



P2.245: Development and Validation of an Immunohistochemistry Scoring Method for BTN1A1 Expression in NSCLC

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INTRODUCTION

- Butyrophilin subfamily 1 member A1 (BTN1A1) is an emerging immune checkpoint that suppresses $\alpha\beta$ T-cell-mediated antitumor immunity and represents a potential therapeutic target distinct from the PD-1/PD-L1 axis. Previous studies have suggested that BTN1A1 may show an expression pattern distinct from PD-L1, raising the possibility that BTN1A1 could identify a tumor population not captured by conventional PD-L1 testing.
- As BTN1A1-targeted therapies enter clinical development, a practical and reproducible method for evaluating BTN1A1 expression by immunohistochemistry (IHC) is needed. However, the distribution and patterns of BTN1A1 expression in NSCLC and the optimal approach to its IHC assessment remain incompletely characterized.
- As a pilot study, we characterized BTN1A1 IHC expression patterns in NSCLC and descriptively explored their variation across selected clinical contexts and in relation to PD-L1 expression, providing a basis for subsequent larger-scale validation studies.

METHODS

Design

Retrospective, single-centre, matched-pair pilot study. Twenty patients with non-small cell lung cancer (Adenocarcinoma), each contributed two formalin-fixed, paraffin-embedded specimens — the primary tumour at initial diagnosis and a subsequent recurrent or metastatic lesion — giving 40 matched specimens.

Cohort

All 20 tumours were adenocarcinoma. Stage at diagnosis: II 7 (35%), IIIA 8 (40%), IIIB 2 (10%), IV 3 (15%). Drivers: EGFR exon 19 deletion 2, EGFR L858R 3, KRAS G12C 1, none identified 14 (70%). Second specimens: lung 7, lymph node 5, brain 4, pleura 2, skin 1, adrenal gland 1. WT for EGFR, ALK, ROS1, BRAF, KRAS, RET, MET and HER2.

Immunohistochemistry

BTN1A1 was detected with the rabbit monoclonal antibody STC43H11-1 (STCube) and PD-L1 with the 22C3 assay, on serial sections of the same block, with controls in every batch.

Scoring

Both markers were scored as the Tumour Proportion Score (TPS) — the percentage of viable tumour cells with membranous staining of any intensity. TPS was analysed as a continuous variable and dichotomised at 1%, 25% and 50%; the 50% threshold matches the enrolment criterion of the ongoing phase 2 STCUBE-004 trial of nelmastobart.

Statistical analysis

Paired comparisons used the Wilcoxon signed-rank statistic with exact permutation p values and Hodges–Lehmann median changes with exact 95% CIs. Agreement between paired specimens was quantified by ICC(2,1), Bland–Altman analysis and Cohen's κ ; the BTN1A1–PD-L1 relationship by Spearman correlation and Fisher exact tests of co-positivity; subgroups by exact Mann–Whitney permutation tests. Two-sided $\alpha = 0.05$; all analyses are descriptive and exploratory, without adjustment for multiplicity.

DISCUSSION AND CONCLUSION

Actionable Take-Away Message

- BTN1A1 was expressed (TPS $\geq 1\%$) in 70% of primary and 85% of recurrent/metastatic specimens, and reached TPS $\geq 50\%$ — the trial enrolment threshold — in 15% and 25%.
 - BTN1A1 was highly concordant between the primary tumour and the matched later lesion (ICC 0.88) with no positive-to-negative conversion.
 - High-level BTN1A1 and PD-L1 rarely coincided (1 of 40 specimens at TPS $\geq 50\%$), and 8 of 9 PD-L1-negative specimens were BTN1A1-positive — BTN1A1 marks a population PD-L1 testing does not capture.
- ### Future Direction for Research
- Larger cohorts scored by two or more independent readers, to establish inter-observer reproducibility and to compare TPS with intensity-weighted scoring.
 - Confirmation of the near-universal BTN1A1/PD-L1 co-expression seen in driver-altered tumours, and what it implies for treatment sequencing after targeted therapy.
 - Correlation of BTN1A1 TPS with outcome in the phase 2 trial of nelmastobart plus docetaxel (TPS $\geq 50\%$), to define a clinically validated cut-off and support companion-diagnostic development.

Limitations

- Single-centre retrospective series of 20 patients; TPS scored without intensity weighting; inter-observer reproducibility not assessed; subgroup analyses exploratory and unadjusted for multiplicity.

