

## **Title**

Richter's Transformation as leptomeningeal infiltration in a Chronic Lymphocytic Leukemia patient receiving Venetoclax. Could blood-brain barrier be a disease "sanctuary" during Venetoclax treatment?

## **Running Title**

Leptomeningeal Richter's Transformation on Venetoclax

## **Keywords**

Chronic Lymphocytic Leukemia, Richter's transformation, Venetoclax, leptomeningeal infiltration, blood-brain barrier

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## Abstract

We present a unique Richter's transformation case in CNS with identical to CLL clonal origin in a patient treated with Venetoclax. With our case we make implications on whether Venetoclax penetrates the blood-brain barrier and we address the debating issue of the appropriate Venetoclax dose in case of drug-drug interactions.

## Intoroduction

Richter's transformation(RT) is the development of high-grade non-Hodgkin lymphoma, prolymphocytic leukemia, Hodgkin disease, acute leukemia in patients with a previous or concomitant diagnosis of chronic lymphocytic leukemia(CLL) or small lymphocytic lymphoma(SLL)<sup>1</sup>.

Clinical features associated with RT include systemic symptoms(59%), progressive lymphadenopathy(64%), extranodal involvement(41%), elevation of lactate dehydrogenase (LDH) (82%), and monoclonal gammopathy(44%). Systemic symptoms include fever, weight loss, and/or drenching night sweats<sup>2</sup>.

Most (80%) RT cases presenting as high grade B cell non-Hodgkin lymphoma have the same clonal origin with the underling CLL/SLL and only 20% arise from a different clone<sup>3,4</sup>. The knowledge of clonal relationship between the non-Hodgkin lymphoma and CLL is important, since the prognosis varies; when the clonal origin is identical, survival is reported to be very short (8-14 months), but when is different survival is reported to be comparable to that of patients with de novo Diffuse Large B Cell Lymphoma(DLBCL)<sup>3</sup>.

RT occurs in approximately 2% to 10% of CLL patients during their disease course, with a transformation rate of 0.5% to 1% per year<sup>5</sup>. Risk factors associated with RT include clinical characteristics(e.g. Rai stage), genetic CLL characteristics(e.g. BCL-2 germ line polymorphisms, NOTCH-1 mutations, TP53 disruption), biological CLL characteristics(e.g. IgVH mutational status) and type of therapy<sup>3-5</sup>.

There is no data to support that novel agents (B cell receptor -BCR-inhibitors or BCL-2 inhibitor) are associated with increased RT incidence in comparison to chemotherapy/chemoimmunotherapy<sup>6-8</sup>. However, in all published clinical-trial and real-world data from these new agents, RT typically appears early, within 1 year of the treatment initiation<sup>6-8</sup>.

Central nervous system (CNS) involvement is rare in RT<sup>2</sup> with unknown definite incidence, since the only existing data is case reports. Half of these cases present as leptomeningeal infiltration<sup>9-11</sup>.

We present a very unusual RT case with leptomeningeal infiltration by high grade B-non Hodgkin lymphoma in a CLL patient treated with Venetoclax.

### Case Presentation

A 56-year-old female patient was diagnosed with CLL (CD5+, CD23+, FMC7<sup>weak</sup>, CD79b<sup>weak</sup>, CD20<sup>weak</sup>, sIgκ<sup>weak</sup>), Binet stage B, asymptomatic in January 2008. She remained under close monitoring until January 2011, when she developed Coomb's positive autoimmune hemolytic anemia (AIHA), which was successfully managed with corticosteroids and cyclosporine. In April 2011 progressive splenomegaly and lymphadenopathy occurred. The IgVH sequencing analysis<sup>12</sup> showed an unmutated VH1-69\*10 clone. FISH analysis for del17p was negative and no TP53 mutations

were identified by Sanger sequencing. The patient received Rituximab-Chlorambucil (10 mg/m<sup>2</sup> for 7d), but progressive disease (PD) was noted after 3 months of therapy (June 2011). She subsequently received 6 cycles of FCR (June 2011-November 2011), achieving partial remission(PR) and then she remained under close follow up. In June 2012, the patient presented serous lesions(bullae) in lower extremities. A biopsy revealed paraneoplastic pemphigus. There was no CLL progression at this time. Corticosteroids and Rituximab was given without improvement and finally treatment with azathioprine(100 mg QD) resulted in resolution of the lesions.

