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Pathway of LCK Tyrosine Kinase and mTOR Signaling in Children with T-Cell Acute Lymphoblastic Leukemia

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Abstract: The aim of this study is to analyze available research on targeting signaling pathways for the development of new drugs in patients with T-cell acute lymphoblastic leukemia (T-ALL). This analysis focuses specifically on the role of LCK tyrosine kinase and mTOR signaling pathways in pediatric patients. Outcome: Current literature suggests that these pathways play a significant role in the regulation of T-cell cycles, making them potential therapeutic targets. However, despite promising findings, there remains a need for further research, particularly in pediatric populations, to fully understand the therapeutic implications and to optimize drug development. The conclusion drawn from this analysis highlights the significant influence of LCK and mTOR on T-cell cycle regulation, underscoring the importance of continued investigation in this area.

Keywords: T-ALL, pediatric, signaling pathways, targeted therapy

Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of pediatric leukemia, accounting for 80% of pediatric leukemia. Acute T-cell lympho-blastic leukemia (T-ALL) accounts for approximately 12% to 15% of diagnosed ALL cases in pediatric patients.[1] Overall survival rates are higher in pediatric patients (80%) than in adult populations (50[2] ALL most commonly occurs in children 2–5 years of age. Although treatment outcomes in patients with T-ALL have improved in recent years, most patients relapse, most often within 2 years of diagnosis. It is estimated that only 25% of patients are in remission.[3,4] The prognosis of T-ALL has improved with cure rates reaching over 75% in children.[5] However, re-lapse is still the problem in this disease as it is associated with lower survival rates, and approximately only 20% of the relapsed patients are expected to be cured with current salvage protocols.[6] Modern procedures of diagnosis require morphology, immunophenotype, and genetic testing for abnormalities.[7] T-ALL is a very heterogeneous disease characterized by various gene mutations, chromosomal aberrations, and impairments in multiple signaling pathways such as phosphoinositide mechanistic target of rapamycin (mTOR) network (about 75–80% of the patients)[8] and which usually is associated with a poorer outcome in T-ALL patients. Over the last decades mTOR has become a promising target for T-ALL patients, and differentiating types of inhibitors have been developed and tested in clinical trials.

LCK Tyrosine Kinase

Lymphocyte-specific protein tyrosine kinase (LCK), whose Lck gene is located in chromosome 1 (1p35.2), is an obligatory enzyme to enable T-cells to develop and mature.[9] Its phosphorylation, which can be done by auto- and trans-phosphorylation, is crucial for T-cell receptor (TRC) signaling to be initiated. A signal pathway of LCK leads to the

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phosphorylation of tyrosine residues of immunoreceptor tyrosine-based activation motifs (ITAMs) based on ζ-chains of the TCR/CD3 complex.[9,10] LCK is set on CD4/CD8 coreceptors on thymocytes as its main location, and it is assumed that this specific coreceptor is responsible for including the MHC in the bonding and providing its LCK to TRC.[10,11] Additionally, free molecules of LCK can also play a key role in TCR/CD3 complex phosphorylation. Interaction between the basic residue-rich part of CD3ζ and the acidic amino acid sequence in a unique place of LCK is requested for initiating the connection of kinase and TCR/CD3 complex, which starts the cascade of reactions in ITAMs activation.[11] Following ITAMs phosphorylation, Zap-70, as a Syk family kinase, is involved in bonding with previously activated TCR via the SH2 domain. Zap-70 was formerly activated as a result of phosphorylation made indirectly by LCK. Afterward, signaling is promoted by Zap-70, which leads to phosphorylation of the transmembrane adaptor protein linker for activation of T cells (LAT). LAT combines TCR with the intracellular signaling pathways and eventually activates T-cells.[11,12] As LCK is necessary for TCR to initiate signaling, it seems like a promising target to control the aggressiveness of T-ALL and potentially prevent its morbidity. That is why the role of LCK in signaling modules of chimeric antigen receptor (CAR)–engineered T cells has become a recent direction of studies. CAR can easily connect to specifically targeted antigens, mostly expressed on tumors, independently from MHC via a single-chain variable fragment (scFv) recognition domain.[13] The most used cytoplasmic domains in ALL treatments are CD28 or CD137, nevertheless the way of activation of CAR signaling and subsequent co-stimulation of CD3ζ ITAM are yet to be widely discovered.[14] The mechanism of action of the LCK tyrosine kinase is presented in Figure 1.

