Demonstration of a Genetic Therapeutic Index for Tumors Expressing Oncogenic *BRAF* by the Kinase Inhibitor SB-590885

Alastair J. King,[1] Denis R. Patrick,[1] Roberta S. Batorsky,[1] Maureen L. Ho,[1] Hieu T. Do,[1] Shu Yun Zhang,[1] Rakesh Kumar,[1] David W. Rusnak,[1] Andrew K. Takle,[6] David M. Wilson,[6] Erin Hugger,[2] Lifu Wang,[7,8] Florian Karreth,[7,9] Julie C. Lougheed,[10] Jae Lee,[10] David Chau,[10] Thomas J. Stout,[10] Earl W. May,[1,3] Cynthia M. Rominger,[3] Michael D. Schaber,[3] Lusong Luo,[3] Ami S. Lakdawala,[5] Jerry L. Adams,[4] Rooja G. Contractor,[11] Keiran S.M. Smalley,[11] Meenhard Herlyn,[11] Michael M. Morrissey,[10] David A. Tuveson,[7,9] and Pearl S. Huang[1]

Departments of [1]Oncology, [2]Drug Metabolism and Pharmacokinetics, [3]Enzymology and Mechanistic Pharmacology, [4]Medicinal Chemistry, MMPD CEDD, GlaxoSmithKline, Collegeville, Pennsylvania; [5]Department of Computational, Analytical and Structural Sciences, Discovery Research, GlaxoSmithKline, King of Prussia, Pennsylvania; [6]Department of Medicinal Chemistry, NGI CEDD, GlaxoSmithKline, Harlow, Essex, United Kingdom; [7]Abramson Family Cancer Research Institute and Abramson Cancer Center at the University of Pennsylvania, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; [8]Department of Gastroenterology in Shanghai Rui Jin Hospital, Shanghai Second Medical University, Shanghai, China; [9]Cambridge Research Institute/Cancer Research UK, Cambridge, United Kingdom; [10]Exelixis, Inc., South San Francisco, California; and [11]The Wistar Institute, Philadelphia, Pennsylvania

Abstract

Oncogenic *BRAF* alleles are both necessary and sufficient for cellular transformation, suggesting that chemical inhibition of the activated mutant protein kinase may reverse the tumor phenotype. Here, we report the characterization of SB-590885, a novel triarylimidazole that selectively inhibits Raf kinases with more potency towards B-Raf than c-Raf. Crystallographic analysis revealed that SB-590885 stabilizes the oncogenic B-Raf kinase domain in an active configuration, which is distinct from the previously reported mechanism of action of the multi-kinase inhibitor, BAY43-9006. Malignant cells expressing oncogenic B-Raf show selective inhibition of mitogen-activated protein kinase activation, proliferation, transformation, and tumorigenicity when exposed to SB-590885, whereas other cancer cell lines and normal cells display variable sensitivities or resistance to similar treatment. These studies support the validation of oncogenic B-Raf as a target for cancer therapy and provide the first evidence of a correlation between the expression of oncogenic *BRAF* alleles and a positive response to a selective B-Raf inhibitor. (Cancer Res 2006; 66(23): 11100-5)

Introduction

The success of novel cancer therapies depends on the identification of functional targets that play an essential role in tumor growth and survival. One strategy for identifying such targets is to consider products of genes that have been amplified or have acquired activating mutations in a tumor genotype. This strategy is attractive if a causal link can be established between these events (gene amplification, mutation) and the disease state, and also if the "gain of function" that is acquired in the tumor cells can be antagonized by traditional small molecule inhibitors.

The activation and amplification of growth factor signaling pathways are commonly observed in many cancers. The clinical utility of therapies targeted to the Erb family growth factor receptors (e.g., trastuzumab, gefitinib, erlotinib, and lapatinib) and Bcr-Abl (e.g., imatinib), shows the dependence of the cancer phenotype on the enzymatic activity of these oncogenes (reviewed in ref. 1). Recently, a subset of patients with lung tumors expressing mutant, activated forms of ErbB1, were shown to be the most responsive to targeted kinase inhibition by gefitinib (reviewed in ref. 2). This correlation, between the presence of an activating mutation in an oncogene and a positive response to an inhibitor, suggests that responding populations to a targeted therapy can be identified based on the genetic signature of the target in tumors.

