SUPPLEMENTAL MATERIAL

METHODS

Determination of dose-limiting toxicity

A dose-limiting toxicity (DLT) was defined as an adverse event (AE) during cycle 1 (28 days) that was possibly related to either study drug and fulfilled any one of the following criteria:

- Grade 4 haematological toxicity of >5 days' duration (except for exclusions listed below)
- Any febrile neutropenia
- Common Terminology Criteria for Adverse Events grade ≥3 thrombocytopenia with clinically significant bleeding that required medical intervention
- Any grade 4 immune-related AE
- Any grade 3 immune-related AE, excluding colitis or pneumonitis, that did not downgrade to grade 2 within 3 days after onset of the event despite optimal medical management, including systemic corticosteroids, or did not downgrade to grade ≤1 or baseline within 14 days
- Any grade ≥3 colitis
- Any grade 3 or 4 non-infectious pneumonitis, regardless of duration
- Any grade 2 pneumonitis that did not resolve to grade ≤1 within 3 days of the initiation of maximal supportive care
- Liver transaminase elevation >8× upper limit of normal or total bilirubin >5× upper limit of normal
- Grade 3 nausea and vomiting that are not manageable with standard treatments
- Grade 4 vomiting or anorexia regardless of duration

- All other grade ≥3 non-haematological toxicity not listed above (except for exclusions listed below)
- Any other significant toxicity deemed by the primary investigator and the sponsor's clinical research personnel to be dose limiting (eg, any toxicity that was possibly related to the study medication that required the withdrawal of the patient from the study during cycles 1 or 2) or resulted in the patient getting <75% of the total doses or led to suspension of galunisertib for >2 weeks.

Toxicity that was clearly and directly related to the primary disease or to another aetiology was excluded from the definition of DLTs. Exceptions were made for:

- Grade 3 constipation <1 week in duration that could be controlled with treatment
- Grade ≥3 fatigue ≤7 days in duration
- Transient (≤2 weeks) grade 3 elevations of alanine aminotransferase and/or aspartate aminotransferase, without evidence of other hepatic injury, in the setting of pre-existing hepatic metastasis. Baseline elevation of these values were not considered a DLT if agreed by the study investigator and the sponsor's clinical research physicians
- Grade 3 endocrine disorder (thyroid, pituitary, and/or adrenal insufficiency) that was managed with or without systemic corticosteroid therapy and/or hormone replacement therapy when the patient was asymptomatic
- Grade 3 inflammatory reaction attributed to a local anti-tumour response (eg, inflammatory reaction at sites of metastatic disease, lymph nodes)

- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolved within 6 hours with appropriate clinical management
- Grade 3 or 4 neutropenia that was not associated with fever or systemic infection that improved by at least one grade within 3 days. Grade 3 or grade 4 febrile neutropenia was a DLT regardless of duration or reversibility
- Grade 3 or 4 lymphopenia
- Grade 3 thrombocytopenia that was not associated with clinically significant bleeding (did not require medical intervention) and improved by at least one grade within 3 days
- Isolated grade ≥3 electrolyte abnormalities that were not associated with clinical signs or symptoms and were reversed with appropriate maximal medical intervention within 3 days
- Concurrent vitiligo or alopecia of any AE grade.

Immune-related AEs were defined per protocol as AEs of an immune nature (ie, inflammatory) in the absence of a clear alternative aetiology. Information on immune-related AEs was not collected on the case report form; therefore, analyses were conducted post hoc.

Although no intra-patient dose escalation or reduction was allowed during cycle 1, for the purposes of patient management, DLTs in cycle 1 would lead to dose interruption.

Safety assessments

AEs were coded according to the Medical Dictionary for Regulatory Activities and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03. Safety analyses were focused on treatment-emergent AEs (the AEs that occurred or worsened after treatment started), treatment-related AEs (the treatment-emergent AEs possibly related to study treatment per the investigators' judgement), serious AEs (the AEs resulting in serious consequences such as death, initial or prolonged inpatient hospitalisation, a life-threatening experience, congenital anomaly/birth defect, persistent or significant disability/incapacity), or any reaction considered significant by the investigator.

