Mouse models in the study of chronic lymphocytic leukemia pathogenesis and therapy

Giorgia Simonetti,1 Maria Teresa Sabrina Bertilaccio,2 Paolo Ghia,3,4 and Ulf Klein1,5,6

1Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; 2Lymphoid Malignancy Unit, Division of Molecular Oncology, IRCCS San Raffaele Scientific Institute, Milan, Italy; 3B cell Neoplasia Unit, Division of Molecular Oncology, IRCCS San Raffaele Scientific Institute, Università Vita-Salute San Raffaele, Milan, Italy; 4Clinical Unit of Lymphoid Malignancies, Department of Oncology-Hematology, IRCCS San Raffaele Scientific Institute, Milan, Italy; and 5Department of Pathology and Cell Biology and 6Department of Microbiology and Immunology, Columbia University, New York, NY

Mouse models that recapitulate human malignancy are valuable tools for the elucidation of the underlying pathogenetic mechanisms and for preclinical studies. Several genetically engineered mouse models have been generated, either mimicking genetic aberrations or deregulated gene expression in chronic lymphocytic leukemia (CLL). The usefulness of such models in the study of the human disease may potentially be hampered by species-specific biological differences in the target cell of the oncogenic transformation. Specifically, do the genetic lesions or the deregulated expression of leukemia-associated genes faithfully recapitulate the spectrum of lymphoproliferations in humans? Do the CLL-like lymphoproliferations in the mouse have the phenotypic, histological, genetic, and clinical features of the human disease? Here we compare the various CLL mouse models with regard to disease phenotype, penetrance, and severity. We discuss similarities and differences of the murine lymphoproliferations compared with human CLL. We propose that the Eμ-TCL1 transgenic and 13q14-deletion models that have been comprehensively studied at the levels of leukemia phenotype, antigen-receptor repertoire, and disease course show close resemblance to the human disease. We conclude that modeling CLL-associated genetic dysregulations in mice can provide important insights into the molecular mechanisms of disease pathogenesis and generate valuable tools for the development of novel therapies. (Blood. 2014;124(7):1010-1019)

Introduction

Chronic lymphocytic leukemia (CLL) originates from the clonal expansion of mature B cells, which show features of antigenic stimulation and express the CD5 cell surface antigen. CLL is a complex disease in which genetic abnormalities cooperate with microenvironmental factors in the malignant transformation of the tumor-cell precursor and in leukemia progression. Compared with most other subtypes of non-Hodgkin lymphoma (NHL), CLL shows a lower frequency of genetic mutations per case and a different spectrum of genetic aberrations, which mostly comprise chromosomal deletions (13q14, ATM, and TP53) or amplifications (trisomy of chromosome 12). More recently, next-generation sequencing (NGS) analyses have identified novel recurrent mutations in CLL, including those that target the NOTCH1, MYD88, SF3B1, and BIRC3 genes. A number of genes are overexpressed in CLL tumor cells compared with normal lymphocytes, presumably as a direct consequence of the genetic aberrations (eg, BCL2 and MCL1 due to deletion of mir-15a/16-1) or through as yet unknown mechanisms (eg, ROR1 or TCL1). Finally, genome-wide association studies have identified several susceptibility loci for familial CLL, including a single nucleotide polymorphism in the IRF4 gene, a known regulator of B-cell developmental processes.

Genetically engineered mouse models of CLL

Several mouse models mimicking genetic lesions found in CLL (13q14 deletion), transgenic for genes that are overexpressed in the disease (including TCLI, APRIL, BCL2 × traf2dn, ROR1), or driven by ectopic oncogene expression (IgH.T and IgH.TEμ) have been generated (Table 1).

Mouse models mimicking the spectrum of deletions of chromosomal region 13q14

Deletion of 13q14 is the most frequent genetic lesion in CLL that occurs in >50% of cases and is clinically associated with an indolent disease with no or delayed need for therapy. The deletion is detected also in monoclonal B-cell lymphocytosis (MBL), an expansion of CD5+ B lymphocytes in the peripheral blood (PB) of otherwise healthy individuals that is thought to precede CLL and is present at a lower frequency in other NHL subtypes.

The 13q14 region corresponds to the murine 14qC3 locus and encodes several genes that are highly conserved among human and mouse (Figure 1). A minimal deleted region (MDR) includes the first exon of the DLEU1 sterile RNA, the deleted in leukemia (DLEU)2 gene, encoding a sterile transcript and the mir-15a/16-1 cluster, located in an intron of DLEU2. A larger 13q14 deletion is found in a sizable number of CLL cases and is hence named the common deleted region (CDR). The functional dissection of the 13q14 tumor suppressor locus by using transgenic mouse models demonstrated that the size of 13q14 deletions influences the phenotype of the developing lymphoproliferations and the severity of disease (Table 1), suggesting a tumor suppressor function for multiple genetic elements encoded in the deleted region. Specifically, the results provided evidence for a causal role of the microRNAs mir-15a/16-1.
and

