Simultaneous inhibition of pan-phosphatidylinositol-3-kinases and MEK as a potential therapeutic strategy in peripheral T-cell lymphomas

Esperanza Martín-Sánchez,[1,2] Socorro M. Rodríguez-Pinilla,[1,3] Margarita Sánchez-Beato,[1,4] Luis Lombardía,[5] Beatriz Domínguez-González,[1] Diana Romero,[5] Lina Odqvist,[1,2] Pablo García-Sanz,[6] Magdalena B. Wozniak,[1] Guido Kurz,[7] Carmen Blanco-Aparicio,[7] Manuela Mollejo,[8] F. Javier Alves,[9] Javier Menárguez,[10] Fernando González-Palacios,[11] José Luis Rodríguez-Peralto,[12] Pablo L. Ortiz-Romero,[13] Juan F. García,[6] James R. Bischoff,[7] and Miguel A. Piris[1,14]

[1]Lymphoma Group and [2]Epithelial Carcinogenesis Group, Molecular Pathology Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [3]Department of Pathology, Fundación Jiménez Díaz, Madrid, Spain; [4]Fundación para la Investigación Biomédica Hospital Universitario Puerta de Hierro, Madrid, Spain; [5]Molecular Diagnostic Unit, CNIO, Madrid, Spain; [6]Translational Research Laboratory, M. D. Anderson Cancer Center Madrid, Spain; [7]Experimental Therapeutics Programme, CNIO, Madrid, Spain; [8]Department of Pathology, Hospital Virgen de la Salud, Toledo, Spain; [9]Department of Pathology, Hospital La Paz, Madrid, Spain; [10]Department of Pathology, Hospital Gregorio Marañón, Madrid, Spain; [11]Department of Pathology, Hospital Ramón y Cajal, Madrid, Spain; [12]Department of Pathology; [13]Department of Dermatology, Instituto i+12, Hospital 12 de Octubre & Medical School Universidad Complutense, Madrid, and [14]Department of Pathology, Hospital Universitario Marqués de Valdecilla – IFIMAV, Santander, Spain

ABSTRACT

Peripheral T-cell lymphomas are very aggressive hematologic malignancies for which there is no targeted therapy. New, rational approaches are necessary to improve the very poor outcome in these patients. Phosphatidylinositol-3-kinase is one of the most important pathways in cell survival and proliferation. We hypothesized that phosphatidylinositol-3-kinase inhibitors could be rationally selected drugs for treating peripheral T-cell lymphomas. Several phosphatidylinositol-3-kinase isoforms were inhibited genetically (using small interfering RNA) and pharmacologically (with CAL-101 and GDC-0941 compounds) in a panel of six peripheral and cutaneous T-cell lymphoma cell lines. Cell viability was measured by intracellular ATP content; apoptosis and cell cycle changes were checked by flow cytometry. Pharmacodynamic biomarkers were assessed by western blot. The *PIK3CD* gene, which encodes the δ isoform of phosphatidylinositol-3-kinase, was overexpressed in cell lines and primary samples, and correlated with survival pathways. However, neither genetic nor specific pharmacological inhibition of phosphatidylinositol-3-kinase δ affected cell survival. In contrast, the pan-phosphatidylinositol-3-kinase inhibitor GDC-0941 arrested all T-cell lymphoma cell lines in the G1 phase and induced apoptosis in a subset of them. We identified phospho-GSK3β and phospho-p70S6K as potential biomarkers of phosphatidylinositol-3-kinase inhibitors. Interestingly, an increase in ERK phosphorylation was observed in some GDC-0941-treated T-cell lymphoma cell lines, suggesting the presence of a combination of phosphatidylinositol-3-kinase and MEK inhibitors. A highly synergistic effect was found between the two inhibitors, with the combination enhancing cell cycle arrest at G0/G1 in all T-cell lymphoma cell lines, and reducing cell viability in primary tumor T cells *ex vivo*. These results suggest that the combined treatment of pan-phosphatidylinositol-3-kinase + MEK inhibitors could be more effective than single phosphatidylinositol-3-kinase inhibitor treatment, and therefore, that this combination could be of therapeutic value for treating peripheral and cutaneous T-cell lymphomas.

Introduction

Peripheral T-cell lymphomas (PTCL) are a heterogeneous group of disorders with a very low incidence and very poor prognosis.[1,2] The molecular biology of PTCL is poorly understood, partially due to the rarity of the disease, the heterogeneity of subtypes and the low number of appropriate models (no representative cell lines are available and no current mouse model recapitulates the human disease). These restrictions have limited understanding of the pathogenesis of PTCL and, therefore, the development of the new targeted therapies that are necessary to improve the outcome of patients with this disease.[3]

The phosphatidylinositol-3-kinase (PI3K) family of proteins participates in one of the most essential pathways for cell survival, proliferation and growth. It is an important mediator of drug resistance and it has been described to be frequently altered in a wide variety of types of human cancer.[4-6] Two subclasses of PI3K have been related to cancer: class IA (p110α, β and δ isoforms of the catalytic subunit) is predominantly acti-

vated by tyrosine kinases, and class IB (p110γ isoform) is mainly activated by G-protein-coupled receptors. Upon extracellular stimulation, PI3K phosphorylate phosphatidylinositol 4,5 biphosphate (PIP2) to form phosphatidylinositol 3,4,5 triphosphate (PIP3). Subsequently, PIP3 can phosphorylate AKT, which initiates a signaling cascade of protein phosphorylation leading to cell survival, growth and proliferation. The activity of PI3K can be inhibited by the phosphatase and tensin homologue (PTEN), which acts as a tumor suppressor protein.[4-6]

