POTENTIAL MECHANISMS OF RESISTANCE IDENTIFIED THROUGH ANALYSIS OF MULTIPLE BIOMARKERS IN IMMUNE-HOT NON-RESPONDERS WITH NON-SMALL CELL LUNG CANCER TREATED WITH TISLELIZUMAB

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Poster: 76
Society for Immunotherapy of Cancer
November 9-14, 2020, Virtual Congress

BACKGROUND

- Programmed cell death protein-1/programmed death-ligand 1 (PD-1/PD-L1) inhibitors have demonstrated clinical benefit and are approved for first- and second-line treatment of advanced stage non-small cell lung cancer (NSCLC)[1]
- High PD-L1 expression on tumor cells, an indicator of an inflamed tumor microenvironment, is associated with improved clinical benefit with anti-PD-(L)1 therapy[1]
  - However, a subset of patients with inflamed tumor microenvironments do not respond
  - The resistance mechanism for such patients needs to be explored
- Tislelizumab has also demonstrated clinical benefit in patients with NSCLC as a single agent (NCT02407990 and NCT04068519)[2,3] and in combination with chemotherapy (RATIONALE 307 [NCT03594747], RATIONALE 304 [NCT03663205])[4,5]
- Here, we analyzed the immune- and tumor-intrinsic gene expression signature profiles (GEPs) and gene mutation status of NSCLC patients treated with tislelizumab monotherapy to explore potential response and resistance mechanisms, especially for patients with inflamed tumor microenvironments

METHODS

Study Design

- Pooled analysis from two clinical trials
  - BGB-A317-001 (NCT02407990): First-in-human, multicenter, phase 1a/1b dose-escalation/ indication-expansion study
    - Samples were analyzed from patients with advanced or metastatic NSCLC
  - BGB-A317-102 (NCT04068519): Chinese, multicenter, phase 1/2 study
    - Samples were analyzed from patients with advanced or metastatic NSCLC

Gene Expression and Mutation Profiling

- Gene expression profiling using the HTG EdgeSeq Precision Immuno-Oncology Panel and next generation sequencing for genetic mutations was performed on baseline tumor samples (formalin-fixed, paraffin-embedded blocks or cut slides)
- Signature scores were calculated using the Gene Set Variation Analysis package with publicly available gene signatures
- Non-responder subgroups were hierarchically clustered by Euclidean distance metrics with average linkage by columns
- Differentially expressed gene signature analysis was performed between responders (Rs) and non-responders (NRs) using a Wilcoxon rank-sum test

Statistical Analysis

- Statistical significance was tested using a two-sided Wilcoxon test
- Survival difference among subgroups were tested using a log-rank test

RESULTS

- Baseline characteristics and clinical outcome of overall and GEP-evaluable NSCLC patients are shown in Table 1

Table 1: Baseline Characteristics and Clinical Outcome of Overall and GEP-Evaluable Patients