APRIL transgenic mice

Table 1. Mouse models of CLL

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<th>Mouse model</th>
<th>Key findings</th>
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<tr>
<td>mir-15a/16-T/- and mir-15a/16-flxed CD19-Cre mice</td>
<td>Germ-line mutations interfering with the normal expression of mir-15a/16-1 are observed in a small fraction of CLL patients. The first indication that a disruption of the physiological expression of mir-15a/16-1 favors CLL development stemmed from the analysis of the N2B strain which is characterized by a germ-line mutation in the 3' flanking region of pre-mir-15a/16-1, resulting in increased expression of mature mir-15a-1. The targeted genetic inactivation of mir-15a/16-1 in mice provided conclusive evidence for a tumor-suppressor role of these microRNAs in CLL development, as proposed. 26-30% of mir-15a/16-1/- and mir-15a/16-TCD19-Cre mice developed, late in life, MBL and CLL, and less frequently CD5-negative NHLs in a B-cell autonomous fashion. The loss of the mir-15a/16-1 cluster led to an earlier entry into the cell cycle and an up-regulation of BCL2 protein levels compared with wild-type B cells, as previously described. 177</td>
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<td>14q3 C minimal deleted region (MDR)/-/- and MDR+/-CD19-Cre mice</td>
<td>MDR-deleted mice that in addition to mir-15a/16-1 lack the deleu2 and deleu5 genes show a higher penetrance of the phenotype and a more aggressive disease course compared with mir-15a/16-1deleted mice, although the pathogenetic mechanism remains to be elucidated. 40-45% of MDR/-/- and MDR+/CD19-Cre mice present with MBL, CLL and CD5-negative NHLs in a B-cell autonomous fashion. MDR/-/- mice, in contrast to mir-15a/16-1/-/- mice, succumbed to the lymphoproliferations earlier than their wild-type littermates.</td>
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<tr>
<td>14q3 common deleted region (CDR)flxed CD19-Cre mice</td>
<td>Homozygous deletion of the CDR in the germ-line causes embryonic lethality. Mice with deletion of the CDR in B cells develop lymphoproliferations at a similar frequency (40-45%) as MDR-deleted mice. However, the mice are characterized by a different spectrum of lymphoproliferations, as they mostly develop CLL with rare instances of MBL and NHLs. In addition, CDR-deleted mice presented with tumor cell infiltrates in spleen, bone marrow and non-lymphoid organs that were generally larger than those observed in the mir-15a/16-1/- and MDR-deleted mice presenting with CLL. CDR/-/- mice developed mainly CD5-positive lymphoproliferations at a penetration of about 25%, which is similar to that observed in MDR/-/- mice. While both CDR/-/- and MDR/-/- mice have a similar disease onset, once the lymphoproliferations develop, deletion of the CDR leads to a more aggressive disease. 177 DLEU7 has been suggested to be a negative regulator of NK-B activity. 118</td>
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<tr>
<td>Eµ-TCL1 transgenic mice</td>
<td>Exogenous expression of the human TCL1 gene under the control of the IGHV promoter and IGH enhancer (Eµ) in vivo (Eµ-TCL1) results in the clonal expansion of CD5+ IgM+ B cells. Between 13 and 18 mo of age, virtually all Eµ-TCL1 mice develop an overt leukemia and massive infiltrations of monoclonal CD5+ B cells in both lymphoid and non-lymphoid tissues. TCL1 is a co-activator of the serine/threonine kinase AKT, activates the NF-κB pathway in CLL cells, and inhibits DNT3A and DNT3B activity. 41 Leukemia development is at least partially dependent on enhanced AKT activity. 30</td>
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<td>APRIL transgenic mice</td>
<td>Based on the finding of elevated levels of tumor necrosis factor (TNF) family member APRIL in sera of CLL patients, APRIL transgenic mice have been generated that accumulate increased levels of the molecule in the sera. 31,32 APRIL induces proliferation of B cells. APRIL transgenic mice develop clonal lymphoproliferations originating from peritoneal CD5+ B cells between 9 and 12 mo of age with a penetrance of 40%. 32</td>
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<td>BCL2 × trafi2dn transgenic mice</td>
<td>Mice transgenic for the anti-apoptotic gene BCL2, which is expressed at high levels in human CLL cells, and a dominant negative form of the adapter protein TNF receptor-associated factor 2 (trafi2), the latter being structurally similar to TRAF1 which is overexpressed in CLL, have been generated to study the synergistic effects of these molecules in CLL pathogenesis. By 14 mo, ~80% of BCL2 × trafi2dn double-transgenic mice had died of a CLL-like disease showing lymphocytosis, lymphoedema, spleen enlargement and involvement of bone marrow and non-lymphoid organs.</td>
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<td>ROR1 transgenic mice</td>
<td>Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is highly expressed on CLL cells in humans. This oncoembryonic antigen is considered a potential target for CLL therapy as it is virtually absent from adult tissues. Transgenic mice with the human ROR1 gene which is controlled by the murine Ig enhancer/promoter to ascertain B cell-restricted expression develop, late in life (≥ 15 mo), clonal lymphoproliferations resembling CLL at a very low penetrance (~5%). However, ROR1 × TCL1 double-transgenic mice succumbed to CLL more rapidly than single Eµ-TCL1 or ROR1-Tg mice, demonstrating that ROR1 expression accelerates disease progression in Eµ-TCL1 mice.</td>
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<td>Eµ-mir-29 transgenic mice</td>
<td>Eµ-mir-29 transgenic mice that overexpress the microRNA cluster miR-29a/b in B cells present with expanded CD5+ B-cell populations and late in life develop an indolent CLL-like leukemia at a penetrance of ~20%. Overexpression of miR-29 in myeloid cells promotes leukemogenesis. By analogy, it is possible that miR-29 may have oncogenic functions in the precursor cells of murine CLL. However, since in human CLL, miR-29 expression is downregulated and thought to exert tumor-suppressor functions, the implications of the findings from the Eµ-mir-29 model regarding a role of this microRNA cluster in human CLL development are presently unclear.</td>
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<td>Vh11 × irf4/-/- mice</td>
<td>To elucidate the proposed role of reduced IRF4 expression in CLL development, mice were crossed to transgenic for the Vh11 heavy chain gene which develop expansions of peritoneal CD5+ B cells. Vh11 × irf4/-/- mice presents with a CLL-like disease in the majority of mice by 12 mo. Since irf4/-/- mice are severely immunodeficient due to critical functions of IRF4 in various immune cell types, the conclusive determination of a B cell-intrinsic role of IRF4 deficiency in CLL pathogenesis needs to await results from Vh11 × irf4 conditional mice crossed to a B cell-specific deleter mouse. With regard to a possible leukemogenic function of altered IRF4 levels, a recent study demonstrated that reduced IRF4 expression altered the migration properties of B cells, most likely by upregulating NOTCH2 activity.</td>
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<td>IgH T and IgH TEµ mice</td>
<td>The SV40 T antigen was suggested to exert an oncogenic function in B-cell malignancies. Sporadic SV40 T antigen expression in mature B cells has been achieved by insertion of a SV40 T antigen gene in opposite transcriptional orientation in the IGH chain locus between the D and JH segments, in presence (IgH TEµ) or absence (IgH T) of an extra copy of the Eµ enhancer. Virtually all aging IgH TEµ and 13% of IgH T mice developed an expansion of CD5- B cells carrying either unmutated IGHV genes with preferential usage of the Vh11 family, or highly mutated IGHV genes.</td>
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AKT, protein kinase B/v-AKT murine thymoma viral oncogene; DNMT, DNA methyltransferase; NF-κB, nuclear factor κB; SV40, simian virus 40; TNF, tumor necrosis factor.

