SCH66336, inhibitor of protein farnesylation, blocks signal transducer and activators of transcription 3 signaling in lung cancer and interacts with a small molecule inhibitor of epidermal growth factor receptor/human epidermal growth factor receptor 2

Afshin Dowlati[a], Amy Kluge[a], David Nethery[b], Balazs Halmos[a] and Jeffrey A. Kern[b]

Signal transducer and activators of transcription 3 (STAT3) is an important transcription factor that is essential for lung cancer cell survival. STAT3 is activated by diverse upstream receptor and nonreceptor tyrosine kinases, and blockade of STAT3 results in tumor growth inhibition. Therefore, a search for STAT3 inhibitors is under way. We demonstrate that SCH66336, at 4 μmol/l, completely blocks STAT3 phosphorlyation in a variety of nonsmall cell lung carcinoma (NSCLC) cell lines, whereas the effect on AKT and extracellular signal-regulated kinase activation is variable. Furthermore, SCH66336 has antiproliferative effects on NSCLC cells. When NSCLC cells are exposed sequentially to SCH66336 and a small molecule dual tyrosine kinase inhibitor of epidermal growth factor receptor and human epidermal growth factor receptor 2, synergistic activity is observed with an increase in the fraction of cells undergoing apoptosis. Concurrent exposure to both agents is, however, associated with antagonism and decreased apoptosis. We conclude that blockade of STAT3 phosphorylation might be one of the mechanisms by which SCH66336 exerts its antitumor activity, and that this can be synergistic *in vitro* when administered sequentially with epidermal growth factor receptor inhibitors. *Anti-Cancer Drugs* 19:9–16 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2008, **19**:9–16

Keywords: epidermal growth factor receptor, lung cancer, signal transducer and activators of transcription 3

Divisions of [a]Hematology/Oncology and [b]Pulmonary and Critical Care, Department of Medicine, Case Western Reserve University and University Hospitals of Cleveland and CASE Comprehensive Cancer Center, Cleveland, Ohio, USA

Correspondence to Afshin Dowlati, MD, Division of Hematology/Oncology, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, Ohio 44106, USA
Tel: +1 216 8441228; fax: +1 216 8445234; e-mail: afshin.dowlati@case.edu

Received 21 March 2007 **Revised form accepted** 31 August 2007

Introduction

Signal transducer and activators of transcription (STAT) are important cytoplasmic proteins that act as transcription factors, which regulate gene expression. STAT proteins, especially STAT3 and 5, are important in tumorigenesis and form homodimers or heterodimers upon activation by upstream tyrosine kinases [1]. STAT proteins can be activated by a variety of upstream receptor and nonreceptor tyrosine kinases, including the epidermal growth factor receptor (EGFR), SRC kinase or Janus kinase 2 (JAK2) [2,3]. STAT3 and 5 then translocate to the nucleus to regulate gene expression by binding to promoter regions of genes implicated in cell cycle progression, apoptosis, angiogenesis, tumor invasion and metastasis [4]. Others and we have demonstrated the expression of both STAT3 and its activated form, phosphorylated STAT3 (p-STAT3), in surgically resected nonsmall cell lung cancer (NSCLC) specimens [5,6]. Furthermore, mutant EGFRs selectively activate STAT signaling [7] and blockade of STAT3 in nonmutated NSCLC cell lines results in extensive apoptosis [8]. Given the importance of STAT3 in lung cancer cell survival, a search for inhibitors of STAT3 pathway is under way [9].

Targeting EGFR has emerged as a therapeutic option in lung cancer. Clinical studies have demonstrated response rates of about 10% to small molecule inhibitors of EGFR in NSCLC patients [10], as well as a much higher response in patients carrying a somatic mutation in the tyrosine kinase domain of EGFR resulting in gefitinib or erlotinib sensitivity [11–13]. Most patients, nevertheless, do not benefit from such an approach, and responding patients ultimately develop resistance to these agents. Cell lines resistant to EGFR inhibitors demonstrate persistent activation of downstream AKT and STAT3 [7,14]. Therefore, in attempts to improve the antitumor efficacy of EGFR inhibitors, these agents are being combined with other targeted agents blocking persistently activated downstream proteins such as AKT and STAT3. Indeed, we have previously demonstrated that combined inhibition of EGFR and STAT3 *in vitro* has synergistic antiproliferative activity [15].

0959-4973

SCH66336 was initially developed as a farnesyl transferase inhibitor (FTI). Functionally, it blocks farnesylation and membrane binding of Ras, thus inhibiting its activity [16]. Although SCH66336 blocks Ras farnesylation both *in vitro* and *in vivo*, it is active in models that are not dependent on Ras for growth, thus casting doubt on its exact mechanism of action [17]. Our preliminary studies suggested that SCH66336 also blocked STAT3 phoshorylation. Furthermore, SCH66336 has also been shown to block AKT phosphorylation in selective cell lines [18]. Given these data, we hypothesized that (i) SCH66336 can inhibit STAT3 activation in NSCLC, (ii) SCH66336 will inhibit NSCLC cell growth, (iii) SCH66336 will have additive or synergistic antiproliferative effects in combination with an inhibitor of EGFR and (iv) activity of SCH66336 is independent of EGFR mutational status.