Since aberrant regulation of the PI3K pathway has been frequently observed in leukemic cells (presumably due to activating signals from the microenvironment rather than mutations in genes belonging to the pathway), PI3K is considered to be a promising target for therapy.[4,6,7] Preclinical experiments indicated that the PI3K inhibitors (PI3Ki) LY294002 and wortmannin induced apoptosis in leukemic cells, but they were also toxic to normal cells, probably because of the low specificity and inhibition of other kinases. Recently, selective isoform-specific inhibitors, such as the PI3Kδ inhibitor CAL-101, have appeared to affect survival of chronic lymphocytic leukemia cells and CAL-101 has entered phase I/II clinical trials. Although the single specificity inhibitors are effective *in vitro*, their effect in patients appears to be modest. Therefore, combination therapy targeting different intermediates of the PI3K signaling pathway is also under investigation. Furthermore, since lymphomagenesis involves aberrant regulation of various signal transduction pathways, simultaneous inhibition of multiple deregulated targets is considered to be a promising therapeutic strategy.[4]

The aims of this study were to determine the efficiency of PI3K inhibition in PTCL, to look for pharmacodynamic biomarkers, and to identify markers that could distinguish responders from non-responders.

Design and Methods

The molecular signature and bioinformatic analysis of peripheral T-cell lymphoma

The PTCL molecular signature was identified by comparing the gene expression profile in a series of 38 frozen PTCL cases and six reactive lymph nodes, and further analyzed using Connectivity Map (Cmap)[8] and Gene Set Enrichment Analysis (GSEA)[9] programs, as described in the *Online Supplementary Design and Methods*. All microarray data are available at the Gene Expression Omnibus under accession number GSE36172. The research was approved by the ethical committees of the Instituto de Salud Carlos III (Madrid, Spain) and the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Cell lines, primary samples and reagents

The cell lines and primary samples are described in the *Online Supplementary Design and Methods*. GDC-0941 was obtained from Chemdea (Ridgewood, NJ, USA). LY294002 and UO126 were purchased from Calbiochem (Darmstadt, Germany). CAL-101 was obtained from Selleck Chemicals (Houston, TX, USA). ETP-45658 was developed[10] and kindly provided by the Experimental Therapeutics Program of the Spanish National Cancer Research Center (Madrid, Spain).

PIK3CD and PIK3CA genetic silencing

MyLa, SR786 and HuT78 cell lines were electroporated with specific small interfering RNA (siRNA) against the *PIK3CD* and *PIK3CA* genes, using the Neon® Transfection System (Invitrogen, Carlsbad, CA, USA), as described in the *Online Supplementary Design and Methods*.

Pharmacological inhibition assays

For drug cytotoxicity experiments, cell lines and primary tumor T cells were treated for 72 or 48 h, respectively, with PI3Ki, MEK inhibitors (MEKi) or their combinations. Cell viability was measured as the intracellular ATP content using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) following the manufacturer's instructions. For drug combination experiments, the combination index (CI) was calculated according to the method of Chou and Talalay.[11] The distribution of cells among different phases of the cell cycle and induction of apoptosis were evaluated by flow cytometry, as described in the *Online Supplementary Design and Methods*. All experiments were done in triplicate and all numerical data are expressed as the average of the values ± the standard error of the mean (SEM).

Results

Phosphatidylinositol-3-kinase as a potential therapeutic target in peripheral T-cell lymphoma

In order to test drugs that could be of therapeutic value in PTCL, we used the Cmap program and a PTCL molecular signature generated in our group of 38 PTCL cases and in six reactive lymph nodes. Cmap identified two PI3K/mTOR pathway inhibitors among the drugs that could most significantly reverse this PTCL signature (Figure 1A). We note that trichostatin A and MS-275, two histone deacetylase inhibitors [like vorinostat and romidepsin, which have already been approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma (CTCL)[12,13]], also appeared on the list.

Furthermore, using the GSEA program we observed in the PTCL molecular signature that the expression of *PIK3CD* (the gene encoding p110δ) was the only PI3K isoform to be significantly correlated with several survival pathways, such as the T-cell receptor, nuclear factor-κB and CD40 pathways (Figure 1B). Moreover, we found that both *PIK3CD* and *PIK3CA* (encoding p110α) were overexpressed in the six PTCL and CTCL cell lines (Figure 1C) and in primary T cells isolated from five patients with Sézary's syndrome (Figure 1D), compared to the expression by normal T cells from healthy donors.

