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Safety and Antitumor Activity of α-PD-L1 Antibody as Monotherapy or in Combination with α-TIM-3 Antibody in Patients with Microsatellite Instability–High/Mismatch Repair–Deficient Tumors FREE

Antoine Hollebecque ; Hyun C. Chung; Maria J. de Miguel ; Antoine Italiano; Jean-Pascal Machiels; Chia-Chi Lin ; Neesha C. Dhani; Marc Peeters ; Victor Moreno ; Wu-Chou Su; Kay Hoong Chow; Violeta R. Galvao; Michelle Carlsen; Danni Yu; Anna M. Szpurka ; Yumin Zhao ; Shelly L. Schmidt; Leena Gandhi; Xiaojian Xu; Yung-Jue Bang

+ Author & Article Information

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Abstract

Purpose:

Immune checkpoint inhibitors show high response rates and durable clinical benefit in microsatellite instability–high/mismatch repair–deficient (MSI-H/dMMR) tumors. However, 50%–60% do not respond to single-agent anti–programmed death-1/programmed death ligand 1 (PD-1/PD-L1) antibodies, and approximately 50% of responders relapse within 6–12 months. This phase Ib trial evaluated safety and

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antitumor activity of anti–PD-L1 antibody LY3300054 monotherapy or in combination with anti–TIM-3 antibody LY3321367 in patients with MSI-H/dMMR advanced solid tumors.

Patients and Methods:

Eligible patients ≥18 years without prior anti–PD-1/PD-L1 therapy received LY3300054 monotherapy (*N* = 40) or combination (*N* = 20); patients with PD-1/PD-L1 inhibitor–resistant/refractory tumors received the combination (*N* = 22). LY3300054 (700 mg) and anti–TIM-3 antibody (cycles 1–2: 1,200 mg, cycle 3 onward: 600 mg) were administered intravenously every 2 weeks. Primary endpoints were safety and tolerability.

Results:

Eighty-two patients were enrolled. Most had colorectal (*n* = 39, 47.6%) or endometrial (*n* = 14, 17.1%) tumors. More than 70% of patients in the PD-1/PD-L1 inhibitor–resistant/refractory combination cohort had received ≥3 treatment lines. Treatment-related adverse events (TRAE) occurred in 22 patients (55.0%) receiving monotherapy, 13 (65.0%) in the PD-1/PD-L1 inhibitor–naïve combination cohort, and 6 (27.3%) in the PD-1/PD-L1 inhibitor–resistant/refractory combination cohort. A total of 2 patients (5.0%) receiving monotherapy and 3 (7.1%) receiving the combination experienced grade ≥3 TRAEs. Objective responses occurred in 13 patients (32.5%) with monotherapy, 9 (45.0%) in the PD-1/PD-L1 inhibitor–naïve combination cohort, and 1 patient (4.5%) in the PD-1/PD-L1 inhibitor–resistant/refractory combination cohort.

Conclusions:

LY3300054 monotherapy and combined LY3300054/anti–TIM-3 had manageable safety profiles. Both regimens showed promising clinical activity against PD-1/PD-L1 inhibitor–naïve MSI-H/dMMR tumors. The combination had limited clinical benefit in patients with PD-1/PD-L1 inhibitor–resistant/refractory MSI-H/dMMR tumors.

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Translational Relevance

This phase I study reported that anti–programmed death ligand 1 (anti–PD-L1) antibody, LY3300054, had a comparable antitumor activity with any other anti–programmed death-1 (anti–PD-1) antibody in patients with microsatellite instability–high/mismatch repair–deficient (MSI-H/dMMR) tumors. This is the first study to combine anti–PD-L1 antibody with the immune checkpoint inhibitor, anti–TIM-3 antibody (LY3321367), for the treatment of patients with MSI-H/dMMR tumors. Combination of the anti–TIM-3 antibody with LY3300054 did not compromise the safety profile of LY3300054 and showed promising durable activity in patients with MSI-H/dMMR tumors who are naïve to prior PD-1/PD-L1 inhibitors. Limited clinical activity was observed in patients with MSI-H/dMMR tumors resistant/refractory to prior anti–PD-1/PD-L1 agents. These findings suggest that LY3300054 monotherapy and combination of LY3300054 and the anti–TIM-3 antibody are safe and have promising clinical activity against PD-1/PD-L1 inhibitor–naïve MSI-H/dMMR tumors.

Introduction

Tumors deficient in DNA mismatch repair (dMMR) represent 17%–31% of endometrial cancers, 9%–19% of gastric cancers, 6%–20% of colorectal cancers, and lower proportions in other cancers (1, 2). The frequency of dMMR may vary according to the stage of disease. High microsatellite instability (MSI-H) in colorectal cancer was reported to have a higher incidence in the early stages (~20% in stages I and II and 12% in stage III) and a lower incidence in the metastatic setting (4%–5%; ref. 3).

The genomes of dMMR tumors have MSI-H, which results in hundreds to thousands of somatic mutations that may encode potential neoantigens. Consequently, MSI-H/dMMR tumors are likely to be immunogenic, as suggested by their high numbers of tumor-infiltrating lymphocytes (TIL; refs. 4, 5).

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Inhibition of the programmed death-1 (PD-1)/programmed death ligand 1 (PD-L1) immune checkpoint axis has shown robust clinical activity in MSI-H/dMMR cancers (6, 7). Single-agent PD-1 inhibition with

pembrolizumab was approved by the FDA in May 2017 for the treatment of patients with unresectable or metastatic MSI-H/dMMR solid tumors, representing the first tumor-agnostic approval for an anticancer agent (8). In the Keynote-164 study, pembrolizumab resulted in an objective response rate (ORR) of 33% in patients with MSI-H/dMMR colorectal cancer, with a median overall survival (OS) of 31.4 months and median progression-free survival (PFS) of 2.3 months in patients who had received ≥2 prior lines of treatment (9). The Keynote-158 study also found pembrolizumab to have robust clinical activity in a non–colorectal cancer cohort of 27 tumor types (ORR, 34.3%; median OS, 23.5 months; median PFS, 4.1 months; ref. 10). Similarly, the PD-1 inhibitor nivolumab resulted in durable clinical benefit in MSI-H/dMMR colorectal cancer in the CheckMate-142 trial (ORR, 31%; median OS not reached; median PFS, 14.3 months; ref. 11) and in MSI-H/dMMR non–colorectal cancer cancers in the NCI-MATCH trial (ORR, 36%; median OS, 17.3 months; median PFS, 6.3 months; ref. 12).

Despite high response rates and durable clinical benefit, 50%–60% of MSI-H/dMMR tumors fail to respond to single-agent anti–PD-1/PD-L1 antibodies, and approximately 50% of patients who initially respond relapse after 6–12 months (9, 10, 13). Combination therapy with different immune checkpoint inhibitors may overcome primary and acquired resistance from single-agent treatment. The CheckMate-142 trial investigated nivolumab in combination with the cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) inhibitor ipilimumab in patients with MSI-H/dMMR colorectal cancer, finding a high ORR of 55%, a 1-year OS of 85%, and a 1-year PFS of 71% (median PFS and OS not reached; ref. 14).

TIM-3 (T-cell immunoglobulin and mucin-domain-containing molecule-3) is another immunoinhibitory checkpoint molecule, and is coexpressed alongside PD-1 in the most exhausted, dysfunctional subsets of $CD8^+$ and $CD4^+$ TILs (15, 16). TIM-3 is also expressed by myeloid cells and regulatory T cells, suggesting it might play a different role to PD-1/PD-L1 in the tumor immune microenvironment (17). Combined blockade of PD-1 and TIM-3 *in vivo* shows greater reactivation of antitumor T-cell responses than PD-1 blockade alone (15, 18). Recent phase I clinical trials have explored combining PD-1/PD-L1 inhibitors with TIM-3

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inhibitors (19–22). Early data show that a combination of the TIM-3 inhibitor TSR-022 with the PD-1 inhibitor dostarlimab has a manageable safety profile, with partial responses observed in several patients with non–small cell lung cancer (NSCLC) who had progressed on PD-1 inhibitor monotherapy (19).