Materials and methods

Cells

All cell lines (H1869, H1650 and H1975) were purchased from the American Type Culture Collection (Rockville, Maryland, USA). H1869 expresses wild-type EGFR, whereas H1650 carries the mutation (delE746A750), rendering cells susceptible to small molecule inhibitors of EGFR [11]. H1975 is an EGFR double mutant cell line (L858R and T790M), rendering cells resistant to EGFR inhibitors [13]. All three cell lines are of the Kras 1 wild-type. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamate, penicillin and streptomycin. They were maintained in a 37°C, 5% CO_2, fully humidified incubator, passed twice weekly and prepared for experimental procedure after reaching 90% confluence.

Reagents

SCH66336 ([+]4-{2-[4-(8-chloro-3,0-dibromo-6,11-dihydroxy-5-benzocyclohepa{1,2-b}pyridine-11-yl)-1-piperidinyl]-2-oxoethyl}-1piperidinecarboxamide) was obtained courtesy of Dr Robert Bishop from Schering-Plough Research Institute (Kenilworth, New Jersey, USA). GW572016 (Lapatinib) was graciously given by Glaxo-Smith Kline (Research Triangle, North Carolina, USA). GW572016 is a selective, reversible dual inhibitor of both EGFR and HER2 receptor tyrosine kinase domains with IC_{50} values of 9–10 nmol/l in an in-vitro kinase assay and IC_{50} of around1 μmol/l in cell lines. Inhibitors were prepared as 20-mmol/l stock solutions in dimethyl sulfoxide and stored at −20°C. Stock solutions were then diluted in DMEM/Ham's F12, to achieve the final desired concentration. The final dimethyl sulfoxide concentrations were less than 0.04%.

Western blotting

All cells were grown to 90% confluence and incubated in serum-free media for 16 h before use. The specific inhibitors were then added to the desired concentration and after 3 h, EGF was added (final concentration of 10 ng/ml) for 10 min. Cells were lysed [0.5% sodium deoxycholate, 0.2% sodium dodecyl sulfate (SDS), 1% Triton X-100, 5 mmol/l EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin and 1 mmol/l phenyl-methylsulfonyl fluoride; all reagents from Sigma Chemical Co., St Louis, Missouri, USA] and sonicated. Samples containing 30–70 μg of protein measured by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, California, USA) were separated by SDS–PAGE consisting of a 5% (w/v) acrylamide stacking gel and a 12.5% (w/v) separating gel containing 0.1% SDS. The running buffer comprised 0.1% SDS, 25 mmol/l Tris and 250 mmol/l glycine (pH 8.3). Electrophoretic fractionation was carried out at a constant current of 100 mA for 90 min. Proteins were then electrotransferred onto an Immobilon P membrane (Milipore Corp., Bedford Massachusetts, USA). The filters were blocked with 5% bovine serum albumin in phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBS–Tween) for 1 h and then incubated at room temperature with primary antibody (1 : 200 dilution) in blocking solution for 1 h. After washing in PBS–Tween, the filters were incubated for 1 h in horseradish peroxidase-conjugated anti-immunoglobulin (1 : 5000). Following three washes in PBS–Tween (5 min each wash), bands were visualized by chemiluminescence and subsequent exposure to hyperfilm-enhanced chemiluminescence (Amersham Life Science Inc., Arlington Heights, Illinois, USA). The antibody to STAT3 is a rabbit polyclonal antibody raised against a recombinant protein mapping to amino acids 50–240. The antibody against phosphorylated STAT3 (p-STAT3) is a mouse monoclonal immunoglobulin G (IgG2b) raised against a peptide corresponding to the amino acid sequence containing phosphorylated Tyr-705 of STAT3 of human origin. All antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, California, USA). Human DnaJ 2 is a mouse monoclonal antibody from NeoMarkers of Lab Vision Corp. (Fremont, California, USA).

Colorimetric cell-proliferation assay

A colorimetric cell-proliferation assay was performed using the CellTiter 96 kit (Promega Corp., Madison, Wisconsin, USA). Briefly, cells were plated in 96-well plates (2000 cells/well) and cultured in DMEM/Ham's F-12 supplemented with 10% FBS for 24 h. Cells were then incubated in the same media with only 2% FBS for the remainder of the experiment. GW572016 and SCH66336 were added at various concentrations alone to determine the IC_{50} values for proliferation for each of these agents in the cell lines tested. Cells were also exposed to each agent for at least 48 h before the colorimetric assay. In the drug combination assays, cells were exposed either for 48 h to both agents concurrently or for 24 h to a single agent sequentially, followed by the next agent. Media and pharmacologic agents were changed daily. At the time of assay, media was aspirated

and CellTiter 96 Aqueous One Solution Reagent (20 µl) was added to each well. The plates were incubated at 37°C for up to 1 h and absorbance recorded at 490 nm using a 96-well plate reader. Data were derived from at least seven wells for each drug concentration and combination. IC_{50} values for SCH66336 were determined as previously described [19]. The growth-inhibitory effects of the drug combinations were quantified according to the method of Chou *et al.* [20] using the Calcusyn software program (Biosoft, Cambridge, UK).

