Exploratory Biomarker Analysis of the Phase 3 RATIONALE-305 Trial: First-Line Tislelizumab + Chemotherapy vs Placebo + Chemotherapy for Advanced Gastric Cancer/Gastroesophageal Junction Adenocarcinoma

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CONCLUSIONS

- Exploratory analyses were performed to identify potentially novel predictive biomarkers in the tumor microenvironment (TME) or tumor genetics using next-generation sequencing in patients with advanced gastric cancer/gastroesophageal junction adenocarcinoma (GC/GEJC)
- From the TME perspective, high cytotoxic T-cell (CTL) and low neutrophil gene expression signatures were associated with improved overall survival (OS) with first-line tislelizumab plus chemotherapy compared with placebo plus chemotherapy in patients with advanced GC/GEJC
- From the tumor intrinsic genomic alterations perspective, increasing clonal tumor mutational burden (cTMB), certain human leukocyte antigen (HLA) genotypes, TP53 wild-type status, ARID1A and NDUFS7 mutations, and absence of 20q13.13 and 11q13.2 amplification were also linked to greater OS benefit in patients receiving tislelizumab plus chemotherapy vs placebo plus chemotherapy for advanced GC/GEJC
- A novel genetic scoring system, based on genomic alterations, stratified patient subtypes and demonstrated a strong correlation with OS, with patients harboring favorable biomarkers experiencing significantly greater survival benefit compared with those with risk-associated signatures
- These findings deepen our understanding of the biological mechanisms underlying the activity of anti–programmed cell death protein-1 (PD-1) antibodies plus chemotherapy and provide actionable insights to guide individualised therapeutic strategies for GC in clinical practice

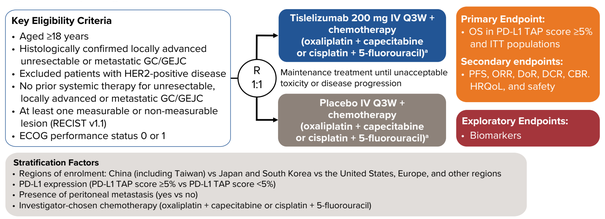
INTRODUCTION

- Advanced GC/GEJC poses significant treatment challenges, with anti–PD-1 antibodies plus chemotherapy as the current standard first-line therapy for human epidermal growth factor receptor 2 (HER2)-negative cases[1-4]
- Variability in patient responses highlights the need for robust biomarkers beyond programmed death-ligand 1 (PD-L1), TMB, and microsatellite instability/deficient mismatch repair status[5]
- Tislelizumab, an anti–PD-1 antibody, showed improved OS when combined with chemotherapy compared with placebo plus chemotherapy in the RATIONALE-305 trial in patients with advanced HER2-negative GC/GEJC (median OS 15.0 vs 12.9 months; HR=0.80; 95% CI: 0.70, 0.92; *P*=.001)[6-8]
- Here we report the comprehensive exploratory biomarker analysis from the RATIONALE-305 trial investigating TME, genomic alterations, and immune-related factors that influence treatment outcomes

METHODS

- RATIONALE-305 (NCT03777657) was a global, randomized, double-blind phase 3 trial of tislelizumab plus chemotherapy vs placebo plus chemotherapy in the first-line setting in patients with HER2-negative advanced GC/GEJC (**Figure 1**)
- Biomarker analysis of baseline tumor samples included sequential assessment of RNA-seq gene expression profiling (GEP) for TME characterization and whole-exome sequencing (WES) of tumor tissue and matching blood samples for TMB, HLA genotyping, significantly mutated gene (SMG) identification, cytoband amplification detection, and genetic subtyping analysis using a novel genetic scoring system of favorable biomarkers (including Epstein-Barr virus [EBV] positivity[9]) and risk factors
- Multiplex immunofluorescence was employed as a validation assay. A five-plex immunofluorescence panel was used to validate immune cell infiltration findings using baseline tumor samples from patients in this study
- The Kaplan–Meier method was used to estimate median OS with 95% confidence intervals (CIs), and the log-rank test was used to evaluate OS differences between treatment arms. Cox proportional hazards models were used to determine hazard ratios (HRs) with 95% CIs. Interaction between biomarkers and treatment was assessed using Cox models, and the Wald test was used to evaluate statistical significance. The Wilcoxon rank-sum test was used to compare the medians of continuous variables between the two treatment groups. P<.15 was considered significant for interaction effects. All P-values are descriptive

