Genomic Alterations of Anaplastic Lymphoma Kinase May Sensitize Tumors to Anaplastic Lymphoma Kinase Inhibitors

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Abstract

Selective kinase inhibitors have had a substantial impact on the field of medical oncology. Whereas these agents can elicit dramatic clinical responses in some settings, their activity is generally limited to a subset of treated patients whose tumor cells harbor a specific genetic lesion. We have established an automated platform for examining the sensitivity to various molecularly targeted inhibitors across a large panel of human tumor-derived cell lines to identify additional genotype-correlated responses that may be clinically relevant. Among the inhibitors tested in a panel of 602 cell lines derived from a variety of human cancers, we found that a selective inhibitor of the anaplastic lymphoma kinase (ALK) potently suppressed growth of a small subset of tumor cells. This subset included lines derived from anaplastic large cell lymphomas, non–small-cell lung cancers, and neuroblastomas. ALK is a receptor tyrosine kinase that was first identified as part of a protein fusion derived from a chromosomal translocation detected in the majority of anaplastic large cell lymphoma patients, and has recently been implicated as an oncogene in a small fraction of non–small-cell lung cancers and neuroblastomas. Significantly, sensitivity in these cell lines was well correlated with specific *ALK* genomic rearrangements, including chromosomal translocations and gene amplification. Moreover, in such cell lines, ALK kinase inhibition can lead to potent suppression of downstream survival signaling and an apoptotic response. These findings suggest that a subset of lung cancers, lymphomas, and neuroblastomas that harbor genomic *ALK* alterations may be clinically responsive to pharmacologic ALK inhibition. [Cancer Res 2008;68(9):3389–95]

Introduction

Tyrosine kinases are now widely recognized as attractive proteins for molecularly targeted cancer therapy. The clinical success of several selective kinase inhibitors including imatinib, erlotinib, sunitinib, and lapatinib has shown that this strategy may be broadly applicable to a variety of hematologic and epithelial malignancies. However, it is also becoming clear that such treatments are largely beneficial to a subset of patients whose tumor cells harbor activating mutations of genes encoding the target kinase (1). Thus, imatinib, which inhibits the ABL, KIT, and platelet-derived growth factor (PDGF) receptor kinases, is effective in chronic myelogenous leukemias, which harbor the BCR-ABL oncogenic kinase fusion, and in gastrointestinal tumors that harbor mutationally activated KIT or PDGF receptors (2). Similarly, most non–small-cell lung cancer patients that respond to the epidermal growth factor receptor (EGFR) kinase inhibitor erlotinib harbor activating EGFR mutations (3). Ongoing cancer genome analyses continue to reveal novel genetic lesions that give rise to activated kinases in a variety of cancers, and many of these may represent attractive targets for therapy.

We have recently reported the development of an automated high-throughput platform for profiling a very large panel of human tumor-derived cell lines to identify subsets that exhibit exquisite sensitivity to a variety of molecularly targeted inhibitors with potential anticancer activity (4). Those findings showed the power of this strategy to reveal genotype-correlated sensitivities that may be useful in guiding clinical testing of novel therapeutic compounds. Here, we describe the profiling of 602 cancer cell lines for sensitivity to a selective inhibitor of the anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase first identified as part of an *NPM-ALK* fusion protein expressed in a subset of patients with anaplastic large cell lymphoma (5). Our studies revealed that a small subset of cell lines harboring *ALK* gene alterations are highly sensitive to ALK inhibition (5, 6). These include cells derived from non–small-cell lung cancers and anaplastic large cell lymphomas, where *ALK* translocations have previously been reported, as well as from neuroblastomas, where *ALK* gene amplification has been described (7, 8). Our findings indicate that selective ALK kinase inhibitors may be useful in the clinical management of a subset of patients with diverse tumor types that harbor *ALK* gene alterations.

Materials and Methods

Human cancer cell lines and cell viability assays. Human cancer cell lines were obtained from commercial vendors and were maintained and tested for viability using an automated platform, as previously described (4).

Protein detection. Immunodetection of proteins following SDS-PAGE was done using standard protocols. Equal lane loading was assessed using a β-tubulin antibody (Sigma). The Akt, ALK, extracellular signal–regulated kinase (Erk)-1/2, phospho-Erk1/2 (T202/Y204), phospho-ALK (Y1604), signal transducers and activators of transcription 3 (STAT3), and phospho-STAT3 (S727) antibodies were from Cell Signaling Technology. The phospho-Akt (S473) antibody was from BioSource International. The poly(ADP-ribose)

polymerase antibody was from BD Biosciences. All antibodies were used at a 1:1,000 dilution, except for the β-tubulin antibody, which was used at 1:10,000 dilution.

Kinase inhibitors. TAE684 and BMS-536924 were synthesized as previously described (9, 10). PF-2341066 was synthesized at Pfizer Pharmaceuticals. WZ-5-126 is a recently developed inhibitor with selective ALK inhibitory activity,[5] and the *in vitro* profile of inhibitory activity against a panel of kinases was done by Ambit Biosciences (Supplementary Table S1).

