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Immune and Genetic Signatures of Breast Carcinomas Triggering Anti-Yo–Associated Paraneoplastic Cerebellar Degeneration

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Abstract

Background and Objectives
Paraneoplastic cerebellar degeneration (PCD) with anti-Yo antibodies is a cancer-related autoimmune disease directed against neural antigens expressed by tumor cells. A putative trigger of the immune tolerance breakdown is genetic alteration of Yo antigens. We aimed to identify the tumors’ genetic and immune specificities involved in Yo-PCD pathogenesis.

Methods
Using clinicopathologic data, immunofluorescence (IF) imaging, and whole-transcriptome analysis, 22 breast cancers (BCs) associated with Yo-PCD were characterized in terms of oncologic characteristics, genetic alteration of Yo antigens, differential gene expression profiles, and morphofunctional specificities of their in situ antitumor immunity by comparing them with matched control BCs.

Results
Yo-PCD BCs were invasive carcinoma of no special type, which early metastasized to lymph nodes. They overexpressed human epidermal growth factor receptor 2 (HER2) but were hormone receptor negative. All Yo-PCD BCs carried at least 1 genetic alteration (variation or gain in copy number) on CDR2L, encoding the main Yo antigen that was found aberrantly overexpressed in Yo-PCD BCs. Analysis of the differentially expressed genes found 615 upregulated and 54 downregulated genes in Yo-PCD BCs compared with HER2-driven control BCs without PCD. Ontology enrichment analysis found significantly upregulated adaptive immune response pathways in Yo-PCD BCs. IF imaging confirmed an intense immune infiltration with an overwhelming predominance of immunoglobulin G–plasma cells.

Discussion
These data confirm the role of genetic alterations of Yo antigens in triggering the immune tolerance breakdown but also outline a specific biomolecular profile in Yo-PCD BCs, suggesting a cancer-specific pathogenesis.

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Paraneoplastic cerebellar degeneration (PCD) is a rare condition but is one of the commonest paraneoplastic neurologic disorders, mainly associated with breast and ovarian carcinomas. The main feature is a rapidly progressive cerebellar ataxia secondary to the specific destruction of Purkinje cells by cytotoxic T cells. About 50% of PCD cases are associated with anti-Yo autoantibodies (Yo-PCD), directed against Yo antigens CDR2 (cerebellar degeneration-related protein 2) and its paralogue CDR2L. The previous description of an uncommon and intense immune attack in Yo-PCD ovarian cancers highlighted that antitumor immunity has a lead role in paraneoplastic syndrome pathogenesis. The presence of specific alterations of genes encoding Yo antigens (copy number gain leading to antigen overexpression and somatic sequence variations) in all Yo-PCD ovarian cancers strongly suggests that neoantigenicity is a pivotal mechanism leading to the immune tolerance breakdown that could in turn drive the immune overreaction spearheading the paraneoplastic syndrome. Hypothesizing the aforementioned Yo gene specificities to be causative in immune tolerance breakdown in Yo-PCD, similar genetic alterations should be found in breast cancer (BC) associated with Yo-PCD. However, the already described overexpression of human epidermal growth factor receptor 2 (HER2) in Yo-PCD BCs leads to believe that antigen specificities are not the only molecular triggers of the immune tolerance breakdown and that other mechanisms, potentially linked to breast oncogenesis, could be involved. To move forward in our understanding of the mechanisms leading to this specific immune reaction, we analyzed the salient clinical, histopathologic, and immunologic features, gene expression profiles, and mutational status of Yo-PCD BCs.

Methods

Patients

Patients with Yo-PCD and a BC diagnosed between January 2005 and March 2021 were identified retrospectively by the French Reference Center for Paraneoplastic Neurological Syndromes (Lyon, France). Patients were included if they had (1) PCD diagnosis according to the international guidelines, (2) presence of Yo antibodies in serum and/or CSF detected using both immunohistochemistry (IHC) on rat brain sections and dot blot using commercial tests (RAVO diagnostika and Euroimmun), and (3) histologically proven BC.

