

## **A blood pact: the significance and implications of eIF4E in lymphocytic leukemia**

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### Summary

Elevated levels of eukaryotic initiation factor 4E (eIF4E) are implicated in neoplasia, with cumulative evidence pointing to its role in the etiopathogenesis of hematological diseases. As a node of convergence for several oncogenic signaling pathways, eIF4E has attracted a great deal of interest from biologists and clinicians whose efforts have been targeting this translation factor and its biological circuits in the battle against leukemia. The role of eIF4E in myeloid leukemia has been ascertained and drugs targeting its functions have found their place in clinical trials. Little is known, however, about the pertinence of eIF4E to the biology of lymphocytic leukemia and a paucity of literature is available in this regard that prospectively evaluates the topic to guide practice in hematological cancer. A comprehensive analysis on the significance of eIF4E translation factor in the clinical picture of leukemia arises, therefore, as a compelling need. This review presents aspects of eIF4E involvement in the realm of the lymphoblastic leukemia status; translational control of immunological function via eIF4E and the state-of-the-art in drugs

**Key words**

Eukaryotic initiation factor 4E • eIF4E • lymphocytic leukemia • translation initiation • protein synthesis  
• hematological diseases • leukemia • ALL • CLL

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## Introduction

Leukemic transformation is a convoluted biological process embracing changes in a gene cascade, which ultimately conduces to the clonal expansion of defective stem cells. Cellular growth and differentiation within the hematopoietic lineage are compromised by the build-up of genetic lesions, frequently resulting in altered tumour suppressive activities. These genetic events—along with epigenetic occurrences—induce leukemogenesis, the course of which has been correlated with deregulated levels of specific proteins. A convincing body of evidence points at aberrant control of protein synthesis as a condition favourable to malignant transformation, ultimately contributing to lymphoid neoplasmas (Ruggero *et al.* 2004). Emerging models reference the eukaryotic translation initiation factor 4E (eIF4E) as a key player in leukemic transformation: eIF4E mRNA and protein expression levels have been observed up-regulated in human malignancies as non-Hodgkin lymphoma (NHL) (Wang *et al.* 1999), acute (AML) and chronic myelogenous leukemia (CML) (Topisirovic *et al.* 2003), and acute lymphoblastic leukemia (ALL) (Zhu *et al.* 2013). Promises exist for novel drug design where the translational apparatus represents a primary therapeutic target. This review explores the significance of eIF4E in the context of lymphoblastic leukemias, delving into eIF4E factor regulation and clinical implications in the light of its long-established role in translation and a newly emerging one in leukemogenesis. Emphasis is on pathways that undergo dysregulation in the leukemic status while upholding a neoplastic phenotype through protein synthesis deregulation. The topic of myeloid leukemia has been dealt with by numerous authors; therefore, it will not be outlined hereinafter. Excellent reviews on the subject are available to interested readers with regard to the biology of myeloid leukemia (Shivarov and Bullinger 2014), its epigenetic landscape (Paluch *et al.* 2016), and current drug therapy (Jabbour 2016, Kadia *et al.* 2016).

## The Lymphoblastic Leukemic State

Acute lymphoblastic leukemia (ALL) is a clonal malignancy that encompasses a group of precursor stage B/T lymphoid cells during the earliest stages of their development, therefore impeding differentiation and maneuvering aberrant cell proliferation. The clinical heterogeneity of the disease progression and outcome, when comparing the pediatric population to adults, portrays quite distinctive biological subtypes. ALL is the most ordinary malignancy diagnosed in children (reviewed in Bhojwani 2015), accounting for over a quarter of all childhood cancers. Subtypes of ALL include both precursor B and T acute lymphoblastic leukemia, Burkitt's leukemia, and acute biphenotypic leukemia, yet this review will focus on B and T lymphoblastic leukemias exclusively. The

preponderance of cases are of B-cell origin; however, ALL can besides arise from lymphoblasts committed to the T-cell lineage, a form of leukemia in which the malignant cells express immature T-cell immunophenotypes. Typified by small to medium-sized blast cells with inadequate cytoplasm, T-ALL portends an unfavourable prognostic outlook than B-precursor ALL in that it is characterized by chemotherapy resistance and frequent relapse (Tariq *et al.* 2009). Current treatment regimens encompass risk-adapted chemotherapy, hematopoietic stem-cell transplantation, and comfort care; improved therapy strategies for T-ALL with reduced long-term toxicities remain nevertheless a compelling need.

Chronic lymphocytic leukemia (CLL) is a condition characterized by a progressive accumulation of monoclonal B lymphocytes that preferentially affects adults over the age of 55, a disproportionate number of whom are males; while it occasionally occurs in younger adults, it appears to leave children mostly unaffected. As a low-grade lymphoproliferative disorder, CLL follows an indolent clinical course and, owing to its relatively longer survival rate, is the most common leukemia within the Western world's adult population (Redaelli *et al.* 2004). B-cell chronic lymphocytic leukemia (B-CLL) has the highest diagnostic recurrence among clinical entities of leukemic nature and is characterized by the progressive accumulation of immunologically competent, phenotypically mature B lymphocytes. T- and B-cells are usually associated with specific CD markers (Montillo *et al.* 2005, Niu *et al.* 2013) that discriminate on cell type (T- from -B) and diverse maturation stages; however, reports exist for cases of B-CLL that aberrantly co-express two T-cell-associated antigens (Espinosa *et al.* 2003, Jani *et al.* 2007). One of the most aggressive CLL subtypes is B-cell prolymphocytic leukemia (PLL), an extremely rare disease comprising less than 1% of B cell leukemias. This kind of leukemia may occur by itself, concurrently with CLL or, in due course, CLL may turn into PLL which progressively tends to worsen more quickly (Yamamoto and Goodman 2008). CLL differs from other leukemic diseases staging since most patients are asymptomatic or only mildly symptomatic at the time of diagnosis and do not require immediate treatment. Once a so-called "incurable" disease in the elderly, CLL can nowadays be fairly effectively alleviated with current standard treatments. Protracted remissions and enhancements in survival rates are improving, albeit a complete remission stage is unlikely to be attained with the current state of medical intervention and novel therapeutic strategies and drug candidates are utterly awaited.