MSI-H/dMMR tumors have higher expression of TIM-3 than microsatellite-stable tumors (23). Moreover, TIM-3 is upregulated during PD-1 blockade, and may form a potential resistance mechanism to PD-1/PD-L1 inhibitors (24). Coinhibition of PD-1/PD-L1 and TIM-3 might therefore improve clinical benefit and overcome both primary and acquired resistance in patients with MSI-H/dMMR tumors, including those whose disease has progressed on prior PD-1/PD-L1 inhibitor therapy.

LY3300054 (Eli Lilly and Company) is a human IgG1 mAb that binds to PD-L1 and blocks interaction with its two cognate receptors PD-1 and CD80 (25). LY3321367 (Eli Lilly and Company) is a human IgG1 mAb that binds TIM-3 and potentially blocks its interaction with phosphatidylserine, and partially blocks its interaction with Gal-9. The molecular basis of LY3321367-mediated blockade of TIM-3 was previously described in Haidar and colleagues (26). LY3321367 partially blocks the TIM-3/GAL-9 complex but does not block TIM-3/CEACAM-1 complex as shown by ELISA assay. A cell-based assay suggested LY3321367 completely blocked the binding of soluble human TIM-3 to phosphatidylserine displayed on the surface of campothecin-treated DO11.10 cells. X-ray crystallography was utilized to solve the structure of the LY3321367-Fab complex with the IgV domain of TIM-3 at 2.0 Å resolution. LY3321367-Fab complementary-determining regions do not undergo any conformational changes upon binding TIM-3. The structural alignment of the human TIM-3/LY3321367-Fab complex with the available murine TIM-3 structure suggests that the 6.0 Å epitope of LY3321367 overlaps the phosphatidylserine binding site on TIM-3. A recent phase I trial has shown the combination of LY3300054 and this anti–TIM-3 antibody is well tolerated in patients with advanced solid tumors, with preliminary evidence of antitumor activity from the combination and from anti–TIM-3 antibody monotherapy (21). Here, we report results from a phase Ib trial that evaluated the safety, tolerability, and preliminary antitumor activity of the PD-L1 inhibitor LY3300054 as

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monotherapy or in combination with the anti–TIM-3 antibody LY3321367 in patients with MSI-H/dMMR, advanced solid tumors. For the PD-L1/TIM-3 inhibitor combination, endpoints were investigated in both PD-1/PD-L1 inhibitor–naïve and PD-1/PD-L1 inhibitor–resistant/refractory MSI-H/dMMR cohorts. In addition, we performed translational analyses to investigate pharmacodynamics and resistance mechanisms, focusing on the tumor immune microenvironment and indoleamine 2,3-dioxygenase 1 (IDO-1) metabolism pathway.

Patients and Methods

Study design

This article reports the results from the phase Ib MSI-H/dMMR expansion cohorts of the multicenter, phase Ia/1b PACT trial (ClinicalTrials.gov identifier NCT02791334; refs. 27, 28). Patients with MSI-H/dMMR tumors were enrolled to receive the anti–PD-L1 antibody LY3300054 (Eli Lilly and Company) alone or combined with the anti–TIM-3 antibody LY3321367 (Eli Lilly and Company) at study sites in Canada, Spain, France, Belgium, South Korea, and Taiwan. The study protocol and all amendments were approved by the ethical review board at each study site, and the study was performed in accordance with the Declaration of Helsinki, national regulations, and Good Clinical Practice. All patients provided written informed consent.

The primary phase Ib objective was to assess the safety and tolerability of LY3300054 monotherapy and LY3300054/anti–TIM-3 antibody combination therapy. Other objectives were to assess the efficacy and pharmacokinetics of LY3300054 monotherapy and the combination therapy, and to characterize relevant blood and tumor tissue biomarkers and whether they correlate with the clinical response. LY3300054 (700 mg) and the anti–TIM-3 antibody (1,200 mg for cycles 1 and 2, 600 mg for cycle 3 onward) were dosed every 2 weeks intravenously. Patients were to receive LY3300054 monotherapy or the combination therapy until confirmed progressive disease (PD), unacceptable toxicity, or discontinuation for any other reason. Enrollment to the monotherapy cohort occurred prior to enrollment to the combination cohorts. The first

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portion of the monotherapy cohort (22 patients, PD-1/PD-L1 treatment naïve) were enrolled during December 2016 and June 2017. After evaluation of the safety and efficacy data from these 22 patients, the protocol was amended to expand the monotherapy cohort sample size up to 40 in May 2018. The second portion of the monotherapy cohort (18 patients, PD-1/PD-L1 treatment naïve) were enrolled between September 2018 and April 2019; 20 PD-1/PD-L1 treatment-naïve patients in the combination cohorts were enrolled during December 2018 and July 2019. PD-1/PD-L1–naïve patients receiving monotherapy or combination treatment were not randomized but rather the treating physician made the decision on monotherapy versus combination for patients. The maximum treatment duration was 12 months, although treatment extension was possible for patients receiving clinical benefit.

Patients

Patients were eligible if they were ages ≥18 years with histologically or cytologically confirmed MSI-H or dMMR, advanced solid tumors.

All enrolled patients had to have: at least one measurable lesion accessible according to RECIST v1.1, Eastern Cooperative Oncology Group performance status of 0 or 1, discontinued all previous treatments for cancer for ≥14 days, and an estimated life expectancy of ≥12 weeks. Patients were excluded if they had: symptomatic or uncontrolled brain metastases, spinal cord compression, or leptomeningeal disease; history of any other condition or abnormality that could interfere with the study; or received a live vaccine within 30 days of starting the study. Women could not be breastfeeding, pregnant, or planning to become pregnant.

MSI-H/dMMR testing was performed at each study site before study entry by PCR, IHC, or both as per local practice (**Table 1**). Patients who had not received prior PD-1/PD-L1 inhibitor therapy (PD-1/PD-L1 inhibitor naïve) were enrolled to receive LY3300054 monotherapy or the combination regimen. Patients whose disease had progressed on prior PD-1/PD-L1 inhibitor therapy were enrolled to receive the combination regimen. A tumor tissue sample from a newly obtained core or excisional biopsy or a recent

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biopsy taken within 3 months prior to study enrollment was required. In addition, patients were required to undergo a biopsy procedure during the study treatment (prior to cycle 2 day 1) if the treating physician considered it safe to perform.

Table 1.

Baseline patient characteristics.

		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	**αPD-1/PD-L1 naïve**	**αPD-1/PD-L1 resistant/refractory**
Characteristic	**(*N* = 40)**	**(*N* = 20)**	**(*N* = 22)**
Median age, y (range)	60.5 (32.0–79.0)	64.0 (36.0–84.0)	54.5 (25.0–76.0)
Male, *n* (%)	20 (50.0)	8 (40.0)	14 (63.6)
Race, *n* (%)			
American Indian or Alaska native	1 (2.5)	0 (0.0)	0 (0.0)
Asian	16 (40.0)	4 (20.0)	8 (36.4)
White	12 (30.0)	7 (35.0)	7 (31.8)
Missing	11 (27.5)	9 (45.0)	7 (31.8)
ECOG status, *n* (%)			
0	19 (47.5)	6 (30.0)	7 (31.8)
1	21 (52.5)	14 (65.0)	15 (68.2)
Smoking history, *n* (%)			