From those included, tumor samples were retrieved when available. Of note, for patients with several nonconcomitant tumors (e.g., patients who had several successive BCs) or tumor sites (e.g., primary BC and lymph node metastasis), we chose—whenever possible—to include all available tumor samples. In this way, we aimed to take into account the potential asynchronism of cancer and PCD onset, and thus, the impossibility to know a priori which tumor sample was responsible for PCD triggering when patients had had several BCs but also the possibility of a “cumulative effect” of different tumors in the same patients. Accordingly, when the primitive tumor and lymph node metastasis were available, we chose to include both samples, as it was possible that only 1 of the 2 tumor sites was responsible for paraneoplastic syndrome pathogenesis. This raise the possibility of potential duplicates or triplicates in the analysis that were systematically taken into account in interpretation of results.

Control Specimens

A cohort of 15 BCs without Yo-PCD from the Biopathology Department of Centre Léon Bérard (Lyon, France) was constituted matched with Yo BCs on their pathologic type and expression of HER2 and hormone receptor (HR).

Tumor Pathology Study

Four-μm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections were stained with hematoxylin-phloxine-saffron. A referent pathologist (I.T.) assessed the subtype of BCs according to the 2019 WHO classification.

Immunohistochemistry

Detailed chromogen IHC protocols and antibodies are described in the eMethods (links.lww.com/NXI/A734). Classical diagnostic markers including estrogen (OR) and progesterone (PR) receptors were obtained by a routine automated protocol. HER2 expression was assessed using prediluted monoclonal anti-HER2 antibody 4B5 (Roche Diagnostics, Basel, Switzerland). CDR2L expression was assessed using an automated IHC protocol. A staining intensity value from 0 (no staining) to 3 (high staining) was given by manual quantification conducted by 2 evaluators blinded to the provenance (patient or control) of the sample.

Multiplex Immunofluorescence Tissue Staining and Digital Image Analysis

Multiplex Immunofluorescence Tissue Imaging

Eight Yo-PCD BC samples were compared with 14 HER2-driven control BC samples. Fully automated seven-color multiplex immunofluorescence (IF) was performed using the Opal system (Akoya Biosciences, Marlborough, MA) and the BOND
RX stainer (Leica Biosystems, Nanterre, France) for patients with available FFPE tumor tissue. The sections were sequentially stained with each primary antibody, followed by OPAL-Horseradish Peroxidase secondary antibody incubation then revealed in the following order: DClnp (dendritic cell lysosomal-associated membrane glycoprotein), CD 20, immunoglobulin (Ig)G, IgA, CD3, and cytokertatin. The sections were then counterstained with spectral 4',6-diamidino-2-phenylindole and mounted with a coverslip. Detailed IF protocols and antibodies are described in the eMethods (links.lww.com/NXI/A734).

Quantification of Immune Cell Densities
Representative regions of interests (6 for tissue <100 mm², 10 otherwise) were defined on whole-slide digital images using the Phenochart software v. 1.0.12 (Akoya Biosciences). After cell segmentation using 4',6-diamidino-2-phenylindole, the different immune cell phenotypes were quantified, and data were processed on phenoptrReports v.0.2.10 R package (Akoya Biosciences) to obtain cell densities.

RNA Sequencing
Fifteen of the 22 Yo-PCD BC samples were eligible for RNA-seq analysis (7 were discarded because of insufficient material or RNA extraction failure). Sequencing was performed (paired end, 2 × 75 cycles) using NextSeq 500/550 High Output V2 kit on a NextSeq 500 machine (Illumina, San Diego, CA). The mean number of reads per sample was around 80 millions. Alignments were performed using STAR11 on the GRCh38 version of the human reference genome. The amount of duplicate reads were assessed using PICARD tools.12 Samples with a number of unique reads below 10 million (5 million paired-reads) were discarded from the analysis. Expression values were extracted using Kallisto version 0.42.5 with GENECODE release 23-genome annotation based on the GRCh38 genome reference.

