The prognosis of lymphoid neoplasms has improved considerably during the last decades. However, treatment response for some lymphoid neoplasms is still poor, indicating the need for new therapeutic approaches. One promising new strategy is the inhibition of kinases regulating key signal transduction pathways, which are of central importance in tumorigenesis. Kinases of the CK1 family may represent an attractive drug target since CK1 expression and/or activity are associated with the pathogenesis of malignant diseases. Over the last years efforts were taken to develop highly potent and selective CK1-specific inhibitor compounds and their therapeutic potential has now to be proved in pre-clinical trials. Therefore, we analyzed expression and mutational status of CK1δ in several cell lines representing established lymphoma entities, and also measured the mRNA expression level in primary lymphoma tissue as well as in non-neoplastic blood cells. For a selection of lymphoma cell lines we furthermore determined CK1δ kinase activity and demonstrated therapeutic potential of CK1-specific inhibitors as a putative therapeutic option in the treatment of lymphoid neoplasms.

Keywords: CK1, lymphoma, therapy, inhibitor, mutation analysis, cell cycle

INTRODUCTION

Lymphoid neoplasms are heterogeneous malignancies of the hematopoietic and lymphoid tissues (Jaffe et al., 2001; Harris et al., 2008). In the United States, the annual incidence of all lymphoid malignancies is about 34 per 100,000.

According to the current WHO classification lymphoid neoplasms are divided into Hodgkin-(HL) and Non-Hodgkin-Lymphomas (NHL) (Jaffe et al., 2001; Harris et al., 2008). HL represents approximately 30% of lymphoid neoplasms and is dichotomized into classical Hodgkin Lymphoma and nodular lymphocyte predominant Hodgkin lymphoma. Although Hodgkin lymphoma is a B cell origin with only very few exceptions, its distinct histology, immunophenotype, and clinical presentation justify separating it from other mature B cell neoplasms (Jaffe et al., 2001; Schmitz et al., 2009). Mature NHL are broadly divided into B and T cell neoplasms and further sub-classified into a large number of distinct entities, according to phenotype, genotype and clinical properties. One of the major entities of mature B cell neoplasms is diffuse large B cell lymphoma, not otherwise specified (NOS), that contains various subtypes and morphologic variants, including immunoblastic lymphoma (Menon et al., 2012). Other B cell lymphoma entities are primary mediastinal B cell lymphoma and Burkitt lymphoma. Primary mediastinal B cell lymphoma is a diffuse large B cell lymphoma typically arising in the mediastinum (Stein et al., 2008). Burkitt lymphoma constitutes an aggressive, highly proliferative mature B cell lymphoma often located at extranodal sites (Leoncini et al., 2008). B and T lymphoblastic lymphomas are primarily pediatric, immature tumors belonging to the group of precursor lymphoid neoplasms (Borowitz and Chan, 2008).

Owing to increasingly elaborate therapy protocols, the prognosis of lymphoid neoplasms has continuously improved during the last decades. Nevertheless, for a significant number of lymphoid neoplasms the response to established treatment remains unsatisfactory, highlighting the need for new therapy strategies. Currently, one of the most promising approaches in oncology seems to be targeting of growth-promoting protein kinases by specific inhibitory substances. Such kinases have already been
identified for a few lymphoid neoplasms, i.e., chronic lymphocytic leukemia, mantle cell lymphoma (Alipour and Advani, 2014) and hairy cell leukemia (Dietrich et al., 2012). However, for the vast majority of malignant lymphomas suitable target molecules are still unknown. Therefore, large research interest is now being focused on the identification of new specific protein kinases to interrupt pivotal signaling pathways in lymphoma cells.