Since the PI3K pathway is frequently altered at the genetic level in many types of human cancer, the mutational status of several genes in the pathway was explored in six PTCL and CTCL cell lines. We did not find any known mutation in *PIK3CA*, *AKT1*, *PTEN*, *KRAS* and *BRAF* genes in any cell line. The described[14] *NRAS* mutation Q61K was confirmed in the HuT78 cell line, but was absent from the other PTCL cell lines (*data not shown*). Although no mutations have yet been reported in the *PIK3CD* gene,[15] two described variants[16] were also studied in PTCL cell lines. We found that the SR786 cell line carried a single nucleotide polymorphism (c935g → S312C, rs61755420) (*data not shown*). Moreover, these *PIK3CD* polymorphisms were analyzed in a series of 27 PTCL cases. Interestingly, we observed two cases (one angioimmunoblastic T cell lymphoma and one anaplastic large cell lymphoma ALK+) harboring the same polymorphism. Additionally, one case of
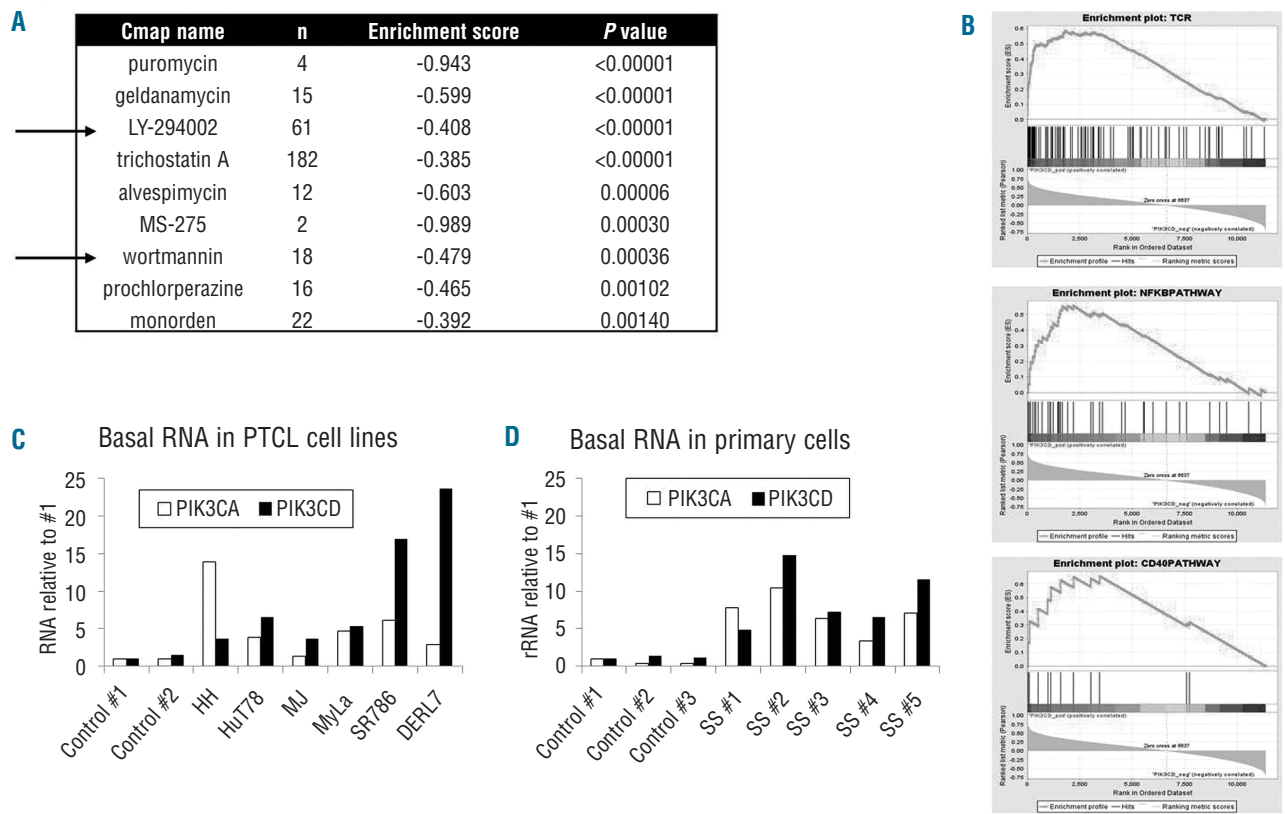
A

Cmap name	n	Enrichment score	*P* value
puromycin	4	-0.943	<0.00001
geldanamycin	15	-0.599	<0.00001
LY-294002	61	-0.408	<0.00001
trichostatin A	182	-0.385	<0.00001
alvespimycin	12	-0.603	0.00006
MS-275	2	-0.989	0.00030
wortmannin	18	-0.479	0.00036
prochlorperazine	16	-0.465	0.00102
monorden	22	-0.392	0.00140

B

C

D

Figure 1. PI3K is a potential therapeutic target in PTCL. (A) Connectivity Map identified PI3K/mTOR pathway inhibitors (indicated by arrows) as the drugs that could potentially reverse (negative enrichment score) the PTCL molecular signature in a very significant manner. (B) The GSEA program revealed that three survival pathways [T-cell receptor (TCR), NF-κB and CD40 signaling] were positively and significantly (false discovery rate <0.25) correlated with *PIK3CD* expression in the PTCL molecular signature. (C and D) Both *PIK3CA* and *PIK3CD* genes were overexpressed in (C) PTCL and CTCL cell lines and (D) Sézary's syndrome (SS) primary T cells compared with normal T cells isolated from healthy donors (control #1-3) measured by quantitative RT-PCR.

PTCL-not otherwise specified was found to have another single nucleotide polymorphism in this region (c2319t → S773S, rs139848768) (*data not shown*).

These data indicate that PI3Kδ is an attractive, potential therapeutic target in PTCL.

Effects of genetic silencing of the delta and alpha isoforms of phosphatidylinositol-3-kinase on peripheral T-cell lymphoma cell lines

To test the hypothesis that targeting PI3Kδ is of therapeutic value in PTCL, we performed genetic silencing experiments to specifically abolish *PIK3CD* and *PIK3CA* expression (because gain-of-function mutations in *PIK3CA* occur very frequently in human tumors[6]). The efficiency of the silencing was 60-90% at the mRNA level, depending on the cell line and the time point (*Online Supplementary Figure S1*). We observed a slight tendency in MyLa and SR786 cell lines, but not in HuT78, to undergo apoptosis when *PIK3CD* (Figure 2A) or *PIK3CA* (Figure 2B) were knocked down, although the differences compared with the non-template control were not statistically significant in any case. No significant changes in the cell cycle profile were observed in any condition or cell line (*data not shown*). The results suggest that the active protein remaining after knockdown could maintain survival of PTCL cell lines.

