Leukemia Research Reports 21 (2024) 100398

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Successful anti-tumor effects with two novel bifunctional chemotherapeutic compounds that combine a LAT1 substrate with cytotoxic moieties in aggressive T-cell lymphomas

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ARTICLE INFO

Keywords:
SLC7A5
LAT1
T-cell lymphomas
QBS10072S
QBS10096S

ABSTRACT

T-cell lymphomas are aggressive neoplasms characterized by poor responses to current chemotherapeutic agents. Expression of the l-type amino acid transporter 1 (LAT 1, SLC7A5) allows for the expansion of healthy T-cell counterparts, and upregulation of LAT1 has been reported in precursor T-cell acute leukemia. Therefore, the expression of LAT1 was evaluated in a cohort of cutaneous and peripheral T-cell lymphomas. The findings demonstrated that LAT1 is upregulated in aggressive variants and absent in low-grade or indolent disease such as mycosis fungoides. In addition, upregulated LAT1 expression was seen in a large proportion of aggressive peripheral T-cell lymphomas, including peripheral T-cell lymphoma not otherwise specific (PTCL-NOS) and angioimmunoblastic T-cell lymphoma (AITL). The anti-tumor effects of two novel non-cleavable and bifunctional compounds, QBS10072S and QBS10096S, that combine a potent cytotoxic chemotherapeutic domain (tertiary N-bis(2-chloroethyl)amine) with the structural features of a selective LAT1 substrate (aromatic β-amino acid) were tested in vitro and in vivo in T-cell lymphoma cell lines. The findings demonstrated decreased survival of T-cell lymphoma lines with both compounds. Overall, the results demonstrate that LAT1 is a valuable biomarker for aggressive T-cell lymphoma counterparts and QBS10072S and QBS10096S are successful therapeutic options for these aggressive diseases.

1. Introduction

Peripheral T-cell lymphomas (PTCLs) account for approximately 10–15 % of non-Hodgkin lymphomas. Most PTCLs are characterized by aggressive behavior, frequent relapses, and 5-year overall survival of less than 30 % [1–5]. Genetic driver mutations and external signals from the tumor microenvironment contribute to the oncogenic transformation and aberrant proliferation of neoplastic T-cells [6–8]. Consistent with this, we and others have demonstrated that elements of the tumor microenvironment can engage the T-cell receptor (TCR) on neoplastic T-cells and promote lymphoma progression [9,10].

After engagement of the TCR, the differentiation and effector functions of healthy T-cells depend on significant metabolic changes and nutrient availability [11,12]. Therefore, an abundant supply of amino acids is required to meet the translational demands of the activated T-cells [12]. Expression of the l-type amino acid transporter 1 (LAT1; SLC7A5) is essential during healthy T-cell activation. Knock-down expression of LAT1 in T-cell lymphocytes is associated with abrogated differentiation and failure to shift to glycolysis after TCR stimulation [11]. Consistent with this, the expression of LAT1 is upregulated in precursor T-cell lymphoproliferative neoplasms, and its inhibition prevents lymphoma/leukemia growth [13]. In addition, LAT1 expression is predominantly detected in aggressive B-cell neoplasms and directly correlates with decreased patient survival [14].

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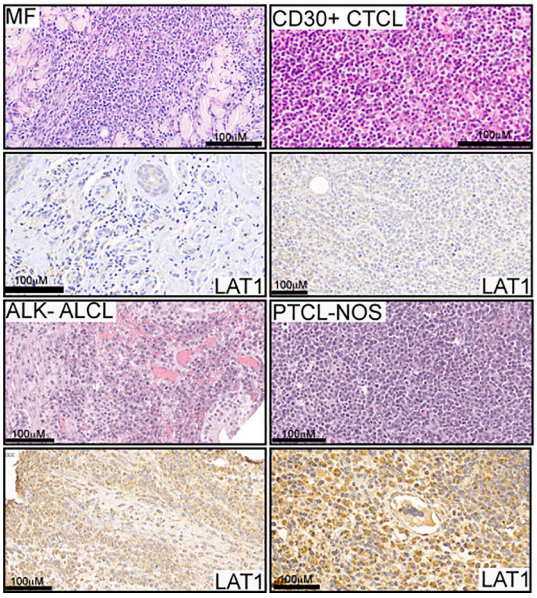
https://doi.org/10.1016/j.lrr.2023.100398
Received 2 May 2023; Received in revised form 5 September 2023; Accepted 21 November 2023
Available online 22 November 2023



