Leukemia (2013) **27,** 534–540

www.nature.com/leu

REVIEW

Xenograft models of chronic lymphocytic leukemia: problems, pitfalls and future directions

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Xenotransplantation of human tumor cells into immunodeficient mice has been a powerful preclinical tool in several hematological malignancies, with the notable exception of chronic lymphocytic leukemia (CLL). For several decades, this possibility was hampered by the inefficient and/or short-term engrafment of CLL cells into available animals. The development of new generations of immunocompromised mice has allowed to partially overcome these constraints. Novel humanized animal models have been created that allow to recapitulate the pathogenesis of the disease and the complex *in vivo* relationships between leukemic cells and the microenvironment. In this review we discuss the development of xenograft models of CLL, how they may help elucidating the mechanisms that account for the natural history of the disease and facilitating the design of novel therapeutic approaches.

Leukemia (2013) **27,** 534–540; doi:10.1038/leu.2012.268

Keywords: immunodeficient mice; CLL xenograft models; primary CLL cells; CLL cell lines

INTRODUCTION

Primary leukemic cells and the rare existing cell lines are the cornerstones of the *in vitro* investigation of the biological and molecular features of chronic lymphocytic leukemia (CLL). Still, a number of major biological questions as well as the validation of new drugs can be only superficially approached by *in vitro* assays. Animal models are therefore essential.

For years, because of the lack of known specific genetic lesions and in contrast with other blood cancers, no animal models of CLL were generated, while CLL or CLL-like disorders appeared to be a 'by-product' of aging animal models (for example, NZB/NZW)[1,2] nonspecifically designed for that purpose. Only at the turn of the century, a transgenic mouse overexpressing the human *Tcl-1* gene under the control of the immunoglobulin heavy chain variable region promoter and immunoglobulin heavy chain enhancer (Eμ-Tcl-1) was generated.[3] Since then it has become a very popular tool of investigation. Nevertheless, though recapitulating a disease very similar to human CLL, this model has some limitations: (i) the disease development is delayed (13–18 months), making it cumbersome to test in reasonable timely schedules the effects of drugs and/or immunotherapeutic strategies; (ii) because of the overexpression of the specific *Tcl-1* gene, the model does not reflect the genetic complexity of human CLL.

In order to reproduce the most frequent genetic aberration of human CLL, the deletion of chromosomal region 13q14, Klein *et al.*[4] developed a transgenic mouse model lacking the minimal deleted region. The minimal deleted region includes the long non-coding RNA deleted in leukemia (*DLEU*)-2 and the first exon of the *DLEU-1* gene (that contains mir15 and mir16 whose deletion was considered to be the main driving lesion). Interestingly, this mouse model develops a full spectrum of lymphoproliferative disorders ranging from monoclonal B-cell lymphocytosis (MBL), to CLL, to more aggressive $CD5^-$ diffuse large B-cell lymphomas, somehow recapitulating the natural history of CLL that may evolve from MBL to CLL to Richter syndrome. However, the disease penetrance is incomplete and the disease onset is again quite delayed, making this model not fully suitable for preclinical use. Conceivably xenograft models based on the direct transplantation of immunodeficient mice with human cells or human cell lines may prove more advantageous for drug testing *in vivo*.

For several decades it proved difficult to establish murine models capable of supporting the expansion of primary human CLL cells and CLL cell lines. Recently, the scenario has significantly changed owing to the use of more severely immunodeficient animals, with a highly restricted, if any, ability to reject xenogeneic cells. It is the purpose of this review to summarize the recent developments in the use of CLL xenograft models, and discuss how these models may help preclinical drug testing, and accelerate the transfer of novel therapeutic strategies into the clinical setting.

IMMUNODEFICIENT MICE: A BRIEF HISTORICAL VIEW

Advances in mammalian immunology have led to develop a number of immunodeficient murine models (Table 1) amenable to the engrafment of human cells, each with its own characteristics but also some caveats.