Figure 1. Study Design



[a]Chemotherapy: oxaliplatin 130 mg/m^2 IV day 1 + oral capecitabine 1000 mg/m^2 BID, days 1-14, Q3W (CAPOX); cisplatin 80 mg/m^2 IV day 1 + 5-fluorouracil 800 mg·m^2/day IV, days 1-5, Q3W. Capecitabine as continuation therapy was optional and only for CAPOX-treated patients. PD-L1 score was determined using the VENTANA PD-L1 (SP263) Assay by TAP score.

Abbreviations: BID, twice daily; CBR, clinical benefit rate; DCR, disease control rate; DoR, duration of response; ECOG, Eastern Cooperative Oncology Group; HRQoL, health-related quality of life; IV, intravenous; ITT, intent-to-treat; ORR, objective response rate; PFS, progression-free survival; Q3W, once every 3 weeks; R, randomized; RECIST, Response Evaluation Criteria in Solid Tumors; TAP, Tumor Area Positivity.

RESULTS

Biomarker-Evaluable Population and Baseline Characteristics

- Baseline tumor tissue samples from the RATIONALE-305 trial (N=997; tislelizumab + chemotherapy: n=501; placebo + chemotherapy: n=496) underwent comprehensive profiling with RNA-seq GEP (tislelizumab + chemotherapy: n=242; placebo + chemotherapy: n=265) and WES (tislelizumab + chemotherapy: n=101; placebo + chemotherapy: n=134)
- Baseline characteristics across treatment arms were generally comparable between GEP or WES biomarker-evaluable populations (BEPs) and the ITT population (data not shown)

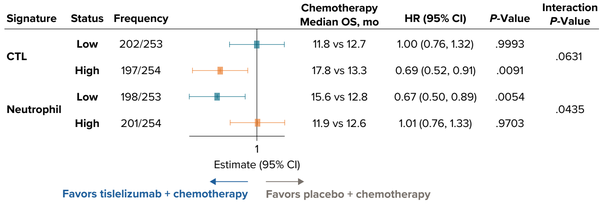
Analysis by TME Features

- High expression of CTL signatures was associated with improved OS for tislelizumab plus chemotherapy vs placebo plus chemotherapy (interaction *P*-value = .0631), while lower expression of neutrophil signatures was associated with improved OS benefit from tislelizumab plus chemotherapy vs placebo plus chemotherapy (interaction *P*-value = .0435) (**Figure 2A**)
- Immunofluorescence validation confirmed that patients within the tislelizumab plus chemotherapy arm with higher baseline intratumoural interferon gamma (IFNγ)+CD8+ T-cells correlated with a trend toward improved OS compared with those with lower IFNγ+CD8+ T-cells (**Figure 2B-2C**)

Figure 2. TME Markers Associated With OS Benefit of Tislelizumab Plus Chemotherapy in GC/GEJC. (A) Forest Plot of OS by Immune Signatures, (B) Multiplex Immunofluorescence of IFNγ+CD8+ T-Cells High vs Low, and (C) Kaplan–Meier Plot of OS by IFNγ+CD8+ T-Cell Infiltration

A

Signature	Status	Frequency	Tislelizumab + Chemotherapy vs Placebo + Chemotherapy Median OS, mo	HR (95% CI)	*P*-Value	Interaction *P*-Value
CTL	Low	202/253	11.8 vs 12.7	1.00 (0.76, 1.32)	.9993	.0631
	High	197/254	17.8 vs 13.3	0.69 (0.52, 0.91)	.0091	
Neutrophil	Low	198/253	15.6 vs 12.8	0.67 (0.50, 0.89)	.0054	.0435
	High	201/254	11.9 vs 12.6	1.01 (0.76, 1.33)	.9703	