LCK in Acute Lymphoblastic Leukemia

As previously mentioned, LCK is a vital enzyme for T-cell differentiation, proliferation, adhesion, and regulation of the cycle.[15] Recent studies have shown that the levels of CD4/LCK and CD8/LCK in ALL are significantly high in thymocytes and might not be meaningfully lower in thymus, which suggests that the highlighted complexes may indicate rapid proliferation and maturing of a greater population of T-cells compared to the standard quantity.[16] However, the exact role of LCK in T-cells maturation and TCR/LCK signaling pathway in T-ALL is yet to be discovered.[9]

The ABL1 gene encodes a ubiquitously expressed tyrosine kinase and has been found to be rearranged in 8% of T-ALL cases. The most common T-ALL-specific ABL1 rearrangement is NUP214-ABL1 episomal amplification (6% of the cases), which has been described in T-ALL in adult and pediatric patients associated with TLX1 or TLX3 expression and CDKN2A deletion.[17] T-ALL containing expression of constitutively phosphorylated tyrosine kinase NUP214-ABL1 are sensitive to selective tyrosine kinase inhibitors (TKi) such as imatinib and dasatinib.[18]

Connection Between LCK and Other Cancers

Additionally, it is important to mention that high LCK expression may lead to other tumors, such as oral squamous cell carcinoma, small cell lung carcinoma, colon cancer, prostate cancer, and human breast cancer, characterized by acquiring a high invasive capacity leading to metastases.[19]

LCK Immunotherapy is a Potential Key to Curing

Leukemia was considered an almost incurable cancer. A huge difficulty in treatment was the lack of one specific placement in the human body, which precluded it from elected treatment as it is used in solid cancer treatment. However, recent studies have shown enormous potential in curing children who suffer from T-cell ALL using immunotherapy including modified CAR. All three generations of CAR own a tumor-targeting domain, which comes from a monoclonal antibody connected to CD3ζ, that acts like a signaling domain inside a cell. In the second generation, either 4–1BB or CD28 is added as a co-stirring domain; meanwhile, the third generation includes elements such as 4–1BB, OX40 (CD134), inducible T-cell costimulator (ICOS), and CD27. The last one's purpose is to increase the number of T-cells together with their stability.[20] Described modifications are meaningfully increasing the effectiveness of all leukemia treatments.

Two drugs containing CAR T products have been approved in the United States and Europe to be used in refractory T-ALL in patients up to 25 years old [KYMRIAH (tisagenlecleucel, CD19 CAR T cells) and YESCARTA (axicabtagene ciloleucel, CD19 CAR T cells).[20] Moreover, recent studies show that growing cell cultures of T-CAR surrounded by interleukins present better anti-tumor function. As IL-2 promotes T-cells multiplying and differentiation, it also enables

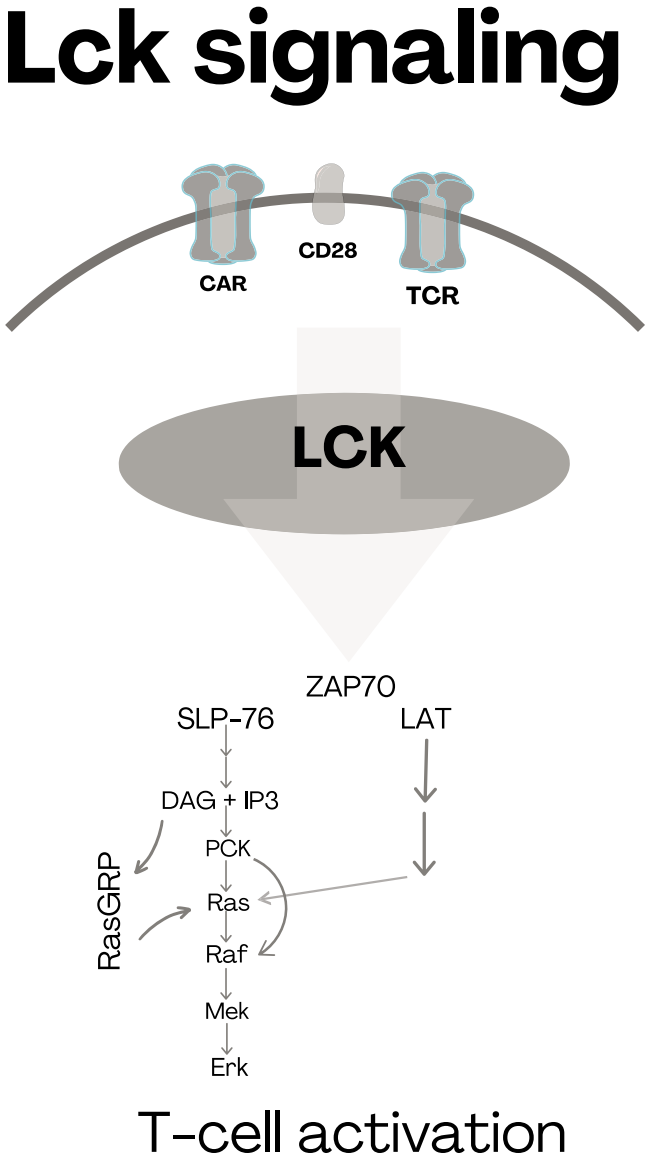

Figure 1 The mechanism of action of the LCK tyrosine kinase.