Athymic nude mice were used for xenotransplantation of human solid human tumors since the seventies,[5] but the transplant of human blood cancers has been, for long time, more problematic.[6,7]

In 1983 the SCID (severe combined immunodeficiency) mouse, homozygous for the *scid* mutation at the protein kinase, DNA activated, catalytic polypeptide ($Prkdc^{scid}$) was developed.[8] SCID mice lack T and B lymphocytes, though with some 'leakiness' in advanced age, and have been frequently used for the transplantation of human leukemias and lymphomas, such as acute lymphoblastic leukemia, acute myeloid leukemia, adult T-cell leukemia and chronic myelogenous leukemia.[9–12] However, these mice still retain normal natural killer (NK) and myeloid cells,

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Received 27 June 2012; revised 5 September 2012; accepted 6 September 2012; accepted article preview online 13 September 2012; advance online publication, 5 October 2012

Table 1. Immunodeficient mouse models: an overview

Strain name	*Characteristics*	*Reference*
Nude	Athymic: lack of T cells; NK cells activity and humoral immunity	Flanagan SP[6]
SCID	Lack of mature T and B cells; NK cells acivity and innate immunity; radiation sensitivity	Bosma GC[8]
Rag1$^{-/-}$	Lack of mature T and B cells; low innate immunity and NK cell activity; radiation resistance	Mombaerts P[19]
Rag2$^{-/-}$	Lack of mature T and B cells; normal development of cells other than lymphocytes	Shinkai Y[20]
NOD/LtSz-scid	Lack of mature B and T cells; decreased innate immunity and NK cells activity;	Shultz LD[14]
NOD/Shi-scid	radiation sensitivity; short-term follow-up due to lethal thymic lymphomas	Koyanagi Y[72]
IL2rg$^{-/-}$	Absence of NK cells; low T and B cells	Cao X[21]
Balb/c-Rag2$^{-/-}$ IL2rg$^{-/-}$	Complete absence of T and B cells, lack of NK cells function	Goldman JP[22]
NOD/Shi-scid IL2rg$^{-/-}$	Lack of mature B and T cells; decreased innate immunity and NK cells absence;	Ito M[23], Shultz LD[24]
NOD/LtSz-scid IL2rg$^{-/-}$	radiation sensitivity	

Abbreviations: IL, interleukin; NK, natural killer cells; NOD, non-obese diabetic; Rag, recombination activation gene; SCID, severe combined immunodeficiency.

and these cells were likely responsible for interfering with the *in vivo* engraftment of some human leukemias/lymphomas.[10]

To overcome these limitations the SCID mutation was back-crossed onto the non-obese diabetic (NOD) background, creating a more promising model for transplantation of lymphoid tumors.[13,14] NOD/SCID mice have low NK cell activity and no circulating complement, which makes them better recipients for several types of human leukemias and lymphomas, including Daudi, Namalwa, Raji and Molt-4 cell lines, and also primary leukemic cells such as adult T-cell leukemia.[15–18]

In 1992 Mombaerts[19] and Shinkai[20] generated mice carrying a germline mutation, whereby a large portion of either the V(D)J recombination activation gene (RAG) 1- or the RAG2-coding region was deleted. This defect prevented the recombination of antigen receptor genes, hence the generation of mature T- and B-lymphocytes. More recently a significant breakthrough in the development of immunodeficient mice has been the introduction of the mutation in the gene encoding the interleukin2 receptor (IL2R) γ-chain (also known as the common cytokine-receptor γ-chain or γc), which is involved in IL2, IL4, IL7, IL9, IL15 and IL21 cytokine receptors signaling.[21] Several immunodeficient Prkdc^{scid} (SCID) and *Rag1* or *2* gene knockout mice with IL2rg locus mutation have been generated, and have facilitated human cell engraftment owing to the complete NK cells deficiency.[22–25]

XENOTRANSPLANTATION OF PRIMARY CLL CELLS

All initial attempts to xenotransplant human primary CLL cells have been hampered by the lack of mice immunodeficient enough to prevent the rejection of human leukemic cells.

In 1997, Shimoni *et al.*[26] transplanted intraperitoneally (i.p.) into lethally irradiated Balb/c or beige/nude/Xid mice radioprotected with bone marrow (BM) cells from NOD/SCID mice, unselected peripheral blood mononuclear cells (PBMCs; 100–1000 $\times$ 10^6) from CLL patients (Table 2). Malignant B cells were engrafted, but remained localized in the peritoneum. That notwithstanding, these experiments showed for the first time a correlation between the engraftment of patients' cells and the stage of the disease according to Rai's criteria. Stage 0 CLL cells poorly engrafted, stage I/II cells partially and cells from stage III/IV patients markedly engrafted.

