INVESTIGATION OF PD-L1 EXPRESSION AND TISLELIZUMAB EFFICACY IN GASTROESOPHAGEAL ADENOCARCINOMA USING A NOVEL TUMOR AND IMMUNE CELL SCORE WITH VENTANA PD-L1 (SP263) ASSAY AND COMBINED POSITIVE SCORE (CPS)

Yee Chao[1], Silu Yang[2], Yun Zhang[2], Zhirong Shen[2], Xikun Wu[2], Jin Wang[2], Michelle Quiroz[3], Alma Nielsen[3], Chunyan Liu[3], Jayesh Desai[4]

[1]Taipei Veterans General Hospital, Taipei City, Taiwan; [2]BeiGene (Beijing) Co., Ltd., Beijing, China; [3]Ventana Medical Systems, Inc., Roche Tissue Diagnostics, Tucson, AZ, USA; [4]Peter MacCallum Cancer Centre, Melbourne, Australia

Poster: 154P
European Society of Medical Oncology
September 19-21, 2020, Virtual Congress

BACKGROUND

- Gastroesophageal adenocarcinoma (GEA), including gastric cancer (GC), gastroesophageal junction (GEJ) adenocarcinoma, and esophageal adenocarcinoma (EAC), is a substantial cause of cancer-related mortality worldwide and has a poor 5-year overall survival rate when diagnosed at advanced stage[1,2]
- Approved programmed cell death protein-1(PD-1) inhibitors have shown encouraging improvements in survival, but many patients do not respond,[3,4] highlighting a potential need for the identification of biomarkers of response
- Programmed death-ligand 1 (PD-L1) protein expression on tumor (TC) and immune cells (IC) may be associated with anti-PD-1 efficacy in GEA and can be assessed via cell counting using the Combined Positive Score (CPS) and Dako 22C3 assay[5,6]; however, the CPS scoring method can be challenging to utilize
- A less time-consuming algorithm based on visual estimation of PD-L1 expression on tumor and immune cells, the visually-estimated Combined Positive Score (vCPS), was developed for the VENTANA PD-L1 (SP263) assay
- Tislelizumab, an engineered human IgG4 anti-PD-1 monoclonal antibody, demonstrated clinical benefit as a single agent in patients with GEA[7,8]
- Clinical utilization of two PD-L1 assays, vCPS (with SP263) and CPS (with 22C3), in samples from the GEA cohort of the tislelizumab first-in-human study (BGB-A317-001) was assessed, as well as potential correlations they may have with efficacy
- Information on the development and analytical validation of the VENTANA PD-L1 (SP263) assay, which is intended for detection of PD-L1 expression in formalin-fixed, paraffin-embedded (FFPE) GC or GEJ adenocarcinoma, is also presented

METHODS

PD-L1 Assessment

- PD-L1 expression in tumor samples from GEA patients (BGB-A317-001) were analyzed post-hoc using the methodologies in Table 1

Table 1: Methodology of PD-L1 Expression Assessment

	vCPS	CPS
Assay	VENTANA PD-L1 (SP263) assay on automated VENTANA Benchmark ULTRA® platform	Dako PD-L1 IHC 22C3 assay on Dako Autostainer Link 48
PD-L1 scoring algorithm	Percent area occupied by PD-L1 staining cells (tumor cell, immune cell*) / Tumor area**	Number of PD-L1 staining cells (tumor cell, macrophage, lymphocyte) / Total number of viable tumor cells ×100
Measurement method	Derived by visual estimation of area occupied by PD-L1 staining TC and IC against tumor area	Derived by cell counting

*Immune cells include lymphocytes, macrophage, histocytes, reticular dendritic cells, plasma cells, and neutrophils.
**Tumor area is defined as the area covered by tumor cells and tumor associated stroma.
Abbreviations: CPS, Combined Positive Score; IC, immune cell; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; TC, tumor cell; vCPS, visually-estimated Combined Positive Score.