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in CLL pathogenesis and demonstrated that the additional deletions of del62 and/or del65 caused a higher disease penetrance and a more severe disease course.26 In both models, CD5-negative NHLs developed along with CD5-positive lymphoproliferations that encompassed MBL and CLL. Importantly, CDR-deleted mice were characterized by a different spectrum of lymphoproliferations compared with MDR and mir-15a/16-1-deleted mice, as they mostly developed CLL (rarely MBL or NHLs), and a more aggressive disease course.27 A commonality among all 13q14 mouse models was the expression of unmutated stereotypic antigen receptors,26,27 indicating a role of antigen in the expansion of the CLL clone.

**Mouse models mimicking the deregulated expression of genes in human CLL**

Several mouse models have been generated that are transgenic for genes overexpressed in the disease (Table 1). The first transgenic mouse that developed a CLL-like disease and that since has been widely used in the study of CLL pathogenesis and therapy is the Eµ-TCL1 transgenic mouse.15,28,29 The TCL1 gene is expressed in the vast majority of human CLL cases.15 Strong TCL1 expression is associated with markers of poor prognosis, including unmutated IGHV genes.15 Eµ-TCL1 mice developed a clonal expansion of CD5+ IgM+ B cells initially in the peritoneal cavity and on progression in the PB, spleen, and bone marrow,28,29 eventually giving rise to a monoclonal B-cell population in virtually 100% of mice (Table 1).28 The B-cell receptors (BCRs) expressed by lymphoproliferations in Eµ-TCL1 mice harbored unmutated IGHV gene rearrangements and exhibited stereotypy in IGHV, IGKV, and IGLV gene rearrangements.30

The characteristics of the other mouse models that develop a CLL-like disease, including APRIL transgenic mice,31,32 BCL2×traf2dn transgenic mice,33 ROR1 transgenic mice,34 Eµ-mir-29 transgenic mice,35 Vh11×irf4−/− mice,36 and IgH.T and IgH. TEL mice37 are summarized in Table 1.

**Commonalities and disparities among CLL mouse models**

In all mouse models, the lymphoproliferations develop late in life and resemble the indolent disease course of CLL. Also, the target cell of the malignant transformation to CLL seems to be the peritoneal B1a cell, as suggested by the expression of CD5 and of unmutated IGHV genes, high levels of IgM, and low levels of IgD and CD23, at least in the models where these parameters were analyzed. Noticeably, whereas virtually all Eµ-TCL1 mice developed CLL, a sizable fraction of B-cell lymphoproliferations in the 13q14 deletion models represented CD5-negative NHLs. The latter observation genuinely reflects the heterogeneous spectrum of B-cell malignancies with the 13q14 deletion occurring in humans.