malformed T-cells death led by their activation. Even better results are shown by using the surroundings with IL-7 and IL-15. The results are auspicious, and more research needs to be done combining both T-CAR and interleukins during clinical trials.[21]

Imatinib is an ABL1 kinase inhibitor, with an impact on T-ALL NUP214-ABL1 positive lines. Due to the small number of patients with oncogenic NUP214-ABL1 fusion kinase, the clinical data remains insufficient. The therapeutic potential in the treatment of T-ALL (NUP214-ABL1-positive) also lies in the inhibition of LCK using dasatinib and bosutinib. In vitro in the pre-clinical trials, both drugs successfully inhibit the activity of the LCK tyrosine kinase.[22] The therapeutic success of these inhibitors is backed by their use in case reports, and in monotherapy with dasatinib complete hematologic remission was obtained.[23]

LCK consists of Y394 and Y505 tyrosines, the key points for regulating LCK. The phosphorylation of the Y394 is required to stabilize the activated LCK catalytic domain. On the contrary, phosphorylation of the Y505 stabilizes the inactive form of the LCK catalytic domain. Dephosphorylation of the mentioned key points is controlled by the transmembrane tyrosine phosphatase CD45. The CD45 indicates dual action, both activating and inactivating LCK activity.[24] The dephosphorylation and inactivation of Y394 can also be promoted by tyrosine phosphatase non-receptor

type 2 (PTPN2), PTPN22, SH2 domain-containing phosphatase 1 (SHP1), dual specificity protein phosphatase 22 (DUSP22).[25,26] The complexity of controlling the mechanisms of activation of the LCK holds promising insights into future discovering of new inhibitors and new therapies of T-ALL.

Disulfiram (originally used for alcohol abuse) is another promising drug to be used in anti-cancer immunotherapy by affecting TCR signaling. Wang. Q et al studies showed that disulfiram directly binds to Cys20/23 of LCK and activates the kinase, thereby boosting CD8+ T cell's immune response to cancer cells. The use of disulfiram is potentially valuable in TCR-T or CAR-T therapies.[10]

The escort Heat shock protein 90 (HSP90) is involved in safeguarding the correct three-dimensional arrangement of proteins. HSP90 was detected to be excessively expressed in leukemia cells, and its elevated presence was essential for the sustenance and proliferation of cancerous cells.[27] Taipale M et al's research has uncovered associations between LCK with HSP90.[28] Although HSP90 blockers were frequently explored as anticancer medications, Mshaik et al observed that NVP-BEP800, functioning as an inhibitor of the ATP binding site of HSP90β48, can hinder LCK in T-ALL Additionally, noted that this medication decreased the viability of primary T-ALL cells in laboratory tests. Furthermore, the growth and proliferation of leukaemia cells were suppressed in NVP-BEP800-treated xenografted mouse models. Moreover, these studies showed that HSP90 served as a crucial controller of SRC kinases, which played a pivotal role in the intracellular signalling pathways crucial for the proliferation and growth of T-ALL cells. These observations indicated that the guidance of SRC kinase by HSP90 contributed to the proliferation and growth of T-ALL cells, presenting innovative targeting approaches for ALL therapy. The encouraging results from preliminary tests need further exploration in clinical trials.[29]

Dasatinib is a tyrosine kinase inhibitor and has a broad spectrum of activity. However, its long-term use is associated with side effects (nausea, vomiting, diarrhoea, rash, and liver toxicity).[30,31] In Yuan et al using a combined photosensitizer zinc(II) phthalocyanine with the small-molecule-targeted drug dasatinib, photocytotoxicity at nanomolar concentrations was demonstrated in an animal model, and remarkable tumour regression was demonstrated in a study on T-ALL cells.[31]

Laukkanen et al's study showed that dasatinib induced a strong suppression of LCK phosphorylation at the activating phosphotyrosine Y394 in Jurkat, MOLT-4, and PF-382 cells. This causes the inhibition of the following components of the TCR signaling pathway, including loss of ZAP70 and LAT phosphorylation, which are needed downstream of LCK for effective signaling.[32]

In the study, Laukkanen et al, dasatinib was shown to suppress LCK kinase activation and subsequently shut down the TCR signalling pathway to halt cell cycle progression and induce T-ALL cell death. It inhibits many downstream components of the TCR signalling pathway, including the loss of ZAP70 and LAT phosphorylation, which are required downstream of LCK for efficient signalling. The study was conducted ex vivo using transgenic danio. In addition, mouse xenografts were used to analyse responses to therapy in 9 diagnostic T-ALL samples from children and 5 from adults. After ex vivo treatment, 4 primary childhood T-ALL responded to combination therapy, of which 1 was resistant to dexamethasone-induced cell killing.[33]

On the other hand, dasatinib therapy causes side effects such as nausea, vomiting, diarrhoea, rashes, and liver toxicity. In the paper, Yuan et al modified dasatinib by synthesising dasatinib and zinc(II) phthalocyanine. The researchers believe that such a photodynamic compound will increase the efficacy of dasatinib by reducing treatment-related complications.[31]

