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First-in-human phase I/Ib open-label dose-escalation study of GWN323 (anti-GITR) as a single agent and in combination with spartalizumab (anti-PD-1) in patients with advanced solid tumors and lymphomas

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ABSTRACT

Background GWN323 is an IgG1 monoclonal antibody (mAb) against the glucocorticoid-induced tumor necrosis factor receptor-related protein. This first-in-human, open-label phase I/Ib study aimed to investigate the safety and tolerability and to identify the recommended doses of GWN323 with/without spartalizumab, an anti-programmed cell death receptor-1 agent, for future studies. Pharmacokinetics, preliminary efficacy and efficacy biomarkers were also assessed.

Methods Patients (aged ≥18 years) with advanced/metastatic solid tumors with Eastern Cooperative Oncology Group performance status of ≤2 were included. GWN323 (10–1500 mg) or GWN323+spartalizumab (GWN323 10–750 mg+spartalizumab 100–300 mg) were administered intravenously at various dose levels and schedules during the dose-escalation phase. Dose-limiting toxicities (DLTs) were assessed during the first 21 days in a single-agent arm and 42 days in a combination arm. Adverse events (AEs) were graded per National Cancer Institute-Common Toxicity Criteria for Adverse Events V.4.03 and efficacy was assessed using Response Evaluation Criteria in Solid Tumors V.1.1.

Results Overall, 92 patients (single-agent, n=39; combination, n=53) were included. The maximum administered doses (MADs) in the single-agent and combination arms were GWN323 1500 mg every 3 weeks (q3w) and GWN323 750 mg+spartalizumab 300 mg q3w, respectively. No DLTs were observed with single-agent treatment. Three DLTs (6%, all grade ≥3) were noted with combination treatment: blood creatine phosphokinase increase, respiratory failure and small intestinal obstruction. Serious AEs were reported in 30.8% and 34.0%, and drug-related AEs were reported in 82.1% and 77.4% of patients with single-agent and combination treatments, respectively. Disease was stable in 7 patients and progressed in 26 patients with single-agent treatment. In combination arm patients, 1 had complete response (endometrial cancer); 3, partial response (rectal cancer, adenocarcinoma of colon and melanoma); 14, stable disease; and 27, disease progression. GWN323 exhibited a pharmacokinetic profile typical of mAbs with a dose-dependent increase in the pharmacokinetic exposure. Inconsistent decreases in regulatory T cells and increases in CD8+ T cells were observed in the combination arm. Gene expression analyses showed no significant effect of GWN323 on interferon-γ or natural killer-cell signatures.

Conclusions GWN323, as a single agent and in combination, was well tolerated in patients with relapsed/refractory solid tumors. The MAD was 1500 mg q3w for single-agent and GWN323 750 mg+spartalizumab 300 mg q3w for combination treatments. Minimal single-agent activity and modest clinical benefit were observed with the spartalizumab combination.

Trial registration number NCT02740270.

INTRODUCTION

With the success of targeted antibody therapies against cytotoxic T-lymphocyte antigen-4 or programmed cell death receptor-1 (PD-1),[1–3] anticancer immunotherapy has evolved and led the way for new and promising approaches that involve activating costimulatory pathways to improve antitumor immune responses. One such strategy targets the costimulatory molecules on T cells, such as the glucocorticoid-induced tumor necrosis factor receptor (GITR), CD40, CD27, 4-1BB and OX40.[4–6]

GITR is a type 1 transmembrane protein belonging to the tumor necrosis factor receptor superfamily that modulates both the adaptive and innate immune responses. It is constitutively expressed at high levels on activated CD4+CD25− effector T (Teff) cells,

Foxp3+ regulatory T (Treg) cells, CD8+ Teff cells, B cells, monocytes and macrophages, natural killer (NK) cells, plasmacytoid dendritic cells, mature dendritic cells, mast cells, eosinophils, basophils and leukocytes.[7–9] On activation, GITR has the capacity to promote the function of Teff cells and to inhibit Treg cells.[10 11] This shift in the balance between the Teff and Treg cells increases the activity of the immune system, making it more effective at tumor cell destruction.[10 11] Studies in GITR transgenic mice suggest that the GITR engagement may increase the levels of memory CD4+ cells (CD44+/CD62L−).[12]

GWN323 is an agonistic Humaneered anti-GITR IgG1 monoclonal antibody (mAb) that binds specifically and with high affinity to the human GITR.[10] GWN323, a human and cynomolgus monkey cross-reactive mAb, showed functional activity in vitro in human T-cell assays and in vivo in syngeneic tumor models in hGITR.hGITRL dKI mice. Preclinical mouse models have demonstrated that GITR agonists have a synergistic antitumor effect when combined with other anticancer therapies, especially PD-1 inhibitors.[9 10 13 14] Spartalizumab is a humanized IgG4 anti-PD-1 mAb that binds to PD-1 and blocks its interaction with programmed death-ligand (PD-L) 1 and PD-L2. Spartalizumab has previously shown favorable pharmacokinetics (PK) and safety and preliminary antitumor activity in patients with anaplastic thyroid cancer and other advanced solid tumors.[15 16]

Here, we report the results of a first-in-human, phase I/Ib, multicenter, open-label study of GWN323 as a single agent and in combination with spartalizumab in adult patients with relapsed or refractory solid tumors and lymphomas. The primary objective was to characterize the safety and tolerability of GWN323 as a single agent and in combination with spartalizumab and to identify recommended doses and schedules for future studies. The secondary objectives were to characterize the PK, assess the pharmacodynamic (PD) effects and evaluate the preliminary antitumor activity of GWN323 as a single agent and in combination with spartalizumab.

METHODS

Patient population

Inclusion criteria

The study enrolled patients aged ≥18 years with histologically confirmed advanced/metastatic solid tumors or lymphomas; with an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2 with measurable or non-measurable disease, as determined by the Response Evaluation Criteria in Solid Tumors (RECIST) V.1.1; and who had progressed on or were intolerant to standard treatment or for whom no standard treatment existed. Eligible patients had a site of disease amenable to biopsy.

Exclusion criteria

Key exclusion criteria included symptomatic central nervous system (CNS) metastases or CNS metastases requiring local CNS-directed therapy, diagnosis of T-cell lymphomas, prior allogeneic transplants, prior anti-GITR therapy or history of severe hypersensitivity reactions to other mAbs. Patients intolerant to prior immunotherapy (unable to continue/receive owing to immune-related adverse events (AEs)); patients with active HIV, hepatitis B virus or hepatitis C virus infections; and patients with impaired cardiac function and inadequate bone marrow or end-organ function during screening were also excluded.

Study design and treatment

The study consisted of two phases: a dose-escalation phase to establish the maximum tolerated dose (MTD) and/or recommended phase 2 dose (RP2D) and a dose-expansion phase at the RP2D. The dose-escalation phase included two parallel dose escalations (GWN323 single agent and combination of GWN323 and spartalizumab (GWN323+spartalizumab) with staggered starts. Patients received GWN323 or GWN323+spartalizumab via intravenous infusion over 30 min once in every 21-day cycle (every 3 weeks (q3w)) until disease progression per the immune-related response criteria (irRC), unacceptable toxicity or treatment discontinuation at the investigator's or patient's discretion. Premedication per institutional standard of care was allowed (except on cycle 1 day 1) at the discretion of the treating physician if the patient experienced infusion reactions.

In the single-agent arm, groups of three to six patients were enrolled in seven dosing cohorts from 10 mg to 1500 mg of GWN323 on day 1 and q3w. The starting dose was 10 mg; selected based on the predicted human minimum anticipated biological effect level using the preclinical PK/PD data and in vitro toxicology studies of GWN323. Similarly, in the combination arm, eight dosing cohorts were evaluated with GWN323 doses ranging from 10 mg to 750 mg and spartalizumab doses ranging from 100 mg to 300 mg. Dose escalations were guided using the Bayesian logistic regression model following escalation with overdose control principle. Dose escalation in the combination arm started only after the completion of the first two dose-level cohorts of the single agent in the q3w schedule. Patients were followed up for 90 days after the last dose in the single-agent arm and for 150 days in the combination arm for safety evaluations.

MTD determination

The MTD in single-agent arm was defined as the highest drug dosage not expected to cause a dose-limiting toxicity (DLT) in ≥33% of the treated patients in the 21 days following the first dose of GWN323 treatment. In the combination arm, MTD was the highest combination drug dose not expected to cause a DLT in ≥33% of the treated patients in the 42 days following the first treatment with the combination. A DLT was defined as any grade ≥3 AE that occurred within the first 21 days of GWN323 treatment or within 42 days of GWN323+spartalizumab treatment, as assessed using the National Cancer

Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE), unless it could be clearly attributed to another cause.

Safety and efficacy assessments

Safety was assessed according to the NCI-CTCAE V.4.03 and included incidence and severity of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs), including changes in laboratory parameters, vital signs and electrocardiograms. Dose interruptions, reductions and intensity were also assessed.

The efficacy assessments included best overall response (BOR) per RECIST V.1.1 and irRC, measured from treatment initiation until disease progression and summarized by treatment arm.

Pharmacokinetic assessments

Blood samples were collected on day 1 of cycle 1 (predose and end of infusion) and during the study visits on days 2, 4, 8 and 15 of cycle 1; on day 1 (predose and end of infusion) of cycles 2 and 3; on days 1 (predose and end of infusion), 2, 4, 8 and 15 of cycle 4; on day 1 (predose) of every subsequent cycle; and at the final visit. The PK parameters were determined using non-compartmental methods for GWN323 and spartalizumab.

Biomarker and PD assessments

Tumor biopsy samples (new or recent (≤3 months from registration) plus two additional biopsies during the course of the study) and peripheral blood mononuclear cells (PBMCs) were used for biomarker and PD assessments. The timings of the tumor sample collections were flexible (at screening, between cycle 2 day 1 and cycle 2 day 15, and between cycle 4 day 1 and cycle 6 day 20). Expression and localization of the biomarkers, including Foxp3, CD8 and PD-L1, were measured using immunohistochemistry or RNA sequencing. In addition, the effector:Treg cell ratio at screening and during treatment was measured using flow cytometry to assess the PD effect of GWN323 alone and in combination with spartalizumab in the PBMC samples.

Statistical analyses

The safety and efficacy analysis population included all patients who received ≥1 full or partial dose of GWN323 or spartalizumab (full analysis set (FAS)). The dose-determining set included all patients from the FAS who completed the minimum exposure requirement (one full dose in the q3w and two-thirds of the planned doses of GWN323 in the weekly schedule plus a full dose of spartalizumab in the combination arm) or had a DLT during the first cycle (21 days) in the single-agent arm or during cycles 1 and 2 (42 days) in the combination arm. Patients who received ≥1 of the planned treatments and provided ≥1 primary PK parameter were included in the PK analysis set.

Descriptive statistics was used to summarize demographic and other baseline data (including disease characteristics and duration of exposure) by treatment arm. All safety assessments were summarized descriptively. DLTs and their incidence were summarized by primary system organ class, preferred term, type and grade of AE and treatment arm. The overall response rate (ORR, defined as the proportion of patients with a BOR of complete response (CR) or partial response (PR)) and disease control rate (DCR, defined as the proportion of patients with a BOR of CR or PR or stable disease (SD)) were presented by treatment arm. Descriptive statistics (mean, SD, coefficient of variation (CV) %) were presented for all serum PK parameters, study arms and study cycles/days.