The most notable difference among the CLL mouse models is the penetrance of the phenotype, which is highest in the Eµ-TCL1 mice (~100%), intermediate in the 13q14-MDR and CDR deletion models and the APRIL transgenic mice (40-50%), and lowest in the mir-15/16-1 deletion model (~25%) and the ROR1 transgenic mice (5%) (Table 2). The different tumor incidence among the CLL mouse models suggests that the various genetic aberrations predispose to, but are not sufficient per se, for the malignant transformation of B cells, a situation similar to that encountered by 13q deletions in low-count MBL, which virtually never progresses into frank leukemia.20 Thus, additional alterations are required to induce leukemia. The heterogeneity of the lymphoproliferation phenotypes observed in mir-15a/16-1−/− and MDR−/− mice (CLL, MBL, and CD5− NHL) may reflect the specific nature of the secondary hits and/or the different B-cell differentiation stages in which the malignant transformation occurs. In particular, the observation that CDR-deleted mice predominantly develop a CLL-like disease27 suggests the existence of tumor suppressor genes in the h13q14/m14qC3 locus in addition to the DLEU2/mir-15a/16-1 cluster. The almost complete disease penetrance in the Eµ-TCL1 model is likely due to a strong oncogenic function of the hTCL1 gene, which affects multiple pathways, including coactivation of the serine/threonine kinase protein kinase B/akt murine thymoma viral oncogene (AKT),38,39 activation of the nuclear factor κB pathway,40 and inhibition of the activity of DNA methyltransferase (DNMT)3a and DNMT3b.41 In the APRIL transgenic model, the sustained microenvironmental stimulation likely facilitates the expansion of B1a cells, which transform in 40% of cases.32 Similarly, prosurvival signals delivered through the RORI receptor may favor the expansion of a preleukemic B-cell population that gives rise to a CLL-like disease in 5% of animals.34 Conversely, an increased proliferative capacity seems to account for leukemia development in 20% of Eµ-mir-29 transgenic mice.35 Overexpression of BCL2 synergizes with a dominant negative form of the adaptor protein tumor necrosis factor (TNF) receptor-associated factor 2 (traf2dn) in the malignant transformation of B cells, resulting in the development of a CLL-like disease in ~80% of BCL2×traf2dn transgenic mice, most likely due to resistance to apoptosis and altered expression of adhesion molecules.32 Apoptosis resistance has also been suggested to account for leukemia development in the Vh11×irf4−/− model,36 although a recent study suggests that the possible leukemogenic function of reduced IRF4 expression may be the alteration of the migration properties of B cells,33 presumably through up-regulation of NOTCH2 activity in IRF4-deficient cells.43 Finally, expression of the simian virus...
Table 2. Features of genetically engineered mouse models of CLL

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Disease penetrance</th>
<th>Time of appearance of circulating leukemic cells</th>
<th>Age of death</th>
<th>IG gene rearrangements</th>
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<tr>
<td>mir-15a/16-1(^{-/-}) and mir-15a/16-1(^{flox}) CD19-Cre</td>
<td>~20% CLL, ~8% MBL, ~2% CD5(^{-/-}) NHL</td>
<td>12-18 mo</td>
<td>15-18 mo</td>
<td>Unmutated and stereotypic IGHV genes</td>
</tr>
<tr>
<td>14q32-CDR(^{-/-}) and MDR(^{flox}) CD19-Cre</td>
<td>~22% CLL, ~12% MBL, ~6% CD5(^{-/-}) NHL</td>
<td>6-18 mo</td>
<td>12-18 mo</td>
<td>Unmutated and stereotypic IGHV genes</td>
</tr>
<tr>
<td>E(_{\mu})-TCL1 tg</td>
<td>100% CLL, ~50% CLL, ~3% MBL</td>
<td>6-18 mo</td>
<td>12-18 mo</td>
<td>Unmutated IGHV genes, stereotypic IGHV and IGLV genes</td>
</tr>
<tr>
<td>APRIL tg</td>
<td>40% CLL, 6% CD5– NHL, 2% CD5– NHL</td>
<td>Not analyzed</td>
<td>12 to &gt;15 mo</td>
<td>Not analyzed</td>
</tr>
<tr>
<td>BCL2 (\times) irf4(^{-/-}) tg</td>
<td>80% CLL, 15% MBL</td>
<td>9-15 mo</td>
<td>&gt;80% dead at 14 mo</td>
<td>Clonal IGHV rearrangements</td>
</tr>
<tr>
<td>ROH1 tg</td>
<td>5% CLL, 15% MBL</td>
<td>&gt;15 mo</td>
<td>&gt;15 mo</td>
<td>Clonal IGHV rearrangements</td>
</tr>
<tr>
<td>E(_{\mu})-mir-28 tg</td>
<td>20% CLL, 15% MBL</td>
<td>16-24 mo</td>
<td>&lt;5 mo</td>
<td>Clonal IGHV rearrangements (determined by FACS)</td>
</tr>
<tr>
<td>Vh11 (\times) irf4(^{-/-})</td>
<td>100% CLL (preceded by MBL in &gt;40% cases)</td>
<td>5-10 mo</td>
<td>&gt;9 mo</td>
<td>Clonal IGHV rearrangements</td>
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<tr>
<td>IgH TEl3</td>
<td>100% CLL</td>
<td>&lt;5 mo</td>
<td>Killed at 2-10 mo</td>
<td>Unmutated IGHV genes (preferentially Vh11) and some highly mutated</td>
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Commonalities among CLL developing in mouse models and humans