REFERENCES & ACKNOWLEDGEMENTS

References

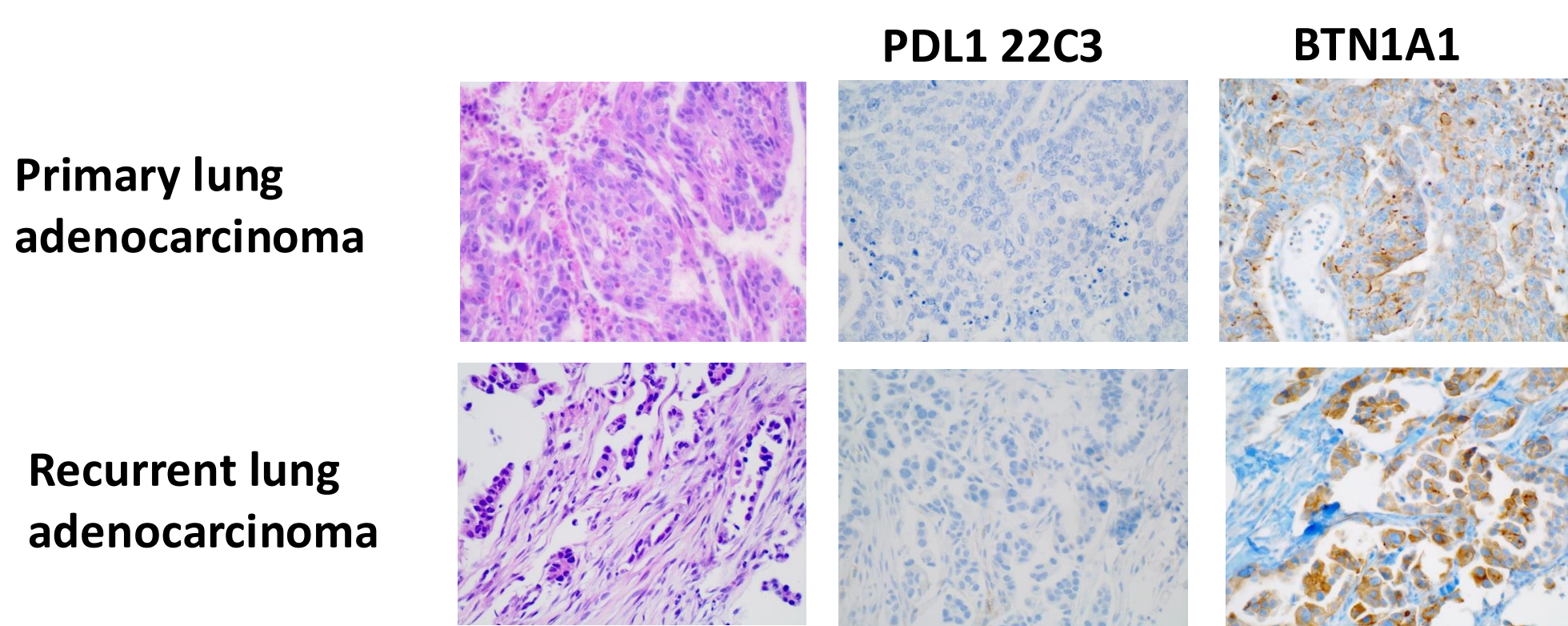
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Acknowledgements

Artificial Intelligence (AI) Use Disclaimer

AI use disclosure: No AI tools were used in the preparation of this work. All data derive from the study dataset; all analyses and text were reviewed and verified by the authors, who take full responsibility for the content.

RESULTS



RESULTS

0.88

ICC for BTN1A1 between the primary tumour and the matched later lesion

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specimens high (TPS $\geq 50\%$) for BTN1A1 and PD-L1 at the same time

Table 1. Cohort (n = 20 patients, 40 specimens)

Characteristic	n (%)
Histology — adenocarcinoma	20 (100)
Stage II / IIIA / IIIB / IV	7 / 8 / 2 / 3
Driver — WT / EGFR / KRAS G12C	14 / 5 / 1
Recurrent specimen — lung / node / brain	7 / 5 / 4
pleura / skin / adrenal	2 / 1 / 1