Effects of specific pharmacological inhibition of phosphatidylinositol-3-kinase-delta on peripheral T-cell lymphoma survival

In order to inhibit the PI3Kδ catalytic activity more efficiently, PTCL cell lines were treated with the specific pharmacological PI3Kδ inhibitor CAL-101. We observed that this drug generally reduced cell viability only at high doses (Figure 2C). Accordingly, CAL-101 was not able to induce apoptosis in any PTCL cell line, only HH suffered an 8% increase in apoptosis compared to the DMSO-treated cells, even when incubated with a dose of 10 μM for 72 h (Figure 2D). Similarly, slight cell cycle arrest at G0/G1 was found after exposure to 5 μM CAL-101 for 24 h in HH and DERL7 cell lines (Figure 2E). These results confirmed that the inhibition of PI3Kδ alone was not sufficient to reduce PTCL cell survival, suggesting that different PI3K isoforms may share redundant functions.

Effects of the pharmacological inhibition of all phosphatidylinositol-3-kinase isoforms on peripheral T-cell lymphoma survival

In order to inhibit all class I PI3K isoforms, six PTCL and CTCL cell lines were treated with three pharmacological PI3Ki (LY294002, ETP-45658 and GDC-0941). Although these drugs have different specificities (LY294002 is a dual
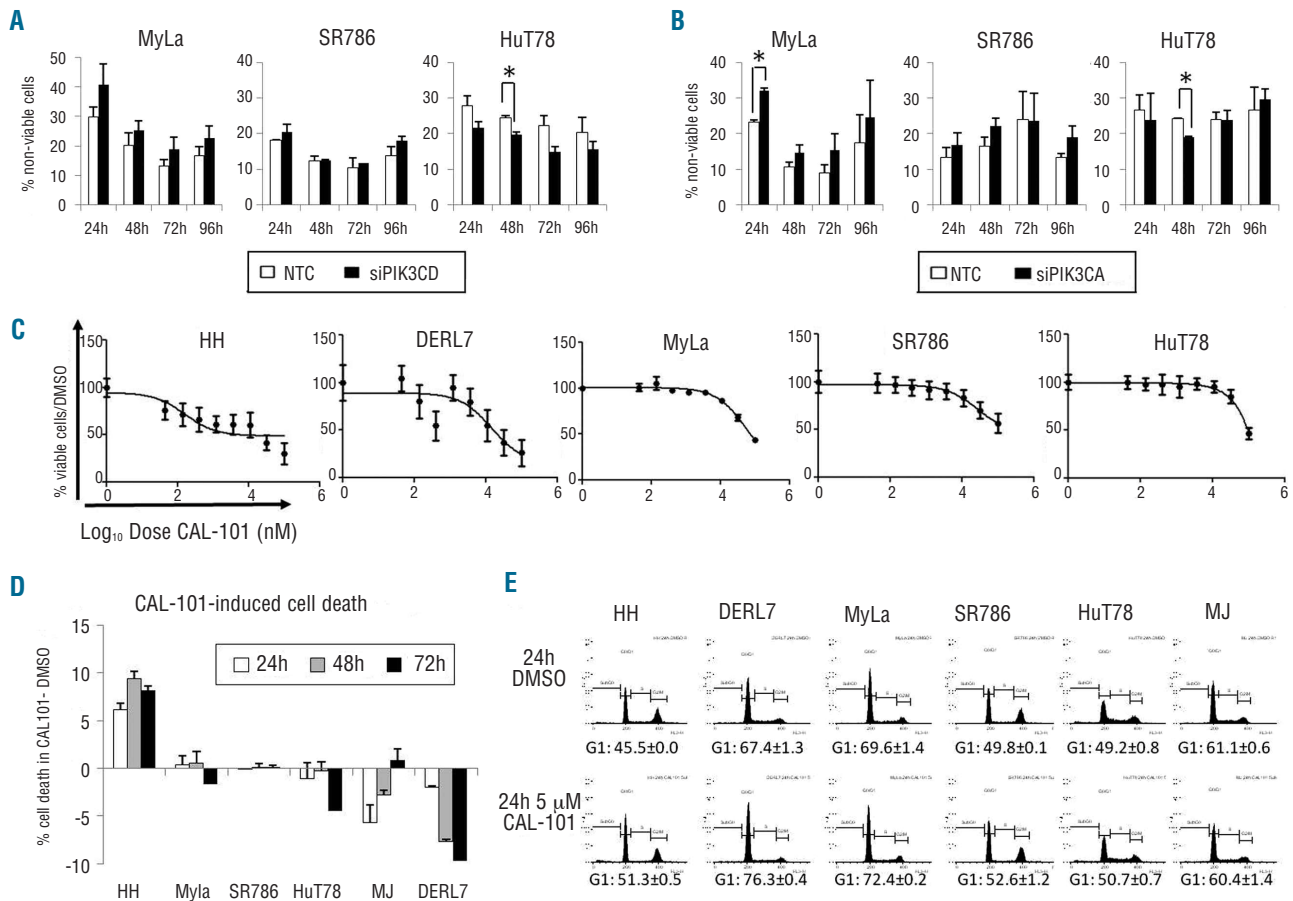
Figure 2. Effects of a single PI3K isoform inhibition on PTCL and CTCL cell line survival. (A) *PIK3CD* knockdown by siRNA slightly, but not significantly, increased induction of apoptosis in MyLa and SR786 cell lines, while no effects were observed in HuT78. * indicates a statistically significant difference (*P*<0.05). (B) A slight, but not significant, increase in apoptosis was found in MyLa and SR786, but not in HuT78, when *PIK3CA* was knocked down. (C) The specific pharmacological PI3Kδ inhibitor CAL-101 was only able to reduce PTCL cell line viability at high doses. (D) Most of the PTCL cell lines did not undergo apoptosis upon treatment with CAL-101. The Y axis indicates the percentage of annexin V$^+$/propidium iodide$^-$ plus annexin V$^+$/propidium iodide$^+$ cells in the treatment with 10 μM CAL-101 minus the cell death in DMSO. (E) After exposure to 5 μM CAL-101 for 24 h most of the PTCL cell lines were not arrested in any cell cycle phase; only HH and DERL7 were slightly arrested at G1.