Pharmacokinetics

Human plasma samples (approximately 4 mL) obtained during this study were analysed for galunisertib using validated liquid chromatography coupled with atmospheric pressured ionisation mass spectrometry (LC-API/MS/MS) methods (BPLY215A and BPLY215B, Project number 31205) at Intertek Pharmaceutical Services (El Dorado Hills, CA, USA; San Diego, CA, USA). For BPLY215A, the lower limit of quantification was 0.0500 ng/mL and the upper limit of quantification was 10.000 ng/mL. For BPLY215B, the lower limit of quantification was 5.000 ng/mL and the upper limit of quantification was 1000.000 ng/mL. All samples were initially analysed using BPLY215B, and all samples below the limit of quantification for this method (5.000 ng/mL) were re-analysed using BPLY215A. Samples above the limit of quantification were diluted and re-analysed with BPLY215B to yield results within the calibrated range. The inter-assay accuracy (% relative error) during validation of BPLY215A ranged from –3.778% to –1.268%. The inter-assay precision (% relative

standard deviation) during validation of BPLY215A ranged from 1.695% to 5.086%. The inter-assay accuracy (% relative error) during validation of BPLY215B ranged from –2.22% to –1.79%. The inter-assay precision (% relative standard deviation) during validation of BPLY215B ranged from 2.21% to 5.07%. Galunisertib was stable for up to 148 days (ARLY215A) and 109 days (ARLY215B) when stored at approximately –20°C. Galunisertib was stable for up to 753 days (ARLY215A) and 1147 days (ARLY215B) when stored at approximately –70°C.

Blood samples for durvalumab immunogenicity were drawn at the onset of, resolution of, and 30 days after infusion-related reactions.

Biomarkers

The percentage of programmed death-ligand 1 expression on the tumour cell membrane was measured by immunohistochemistry using the Ventana PD-L1 (SP263) kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). Cancer gene sequencing was performed by Foundation Medicine using the FoundationOne T7 assay (Foundation Medicine, Cambridge, MA, USA). Analysis focused only on genetic variants with known or likely functional consequences as defined by Foundation Medicine.[1] We report results for genes with variants detected in ≥4 samples.

Serum proteins were assessed at baseline using the Inflammation Multi-analyte Immunoassay panel developed by Myriad RBM (Austin, TX, USA) as it represents the most relevant immune-related circulating biomarkers of any Myriad panel. The panel was supplemented with assay panels for interferon gamma-induced protein 10, follicle-stimulating hormone, plasminogen activator inhibitor 1, and macrophage inflammatory protein-1 alpha based on the findings of a prior study

of galunisertib treatment response in pancreatic cancer.[2] Analysis focused on patients treated with galunisertib 150 mg twice daily plus durvalumab 1500 mg every 4 weeks.

Statistical analyses

The efficacy endpoints were objective response rate (the percentage of patients with a complete response [CR] or partial response [PR] as their best overall response), disease control rate (CR, PR, or stable disease as their best overall response), and time-to-event variables (progression-free survival [PFS], overall survival [OS], and duration of response [DoR]). The Kaplan-Meier method was used to analyse time-to-event variables. Median and 95% confidence intervals were estimated where data were available.

PFS was defined as the time from the start of study treatment until the first occurrence of documented disease progression per Response Evaluation Criteria in Solid Tumours version 1.1, or death from any cause in the absence of progressive disease. Patients known to be alive and without disease progression as of the data inclusion cut-off date were censored at the time of the last adequate tumour assessment. OS was defined as the time from the start of study treatment until death from any cause. If the patient was alive, lost to follow-up, or withdrawn from the study at the time of data analysis, OS data were censored on the last date the patient was known to be alive.

DoR was defined as the time from the date that measurement criteria for CR or PR (whichever was recorded first) were first met until the first occurrence of documented disease, or death from any cause in the absence of progressive

disease. The DoR followed the same censoring scheme as PFS, as described above.