B.

Diagnosis	Number of cases	% cases with LAT-1 upregulation
Mycosis Fungoides	78	0
Lymphomatoid Papulosis	17	0
CD30+ CTCL	21	0
Sèzary Syndrome	11	10 (90%)
AITL	6	6 (100%)
ALK- ALCL	6	4 (66%)
PTCL-NOS	10	6 (60%)

Fig. 1. A. LAT1 is predominantly upregulated in aggressive T-cell lymphoma variants. Upper panel: representative pictures from cutaneous T-cell lymphomas with negative expression of LAT1. Mycosis Fungoides; Myc. Fung. and CD30+ cutaneous T-cell lymphoma (CTCL). Lower panel: representative pictures of aggressive peripheral T-cell lymphomas with upregulated expression of LAT1. ALK- anaplastic large cell lymphoma (ALCL) and Peripheral T-cell lymphoma non-otherwise specified (PTCL-NOS). B. Upregulation of LAT-1 expression was evaluated in a cohort of cutaneous and peripheral T-cell lymphomas. The percentage of cases with upregulation of LAT-1 is indicated. C. Immunoblot analysis demonstrates that stimulation of the T-cell receptor (TCR) in T-cell lymphoma lines (T8ML1) and T-cell acute lymphoblastic leukemia (Jurkat) cell lines (D) is associated with the upregulation of the LAT1 protein levels.

2. Results

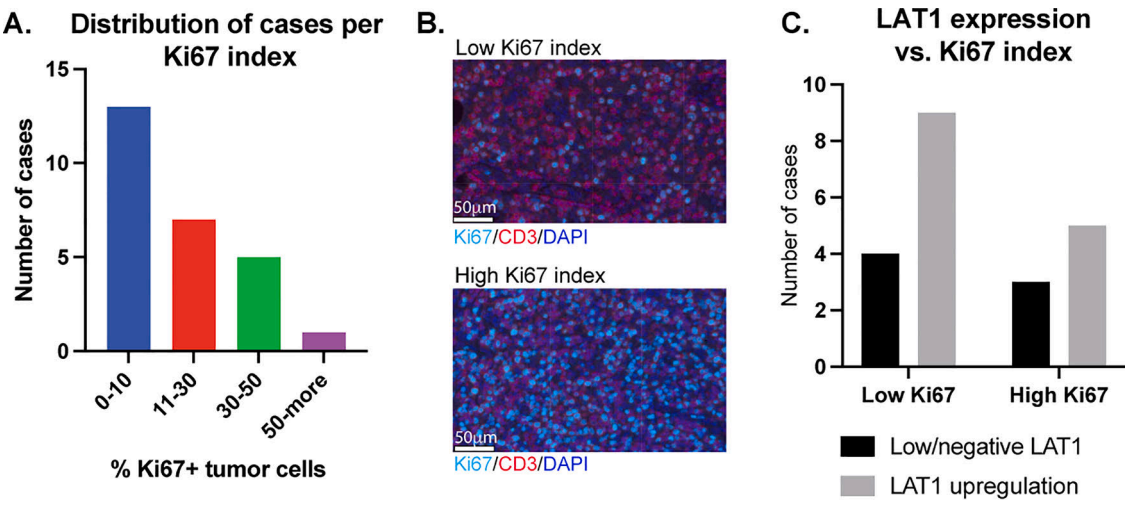
The expression of LAT1 was tested by immunohistochemistry in a cohort of T-cell neoplasms that included non-aggressive cutaneous T-cell lymphomas, including mycosis fungoides and CD30+ cutaneous T-cell lymphoproliferative neoplasms. LAT1 expression was also evaluated in aggressive T-cell lymphoma variants such as Sézary Syndrome, peripheral T-cell lymphoma non-otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), and ALK-negative anaplastic large cell lymphoma (ALK- ALCL). Upregulated expression of LAT1 was identified in 80 % of PTCL-NOS cases ($n = 10$), 66 % of ALK- ALCL cases ($n = 6$), and 90 % of AITL cases ($n = 6$). Expression of LAT1 was also detected in 90 % of Sèzary Syndrome cases ($n = 11$). LAT1 expression was not detected in CD30+ cutaneous T-cell lymphomas ($n = 21$) or mycosis fungoides cases ($n = 78$). Overall, the findings indicated that upregulation of LAT1 expression is predominantly detected in T-cell lymphoma variants with aggressive behavior (Fig. 1, and supplementary material).

