

## Genetics of “high-risk” chronic lymphocytic leukemia in the times of chemoimmunotherapy

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Chronic lymphocytic leukemia (CLL) is a common leukemic B-cell lymphoma driven by distinct molecular features such as autonomous B-cell receptor signaling and genetic alterations including mutations targeting the DNA damage machinery, RNA processing and splicing, oncogenic signaling pathways (such as Notch) as well as epigenetic and chromatin modification.<sup>1-3</sup> In a simplified model, the “sum” of autonomous B-cell receptor signaling and driver mutations govern CLL progression. In addition, mutations in individual genes, such as *TP53*<sup>4,5</sup> are tightly linked to refractoriness to chemotherapy.<sup>6</sup> This model summarizes current knowledge, but we cannot exclude the possibility that additional (maybe unknown) mechanisms contribute significantly to proliferative drive and may thus predispose (or select for?) driver mutations. The current emergence of additional data and more appropriate statistical tools to query complex molecular data can be expected to provide novel insights into the pathogenesis of CLL.<sup>7</sup>

The study by Jennifer Edelmann and colleagues published in this issue of *Haematologica* is an informative addition to the catalogs of gene mutations in CLL. In this study, Edelmann and colleagues use cohorts of patients treated with chemotherapy/alemtuzumab in multiple trials and high-resolution single nucleotide polymorphism-array profiling and sequencing to characterize copy number variants and a limited mutational landscape of high-risk CLL cases.<sup>8</sup> The analysis summarizes data from 146 patients from CLL trials (CLL8, CLL11, CLL20), in which high risk was defined as either a *TP53* deletion/mutation genotype, “complex” karyotype/ increased genomic complexity or purine-analog refractory cases (progression-free survival <6 months). The authors thus provide a comprehensive description of genomic alterations in high-risk CLL patients that are selected for in the context of chemo(immuno)therapy, by building groups and individually testing for unbalanced incidences of mutations. The results lead to a description of well-known tumor drivers, which appear to contribute to high-risk CLL in addition to *TP53*; *MYC*, [gain (8)(q24)], *CDKN2A/B* [del(9)(p21)] and Notch pathway mutations.

The authors describe mutations in Notch-associated genes and known negative regulators (i.e. *SPEN*, *RBPJ*). They found that the above genes were mutated/deleted in 3.7-8.2% of high-risk CLL patients and showed that mutated cases had higher levels of expression of Notch target genes (e.g. *HES1*, *DTX1* and *MYC*). Furthermore, they revealed that *SNW1* is a potential negative regulator of the Notch signaling pathway. *SNW1* has also been shown to act as a co-activator of Notch-driven transcription.<sup>9</sup>