RESULTS

A total of 92 patients with relapsed/refractory solid tumors and lymphomas were enrolled between July 2016 and November 2018 in Canada, Israel, Japan, Singapore, Spain, the USA and the UK. Owing to minimal antitumor activity (and not owing to safety concerns), the enrollment in the study was terminated at the end of the dose-escalation phase, and the dose-expansion phase was not initiated. Based on the early signal of antitumor activity during initial dose escalation, the GWN323 150 mg+spartalizumab 300 mg dose level in the combination arm was enriched for patients with high microsatellite instability (MSI-H) cancers and for patients with melanoma.

Patient characteristics

Overall, 92 patients were treated with GWN323 as a single agent (n=39) or in combination with spartalizumab (n=53) and were included in the FAS. The baseline characteristics and demographics of the patients are listed in table 1. The median age of the patients in the single-agent arm was 61 (range 31–79) years; 56.4% of the patients were female and 61.5% were Caucasian. Most had an ECOG performance status of 0 (53.8%) or 1 (43.6%). The most common cancer types included were colorectal cancer (12.8%) and ovarian cancer (10.3%). All the patients (100%) in the single-agent arm received ≥1 regimen of antineoplastic therapy before entering the study; 28.2% of the patients had two prior antineoplastic regimens, and 82.1% had ≥2 prior antineoplastic regimens. The median age in the combination arm was 60.0 (range 34–84) years; 56.6% of the patients were male and 67.9% were Caucasian (table 1).

Of the 39 patients in the single-agent arm, 33 (84.6%) discontinued treatment owing to progressive disease (online supplemental table 1). The major reason for discontinuation in the post-treatment follow-up phase was completion of study treatment (12 (30.8%)). In the combination arm, 41 patients (77.4%) discontinued owing to progressive disease (online supplemental table 2). Of the 40 patients who entered the post-treatment follow-up phase, 28 (52.8%) discontinued owing to new therapy for study indication.

Treatment exposure

The median (range) duration of exposure to GWN323 was 9 (2–72) weeks in the single-agent arm and 12 (3–139)

Table 1 Demographic and baseline characteristics by treatment arm

	Single-agent arm	Combination arm
Demographic variables	N=39	N=53
Age (years), median (range)	61.0 (31–79)	60.0 (34–84)
Female, n (%)	22 (56.4)	23 (43.4)
Race, n (%)		
Caucasian	24 (61.5)	36 (67.9)
Asian	10 (25.6)	10 (18.9)
Other	2 (5.1)	1 (1.9)
Unknown	2 (5.1)	3 (5.7)
Black or African–American	1 (2.6)	3 (5.7)
ECOG performance status, n (%)		
0	21 (53.8)	19 (35.8)
1	17 (43.6)	34 (64.2)
2	1 (2.6)	0 (0.0)
Diagnosis, n (%)		
Colorectal cancer	5 (12.8)	10 (18.9)
Cutaneous melanoma	1 (2.6)	6 (11.3)
Ovarian cancer	4 (10.3)	4 (10.3)
Breast cancer	3 (7.7)	2 (3.8)
Cervical cancer	3 (7.7)	2 (3.8)
Mesothelioma	3 (7.7)	0 (0.0)
Pancreatic cancer	3 (7.7)	2 (3.8)
Others	16 (41.0)	31 (58.4)
Number of prior antineoplastic regimens, n (%)		
1	7 (17.9)	9 (17.0)
2	11 (28.2)	13 (24.5)
3	5 (12.8)	9 (17.0)
4	5 (12.8)	8 (15.1)
≥5	11 (28.2)	18 (33.9)

ECOG, Eastern Cooperative Oncology Group.

weeks in the combination arm. Most patients (41.0%) in the single-agent arm were exposed to GWN323 for 6–12 weeks. In the combination arm, 35.8% of the patients had an exposure of ≥18 weeks for GWN323. The median (range) exposure to spartalizumab in the combination arm was 12 (3–139) weeks.

MTD and maximum administered dose (MAD)

No DLTs were observed with the single-agent treatment, and MTD was not reached (table 2). MAD was GWN323 1500 mg q3w for the single-agent treatment as further dose escalation was not deemed to significantly increase GITR receptor occupancy and elevate biological response.

DLTs were reported in three patients (6%) in the combination arm; blood creatine phosphokinase increase (grades 3–4) in the GWN323 10 mg+spartalizumab 200 mg dose-escalation cohort, respiratory failure (grade 3) in the

Table 2 Summary of DLTs

	Single-agent arm—no DLTs were reported																			
	Combination arm																		All patients	
	GWN323 10 mg+spartalizumab 100 mg q3w		GWN323 10 mg+spartalizumab 200 mg q3w		GWN323 30 mg+spartalizumab 100 mg q3w		GWN323 30 mg+spartalizumab 300 mg q3w		GWN323 75 mg+spartalizumab 300 mg q3w		GWN323 150 mg+spartalizumab 300 mg q3w		GWN323 300 mg+spartalizumab 300 mg q3w		GWN323 750 mg+spartalizumab 300 mg q3w			
n (%)	N=6		N=4		N=4		N=5		N=4		N=17		N=7		N=3		N=50	
	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3	All grades	Grade ≥3
Patients with ≥1 event	0	0	1 (25.0)	1 (25.0)	1 (25.0)	1 (25.0)	0	0	0	0	1 (5.9)	1 (5.9)	0	0	0	0	3 (6.0)	3 (6.0)
Blood creatine phosphokinase increased	0	0	1 (25.0)	1 (25.0)	0	0	0	0	0	0	0	0	0	0	0	0	1 (2.0)	1 (2.0)
Respiratory failure	0	0	0	0	1 (25.0)	1 (25.0)	0	0	0	0	0	0	0	0	0	0	1 (2.0)	1 (2.0)
Small intestinal obstruction	0	0	0	0	0	0	0	0	0	0	1 (5.9)	1 (5.9)	0	0	0	0	1 (2.0)	1 (2.0)

DLT, dose-limiting toxicity; q3w, every 3 weeks.

Table 3 Summary of adverse events reported in ≥10% of the patients

Single-agent arm			Combination arm		
N=39			N=53		
n (%)	All grades	Grade ≥3	n (%)	All grades	Grade ≥3
Patients with ≥1 event	39 (100)	17 (43.6)	Patients with ≥1 event	51 (96.2)	25 (47.2)
Constipation	12 (30.8)	0	Fatigue	15 (28.3)	1 (1.9)
Fever	11 (28.2)	0	Decreased appetite	14 (26.4)	1 (1.9)
Fatigue	10 (25.6)	0	Nausea	13 (24.5)	1 (1.9)
Abdominal pain	9 (23.1)	0	Abdominal pain	11 (20.8)	3 (5.7)
Chills	8 (20.5)	0	Diarrhea	11 (20.8)	0
Myalgia	8 (20.5)	0	Fever	11 (20.8)	0
Nausea	8 (20.5)	0	Cough	10 (18.9)	0
Cough	7 (17.9)	0	Anemia	9 (17.0)	4 (7.5)
Decreased appetite	7 (17.9)	1 (2.6)	Back pain	9 (17.0)	0
Anemia	6 (15.4)	5 (12.8)	Constipation	9 (17.0)	0
Diarrhea	6 (15.4)	0	Rash	9 (17.0)	0
Dyspnea	6 (15.4)	1 (2.6)	Vomiting	9 (17.0)	1 (1.9)
Ascites	5 (12.8)	3 (7.7)	Chills	8 (15.1)	0
Asthenia	5 (12.8)	0	Headache	8 (15.1)	0
Rash	5 (12.8)	0	Pruritus	8 (15.1)	0
Lymphopenia	4 (10.3)	1 (2.6)	Blood creatinine increased	7 (13.2)	0
Edema peripheral	4 (10.3)	0	Edema peripheral	7 (13.2)	0
Vomiting	4 (10.3)	0	Aspartate aminotransferase increased	6 (11.3)	0
			Hyperglycemia	6 (11.3)	1 (1.9)

GWN323 30 mg+spartalizumab 100 mg dose-escalation cohort and small intestinal obstruction (grade 3) in the GWN323 150 mg+spartalizumab 300 mg dose-escalation cohort (table 2).The number of DLTs in these cohorts did not exceed the threshold set for MTD (>33%), and MTD was not reached. GWN323 750 mg+spartalizumab 300 mg q3w was determined as MAD for the combination treatment. Owing to minimal antitumor activity, no dose escalation beyond GWN323 750 mg was carried out in the combination arm.

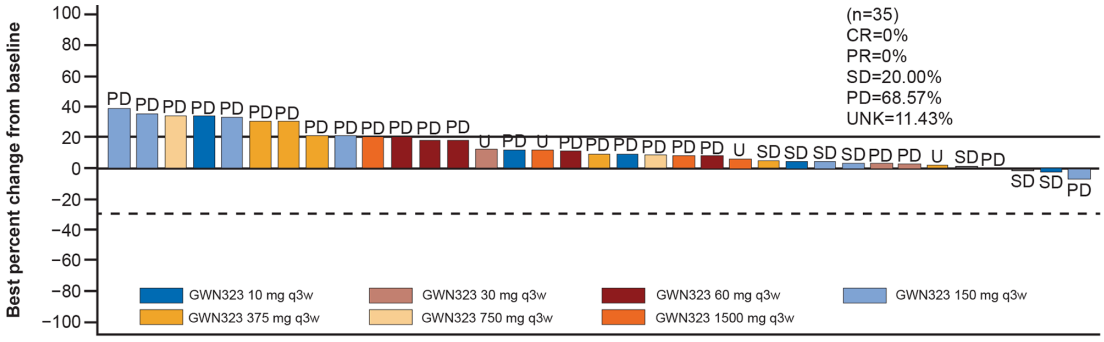
Safety

All 39 patients (100%) in the single-agent arm had ≥1 TEAE, including 17 (43.6%) with grade ≥3 TEAEs (table 3). The most frequently reported TEAEs were constipation (30.8%), fever (28.2%) and fatigue (25.6%). In the combination arm, 51 patients (96.2%) reported ≥1 TEAE, including 25 patients (47.2%) with grade ≥3 TEAEs; the most frequently reported TEAEs were fatigue (28.3%), decreased appetite (26.4%) and nausea (24.5%; table 3).

SAEs occurred in 30.8% and 34.0% of the patients in the single-agent and combination arms, respectively (online supplemental tables 3 and 4). Most common SAEs were anemia, pulmonary embolism, nausea, vomiting and small intestinal obstruction in the single-agent arm (5.1% each), and abdominal pain, sepsis and vomiting in the combination arm (5.7% each, online supplemental table 4). No SAEs in the single-agent arm were related to the study drug. However, 7.5% of the patients in the combination arm reported SAEs related to the study drug (infusion-related reaction (1.9%), small intestinal obstruction (1.9%), type II respiratory failure (1.9%) and abdominal pain (1.9%)). AEs suspected to be related to the study drug were reported in 82.1% and 77.4% of the patients (online supplemental tables 3 and 5) and AEs leading to treatment discontinuation were reported in 7.7% and 5.7% of the patients in the single-agent and combination arms, respectively. Most common AEs suspected to be related to the study drug were fever (25.6%), chills (17.9%) and myalgia (15.4%) in the single-agent arm and fatigue (18.9%), fever (18.9%) and chills (15.1%) in the combination arm (online supplemental table 5); 10.3% and 20.8% of the patients in the single-agent and combination arms, respectively, required no dose reduction. One patient died on treatment owing to renal failure and one died owing to disease progression in the single-agent and combination arms, respectively. However, neither death was suspected to be related to the study drug (online supplemental table 3).

