

Distinct biomarker features in triple-negative breast cancer metastases to the brain, liver and bone

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Abstract

Background: Triple-negative breast cancer (TNBC) is characterized by its aggressive nature and accounts for a disproportionate number of metastatic disease cases and breast cancer-related deaths. Despite recent improvements, TNBC patients who develop metastatic diseases have limited treatment options. We investigated biomarkers from brain, liver and bone metastases collected from TNBC patients to identify therapeutic options and to examine molecular differences between the metastatic sites.

Method: Triple-negative breast cancer tumors referred to Caris Life Sciences (Phoenix, AZ) between 2009 and 2015 were tested with a combination of immunohistochemistry (IHC), fluorescent/chromogenic in-situ hybridization (FISH/CISH) and sequencing (Next-generation sequencing, or NGS and Sanger).

Result: 1570 TNBC tumors were analyzed, including 1297 tumors taken from breast, 54 from brain, 172 from liver and 47 from bone. Select biomarker frequencies of protein overexpression (IHC), gene amplification (ISH) and mutations (SEQ) are summarized in Table 1. Brain metastases showed the highest protein expression of TOPO2A and PDL1; liver metastases showed the highest expression of AR and SPARC, as well as the highest mutation rate of PIK3CA. Bone metastases showed the lowest expression of TS, RRM1 and ERCC1. BRCA1 and BRCA2 mutation rates ranged from 0-11% in various specimen sites.

Biomarker And Method	Breast specimens (%)	Brain Metastases (%)	Liver Metastases (%)	Bone Metastases (%)	P value [§]
TOPO2A IHC	76	100	73	39	<0.0001
PDL1 IHC	15	40	8	17	0.03
AR IHC	15	10	36	26	0.0005
SPARC IHC	17	30	40	15	0.0027
PIK3CA SEQ	16	5.3	29	25	0.036
TS IHC*	49	54	24	15	<0.0001
RRM1 IHC*	39	43	32	16	0.006
ERCC1 IHC*	35	55	48	16	0.002
BRCA1 SEQ	7	0	8	0	ns
BRCA2 SEQ	11	11	4	0	ns

n/a: data not available due to low N. * low levels predict response to associated drugs. §p values are calculated from comparing the group with the highest frequency with the lowest frequency. ns: non-significant, i.e., p>0.05

Conclusion: Distinct biomarker features identified in different metastatic sites in TNBC present the rationale to investigate differential treatment strategies. Based on biomarker results, etoposide, immune-modulatory agents may seem promising for brain metastases; anti-androgen therapies and nab-paclitaxel may be promising in treating liver metastases; while fluoropyrimidines, gemcitabine and platinum may be considered for TNBC patients with bone metastases.