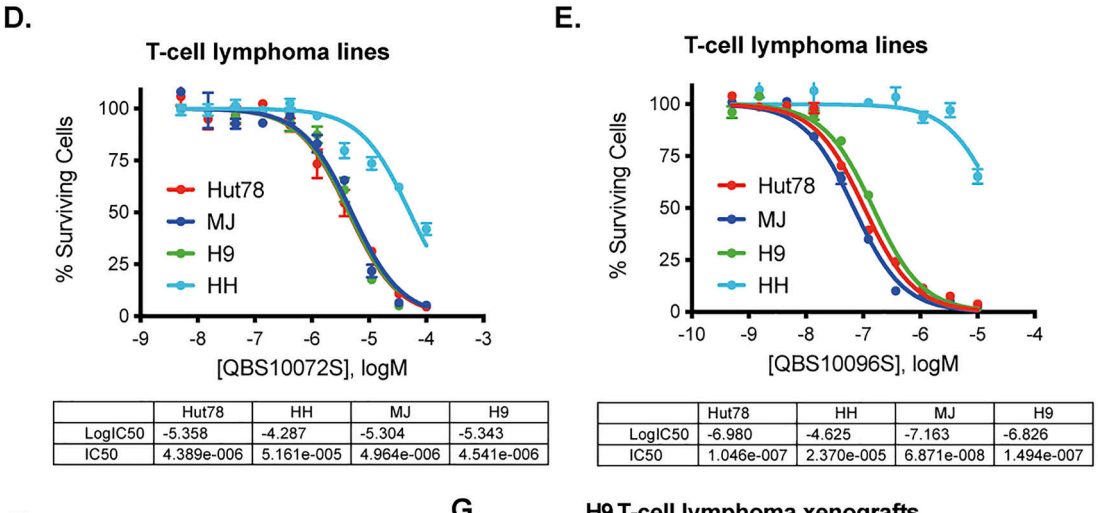
T-cell receptor (TCR) stimulation in peripheral T-cell lymphomas is associated with increased growth and chemotherapy resistance [9,10, 15]. Therefore, the protein levels of LAT1 were evaluated upon TCR stimulation in a peripheral T-cell lymphoma cell line (T8ML1). Increased protein expression of LAT1 was detected after 24 h of TCR stimulation (Fig. 1). These results indicate steady upregulation of LAT1 upon TCR stimulation in T-cell lymphomas.

Previous studies demonstrated that LAT1 expression in tumor cells correlates positively with biomarkers associated with high proliferation rates [16]. Therefore, the expression of LAT1 was correlated with the Ki-67 proliferation index in peripheral T-cell lymphomas ($n = 21$). The findings did not demonstrate any significant correlation between the expression of LAT1 and the Ki-67 proliferation index of the cases analyzed (Fig. 2). This contrasts with a prior study demonstrating a positive correlation between the Ki-67 proliferation index and LAT1 expression in B-cell lymphomas [14], and this may be secondary that the current analysis was restricted to peripheral T-cell lymphomas.