The requirement for growth factor signaling in tumor cells can be replaced by activating mutations in downstream effectors of the signaling pathway. In particular, the *KRAS* gene is frequently mutated to activated forms in pancreatic cancer, and activated forms of the *BRAF* gene have been identified in a number of neoplasms, including melanoma, ovarian, colorectal, thyroid, cholangiocarcinoma, and lung adenocarcinoma (reviewed in ref. 3). Activating mutations in the regulatory domain of B-Raf, most frequently, V600E, bypass the need for growth factor stimulation and create a constitutively activated kinase that has a 500-fold higher kinase activity, relative to the basal activity of wild-type protein (4). Expression of the activated *BRAF* gene has been shown to be both sufficient and necessary to maintain tumorigenesis in experimental settings. Introduction of activated $BRAF^{V600E}$ into immortalized melanocytes is sufficient to impart a transformed phenotype (5) and ablation of $BRAF^{V600E}$ mRNA by RNA interference induces growth inhibition and cell death in human tumor cell lines that express activated B-Raf (6, 7). Although it is clear that knock-down of B-Raf protein by RNA interference can impede the survival of tumor cells, these experiments do not address the utility of an enzyme inhibitor for this kinase in tumor cells.

A potent and specific inhibitor of the B-Raf kinase, SB-590885, was evaluated as a candidate therapeutic agent in cell culture models and used here to address the significance of an activating *BRAF* mutation in cancer. Importantly, SB-590885 showed potent and selective growth inhibition of tumor cells harboring a mutant

Requests for reprints: Denis R. Patrick, Department of Oncology, GlaxoSmithKline, Collegeville, PA 19426. E-mail: denis.r.patrick@gsk.com or David A. Tuveson, Cambridge Research Institute/Cancer Research UK, Cambridge, United Kingdom. E-mail: david.tuveson@cancer.org.uk.

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BRAF allele, both in human tumor cell lines and primary tumor samples, validating oncogenic B-Raf as an attractive therapeutic target in human malignancies.

Materials and Methods

Compounds and B-Raf/SB-590885 complex crystallization. SB-590885 and CI-1040 were synthesized (8, 9) and characterized to ensure purity. Human B-Raf kinase domain (residues 445–723) was expressed in Sf-9 insect cells using a modified pAcGP67 baculovirus DNA transfer vector (BD PharMingen, San Diego, CA). B-Raf produced from this vector contained an NH_2-terminal hexahistidine tag and TEV protease cleavage site (MLLGSH_6GENLYFQGS) and is referred to as N-His_6-B-Raf. Production of N-His_6-B-Raf in the soluble fraction was increased by coexpression with human Cdc37. After recovery over Ni-NTA resin (Qiagen, Valencia, CA), N-His_6-B-Raf was exchanged into 20 mmol/L Bis-Tris Propane (pH 7.0), 10% glycerol, 5 mmol/L 2-mercaptoethanol for cleavage with recombinant TEV protease. Cleaved B-Raf was then purified away from cleavage products over Ni-NTA. B-Raf [2 mg/mL in 20 mmol/L Bis-Tris Propane (pH 7.0), 15% glycerol, and 1 mmol/L DTT] was incubated with 0.5 mmol/L of SB-590885 for 30 minutes at room temperature, before mixing 1 μL of this solution with 1 μL of a reservoir solution of 8% PEG 4000, 0.1 mol/L of Tris (pH 8.5), and 0.4 mol/L of LiCl containing *apo* B-Raf micro-seeds. Complex crystals were grown in sitting drops at 18°C and transferred to a cryoprotectant solution, consisting of reservoir solution supplemented with 4% PEG 4000 and 35% ethylene glycol, before being flash-cooled by immersion in liquid nitrogen. Crystals belonged to the space group P41212 (a = 95.638 Å, c = 159.222 Å) and contained two complexes in the asymmetric unit. X-ray diffraction data were collected from a single crystal at beamline 9-1 of the Stanford Synchrotron Radiation Laboratory. Resultant images were processed with MOSFLM (10) and scaled with SCALA (Collaborative Computational Project, 1994). A molecular replacement solution was found with MOLREP (11) using a previously solved structure of B-Raf bound to another inhibitor as a search model. The model was subsequently refined in REFMAC (12) using rigid body refinement and maximum likelihood procedures. Fourier syntheses with coefficients of Fo-Fc and 2Fo-Fc yielded clearly interpretable electron density for the ligand, however, portions of the protein were adjusted by iterative cycles of manual rebuilding in QUANTA (Accelrys, Inc., San Diego, CA) and refinement in REFMAC before the ligand was placed into the Fo-Fc map using QUANTA/X-Ligand. The complex was subsequently refined using maximum likelihood procedures in REFMAC. Water molecules were built and manually inspected in QUANTA.

Cell lines. Human cell lines were obtained from the American Type Culture Collection (Manassas, VA), the Coriell Institute for Medical Research (Camden, NJ), and provided by M. Herlyn. *BRAF* mutations were determined by cDNA sequencing of the *BRAF* (GenBank accession no. M95712) mRNA isolated from growing cells using SuperScript III first-strand synthesis reverse transcription-PCR (Invitrogen, Carlsbad, CA) and HotStar Taq DNA polymerase (Qiagen). The primers used for amplification can be provided upon request. Amplicons were purified using Qiagen QIAquick PCR purification kits, sequenced with BigDye Terminator chemistry (Applied Biosystems, Foster City, CA). Newborn human melanocytes were prepared as described in http://www.wistar.org/herlyn/resource_culture_isolation.htm.