Interestingly, the authors concomitantly demonstrated an inverse correlation between the engraftment of leukemic cells and autologous T cells with a marked engraftment of T lymphocytes in stage 0 patients, and the total absence of T-cell engraftment when PBMCs from advanced-stage patients were used. These results suggested a role for T cells in controlling the expansion of leukemic B cells *in vivo*.

Human T-cell depletion experiments performed in human→mouse chimeras by the same group confirmed this hypothesis.[27] *In vivo* T-cell depletion by means of the monoclonal antibody, OKT3, enhanced the engraftment of CLL lymphocytes from the PBMCs of early-stage patients. On the contrary, T-cell enrichment of PBMCs from advanced-stage CLL patients caused a relevant reduction of CLL engraftment. These data set the concept that T cells from CLL patients can actively suppress the expansion of leukemic B cells by inhibiting xenotransplantation, particularly in early stage disease.

The picture radically changed with more immunocompromised animals that did not need relevant manipulation to obtain a successful transplantation.

Durig *et al.*[28] transplanted primary unselected CLL PBMC directly into NOD/SCID mice. Using combined intravenous (i.v.) and i.p. injection, they showed a robust and stable recovery of CLL PBMC in various murine tissues over 12 weeks. The majority of the engrafted human CD45$^+$ cells were found in the spleen, followed by the peritoneal cavity, BM and peripheral blood. CLL cells recovered from the spleens stained positive for Ki67, thus showing proliferative activity. The splenic engraftment capacity correlated with the clinical and molecular features of the patients at the time of transplantation, with leukemic cells from advanced-stage patients having a higher engraftment potential than early-stage CLL cells. The mutually exclusive B- and T-cell patterns of engraftment first described by Shimoni *et al.*[27] was confirmed, and the possibility was raised that T cells deriving from early-stage patients might maintain the ability to suppress tumor cell growth and to exert immunosurveillance as well, while T cells from Binet stage C patients might not.

More recently the capacity of leukemic cells to engraft in the NOD/SCID model was correlated with a number of CLL biological prognostic markers.[29] CLL cells from patients with unmutated *IGHV* genes engrafted better than those from mutated cases, and the expression of CD38, ZAP70 and CD49d was associated with a successful engraftment.

Concomitantly, Bagnara *et al.*[30] proposed a novel adoptive transfer model of human CLL by using for the first time the NOD/Sci-scid IL2rg$^{-/-}$ (NSG) mouse, a NOD/SCID-derived strain completely deficient in lymphocytes, including NK cells. In this model they assessed the role of different bystander elements, including myeloid and mesenchymal cells. It became evident that human mesenchymal stem cells were not necessary as the endogenous murine BM mesenchymal microenvironment was sufficient for the growth of human CLL cells, confirming the *in vitro* findings that murine stroma is able to support CLL cell survival.[31]

In contrast, the cotransfer of CLL PBMCs (either i.v. or intrabone) and normal antigen presenting cells (APCs; CD14$^+$ or CD19$^+$ cells) from unrelated donors elicited an increased CLL cell survival and proliferation *in vivo*.

This model underscored the important role of autologous T lymphocytes and allogeneic APCs in CLL engraftment. Bagnara *et al.*[30] observed a direct correlation between T-cell levels in mouse blood and leukemic cell proliferation, as in animals without T-cell expansion, CLL cell proliferation did not occur. Accordingly,

Table 2. Xenograft models of primary CLL cells

Model	*Description*	*Reference*
human/mouse radiation chimera	Transplantation of CLL PBMC into peritoneal cavity of irradiated Balb/c or BNX mice, radioprotected with bone marrow from SCID mice	Shimoni A[26]
NOD/SCID	Transplantation of CLL PBMC NOD/SCID mice and combining intravenously and intraperitoneally injection	Durig J[28]
NOD/SCID- IL2rg$^{-/-}$	Co-transfering of CLL PBMCs intravenously with allogeneic APCs (CD14^{+} or CD19^{+} cells)	Bagnara D[30]
NOD/SCID- IL2rg$^{-/-}$	Transplantation of hematopoietic stem cells purified from CLL patients into newborn mice (by facial vein)	Kikushige Y[33]