Paired change in BTN1A1 and PD-L1 expression, primary → recurrence/metastasis

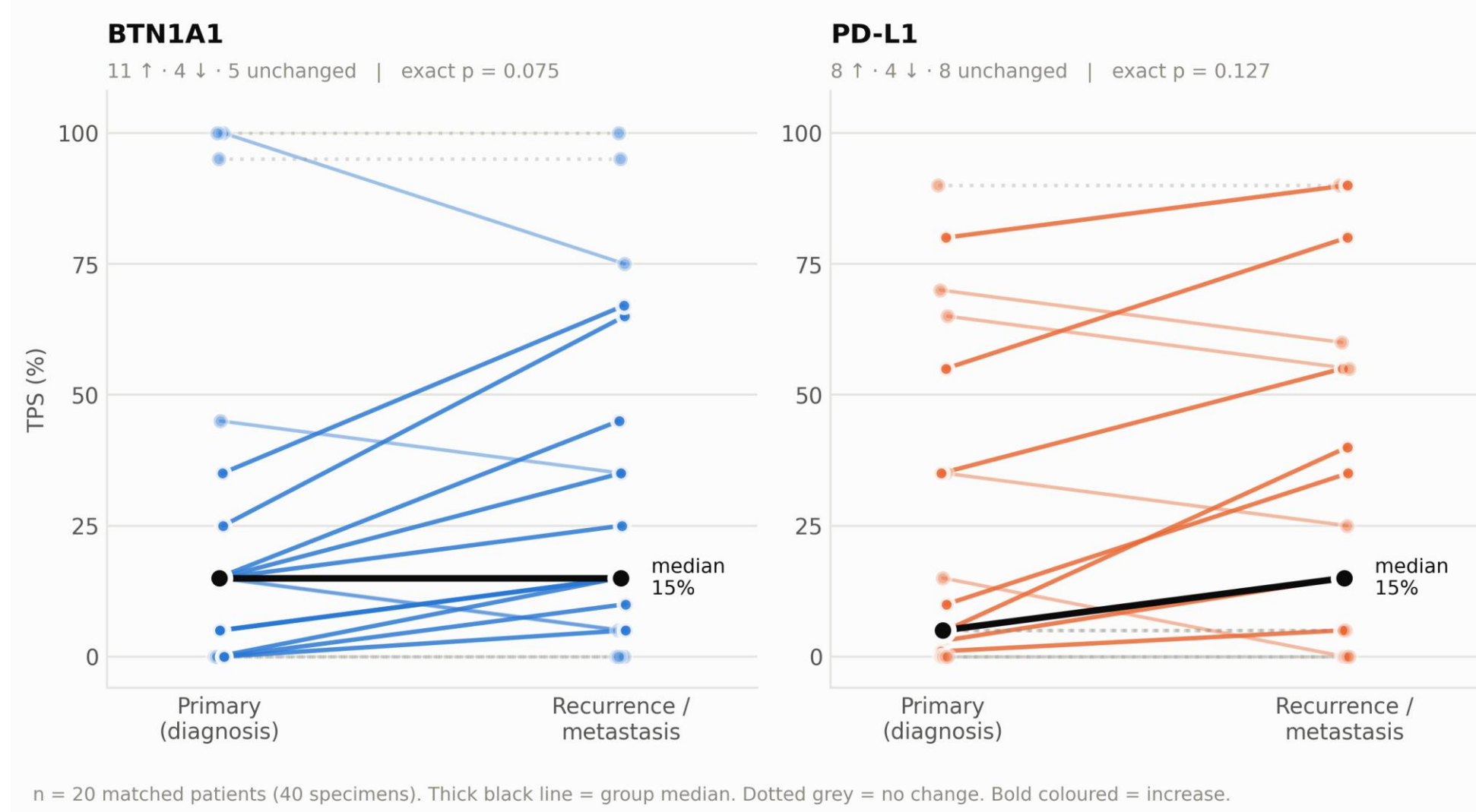


Figure 1. Paired change from the primary tumour to the matched recurrent/metastatic lesion. BTN1A1 rose in 11 patients, fell in 4 and was unchanged in 5 (Hodges–Lehmann +5.0 pp, exact p = 0.076); PD-L1 in 8, 4 and 8 (+5.0 pp, p = 0.126).