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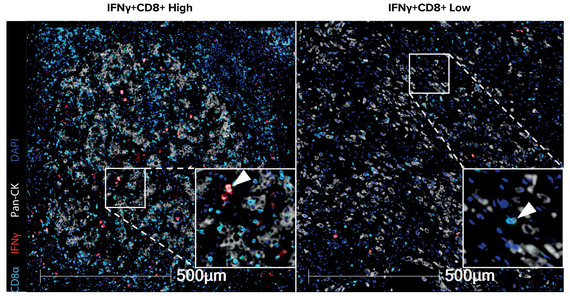

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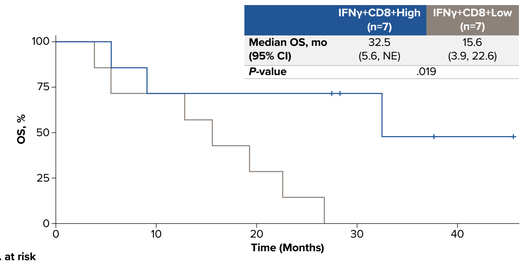


	IFNγ+CD8+High (n=7)	IFNγ+CD8+Low (n=7)
Median OS, mo (95% CI)	32.5 (5.6, NE)	15.6 (3.9, 22.6)
P-value	.019	

No. at risk					
IFNγ +CD8+ high	7	5	5	3	1
IFNγ+CD8+ low	7	5	2	0	0

Representative images of multiplex immunofluorescence staining from IFNγ+CD8+ T-cell high vs low and Kaplan–Meier plot of OS stratified by median intratumoral IFNγ+CD8+ T-cell density. High vs low used median cutoff of GEP BEPs. Scale bars, 500 μm of the main images (N=14).

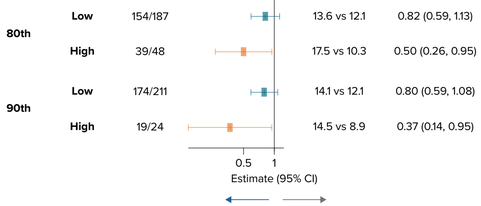
Abbreviations: IFNγ, interferon gamma; mo, months; NE, not estimable.

Analysis by cTMB and HLA Class I Genotype

- Although total TMB was not associated with OS benefit (data not shown), increasing cTMB cutoffs correlated with greater OS benefit from tislelizumab plus chemotherapy vs placebo plus chemotherapy, peaking at the 90th percentile cutoff (**Figure 3**)
  - cTMB is the number of clonal non-synonymous mutations present in all tumor cells and represents those mutations less likely to be eliminated during immune editing

Figure 3. OS With Tislelizumab Plus Chemotherapy vs Placebo Plus Chemotherapy in Patients With cTMB High vs Low at Different Cutoffs

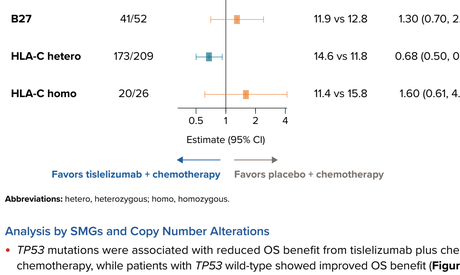
cTMB Cutoff	Status	Frequency	Tislelizumab + Chemotherapy vs Placebo + Chemotherapy Median OS, mo	HR (95% CI)	*P*-Value	Interaction *P*-Value
60th	Low	112/139	12.6 vs 12.3	0.85 (0.58, 1.24)	.3940	.2469
	High	81/96	16.2 vs 11.8	0.60 (0.39, 0.94)	.0240	
70th	Low	133/164	13.4 vs 11.8	0.74 (0.52, 1.05)	.0954	.8910
	High	60/71	14.9 vs 11.8	0.71 (0.43, 1.18)	.1865	
80th	Low	154/187	13.6 vs 12.1	0.82 (0.59, 1.13)	.2180	.1779
	High	39/48	17.5 vs 10.3	0.50 (0.26, 0.95)	.0337	
90th	Low	174/211	14.1 vs 12.1	0.80 (0.59, 1.08)	.1440	.1247
	High	19/24	14.5 vs 8.9	0.37 (0.14, 0.95)	.0379	