Comparative Genomic Hybridization Array
Comparative genomic hybridization array (CGHa; accession number in NCBI’s Gene Expression Omnibus: GSE96039) was performed on 10 Yo-PCD BC samples. Fragmentation, labeling, cohybridization on 4 × 180 K Agilent SurePrint G3 Human whole-genome oligonucleotide arrays (Agilent Technologies), scanning, and analysis are fully described in the eMethods (links.lww.com/NXI/A734).

DNA Sequencing
CDR2 and CDR2L genes were sequenced on 8 Yo-PCD BC samples using the MiSeq next-generation sequencing platform (Illumina) according to the manufacturer’s instructions and described in the eMethods (links.lww.com/NXI/A734). The sequence data generated were aligned using NextGENe (SoftGenetics, State College, PA) on human reference sequences hg38 for CDR2 (NM_001802.1) and CDR2L (NM_014603.2).

Fluorescent In Situ Hybridization
Copy number alterations of the CDR2L gene were assessed on 12 Yo-PCD BC samples and 15 control BC samples with a
### Table 2 Histologic and Biomolecular Characteristics of Yo-PCD BCs

| Patient no. | Sample | Sample type | ErbB2 CNV | HER2 IHC expression | OR | PR | PCD-cancer interval, mo | Genetic alteration on the CDR2 or CDR2L gene | CDR2L CNV | CDR2 CNV | CDR2L expression (IHC) |
|-------------|--------|-------------|-----------|---------------------|----|----|------------------------|-----------------------------------------------|-----------|-----------|------------------------|
| 1           | A      | PBT         | NA +      | + +                 | 44 | None | None                  | None                                           | NA        | NA        | +                      |
| 2           | B      | PBT         | Amplification +++ | 0 0 | 6 | p.R394Q (CDR2L) | None                                           | None      | None      | +                      |
| 3           | C      | MLN         | None −     | 0 0                 | 1  | p.V176M (CDR2) | Gain                                             | None      | None      | ++                     |
| 4           |        | PBT         | NA +       | 0 0                 | 24 | NA   | Amplification          | NA                                               | NA        | +++       |                        |
| 5           | A      | MLN         | NA +       | 0 0                 | 152 | NA | Gain                   | None                                             | NA        | ++        |                        |
| 6           | B      | MLN         | NA +       | 0 0                 | 22 | NA | Amplification          | NA                                               | NA        | +++       |                        |
| 7           |        | PBT         | NA +++     | 0 0                 | 6  | NA   | Amplification          | NA                                               | NA        | +++       |                        |
| 8           |        | PBT + MLN   | NA +++     | 0 0                 | 1  | NA | Amplification          | NA                                               | NA        | ++        |                        |
| 9           |        | PBT         | Amplification +++ | 0 0 | −1 | p.Q50X (CDR2L) | Amplification | Gain | ++        |                        |
| 10          |        | PBT         | Amplification +++ | 0 0 | −5 | NA | Amplification | None | +++       |                        |
| 11          |        | MLN         | NA +++     | 0 0                 | 0  | NA | Amplification          | NA                                               | NA        | ++        |                        |
| 12          |        | PBT         | None −     | 0 0                 | 105 | p.R216W (CDR2) | Gain                                             | None      | −         |                        |
| 13          | A      | PBT         | None −     | + +                 | 8  | None | Strong gain            | None                                             | Strong gain | −         |                        |
| 14          | B      | PBT         | Amplification +++ | 0 0 | 8  | None | Amplification | Gain | +++       |                        |
| 15          |        | PBT         | NA −       | 0 0                 | 111 | p.S138L (CDR2) | NA                                               | NA        | ++        |                        |
| 16          |        | MLN         | Amplification +++ | 0 0 | −2 | None | Amplification | None | +++       |                        |
| 17          |        | MLN         | NA +++     | 0 0                 | 3  | NA | NA                    | NA                                               | NA        | +++       |                        |

Abbreviations: CNV = copy number variation; HER2 = ErbB2 protein; MLN = metastatic lymph node; NA = not available; OR = estrogen receptor; PBT = primary breast tumor; PCD = paraneoplastic cerebellar degeneration; PR = progesterone receptor.