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		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	**αPD-1/PD-L1 naïve**	**αPD-1/PD-L1 resistant/refractory**
Characteristic	**(*N* = 40)**	**(*N* = 20)**	**(*N* = 22)**
Current or former smoker	17 (42.5)	4 (20.0)	9 (40.9)
Never smoked	23 (57.5)	16 (80.0)	13 (59.1)
Tumor type, *n* (%)			
Colorectal	15 (37.5)	7 (35.0)	17 (77.3)
Endometrial	8 (20.0)	5 (25.0)	1 (4.5)
Gastric	4 (10.0)	3 (15.0)	0 (0.0)
Small intestine	3 (7.5)	2 (10.0)	2 (9.1)
Esophageal	2 (5.0)	0 (0.0)	0 (0.0)
Pancreatic	1 (2.5)	1 (5.0)	0 (0.0)
Sarcoma	1 (2.5)	1 (5.0)	0 (0.0)
Ovarian	1 (2.5)	0 (0.0)	0 (0.0)
Cholangiocarcinoma	1 (2.5)	0 (0.0)	0 (0.0)
Other	4[a] (10.0)	1[b] (5.0)	2[c] (9.1)
Prior systemic therapy, *n* (%)	40 (100.0)	20 (100.0)	22 (100.0)
1 regimen	18 (45.0)	9 (45.0)	1 (4.5)

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		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	**αPD-1/PD-L1 naïve**	**αPD-1/PD-L1 resistant/refractory**
Characteristic	**(*N* = 40)**	**(*N* = 20)**	**(*N* = 22)**
2 regimens	10 (25.0)	6 (30.0)	5 (22.7)
3 regimens	6 (15.0)	4 (20.0)	7 (31.8)
≥4 regimens	6 (15.0)	1 (5.0)	9 (40.9)
Prior I-O therapy, *n* (%)	0 (0.0)	0 (0.0)	22 (100.0)
αPD-1 antibody	0 (0.0)	0 (0.0)	13 (59.1)
αPD-L1 antibody	0 (0.0)	0 (0.0)	10 (45.5)
MSI-H/dMMR detection method, *n* (%)[d]			
PCR	18 (45.0)	5 (25.0)	9 (40.9)
IHC	4 (10.0)	7 (35.0)	1 (4.5)
Both	18 (45.0)	8 (40.0)	12 (54.5)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; I-O, immuno-oncology; PD-1, programmed cell death-1; PD-L1, programmed death ligand 1.

[a]Includes one case each of adrenal carcinoma, adenocarcinoma of unknown primary site, well-differentiated adenocarcinoma, and carcinoma of unknown primary site.

[b]One case of prostate adenocarcinoma.

[c]Includes one case each of adrenal carcinoma and adenocarcinoma of the cardia.

[d]As reported by the sites and per local testing.

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Endpoints and assessments

The primary endpoint was safety for all cohorts. Investigators evaluated adverse events (AE) using the criteria of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Patients were followed for safety and tolerability assessments for at least 24 weeks of treatment after the last patient was enrolled. Patients were also evaluated through physical examinations, laboratory tests, and electrocardiograms during the study. Efficacy endpoints were ORR, disease control rate (DCR), PFS, and OS. Assessment of tumor responses were based on RECIST v1.1 guidelines. Local tumor imaging was performed at baseline (within 28 days of starting cycle 1), every 8 weeks (±7 days) over the next 12 months, and thereafter every 12 weeks. Tumor responses and disease progression were confirmed by repeat imaging performed ≥4 weeks later. To assess PFS and OS, patients were followed until death or study completion. Blood samples were collected from all patients to assess pharmacokinetic characteristics following LY3300054 and anti–TIM-3 antibody administration. As an exploratory endpoint, blood and intratumoral immune markers, and plasma kynurenine (Kyn) and tryptophan (Trp) concentrations were assessed before and during treatment to determine biomarkers of response and treatment resistance mechanisms.

Pharmacokinetic analysis

Human plasma samples were analyzed for LY3300054 by PPD Laboratories using a validated ECL immunoassay. A population pharmacokinetic model was developed previously using data from 61 patients with 268 samples that were available at the time of analysis. For each cohort, samples were taken at pre-dose and 2, 24, 72, and 168 hours following the start of the first dose of PD-L1 inhibitor; at pre-dose on day 15 of cycle 1; at day 1 of cycles 2–4; and subsequently every 3 cycles.

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Tumor biomarkers

Approximately 30%–40% of patients had on-treatment tissue biopsies. The most common reason that tumor tissue was not collected was due to (i) on-treatment biopsy was optional before a protocol

amendment (approved in May 2018) which made on-treatment biopsy a requirement after May 2018, (ii) the investigator considered it unsafe to perform a biopsy, (iii) the patient refused an on-treatment biopsy, or (iv) the patient discontinued from study treatment before their scheduled on-treatment biopsy. Among all the tumor tissue collected at baseline and on-treatment, some did not have malignant tumor cells with which we could perform biomarker testing. A total of 21 paired tumor tissue samples were evaluable for biomarker testing from the entire study. Tissue immunoreactivity for PD-L1 was assessed using the Dako PD-L1 IHC 22C3 pharmDx kit (RRID:AB_2833074; Agilent). CD8 IHC was performed using a monoclonal mouse anti-human CD8 ready-to-use antibody (RRID:AB_2075537; Dako C8-144B) on an automated staining platform. TIM-3 IHC was performed using a clone against the TIM-3 extracellular domain (RRID:AB_2716862; Cell Signaling Technology). Samples were analyzed at Clinical Diagnostics Laboratory, Eli Lilly and Company, and results were evaluated by a qualified pathologist according to prespecified interpretation guidelines. The average number of positive cells counted approximately within five randomly selected representative high-power microscopic fields (hpf) were reported for CD8 and TIM-3. PD-L1 expression was reported using tumor proportional score (TPS), which is the percentage of viable tumor cells showing partial or complete membranous staining at any intensity. The specimen was considered negative for PD-L1 expression if TPS was < 1%, and positive if TPS was ≥ 1%.

Pharmacodynamic analysis

Peripheral blood was collected in sodium heparin tubes to monitor circulating quantities of $CD4^+$ and $CD8^+$ T-cell subpopulations expressing the activation marker HLA-DR or the intracellular proliferation marker Ki67. Blood samples were obtained at baseline (within 7 days before cycle 1 day 1) and pre-dose cycle 1 day 1, then at cycle 1 day 8, cycle 1 day 15, cycle 2 day 1, and cycle 3 day 1. Immunophenotyping was centrally assessed from peripheral blood using qualified flow cytometry–based assays (Q2 Solutions). Only patients with available and evaluable confirmed best overall response status were included in the analysis.

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Plasma Kyn and Trp

To explore whether circulating Kyn and Trp levels changed on therapy, plasma samples from 14 patients with pairs of measurable results in the PD-L1 inhibitor monotherapy cohort were analyzed at baseline and day 1 of cycle 3. Kyn and Trp levels were not assessed in patients receiving combination therapy. Plasma Kyn and Trp levels were measured using LC/MS-MS (Q2 Solutions).

Statistical analysis

Safety and efficacy were analyzed in all patients receiving any quantity of study treatment.

A sample size of 20–40 patients per cohort was selected to allow adequate assessment of safety and preliminary antitumor activity. The 95% confidence interval (CI) for the estimated incidence rates of specified AEs or response to treatment is approximately equal to the observed incidence rate ± 17%–36% for $N = 10$, ± 16%–24% for $N = 20$, and ± 9%–16% for $N = 40$.

The protocol was amended in May 2018 to expand the monotherapy cohort size from 20 to 40. This was based on the safety and efficacy data from the 22 patients enrolled in the monotherapy cohort between December 2016 and June 2017. The combination cohort sample size remained at 20.

The frequency and percentage of AEs are presented using descriptive statistics by cohort. ORR was defined as the proportion of enrolled patients in each cohort who achieved a best overall response of confirmed complete response (CR) or partial response (PR). DCR was defined as the proportion who had a best overall response of confirmed CR, confirmed PR, or stable disease (SD). PFS and OS were summarized by Kaplan–Meier methodology. Median and 95% CI were estimated where data were available.

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Results

Patients

Between December 13, 2016 and December 6, 2019, 82 eligible patients with MSI-H/dMMR advanced refractory solid tumors were enrolled and treated. Of these, 40 anti–PD-1/PD-L1–naïve patients were enrolled to receive LY3300054 monotherapy, 20 anti–PD-1/PD-L1–naïve patients were enrolled to receive the combination treatment, and 22 patients who had progressed after prior PD-1/PD-L1 inhibitor treatment were assigned to receive the combination treatment.