Abbreviations: APC, antigen presenting cell; BNX, Balb/c or beige/nude/Xid; CLL, chronic lymphocytic leukemia; IL, interleukin; NOD, non-obese diabetic; PBMC, peripheral blood mononuclear cell; SCID, severe combined immunodeficiency.

in vivo depletion of CD3^{+} or CD4^{+} cells completely abrogated leukemic cell survival and proliferation.

Indeed these results are in contrast with those previously obtained by other groups who found that elimination of T cells by anti-CD3 monoclonal antibody increased CLL engraftment, and also that early-stage CLL PBMCs transfer did not allow successful leukemic engraftment because of a T-cell activity that appeared capable of preventing CLL cells growth.[27,28] Furthermore in the NOD/SCID model, Aydin *et al.*[29] observed a role for CD38 expression in CLL engraftment and tumor proliferation, whereas Bagnara *et al.*[30] did not observe a statistical correlation between markers of clonal aggressiveness, such as CD38, and *in vivo* CLL cell growth. Though the different results may simply reflect the use of different immunodeficient mouse models, these discrepancies need to be further investigated, likely in parallel experiments.

Besides T cells, a second necessary human hematopoietic cell type of the NSG model appears to be represented by normal mature allogeneic APCs (CD14^{+} or CD19^{+} cells), thus further supporting the concept that the activation of autologous T cells by allogeneic APCs might be central to CLL survival and expansion. This data are well in line with recent findings on the relevance of myeloid cell function in the pathogenesis of CLL patients.[32]

Interestingly, the use of the same mouse model, NOD/SCID/IL2rg null (NSG), has led to another challenging result in CLL, as published by Kikushige *et al.*[33] The transplant of hematopoietic stem cells (HSC) purified from CLL patients into newborn NSG mice allowed to show the propensity of these animals to develop monoclonal B-cell populations, though not a full-blown CLL.

HSC (CD34^{+}CD38^{-} or CD34^{+}CD38^{-}CD90^{+}) from CLL patients and healthy donors transplanted into irradiated NSG 48 h newborn mice via a facial vein gave rise to secondary CD34^{+}CD38^{-} HSCs, CD34^{+}CD38^{+} progenitors, CD34^{-}CD19^{+} B cells and CD34^{-}CD33^{+} myeloid cells in the murine BM. The number of CLL-HSC-derived human pro-B cells expressing CD5 was significantly higher as compared with the healthy HSC donors. In addition, the mature B-cell progeny showed mono or oligoclonal immunoglobulin heavy genes rearrangements, though VDJ recombinations were always independent from the recombinations originally expressed by the patient's CLL clone. Along the same line, when CLL-HSCs from an individual patient were transplanted into different mice, the B-cell clone population arising in each mouse was moncolonal, yet characterized by a different VDJ recombination. Similarly, after serial transplantation experiments, secondary recipients developed B-cell clones with their own VDJ recombination, which was different from the original B-cell clone. As the engrafting HSC, as well as the B-cell clones, had normal karyotypes, these xenogeneic studies suggest that the propensity of CLL-HSCs to generate clonal B cells is independent of the oncogenic events triggered by the known chromosomal abnormalities. The implication is that the latter abnormalities are likely acquired as later oncogenic events at the mature B-cell stage *en route* towards CLL. Still, HSC from CLL patients appear to be skewed toward B-cell lineage, thereby suggesting the existence of other, so far undetected, intrinsic abnormalities.

XENOTRANSPLANTATION OF CLL CELL LINES AND APPLICATIONS

Xenograft models with tumor cell lines are easily reproducible tools for the cellular and molecular analyses required to develop novel therapeutic strategies (Table 3).