The interval between PCD and cancer is calculated with cancer diagnosis as day 0; the negative interval thus corresponds to patients for whom PCD was diagnosed before cancer.
dual color probe where the CDR2L gene is labeled in orange, and the centromere of chromosome 17 is labeled in green (Empire Genomics, Buffalo, NY). The CDR2L gene was considered amplified if the mean number of orange signals was ≥6.

**Statistical and Bioinformatical Analysis**

Statistical analysis was performed using R v.4.0.3. Comparisons were made using the χ² or Fisher exact test according to preanalytical conditions. Cell densities evaluated with multi-IF were compared using the Mann-Whitney U test.

Bioinformatical analyses are detailed in the eMethods (links.lww.com/NXI/A734). Differential gene expression analysis was performed using DESeq2 v1.32.0 R package by comparing gene expression between Yo-PCD and controls. Hierarchical clustering was performed using the ComplexHeatmap v2.8.0 R package using the Euclidean distance and the Ward clustering method. Gene Ontology (GO) enrichment was performed using clusterProfiler v4.0.0 R package on overexpressed and underexpressed Yo-PCD genes separately, and all genes used for differential gene expression were used as background. Immune cell populations were analyzed using the Microenvironment Cell Populations-counter (MCP-counter) method and the MCPCounter v1.1 R package.

**Study Approval**

This study is part of the project Gene PNS (NCT03963700) and was approved by the institutional review board of the Hospices Civils de Lyon. Tumors and other biological samples were collected after patients gave informed and written consent.

**Data Availability**

RNA-seq expression, differential expression results, and GO enrichment analysis data are available online (zenodo.org/record/6477807, doi: 10.5281/zenodo.6477807). RNA-seq raw data (FASTQ) will be shared with any qualified investigator on request.

**Results**

**Clinical and Pathologic Cohorts**

Thirty patients with Yo-PCD associated with a BC were identified; 1 patient was excluded because of missing clinical data. For the 29 patients with Yo-PCD included, the histopathologic data of 34 BCs were collected (Table 1); 17 patients had available FFPE tissue samples: 6 axillary lymph nodes and 16 primary BCs. One patient had 3 available samples (patient 1), and 3 patients had 2 available samples (patients 5, 6, and 13); a total of
22 samples were analyzed. Histologic data on each available tumor sample are detailed for each patient in Table 2, and the study flowchart summarizing the methods and analyses applied to characterize Yo-PCD BCs compared with control BCs is presented in Figure 1.

Yo-PCD BCs Are Mainly HER2-Positive and HR-Negative Invasive Carcinomas of No Special Type

Thirty-one of the 34 (91.1%) Yo-PCD BCs were invasive carcinomas of no special type (NST). The Nottingham grade\(^1\) was available for 25 samples: 19 BCs were grade 3 (55.9%), and the other 6 were grade 2 (17.6%). IHC found that HER2 was overexpressed in 29 (85.3%) Yo-PCD BCs and amplification of ErbB2, encoding HER2, was found in 17 of 22 (77.2%). HRs, namely PR and OR, were both negative in 26 BCs (76.4%). Overall, the majority (67.6%) of Yo-PCD BCs were HER2-positive and HR-negative invasive carcinomas of NST, also called HER2-driven BCs (Table 1).

