TKI258, a Multi-tyrosine Kinase Inhibitor Is Efficacious Against Human Infant/Childhood Lymphoblastic Leukemia *In Vitro*

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Abstract. *Background/Aim: The goal of the present study was to evaluate if the multiple tyrosine kinase inhibitor (TKI) TKI258 has any treatment value for infant/childhood acute lymphoblatic leukemia (ALL), especially those ALLs bearing the mixed lineage leukemia (MLL) genes. Materials and Methods: Cell proliferation was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; cell apoptosis and cell-cycle distribution with flow cytometry. Gene expression at the protein level was determined by western blotting. Results: These ALL cells were extremely sensitive to TKI258 treatment with a concentration for 50% inhibition of cell proliferation (IC_{50}) values in the nanomolar range in vitro. By combination with mTOR inhibitor RAD001, a synergistic effect on cell death and cell proliferation was observed in these cells. Conclusion: TKI258 may become a potent therapeutic agent, either alone or in combination with RAD001, for treatment of ALL, especially the entity with MLL genes.*

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood, which represents 34% of all cancers in this age group with a peak incidence at 2 to 5 years of age. ALL can be now successfully treated in the great majority of patients by the application of intensive combination chemotherapy regimens (1). Unfortunately, certain clinically- and biologically-distinct patient sub-groups with ALL have a particularly poor outcome with standard ALL treatment. One of such sub-groups is infant ALL patients (*i.e.* children <1 year of age). Due to the high relapse rate, difficulty to carry out the stem cell transplantation and therapy-related mortality, the overall survival rate remains a dismal 30-50% (2). Infant ALL is characterized by an exceptionally high incidence of leukemia-specific rearrangements involving the mixed lineage leukemia (*MLL, ALL-1* or *HRX*) gene on chromosome 11q23, which occur in about 80% of the cases (3). These chromosomal abnormalities, which were believed to be the initiating events in leukemogenesis, usually involve reciprocal translocations fusing the N-terminal portion of the *MLL* gene to the C-terminal region of one of its translocation partner genes. Such fusion genes encode chimeric transcripts that give rise to oncogenic fusion proteins with pronounced transforming potential. So far the most common *MLL* translocations found in infant ALL patients are t(4;11), t(11;19) and t(9;11), fusing MLL to the transcription factors AF4, ENL and AF9, respectively. Among several prognostic factors identified in infant ALL, the presence of *MLL*-rearrangement seems to be the most important independent predictor of an adverse outcome (4, 5). Micro-array studies demonstrated that *MLL*-rearranged leukemia displays characteristic gene expression profiles that distinguish them from other childhood ALL subtypes. When gene expression in *MLL*-rearranged leukemia cells was compared to other acute leukemia, the tyrosine kinase FMS-like tyrosine kinase 3 (Flt3) was found consistently highly expressed. This resulted from either activating mutations (6, 7) or overexpression (8, 9) of this kinase gene. High-level Flt3 expression renders high response of this type of ALL to Flt3 inhibitors. These data showed that Flt3 inhibition may represent a novel targeted-therapeutic strategy for infant *MLL*-rearranged ALL.

It is clear that new tyrosine kinase inhibitors (TKI), especially those that inhibit multiple tyrosine kinases, hold promise the in treatment of leukemias with aberrant tyrosine kinase activities. However, as we have learned from imatinib resistance, use of TKI inhibitors as a single-agent may lead to the quick emergence of drug resistance. Simultaneous

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targeting of multiple aberrantly regulated signal transduction pathways is a promising therapeutic strategy to enhance the treatment efficacy and overcome drug resistance. Aberrant tyrosine kinases activate several downstream pathways including the mammalian target of rapamycin (mTOR) signaling. MTOR is a central regulator of cell growth and proliferation. This signaling is frequently dysregulated in most of tumors, including infant ALL, indicating that the integration of mTOR inhibitor in the treatment regime would provide an attractive therapeutic strategy in leukemia treatment.

TKI258 (dovitinib®) is an investigational new drug developed by Novartis that inhibits multiple receptor tyrosine kinases (RTKs) involved in tumor growth as well as tumor angiogenesis of solid and hematological cancers. TKI258 has a great potency as an inhibitor of class III/IV/V RTKs associated with vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), stem cell factor (c-KIT), Flt3 and colony-stimulating factor-1 (CSF-1) (10) with concentration for 50% inhibition of cell proliferation (IC_{50}) values of approximately 10 nM. Due to its super activity and oral availability, TKI258 is now in phase III development for renal cell carcinoma, in phase II development in advanced breast cancer, relapsed multiple myeloma and urothelial cancer.

Based on the tyrosine kinase profiles that are inhibited by TKI258 and the published data available so far, we expected that this agent would be effective against infant *MLL*-rearranged ALLs with dysregulated Flt3 activity. In addition, in this study we tested the possibility to intensify the treatment effect of TKI258 by combining it with the mTOR inhibitor RAD001 to evaluate if this combination had the potential to become a new treatment regimen for this leukemia entity.