As for CLL, many have been the past failures. At present a number of reliable models are available, thanks to the use of severely immunocompromised animals and a more accurate characterization of the cell lines utilized. As an example, few xenotransplantation models supposedly using CLL cell lines (WSU-CLL) turned out being either contaminated with cells more aggressive (pre B-acute lymphoblastic leukemia cell line REH)[34,35] or derived from a different disease[36,37] (JOK-1, a HCL cell line).

Failures in establishing CLL cell lines are well known, and all the existing *bona fide* CLL cell lines are either derived from Epstain-Barr virus (EBV)-seropositive patients or were established by *in vitro* EBV infection. Admittedly, the *in vivo* growth of EBV-CLL cell lines in immunocompromised mice could be due to the EBV-induced stimulus for uncontrolled proliferation. However it has also been recognized that EBV-transformed normal B cells, though having trisomy 12, were not tumorigenic in immunosuppressed mice, and, in addition, trisomies 7, 8 (8 q) and 9 appear to induce tumorigenicity only in a very limited number of mice (around 15%).[38] This observation again underlines the importance of the recipient mice.

The first effort to transplant a CLL cell line was done into nude mice by injecting two irradiated cell lines EBV-CLL[1] and EBV-CLL[2] established from the peripheral blood lymphocytes of a CLL patient.[39] A successful subcutaneous (s.c.) engraftment was described in 36% of the injected nude mice with splenic and nodal dissemination of the leukemic cells, but without any sign of involvement of peripheral blood and of other tissues. Numerous studies have utilized irradiated nude mice transplanted s.c. with D10-1,[40] derived from the subline 85-4 LN[41] of the EBV-CLL.[1] This model was characterized by a very fast tumor growth, and again nodal and splenic dissemination, but no peripheral blood or other tissue involvement.

That notwithstanding, the D10-1 subclone transplanted either into nude or scid mice proved useful for the *in vivo* testing of monoclonal antibodies[42,43] and of radioimmunotherapy approaches.[44]

In 1993, a new CLL cell line, MO1043, with trisomy 12 and CD5 expression was established from the peripheral blood of a CLL patient by the co-incubation with EBV and cyclosporine A.[45] These cells, adapted *in vivo,* as described for the NALM cell line,[46] were transplanted into nude as well as SCID mice by i.p. and s.c. routes of administration. In non-conditioned nude mice injected i.p. with 54×10^6 MO1043 cells, 40% of mice formed tumors. In X-irradiated nude mice injected s.c. or i.p., the engraftment occurred in around 80 and 100% of mice. In the latter case, the

Table 3. Xenograft models of CLL cell lines

Model	*Cell line*	*Cells source and/or reference*	*Cells phenotypic features*	*Cytogenetics*	*Cells Ig gene rearrangement*	*Model reference*
Nude	EBV-CLL(1) and EBV-CLL(3)	Lee CL[39]	EBV-CLL(1): ND; EBV-CLL(3): ND	EBV(1): trisomy 12; EBV(3): 11q +	EBV(1): polyclonal EBV-CLL(3): ND	Lee CL[39]
Nude or SCID	MO1043	Kawata A[45]	$CD19^+$, $CD20^+$ $CD5^+$, HLA-DR^+, IgK^+	Trisomy 12	ND	Kawata A[45]
Nude	JVM-3	DSMZ/ Melo JV[73]	$CD19^+$, $CD20^+$ $CD5^-$, HLA-DR^+, IgK^+	Trisomy 12	Monoclonal	Loisel S[48]
Balb/c-Rag2 $^{-/-}$ IL2rg $^{-/-}$	MEC-1	DSMZ/ Stacchini A[47]	$CD19^+$, $CD20^+$ $CD5^-$,HLA-DR^+, IgK^+, $sIgM^+$, $sIgD^+$, $CD23^+$, $CD38^+$	del(17), del(12)	Monoclonal	Bertilaccio MT[49]

Abbreviations: CLL, chronic lymphocytic leukemia; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin; ND, not described; Rag, recombination activation gene; SCID, severe combined immunodeficiency.

tumor growth was observed mainly in the abdominal cavity as ascites, and the pattern of dissemination was limited to abdominal lymph nodes, pancreas and, sometimes, peribronchial lymph nodes.

