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Preclinical modeling of novel therapeutics in chronic lymphocytic leukemia: the tools of the trade

Sarah E.M. Herman*, Adrian Wiestner

Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD

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ABSTRACT

In the last decade our understanding of chronic lymphocytic leukemia (CLL) biology and pathogenesis has increased substantially. These insights have led to the development of several new agents with novel mechanisms of action prompting a change in therapeutic approaches from chemotherapy-based treatments to targeted therapies. Multiple preclinical models for drug development in CLL are available; however, with the advent of these targeted agents, it is becoming clear that not all models and surrogate readouts of efficacy are appropriate for all drugs. In this review we discuss in vitro and in vivo preclinical models, with a particular focus on the benefits and possible pitfalls of different model systems in the evaluation of novel therapeutics for the treatment of CLL.

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1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world [1]. It is characterized by the expansion of monoclonal, auto-reactive B cells that display decreased cell death and increased proliferation rates [1,2]. Standard treatment for physically fit symptomatic patients is chemo-immunotherapy (such as FCR—fludarabine, cyclophosphamide, and rituximab); however, these agents are not well tolerated by elderly patients and do not perform well in patients with adverse cytogenetic profiles (such as deletion of the short of arm of chromosome 17) [3,4]. In the past few years treatment of CLL has started to undergo dramatic changes; moving away from traditional chemotherapeutics towards targeted agents. While there are multiple preclinical models for drug development, evaluation of therapeutics for the treatment of CLL is typically done using in vitro cultures of either cell lines or primary CLL cells collected from the peripheral blood, using cell death as the preferred readout. With the advent of targeted agents, it is becoming clear that not all models and measures of activity are appropriate for the evaluation of all drugs.

In the context of this review we define novel therapeutics as kinase inhibitors (such as ibrutinib and idelalisib), immunomodulatory agents (such as antibodies against PD-1, PD-L1, or CTLA4), and BH3-mimetics (such as ABT-199). Kinase inhibitors are at the forefront of investigation for the treatment of CLL; with ibrutinib and idelalisib demonstrating impressive clinical activity as single agents [5–7], as well as in combination with anti-CD20 monoclonal antibodies [8,9]. These agents work by inhibiting both intrinsic signaling pathways, as well as disrupting tumor-microenvironment interactions [10–15]. Because of this latter mechanism, these drugs differ greatly from traditional chemotherapy, which works primarily through the direct induction of cell death, suggesting that changes in cell viability may not be the most appropriate readout to evaluate these agents. Similarly, immunomodulating agents are used to enhance immunity, for example by blocking PD-1 signaling in T cells leading to a reversal of T-cell anergy [16,17]. Unlike many agents currently used in the treatment of CLL, this latter class of agents does not necessarily target the CLL cell, but rather accessory cells such as T cells. Lastly, BH3-mimetics target anti-apoptotic proteins key to CLL cell survival [18,19]. Although these agents act directly on the CLL cells and are cytotoxic, preclinical evaluation of these agents requires a culture system that mimics the upregulation of anti-apoptotic proteins observed in vivo [20].

Herein we discuss in vitro and in vivo preclinical models, with a particular focus on the benefits and potential pitfalls of different model systems to evaluate novel therapeutics for the treatment of CLL.

2. Preclinical modeling in vitro

Most preclinical modeling of CLL is performed in vitro using either primary CLL cells or tumorigenic cell lines mimicking the

* Corresponding author. Hematology Branch, NHLBI, NIH, Bldg 10, CRC 3-5256, 10 Center Dr, Bethesda, MD 20892-1202. Tel.: +1 (301) 496 5328; fax: +1 (301) 496 8396.
E-mail address: hermanse@mail.nih.gov (S.E.M. Herman).

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biological properties of CLL. In recent years, the importance of the microenvironment in the pathogenesis of CLL has become more evident [21–25]. Consequently, many novel therapeutic agents currently under development for CLL target not only intrinsic CLL signaling pathways, but also disrupt key tumor–microenvironment interactions. Because of this, traditional read-outs, such as cell death in vitro, may be less useful to evaluate these agents. For example, the low rate of apoptosis ($< 20\%$) induced by ibrutinib in vitro would not have identified this agent as a leading therapeutic [11]. To better model the activity of such novel therapeutics, microenvironmental conditions need to be recapitulated in vitro (Fig. 1).

2.1. Mimicking the microenvironment

It has been shown that CLL cells depend on signaling from the microenvironment. This is evident by the fact that CLL cells undergo apoptosis in vitro unless substitutes of survival signals found in the tumor microenvironment are provided. To this end multiple systems to recreate the microenvironment in vitro have been developed as model systems for CLL. Among the most widely utilized and probably most relevant to the evaluation of novel therapeutics are the stromal cell and the nurse-like cell (NLC) co-culture systems. The primary benefits and potential drawbacks of these co-culture systems are summarized in Table 1.