### **The Eukaryotic Initiation Factor 4E**

In eukaryotes, a majority of cellular mRNAs is translated via cap-dependent translation (reviewed in Merrick *et al.* 2004, Sonenberg and Hinnebusch 2009). The m7GpppN cap structure present at the

5'-end of messenger ribonucleic acids is bound by eIF4E as a part of the eIF4F complex (Grifo *et al.* 1983, Sonenberg *et al.* 1978). A multisubunit structural entity, eIF4F consists of the rate limiting factor eIF4E and its interaction partners, the scaffolding protein eIF4G and the DEAD-box helicase eIF4A. eIF4E role is, however, not constrained to the pre-initiation complex: this key translation factor also functions in mRNA-related events as nucleocytoplasmic transport of mRNA, its sequestration and protection against exonuclease decay. Critical to gene expression, the step of translation initiation presides over the making of individual protein components by restraining the levels of cap-binding complex elements and mRNAs. Perturbations over translation control have dramatic biological effects and are a primary occurrence in malignancies (Ruggero *et al.* 2004). The least abundant of the initiation factors, eIF4E is overexpressed in many cancers and plays pivot roles in the development and progression of hematological malignancies in animal models and humans (Wang *et al.* 1999), rendering it an alluring target for the treatment of leukemias. The transformation activity of eIF4E is, however, not limited to its role in translation: close ties exist between eIF4E-dependent mRNA export and oncogenic transformation (Culjkovic-Kraljacic *et al.* 2012). The stability of eIF4E mRNA (Topisirovic *et al.* 2009) and dysregulation of related cellular processes as P-bodies and stress granules formation (Andrei *et al.* 2005, Kedersha *et al.* 2005, Frydryskova *et al.* 2016) could also contribute to eIF4E transformative capacity and deserve further investigation. Translational control of eIF4E activity is multi-level regulated by diverse cellular processes (Fig. 1). Among the better understood are the phosphorylation event which allows eIF4E to bind the 5' cap structure, the regulation of eIF4E expression level, and its interaction with translational repressor 4E-BPs. Translational control of eIF4E expression is implicated in many cancers; concordantly, eIF4E transforming properties have been linked to its ability to promote translation of genes involved in proliferation and survival. Based upon this evidence eIF4E has been acknowledged as an oncoprotein (Mamane *et al.* 2007). eIF4E overexpression leads to increased expression of a subset of mRNAs, many of which encoding mediators of the mitogenic response. Among them are the proto-oncogene c-Myc, the core component of the cell cycle machinery cyclin D1, B-cell lymphoma 2 (Bcl-2) protein, cyclin-dependent kinase 2 (CDK-2), the apoptosis inhibitor survivin, and the ornithine decarboxylase enzyme (Culjkovic *et al.* 2005, Culjkovic *et al.* 2013, De Benedetti *et al.* 1994, Rosenwald *et al.* 1995). At the nuclear level, eIF4E regulates the export—and subsequent expression—of many messenger RNAs implicated in cell cycle progression via the existence of a nucleotide structural element in the 3' UTR of the given transcripts (Culjkovic *et al.* 2005). Deregulation of eIF4E-mediated mRNA export has been shown to play a significant role in lymphomagenesis (Culjkovic-Kraljacic *et al.* 2016). In AML cells, most of the eIF4E is found in the nucleus, highlighting the importance to target nuclear mRNA export in addition to effects on

translation. Importantly, the movement of eIF4E to the cytoplasmic compartment correlated with remissions (Assouline *et al.* 2015). Although prominently relevant, not all dysregulated translation in cancer is driven by eIF4E. Other translation factors play a role in neoplastic transformation via a cap-dependent mechanism: for instance, overexpression of a number of eIF3 subunit proteins has been proposed to contribute to malignant phenotypes (Zhang *et al.* 2007).

### **Translational control of immunological function via eIF4E**

As a site of control over the immune system's activity, eIF4E has attracted the attention of cancer immunologists. Translational control of immunological function via the eIF4E node endures modulation by mammalian target of rapamycin complex 1 (mTORC1)-dependent inactivation of 4E-BPs and direct phosphorylation by the Mnk1/2 kinases (Beretta *et al.* 1996, Ueda *et al.* 2004). Besides, increases in eIF4E activity can be achieved by affecting its mRNA stability (Topisirovic *et al.* 2009) and/or stimulating its expression at the transcriptional level (e.g., via the transcription factor c-Myc). Given that the majority of eIF4E-sensitive 5' capped transcripts encode proliferation and antiapoptotic polypeptides, it is not surprising that leukemia research efforts have been directed towards modulating eIF4E expression, functions, and inter-related routes. So far, the most satisfactory results have been achieved in the treatment of AML however, a number of exciting research efforts are raising the prospect of significant headways in the cure of lymphocytic leukemias.

Among its reported activities, eIF4E acts as a downstream effector of the serine/threonine kinase mTOR complex 1 (mTORC1) the elevated activity of which is a prominent feature of cancer cells, including hematological malignancies (Vu and Fruman 2010). As anticipated in the text, eIF4E binds the 5' cap structure present on cellular mRNAs and initiates protein synthesis in virtue of its ability to associate with eukaryotic initiation factor 4G (eIF4G) and helicase eIF4A. Binding of eIF4E to scaffold protein eIF4G is restrained in a competitive manner by translational inhibitors, 4E-binding proteins (4E-BPs); upon mTOR-mediated phosphorylation, 4E-BP1 decreases its affinity for eIF4E and, by freeing the binding site, facilitates the joining of eIF4G thereby enhancing cap-dependent translation. Conversely, induction or activation of the tumour suppressor protein p53 rapidly lead to 4E-BP1 dephosphorylation (Constantinou *et al.* 2008), resulting in sequestration of eIF4E, decreased formation of the eIF4F complex and consequent inhibition of protein synthesis. The mTORC1–4E-BP–eIF4E pathway is regarded as an important axis that affects the translation of several proteins with central roles in immunology, e.g., the interferon regulatory factor IRF7, trans-acting T-cell-specific

transcription factor GATA-3, and the cytokine interleukin 4 (IL-4) (Colina *et al.* 2008, Cook and Miller 2010, Gigoux *et al.* 2014).

- eIF4E levels and T cells activation

Signals generated from the T-cell receptor (TCR) can trigger the activation of T cells from their naïve or quiescent state with a subsequent increase in processes as transcription, synthesis of DNA, and protein synthesis (Cooper 1969, Crabtree and Clipstone 1994). Polysome profiling analysis shows that activated T cells undergo a rapid increase of their protein synthesis levels, along with an augmented ratio of translation/ribosome (Cooper and Braverman 1977). An investigation over translation rates looking at eIF4E phosphorylation and effects of TCR stimulation conducted on thymocytes demonstrated a severe change in eIF4E activity following activation of these hematopoietic progenitor cells (Beretta *et al.* 1998). Furthermore, activation of human peripheral blood T cells by cross-linking of TCR-CD3 results in a strong increase in translation rates and expression of initiation factors (Mao *et al.* 1992). T cell development and activation are usually accompanied by assembly of proteins that require active translation, an early happening in activated T cells; eIF4E protein level increases during the first 24 hours (Boal *et al.* 1993) and later even double in T cell activation (Nikolcheva *et al.* 2002). This event occurs before or concurrently with the cellular appearance of RFLAT-1, a transcription factor of late-activated T lymphocytes-1, and hints to a role for eIF4E in the induction of RFLAT-1 protein expression. Overexpression of eIF4E increases RFLAT-1 protein level, whereas inhibition of Mnk1 —a kinase which phosphorylates eIF4E increasing its affinity for the mRNA cap— diminishes RFLAT-1 production rate (Nikolcheva *et al.* 2002). Echoing the notion that RFLAT-1 expression endures a cap-dependent regulation mechanism, involving eIF4E and Mnk1, are data evidencing that Mnk1 and Mnk2 kinases are required for T cell development and activation (Gorentla *et al.* 2013). In consideration of these findings, the prospect that eIF4E and its physiological Mnk1/2 kinases might be selectively required for efficient translation of specific protein subsets affecting T cell responses cannot be dismissed.