Cell cycle analysis. Cells were pulsed with 10 μmol/L bromodeoxyuridine (BrdUrd) for 1 to 2 h before collection, centrifuged to remove supernatant, and fixed in ice-cold 70% ethanol. The cells were washed with PBS/0.5% bovine serum albumin (BSA) and incubated in denaturing solution (2 mol/L HCl) for 20 min at room temperature. After a further wash with PBS/0.5% BSA, the cells were resuspended in 0.1 mol/L sodium borate for 2 min at room temperature. After an additional wash, the cells were suspended in anti-BrdUrd monoclonal antibody for 20 min (1:500; Becton Dickinson) per manufacturer's instructions. Cells were washed in PBS/0.5% BSA and the pellet was resuspended in FITC-conjugated antimouse IgG (1:50; Vector Laboratories) for 20 min. After an additional wash in PBS/0.5% BSA, the cells were stained with 10 μg/mL propidium iodide (Sigma) and treated with RNase A (Sigma) before two-dimensional fluorescence-activated cell sorting analysis using CellQuest software (Becton Dickinson).

RNAi studies. Two shRNA species targeting sequences downstream of the common *ALK* breakpoint were expressed from the pLKO1 lentiviral vector (target sequences: 5′-GTGATAAATACAAGGCCCAGA-3′ and 5′-GAGCTGGTCATTACGAGGATA-3′). Cells were infected with the viruses overnight in the presence of polybrene (8 μg/mL) and then maintained in the presence of 2 μg/mL puromycin for an additional 6 days. A cell line resistant to the ALK inhibitor (A549) was used to show the infection efficiency and specificity of the effect seen in the NCH-H3122 and KELLY cell lines.

Fluorescence *in situ* hybridization. Two-color fluorescence *in situ* hybridization (FISH) was done on 3:1 methanol/acetic acid–fixed cell lines or on formalin-fixed paraffin-embedded tumor tissue using the LSI *ALK* Dual Color, Break Apart Rearrangement Probe (Abbott-Vysis) following the manufacturer's protocols. Images were captured with an Olympus BX61 fluorescent microscope equipped with a charge-coupled device camera, and analysis was done with Cytovision software (Applied Imaging).

PCR detection of ALK fusion products. RNA was extracted from cell lines using RNA STAT-60 according to the manufacturer's instructions (Tel-Test, Inc.) and reverse transcription was carried out with the AffinityScript Multi-Temperature cDNA Synthesis kit (Stratagene). PCR was then done using the AmpliTaq Gold PCR Master Mix (Applied Biosystems). Primer sequences are listed in Supplementary Fig. S1.

DNA sequencing. Genomic DNA was isolated from cell lines using the Gentra purification system according to the manufacturer's protocol. The entire *ALK* coding sequence (exons 1–29) was amplified from genomic DNA by PCR with primers (Supplementary Fig. S2). PCR products were purified and subjected to bidirectional sequencing using BigDye v1.1 (Applied Biosystems) in combination with an ABI3100 sequencer (Applied Biosystems). Electropherograms were analyzed using Sequence Navigator software (Applied Biosystems).

Data analysis. The sensitivity of each cell line to various concentrations of kinase inhibitors was calculated as the fraction of viable cells relative to untreated cells. Data were subjected to nonlinear regression analysis using GraphPad Prism Software version 3.0 (GraphPad Software, Inc.) to obtain IC_{50} values.

Results and Discussion

A small subset of human cancer cell lines are sensitive to a selective ALK kinase inhibitor. Using an automated platform to examine drug sensitivity in cancer cell lines (4), we tested the sensitivity of 602 established cancer cell lines derived from a wide variety of tumor types (Supplementary Fig. S3) to TAE684, a selective inhibitor of the ALK kinase (9). Cells were treated for 72 hours with a range of TAE684 concentrations and then assayed for potential cytostatic or cytotoxic responses. Whereas the vast majority of tested cell lines were largely refractory to treatment, a small subset of lines (∼2%) displayed marked sensitivity to TAE684, as indicated by a significant reduction in cell number following treatment (Fig. 1*A*).

The subset of TAE684-sensitive cells was notably enriched with cell lines derived from non–small-cell lung cancer, neuroblastoma, and anaplastic large cell lymphoma, tumor types where genomic ALK activation has previously been reported (5, 6, 8, 11). Chromosomal translocations involving gene sequences encoding the intracellular domain of ALK have been detected in anaplastic large cell lymphoma (50–60%), inflammatory myofibroblastic tumors (27%), and non–small-cell lung cancer (7%; refs. 5, 12). The majority of ALK translocations involve a common breakpoint that yields a fusion protein comprising the complete intracellular portion of ALK, including the kinase domain. At least 15 different ALK fusion partners have been discovered in human cancers, and in each case, the NH2-terminal region of the protein contains an oligomerization domain, which is believed to cause dimerization of the fusion protein and ALK kinase–mediated autophosphorylation (13). Activating point mutations of ALK have not been reported.