The usefulness of CLL mouse models for the study of the human disease depends on the grade of relatedness of the disease characteristics between animal models and patients. There are 2 issues to consider. First, with respect to the involvement of the genetic lesions or the deregulated expression of leukemia-associated genes, do the models faithfully recapitulate the spectrum of lymphoproliferations observed in the human disease? Second, do the CLL-like lymphoproliferations in the mouse have the phenotypic, histological, genetic, and clinical features of the disease developing in humans? Evidently, a major factor in this is the phenotypic and functional relatedness of the cells targeted by the oncogenic transformation in humans and mice. In the following, we will discuss the commonalities among CLL in mice and humans with respect to disease origin, repertoire of antigen receptors, and phenotypic and epigenetic characteristics of the tumor cells.

Disease origin

Possibly all CD5-positive lymphoproliferations developing in CLL mouse models derive from the transformation of peritoneal, self-replenishing CD5\(^{+}\) B1a cells. Clonal populations of B1a cells develop spontaneously within the peritoneal cavity of aging mice and eventually disseminate to and expand in other lymphoid organs. This suggests that over time, B1a cells become susceptible to malignant transformation as the results of genetic abnormalities that accumulate during their self-replenishment, eventually giving rise to a CLL-like disease. Protumorigenic forces such as deletion of 13q14, overexpression of TCL1, or high serum levels of APRIL that confer a proliferation and/or survival advantage may accelerate the disease onset, increase the penetrance of the phenotype, and impact disease course and severity (Table 2).

What is the relationship between the CLL precursor cells in the human and mouse? The longstanding discussion about the putative
cell of origin of CLL in humans has recently been revisited by Chiorazzi and Ferrari, who provide conceivable arguments for the not mutually exclusive possibilities that the oncogenic transformation may occur in a marginal zone B cell, a transitional B cell, or a human B1-like cell, and independent evidence suggests that CLL cases with unmutated IGHV genes may originate from transformed naïve B cells. The notion of a B1a-like cell of origin of CLL recently gained traction through the identification of a previously unrecognized CD5-expressing B-cell population in the adult. These cells comprise only about 1% of PB B cells, but their gene expression profile is closely related to that of CLL cells. One notable aspect of this new hypothesis is that it would be consistent with the fact that the CD5 antigen is invariably expressed on human and murine CLL cases. However, because the function of these newly identified CD5+ B cells is unknown, it is presently impossible to draw a functional relationship between these cells and murine B1a cells.

Stereotypic antigen receptors

A recent study provided a fascinating example that the expansion of the CLL clone can be driven by antigen-independent cell autonomous signaling. Nevertheless, there is clear evidence for the role of antigen-BCR stimulation via autoregions or external antigens in CLL pathogenesis. A characteristic of CLL is the expression of stereotypic antigen receptors among unrelated CLL cases that show highly similar heavy-chain complementarity-determining (HCDR3) regions, presumably as the result of strong selection forces because the normal HCDR3 repertoire is extremely diverse due to IGHV-IGHD-IGHJ combinatorial and junctional diversity. Sequence analyses of antigen-receptor rearrangements from \( \text{E}_{\mu}-\text{TCL1} \) transgenic and 13q14 deletion models demonstrated a skewed IGHV-IGHD-IGHJ rearrangement repertoire in the leukemic clones derived from these mice.

Phenotypic and epigenetic characteristics

The ZAP70 tyrosine kinase is recruited to the cell surface of CLL cells on stimulation through the BCR and promotes activation of the downstream signaling pathway. In addition, ZAP70+ CLL cells display increased migration properties. ZAP70 expression is upregulated in splenic B cells of \( \text{E}_{\mu}-\text{TCL1} \) mice already in the early stage of the disease. Deletions of the \( \text{E}_{\mu}-\text{TCL1} \) mice in the B-cell compartment of \( \text{E}_{\mu}-\text{TCL1} \) mice may provide new insights into the role of ZAP70 in the dynamics of disease onset, progression, and dissemination.

Recent studies suggest that CLL cells have the functional capacity to express and secrete interleukin (IL)-10, a property that characterizes a subset of B cells with immune regulatory functions (B-10 cells). Based on the observation in \( \text{E}_{\mu}-\text{TCL1} \) mice that the expansion of IL-10–competent malignant B cells precedes the development of overt leukemia, IL-10 production was suggested to participate in the immunoregulatory capacity of the malignant cells and therefore influence disease progression, outcome, and response to treatment, possibly through suppression of the antitumor response.

As observed in CLL patients, leukemic \( \text{E}_{\mu}-\text{TCL1} \) mice display multiple T-cell alterations, including the shift from a naïve to a memory T-cell subtype and defective signal transduction at the immune synapse. Also, similar to human CLL, leukemic mice present with an increased number of T cells in the PB. Interestingly, transfer of leukemic B cells into disease-free \( \text{E}_{\mu}-\text{TCL1} \) mice caused the development of T-cell dysfunctions that allowed the malignant clone to escape from the host immune system and expand in the recipient mice. Together, these findings suggest that the \( \text{E}_{\mu}-\text{TCL1} \) model represents a suitable tool to study the interplay between CLL cells and T cells in disease development and progression.