		NSCLC		NSQ		SQ	
		Overall (n=105)	GEP (n=52)	Overall (n=59)	GEP (n=26)	Overall (n=41)	GEP (n=24)
Age ≥65 years, n (%)		35 (33.3)	16 (30.8)	20 (33.9)	6 (23.1)	14 (34.1)	9 (37.5)
Male, n (%)		67 (63.8)	36 (69.2)	28 (47.5)	13 (50.0)	35 (85.4)	21 (87.5)
ECOG PS=1, n (%)		82 (78.1)	38 (73.1)	45 (76.3)	17 (65.4)	33 (80.5)	19 (79.2)
Smoking, n (%)	Current	9 (8.6)	6 (11.5)	4 (6.8)	3 (11.5)	4 (9.8)	2 (8.3)
	Former	58 (55.2)	28 (53.8)	26 (44.1)	9 (34.6)	28 (68.3)	18 (75.0)
	Never	38 (36.2)	18 (34.6)	29 (49.2)	14 (53.8)	9 (22.0)	4 (16.7)
Brain metastasis, n (%)	No	50 (47.6)	37 (71.2)	24 (40.7)	18 (69.2)	26 (63.4)	19 (79.2)
	Yes	9 (8.6)	10 (19.2)	7 (11.9)	5 (19.2)	1 (2.4)	4 (16.7)
	Not evaluable	46 (43.8)	5 (9.6)	28 (47.5)	3 (11.5)	14 (34.1)	1 (4.2)
Liver metastasis, n (%)	No	41 (39.0)	32 (61.5)	19 (32.2)	15 (57.7)	22 (53.7)	17 (70.8)
	Yes	15 (14.3)	11 (21.2)	10 (16.9)	5 (19.2)	5 (12.2)	4 (16.7)
	Not evaluable	49 (46.7)	9 (17.3)	30 (50.8)	6 (23.1)	14 (34.1)	3 (12.5)
Race, n (%)	Non-Asian	28 (26.7)	10 (19.2)	16 (27.1)	4 (15.4)	9 (22.0)	4 (16.7)
	Asian	77 (73.3)	42 (80.8)	43 (72.9)	22 (84.6)	32 (78.0)	20 (83.3)
Prior lines of therapy*, n (%)	1	6 (5.7)	2 (3.8)	4 (6.8)	2 (7.7)	2 (4.9)	0 (0.0)
	2	43 (41.0)	22 (42.3)	22 (37.3)	7 (26.9)	19 (46.3)	13 (54.2)
	≥3	56 (53.3)	28 (53.8)	33 (55.9)	17 (65.4)	20 (48.8)	11 (45.8)
Median PFS, months (95% CI)		4.07 (2.20, 6.11)	4.12 (2.17, 8.18)	3.94 (2.10, 6.18)	3.55 (2.04, 12.29)	4.57 (2.14, 8.18)	4.17 (2.04, 10.45)
Median OS, months (95% CI)		15.54 (11.00, 32.76)	21.62 (10.09, NE)	32.75 (9.89, NE)	NE	12.48 (8.31, 22.14)	12.48 (6.18, 25.00)
ORR, % (95% CI)		15.24 (8.97, 23.56)	21.15 (11.06, 34.70)	13.56 (6.04, 24.98)	23.08 (8.97, 43.65)	19.51 (8.82, 34.87)	20.83 (7.13, 4.22)

Median follow-up time was 15.3 months (95% CI: 14.06, 15.90).
*Prior lines of therapy were manually reviewed and defined from BGB-A317-001 and BGB-A317-102 studies. BGB-A317-001 study defined therapy used for metastatic, locally advanced, or palliative as a line of systemic therapy. BGB-A317-102 study defined adjuvant or neoadjuvant therapy as a line of systemic therapy in 8 patients.
Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; GEP, gene expression profile; NE, not evaluable; NSCLC, non-small cell lung cancer; NSQ, nonsquamous non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SQ, squamous non-small cell lung cancer.

- Gene expression signatures representing immune infiltration status and tumor-intrinsic factors (Table 2) were utilized to give a comprehensive profile of the tumor and to explore potential response and resistance mechanisms

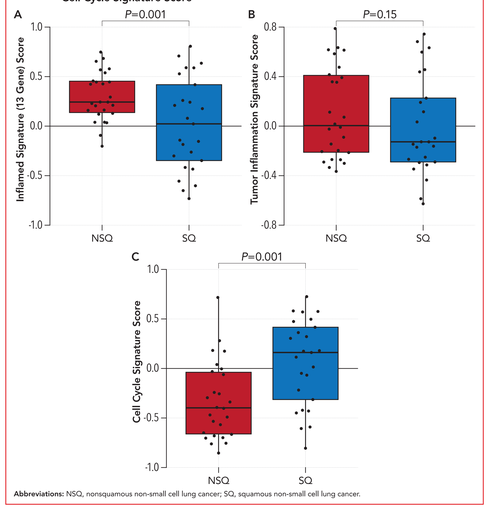
Table 2: Tumor and Immune Gene Signatures

