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Expression of TCL-1 as a Potential Prognostic Factor for Treatment Outcome in B-Cell Chronic Lymphocytic Leukemia

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Abstract

TCL-1 expression is variable in CLL, and no study has examined its association with treatment response. We measured TCL-1 protein in CLL cells from 51 patients who then received pentostatin, cyclophosphamide, and rituximab. TCL-1 expression did not correlate with any pre-treatment characteristics. Lower TCL-1 levels were associated with higher probability of attaining flow cytometry-negative status post-treatment (52% versus 17%, $p=0.046$). Trends toward improved complete remission rate (49% versus 19%, $p=0.064$) and progression-free survival (medians: 33 versus 20 months, $p=0.199$) were noted with lower TCL-1 expression. These data suggest TCL-1 expression may help predict treatment outcome in CLL patients following chemoimmunotherapy, and examination in larger studies is warranted.

Keywords

Chronic Lymphocytic Leukemia (CLL); TCL-1; prognostic factor; pentostatin; chemoimmunotherapy

Introduction

Chronic Lymphocytic Leukemia (CLL) is characterized by the expansion of clonal CD5+/CD19+ lymphocytes. A variety of genetic, epigenetic, and biochemical features have been described that may contribute to the pathogenesis of CLL and may be associated with disease progression and inferior survival. These include immunoglobulin (Ig) V_H gene mutational status, ZAP-70 expression, CD38 expression, interphase cytogenetic abnormalities, *p53* or *ATM* mutations, thymidine kinase activity, and β_2 M level. However, many of these features have not been definitively shown to predict efficacy of therapy once treatment becomes necessary.

TCL-1 is expressed normally in B cells up to the germinal center stage, and in precursor and activated T cells. TCL-1 activates Akt to provide survival signals through phosphorylation of pro-survival kinases and inactivation of apoptotic pathway components [1,2]. Over-expression

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of TCL-1 in transgenic mouse models results in leukemic phenotypes, including a CLL-like disease when restricted to B-cell expression, indicating a primary role for TCL-1 in tumorigenesis [2,3,4]. Herling *et al.* showed that TCL-1 protein is expressed in 90% of human CLL, but that its expression is variable and correlates with other biological parameters used in prognosis such as ZAP-70 expression, IgV_H gene rearrangement, and interphase cytogenetic abnormalities [5]. To our knowledge, no study has examined the relevance of TCL-1 expression in predicting response or response duration to any type of CLL therapy.

We recently reported a phase II study of pentostatin, cyclophosphamide and rituximab [6] demonstrating a high complete response and extended progression-free survival (PFS). In samples from this study we also performed pre-treatment analyses of interphase cytogenetics, IgV_H mutational status, ZAP-70 expression, CD38 expression, and mutational status of *p53* and *ATM*. We hypothesized that TCL-1 expression might influence treatment outcome due to its ability to inhibit apoptosis.

Materials and Methods

Untreated patients with progressive CLL were enrolled on an Institutional Review Board-approved Phase II trial evaluating pentostatin, cyclophosphamide, and rituximab combination treatment. Details of treatment, response assessment, and measurement of minimal residual disease have been described [6]. Peripheral blood mononuclear cells were obtained before treatment after receiving written informed consent, and TCL-1 protein expression was measured by standard immunoblot with anti-TCL-1 (MBL, Woburn MA). GAPDH (Chemicon, Temecula CA) was used as a normalization control across lanes, and aliquotted lysate from a single TCL-1 transgenic mouse spleen [3] was used as a normalization control across immunoblots. All samples were run at least in duplicate and digital quantification was performed using a ChemiDoc instrument (BioRad, Hercules CA). TCL-1 expression was averaged between duplicates and calculated relative to expression in the mouse lysate, arbitrarily set to 1.0. Data were analyzed as continuous measures and by various cutpoints relative to clinical outcomes. Given the paucity of information on TCL-1 expression in this patient population, cutpoints explored were based approximately on data-driven measures such as medians, tertiles, quartiles, or other natural breakpoints that may have appeared in the data. Fisher's exact tests were used to compare categorical variables, Wilcoxon rank sum tests to compare categorical versus continuous variables, and Cox proportional hazards models and log rank statistics to compare PFS data.

Results and Discussion

Samples were available from 51 of 64 enrolled evaluable patients. There were no significant differences in clinical outcome features between the subset of patients studied and the entire 64-patient group. The only observed notable difference was in IgV_H gene mutation status between the 51 patients with TCL-1 expression data available versus the remaining 13 patients (34% vs. 8%; $p=0.15$). A typical immunoblot result is shown in Figure 1a, with graphic representation of duplicates shown in Figure 1b. No previously established pre-treatment risk factors were significantly associated with baseline TCL-1 expression in the 51-patient sample set. These included age greater than 70 ($p=0.59$), gender ($p=0.67$), occurrence of del(17p) or del(11q) interphase cytogenetic abnormalities ($p=0.15$), IgV_H mutational status ($p=0.94$), ZAP-70 expression in greater than 20% of cells ($p=0.51$), or CD38 expression above 30% ($p=0.40$). The same lack of significant association with these risk factors was found even when TCL-1 was dichotomized (see Table 1).

