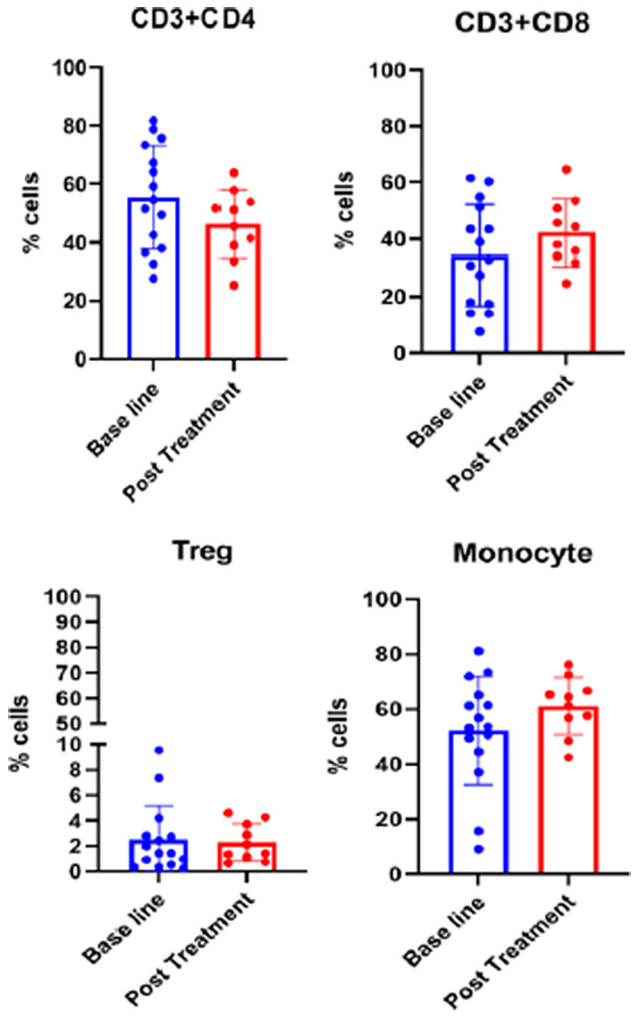

Figure 2. The proportion of circulating immune cells in paired pre- and on-treatment samples. *p*-value for the Mann-Whitney test was 0.22 for CD4 T-cells, 0.26 for CD8 T-cells, 0.60 for Treg, and 0.29 for monocytes.

additional signal was identified for toxicity. Clinical activity for the trial combination was modest with 25% of patients with stable disease as the best response. There is a need for prospectively validated biomarkers for immunotherapy-resistant NSCLC to better guide future trials for these patients.

CRediT Authorship Contribution Statement

Jyoti Malhotra: Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft.

Yong Lin: Formal analysis, Writing - original draft.

Malini Patel: Investigation, Writing - original draft.

Michael J. Yellin: Conceptualization, Funding acquisition.

Emmanuel Zachariah: Formal analysis, Investigation.

Curtis Krier: Formal analysis, Investigation.

Ankit Saxena: Formal analysis, Investigation.

Salma K. Jabbour: Conceptualization, Investigation, Supervision, Writing - original draft.

Disclosure

Dr. Yellin is employed by Celldex. The remaining authors declare no conflict of interest.

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Supplementary Data

To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at [https://doi.org/10.1016/j.jtocrr.2024.100687].

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