MO1043 cells were also transplanted i.p. into SCID mice, and the engraftment was observed in 100% of the animals. SCID mice developed less ascites than nude mice, but had massive enlargement of para-aortic lymph nodes together with mesentery, liver, spleen and abdominal lymph nodes involvement. The BM of the tumor-bearing mice was slightly infiltrated with CLL cells, and no leukemic cells were described in the peripheral blood.

Later, two cell lines, MEC-1 and MEC-2, were established from the peripheral blood of a EBV-seropositive CLL patient in prolymphocytoid transformation.[47] It is interesting to note that both these cell lines as well as the JVM-3 (see next and Table 3) were derived from CLL patients either in prolymphocytoid transformation or with a diagnosis of prolymphocytic leukemia. This might explain the lack of expression of the CD5 molecule, a typical marker of CLL pathology which is frequently lost when CLL progresses into prolymphocytic leukemia. Therefore, one has to consider that the *in vitro* establishment of cell lines, maintaining all the typical markers of CLL, still remains an unsolved issue.

Having said that, these cell lines have been extensively used as a surrogate for CLL models and Loisel *et al.*[48] transplanted nude mice with MEC-2 or with the human JVM-3, and described the successful establishment of a CLL xenograft model with the s.c. or i.v. injection of JVM-3 but not of MEC-2. The transplantability of JVM-3 cells was 100%, and the tumor disseminated into the lungs, liver and abdominal organs, while no lymph node infiltration was apparent at histological analysis. BM and peripheral blood involvement were not reported in this model.

Our group explored the ability of Rag2 $^{-/-}\gamma_c^{-/-}$ mice, which lack B, T and NK cells, to support the growth of the MEC-1 cell line.[49] We generated a reproducible model of aggressive human CLL that develops rapidly and closely recapitulates its human counterpart by spreading systemically. MEC-1 cells injected either s.c. or i.v. into Rag2 $^{-/-}\gamma_c^{-/-}$ mice localize in several lymphoid and non-lymphoid organs with a relevant expansion of the leukemic clone in the BM, peripheral blood, lymph nodes and peritoneum.

The engrafment efficacy is 100% and the model has proved useful for evaluating both the biological basis of CLL growth and dissemination,[50] and for testing new therapeutic strategies.[51]

CONCLUDING REMARKS

Xenograft models are becoming master tools in CLL. First, they contribute to formulate a conceivable and testable CLL model, possibly providing the opportunity to observe the development of the whole CLL process from stem cell to the emergence of monoclonal B-cell expansions (Figure 1). The most intriguing example is the model reported by Kikushige *et al.*,[33] which aims at answering a very relevant question: at what stage does the first oncogenic event occur in CLL? The experimental evidence demonstrate that CLL-HSCs, though having a normal karyotype, possess a 'cell-intrinsic' capacity to develop polyclonal B-cells progenitors frequently progressing into MBL. This finding, places the initial intrinsic defect at the level of HSC that have not yet rearranged immunoglobulin heavy genes. The implication is that the well-known chromosome abnormalities, such as deletion of 13q14, which causes loss of miR15a and miR16-1,[52,4] are likely secondary oncogenic events that accumulate late in the natural history of CLL, while other insofar unknown primary transforming events are occurring at the HSC level. This xenograft model of CLL-HSCs into NSG mice reasonably recapitulates MBL development, but the primary genetic hit, taking place in the self-renewing CLL-HSC level, is still unknown.