Biochemical and cellular assays. Western blot analysis was done as described using a LI-COR Odyssey IR imager (13). For proliferation assays, cells were treated with compounds in 0.1% DMSO and incubated for 72 hours at 37°C, 5% CO_2. Viable cells were quantified using CellTiter-Glo reagent (Promega, Madison, WI) and luminescence detection on a Victor 2^V plate reader (Perkin-Elmer, Turku, Finland). Cells were prepared for cell cycle analysis on a Becton Dickinson FACScan, according to the manufacturer's instructions. Data was acquired and analyzed using CellQuest v3.3 software. Anchorage-independent growth assays were done as described elsewhere (6), with inhibitors or DMSO vehicle included in the agar layer. Cultures were re-fed with media and inhibitor or DMSO every 5 to 7 days for a total of 28 days. Colonies were visualized and photographed by conventional light microscopy and quantified by counting on a grid in triplicate.

Spheroid preparation and growth assay. Human melanoma cells were isolated from metastatic lesions and grown to form spheroids, as previously described (14). Spheroid cultures were treated with compounds or DMSO and incubated for 72 hours. Metabolic activity was measured by conversion of the tetrazolium salt, WST-1 (Roche Diagnostics, Indianapolis, IN), in cultures for 4 hours at 37°C. Lack of metabolic activity in spheroid cultures was determined using the "Live/Dead cell kit" (Molecular Probes, Invitrogen, Carlsbad, CA), which does not distinguish between senescent cells and cells undergoing early apoptosis. Spheroids were photographed under inverted fluorescence microscopy using FITC/TRITC channels.

A375P xenograft model. The pharmacokinetic properties and safety of SB-590885, following i.p. injection, were determined and 50 mg/kg daily injections were found to give therapeutic levels with minimal body weight changes. Tumors were initiated in 8- to 12-week-old female nude mice by s.c. injection of 5×10^6 A375P cells in Matrigel suspension, and 3 weeks after tumor induction when the tumors had reached a volume of 150 to 250 mm^3, mice were randomized into groups of eight prior to treatment. Animals were treated with vehicle [2% *N,N*-dimethylacetamide, 2% Cremophor EL, and 96% acidified water (pH ~4–5)], or vehicle containing 50 mg/kg of SB-590885 daily for 21 days. A cohort of mice treated with

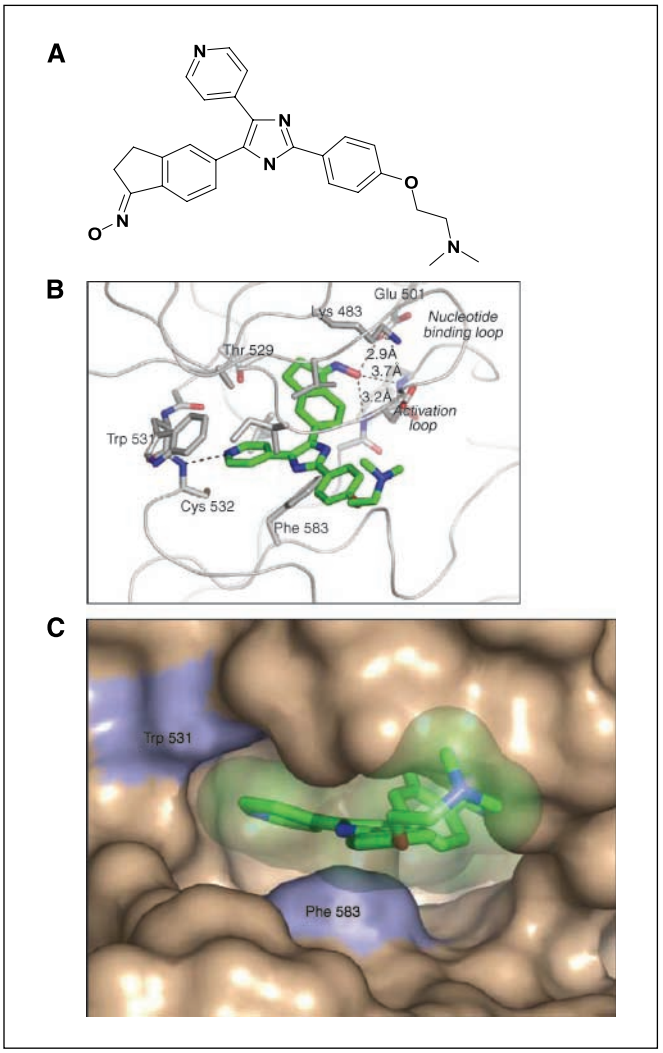


Figure 1. SB-590885 is a potent and selective inhibitor of B-Raf kinase. *A,* SB-590885 is a small molecule kinase inhibitor of the triarylimidazole class. *B,* the crystal structure of SB-590885 bound to B-Raf^{V600E} reveals competition for the ATP-binding pocket and interaction with nearby residues. *Dashed lines,* hydrogen bonds and electrostatic interactions. *C,* view of the packing between B-Raf and SB-590885. *Light blue,* F583 and W531 side chain surfaces; *beige,* protein surfaces. The ligand is shown with a transparent surface.