Materials and Methods

Cell lines, reagents and cell cultures. Human ALL cell lines Nalm-6, CCRF-CEM, CEM/C2 were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA) and maintained in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (PS). Cells in logarithmic growth phase were used for experiments. Human *MLL*-rearranged ALL cell lines SEM-K2, HB-1119 and RS4:11 were kindly provided by Dr. P. Brown from the Department of Oncology and Pediatrics, Johns Hopkins University, Baltimore, USA and maintained in the same media. The characteristics of cell lines used in this study are summarized in Table I.

TKI258 and everolimus (RAD001) were kindly provided by Novartis Institutes for Biomedical Research (Basel, Switzerland). A 10 mM stock solution for RAD001 and 1mM for TKI258 were prepared in dimethyl sulfoxide (DMSO) (Sigma–Aldrich, Munich, Germany), stored at −20˚C and diluted with fresh culture medium immediately before use.

Determination of cell proliferation by 3,[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. Different leukemic cells were seeded into a 96-well plate at a density of 5×10^3 cells per well and exposed to different concentrations of TKI258, with or without RAD001, in culture medium. After incubation for indicated time points, cell proliferation was carried out as previously described (11).

Cell-cycle analysis. For cell-cycle analysis, cells were exposed to different concentrations of TKI258, with or without RAD001, in growth medium. After different culture durations, cells were harvested and fixed in 70% ethanol at 4˚C for over 30 min. After incubation for indicated time points, DNA contents were stained and cell cycle distribution was carried out as previously described (11).

Apoptosis analysis. Cell apoptosis was detected by determining phosphatidylserine expression on the cell surface with the Annexin V kit (BD Biosciences, Heidelberg, Germany) by using flow cytometry according to the instructions of the manufacturers.

Western blot analysis. Protein concentrations of whole-cell lysates were measured using a BCA protein assay kit (Pierce, Bonn, Germany). Western blot analysis was performed as described previously (11). Antibodies against p-Flt3 was from Cell Signaling Technology (New England Biolabs, Frankfurt am Main, Germany); Antibodies against c-Myc, Mcl-1 and β-Actin were from Santa Cruz (Heidelberg, Germany).

Statistical analysis. Numerical data were reported as mean±standard deviation (SD) unless otherwise specified. All data represent the results of at least three independent experiments. Synergistic, additive effects on cell proliferation by the combination of TKI258 and RAD001 as well as the IC_{50} values were assessed using the Chou-Talalay method (12) and CalcuSyn software (Biosoft, Ferguson, MO, USA) as described previously (11).

Results

The multi-tyrosine kinase inhibitor TKI258 inhibited cell proliferation of ALL cell lines with MLL rearrangement. Different human ALL cell lines, including the B-ALL cell line Nalm-6, CCRF-CEM and its chemotherapy-resistant counterpart cell line CEM/C2, as well as infant ALL cell lines with *MLL* translocation gene (SEM-K2, HB-1119, RS4:11) were exposed to different concentrations of TKI258 (Table I). Among these cell lines, TKI258 moderately inhibited the proliferation of ALL cell lines, Nalm-6 and CCRF-CEM with IC_{50} at about 0.4 μM. It seems that the chemotherapy-resistant cell line CEM/C2 was relatively resistant to TKI258 treatment (IC_{50}=1.125 μM). All these cell lines above mentioned express very low level of Flt3 (data not shown). In sharp contrast, infant ALL cell lines SEM-K2 and HB-1119 that have constitutively activated Flt3 caused by *MLL*-rearrangements were extremely sensitive to the TKI258 treatment (IC_{50}=22 nM and 28 nM respectively). The infant ALL cell line RS4:11 with rearrangements of the *MLL* gene but wild-type Flt3 (13) showed relative resistance to TKI258 with IC_{50} at about 3 μM (Table I).

Table I. *Growth inhibitory profile of TKI258.*

Cell line	Characteristics	IC_{50} (TKI258, μM)
CCRF-CEM	Human T-ALL	0.398
CEM/C2	Camptothecin resistant derivative of CCRF-CEM and cross resistant to etoposide, dactinomycin, bleomycin, mitoxantrone, 4'-(9-acridinylamino) methanesulfon-m-anisidide, and the anthracyclines daunorubicin and doxorubicin.	1.125
Nalm-6	Human pre-B ALL	0.382
SEM-K2	Infant Pro-B ALL with a t(4;11) translocation (resulting in the MLL-AF4 fusion protein)	0.022
HB-1119	Infant Pre-B ALL with a t(11;19) translocation (resulting in the MLL-ENL fusion protein)	0.028
RS4:11	Adult ALL with a t(4;11)/MLL-AF4 fusion protein	2.81

Different human ALL cells were seeded into 96-well plates at a density of 5×10^3 cells per well and were exposed to different concentrations of TKI258 in growth medium. After a 5-day incubation, cell proliferation was determined with MTT assay. The concentrations necessary to induce a 50% decrease in the number of various cells (IC_{50}) was calculated by the CalsuSyn software.