Flow cytometric analysis

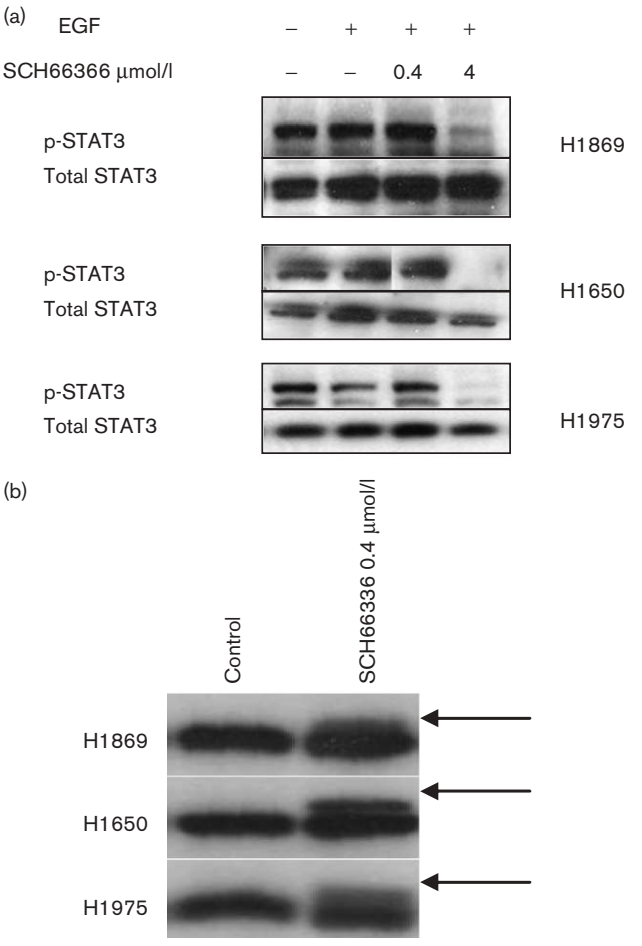
Cells were grown in flasks and allowed to reach around 50% confluence. The inhibitors (SCH66336 and GW572016) were then added at their respective concentrations for 2 days (media and inhibitors changed daily). In the case of single-agent or concurrent treatment, the cells were exposed to the agents for 48 h; in the case of sequential treatment, cells were exposed to each agent for 24 h. On the third day, cells were collected by trypsinization. Cells were washed twice in buffer containing PBS, 0.1% bovine serum albumin and 2% FBS. Cells were resuspended in 1 ml of the same buffer and fixed by adding 3 ml of −20°C absolute ethanol. Fixed cells were then centrifuged and resuspended in 1 ml of PBS. A volume of 100 µl of DNase-free, RNaseA was then added and incubated at 37°C for 30 min. Propidium iodide at a volume of 100 µl (1 mg/ml) was then added and incubated at room temperature for 5–10 min. Samples were placed in 12 × 75 Falcon tubes and read on Becton Dickson FACStarPLUS (Franklin Lakes, New Jersey, USA). For the Annexin-V staining and flow cytometry studies Annexin-V-FLUOS staining kit from Roche Applied Science (Indianapolis, Indiana, USA) was used according to protocol. Briefly, cell lines were treated with inhibitor as outlined above. Cells were prepared as follows: cells were transferred to conical tubes, centrifuged at about 1500 rpm for 5 min and media was aspirated off. Cells were then washed with PBS, repelleted by centrifugation and PBS was aspirated off. Annexin-V-FLUOS was prepared according to protocol and 100 µl of it was used to resuspend pelleted cells. Cells were then transferred to 5-ml polystyrene tubes with cell-strainer caps by pipetting cell slurry through the cap. Incubation buffer at a volume of 500 µl was added to increase the sample volume. Samples were read on a Coulter EPICS XL-MCL benchtop flow cytometer (Fullerton, California, USA).

Results

SCH66336 blocks signal transducer and activators of transcription3 phosphorylation in nonsmall cell lung carcinoma cell lines

Three NSCLC cell lines (H1869, H1650 and H1975) were chosen on the basis of their EGFR mutational status. They were first studied to determine their STAT3 activation status. Cells were cultured under serum-free conditions or exposed to 10 ng/ml of EGF. In the serum-free state, all three cell lines had constitutive STAT3 phosphorylation (Fig. 1b). Cells were then exposed to increasing concentrations of SCH66336 for 3 h and stimulated with EGF. In dose-finding studies, we found that SCH66336 at a concentration of 4 µmol/l resulted in a complete blockade (> 99%) of STAT3 phosphorylation in all three cell lines (Fig. 1). At a lower dose (0.4 µmol/l), SCH66336 failed to inhibit STAT3 activation. Given the effect of SCH66336 on p-STAT3 within 3 h of exposure, we looked to see whether, at this time point, SCH66336 results in farnesyl transferase inhibition. Figure 1b demonstrates by Western blotting the appearance of a slower migrating species of human DnaJ 2, indicating that at this 3-h time point, SCH66336 results in inhibition of farnesyl transferase [21]. To exclude a nonspecific