- eIF4E and memory T lymphocytes generation

The vast majority of lymphocytes is represented by CD4<sup>+</sup> T and CD8<sup>+</sup> T cells, the immunological memory of which is an essential component of protective immunity. In CLL patients, these cells are functionally impaired and exhibit features of T cells exhaustion (Riches *et al.* 2013). Therefore, developing strategies to induce an effective response in memory CD4<sup>+</sup> T and CD8<sup>+</sup> T cells represents a major ambition in cancer immunology. CD4<sup>+</sup> T helper cell lineage differentiation is defined by expression of specific transcription factors required for cell subset identity (Wong *et al.* 2011).

Among them Foxp3, a member of the forkhead box family, acts a key regulator by effecting lineage commitment and impacting T regulatory cell (Treg) development and function.

Bjur *et al.* (2013) reported the first genome-wide investigation on translational control in primary CD4<sup>+</sup> cell subsets, unveiling mRNA specific quanti- and qualitative differences in the translational profiles of these cellular subgroups. eIF4E mRNA was favourably translated in activated effector T (Teff) cells compared to activated Treg cells; in agreement with the mRNA level, eIF4E protein level was also greater in activated Teff cells. Importantly, polypeptides translated from eIF4E-sensitive, cell cycle-related mRNAs showed higher level of expression in activated T<sub>Foxp3-</sub> cells versus activated T<sub>Foxp3+</sub> cells. Thus, to an extent, the diverse translational landscapes of activated T cell subsets can be rationalized as a consequence of different eIF4E levels. In parallel to a greater eIF4E expression, translation of mRNAs comprising cyclins was augmented in activated Teff cells, echoing to the rationale that mRNAs engaging in cell cycle progression are more susceptible to eIF4E regulation. Along with cell cycle, other biological functions (i.e., ubiquitination and chromatin modification) were found to be co-regulated by eIF4E, strongly suggesting that a module of gene expression exists at eIF4E-dependent translational level in CD4<sup>+</sup> T cell subsets. Making use of an eIF4E cap binding inhibitor, the pro-drug 4Ei-1, Foxp3 expression could be induced in activated Teff cells upon inhibition of eIF4E activity under undifferentiating conditions, a finding that mirrors the lower eIF4E level observed in activated Treg cells. Differential thresholds of eIF4E levels between Teff and Treg cells seem to dictate translational pattern of eIF4E-sensitive, cell cycle-related mRNAs that, in turn, direct proliferation and ultimately define CD4<sup>+</sup> T cells lineage commitment. Translational control permits to promptly induce or terminate protein synthesis of specific polypeptides required during the inflammatory process. Activation of mTOR signaling by diverse environmental triggers might modulate eIF4E activity, regulate its translational activity on eIF4E-sensitive mRNA, and ultimately orchestrate T cell subset-specific responses. Bjur and colleagues' insightful investigation attests to the significance of translational control in the regulation of proliferation of T cell subgroups; it remains yet to be defined whether eIF4E activity modulates T cell lineage identity via a direct or an indirect fashion.

Lindquist *et al.* (2011) evaluated the presence of cytotoxic CD4<sup>+</sup> T cells—including Tregs—in the peripheral blood of patients affected by B-CLL, pre-B ALL, and B-cell lymphoma. Although these hematological cancers share the same B-cell origin, they differ with respect to the B cell maturation stage and the anatomical location of the leukemic cells. Elevated levels of Tregs and CD127<sup>high</sup> FoxP3<sup>+</sup> cells were determined in patients affected by CLL and both CD4<sup>+</sup> FoxP3<sup>+</sup> and CD4<sup>+</sup> FoxP3<sup>-</sup> T cells from patients with B-cell malignancy express cytolytic markers. Overall, cytotoxic



populations of CD4<sup>+</sup> T cells, including Tregs, are present in patients with B-cell malignancies and may be an important factor in immune-related disease control. A scenario is conceivable in which hematopoietic cancer cells are regarded as immune cells by the immune system. Under this assumption, the malignant B cells would be susceptible to regulation by CD4<sup>+</sup> T cells as well as Tregs. Echoing this hypothesis is a report on more favourable survival outcomes for patients with B-cell lymphoma who have a greater number of FoxP3<sup>+</sup> cells within the tumour (Tzankov *et al.* 2008). It seems plausible to postulate that eIF4E levels could be regulated in response to changes in the local inflammatory environment and, by this means, spanning the extracellular microenvironment, genetic programming, and consequential biological responses. Giving the significance of CD4<sup>+</sup> T cell subsets and their roles in immunity mechanisms, exposing regulatory processes that lie beneath gene expression affecting T cell function is of paramount relevance for therapeutic discovery.

mTOR, regulates memory CD8 T cell differentiation and, counterintuitively to its immunosuppressive nature, the mTOR specific inhibitor rapamycin shows unexpected immunostimulatory effects on the generation of memory CD8 T cells (Araki *et al.* 2009). Taking advantage of a retrovirus based RNAi system, Araki *et al.* (2009) investigated the consequences of distinctively knock-down various genes within the mTOR pathway (i.e., mTOR, its regulatory-associated protein raptor, ribosomal protein S6 kinase 1 (S6K1), eIF4E, and the immunophilin FKBP12) in antigen specific CD8 T lymphocyte cells. Retroviruses marked by GFP and expressing RNAi for a given gene or a control retrovirus were used to infect lymphocytic choriomeningitis virus (LCMV) specific transgenic CD8 T cells and these transduced cells were then transferred into naïve mice, followed by LCMV infection. Pursing more insight into mTOR-regulated cell memory formation, the authors examined the role of S6K1 and eIF4E: knockdown of such mTORC1 downstream effectors significantly enhanced memory CD8 T cell differentiation, thereby suggesting that mTOR exerts its effect through these downstream proteins (Araki *et al.* 2009).

- The mTORC1–4E-BP–eIF4E signaling pathway and cell cycle in lymphocytes

Cellular protein synthesis plays an explicit role in cell cycle progression. Appropriate cell size and geometry are prerequisites to enter and successfully complete the cell division process. Altogether, a growing body of literature indicates eIF4E as a mediator of mTOR-dependent cell cycle control.

Fingar and colleagues (2004) identified the 4E-BP–eIF4E pathway as one of the major mTOR-dependent downstream signaling routes in mediating G1 phase progression. The expression level of eIF4E is the determining factor in whether eIF4E triggers a negative feedback loop to constrain deviant cell cycle progression and proliferation. Interestingly, cells overexpressing eIF4E displayed: a) a minor, however significant, acceleration of S phase entry when rapamycin-treated; b) an increase

rate of G1 phase progression from quiescence to S phase in a rapamycin-free environment. Simultaneous downregulation of both S6K1 and 4E-BP-eIF4E pathways impedes G1 phase progression to a degree comparable to that elicited by mTOR inhibitor rapamycin (Fingar *et al.* 2004).