RESULTS

Supplementary table 1 Overview of AEs (safety population)

n (%)	Galunisertib 50 mg QD + durvalumab 1500 mg Q4W n=3	Galunisertib 50 mg BID + durvalumab 1500 mg Q4W n=4	Galunisertib 80 mg BID + durvalumab 1500 mg Q4W n=3	Galunisertib 150 mg BID + durvalumab 1500 mg Q4W n=32	Total N=42
Subjects with ≥1 TEAE	3 (100)	4 (100)	3 (100)	31 (96.9)	41 (97.6)
Related to study treatment	2 (66.7)	2 (50.0)	3 (100)	13 (40.6)	20 (47.6)
Subjects with ≥1 grade ≥3 TEAE	0	3 (75.0)[a]	2 (66.7)	24 (75.0)	29 (69.0)[a]
Related to study treatment	0	0	0	5 (15.6)	5 (11.9)
Subjects with ≥1 SAE	1 (33.3)	1 (25.0)	0	12 (37.5)	14 (33.3)
Related to study treatment	0	0	0	2 (6.3)	2 (4.8)
Subjects who discontinued study treatment due to AE	0	0	0	2 (6.3)	2 (4.8)
Related to study treatment	0	0	0	1 (3.1)	1 (2.4)
Subjects who died due to AE on study treatment	0	0	0	0	0

Subjects who died due to AE within 30 days of discontinuation from study treatment	0	0	0	0	0

AE, adverse event; BID, twice daily; Q4W, every 4 weeks; QD, once daily; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

Supplementary table 2 Tumour response

	Galunisertib 50 mg QD + durvalumab 1500 mg Q4W n=3	Galunisertib 50 mg BID + durvalumab 1500 mg Q4W n=4	Galunisertib 80 mg BID + durvalumab 1500 mg Q4W n=3	Galunisertib 150 mg BID + durvalumab 1500 mg Q4W n=32
Best overall response, n (%)				
Complete response	0	0	0	0
Partial response	0	0	0	1 (3.1)
Stable disease	1 (33.3)	0	1 (33.3)	7 (21.9)
Progressive disease	2 (66.7)	4 (100)	2 (66.7)	15 (46.9)
Non-evaluable	0	0	0	9 (28.1)
Overall response rate, n (%)[a]	0	0	0	1 (3.1)
Disease control rate, n (%)[b]	1 (33.3)	0	1 (33.3)	8 (25.0)

BID, twice daily; Q4W, every 4 weeks; QD, once daily.

[a]Overall response rate = complete response/partial response.

[b]Disease control rate = complete response/partial response/stable disease.

Supplementary table 3 Common[a] genetic variants detected

					Gene						PD-L1	
Galunisertib dose BID	PFS (months)	OS (months)	% Change in tumour size	BOR	*KRAS*	*TP53*	*CDKN2A*	*CDKN2B*	*SMAD4*	*ARID1A*	Tumour membrane %	Immune membrane %
150 mg	8.3	14.3	−42.55	PR							0	30
80 mg	7.6	10.4	−5.76	SD							0	2
150 mg	5.4	12.2	−7.54	SD							NA	NA
150 mg	3.6	12.8	0	SD	SV						0	2
150 mg	3.6	6.0	−10.84	SD	CN (amp) + SV	SV	HD	HD		SV	40	40
150 mg	2.2	2.2	NA	NA							0	15
150 mg	1.9	2.8	−8.51	PD	SV		HD	HD	HD	SV	0	30
80 mg	1.8	2.8	0	PD	SV	SV	SV		SV	SV	0	70
150 mg	1.8	8.1	0	PD	SV	SV					0	5
150 mg	1.8	3.6	36.8	PD	SV	SV				SV	30	70
150 mg	1.8	3.1	9.95	PD		SV			SV		0	10
150 mg	1.7	10.2	−8.51	PD	SV	SV	SV				0	40
80 mg	1.7	3.6	77.03	PD	SV	SV					2	10
150 mg	1.7	5.7	77.52	PD	SV		HD	HD			0	5
150 mg	1.6	4.0	20.64	PD	SV	SV	SV		SV		1	25
150 mg	0.9	4.9	33.33	PD	SV	SV	HD	HD	SV		40	70
150 mg	0.8	1.3	46.57	PD	SV	SV	HD			SV	3	30
80 mg	0.8	1.1	72.88	PD	SV	SV	HD	HD	HD		0	20
150 mg	0.0	4.0	NA	NA	SV				SV		2	30
150 mg	0.0	6.1	NA	NA	SV	SV	SV				0	30

BID, twice daily; BOR, best overall response; CN (amp), copy number alteration amplification; HD, homozygous deletion; NA, not applicable; OS, overall survival; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PR, partial response; SD, stable disease; SV, short variant.