Efficacy

None of the patients in the single-agent arm had CR or PR. Of the 39 evaluable patients, 7 (17.9%) achieved



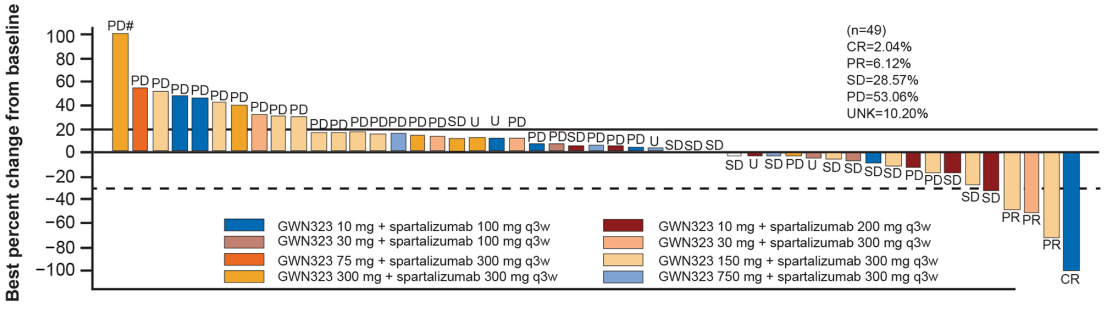
	GWN323 10 mg q3w N=6	GWN323 30 mg q3w N=3	GWN323 60 mg q3w N=5	GWN323 150 mg q3w N=9	GWN323 375 mg q3w N=7	GWN323 750 mg q3w N=5	GWN323 1500 mg q3w N=4	All patients N=39
Overall response rate, mean (standard deviation); 95% CI	0 (0.0), [0.0, 45.9]	0 (0.0), [0.0, 70.8]	0 (0.0), [0.0, 52.2]	0 (0.0), [0.0, 33.6]	0 (0.0), [0.0, 41.0]	0 (0.0), [0.0, 52.2]	0 (0.0), [0.0, 60.2]	0 (0.0), [0.0, 9.0]
Disease control rate, mean (standard deviation); 95% CI	3 (50.0), [11.8, 88.2]	0 (0.0), [0.0, 70.8]	0 (0.0), [0.0, 52.2]	2 (22.2), [2.8, 60.0]	1 (14.3), [0.4, 57.9]	1 (20.0), [0.5, 71.6]	0 (0.0), [0.0, 60.2]	7 (17.9), [7.5, 33.5]

Figure 1 Best percentage change and best overall response by investigator assessment (RECIST V.1.1) in patients with target lesions in the single-agent arm. n is the number of patients with ≥1 baseline and postbaseline assessment of target lesions. CR, complete response; GWN, GWN323; PD, progressive disease; PR, partial response; q3w, every 3 weeks; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; UNK, unknown.

SD (patients remained free of disease progression for a period of 1.4–5.8 months after showing SD) and 26 (66.7%) had disease progression per RECIST V.1.1. DCR was 17.9% (95% CI 7.5% to 33.5%, figure 1).

In the combination arm, one patient (1.9%) with Lynch syndrome (*PMS2* mutation) with MSI-H endometrial cancer (PD-1/PD-L1 naïve) had CR, and three patients (5.7%) had PR. Tumor type and pretreatment were (1) MSI-H rectal cancer, PD-1/PD-L1 naïve; (2) Lynch syndrome with poorly differentiated adenocarcinoma of the colon (MSI-H adenocarcinoma of the colon), PD-1/PD-L1 naïve; and (3) melanoma pretreated with two different lines of PD-1/PD-L1 immune checkpoint inhibitors (pembrolizumab and nivolumab). The disease was stable in 14 patients (26.4%) and progressed in 27 patients (50.9%). The ORR was 7.5% (95% CI 2.1% to 18.2%), and DCR was 34% (95% CI 21.5% to 48.3%, figure 2). Efficacy results based on the irRC were identical to those observed with RESIST V.1.1.

After the inclusion of the enrichment cohort, of the nine patients treated for microsatellite instability tumors, one had CR (MSI-H endometrial cancer treated with GWN323 10 mg+spartalizumab 100 mg) and two had PR (both MSI-H colorectal cancer treated with GWN323 150 mg+spartalizumab 300 mg). The three patients were anti-PD-1/PD-L1 treatment naïve. Of the eight patients with melanoma, one patient had PR (previously treated with immune checkpoint inhibitors and was treated at a



	GWN323 10 mg +spartalizumab 100 mg q3w N=6	GWN323 10 mg +spartalizumab 200 mg q3w N=4	GWN323 30 mg +spartalizumab 100 mg q3w N=4	GWN323 30 mg +spartalizumab 300 mg q3w N=5	GWN323 75 mg +spartalizumab 300 mg q3w N=5	GWN323 150 mg +spartalizumab 300 mg q3w N=17	GWN323 300 mg +spartalizumab 300 mg q3w N=7	GWN323 750 mg +spartalizumab 300 mg q3w N=5	All Patients N=53
Overall response rate, mean (standard deviation); 95% CI	1 (16.7), [0.4, 64.1]	0 (0.0), [0.0, 60.2]	0 (0.0), [0.0, 60.2]	1 (20.0), [0.5, 71.6]	0 (0.0), [0.0, 52.2]	2 (11.8), [1.5, 36.4]	0 (0.0), [0.0, 41.0]	0 (0.0), [0.0, 52.2]	4 (7.5), [2.1, 18.2]
Disease control rate, mean (standard deviation); 95% CI	2(33.3), [4.3, 77.7]	2 (50.0), [6.8, 93.2]	2 (50.0), [6.8, 93.2]	2 (40.0), [5.3, 85.3]	2 (40.0), [5.3, 85.3]	6 (35.3), [14.2, 61.7]	1 (14.3), [0.4, 57.9]	1 (20.0), [0.5, 71.6]	18 (34.0), [21.5, 48.3]

Figure 2 Best percentage change and best overall response by investigator assessment (RECIST V.1.1) in patients with target lesions in the combination arm. # indicates percentage changes from baseline of >100% are set to 100%. n indicates the number of patients with ≥1 baseline and postbaseline assessment of target lesions. CR, complete response; GWN, GWN323; PD, progressive disease; PR, partial response; q3w, every 3 weeks; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; UNK, unknown.

dose of GWN323 150 mg+spartalizumab 300 mg) and one had unconfirmed PR (treated with GWN323 10 mg+spartalizumab 200 mg).

Pharmacokinetics

The PK variability for single-agent GWN323 was moderate at cycle 1 day 1, as illustrated by the between-patient variability (CV%) for maximum concentration (C_{max}, 8.6%–54.3%) and area under the curve from time=0 to last measurable concentration (AUC_{last}, 22.6%–45.9%). The median terminal half-life ($t_{½}$) of GWN323 was 7.3 days (range 5.6–9.5 days) in the single-agent arm (online supplemental table 6). A higher PK exposure was observed at cycle 4 day 1 compared with cycle 1 day 1. The $t_{½}$ of GWN323 in the combination arm was 8.7 days (range 4.6–12.6 days, online supplemental table 7). A dose-dependent increase in PK exposure (C_{max} and AUC_{last}) was observed with the increasing dose of GWN323. Similarly, the PK exposure in the combination arm increased with increasing GWN323+spartalizumab doses. The PK exposure of GWN323 in the combination setting was comparable to that of the single agent, indicating no significant drug–drug interaction between GWN323 and spartalizumab.

Biomarkers

Flow cytometry of the PBMC samples was conducted to examine several immune cell subsets. Transient increases in proliferating CD8, NK and effector memory CD8 cells were observed during treatment in patients in both the single-agent and combination arms (figure 3A,B). Some patients in the single-agent arm exhibited on-treatment decreases in peripheral effector Treg cells (online supplemental figure 1), although these changes were not dose dependent. No effects of GWN323 treatment on the total Treg cells were observed by flow cytometry analysis (data not shown).

Immunohistochemistry was performed to assess the levels of the PD-L1, Foxp3 and CD8 markers in paired tumor biopsies. The CD8 levels were low in most patients; only five patients in the single-agent arm exhibited CD8 levels of >2% at baseline (figure 4A). Similarly, low levels of Foxp3 staining were observed in most patients, with four patients exhibiting >1% Foxp3 levels at baseline. Of these four patients, three exhibited an on-treatment decrease in the Foxp3 level. Levels of PD-L1 were low in most patients. One patient with SD had a modest on-treatment increase in the levels of all three markers (figure 4A). Most patients in the combination arm had generally low baseline CD8 (<2%) and Foxp3 (<1%) levels (figure 4B). Three patients with >1% Foxp3 level at baseline exhibited an on-treatment decrease in this marker (figure 4B). One patient in the combination arm with a confirmed CR exhibited on-treatment increases in the levels of all three markers.

Combined RNA sequencing data from all the patients in the single-agent arm (n=15 paired samples) indicated no significant correlations between GWN323 dosing and changes in T-cell function (as measured by the interferon (IFN)-γ expression levels) or NK cell signatures (online supplemental figure 2). Analysis of paired samples from the combination arm (n=13 paired samples) indicated that IFN-γ and Treg signatures were upregulated in patients who had a 30% on-treatment decrease in tumor volume (online supplemental figure 3).

DISCUSSION

Agonistic antibodies targeting GITRs are expected to have a dual mechanism of action, involving both elimination of Treg cells and enhanced activation of Teff cells.[10 11] Several human GITR agonists are currently in early clinical development for the treatment of solid tumors, for example, TRX518, INCAGN01876, AMG 228, MEDI1873 and MK-4166 among others.[9 10 17–19] However, most GITR agonists failed to show sustainable antitumor activity, especially as monotherapy, in phase I trials.[9 10] Here, we report the results of a first-in-human phase I/Ib study of GWN323, a highly selective anti-GITR antibody. In this study, GWN323 was well tolerated at all dose levels, including the highest dose level (1500 mg q3w). Although no formal MTD was reached in the study for either single-agent or combination treatments, MADs of GWN323 1500 mg q3w for single-agent treatment and GWN323 750 mg+spartalizumab 300 mg q3w for combination treatment were considered to be tolerated based on the nature and severity of the DLTs observed at that dose level.

The AE profile of GWN323 observed in this study as monotherapy or in combination was as expected and consistent with that observed in the early clinical studies of other GITR agonists.[17–19] The safety profile of GWN323 in combination with spartalizumab at all dose levels was tolerable and identical to that of spartalizumab alone.[15] No on-treatment deaths related to the study drug were reported.