Statistical Analysis

- Objective response rate (ORR) and the Clopper-Pearson 95% CIs are provided
- The Kaplan-Meier method was used to estimate medians of OS and progression-free survival (PFS) along with the 95% CIs (constructed using Brookmeyer and Crowley method with Log-Log transformation); the reverse Kaplan-Meier method was used to estimate the median follow-up
- Kaplan-Meier curves of PD-L1 subgroups were compared by log-rank test; the hazard ratio was estimated using Cox proportional hazard model

Analytical Validation of VENTANA PD-L1 (SP263) Assay in GC and GEJ Adenocarcinoma

- The VENTANA PD-L1 (SP263) assay was validated for use in GC/GEJ adenocarcinoma FFPE samples in a series of studies that addressed assay repeatability, intermediate precision, reader precision, and inter-laboratory reproducibility

RESULTS

- Of the 81 patients enrolled in BGB-A317-001 GEA cohort, PD-L1 expression was evaluable by vCPS (by VENTANA PD-L1 SP263) and CPS (by Dako 22C3) in 74 and 49 patients with available FFPE tumors, respectively; 45 were evaluable by both assays
  - Patient baseline characteristics and clinical efficacy are shown in Table 2

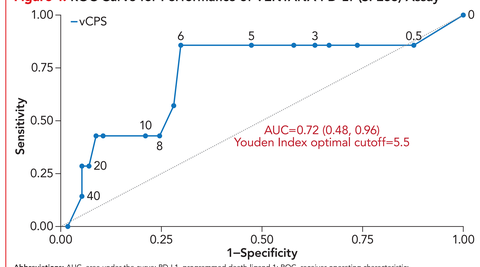
Table 2: Baseline Characteristics and Clinical Outcome

Characteristic		vCPS Evaluable N=74	CPS Evaluable N=49	All GEA Patients N=81
Age, n (%)	<65	45 (60.8)	33 (67.3)	48 (59)
	≥65	29 (39.2)	16 (32.7)	33 (41)
Sex, n (%)	Male	48 (65)	33 (67)	54 (67)
	Female	26 (35)	16 (33)	27 (33)
Tumor type, n (%)	GC/GEJ adenocarcinoma	48 (65)	27 (55)	54 (67)
	EAC	26 (35)	22 (45)	27 (33)
Tumor stage, n (%)	III	4 (5.4)	1 (2.0)	5 (6.2)
	IV	70 (95)	48 (98)	76 (94)
Response, n (%)	PR	7 (9.5)	4 (8.2)	8 (9.9)
	SD	14 (19)	10 (20)	17 (21)
	PD	43 (58)	30 (61)	46 (57)
	NA	1 (1.4)	1 (2.0)	1 (1.2)
ORR, % (95% CI)		10.9 (4.5, 21.2)	9.1 (2.5, 21.7)	11.3 (5, 21)
Median PFS, months (95% CI)		2.0 (1.7, 2.1)	2.0 (1.5, 2.1)	2.0 (1.8, 2.1)
Median OS, months (95% CI)		5.6 (3.9, 6.7)	5.6 (3.8, 8.6)	5.9 (4.2, 9.1)
Median follow-up, months (95% CI)		14.2 (10.9, 21.2)	NE (13.9, NE)	17.4 (13.9, NE)

Abbreviations: CI, confidence interval; CPS, Combined Positive Score; EAC, esophageal adenocarcinoma; GC, gastric cancer; GEJ, gastroesophageal junction; NA, not applicable; NE, not estimable; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD; stable disease; vCPS, visually-estimated Combined Positive Score.

- vCPS 5% was determined as the optimal cutoff based on the receiver operating characteristic analysis depicted in Figure 1, prevalence, and pathological feasibility

Figure 1: ROC Curve for Performance of VENTANA PD-L1 (SP263) Assay

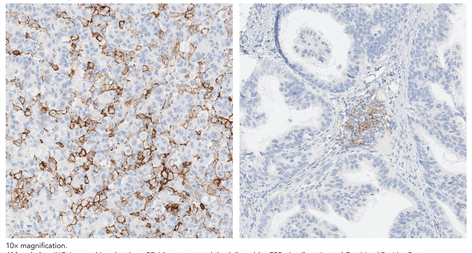


Abbreviations: AUC, area under the curve; PD-L1, programmed death-ligand 1; ROC, receiver operating characteristic; vCPS, visually-estimated combined positive score.