TAE684-sensitive non-small-cell lung cancer–derived cell lines harbor genomic *ALK* rearrangements. Among 134 non–small-cell lung cancer cell lines tested with TAE684, substantial drug sensitivity was observed in three of the lines (Fig. 1*A*). Interphase FISH analysis with an *ALK* FISH probe revealed that of the three TAE684-sensitive cell lines, the two most sensitive cell lines (NCI-H2228 and NCI-H3122) displayed unbalanced rearrangements of *ALK* signified by loss of the 5′ centromeric (green signal) and extra copies of the 3′ telomeric (red signal) portions of the gene (Fig. 1*B*). In addition, immunoblotting with an antibody recognizing an epitope in the preserved 3′ end of *ALK* revealed that both lines express significant levels of a protein considerably smaller than the expected 200-kDa full-length ALK protein (Fig. 1*C*). To determine the identity of the 5′ fusion partners in both cell lines, we carried out PCR analysis using primers 5′ and 3′ to the common translocation breakpoint in eight known fusion partners and *ALK*, respectively (Supplementary Fig. S4). There was no evidence of either of the *EML4-ALK* fusion mRNAs (variant 1 or 2) previously detected in non–small-cell lung cancer patients in the NCI-H2228 cell line (6), and the identity of the fusion partner in this line remains unknown. However, in the NCI-H3122 cell line, we detected the *EML4-ALK* variant 1 fusion mRNA in which intron 13 of *EML4* is fused to intron 20 of *ALK*. The HCC-78 cell line, which displayed moderate TAE684 sensitivity, does not seem to harbor *ALK* gene abnormalities or detectable ALK protein expression, and thus the basis for its sensitivity is not known (data not shown). Significantly, a very recent study of global phosphotyrosine signaling in a large panel of lung cancer cell lines and primary tumors identified a chromosomal translocation in HCC-78 cells that yields a fusion protein containing the kinase domain of the receptor tyrosine kinase ROS, which is activated (14). The fact that there is a high level of homology between the kinase domains of ALK and ROS raises the possibility that the TAE684 sensitivity of HCC-78 cells reflects the inhibition of ROS signaling.

In both non–small-cell lung cancer lines with *ALK* gene rearrangements, ALK protein was expressed and phosphorylated,
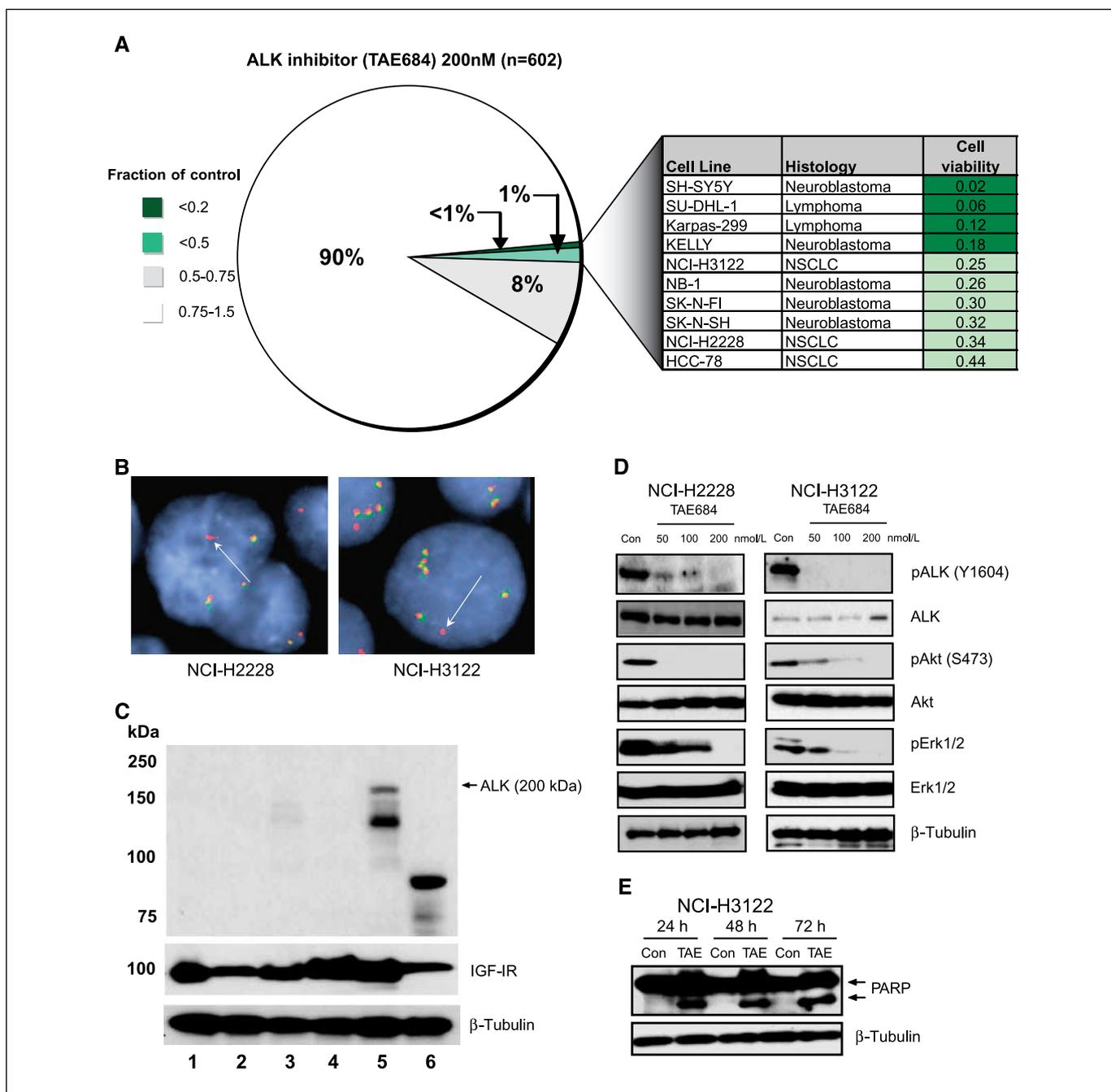
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[5] N.S. Gray, unpublished data.