BTN1A1 and PD-L1 are rarely co-high in the same specimen

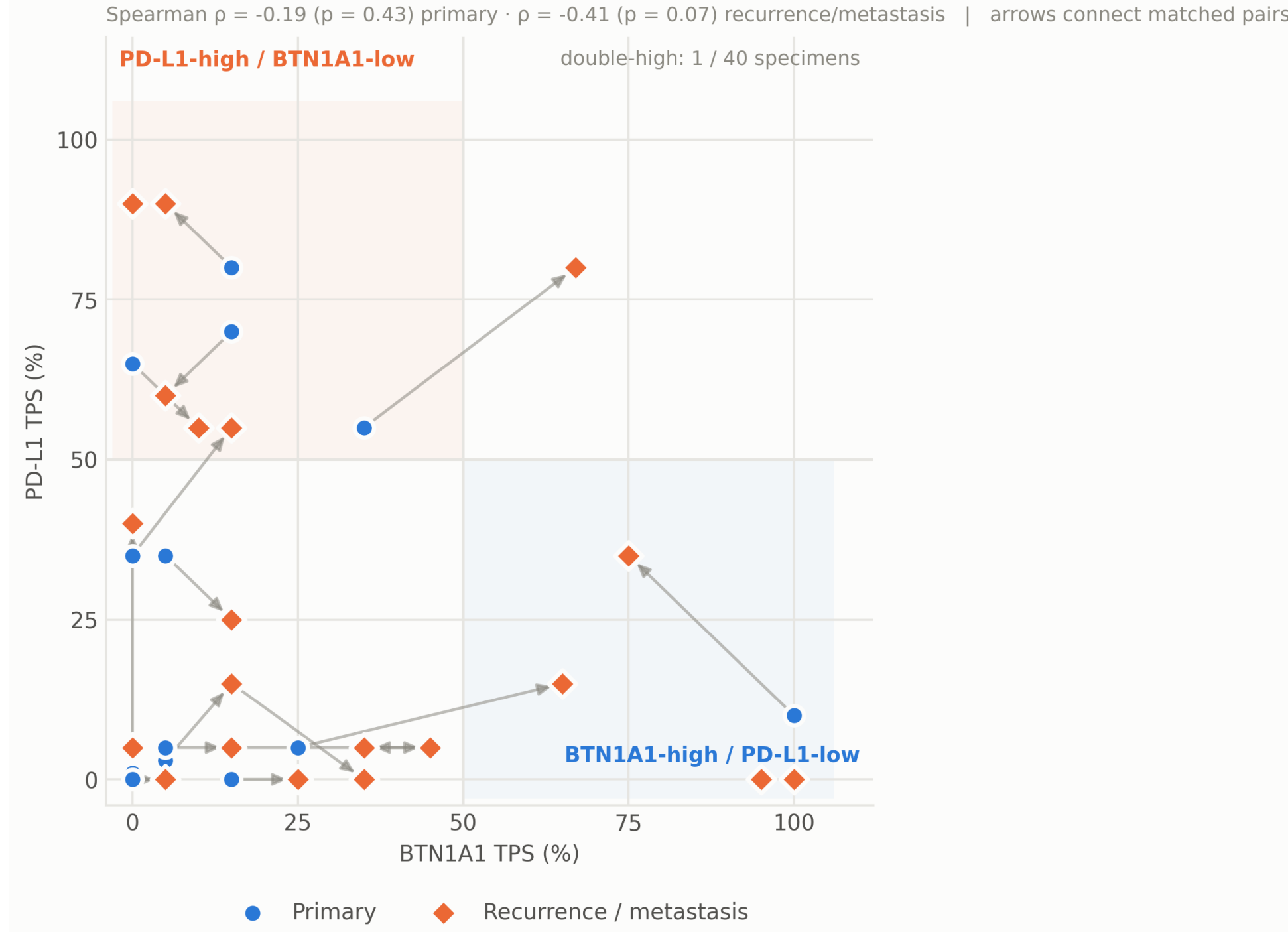


Figure 2. BTN1A1 (STC43H11-1) versus PD-L1 (22C3) TPS in all 40 specimens; arrows connect matched pairs. Only 1 of 40 specimens was high ($\geq 50\%$) for both markers; 3 of 40 were double-positive at $\geq 25\%$ versus 6.0 expected under independence (p = 0.056).