Casein kinase 1 (CK1) isoforms are ubiquitously expressed serine/threonine-specific kinases. In human, at least six different isoforms (α, γ1, γ2, γ3, β, and ε) and a number of related splice variants have been identified (Green and Bennett, 1998; Fu et al., 2001; Burzio et al., 2002; Knippschild et al., 2014). While all isoforms and variants significantly differ in length and composition of their N- and C-terminal domains, the kinase domain is highly conserved within all isoforms. CK1δ and ε show the highest sequence homology of about 98%. CK1 family members are able to phosphorylate a broad range of substrates regulating important cellular processes like cytokinesis, DNA repair, cell cycle progression, differentiation processes, and apoptosis (Knippschild et al., 2005, 2014; Price, 2006; Cheong and Virshup, 2011). CK1 activity is also linked to pathways involving the central signal transduction molecules p53 or β-catenin (Cheong and Virshup, 2011; Cruciat, 2014; Knippschild et al., 2014). Accordingly, changes in cellular CK1 expression and/or activity contribute to the pathogenesis of various diseases including cancer. In recent years considerable research effort has addressed the development and characterization of CK1-specific inhibitors (for review see Knippschild et al., 2014). As a result, new inhibitor compounds specifically targeting CK1 isoforms are more and more proving their therapeutic potential for a variety of malignancies (Perez et al., 2010; Knippschild et al., 2014).

To investigate whether CK1δ could be a potential therapeutic target in lymphoid malignancies, we characterized the role of CK1δ in 18 human cell lines representing a number of established lymphoma entities, i.e., acute lymphoblastic B cell lymphoma, acute lymphoblastic T cell lymphoma, diffuse-large B cell lymphoma (NOS), primary mediastinal B cell lymphoma, Burkitt lymphoma, classical Hodgkin-lymphoma, and nodular lymphocyte-predominant Hodgkin Lymphoma. In the present study, we tested the effects of established and novel CK1-specific inhibitor compounds on selected lymphoma cell lines in order to evaluate the therapeutic potential of CK1 inhibition. Prior to functional testing, we determined expression, mutational status, and kinase activity of CK1δ in several established lymphoma cell lines to estimate the effects of CK1-specific kinase inhibition and to correlate kinase inhibition-mediated effects with CK1δ expression and/or activity levels or possible mutations in the CK1δ coding sequence.

**MATERIALS AND METHODS**

**HUMAN LYMPHOMA TISSUE**

In summary 12 patients suffering from diffuse large B cell lymphomas whose informed consent was obtained prior to surgery were included in the study. Lymphoma diagnosis was in accordance with the current World Health Organization classification. All samples were drawn from our archive of frozen tissues and pseudonymized to comply with the German law for correct usage of archival tissue for clinical research (Deutsches Ärzteblatt 2003; 100 A1632). Frozen tissue samples were used for gene expression analysis. The project was performed with the permission of the independent local ethics committee of the University of Ulm.

**CELL LINES**

Within the present study the following established lymphoma cell lines were used: DAUDI (Klein et al., 1968), DEV (Poppema et al., 1985), DOHH-2 (Kluin-Nelemans et al., 1991), HDLM2 (Drexler et al., 1986), JIYOYE (Pulvertaft, 1965), Jurkat (Schneider et al., 1977), KARPAS-1106P (Nacheva et al., 1994), KM-H2 (Kamesaki et al., 1986), L-1236 (Kanzler et al., 1996), L-428 (Diehl et al., 1981), L-540 (Diehl et al., 1981), MedB-1 (Moller et al., 2001), NALM-6 (Hurwitz et al., 1979), RAJI (Pulvertaft, 1964), RAMOS (Klein et al., 1975), SU-DHL-6 (Epstein et al., 1978), SU-DHL-8 (Epstein et al., 1978), U-H01 (Mader et al., 2007). Furthermore, the Epstein-Barr virus (EBV) transformed lymphoblastoid B cells from peripheral blood of healthy donors LCL1, LCL2, and BSM (Halder et al., 2000) were used. All cell lines were maintained in RPMI-1640 medium (Invitrogen, Karlsruhe, Germany) except for U-H01 (4:1 mixture of DMEM and RPMI-1640; Invitrogen, Karlsruhe, Germany) and MedB-1 (IMDM; Invitrogen, Karlsruhe, Germany). Media were supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated fetal calf serum (FCS) except for DEV, HDLM2, Jurkat, L-540, for which 20% FCS was used. Cells were kept in a humidified 5% carbon dioxide atmosphere at 37°C.