Yo-PCD BCs Are Small-Sized BCs Early Metastasizing in Regional Lymph Nodes

Overall, 20 of 34 (58.8%) Yo-PCD BCs were T1 or lower and 23 of 34 BCs (67.6%) had ipsilateral axillary lymph node metastasis at diagnosis (Table 1). Seven of the 34 BCs (20.5%) had an occult BC (OBC) with axillary lymph node metastasis for which a definitive pathologic diagnosis was obtained after lymph node biopsy without detection of any primary breast tumor following appropriate radioclinical
Figure 3 Differentially Expressed Genes and Immune Cell Type Estimates Between Control and Yo-PCD Breast Cancers

(A) Heatmap of the differentially expressed genes (in rows) between Yo-PCD (red) and control (dark blue) samples (in columns). Tumor sites are separated in primary breast cancer (BC; yellow) and metastatic lymph node (light blue). TPM expression values were first transformed into log10(TPM + 0.01) and then transformed into a Z score per gene. Panels B and C show boxplots of the median value and interquartile range (IQR) for the concerned variable of control (on the left, in blue) and Yo-PCD BC samples (on the right, in red); the upper whisker extends from the hinge to the largest value no further than 1.5 × IQR, and the lower whisker extends to the smallest value at most 1.5 × IQR from the hinge; each dot represents the value of a sample. *p* Values of comparisons between Yo-PCD and control groups using the Wilcoxon rank-sum test are adjusted with the Benjamini and Hochberg method (see eMethods for detail, links.lww.com/NXI/A734) and shown on the top of each couple of boxplots. Yo-PCD samples from metastatic lymph nodes have been removed. (B.a–B.j) Boxplots of MCP-counter estimates for 3 classical cell types in control and Yo-PCD samples. (C.a-b) Boxplots of TPM expression of 2 typical plasma cell markers in control and Yo-PCD samples. PCD = paraneoplastic cerebellar degeneration.
Yo Genes Are Mutated, Amplified, and Overexpressed in Yo-PCD BCs

As CDR2 and CDR2L are the targets of the anti-Yo antibodies, a first focus was made on these 2 proteins. The mutational status of Yo genes (CDR2 and CDR2L) was assessed by DNA and/or RNA sequencing on 12 Yo-PCD BC samples from 8 different patients (1A, B; C; 3; 6A, B; 10; 12; 13A, B; 14; and 15). Sequence alterations were found in 6 Yo-PCD BCs (1B, 1C, 3, 9, 12, and 14; Table 2): 3 in CDR2 (n = 3) and 3 in CDR2L including an in-frame fusion (Figure 2A). Only one of these variants (R216W, patient 12) has been reported in the Single-Nucleotide Polymorphism Catalogue Of Somatic Mutations In Cancer (COSMIC) database. This, together with the absence of mutations in the matched constitutional counterpart (available for patients 3 and 9) and in the blood DNA of 10 patients with Yo-PCD without evaluable tumor tissue, indicated that these mutations were somatic.

Four of these mutations resulted in amino acid substitutions in the protein sequence, and 1 leads to protein truncation. Overall, 5 of 8 (62.5%) patients with Yo-PCD displayed at least 1 alteration in the CDR2 or CDR2L gene.

Information on CDR2L CNV was available for 13 samples by CGHa and fluorescent in situ hybridization (FISH; n = 9), CGHa alone (n = 1), or FISH alone (n = 3), 8 of which displayed amplification (61.5%) and 2 had a significant gain in 17q25 carrying the CDR2L gene (Figure 2B). By contrast, amplification of CDR2L was found in 1 of 15 (6.6%) control samples (p < 0.0001). Of note, CDR2L and ErbB2 both are on 17q chromosome, but, when associated, amplifications of both genes were not dependent on the same amplicon. Information on CDR2 CNV was available for 10 samples by CGHa: 3 patients had a significant gain, but no amplification of this gene was found. Overall, all included patients with Yo-PCD had at least 1 BC sample harboring at least 1 alteration in CDR2 or CDR2L: mutations (62.5%), amplifications (61.5%), or gains (38.4%) irrespective of the tumor site (primary tumor or metastasis). These alterations concerned only CDR2L for 9
An exhaustive list of differentially expressed genes is available in eTable 1 (links.lww.com/NXI/A734). Hierarchical clustering based on the RNA expression profile of the 669 differentially expressed genes separates the samples into 2 main clusters: Yo-PCD BC samples including both primary (n = 9) and metastatic (n = 3) sites on one side and HER2-driven matched control BC samples on the other side. A core set of genes was constantly downregulated in Yo-PCD BCs, making it a putative hallmark of Yo-PCD transcriptomic profiles (Figure 3A). Functional GO enrichment analysis on this downregulated cluster found a predominance of extracellular matrix (ECM)-related genes, whereas overexpressed genes were mainly related to adaptive immune activation. Detailed GO enrichment analysis is provided in eTable 2 and eTable 3 (links.lww.com/NXI/A734).

