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Blocking TIM-3 in Treatment-refractory Advanced Solid Tumors: A Phase Ia/b Study of LY3321367 with or without an Anti-PD-L1 Antibody

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ABSTRACT

Purpose: T-cell immunoglobulin and mucin-domain–containing molecule-3 (TIM-3) blunts anticancer immunity and mediates resistance to programmed death 1 (PD-1) and PD ligand 1 (PD-L1) inhibitors. We assessed a novel, first-in-class, TIM-3 mAb, LY3321367, alone or in combination with the anti-PD-L1 antibody, LY300054 in patients with advanced solid tumor.

Patients and Methods: This open-label, multicenter, phase Ia/b study aimed to define the safety/tolerability and recommended phase II dose (RP2D) of LY3321367 with or without LY300054. Secondary objectives included pharmacokinetics/pharmacodynamics, immunogenicity, and efficacy. Biomarkers were assessed in exploratory analysis.

Results: No dose-limiting toxicities were observed in the monotherapy ($N = 30$) or combination ($N = 28$) dose escalation. LY3321367 treatment-related adverse events (≥2 patients) included pruritus, rash, fatigue, anorexia, and infusion-related reactions. Dose-proportional increase in LY3321367 concentrations was not affected by either LY300054 or antidrug antibodies (observed in 50%–70% of patients). Pharmacokinetic/pharmacodynamic modeling indicated 100% target engagement at doses ≥600 mg. LY3321367 RP2D was 1,200 mg biweekly for four doses followed by 600 mg every 2 weeks thereafter. In the non–small cell lung cancer monotherapy expansion cohort, outcomes varied by prior anti-PD-1 therapy response status: anti-PD-1/L1 refractory patients [$N = 23$, objective response rate (ORR) 0%, disease control rate (DCR) 35%, progression-free survival (PFS) 1.9 months] versus anti-PD-1/L1 responders ($N = 14$, ORR 7%, DCR 50%, PFS 7.3 months). In combination expansion cohorts ($N = 91$), ORR and DCR were 4% and 42%; CD8 infiltration in paired biopsies increased in approximately half these patients.

Conclusions: LY3321367 exhibited acceptable safety profile with favorable pharmacokinetics/pharmacodynamics but only modest antitumor activity. The therapeutic relevance of TIM-3 blockade requires further investigation.

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Introduction

Antagonist antibodies to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death receptor 1/ligand (PD-1/L1), alone or in various combinations, exhibit a manageable safety profile, provide durable antitumor activity, and improve survival for several advanced solid tumors (1, 2). Nevertheless, most patients do not respond to first-generation immune checkpoint inhibitors, indicating that innate resistance mechanisms interfere with an effective anticancer immune response. Subsets of patients who initially attain disease control appear to acquire resistance to anti-PD-1 therapy (3, 4). Thus, there is an urgent need to explore novel immune checkpoint molecules to augment outcomes in patients with cancer (5, 6).

T-cell immunoglobulin and mucin-domain–containing molecule-3 (TIM-3), a membrane-bound and soluble immune checkpoint molecule, is expressed on CD4/8$^+$ T lymphocytes, regulatory T cells, myeloid lineages, and natural killer cells (7, 8). TIM-3 drives diverse immunologic pathways including immunosuppression (9, 10). TIM-3 negatively regulates IFN gamma–secreting T cells leading to T-cell death and anergy (11). TIM-3 also defines an "exhausted" T-cell phenotype (12) and associates with poor patient outcomes in non–small cell lung cancer (NSCLC; ref. 13), gastric/gastroesophageal junction (GEJ) adenocarcinoma (14), hepatocellular carcinoma (HCC), urothelial carcinoma (UC) (15), squamous cell carcinoma of the head and neck (SCCHN; ref. 16) and other malignancies (17, 18).

In preclinical models, TIM-3 interference reconstitutes T-cell activity, which propagates an effective anticancer immune response. Unlike CTLA-4 or PD-1, TIM-3 loss does not induce autoimmunity,

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Translational Relevance

T-cell immunoglobulin and mucin-domain–containing molecule-3 (TIM-3) is an immune checkpoint molecule implicated in immune evasion and acquired resistance to anti-PD1/PD-L1 therapy. LY3321367 is a novel, first-in-class, humanized (IgG1 λ, Fc-null) mAb to TIM-3 that inhibits ligand-dependent activation. In this phase Ia/1b study, the safety, efficacy, and pharmacokinetics/pharmacodynamics of LY3321367, an anti-TIM-3 antibody, either alone or in combination with an anti-PD-L1 antibody (LY3300054) were evaluated in patients with advanced cancers. Monotherapy and combination therapy were safe and tolerable with favorable pharmacokinetics and near saturation of TIM-3 at therapeutic dose levels. Antidrug antibodies were identified in most patients but did not affect drug exposure. Modest antitumor activity to TIM-3 blockade was evident in a subset of patients with lung cancer. Correlative analysis indicated a nonstatistically significant increase of CD8 expression in paired fresh biopsies with treatment, though baseline PD-L1, TIM-3, and CD8 expression did not correlate with response.

suggesting that antibodies to TIM-3 may have a more favorable safety profile than first-generation immune checkpoint inhibitors. LY3321367 is a novel, first-in-class, humanized (IgG1 λ, Fc-null) mAb to TIM-3 that inhibits both galactin-9 and phosphatidylserine ligand-dependent activation (19). LY3321367 alone and in combination with LY3300054 enhance T-cell activation in cellular systems (19). These data support evaluating LY3321367 as an immunotherapy for patients with cancer in the clinic.

TIM-3 is upregulated in response to anti-PD-1 therapy and is proposed as an acquired resistance mechanism to anti-PD1 therapy (20). Cotargeting TIM-3 and PD-1 overcomes resistance to anti-PD-1 therapy in preclinical models, and combination treatment confers a survival advantage over monotherapy *in vivo* (11, 20–22). Thus, combining the anti-TIM-3 antibody (LY3321367) with PD-1/L1 blockade is a reasonable therapeutic strategy. LY3300054, a full human (IgG1λ Fc-null) mAb, binds to human PD-L1 with high affinity and inhibits interactions of PD-L1 with its two cognate receptors, PD-1 and CD80 (23). LY3300054 is well tolerated and has shown durable antitumor activity (24).

To assess the operating characteristics of TIM-3 blockade in patients with advanced solid tumors, we conducted a phase Ia/b study to define the safety, pharmacokinetics, pharmacodynamics, and efficacy of LY3321367 administered as monotherapy and in combination with LY3300054.

Patients and Methods

Study design and treatment

This phase I, open-label, multicenter, two-part study consisted of a dose-finding (phase Ia) and a dose-expansion portion (phase Ib) in patients with advanced, treatment-refractory, solid tumors (Supplementary Fig. S1). The primary objective was to determine the safety/tolerability and recommended phase II dose (RP2D) of LY3321367 as monotherapy and in combination with LY3300054. Secondary objectives included pharmacokinetics/pharmacodynamics, immunogenicity, and efficacy. Correlatives explored pretreatment tumor PD-L1, TIM-3, and CD8 expression by IHC at baseline and in response to treatment.