Blinatumomab is a CD19/CD3 bispecific monoclonal antibody T cell engager, which recognizes and eliminates CD19-positive acute lymphoblastic leukemia (ALL) blasts.[9,34] In Leonard JT study, compound ABL inhibitors and Src/ABL inhibitors with blinatumomab in vitro from healthy donor and patients with Ph+ ALL. Blinatumomab alone led to both T cell proliferation and elimination of CD19+ target cells and increased interferon-γ (IFN-γ) production.[32]

mTOR Signaling

The mechanistic (or mammalian) target of rapamycin (mTOR) is a serine/threonine protein kinase belonging to the PI3K-related kinases (PIKK) family, which is encoded by the MTOR gene, located in chromosome 1 (1p36.22). The mTOR pathway has a significant role in cellular physiology as a central point of growth controlling network.[35] The changes in the activity of the mTOR are involved in many pathologies, and hyperactivity of the mTOR signaling is present in the majority of cancers. The mTOR protein is a component of two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[36,37]

mTORC1 consists of three core components: mTOR, Raptor (regulatory protein associated with mTOR), and mLST8 (mammalian lethal with Sec13 protein 8)[38] mTORC1 has a crucial role in regulating the balance between anabolism and catabolism. mTORC1 promotes protein synthesis, through the phosphorylation of p70S6 Kinase 1 (S6K1) and eIF4E Binding Protein (4EBP), de novo synthesis of lipids, through the sterol responsive element-binding protein (SREBP), and de novo synthesis of pyrimidine nucleotides through a posttranslational mechanism, that may result in higher RNA and DNA synthesis.[35,39] Recent studies have shown a role for mTORC1 in T-cell maturation.[40] The regulation of the mTORC1 activity depends on growth factors, cellular energy, amino acids, oxidative stress, and many more.[41]

mTORC2 contains mTOR, mLST8, and Rictor (rapamycin-insensitive companion of mTOR). The main function of these complexes is to control cell proliferation and survival. mTORC2 phosphorylates mainly AGC kinases (PKA/PKG/PKC) members protein kinase C (PKC) family, serum- and glucocorticoid-induced kinases 1 (SGK1). Likely to mTORC1, mTORC2 plays a key role in cell metabolism which involves lipids, glucose, nucleotides, fatty and amino acids.[42,43]

As hyperactive mTOR signaling occurs in a wide range of pediatric cancers, it makes mTOR an attractive target for therapy. Inhibitors of mTOR are Rapamycin and its analogues (also called rapalogs). Rapamycin is an antibiotic, produced by the Streptomyces hygroscopicus. Due to its immunosuppressive properties, it has been established as an immunosuppressant, but the anti-cancer activity of rapamycin has also been proven.[44–46] Rapalogs have been the subject of clinical trials and were approved by the Food and Drug Administration (FDA) for some types of cancers. The next-generation inhibitors such as ATP-competitive mTOR inhibitors and Rapalink have been developed and are showing promising results, but larger-scale trials need to be conducted.[47]

The role of mTOR pathway metabolism and physiology is essential, proper regulation would be the key to settling cellular homeostasis, and mTOR is a centre for controlling cell growth, apoptosis, and metabolism. Studies have proven the influence of this pathway in the differentiation and functioning of T-cells. Freshly activated T-cells aerobic processes to meet their energetic needs. These processes are regulated by mTORC1 signaling which regulates transcription of many glycolytic enzymes.[48]

Activation of mTOR depends on growth factors, cytokines, stimulation of T-cell receptor (TCR), nutrient availability, and energy status of the cells, immature thymocytes migrate from the bone marrow to the thymus, where the dynamic process of proliferation and metabolic activity occurs. Development is accompanied by increased mTOR activity and higher expression of transmembrane transporters dependent on mTOR. Inhibition of the mTOR pathway in T-cells leads to poorer proliferation and changes in differentiation that can be associated with non-efficient glycolytic energy uptake.[49] Th1 cells' development depends on the mTORC1 complex. CD4+ cells without mTORC1 activating GTRase Rheb do not differentiate from the Th1 cells. Moreover, Rictor deficiency reduces the differentiation of Th1 cells due to the downregulation of the AKT pathway Unlike Th1 Th2 cells do not depend on mTORC1, but they fail to develop without TORC2 activity (Laboratory tests have shown that Th1 and Th17 differentiation is mTORC1 dependent but not Th2).[50] The use of rapamycin has shown inhibition of the CD8+ (memory) cells at the cost of other effector cells. The mechanism of action of the mTOR is presented in Figure 2.