PI3K/mTOR inhibitor and the other two are specific pan-PI3Ki[10,17]), all of them had similar effects on PTCL cell line survival (*Online Supplementary Figure S2*). We found that GDC-0941 was able to reduce cell viability in all cell lines in a dose-dependent manner, but the effect was stronger in some cell lines than in others, such as HuT78 and MJ, which were considered to be resistant (Figure 3A). Furthermore, GDC-0941 promoted cell cycle arrest at the G0/G1 phase in all PTCL cell lines, being stronger in sensitive cells lines than in resistant ones (Figure 3B). These differential responses were confirmed at the level of apoptosis induction: only the sensitive cell lines underwent slight apoptosis upon treatment with pan-PI3Ki (Figure 3C), while the resistant cell lines HuT78 and MJ did not. These results indicate that the pharmacological inhibition of all PI3K isoforms was more effective than isoform-specific inhibition.

Biomarkers for pan-phosphatidylinositol-3-kinase inhibitors in peripheral T-cell lymphoma

In order to test the activity of the pan-PI3Ki in PTCL cell lines, the phosphorylation status of several proteins belonging to the PI3K pathway was examined following GDC-0941 treatment. First, we found by flow cytometry that AKT phosphorylation, the most direct target of PI3K,[6] was reduced overall in all cell lines after 4 h of treatment with GDC-0941 (Figure 4A), confirming that the drug was indeed inhibiting PI3K even in the resistant cell line HuT78, although the reduction was not very substantial. Next, we studied other downstream proteins and observed that GSK3β and p70S6K phosphorylation was reduced in the sensitive cell lines, but not in the resistant ones, even after high-dose treatment with GDC-0941 (Figure 4B). These data suggest that pGSK3β and p-p70S6K could act as biomarkers for the response to PI3Ki in PTCL cell lines.

Cooperation between phosphatidylinositol-3-kinase and ERK pathways in peripheral T-cell lymphoma

Although the pharmacological pan-PI3Ki strategy seemed to be more effective than the isoform-specific inhibitor approach, the effects were not very dramatic. In order to determine whether another cellular pathway could compensate for the reduced PI3K activity and sus-
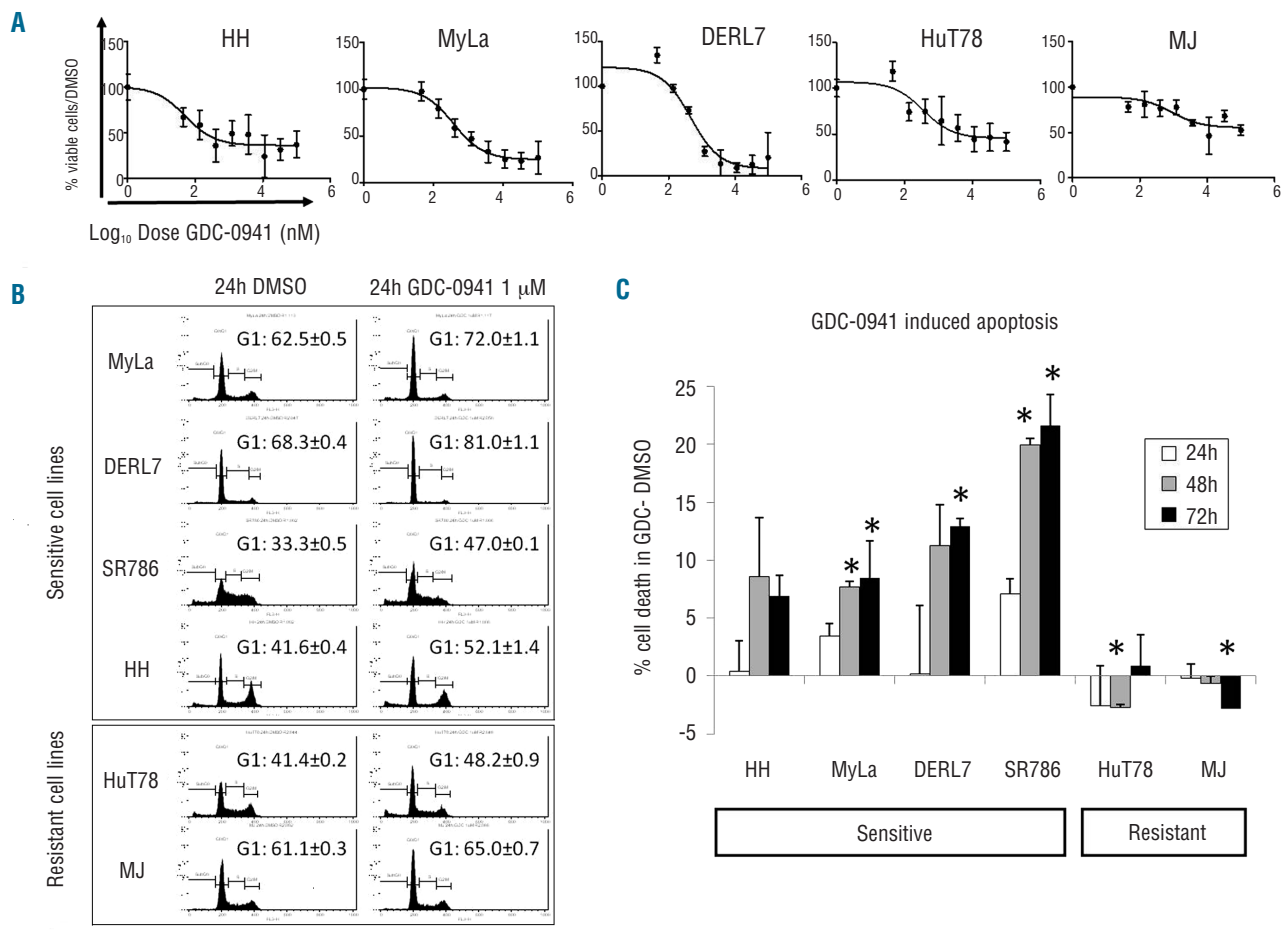
Figure 3. Effects of the pharmacological pan-PI3Ki GDC-0941 on PTCL and CTCL cell line survival. (A) GDC-0941 reduced PTCL cell viability after 72 h treatment in all cell lines, but HuT78 and MJ cell lines were more resistant to the PI3Ki. (B) GDC-0941 promoted cell cycle arrest at the G1 phase in all studied PTCL cell lines. The arrest was stronger in sensitive cell lines than in resistant ones. (C) GDC-0941 significantly induced apoptosis in sensitive cell lines, but not in resistant ones. The Y axis indicates the percentage of annexin V^+/propidium iodide$^-$ plus annexin V^+/propidium iodide$^+$ cells following treatment with 1 μM GDC-0941 minus the cell death in DMSO. *Indicates a statistically significant difference compared with DMSO ($P<0.05$).