Table 1. Kinase inhibition selectivity profile for SB-590995

Kinase	Fold selectivity	Kinase	Fold selectivity	Kinase	Fold selectivity
Abl	>17,000	GSK3β	>17,000	p38δ	>17,000
AMPK	>17,000	IGFIR	>17,000	p70 S6K	>17,000
AurB/Aur1	>17,000	InsR	>17,000	PDGFRα	>17,000
B-Raf	1	JNK1	>17,000	PDGFRβ	>17,000
CDK2/cyclin A	>17,000	Kit	>17,000	PDK1	>17,000
CHK1	>17,000	Lck	~17,000	PhK	>17,000
CK1	~17,000	MAPKAP-K1α	>17,000	PI3 kinase	>17,000
CK2	>17,000	MAPKAP-K1β	>17,000	PKA	>17,000
c-Raf	11	MAPKAP-K2	>17,000	PKB1/PKBα	>17,000
CSK	>17,000	Met	>17,000	PKCα	>17,000
DYRK1A	>17,000	MKK1	>17,000	PKCβ1	>17,000
ERK2	>17,000	MSK1	>17,000	PRAK	>17,000
FGFR1	>17,000	Nek6	>17,000	ROCKα	>17,000
FGFR3	>17,000	p38α	>17,000	SGK	>17,000
FGFR4	>17,000	p38β	>17,000	TIE2	3,000
FLT3	>17,000	p38γ	>17,000	VEGFR2/KDR	>17,000

NOTE: SB-590885 displays selectivity for the B-Raf kinase *in vitro*. SB-590885 was evaluated *in vitro* against a panel of kinases in the ProQinase GmbH and Dundee University collections. Examples tested collectively covered the major kinase subfamilies and data are presented in terms of fold selectivity of the IC_{50} for SB-590885 versus B-Raf.

SB-590885 were then observed an additional 14 days following cessation of treatment. Tumor volume was measured for 55 days by calipers twice weekly. All *in vivo* procedures were carried out in accordance with protocols approved by the GSK Institutional Animal Care and Use Committee.

