Mutational Complexity Increases in Lung Adenocarcinoma (LADC) with the Development of Brain Metastasis (BM)

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BACKGROUND

Up to 40% of LADC patients develop BM[1,2] but little is known about the inciting molecular events.

METHODS

- We compared mutational profiles of LADC BM patients (pts) with primary (P) LADC submitted to Caris Life Sciences[3] from 2015-2017.

- Testing included[4]:
- **Next-generation sequencing (NGS)** on genomic DNA of 592 cancer-related genes isolated from formalin-fixed, paraffin-embedded (FFPE) tumor samples utilizing NextSeq platform (Illumina Inc., San Diego, CA). The 592 genes were enriched with a custom-designed SureSelect XT assay (Agilent Technologies, Santa Clara, CA, USA). Variants were detected with >99% confidence based upon allele frequency and amplicon coverage, with an average sequencing depth of coverage >500X and an analytic sensitivity of 5% variant frequency. NGS aberrations were test-defined as pathogenic (PATH), variants of undetermined significance or unclassified mutations (VUS).

- **PD-L1** immunohistochemistry was completed on slides of FFPE tumor sections utilizing automated staining techniques with procedures in accordance with the requirements of the College of American Pathologists. Prior to January 2016, the antibody used against PD-L1 was SP142 (Spring Bioscience, Pleasonton, CA). Starting in January 2016 the primary antibody against PD-L1 was 22c3 (Dako, Santa Clara, CA) in non-small cell lung cancer (NSCLC) tumors, including LADC.

- **Tumor mutational burden (TMB)**, reported in mutations per megabase (Mb), was determined by calculating the number of nonsynonymous somatic mutations identified by NGS after removing single nucleotide polymorphisms (SNP) identified in dbSNP (version 137) or in 1000 Genomes Project database (phase 3). 1.4 Mb were sequenced per tumor. TMB was test-defined as: high (H; ≥17 mutations/megabase), intermediate (I; 7-16) and low (L; 0-6).

RESULTS

Characteristic		Brain Metastasis (N=145)	Primary (N=1145)
Median Age (range)		64 (31-86)	70 (25-90)
Number Female (%)		83 (57)	669 (58)
PATHs (%)	*KRAS*	55 (38)	430 (38)
	STK11	33 (23)*	120 (11)*
	EGFR	17 (12)	183 (16)
	BRAF	5 (3)	28 (2)
MET-amplified (%)		3 (2)	17 (2)
ALK-rearranged (%)		3 (2)	10 (1)
ROS1-rearranged (%)		1 (1)	5 (0)
PD-L1 IHC (%)	≥1%	65/142 (46)	518/1060 (49)
	≥50%	34/142 (24)	241/1060 (23)

Table 1. Characteristics of LADC BM vs. P samples.

- 145 BM (57% female (f)) and 1145 P (58% f) cases were identified (Table 1).
- BM median age was 64 (range 31-86) v. 70 (25-90) in P.
- BM had 55 PATHs (38% pts) in *KRAS*, 34 *STK11* (23%), 17 *EGFR* (12%), 5 *BRAF* (3%); 3 were *MET*-amplified (2%), 3 *ALK* (2%) and 1 *ROS1*-rearranged (1%).
- Compared to P, more BM pts harbored *STK11* PATHs (23% vs. 11%, P<0.0001); no other difference in PATHs was observed.
- Of 142 BM and 1060 P with PD-L1 testing, incidence of ≥1% (46% BM vs. 49% P) and ≥50% (24% vs. 23%) cases were similar.

- 143 BM and 1102 P pts had TMB data (Figure 1).
- BM cases were more-frequently TMB-H compared to P (39% (N=56) vs. 12% (132), P<0.0001) and less likely to be TMB-L (8% (12) vs. 33% (366), P<0.0001).
- 131 (92%) BM pts were TMB-I or H.

- No significant difference was observed between BM specimen site and *EGFR*, *KRAS*, TMB, and PD-L1 status.

