



# Cysteine Cathepsins and Their Prognostic and Therapeutic Relevance in Leukemia

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## Abstract

Cysteine cathepsins are lysosomal proteases that require Cys-His ion pair in their catalytic site for enzymatic activity. While their aberrant expression and oncogenic functions have been widely reported in solid tumors, recent findings suggest that these proteases also play an important role in the pathogenesis of hematological malignancies. In this review, we summarize the potential clinical implications of cysteine cathepsins as diagnostic and prognostic markers in leukemia, and present evidences which supports the utility of these proteases as potential therapeutic targets in hematological malignancies. We also highlight the available information on the expression patterns, regulation, and potential functions of cysteine cathepsins in normal hematopoiesis and hematological malignancies. In hematopoiesis, cysteine cathepsins play a variety of physiological roles including regulation of hematopoietic stem cell adhesion in the bone marrow, trafficking, and maturation. They are also involved in several functions of immune cells which include the selection of lymphocytes in the thymus, antigen processing, and presentation. However, the expression of cysteine cathepsins is dysregulated in hematological malignancies where they have been shown to play diverse functions. Interestingly, several pieces of evidence over the past few years have demonstrated overexpression of cathepsins in leukemia and their association with worst survival outcomes in patients. Strategies aimed at altering the expression, activity, and subcellular localization of these cathepsins are emerging as potential therapeutic modalities in the management of hematological malignancies. Recent findings also suggest the involvement of these proteases in modulating the immune response in leukemia and lymphomas.

## Keywords

- ▶ cathepsins
- ▶ leukemia
- ▶ proteases
- ▶ acute myeloid leukemia
- ▶ CTSL
- ▶ CTSB

## Introduction

Cysteine cathepsins are endo-lysosomal proteases that are primarily responsible for the turnover and degradation of intracellular proteins. These proteases are also involved in various other physiological processes including antigen presentation,<sup>1</sup> sperm maturation,<sup>2</sup> pro-hormone activation,<sup>3</sup>

ovulation,<sup>4</sup> and fetal implantation.<sup>5,6</sup> Altered expression of these proteases underlie various pathological processes such as atherosclerosis,<sup>7</sup> rheumatoid arthritis,<sup>8</sup> autoimmune disorders,<sup>9</sup> and several types of malignancies.<sup>10,11</sup>

Cysteine cathepsins contain a highly conserved cysteine residue in their active sites. Due to a high degree of homology to the plant enzyme papain, these proteases have been

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included in the C1 family of the clan CA proteases.<sup>12</sup> HUGO Gene Nomenclature Committee have recognized a total of 15 functional genes for cathepsins in the human genome (<https://www.genenames.org/>). These include 11 cysteine cathepsins (CTS-B, C, F, H, K, L, O, S, V, W, and Z) and four noncysteine cathepsins (CTS-A, G, D, and E). They are synthesized as catalytically inactive pre-pro-enzymes (zymogens) on endoplasmic reticulum (ER) bound ribosomes and acquire mannose 6-phosphate tag while passing through the ER-Golgi network.<sup>13</sup> This tag along with the vesicular mannose 6-phosphate plays a key role in targeting them to lysosomes. In the acidic environment of lysosomes, these zymogens are proteolytically processed into their catalytically active form. The processed form of cysteine cathepsins ranging from 20 to 35 kDa in size consists of a heavy and a light chain subunit which are linked by disulphide bridges. However, unlike other cysteine cathepsins, the processed form of CTSC exists as an oligomer. While most cathepsins are active at acidic pH, CTSS and CTSL display their optimal activities at alkaline and neutral pH, respectively.<sup>14</sup>

Although a majority of human cysteine cathepsins (CTS-B, H, L, C, X, F, O, and V) are ubiquitously expressed, cathepsin K, W, and S display tissue-specific pattern.<sup>14</sup> CTSC is expressed in osteoclasts, most epithelial cells, and also in the synovial fibroblasts of rheumatoid arthritis patients, whereas CTSV (named as L2, a variant of CTSL) is found in thymus and testis. CTS such as K, L, S, F, V, and B which display high elastolytic and collagenolytic activities are involved in ECM remodeling. Since cathepsin B, C, H, and L are regulated by common transcription factors, they are often coexpressed in a variety of tissues and cell types. Studies using oligopeptide libraries have revealed overlapping substrate specificities of cysteine cathepsins but individual preferences to different peptide sequence.<sup>15,16</sup>