TKI258 inhibited cell proliferation of ALL cells by inducing apoptosis and G_1 phase cell cycle arrest. To investigate whether the decreased cell proliferation was caused by cell death, cell apoptosis was detected by measuring the Annexin V-FITC/PI-staining with flow cytometry. TKI258 induced significant apoptosis in all tested ALL cell lines after 24 h or 48 h treatment (Figure 1A, 1B). In cell line Nalm-6, 2 μM TK1258 treatment induced apoptosis resulting in about 72% of cell death after 24 h treatment and 81% after 48 h (Figure 1A). The apoptosis-inducing effect of TKI258 was also observed in *MLL*-rearranged ALL cell lines in a concentration-dependent manner. As a representative result, it was shown here that TKI258 induced early apoptosis of SEM-K2 cells at 0.1 μM after 24 h. At 1 μM, almost all of SEM-K2 cells were apoptotic with more than 70% cells in early apoptosis and others in late apoptosis (Figure 1B). A significant apoptosis was also observed in RS4:11 cells when higher concentrations (about 2 μM) of TKI258 were employed (data not shown). In addition to apoptosis, TKI258 treatment also induced G_0/G_1 cell cycle arrest with concomitant reduction of S and G_2/M phases in a dose-dependent manner. In TKI258-sensitive cell line SEM-K2, 10 nM of TKI258 were enough to induce significant G_1 phase arrest after 48 h (Figure 1C), whereas in chemotherapy-resistant CEM/C2 cells, a higher dose of TKI258 (1 μM) was required for reaching the similar effect (Figure 1D).

Combination of TKI258 and mTOR inhibitor RAD001 at low concentrations resulted in a synergistic effect on cell proliferation and apoptosis. The phosphatidylinositol 3'–kinase (PI3K)/protein kinase B (PKB/Akt)/mammalian target of rapamycin (mTOR) pathway is a crucial downstream signaling of different RTKs. We believe that simultaneously targeting this pathway will enhance the cytotoxic effect of TKI258 and overcome drug resistance. To evaluate this hypothesis, we investigated the combination effect of TKI258 and mTOR inhibitor RAD001 on cell proliferation and apoptosis of ALL cells. Figure 2 shows that RAD001 dramatically enhanced the cytotoxic effect of low doses of TKI258 (0.5 μM and 1 μM) on the chemotherapy-resistant ALL cell line CEM/C2 (Figure 2A). Cell proliferation rate was reduced to 42% of the control for the CEM/C2 cell line when 0.5 μM of TKI258 was combined with 10 nM of RAD001. For reaching the same inhibitory effects as with the combination treatment regimen, about 3- to 4-fold higher concentrations of TKI258-alone were required. These combination effects were not only observed in cells that were relatively insensitive to TKI258 treatment, but also in *MLL*-rearranged and TKI258-sensitive ALL cell lines SEM-K2 and HB-1119. Combination effects with 10 nM RAD001 were noticed even in lower dose ranges of TKI258 (0.05-1 μM for SEM-K2 and HB-1119 cell lines and data not shown) (Figure 2B-C).

Synergistic inhibitory effects of TKI258 and RAD001 on cell proliferation were further demonstrated by Combination Index (CI) values for 50%, 75% or 90% of cell proliferation inhibition, which were calculated based on the experimental data with the CalsuSyn software (Table II). The synergistic effect was shown as CI<1.0 for 50% and 75% proliferation inhibition. However, almost no combination effects were noticed at higher doses for reaching 90% of proliferation inhibition (Table II) since TKI258-alone already induced significant cell growth arrest and apoptosis at these doses as showed above.

By measuring cell apoptosis with Annexin V/PI staining, significant apoptosis induction was achieved by even low dose of TKI258 in combination with RAD001 in the tested cell lines (Figure 2D). These results together indicated that mTOR inhibitors could synergistically enhance the treatment efficacy of TKI258 on ALLs.