Fig. 1



(a) Effects of SCH66336 on activation of signal transducer and activators of transcription 3 (STAT3). At 4 µmol/l, complete disappearance of p-STAT3 can be seen. (b) Effect of SCH66336 on migration of human DnaJ 2 (HDJ-2). HDJ-2 is a protein used as a marker of farnesyl transferase inhibition. The arrows indicate the appearance of a slower migrating band with SCH66336 exposure.

decrease in protein phosphorylation as a mechanism for decrease in p-STAT3, we looked for the effects of SCH66336 on global phosphorylation status. Using an antibody against phospho-tyrosine residues, we investigated whether SCH66336 affected the global phosphorylation status of proteins. SCH66336 at these concentrations failed to effect total phosphorylation status, suggesting that the decrease in p-STAT3 observed is not related to a global decrease in protein phosphorylation (Fig. 2).

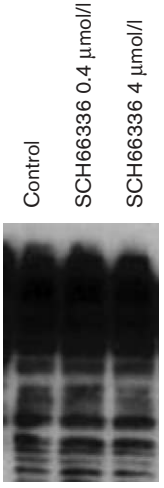
SCH66336 has fewer effects on AKT and extracellular signal-regulated kinase activation

In a further attempt to characterize the effects of SCH66336 on proliferative and apoptotic pathways, the same model system was used to determine the ability of SCH66336 to block EGF-induced activation of the ERK and AKT pathways. Although dose-dependent effects were seen in some cell lines, there was significant variability among the cell lines compared with the effects on p-STAT3 (Fig. 3). SCH66336 had no effect on AKT or ERK activation in the H1869 cell line. In H1650 and H1975, a dose-dependent blockade of phosphorylation was seen in both AKT and ERK; however, it was not of the same degree as the STAT3 blockade. This suggests that the biologic effect of SCH66336 might mainly reside in its capacity to block STAT3 activation.

SCH66336 has antiproliferative effects on nonsmall cell lung cancer cells with constitutive signal transducer and activators of transcription 3 activation

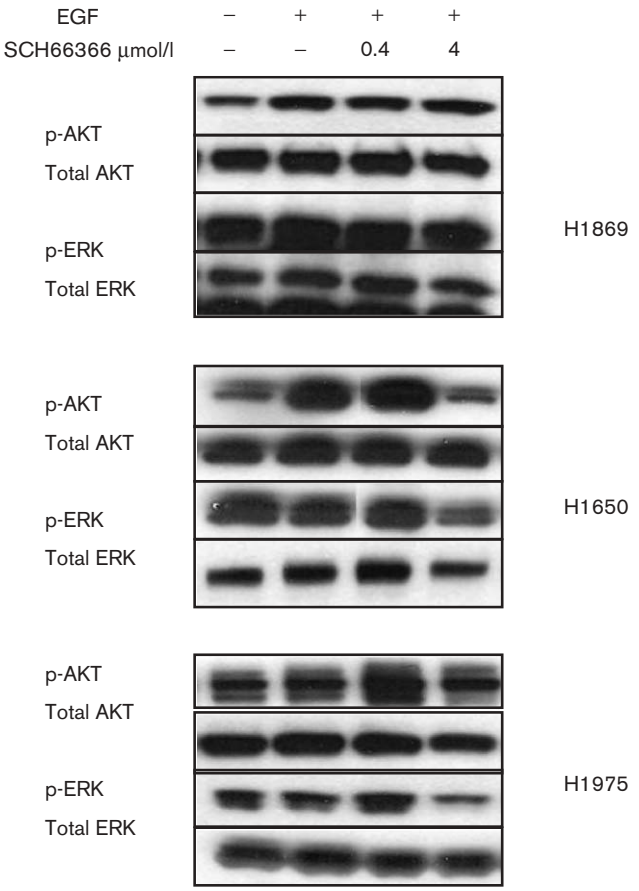
All three cell lines were exposed to increasing doses of SCH66336 and cell counts were performed to determine the effect on proliferation. A clear antiproliferative effect emerged. H1869, H1650 and H1975 had IC_{50} values of 2, 4 and 5 μmol/l, respectively. The similar IC_{50} values for EGFR wild-type (H1869), mutant (H1650) or double mutant cells (H1975) suggest that SCH66336 activity on NSCLC is independent of EGFR mutational status.

Fig. 2



Absence of the effect of SCH66336 on global protein phosphorylation using a panphosphotyrosine antibody in H1869 cells.