**GENE EXPRESSION ANALYSIS**

Total RNA was isolated from human lymphoma cells, blood cells, and tumors from lymphoid tissue using the RNaseasy Mini kit (Qiagen, Hilden, Germany). Equal amounts of total RNA were reverse transcribed into complementary DNA using the AffinityScript cDNA Synthesis kit (Agilent Technologies, Waldbronn, Germany). Quantitative reverse-transcription PCR (qRT-PCR) data presented in Figure 1 were generated using an iCycler real-time PCR system and iQ SYBR green supermix according to the manufacturer’s instructions (both Biorad, Munich, Germany). Glycerinaldehyde-3-phosphat-dehydrogenase (GAPDH) was used as external standard, cryopreserved tissue of human tonsils as reference material. qPCR-conditions were generated using the ΔΔCt method. Real-time RT-PCR data shown in Figure 7 were generated using the QuantiTect™ SYBR Green PCR kit (Qiagen, Hilden, Germany) and a LightCycler® 480 (Roche, Mannheim, Germany) real-time PCR instrument. QuantiTect Primer Assays for CK1δ and HPRT were purchased from Qiagen (Qiagen, Hilden, Germany) and used according to the manufacturer’s instructions using the following cycling conditions: heat activation at 95°C for 15 min, 55 cycles of 15 s denaturation at 94°C, 20 s annealing at 55°C and 30 s synthesis at 72°C. Slope was always set at 20°C/s throughout amplification. The fluorescence data collection of the color signal was measured in real-time model.

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at the end of each DNA synthesis cycle, and the crossing point was calculated. The specificity of DNA amplification for CK1δ was examined analyzing the melting curve. mRNA contents were normalized to HPRT mRNA levels.

**ISOLATION OF PRIMARY HUMAN IMMUNE CELLS**

Human peripheral blood mononuclear cells (PBMCs) were freshly isolated from buffy coats of healthy blood donors by density gradient centrifugation. Conventional dendritic cells (cDC, CD1c+), monocytes (CD14+), T regulatory cells (CD4+, CD25+), cytotoxic T lymphocytes (CD8+), natural killer cells (NK cells, CD56+), and B cells (CD19+) were positively selected using the appropriate magnetic cell separation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s protocol. Use of patient material in this study was approved by the ethics committee at the University of Ulm.

**SEQUENCE ANALYSIS**

CK1δ was amplified from cDNA prepared as described above using PrimeSTAR HS DNA Polymerase. PCR was performed in a Primus 96 Plus Thermal Cycler (MWG Biotec, Ebersberg, Germany). To obtain adequate amounts of specific amplification product, a nested PCR was carried out applying the following conditions: denaturation at 98°C, annealing at 56°C, and elongation at 72°C in 35 cycles. For the first round of PCR the primers GCCCTTCAGAGCAATAAGGA and CCAGATTCAGACCCAGGAA were used in 35 cycles. For the second round the primers GCACGACAGACTGAAGACCA and CCAGATTCAGACCCAGGAA were used in 20 cycles. Prior to sequencing, amplified CK1-products were separated on a 2% agarose gel and cleaned up using thepeqGOLD MicroSpin gel elution kit (peqlab Biotechnologie GmbH, Erlangen, Germany). Sequencing primers for CK1δ were CCAGATTCAGACCC AGAA, AGACATCCCCCTATCGTAG, and GCACGACAGACT GAAGACCA. Sequencing of DNA was accomplished by LGC genomics (Berlin, Germany). Received CK1δ cDNA sequence data were evaluated using the database sequence for human CK1δ transcription variant 2 (accession ID: NM_139062) and the multiple sequence alignment tool ClustalW (Larkin et al., 2007; Goujon et al., 2010).