Single-agent GWN323 exhibited a PK profile that is typical of a mAb with a dose-dependent increase in the PK exposure and a higher PK exposure at cycle 4 day 1 compared with cycle 1 day 1. The variability of the PK exposure was moderate. The PK exposure of GWN323 in the combination setting was comparable to that of a single agent, while the PK exposure of spartalizumab in the combination setting was comparable to the published data of single-agent spartalizumab study (data not shown).[15] Taken together, these observations suggest that no significant drug–drug interaction exists between GWN323 and spartalizumab.

During the dose escalation, no patients had objective responses with GWN323 monotherapy. The limited antitumor activity of GWN323 monotherapy with overall good tolerability observed in this study is consistent with early clinical data available for the other GITR agonists, MEDI1873, TRX518 and AMG 228, in patients with advanced solid tumors.[17 19 20] Dose-escalation results from the AMG 228 studies showed no antitumor activity but

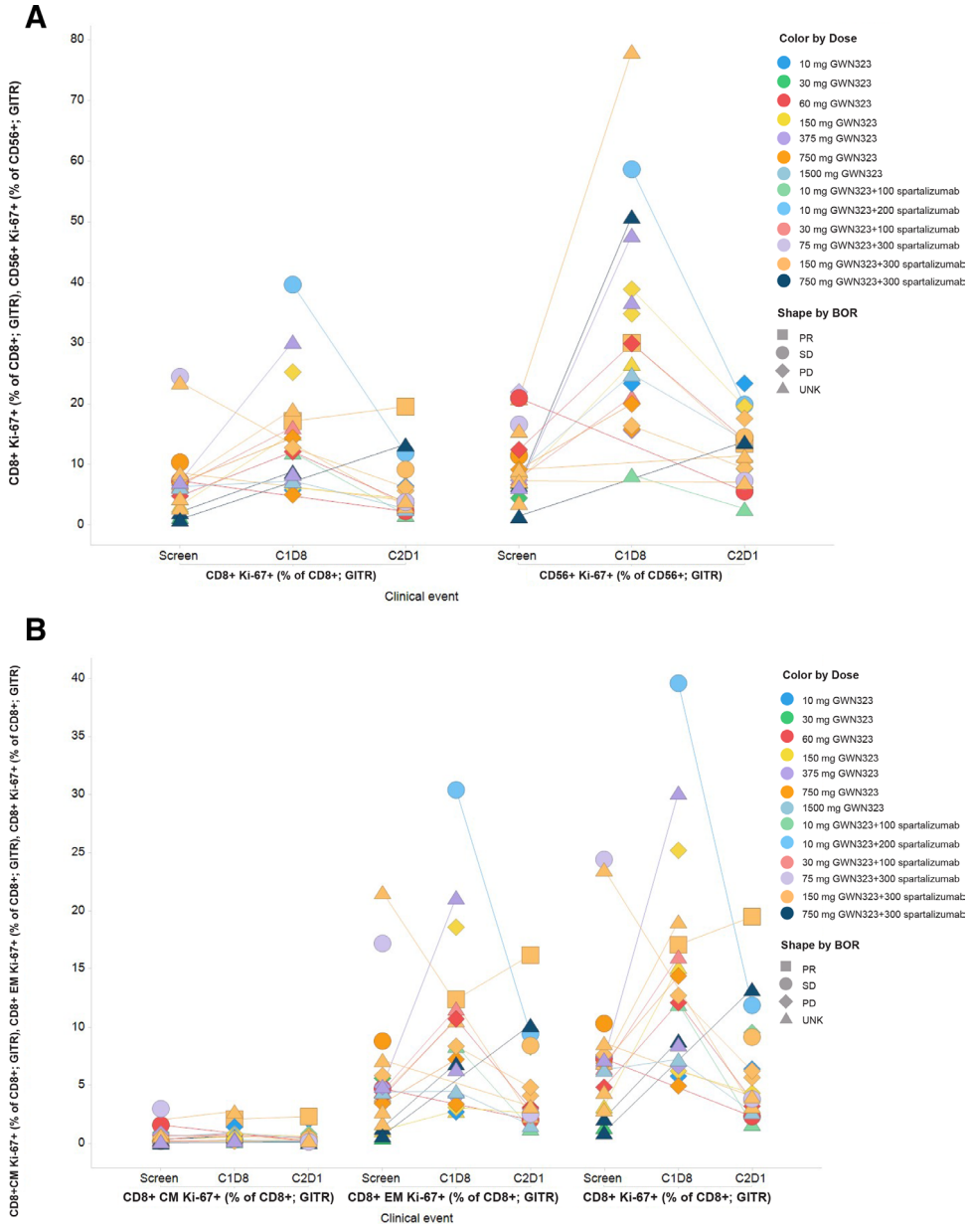


Figure 3 Effect of treatment on (A) proliferating CD8 and NK cells and (B) effector memory CD8 cells (flow cytometry data). Blood was collected and PBMCs were isolated at screening, C1D8 and C2D1. PBMCs were stained to identify the percentages of proliferating CD8 and NK cells (A) as well as effector memory T cells (B). BOR categories are indicated by shape, and doses of GWN±spartalizumab are color coded. BOR, best overall response; C, cycle; CR, complete response; D, day; GITR, glucocorticoid-induced tumor necrosis factor receptor; GWN, GWN323; NK, natural killer; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PR, partial response; SD, stable disease; UNK, unknown.

good overall tolerability in patients with advanced solid tumors.[21] One patient in the combination arm achieved CR; 3 patients achieved PR; and 14 had SD. However, the clinical activity observed in the combination arm was mostly in patients with PD-1/PD-L1-sensitive tumors who had not received prior checkpoint inhibitor therapy, and we were unable to ascertain the additional benefit with GWN323. The antitumor activity observed in the enrichment cohort with immune checkpoint inhibitor-responsive tumors did not clearly demonstrate additional benefit from GWN323 in combination with spartalizumab.

It was hypothesized that treatment with GWN323 would suppress the Treg cells and enhance the activation of Teff cells in the tumor microenvironment. However, based on the current biomarker data, conclusive evidence of T-cell activation with GWN323 monotherapy was not observed. This result is consistent with the observation that GITR agonists are not effective as monotherapy to produce robust antitumor effects compared with that of combination therapy with a PD-1 antibody.[9] Recent studies on GITR agonists MK-4166, MK-1248 and BMS-986156 showed good tolerability in patients with advanced solid

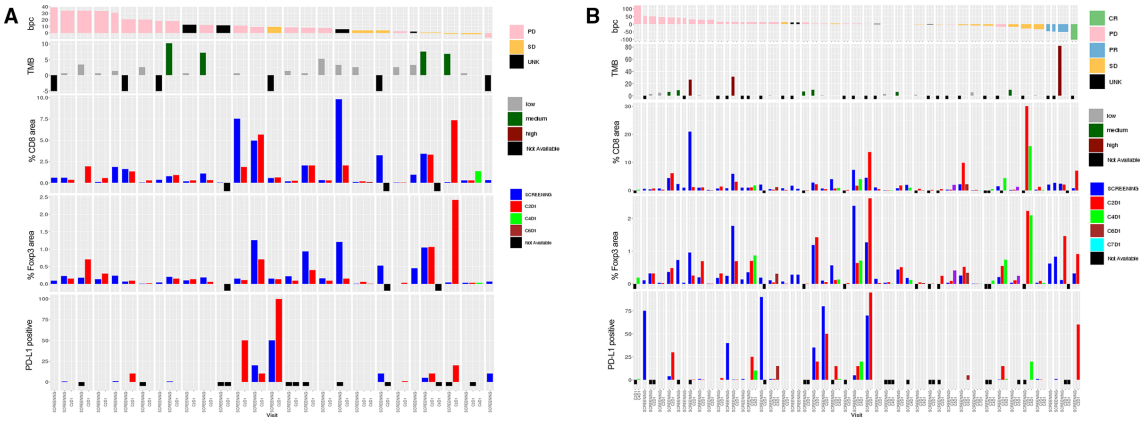


Figure 4 Immunohistochemistry data for the (A) single-agent and (B) combination arms. Immunohistochemistry was performed on paraffin-embedded sections of the tumor samples collected at screening, C2D1 and C4/5/6D1. Visits are color coded. BPC, best percent change in tumor size; C, cycle; CR, complete response; D, day; PD, progressive disease; PR, partial response; SD, stable disease; TMB, tumor mutational burden; UNK, unknown.

tumors alone and in combination with checkpoint inhibitors, but objective responses were seen only for combination treatment.[22–24]

The flow cytometry analysis showed modest reductions in peripheral Teff cells in patients with single-agent treatment; however, these changes were not dose dependent and were not observed in all patients. The decreases in Teff cells and increases in proliferating NK and CD8+ cells observed in the combination arm could potentially be attributed to spartalizumab treatment. The gene expression analysis by RNA sequencing in paired tumor samples from the single-agent arm showed lack of correlation between GWN323 treatment and upregulation of IFN-γ or NK signatures. An upregulation of IFN-γ and Treg signatures was observed in patients in the combination arm who showed a decrease in tumor volume; however, this could be due to the effects of spartalizumab. Importantly, immunohistochemistry staining of paired tumor biopsies showed very low levels (<1%) of the Foxp3 marker at baseline in most patients in both arms. On-treatment decreases in Foxp3 were observed in only three patients treated with single-agent GWN323; all of these patients had >1% Foxp3 staining at baseline. This observation highlights the potential explanation for the lack of effect of GWN323 on Treg cell levels in the tumor; if most patients have no Foxp3 Treg cells in the tumor microenvironment at baseline, it may be impossible to test the hypothesis that GWN323 decreases Treg cells. In addition, several patients demonstrated, by RNA sequencing analysis, higher levels of inhibitory markers (ie, indoleamine 2,3-dioxygenase 1). It is possible that the complex pattern of immune stimulatory/inhibitory factors in combination with tumor heterogeneity may have contributed to limited clinical efficacy. Future efforts to select patients with higher baseline levels of Treg cells may allow us to test the effects of GWN323 more effectively.

CONCLUSIONS

In patients with relapsed or refractory solid tumors and lymphomas, GWN323 q3w monotherapy was well tolerated at all dose levels tested, including the highest dose level (1500 mg q3w). GWN323 in combination with spartalizumab was tolerable up to the highest tested dose (GWN323 750 mg+spartalizumab 300 mg q3w). MAD was GWN323 1500 mg q3w for single-agent treatment and GWN323 750 mg+spartalizumab 300 mg q3w for combination treatment with spartalizumab. GWN323 exhibited a PK typical of mAbs with no drug–drug interaction between GWN323 and spartalizumab. No evidence of T-cell activation or antitumor activity with GWN323 monotherapy was reported.

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Ethics approval The study protocol and amendments were reviewed by an independent ethics committee or institutional review board for each site. The study was conducted in compliance with the Declaration of Helsinki and applicable regulatory requirements. Written informed consent was obtained from all patients.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information. Novartis will not provide access to patient-level data, if there is a reasonable likelihood that individual patients could be reidentified. Phase I studies, by their nature, present a high risk of patient reidentification; therefore, patient individual results for phase I studies cannot be shared. In addition, clinical data, in some cases, have been collected Patient to contractual or consent provisions that prohibit transfer to third parties. Such restrictions may preclude granting access under these provisions. Where codevelopment agreements or other legal restrictions prevent companies from sharing particular data, companies will work with qualified requestors to provide summary information where possible.