Yo-PCD BCs Are Distinguished by Their Immune Cell Infiltration

GO enrichment analysis on overexpressed genes found an enrichment in B-cell and T-cell activation and proliferation pathways and adaptive immune response final pathways in Yo-PCD BCs (eTable 2 and eTable 3, links.lww.com/NXI/A734). The striking proportion of immune-related gene overexpression suggested a massive infiltration by immune cells in Yo-PCD compared with controls. To further analyze and quantify the implicated cell populations, we conducted an MCP-counter analysis exclusively on primary BCs in both groups. As expected, we found no difference in innate immunity components (neutrophils, NK cells, and monocyte lineage; Figure 3B). Analysis of B lineage and T cells revealed a strong intersample variability in Yo-PCD BCs. Three samples (1A, 1B, and 13A) had both low T-cell and B-cell counts and corresponded to samples of patients with another prevalent tumor (namely, 1C and 13B) with a higher MCP-score of adaptive immune cells. Indeed, a markedly elevated B- and/or T-cell MCP-score is found in the remaining 6 samples, probably accounting for the aforementioned adaptive immune pathway enrichment. However, all Yo samples, irrespective of their B- and T-cell MCP-score, were characterized by a significant overexpression of plasma cells/plasma blast genes (MZB1 and TNFRSF17; Figure 3C), suggesting that the latter cells may have a pivotal role in this strong adaptive immune reaction in Yo-PCD BCs.

An IgG-Plasma Cell Attack Characterizes Yo-PCD HER2-Driven BCs

Seven-color IF staining and digital image analysis were used to quantify tumor-infiltrating T cells, B cells, myeloid dendritic cells (mDCs), and IgG- and IgA-plasma cells in patients with available primary FFPE tumor tissue. These found a clearly more intense and diffuse infiltration by a wide variety of immune cells within and around the tumor tissue of Yo-PCD BCs compared with HER2-driven controls (Figure 4A) and confirmed the considerable proportion of IgG plasma cells in Yo-PCD BCs. Indeed, the median density (number of cells/mm²) of T cells (CD3; Figure 4B) and mDC (DC-LAMP, data not shown) did not significantly differ between Yo and controls, whereas CD20 + B cells and most prominently IgG (but not IgA) plasma cell densities were significantly higher in Yo-PCD BCs compared with controls (Figure 4C and D).