**WESTERN BLOT**

Cells were lysed in radio immunoprecipitation assay (RIPA) lysis buffer by incubation on ice for 30 min. Extracts were clarified by centrifugation and protein concentration was determined using the BCA protein assay (Thermo Fisher Scientific, Rockford, USA). Equal amounts of protein extracts were separated by SDS-PAGE and transferred to a PVDF membrane by semidry Western blotting. After blocking with 5% (w/v) milk the membrane was incubated with the specific antibody for CK1δ (C–18; Santa Cruz biotechnology, Heidelberg, Germany). Immunocomplexes were detected using HRP-conjugated anti-goat IgG (Santa Cruz biotechnology, Heidelberg, Germany), followed by chemiluminescence detection with SuperSignal West Dura (Thermo Fisher Scientific, Rockford, USA) and exposure to X-ray films. Densitometric quantification was performed using TINA 2.09 software. Equal loading of extracted proteins was determined by re-probing the membrane with a β-actin specific monoclonal antibody.

**FRACTIONATION OF PROTEINS**

Protein extracts for anion exchange chromatography were prepared in sucrose lysis buffer (20 mM Tris–HCl pH 7.0, 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 1% (v/v) Triton X-100, 1 mM benzamidine, 25 μg/ml aprotinin, and 5 mM DTT) by incubation on ice for 30 min. Extracts were cleared by centrifugation and protein concentration was determined using the BCA protein
expression (Thermo Fisher Scientific, Rockford, USA). Prior to column loading protein lysates were passed through a 0.4 μm filter. Fractionation of equal protein amounts was performed using an anion-exchange column (Resource Q; GE Healthcare, Chalfont St Giles, UK) attached to an ETTAN LC purifier (GE Healthcare, Chalfont St Giles, UK). Bound proteins were eluted with a linear ascending gradient between 0 and 1000 mM NaCl in fractionation buffer (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 5% (v/v) glycerol, 0.04% Brij, 1 mM benzamidine, 4 μg/ml leupeptin, and 0.1% (v/v) β-mercaptoethanol) and 250 μl of each protein fraction was collected.

**IN VITRO KINASE REACTIONS**

In order to detect cellular CK1-specific kinase activity in vitro kinase assays were carried out using selected fractions of anion-exchange fractionated cellular protein extracts as source of kinase while the GST-p53δ–64 fusion protein (FP267) (Milne et al., 2001) has previously been described in detail (Knippschild et al., 1997).

**EXPRESSION AND PURIFICATION OF GLUTATHIONE S-TRANSFERASE FUSION PROTEINS**

Expression and purification of glutathione S-transferase (GST)-p53δ–64 fusion protein (FP267) (Milne et al., 2001) has previously been described in detail (Knippschild et al., 1997).

**RESULTS**

**ANALYSIS OF CK1δ mRNA AND PROTEIN LEVELS IN ESTABLISHED LYMPHOMA CELL LINES**

Several studies indicate that deregulated expression and/or activity of CK1δ is associated with tumorigenesis in a number of malignancies (Iinuzuka et al., 2010; Elyada et al., 2011; Knippschild et al., 2014). However, for human malignant lymphoma the impact of CK1δ on tumor development or progression has not been systematically investigated so far. In order to determine CK1δ expression levels, we first conducted quantitative reverse-transcription PCR (qRT-PCR). CK1δ mRNA was found in all 18 cell lines investigated. Both PMBL (mediastinal large B cell lymphoma) cell lines, MedB-1 and KARPAS-1066P, showed about twofold higher amounts of CK1δ mRNA than the other lymphoma cell lines included in our study (Figure 1A). CK1δ protein expression was quantified by Western blotting analysis and could be detected in all tested cell lines of the various lymphoma entities. In contrast to the mRNA findings, PMBL cell lines did not show elevated CK1δ protein expression, whereas the cHL (classical Hodgkin lymphoma) group and the single tested T-ALL (T cell acute lymphoblastic lymphoma) cell line displayed slightly increased CK1δ protein levels in comparison to the other lymphoma entities (Figure 1B).