Potential Response Mechanisms: Pre-Existing Tumor Immunity			Potential Resistance Mechanisms: Immunosuppressive and Tumor Intrinsic Factors		
Interferon Signaling	Tumor Reactive T Cell	Immunity Cycle	Inhibitory Immune Cells	Proliferation	Key Features of Tumor
IFNγ sig	CD8 T cell	Antigen presentation sig	Macrophage	DNA repair sig	CAF
Tumor inflammation sig[6]	Exhausted CD8	Trafficking and infiltration sig	Mast cell	Cell cycle sig[8]	NFκB sig
Inflamed sig (13 gene)[7]	T cell	Dendritic cell	M1 macrophage	G1_S sig	EMT mesenchymal sig
	Exhausted T cell		M2 macrophage	G2_M sig	Hypoxia sig
			Th2 cell		TGFβ sig
			Th17 cell		Cell adhesion sig
			Treg		Angiogenesis sig

Abbreviations: CAF, cancer-associated fibroblast; EMT, epithelial-mesenchymal transition; IFNγ, interferon gamma; NFκB, nuclear factor kappa B; sig, signature; TGFβ, transforming growth factor beta; Th, T helper; Treg, regulatory T cell.

- The immune- and tumor-intrinsic GEPs in nonsquamous NSCLC (NSQ) and squamous NSCLC (SQ) were analyzed first
- NSQ showed significantly higher inflamed signature score and a lower cell cycle signature score than SQ (Figure 1), indicating different immune- and tumor-intrinsic characteristics of NSQ and SQ; therefore, NSQ and SQ were analyzed separately to explore their response and resistance mechanisms

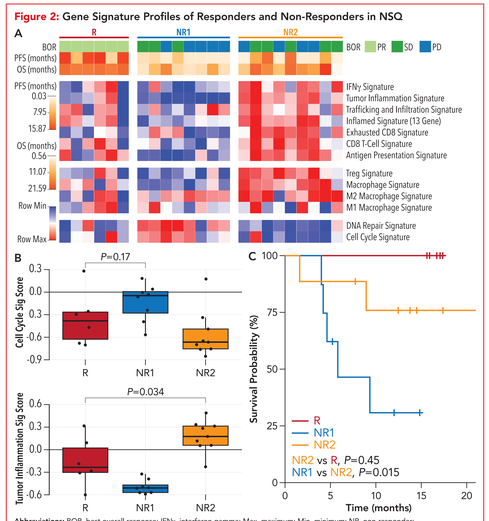
Figure 1: NSQ Showed a Higher Inflamed Signature Score and SQ Featured a Higher Cell Cycle Signature Score



Abbreviations: NSQ, nonsquamous non-small cell lung cancer; SQ, squamous non-small cell lung cancer.

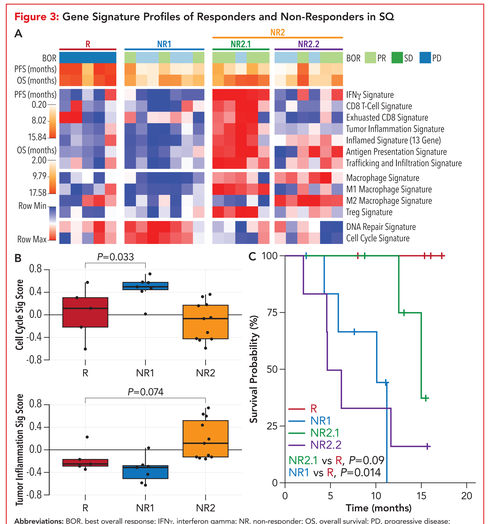
- In both NSQ and SQ, NRs could be clustered into two subgroups (NR1 and NR2; Figure 2 and Figure 3) with different immune- and tumor-intrinsic GEPs
  - Compared with Rs, NR1 had a trend of elevated cell cycle signature scores (Figure 2B and Figure 3B), and a trend of decreased inflamed gene signature profiles
  - However, NR2 showed even higher tumor inflammation signature scores and could be classified as immune hot (Figure 2B and Figure 3B)
    - A subset of NR2 patients in SQ were especially immune hot (NR2.1; Figure 3A)
- In NSQ, immune-hot NR2 showed comparable overall survival (OS) with Rs (P=0.45) and longer than NR1 (P=0.015); in SQ, NR2.1 tended to have longer OS compared with NR1 (Figure 2C and Figure 3C)