mTOR in T-Cell Acute Lymphoblastic Leukemia

(PI3K)/Akt/mTORC pathways are active in many T-ALL (detectable in 70–85% of the patients). It leads to higher cell proliferation and an increase in the stem cell population but is also associated with a poorer outcome (and resistance to the treatment).[51] In T-cells, acute lymphocytic leukemia has been identified as a mutation that causes hyperactivation of both mTOR complexes. This mutation (C1483Y) occurs in the FRAP, ATM, and TRRAP domains, and as a result, a decrease in the level of Raptor and an increase in the level of Rictor are observed.[9] T-ALL clones are also identified with PTEN (phosphatase and tensin homologue), and NOTCH1 (neurogenic locus notch homolog protein 1) mutations, PTEN dephosphorylates PIP3, thus yielding PIP2 and blunting PI3K activity. NOTCH1 mutations are detected in more than 50% of T-ALL. NOTCH1 is a factor controlling both mTOR components' activity.[52] The mTOR signaling pathway in Clinical Trials was showed in Table 1.

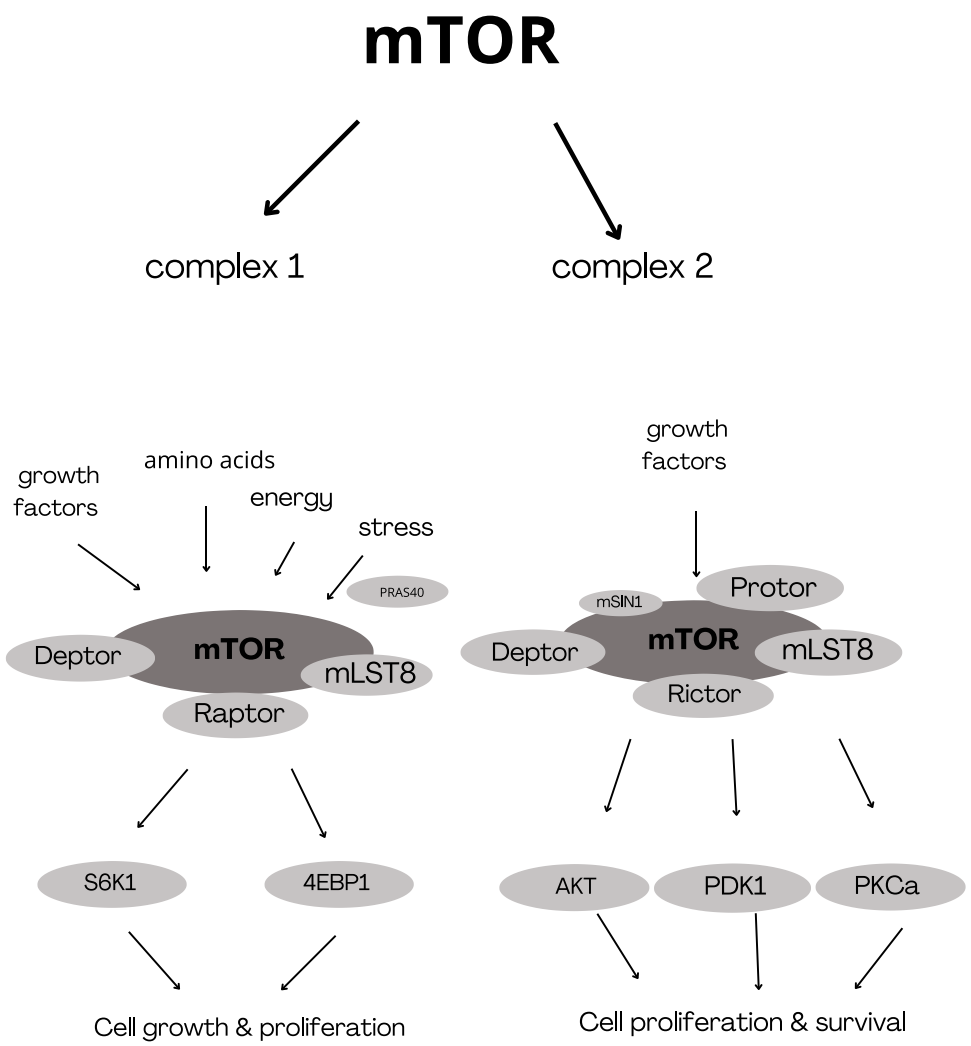

Figure 2 The mechanism of action of the mTOR.