tain PTCL survival, we studied the phosphorylation status of ERK, because the Ras/Raf/MEK/ERK pathway is one of the most essential pathways in cell survival.[18] Following treatment with GDC-0941 for 24 h, strong ERK activation was observed in the MyLa and SR786 cell lines, but not in HuT78 (Figure 5A). This result could explain the marginal effects on cell survival we had observed when PI3K was pharmacologically or genetically inhibited. The differential behavior of HuT78 could be explained by its resistance to PI3Ki: perhaps the ERK pathway did not need to be activated in this cell line in the presence of GDC-0941, because even under these conditions the downstream members of PI3K were still activated, sustaining cell survival (Figure 4B). More importantly, these results could support the use of a combined pan-PI3Ki + MEKi treatment in PTCL.

PTCL and CTCL cell lines were, therefore, treated with the pan-PI3Ki GDC-0941 plus the MEKi UO126 for 72 h. We found that the combination was highly synergistic (CI <1) in four of the six PTCL cell lines at doses equivalent to IC$_{50}$ values (Figure 5B). On the other hand, we observed a slight antagonism (CI >1) in the HuT78 cell line. Moreover, the combination enhanced the cell cycle arrest at G0/G1 in all PTCL cell lines (Figure 5C). In contrast, overall, the drug combination did not increase the apoptosis induced by each drug alone (*Online Supplementary Figure S3*). This is probably due to the levels of pAKT and pERK, which remained even in the presence of the drug combination (*Online Supplementary Figure S4*). Importantly, simultaneous PI3K and MEK inhibition significantly reduced cell viability in primary tumor T cells *ex vivo*; at higher doses, the drug combination had a stronger effect than the pan-PI3Ki alone (Figure 5D).

These results suggest that combined treatment with pan-PI3Ki + MEKi could be more effective than treatment with a PI3Ki alone and that the combination could be of therapeutic value in the treatment of PTCL and CTCL.

Discussion

Here we hypothesized that PI3K inhibition, especially inhibition of the delta isoform, could be of therapeutic

value in PTCL because: (i) our *in silico* approach identified PI3K pathway inhibitors as drugs that potentially reverse the PTCL molecular signature; (ii) PI3Kδ has been described to play a role in the differentiation, survival and activation of normal T cells, and so is probably also important for T-cell transformation;[19-21] (iii) *PIK3CD* was the only isoform to be significantly correlated with survival pathways in this PTCL signature; and (iv) we found that *PIK3CD* was much more overexpressed than *PIK3CA* in PTCL and CTCL cell lines and in Sézary's syndrome primary T cells.

However, our results indicated that the genetic or specific pharmacological inhibition of PI3Kδ had no effects on the survival of PTCL cell lines. CAL-101 is a PI3Kδ inhibitor that has been reported to have very promising preclinical activity in chronic lymphocytic leukemia (either alone[22,23] or in combination with other agents[24]) and very recently, also in Hodgkin's lymphoma.[25] The initial clinical data in phase I/II studies were most impressive in chronic lymphocytic leukemia, and have created some excitement about PI3K drug development. Nevertheless, some controversy exists: leukemia and lymphomas generally express all class I PI3K isoforms and rarely carry mutations in *PI3K* genes or have *PTEN* loss. In addition, the first set of preclinical studies with CAL-101 did not clearly predict the dramatic efficacy of the drug in clinical trials: cytotoxic effects on human chronic lymphocytic leukemia samples *in vitro* were generally achieved only at high CAL-101 doses (10 μM). These results suggest that selective PI3Kδ inhibition has a minimal impact on cell-intrinsic survival signals in B-lineage cancer cells, where it seems to act mainly by perturbing the signals received from the tumor microenvironment.[26,27]

Moreover, even in cancers that seem to be specifically reliant on either PI3Kα or β, there is the concern that other non-targeted PI3K isoforms might eventually compensate for the decreased activity of the targeted isoform.[6] It has been described that removal of more than 90% of p85-associated PI3K activity (α, β and δ isoforms) did not compromise the proliferation in hematopoietic progenitor cells.[28] These data could explain the absence of effect that we observed when a single PI3K isoform was inhibited in PTCL cell lines, because our knockdown efficiency was 80% at the mRNA level, and thus, more than 10% of the protein was still active, along with the other intact isoforms, allowing the survival of PTCL cell lines.