The transitional repressors 4E-BPs are key mTOR effectors controlling growth and proliferation in activated B and T cells. Making use of an inducible *in vivo* system So *et al.* (2016) showed that inhibiting eIF4E was sufficient to impede lymphocytes growth and proliferation. Accordingly, deletion of 4E-BPs partially rescued B cells from the effects of mTOR inhibition. In lymphocytes, rapamycin disrupted eIF4E function due to an abundance of 4E-BP2 over 4E-BP1 in these specific cells and their greater sensitivity to this drug. It was concluded that 4E-BP1/eIF4E route is rapamycin sensitive in lymphocytic cells only where it affects clonal expansion by coordinating both growth and proliferation (So *et al.* 2016).

- eIF4E and Mnk1/2-direct phosphorylation events

Besides the modulation exerted by the mTORC1-4E-BP pathway, studies have also linked the Mnk1/Mnk2-eIF4E route to mechanisms controlling immunological functions. Indispensable for eIF4E phosphorylation but not for cell growth, Mnk1 and Mnk2 are eIF4E kinases (Ueda *et al.* 2004) that control protein synthesis via regulating its activity. In genetically engineered mouse lymphoma models, eIF4E-mediated transformation is dependent of its ability to start translation via phosphorylation on serine 209 (Ser209). While the precise mechanism through which eIF4E phosphorylation mediates malignant transformation in these models is yet elusive, no uncertainty surrounds the absolute requirement for Mnk1/2-mediated Ser209 phosphorylation for eIF4E oncogenic activity (Wendel *et al.* 2007). Non-phosphorylatable forms of eIF4E are less efficient in causing *in vivo* transformation, highlighting the therapeutic potential for targeting the Mnk1/2 kinases to impede eIF4E-mediated transformation (Konicek *et al.* 2011, Ueda *et al.* 2010). Notably, targeting mTOR and Mnk1/2-eIF4E routes concurrently conducts to a more potent antileukemic response than the solo targeting of each pathway (Altman *et al.* 2010). A recent investigation from Lim *et al.* reports the Mnk1/2-eIF4E axis as a significant controller of blast crisis (BC) self-renewal (Lim *et al.* 2013), endorsing therapeutic exploration into Mnk1/2 kinase pharmacologic inhibition. To date, however, promising results seem to be confined to BC chronic myeloid leukemia. The effect of Mnk1/2 on tumorigenesis occurs probably through eIF4E phosphorylation (Furic *et al.* 2010); however, Mnk1/2 affects the malignant process also via other targets e.g., hnRNPA1, a nuclear ribonucleoprotein which is phosphorylated in response to T cell activation (Buxad  t *et al.* 2005), Sprouty2 (DaSilva *et al.* 2006) and cPLA2 (Hefner *et al.* 2000).

- eIF4E and proto-oncogene c-Myc-induced protein synthesis

Neoplastic conditions are commonly associated with elevated levels of eIF4E (Zhu *et al.* 2013); a remarkable increase in its activity is achieved by enhancing its expression via activation of given transcription factors (Jones *et al.* 1996). Elements of the protein synthesis machinery are established targets of c-Myc whose ability to regulate the transcription of such components has been validated in several cell types. eIF4E itself is a long-known c-Myc target (Rosenwald *et al.* 1993) and more recent work from Lin *et al.* (2008) establishes the remaining two components of the eIF4F complex, eIF4A and eIF4G, as bona fide c-Myc targets. The same authors suggested a positive cascade loop between c-Myc and the eIF4F complex as a contributing factor to lymphoma development (Lin *et al.* 2008). Nevertheless, the cellular consequences, the specifically involved messenger RNAs, and the stages guiding neoplastic transformation triggered by c-Myc-dependent augmented protein synthesis remain ill-defined. Transcription factor c-Myc has been found implicated in B-cell lymphomagenesis (Ruggero *et al.* 2004) and is frequently overexpressed in T-ALL (Palomero and Ferrando 2008) where it acts in cooperation with eIF4E in the development of such malignancy. C-Myc mRNA is itself a translational target of eIF4E exerting a feedback mechanism on the so-called RNA regulon (reviewed in Culjkovic *et al.* 2007): c-Myc promotes the transcription of eIF4E (Schmidt 2004), which, in turn, translationally stimulates c-Myc mRNA expression (Lin *et al.* 2008). Furthermore, eIF4E activity is regulated by c-Myc via stimulation of mTOR-mediated 4E-BPs phosphorylation (Pourdehnad *et al.* 2013). The reciprocal dependency of eIF4E and c-Myc has the potential to be exploited therapeutically to take action against c-Myc driven malignancies, expressly c-Myc driven lymphomas which are highly dependent on 4E-BP1.

An investigation carried out on transgenic mice in which eIF4E expression is driven by the  $\beta$ -actin promoter (BetaT-eIF4E) found B-cell lymphoma among the most represented cancer types (Ruggero *et al.* 2004). Tumour initiation occurs relatively late during the life time of these animals, a latency suggesting that eIF4E may cooperate with additional oncogenic events in tumour development. One contribution towards leukemogenesis comes from the circuitual activity of eIF4E and the critical gene regulator c-Myc. An effective, genetic cooperation lies behind: eIF4E suppresses c-Myc proapoptotic function, therefore resulting in a marked acceleration of B-cell lymphomagenesis; on the other hand, c-Myc antagonizes the proliferative disadvantage resulting from eIF4E-dependent senescence (Ruggero *et al.* 2004).

Mechanisms of protein translation control through lncRNAs have been recently described (Gumireddy *et al.* 2013, Yoon *et al.* 2012) and some clarification on the regulation of c-Myc

translation was presented: growth arrest-specific 5 (GAS5), a lncRNA linked to normal growth arrest in both leukemic and untransformed human lymphocytes, interacts and cooperates with eIF4E to regulate c-Myc translation through lncRNA-mRNA interaction. The same publication informs on how, under mTOR regulation, GAS5 lncRNA is recruited to the translation initiation complex eIF4F via direct binding with eIF4E (Hu *et al.* 2014).