  - Representative photomicrographs of GEA displaying vCPS ≥5% and vCPS <5% are shown in Figure 2

Figure 2: Representative IHC Images of VENTANA PD-L1 (SP263) Staining

A. Tumor Specimens with vCPS ≥5% B. Tumor Specimens with vCPS <5%



10x magnification.
Abbreviation: IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; vCPS, visually-estimated Combined Positive Score.

Clinical Utility of vCPS and CPS In the Tislelizumab First-In-Human Study

- Response, prevalence, positive predictive value (PPV), and negative predictive value (NPV) for vCPS ≥5% and CPS ≥1 are shown in Table 3
  - Enriched ORR was observed in patients with vCPS ≥5% tumors versus vCPS <5% tumors (ORR=18.2% vs 3.2%), which is similar to those using a CPS ≥1 cutoff

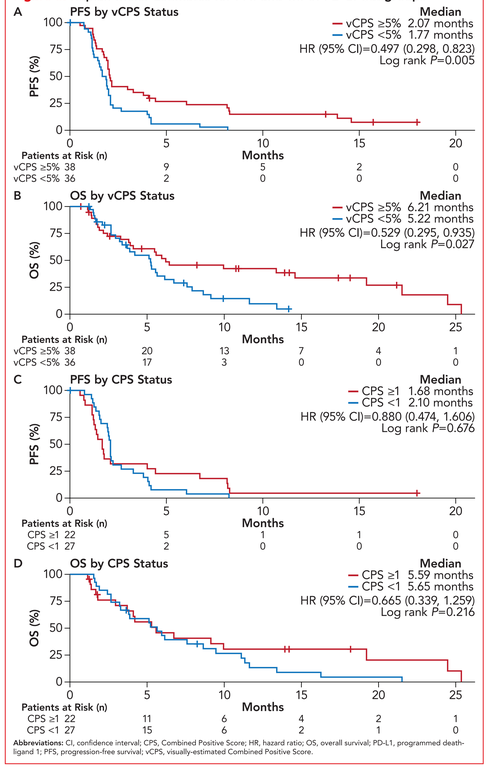
Table 3: Response, Prevalence, and Assay Performance

Scoring Method	PD-L1 Expression	BEP	ORR (%)	PD-L1 Prevalence (%)	Response Odds Ratio	PPV (%)	NPV (%)
vCPS (SP263)	≥5%	38	18.2	51	6.67	15.8	83.3
	<5%	36	3.2				
CPS (22C3)	≥1	22	20.0	45	∞*	18.2	88.9
	<1	27	0				

*Odds ratio could not be estimated due to no responders in CPS <1.
Abbreviations: BEP, biomarker evaluable population; CI, confidence interval; CPS, Combined Positive Score; NPV negative predictive value; ORR, objective response rate; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PPV, positive predictive value; vCPS, visually-estimated Combined Positive Score.

- At a 17.4-month median follow-up, patients with vCPS ≥5% or CPS ≥1 tumors showed survival benefit (Figure 3)
  - More favorable PFS and OS were seen in patients with vCPS ≥5% tumors (PFS HR=0.497, OS HR=0.529) and CPS ≥1 tumors (PFS HR=0.880, OS HR=0.665)

Figure 3: Kaplan-Meier Estimates for PFS and OS of PD-L1 Subgroups



Abbreviations: CI, confidence interval; CPS, Combined Positive Score; HR, hazard ratio; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; vCPS, visually-estimated Combined Positive Score.

Analytical Validation of VENTANA PD-L1 (SP263) Assay in GC and GEJ Adenocarcinoma

- Within-run, between-day repeatability, and intermediate precision (between antibody, detection kit lot, and instrument) for the VENTANA PD-L1 (SP263) assay showed 100% overall percent agreement (OPA) with vCPS in gastric and GEJ adenocarcinoma, respectively (Table 4)