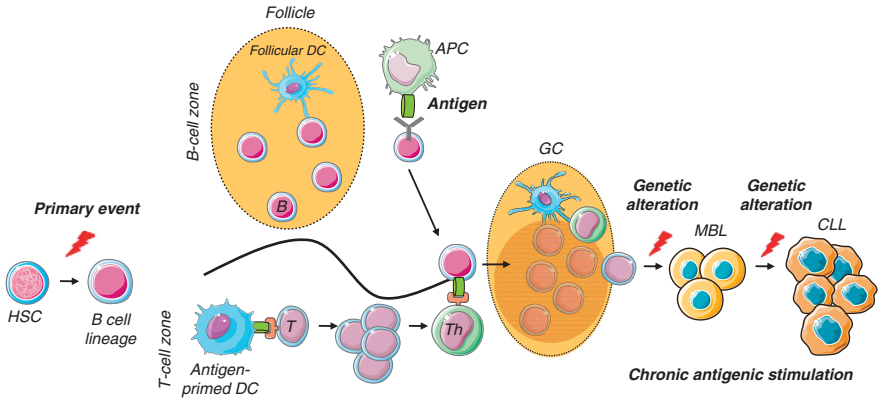

Figure 1. Schematic model of CLL development based on Xenograft Studies An initial, yet unknown, genetic event predisposing to the evolution into MBL, may occur very early during B-cell development, even at the stage of HSCs.[33] Accumulating genetic abnormalities,[71] together with microenvironmental interactions,[27,28,30] including antigen stimulation,[59–61] may then cooperate for the progression into fully-blown CLL.

Another relevant aspect of CLL biology elucidated by xeno-transplantation studies is the role of normal bystander cells present within the microenvironment. It is known that the proliferative drive of genetically abnormal CLL B cells is also dependent upon external signals, originating from different cellular elements of the microenvironment including soluble (for example, cytokines and chemokines) and surface-bound (that is, cell – cell interactions) factors.[53] Several groups through different xenograft models confirmed the relevant role of autologous T cells.[26,28,30] B – T cell interactions, either directly or indirectly, are critical for the growth of CLL cells, though it is yet to be fully clarified which interactions are advantageous for the leukemic cells and which are detrimental. Conceivably the different outcome is accounted for by the stage of the disease, as well as by the site of the interaction with notable differences between tissues (BM vs peripheral lymphoid organs) being plausible.

A recent model revealed that APCs are another important piece of the puzzle that favours the growth of CLL cells, probably again facilitating the T-cell action toward CLL cells.[30]

All these evidences confirm and expand a wealth of *in vitro* findings, showing that cells of the lymphoid,[54,55] myeloid[56] and mesenchymal lineages[57,58] are involved in CLL survival and growth. The intervention of all these components is reminiscent of the complexity of the cellular interactions that take place when normal B cells encounter their specific antigen, and that may be reproduced in CLL B cells, where gene expression profiling studies[59] and the skewed *IGHV* gene repertoire characteristic of CLL[60–65] have underlined the importance of antigen stimulation. The use of xenograft models may help dissecting the complexity of the whole process by selectively adding or removing potential cellular culprits.

Xenograft models of CLL may have useful clinical applications, considering both the rapid development of a wealth of new drugs and the increasing importance of the new immunotherapeutic approaches based upon chimeric receptor-modified T cells.[66] First of all xenograft models may allow the rapid and reliable *in vivo* testing of novel drugs, such as those aimed at targeting crucial steps of B-cell receptor signaling,[67,68] and those that interfere with or modify the leukemic microenvironment.[69] As proper CLL xenograft models were not available in the past, many preclinical studies were performed on mouse xenograft models of leukemias/lymphomas other than CLL. As an example TRU-016, a humanized anti-CD37 immunoglobulin G fusion protein, was tested in Raji – Ramos – Daudi cell-based xenografts models.[70] As for the preclinical testing of new immunotherapeutic approaches, we have recently, successfully utilized the Rag2$^{-/-}\gamma_c^{-/-}$ mice injected with MEC-1 cells to probe a novel immunotherapy approach based on anti-CD23 chimeric receptors.[51] Accordingly, within this context, CLL xenograft models may become useful tools not only to evaluate innovative strategies, but also to help designing patient-specific treatment options.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This project was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC; Investigator Grant to PG and Special Program Molecular Clinical Oncology—5 per mille #9965 to PG and FC-C), Milano, Italy; 'Fondazione Piera, Pietro e Giovanni Ferrero', Alba, Italy; Fondazione CARIPLO, Milano, Italy; 'CLLGRF—U.S./European Alliance for the Therapy of CLL', FIRB and PRIN—Ministero Istruzione, Università e Ricerca (MIUR), Roma, Progetti Integrati Oncologia (PIO) e Progetto Finalizzato 2010—Ministero della Salute, Roma. CS is supported by the EHA Fellowship Program (2009/18). Figure was produced using Servier Medical Art: www.servier.com.

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