In the dose-escalation portion, patients were treated with increasing doses of LY3321367 monotherapy either every 2 or 3 weeks starting at 3 mg flat dose intravenously (A cohorts). In the combination dose escalation (B cohorts), patients received increasing doses of LY3321367 (starting dose at 70 mg) in combination with LY3300054 (200, 700, or 1,400 mg) either every 2 or 3 weeks. Tumor-specific expansions enrolled at the RP2D to further define safety, tolerability, and efficacy: LY3321367 monotherapy in NSCLC who were refractory to prior anti-PD-1/L1 (cohort M1) or who had a prior response to prior anti-PD-1/L1 therapy and later progressed (cohort M2); combination therapy for gastric/GEJ (cohort C1), NSCLC (cohort C2), UC (cohort C3), HCC (cohort C4-HCC), SCCHN, (cohort C4-SCCHN), and small-cell lung cancer (SCLC; cohort C5).

Patients received study treatment until confirmed progressive disease, unacceptable toxicity, or withdrawal of consent. Intrapatient dose escalation was not allowed with the exception in cohort A1.

All patients provided written informed consent, and local Institutional Review Board approvals were obtained. The study was conducted in accordance with the Declaration of Helsinki and in compliance with the International Council for Harmonization guidelines on Good Clinical Practice.

Patients

Eligible patients were ≥18 years of age with a histologic diagnosis of advanced solid tumor who had adequate organ function, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and measurable disease as defined by RECIST version 1.1 (25). Prior treatment with anti-PD-l with or without a prior anti-CTLA-4 antibody was allowed, though those patients with grade ≥3 immune-related adverse events (irAE) and any grade neurologic or ocular irAE were excluded. Patients could not have had a serious, uncontrolled, intercurrent medical illness, including autoimmunity or brain metastasis. Dose-escalation patients (phase Ia: cohorts A and B) provided archival tumor tissue, while patients in expansion (phase Ib: cohorts M and C) were required to provide paired pretreatment tumor sample prior to study treatment and during study treatment.

Other key inclusion criteria specific to the dose-escalation or expansion cohorts included: any solid tumor diagnosis with progression on all prior lines of therapy with known clinical benefit in the phase Ia monotherapy or combination dose escalation (cohorts A and B); confirmed diagnosis of NSCLC with at least two to four prior lines of therapy and progression on prior immune checkpoint blockade as assessed by RECIST version 1.1 (cohorts M1 and M2)—investigators were required to adjudicate patients with NSCLC with primary progression of disease (M1) or prior response/disease control to prior immune checkpoint inhibitors (M2); metastatic or locally advanced gastric/GEJ (Siewert type I, II, or III) with at least one, but no more than three, prior lines of systemic therapy (cohort C1); metastatic NSCLC with at least one, but no more than three, prior lines of systemic therapy (cohort C2); locally advanced, unresectable, or metastatic urothelial carcinoma (bladder, urethra, ureter, or renal pelvis) with at least one, but no more than three, prior lines of systemic therapy, which included a platinum-based chemotherapy (cohort C3); locally advanced, unresectable, or metastatic HCC with Child-Pugh stage A or locoregional advanced or metastatic SCCHN after available standard therapies failed in the judgment of the investigator (cohort C4); and extensive stage SCLC, with no more than two lines of prior therapy (cohort C5).

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Study assessments

Safety

Patients were evaluated for medical history (baseline), physical examination (baseline and day 1 of each cycle), and laboratories (day 1 of first cycle and then every 2 or 3 weeks depending on the dosing cohort). All AEs were graded by the NCI Common Terminology Criteria for Adverse Events (version 4.0).

A dose-limiting toxicity (DLT) was defined as an AE during first 4 weeks of treatment (cycle 1) that was related to LY3321367 and fulfilled any one of the following criteria: hematologic toxicity (grade 4 toxicity lasting >7 days, grade 3 thrombocytopenia with clinically significant bleeding and requiring platelet transfusion or grade 4 thrombocytopenia of any duration, grade ≥3 febrile neutropenia, and grade ≥3 anemia requiring a blood transfusion); and nonhematologic toxicity (grade 4 irAE, grade ≥3 colitis or pneumonitis, other grade 3 irAE that did not downgrade to grade ≤1 within 14 days after onset, grade 2 pneumonitis not resolved to grade ≤1 within 3 days after initiation of medical management, grade ≥3 toxicity lasting an extended period of time, and grade ≥3 hypertension, grade 3 or 4 amylase not resolved to grade ≤2 within 7 days after corticosteroid therapy, other grade 3 or 4 laboratory value lasting 14 days, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) values >8 × upper limit of normal (ULN) in patients with no HCC or liver metastasis or ≥2-fold above the patient's baseline value that lasts >7 days in patients with HCC or liver metastasis, and total bilirubin >5 × ULN).

Pharmacokinetic/pharmacodynamic analysis

Serum samples for pharmacokinetic and pharmacodynamic analysis of LY3321367 and LY3300054 were obtained prior to infusion on cycle 1 day 1 and at 2, 4, 24, 72, 120, and 168 hours after treatment, and day 15 preinfusion and at 2, 4, 24, and 168 hours after infusion. As TIM-3 exists as a soluble receptor in plasma, soluble TIM-3 concentration was assayed for pharmacodynamics at each pharmacokinetic timepoint to determine the fraction of free to bound soluble TIM-3 over the total soluble target using a validated electrochemiluminescence assay.

Simultaneous population pharmacokinetic/pharmacodynamic model for pharmacokinetic and TIM-3 soluble target concentrations was performed using NONMEM 7.4 (Icon Development Solutions). A proportional error model was applied to describe residual variability for pharmacokinetic and pharmacodynamic observations (soluble target concentrations). Plots were developed in MATLAB (The Mathworks). The disposition of LY3321367 in peripheral blood (plasma) was described by compartmental models. Model selection was made from one-, two-, or three-compartment models.

Soluble target accumulation is attributed to the decrease in the clearance rate of soluble target when bound to the antibody. Therefore, soluble target was described by an indirect response model with an E_{max} effect (E_{max} represents the maximum effect attributable to the drug). Half-maximal effective concentration (EC_{50}), parameter that denotes the drug concentration that produces half of E_{max}, was used to calculate soluble target engagement as percentage:

$$sTE\% = 100 \times [C/(EC_{50} + C)]$$

where C is total drug concentration in central compartment (plasma).

Immunogenicity

Samples for immunogenicity were drawn for every 2 weeks dosing on days 1, 8, and 15 of cycle 1; day 1 of cycles 2, 3, and 4; day 1 of cycle 6, and then every six cycles and, for every 3 weeks dosing, on day 1 and 8 of cycle 1; and day 1 of cycles 2, 3, 4, and 8, and then every eight cycles. The formation of antidrug antibodies (ADA) was analyzed at BioAgilytix Labs using a validated upfront acid treatment affinity capture elution bridge ELISA following a 4-tier approach to screen, confirm, titer, and assess neutralizing antibodies against LY3321367. The ADA assay was validated in accordance with the FDA Guidance for Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins (26).

Antitumor activity

Cross-sectional imaging was completed every 6 weeks (± 7 days) with CT of chest, abdomen, and pelvis with contrast and, when applicable, neck (SCCHN) or liver three phase (HCC). Radiographic response was adjudicated at the treating institution according to RECIST v 1.1 for all patients.

Biomarker assessments

PD-L1, TIM-3, and CD8 IHC assays were validated in the Clinical Diagnostics Laboratory (Eli Lilly and Company) prior to use, and the results were evaluated by a qualified pathologist according to prespecified interpretation guidelines. Tissue immunoreactivity for PD-L1 was assessed using the Dako PD-L1 IHC 22C3 pharmDx Kit (Agilent). CD8 IHC was performed using a monoclonal mouse anti-Human CD8 antibody (Dako C8-144B). TIM-3 IHC was performed using a clone against TIM-3 extracellular domain (Cell Signaling Technology). The average number of positive cells counted approximately within randomly selected five representative high-power microscopic fields were reported for CD8 and TIM-3. PD-L1 expression was assessed per the scoring algorithm specified in labels using tumor proportional score (TPS), which was the percentage of viable tumor cells showing partial or complete membranous staining at any intensity. The specimen was negative or positive for PD-L1 expression if TPS was lower than 1% or at least 1%, respectively.