[a]Defined as genes containing known or likely functional variants observed in ≥4 samples.

Supplementary table 4 Baseline serum proteins

Protein	Cut point	OR[a] (95% CI)	p value[a]	Adjusted p value[b]
Fibrinogen	0	0 (0-Inf)	0.001	0.063
Interleukin-6 (IL-6)	0	0 (0-Inf)	0.001	0.063
Latency-associated peptide of transforming growth factor beta 1 (LAP TGFβ1)	7.8	0.05 (0.01-0.049)	0.001	0.063
Interleukin-8 (IL-8)	5.2	0.12 (0.02-0.7)	0.01	0.6
Interferon gamma-induced protein 10 (IP-10)	184	7 (1.18-41.36)	0.019	1

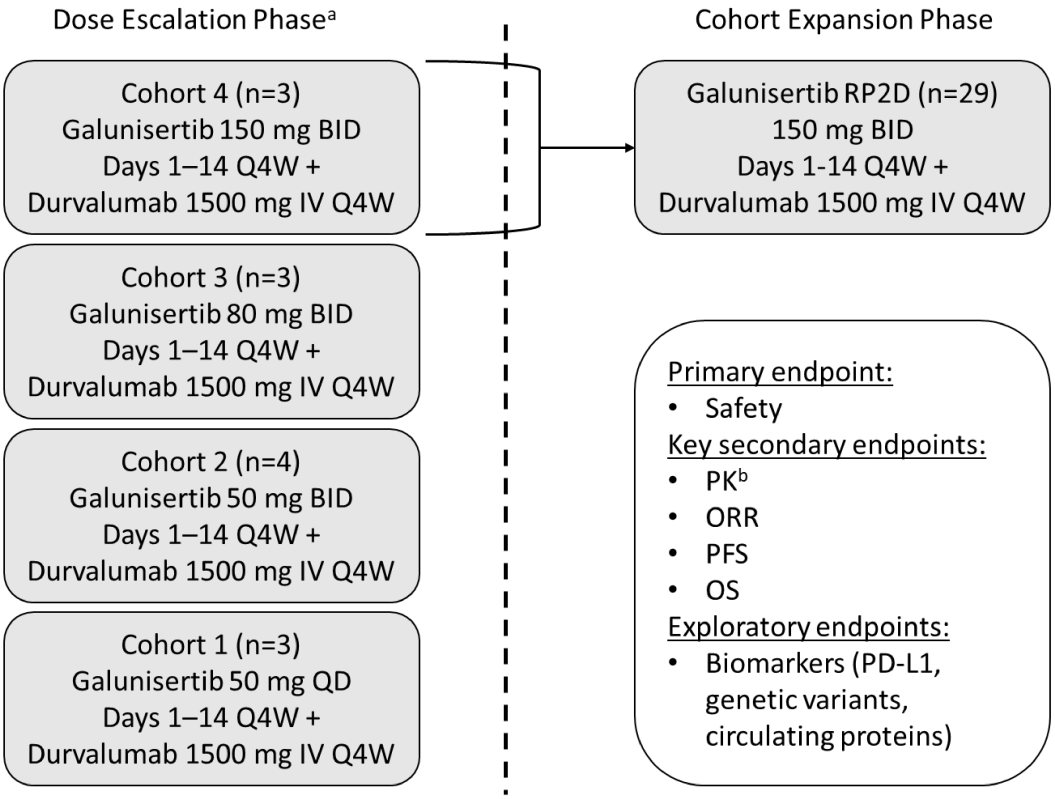
Markers with p values ≤1 (unadjusted p value: no covariates) are included and sorted in the table. Multiplicity adjustment (Holm's method) was applied.

CI, confidence interval; CR, complete response; OR, odds ratio; PR, partial response; SD, stable disease.