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<SUPPLEMENT>

Table S1. Patient disposition in the single-agent arm

n (%)	GWN323 10 mg q3w	GWN323 30 mg q3w	GWN323 60 mg q3w	GWN323 150 mg q3w	GWN323 375 mg q3w	GWN323 750 mg q3w	GWN323 1500 mg q3w	All patients
	N=6	N=3	N=5	N=9	N=7	N=5	N=4	N=39
Treated	6 (100)	3 (100)	5 (100)	9 (100)	7 (100)	5 (100)	4 (100)	39 (100)
Discontinued from treatment	6 (100)	3 (100)	5 (100)	9 (100)	7 (100)	5 (100)	4 (100)	39 (100)
Reason for discontinuation								
Adverse event	0	0	0	1 (11.1)	0	1 (20.0)	0	2 (5.1)
Physician decision	0	0	0	1 (11.1)	0	0	0	1 (2.6)
Progressive disease	6 (100)	3 (100)	4 (80.0)	7 (77.8)	6 (85.7)	3 (60.0)	4 (100)	33 (84.6)
Patient/guardian decision	0	0	1 (20.0)	0	1 (14.3)	0	0	2 (5.1)
Death	0	0	0	0	0	1 (20.0)	0	1 (2.6)
Post-treatment follow-up for patients who discontinued from treatment								
Did not enter post-treatment follow-up	1 (16.7)	1 (33.3)	1 (20.0)	0	0	2 (40.0)	1 (25.0)	6 (15.4)
Entered post-treatment follow-up, discontinued	5 (83.3)	2 (66.7)	4 (80.0)	9 (100)	7 (100)	3 (60.0)	3 (75.0)	33 (84.6)
Reason for discontinuation								
Completed	2 (33.3)	1 (33.3)	1 (20.0)	2 (22.2)	4 (57.1)	0	2 (50.0)	12 (30.8)
Death	2 (33.3)	0	1 (20.0)	3 (33.3)	2 (28.6)	2 (40.0)	0	10 (25.6)
New therapy for study indication	1 (16.7)	1 (33.3)	2 (40.0)	3 (33.3)	1 (14.3)	1 (20.0)	1 (25.0)	10 (25.6)
Progressive disease	0	0	0	1 (11.1)	0	0	0	1 (2.6)

q3w, every 3 weeks.

Table S2. Patient disposition in the combination arm

n (%)	GWN323 10 mg + spartalizumab 100 mg q3w	GWN323 10 mg + spartalizumab 200 mg q3w	GWN323 30 mg + spartalizumab 100 mg q3w	GWN323 30 mg + spartalizumab 300 mg q3w	GWN323 75 mg + spartalizumab 300 mg q3w	GWN323 150 mg + spartalizumab 300 mg q3w	GWN323 300 mg + spartalizumab 300 mg q3w	GWN323 750 mg + spartalizumab 300 mg q3w	All patients
	N=6	N=4	N=4	N=5	N=5	N=17	N=7	N=5	N=53
Patients treated									
Treated	6 (100)	4 (100)	4 (100)	5 (100)	5 (100)	17 (100)	7 (100)	5 (100)	53 (100)
Discontinued from treatment	6 (100)	4 (100)	4 (100)	5 (100)	5 (100)	17 (100)	7 (100)	5 (100)	53 (100)
Reason for discontinuation									
Adverse event	0	1 (25.0)	1 (25.0)	0	0	0	0	0	2 (3.8)
Lost to follow-up	0	0	0	0	1 (20.0)	0	0	0	1 (1.9)
Physician decision	0	0	0	0	0	0	1 (14.3)	1 (20.0)	2 (3.8)
Progressive disease	4 (66.7)	3 (75.0)	3 (75.0)	4 (80.0)	2 (40.0)	15 (88.2)	6 (85.7)	4 (80.0)	41 (77.4)
Study terminated by sponsor	0	0	0	1 (20.0)	0	1 (5.9)	0	0	2 (3.8)
Patient/guardian decision	1 (16.7)	0	0	0	1 (20.0)	1 (5.9)	0	0	3 (5.7)
Death	1 (16.7)	0	0	0	1 (20.0)	0	0	0	2 (3.8)
Post-treatment follow-up for patients who discontinued from treatment									
Did not enter post-treatment follow-up	2 (33.3)	0	1 (25.0)	1 (20.0)	2 (40.0)	4 (23.5)	0	3 (60.0)	13 (24.5)
Entered post-treatment follow-up, discontinued	4 (66.7)	4 (100)	3 (75.0)	4 (80.0)	3 (60.0)	13 (76.5)	7 (100)	2 (40.0)	40 (75.5)
Reason for discontinuation									
Completed	1 (16.7)	1 (25.0)	0	0	0	3 (17.6)	1 (14.3)	0	6 (11.3)
Death	1 (16.7)	0	1 (25.0)	0	2 (40.0)	0	1 (14.3)	0	5 (9.4)
New therapy for study indication	2 (33.3)	3 (75.0)	2 (50.0)	4 (80.0)	1 (20.0)	9 (52.9)	5 (71.4)	2 (40.0)	28 (52.8)
Patient/guardian decision	0	0	0	0	0	1 (5.9)	0	0	1 (1.9)

q3w, every 3 weeks.

Table S3. Overview of AEs

	Single-agent arm															
n (%)	**GWN323 10 mg q3w N=6**		**GWN323 30 mg q3w N=3**		**GWN323 60 mg q3w N=5**		**GWN323 150 mg q3w N=9**		**GWN323 375 mg q3w N=7**		**GWN323 750 mg q3w N=5**		**GWN323 1500 mg q3w N=4**		**All patients N=39**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
AEs	6 (100)	3 (50.0)	3 (100)	2 (66.7)	5 (100)	1 (20.0)	9 (100)	4 (44.4)	7 (100)	4 (57.1)	5 (100)	2 (40.0)	4 (100)	1 (25.0)	39 (100)	17 (43.6)
Treatment related	6 (100)	0	3 (100)	0	4 (80.0)	0	5 (55.6)	0	7 (100)	1 (14.3)	4 (80.0)	0	3 (75.0)	0	32 (82.1)	1 (2.6)
SAEs	2 (33.3)	2 (33.3)	2 (66.7)	2 (66.7)	0	0	3 (33.3)	3 (33.3)	2 (28.6)	2 (28.6)	2 (40.0)	2 (40.0)	1 (25.0)	1 (25.0)	12 (30.8)	12 (30.8)
Fatal SAEs	0	0	0	0	0	0	1 (11.1)	1 (11.1)	0	0	0	0	0	0	1 (2.6)	1 (2.6)
AEs leading to discontinuation	0	0	1 (33.3)	0	0	0	1 (11.1)	1 (11.1)	0	0	1 (20.0)	1 (20.0)	0	0	3 (7.7)	2 (5.1)
AEs leading to dose adjustment/interruption	0	0	1 (33.3)	1 (33.3)	0	0	0	0	1 (14.3)	1 (14.3)	2 (40.0)	1 (20.0)	0	0	4 (10.3)	3 (7.7)
AEs requiring additional therapy	6 (100)	2 (33.3)	3 (100)	2 (66.7)	5 (100)	0	9 (100)	4 (44.4)	7 (100)	3 (42.9)	4 (80.0)	2 (40.0)	3 (75.0)	1 (25.0)	37 (94.9)	14 (35.9)

	Combination arm															
n (%)	**GWN323 10 mg + spartalizumab 100 mg q3w**		**GWN323 10 mg + spartalizumab 200 mg q3w**		**GWN323 30 mg + spartalizumab 100 mg q3w**		**GWN323 30 mg + spartalizumab 300 mg q3w**		**GWN323 75 mg + spartalizumab 300 mg q3w**		**GWN323 150 mg + spartalizumab 300 mg q3w**		**GWN323 300 mg + spartalizumab 300 mg q3w**		**GWN323 750 mg + spartalizumab 300 mg q3w**		**All patients**	
	N=6		**N=4**		**N=4**		**N=5**		**N=5**		**N=17**		**N=7**		**N=5**		**N=53**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
AEs	6 (100)	4 (66.7)	3 (75.0)	2 (50.0)	4 (100)	3 (75.0)	5 (100)	2 (40.0)	5 (100)	3 (60.0)	17 (100)	6 (35.3)	6 (85.7)	3 (42.9)	5 (100)	2 (40.0)	51 (96.2)	25 (47.2)
Treatment related	5 (83.3)	0	3 (75.0)	1 (25.0)	4 (100)	2 (50.0)	4 (80.0)	0	4 (80.0)	0	15 (88.2)	2 (11.8)	3 (42.9)	1 (14.3)	3 (60.0)	0	41 (77.4)	6 (11.3)
SAEs	3 (50.0)	3 (50.0)	0	0	3 (75.0)	3 (75.0)	1 (20.0)	1 (20.0)	3 (60.0)	3 (60.0)	4 (23.5)	3 (17.6)	2 (28.6)	2 (28.6)	2 (40.0)	2 (40.0)	18 (34.0)	17 (32.1)

Treatment related	0	0	0	0	1 (25.0)	1 (25.0)	0	0	0	0	3 (17.6)	2 (11.8)	0	0	0	0	4 (7.5)	3 (5.7)
Fatal SAEs	1 (16.7)	1 (16.7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1 (1.9)
AEs leading to discontinuation	0	0	0	0	1 (25.0)	1 (25.0)	0	0	1 (20.0)	1 (20.0)	0	0	1 (14.3)	1 (14.3)	0	0	3 (5.7)	3 (5.7)
Treatment related	0	0	0	0	1 (25.0)	1 (25.0)	0	0	0	0	0	0	0	0	0	0	1 (1.9)	1 (1.9)
AEs leading to dose adjustment/interruption	1 (16.7)	1 (16.7)	1 (25.0)	0	1 (25.0)	0	0	0	2 (40.0)	0	5 (29.4)	3 (17.6)	0	0	1 (20.0)	0	11 (20.8)	4 (7.5)
AEs requiring additional therapy	5 (83.3)	3 (50.0)	3 (75.0)	2 (50.0)	4 (100)	3 (75.0)	5 (100)	2 (40.0)	5 (100)	3 (60.0)	15 (88.2)	5 (29.4)	5 (71.4)	3 (42.9)	4 (80.0)	2 (40.0)	46 (86.8)	23 (43.4)

AE, adverse event; q3w, every 3 weeks; SAE, serious AE.