Accordingly, a stronger effect was found with the pan-PI3Ki GDC-0941. All cell lines were arrested at the G0/G1 phase and a subset of them underwent apoptosis. Overall, the response was mainly cytostatic, as widely reported.[6,28,29] Almost complete class IA inhibition is required to produce cell death, but such full inhibition of PI3K activity might be difficult to achieve with ATP-competitive inhibitors. This could partially explain why PI3Ki do not induce apoptosis but rather cytostasis.[28] However, this outcome could also be explained by alternative mechanisms of activation of downstream effectors through feedback loops,[4] especially because of the inconsistent reduction of pAKT we observed after GDC-0941 treatment, which might also suggest an AKT-independent mechanism of action. PI3K pathway members are able to interact very actively with a number of proteins, creating a very complex network.[30] Cooperation between the PI3K/AKT and the Ras/Raf/MEK/ERK pathways has been extensively described in many types of cell types.[6,28,31] Interestingly, we
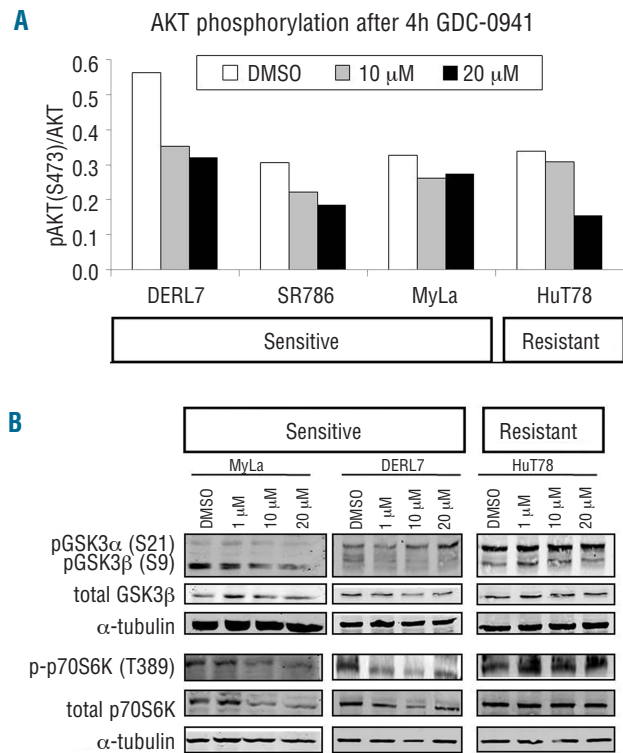

Figure 4. PI3Ki activity and response biomarkers in PTCL and CTCL cell lines. (A) GDC-0941 diminished AKT phosphorylation in a dose-dependent manner in all cell lines after 4 h of treatment. (B) GSK3β and p70S6K phosphorylation was reduced only in sensitive cell lines after 4 h exposure to GDC-0941, but not in resistant cell lines.

found that ERK phosphorylation was increased upon PI3Ki treatment in PTCL cell lines, probably as a part of a compensatory up-regulation of interconnected pathways. More importantly, the combination of PI3Ki + MEKi was highly synergistic overall (as reported already[6,28,31]) *in vivo* and *ex vivo*, enhancing the cytostatic effect although without induction of apoptosis.

Unfortunately, the biological basis of the poor outcome in patients with PTCL, in contrast to that of patients with aggressive B-cell lymphomas, is not well understood.[3] Biomarkers that predict the response to a given therapy are, therefore, needed. Very recently, a study in primary PTCL cases highlighted the clinical relevance of pAKT, which was negatively correlated with the response rate and survival,[32] thus supporting the use of PI3Ki in PTCL therapy. Here, we present preclinical evidence suggesting that PI3Ki could be potential therapeutic agents for the treatment of PTCL. Furthermore, we found different sensitivities among the PTCL cell lines and observed that phosphorylation of GSK3β and p70S6K was only reduced in the most sensitive cell lines, while remaining unchanged in the resistant cell lines. This result suggests that these phospho-proteins could act as potential pharmacodynamic biomarkers for the response to PI3Ki in PTCL.

In addition, we looked for biomarkers that could distinguish potential responders before treatment, such as mutations in some parts of the PI3K pathway. Although mutations in *PIK3CA*, *AKT1* and *PTEN* have been detected in