Figure 1. *A,* pie chart representation of the sensitivity of 602 human cancer cell lines to treatment with 200 nmol/L TAE684. The drug effect was calculated as the fraction of untreated cells present after 72 h of treatment. The color scheme corresponds to the relative inhibitory effect of treatment, with ratios reflecting the number of cells remaining following exposure to inhibitor. Details on the most sensitive cell lines identified are shown in the chart, and the cell lines are shown in order of decreasing sensitivity (from top to bottom). *B,* FISH analysis of the two most TAE684-sensitive non–small-cell lung cancer cell lines, NCI-H2228 and NCI-H3122, using the LSI *ALK* Dual Color, Break Apart Rearrangement Probe. *White arrows, ALK* translocations. *C,* immunoblots showing expression of ALK and IGF-IR proteins in a variety of non–small-cell lung cancer cell lines, including the two most TAE684-sensitive lines, NCI-H3122 and NCI-H2228. *Lane 1,* NCI-H460; *lane 2,* NCI-H1650; *lane 3,* NCI-H1975; *lane 4,* PC9; *lane 5,* NCI-H3122; *lane 6,* NCI-H2228. *D,* immunoblots showing the effect of treating the NCI-H2228 and NCI-H3122 cell lines with the indicated concentrations of the ALK inhibitor TAE684 for 6 h on ALK autophosphorylation and on the downstream survival effectors, Erk1/2 and Akt. *E,* effect of treatment of the NCI-H3122 non–small-cell lung cancer cell line with 200 nmol/L TAE684 for 24, 48, and 72 h on poly(ADP-ribose) polymerase (*PARP*) cleavage.

and phosphorylation was completely abolished following treatment with TAE684. Thus, the ALK kinase seems to have become activated by virtue of genomic rearrangement in these cells. Autophosphorylation of ALK leads to the activation of multiple signaling pathways that contribute to cell survival and transformation (15). Significantly, treatment of each of these lines with TAE684 resulted in a dramatic inhibition of Akt and Erk1/2 phosphorylation (Fig. 1*D*), suggesting that ALK activation in these cells is coupled to the engagement of downstream survival effectors.

ALK shares a high degree of homology with the insulin-like growth factor receptor (IGF-IR), which has also been implicated

in tumorigenesis (16), and significant expression of IGF-IR was detected in both of the TAE684-sensitive non–small-cell lung cancer cell lines (Fig. 1*C*). However, treatment of both lines with an IGF-IR inhibitor, BMS-536924 (IC_{50} 100 nmol/L), had no effect on cell viability (Supplementary Fig. S5). Moreover, these cells were similarly sensitive to another selective ALK inhibitor, WZ-5-126 (IC_{50} 3.4 nmol/L; Supplementary Table S1), suggesting that the observed effects of TAE684 in these cells are mediated through ALK inhibition (Supplementary Fig. S5). Cell cycle analysis of the NCI-H3122 cell line following treatment with TAE684 revealed a dramatic increase in the sub-G_1 apoptotic fraction of cells as early as 24 hours after treatment, suggesting

a cytotoxic response to ALK inhibition (Supplementary Fig. S6*A*). Poly(ADP-ribose) polymerase cleavage was also evident in this cell line following treatment with TAE684 (Fig. 1*E*). Notably, the TAE684 response in the NCI-H2228 cell line seems to be cytostatic rather than apoptotic (data not shown). Thus, ALK kinase inhibition in tumor cells harboring *ALK* genomic lesions may lead to either a cytostatic or cytotoxic outcome, potentially depending on additional genetic features.