Fig. 3

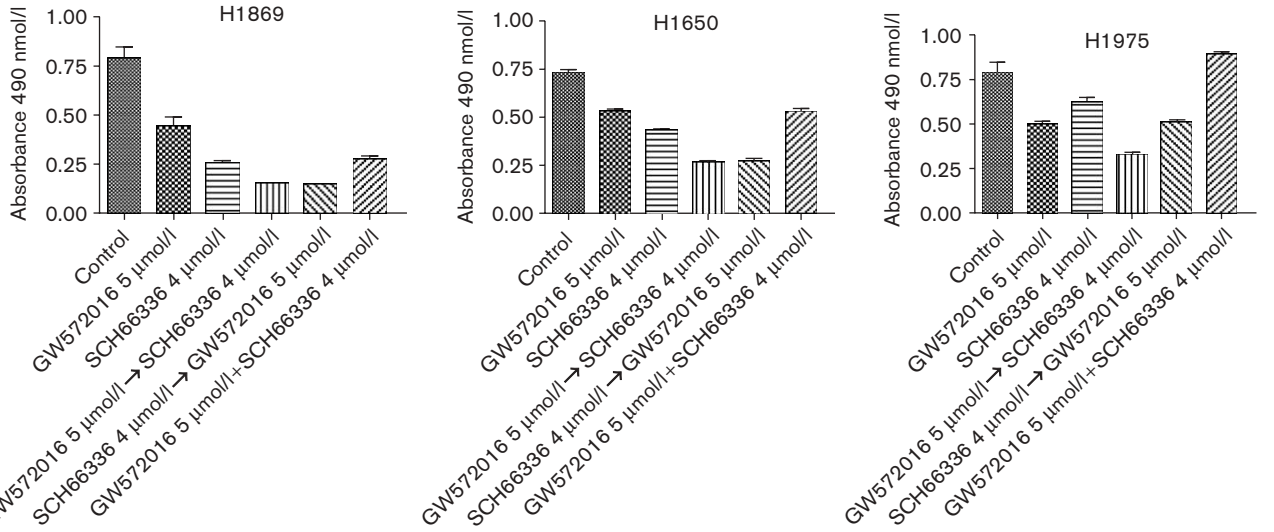


Effects of SCH66336 on AKT and ERK phosphorylation. No effect is seen in H1869, and variable effect is seen in H1650 and H1975.

Epidermal growth factor receptor/human epidermal growth factor receptor 2 inhibitor and SCH66336 synergistically inhibit when exposed sequentially, but are antagonistic when exposed concurrently

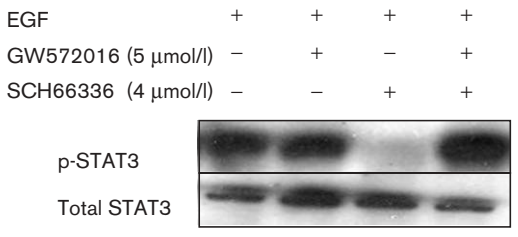
We had previously shown that EGFR inhibition when combined with STAT3 blockade acts synergistically to inhibit the growth of A431 cells. Therefore, we exposed H1869, H1650 and H1975 cells to SCH66336 (4 μmol/l) or an EGFR/HER2 dual inhibitor, GW572016 (5 μmol/l), alone, sequentially or concurrently (Fig. 4). SCH66336 alone, at 4 μmol/l, resulted in 68% (± 2 SD), 42% (± 2 SD) and 25% (± 7 SD) growth inhibition by day 3 in H1869, H1650 and H1975, respectively. In all the three cell lines, sequential exposure resulted in greater growth inhibition than with SCH66336 alone. When cells were

Fig. 4



MTS assay 48 after exposure of H1869, H1650 and H1975 cells to inhibitors in media containing 2% fetal bovine serum (FBS). In the sequential arms, each inhibitor was given for 24 h, then washed off and followed by the second inhibitor. Differences among sequential and concurrent exposures are statistically significant ($P<0.05$) in all three cell lines. $N=7$ for each group; data are expressed as mean ± SE.

Fig. 5



Effects of SCH66336, with or without concurrent exposure to GW572016, on phosphorylation of signal transducer and activators of transcription 3 (STAT3) in H1869 cells. Concurrent exposure abrogates the capacity of SCH66336 to block phosphorylation of STAT3. Effects of sequential exposure are similar to the effects of SCH66336 alone.

exposed sequentially to GW572016 and SCH66336, i.e. when GW572016 was followed by SCH66336 [H1869, 81% ± 0.6 inhibition; combination index (CI) = 0.7 ± 0.3 or vice versa (82% ± 0.3 inhibition, CI = 0.6 ± 0.2), a synergistic effect was seen in both sequences. With concurrent exposure of both agents, however, a significant degree of antagonism was seen (66% ± 6 inhibition, CI = 5 ± 1). Interestingly, when H1869 are exposed to both agents, the ability of SCH66336 to decrease STAT3 phosphorylation disappears (Fig. 5). Similar results were seen in H1975 and H1650 cells.