Background

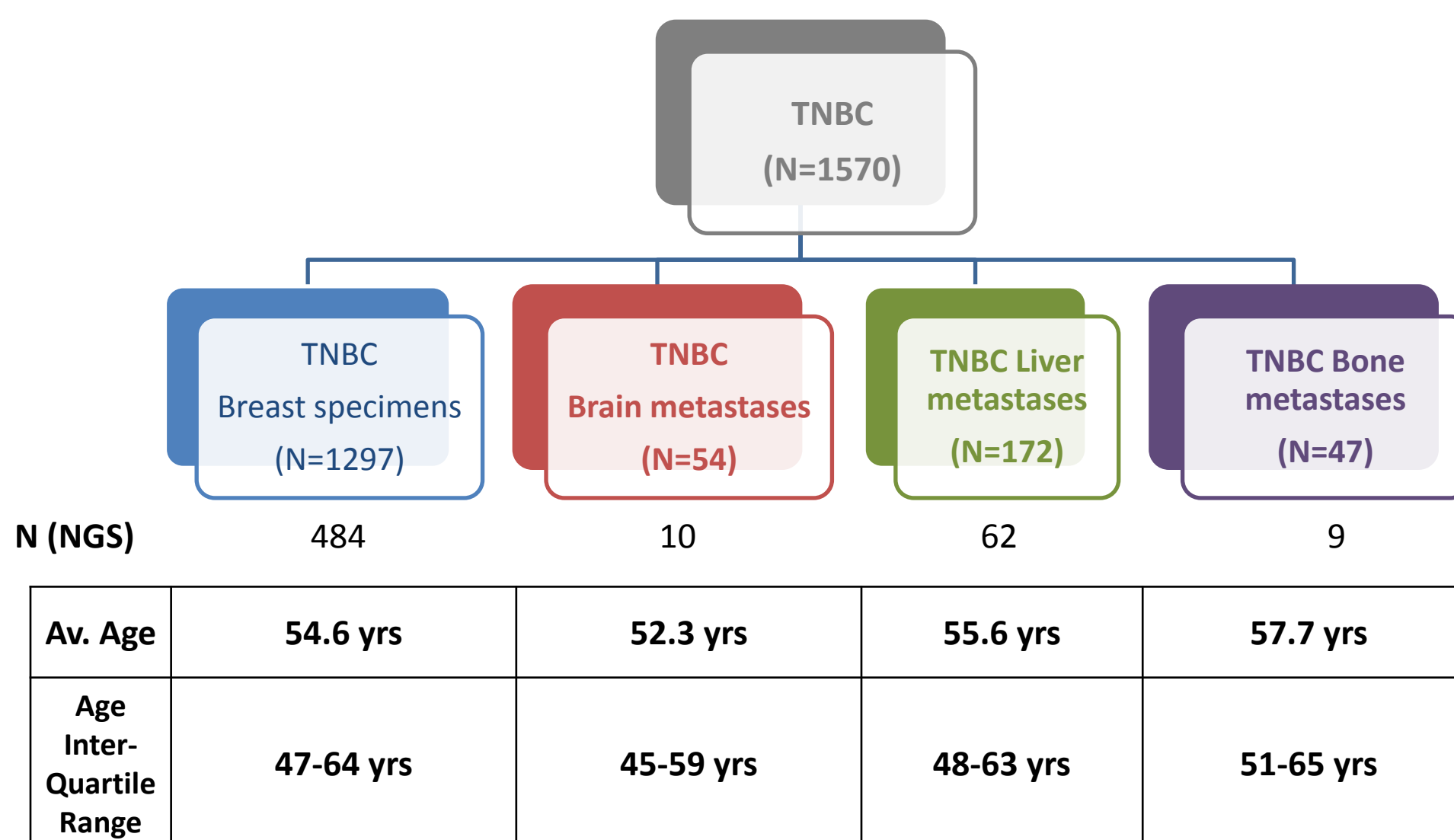
TNBC is known for its highly aggressive behavior and resistance to current standard therapies. It's a well-defined subtype of breast cancer immunohistochemically however it remains a molecularly heterogeneous group (1). Clinical data suggest that TNBC patients with metastases to different organs experience different survival times however different responses to therapies haven't been systematically explored (2). Multiplex tumor-profiling has shown to effectively prolong patients' progression-free survival in metastatic heavily pre-treated breast cancer (3), therefore we investigated the profiles in TNBC tumors with different metastases.

Methods

IHCs were performed on formalin-fixed paraffin-embedded (FFPE) tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies. FISH was used for evaluation of the HER-2/neu [HER-2/CEP17 probe] and EGFR [EGFR/CEP7 probe] (Abbott Molecular/Vysis). CISH was also used (INFORM HER-2 Dual ISH DNA Probe Cocktail; EGFR DNP probe, Ventana). NGS was performed using the Illumina MiSeq platform. Specific regions of 47 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel. Sanger sequencing included selected regions of BRAF, KRAS, NRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences. TNBC was defined as ER IHC negative (Ab: SP1; =0+ or <10%); PR IHC negative (Ab: 1E2; =0+ or <10%), Her2 IHC negative (Ab:4B5) and Her2 ISH negative (Her2 results and interpretation follow the ASCO/CAP scoring criteria. Wolff, AC. et al. (2013) J Clin Oncol: 31 (31):3997-4013).

Results:

Figure 1: Patient Characteristics



Results

Figure 2: IHC and ISH marker comparison of TNBC tumors taken from the breast, bone, liver and the brain. Bars represent the frequency of the biomarker measurement observed. Therapeutic agents are listed for corresponding biomarkers that show significantly different frequencies. A star indicates statistical significance when the highest mutation rate is compared to the lowest.