Results

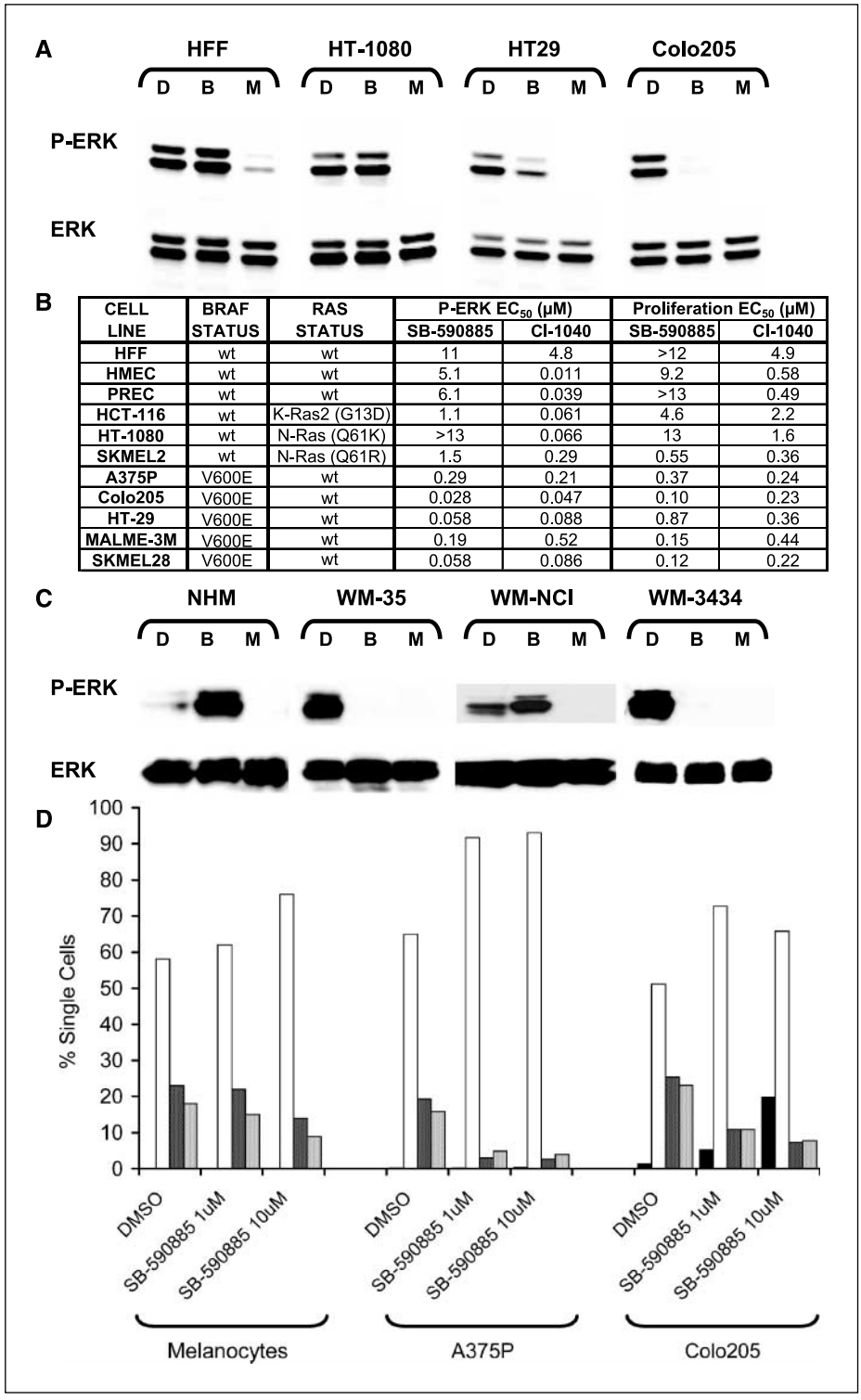
SB-590885 is a novel inhibitor of B-Raf that stabilizes the open conformation of the enzyme. Chemical inhibitors of B-Raf were designed as described previously (8). Using a coupled assay in which MEK activity was dependent on B-Raf activity, the triarylimidazole, SB-590885 (Fig. 1*A*), was shown to potently inhibit the oncogenic B-Raf protein kinase with a $K_{i\ app}$ of 0.16 ± 0.03 nmol/L.[12] Interestingly, selectivity towards B-Raf was shown as SB-590885 inhibited c-Raf with a $K_{i\ app}$ of 1.72 ± 0.65 nmol/L. These results showed that SB-590885 was a more potent inhibitor than the previously described Raf/VEGFR kinase inhibitor BAY 43-9006 ($K_{i\ app}$ = 38 nmol/L for mutant B-Raf, 6 nmol/L for c-Raf) and exhibited more selectivity towards B-Raf inhibition (15). SB-590885 was further profiled against a panel of 48 other human kinases, comprising representatives from all of the main branches of the human kinome (16). Strikingly, this compound displayed remarkable specificity for B-Raf inhibition, with significant activity measured only against c-Raf and minimal off-target activity (Table 1). Therefore, SB-590885 is a novel, low molecular weight compound that potently and selectively inhibits mutant B-Raf kinase activity.

The structural basis for B-Raf enzyme inhibition by SB-590885 was investigated by preparing co-crystals of the $B\text{-}Raf^{V600E}$ kinase domain and SB-590885, with X-ray diffraction patterns resolved at a resolution of 2.9 Å. Analysis determined that SB-590885 occupies the ATP-binding pocket and binds to an active conformation of B-Raf (Fig. 1*B* and *C*). The origin of the selectivity of SB-590885 for B-Raf seems to be due to interactions with several B-Raf amino acids, as well as the presence of the indane-oxime. In particular, a phenylalanine residue (F583) in the COOH-terminal lobe forms favorable π-stacking interactions with the imidazole and pyridine rings of SB-590885. Furthermore, a tryptophan residue (W531) is located above the opposing face of the pyridine, also forming favorable π-stacking interactions. Additionally, the indane-oxime forms a strong interaction with the salt bridge of B-Raf as well as a hydrogen bond with a backbone amide in the activation loop, thus likely contributing to the selectivity and potency of SB-590885. The stabilization of an active conformation of B-Raf by SB-590885 is in contrast to the report that BAY 43-9006 binds to the inactive conformation of B-Raf (4), demonstrating an alternative means to inhibit B-Raf kinase.