Figure 2: Gene Signature Profiles of Responders and Non-Responders in NSQ



Abbreviations: BOR, best overall response; IFNγ, interferon gamma; Max, maximum; Min, minimum; NR, non-responder; NSQ, nonsquamous non-small cell lung cancer, OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; R, responder; SD, stable disease; Sig, signature; Treg, regulatory T cell.

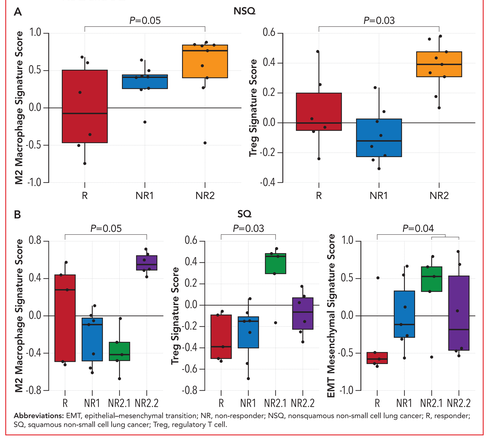
Figure 3: Gene Signature Profiles of Responders and Non-Responders in SQ



Abbreviations: BOR, best overall response; IFNγ, interferon gamma; NR, non-responder; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; R, responder; Sig, signature; SQ, squamous non-small cell lung cancer; Treg, regulatory T cell.

- Immune- and tumor-intrinsic gene expression signatures were analyzed to explore the potential resistance mechanisms of immune-hot NRs (NR2) in the NSQ and SQ cohorts
- Higher M2 macrophage and Treg signature scores were found in immune-hot NRs in NSQ and also in a subset of NR2 patients in SQ (P≤0.05; Figure 4A and Figure 4B)
  - Higher epithelial–mesenchymal transition (EMT) mesenchymal signature scores were found in NRs in SQ, but not in NSQ (Figure 4B), implying the tumor-intrinsic factors may also be related to the resistance to immunotherapy in SQ

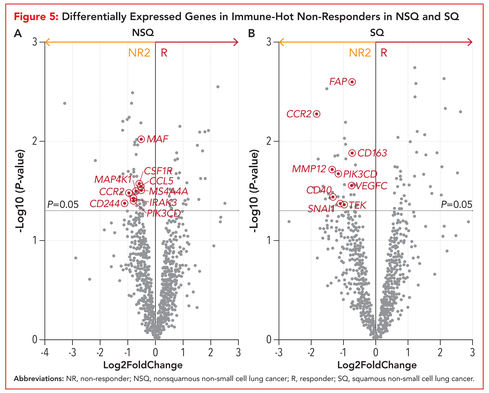
Figure 4: Differentially Expressed Gene Signatures in Immune-Hot Non-Responders (NR2) in NSQ and SQ



Abbreviations: EMT, epithelial–mesenchymal transition; NR, non-responder; NSQ, nonsquamous non-small cell lung cancer; R, responder; SQ, squamous non-small cell lung cancer; Treg, regulatory T cell.