In June 2015(48 months after FCR) PD with progressive splenomegaly(7 cm below left costal margin-blcm), lymphadenopathy and thrombocytopenia(PLTs=78 10<sup>9</sup>/L) was noticed. No del17p/TP53 mutations were detected and bone marrow karyotype was normal. The patient received Ibrutinib(starting dose 420 mg QD) but severe bruising of upper and lower extremities occurred. Until December 2015 the patient was receiving Ibrutinib at the lower dose of 140 mg QD due to recurrence of bruising at higher doses. CLL remained stable during Ibrutinib treatment and the patient was switched to Idelalisib/Rituximab in February 2016. No del17p/TP53 mutations were detected at that time and bone marrow karyotype was normal. With Idelilisib/Rituximab, an initial improvement of splenomegaly(best response 3 cm blcm) and lymphadenopathy was noted, but 6 months later(September 2016) PD was noticed, with fever, bulky and progressive splenomegaly(20 cm blcm), lymphadenopathy and thrombocytopenia. A new bone marrow karyotype at that time was complex (more than 5 chromosomal abnormalities, including del17p):

46,XX,del(6)(q21q24),-17,+mar[3]/46,XX,-6,+der(?)t(1;?)(q21;?)[4]/46,XX[16].

We decided to treat the patient with Venetoclax(available through an early access program). The ramp up period of Venetoclax started on 24-Oct-2016. Her blood counts(CBC) were: WBC=21.5  $10^9/L$ , Lymph=95%, Hb=8.6 g/dL, PLTs=52  $10^9/L$ . The patient received the final dose of 400 mg QD on 29-Nov-2016. At this time there was considerable improvement of the disease burden: no fever, good performance status (PS), spleen size reduction to 7 cm blcm, no peripheral lymphadenopathy. CBC: WBC= 2.2  $10^9/L$ , PMN=1.3  $10^9/L$  (with GCSF support), Hb=8.9 g/dL, PLTs=75  $10^9/L$ .

On 20-Mar-2017 reactivation of AIHA was noticed. Venetoclax was discontinued and the patient received corticosteroids and cyclosporine, which managed to control AIHA.

On 27-Apr-2017 Venetoclax was reinitiated through a new ramp up period with cyclosporine, to a final dose of 200 mg QD, due to CYP3A interactions. Despite Venetoclax dose reduction, neutropenia grade 4 occurred mandating continuous GCSF administration and further dose reduction of Venetoclax to 100 mg QD. There was adequate control (PR) of CLL for almost 20 months, with further reduction of splenomegaly to 2 cm blcm, no B symptoms, improved PS:0, no need for transfusions, adequate PLT: 70-90.000/ $\mu L$ , Hb between 11-12 g/dL and PMN: 1000-1500/ $\mu L$ , with frequent GCSF support.

In June 2018(20 months after Venetoclax initiation) the patient developed walking and speech difficulties. She was admitted to the hospital with confusion and impaired mental status on 23-Jun-2018. On physical examination there was reappearance of splenomegaly(8 cm blcm), with no palpable lymphadenopathy. She was afebrile without significant deterioration of CBC: WBC=2,7  $10^9/L$ , PMN=2.2  $10^9/L$ ,

Lymph=0.3  $10^9$ /L, Hb=9.6 g/dL, PLTs=51  $10^9$ /L. Serum LDH was normal and no serum monoclonal protein was detected. Serum biochemistry analysis was within normal limits. No lesions were found in brain MRI. The cerebrospinal fluid(CSF) aspiration analysis showed 2.4  $10^9$  cells/L and increased protein level=1510 mg/L (range: 150-450). The cytopsin of the aspirate stained with May-Gruenwald-Giemsa (figure 1,A) revealed monomorphous large, cerebriform lymphoid cells with nucleoli. The CSF's cells immunophenotype showed a large clonal B lymphoid population(98% of cells): sIg  $\kappa^{weak}$ , CD20<sup>dim</sup>, CD79b neg, CD5neg, CD23+, CD200+ (figure 1, B). Lymphocytes with the same morphology and immunophenotype were present in peripheral blood (1%) and in bone marrow (50%).