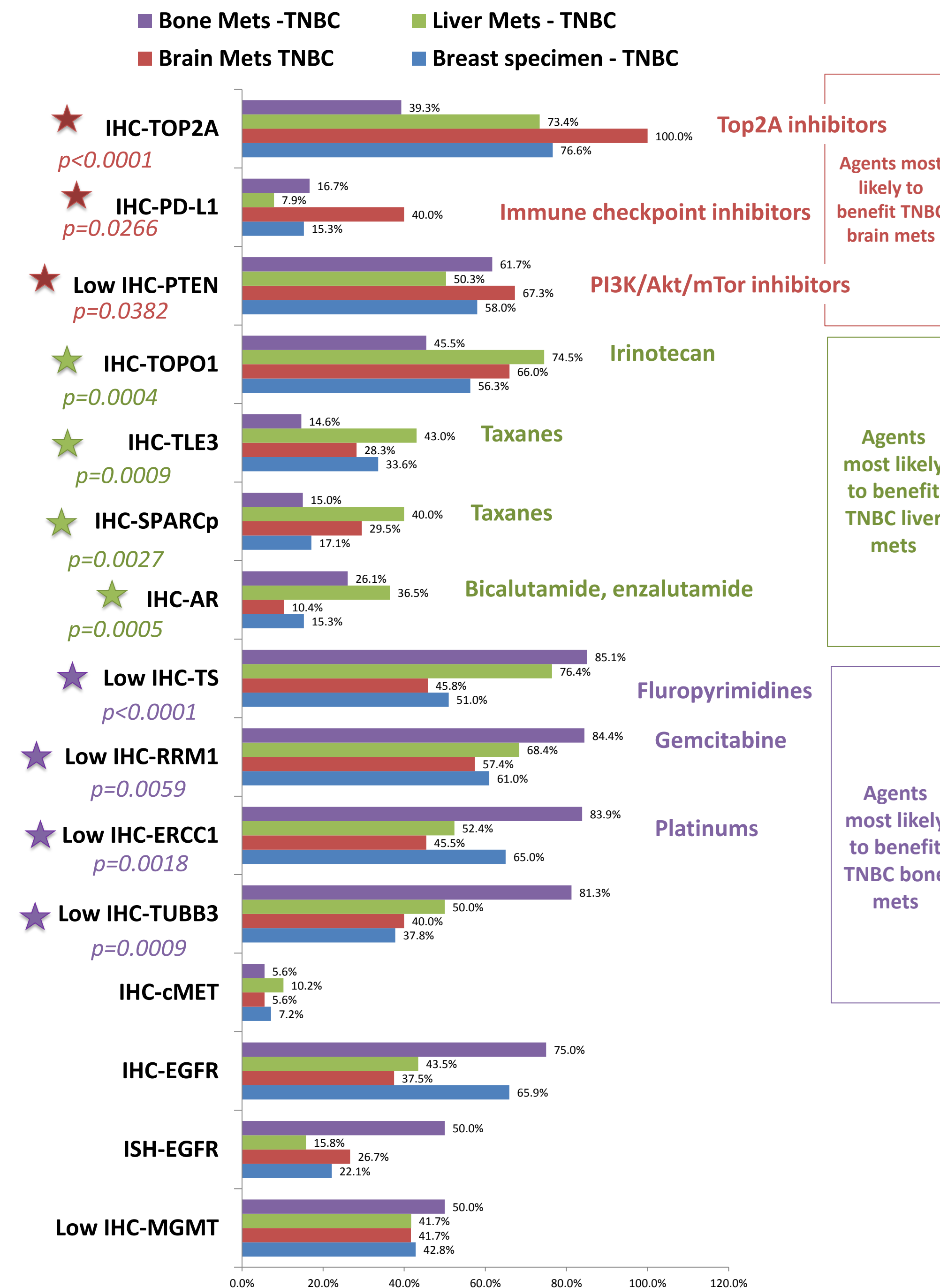
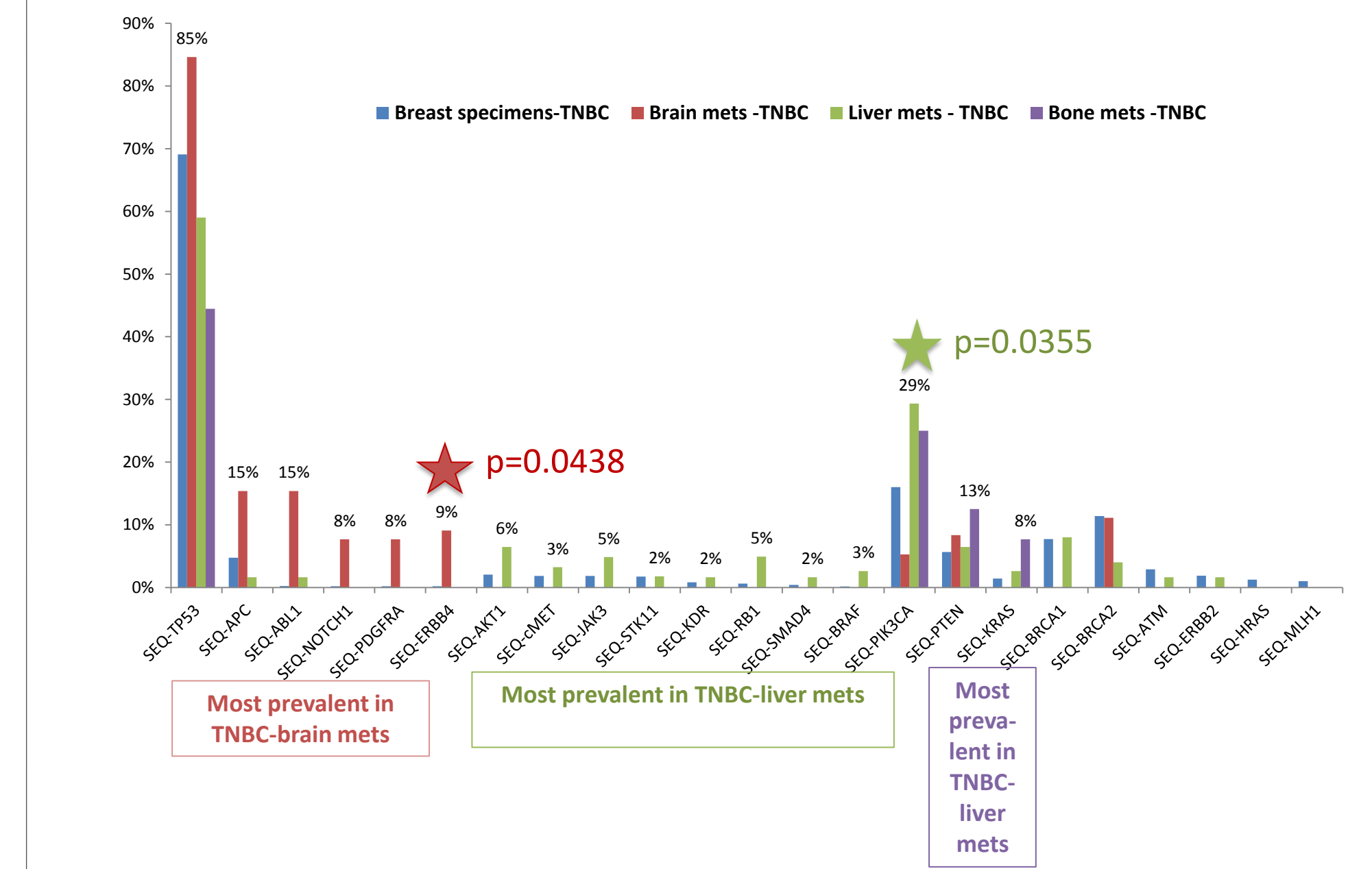


Figure 3: Comparison of gene mutations rates in primary TNBC and metastases to the brain, liver and bone. No statistical significance was seen with the exception of ERBB4 and PIK3CA with borderline significant p values.



Conclusions

- The investigation of biomarkers by IHC, ISH and NGS shows molecular heterogeneity of TNBC tumors metastasized to the brain, liver and bone.
- These results suggest agents that can potentially deliver increased benefit for TNBC patients with metastases to different organs, including TOP2A inhibitors and immune checkpoint inhibitors for brain metastases, taxanes and anti-androgen agents for liver metastases and fluoropyrimidine, gemcitabine and platinum agents for bone metastases.
- The mutation data show promising results including high mutation rates of ERBB4 in brain mets and PIK3CA in liver mets, however, this portion of the study is from a limited NGS dataset.
- Distinct molecular profiles identified in different metastases support the rationale of tailoring treatments of TNBC cancers to the biomarker features of individual patients.

References

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