- p53 regulation of eIF4E

Activation of mTOR1 can reduce the levels of cellular tumour antigen p53 via eIF4E (Mungamuri *et al.* 2006); in turn, p53 is involved in the regulation of eIF4E expression through its interaction with the proto-oncogene c-Myc, thereby preventing c-Myc binding to eIF4E promoter (Zhu *et al.* 2005). Inactivation of the p53 pathway is a universal event in human cancers and promotes tumourigenesis and resistance to chemotherapy. Mutations in p53 have been linked to hematological malignant diseases, although with a minor incidence than in solid tumours (Krug *et al.* 2002). Direct inactivating p53 mutations are infrequent in non-complex karyotype leukemias, suggesting that the p53 pathway must endure inactivation via alternative mechanisms (Schittenhelm *et al.* 2013). Following finding of induction of wt-p53 repressed eIF4E expression in acute lymphoblastic EU-3 cells, Zhu and colleagues (2005) deduce an association between elevated eIF4E expression levels, its tumourigenicity, and defects in p53. The same authors suggest eIF4E expression as being under the mutual regulation of p53 and c-Myc; hence, loss of p53-mediated control over c-Myc-dependent transactivation of eIF4E might promote neoplastic transformation in an eIF4E-dependent fashion (Zhu *et al.* 2005). EIF4E promoter activity is constrained by p53 gene transfection, whereas inactivation of p53 following overexpression of the murine double-minute 2 oncoprotein (MDM2) human homolog results in stimulation of EIF4E promoter activity (Zhu *et al.* 2005). A Tumour-Associated Antigen (TAA), MDM2 is overexpressed in hematological malignancies (Jones *et al.* 1988) where it prompts an immune response and is presented as TAA in CLL human cells aiding tumour-specific T cells proliferation (Mayr *et al.* 2006). Hsu *et al.* (2011) described a strong association between elevated eIF4E levels and increased expression of Mdm2 in esophageal cancer, providing further evidence of the balanced regulatory loop

- eIF4E-Notch regulation in T/B cell lymphocytic leukemia

The family of transmembrane Notch receptors plays a pivotal role in cellular fate along with a recognized importance in ordinary lymphocyte function (Radtke *et al.* 1999, Wolfer *et al.* 2002). Abnormal regulation of Notch is held as responsible in the development and progression of human

malignancies including leukemia, where Notch aberrant signaling exhibits either an oncogenic or tumour suppressive nature with conceivable context-dependent features (Gothert *et al.* 2007). Notch1 mutations triggering its continuous signaling were uncovered in nearly 60% of T-ALL patients, making Notch1 the most prominent oncogene involved in the pathogenesis of T-ALL (Weng *et al.* 2004). Studies from Zou *et al.* (2013) have unveiled that Notch1 signaling is required for multiple pathogenic processes in T-ALL (Zou *et al.* 2013), suggesting that pharmacological inhibitors of Notch1 signaling may be attractive interventions for T-ALL treatment. The exact mechanism through which Notch1 induces leukemogenesis remains to be fully characterized since it has become apparent that the NOTCH1 mutations uncovered in human T-ALL display only a feeble oncogenic activity (Ntziachristos *et al.* 2012). Thus, within the Notch pathway, additional initiating and collaborating genetic events may be involved in the initiation and progression of T-ALL.

The Notch pathway exerts control over eIF4E directly via c-Myc and indirectly via the c-Myc-tuberous sclerosis complex (TSC)-mTOR-4E-BP1 axis (Schmidt 1999). Notch further regulates post-transcriptional events by promoting the expression of HuD (Bellavia *et al.* 2007), an RNA-binding protein expressed in human T-ALL which co-localizes with both eIF4E and the translation initiation complex component poly(A)-binding protein (PABP) (Bolognani *et al.* 2010). Notch signaling contributes to apoptosis resistance in CLL in part by sustaining antiapoptotic protein Mcl-1 expression. Mcl-1 downregulation by Notch targeting is accompanied by reduced phosphorylation of eIF4E, indicating this key translation initiation factor as an additional target of the Notch pathway in CLL cells (De Falco *et al.* 2015).

### **Leading the path towards cure**

Significant progress has been attained in understanding the biological core behind leukemia occurrence. Within clinical research, efforts are spent on elucidating the mechanisms of resistance to current available medications, while advancements in medical therapies stemming from molecular biology lead the quest for better drugs. In the context of clinical stage, outcomes are still substantially inconsistent, demanding for additional markers to predict survival and direct management in early staged leukemic patients. CLL is considered incurable, except possibly in a minority of patients whose physical conditions make a stem cell transplant bearable. New treatments, or better combinations of the existing ones, are demanded to overcome the deadlock we appear to have come in the treatment of leukemia at its acute stage of development. The prospect of effectively targeting eIF4E and its interrelated signaling routes in leukemia therapy is

alluring, given that eIF4E is a downstream node on which multiple oncogenic signaling pathways converge. To date, however, efforts in this direction have yielded limited clinical success.

- mTOR Kinase Inhibitors

A variety of rapamycin analogue agents (rapalogs) have been employed with success to target mTOR signaling pathway in a number of malignant hematological occurrences and demonstrated significant preclinical activity against ALL, thereby prompting a considerable number of clinical trials. Owing to the synergistic effects shown with conventional cytotoxic agents (e.g., methotrexate and corticosteroids), mTOR inhibitors are able to overcome pathways of chemotherapeutic resistance affecting conventional ALL treatment. Whereas first generation rapalogs only achieve an incomplete block of mTOR downstream signalling by targeting one element of the pathway (i.e., mTORC1), newer molecules are designed to target different kinases within the PI3K/AKT/mTOR route (e.g., PI3K, mTORC1 or mTORC2). A number of pharmaceutical agents under development have their target(s) extended to other path components, comprising eIF4E and the phosphoinositide-dependent protein kinase 1 (PDK1). Recent studies on T-ALL cells reveal that, although mTORC1 or mTORC2 knock-down diminishes proliferation of blasts, only the severe constraint of both mTOR complexes via eIF4E knockdown or inhibitor compounds instigate their death (Schwarzer *et al.* 2014), corroborating the notion that mTOR-driven leukemogenesis crucially hinges upon augmented cap-depend translation.

A novel dual mTOR ATP active site inhibitor, PP242, targets mTOR kinase functions in both complexes (TOR1 and TOR2) and modulates the activity of key components of the translational apparatus. This compound encouragingly exhibits elevated selectivity towards leukemic cells versus normal bone marrow and peripheral blood lymphocytes (Janes *et al.* 2010). Notably, PP242 attains inhibition of cap-dependent translation under conditions in which rapamycin is ineffective (Feldman *et al.* 2009) and its antileukemic activity is reported to be influenced by eIF4E phosphorylation levels at its major phosphorylation Ser209 site (Shi *et al.* 2015). A study on human leukemic cells revealed PP242 prominent cytotoxic effect on the Philadelphia-positive (Ph+) ALL SUP-B15 cell line, expressly in combination with the anthracycline daunorubicin (DNR). DNR activates the Akt/mTORC1/eIF4E cascade, a detrimental side effect ablated by PP242, which likewise strengthens the antiproliferative feature of DNR therapy in a synergistic fashion. Moreover, PP242 acts as a translation inhibitor of the antiapoptotic protein Mcl-1 by downregulating the Akt/mTORC1/eIF4E signaling pathway (Shi *et al.* 2015).