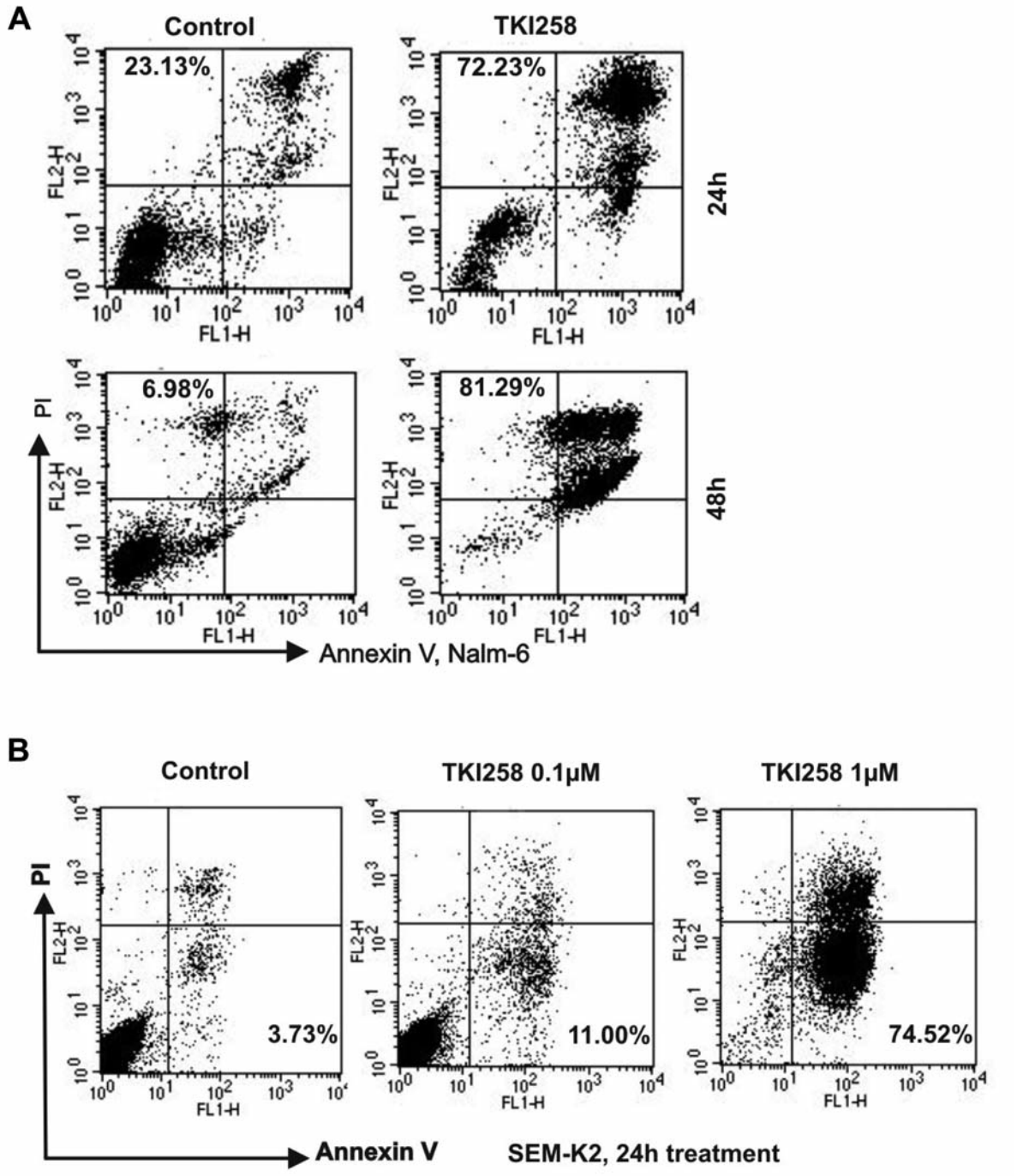

Figure 1. *continued*

Inhibition of oncoproteins by TKI258 or TKI258 in combination with RAD001 in MLL-rearranged ALL. The constitutively activated Flt3 is one of the characteristics of *MLL*-rearranged ALL which distinguish this entity from other ALLs. Targeting Flt3 signaling can be a new approach for developing new drugs specific for this type of ALL. The cell lines SEM-K2 and HB-1119, harboring activating mutations of the *Flt3* gene, were extremely sensitive to TKI258 whereas RS4:11 with wild-type *Flt3* and moderate Flt3 activity (13) were only moderately sensitive to TKI258. It seems that Flt3 is a direct target of TKI258 in *MLL*-rearranged ALL. Western blot analysis showed the dramatically reduced phosphorylation of *Flt3* with the TKI258 treatment at increased concentrations in SEM-K2 and RS4:11 cell lines (Figure 3A). In good