Baseline patient characteristics are given in **Table 1**. Colorectal tumors were the most frequent tumor type overall (*n* = 39, 47.6%), especially in the anti–PD-1/PD-L1–resistant/refractory cohort receiving combination therapy (17/22, 77.3%). Most patients in the anti–PD-1/PD-L1–naïve monotherapy (70.0%) and combination therapy (75.0%) cohorts had received one or two prior treatment lines, whereas >70% of patients in the anti–PD-1/PD-L1–resistant combination therapy cohort had received three or more lines. In the anti–PD-1/PD-L1–resistant cohort, prior anti–PD-1 agents (13/22, 59.1%) or anti–PD-L1 agents (10/22, 45.5%) had generally been received as monotherapy (in 18/22 patients, 81.8%). In most cases, regimens had ended immediately before entering the study (18/22, 81.8%; Supplementary Table S1).

At the analysis cut-off date (June 1, 2020), 28 of 40 patients had discontinued monotherapy (*n* = 23 for PD, *n* = 2 for physician decision, *n* = 3 for AE) with a median (range) follow-up time of 19.1 (1.2–40.3) months. A total of 2 patients discontinued monotherapy based on the physician's decision; both patients had received approximately 2-year study treatment and had CR at the time of discontinuation. Thirteen of 20 with anti–PD-1/PD-L1–naïve tumors (*n* = 8 for PD, *n* = 2 for AE, *n* = 2 for physician decision, *n* = 1 for study protocol deviation) and 19 of 22 with anti–PD-1/PD-L1–resistant/refractory tumors (*n* = 17 for PD, *n* = 1 for AE, and *n* = 1 for sudden death) had discontinued combination therapy. A total of 2 patients discontinued combination treatment as per physician decision and 1 patient underwent primary tumor resection to control lesion bleeding. The other patient received 1-year study treatment. Treatment extension over 1 year was not requested by the treating physician. For combination therapy, median (range) follow-up time was

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14.8 (1.1–17.6) months and 11.9 (0.6–18.4) months for patients with anti–PD-1/PD-L1–naïve and anti–PD-1/PD-L1–resistant/refractory tumors, respectively.

Safety and tolerability

The median LY3300054 treatment duration was 9.0 months (range, 0.92–40.7 months) for the anti–PD-1/PD-L1–naïve monotherapy cohort, 8.1 months (0.92–17.9 months) for the anti–PD-1/PD-L1–naïve combination therapy cohort, and 2.6 months (0.46–18.4 months) for the combination therapy cohort with anti–PD-1/PD-L1–resistant/refractory tumors. Treatment was continued beyond 12 months (≥13 cycles) in 13 patients (32.5%) receiving monotherapy, 6 (30.0%) with PD-1/PD-L1 inhibitor–naïve tumors receiving combination therapy, and 2 (9.1%) with anti–PD-1/PD-L1–resistant/refractory tumors receiving combination therapy.

In the PD-1/PD-L1 inhibitor–naïve cohorts, treatment-related AEs (any grade) occurred in 22 patients (55.0%) receiving LY3300054 monotherapy and 13 patients (65.0%) receiving combination therapy (Table 2). Diarrhea was the most frequent treatment-related AE in the monotherapy cohort (7/40, 17.5%). Pruritus, hyperthyroidism, and hypothyroidism were other frequent treatment-related AEs in both PD-1/PD-L1 inhibitor–naïve cohorts (≥7.5% in each cohort). Fewer patients (6/22, 27.3%) had treatment-related AEs in the PD-1/PD-L1 inhibitor–resistant/refractory combination cohort, the most common being fatigue (2/22, 9.1%).

Table 2.

Safety summary.

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		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	αPD-1/PD-L1 naïve	αPD-1/PD-L1 resistant/refrac

Event, *n* (%)	(*N* = 40)	LY3300054 + αTIM-3 combination (*N* = 20)	(*N* = 22)
	LY3300054 monotherapy	αPD-1/PD-L1 naïve	αPD-1/PD-L1 resistant/refrac

Event, *n* (%)	(*N* = 40)	(*N* = 20)	(*N* = 22)
Any AE	39 (97.5)	20 (100.0)	20 (90.9)
Treatment-related AE	22 (55.0)	13 (65.0)	6 (27.3)
Diarrhea	7 (17.5)	0 (0.0)	0 (0.0)
Hyperthyroidism	5 (12.5)	2 (10.0)	0 (0.0)
Fatigue	4 (10.0)	1 (5.0)	2 (9.1)
Pruritus	3 (7.5)	5 (25.0)	1 (4.5)
Asthenia	3 (7.5)	1 (5.0)	0 (0.0)
Myalgia	3 (7.5)	1 (5.0)	0 (0.0)
Nausea	3 (7.5)	1 (5.0)	0 (0.0)
Blood creatine phosphokinase increase	3 (7.5)	0 (0.0)	0 (0.0)
Hypothyroidism	3 (7.5)	2 (10.0)	0 (0.0)
Pyrexia	2 (5.0)	2 (10.0)	1 (4.5)
Dry skin	1 (2.5)	2 (10.0)	0 (0.0)
ALT increase	1 (2.5)	2 (10.0)	1 (4.5)

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		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	αPD-1/PD-L1 naïve	αPD-1/PD-L1 resistant/refrac
Event, *n* (%)	**(*N* = 40)**	**(*N* = 20)**	**(*N* = 22)**
AST increase	0 (0.0)	2 (10.0)	1 (4.5)
Maculopapular rash	0 (0.0)	2 (10.0)	0 (0.0)
Any grade ≥3 AE	16 (40.0)	11 (55.0)	4 (18.2)
Treatment-related grade ≥3 AE	2 (5.0)	2 (10.0)	1 (4.5)
Blood creatine phosphokinase increase	1 (2.5)	0 (0.0)	0 (0.0)
Diarrhea	1 (2.5)	0 (0.0)	0 (0.0)
Hyponatremia	1 (2.5)	0 (0.0)	0 (0.0)
ALT increase	0 (0.0)	1 (5.0)	1 (4.5)
AST increase	0 (0.0)	1 (5.0)	1 (4.5)
Lipase increase	0 (0.0)	1 (5.0)	0 (0.0)
Serious AE	13 (32.5)	5 (25.0)	3 (13.6)
Treatment-related serious AE	3 (7.5)	1 (5.0)	0 (0.0)
Treatment-related AE leading to discontinuation	2 (5.0)	1 (5.0)	0 (0.0)

Note: Treatment-related AEs occurring in >5% of one or more cohorts and all treatment-related grade ≥3 AEs are shown.

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Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PD-1, programmed cell death-1; PD-L1, programmed death ligand 1.

Four patients experienced treatment-related serious AEs (SAE): 3 receiving LY3300054 monotherapy and 1 receiving combination therapy (PD-1/PD-L1 inhibitor–naïve cohort). In the monotherapy cohort, one treatment-related SAE occurred in each of the 3 patients: grade 2 pleural effusion, grade 3 blood creatine phosphokinase increase, and grade 2 pneumonitis that led to study discontinuation. The grade 2 pneumonitis occurred after cycle 18 and the patient recovered after corticosteroid treatment. The patient in the combination therapy cohort experienced grade 3 alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increase, which resulted in discontinuation from the study. The patient received corticosteroids, which alleviated the SAEs to grade 1 within 3 days, and was fully recovered in 14 days. Three additional patients had treatment-related grade ≥3 AEs that were not SAEs: 1 patient in the monotherapy cohort who developed grade 3 diarrhea and hyponatremia, 1 in the PD-1/PD-L1 inhibitor–naïve combination therapy cohort who developed grade 3 lipase increase, and 1 in the PD-1/PD-L1 inhibitor–resistant/refractory cohort who developed grade 3 ALT and AST increase. All treatment-related grade 3 AEs resolved. No treatment-related AEs led to death. One sudden death was reported in a patient with colorectal cancer in the PD-1/PD-L1 inhibitor–resistant/refractory combination therapy cohort. The patient had grade 3, worsening thrombosis (not related to study treatment) around the time of death.