Concurrent exposure results in a decreased fraction of apoptotic cells

We then evaluated the cell cycle using flow cytometry with propidium iodide staining in H1650 cells. The proportion of cells in sub-G_1 with the use of SCH66336 alone was 19%. The proportion of cells in sub-G_1 (indicative of cells undergoing apoptosis) significantly increased (to 69% of cells) when cells were exposed sequentially to both agents (Fig. 6a). Concurrent exposure led to a less than additive (indicative of antagonism) increase of the fraction of cells in sub-G_1 (41% of cells). To further investigate our findings, flow cytometry using Annexin-V staining was used in H1975 cells (Fig. 6b). The population of cells staining for Annexin-V increased from 2.8% in control cells to 6.4% with GW572106 alone and to 14.5% with SCH66336 alone. When given sequentially, there was substantial increase in cells staining for Annexin-V (21.6 and 35.9%) but this decreased when agents were used concurrently (11.7%).

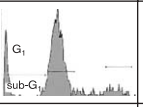
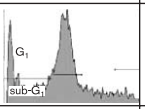
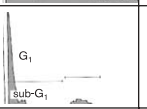
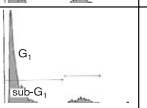
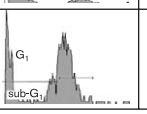
Discussion

STAT signaling has emerged as a promising target for therapeutic development in lung cancer [1]. STAT3 and its activated (phosphorylated) form are expressed in NSCLC cell lines and human NSCLC resected tumors [5,6]. It is an important survival pathway for NSCLC cells and blockade results in cell death [7]. Blockade of STAT3 is synergistic with EGFR inhibition in the A431 cell line [15].

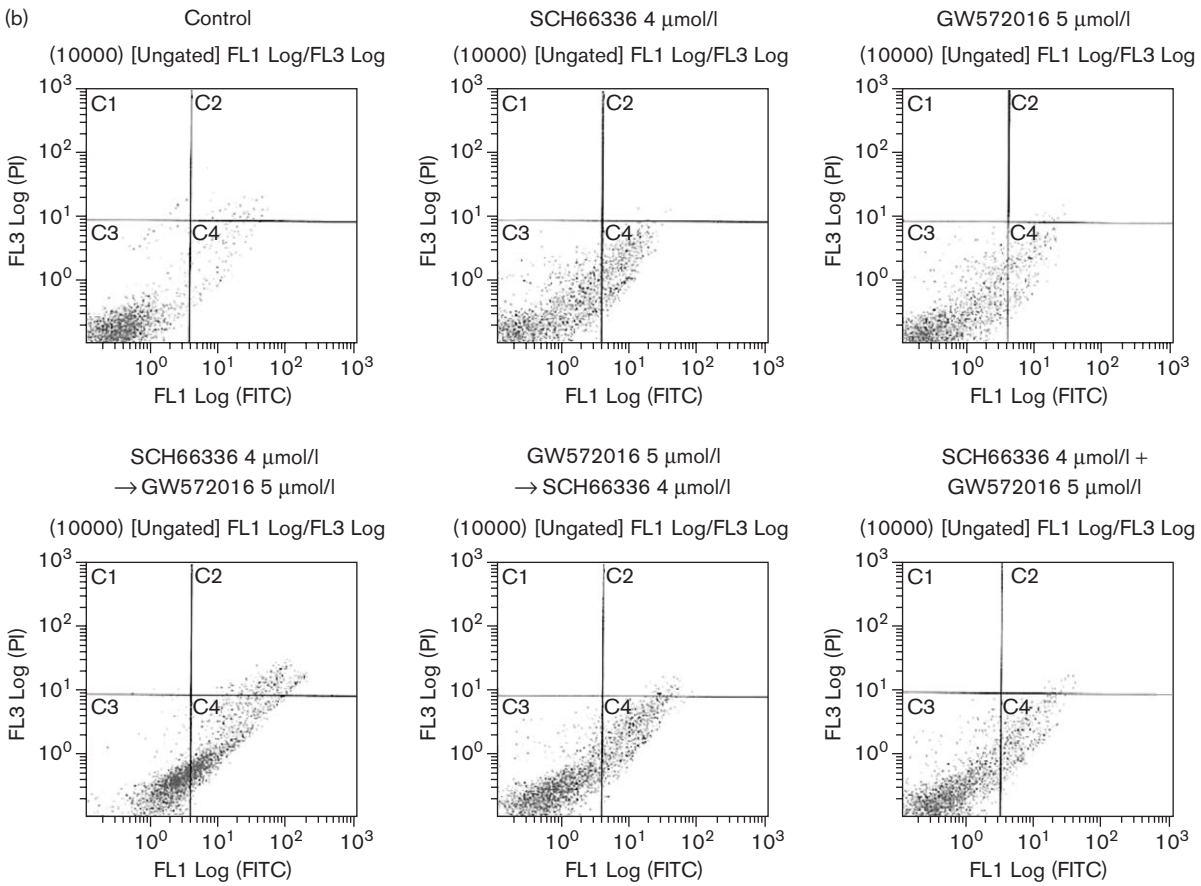
In this study, we demonstrate that SCH66336 blocks STAT3 activation. Several studies show that inhibition of Ras farnesylation is not sufficient to explain the antitumor activity of FTIs, including SCH66336. For