- Differentially expressed genes were further analyzed in immune-hot NRs in the NSQ and SQ cohorts
  - Significantly higher expression of immune regulatory genes included *PIK3CD, IRAK3,* and *MAP4K1* (P<0.05), regarded as potential cancer targets, were found in immune-hot NRs (NR2) in NSQ; macrophage-related genes, including *CSF1R, CCR2, CCL5, CD244, MAF,* and *MS4A4A* (P<0.05), were also highly expressed in NSQ NR2 (Figure 5A) and could also be potential drug targets
  - For SQ, *PIK3CD, CCR2, CD40, CD163,* and *MMP12* (P<0.05) showed higher expression in the immune-hot NRs; moreover, significantly higher EMT and angiogenesis gene expression, including *SNAI1, FAP, VEGFC,* and *TEK* (P<0.05) genes, were also observed in SQ (Figure 5B)
  - High expression of macrophage-related genes in NSQ and SQ NR2 and EMT-related genes in SQ NR2 is in concordance with signature analysis results (Figure 4)

Figure 5: Differentially Expressed Genes in Immune-Hot Non-Responders in NSQ and SQ



Abbreviations: NR, non-responder; NSQ, nonsquamous non-small cell lung cancer; R, responder; SQ, squamous non-small cell lung cancer.

CONCLUSIONS

- In this study, we analyzed multiple immune- and tumor-intrinsic gene expression signatures and identified signatures associated with resistance to tislelizumab monotherapy in NSCLC
  - A subgroup of tislelizumab NRs (NR2) were characterized as immune-hot with high tumor inflammation signature scores in both NSQ and SQ cohorts
  - M2 macrophage and Treg signatures, as well as negative immune regulation genes, were highly expressed in immune-hot NRs in both NSQ and SQ cohorts, reflecting that the immune suppressive microenvironment might be related to the resistance mechanism
    - For SQ, multiple tumor-intrinsic factors, including EMT and angiogenesis-related genes may associate with resistance
    - Moreover, the existence of driver/resistance mutations in immune-hot NRs may indicate resistance
- This resistance mechanism would suggest there are potential advantages to combinatorial immunotherapy strategies
- Due to the limited GEP-evaluable population in the NSCLC patients from these two studies, results may be biased, therefore resistance mechanisms will be further explored and validated in ongoing tislelizumab phase 3 studies in NSCLC (BGB-A317-307, RATIONALE 307 [NCT03594747]; BGB-A317-304, RATIONALE 304 [NCT03663205]; BGB-A317-303, RATIONALE 303 [NCT03358875])

- Gene mutations were also investigated in evaluable patients (8/20 in immune-hot NRs) to explore their potential role in resistance to immunotherapy (Table 3)
  - In NSQ, 4/5 genetic mutation-evaluable immune-hot NRs had resistance (*JAK2* and *STK11* loss of function mutation) or driver mutations (*RET* and *ROS1* fusion)
  - In SQ, all the three genetic mutation-evaluable immune-hot NRs had resistance (*MDM2* amplification) or driver mutations (*FGFR* amplification, *PIK3CA* amplification concurrent with *BRAF*-activating mutation)
  - Such driver or resistance mutations were not found in Rs

Table 3: Resistance and Driver Mutations Were Found in Immune-Hot Non-Responders

	Histology	Gene	Mutation	TMB (mut/Mb)	BOR
Immune-hot NRs in NSQ	NSQ	*JAK2*	p.V441L	23.93	SD
	NSQ	*STK11*	p.D194Y	2.99	PD
	NSQ	*RET*	CCDC6-RET fusion	1	SD
	NSQ	*ROS1*	CD74-ROS1 fusion	1.99	PD
Immune-hot NRs in SQ	SQ	*MDM2*	amplification	8.83	SD
	SQ	*PIK3CA*	amplification	1.99	PD
	SQ	*FGFR1*	amplification	4.99	PD
		BRAF	p.L597R p.T589S		

Abbreviations: BOR, best overall response; Mb, megabase; mut, mutation; NRs, non-responders; NSQ, nonsquamous non-small cell lung cancer; PD, progressive disease; SD, stable disease; SQ, squamous non-small cell lung cancer; TMB, tumor mutational burden.

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ACKNOWLEDGMENTS

The authors wish to acknowledge the investigative center study staff, the study patients, and their families. BeiGene, Ltd. provided financial support for this presentation, including editorial assistance by Agnieszka Laskowski, PhD, and Elizabeth Hermans, PhD (OPEN Health Medical Communications, Chicago, IL).

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