Statistical analysis

All patient-level data were reported and tabulated in a descriptive manner. Any patient who received one dose of study drug was included in the assessment for safety and efficacy. For the safety analysis, the frequency and percentage of patients with DLTs and AEs are presented for each cohort. A 3 + 3 dose-escalation design was employed to determine the MTD, which was defined as the highest tested dose that had <33% probability of causing a DLT during cycle 1. The RP2D was selected based on a composite of safety and pharmacokinetic/pharmacodynamic modeling. The expansion cohorts were exploratory in nature to evaluate safety and antitumor activity in histology-specific indications and allowed enrollment of up to 20 patients each.

We estimated objective response rate (ORR) and disease control rate (DCR) according to RECIST (version 1.1). For the time-to-event variable, progression-free survival (PFS), the medians, and survival rates at various time points with 90% confidence interval (CI) were estimated by Kaplan–Meier methodology. Individual changes in the tumor burden over time are presented graphically by cohort. ADAs and biomarker data were analyzed using available and measurable samples. Descriptive statistical analyses were implemented without any imputation on missing values.

Results

Patient demographics

One hundred eighty-six patients with advanced solid tumors were enrolled between April 12, 2017 and December 10, 2019; 30 patients in

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the monotherapy dose escalation (A cohorts), 28 patients in the combination therapy dose escalation (B cohorts), 37 patients in the monotherapy expansions (M cohorts), and 91 patients in the combination therapy expansion (C cohorts; **Table 1**). Patients were predominantly male (67%) and white (72%) with a median age of 61.5 years (range: 19–90 years). Fifty-seven percent patients underwent surgery for their primary tumor. The majority of the patients received prior systemic therapy and 47.3% had ≥3 prior lines of treatment. Seventy-eight (42%) patients received a prior immune checkpoint molecule. As the dose escalation allowed any solid tumor, patients had varied tumor types; the most common tumor type being SCLC (A cohorts, $n = 4$; B cohorts, $n = 5$). NSCLC was the most common tumor type in the expansion cohorts. In total, 37 patients with NSCLC who progressed on prior immune checkpoint inhibitors were treated with monotherapy in M1 and M2 cohorts (Supplementary Table S1). For combination treatment, 29 patients with gastric/GEJ (cohort C1), 21 patients with NSCLC (cohort C2), 9 patients with UC (cohort C3), 16 patients with HCC (cohort C4-HCC), 14 patients with SCCHN (cohort C4-SCCHN), and 2 patients with SCLC (cohort C5) were enrolled.

Safety

No DLT or DLT-equivalent toxicities were reported up to the maximum studied dose for monotherapy or combination therapy. AEs of any grade and any attribution for each cohort are presented in **Table 2**.

Treatment-related AEs (TRAE) of any grade for monotherapy (A or M cohorts) occurring at least in 2 patients in cohort A were pruritus

Table 1. Baseline patient and disease characteristics.

	Dose escalation		Expansion		
	Cohort A (*N* = 30)	Cohort B (*N* = 28)	Cohort M (*N* = 37)	Cohort C (*N* = 91)	Total (*N* = 186)
Gender, *n* (%)					
Male	15 (50.0)	15 (53.6)	24 (64.9)	70 (76.9)	124 (66.7)
Age, years					
Median	54.5	67.5	61.0	61.0	61.5
Min	26	40	37	19	19
Max	76	90	77	84	90
Race, *n* (%)[a]					
White	27 (93.1)	23 (85.2)	27 (73.0)	52 (60.5)	129 (72.0)
Asian	2 (6.9)	3 (11.1)	10 (27.0)	31 (36.0)	46 (25.7)
Black or African American	—	1 (3.7)	—	1 (1.2)	2 (1.1)
Multiple	—	—	—	2 (2.3)	2 (1.1)
Missing	1	1	—	5	7
ECOG PS[a], *n* (%)					
0	17 (56.7)	12 (42.9)	15 (40.5)	30 (33.0)	74 (40.2)
1	13 (43.3)	16 (57.1)	22 (59.5)	59 (64.8)	110 (59.8)
Missing	—	—	—	2	2
Pathological diagnosis, *n* (%)					
Non–small cell lung cancer	5 (16.7)	2 (7.1)	37 (100.0)	21 (23.1)	65 (34.9)
Small cell lung cancer	4 (13.3)	5 (17.9)	—	2 (2.2)	11 (5.9)
Hepatocellular carcinoma	—	—	—	16 (17.6)	16 (8.6)
Gastric cancer/GEJ	2 (6.7)	—	—	29 (31.9)	31 (16.7)
Urothelial carcinoma	—	1 (3.6)	—	9 (9.9)	10 (5.4)
Head and neck squamous cell carcinoma	—	—	—	14 (15.4)	14 (7.5)
Other[b]	19 (63.3)	20 (71.4)	—	—	39 (21.0)
Prior therapy, *n* (%)					
Radiotherapy	19 (63.3)	13 (46.4)	21 (56.8)	39 (42.9)	92 (49.5)
Surgery	20 (66.7)	19 (67.9)	19 (51.4)	48 (52.7)	106 (57.0)
Systemic therapy	30 (100.0)	27 (96.4)	37 (100.0)	90 (98.9)	184 (98.9)
Prior systemic treatments, *n* (%)					
1	5 (16.7)	7 (25.0)	—	25 (27.5)	37 (19.9)
2	11 (36.7)	5 (17.9)	12 (32.4)	31 (34.1)	59 (31.7)
≥3	14 (46.7)	15 (53.6)	25 (67.6)	34 (37.4)	88 (47.3)
Prior immune checkpoint therapy, *n* (%)					
Anti-PD1/L1	8 (26.7)	3 (10.7)	36 (97.3)	31 (34.1)	78 (41.9)
Anti-CTLA4 and/or anti-PD1/L1	8 (26.7)	3 (10.7)	36 (97.3)	31 (34.1)	78 (41.9)

Abbreviations: CTLA-4, cytotoxic T-lymphocyte–associated protein 4; ECOG, Eastern Cooperative Oncology group; GEJ, gastroesophageal junction; Max, maximum; Min, minimum; *N*, total population; *n*, number of patients; PD-1/PD-L1, programmed death receptor-1/ligand-1; PS, performance status.

[a]Number of subjects with nonmissing data, used as denominator.

[b]Includes adenoid cystic carcinoma of salivary gland, adenocarcinoma of the colon or rectum, bone cancer, breast cancer, cholangiocarcinoma, chondrosarcoma, chordoma, renal cell carcinoma, gallbladder adenocarcinoma, neuroendocrine/small cell carcinoma, esophageal carcinoma, pancreatic carcinoma, pleural mesothelioma, prostate cancer, serous cystadenocarcinoma ovary, small intestine carcinoma, squamous cell carcinoma of cervix, squamous cell carcinoma of vulva, tonsil cancer.

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[2 (6.7%) patients] and maculopapular rash [2 (6.7%) patients]. In cohort M, TRAEs were asthenia [3 (8.1%) patients], arthralgia [2 (5.4%) patients], nausea [2 (5.4%) patients], chest pain [2 (5.4%) patients], fatigue [2 (5.4%) patients], and pneumonitis [2 (5.4%) patients]. At the RP2D for monotherapy (M cohort), grade ≥3 TRAEs occurred in 2 patients; asymptomatic lipase increase (1 patient) and dyspnea (1 patient).