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PD-L1-negative specimens (TPS $< 1\%$) that were BTN1A1-positive

100% vs 39%

co-positivity for both markers, actionable-driver vs wild-type specimens

Table 2. BTN1A1 and PD-L1 expression by time point

	Primary (n = 20)	Recurrence / metastasis (n = 20)
BTN1A1 — median TPS, % (IQR)	15 (0–28)	15 (5–50)
TPS $\geq 1\%$	14 (70%)	17 (85%)
TPS $\geq 25\%$	6 (30%)	9 (45%)
TPS $\geq 50\%$	3 (15%)	5 (25%)
PD-L1 — median TPS, % (IQR)	5 (3–40)	15 (4–55)
TPS $\geq 1\%$	16 (80%)	15 (75%)
TPS $\geq 50\%$	5 (25%)	6 (30%)

Expression is highly concordant between matched primary and metastatic tissue

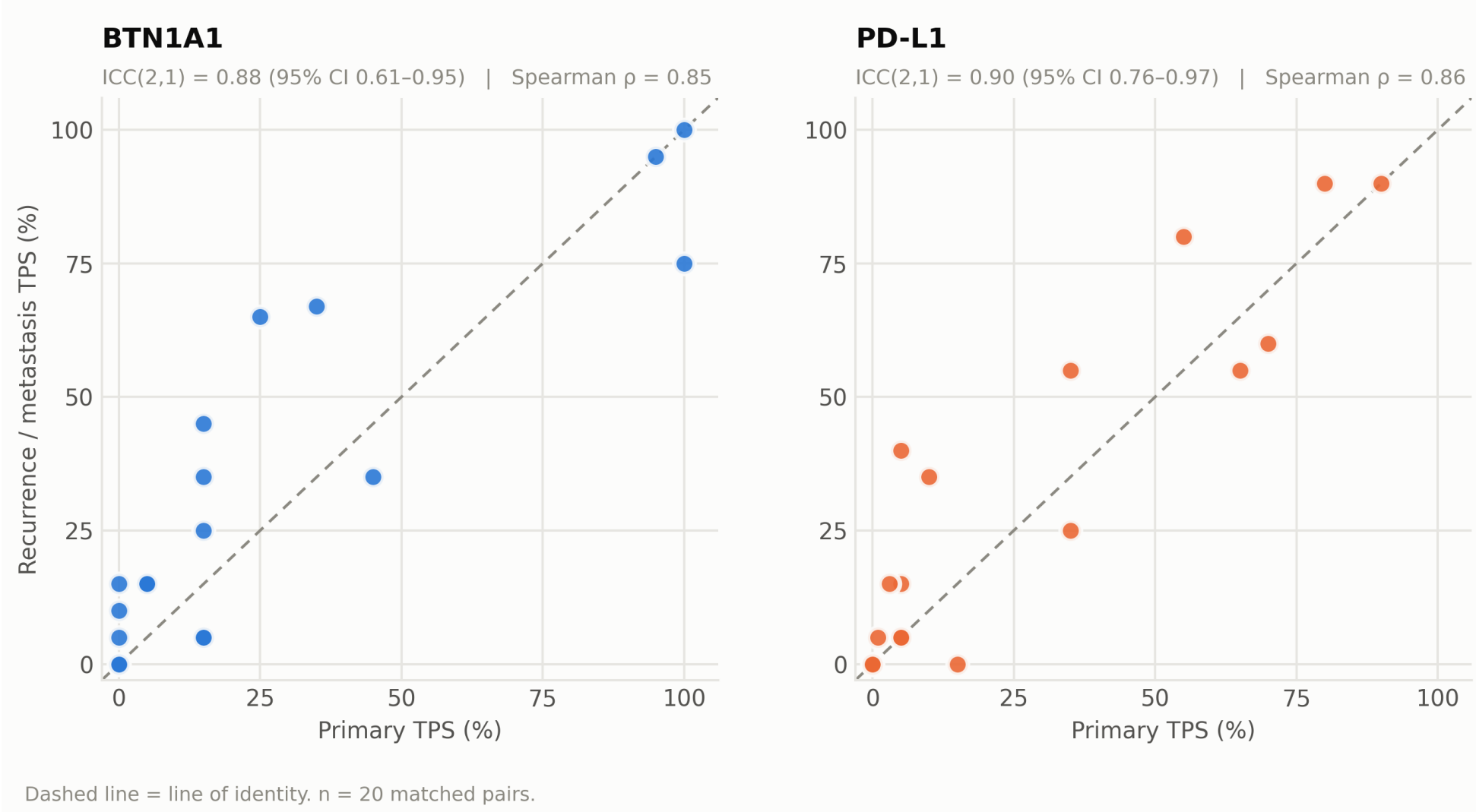


Figure 3. Concordance between the matched specimens; dashed line, line of identity. BTN1A1 ICC(2,1) = 0.88 (95% CI 0.61–0.95); PD-L1 ICC(2,1) = 0.90 (95% CI 0.76–0.97). No primary-positive case became negative at relapse.