A

B

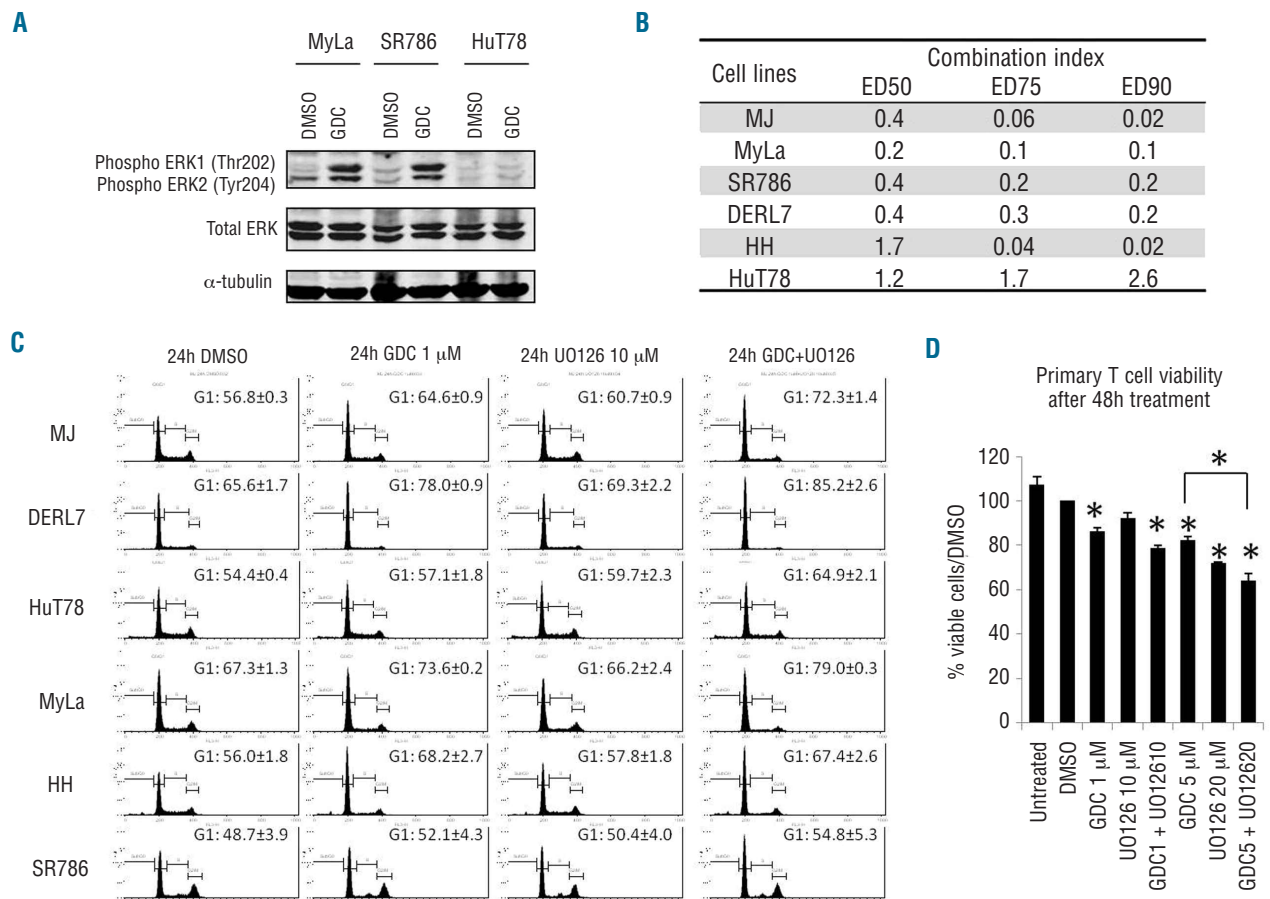| Cell lines	Combination index		
	ED50	ED75	ED90
MJ	0.4	0.06	0.02
MyLa	0.2	0.1	0.1
SR786	0.4	0.2	0.2
DERL7	0.4	0.3	0.2
HH	1.7	0.04	0.02
HuT78	1.2	1.7	2.6

C

D

Figure 5. Cooperation between PI3K and ERK pathways in PTCL and CTCL *in vitro* and *ex vivo*. (A) ERK phosphorylation was increased in MyLa and SR786 cell lines after 24 h of treatment with GDC-0941 10 μM. (B) The combination index (CI) was calculated for different doses of the combined pan-PI3Ki (GDC-0941) + MEKi (UO126): CI <1 indicates synergism between the two drugs, CI ≈1 indicates an additive effect, CI >1 indicates antagonism. (C) The combination enhanced cell cycle arrest at G1 in all PTCL cell lines when compared with single treatments. (D) The PI3Ki + MEKi combination significantly reduced cell viability in primary tumor T cells from eight CTCL patients (5 with Sézary's syndrome and 3 with mycosis fungoides). *indicates statistically significant differences compared with DMSO (*P*<0.05).

patients with leukemia, these mutations appear to be rare in hematologic neoplasms.[4,33] None of the known activating mutations in *PIK3CA*, *AKT1*, *KRAS* or *BRAF* or deletions in *PTEN* was found in any PTCL and CTCL cell line. Consistent with this, PTEN alterations were not observed at the genetic or protein levels in a series of cases of PTCL-not otherwise specified.[34] The only gene variant found in one of the six PTCL cell lines and two of the 27 PTCL cases was *PIK3CD*, which harbored two single nucleotide polymorphisms (c935g = rs61755420 and c2319t = rs139848768). The first polymorphism (c935g) might be somehow related to the incidence of anaplastic large cell lymphoma ALK[+], because it was present in the only anaplastic large cell lymphoma ALK[+] cell line available and in one of the three cases of anaplastic large cell lymphoma ALK[+]. Nevertheless, a case-control study reporting a significant association in a large series would be required to explore this possibility.

In conclusion, collectively these results suggest that the simultaneous inhibition of all PI3K isoforms, instead of specific PI3Kδ inhibition, could be a therapeutic strategy in a subset of PTCL and CTCL. More importantly, the cytostatic effects can be enhanced in combination with MEKi, and might improve the poor outcome in PTCL patients, who lack efficient therapies.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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