TRAEs of any grade occurring at least in 3 patients in cohort B were fatigue [4 (14.3%) patients], decreased appetite [3 (10.7%) patients], and infusion-related reaction (IRR) [3 (10.7%) patients], and in cohort C, TRAEs were fatigue [5 (5.5%) patients], rash [4 (4.4%) patients], and aspartate aminotransferase increased [3 (3.3%) patients]. At the RP2D for combination therapy (C cohorts), treatment-related grade ≥3 of diarrhea, hyperglycemia, and renal failure were reported in 1 (1.1%) patient each.

A total of 6 patients had grade 1–2 IRR related to the anti-TIM-3 antibody LY3321367; 1 patient in A cohort, 3 patients in B cohorts, and 2 patients in C cohorts. In all cases, reactions were mild, resolved with supportive measures, and did not preclude retreatment.

Eight deaths occurred on study due to AE, which were unrelated to study treatment and related to underlying cancer [1 patient due to dyspnea in cohort A2; 2 patients due to sepsis and acute respiratory failure in the monotherapy expansion cohorts (cohort M1); and 5 patients due to pulmonary hemorrhage, dyspnea, bronchial aspiration, cardiac arrest, and biliary obstruction in the combination expansion cohorts].

Pharmacokinetics and pharmacodynamics

The observed C_{max} for the anti-TIM-3 antibody LY3321367 was reached at approximately 2 hours after dose across all dose levels, and C_{max} linearly increased with increasing doses in both cohorts A (monotherapy) and B (combination; **Fig. 1A** and **C**; Supplementary Table S2). Clearance of LY3321367 was 0.0117 L/hour, indicating an approximate half-life of 21 days.

In both cohorts A and B, bound soluble TIM-3 increased with increasing doses of LY3321367 (**Fig. 1B**) with no impact by the addition of LY3300054 (**Fig. 1D**). Pharmacokinetic/pharmacodynamic modeling showed complete (≥99%) target engagement at doses ≥600 mg LY3321367 at steady-state, which was achieved after multiple dosing (**Fig. 1E**). At the 1,200-mg dose, complete soluble TIM-3 target engagement was reached after the first dose for most patients. As a result, RP2D for LY3321367 was determined to be 1,200 mg every 2 weeks for cycles 1 and 2, followed by 600 mg every 2 weeks cycle 3 onward. This dose regimen enabled reaching steady state faster with initial loading dose and using lower maintenance dose thereafter.

Addition of the anti-PD-L1 antibody LY3300054 did not affect pharmacokinetics of the anti-TIM-3 antibody LY3321367, and vice versa (Supplementary Table S2). LY3321367 C_{max} remained consistent across the 70-mg dose of LY3321367 (cohorts B2 and B3), regardless of increase in the LY3300054 dose. Similarly, the C_{max} for LY3300054 increased with increasing dose of LY3300054 and remained consistent across the 700-mg dose of LY3300054 (cohorts B3, B4, B5, and B7), regardless of increase in the LY3321367 dose.

Immunogenicity

A high proportion of treatment-emergent ADA against LY3321367 was observed: 65.5% and 51.4% in the A and M cohorts (monotherapy); 70.4% and 58.1% in the B and C cohorts (combination cohorts). Maximum ADA titers were detected in approximately the first 2 weeks after dose with median titer ranging from 1:80 to 1:160. Most ADA titers returned to baseline status by cycle 3. Favorable pharmacokinetic profile and soluble TIM-3 target engagement suggest that ADA titer values did not appear to have an impact on LY3321367 exposure and soluble TIM-3 target engagement. A total of 6 patients had IRRs (not

Table 2. Treatment-emergent AEs (≥10% of patients) by preferred terms.

Dose escalation				Expansion			
Cohort A monotherapy dose escalation (*N* = 30)		**Cohort B combination dose escalation (*N* = 28)**		**Cohort M NSCLC monotherapy expansion (*N* = 37)**		**Cohort C multi-histology expansion (*N* = 91)**	
Fatigue	8 (26.7)	Fatigue	11 (39.3)	Dyspnea	11 (29.7)	Fatigue	18 (19.8)
Back pain	5 (16.7)	Nausea	7 (25.0)	Fatigue	7 (18.9)	Dyspnea	15 (16.5)
Cough	5 (16.7)	Abdominal pain	6 (21.4)	Pneumonia	7 (18.9)	Decreased appetite	14 (15.4)
Nausea	5 (16.7)	Constipation	6 (21.4)	Arthralgia	6 (16.2)	Anemia	12 (13.2)
Abdominal pain	4 (13.3)	Diarrhea	6 (21.4)	Cough	6 (16.2)	Abdominal pain	9 (9.9)
Arthralgia	4 (13.3)	Anemia	5 (17.9)	Back pain	5 (13.5)	Aspartate aminotransferase increased	9 (9.9)
Dehydration	4 (13.3)	Tumor pain	5 (17.9)	Decreased appetite	5 (13.5)		
Dyspnea	4 (13.3)	ALT increased	4 (14.3)	Nausea	5 (13.5)		
Pruritus	4 (13.3)	AST increased	4 (14.3)	Pyrexia	5 (13.5)	Constipation	9 (9.9)
Pyrexia	4 (13.3)	Decreased appetite	4 (14.3)	Diarrhea	4 (10.8)		
Rash maculopapular	4 (13.3)	Cough	3 (10.7)	Musculoskeletal pain	4 (10.8)		
Anemia	3 (10.0)	Dyspnea	3 (10.7)	Respiratory tract infection	4 (10.8)		
Constipation	3 (10.0)	Infusion-related reaction	3 (10.7)	Vomiting	4 (10.8)		
Diarrhea	3 (10.0)	Myalgia	3 (10.7)				
Dysphonia	3 (10.0)	Pyrexia	3 (10.7)				
Headache	3 (10.0)						
Hyperglycemia	3 (10.0)						
Pain	3 (10.0)						
Vomiting	3 (10.0)						

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; *N*, total population; NSCLC, non–small cell lung cancer.

