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COMMENTARY

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Theranostic molecular profiling of pleomorphic ductal carcinoma of the breast

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Pleomorphic ductal carcinoma (PDC) is a very rare subtype of invasive ductal carcinoma of no-special type (NST), characterized by the presence of highly atypical/bizarre (>6-fold variation in nuclear size) and multinucleated (giant) neoplastic cells comprising >50% of the tumor cell population[1] (Figure 1A). PDC is typically triple-negative breast cancer (TNBC), associated with an aggressive clinical course and a poor outcome.[2–4] So far, no single study explored novel predictive biomarkers for the precision medicine purposes in the patients with PDC. Formalin-fixed paraffin-embedded tissue samples of the six PDC patients (four primary and two metastatic cases) were sequenced for 592-genes using NextSeq platform (Illumina, La Jolla, CA, USA). Tumor mutational load (TML) was calculated using only somatic nonsynonymous missense mutations; high TML was considered when it was ≥17 mutations/Mb. Microsatellite instability (MSI) status was explored by the direct analysis of known MSI loci in the target regions of the sequenced genes. Cases were considered microsatellite instable (MSI-H) if they exhibited ≥46 altered microsatellite loci (the threshold was established by comparing to the PCR-based MSI FA result from ~2100 cases[5]). Copy number variations (CNVs) were determined by comparing the depth of sequencing of genomic loci with a diploid control. Calculated gains ≥6 copies were considered amplified. ArcherDx FusionPlex Assay was used to detect gene fusions (52 gene targets). Immunohistochemistry was used to detect expression of PD-L1 (SP142 antibody, Ventana) in tumor cells (TC) and immune cells (IC). PD-L1 positivity in TC was defined as 2+ intensity in ≥5% of tumor cells.[6] PD-L1 status

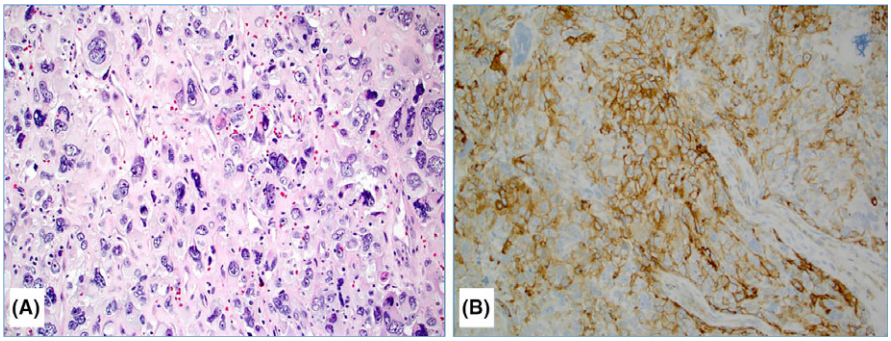

FIGURE 1 A case of pleomorphic ductal carcinoma with a diffuse infiltration of highly pleomorphic neoplastic cells some of which with a bizarre and multinuclear appearance (Hematoxylin & Eosin stain, 20×) (A); this was the only pleomorphic ductal carcinoma with a significant (20% of tumor cells) PD-L1 expression (20×) (B) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 A summary of the molecular profiling results on six pleomorphic ductal carcinomas of the breast

PDC	TML	MSI	*BRCA1*	*BRCA2*	*TP53*	Other NGS	PD-L1 (TC)	PD-L1 (IC)	CNV	Archer
Case#1 (primary)	6/Mb	Stable	E143X	wt	R273C	None	20% (2+ intensity)	IC2	None	None
Case#2 (primary)	11/Mb	Stable	wt	wt	R342P	*KRAS* G12A	1% (2+ intensity)	N/E (necrosis)	*CDKN1B*	None
Case#3 (primary)	4/Mb	Stable	wt	wt	H193R	None	Negative	IC1	None	None
Case#4 (primary)	4/Mb	Stable	wt	wt	E294fs	VUS	Negative	IC2	*FGFR1*	Failed
Case#5 (metastatic)	7/Mb	Stable	wt	wt	wt	SNPs	3% (2+ intensity)	IC2	None	None
Case#6 (metastatic)	7/Mb	Stable	wt	wt	R248Q	*SMARCA4* *FH*	Negative	Negative	None	Failed

CDKN1B, cyclin-dependent kinase inhibitor 1B; CNV, copy number variations; *FGFR1*, fibroblast growth factor receptor 1; *FH*, fumarate hydratase gene; IC, immune cells; Mb, megabase; MSI, microsatellite instability; N/E, not evaluated; NGS, next-generation sequencing; PDC, pleomorphic ductal carcinoma; SNP, single-nucleotide polymorphism; TC, tumor cells; TML, tumor mutational load; VUS, variant of unknown significance; wt, wild type; ↑: amplified.

in ICs was categorized as IC0 (<1%), IC1 (≥1% but <5%), and IC2/3 (≥5%).[7] All tests were performed at Caris Life Sciences (Phoenix, AZ), and details are available at https://www.carismolecularintelligence.com/tumor-profiling-menu/mi-profile-usa-excluding-new-york/).

All PDCs were confirmed to be of the breast origin and positive for epithelial markers (eg, AE1/AE3, Cam5.2). Estrogen receptor (ER), progesterone receptor (PR), and Her-2/neu protein were negative in all cases. *TP53* mutations were detected in five of six cases, with one case harboring two additional pathogenic mutations (*SMARCA4* R1093X and *Fumarate Hydratase* K477dup), and two cases with pathogenic *BRCA1* (E143X) or *KRAS* (G12A) mutations (Table 1). No pathogenic mutation was detected in one case. No gene fusions were detected in any of the cases successfully analyzed (0/4). Gene amplification of cyclin-dependent kinase inhibitor 1B (*CDKN1B*) and fibroblast growth factor receptor 1 (*FGFR1*) genes was detected in one case, each. These results indicate that PDCs exhibit significantly less targetable genetic alterations in contrast to related TNBC and metaplastic breast carcinomas.[8,9] A single case with a mutation in *BRCA1* gene indicates a potential benefit to platinum compounds and PARP inhibitors.[10] Tumor expression of PD-L1 (TC) was negative/low in all but one case that exhibited 20% positive tumor cell population (Figure 1B), while IC expressing PD-L1 were detected at potentially significant levels (IC2; ≥5%<50%) in three cases. Total mutational load (TML) was low in all cases (range, 4-11/Mb), and no DNA microsatellite instability was detected in any case (all five cases were microsatellite stable) (Table 1). Low TML, rare PD-L1 expression (1/6 TC+ and 3/5 IC+), and absence of mismatch repair deficiency make this tumor an inconsistent candidate for treatment with the current immune checkpoint inhibitors.[5] We encourage further studies on PDC to reveal novel predictive biomarkers for this rare and difficult-to-treat breast cancer subtype.

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