Therapeutic Targeting

The mTOR signaling pathway plays a key role in T-ALL cell survival; these complexes are crucial for antileukemic therapy. As for this day, three classes of inhibitors are in use:

- Allosteric inhibitors (targeting mTORC1)
- Rapamycin (Sirolimus)
- RAD001 (everolimus)
- CCI-779 (temsirolimus)
- ATP-competitive dual PI3K/mTOR inhibitors (targeting PI3K, mTORC1/2)
- PKI-587 (Gedatolisib)
- BEZ235
- ATP-competitive mTOR kinase inhibitors (targeting mTORC1/2)[53–55]
- AZD8055
- OSI-027

Rapamycin cooperates with the FK506-binding protein 12 (FKBP12). Due to this interaction, the signalling of growth cytokinins interferes. Rapamycin associates with the FKBP12-rapamycin-binding (FRB) domain of mTORC1 and

Table 1 mTOR Signaling Pathway in Clinical Trials

Drug	Clinical Trials	Phase	
Rapamycin	NCT01184885	Early 1	Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone (Hyper-CVAD) with Sirolimus
Everolimus	NCT03328104	1	Safe and effective in adults and children for the treatment of T-cell ALL
Everolimus	NCT00081874	1and2	
Everolimus	NCT009682253	1and2	Including children (10 years and older) with relapsed ALL. Plus HyperCVAD chemotherapy
Everolimus	NCT01523977	1	18 months and older with relapsed ALL. Combined with prednisone, vincristine, PEG-asparaginase, doxorubicin
Temsirolimus	NCT01614197	1	Combination with Etoposide and Cyclophosphamide in Children
Temsirolimus	NCT01614197	1	Combined with etoposide and cyclophosphamide
Sirolimus	NCT01087554	1	Combination of Sirolimus (designed to block a protein mTOR) or Everolimus or Temsirolimus (designed to block a protein mTOR) and Vorinostat

downregulates the activity of this mTOR complex.[56] However, the use of rapamycin has its limitations, in ensuring the further of ALL treatment rapalogs, like RAD001 and CCI-779 were developed. The effects of inducing apoptosis of this group of inhibitors could be elevated by combining them with glucocorticoids (GCs). Yet, children with T-ALL resist GCs[54] methotrexate, cyclophosphamide, doxorubicin, and idarubicin.[56–59] The effective treatment with allosteric inhibitors in T-ALL has been proven in preclinical trials, blocking T-ALL cell proliferation depending on interleukin 7 (IL7) and inducing apoptosis of these cells.[60] The clinical trials of Everolimus, other drugs, and Temsirolimus evaluate their use in patients with ALL (including children).[61,62]

The metabolic patterns of ALL still need to be more adequately comprehended. Nevertheless, there have been indications of a significant elevation of Glut1 and Hexokinase 1 and 2 (HK1 and HK2) in primary human peripheral T-ALL blood specimens compared to T cells sourced from healthy donors. The adjustment of energy demand is regulated via mTORC1 inhibition, which governs anabolic growth, resulting in reduced aerobic glycolysis. The modulation of metabolic stress and apoptosis by AMPK could potentially offer novel therapeutic avenues for managing T-ALL. Recent analyses have investigated the transcriptional profiles of several glucocorticoid-resistant T-ALL cells exhibiting heightened expression of genes associated with metabolic pathways. Treatment with rapamycin has been observed to enhance cellular sensitivity to the glucocorticoid-dexamethasone, indirectly linking metabolic upregulation with mTOR function. In contrast to sensitive ALL cells, recent research has outlined the metabolic profile of daunorubicin-resistant T-ALL cells, revealing a heightened reliance on glucose and a reduced dependence on glutamine and fatty acids. The diminished expression of glutamate-ammonia ligase (GLUL), as well as low levels of glutamine metabolism genes ASNS and ASS1, and reduced expression of transporter SLC1A5 and pantothenic acid, may indicate a broader adaptation based on a metabolic reprogramming characteristic of drug resistance in tumour cells.[63]

The obstacle presented by GC resistance significantly impedes the effectiveness of T-ALL chemotherapy.[64] Silic-Benussi et al in a study delved into the intricate interplay between mTOR and reactive oxygen species (ROS) equilibrium in GC-resistant T-ALL cells. Their findings unveiled that inhibition of mTOR decreased the activity of glucose-6-phosphate dehydrogenase (G6PD), a pivotal enzyme in the pentose phosphate pathway (PPP). Consequently, NADPH levels declined, fostering elevated ROS levels and the eventual demise of T-ALL cells.

This revelation sheds light on the interconnectedness of mTOR and ROS equilibrium within T-ALL cells, offering substantive mechanistic backing for the combined use of glucocorticoids and mTOR inhibitors as a viable therapeutic strategy against refractory T-ALL. Notably, experiments demonstrated that while Jurkat cells exhibited complete dexamethasone resistance, they displayed responsiveness to everolimus. Furthermore, the concurrent administration of dexamethasone and everolimus significantly augmented cell death.[65]

A notable repercussion of ROS accumulation in everolimus-treated T-ALL cells is the proteolytic cleavage of OPA1, an inner mitochondrial membrane protein pivotal in mitochondrial dynamics, cristae remodelling, and apoptosis regulation. This discovery builds upon prior Silic-Benussi et al research where it was showcased that heightened ROS levels in T-ALL cells prompted the activation of OMA1, a ROS-sensitive protease responsible for OPA1 cleavage, thus sensitizing cells to proapoptotic stimuli.[66] This study provides compelling support for the synergistic deployment of glucocorticoids alongside mTOR inhibitors to combat refractory T-ALL by attenuating G6PD activity is the rate-limiting step in the pentose phosphate pathway.[65]