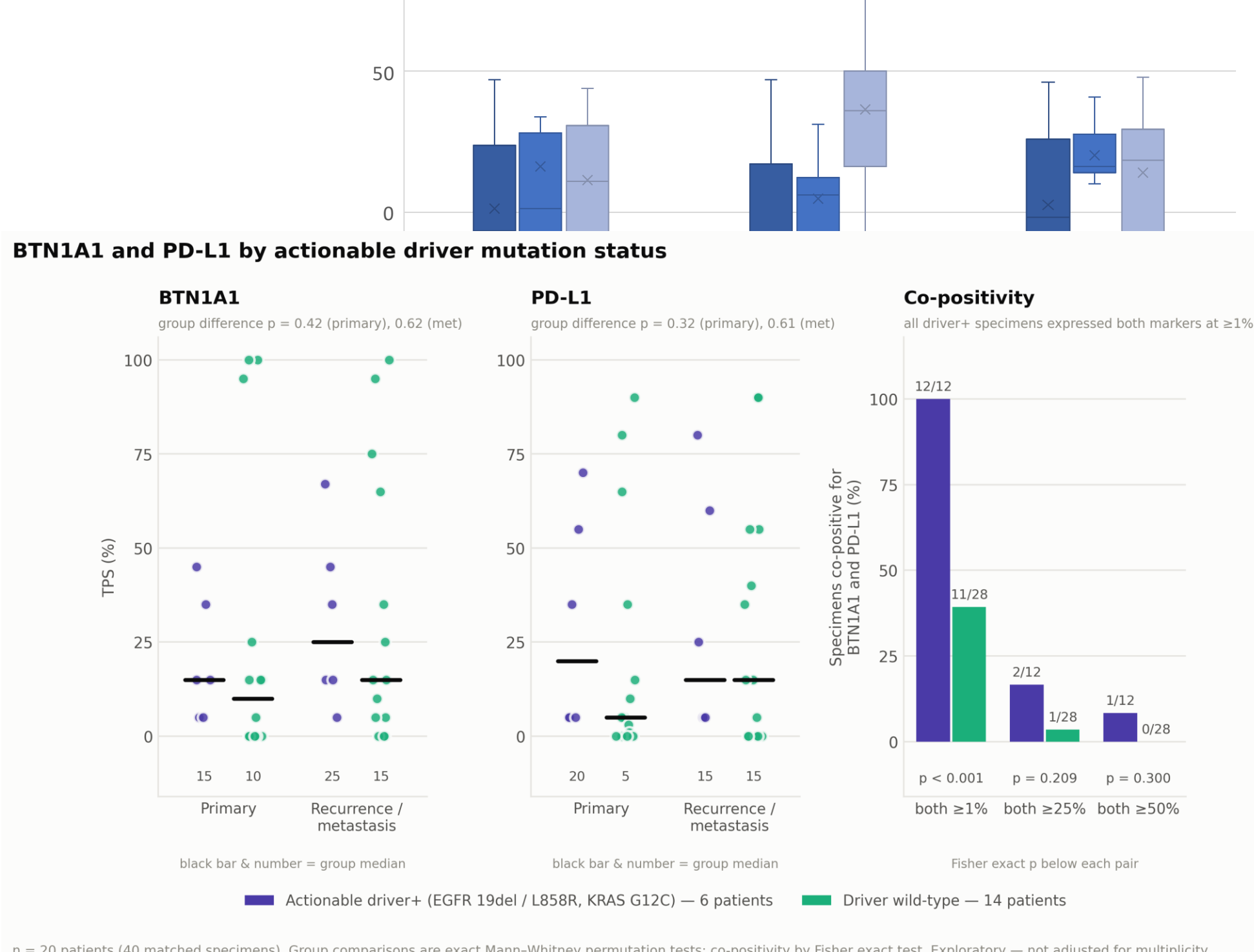


Figure 4. Expression by actionable driver status. Levels did not differ (all p ≥ 0.32), but all 12 driver-positive specimens were co-positive for both markers at TPS $\geq 1\%$ versus 11 of 28 wild-type specimens (Fisher p < 0.001).