Antitumor activity

LY3300054 monotherapy resulted in an ORR of 32.5% and DCR of 60.0% in patients with PD-1/PD-L1 inhibitor–naïve tumors (**Table 3**). Five patients (12.5%) receiving monotherapy, 1 each with ovarian, small intestine, colon, gastric, and endometrial cancer, achieved CRs lasting a median of 32.2 months (range, 7.4–36.9 months; **Fig. 1**). A further 8 patients (20.0%) achieved PRs lasting a median of 13.6 months (range, 5.6–37.0 months), and 11 (27.5%) had stable disease as best response. Among patients with stable disease, 9 of 11 patients had target lesion shrinkage. The median PFS for the monotherapy cohort

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was 7.4 months (95% CI: 1.8–23.8 months) and the median OS was not reached [OS at 12 months, 71.0% (95% CI: 53.5–82.9)].

Table 3.

Confirmed efficacy results per RECIST v1.1.

		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	**αPD-1/PD-L1 naïve**	**αPD-1/PD-L1 resistant/refractory**
	(*N* = 40)	**(*N* = 20)**	**(*N* = 22)**
Best overall response			
Complete response	5 (12.5)	2 (10.0)	0 (0.0)
Partial response	8 (20.0)	7 (35.0)	1 (4.5)
Stable disease	11 (27.5)	5 (25.0)	6 (27.3)
Progressive disease	14 (35.0)	6 (30.0)	12 (54.5)
Nonevaluable	2 (5.0)	0 (0.0)	3 (13.6)
Objective response rate[a]	13 (32.5)	9 (45.0)	1 (4.5)
Disease control rate[b]	24 (60.0)	14 (70.0)	7 (31.8)
Median time to response, months (95% CI)	1.8 (1.6–3.4)	1.9 (1.8–5.4)	3.5 (NC)
Progression-free survival			
Events, *n* (%)	25 (62.5)	11 (55.0)	18 (81.8)
Median, months (95% CI)	7.4 (1.8–23.8)	7.6 (1.9–NR)	1.9 (1.6–3.7)

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		LY3300054 + αTIM-3 combination	
	LY3300054 monotherapy	**αPD-1/PD-L1 naïve**	**αPD-1/PD-L1 resistant/refractory**
	(*N* = 40)	**(*N* = 20)**	**(*N* = 22)**
6-month rate, % (95% CI)	52.5 (36.1–66.5)	69.3 (44.0–84.9)	24.0 (8.7–43.3)
12-month rate, % (95% CI)	40.7 (25.0–55.8)	39.6 (17.6–61.0)	12.0 (2.2–30.7)
Overall survival			
Deaths, *n* (%)	13 (32.5)	6 (30.0)	9 (40.9)
Median, months (95% CI)	NR (14.4–NR)	NR (11.1–NR)	9.1 (6.2–NR)
6-month rate, % (95% CI)	77.0 (60.5–87.3)	85.0 (60.4–94.9)	79.4 (53.9–91.7)
12-month rate, % (95% CI)	71.0 (53.5–82.9)	63.8 (34.9–82.5)	44.1 (18.7–67.0)

Abbreviations: CI, confidence interval; NC, not calculable; NR, not reached.

[a]Objective response defined as complete response or partial response.

[b]Disease control defined as complete response, partial response, or stable disease.

Figure 1.

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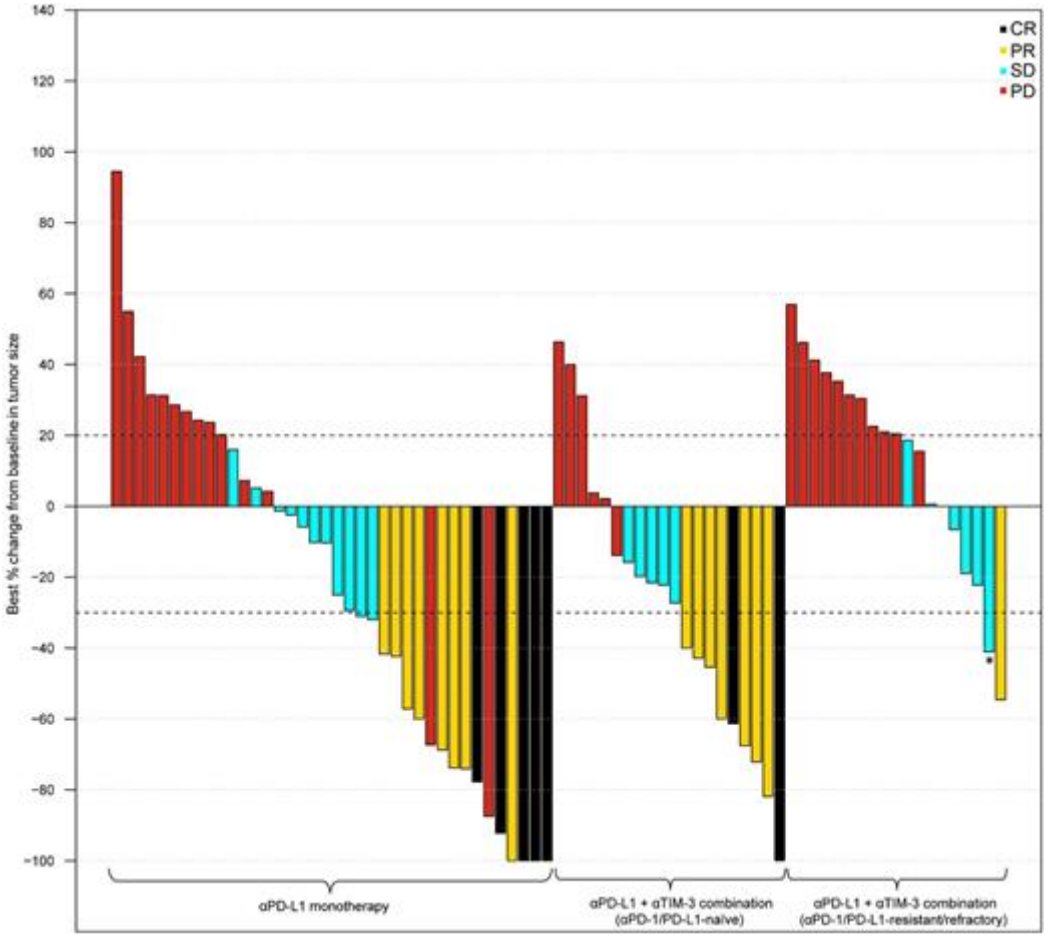

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Waterfall plot. Each bar represents 1 patient. Excludes patients with tumors that could not be evaluated post baseline. In 1 patient [indicated by the asterisk (*)], a PR was not confirmed in the subsequent assessment despite a best overall tumor size reduction >30%. CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

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Combination therapy with LY3300054 and the anti–TIM-3 antibody resulted in an ORR of 45.0% and DCR of 70.0% in the PD-1/PD-L1 inhibitor–naïve combination cohort. Two patients (10.0%) achieved CRs: 1

patient with colorectal cancer with a response duration of 14.8 months, and 1 patient with endometrial cancer with a response duration of 12.8 months. Seven patients (35.0%; 3 with colorectal cancer, 2 with gastric cancer, 1 each with intestinal or endometrial cancer) achieved PRs of median duration 4.9 months (range, 1.0–14.8 months). Five patients (25.0%) achieved a best response of SD (target lesion reduction range, −15.2% to −22.2%). The median PFS was 7.6 months (95% CI: 1.9 months–not reached) and the median OS was not reached [OS at 12 months, 63.8% (95% CI: 34.9–82.5)].