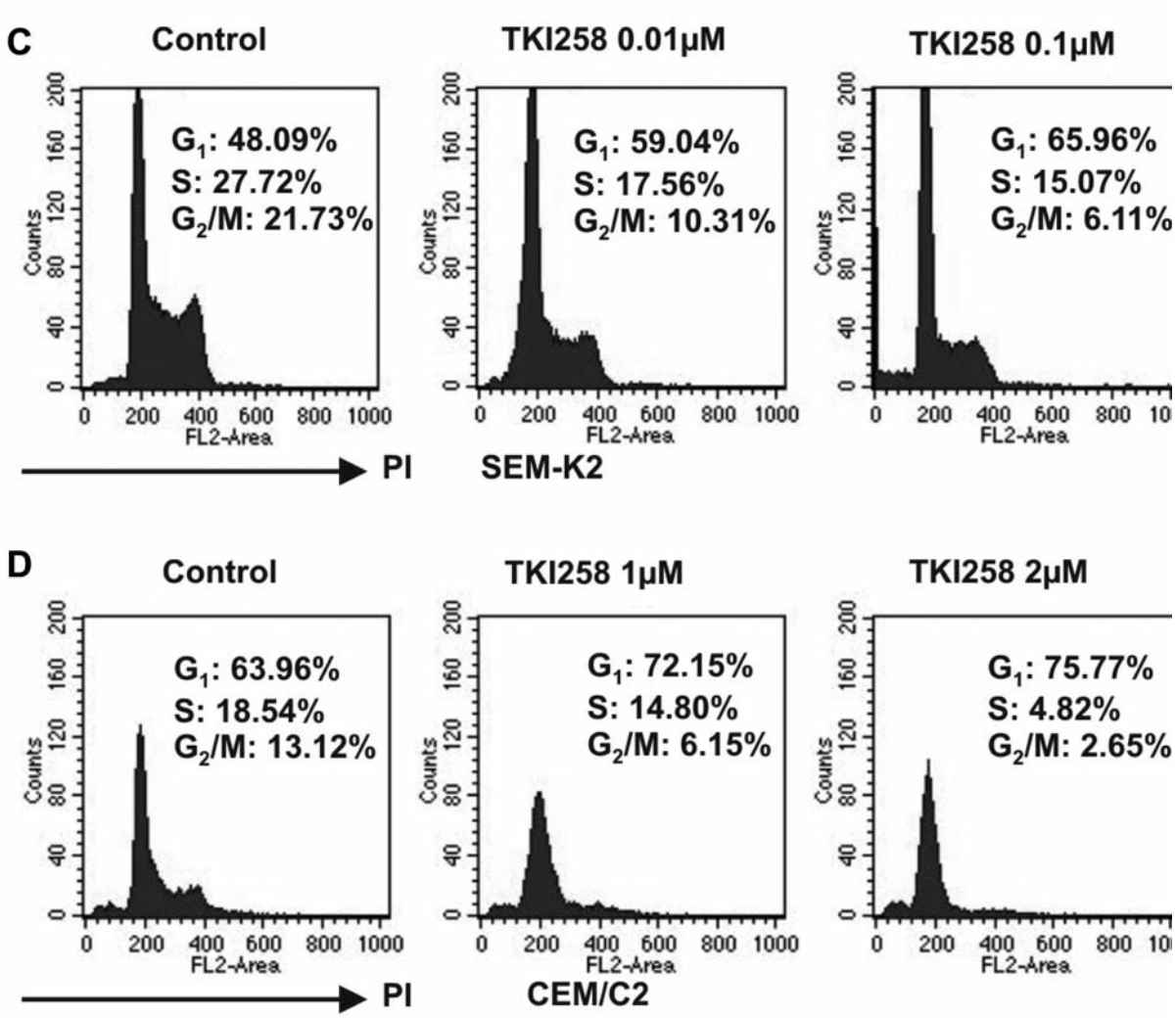

Figure 1. *TKI258 inhibited cell proliferation of ALL cells by inducing apoptosis and G_1 phase to S phase arrest of cell cycle. A-B: Cells were cultured in the presence of TKI258 at the indicated concentrations for the indicated durations, respectively. Cell apoptosis was measured with Annexin V/PI-staining and flow cytometry as described in the Materials and Methods. (A): ALL cell line Nalm-6; (B): MLL-rearranged ALL cell line SEM-K2. C-D: Cells were incubated either in the presence or absence of TKI258 for 48 h, fixed in 70% ethanol, treated with RNAse and stained for measurement of DNA with PI. Cell-cycle distribution was determined by FACS analysis, as described in Materials and Methods. (C): SEM-K2; (D): CEM/C2.*

correlation with the sensitivity of these cells to TKI258, reduction of Flt3 phosphorylation in SEM-K2 cells was stronger and required lower concentrations of TK1248 than that in RS4:11 cells. Next, we performed western blot analysis to further investigate how the combination of TKI258 and RAD001 impact on the downstream signaling of Flt3. It is evidenced that c-Myc and Mcl-1 are two oncoproteins which are regulated by the mTOR signaling and are closely related to the leukemogenesis caused by *MLL*-rearrangement (4). Indeed, the combination of TKI258 and RAD001 resulted in decreases of c-Myc and Mcl-1 levels in SEM-K2 cells (Figure 3B) suggesting that c-Myc and Mcl-1 are possible targets of the combination treatment of TKI258 and RAD001.

Discussion

The infant ALL harboring MLL-rearrangements has very poor prognosis due to the low response rate and quick relapse after current chemotherapy protocols. In addition, the difficulties to carry-out the stem cell transplantation and the high therapy-related mortalities in infants make this disease even harder to treat. Novel therapeutic strategies that will hopefully result in better treatment outcomes are urgently required. Several genetic studies identified a high Flt3 activity in *MLL*-rearranged ALL due to either activating mutations or overexpression of this gene (6) suggesting that Flt3 may present a promising target for this ALL entity.