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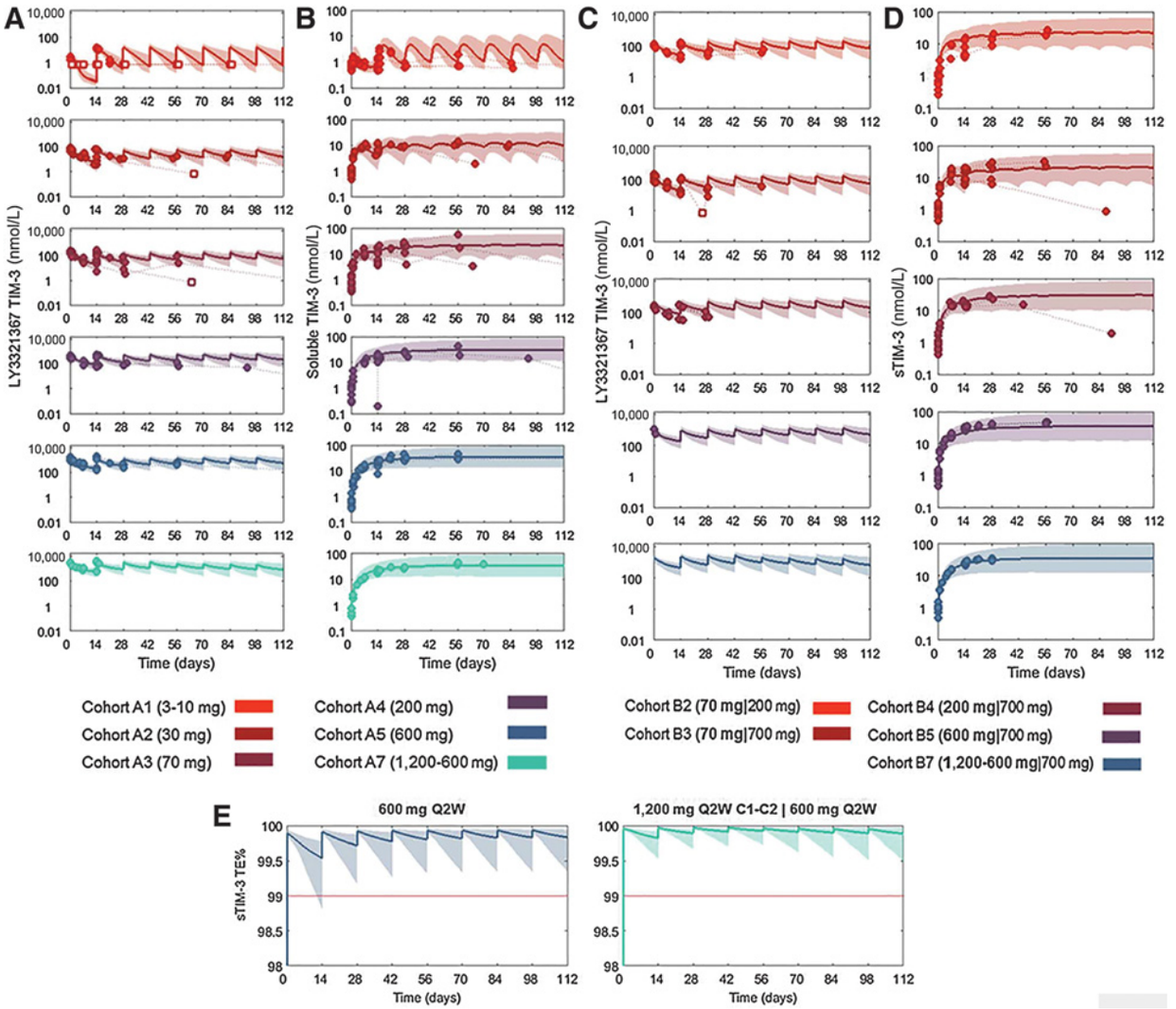

Figure 1.

Population pharmacokinetic/pharmacodynamic model for anti-TIM-3 antibody in the dose-escalation study. Concentration of LY3321367 over time in A cohorts (**A**) and B cohorts (**C**). Bound soluble TIM-3 concentration over time in A cohorts (**B**) and B cohorts (**D**). Percent target engagement for the 600 mg and 1,200 mg dose cohorts (**E**). Q2W, every 2 weeks. TIM-3, T-cell immunoglobulin and mucin-domain–containing molecule-3.

serious and all recovered or resolved), and 5 of those patients had detectable ADA.

Antitumor activity

In the monotherapy dose-escalation A cohorts, the ORR and DCR were 3.3% and 40%, respectively, in 28 evaluable patients. One nonsmoking patient with SCLC [microsatellite instability-stable and TMB low (0.9 mutations per megabase) by next-generation sequencing and PD-L1 negative by IHC] achieved a confirmed partial response (PR) and remained on therapy for more than 12 months at the RP2D. Directly prior to study enrollment, the patient received nivolumab and ipilimumab for approximately 12 months with disease progression prior to study entry. There were 11 patients with stable disease (SD); specifically, 1 patient (SCLC) remained on the study for >2 years, and 2 patients (lung adenocarcinoma and bone cancer) remained on study for >6 months. In the combination dose-escalation B cohorts, no objective responses were observed, though 8 patients had SD with a DCR of 28.6%, and only 2 patients had stable disease for more than 6 months.

As preclinical and clinical data suggested TIM-3–mediated acquired resistance to anti-PD1/PD-L1 therapy in patients with lung cancer, M cohorts evaluated monotherapy in 37 patients with NSCLC who had previously received prior standard-of-care immunotherapy (Supplementary Table S1). M1 patients must have had confirmed progressive disease as best response to prior immunotherapy, while M2 patients were required to have disease control or response to prior immunotherapy. In patients with progressive disease as best response to prior immunotherapy (cohort M1), ORR was 0%, DCR was 34.8%, and median PFS was 1.9 months (90% CI, 1.7–2.7). In cohort M1, 8 patients had SD of whom 6 remained on therapy for >3 months (**Fig. 2**).

In patients who had prior disease control or response on immunotherapy but later progressed (cohort M2), 7.1% ORR, 50% DCR, and

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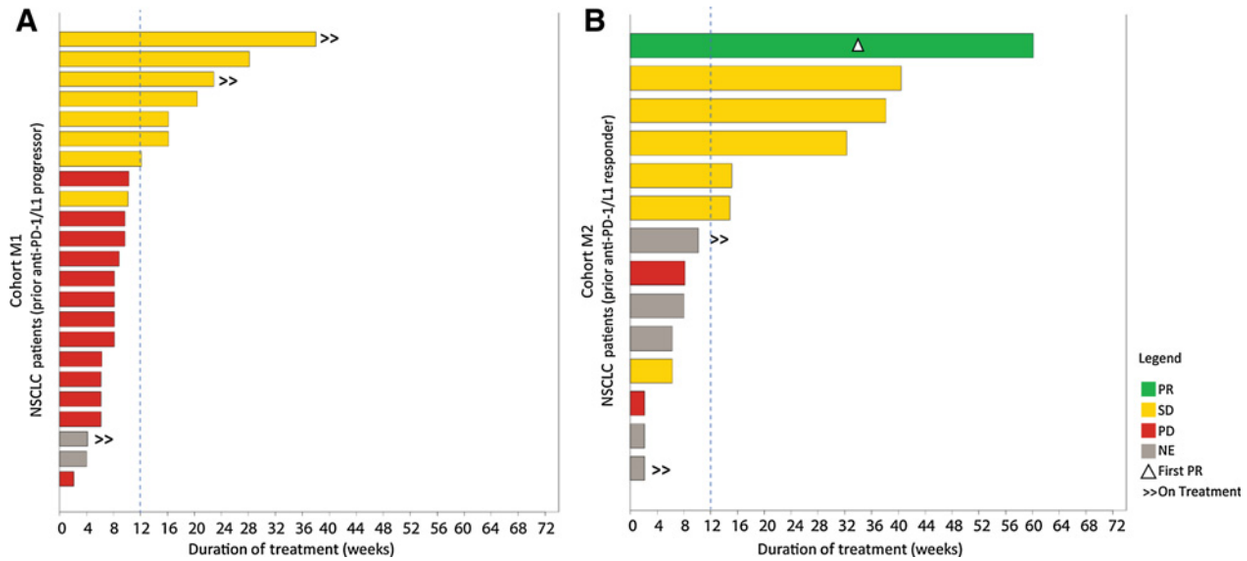

Figure 2.

Treatment duration and response for patients in cohort M1 (**A**) and cohort M2 (**B**). Each horizontal bar represents a patient. Colors of the bar denote categories of best response. NE, nonevaluable; NSCLC, non–small cell lung cancer; PD, progressive disease; PR, partial response; SD, stable disease.

a median PFS of 7.3 months (90% CI, 1.5–9.2) were observed (**Table 3**). Seven of the patients from cohort M2 had progressed on prior immune checkpoint inhibitor–based therapy immediately prior to enrollment in this study. One patient had confirmed PR and remained on therapy for about 15 months and had progressed on nivolumab immediately prior to this study. Of the 6 patients who had SD, 3 patients had received checkpoint inhibitor–based therapy immediately prior to this study (2 of these SD were greater than 6 months) and 3 had received intervening therapy prior to joining this study (1 of these SD was more than 6 months).

