LETTER TO THE EDITOR

Diffuse large B cell lymphomas relapsing in the CNS lack oncogenic MYD88 and CD79B mutations

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Diffuse large B cell lymphoma (DLBCL) is a clinically and molecularly heterogeneous disease. The majority of patients respond to immunochemotherapy, generally consisting of rituximab in combination with cyclophosphamide, doxorubicin, vincristine, prednisone and 60–70% can be cured. Approximately 30% will develop a relapse, of which relapses in the central nervous system (CNS), although relatively rare (~1%), carry a particularly poor prognosis. The factors that determine homing to extranodal sites such as the CNS are largely unknown, but a much higher incidence of CNS relapse is observed in very aggressive lymphoma types such as Burkitt lymphoma, lymphoblastic lymphoma, and primary testicular lymphoma (PTL).

We and others have previously demonstrated that primary CNS lymphomas (PCNSLs) and PTLs, both arising at immune-privileged sites, are characterized by a high frequency of oncogenic mutations in both MYD88 and CD79B, causing chronic active B cell receptor (BCR) signaling, and in MYD88, an adapter protein that mediates toll-like receptor (TLR) and interleukin-1 receptor signaling. Both mutations ultimately lead to activation of the NF-kB pathway. Although they are found almost exclusively in activated B cell type (ABC-type) DLBCL, there is a striking difference in the prevalence of MYD88 mutations in PCNSL (75%) and PTLs (71%) versus nodal lymphomas (17%) and gastrointestinal (11%) lymphomas.

To explore whether MYD88 and CD79B mutations are preferentially associated with DLBCL originating in the CNS or testis, or whether they are also present in DLBCL relapsing in the CNS, we tested a panel of 14 patients with CNS relapse of a DLBCL. These patients, with either leptomeningeal and/or brain parenchymatous relapse, were treated in the phase II HDVON08 NHL trial with reinduction chemotherapy (consisting of three cycles of R-DHAP-MTX (dexamethasone 40 mg on days 1–4, cisplatin 100 mg/m² on day 1, cytarabine 2 x 2 g/m² on day 2, rituximab 375 mg/m² on day 5, methotrexate 3 g/m² on day 15) and intrathecal rituximab (registered at www.trialregister.nl as NTR1757, EudraCT number 2006-002141-37). Patients with either a partial or a complete response received consolidation with busulfan/cyclophosphamide and autologous stem cell transplantation; all others went off protocol. A total of 36 eligible patients, aged 23–65 years (median 57 years) were treated between 2007 and 2011, 24 of whom had parenchymal localizations on MRI and 18 of whom had a leptomeningeal relapse. The overall response rate for these patients was 53% (28% of patients reached a complete response), with a median response duration of 6 months. Mutation analysis was performed in the 14 patients for whom either brain biopsy material or tumor-positive cerebrospinal fluid (on the basis of pathology and/or immunophenotyping results) was available. For 13 of these patients the biopsy material obtained at primary diagnosis could also be retrieved.

We used a panel of allele-specific PCRs covering all major mutation (hot) spots to detect somatic mutations in MYD88 and CD79B. As recently reported, this strategy permits efficient and sensitive detection of mutations. The detected mutations were verified by Sanger sequencing.

None of the 27 tested samples (13 primary material, 14 relapse material) showed CD79B mutations and a MYD88 mutation was found in 3/14 CNS relapse patients only (21%; Table 1).

Table 1. Clinical characteristics of DLBCL relapsing in the CNS and results of MYD88 and CD79b PCR