Elevated expression of cysteine cathepsins in solid tumors has been extensively documented.<sup>11,17</sup> They promote tumor cell proliferation, inhibit apoptosis, and mediate metastasis. Higher enzymatic activities of cysteine cathepsins are also associated with poor overall survival outcomes in leukemia patients.<sup>18,19</sup> In this review, we attempt to present current knowledge about the role of cysteine cathepsins in the pathogenesis of leukemia and their utility in its management.

## Cysteine Cathepsins in Hematopoiesis and Immune Cells Functions

Under physiological conditions, a majority of cysteine cathepsins are primarily localized to lysosomes and play a key role in the turnover and degradation of intracellular proteins. However, localization of these proteases in the nucleus, cell surface, and extracellular milieu is also documented where they perform more specialized functions.<sup>14</sup> Several cysteine cathepsins are expressed in cells at different stages of hematopoiesis. In the bone marrow, hematopoietic stem cells (HSCs) and other progenitor cells are trapped in complex microenvironment containing osteoblasts and stromal cells. This adhesion is partially mediated by chemoattractant CXCL12.<sup>20</sup> Osteoblasts and stromal cells synthesize and

secrete large quantities of CTSZ, which in turn degrade CXCL12 and thereby regulate hematopoietic stem and progenitor cell trafficking.<sup>21</sup> Similarly, cathepsin S and D coordinate to facilitate the commitment of HSCs to differentiate into dendritic cells.<sup>22</sup> Moreover, CTSZ has been shown to control phagocytosis in macrophages and maturation of dendritic cells via proteolytically activating  $\beta 2$  integrin receptor Mac-1 (CD11b/CD18).<sup>23</sup> Interestingly, Lalanne et al through a series of elegant experiments, established that toll-like receptor-9 (TLR-9) signaling inhibits expansion of pro-B cells via CTSC dependent apoptosis, thereby demonstrating the involvement of this cysteine cathepsin in the regulation of lymphopoiesis.<sup>24</sup>

Several studies demonstrating the role of cysteine cathepsins in antigen processing in both professional and non-professional antigen presenting cells have been reviewed previously.<sup>25,26</sup> Multiple cathepsins are involved in invariant chain processing and major histocompatibility complex class II peptide loading by macrophages.<sup>27</sup> During thymic selection, several proteases are differentially expressed in thymic antigen presenting cells to process autoantigens, which governs the selection of autoreactive T-cells. Cathepsin S, L, B, and D are involved in antigen processing in APCs.<sup>28</sup> CTSV is the dominant cysteine protease in cortical thymic epithelial cells, while CTSL and CTSS are restricted to dendritic and macrophage-like cells.<sup>29</sup> Interestingly, CTSS plays a central role in the processing of the autoantigens in the thymus.<sup>30</sup> However, CTSW, the predominant cathepsin of natural killer cells and CD8<sup>+</sup> T-lymphocytes is secreted during cytotoxic T cell effector functions.<sup>31</sup> Recently, functions of cysteine cathepsins in tumor-associated immune cells have been reviewed in detail.<sup>32</sup>

## Prognostic Significance of Cysteine Cathepsins in Leukemia

The elevated expression of cysteine cathepsins in pathological conditions such as inflammatory diseases, cancer cell proliferation, and metastasis has prompted researchers over the years to explore the clinical significance of these proteases. The utility of circulating cysteine cathepsins as a potential diagnostic biomarker has been explored in solid tumors such as nasopharyngeal,<sup>33</sup> gastric,<sup>34</sup> and lung cancer.<sup>35</sup>

In 2003, overexpression of cysteine cathepsin W and C was demonstrated in peripheral blood mononuclear cells (PBMCs) of large granular lymphocytic (LGL) leukemia patients.<sup>36</sup> However, protease inhibitors such as cystatin C, A, alpha-1 antitrypsin, and metalloproteinase inhibitors were observed to be downregulated in these patients. A pilot study in our laboratory was undertaken to assess the expression of cysteine cathepsin B and L in the (PBMCs) of pediatric acute myeloid leukemia (AML) patients and age-matched healthy controls by real-time PCR, and enzymatic assays for both these proteases.<sup>37</sup> The results of this study revealed significantly higher activity and expression of both these proteases in AML patients compared to the healthy controls. Furthermore, the expression of cystatin C displayed a strong negative correlation with the activities of both CTSC and