Dual PI3K/mTOR inhibitors were developed to overcome some of the shortcomings of the allosteric inhibitors such as partial inhibition of the translation depending on mTOR complex 1 or activation of Akt (observed in rapamycin/rapalogs use).[53] PI-103, NVP-BEZ235 showed higher potential in inducing apoptosis in T-ALL than rapamycin[52,67] However, to reduce the toxicity present after their use ATP-competitive inhibitors were developed.[68]

In separate studies of acute leukemias, particularly ALL, Tensirolimus was used as an mTOR inhibitor. Rheingold et al combined it with reinduction chemotherapy and observed significant toxicity and poor tolerance in children, though many participants achieved a complete response with minimal residual disease (MRD). Tasian et al paired Tensirolimus with Cyclophosphamide and Etoposide, reporting similar response rates but with reduced toxicity, using fewer doses. Both studies highlighted varying degrees of effectiveness and safety, with some participants showing very low levels of MRD post-treatment.[62,69]

A Phase I trial examined temsirolimus combined with etoposide and cyclophosphamide in children with relapsed acute lymphoblastic leukemia (ALL) (NCT01614197). Mammalian targets of rapamycin inhibitors have been shown to inhibit the growth of pre-B and T-cell ALL lines both in vitro and in xenograft models. Temsirolimus was selected due to its weekly IV administration, consistent blood levels, established pediatric MTD (maximum tolerated dose) as a single agent, and its sustained effect via conversion to sirolimus. The study aimed to determine the MTD of temsirolimus combined with dexamethasone, cyclophosphamide, and etoposide in relapsed ALL cases.[70]

In a study involving pediatric patients with relapsed acute lymphoblastic leukemia (ALL), treatment with a combination of Everolimus, Vincristine, Prednisone, Pegaspargase, and Doxorubicin resulted in complete remission for 19 out of 22 consecutive patients.[61]

The treatment regimen combining Temsirolimus with Dexamethasone, Vincristine, Mitoxantrone, Pegaspargase, and intrathecal Methotrexate was evaluated in pediatric patients (median age 9 years) with relapsed or refractory ALL. In the study, nearly half of the participants achieved remission. As for therapy-related adverse events, the majority of the children (73%) developed neutropenic fever and over half experienced severe infections, including one case of life-threatening bacterial sepsis. In this study, incorporating temsirolimus into re-treatment led to intolerable toxicity in children with relapsed ALL. Dose-limiting toxicities were observed at every dosage level.[69]

Daver et al's study evaluates the safety and efficacy of combining the mTOR inhibitor everolimus with HyperCVAD in patients aged 10 years or older with relapsed or refractory ALL. The combination was feasible, showing promising results, particularly in heavily pretreated T-ALL patients, with half responding and a median survival of 23 weeks.

Hematologic effects, infections, and mucositis were the main toxicities, with the maximum tolerated everolimus dose set at 5 mg/day. The everolimus-HyperCVAD regimen did not increase toxicity compared to HyperCVAD alone, suggesting that targeting ALL with mTOR inhibitors, especially in T-ALL, is a promising approach.[71]

The mTOR inhibitors were among the initial PI3-K-AKT-mTOR pathway blockers tested in pediatric patients. Early mTOR inhibitors comprised drugs like everolimus, temsirolimus, sirolimus (also known as rapamycin), ridaforolimus, umirolimus, and zotarolimus. Later, second-generation inhibitors emerged, including ATP-competitive mTOR kinase inhibitors. These included dual mTORC1/2 inhibitors such as torin-1, torin-2, and vistusertib, as well as mTOR/PI3-K dual inhibitors like paxalisib, samotolisib, and SF-1126.[72]

Several clinical trials involving everolimus in pediatric cancer have been published, including two specifically focused on hematological malignancies. These trials explored the efficacy and safety of everolimus as a treatment option for children with blood-related cancers, investigating its potential role in improving outcomes either as a single agent or in combination with other therapies. The studies aimed to assess how well pediatric patients tolerate everolimus, its effects on disease progression, and its overall therapeutic value in managing hematological cancers.[61]

Sirolimus (Rapamycin) is an immunosuppressant that acts by inhibiting the mTOR pathway. *MYCN* proto-oncogene overexpression has been reported to be associated with poor prognosis in pediatric T-ALL.[73]

The outcomes propose that the effect of rapamycin on adult T-ALL is probably mediated by downregulation of MYCN. MYCN may be a potential target for the treatment of adult T-ALL.[74]

Curcumin

Curcumin, a natural compound, has been shown to induce apoptosis in chemotherapy-resistant leukemia cells by inhibiting mTOR signaling.