In the combination cohorts (C cohorts; **Fig. 3**; **Table 3**), ORR and DCR were 4.4% (gastric/GEJ cohort, 3.4%; SCCHN cohort, 14.3%; other cohorts, 0%) and 41.8% (gastric/GEJ cohort, 20.7%; NSCLC cohort, 66.7%; UC cohort, 44.4%; HCC cohort, 56.3%; SCCHN cohort, 35.7%; SCLC cohort, 0%) respectively, in 91 evaluable patients. Four patients had confirmed PR (1 GEJ, 1 urothelial carcinoma, and 2 SCCHN) and remained on therapy between 6.7 months and 13.5+ months. There were 34 patients with SD, and 6 remained on therapy for >6 months. The median PFS was 1.5 months (90% CI, 1.2–1.7) for patients in the gastric/GEJ cohort (C1), 3.7 months (90% CI, 3.3–6.7) for patients in the NSCLC cohort (C2), 2.0 months (90% CI, 1.0–3.5) for patients in urothelial carcinoma (C3), 2.6 months (90% CI, 1.8–5.4) for patients in HCC cohort, and 1.8 months (90% CI, 1.4–3.5) for patients in SCCHN cohort.

Exploratory biomarker analyses

Tumor tissue samples were collected at baseline from all enrolled patients and analyzed for TIM-3 (combination therapy, $n = 67$; monotherapy, $n = 26$); PD-L1 (combination therapy, $n = 68$; monotherapy, $n = 26$); and CD8 (combination therapy, $n = 68$; monotherapy, $n = 26$) expression whenever possible (Supplementary Fig. S2). The median TIM-3–positive (TIM-3$^+$) immune cells at baseline was 6.5 and 5 for the patients in monotherapy and combination therapy, respectively (Supplementary Fig. S3A). The average number of TIM-3$^+$ cells varied among the different tumor types with the highest levels observed in patients with NSCLC (cohort C2; median 18). When comparing NSCLC M1 cohort (patients who never responded to prior immune checkpoint inhibitor) and M2 cohort (patients who had disease control or response to prior immune checkpoint inhibitor), the median baseline TIM-3$^+$ cells per high-power field was 8 and 5, respectively (Supplementary Table S3). PD-L1 expression was negative in most patients (combination therapy, 53/68 patients; monotherapy, 16/26 patients; Supplementary Fig. S3B). High PD-L1 expression (>50%) was observed in evaluable tumor specimens from the following patients: cohort C4/SCCHN (2/10), cohort M1 (2/14), and cohort M2 (3/12) patients (Supplementary Table S4). Comparable median intratumoral CD8$^+$ T cell at baseline was observed in the patients treated with combination and monotherapy (26 vs. 21; Supplementary Fig. S3C). The average number of CD8$^+$ cells varied among the different tumor types with higher pretreatment CD8$^+$ tumor infiltration observed in cohort C2 (median 68), cohort M1 (median 43.5), and cohort C4/SCCHN (median 40; Supplementary Table S3).

To look at the immune expression changes observed in the tumor, CD8 IHC was used for pretreatment and on-treatment tissue samples with paired biopsies (combination therapy, $n = 30$; monotherapy, $n = 12$; Supplementary Fig. S3D and S3E). Intratumoral CD8$^+$ T-cell increases were observed in almost half these patients [combination therapy, 14 (46.7%) patients; monotherapy, 6 (50%) patients; Supplementary Fig. S3D and S3E].

Baseline TIM-3, PD-L1, and CD8 expression by IHC did not correlate with response to therapy (Supplementary Table S5; Supplementary Fig. S2). Patients with SD in cohorts C4/SCCHN and C2 had a higher median expression of TIM-3 at baseline compared with patients with PD (Supplementary Fig. S2A). A potential trend of positive correlation between CD8$^+$ cells and best overall response was mostly observed in nonlung cohorts (C1, C3, and C4), whereby CD8 levels had higher medians in PR/SD patients compared with those with PD (Supplementary Fig. S2B). Baseline PD-L1 expression, when analyzed with respect to best overall response, was positive in 2 patients with PR,

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Table 3. Summary of best overall response with confirmation in cohort M and cohort C.

	Cohort M1 NSCLC (*N* = 23) *n* (%)	Cohort M2 NSCLC (*N* = 14) *n* (%)	Total (*N* = 37) *n* (%)
Best overall response			
Complete response (CR)	0	0	0
Partial response (PR)	0	1 (7.1)	1 (2.7)
Stable disease (SD)	8 (34.8)	6 (42.9)	14 (37.8)
Progressive disease	13 (56.5)	2 (14.3)	15 (40.5)
Objective progressive disease	13 (56.5)	2 (14.3)	15 (40.5)
Nonevaluable	2 (8.7)	5 (35.7)	7 (18.9)
Overall response rate (CR/PR)	0	1 (7.1)	1 (2.7)
Disease control rate (CR/PR/SD)	8 (34.8)	7 (50.0)	15 (40.5)
Progression-free survival, months			
Median (90% CI)	1.9 (1.7–2.7)	7.3 (1.5–9.2)	

	Cohort C1 Gastric/GEJ (*N* = 29) *n* (%)	Cohort C2 NSCLC (*N* = 21) *n* (%)	Cohort C3 UC (*N* = 9) *n* (%)	Cohort C4 HCC (*N* = 16) *n* (%)	Cohort C4 SCCHN (*N* = 14) *n* (%)	Cohort C5 SCLC (*N* = 2) *n* (%)	Total (*N* = 91) *n* (%)
Best overall response							
Complete response (CR)	0	0	0	0	0	0	0
Partial response (PR)	1 (3.4)	0	1 (11.1)	0	2 (14.3)	0	4 (4.4)
Stable disease (SD)	5 (17.2)	14 (66.7)	3 (33.3)	9 (56.3)	3 (21.4)	0	34 (37.4)
Progressive disease	16 (55.2)	4 (19.0)	4 (44.4)	5 (31.3)	5 (35.7)	0	34 (37.4)
Objective progressive disease	16 (55.2)	4 (19.0)	4 (44.4)	5 (31.3)	5 (35.7)	0	34 (37.4)
Nonevaluable	7 (24.1)	3 (14.3)	1 (11.1)	2 (12.5)	4 (28.6)	2 (100)	19 (20.9)
Overall response rate (CR/PR)	1 (3.4)	0	1 (11.1)	0	2 (14.3)	0	4 (4.4)
Disease control rate (CR/PR/SD)	6 (20.7)	14 (66.7)	4 (44.4)	9 (56.3)	5 (35.7)	0	38 (41.8)
Progression-free survival, months Median (90% CI)	1.5 (1.2–1.7)	3.7 (3.3–6.7)	2.0 (1.0–3.5)	2.6 (1.8–5.4)	1.8 (1.4–3.5)		

Abbreviations: CI, confidence interval; CR, complete response; GEJ, gastroesophageal junction; HCC, hepatocellular carcinoma; *N*, total population; *n*, number of patients; NSCLC, non–small cell lung cancer; PR, progressive response; SCCHN, squamous cell carcinoma of the head and neck; SCLC, small cell lung cancer; SD, stable disease; UC, urothelial carcinoma.

specifically TPS score of 1%–49% was observed in a cohort M2 patient, and TPS score >50% in a cohort C4 SCCHN patient (Supplementary Table S4).