**CK1δ-SPECIFIC KINASE ACTIVITY DIFFERS IN ESTABLISHED LYMPHOMA CELL LINES**

CK1δ activity is tightly regulated by various cellular mechanisms. Having demonstrated CK1δ expression, we therefore tested CK1δ-specific kinase activity in selected lymphoma cell lines: the two classical HL lines KM-H2 and U-H01, the diffuse large B cell lymphoma line DOHH-2, and the Burkitt lymphoma line RAMOS. Equal amounts of protein extracts were fractionated by ion-exchange chromatography in order to separate CK1δ from other protein kinases as well as cellular modulators of kinase activity like activating/inhibiting binding partners and phosphatases. Resulting fractions were checked for CK1δ kinase activity using the substrate GST-p53δ–64. Strong CK1δ-specific activity could be detected in extracts of KM-H2 and RAMOS cells.
while U-H01 and DOHH-2 cells only showed weak detectable activity (Figure 2A). In order to confirm a major contribution of CK1δ to the detected activity in RAMOS and KM-H2 cells, kinase activity in the peak fractions was assayed in the presence of different CK1-specific inhibitors, including IC261 (Mashhoon et al., 2000), compound 1 (Richter et al., 2014a), and compound 17 (Peifer et al., 2009). For both cell lines kinase activity in the peak fractions could be inhibited by all three substances. At least peak indeed mainly was caused by CK1δ demonstrated with compound 1, confirming, that the detected activity reported. Strongest activity-reduction of up to 70% was demonstrated for U-H01 and DOHH-2 cells. In contrast IC261 treatment only led to minor effects on in G2 phase for an increased number of KM-H2 and DOHH-2 cells by either increasing the amount of specific inhibitor IC261 showed obvious effects on RAMOS, KM-H2, and DOHH-2 cells by either increasing the amount of dead cells in case of RAMOS cells or by leading to cell cycle arrest in G2 phase for an increased number of KM-H2 and DOHH-2 cells. In contrast IC261 treatment only led to minor effects on U-H01 cells (Figure 4). While compound 17 showed no obvious effects on the tested cell lines treatment with only low concentrations of compound 1 resulted in an increase of dead cells already after 24 h (Figures 5, 6). Except for RAMOS these effects of compound 1 could not be potentiated but remained stable while treatment duration or inhibitor concentration was increased. All compounds showed most obvious effects on RAMOS cells after 24 h which could be enhanced by either increasing inhibitor concentration or treatment duration to 48 h.

CK1δ mRNA EXPRESSION LEVELS IN LYMPHOMA TISSUES CORRESPOND TO TUMOR CELL LINES BUT DIFFER FROM NON-NEOPLASTIC LYMPHOID CELLS

So far, our results demonstrate that CK1-specific inhibitors differently affect the growth of various lymphoma cell lines. Their use in novel therapeutic concepts could be considered, especially if alterations in the expression levels of CK1δ in tumor tissue are detectable. Therefore, we now analyzed CK1δ mRNA expression in 12 tissue samples of diffuse large B cell lymphoma, which is the most prevalent aggressive lymphoma type. We also measured the expression levels in EBV-immortalized lymphoid cells, and non-neoplastic lymphoid cell populations from healthy donors. In the tested tumor tissue samples the expression levels of CK1δ varied up to 5.9-fold (Figure 7A, Table 1). Similarly, the expression levels of CK1δ among blood cells and lymphocytes from healthy donors also varied up to 5.4-fold (Figure 7B, Table 1). However, CK1δ expression in non-neoplastic blood cells (i.e., B cells, Tregs, cytotoxic T cells, and monocytes) were markedly increased compared to expression levels in tumor samples, lymphoma cell lines, and EBV-transformed lymphoblastoid B cells (Figures 7A,B).