We performed mutational analysis of the IgVH genes according to BIOMED-2 recommendations<sup>12</sup> in the CSF sample and we found the identical unmutated VH sequence (VH1-69\*10) as in the diagnosis sample (Jan-2008, 99.18% identity to germ line sequence).

The patient received high-dose dexamethasone(40 mg QD) for 4 days and intrathecal infusion of dexamethasone(8 mg) and methotrexate(15 mg), but she died in coma 7 days later, on 4-Jul-2018.

## Discussion

The best of our knowledge this is the first case of RT presenting as leptomeningeal infiltration by high grade B-non Hodgkin lymphoma in a CLL patient, treated with Venetoclax. It is also the first reported case that IgVH mutational analysis has been performed and revealed identical clonal origin of CLL and RT during Venetoclax

treatment<sup>7</sup>. Morphologically the lymphoma cells were large with nucleoli and had lost CD5 antigen positivity. This unusual type of RT has been anecdotally described previously in CLL patients treated with chemotherapy (mainly purine analogs)<sup>9-11</sup>.

RT has been described in CLL patients treated with novel agents (Ibrutinib, Idelalisib, Venetoclax)<sup>5-7</sup>. The incidence of RT for these patients ranges between 4,5% and 16%<sup>10</sup> and has been found to be higher during the 1<sup>st</sup> year of treatment and among patients with del17p<sup>5-8</sup>. This observation implies that some patients enter treatment with preexisting occult features of RT<sup>7,8</sup>. Most of the cases involve transformation to high grade B cell lymphomas(mainly DLBCL)<sup>5-8</sup>. Our patient had high risk characteristics when Venetoclax was initiated: complex karyotype, del17p, heavily pretreated(4 previous lines of therapy), excessive splenomegaly, failure to both Ibrutinib and Idelalisib. With these high risk characteristics one could suggest that RT “foci” might be already present. However, sustained PR was obtained with Venetoclax for almost 2 years, with improved quality of life to the patient.

Another question arising from this unusual RT case is whether Venetoclax penetrates the CNS blood-brain barrier, since RT occurrence mainly affected CNS and the patient finally died due to neurological complications. Very few published data exists: in animal studies with mice receiving radiolabeled intravenous Venetoclax, CSF levels of the drug were absent(below measurable limits)<sup>13</sup>. However, in a recent case report of a patient receiving Venetoclax and intrathecal therapy (cytarabine and methotrexate) for CLL relapse in CNS, clearance of CNS disease was observed. In this patient, Venetoclax CNS levels were measured at 0.1% of the plasma Venetoclax concentrations<sup>14</sup>. Our RT case with CNS localization during Venetoclax treatment, implies that CSF concentrations of the drug may be suboptimal, allowing a “sanctuary” for CLL evolution. One of course might argue that low Venetoclax



dose(100 mg/d) could be a reason for low/absent CSF concentrations. As mentioned, the Venetoclax dose was reduced to our patient due to the concomitant use of the moderate CYP3A inhibitor cyclosporine and severe neutropenia. Our case suggests that Venetoclax dose reductions in CLL treatment, in the setting of toxicity and /or CYP3A interactions, should be very carefully done, in multi -resistant CLL patients with adverse cytogenetic and molecular features.

Finally, our case confirms that RT remains an unmet therapeutic need in CLL patients, even in the era of new “targeted” therapies.

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Figure 1 Legend

Cerebrospinal fluid (CSF): A. cytopsin, stained with May-Gruenwald-Giemsa:

monomorphous large cerebriform lymphoid cells with nucleoli. B. CSF

Immunophenotype: large B lymphocytes, CD5 neg, CD79b neg,  $k^{weak}$ ,  $CD20^{dim}$ ,

CD200 +