Recent studies have demonstrated potent anti-tumor effects and survival with the novel compound QBS10072S that targets LAT1 positive neoplasms, such as glioblastoma (GBM) or triple-negative breast cancer metastasized to the brain [17–19]. Small molecule LAT1 inhibitors such as JPH203, KMH-233, SKN-103, BCH, and others [20] inhibit the cellular uptake of essential neutral amino neutral acids, i.e., leucine or phenylalanine, interrupting vital biological processes through amino acid deprivation. In contrast, the novel cytotoxic LAT1 substrates QBS10072S and QBS10096S mimic endogenous natural neutral amino acids, utilize LAT1 as a port for cellular entry, and capitalize on the oncogenic LAT1 overexpression for tumor-selective targeting (supplementary material). Our findings demonstrate that upregulation of LAT1 is detected in a significant proportion of aggressive PTCL variants. Therefore, these two novel compounds (QBS10072S and QBS10096S) were tested during T-cell lymphoma growth [17]. Dose-dependent decreased survival was observed in four T-cell lymphoma cell lines with positive expression of LAT1 (H9, HH, MJ, and Hut78) after one-week incubation with both cytotoxic compounds (Fig. 2). The IC50s for QBS10072s ranged from 4.4mM (Hut78) to 51mM (HH). More potent effects were observed with QBS10096S, and the IC50s went from 68 nM (Hut78 cell lines) to 23mM (HH cell lines). The findings indicate that cytotoxic compounds that exploit LAT1 upregulation in T-cell lymphoma lines are associated with decreased survival (Fig. 2) and constitute potential therapeutic agents for T-cell lymphomas. Interestingly HH cell lines featured reduced sensitivity to both compounds tested, although comparable protein expression levels of LAT1 were detected in cell lines with increased sensitivity. This may result from the genetic complexity of these cell lines or artifactual changes introduced by their specific growth rate.

A complementary approach using murine xenografts of T-cell lymphoma cell lines was utilized to test the in vivo effects of these cytotoxic LAT1 substrates. Two doses (2 mg/kg and 8 mg/kg) of each compound (QBS10072S and QBS10096S) were tested after H9 xenograft engraftment. A significant decrease in xenograft growth was observed with both compounds at 8 mg/kg doses. However, no significant effects were observed at 2 mg/kg. No adverse toxic effects were observed in the experimentation animals secondary to the administration of the





	Hut78	HH	MJ	H9
LogIC50	-5.358	-4.287	-5.304	-5.343
IC50	4.389e-006	5.161e-005	4.964e-006	4.541e-006

	Hut78	HH	MJ	H9
LogIC50	-6.980	-4.625	-7.163	-6.826
IC50	1.046e-007	2.370e-005	6.871e-008	1.494e-007