Table II. *CI-values (combination index) at effective doses (ED) for 50%, 75% and 90% proliferation inhibition of different cell lines.*

	CI Values		
Cell line	ED_{50}	ED_{75}	ED_{90}
SEM-K2	0.00686	0.286	1.242
HB-1119	0.115	0.377	1.230
RS4:11	0.344	0.542	1.068
CEM-C2	0.451	0.763	1.326

Cells of different MLL-rearranged ALL cell lines (SEM-K2, HB-1119, RS4:11) and the chemotherapy-resistant ALL cell line CEM/C2, were cultured in the presence of either TKI258 (0-5 μM) alone, RAD001 (0-100 nM) alone or the combination of both drugs for 3 days. After incubation, cell proliferation was determined by the MTT assay. The combination effect of the two agents on cell proliferation was analyzed with the CalsuSyn software. Presented are the representative values of three independent experiments.

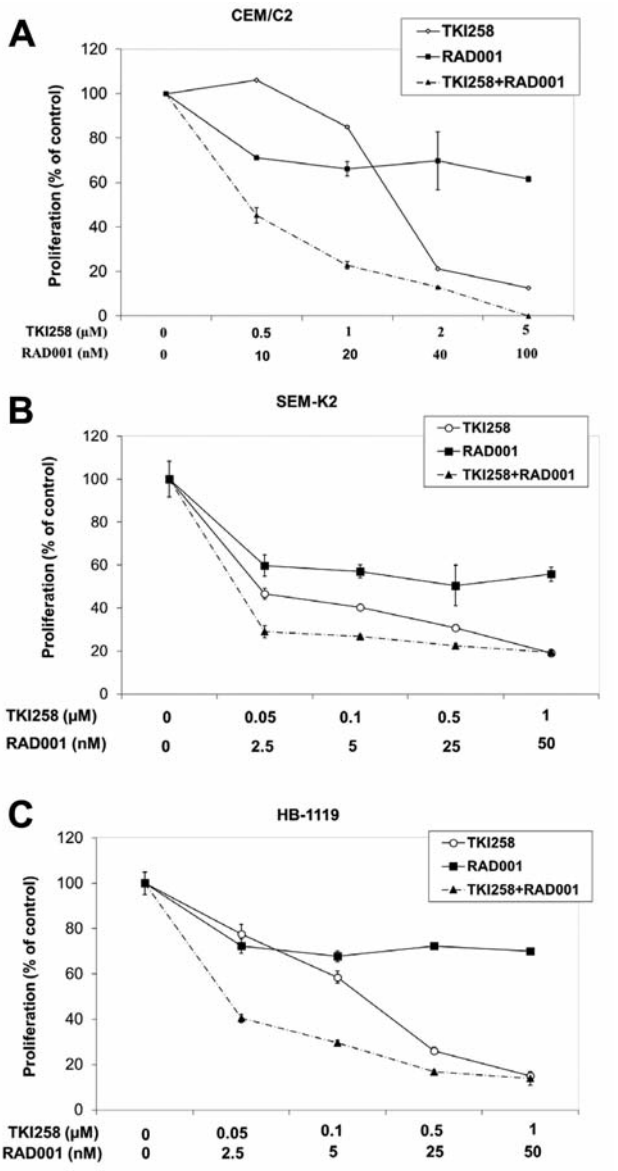

Figure 2. *continued*

TKI258, a multiple inhibitor of several RTKs including Flt3, may possess the potential to be a targeted therapy. We found in this study that TKI258 inhibited the growth of all of tested ALL cell lines in the low-dose range (IC_{50}<0.5 μM) by inducing G_1 cell-cycle arrest and apoptosis. Among these ALL cell lines, the infant ALL cell lines with *MLL*-rearrangement and highly activated Flt3 (HB-1119, SEM-K2) (13) were extremely sensitive to the TKI258 (IC_{50}<0.05 μM); in contrast, the infant ALL cell line RS4:11 with rearrangements of the *MLL* gene but wild-type of Flt3 (13) and the Nalm-6 cells, which express very low levels and not constitutively phosphorylated Flt3 (8), were relatively resistant to TKI258 treatment. There appears to be a strong correlation between cytotoxic response to TKI258 and constitutively activated Flt3 in ALL. In line with this, the same correlation was shown by Brown in the investigation of cytotoxic effects of the Flt3-specific inhibitor CEP-701 in infant and childhood ALL (8, 13). We further showed that TKI258 was able to inhibit the phosphorylated (activated) Flt3 directly in infant *MLL*-rearranged ALL. Again, this inhibitory effect was much stronger in cells with Flt3-activating mutations than cells with wild-type and moderate activity of Flt3. These data strongly suggest that Flt3 may be one of the direct targets of TKI258. Although this recognition was obtained using *MLL*-rearranged ALL, we can speculate that TKI258 would have strong activities in other Flt3-overexpressed leukemias.