Using a pharmacogenetic approach, Pourdehnad *et al.* (2013) proved that mTOR-dependent phosphorylation of 4E-BP1 is required for tumour cell survival in c-Myc-dependent hematological malignancies. Accordingly, hyperactivation of 4E-BP1 renders c-Myc-driven lymphomas and myelomas druggable by a class of mTOR active site inhibitors capable of impeding 4E-BP1 phosphorylation. The second generation, ATP-competitive mTOR kinase inhibitor MLN0128 impedes mTOR-dependent 4E-BP1 phosphorylation (Pourdehnad *et al.* 2013). Confined so far to faithful models of diffuse large B-cell lymphoma, Burkitt's lymphoma, and multiple myeloma this mTOR active site inhibitors show promising therapeutic efficacy across an extensive range of blood-related cancers typified by c-Myc overexpression (Pourdehnad *et al.* 2013).

- Immunomodulating Agents

Immunomodulatory drugs are currently employed in medication management of chronic lymphoblastic leukemia. In monotherapy applications, Lenalidomide and Ofatumumab have proven themselves clinically efficient in patients with episodes of CLL relapse (Ferrajoli *et al.* 2008, Strati *et al.* 2016). Lenalidomide pro-apoptotic benefit is exerted via disturbance of the phosphatidylinositol pathway (Herman *et al.* 2011) and decreased activation of distinctive enzymes; two of such are the oncogenic kinase Akt2 and the pro-survival cascade ERK, both involved in tumourigenesis via eIF4E-mediated mechanism. Importantly, an established mechanism through which Lenalidomide exerts its activity is by decreasing the expression of the anti-apoptotic agent Bcl-2 and of the translation checkpoint protein eIF4E (Li *et al.* 2011). When employed as first medical intervention in CLL patients, this immunomodulating agent showed an encouraging response in long term responder subjects, normalizing the CD4+/CD8+ cells ratio as well as circulating T lymphocytes (Strati *et al.* 2013). Although promising altogether (Buhler *et al.* 2016, Wendtner *et al.* 2016), warnings on the appearance of secondary cancers following lenalidomide administration, at least in multiple myeloma patients (Usmani *et al.* 2012), require further enquiry on the safety of this compound.

- Multikinase inhibitors

Inhibition of the mTOR pathway—better if in combination with class I histone deacetylase inhibitors—represents a promising treatment strategy for mature B cell neoplasms like mantle cell lymphoma (Simmons *et al.* 2014), a leukemic condition that shares many features with CLL as a common CD5+ B-cells origin, specific chromosomal aberrations, and clinical presentation (Bentz *et al.* 2000, Hoeller *et al.* 2013). The natural polyphenol Resveratrol (RSV) induces significant

dephosphorylation of 4E-BP1, cell cycle arrest, apoptosis, and autophagy in T-ALL cells via inhibition of the Akt/mTOR/4E-BP1 pathway (Ge *et al.* 2013); disappointingly, RSV administration in immunodeficient NOD/SCID mice engrafted with human ALL failed to delay leukemia progression (Zunino *et al.* 2012).

Sorafenib is an oral drug co-marketed by Bayer and Onyx Pharmaceuticals under the commercial name of Nexavar®; as a multikinase inhibitor, it reduces eIF4E phosphorylation level and downregulates the Bcl-2 family member Mcl-1. This small molecule inhibitor significantly downregulates the PI3K/Akt/mTOR pathway, a circuit that controls the activation of eIF4E by regulating the phosphorylation of translation inhibitors 4E-BPs. FDA approved for therapy of several carcinomas, Sorafenib was moreover proven to display significant antileukemic activity in vitro, prospecting opportunities for therapeutic intervention in both ALL (Schult *et al.* 2010) and CLL (López-Guerra *et al.* 2012).

- Ribavirin

Whereas active-site inhibitors of mTOR show some promise and inhibitors of eIF4E phosphorylation may emerge as clinical candidates, to date, the sole compound to demonstrate antitumour activity associated with eIF4E inhibition in patients is ribavirin; this same drug is the only direct inhibitor of eIF4E to reach clinical trials (Assouline *et al.* 2009, Assouline *et al.* 2015). A guanosine analog, ribavirin suppresses eIF4E-induced transformation by lodging in the binding site devoted to the eIF4E-5' cap interaction (Kentsis *et al.* 2004, Volpon *et al.* 2013); consequently, it competes for eIF4E:RNA association, changes the eIF4E subcellular localization and disrupts eIF4E-mediated nuclear-cytoplasmic mRNA export (Culjkovic *et al.* 2005, Kentsis *et al.* 2004). A ribavirin-induced switch from nuclear to cytoplasmic eIF4E localization has also been observed in blast cells during clinical studies (Assouline *et al.* 2009, Assouline *et al.* 2015). Ribavirin was proposed to act as a physical mimic of the m7G moiety thereby blocking eIF4E activity (Kentsis *et al.* 2004); controversially, two independent groups reported on its incapability to function as an mRNA cap analog (Westman *et al.* 2005, Yan *et al.* 2005). Incongruences between the original findings and Westman and Yan's results have been later explained as dependent on the experimental conditions (Kentsis *et al.* 2005). Specific ribavirin loading to the eIF4E cap-binding pocket was further supported by NMR studies with human eIF4E and its non-functional W56A mutant (Volpon *et al.* 2013). Zahreddine *et al.* identified a ribavirin glucuronidation that prevents its binding to eIF4E as a possible causation of drug resistance that developed in patients involved in clinical trial using ribavirin monotherapy to treat refractory AML. This observation provides another evidence for specific



interaction between eIF4E and ribavirin *in vivo* (Zahreddine *et al.* 2014). However, other ribavirin targets have also been identified. Ribavirin inhibits an inosine monophosphate dehydrogenase (IMPDH) (Yamada *et al.* 1988), the rate-limiting catalyst of the GTP biosynthesis pathway, leading to reduced levels of cellular guanosine nucleotides. Lowering of intracellular guanosine levels by submillimolar ribavirin treatment was evidenced by Suzuki *et al.* who were able to reverse ribavirin-induced expression of coagulation Factor VII in HepG2 human hepatoma cells to normal level by adding guanosine to the culture medium (Suzuki *et al.* 2013). Recently, ribavirin was reported to inhibit histone methyltransferase zeste homolog 2 (EZH2). The same study showed that siRNA-mediated knock-downs of EZH2, eIF4E, IMPDH1 and IMPDH2 led to the substantial growth inhibition of the MCF-7 human breast adenocarcinoma cells. Reduced cell proliferation was not further significantly intensified by ribavirin, giving additional evidence that all these proteins can serve as a ribavirin target (De la Cruz-Hernandez *et al.* 2015). Recent clinical data from a small cohort of lymphoma patients receiving ribavirin therapy to treat viral infection prior or immediately after transplantation suggested that ribavirin may have a significant anti-lymphoma activity (Rutherford *et al.* 2018). Plausibly, the favourable outcomes of ribavirin treatments are to be attributed to its pleiotropic effects on at least all three known targets (De la Cruz-Hernandez *et al.* 2015), importance of each of them may differ from tumour to tumour.