TAE684 sensitivity in neuroblastoma cells correlates with *ALK* gene amplification and rearrangement. The cell line profiling data also revealed a preponderance of neuroblastoma-derived cell lines among the most TAE684-sensitive lines (Fig. 1*A*).
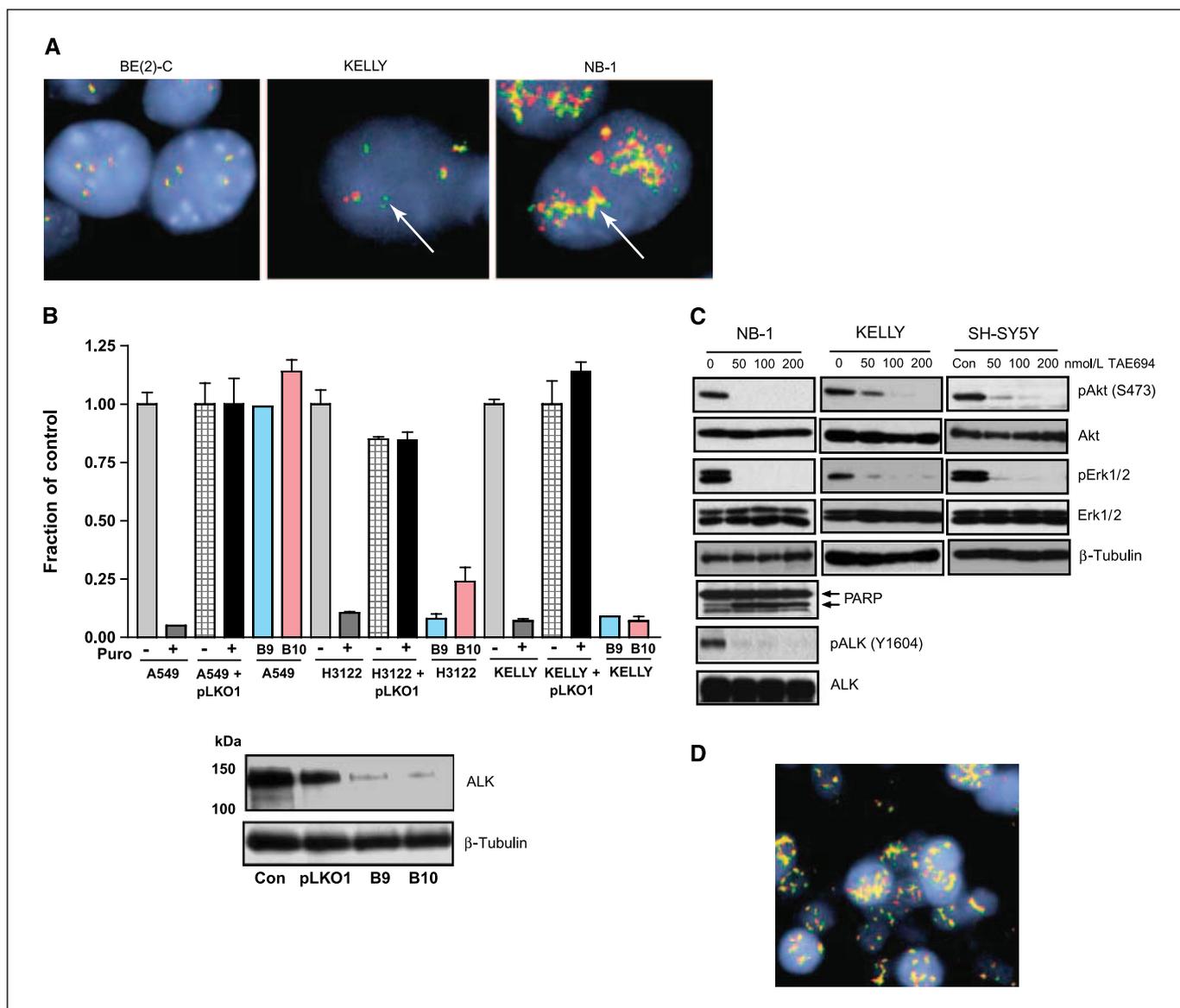