CTSL in AML patients. Interestingly, AML patients with higher activities and expression of these cathepsins exhibited an inferior overall, and event-free survival as compared to the patients with lower activities of the same. These results suggested the prognostic significance of both CTSL and CTSB in pediatric AML. Subsequent studies were undertaken to validate these findings by using a large cohort of 101 newly diagnosed pediatric AML patients and 35 healthy controls. In these studies, the expression and activities of both the proteases were assessed by real-time PCR, Western blotting, and enzyme assays, respectively before the induction of chemotherapy and postinduction. A detailed plan of the same is summarized in ►Fig. 1. The results of these studies established significantly higher expression and activities of both these proteases not only in PBMCs but also in bone marrow mononuclear cells (BMMCs) of pediatric AML patients as compared to healthy controls.<sup>18,19</sup> Activity of both the proteases displayed a dramatic reduction in response to chemotherapy, thereby suggesting the association of these proteases with the status of malignancy. By multivariate analysis, CTSL and CTSB in BMMCs emerged as independent prognostic markers for predicting the poor outcome of the disease. Survival analysis based on median CTSL/CTSB activity in pediatric AML patients is summarized in ►Table 1.<sup>18,19</sup> As evident from this table, the overall survival (OS) and event-free survival (EFS) in AML patient group with low CTSL activity (lower than the median value) were observed to be 20.4 and 12.5 months, respectively. Whereas OS and EFS in patient group with high activity (higher than the median value) of this protease were observed to be 8.0 and 9.8 months, respectively. Similarly, OS and EFS in group of patients with low activity of CTSB (in PBMC) were estimated to be 26.2 and 14.3 months, respectively. On the other hand, OS and EFS in patients with high CTSB activity were found to be 8.23 months and 9.8 months, respectively. Thus these results conclusively demonstrated significantly higher OS and EFS in patients with low activities of CTSL and CTSB as compared to the patient group with high activity of these proteases. Similar findings were observed in this study when patients were divided based on the activity of these cysteine cathepsins in BMMCs. Thus, Pandey et al convincingly demonstrated significantly inferior OS and

EFS in AML patients with high CTSB and CTSL activities as compared to the low activity group, thereby establishing their utility as potential prognostic markers in this malignancy.<sup>18,19</sup> Although the activities of these proteases were significantly higher in patient's BMMCs as compared to PBMCs, they displayed a strong positive correlation in both the cell types. These findings confirmed that the expression/activity of CTSB and CTSL in PBMCs reflects their expression in BMMCs of AML patients, which enhances the feasibility of assaying these proteases in peripheral blood samples for predicting the outcome of the disease or to monitor the progress of the therapy.

Chromosomal localization studies mapped human cathepsin L gene to 9q 21-22.<sup>38</sup> Altered expression of genes located on the long arm (q arm) of chromosomes 9 was previously demonstrated in chronic myelogenous leukemia (CML) patients.<sup>39</sup> Given these reports and observed overexpression of CTSL in AML patients, Samaiya et al measured the activity and expression of this protease in the PBMCs of 47 CML patients.<sup>40</sup> In total, 37 adults with systemic infection as well as 50 healthy volunteers served as controls in this study. This investigation revealed significantly higher expression and activity of CTSL in CML patients compared to patients with systemic infection and healthy volunteers. However, CML patients in the accelerated phase/blast crisis (AP/BC) displayed a drastic decrease in CTSL expression and activity as compared to the patients in the chronic phase of the disease. Promoter methylation studies revealed that this drastic decrease in CTSL expression in AP/BC patients was due to hypermethylation of its promoter. These findings were further corroborated by the treatment of human blast crisis cells K562 by DNA methyltransferase inhibitor 5-Azacytidine, which restored the expression of CTSL in these cells to the level of chronic phase. These results were further confirmed by the treatment of PBMCs isolated from blast crisis patients with 5-Azacytidine. In chronic phase patients, a CTSL expression exhibited a very strong positive correlation with the expression of vascular endothelial growth factor (VEGF). Thus, in view of the previous reports on transcriptional upregulation of CTSL expression by VEGF,<sup>41</sup> elevated levels of CTSL in chronic phase patients were attributed to