| Patient | Year of primary lymphoma diagnosis | Localization of biopsy | Year of CNS relapse | Age at CNS relapse | Sex | Parenchymal, CSF or other CNS site of relapse | MYD88 primary localization | MYD88 relapse brain/CSF | CD79B primary localization | CD79B relapse | If CSF, clonal BCR in CSF? |
|---------|-----------------------------------|------------------------|---------------------|-------------------|-----|---------------------------------------------|-----------------------------|---------------------------|--------------------------|----------------|---------------------------|
| 001     | 2005                              | Bone                   | 2007                | 64                | F   | Brain                                      | --                         | --                        | --                       | --             | n.a.                      |
| 002     | 2006                              | Lung                   | 2007                | 62                | F   | Brain/CSF                                  | --                         | --                        | --                       | --             | --                        |
| 003     | 2003                              | Testis                 | 2008                | 58                | M   | Brain                                      | +                          | --                        | --                       | --             | n.a.                      |
| 004     | 2002                              | LN                     | 2009                | 58                | F   | Brain                                      | n.d.                       | --                        | --                       | --             | n.a.                      |
| 005     | 2009                              | Bone                   | 2009                | 56                | M   | Brain                                      | --                         | --                        | --                       | --             | n.a.                      |
| 006     | 2009                              | LN                     | 2009                | 58                | M   | Brain                                      | --                         | --                        | --                       | --             | n.a.                      |
| 007     | 2009                              | BM                     | 2009                | 47                | M   | CSF                                        | --                         | --                        | --                       | --             | n.a.                      |
| 008     | 2001 LPLa                         | LN                     | 2010                | 58                | M   | Brachial nerve                              | + (LN)                     | --                        | --                       | --             | n.a.                      |

Abbreviations: BCR, B cell receptor; CNS, central nervous system; CSF, cerebrospinal fluid; DLBCL, diffuse large B cell lymphoma; LN, lymph node; n.a., not applicable; n.d., not done. *Patient was diagnosed with lymphoplasmacytic lymphoma (LPL)/Waldenstrom Macroglobulinemia (IgM Mprotein present) in 2001 and with nodal transformation to DLBCL in 2010, with subsequent neurological symptoms, brain parenchymal lesions and a lesion in the brachial nerve. The brachial nerve biopsy was positive for DLBCL.
Remarkably, of the three samples containing a MYD88 mutation, two patients had been originally diagnosed with PTL, whereas the third patient had been diagnosed with a lymphomatous lymphoma (LPL), with subsequent transformation to a (nodal) DLBCL. In all the three positive cases a leucine-to-proline exchange at position 265 (L265P) was demonstrated, the most frequently found ‘hotspot’ mutation in both DLBCL and Waldenstrom’s macroglobulinemia.\(^1\)\(^,\)\(^2\) In all three cases the primary material also carried the mutation. Of the 11 DLBCL not originating from either PTL or LPL none displayed a MYD88 mutation.

We have previously demonstrated that MYD88 mutations are highly prevalent in both PCNSL and PTLs, but not in lymphomas originating in ‘professional’ lymphoid organs/tissues such as the lymph nodes or the Peyer’s patches in the gastrointestinal tract. Frequently, in these tumors a CD79B mutation could also be found.\(^6\)\(^,\)\(^7\) These findings support the concept that IP-DLBCLs present a pathogenetically distinct group of lymphomas and we propose that mutational activation of TLR/MYD88-signaling endows lymphoma-initiating cells with a selective growth advantage at immune-privileged sites. These tissues are barrier-protected and immunologically silent and, in marked contrast to lymph nodes and mucosa-associated lymphoid tissues, will presumably provide only limited stimulation by TLR ligands. The (concomitant) presence of CD79B (or other BCR-pathway) mutations, causing chronic active BCR signaling, may further promote the selective outgrowth of the tumor cells within these relatively stimulus-poor microenvironments. Our current finding that DLBCL relapsing in the CNS lack these mutations (unless the primary lymphoma was either a PTL or an LPL) supports the hypothesis that these molecular alterations are instrumental for tumor initiation at immune-privileged sites but not for homing of lymphoma cells to the CNS. Mechanisms guiding the latter process remain to be unraveled.

In conclusion, previous studies by us and others indicate that MYD88 mutations, and to a lesser extent CD79B mutations, are important drivers of lymphomagenesis in PCNSL and PTL, but our current results imply that these mutations do not play a role in lymphomas relapsing in the CNS. This may have important therapeutic consequences, as the patients with tumors containing MYD88 and/or CD79B mutations will more likely benefit from therapies targeting MYD88-signaling components like the IRAK kinase inhibitors, either alone or in combination with drugs blocking key mediators of BCR signaling such as Bruton’s tyrosine kinase.\(^12\)\(^,\)\(^13\)

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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