Currently, a variety of Flt3-specific inhibitors and broad spectrum tyrsosine kinase inhibitors has been developed and are being tested in clinical trials for treatment of Flt3-expressing leukemia cells. The N-benzoylstaurosporine PKC412 (Novartis Pharma AG, Basel, Switzerland), for instance, is an orally bioavailable inhibitor of Flt3, PDGFR-β, c-KIT, and c-FMS and is being investigated in phase II trial in newly diagnosed acute myeloid leukemia (AML) with *Flt3*-mutation. The indolinone SU5416 (SUGEN; Pfizer, NY, USA), which inhibits kinases associated with Flt3, c-KIT, VEGFR1/2 and SCF, was demonstrated in a multicenter phase II clinical trial to inhibit phosphorylation of Flt3 in patients with refractory AML (14). The novel, orally-administered Flt3 inhibitor CEP-701 (Cephalon, Frazer, PA, USA) showed effectiveness against Flt3-ITD–expressing AML cells, and induced clinical responses of short duration in a phase I/II clinical trial in patients with relapsed or refractory disease (15). Although these clinical studies

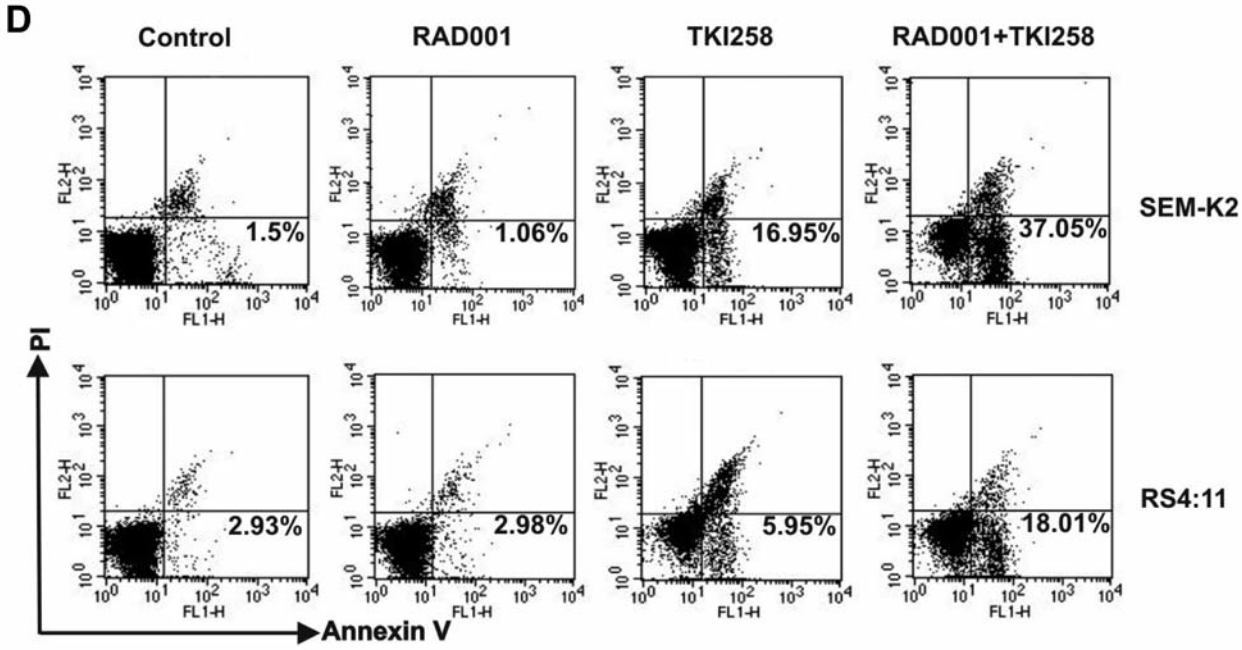

Figure 2. *Combination effect of TKI258 and mTOR inhibitor RAD001 at low concentrations on cell proliferation and cell apoptosis of ALL cells. A-C: Cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well and were exposed to different concentrations of TKI258 alone, RAD001 alone and TKI258 in combination RAD001 respectively. After a 3-day incubation, cell proliferation was determined by the MTT assay. Data were analyzed with the CalcuSyn software. (A): Chemotherapy-resistant ALL cell line CEM/C2; (B): SEM-K2; (C): HB-1119. D: SEM-K2 and RS4:11 cells were exposed to TKI258 (0.5 μM) alone, RAD001 (10 nM) alone and TKI258 in combination RAD001, respectively. After incubation for 24 h, cell apoptosis was determined with Annexin V/PI-staining and flow cytometry, as described in Materials and Methods.*