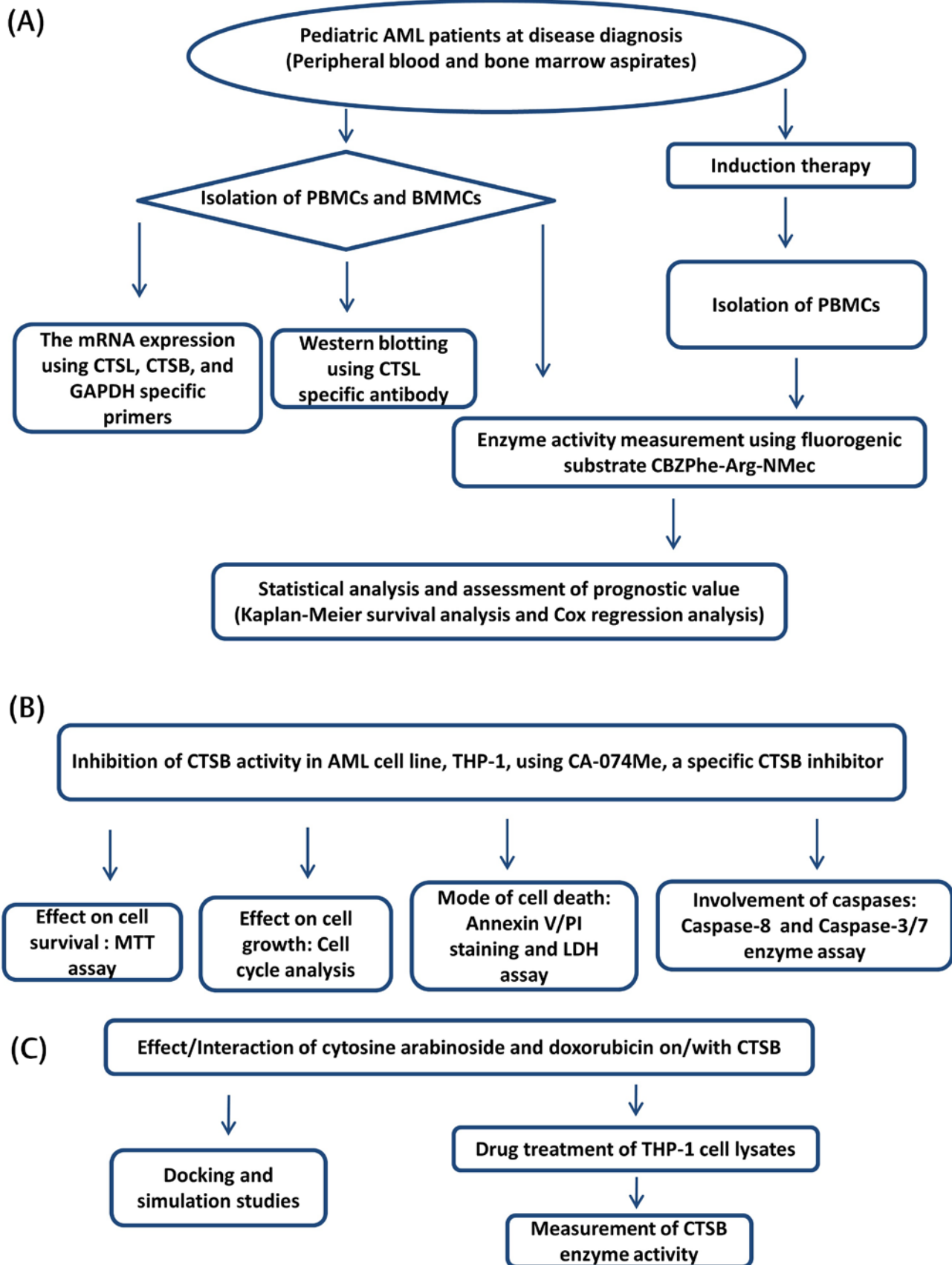
**Table 1** Survival analysis of pediatric acute myeloid leukemia patients by the median activity of cysteine cathepsins.