DISCUSSION

Protein kinases of the CK1 family are involved in essential cellular processes and CK1 dysregulation can be associated with the pathogenesis of certain diseases as well as tumorigenesis...
CK1δ kinase domain amplified from cell lines RAMOS, KM-H2, U-H01, and DOHH-2.

Messenger RNA from cell lines RAMOS, KM-H2, U-H01, and DOHH-2 was isolated, transcribed into cDNA, and the CK1δ coding sequence was amplified as described in Material and Methods. DNA sequences obtained from reactions using primers listed in Material and Methods were translated into protein sequences and the alignment was generated using ClustalW 2.1 (Larkin et al., 2007; Goujon et al., 2010).

Knippschild et al., CK1δ in lymphoma

Recently, CK1δ was suggested as a therapeutic target for innovative strategies to treat malignancies arising from the hematopoietic system like multiple myeloma (Hu et al., 2014), acute myeloid leukemia (Jaras et al., 2014), or del(5q) myelodysplastic syndrome (Schneider et al., 2014). In the present study, we demonstrate the therapeutic potential of CK1-specific kinase inhibition by treating established lymphoma cell lines with CK1-specific small molecule inhibitors. In order to characterize the CK1-specific status and to estimate the expected treatment effects, we first analyzed CK1δ expression and mutation status in 18 cell lines covering a broad spectrum of lymphoid neoplasms. In all cell lines CK1δ mRNA and protein were found to be expressed strongly and at a similar level (except mRNA expression in PMBL cell lines) pointing to essential functions of CK1 in the regulation of apoptosis, NFκB, and Wnt-related signaling (Cruciat, 2014; Knippschild et al., 2014). Previous studies carried out in a murine model system already detected increased expression of CK1δ in hyperplastic B cell follicles and progressed B cell lymphomas in p53-deficient mice (Maritzen et al., 2003).

Mutations in the coding sequences of central signal transduction molecules play a pivotal role in tumorigenesis. In a number of genes, somatic mutations are quite common in malignant lymphoma and can even be characteristic for certain lymphoma entities (Kuppers, 2005). Mutations in the CK1δ protein may lead to altered binding and potency of certain inhibitor molecules (Peifer et al., 2009; Richter et al., 2014b). Therefore, mutation analysis is essential when determining the therapeutic potential of CK1-specific inhibitor compounds. Remarkably however, the analyzed human lymphoma cell lines without exception showed an unmutated state of the CK1δ coding sequence. This finding may point to an essential need for unmutated and functional CK1δ in signaling pathways required for intact lymphoid neoplasm cell function. Further analysis would be needed to validate this conclusion. An important mechanism leading to mutations...
FIGURE 4 | Cell cycle analyses of RAMOS, KM-H2, U-H01, and DOHH-2 cells after treatment with the CK1δ/ε-specific inhibitor IC261. Cells were treated with different concentrations of IC261 for 24 h and 48 h and stained with propidium iodide in order to perform cell cycle analysis as described in Material and Methods. DMSO treated cells served as control. Each 20,000 cells in two replicates per condition were counted. One representative graph is shown. The determined fractions of cells in various phases of the cell cycle are given as percent values (%). Flow cytometry plots show the number of cells (y-axis) with each observed intensity of propidium iodide DNA staining (x-axis). DMSO treated cells showed a normal cell cycle distribution of asynchronously proliferating cells. Treatment with IC261 led to an increase of dead RAMOS cells, whereas in KM-H2, U-H01, and DOHH-2 cells an increase of G2 arrested cells could be observed.
FIGURE 5 | Cell cycle analyses of RAMOS, KM-H2, U-H01, and DOHH-2 cells after treatment with the CK1δ-specific inhibitor compound 1. Cells were treated with different concentrations of compound 1 for 24 h and 48 h and stained with propidium iodide in order to perform cell cycle analysis as described in Material and Methods. DMSO treated cells served as control. Each 20,000 cells in two replicates per condition were counted. One representative graph is shown. The determined fractions of cells in various phases of the cell cycle are given as percent values (%). Flow cytometry plots show the number of cells (y-axis) with each observed intensity of propidium iodide DNA staining (x-axis). DMSO treated cells showed a normal cell cycle distribution of asynchronously proliferating cells. Treatment with compound 1 led to an increase of RAMOS, KM-H2, and U-H01 cells in G2 phase. Additionally, RAMOS cells accumulate in Sub G1 phase as well. No obvious effects could be observed in DOHH-2 cells.