Drug resistance is a major clinical difficulty in addressing medical strategies, posing serious public health threats. In the realm of leukemia, developed resistance to chemotherapy interventions can be ascribed, to a degree, to the lymph node microenvironment via induction of pro-survival BCL2 family (Morin 2003). Analog-based nucleosides represent the standard regimen for many leukemic patients; within this framework, it seemed conceivable to assess the contribution of eIF4E to the purine analog fludarabine (FLU) resistance in primary CLL lymphocytes. Genuine eIF4E inhibitors (i.e., Ribavirin and CGP57380) have proven effective adjuvants in sensitizing primary CLL lymphocytes to fludarabine treatment *in vitro* (Martinez-Marignac *et al.* 2013). Besides sparse experimental enquiries, the role of eIF4E in drug resistance remains inadequately explored.

- 4EGI-1, an inhibitor of cap-dependent translation

As earlier reviewed in the text, the assembly of the eIF4E/eIF4G protein complex has a central role in the regulation of gene expression at the translational level. The joining of these two initiation factors is regulated by 4E-BP translational repressors, which compete with eIF4G for binding to the dorsal surface on EIF4E. With the intent to pharmacologically mimic 4E-BP function, Moerke and colleagues (2007) developed a high-throughput screening assay to characterize small-molecule

inhibitors of the eIF4E/eIF4G interaction. The most potent compound identified was 4EGI-1, a synthetic cap-translation inhibitor that binds eIF4E, disrupts eIF4E/eIF4G association, and restrains cap-dependent translation. While 4EGI-1 displaces eIF4G from its eIF4E binding site, it promotes 4E-BP1 association as observed in model systems *in vitro* (Moerke *et al.* 2007). Echoing 4E-BPs tumour suppressor activity, this translation inhibitor shows great promise: it decreases cellular expression of oncogenic proteins encoded by weak mRNAs, exhibits activity against multiple cancer cell lines, and preferentially affects transformed cells rather than non-transformed ones (Chen *et al.* 2012). A recent study (Schwarzer *et al.* 2014) reports that exposure of T-ALL clones to 4EGI-1 triggers a rapid induction of apoptotic mechanism, while gene set enrichment analysis reveals that mRNAs sensitive to such compound regulate core oncogenic pathways in T-ALL. Some basal mRNA translation is retained following treatment with 4EGI-1, a finding which may justify the low toxicity of this compound on noncancerous tissues (Chen *et al.* 2012). Synergistic drugs combinations have also been a focus of study. BH3-mimetic inhibitors of the Bcl-2 family of pro-survival proteins belong to a class of chemical compounds currently explored for CLL. When used in a synergistic approach with homology domain 3 (BH3) mimetic ABT-737 agent, 4EGI-1 restores sensitivity to the BCL2 apoptosis via both cap-dependent and cap-independent mechanisms in CLL (Willimott *et al.* 2013), laying the basis for a therapeutically valuable drug combination. The identification of 4EGI-1 inhibitory activity is not only promising for drug discovery but it also equips us with a powerful tool to scrutinize translational control, opening up to new anti-cancer strategies.

- A lesson from a failed clinical trial

Late in 2014, Denovo Biopharma announced the acquisition of enzastaurin, a late-stage oncology drug candidate the development of which was abandoned from Eli Lilly. A serine/threonine kinase inhibitor, this acyclic bisindolylmaleimide suppresses angiogenesis and induces apoptosis in several human cancer cell lines—including B-lymphoid cell lines—by targeting protein kinase C and PI3K/AKT routes; this latter pathway signaling frees eIF4E via the hierarchical phosphorylation of translational repressors 4E-BPs. Enzastaurin treatment affects signaling through the AKT/mTOR pathway leading to hypophosphorylation of 4E-BP in cancer cells of diverse lineages and showed promises for combination therapy in patients refractory to single drugs alone (Liffraud *et al.* 2012). Treatment with Enzastaurin increases the amount of eIF4E bound to 4E-BP1 and decreases association of eIF4E with eIF4G, thereby reducing eIF4F translation initiation complex levels. Remarkably, enzastaurin-induced apoptosis is blocked in cancer cells depleted of 4E-BP1 by siRNAs or in 4E-BP1/2 KO murine embryonic fibroblasts cells. Moreover, eIF4E and 4E-BP1 expressions are respectively augmented

and diminished in cancer cells exhibiting reduced sensitivity to enzastaurin (Dumstorf *et al.* 2010). Although enzastaurin did not meet primary endpoint in Phase III PRELUDE clinical trial, patients' responsiveness trend in progression-free survival led to its re-evaluation in the realm of genetic analysis. This attests to an emerging trend in the identification of biomarkers correlated with patients' positive responses as late-stage trial candidates may enable personalized targeted therapies and optimize further potential treatments. It further reiterates the importance of modulating eIF4E function—and all together eIF4F levels—indicating 4E-BP1 as a determinant of antileukemic compounds activity in hematological tumour types.

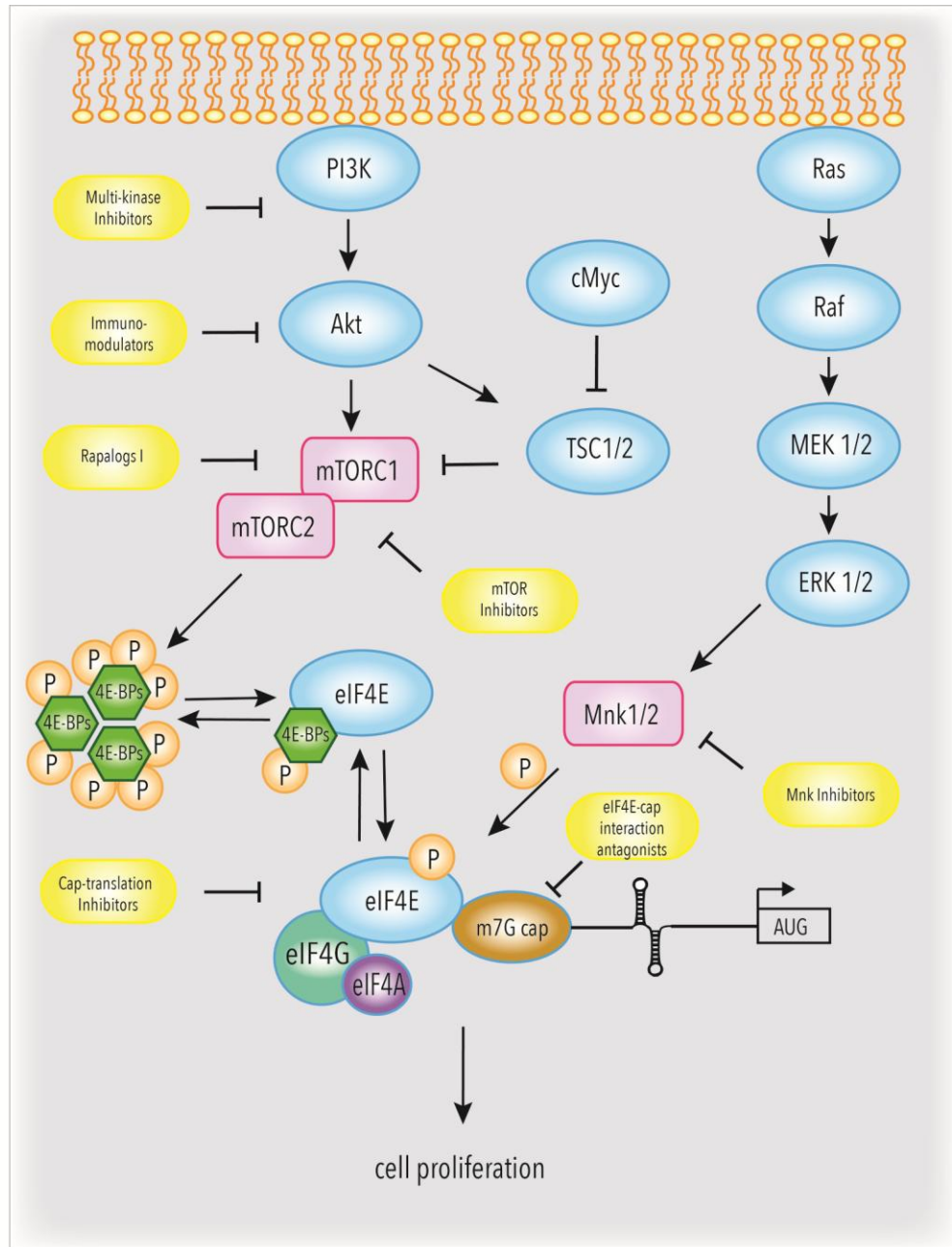
- Additional eIF4E family members complicate the therapeutic targeting of the Akt/mTOR/eIF4E pathway