Fig. 6

(a)

Treatment	Flow PI staining	% Cells in sub-G_1
SCH66366 4 μmol/l		19%
GW572016 5 μmol/l		31%
SCH66366 4 μmol/l → GW572016 5 μmol/l		69%
GW572016 5 μmol/l → SCH66366 4 μmol/l		69%
SCH66366 4 μmol/l + GW572016 5 μmol/l		41%

(b)



(a) Flow cytometric analysis of cell cycle using propidium iodide (PI) staining in H1869 cells. (b) Flow cytometric analysis using Annexin-V staining in H1975 cells.

example, FTIs seem to inhibit tumor cell growth independently of the mutation status of Ras in human cancer cell lines [22,23]. Furthermore, the kinetics of FTI-induced reversal of the transformed phenotype does not correlate with the kinetics of FTI-mediated inhibition of Ras farnesylation in H-Ras-transformed rodent fibroblasts [24]. Finally, despite cross-prenylation (geranylgeranylation) of K-Ras and possibly N-Ras in human cancer cells treated with FTIs, the growth of these cells continues to be inhibited [25,26]. In this study, we demonstrate that an alternative mechanism of action for SCH66336 might lie in its ability to block STAT3 phosphorylation. Indeed, a recent study of another FTI, R115777, has shown that this agent also inhibits tyrosine phosphorylation of STAT3 in human pancreatic cell lines [27]. The authors suggest that this effect is mediated via ERK. Given that the major input for STAT3 activation in NSCLC resides in upstream receptor tyrosine kinase activity, mainly EGFR, we looked at three cell lines with different EGFR status: wild-type, EGFR mutant and double mutant lines. SCH66336 was able to block STAT3 phosphorylation to the same degree in all three lines. Contrary to the profound effect on STAT3, the effects on AKT and ERK activation were less significant and uniform among the three cell lines. This suggests that STAT3 blockade might be an important mechanism by which SCH66336 mediates its antiproliferative effects. Indeed, SCH66336 resulted in substantial growth inhibition in our three cell lines that had constitutive p-STAT3 expression, with IC_{50} values in the low micromolar range. This concentration of SCH66336 is clinically achievable in patients [28].

We had previously shown that EGFR and STAT3 blockade in combination is synergistic in inhibiting A431 proliferation. Recent studies also demonstrated that combining SCH66336 with other inhibitors of growth factor signaling might be synergistic or additive [18]. In a recent study targeting insulin growth factor signaling by upregulation of its binding protein, a synergistic activity was seen with SCH66336 when treatment *in vitro* was performed simultaneously. We thus tested this concept with SCH66336 and a small molecule inhibitor of EGFR/HER2, GW572016. GW572016 was chosen for the experiments on the basis of our earlier studies, in which we demonstrated that dual inhibition of EGFR and HER2 had superior antiproliferative effects compared with EGFR-specific agents. Furthermore, the doses we chose for GW572016 were based on the IC_{50} of this agent (4 μmol/l) in the most resistant cell line (i.e H1975) [29]. We were thus able to demonstrate synergy with this combination in multiple cell lines when the agents were used sequentially. When used in combination in a concurrent fashion, however, antagonism appeared in the growth assays. Flow cytometry shows that an increase in the sub-G_1 population indicative of apoptotic frequency was greatly decreased with concurrent exposure, but increased with sequential exposure. The explanation for this antagonism is not clear; however, our preliminary results show that concurrent treatment with SCH66336 and GW572016 results in a disappearance of STAT3 blockade, seen with SCH66336 alone or with sequential exposure. One potential hypothesis to explain this finding is related to the ability of EGFR inhibitors to block ERK signaling. Indeed, in the study by Venkatasubbarao *et al.* [27] blocking ERK signaling by PD98059 reversed the inhibitory effects of a farnesyltransferase inhibitor on STAT3 phosphorylation.

In conclusion, we demonstrate that SCH66336 can block STAT3 activation and growth in NSCLC cell lines, independent of EGFR mutational status. We also demonstrate *in vitro* that combined EGFR/HER2 blockade with SCH66336 might be synergistic when given sequentially (with increased cellular apoptosis), but is antagonistic with concomitant exposure.

Acknowledgements

This study was supported by 5K23 CA109348-01 (to AD) from the National Institutes of Health, the Coburn Haskill Endowment and Blumenthal Lung Cancer Research Fund, both from University Hospitals Case Medical Center.