- HLA Class I genotype B62 supertype was associated with improved OS benefit, while B27 supertype and HLA-C homozygosity correlated with reduced OS benefit with tislelizumab plus chemotherapy vs placebo plus chemotherapy (**Figure 4**)

Figure 4. Correlation of HLA Genotypes With OS Benefit of Tislelizumab Plus Chemotherapy vs Placebo Plus Chemotherapy

Group	Frequency	Tislelizumab + Chemotherapy vs Placebo + Chemotherapy Median OS, mo	HR (95% CI)	*P*-Value	Interaction *P*-Value
non-B62	30/157	12.5 vs 12.1	0.89 (0.63, 1.26)	.5123	.1128
B62	63/78	18.6 vs 11.8	0.54 (0.33, 0.90)	.0176	
Non-B27	152/183	15.5 vs 11.1	0.64 (0.46, 0.89)	.0072	.0454
B27	41/52	11.9 vs 12.8	1.30 (0.70, 2.41)	.4012	
HLA-C hetero	173/209	14.6 vs 11.8	0.68 (0.50, 0.92)	.0124	.0951
HLA-C homo	20/26	11.4 vs 15.8	1.60 (0.61, 4.16)	.3375	



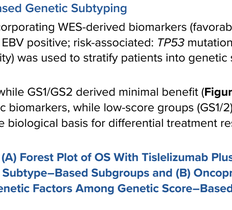
Abbreviations: hetero, heterozygous; homo, homozygous.

Analysis by SMGs and Copy Number Alterations

- *TP53* mutations were associated with reduced OS benefit from tislelizumab plus chemotherapy vs placebo plus chemotherapy, while patients with *TP53* wild-type showed improved OS benefit (**Figure 5**)
- Patients with *ARID1A* or *NDUFS7* mutations showed improved OS benefit with tislelizumab plus chemotherapy vs placebo plus chemotherapy compared with those with wild-type status for these genes
- Amplification of 20q13.13 or 11q13.2 was associated with reduced OS benefit from tislelizumab plus chemotherapy vs placebo plus chemotherapy compared to the respective non-amplified loci

Figure 5. OS Analysis by SMGs or Copy Number Alterations With ≥10% Frequency

Gene	Status	Frequency	Tislelizumab + Chemotherapy vs Placebo + Chemotherapy Median OS, mo	HR (95% CI)	*P*-Value	Interaction *P*-Value
TP53	Wild-type	69/84	17.0 vs 10.7	0.48 (0.29, 0.78)	.0033	.0245
	Mutation	121/148	13.4 vs 12.3	0.96 (0.67, 1.38)	.8246	
ARID1A	Wild-type	166/200	13.4 vs 12.3	0.84 (0.62, 1.14)	.2697	.1027
	Mutation	21/28	18.9 vs 8.0	0.39 (0.17, 0.93)	.0336	
NDUFS7	Wild-type	150/182	12.8 vs 12.3	0.89 (0.64, 1.23)	.4818	.0653
	Mutation	23/28	16.0 vs 7.2	0.39 (0.17, 0.88)	.0243	
20q13.13	Non-amplification	168/205	16.0 vs 11.8	0.64 (0.47, 0.88)	.0052	.0017
	Amplification	25/30	10.0 vs 12.1	2.58 (1.15, 5.79)	.0211	
11q13.2	Non-amplification	170/208	14.9 vs 12.1	0.70 (0.52, 0.96)	.0255	.1285
	Amplification	23/27	5.6 vs 10.7	1.41 (0.61, 3.26)	.4233	



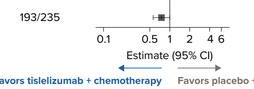
Analysis by Genomic Alteration–Based Genetic Subtyping

- A novel genetic scoring system incorporating WES-derived biomarkers (favorable: cTMB-high, *ARID1A* mutation, *NDUFS7* mutation, B62 supertype, EBV positive; risk-associated: *TP53* mutation, 20q13.13/11q13.2 amplifications, B27 supertype, HLA-C homozygosity) was used to stratify patients into genetic subtypes (GS1-GS4), with varying treatment benefit
- GS4 showed greatest OS benefit, while GS1/GS2 derived minimal benefit (**Figure 6A**); high-score groups (GS3/4) were enriched for favorable genetic biomarkers, while low-score groups (GS1/2) harbored risk-associated features (**Figure 6B**), confirming the biological basis for differential treatment response