Discussion

LY3321367, a novel, first-in class, antibody to TIM-3, was safe and tolerable in advanced solid tumor patients. AEs typical of immune checkpoint inhibitors occurred at a low frequency and rarely resulted in severe symptoms or dose discontinuation. LY3321367 exposure was dose proportional despite a high proportion of ADAs. The LY3321367 clearance supported dosing every 2 to 3 weeks. Embedded PK/PD analyses revealed near complete target engagement at the highest doses tested at steady state, and a loading dose of 1,200 mg led to rapid TIM-3 receptor occupancy after a single dose. Thus, the RP2D was defined as 1,200 mg for cycles 1–2 followed by 600 mg every 2 weeks from cycle 3 onward and was implemented in monotherapy and combination treatment expansions. Combination with anti-PD-L1 antibody LY3300054 did not affect LY3321367 safety profile or drug exposure. Expansion cohorts demonstrated limited efficacy in most tumor types, though a modest and intriguing signal was observed in an anti-PD-L1 antibody refractory patient with NSCLC, as well as in heavily pretreated SCLC.

Preclinical data support a clear hierarchy regarding immune checkpoint molecules on maintenance of self-tolerance—loss of CTLA-4 results in exuberant lymphoproliferation and death; loss of PD-1 results in autoimmunity; while loss of LAG-3, TIGIT, and TIM-3 has no appreciable effects *in vivo*. In line with these observations, our clinical experience demonstrates minimal drug-related toxicity with TIM-3 blockade. Indeed, only two ≥grade 3 TRAEs (dyspnea and lipase increase) were noted in 67 patients treated with monotherapy. Other low-frequency and low-grade AEs, such as maculopapular rash, pruritus, and IRRs, may represent a class effect of targeting TIM-3, as TIM-3 serves to control $CD8^+$ memory T cells known to prevent allergic reactions (27). Given that a high proportion of patients had developed ADA, it is also possible these reactions are specific to LY3321367.

While treatment-emergent ADA were observed in 51.4% to 70.4% of patients, ADA were low titer and short lived. No apparent impact on LY3321367 pharmacokinetics at steady state or soluble TIM-3 target engagement were observed in ADA positive versus negative patients. Furthermore, toxicity that could potentially be observed with ADA, namely, IRR, occurred in <5% of patients on this study. Outside of the context of this study, the humoral response to humanized antibodies to immune checkpoint inhibitors is well documented; ADA as high as 54% is reported for FDA-approved anti-PD-L1 inhibitors (28).

Although the study did not embed *a priori* rules for efficacy or futility, antitumor activity was limited for both monotherapy and combination therapy. LY3321367 monotherapy had no activity in patients with PD-1/L1 refractory NSCLC, and combination therapy

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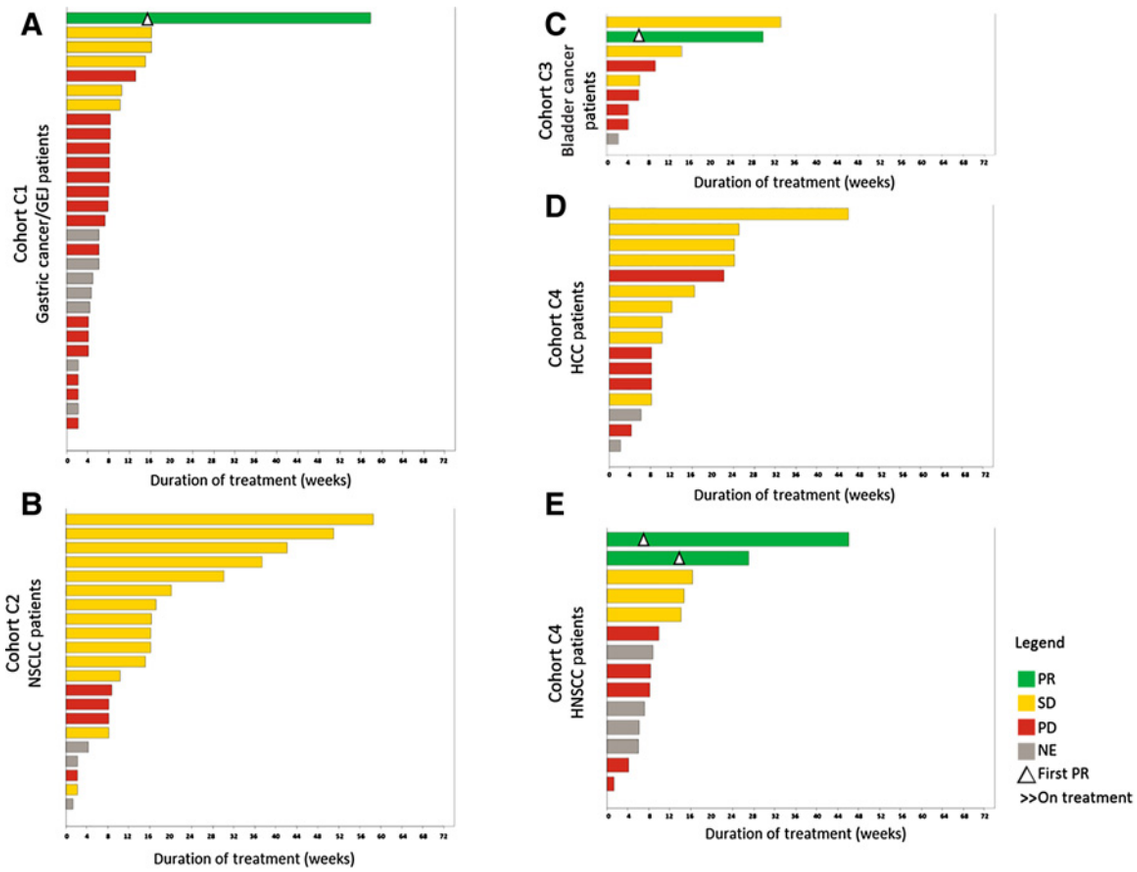

Figure 3.

Treatment duration and response for patients in expansion cohort C1 (**A**), cohort C2 (**B**), cohort C3 (**C**), cohort C4 HCC (**D**), and cohort C4 SCCHN (**E**). Each horizontal bar represents a patient. Colors of the bar denote categories of best response. HCC, hepatocellular carcinoma; PD, progressive disease; PD-1/L1, programmed death receptor-1/ligand; PR, partial response; SCCHN, squamous cell carcinoma of the head and neck; SD, stable disease.

did not appear to augment anti-PD-1 therapy activity in any of the expansion cohorts. TIM-3 blockade appears to have some antitumor activity in a small subset of patients with NSCLC who had a prior response to FDA-approved immune checkpoint blockade, and this observation is supported by prior translation and preclinical data. In addition, a possible signal was also observed in patients with SCLC albeit such events might represent outliers with more favorable biology. Our data, along with other reports, support further investigation into TIM-3 blockade in lung cancer with emphasis on patients with acquired resistance to prior PD-L1 therapy.