References

1 Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel targets for therapeutic intervention. *Clin Cancer Res* 2002; **8**:945–954.
2 Levy DE, Darnell JE Jr. STATs: transcriptional control and biological impact. *Nat Rev* 2002; **3**:651–662.
3 Alvarez JV, Greulich H, Sellers WR, Meyerson M, Frank DA. Signal transducer and activator of transcription 3 is required for the oncogenic effects of non-small cell lung cancer associated mutations of the epidermal growth factor receptor. *Cancer Res* 2006; **66**:3162–3168.
4 Haura EB, Turkson J, Jove R. Mechanisms of disease: insights into the emerging role of signal transducers and activators of transcription in cancer. *Nat Clin Pract Oncol* 2005; **2**:315–324.
5 Cortas T, Eisenberg R, Fu P, Kern JA, Patrick L, Dowlati A. Activation state EGFR and STAT3 as prognostic markers in resected non-small cell lung cancer. *Lung Cancer* 2007;**55**:349–355.
6 Haura EB, Zheng Z, Song L, Cantor A, Bepler G. Activated epidermal growth factor receptor signaling promotes tumor survival *in vivo* in non-small cell lung cancer. *Clin Cancer Res* 2005; **11**:8288–8294.
7 Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004; **305**:1163–1167.
8 Song L, Turkson J, Karras JG, Jove R, Haura EB. Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells. *Oncogene* 2003; **22**:4150–4165.
9 Turkson J, Zhang S, Mora LB, Burns A, Sebti S, Jove R. A novel platinum compound inhibits constitutive Stat3 signaling and induces cell cycle arrest and apoptosis of malignant cells. *J Biol Chem* 2005;**280**:32979–32988.
10 Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, *et al.* Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005; **353**:123–132.
11 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; **350**:2129–2139.
12 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; **304**:1497–1500.

13 Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, *et al*. EGFR mutation and resistance of non-small cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**:786–792.
14 Engelman JA, Janne PA, Mermel C, Pearlberg J, Mukohara T, Fleet C, *et al*. ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. *Proc Natl Acad Sci U S A* 2005; **102**:3788–93.
15 Dowlati A, Nethery D, Kern JA. Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition *in vitro* than single agent therapy. *Mol Cancer Ther* 2004; **3**:459–463.
16 Baum C, Kirschmeier P. Preclinical and clinical evaluation of farnesyltransferase inhibitors. *Curr Oncol Rep* 2003; **5**:99–107.
17 Sebti SM. Blocked pathways: FTIs shut down oncogene signals. *The Oncologist* 2003; **8** (S**uppl 3**):30–38.
18 Lee HY, Moon H, Chun KH, Chang YS, Hassan K, Ji L, *et al*. Effects of insulin-like growth factor binding protein-3 and farnesyltransferase inhibitor SCH66336 on Akt expression and apoptosis in non-small-cell lung cancer cells. *J Natl Cancer Inst* 2004; **96**:1536–1548.
19 Tsai CM, Gazdar AF, Venzon DJ, Steinberg SM, Dedrick RL, Mulshine JL, *et al*. Lack of *in vitro* synergy between etoposide and *cis*-diammine-dichloroplatinum III. *Cancer Res* 1989; **49**:2390–2397.
20 Chou TC, Motzer RJ, Tong Y, Bosl GJ. Computerized quantification of synergism and antagonism of taxol, topotecan, and cisplatin against human teratocarcinoma cell growth: a rational approach to clinical protocol design. *J Natl Cancer Inst* 1994; **86**:1517–1524.
21 Adjei AA, Davis JN, Erlichman C, Svingen PA, Kaufmann SH. Comparison of potential markers of farnesyltransferase inhibition. *Clin Cancer Res* 2000; **6**:2318–2325.
22 Prendergast GC, Davide JP, deSolms SJ, Giuliani EA, Graham SL, Gibbs JB, *et al*. Farnesyltransferase inhibition causes morphological reversion of ras-transformed cells by a complex mechanism that involves regulation of the actin cytoskeleton. *Mol Cell Biol* 1994; **14**:4193–4202.
23 Lerner EC, Zhang TT, Knowles DB, Qian Y, Hamilton AD, Sebti SM. Inhibition of the prenylation of K-Ras, but not H- or N-Ras, is highly resistant to CAAX peptidomimetics and requires both a farnesyltransferase and a geranylgeranyltransferase I inhibitor in human tumor cell lines. *Oncogene* 1997; **15**:1283–1288.
24 Rowell CA, Kowalczyk JJ, Lewis MD, Garcia MD. Direct demonstration of geranylgeranylation and farnesylation of Ki-Ras *in vivo*. *J Biol Chem* 1997; **272**:14093–14097.
25 Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, *et al*. K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J Biol Chem* 1997; **272**:14459–14464.
26 Sun J, Qian Y, Hamilton AD, Sebti SM. Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. *Oncogene* 1998; **16**:1467–1473.
27 Venkatasubbarao K, Choudary A, Freeman JW. Farnesyl transferase inhibitor (R115777)-induced inhibition of STAT3 (Tyr705) phosphorylation in human pancreatic cancer cell lines require extracellular signal-regulated kinase. *Cancer Res* 2005; **65**:286171.
28 Eskens FA, Awada A, Cutler DL, de Jonge MJ, Luyten JP, Faber MN, *et al*. Phase I and pharmacokinetic study of the oral farnesyl transferase inhibitor SCH 66336 given twice daily to patients with advanced solid tumors. *J Clin Oncol* 2001; **19**:1167–1175.
29 Carter TA, Wodicka LM, Shah NP, Velasco AM, Fabian MA, Treiber DK, *et al*. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci U S A* 2005; **102**:11011–11016.