demonstrated somehow activities of these agents against Flt3-overexpressed leukemia, the efficacy was limited and lasted shortly when they were used as single agents (14, 16). A possible explanation for this limitation is perhaps the inefficiency of these inhibitors to hinder the downstream signaling of Flt3 or the inhibition is compensated by other survival pathways. To this regard, concomitant inhibition of Flt3 and its possible downstream signaling will enhance the cytotoxicity of these inhibitors. Chen *et al*. demonstrated that activating mutations of the Flt3 gene mediated leukemogenesis, at least in part, through activation of PI3K/AKT/mTOR (17). In their publication, mTOR effectors such as 4EBP1 and p70S6K were found highly phosphorylated in cultured and primary Flt3-mutated AML cells. Inhibition of the Flt3 kinase resulted in down-regulation of mTOR signaling associated with decreased survival of Flt3-mutated cells. These findings suggest that mTOR signaling operates downstream of the activated Flt3 kinase and makes an essential contribution to tumor cell survival. In our study, we observed the synergy of TKI258 and RAD001 only if TKI258 was used in low doses, and it seems that TKI258 alone at low doses was insufficient to block mTOR signaling downstream of Flt3. Concurrent inhibition of Flt3 and mTOR signaling resulted in both synergistically-

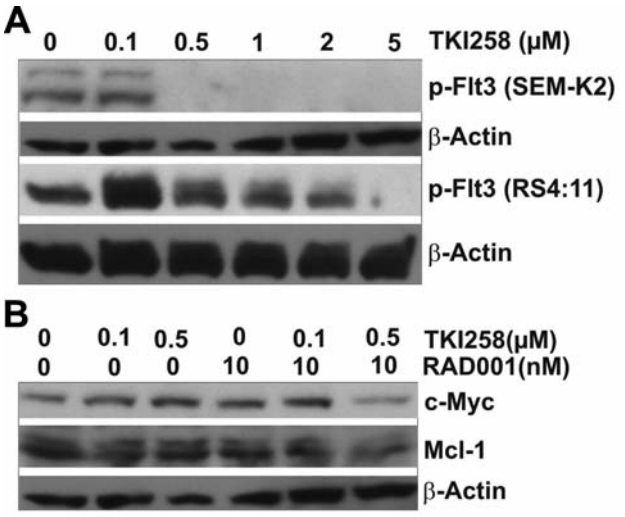

Figure 3. *Inhibition of oncoproteins by TKI258 or TKI258 in combination with RAD001 in MLL-rearranged ALL. A: MLL-rearranged ALL cells SEM-K2 were cultured in the presence of TKI258 (0.1, 0.5, 1, 2, 5 μM) for 24 h, respectively. Cells were lysed, p-Flt3 was determined by western blot. B: MLL-rearranged ALL cells SEM-K2 were cultured in the presence of TKI258 (0.1 μM, 0.5 μM), RAD001 (10 nM) or the combination of the two drugs for 24 h, respectively. Cells were lysed and c-Myc as well as Mcl-1 were determined by western blot.*

increased cell proliferation inhibition and cell apoptosis, as well as an additional reduction of phosphorylation of mTOR effector 4EBP1. Therefore, in the presence of mTOR inhibitors, the doses of TKI258 can be significantly reduced without sacrificing the inhibitory effects on these leukemias. This combination strategy is especially attractive for therapeutic applications because it not only enhances the efficacy of TKI258 but could also reduce the possible TKI258-associated side-effects. Thus, this new treatment concept deserves further pre-clinical and clinical investigation.

Several oncoproteins are controlled by mTOR signalling including Myc and Mcl-1, two molecules that are frequently up-regulated and involved in survival and therapy-resistance in *MLL*-associated leukemia (4, 18, 19). By immunoblotting analysis we demonstrated that expression of these two proteins was significantly reduced by the combination of TKI258 and RAD001 but not by each drug alone in SEM-K2 cells. This result suggests that the intensified inhibition of these two oncoproteins maybe responsible for, at least in part, the synergistic effects of the two drugs on cell proliferation and cell apoptosis.

In addition to its effects in MLL-rearranged ALL, TKI258 also demonstrated activity on growth inhibition and apoptosis in childhood ALL cell lines, CCRF-CEM and Nalm-6 with almost equal efficiency, suggesting that this agent will have the potential to be integrated into the treatment regimen for childhood ALL. Interestingly, CEM/C2 cells, which are resistant to most of chemotherapeutics, responded to TKI258 treatment, albeit to a lesser extent as compared to their naïve counterpart CCRF-CEM cells.

In conclusion, our data strongly demonstrated that TKI258 induces growth arrest and apoptosis of infant/childhood ALLs, especially *MLL*-rearranged ALLs. Notably, the infant MLL-rearranged ALLs with *Flt3*-activating mutations are extremely sensitive to TKI258. Finally, of more clinical importance, addition of the clinically-approved RAD001 synergistically enhances the cytotoxic effects of TKI258 in *MLL*-rearranged ALLs. Overall, our data suggest that TKI258, either alone or with mTOR inhibitors, could represent a novel targeted-therapy for infant/child ALLs, particularly ALLs with MLL rearrangement.

Conflicts of Interest

The Authors declare no conflicts of interests.

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