Figure 2. *A*, FISH analysis of the BE(2)-C, KELLY, and NB-1 neuroblastoma cell lines using the LSI *ALK* Dual Color, Break Apart Rearrangement Probe. *White arrows, ALK* translocations and amplification in KELLY and NB-1 cells, respectively. *B*, cell viability assay of the indicated cell lines 7 d following lentiviral-mediated delivery of ALK-specific shRNAs showing potent suppression of viability in two TAE684-sensitive lines (H3122 and KELLY), but not in a TAE684-insensitive line (A549). B9 and B10 are two independent RNAi sequences corresponding to different regions of the 3′ end of the gene (contained within the fusion) and pLKO1 is a control vector. Below the graph are immunoblots showing ALK protein levels in the NCI-H3122 cell line 4 d following infection with either a control vector (pLKO1) or ALK-specific shRNAis B9 and B10. *C*, immunoblots showing the effect of treating the NB-1, KELLY, and SH-SY5Y cell lines with the indicated concentrations of the ALK inhibitor TAE684 for 6 h on ALK phosphorylation and phosphorylation of the indicated downstream effectors. Note the poly(ADP-ribose) polymerase cleavage in TAE684-treated NB-1 cells, indicative of apoptosis. *D*, FISH analysis of a pediatric neuroblastoma case using the LSI *ALK* Dual Color, Break Apart Rearrangement Probe.
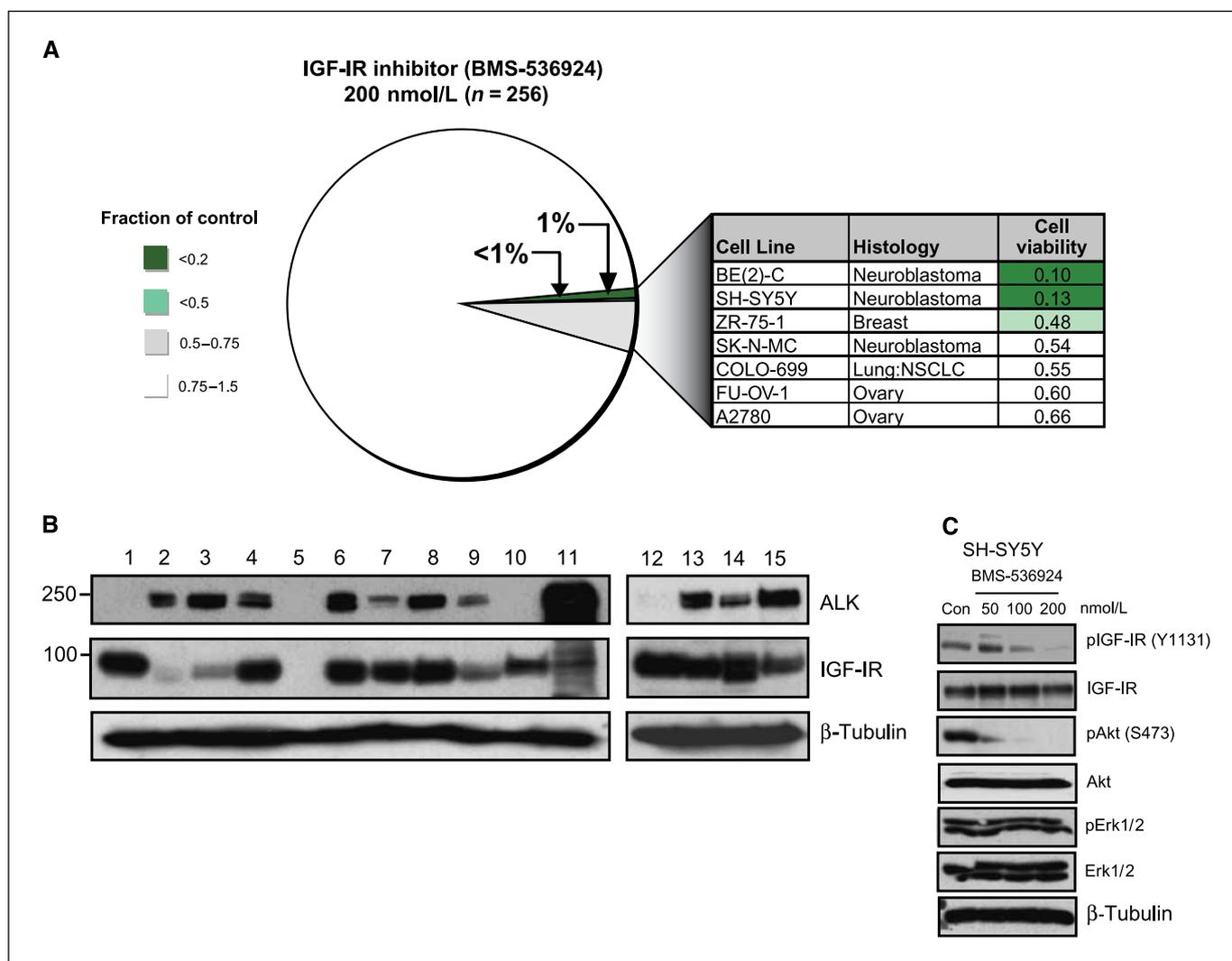
Figure 3. *A,* pie chart representation of the sensitivity of 256 human cancer cell lines to 200 nmol/L of the IGF-IR inhibitor BMS-536924 following 72 h of treatment. *B,* immunoblots of total ALK and IGF-IR protein expression in a panel of 15 neuroblastoma cell lines [*lanes: 1,* SK-N-AS; *2,* MC-IX-C; *3,* SK-N-SH; *4,* BE(2)-M17; *5,* SCCH-26; *6,* SK-N-BE(2); *7,* SK-N-FI; *8,* IMR-32; *9,* SIMA; *10,* NB69; *11,* NB-1; *12,* BE(2)-C; *13,* MHH-NB-11; *14,* KELLY; *15,* SH-SY5Y]. *C,* immunoblots showing the effect of various concentrations of BMS-536924 on IGF-IR, Akt, and Erk activation in SH-SY5Y neuroblastoma cells 6 h after treatment.

ALK expression has previously been reported in a large fraction of neuroblastomas (11), and rare cases of *ALK* gene amplification have also been described (7, 8). Therefore, we examined the 17 neuroblastoma cell lines that were screened with the ALK inhibitor using an *ALK* FISH probe to detect gene rearrangements. Two of the most TAE684-sensitive cell lines showed either *ALK* gene rearrangement (KELLY) or substantial amplification of intact *ALK* (NB-1; Fig. 2*A*). Although FISH analysis of the KELLY line revealed a clear chromosomal split within the *ALK* gene, the molecular nature of the gene rearrangement remains unknown. Curiously, phosphorylated ALK was difficult to detect in the KELLY cell line, suggesting that very low levels of protein may be driving downstream signaling in these cells. However, KELLY cells, as well as H3122 non–small-cell lung cancer cells, were effectively killed following infection with either of the two different lentiviruses that encode *ALK*-specific shRNAs, confirming the requirement for ALK in these cells (Fig. 2*B*). Cell cycle analysis of the KELLY cell line following treatment with TAE684 revealed a small but significant increase in the sub-G_1 apoptotic fraction of cells as early as

24 hours after treatment, suggesting a cytotoxic response to ALK inhibition (Supplementary Fig. S6*B*).

Furthermore, TAE684 treatment potently suppressed Akt and Erk1/2 phosphorylation in the KELLY and NB-1 cell lines (Fig. 2*C*). Thus, in these cell lines with genomic ALK alterations, ALK signaling seems to be coupled to key downstream survival effectors. Moreover, as early as 6 hours after treatment with TAE684, there was evidence of poly(ADP-ribose) polymerase cleavage in the NB-1 cell line (Fig. 2*C*), indicating that, as in non–small-cell lung cancer cells harboring *ALK* translocations, neuroblastoma cells with activated ALK also undergo an apoptotic response to kinase inactivation by TAE684. Previous studies that made use of *ALK*-specific siRNAs to reduce ALK protein expression showed a similar requirement for ALK in a neuroblastoma cell line exhibiting *ALK* gene amplification (7).