Table S4. Serious adverse events reported in ≥5% of patients

	Single-agent arm															
n (%)	**GWN323 10 mg q3w N=6**		**GWN323 30 mg q3w N=3**		**GWN323 60 mg q3w N=5**		**GWN323 150 mg q3w N=9**		**GWN323 375 mg q3w N=7**		**GWN323 750 mg q3w N=5**		**GWN323 1500 mg q3w N=4**		**All patients N=39**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Patients with ≥1 event	2 (33.3)	2 (33.3)	2 (66.7)	2 (66.7)	0	0	3 (33.3)	3 (33.3)	2 (28.6)	2 (28.6)	2 (40.0)	2 (40.0)	1 (25.0)	1 (25.0)	12 (30.8)	12 (30.8)
Anaemia	0	0	2 (66.7)	2 (66.7)	0	0	0	0	0	0	0	0	0	0	2 (5.1)	2 (5.1)
Nausea	0	0	0	0	0	0	0	0	0	0	1 (20.0)	0	1 (25.0)	0	2 (5.1)	0
Pulmonary embolism	0	0	0	0	0	0	1 (11.1)	1 (11.1)	0	0	1 (20.0)	1 (20.0)	0	0	2 (5.1)	2 (5.1)
Small intestinal obstruction	1 (16.7)	1 (16.7)	0	0	0	0	0	0	0	0	0	0	1 (25.0)	1 (25.0)	2 (5.1)	2 (5.1)
Vomiting	0	0	0	0	0	0	0	0	0	0	1 (20.0)	0	1 (25.0)	0	2 (5.1)	0

	Combination arm															
n (%)	**GWN323 10 mg + spartalizumab 100 mg q3w N=6**		**GWN323 10 mg + spartalizumab 200 mg q3w N=4**		**GWN323 30 mg + spartalizumab 100 mg q3w N=4**		**GWN323 30 mg + spartalizumab 300 mg q3w N=5**		**GWN323 75 mg + spartalizumab 300 mg q3w N=5**		**GWN323 150 mg + spartalizumab 300 mg q3w N=17**		**GWN323 300 mg + spartalizumab 300 mg q3w N=7**		**GWN323 750 mg + spartalizumab 300 mg q3w N=5**		**All patients N=53**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Patients with ≥1 event	3 (50.0)	3 (50.0)	0	0	3 (75.0)	3 (75.0)	1 (20.0)	1 (20.0)	3 (60.0)	3 (60.0)	4 (23.5)	3 (17.6)	2 (28.6)	2 (28.6)	2 (40.0)	2 (40.0)	18 (34.0)	17 (32.1)
Abdominal pain	1 (16.7)	1 (16.7)	0	0	0	0	0	0	0	0	1 (5.9)	1 (5.9)	0	0	1 (20.0)	1 (20.0)	3 (5.7)	3 (5.7)
Sepsis	1 (16.7)	1 (16.7)	0	0	1 (25.0)	1 (25.0)	0	0	1 (20.0)	1 (20.0)	0	0	0	0	0	0	3 (5.7)	3 (5.7)
Vomiting	1 (16.7)	0	0	0	0	0	0	0	0	0	1 (5.9)	1 (5.9)	1 (14.3)	0	0	0	3 (5.7)	1 (1.9)

q3w, every 3 weeks.

Table S5. Adverse events suspected to be related to the study drug reported in ≥10% of patients

	Single-agent arm															
n (%)	**GWN323 10 mg q3w N=6**		**GWN323 30 mg q3w N=3**		**GWN323 60 mg q3w N=5**		**GWN323 150 mg q3w N=9**		**GWN323 375 mg q3w N=7**		**GWN323 750 mg q3w N=5**		**GWN323 1500 mg q3w N=4**		**All patients N=39**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Patients with ≥1 event	6 (100)	0	3 (100)	0	4 (80.0)	0	5 (55.6)	0	7 (100)	1 (14.3)	4 (80.0)	0	3 (75.0)	0	32 (82.1)	1 (2.6)
Pyrexia	2 (33.3)	0	1 (33.3)	0	1 (20.0)	0	1 (11.1)	0	5 (71.4)	0	0	0	0	0	10 (25.6)	0
Chills	1 (16.7)	0	0	0	1 (20.0)	0	2 (22.2)	0	0	0	1 (20.0)	0	2 (50.0)	0	7 (17.9)	0
Myalgia	2 (33.3)	0	0	0	0	0	2 (22.2)	0	1 (14.3)	0	0	0	1 (25.0)	0	6 (15.4)	0
Diarrhoea	1 (16.7)	0	1 (33.3)	0	1 (20.0)	0	1 (11.1)	0	0	0	1 (20.0)	0	0	0	5 (12.8)	0
Rash	0	0	2 (66.7)	0	1 (20.0)	0	0	0	0	0	0	0	1 (25.0)	0	4 (10.3)	0

	Combination arm															
n (%)	**GWN323 10 mg + spartalizumab 100 mg q3w N=6**		**GWN323 10 mg + spartalizumab 200 mg q3w N=4**		**GWN323 30 mg + spartalizumab 100 mg q3w N=4**		**GWN323 30 mg + spartalizumab 300 mg q3w N=5**		**GWN323 75 mg + spartalizumab 300 mg q3w N=5**		**GWN323 150 mg + spartalizumab 300 mg q3w N=17**		**GWN323 300 mg + spartalizumab 300 mg q3w N=7**		**GWN323 750 mg + spartalizumab 300 mg q3w N=5**		**All Patients N=53**	
	All grades	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**	**All grades**	**Grade ≥3**
Patients with ≥1 event	5 (83.3)	0	3 (75.0)	1 (25.0)	4 (100)	2 (50.0)	4 (80.0)	0	4 (80.0)	0	15 (88.2)	2 (11.8)	3 (42.9)	1 (14.3)	3 (60.0)	0	41 (77.4)	6 (11.3)
Fatigue	2 (33.3)	0	0	0	0	0	1 (20.0)	0	3 (60.0)	0	3 (17.6)	0	1 (14.3)	0	0	0	10 (18.9)	0
Pyrexia	1 (16.7)	0	1 (25.0)	0	0	0	0	0	1 (20.0)	0	5 (29.4)	0	1 (14.3)	0	1 (20.0)	0	10 (18.9)	0
Chills	0	0	0	0	0	0	1 (20.0)	0	1 (20.0)	0	4 (23.5)	0	1 (14.3)	0	1 (20.0)	0	8 (15.1)	0
Nausea	2 (33.3)	0	2 (50.0)	0	1 (25.0)	0	1 (20.0)	0	0	0	1 (5.9)	0	0	0	1 (20.0)	0	8 (15.1)	0
Rash	2 (33.3)	0	0	0	1 (25.0)	0	0	0	0	0	3 (17.6)	0	1 (14.3)	0	0	0	7 (13.2)	0
Pruritus	0	0	0	0	1 (25.0)	0	2 (40.0)	0	0	0	3 (17.6)	0	0	0	0	0	6 (11.3)	0

q3w, every 3 weeks.

Table S6. Pharmacokinetic parameters for the GWN323 single-agent arm

Parameter	Statistics	GWN323 10 mg q3w N=6	GWN323 30 mg q3w N=3	GWN323 60 mg q3w N=5	GWN323 150 mg q3w N=6	GWN323 375 mg q3w N=3	GWN323 750 mg q3w N=5	GWN323 1500 mg q3w N=4	All patients N=32
				Cycle 1 day 1					
AUC_{last} (h*µg/mL)	Mean (SD)	669 (263)	1830 (406)	3820 (1160)	5740 (2270)	15 000 (4610)	43 700 (16 100)	82 600 (34 200)	20 400(31 000)
	CV%	39.3	22.2	30.4	39.5	30.8	36.7	41.4	151.9
	Geo-mean	630	1810	3680	5360	14 400	41 800	77 100	6320
	Geo-CV%	38.8	22.6	31.8	42.3	34.8	34.8	45.9	392.2
AUC_{inf} (h*µg/mL)	Mean (SD)	660 (176)	2390 (NA)	NA	5870 (3180)	NA	39 200 (NA)	NA	6290 (12 600)
	CV%	26.6	NA	NA	54.1	NA	NA	NA	200.0
	Geo-mean	640	2390	NA	5430	NA	39 200	NA	1880
	Geo-CV%	28.1	NA	NA	62.0	NA	NA	NA	290.4
C_{max} (ng/mL)	Mean (SD)	5470 (2960)	9320 (3700)	20 000 (6400)	37 600 (9290)	127 000 (37 000)	248 000 (21 500)	474 000 (221 000)	122 000 (174 000)
	CV%	54.2	39.7	31.9	24.7	29.2	8.7	46.6	142.9
	Geo-mean	4880	8820	19300	36 500	123 000	247 000	438 000	40 900
	Geo-CV%	54.3	43.5	31.5	27.8	32.6	8.6	47.6	365.4
t_{max} (h)	Median	0.583	0.583	0.5	0.583	0.583	0.583	0.625	0.583
	Min-max	0.5-1.58	0.5-0.583	0.5-0.567	0.550-0.750	0.583-0.750	0.5-0.833	0.567-0.750	0.5-1.58
$t_{½}$ (h)	Median	171	169	NA	198	NA	136	NA	175
	Min-Max	140-227	169-169	NA	191-206	NA	136-136	NA	136-227
CL (L/h))	Mean (SD)	0.0161 (0.004 45)	0.0125 (NA)	NA	0.0299 (0.0162)	NA	0.0191 (NA)	NA	0.0191(0.0091)
	CV%	27.7	NA	NA	54.1	NA	NA	NA	47.6
V_z (L)	Mean (SD)	4.06 (1.09)	3.06 (NA)	NA	8.43 (4.17)	NA	3.76 (NA)	NA	4.89 (2.63)
	CV%	26.7	NA	NA	49.5	NA	NA	NA	53.7
				Cycle 4 day 1					
AUC_{last} (h*µg/mL)	Mean (SD)	1420 (546)	NA	5140 (2000)	12 300 (4520)	NA	102 000 (NA)	115 000 (29 200)	29 100 (47 300)
	CV%	38.4	NA	39.0	36.9	NA	NA	25.5	162.8
	Geo-mean	1310	NA	4820	11 800	NA	102 000	113 000	6890
	Geo-CV%	52.1	NA	48.8	39.1	NA	NA	26.3	502.4

AUC_{inf} (h*µg/mL)	Mean (SD)	1740 (326)	NA	NA	16 000 (NA)	NA	NA	NA	5310 (7150)
	CV%	18.8	NA	NA	NA	NA	NA	NA	134.7
	Geo-mean	1720	NA	NA	16 000	NA	NA	NA	3000
	Geo-CV%	20.2	NA	NA	NA	NA	NA	NA	160.6
C_{max} (ng/mL)	Mean (SD)	6670 (1740)	NA	25 400 (4450)	52 000 (6010)	NA	304 000 (NA)	699 000 (291 000)	147 000 (271 000)
	CV%	26.2	NA	17.5	11.6	NA	NA	41.6	183.7
	Geo-mean	6450		25 200	51 800		304 000	668 000	33 400
	Geo-CV%	30.3		17.9	11.6		NA	44.9	467.8
t_{max} (h)	Median	0.583	NA	0.550	0.550	NA	1.13	0.5	0.550
	Min-max	0.5-0.6	NA	0.5-0.783	0.550-0.550	NA	1.13-1.13	0.5-0.5	0.5-1.13
$t_{½}$ (h)	Median	256	NA	NA	341	NA	NA	NA	272
	Min-max	233-288	NA	NA	341-341	NA	NA	NA	233-341
V_z (L)	Mean (SD)	3.12 (0.976)	NA	NA	6.95 (NA)	NA	NA	NA	4.08 (2.07)
	CV%	31.3	NA	NA	NA	NA	NA	NA	50.9

AUC_{last}, area under the curve from time = 0 to last measurable concentration; AUC_{inf}, area under the plasma concentration-time curve extrapolated to infinity; CL, total clearance uncorrected for absolute bioavailability; C_{max}, maximum concentration; CV, Coefficient of variation; Geo, geometric; Min-max, minimum-maximum; q3w, every 3 weeks; SD, standard deviation; $t_{½}$, elimination half-life; t_{max}, time to reach C_{max}; V_z, apparent volume of distribution during terminal elimination phase.