Molecules targeting specific mechanisms of translation upon which selected cells are dependent may represent an unprecedented opportunity for therapeutic success. In this perspective, two additional eIF4E family members termed eIF4E2 and eIF4E3 (Joshi *et al.* 2004) are worth mentioning. They are speculated to meet specialized roles in regulating mRNA recruitment to the ribosomal structure, conceivably as a result of their different cap binding abilities and interactions with eIF4G and 4E-BPs. eIF4E2 is assumed to be mostly involved in translation repression (Tao *et al.* 2015). Under hypoxic conditions, eIF4E2 takes part in active translation (Uniacke *et al.* 2012), congruently with being part of a gene expression signature which underlies an ability of solid primary tumours to form metastases (Ramaswamy *et al.* 2003). Novel roles are emerging for the least studied eIF4E family member. Overexpression of mouse eIF4E3 reduced growth and foci formation in mouse NIH 3T3 fibroblasts and human U2OS osteosarcoma cell line. Interestingly, the same group observed lower expression of eIF4E3 mRNA in primary human blood specimens from M4/M5 AML patients relative to healthy volunteers (Osborne *et al.* 2013). In diffuse large B-cell lymphoma, enhanced eIF4E3 expression partly suppresses eIF4E-driven translation while exhibiting a unique translome (Landon *et al.* 2014). Recent work on two major variants of the human eIF4E3 isoform, has unveiled that eIF4E3\_A but not eIF4E3\_B associates with the translation initiation complex (Frydryskova *et al.* 2016). At the same time, inability of eIF4E3\_A to bind 4E-BPs (Joshi *et al.* 2004) might spare it from the canonical translation control. This raises speculations on whether eIF4E3\_A carries out basal translation initiation when eIF4E is repressed. This hypothesis is supported by relocalization of eIF4E3\_A to stress granules but not to P-bodies upon a stress insult, whereas canonical eIF4E localizes to both these RNA granules in stress conditions (Frydryskova *et al.* 2016). It is tempting to

hypothesize that eIF4E3\_A might secure translation of specific subset of mRNAs which do not respond to changes directed by cellular pathways controlling eIF4E function in translation initiation.

### **Final remarks**

A thorough understanding of protein synthesis control has a far-reaching potential to shed light on the complex pathogenesis of leukemia, address its clinical management, and develop innovative therapies. We are witnessing an appreciation for targeting factors functioning within the framework of translation initiation as a new opportunity for therapeutic intervention in the realm of the leukemic state. Challenging questions remain yet unanswered concerning leukemic lesions that directly impinge on dysregulated activities of the translational machinery. To identify messenger RNAs pertinent to leukemic transformation, along with components of the translational apparatus that hold leukemogenic potential, would add a crucial piece to the puzzle. Elucidating the clinical significance of eIF4E expression in both ALL and CLL would represent a predictor of clinical outcome and meet a burning medical need for novel targeted therapies and synergistic interventions. Undoubtedly, it would ease the development of drug combinations and contribute to a refinement in clinical management with the prospect of chemo-free strategies as concrete therapeutic alternatives in patients affected by lymphoblastic leukemias.



**Fig 1. Mechanisms of translation initiation control via the eIF4E hub are pertinent to deregulated lymphoblastic activity.** In the realm of lymphoblastic leukemia therapeutical interventions targeting eIF4E have been attempted by mimicking of the 5' cap structure (Ribavirin, 4Ei-1), inhibition of eIF4E specific MNK1/2 kinases, cap-translation inhibitors disrupting 4E-4G factors binding (4EGI-1), immunomodulators (Lenalidomide and Ofatumumab), inhibition of the PI 3K-Akt-eIF4E axis (Sorafenib), and of one (first generation Rapalogs) or both (PP242) mTOR complexes. eIF4E hub inhibitors are discussed in detail in the text.

## **Abbreviations**

eIF4E, eukaryotic initiation factor 4E; NHL, non-Hodgkin lymphoma; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, Acute lymphoblastic leukemia; CLL, Chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; Bcl-2, B-cell lymphoma 2; CDK-2, cyclin-dependent kinase 2; mTORC, mammalian target of rapamycin complex 1; Mnk, mitogen-activated protein kinase interacting protein kinases; eIF4G, initiation factor 4G; IRF7, interferon regulatory factor 7; IL-4, interleukin 4; TCR, T-cell receptor; R-FLAT-1, RANTES factor of Late Activated T Lymphocytes-1; Treg, T regulatory cell; Teff, effector T; S6K1, S6 kinase 1; FKBP12, FK506-binding protein; LCMV, lymphocytic choriomeningitis virus; BC, blast crisis; GAS5, growth arrest-specific 5; MDM2, murine double-minute 2; TAA, Tumour-Associated Antigen; TSC, tuberous sclerosis complex; PABP, poly(A)-binding protein; PI3K, phosphoinositide 3-kinase; PDPK1, phosphoinositide-dependent protein kinase 1; RSV, Resveratrol; IMPDH, inosine monophosphate dehydrogenase; FLU, fludarabine.

## **Conflict of Interest**

The authors declare that there are no conflicts of interest.

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