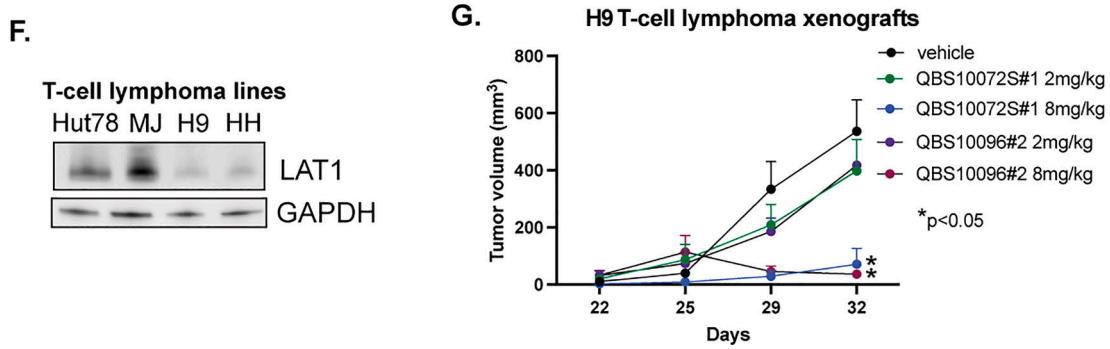


Fig. 2. A. Distribution of the number of cases with the percentage of positive tumor cells for Ki-67. B. Representative pictures of one case with a low Ki-67 index (<25% tumor cells positive for Ki-67) and one case with a high Ki-67 index (≥25% Ki-67 positive tumor cells). C. Contingency analysis of the level of LAT1 expression and the Ki-67 proliferation index. The cutoff point for high LAT1 expression was set at>50% tumor cells. D-E. Two novel compounds, QBS10072S and QBS10096S, recognized as substrates by LAT1 through their amino acid architecture importing potent non-cleavable cytotoxic moieties, were tested in T-cell lymphoma lines. Decreased survival is observed in a dose-dependent manner. F. Immunoblot analysis demonstrates positive expression of LAT1 in the indicated T-cell lymphoma cell lines. G. A single xenograft model of T-cell lymphoma cell lines (H9) was treated with two LAT1 substrates (QBS10072S and QBS10096S) at two different doses (2 mg/kg or 8 mg/kg). The mean tumor volume is depicted over the indicated time points.

experimental compounds. These results are consistent with the in vitro findings and support using these compounds to decrease T-cell lymphoma proliferation (Fig. 2).

Our findings demonstrate that upregulated LAT1 expression is predominantly detected in aggressive T-cell lymphoma variants, likely as a response to the elevated metabolic needs of these groups of neoplasms. Therefore, as in the aggressive B-cell counterparts, the expression of LAT1 constitutes a novel biomarker of aggressive T-cell lymphoma subtypes [14]. The findings indicate that LAT1 expression is predominantly detected in aggressive nodal peripheral T-cell lymphoma variants, and therapeutically exploiting LAT1 upregulation may also benefit high-risk T-cell lymphomas.

Informed consent statement

Not applicable.

Data availability statement

Not applicable.

Declaration of artificial intelligence (AI) technologies

No AI-assisted technologies were utilized for this manuscript.

CRediT authorship contribution statement

Carlos Murga-Zamalloa: Conceptualization, Investigation, Writing – original draft. **Shaun Webb:** Conceptualization, Investigation. **John Reneau:** . **Alejandro Zevallos:** . **Pierina Danos-Diaz:** Conceptualization, Investigation. **Vanessa Perez-Silos:** Conceptualization, Investigation. **Mirna Rodriguez:** Conceptualization, Investigation. **Guangyao Gao:** Methodology, Investigation. **Wolf-Nicolas Fischer:** Conceptualization, Investigation. **Bernd Jandeleit:** Methodology, Investigation, Writing – review & editing. **Ryan Wilcox:** Conceptualization, Investigation, Writing – original draft.

Declaration of Competing Interest

This study received funding from Quadriga Biosciences. Inc. At the time of the study, Bernd Jandeleit, Wolf-Nicolas Fischer, and Mirna Rodriguez were paid employees of Quadriga Biosciences, Inc. Mirna Rodriguez and Wolf-Nicholas Fisher (Quadriga Biosciences) designed and conducted in-vitro cytotoxicity experiments. At the time of the study, Bernd Jandeleit and Wolf-Nicolas Fischer were named inventors on the US patent application Jandeleit, B, et al. *Beta-substituted beta-amino acids and analogs as chemotherapeutic agents and uses thereof*. US patent publication US 9783,487. Guangyao Gao was a paid contractor for Quadriga Biosciences Inc. All authors declare no other competing interests

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2023.100398.

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