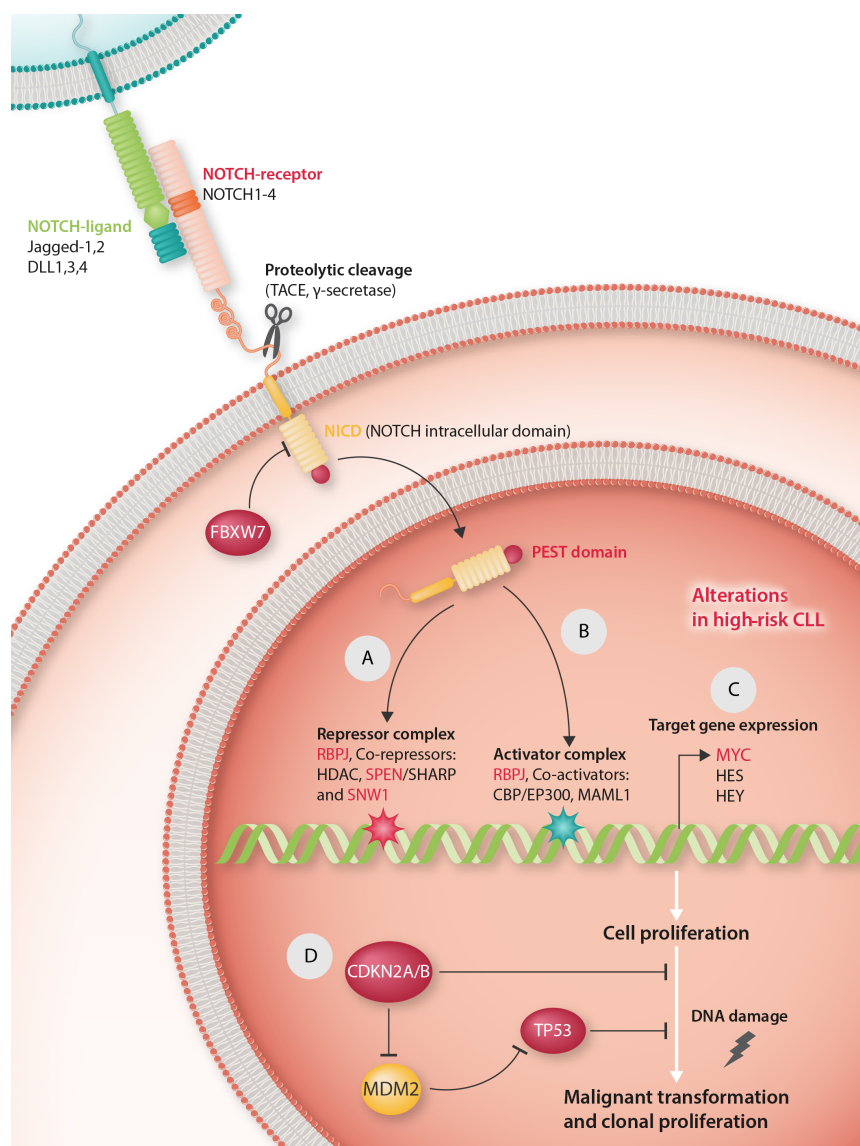
Notch signaling is an evolutionarily conserved signaling pathway that allows cell-cell interactions regulating a wide range of biological functions.<sup>10</sup> There are four mammalian

members of NOTCH transmembrane proteins or receptors (NOTCH1 - 4) which have only partially overlapping functions despite similar structures. These receptors function as ligand-activated transcription factors, interacting with transmembrane ligands (Delta-like1, 3 and 4, and Jagged1 and 2) (Figure 1A, B).

While Notch signaling plays an important physiological role in hematopoiesis and hematopoietic stem cell biology,<sup>11,12</sup> aberrant Notch signaling has been found to be an oncogenic driver in precursor lymphoid and myeloid neoplasms as well as mature B-cell neoplasms with different mechanisms of oncogenic pathway activation including mutations in Notch receptors, mutations in negative regulators (e.g. *FBXW7*) or overexpression of ligands and receptors.<sup>13-15</sup> *NOTCH1* is one of the most frequently mutated genes in CLL,<sup>16</sup> affecting approximately 12% of cases.<sup>17,18</sup> The majority of mutations occur in coding regions leading to stabilization of the Notch intracellular domain (NICD) via loss of the PEST [proline (P), glutamic acid (E), serine (S), and threonine (T)] domain. *NOTCH1* gain-of-function mutations in CLL were first described by Ianni *et al.*<sup>19,20</sup> and were later found in large-scale sequencing studies.<sup>21,22</sup> Additionally, mutations in the non-coding 3' untranslated region have been described.<sup>17,23</sup> These mutational events ultimately lead to the accumulation of NICD and increase Notch signaling activity. Notch activation through mechanisms other than activating mutations also frequently occur in CLL,<sup>18</sup> with roughly 50% of CLL cases exhibiting high levels of NICD without detectable *NOTCH1* mutations.<sup>18,20</sup> Although potential mechanisms of *NOTCH1* mutation-independent pathway activation have been proposed (e.g. *MED12* mutations<sup>24</sup>), the biology remains incompletely understood. Mutations in the negative regulator *FBXW7* have been described in CLL.<sup>25</sup>

*NOTCH1* has been found to be an adverse prognostic marker in CLL<sup>26-29</sup> and has been associated with the co-occurrence of other adverse prognostic factors in CLL, such as *IGHV* mutational status<sup>30</sup> and trisomy 12.<sup>31</sup> While *NOTCH1* mutations are more frequently found in CLL with unmutated *IGHV*, the accumulation of NICD without *NOTCH1* mutations seems similarly distributed in CLL with unmutated and mutated *IGHV* genes.<sup>18</sup> Integration of information about the presence or absence of *NOTCH1* mutations into prognostic scoring systems improved survival predictions.<sup>32</sup> *NOTCH1* mutations have not only been linked to progressive disease, but also to the earliest stages of development of CLL.<sup>33</sup>