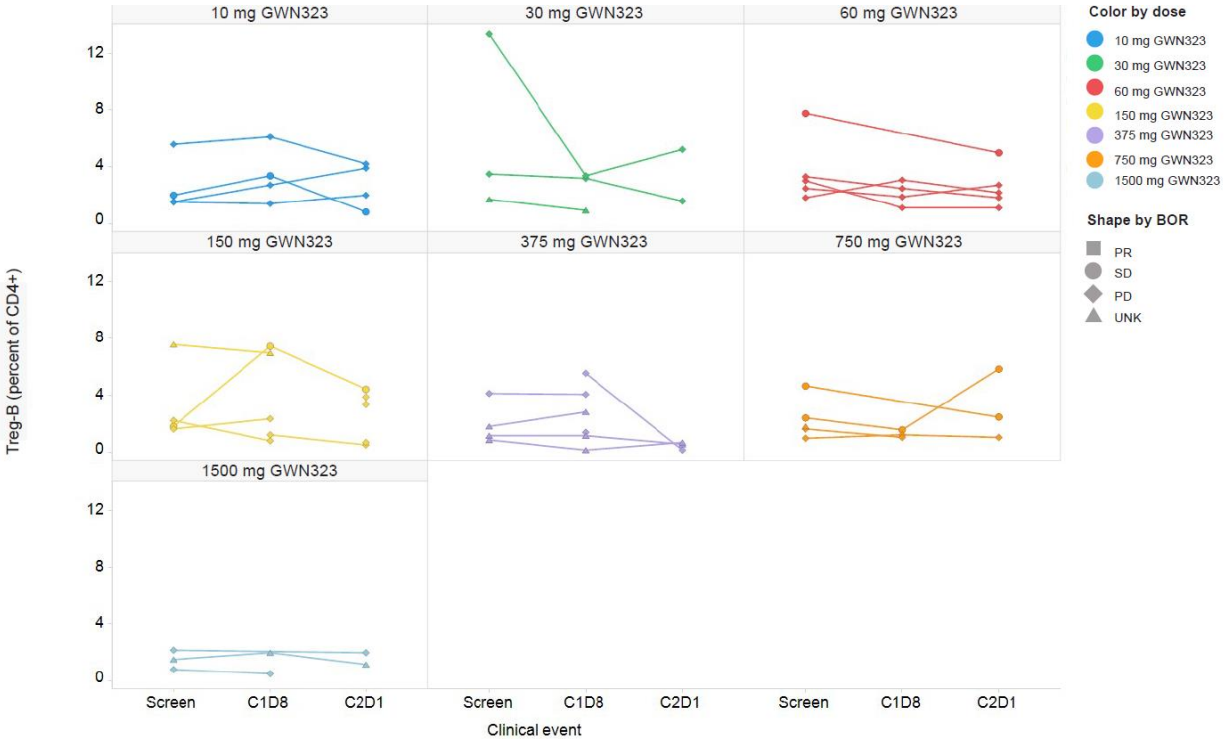
Table S7. Pharmacokinetic parameters for GWN323 (cycle 1 day 1): combination arm

		Cycle 1 day 1							
Parameter	**Statistics**	**GWN323 10 mg + spartalizumab 100 mg q3w N=5**	**GWN323 10 mg + spartalizumab 200 mg q3w N=4**	**GWN323 30 mg + spartalizumab 100 mg q3w N=4**	**GWN323 30 mg + spartalizumab 300 mg q3w N=5**	**GWN323 75 mg + spartalizumab 300 mg q3w N=5**	**GWN323 150 mg + spartalizumab 300 mg q3w N=12**	**GWN323 300 mg + spartalizumab 300 mg q3w N=6**	**GWN323 750 mg + spartalizumab 300 mg q3w N=1**
AUC_{last} (h*µg/mL)	Mean (SD)	395 (95.9)	524 (210)	1210 (586)	1310 (268)	4870 (1450)	6680 (2100)	23 500 (10 600)	NA
	CV%	24.3	40.1	48.3	20.5	29.8	31.4	44.9	NA
	Geo-mean	385	491	1100	1290	4700	6400	22100	
	Geo-CV%	26.3	43.9	64.6	21.5	32.7	34.8	45.8	
AUC_{inf} (h*µg/mL)	Mean (SD)	456 (149)	390 (NA)	NA	1500 (405)	4830 (1860)	9690 (528)	30300 (18200)	NA
	CV%	32.7	NA	NA	26.9	38.4	5.5	60.0	NA
	Geo-mean	437	390	NA	1480	4650	9680	27400	NA
	Geo-CV%	35.2	NA	NA	27.7	41.0	5.5	71.2	NA
C_{max} (ng/mL)	Mean (SD)	2690 (509)	2690 (974)	7240 (1070)	8300 (2100)	24100 (8540)	39000 (11800)	109000 (36600)	NA
	CV%	18.9	36.3	14.7	25.3	35.5	30.3	33.7	NA
	Geo-mean	2650	2550	7190	8090	22900	37000	104000	NA
	Geo-CV%	20.3	39.3	15.5	27.1	37.5	40.1	36.2	NA
t_{max} (h)	Median	1.58	1.71	1.52	1.70	11.7	2.17	2.09	NA
	Min-max	0.5-1.85	0.5-1.92	0.550-1.75	1.50-2.53	1.50-25.9	1.98-2.37	1.58-21.8	NA
$t_{½}$ (h)	Median	181	213	NA	150	182	273	203	NA
	Min-max	124-220	213-213	NA	110-191	147-218	244-303	180-227	NA
CL (L/h))	Mean (SD)	0.0239 (0.0083)	0.0256 (NA)	NA	0.0207 (0.00557)	0.0168 (0.00643)	0.00805 (0.0114)	0.0121 (0.00725)	NA
	CV%	34.7	NA	NA	26.9	38.4	141.4	60.0	NA
V_z (L)	Mean (SD)	5.80 (1.40)	7.86 (NA)	NA	4.26 (0.493)	4.64 (2.91)	3.52 (4.97)	3.37 (1.55)	NA
	CV%	24.1	NA	NA	11.6	62.7	141.4	46.0	NA
		Cycle 4 day 1							
AUC_{last} (h*µg/mL)	Mean (SD)	915 (644)	2160(790)	3730 (2130)	2480 (366)	33100 (24900)	15300 (NA)	NA	NA
	CV%	70.4	36.5	57.1	14.8	75.3	NA	NA	NA
	Geo-mean	771	2090	3310	2470	28000	15300	NA	NA
	Geo-	82.4	38.7	67.7	14.6	101.2	NA	NA	NA

	CV%								
AUC_{inf} (h*µg/mL)	Mean (SD)	1230 (676)	3090 (NA)	6520 (NA)	2700 (288)	35600 (25000)	NA	NA	NA
	CV%	55.0	NA	NA	10.7	70.3	NA	NA	NA
	Geo-mean	1130	3090	6520	2690	30900	NA	NA	NA
	Geo-CV%	63.3	NA	NA	10.7	90.2	NA	NA	NA
C_{max} (ng/mL)	Mean (SD)	2890(1280)	5770(2470)	10500(3290)	10600(1880)	60000(16300)	47800(13000)	NA	NA
	CV%	44.4	42.8	31.3	17.7	27.2	27.2	NA	NA
	Geo-mean	2700	5490	10100	10500	58800	46900	NA	NA
	Geo-CV%	47.1	46.4	34.4	18.8	28.1	28.1	NA	NA
t_{max} (h)	Median	1.52	11.0	1.67	2.15	230	0.842	NA	NA
	Min-max	0.767-1.55	0.517-21.6	1.58-23.6	1.57-2.57	2.17-458	0.667-1.02	NA	NA
$t_{½}$ (h)	Median	355	751	598	209	465	NA	NA	NA
	Min-max	354-356	751-751	598-598	203-215	356-575	NA	NA	NA
V_z (L)	Mean (SD)	8.36(5.34)	10.3(NA)	8.36(NA)	4.22(0.321)	3.11(0.658)	NA	NA	NA
	CV%	63.9	NA	NA	7.6	21.2	NA	NA	NA

AUC_{last}, area under the curve from time = 0 to last measurable concentration; AUC_{inf}, area under the plasma concentration-time curve extrapolated to infinity; CL, total clearance uncorrected for absolute bioavailability; C_{max}, maximum concentration; CV, Coefficient of variation; Geo, geometric; Min-max, minimum-maximum; q3w, every 3 weeks; SD, standard deviation; $t_{½}$, elimination half-life; t_{max}, time to reach C_{max}; V_z, apparent volume of distribution during elimination phase

Figure S1: Peripheral effector Treg cells in patients treated with single-agent GWN323

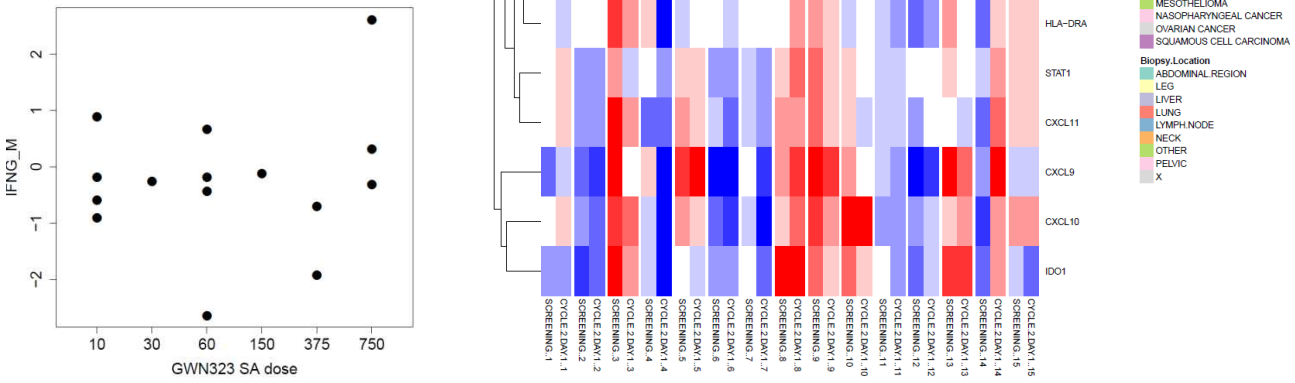


Blood was collected and PBMCs isolated at screening, C1D8 and C2D1. PBMCs were stained to identify the percentages of peripheral effector Treg cells. BOR categories are indicated by shape and doses of GWN323 +/- spartalizumab are colour coded. Immune cell identification markers were: Proliferating T cells: CD8+Ki67+ % of CD8+, proliferating NK cells: CD56+Ki67+ % of CD56+Total Tregs: (CD4+ CD127lo $CD25^{hi}$ Foxp3+ (% of CD4+ $CD127^{lo}$ $CD25^{hi}$)* CD4+ CD127lo CD25hi (% of CD4+))/100, eTregs: CD4+ $CD127^{lo}$ $CD25^{hi}$ $Foxp3^{hi}$ CD45RA-% of CD4+ and nTregs: CD4+ $CD127^{lo}$ $CD25^{hi}$ $Foxp3^{lo}$ CD45RA+% of CD4+.