in malignant lymphoma is cytidine deamination by activation induced cytidine deaminase (AID) and the subsequent incorrect repair of the affected base. Since chromatin-immunoprecipitation studies in murine B-lymphocytes revealed that AID is not able to substantially bind to CK18 DNA sequence (Yamane et al., 2011), this could provide one explanation for the absence of CK18 mutations while some of the analyzed cell lines carry mutations in multiple genes (Kuppers, 2005; Mader et al., 2007).
For further analyses on the role of CK1δ in human lymphoma cell lines we concentrated on four cell lines, representing classical Hodgkin lymphoma (KM-H2 and U-H01), diffuse large B cell lymphoma (DOHH-2) and Burkitt lymphoma (RAMOS). Although all four cell lines share rather similar CK1δ expression levels, differences in the cellular kinase activity of CK1δ could be detected. Using the established CK1-specific small molecule inhibitor IC261 and two structurally independent CK1δ-specific inhibitors (Peifer et al., 2009; Richter et al., 2014a) the kinase activity detected using substrate GST-p531-64 (Knippschild et al.,...
Mechanisms that regulate CK1δ activity are intramolecular autophosphorylation and phosphorylation through other cellular kinases (Rivers et al., 1997) could clearly be assigned to CK1δ. Cell line specific differences might be explained by posttranslational modifications of CK1δ and have already been reported in previous studies (Maritzen et al., 2003; Giamas et al., 2007). Mechanisms that regulate CK1δ activity are intramolecular autophosphorylation and phosphorylation through other cellular kinases (Rivers et al., 1997; Gietzen and Virshup, 1999; Giamas et al., 2007; Bischof et al., 2013).

In all four cell lines, FACS analyses demonstrated changes in cell cycle distribution after treatment with CK1δ-specific inhibitor compounds. Due to reduced stability and solubility of inhibitor compounds 1 and 17 much higher concentrations of these compounds have to be used for the treatment of cultured cells when being compared to in vitro application and to the use of IC261. The observed effects are dependent on inhibitor concentration, cell line, and duration of inhibitor exposure. Cell cycle arrest, as observed for some cell lines being treated with IC261 and compound 1, can be explained by CK1δ effects on microtubule dynamics and chromosome segregation (Behrend et al., 2000; Stoter et al., 2014). However, Cheong and colleagues meanwhile demonstrated, that IC261 could have a similar effect on cellular microtubule dynamics as the spindle poison colchicine (Cheong et al., 2011). In order to exclude unspecific effects, we therefore performed all experiments with three structurally different CK1δ-inhibitors. Previous studies showed that the status of the tumor suppressor p53 plays a central role for the effects induced by inhibitor IC261. While treatment of wildtype p53 expressing cells with CK1δ-inhibitors leads to postmitotic arrest or apoptosis, the treatment of cells expressing mutated or no p53 at all is followed by endoreduplication (Behrend et al., 2000; Stoter et al., 2005). The present study is in partial accordance to these reports. Cell lines KM-H2 and DOHH-2 (wt p53, Drakos et al., 2007; Wang et al., 2011) showed increased apoptosis upon treatment with

Table 1 | CK1δ expression levels in tumors from lymphoid tissue, established lymphoma cell lines, EBV-immortalized lymphoblastoid B cells, and peripheral blood cell populations of healthy donors.