SB-590885 potently inhibits ERK phosphorylation and proliferation in tumor cells expressing $B\text{-}Raf^{V600E}$. The effects of B-Raf kinase inhibition on biochemical and cellular characteristics were determined by incubating cultures of normal and malignant human cell lines with SB-590885 (Fig. 2). Mitogen-activated protein kinase pathway inhibition was most apparent in established colorectal carcinoma (Colo205 and HT29) and melanoma cell lines (A375P, SKMEL28, and MALME-3M) that expressed $B\text{-}Raf^{V600E}$ following brief incubation with SB-590885 (Fig. 2*A* and *B*; data not shown), whereas normal cells (HFF, HMEC, and PREC) and malignant cells not expressing mutant B-Raf (HT-1080, HCT-116, and SKMEL2) showed intermediate sensitivity or resistance. Furthermore, prolonged incubation with SB-590885 preferentially decreased the proliferation of cells expressing oncogenic $B\text{-}Raf^{V600E}$ (Fig. 2*B*). As previously reported (17), the MEK inhibitor, CI-1040, also potently inhibited ERK signaling and proliferation in cells expressing *BRAF* mutations. However, in contrast to SB-590885, CI-1040 showed significant inhibition of ERK signaling and cellular proliferation in normal and malignant cells that do not express mutant B-Raf (Fig. 2*A* and *B*). Cell cycle analysis revealed that a G_1 phase cell cycle block was the predominant mechanism of

[12] E. May, C. Rominger, M. Schaber, and P. Huang, unpublished data.

decreased proliferation, with only minimal induction of apoptosis and a cytostatic growth arrest apparent in adherent cell cultures (Fig. 2*D*; data not shown). A panel of normal melanocytes (newborn human melanocytes), early stage melanoma cells (RGP line WM-35), and primary melanoma cells (WM-NCI and WM-3434) also showed decreased phosphorylated ERK levels following treatment with CI-1040; and, as expected, only those cells harboring oncogenic B-Raf^{V600E} showed decreased ERK signaling following SB-590885 treatment (Fig. 2*C*). Interestingly, both normal melanocytes and primary melanoma cells that express wild-type B-Raf (WM-NCI) paradoxically showed increased phosphorylated ERK levels following brief incubation with SB-590885 (Fig. 2*C*). Importantly, the increased phosphorylated ERK levels in normal melanocytes did not correlate with any appreciable changes in the cell cycle profile, with partial G_1 arrest noted at high concentrations of SB-590885 (Fig. 2*D*). Therefore, SB-590885 may represent an



CELL LINE	BRAF STATUS	RAS STATUS	P-ERK EC_{50} (μM)		Proliferation EC_{50} (μM)	
			SB-590885	CI-1040	SB-590885	CI-1040
HFF	wt	wt	11	4.8	>12	4.9
HMEC	wt	wt	5.1	0.011	9.2	0.58
PREC	wt	wt	6.1	0.039	>13	0.49
HCT-116	wt	K-Ras2 (G13D)	1.1	0.061	4.6	2.2
HT-1080	wt	N-Ras (Q61K)	>13	0.066	13	1.6
SKMEL2	wt	N-Ras (Q61R)	1.5	0.29	0.55	0.36
A375P	V600E	wt	0.29	0.21	0.37	0.24
Colo205	V600E	wt	0.028	0.047	0.10	0.23
HT-29	V600E	wt	0.058	0.088	0.87	0.36
MALME-3M	V600E	wt	0.19	0.52	0.15	0.44
SKMEL28	V600E	wt	0.058	0.086	0.12	0.22

Figure 2. SB-590885 preferentially inhibits ERK phosphorylation and proliferation in tumor cells expressing B-Raf^{V600E}. *A,* SB-590885 and CI-1040 were compared for their ability to inhibit ERK phosphorylation in normal and malignant cell lines at single 2 μmol/L concentrations following 60-minute incubations by Western blot analysis (*D,* DMSO; *B,* SB-590885; *M,* CI-1040). *B,* ERK phosphorylation and cellular proliferation are selectively inhibited in cells expressing oncogenic B-Raf. SB-590885 or CI-1040 was used at a concentration range of 1 nmol/L to 20 μmol/L, and a tabular summary of EC_{50} values determined from calculated ratios of phospho-ERK1/2 to total ERK1/2 and cellular proliferation is given. Data are representative of two or more runs and are shown with the *BRAF* and *RAS* status of each cell line for reference. *C,* effects of SB-590885 and CI-1040 on ERK phosphorylation in normal and malignant melanocytes at single 2 μmol/L concentrations following 60 minutes of incubation by Western blot analysis (*D,* DMSO; *B,* SB-590885; *M,* CI-1040). *D,* SB-590885 inhibits cell growth in normal melanocytes and adherent tumor cells bearing B-Raf^{V600E} predominantly by induction of a G_1 arrest. The effect of incubation with 1 and 10 μmol/L of SB-590885 for 24 hours was examined in normal human melanocytes, A375P and Colo205 tumor cells. Staurosporine was used at 5 μmol/L for 6 hours as a positive control for the induction of apoptotic cell death (data not shown). Sub-G_1 (*solid columns*), G_1 phase (*open columns*), S phase (*hatched columns*), and G_2-M phase (*speckled columns*); representative of two independent experiments.

improved therapeutic approach for the treatment of tumors expressing oncogenic B-Raf, as it spares more normal cells compared with MEK inhibition, and it is the first B-Raf kinase inhibitor described with selectivity towards tumor cells expressing oncogenic B-Raf.