CLL cells in humans show multiple epigenetic alterations that have been detected also in the \( \text{E}_{\mu}-\text{TCL1} \) model. Aberrant methylation of promoter sequences was observed in B cells of \( \text{E}_{\mu}-\text{TCL1} \) mice starting at 3 months of age, and the level of DNA methylation increased with time, suggesting a causative role in the development and progression of CLL. In addition, splenic B cells from \( \text{E}_{\mu}-\text{TCL1} \) mice showed an elevated number of hypomethylated regions compared with wild-type B cells.

**CLL mouse models in the study of disease pathogenesis and therapy**

**\( \text{E}_{\mu}-\text{TCL1} \) transgenic mouse model as a tool to investigate pathogenic mechanisms**

Due to the similarity of the disease characteristics of CLL in humans and mice and the complete penetrance of the model, \( \text{E}_{\mu}-\text{TCL1} \) mice have been extensively used in the dissection of the pathogenic mechanisms leading to CLL. Several transgenic and knockout mouse models have been crossed with \( \text{E}_{\mu}-\text{TCL1} \) mice to elucidate the functional role of specific molecules in the onset and progression of CLL in vivo (Figure 2; supplemental Table 1), providing important new insights into the pathogenic role of those genes in the dysregulation of signaling, proliferation, and apoptosis, in mediating altered trafficking and homing, and in the aberrant cross-talk with the microenvironment.

As predicted from the study of CLL in humans, establishment of suitable \( \text{E}_{\mu}-\text{TCL1} \) compound mouse models could validate the critical role of BCR signaling in CLLogenesis, provided evidence for the importance of continuous autoantigenic stimulation in CLL development, and suggested a proleukemogenic function of Toll-like receptor signaling. Interestingly, a recent study found that B cell-specific inhibition of the endoplasmic reticulum stress response, which is coupled to defective spleen tyrosine kinase and Bruton agammaglobulinemia tyrosine kinase activation on BCR stimulation, reduces leukemia development in \( \text{E}_{\mu}-\text{TCL1} \) mice.

As expected from the known tumor suppressor function of TP53, genetic ablation of tp53 in mice, thus mimicking 17p deletion, the most deleterious genetic abnormalities in CLL, accelerated leukemia onset and progression in the \( \text{E}_{\mu}-\text{TCL1} \) model. Similar observations were made for a putative CLL tumor suppressor gene: inhibitor of DNA binding protein 4 (\( \text{Id}4 \)).

The notion that aberrant cytoskeletal organization and cell trafficking contribute to CLL pathogenesis potentially through a disrupted interaction of the malignant cells with the bone marrow microenvironment was experimentally confirmed in vivo in hematopoietic cell-specific Lyn substrate (hsL) \( \text{E}_{\mu}-\text{TCL1} \) mice.
addition, another Eμ-TCL1 compound mouse model provided evidence that cellular motility plays an important role in bone marrow infiltration of CLL cells.

In CLL patients, endothelial, nurse-like, and antigen-presenting cells release the TNF family members B-cell activating factor (BAFF) and APRIL, which activate the transformed B cells. In accordance, ectopic expression of the corresponding transgenes in Eμ-TCL1 mice revealed that BAFF and APRIL protect leukemic cells from apoptosis in vivo, resulting in a rapid expansion of the malignant clone. The importance of microenvironmental interactions in the maintenance of the CLL clone could further be demonstrated by 2 independent Eμ-TCL1 compound mouse models (mif−/− Eμ-TCL1 and cd44−/− Eμ-TCL1 mice). Moreover, the potential role of the tumor microenvironment in signaling to malignant B cells through wingless-type MMTV integration site family (WNT) receptors was addressed by genetically ablating the WNT receptor frizzled 6 (fzd6) in Eμ-TCL1 mice. The results suggested that dysregulated FZD6 expression modulates the disease course as it delayed but did not abrogate tumor growth.

In summary, Eμ-TCL1 compound mouse models have proved extremely useful in determining the pathogenic functions of CLL-associated genes in an in vivo context. It will be interesting to explore the leukemogenic role of the newly identified oncogenic mutations in the NOTCH1, MYD88, SF3B1, and BIRC3 genes by modeling these mutations on the background of Eμ-TCL1, as well as other CLL mouse models.

Figure 2. Study of novel pathogenic mechanisms of CLL in the TCL1-driven leukemia model. Overexpression (transgenic [tg]) or deficiency of different molecules in the Eμ-TCL1 transgenic mouse model variably affects the disease phenotype (BM, bone marrow; TAM, tumor-associated macrophages). The following mouse models have been crossed with Eμ-TCL1 mice: xid, pkcθ−/− (or pkcθ−/−), xbp1fl/flCD19-Cre, tir8−/−, drnag1-tg, ha1−/−, rhoh−/−, p53−/−, id4−/−, id4−/−, mif29a/b-tg, fzd6−/−, cd44−/−, mif−/−, ROR1-tg, APRIL-tg, and bak-tg.