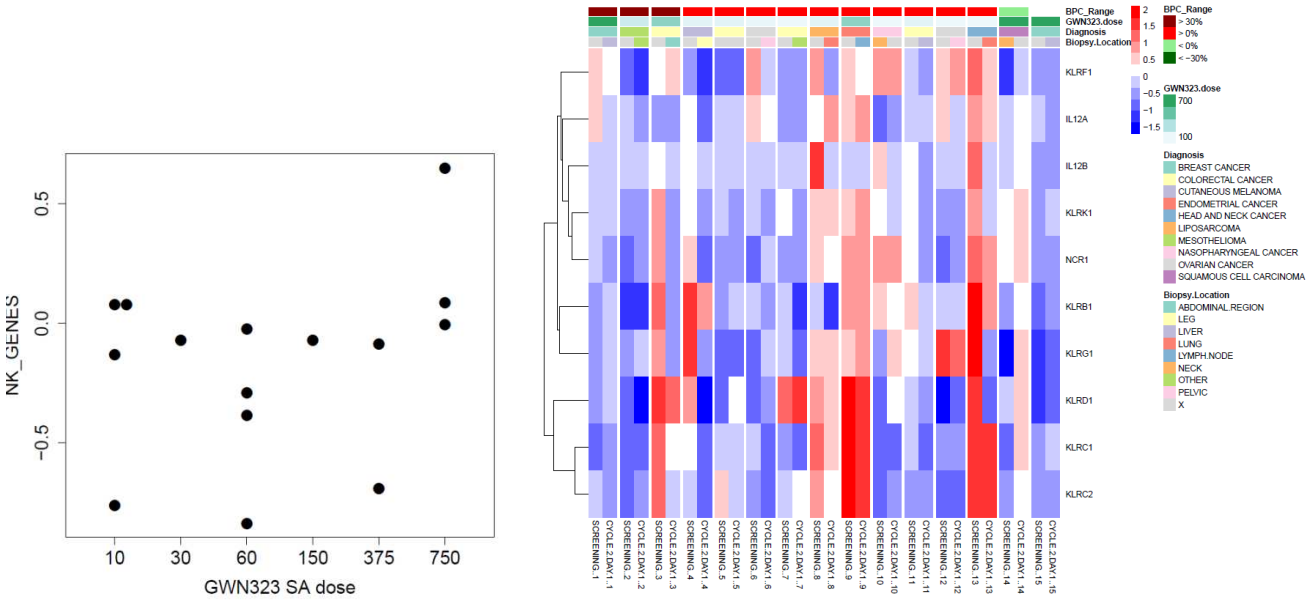
BOR, best overall response; C, cycle; D, day; PBMC, peripheral blood mononuclear cell; PD, progressive disease; SD, stable disease; Treg, regulatory T; UNK, unknown.

Figure S2: Combined RNA sequencing data from all single-agent patients: (A) effect of treatment on IFNγ and (B) NK cell signature

(A)



(B)

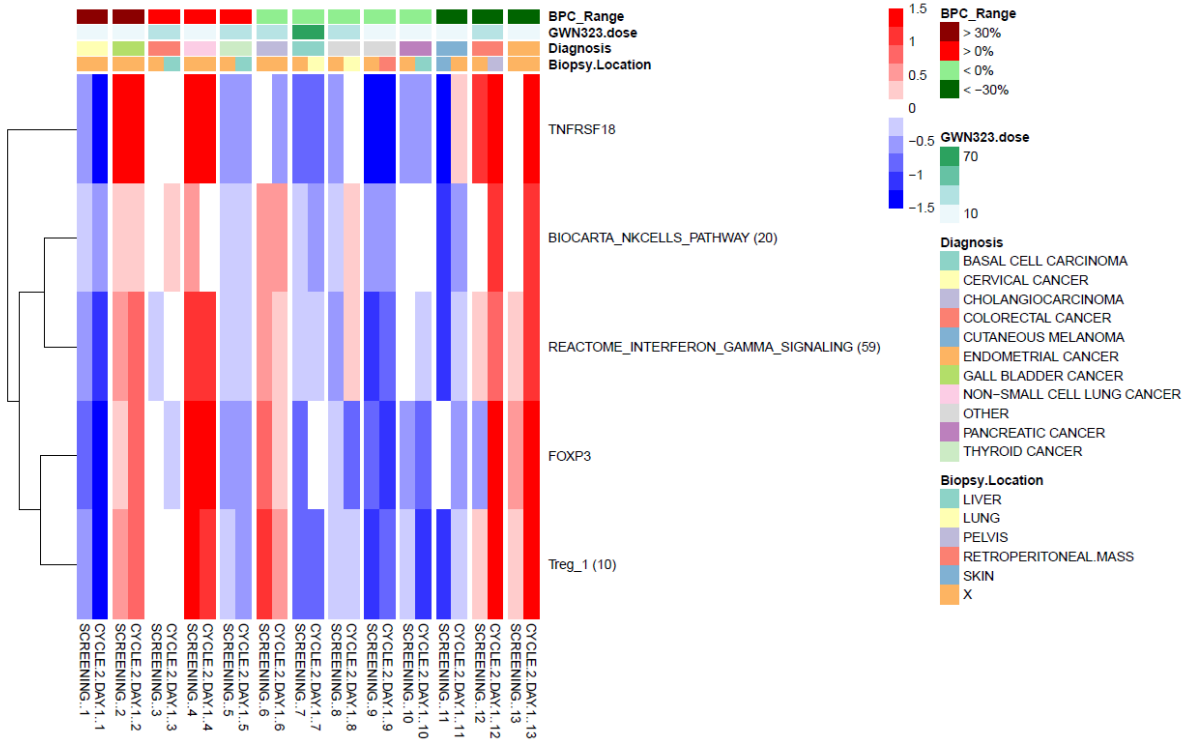


RNA sequencing analysis was performed on paraffin-embedded sections of tumour collected at screening and cycle 2 day 1 for the single-agent treatment patients. Gene expression heatmaps and correlation plots between GWN323 dose and IFNɣ gene signatures (A) and NK cell gene signatures (B) are shown. The heatmaps on the right show the gene expression values for the screening/cycle 2 day 1 pairs. The GWN323 dose, diagnosis and biopsy locations are colour coded and sorted on the range of BPC (BPC_Range) in tumour size. Biopsy location is important as normal cells from nearby tissue can impact differential gene expression differently. The boxplots on the left show the distribution of fold change for the signatures for all the sets of GWN323 doses. The gene signature expression levels in the single arm indicate no significant correlations between the GWN323 dosing and changes in T-cell expression. A single outlier in the 750

mg dosage arm is likely owing to difference in biopsy location between the screening and on-treatment samples, the on-treatment sample being sourced from the lymph node resulting in higher gene expression levels in general.

BPC, best percent change; IFN, interferon; NK, natural killer; RNA, Ribonucleic acid; SA, single agent

Figure S3: Combined RNA sequencing data from patients treated with GWN323 and spartalizumab



RNA sequencing analysis was performed on paraffin-embedded sections of tumour collected at screening and cycle 2 day 1 for the combination arm. Gene signature expression heatmaps for the screening/cycle 2 day 1 pairs are shown for a number of IFNɣ, Treg and NK cell gene signatures and more. The GWN323 dose, diagnosis and biopsy locations are colour coded and sorted on the range of BPC (BPC_Range) in tumour size. Biopsy location is important as normals cells from nearby tissue can impact differential gene expression differently. Analysis of the paired samples in the combination arm in patients with a 30% on-treatment

decrease in tumour volume show upregulation of multiple IFN-γ signatures and Treg signatures. The numbers next to each signature name indicate the number of genes contained in the respective signature.

BPC, best percent change; IFN, interferon; NK, natural killer; RNA, Ribonucleic acid; Treg, T regulatory. TNFRSF, tumor necrosis factor receptor superfamily

SUPPLEMENTAL METHODS

Biomarker assessments

Assessments were performed on archival or newly obtained tumour biopsies via immunohistochemistry and ribonucleic acid (RNA) sequencing. Immunohistochemistry staining for spartalizumab was performed on the Dako Autostainer Link 48 system with the 22C3 mouse monoclonal primary antibody and EnVision FLEX visualisation system, as described in the spartalizumab immunohistochemistry 22C3 pharmDx package insert. The percentage of tumour cells with partial or complete membranous staining of spartalizumab was assessed. Immunohistochemistry staining for CD8 was performed on the Ventana Benchmark XT system with the Dako CD8 mouse monoclonal primary antibody (clone C8/144B). Images of whole tumour sections were captured using a Mirax scanner (Zeiss) and Meso Scale Discovery (MSD) with Definiens (Definiens AG, Munich, Germany). DAB (3,3′-Diaminobenzidine) intensity was quantified as percent positive pixels. For RNA sequencing, RNA was extracted from formalin-fixed paraffin-embedded tissue biopsies. To enrich for messenger RNA (mRNA), ribosomal RNA (rRNA) was depleted using RNase H digestion. The rRNA-depleted RNA was fragmented, converted to complementary DNA and constructed into sequencing libraries with the TruSeq RNA Library Preparation Kit v2 (Illumina #RS-122-2001 and #RS-122-2002). The resulting libraries were sequenced with 100-base pair (bp) paired-end reads to a target depth of 50 million total reads per sample on an Illumina HiSeq sequencing system. Next-generation sequencing data processing sequencing reads were aligned to the human reference genome (hg19) using STAR. HTSeq was used to quantify the number of reads aligned to each gene in the RefSeq transcriptome. Sequencing data were evaluated for quality, and low complexity libraries with <2 million estimated unique read pairs were excluded from the downstream analysis. Gene count

data were normalised using the trimmed mean of M values method as implemented in edgeR. All downstream gene signature analyses were performed on the log2 of the normalised gene count data. Flow cytometry (using a BD Fortessa) was performed on peripheral blood mononuclear cell samples taken before treatment and at several on-treatment timepoints to quantify immune cell subsets. Immune cell identification markers were as follows: Proliferating T cells: CD8+Ki67+ % of CD8+, proliferating NK cells: CD56+Ki67+ % of CD56+Total Tregs: (CD4+ CD127lo $CD25^{hi}$ Foxp3+ (% of CD4+ $CD127^{lo}$ $CD25^{hi}$)* CD4+ $CD127^{lo}$ $CD25^{hi}$ (% of CD4+))/100, eTregs: CD4+ $CD127^{lo}$ $CD25^{hi}$ $Foxp3^{hi}$ CD45RA-% of CD4+ and nTregs: CD4+ $CD127^{lo}$ $CD25^{hi}$ $Foxp3^{lo}$ CD45RA+% of CD4+.