Figure 6. Genetic Subtype Analysis. (A) Forest Plot of OS With Tislelizumab Plus Chemotherapy vs Placebo Plus Chemotherapy Among Genetic Subtype–Based Subgroups and (B) Oncoprint Showing the Distribution of Favorable and Risk-Associated Genetic Factors Among Genetic Score–Based Subtypes

A

Genetic Subtype	Frequency	Tislelizumab + Chemotherapy vs Placebo + Chemotherapy Median OS, mo	HR (95% CI)	*P*-Value
GS1	45/59	11.4 vs 12.9	2.32 (1.26, 4.27)	.007
GS2	53/59	10.1 vs 12.1	0.90 (0.52, 1.56)	.712
GS3	60/71	16.2 vs 9.1	0.53 (0.32, 0.89)	.017
GS4	35/46	22.5 vs 8.1	0.27 (0.13, 0.53)	<.001
All	193/235	14.1 vs 11.8	0.75 (0.56, 1.00)	.047



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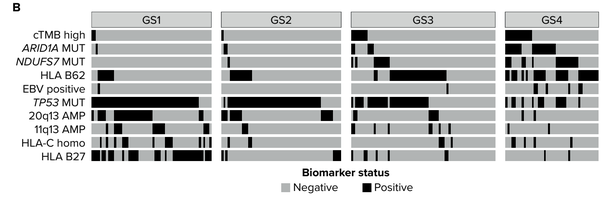

High vs low cTMB was determined using the 90th percentile cutoff.

Abbreviations: AMP, amplification; MUT, mutation.

REFERENCES

1. Zhan T, et al. *Signal Transduct Target Ther.* 2025;10:24.
2. Filho AM, et al. *Int J Cancer.* 2025;156:1336-1346.
3. Obermannová R, et al. *Ann Oncol.* 2022;33:992-1004.
4. Lordick F, et al. *Ann Oncol.* 2022;33:1005-1020.
5. Lefler DS, et al. *Immunotherapy.* 2022;14:885-902.
6. Hong Y, et al. *FEBS Open Bio.* 2021;11:782-792.
7. Zhang T, et al. *Cancer Immunol Immunother.* 2018;67:1079-1090.
8. Qiu MZ, et al. *BMJ.* 2024;385:e078876.
9. Kim ST, et al. *Nat Med.* 2018;24:1449-1458.

DISCLOSURES

KW, **JS**, **YS**, **RH**, **DSS:** Employment with, and may own stocks or shares in, BeOne Medicines, Ltd. **D-YO:** Consulting or advisory role for AbbVie, Alligator Bioscience AB, Arcus Biosciences, ASLAN Pharmaceuticals, Astellas Pharma, AstraZeneca, Basilea, Bayer, BeOne Medicines, Ltd., Celgene, Eutilex, Genentech/Roche, Halozyme, Hana Pharm, Idience, IQVIA, J-Pharma, LG Chem, Merck Serono, Mirati Therapeutics, Moderna Therapeutics, MSD Oncology, Novartis, Taiho Pharmaceutical, Turning Point Therapeutics, Yuhan, and Zymeworks; Research funding from Array BioPharma, AstraZeneca, BeOne Medicines, Ltd., Handok, Lilly, MSD, Novartis, and Servier Laboratories. **M-ZQ**, **ML**, **JY**, **YB**, **R-HX**: No disclosures.

ACKNOWLEDGMENTS

The authors thank the patients and their families, investigators, co-investigators, and the study teams at each of the participating centers. This study was sponsored by BeOne Medicines, Ltd. The authors acknowledge the valuable bioinformatics guidance provided by Pei Zhang, PhD, Director of Bioinformatics at BeOne Medicines. Medical writing support was provided by Nitya Venkataraman, PhD, of Parexel, and supported by BeOne Medicines.

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Presented at AACR, April 17-22, 2026, San Diego, CA, USA