| Sample | Fold change expression of CK1δ |
|--------|-------------------------------|
| P1     | 0.34 ± 0.06                   |
| P2     | 1.39 ± 0.30                   |
| P3     | 0.82 ± 0.23                   |
| P4     | 2.00 ± 0.76                   |
| P5     | 0.64 ± 0.01                   |
| P6     | 1.05 ± 0.01                   |
| P7     | 0.85 ± 0.30                   |
| P8     | 0.81 ± 0.03                   |
| P9     | 0.96 ± 0.05                   |
| P10    | 0.88 ± 0.09                   |
| P11    | 0.91 ± 0.07                   |
| P12    | 0.94 ± 0.23                   |
| HDLM2  | 0.22 ± 0.04                   |
| KM-H2  | 0.66 ± 0.16                   |
| LCL1   | 0.74 ± 0.12                   |
| LCL2   | 1.34 ± 0.05                   |
| BSM    | 0.33 ± 0.02                   |
| B cells| 14.88 ± 2.17                  |
| Cytotoxic T cells | 8.30 ± 1.35 |
| Tregs  | 3.03 ± 0.26                   |
| NK cells | 15.35 ± 1.87               |
| Dendritic cells | 4.64 ± 0.05 |
| Monocytes | 16.31 ± 5.34          |

Values represent fold change of relative CK1δ expression ± SD compared to HPRT mRNA levels.
IC261 and compound 1. Treatment with IC261, however, also arrested an increased number of KM-H2 and DOHH-2 cells in G2-phase of the cell cycle. Albeit expressing mutated p53 (Farrell et al., 1991), RAMOS cells showed a remarkable increase of apoptotic cells after treatment with the CK1-specific inhibitors. In DOHH-2 cells the chromosomal translocation t(14:18) results in overexpression of Bcl-2 which is associated with chemoresistance (Tsujimoto et al., 1984; Khlin-Nelemans et al., 1991; Reed, 2008). The anti-apoptotic effect of Bcl2 may explain the low level of apoptosis induction that could be achieved in DOHH-2 treated with CK1-specific inhibitors. Similarly, the reduced reactivity to inhibitor treatment of cell line U-H01 could be explained by mutation of the phosphatase PTPN1 which protects the cells from apoptosis and therefore provides an advantage for cell survival (Knecht et al., 2010). Generally, the low activity of CK1δ detected in U-H01 and DOHH-2 provides another reason why the observed effects are lower than in KM-H2 or RAMOS, two cell lines showing high cellular CK1α kinase activity.

After having demonstrated significant effects of CK1-specific inhibitors on established lymphoma cell lines, the mRNA expression levels of CK1δ in primary lymphoid tumor tissue, EBV-immortalized lymphoid cells, and blood cells from healthy donors were compared. While the variation of CK1δ mRNA levels was nearly the same in primary tumor tissue and normal blood cells, CK1δ mRNA levels in general were remarkably low in all tested samples in comparison to blood cells from healthy donors. Cell lines HDLM2 and KM-H2, which were also included in this comparison, showed similarly low CK1δ mRNA levels as the analyzed primary tumor tissue samples. Therefore, these data provide further evidence that these cell lines may be used as a model for the validation of CK1δ-specific inhibitors in new lymphoma treatment strategies.

As a conclusion, its multiple functions may qualify CK1δ as a potential new target molecule to treat lymphoid neoplasms. The results of the present study suggest for the first time that CK1δ could have adjuvant effects in the therapy of malignant lymphoma. However, these effects are strongly dependent on the cellular background of the individual lymphoma cell population and for future application in novel concepts of personalized medicine still large efforts have to be made to increase solubility and selectivity of the inhibitor compounds as well as to improve their safe delivery to the target tissue to avoid severe side effects.

**AUTHOR CONTRIBUTIONS**

Project work was designed by UK and FL. Experimental work was performed by BW, FO, JR, JB, PX, and TB. Data analysis and interpretation was done by BW, FO, JR, JB, PX, TB, FL, and UK. All Authors (BW, FO, JR, JB, PX, TB, FL, and UK) were involved in writing passages of the present manuscript and participated in final approval and revision. Figures were created by BW, FO, JR, and JB.

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