We next examined treatment outcomes relative to TCL-1 expression. The percent residual CD5⁺/CD19⁺ leukemic B-cells at followup (two months after completion of six cycles of treatment)

correlated with the pretreatment TCL-1 level ($r=0.33$, $p=0.03$). Since these were studied as continuous variables, we next evaluated which threshold of TCL-1 best categorized patients with respect to clinical outcomes. After exploring various cutpoints, we found the second tertile of TCL-1 expression best segregated clinical outcomes. Table 2 shows the association of TCL-1 levels in pre-treatment samples with treatment outcome, analyzed using a dichotomized cutoff of a relative TCL-1 expression level of 0.275 (approximately the second tertile). This variable was significantly associated with whether or not peripheral blood CD5+/CD19+ leukemic B-cells were less than 1.0% at followup ($p=0.046$). Using this same cutoff, there was also a suggestion that patients with reduced TCL-1 were more likely to achieve a complete response by NCI criteria (49% vs. 19%; $p=0.064$). Finally, the median PFS appeared to be shorter in patients with increased TCL-1 expression versus those with $TCL-1 < 0.275$ (20.3 vs. 32.6 months; $p=0.199$). Although these differences did not reach statistical significance, the observed differences in the curves and corresponding medians encourage exploration of this novel marker in larger studies with better statistical power.

While preliminary, our data add to the report of Herling *et al* [5] indicating that elevated TCL-1 protein expression might be associated with adverse treatment outcome in symptomatic CLL. In contrast to their results, however, statistically significant associations with other parameters including Rai stage, IgV_H mutation status, ZAP-70 expression, CD38 expression, and interphase cytogenetics abnormalities were not detected in this sample set. This discordance may be due to the relatively small sample size as well as variability inherent in immunoblot analyses. Additionally, Herling *et al.* reported dynamic regulation of TCL-1 by cytokines such as IL-4 as well as variation according to microenvironment that could further complicate analysis of patient samples. Although cytokine assessments were not performed in this study, only peripheral blood cells were examined and all samples were obtained prior to therapy, hopefully reducing the impact of such factors. Given these points, it is possible that if TCL-1 proves to be a significant indicator of clinical outcome in CLL, it may reflect the ability of the microenvironment to inhibit removal of CLL cells.

A limitation of our data set is the relatively small sample size and thus limited power to detect modest differences in endpoints such as PFS. Still, the preliminary analyses in this study on both complete response rate and PFS yielded interesting and consistent differences in relation to TCL-1 expression and warrant further investigation in larger populations. Because the clinical study has been completed, it is not possible to add more subjects to confirm this result. We therefore believe it is important to report our TCL-1 data so this prognostic feature can be assessed by other groups using larger, uniformly treated populations of patients with symptomatic CLL.

Overall, these data provide preliminary evidence that TCL-1 expression correlates with clinical outcome of CLL patients treated with chemoimmunotherapy. If validated in larger studies, especially by using rigorous protein quantification techniques on carefully obtained samples, TCL-1 expression may serve as a novel prognostic factor with relevance at the point when therapy is needed. Additionally, efforts to target TCL-1, through down-regulation or interference with TCL-1/AKT interactions [7] might represent a novel therapeutic approach.

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Figure 1.

a: Representative duplicate immunoblots of TCL-1 in CLL patient samples. Lysates from patient samples were separated by 10% SDS-PAGE and immunoblotted simultaneously for GAPDH and TCL-1. Lysate from the spleen of a single TCL-1 transgenic mouse was used to normalize signal across all immunoblots. **b:** Graphic representation of data from the immunoblots shown in (a).

Table 1Association of pre-treatment TCL-1 expression (<0.275 vs. ≥ 0.275) with various established risk factors.

Risk Factor	% with TCL-1 ≥ 0.275	p-value
<i>Mutation Status</i>		
Mutated	5/17=29%	0.99
Non-mutated	11/33=33%	
<i>CD38 positivity</i>		
$\leq 30\%$	9/34=26%	0.35
$> 30\%$	7/17=41%	
<i>ZAP-70 positivity</i>		
$< 20\%$	9/30=30%	0.99
$> 20\%$	4/14=29%	
<i>Poor prognosis FISH</i>		
11q- or 17p-	6/14=43%	0.31
Other	9/35=26%	
<i>Age</i>		
< 70 yrs	12/38=32%	0.99
≥ 70 yrs	4/13=31%	
<i>Rai risk</i>		
Low/Intermediate	6/25=24%	0.37
High	10/26=38%	
<i>Gender</i>		
Male	12/39=31%	0.99
Female	4/12=33%	

Table 2

TCL-1 expression and clinical outcome features in previously untreated CLL patients treated with pentostatin, cyclophosphamide, and rituximab.

Clinical Feature	TCL-1 < 0.275 N (%)	TCL-1 ≥ 0.275 N (%)	p-value
CD5+/CD19+ % < 1.0	16 (52%)	2 (17%)	0.046
CD5+/CD19+ % ≥ 1.0	15 (48%)	10 (83%)	
Pts with CR	17 (49%)	3 (19%)	0.064
Pts without CR	18 (51%)	13 (81%)	
Pts with any response (CR, nPR, PR)	33 (94%)	14 (88%)	0.58
Pts never achieving any response	2 (6%)	2 (12%)	
Median PFS, months	32.6	20.3	0.199

CD5+/CD19+ %: percentage of cells positive for both markers two months after the completion of 6 cycles of treatment, as a measure of minimal residual disease; Pts: Number of Patients; CR: Complete Response; PR: Partial Response; nPR: nodular partial response; PFS: Progression-Free Survival.