Discussion

In our understanding of immune tolerance breakdown leading to paraneoplastic neurologic diseases, genetic alterations of onconeural antigens stand as a key element. Here, as in Yo-PCD ovarian cancer,⁷ nearly two-thirds of the patients with Yo-PCD with a BC presented with at least 1 somatic alteration of a Yo gene, although alterations of these genes are very rare in BCs, including in HER2-positive ones, according to COSMIC database (<1%).¹⁹ Although somatic mutations were also described in Yo ovarian cancers,⁷ none was common to BCs and ovarian cancers, but all mutations have direct consequences on the corresponding protein and are thus likely to generate neoepitopes. However, this scenario of neoantigen creation does not fully apply to each Yo-PCD BC in this study. In that respect, gain in copy number leading to onconeural antigen overexpression itself may be a sufficient trigger for the occurrence of an immune tolerance breakdown, irrespective of the creation of neoepitopes, as it has been suggested by others concerning paraneoplastic anti-THSD7A membranous nephropathy.²⁰ Amplification (≥6 copies) mainly concerned CDR2L, and it was demonstrated herein that these CDR2L amplifications result in CDR2L transcript and protein overexpression in Yo-PCD BCs. Conversely, there were only infrequent copy number gains (<6) for CDR2, and these did not produce any detectable increase of CDR2 expression. Thus, as already highly suspected,²¹ the data presented herein support that CDR2L could be the major antigen in Yo-PCD, irrespective of the nature of the associated tumor. Yet, if strong similarities exist between Yo-PCD ovarian tumors and BCs, from the frequency of Yo genetic alteration to the intensity of the intratumor immune attack,⁷ 2 specificities of Yo BCs suggest mechanistic differences in the immune tolerance breakdown triggering PCD between these 2 tumor types. First,
CDR2L amplification seems to be pivotal in Yo-PCD BCs, whereas ovarian Yo-PCD cancers only present gain in copy number. Second, as previously described, a strong over-representation of HER2-positive BCs harboring both ERBB2 gene amplification and HER2 overexpression in Yo-PCD BCs was found herein, whereas Yo-PCD ovarian cancers do not display ERBB2 amplification. Although ERBB2 and CDR2L are located at the same chromosomal region (17q21-25), CDR2L amplification does not seem to be a consequence of ERBB2 amplification, and FISH analysis ruled out double-minute chromosome; hence, the mechanism of this double amplification remains elusive. Further studies are needed to decipher this mechanism, as well as the interactions of these 2 proteins in PCD pathogenesis in Yo-PCD BCs, which are not only HER2-positive but also HR-negative invasive carcinomas of NST. This HER2-driven histopathologic signature is otherwise very marginally represented among BCs (around 3.2%), which makes it a hallmark of Yo-PCD BCs. From a clinical perspective, HER2 negativity should thus raise suspicion of an alternative tumor site in the diagnostic workup for the underlying neoplasm in Yo-PCD. From a mechanistic perspective, this HER2-driven signature of Yo-PCD BCs is most probably implicated in the peculiar immune attack characterizing these BCs; several studies have described an overrepresentation of tumor-invasive CD4+ and CD8+ T cells and B cells in HR-negative BCs. A potential explanation for this extraimmunogenic feature lies in the mutational load (number of somatic mutations per tumor), which is substantially higher in HR-negative than in HR-positive BCs and mirrors the neoantigen load. The implication of HER2 positivity per se in tumor immunogenicity is, for its part, still elusive. But beyond this HER2-driven feature, other mechanisms might support Yo-PCD development because the tumors investigated herein presented a markedly more intense immune infiltration compared with HER2-driven controls. The most overrepresented immune cells were IgG-plasma cells suggesting the prominence of humoral immune attack in Yo-PCD BCs, probably linked with the production of Yo autoantibodies. This may also be related to a potential earlier diagnosis of Yo BCs allowing to capture the early phenomenon of the immune attack, although the equivalent tumor size distribution between Yo and control BCs paves for the equivalent time of evolution. Taken together, this suggests that immune cells participate in an effective antitumor attack; however, as functional aspects could not be analyzed herein because retrospective collection can only concern fixed samples, it is also possible that these cells or a subset of these are exhausted. This is supported by the overwhelming proportion of lymph node–positive BCs among Yo-PCD BCs (80% vs 43% in HER2-driven BC series with a similar tumor size distribution), implying that cancer cell migration escapes from immune surveillance. Even more striking is that more than a fifth of patients with Yo-PCD had OBC, whereas such a situation is rare in the general population of patients with BC (prevalence estimated to be 0.1%). Considering OBC, HER2 positivity and HR negativity are 2 known risk factors, but, taken together, HER2-driven BC is only slightly overrepresented in OBC cohorts (3.1% in OBC vs 2.3% in non-OBC in the study reported in reference 29). Isolated DCIS concomitant to lymph node metastasis is another rarity (4.4% of DCIS), not so infrequent in the present cohort of Yo-PCD BCs (2/3 DCIS with lymph node metastasis). Taken together, this suggests that Yo-PCD BCs show a strong and early propensity for lymphatic metastasis in their lifespan. In practice, awareness about this peculiar behavior of Yo-PCD BCs is critical to optimize the search for the underlying tumor: axillary lymph nodes should be promptly analyzed even without evidence of ipsilateral BCs. Such regression of the primary tumor (occult primary tumor) with concomitant lymph node metastasis is regularly described in melanoma and interpreted as the result of an effective past immune attack of the primary site. A particularity of Yo-PCD BCs is the concomitant observation of an intense intratumor immune response and early metastasis, which is not described in regressive melanomas. This could suggest that tumor microenvironment is particularly permissive to tumor cell migration in Yo-PCD BCs that may be related to the under-expression of ECM compounds characterizing Yo-PCD BC transcriptomic profiles.