	CTSL activity PBMCs (Low)	CTSL activity PBMCs (High)	CTSL activity BMMCs (Low)	CTSL activity BMMCs (High)	CTSB activity PBMCs (Low)	CTSB activity PBMCs (High)	CTSB activity BMMCs (Low)	CTSB activity BMMCs (High)
EFS (mo)	12.5 <sup>a</sup>	8.0	12.4 <sup>a</sup>	7.9	14.3 <sup>a</sup>	8.23	13.8 <sup>a</sup>	8.2
OS (mo)	20.4 <sup>a</sup>	9.8	18.1 <sup>a</sup>	9.4	26.2 <sup>a</sup>	9.8	26.2 <sup>a</sup>	9.5
References	18				19			

Abbreviations: BMMC, bone marrow mononuclear cell; CTSB, cathepsin B; CTSL, cathepsin L; EFS, event free survival; OS, overall survival; PBMC, peripheral blood mononuclear cell.

Note: Peripheral blood mononuclear cells and bone marrow mononuclear cells were isolated by percol density gradient centrifugation from 103 newly diagnosed acute myeloid leukemia patients. The cells were lysed and the activities of CTSB and CTSL were assayed in the cell lysates. The median value of each of the above mentioned cysteine cathepsin was determined. Patients were divided into two groups based on the median value of enzyme activities. The patients with CTSB activities higher or equal to the median values were placed in the high enzyme activity group and rest in the low enzyme activity group. A similar criterion was used for placing patients in high or low CTSL groups. Kaplan–Meier survival analysis was used to determine the association of protease expression with event-free survival and overall survival. The relapse or death of the patient was considered as an event.

<sup>a</sup>Values significantly different from high cysteine cathepsin group.



**Fig. 1 (A-C)** Experimental plan used to investigate the prognostic and therapeutic significance of cathepsins B and cathepsin L in acute myeloid leukemia. Figure edited and reproduced from Pandey et al.<sup>18,19</sup>

elevated expression of VEGF during this phase of malignancy. Elevation in CTSL expression by the treatment of PBMCs isolated from healthy volunteers with recombinant VEGF corroborated these findings. This study although did not evaluate the prognostic significance of CTSL, demonstrated that unlike in AML, this protease is overexpressed in CML patients in chronic phase and thus associated with better outcome of the disease.<sup>40</sup>

While the above mentioned studies strongly support prognostic implications of cysteine cathepsins in individual hematological malignancies, correlating the expression and activities of these proteases with clinical/molecular subtype may further confirm the utility of cysteine cathepsins as prognostic markers. In line with this view, Wrona et al compared the expression of CTSB in different genetic subtypes of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and observed overexpression of CTSB in BMMCs of BCP-ALL patients with deletion of paired box 5 (*PAX5*) gene, but no other primary genetic abnormality.<sup>42</sup> However, this differential expression of CTSB did not have any significant impact on the 5-year recurrence-free survival in these patients.

In the past few years, several studies have explored the utility of cysteine cathepsins in diagnostic imaging for the detection of cancers.<sup>43</sup> Activity-based fluorescence probes for these proteases have been successfully used for image-guided surgery of multiple cancers<sup>44,45</sup> and bone diseases.<sup>46-48</sup> A similar strategy might prove beneficial for less invasive *in vivo* detection of leukemic cells in the bone marrow.

Although fewer studies assessing the role of cysteine cathepsins in blood cancer are available as compared to solid tumors, they certainly provide a rationale to further explore their potential as diagnostic/prognostic markers in leukemia. Moreover, the systemic nature of leukemic cells may allow

their usage as routine noninvasive biomarkers in clinical practice. Various studies which have been carried out to investigate the utility of cysteine cathepsins as biomarkers in leukemia are listed in ►Table 2.