Three patients (13.6%) in the PD-1/PD-L1 inhibitor–resistant/refractory cohort had disease progression before first tumor assessment and were considered not evaluable for best overall response (Supplementary Table S2). Twelve (54.5%) had PD as best overall response. One patient (4.5%) with PD-1/PD-L1 inhibitor–resistant/refractory intestinal adenocarcinoma achieved a confirmed PR of 13.1 months and continues on combination therapy. This patient previously received FOLFOX and later FOLFIRI chemotherapy regimens, and subsequently an investigational anti–PD-1 agent. The patient did not receive any other systemic treatment before entering the study. The remaining 6 patients (27.3%) had stable disease, 4 of which had target lesion shrinkage. One of these 4 patients had received 12 cycles of treatment and had a 41.1% target lesion size reduction; however, a PR was not confirmed in the following assessment. Another had a 22.2% target lesion size reduction and continues treatment after 20 cycles. The median OS was 9.1 months (95% CI: 6.2 months–not reached), with survival rates of 79.4% at 6 months and 44.1% at 12 months.

Kaplan–Meier curves of PFS and OS are given for each cohort in Supplementary Figs. S1 and S2 and confirmed best overall response by tumor type is given in Supplementary Table S2.

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Response rate (CR + PR) was compared for PD-L1 TPS <1% and TPS ≥1% using Fisher exact test within each treatment group. No statistically significant associations were found for any treatment group

(LY3300054 monotherapy, $P = 0.224$; PD-1/PD-L1 inhibitor–naïve combination cohort, $P = 0.627$; PD-1/PD-L1 inhibitor–resistant/refractory combination cohort, $P = 0.999$).

Pharmacokinetics

We previously reported LY3300054 to have an approximately dose-proportional pharmacokinetic profile described by a two-compartment model, with first-order linear clearance, a low clearance (8.9 mL/hour), limited volume of distribution (5.92 L), and approximately 3-week half-life. The pharmacokinetics of LY3300054 as monotherapy and in combination with anti–TIM-3 antibody were adequately described by the population pharmacokinetic model, indicating no effect of the anti–TIM-3 antibody on the pharmacokinetics of LY3300054 (Supplementary Fig. S3).

Baseline and on-treatment tumor immune marker expression versus clinical activity

Tumor tissue baseline CD8 and TIM-3 expression was comparable between the PD-1/PD-L1 inhibitor–naïve and PD-1/PD-L1 inhibitor–resistant/refractory cohorts (**Fig. 2**, see the median value listed in the right of each plot; Supplementary Table S3). Most patients had low tumor baseline PD-L1 expression: 45 (71.4%) patients had PD-L1 TPS <1% versus 18 (28.6%) with TPS ≥1% (Supplementary Table S3). Although not statistically significant, median tumor CD8 expression at baseline was higher in responders than in patients with PD in the monotherapy (median count, 76.0 vs. 38.5; $P = 0.4$) and anti–PD-1/PD-L1–naïve combination therapy (64.5 vs. 13.0; P value not calculable) cohorts (**Fig. 2**, left and middle panel on the top). The patient with a PR in the PD-1/PD-L1 inhibitor–resistant/refractory cohort had a median CD8 level of 69, which was higher than most other patients with SD or PD in this cohort (**Fig. 2**, right panel on the top). Baseline tissue TIM-3 expression did not differ by confirmed best overall response in any of these three cohorts. Baseline median tissue TIM-3 count was 16, 1, and 24.5, respectively, in patients receiving monotherapy treatment with best overall response of CR/PR, SD, and PD; baseline median TIM-3 count

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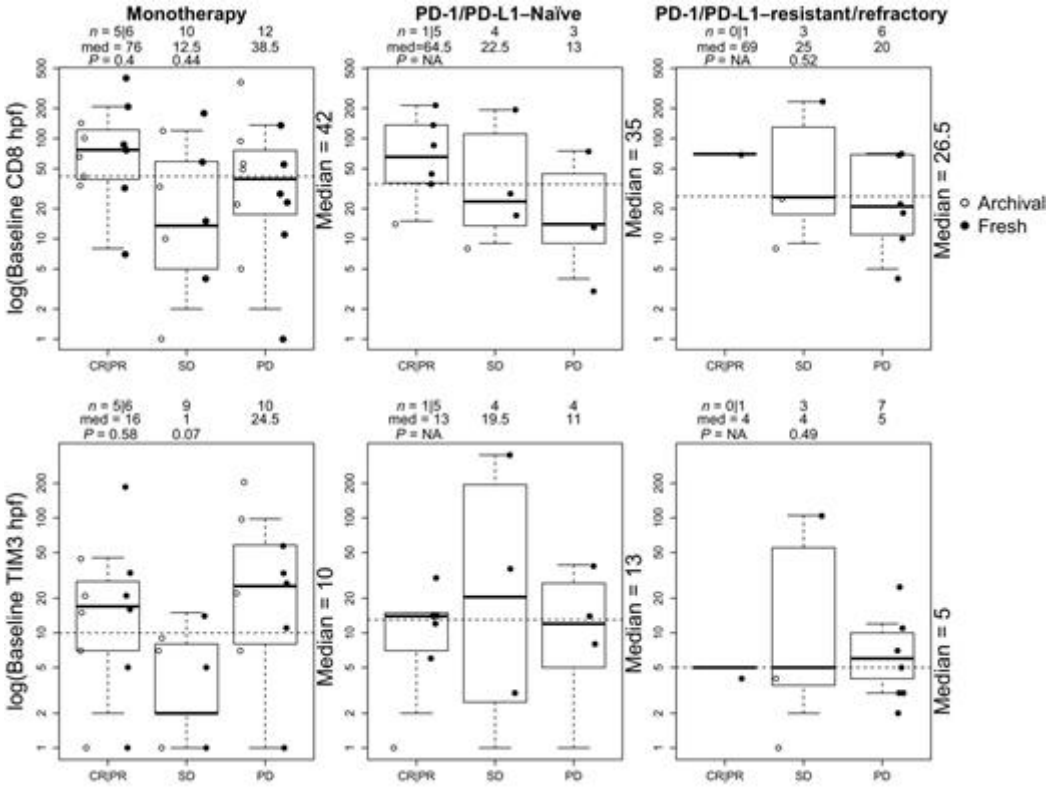
was also similar for PD-1/PD-L1–naïve patients receiving combination treatment: 13 versus 19.5 versus 11 for patients with CR/PR, SD, and PD, respectively (Fig. 2).

Figure 2.



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Baseline tumor tissue CD8 and TIM-3 levels grouped by confirmed best overall response. CD8 and TIM-3 levels were correlated to clinical responses in baseline fresh and archival tumor biopsies for patients receiving monotherapy (left), PD-1/PD-L1–naïve patients receiving combination (middle), and PD-1/PD-L1–resistant/refractory patients receiving combination treatment (right). Two-sided two-group *t* tests were used

to evaluate whether the median expression of markers were equal between patients with SD or CR/PR versus patients with PD. Only patients with available and evaluable best overall response status were included in the analysis. At the top of each plot, sample size [*n* includes patients with archival tumor tissues (represented by white circles) and patients with fresh tumor tissues (represented by black circles)], median level (IHC value), and *P* values are listed. Overall medians (for all patients enrolled in each cohort) are given in the bottom right of each plot. CR, complete response; hpf, high-power microscopic fields; IHC, immunohistochemistry; NA, not analyzed; PD, progressive disease; PR, partial response; SD, stable disease.

On the basis of limited paired tumor tissues, we did not observe consistent on-treatment changes in intratumoral CD8 or TIM-3 expression with LY3300054 monotherapy or LY3300054/anti–TIM-3 antibody combination therapy, and changes appeared to be independent of clinical response (Supplementary Fig. S4).

Pharmacodynamic activity

At cycle 1 day 8, Ki67$^+$ HLA-DR$^+$ (proliferating and activated) CD4$^+$ and CD8$^+$ T cells showed a trend of transient increase in peripheral blood from most patients in the monotherapy and PD-1/PD-L1 inhibitor–naïve combination cohorts. These observations indicate pharmacodynamic activity of PD-L1 pathway blockade (**Fig. 3**).

Figure 3.