Conclusion

This study indicates that there is a morphophenotypical signature of Yo-PCD BCs: invasive HER2-driven carcinoma overexpressing mutated CDR2L and metastasizing early to regional lymph nodes despite massive infiltration by effector immune cells with an overwhelming predominance of IgG-plasma cells. This specific BC profile, closely linked to HER2-driven carcinogenesis, undoubtedly participates in triggering the immune tolerance breakdown, leading to Yo-PCD autoimmunity.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/NN for full disclosures.

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| Name                  | Location                                                                 | Contribution                                                                 |
|-----------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Elise Peter, MSc      | Synaptopathies and Autoantibodies (SynatAc) Team, Institut NeuroMyoGène-MeLiS, INSERM U1314/CNRS UMR 5284, Université de Lyon; French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon; University of Lyon, Université Claude Bernard Lyon 1, France | Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data |
| Isabelle Treilleux, MD, PhD | Department of Biopathology, Centre Leon Berard, Lyon, France | Major role in the acquisition of data and analysis or interpretation of data  |
| Valentin Wucher, PhD   | University of Lyon, Université Claude Bernard Lyon 1; INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon, France | Drafting/revision of the manuscript for content, including medical writing for content, and analysis or interpretation of data |
| Emma Jouglia, MSc      | University of Lyon, Université Claude Bernard Lyon 1; INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon, France | Major role in the acquisition of data |
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| Justine Berthet, MSc   | Department of Biopathology, Centre Leon Berard; Cancer Genomics Platform, Department of Translational Research, Centre Leon Berard; Laboratoire d’Immunothérapie des Cancers de Lyon (LICL), France | Major role in the acquisition of data |

### Appendix (continued)

| Name                  | Location                                                                 | Contribution                                                                 |
|-----------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Géraldine Picard      | French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon, France | Major role in the acquisition of data |
| Véronique Regemondon, PhD | University of Lyon, Université Claude Bernard Lyon 1; INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon, France | Major role in the acquisition of data |
| Marine Villard        | French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon, France | Major role in the acquisition of data |
| Clémentine Vincent, PhD | University of Lyon, Université Claude Bernard Lyon 1; INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon, France | Major role in the acquisition of data |
| Laurie Tonon, PhD     | Synergie Lyon Cancer-Bioinformatics Platform-Gilles Thomas, Centre de Recherche en Cancérologie de Lyon | Analysis or interpretation of data |
| Alain Viari, PhD      | Synergie Lyon Cancer-Bioinformatics Platform-Gilles Thomas, Centre de Recherche en Cancérologie de Lyon | Analysis or interpretation of data |
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| Bertrand Dubois, PhD  | INSERM 1052, CNRS 5286, Centre Leon Berard, Centre de Recherche en Cancérologie de Lyon; Laboratoire d’Immunothérapie des Cancers de Lyon (LICL), France | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data |
| Virginie Desestret, MD, PhD | Synaptopathies and Autoantibodies (SynatAc) Team, Institut NeuroMyoGène-MeLiS, INSERM U1314/CNRS UMR 5284, Université de Lyon; French Reference Center on Paraneoplastic Neurological Syndrome, Hospices Civils de Lyon; University of Lyon, Université Claude Bernard Lyon 1, France | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data |
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