To assess the potential clinical significance of these cell line findings in primary neuroblastomas, we used FISH to detect *ALK* gene abnormalities in 10 pediatric neuroblastoma samples. Among the 10 cases analyzed, we identified 1 case with marked

amplification of *ALK*, similar to that seen in the NB-1 cell line (Fig. 2*D*). Although this represents a small sample size, a previous report identified *ALK* gene amplification in 8 of 85 primary neuroblastoma specimens, suggesting an ∼10% frequency of this genotype in human neuroblastomas (7).

Surprisingly, the most TAE684-sensitive neuroblastoma cell line identified in our panel, SH-SY5Y, showed no evidence of either *ALK* gene rearrangement by FISH or *ALK* coding sequence mutation by DNA sequencing (data not shown). However, TAE684 treatment of these cells effectively suppressed Akt and Erk1/2 phosphorylation (Fig. 2*C*). Significantly, a separate analysis of tumor cell sensitivity to the IGF-IR inhibitor BMS-536924 in 256 cell lines from a variety of tissue types revealed that, as with TAE684, the majority of cell lines were drug resistant, but SH-SY5Y was notably among the most sensitive cell lines (Fig. 3*A*). As mentioned above, the ALK kinase domain exhibits a high degree of sequence homology with the IGF-IR kinase, and TAE684 inhibits phosphorylation of IGF-IR in *in vitro* kinase assays at concentrations of 10 to 20 nmol/L (9). In addition to expressing ALK, a large fraction of the neuroblastoma cell lines also express IGF-IR (Fig. 3*B*). Although KELLY and SH-SY5Y both express significant levels of IGF-IR, a comparison of their sensitivities to TAE684, WZ-5-126, and BMS-536924 showed that in KELLY cells the predominant target of TAE684 is ALK, whereas in the SH-SY5Y cell line it appears to be IGF-IR (data not shown). Indeed, treatment of SH-SY5Y cells with the IGF-IR inhibitor BMS-536924 resulted in a dramatic suppression of Akt phosphorylation (Fig. 3*C*). Previous studies have also implicated IGF-IR as a potential therapeutic target in neuroblastoma cells, including SH-SY5Y cells (17). We also noted that two of the neuroblastoma lines without obvious *ALK* gene alterations (SK-N-FI and SK-N-SH) exhibited TAE684 sensitivity but did not respond to BMS-536924, raising the possibility that these cells harbor more subtle *ALK* lesions or that another target of TAE684 confers sensitivity in those lines. Taken altogether, these findings suggest that a subset of neuroblastomas with *ALK* gene amplification or rearrangement may be clinically responsive to selective ALK kinase inhibitors. Moreover, our findings raise the possibility that a dual inhibitor of ALK and IGF-IR, such as TAE684, may be clinically active in a subset of neuroblastomas that includes those with either ALK or IGF-IR dependency.

Anaplastic large cell lymphoma–derived cells with *ALK* translocations are sensitive to ALK kinase inhibition. Anaplastic large cell lymphoma is the tumor type where *ALK* translocations have been most frequently detected (5). Our cell line profiling screen with TAE684 included two anaplastic large cell lymphoma–derived cell lines (SU-DHL-1 and Karpas-299), and both have previously been shown to express a fusion protein resulting from the *NPM-ALK* translocation. Significantly, these lines were among the most TAE684-sensitive cell lines detected in our screen (Fig. 1*A*), and we confirmed the presence of the *NPM-ALK*
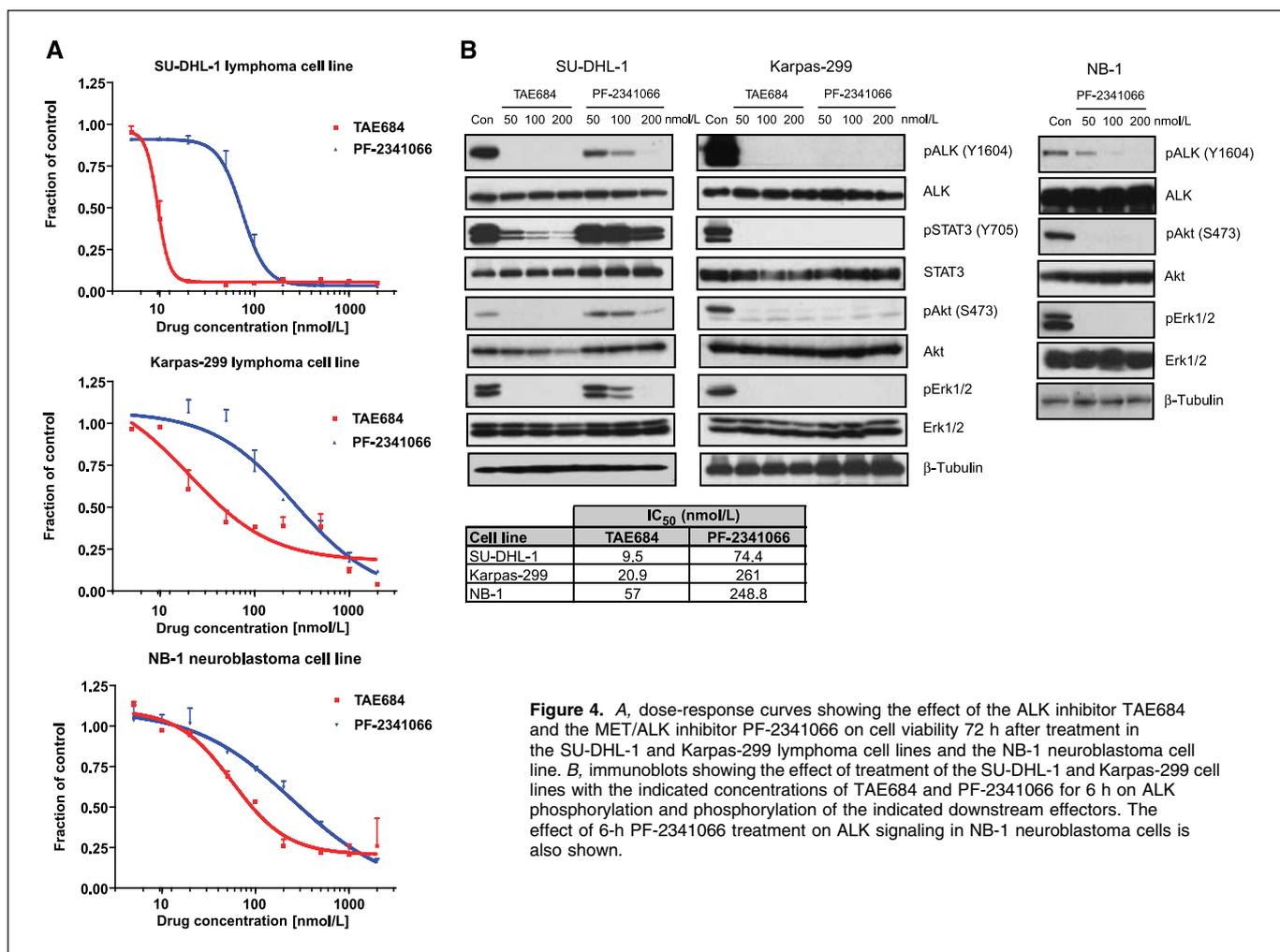