Unlike conventional mTOR inhibitors, curcumin targets both the mTOR and BCAT1 pathways, enhancing its apoptotic effect. Studies have highlighted that long-term mTOR inhibition can trigger compensatory survival mechanisms, but curcumin may avoid these issues by blocking downstream components, making it a more effective and less toxic treatment option.

Additionally, curcumin influences epigenetic modifications, such as histone methylation and acetylation, which further suppress mTOR activity. This multi-targeted approach broadens the potential for using mTOR inhibitors in cancer treatment, particularly in cases of chemotherapy resistance.[75]

ATP-Competitive mTOR Kinase Inhibitors (TORKIs)

Inhibition of RNA translation is induced by these molecules, which leads to a decrease in the level of oncogenic proteins. Clinical trials of the TORKI TAK-228 (NCT02484430) in combination with chemotherapeutics are conducted.[52]

Other Promising Therapies

Ginsenoside Rh2 with a Hydroxyl Group at the C-20 Position (20(S)-GRh2)

Using 20(S)-GRh2 lowers the levels of phosphorylated PI3K, Akt, and mTOR. The studies suggest that the PI3K/Akt/mTOR pathway is inhibited in Jurkat cells. Also, studies have shown that 20(S)-GRh2 accelerates apoptosis by blocking the PI3K/Akt/mTOR. The 20(S)-GRh2 might be considered a new natural targeted therapy for T-ALL patients.[76]

Krüppel-Like Transcription Factor 9 (KLF9)

Conducted studies have shown that the overexpression of KLF9 seems to suppress the level of mTOR phosphorylation, and KLF9 stimulates autophagy and apoptosis of T-ALL cells via the impact on the Akt/mTOR signaling pathway.[77]

Nelfinavir

Research has shown that might inhibit the mTOR pathway in T-ALL cells, because of upregulation of the expression of the stress-responsive gene (SESN2) via triggering of ER stress by increasing phosphorylated eIF2α.[78]

Conclusion

LCK and mTOR significantly influence the processes of T cell cycle regulation. The studies showed that drugs using LCK as a Disulfiram, Dasatinib, and Blinatumomab have potential key in T-ALL treatment. It is estimated that the pathway PI3K/mTOR is active up to 85% of patients with T-ALL, leading to increased cell proliferation and worsening treatment effects. Rapamycin is a mTOR kinase inhibitor with a potential therapeutic strategy in T-ALL due to an immunosuppressive effect by inhibiting the activation and proliferation of T lymphocytes. Moreover, Everolimus (a sirolimus derivative based on mTOR kinase inhibition) combined with other treatment strategies (steroids, HyperCVAD, chemotherapy) is well tolerated in relapsed T-ALL. In NCT01614197 trials study, temsirolimus mTOR inhibitor could be safely used in two weekly doses in combination with 5 days of cyclophosphamide and etoposide. It is important to develop and conduct more clinical trials of inhibitors, specifically for pediatric patients whose physiology is not analogous to adults.

Abbreviations

ALL, acute lymphoblastic leukemia; T-ALL, Acute T-cell lympho-blastic leukemia; mTOR, mechanistic target of rapamycin; LCK, lymphocyte-specific protein tyrosine kinase; TRC, T-cell receptor; ITAMs, immunoreceptor tyrosine-

based activation motifs; LAT, transmembrane adaptor protein linker for activation of T cells; CAR, chimeric antigen receptor; scFv, single-chain variable fragment; ICOS, inducible T-cell costimulator; PTPN2, tyrosine phosphatase non-receptor type 2; SHP1, SH2 domain-containing phosphatase 1; DUSP22, dual specificity protein phosphatase 22; HS90, heat shock protein; ALL, acute lymphoblastic leukemia; IFN-γ, interferon-γ; mTOR, mechanistic target of rapamycin; PIKK, PI3K-related kinases; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; mTOR Raptor, regulatory protein associated with mTOR; mLST8, mammalian lethal with Sec13 protein; S6K1, p70S6 Kinase 1; 4EBP, eIF4E Binding Protein; SREBP, sterol responsive element-binding protein; Rictor, rapamycin-insensitive companion of mTOR; PKC, protein kinase C family; SGK1 serum- and glucocorticoid-induced kinases; FDA, food and drug administration; TCR, T-cell receptor; PTEN, phosphatase and tensin homologue; NOTCH, neurogenic locus notch homolog protein; FKBP12, FK506-binding protein 12; FRB, FKBP12-rapamycin-binding domain; GCs, glucocorticoids; IL-7, interleukin 7; HK1 and HK2, Hexokinase 1 and 2; GLUL, glutamate-ammonia ligase; ROS, reactive oxygen species; G6PD, glucose-6-phosphate dehydrogenase, PPP, pentose phosphate pathway; KLF9, Krüppel-like transcription factor 9; 20(S)-GRh2, Ginsenoside Rh2 with a hydroxyl group at the C-20 position; SESN2, stress-responsive gene.

Disclosure

The authors report no conflicts of interest in this work.

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