Eμ-TCL1 transgenic mouse model as a preclinical model for novel therapeutics

Mouse models represent a useful tool to evaluate efficacy, pitfalls, and potential side effects of novel therapeutic strategies. The Eμ-TCL1 mouse has been validated as a suitable preclinical model by demonstrating that the response of leukemic mice to fludarabine, a cytotoxic drug used in first-line treatment of CLL, resembles that observed in human patients. The disadvantage of the long disease latency was overcome by transferring splenic leukocytes or B cells from leukemic mice into syngeneic or immunodeficient recipient mice. Together with xenograft models of CLL, such transfer experiments represent reproducible systems for the elucidation of the efficacy and mechanism of action of novel therapies. As such, preclinical studies with Eμ-TCL1 mice were instrumental in understanding the pathogenic relevance of molecular targets in disease biology (supplemental Table 2), as summarized in the following.

Inhibition of signaling. Based on the known function of the TCL1 oncogene as an AKT coactivator, the dependence of TCL1-driven leukemia on AKT signaling has been investigated by inhibiting the mTOR effector with rapamycin in a transfer model. The study demonstrated a critical role of the AKT pathway in the disease onset rather than progression. The fact that CLL cells are chronically stimulated and in lymphoid tissues show a gene expression signature of lymphocytes activated
through the BCR\textsuperscript{101} provided a rationale for the development of strategies based on BCR signaling inhibitors. In clinical practice, the Bruton agammaglobulinemia tyrosine kinase inhibitor ibrutinib (PCI-32765), the spleen tyrosine kinase inhibitor fostamatinib, and the phosphatidylinositol 3-kinase-\(\delta\) inhibitor idelalisib (GS-1101) caused rapid lymph node shrinkage and transient lymphocytosis in patients\textsuperscript{102,103} by impairing the retention of CLL cells in the tissue microenvironments.\textsuperscript{104} Importantly, a transient increase in PB B-cell counts has also been reported early after treatment of mice transplanted with \(\text{E}_{\mu}-\text{TCL1}\)-derived leukemias with fostamatinib and ibrutinib.\textsuperscript{92,93}

**Inhibition of cytoskeletal functions and cell trafficking.** The findings that BCR inhibitors cause rapid tissue mobilization of CLL cells established a new paradigm in the CLL field, which identifies the disruption of the homing properties of leukemic cells as a therapeutic approach. This notion is supported by results from HS1 or RHOH-deficient \(\text{E}_{\mu}-\text{TCL1}\) compound mouse models (supplemental Table 1),\textsuperscript{79,80} by recent phase 3 studies,\textsuperscript{105} and by the Food and Drug Administration approval of ibrutinib for the treatment of CLL. In line with these assertions, interfering with the proper cytoskeleton function of CLL cells via the dual v-\text{src} avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC) kinase/BCR-Abelson murine leukemia viral oncogene homolog 1 (ABL) inhibitor dasatinib may be a conceivable therapeutic option for the subset of patients with an active v-\text{yes}-1 yamaguchi sarcoma viral related oncogene homolog (LYN)/HS1 axis.\textsuperscript{98} Indeed, inhibition of LYN/HS1 signaling impaired leukemia progression and lymphoid organ infiltration in a transfer model of TCL1-driven leukemia. Moreover, an indirect effect on leukemic cell trafficking may contribute to the efficacy of lenalidomide, an immunomodulatory agent that is currently under investigation as salvage therapy for patients with relapsed or refractory CLL\textsuperscript{106} and as a consolidation strategy in progressive cases.\textsuperscript{107} Lenalidomide treatment reduced the chemokine response of CLL cells in vitro and down-modulated the expression of RHOH,\textsuperscript{80} which, in the \(\text{E}_{\mu}-\text{TCL1}\) model, is indispensable for the homing of malignant B cells to the bone marrow.\textsuperscript{80}

**Inhibition of tumor-microenvironmental interactions.** Several novel therapeutics target the cross-talk between the malignant cells and the tumor microenvironment through neutralization of protumorigenic factors, inhibition of surface receptors, or nuclear transport. Treatment with the anti-CD44 antibody IM7 that exerts a proapoptotic function prevented the accumulation of CD44-expressing leukemic B cells in the PB of an adoptive transfer model of TCL1-driven leukemia early after injection.\textsuperscript{87} Similarly, administration of the anti-ROR1 monoclonal antibody D10 inhibited the engraftment of leukemic cells from \(\text{ROR1} \times \text{E}_{\mu}-\text{TCL1}\) transgenic mice into \(\text{ROR1}\) transgenic recipients, which was accompanied by a strong reduction of PB and splenic B-cell counts.\textsuperscript{34} A reduction in the number of circulating malignant B cells was also observed on neutralizing the prosurvival effect of BAFF with CD268-Fc and CD269-Fc decay receptors in a model obtained by intraperitoneal injection of CDS\textsuperscript{+} leukemic B cells from \(\text{baff} \times \text{E}_{\mu}-\text{TCL1}\) transgenic mice into \(\text{baff}\) transgenic recipients.\textsuperscript{84} Finally, marked antileukemic effects have recently been reported for the inhibitor of nuclear export KPT-185 and the antibody drug conjugate IMGN529.\textsuperscript{35,97} KPT-185 acts by blocking the activity of the nuclear exporter chromosome region maintenance 1 protein, or exportin1 (CRM1/ XPO1), which is a downstream mediator of microenvironmental signals sustaining CLL cell survival and growth.\textsuperscript{97} Administration of the small molecule to SCID mice engrafted with leukemic B cells from \(\text{E}_{\mu}-\text{TCL1}\) mice improved survival of those mice. A benefit in terms of overall survival has also been observed in CD37 transgenic mice engrafted with \(\text{CD37} \times \text{E}_{\mu}-\text{TCL1}\) leukemia on treatment with IMGN529, which consists of the cytotoxic anti-CD37 antibody and the antimicrotubule agent DM1 that exerts an antiproliferative effect.\textsuperscript{95}