Table 4: Repeatability and Intermediate Precision Studies

Design	Study Outline	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
• 24 GC or GEJ adenocarcinoma cases representing a range of PD-L1 expression levels – 12 with vCPS ≥5% (including 2 borderline cases) – 12 with vCPS <5% (including 2 borderline cases) • One reader evaluated all the cases	**Within-Run Repeatability** (single run on a BenchMark ULTRA instrument)	100% (96.6, 100.0)	100% (96.6, 100.0)	100% (98.3, 100.0)
	Between-Day Repeatability (3 non-consecutive days on the same Benchmark ULTRA instrument)	100% (94.9, 100.0)	100% (94.9, 100.0)	100% (97.4, 100.0)
	Between-Antibody Intermediate Precision (3 lots of PD-L1 SP263 antibody)	100% (94.9, 100.0)	100% (94.9, 100.0)	100% (97.4, 100.0)
	Between-Detection Kit Intermediate Precision (3 lots of OptiView IHC Detection Kit)	100% (94.9, 100.0)	100% (94.9, 100.0)	100% (97.4, 100.0)
	Between-Instrument Intermediate Precision (3 BenchMark ULTRA instruments)	100% (94.9, 100.0)	100% (94.9, 100.0)	100% (97.4, 100.0)

Abbreviations: CI, confidence interval; GC, gastric cancer; GEJ, gastroesophageal junction; NPA, negative predictive value; OPA, overall percent agreement; PD-L1, programmed death-ligand 1; PPA, positive predictive value; vCPS, visually-estimated Combined Positive Score.

- The VENTANA PD-L1 (SP263) assay demonstrated between-reader precision and within-reader precision (OPA) with vCPS of 99.3% and 99%, respectively (Table 5)

Table 5: Between-Reader and Within-Reader Precision Studies

Design	Study Outline	APA (95% CI)	ANA (95% CI)	OPA (95% CI)
• 100 GC or GEJ adenocarcinoma cases representing a range of PD-L1 expression levels – 50 with vCPS ≥5% (including 5 borderline cases) – 50 with vCPS <5% (including 5 borderline cases)	**Between-Reader Precision** (3 readers within 1 assessment)	99.3% (98.0, 100.0)	99.3% (98.0, 100.0)	99.3% (98.0, 100.0)
	Within-Reader Precision (2 assessments with a minimum 2-week wash-out period)	99.0% (98.0, 100.0)	99.0% (98.0, 100.0)	99.0% (98.0, 100.0)

Abbreviations: ANA, average negative agreement; APA, average positive agreement; CI, confidence interval; GC, gastric cancer; GEJ, gastroesophageal junction; OPA, overall percent agreement; PD-L1, programmed death-ligand 1; vCPS, visually-estimated Combined Positive Score.

- Inter-laboratory reproducibility testing, performed across two readers at each of three external laboratories, demonstrated OPA of 95% between readers and 92.5% between sites (Table 6)

Table 6: Inter-laboratory Reproducibility

Design	Study Outline	APA (95% CI)	ANA (95% CI)	OPA (95% CI)
• 28 GC or GEJ adenocarcinoma cases representing a range of PD-L1 expression levels – 14 with vCPS ≥5% (including 2 borderline cases) – 14 with vCPS <5% (including 2 borderline cases) • Each of 3 external clinical sites tested 5 sets of the same cases using the same reagent lots. Each site stained 28 cases with VENTANA PD-L1 (SP263) CDx Assay on BenchMark ULTRA instrument, 1 staining set per day, on each of 5 non-consecutive days, over a period of at least 20 days • 2 qualified readers per site independently read each of the 28 cases	**Between-Reader Precision** (2 readers per site, 3 external sites)	94.6% (90.8, 98.0)	95.3% (91.5, 98.5)	95.0% (91.2, 98.3)
	Between-Site Reproducibility (3 external sites)	92.0% (87.7, 96.3)	92.9% (88.2, 97.0)	92.5% (88.1, 96.6)

Abbreviations: ANA, average negative agreement; APA, average positive agreement; CI, confidence interval; GC, gastric cancer; GEJ, gastroesophageal junction; OPA, overall percent agreement; PD-L1, programmed death-ligand 1; vCPS, visually-estimated Combined Positive Score.