SB-590885 inhibits cellular transformation and tumorigenesis. The promising results obtained with SB-590885 treatment of adherent cell cultures suggested that SB-590885 may have therapeutic relevance in three-dimensional preclinical model systems. Accordingly, the potential antineoplastic effects of SB-590885 were directly examined using several *in vitro* and *in vivo* assays of cellular transformation. Consistent with the previously reported requirement for B-Raf^{V600E} protein in melanoma cells (6, 7), inhibition of B-Raf kinase with SB-590885 decreased anchorage-independent growth of established melanoma cell lines in a *BRAF* mutant–selective manner over a dose range reflecting the potency of SB-590885 in two-dimensional culture (Figs. 2*B* and

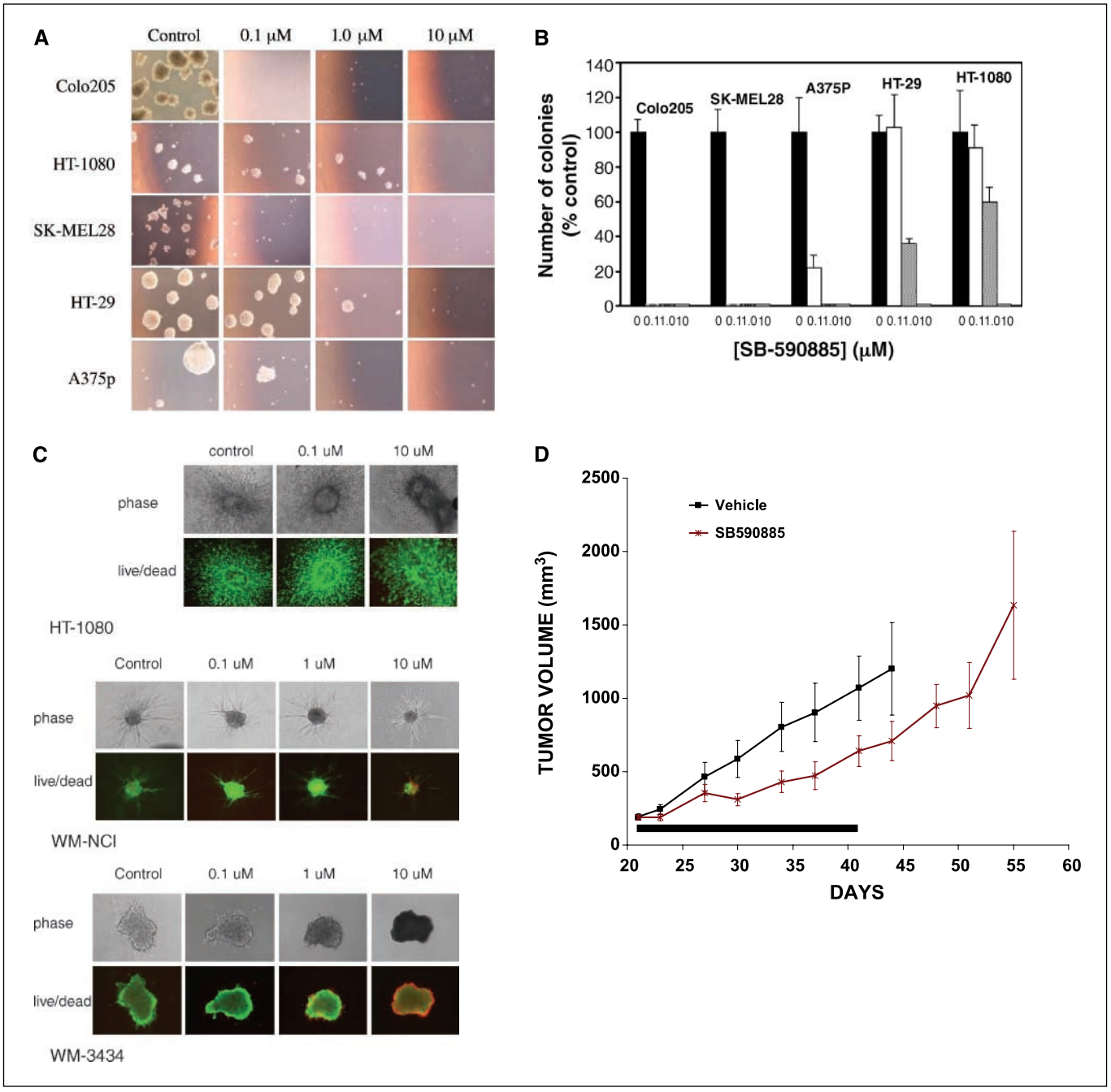


Figure 3. Prolonged exposure to SB-590885 inhibits cellular transformation and proliferation in three-dimensional culture. *A,* SB-590885 inhibits anchorage-independent tumor colony growth selectively in tumor cell lines expressing B-Raf^{V600E} (light microscopy, 4×). *B,* anchorage-independent growth was quantified by counting individual colonies on a grid in triplicate and normalizing to vehicle controls (±SE) for each cell line. *C,* spheroid cultures of HT-1080, WM-NCI, and WM-3434 were treated with a range of SB-590885 for 72 hours, and assessed for invasiveness by light microscopy (*top*) and metabolic activity by using the "Live/Dead cell assay" as indicated by the green and red fluorescent images, respectively. *D,* decreased tumorigenicity of A375P cells following treatment with SB-590885. *Points,* tumor growth for cohorts of animals treated with vehicle alone (■) or with SB-590885 (X); *bars,* ±SE. Mice were treated for 21 days, and following this, the mice treated with SB-590885 were observed for an additional 14 days.

3*A* and *B*). This result was extended to primary human melanoma cell spheroids to determine the effects of B-Raf inhibition on melanoma cell invasion and metabolic activity. As expected, when treated with SB-590885, spheroids comprised of melanoma cells expressing mutant B-Raf (WM-3434) showed decreased matrix invasion and metabolic activity as compared with spheroids comprised of HT-1080 fibrosarcoma cells or melanoma cells expressing wild-type B-Raf (WM-NCI; Fig. 3*C*), over a dose range reflecting the potency of SB-590885 in two-dimensional culture (data not shown). Finally, SB-590885 decreased tumorigenesis in murine xenografts established from mutant B-Raf-expressing A375P melanoma cells (Fig. 3*D*). Although the effect on tumor growth was modest, it is consistent with the average degree of target inhibition observed in treated tumor tissues (~ 50–80% reduction in pERK levels; data not shown). The regrowth of tumors after treatment cessation is also consistent with work in cell culture, as tissue analysis showed no overt evidence of increased apoptosis (data not shown). Therefore, SB-590885 showed important characteristics of a targeted therapeutic by decreasing the transformed and tumorigenic properties of malignant cells expressing oncogenic B-Raf.