**Forced induction of apoptosis.** A major pathogenic mechanism in CLL development constitutes the resistance of the tumor cells to apoptosis. Therefore, therapeutics that target the antiapoptotic pathway represent valuable strategies for CLL treatment. Preclinical studies of actinomycin D\textsuperscript{108} and silvestrol (a plant-derived cyclopenta[b] benzofuran)\textsuperscript{109} that, respectively, inhibit transcription and translation, and of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) (a derivative of the heat shock protein 90 inhibitor geldanamycin)\textsuperscript{94} showed promising results and were indeed associated with a reduction in the levels of antiapoptotic proteins. The studies may provide a rationale for the treatment of CLL cases carrying a deletion of the \(\text{i7p}\) locus (that are resistant to standard chemotherapy including fludarabine)\textsuperscript{110} with actinomycin D or silvestrol, which can induce apoptosis in a P53-independent fashion.\textsuperscript{108,109}

**Epigenome targeting.** Finally, CLL cells display epigenetic alterations, and evidence suggests that overexpression of histone deacetylase enzymes contributes to CLL pathogenesis.\textsuperscript{65,111} The histone deacetylase inhibitor MGCD0103 induced apoptosis\textsuperscript{112} and autophagy suppression\textsuperscript{113} in CLL cells in vitro and showed antitumor activity in a phase 2 clinical trial.\textsuperscript{114} Moreover, the class I and II deacetylase (DAC) inhibitor, AR-42, prolonged the overall survival of mice transplanted with TCL1-driven leukemia.\textsuperscript{99} Class I and II inhibitors such as AR-42 that target both histone and nonhistone substrates may be promising therapeutics for a multitarget therapy.

**Conclusions and outlook**

Several genetically engineered mouse models of CLL have been generated that mimic the human disease. The \(13q14\) deletion models provided the first in vivo evidence of the tumor-suppressor function of a CLL-associated genetic lesion. As numerous studies revealed similarities between CLL developing in humans and \(\text{E}_{\mu}-\text{TCL1}\) mice, this transgenic model has become a suitable tool in the investigation of pathogenic mechanisms of CLL and represents a convenient preclinical model, particularly due to its complete disease penetrance.

Based on the stereotypic antigen receptors in the \(\text{E}_{\mu}-\text{TCL1}\) mice that recall those in some aggressive forms of human CLL, and the association of the \(13q14\)-deletion in humans with CLL bearing somatically mutated IGHV genes that have a more favorable disease course, it has been suggested that the corresponding mouse models mimic the aggressive and indolent forms of CLL, respectively. However, this view may have to be revisited due to the circumstance that the CD5\textsuperscript{+} lymphoproliferations developing in the \(\text{E}_{\mu}-\text{TCL1}\) transgenic and \(13q14\) deletion models are similar regarding their phenotype and their expression of unmutated IGHV genes and of stereotypic antigen receptors, indicating that the target cell of the oncogenic transformation may be the same in the 2 models. Two implications ensue from this: first, in lieu of knowledge on the precise cell of origin of CLL in humans, it is impossible to confidently assign the CLL developing in the mouse models to a particular human CLL subtype. Second, the virtual absence of somatically mutated IGHV genes in CLLs developing in the mouse models may suggest that the normal cellular counterpart(s) of CLL differ among mice and humans, eg, that a somatically mutated CD5\textsuperscript{+} subset may simply not exist in the mouse. In support
of the latter assumption is the observation that, similar to humans, CD5-negative NHL with somatically mutated IGHV genes do develop in 13q14 deletion models.

Regardless of these unresolved issues, genetically engineered CLL mouse models, together with xenograft models, have emerged as valuable tools for the first-line testing of new therapies. A downside of the transgenic CLL models in their use as preclinical models is the long latency until disease develops. The presence of stereotypic receptors and active BCR signaling in the leukemic cells of those mice may provide a rationale for the development of a CLL mouse model with potentially shorter latency by combining an antigen or BCR-driven mechanism with the commonly known, or newly identified, CLL-associated genetic aberrations.

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References
35. Widhopf GF II, Cui B, Ghia EM, et al. ROR1 can interact with TCL1 and enhance leukemogenesis.

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Giorgia Simonetti, Maria Teresa Sabrina Bertilaccio, Paolo Ghia and Ulf Klein