CONCLUSIONS

- vCPS ≥5% stained by the VENTANA PD-L1 (SP263) assay was determined as the optimal cutoff based on statistical analysis, prevalence, and pathological feasibility and was further developed and analytically validated in tumor samples of GC/GEJ adenocarcinoma
- At evaluated cutoffs, both the VENTANA PD-L1 (SP263) assay with vCPS ≥5% and the commercialized Dako 22C3 assay with CPS ≥1 aided in the identification of patients with PD-L1 high tumors who were more likely to gain favorable clinical efficacy than those with PD-L1 low tumors
- The VENTANA PD-L1 (SP263) assay is a robust and reproducible tool for assessing and quantifying PD-L1 expression in GC and GEJ adenocarcinoma
- The reproducibility of the VENTANA PD-L1 (SP263) assay with vCPS by differing pathologists, materials, and laboratories points to the highly trainable nature of the assay as well as its consistency in GC and GEJ adenocarcinoma
- Further clinical validation is underway for vCPS ≥5% in patients with GC and GEJ adenocarcinoma from a phase 3 study designed to compare tislelizumab plus platinum/fluoropyrimidine versus placebo plus platinum/fluoropyrimidine as first-line therapy (RATIONALE 305; BGB-A317-305)

REFERENCES

1. Ajani JA, Lee J, Sano T, Janjigian YY, Fan D, Song S. Gastric adenocarcinoma. *Nat Rev Dis Primers*. 2017;3(17036). doi:10.1038/nrdp.2017.36
2. Rubenstein JH, Shaheen NJ. Epidemiology, diagnosis, and management of esophageal adenocarcinoma. *Gastroenterology*. 2015;149(2):302-317. doi:10.1053/j.gastro.2015.04.053
3. Kang, YK, Satoh T, Ryu MH, et al. Nivolumab (ONO-4538/BMS-936558) as salvage treatment after second or later-line chemotherapy for advanced gastric or gastro-esophageal junction cancer (AGC): A double-blinded, randomized, phase III trial. *J Clin Oncol*. 2017;35(suppl; abstr 2).
4. Fuchs CS, Doi T, Jang RW-J, et al. KEYNOTE-059 cohort 1: Efficacy and safety of pembrolizumab (pembro) monotherapy in patients with previously treated advanced gastric cancer. *J Clin Oncol*. 2017;35(suppl; abstr 4003).
5. Kulangara K, Zhang N, Corigliano E, et al. Clinical utility of the combined positive score for programmed death-ligand 1 expression and the approval of pembrolizumab for treatment of gastric cancer. *Arch Pathol Lab Med*. 2019;143(3):330-337. doi: 10.5858/arpa.2018-0043-OA
6. Kato K, Kojima T, Hochhauser D, et al. Pembrolizumab in previously treated metastatic esophageal cancer: Longer term follow-up from the phase 2 KEYNOTE-180 Study. *J Clin Oncol*. 2019;37(suppl 15):4032.
7. Desai J, Millward M, Chao Y, et al. Preliminary results from subsets of patients (pts) with advance gastric cancer (GC) and esophageal carcinoma (EC) in a dose-escalation/expansion study of BGB-A317, an anti-PD-1 monoclonal antibody (MAB). *Ann Oncol*. 2017;28(suppl 5):v122-v141.
8. Shen L, Yuan Y, Bai Y, et al. Tislelizumab in Chinese patients with esophageal cancer (EC), gastric cancer (GC), hepatocellular carcinoma (HCC), and microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) tumors. Presented at: 22nd Annual Chinese Society for Clinical Oncology Congress; September 18-22, 2019; Xiamen, China.

CONFLICTS OF INTEREST

JD reports a consulting or advisory role for Amgen, BeiGene, Biocon, Eisai, Lilly; Research Funding for his institution from AstraZeneca/MedImmune, BeiGene, Bristol-Myers Squibb, GlaxoSmithKlin, Lilly, Novartis, and Roche. **CL**, **AN**, and **MQ** are employee with stock options at Roche Tissue Diagnostics. **SY**, **YZ**, **ZS**, **XW**, and **JW** are employees with stock options at BeiGene, Ltd. **YC** has nothing to disclose.

ACKNOWLEDGMENTS

The authors wish to acknowledge the investigative centers' study staff and study patients, and to recognize those from BeiGene, Ltd. who have substantially contributed to the development of this presentation. This study was sponsored by BeiGene, Ltd. Writing and editorial assistance was provided by Stephan Lindsey, PhD, and Elizabeth Hermans, PhD (OPEN Health Medical Communications, Chicago, IL), and funded by the study sponsor.

Please address any questions or comments regarding this poster to Clinicaltrials@beigene.com