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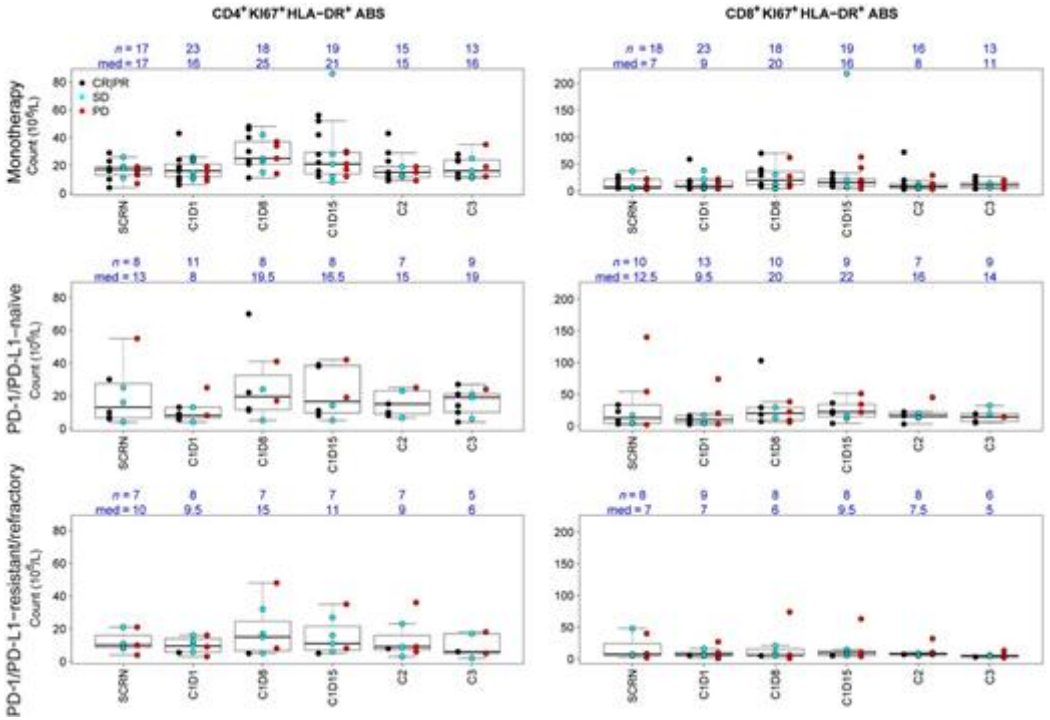

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Peripheral blood CD4+ Ki67+HLA-DR+ and CD8+Ki67+HLA-DR+ changes after treatment. Peripheral blood T cells were analyzed by flow cytometry. Absolute count (10^6/L) was represented in *Y* axis. Different timepoints were presented in *X* axis. Sample size and median value are given at the top of each plot. ABS, absolute count; C1D1, cycle 1 day 1; C1D8, cycle 1 day 8; C1D15, cycle 1 day 15; C2, cycle 2; C3, cycle 3; SCRN, screening.

The transient increases in proliferating and activated T cells were modest at cycle 1 day 8 in the PD-1/PD-L1 inhibitor–resistant/refractory cohort (**Fig. 3**).

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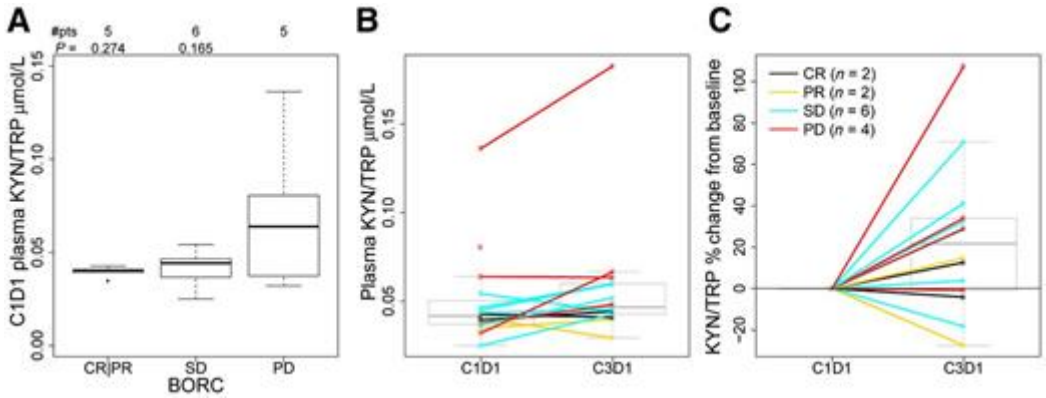
Increases in proliferating and activated CD4+ or CD8+ T cells did not correlate with confirmed best overall response.

Baseline and on-treatment Kyn/Trp ratio changes among patients receiving LY3300054 monotherapy

IDO-1 is a heme-containing enzyme that catalyzes the degradation of Trp into Kyn (29). The plasma Kyn/Trp ratio is frequently used to express or reflect the activity of the extrahepatic Trp-degrading enzyme IDO (30).

Upregulated IDO-1 activity has been reported in patients receiving immunotherapy of different tumor types such as melanoma, renal cell carcinoma, and NSCLC (31–33). It remains unclear whether IDO-1 activity may comprise a potential resistance mechanism for MSI-H/dMMR patients receiving single-agent anti–PD-1/PD-L1 treatment (34). Patients with a confirmed best overall response of PD on monotherapy had a trend for higher plasma Kyn/Trp ratios at baseline, signaling potentially increased IDO-1 activity (Fig. 4). In most patients with measurable samples, the Kyn/Trp ratio increased at cycle 3 day 1 from baseline levels (10/14, 71.4%). Patients with CRs or PRs tended to have lower percentage changes in plasma Kyn/Trp ratio at cycle 3 day 1 than at baseline.

Figure 4.



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Kyn/Trp ratio at baseline and on-treatment in LY3300054 monotherapy cohort. **A,** Plasma Kyn/Trp ratio at cycle 1 day 1 (C1D1; baseline) for patients with CRs|PRs, SD, and PD. One-sided two-group Wilcoxon tests were applied to determine whether the averages were smaller in the CR|PR group or SD group versus the PD group. Sample size and *P* values are given above the plot. Kyn/Trp ratios at C1D1 and C3D1 (**B**) and % change from baseline (**C**) are shown by confirmed best overall response (BORC) grouping. Analyses only included patients in the LY3300054 monotherapy cohort with measurable samples.

Discussion

The results indicate that a new anti–PD-L1 antibody, LY3300054, has a manageable safety profile in patients with MSI-H/dMMR tumors as well as clinical activity, with an ORR of 33%, DCR of 60%, and 1-year OS rate of 71%. This study is the first to describe the safety and clinical activity of an anti–PD-L1/anti–TIM-3 antibody combination in patients with MSI-H/dMMR tumors. Combining TIM-3 inhibition with LY3300054 did not compromise safety and tolerability, and may have led to numerically higher response rates in anti–PD-1/PD-L1 inhibitor–naïve MSI-H/dMMR tumors (ORR, 45%; DCR, 70%; 1-year OS, 64%).

The AEs related to LY3300054 monotherapy and their frequency are consistent with the known safety profile for PD-L1 inhibitors in various cancers (**35–38**). Importantly, the frequencies and types of treatment-related AEs did not appear to change when LY3300054 was combined with the anti–TIM-3 antibody. In contrast, greater frequencies of immune-related toxicities are seen when combining CTLA-4 and PD-1 inhibitors than monotherapy with either agent (**14, 39**). It has been argued that targeting TIM-3 alongside other checkpoint inhibitors may prevent some of the immune-related toxicities observed with PD-1/PD-L1 or CTLA-4 inhibitors (**17**). This reasoning is due to TIM-3 expression being limited to terminally differentiated IFNγ-producing T cells, whereas PD-1 and CTLA-4 are expressed on all activated T cells (**17**). The particularly low incidence of treatment-related AEs in the anti–PD-1/PD-L1–resistant/refractory

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cohort might be linked with the relative lack of proliferating and activated peripheral T cells at cycle 1 day 8, although other explanations could be different disease types and treatment duration.