Discussion

The generation of specific agents that neutralize required oncogenic signaling pathways in tumors is a major thrust of cancer drug discovery, and here, we describe the characteristics of the first specific B-Raf inhibitor, SB-590885. Both the potency and specificity of SB-590885 show attractive properties for further development of this and related compounds as therapeutics in cancer medicine.

SB-590885 inhibits B-Raf kinase enzymatic activity ~ 100-fold more potently than the Raf/VEGFR inhibitor, BAY 43-9006. The structural basis of B-Raf inhibition by SB-590885 is due to stabilization of the active conformation of B-Raf by SB-590885, in contrast to stabilization of the inactive conformation of B-Raf by BAY 43-9006 (4). This alternative mechanism for kinase inhibition offers an additional method to target oncogenic B-Raf in patients with melanoma and the potential for overcoming generated resistance to inhibitors which bind to the inactive conformation of B-Raf, as has been shown for other oncogene-dependent malignancies (18).

Preferential inhibition by SB-590885 of biochemical signaling, proliferation, survival, and transformation was noted for human tumor cell lines expressing oncogenic B-Raf. The correlation between $B\text{-}Raf^{V600E}$ expression and the inhibition of proliferation induced by SB-590885 was striking. Two tumor cell lines harboring mutant *KRAS* (HCT-116) or *NRAS* (SKMEL2) alleles exhibited moderate sensitivity to SB-590885 and the MEK inhibitor CI-1040, consistent with prior work (17). These data suggest a primary role for B-Raf as a downstream effector of Ras in these tumor cell lines. Importantly, little to no activity was observed for SB-590885 in three normal human cell types. The mechanism for the paradoxical increase in phosphorylated ERK in normal melanocytes and melanoma cells expressing wild-type B-Raf following treatment with SB-590885 (Fig. 2*C*) is currently unknown, but may reflect alterations in biochemical feedback inhibition loops (19, 20). Nonetheless, this change in the phosphorylation state for ERK did not correlate with increased cell proliferation in either case. In contrast, the MEK inhibitor, CI-1040, inhibited ERK phosphorylation and cellular proliferation in a wide array of malignant and normal cells in the panel tested. The lack of selectivity between the different tumor cell types and primary cells following treatment with CI-1040 is consistent with the fact that MEK is an effector of multiple signaling pathways in mammalian cells (9, 19), and predicts that direct B-Raf inhibition will be advantageous in the treatment of patients suffering from oncogenic BRAF-driven tumors. Therefore, by exhibiting this "genetic therapeutic index" towards mutant B-Raf-expressing malignant cells, SB-590885 represents an additional compound that should be considered in the clinical development of small molecule therapeutics targeting oncogenic B-Raf.

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