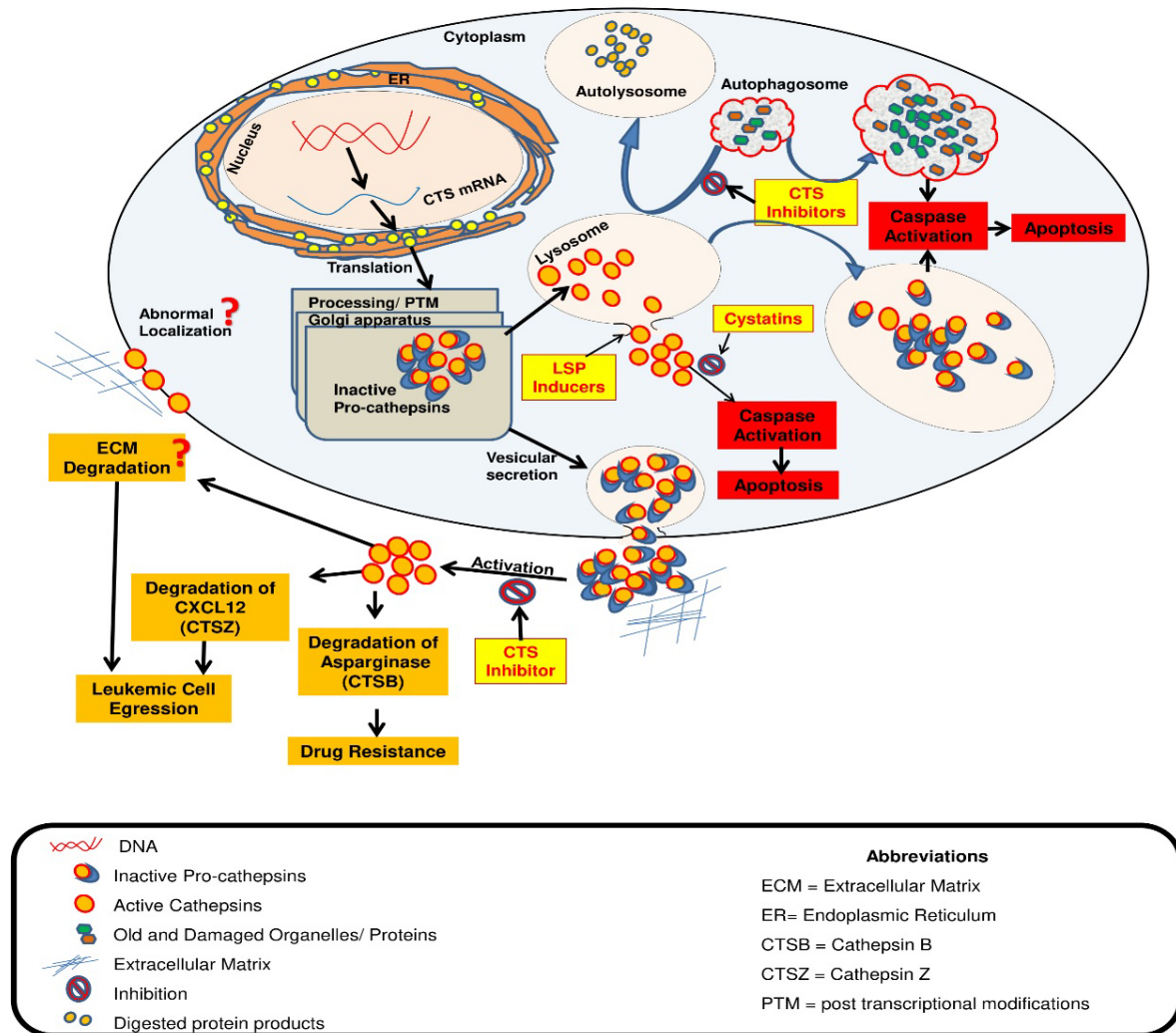
## Cathepsins as Therapeutic Targets in Leukemia