IC$_{50}$ (nmol/L)		
Cell line	**TAE684**	**PF-2341066**
SU-DHL-1	9.5	74.4
Karpas-299	20.9	261
NB-1	57	248.8

Figure 4. *A*, dose-response curves showing the effect of the ALK inhibitor TAE684 and the MET/ALK inhibitor PF-2341066 on cell viability 72 h after treatment in the SU-DHL-1 and Karpas-299 lymphoma cell lines and the NB-1 neuroblastoma cell line. *B*, immunoblots showing the effect of treatment of the SU-DHL-1 and Karpas-299 cell lines with the indicated concentrations of TAE684 and PF-2341066 for 6 h on ALK phosphorylation and phosphorylation of the indicated downstream effectors. The effect of 6-h PF-2341066 treatment on ALK signaling in NB-1 neuroblastoma cells is also shown.

translocation in these cells by both PCR and FISH analysis (Supplementary Fig. S7). Furthermore, TAE684 potently suppressed cell viability and ALK phosphorylation, as well as the phosphorylation of downstream survival effectors, in both lines (Fig. 4*A* and *B*).

Because TAE684 is currently not being tested as a clinical agent, we also examined the activity of PF-2341066, a dual MET/ALK kinase inhibitor currently undergoing phase I clinical testing (18). In the two anaplastic large cell lymphoma lines tested, as well as the neuroblastoma line NB-1, PF-2341066 was able to inhibit proliferation and ALK-mediated signaling in these cell lines at clinically achievable doses, although the inhibitory effects were not as substantial as those seen with TAE684 (Fig. 4*A* and *B*). Moreover, potent suppression of Akt and Erk signaling was also seen in PF-2341066–treated NB-1 neuroblastoma cells. Similar trends in sensitivity to both TAE684 and PF-2341066 were also evident in the non–small-cell lung cancer cell line NCI-H3122 and the neuroblastoma line KELLY (Supplementary Fig. S8). Together, our cell line findings suggest that *ALK* gene rearrangements associated with specific chromosomal translocations or gene amplification are well correlated with sensitivity to selective ALK kinase inhibition, and that clinical testing of PF-2341066 in anaplastic large cell lymphoma, non–small-cell lung cancer, and neuroblastoma may be warranted.

Concluding remarks. Our collective observations from cell line profiling analysis with the selective ALK kinase inhibitor TAE684 have revealed that a subset of human cancer-derived cell lines harboring *ALK* gene rearrangements and/or amplifications are exquisitely sensitive to ALK kinase inhibition (Supplementary Fig. S9). Moreover, in these cells, ALK activation seems to be coupled to critical downstream survival effectors including Erk and

Akt. Although the correlation between TAE684 sensitivity and *ALK* gene status among cell lines was strong, it was not perfect, suggesting that *ALK* genomic status may not be the sole determinant of sensitivity to kinase inhibition. Moreover, because it was not readily feasible to examine the *ALK* genomic status in all of the cell lines in our large panel, it is possible that there are additional tumor cells with ALK activation that did not score as TAE684 sensitive. However, the overall findings suggest that clinical studies of selective ALK kinase inhibitors are likely to benefit from preselection of patients with anaplastic large cell lymphoma, non–small-cell lung cancer, or neuroblastoma whose tumors exhibit *ALK* gene amplification or translocation. The identification of a kinase activation event that contributes to oncogenicity in three diverse human cancer types, including both hematologic and solid tumors, is unusual, and highlights the potential importance of considering specific genotypes, as opposed to tissue types, in future strategies to develop and clinically evaluate molecularly targeted cancer drugs.

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