ORRs in the range of 31%–36% and DCRs in the range of 52%–69% have been observed for PD-1 inhibitors in MSI-H/dMMR cohorts (**9–12**). The ORR (33%) and DCR (60%) with monotherapy in our study are comparable with these earlier observations, although direct comparisons of clinical activity between studies are complicated by differences in patient, disease, immunotherapy, and study design characteristics. A further caveat is that MSI-H/dMMR tumors respond differently to immunotherapy depending on the tumor type and the magnitude of the mutational burden (**2**).

Response rates were numerically greater in PD-1/PD-L1 inhibitor–naïve patients when combining LY3300054 with inhibition of TIM-3 (ORR, 45%; DCR, 70%), which has been identified as a potential resistance mechanism to PD-1/PD-L1 blockade (**17, 24**). However, baseline tumor immune markers, including TIM-3 expression, appeared to be no different between responders and nonresponders. The monotherapy and combination therapy cohorts in this study were neither randomly assigned nor powered for direct comparison, limiting the conclusions that can be drawn from the clinical activity endpoints. A randomized trial would be required to address whether co-blockade of PD-L1 and TIM-3 can overcome primary resistance and improve ORRs in anti–PD-1/PD-L1–naïve MSI-H/dMMR populations.

CheckMate-142 reported nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) once every 3 weeks for four doses and then nivolumab 3 mg/kg once every 2 weeks had achieved ORR of 55% (95% CI, 45.2–63.8) in patients with colorectal cancer with dMMR/MSI-H who had received (or were ineligible for) prior systemic treatments. Twelve-month PFS and OS rates were 71% and 85%, respectively (**14**). Therefore, a patient selection strategy to further enrich for responders is needed for LY3300054 in combination with anti–TIM-3 in this similar population. It would be intriguing to explore patient characteristics including clinical status, genomic features, and tumor microenvironment to understand their response and resistance to anti–

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PD1/PD-L1 in combination with either anti–CTLA-4 or anti–TIM-3. There may be some population who respond to anti–PD1/PD-L1 in combination with anti–TIM-3 but may not respond to anti–PD-1/PD-L1 in combination with anti–CTLA-4 and vice versa, considering the distinct mechanisms of CTLA-4 and TIM-3.

The LY3300054/anti–TIM-3 antibody combination showed limited clinical activity in patients with tumors resistant/refractory to prior PD-1/PD-L1 treatment (ORR, 4.5%; DCR, 32%). Only one PR of 13-month duration occurred in this cohort, in a patient with intestinal adenocarcinoma, although another patient with colorectal cancer had SD with a 22% target lesion size reduction and continues treatment after 20 cycles. Comprehensive immune biomarker and molecular profiling may reveal which protein, genomic, and cellular characteristics predict responders. A patient-tailored therapy will be critical for the LY3300054/anti–TIM-3 antibody combination in the PD-1/PD-L1 inhibitor–resistant MSI-H/dMMR patients if there is any further clinical development plan in this population.

Blood samples and tumor tissues were analyzed to understand pharmacodynamic effects and to help with a predictive biomarker strategy for monotherapy/combination treatment. Blood-based biomarker changes identified in this study are consistent with the findings of previous studies (**28**). Peripheral proliferating and activated immune cells increased upon treatment. Plasma Kyn levels also increased in most patients receiving monotherapy treatment; however, the expression levels of tumor tissue–based immune biomarkers including CD8 and TIM-3 did not demonstrate consistent on-treatment effects. CD8 levels at baseline were higher in responders compared with progressors receiving monotherapy and combination treatment. Interpretation of the tumor tissue biomarker data was limited by the relatively low number of evaluable samples that could be collected.

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This study had several limitations. No central testing was performed to confirm MSI-H/dMMR status, *RAS* or *BRAF* mutation status, or patient clinical history of Lynch syndrome and no comprehensive genomic

profiling was undertaken, which meant we could not correlate tumor mutational burden or genomic alterations with clinical efficacy.

Conclusions

LY3300054 monotherapy and LY3300054/anti–TIM-3 antibody combination therapy each had a manageable safety profile in patients with advanced MSI-H/dMMR solid tumors. Both monotherapy and the combination showed considerable clinical activity, durable responses, and encouraging survival rates against MSI-H/dMMR solid tumors naïve to prior PD-1/PD-L1 inhibitor therapy. The results also suggest PD-L1/TIM-3 coinhibition had limited clinical activity in patients with MSI-H/dMMR tumors resistant/refractory to prior PD-1/PD-L1 inhibitor treatment. Further clinical and translational studies are needed to explore how TIM-3 and other pathways contribute to PD-1/PD-L1 inhibition–related primary and acquired resistance.

Authors' Disclosures

A. Hollebecque reports non-financial support from Eli Lilly and Company during the conduct of the study. A. Hollebecque also reports personal fees from Amgen, BMS, Eisai, Debiopharm, QED Therapeutics, and Basilea; grants, personal fees, and non-financial support from Incyte; grants and non-financial support from AstraZeneca; and non-financial support from Roche and Servier outside the submitted work. H.C. Chung reports grants from Eli Lilly and Company, GSK, MSD, Merck-Serono, BMS-Ono, Taiho, Amgen, BeiGene, Incyte, and Zymework, as well as personal fees from Merck-Serono, Eli Lilly and Company, Taiho, Celltrion, MSD, BMS, Gloria, BeiGene, Amgen, and Zymework outside the submitted work. M.J. de Miguel reports other support from Pharmamar, Sanofi, Roche, Novartis, AbbVie, Eli Lilly and Company, MSD, Cytomex, Array, Basilea, Bayer, Genmab, Faron, Genentech, Janssen, and Menarini outside the submitted work. A. Italiano reports grants and personal fees from Bayer, Roche, and Ipsen, as well as grants from AstraZeneca, BMS, MSD, and SpringWorks outside the submitted work. J.-P. Machiels reports other

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Authors' Contributions

A. Hollebecque: Data curation, investigation, project administration, writing–review and editing. **H.C. Chung:** Data curation, investigation, project administration, writing–review and editing. **M.J. de Miguel:** Data curation, investigation, project administration, writing–review and editing. **A. Italiano:** Data curation, investigation, project administration, writing–review and editing. **J.-P. Machiels:** Data curation, investigation, project administration, writing–review and editing. **C.-C. Lin:** Data curation, investigation,

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project administration, writing–review and editing. **N.C. Dhani:** Data curation, investigation, project administration, writing–review and editing. **M. Peeters:** Data curation, investigation, project administration, writing–review and editing. **V. Moreno:** Data curation, investigation, project administration, writing–review and editing. **W.-C. Su:** Data curation, investigation, project administration, writing–review and editing. **K.H. Chow:** Data curation, formal analysis, investigation, methodology, writing–review and editing. **V.R. Galvao:** Formal analysis, investigation. **M. Carlsen:** Data curation, formal analysis, investigation, writing–review and editing. **D. Yu:** Data curation, formal analysis, investigation, writing–review and editing. **A.M. Szpurka:** Conceptualization, data curation, formal analysis, supervision, investigation, project administration, writing–review and editing. **Y. Zhao:** Data curation, formal analysis, supervision, investigation, methodology, project administration, writing–review and editing. **S.L. Schmidt:** Data curation, formal analysis, writing–review and editing. **L. Gandhi:** Data curation, investigation, project administration, writing–review and editing. **X. Xu:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–original draft, project administration, writing–review and editing. **Y.-J. Bang:** Data curation, investigation, project administration, writing–review and editing.

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Supplementary data

Supplementary Figure S1

Figure S1. Progression-free survival

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Supplementary Figure S2

Figure S2. Overall survival

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Supplementary Figure S3

Figure S3. Pharmacokinetics for the anti-PD-L1 antibody LY3300054 alone and in combination with anti-TIM-3 antibody.

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Supplementary Figure S4

Figure S4. Changes in intratumoral immune markers after treatment

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Supplementary Table S1

Table S1. Prior immunotherapies in anti-PD-1/PD-L1-resistant/refractory cohort

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Supplementary Table S2

Table S2. Confirmed best overall response per RECIST-1.1 by tumor type

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Supplementary Table S3

Table S3. Baseline distribution of tumor microenvironment biomarkers in MSI-H/dMMR patients

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