Initial studies to understand the role of cysteine cathepsins in different pathologies by inhibition of their activities proved difficult because of the broad specificity of inhibitors. However, computational approaches eventually lead to the identification of specific inhibitors. The details of these inhibitors have been extensively reviewed.<sup>49</sup> Active site mapping strategies combined with inhibitor library screenings lead to the identification of several specific inhibitors for CTSB and CTSL.<sup>50-52</sup> Using *in vitro* and mouse xenograft models, several studies have demonstrated the potential therapeutic utility of cysteine cathepsin inhibition in the management of cancers of the pancreas, prostate, and brain.<sup>53-55</sup> A combination of cysteine cathepsins with pre-existing targeted therapies have also been reported to improve therapeutic effectiveness. For example, the combined use of anti-VEGF antibody and anti-cathepsin S antibody synergistically inhibits angiogenesis in solid tumors.<sup>56</sup> Similarly, CTSS inhibitor (*Z*-FL-COCHO) sensitized renal carcinoma cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis *in vitro*.<sup>57</sup> Interestingly, cysteine cathepsins are also expressed at high levels in stromal cells of the tumor microenvironment (TME), particularly in tumor-associated macrophages (TAMs). TAMs exhibit high plasticity in their function and display both pro- and anti-tumor properties depending upon cues received from the

**Table 2** Studies on potential utility of cysteine cathepsins as biomarkers in leukemia

Sample/Tissue	Detection	Method	Feasibility of implementation in clinical practice	Reference(s)
BMMC/PBMC	Total mRNA	qRT-PCR	Feasible and rapid	19
	Cell specific mRNA level	Cell enrichment by FACS/MACS followed by qRT-PCR	Less feasible in the routine clinical setting	NA
	Cell specific protein level	Flow cytometry	Already used in the routine clinical setting	70
	Enzyme activity	Biochemical assay	Feasible for some cathepsins, including CTSB and CTSL	18
Bone marrow cells	Enzyme activity	<i>In vivo</i> imaging using activity-based probes	Potentially feasible	44,45
Serum/body fluids	Circulating total cathepsin level	ELISA	Feasible and rapid	71
	Cathepsin/cystatin complex level	Sandwich ELISA with simultaneous recognition of cathepsin and cystatin present as complex	Feasible and rapid	72

Abbreviations: BMMC, bone marrow mononuclear cell; CTSB, cathepsin B; CTSL, cathepsin L; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell sorting; MACS, magnetic activated cell sorting; NA, not available; PBMC, peripheral blood mononuclear cell; qRT-PCR, quantitative real time polymerase chain reaction.



**Fig. 2** Putative role of cysteine cathepsins in leukemia. The mRNA of cysteine cathepsins are synthesized in the nucleus, transported to the cytosol, and translated into pre-pro-cathepsins on ER bound ribosomes. These pre-pro-cathepsins further undergo post-translational modifications in the ER-Golgi network to form pro-cathepsins. These pro-cathepsins are either packaged into lysosomes or secreted out of the cells. In lysosomes or acidic leukemic microenvironment, these pro-cathepsins are proteolytically processed to their enzymatically active forms, where they function in the degradation and turnover of intracellular proteins and extracellular matrix, respectively. During protein degradation, autophagic phagosomes fuse with lysosomes (forming autophagosomes), where active cathepsin degrade these dead/damaged organelles and proteins for recycling. As cathepsins are overexpressed in leukemia, treatment with cathepsin inhibitors leads to the accumulation of cellular debris and enlarged lysosomes with inactive cathepsins, and subsequently, induce caspase-mediated apoptosis. Further, it may also inhibit the degradation of asparaginase and CXCL12 in the leukemic microenvironment, thereby regulating the egression and drug response of leukemic cells. Lysosome permeabilization inducers disrupt lysosomal membrane integrity leading to the release of cathepsins into the cytosol, which also induces apoptosis. Cystatins are endogenous inhibitors of cysteine cathepsins and protect cells from cytosolic cathepsin induced apoptosis. LSPs, lysosome permeabilization.

cellular microenvironment.<sup>58</sup> Therefore, an important consideration for cysteine cathepsin inhibition may also be to first determine the cell-specific role of these proteases in the TME. In line with this, a study using a murine model of breast cancer reported that CTSB overexpression in tumor cells, not stromal cells to be associated with tumor progression.<sup>59</sup> The secretion of cysteine cathepsins by TAMs has also been demonstrated to induce resistance to taxol, doxorubicin, and etoposide in breast cancer.<sup>60</sup> Inhibition of cysteine cathepsins B, L, and S by a novel inhibitor GB111-NH2 has been shown to induce oxidative stress and apoptosis in macrophages.<sup>61</sup> Interestingly,

apoptosis in TAMs also induced death of neighboring breast cancer cells *in vivo* and hence resulted in regression of the primary growth. In leukemia, the determination of the cellular origin of protease expression could be comparatively easier than solid tumors because of the ready availability of single cells suspensions. Additionally, the implementation of cysteine cathepsins as prognostic markers or in predicting the therapeutic response in future might be comparatively easier in leukemia than in solid tumors since flow cytometry is routinely used for the diagnosis of this malignancy. It may further facilitate determination of their cell specific

expression pattern along with established clinical markers and thus help in identification of molecular subtypes of leukemia.