Stromal co-culture systems were first described by Panayiotidis et al in 1996. They demonstrated that culturing CLL cells on top of bone marrow–derived stromal cells (BMSCs) could increase the percent of viable cells after 10 days in culture by more than 30% compared to control [26]. Additionally, they demonstrated that BMSCs could maintain CLL cells for up to 30 days in 70% of patients [26]. The protection afforded by these co-culture systems was shown to be mediated by cell–cell contact. This was determined using a transwell system where CLL cells were separated by a porous membrane from stromal cell cultures—preventing any direct cell–cell contact. Under these conditions, no survival advantage was observed [26]. Further, conditioned media from stromal cells did not induce pro-survival signals in CLL cells [27]. More recently, it has been demonstrated that in addition to BMSCs, stromal cell lines (such as the murine bone marrow cell lines M210B4, SUM4, and KUSA-H1 and the human stromal cell lines: Hs5 and StromaNKTert) can protect CLL cells from drug-induced apoptosis [28]. In addition to providing a survival benefit stromal co-culture systems have been demonstrated to induce pro-survival signaling and upregulate anti-apoptotic proteins. Using the M210B4 stromal cell line, Edelmann et al demonstrated that co-cultured CLL cells display increased phosphoinositide 3-kinase (PI3K) and nuclear factor-κB (NF-κB) signaling as well as signs of a pro-angiogenic switch [29]. Additionally, an increase in the expression of the oncogene TCL-1, a key protein in CLL pathogenesis, was shown to be upregulated in these co-culture systems [30,31]. Kay et al developed a unique stromal co-culture composed of four stromal elements: epithelial fibroblast like cells, endothelial cells, phagocytic cells and large adipocytes. Using this system CLL cells were able to be maintained in vitro for more than 12 months [32]. The survival advantage afforded by co-culturing CLL cells on a stromal cell layer occurred concurrently with increases in expression of key anti-apoptotic proteins, including XIAP, MCL-1, BCL-2, and Survivin in the CLL cells [32]. Additionally a shift towards pro-angiogenesis was also observed in this model. Interestingly, stereotypic CLL B-cell receptors recognize stromal cell antigens suggesting that stromal cell co-culture systems can induce BCR-mediated survival signals [33]. This concept has been validated by

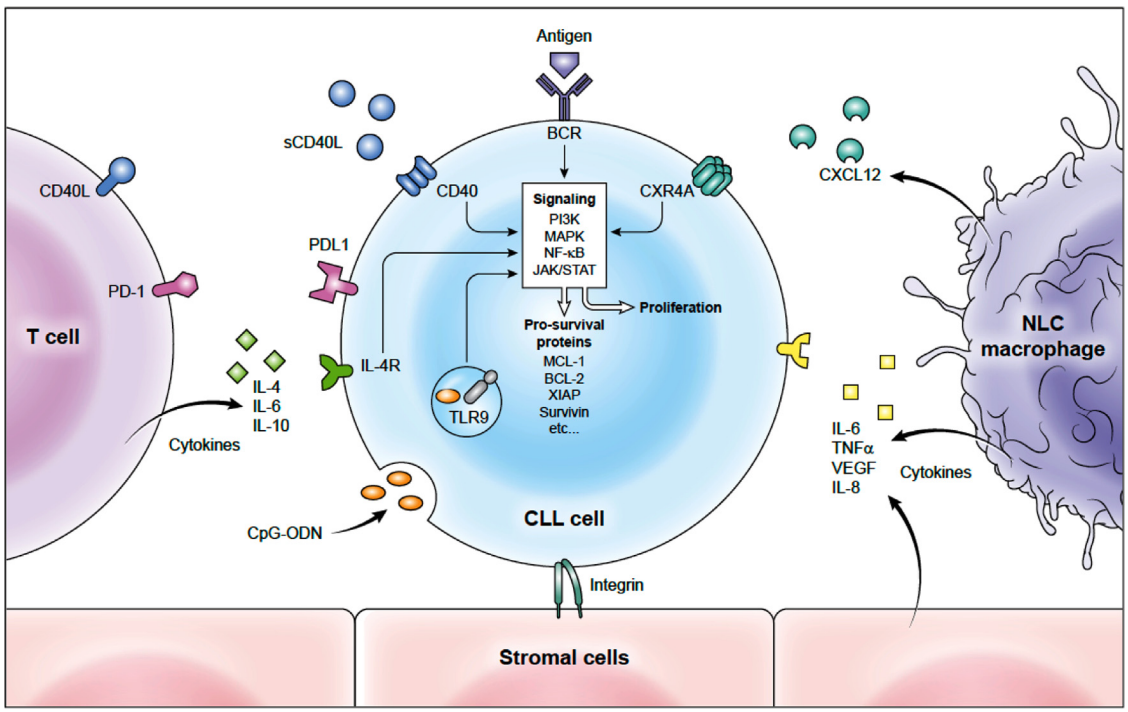

Fig. 1. Recapitulation of the CLL tissue microenvironment in vitro. In vivo CLL cells receive signals from multiple cell types leading to cellular activation, proliferation and survival. This can be recreated in vitro by the addition of accessory cells such as stromal cells, NLCs or other PBMC subsets that alter CLL cell signaling through either direct cell–cell contact or through the release of cytokines or chemokines such as IL-4, IL-8, or CXCL12. Additionally, CLL cell activation and signal transduction that occurs in the microenvironment can be replicated by the addition of soluble factors such as anti-IgM, which ligates the B-cell receptor (BCR), CpG-ODN, which activates Toll-like receptor 9 (TLR9) in endosomes, or cytokines such as sCD40L or IL-4. Binding of these soluble factors to their respective receptors leads to activation of key CLL signaling pathways, including BCR, PI3K, NF-κB, and JAK/STAT, leading to a multitude of downstream events including the up-regulation of pro-survival proteins (such as MCL-1, BCL-2, XIAP, and survivin).