- 327 VUS in 28 receptor tyrosine kinases (RTK) were observed in 117 BM (median 1 (0-12)) vs. 1648 VUS in 807 P pts (median 1 (0-13); 79% vs. 70%, P=0.007).

- RTK VUS more frequently observed in BM included: 31 *EPHA3* VUS (17% pts vs. 8%. P=0.0002), 25 *EPHA5* (15% vs. 8%, P=0.004), 26 *NTRK3* (14% vs. 6%, P=0.0002), 22 *EPHB1* (13% vs. 6%, P=0.0001), 17 *PDGFRA* (12% vs. 5%, P=0.0001), *KDR* 13 (9% vs. 5%, P=0.0497), and *NTRK1* 11 (8% vs. 4%, P=0.0168).

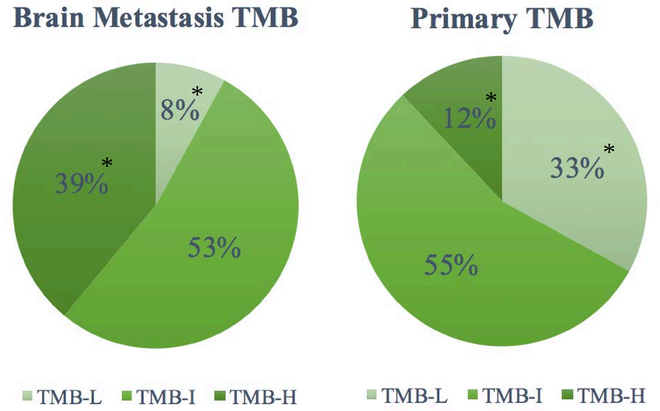

Figure 1. TMB assessment of BM vs. P.

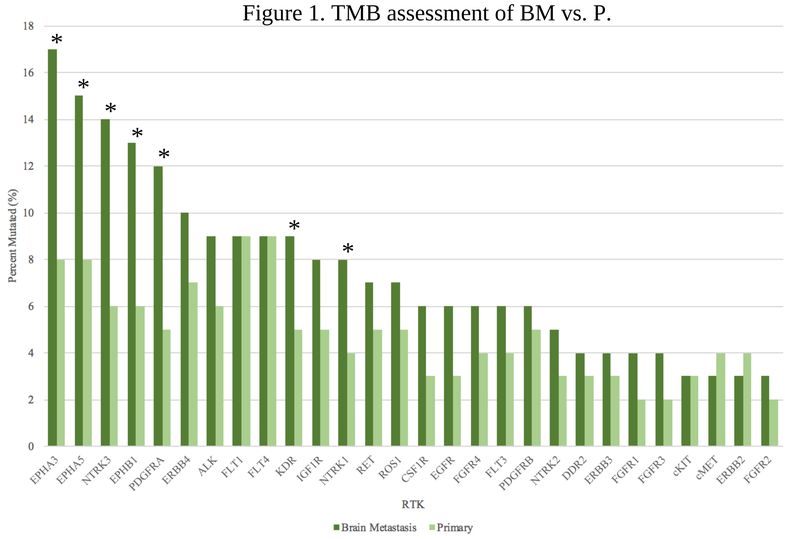

Figure 2. Comparison of RTK frequency between BM and P.

CONCLUSIONS

- Classic LADC biomarkers including PD-L1 (≥1% and ≥50%), *EGFR*, and *KRAS* were similar between BM and P cases.

- However, nearly 40% BM patients were TMB-H (≥25% more than P) and >90% either TMB-I or H, indicating an increased mutational complexity in BM development, suggesting immune checkpoint inhibitor use.[5]

- In addition to *STK11*[6] PATHs, RTK VUSs including: *EPHA3*, *EPHA5*, *NTRK3*, and *EPHB1* were more-frequently mutated and warrant further evaluation as biomarkers or targets in BM.

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