Several factors account for suboptimal antitumor activity including the target, the agent, and the study design. It is still unclear which TIM-3 binding partner (i.e., phosphatidylserine, HMGB-1, Galectin-9, and CEACAM-1) is most relevant to cancer immune evasion. Galectin-9, which is blocked by LY3321367, plays a dominant role in modulating the adaptive immune responses to cancer. CEACAM-1, not bound by LY3321367, is expressed on several solid tumors and may contribute to blunting immune response by direct TIM-3 engagement. Thus, it is possible that TIM-3 immunosuppressive signals are not fully abrogated with LY3321367 treatment. Alternatively, TIM-3 may function as a costimulatory receptor, augmenting T-cell activation. TIM-3 promotes the development of short-lived effector T cells, while TIM-3 deficiency impairs T-cell responsiveness (29), although no such activation was observed in the preclinical or clinical development of LY3321367. The high proportion of ADA may have impacted antitumor activity as has been observed with atezolizumab (28), which also has a high prevalence of treatment-emergent ADA. Retrospective assessment of LY3321367 with more advanced immunogenicity risk assessment tools suggests that additional protein engineering could generate a less immunogenic next-generation molecule. Although we did not observe a negative effect on AEs or pharmacokinetics, we did not have the sample size nor a high number of responses to formally compare ADA level with efficacy. Finally, the study design may have confounded our ability to observe a signal. Some expansion cohorts were of small sample size making estimation of efficacy difficult (i.e., SCLC, urothelial carcinoma). In addition, outside of M1/M2 cohorts, where prior PD-1 therapy and RECIST progression adjudicated by the investigator was required, the other cohorts were more flexible in their entry criteria related to prior immunotherapy and prior response.

Correlative analyses did not reveal meaningful differences in pretreatment TIM-3 expression based on prior immune checkpoint inhibitor responses. Furthermore, we did not find that baseline level of immune activation by PD-L1, TIM-3, or CD8 influenced outcomes to treatment, but these assessments are problematic given the few responders and the diversity of histology. We did not see a statistically

significant increase in CD8 expression on paired biopsies with TIM-3 blockade. Acknowledging the caveats of tumor heterogeneity and small sample size, these findings suggest insufficient immune activation to TIM-3 blockade (30).

To our knowledge, we present the first comprehensive report on the clinical operating characteristics of a novel anti-TIM-3 antibody. Although LY3321367 administered as both monotherapy and combination therapy had an acceptable safety profile, limited clinical activity resulted in early termination of the study. The therapeutic relevance of TIM-3 requires further investigation in select tumor types (particularly in lung cancer) and is likely to be clarified by multiple ongoing studies.

Conclusions

LY3321367 administered alone and in combination with LY3300054 in patients with solid tumors had an acceptable safety profile. Further studies with larger populations of patients with evaluable tumor samples for biomarker assessments will help determine the efficacy of these inhibitors in cancer patients.

Authors' Disclosures

J.J. Harding reported personal fees from Bristol Myers Squibb, Merck, Eisai, Exelexis, Eli Lilly, QED, and Imvax and grants from Bristol Myers Squibb outside the submitted work. V. Moreno reported personal fees from Roche, BMS, Bayer, Janssen, and Basilea outside the submitted work. Y.-J. Bang reported personal fees from AstraZeneca, Novartis, Genentech/Roche, MSD, Merck Serano, Bayer, BMS, Eli Lilly, Taiho, Daiichi-Sankyo, Astellas, BeiGene, GreenCross, Samyang Biopharm, Hanmi, and Genexine and grants from AstraZeneca, Novartis, Genentech/Roche, MSD, Merck Serano, Bayer, BMS, GSK, Pfizer, Eli Lilly, Boehringer-Ingelheim, MacroGenics, Boston Biomedical, FivePrime, Curis, Taiho, Takeda, Ono, Daiichi Sankyo, Astellas, BeiGene, Green Cross, CKD Pharma, and Genexine outside the submitted work. A. Patnaik reported other from Merck, Pfizer, Lilly, Plexxikon, Corvus Pharmaceuticals, Tesaro, Abbvie, Forty Seven, Five Prime Therapeutics, Infinity Pharmaceuticals, Pieris Pharmaceuticals, Surface Oncology, Livzon, Vigeo Therapeutics, Astellas Pharma, Klus Pharma, Symphogen, Syndax, Arcus, Fochon, Upsher-Smith, Exelixis, Seattle Genetics, Bolt, Ionova, Daiichi Sankyo, Sanofi, Bayer, Novartis, Genentech/Roche, Seattle Genetics, Merck, Bristol-Myers Squibb, and Silverback Therapeutics during the conduct of the study. J. Trigo reported personal fees from BMS, AstraZeneca, MSD, and Boehringer outside the submitted work. A.M. Szpurka reported other from Eli Lilly and Company outside the submitted work; and salary and stocks holder (employee of Eli Lilly). N. Yamamoto reported other from ONO, Janssen Pharma, MSD, Boehringer Ingelheim, Cimic, Chugai, and Otsuka outside the submitted work. T. Doi reported grants from Lilly, Merck Serono, Janssen Pharma, Eisai, Pfizer, Sumitomo Dainippon Pharma, and IQVIA; grants and personal fees from Taiho, Novartis, MSD, Boehringer Ingelheim, Daiichi Sankyo, Bristol Myers Squibb, and Abbvie; personal fees from Amgen, Rakuten Medical, Takeda, Ono Pharma, Oncolys BioPharma, Astellas Pharma, and Bayer outside the submitted work. N. Velez de Mendizabal reported a patent for TIM-3 mAb pending. E. Calvo reported personal fees from Nanobiotix, Novartis, BMS, Roche/Genentech, Servier, OncoDNA, Alkermes, and PharmaMar and grants from BeiGene outside the submitted work; and is a scientific board member of PsiOxus Therapeutics, is an employee of Clinical Research HM Hospitals Group and START Program of Early Phase Clinical Drug Development in Oncology (medical oncologist, director), and is a founder and president with Non-for-profit Foundation INTHEOS (Investigational Therapeutics in Oncological Sciences). L. Gandhi reported other from Lilly during the conduct of the study; other from Lilly outside the submitted work. Z.T. Liu reported personal fees from Eli Lilly and Company outside the submitted work. C.C. Leow reported other from Lilly outside the submitted work. M.J. de Miguel reported other from START during the conduct of the study; other from Roche, Novartis, MSD, Pharmamar, Eisai, Lilly, Faron, Janssen, Abbvie, Bayer, BMS, and Boehringer outside the submitted work. No disclosures were reported by the other authors.

Disclaimer

Employees of Eli Lilly and Company participated in the study design; in the collection, analysis, and interpretation of the data; in the writing of this article; and in the decision to submit this article for publication.

Authors' Contributions

J.J. Harding: Conceptualization, data curation, formal analysis, supervision, writing–original draft, writing–review and editing. **V. Moreno:** Data curation, writing–review and editing. **Y.-J. Bang:** Data curation, methodology, writing–review and editing. **M.H. Hong:** Data curation, writing–review and editing. **A. Patnaik:** Data curation, writing–review and editing. **J. Trigo:** Data curation, writing–review and editing. **A.M. Szpurka:** Data curation, writing–original draft, writing–review and editing. **N. Yamamoto:** Data curation, writing–review and editing. **T. Doi:** Conceptualization, data curation, writing–review and editing. **S. Fu:** Data curation, writing–review and editing. **B. Calderon:** Data curation, writing–original draft, writing–review and editing. **N. Velez de Mendizabal:** Conceptualization, data curation, writing–original draft, writing–review and editing. **E. Calvo:** Data curation, writing–review and editing. **D. Yu:** Data curation, writing–original draft, writing–review and editing. **L. Gandhi:** Data curation, methodology, writing–review and editing. **Z.T. Liu:** Conceptualization, data curation, writing–original draft, writing–review and editing. **V.R. Galvao:** Data curation, writing–original draft, writing–review and editing. **C.C. Leow:** Conceptualization, data curation, formal analysis, supervision, methodology, writing–original draft, writing–review and editing. **M.J. de Miguel:** Data curation, writing–review and editing.

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