Previous studies have demonstrated CTSB-mediated degradation of asparaginase, a drug used to treat childhood ALL, thereby rendering it ineffective in the treatment of this leukemia.<sup>62,63</sup> This observation was further supported by increased half-life of asparaginase in patients harboring germline mutation in the CTSB gene, resulting in the deletion of a highly conserved lysine residue in its carboxy terminus (p.K237del).<sup>62</sup> This may explain the extensive variations in response to asparaginase therapy observed in leukemic patients and suggests that CTSB inhibition may augment therapeutic response and overcome drug resistance in ALL.

Few studies have been undertaken to elucidate the mechanism of cysteine cathepsin inhibition mediated cell death in leukemia. Zhu and Uckun demonstrated induction of apoptosis in multiple human leukemia and lymphoma cell lines by the inhibition of cysteine cathepsins with pan cathepsin inhibitor I (CATI-I).<sup>64</sup> This selective inhibitor of cysteine cathepsins triggered apoptosis in a caspase-independent, p53-independent, BAX-independent as well as MAP kinase-independent fashion. With the recent advancements in the understanding of cell death pathways, determination of the full potential of targeting individual cysteine cathepsins may prove useful in the management of leukemia. With this objective, Pandey et al sought to investigate the therapeutic potential of CTSB inhibition in AML using an AML derived human cells line THP-1.<sup>19</sup> The strategy used in this study is given in ►Fig. 1B, C. The results of this study conclusively demonstrated the abrogation of cell proliferation and induction of caspase-dependent apoptosis in THP-1 cells by inhibition of CTSB with CA-074 Me.<sup>19</sup> Furthermore, doxorubicin—a conventional drug used to treat this malignancy—efficiently inhibited CTSB activity *in vivo* as well as *in vitro*, thereby suggesting that this therapeutic agent imparts some part of its cytotoxic effects on leukemic cells by inhibiting CTSB. Docking and simulation studies confirmed the binding of doxorubicin to the active site of CTSB with higher binding energy than its specific inhibitor CA-074 Me. These results establish the potential utility of CTSB as a therapeutic target. Consistent with these findings, benzyl-oxycarbonyl-phenylalanine-alanine-chloromethyl ketone (z-FA-CMK), a specific CTSB inhibitor has recently been shown to induce oxidative stress mediated apoptosis in leukemic T-cells at low concentrations, while at high concentrations, it induced necrotic cell death.<sup>65,66</sup> The putative roles of cysteine cathepsins in leukemia have been summarized in ►Fig. 2.

In addition of being therapeutic targets in leukemia, cysteine cathepsins have also been shown to be mediators of cell death in certain conditions. Ivanov et al observed that certain CD20-specific mAbs and HLA-DR specific mAb induce a nonapoptotic cell death that is rapid, non-autophagic, and involve disintegration of lysosomal membrane, thereby releasing its contents including CTSB into the cytoplasm.<sup>67</sup> Similarly, in another study, Puissant et al

demonstrated that imatinib induced death of CML cells is associated with lysosomal membrane permeabilization leading to release of CTSB into the cytosolic compartment which in turn cleaves BCR-ABL and thereby induces apoptosis.<sup>68</sup> Consistent with these findings, treatment of chronic lymphocytic leukemia cells with lysosomotropic agents also induced cell death following CTSB release.<sup>69</sup>

In summary, elevated levels of cysteine cathepsins in different types of leukemia and their association with poor survival outcomes in patients suggest their potential utility as a prognostic marker. Additionally, targeting specific cysteine cathepsins in combination with existing targeted therapies in leukemia may improve patient survival. Therefore, future studies aimed at delineating the cathepsin-mediated cell survival/apoptotic pathways in leukemia may open new therapeutic avenues.

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### Conflict of Interest

None declared.

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