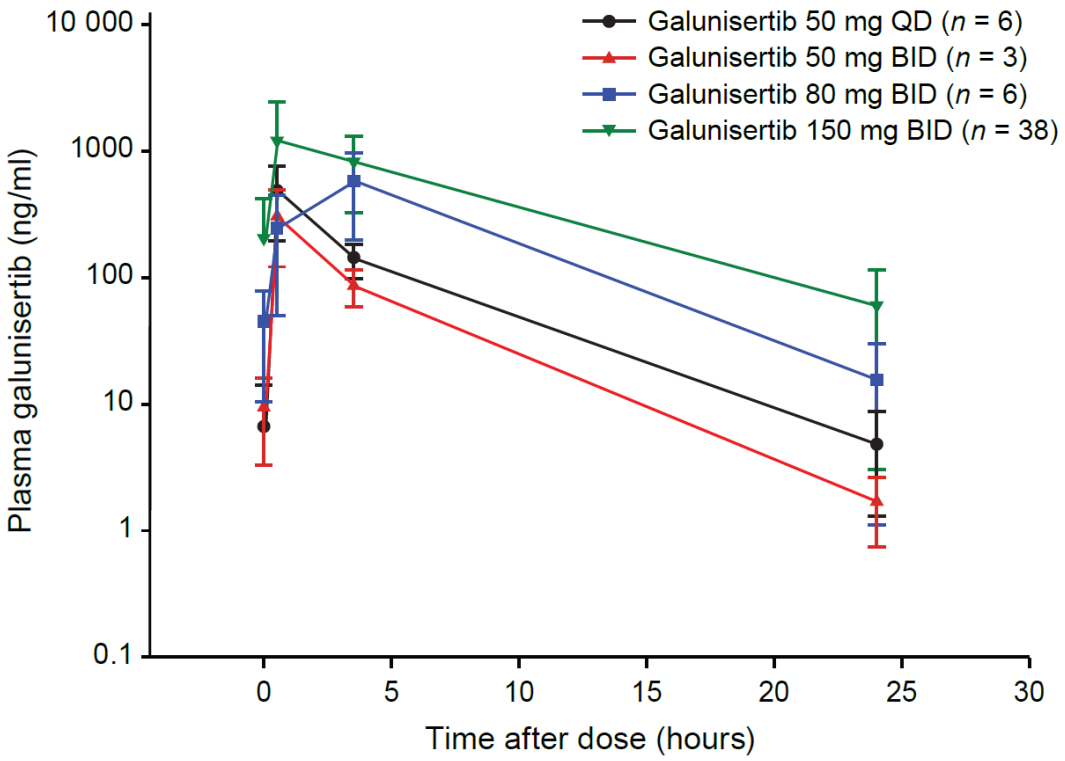
[a]OR and p value (likelihood ratio chi-square test) obtained from logistic regression of clinical benefit (CR or PR, or SD ≥3 months) as function of marker as continuous variable.

[b]Adjusted p value across markers for the specific endpoint.

Supplementary figure S1 Study design. [a]A 3+3 dose escalation design was used; if no dose-limiting toxicities occurred in a cohort of three patients, a new cohort of three patients was treated at the next higher-dose level. [b]The pharmacokinetics of galunisertib were assessed in venous blood samples collected on day 1 (pre-dose and between 0.5 and 3 hours) and day 14 (pre-dose, between 0.5 and 2 hours, between 3.5 and 5 hours, and 24 hours post-dose) in cycles 1 and 2, followed by pre-dose samples in cycles 3, 4, and 7. BID, twice daily; IV, intravenously; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PK, pharmacokinetics; Q4W, every 4 weeks; QD, once daily; RP2D, recommended phase II dose.



Supplementary figure S2 Mean concentration–time plots for plasma galunisertib at steady state. BID, twice daily; QD, once daily.



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2 Melisi D, Garcia-Carbonero R, Macarulla T, et al. TGFbeta receptor inhibitor galunisertib is linked to inflammation- and remodeling-related proteins in patients with pancreatic cancer. *Cancer Chemother Pharmacol* 2019;83:975–91.