Current approaches targeting Notch signaling include  $\gamma$ -secretase inhibitors, which block the proteolytic cleavage of NICD. More than 100  $\gamma$ -secretase inhibitors have been developed,<sup>34</sup> with some demonstrating effects in CLL as single agents or in combination with other drugs.<sup>35,36</sup> Monoclonal antibodies targeting Notch receptors (e.g. OMP-52M51) have been tested in pre-clinical<sup>37</sup> and clinical



**Figure 1. Molecular drivers of high-risk chronic lymphocytic leukemia.** (A, B) Notch signaling. In its inactive state the Notch transcriptional complex is bound by co-repressors such as SPEN, histone deacetylases (HDAC) and, potentially, SNW1 (A). Binding of Notch ligands (Jagged-1,2, DLL1, 3, 4) to Notch receptors leads to proteolytic cleavage of the intracellular domain (NICD) via  $\gamma$ -secretase and translocation of NICD to the nucleus to form a transcriptionally active complex with MAML1 (Mastermind-like protein 1), RBPJ (Recombination signal binding protein for immunoglobulin kappa J region) and transcriptional co-activators such as the histone acetyl transferases CBP/EP300, leading to Notch target gene expression (including MYC, HES, HEY) (B). Termination of Notch signaling is achieved mainly via ubiquitination of the PEST degradation domain of NICD by FBXW7 (F-box/WD repeat-containing protein 7). (C) Alterations in MYC activity. MYC is a direct target of Notch signaling driving cell proliferation. Gain of the MYC locus (8)(q24) enhances activity. (D) DNA damage checkpoint. TP53 is frequently altered and a hallmark of high-risk chronic lymphocytic leukemia (CLL). Loss of function in CDKN2A/B impairs TP53 tumor suppressor function and cell cycle control. (Gene symbols and gene names in red represent altered/mutated genes in high-risk CLL).

studies (NCT01778439, NCT 01703572). Indirect targeting approaches are also under investigation (e.g. bepridil).<sup>38</sup>

Dysregulation of MYC in B-cell tumors has been well established and comprehensively reviewed.<sup>39,40</sup> Edlmann *et al.* have demonstrated that gain of the MYC locus [gain (8)(q24)] frequently occurs in high-risk CLL. MYC has also been shown to be a direct target of NOTCH1<sup>41</sup> (Figure 1C).

Disruption of the DNA damage repair complex and associated cell cycle control or arrest is a hallmark of high-risk CLL. The protein products of both CDKN2A and CDKN2B (i.e. p16INK4a, p14ARF and p15INK4b, respectively) are central to DNA damage-related cell cycle control by interacting with both p53 and RB1 as well as direct inhibitors of cyclin-dependent kinases (CDK). Both p16INK4a and p15INK4b inhibit CDK4 and CDK6 and lead to the activation of RB1.<sup>42</sup> The alternate reading frame product p14ARF inhibits MDM2, thereby stabilizing p53.<sup>43</sup> (Figure 1D). These mechanisms have important tumor suppressor function guarding against DNA damage with potentially tumorigenic mutations and loss of these

tumor suppressors may exert deleterious effects similar to loss of TP53. Cases of transformed CLL into aggressive lymphoma (Richter transformation) often exhibit CDKN2A/B disruption.<sup>44</sup>

With the advent of chemotherapy-free treatments, it remains to be seen if the results presented will be applicable to current standards of care. For a more comprehensive understanding of CLL, clonal evolution and predictive markers, future studies will leverage comprehensive proteomic, methylation and RNA expression in addition to DNA-level investigations in a genome-wide manner. As these data emerge and are analyzed with more complex statistical models<sup>7</sup> the mechanisms underlying aggressive disease will become clearer. We hope this will have direct implications for the clinical management of CLL patients. One simple step in this direction is an open approach to data sharing and access, a prerequisite to advance knowledge on rare variants. We are certain that the study groups involved in the analysis by Edlmann *et al.* will also take a lead in this area.

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