

Bosutinib Reduces the Efficacy of Dasatinib in Triple-negative Breast Cancer Cell Lines

MIKE TARPLEY¹, TEMESGEN T. ABDISSA¹, GARY L. JOHNSON^{2,3} and JOHN E. SCOTT^{1*}

¹Department of Pharmaceutical Sciences, Biomanufacturing Research Institute and Technology Enterprise, North Carolina Central University, Durham, NC, U.S.A.;

²Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, U.S.A.;

³Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, U.S.A.

Abstract. *Background:* Triple-negative breast cancer (TNBC) is an aggressive sub-type of breast cancer. Dasatinib and bosutinib are FDA-approved Src/Abl kinase inhibitor drugs. Dasatinib potently inhibits the proliferation of many TNBC cell lines. *Materials and Methods:* The cell viability/proliferation for a panel of 4 TNBC cell lines was measured by detection of cellular ATP levels and cell numbers were directly determined by automated cell counting. *Results:* Bosutinib ($\leq 1 \mu\text{M}$) had little to no inhibitory activity on cell viability/proliferation, while dasatinib-alone generated potent IC_{50} values of $<100 \text{ nM}$. Combination treatment of cells with both dasatinib and bosutinib resulted in reduced efficacy of dasatinib in all four cell lines, with two of them displaying a dramatic loss of efficacy. Direct cell counting confirmed that bosutinib enhanced cell proliferation in the presence of dasatinib. *Conclusion:* Bosutinib potently reduced the *in vitro* anti-proliferative efficacy of dasatinib in TNBC cell lines. We, hereby, report on a novel drug-induced loss in dasatinib sensitivity.

Approximately 15-20% of breast cancers are negative for the expression of estrogen receptor (ER) and progesterone (PR) expression and lack ErbB2 overexpression (1). Hence, this clinical sub-type of breast cancer is referred to as triple-negative breast cancer (TNBC). This clinical phenotype significantly overlaps with the basal-type breast cancer sub-type established, based on whole-transcriptome microarray gene expression analysis of breast cancers (2). The diagnosis

of TNBC correlates with aggressive cancer and there are limited treatment options, with no efficacious targeted-therapies.

TNBC has the general characteristic of aggressive relapses *i.e.* initially the cancer responds to chemotherapy, but then rapidly gains resistance and grows back (3). There are at least three general mechanisms for acquired resistance to targeted or traditional anticancer therapy. One of the most straightforward mechanisms is mutation of the drug target such as mutations in the kinase inhibitor targets EGFR, Abl and ALK (4-8). Another mechanism for acquired resistance is the activation of alternate pathways that bypass the original target which allow cells to proliferate and/or survive, as has been demonstrated for Met and ERBB2 (9, 10). Resistance to chemotherapy can also arise due to up-regulation of ABC transporters that actively pump drugs out of the cell, thereby reducing the intracellular drug concentration (11).

Dasatinib (BMS-354825) is a Food and Drug Administration (FDA)-approved cancer drug which has been approved for treatment of acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML) (12). Dasatinib is an orally-active ATP-competitive small-molecule kinase inhibitor that potently inhibits Abl kinase, Src family kinases and other kinases (13). From *in vitro* biochemical kinase profiling data, this drug also potently ($\text{IC}_{50} \leq 1 \text{ nM}$) inhibits various other kinases including c-Kit, PDGFR and kinases in the EPH family. In addition to its activity against leukemia cells, dasatinib has also been shown to inhibit cell proliferation in numerous different types of tumor cell lines, including basal-type/TNBC (14), gastric (15), pancreatic (16), head and neck (17), ovarian (18), prostate (19) and lung cell lines (20, 21). The activity in solid tumors has been proposed to be due to the inhibition of Src family kinases. However, the DDR2 kinase was identified as a target of dasatinib in squamous cell lung cancer cells (22, 23). In mouse models of prostate and pancreatic cancer, dasatinib

Correspondence to: John E. Scott, Ph.D., 1801 Fayetteville St, BRITE Bldg, Rm 1019, North Carolina Central University, Durham, NC 27707, U.S.A. Tel: +1 9195307569, Fax: +1 9195306600, e-mail: jscott@nccu.edu

Key Words: Bosutinib, dasatinib, breast cancer.

inhibited tumor growth and metastasis (24, 25). Therefore, numerous clinical trials are on-going in which dasatinib is being assessed as monotherapy or in combination with other cancer drugs for efficacy against many cancer types.

Most basal-type and post-EMT cell lines have been discovered to be highly sensitive to cell growth inhibition by dasatinib *in vitro* with IC₅₀ values <1 μ M (14, 20, 26). In contrast, luminal-subtype breast cancer cell lines are generally resistant (IC₅₀ >1 μ M) (14). In general, the effect of dasatinib on dasatinib-sensitive breast cancer cell lines is predominately anti-proliferative with modest to no induction of apoptosis and cell death (27, 28). In the dasatinib sensitive TNBC cell line MDA-MB-231, dasatinib treatment induces G₁ accumulation with little apoptosis, disrupts cell morphology and blocks *in vitro* migration and invasion (28). Despite this potent *in vitro* activity, dasatinib as a monotherapy failed to demonstrate significant efficacy against TNBC in a Phase 2 clinical trial (29). Other clinical trials are on-going using dasatinib in combination with other chemotherapies for TNBC.

Bosutinib (SKI-606) is also an FDA-approved drug for the treatment of Philadelphia chromosome positive (Ph+) CML in adult patients with resistance or intolerance to prior therapy (30). Like dasatinib, bosutinib is an ATP competitive Abl and Src inhibitor (31). However, chemical proteomics approaches have identified 40 to >45 target kinases for bosutinib as well as dasatinib (32, 33). Bosutinib is also being studied for use in many other cancers.

We report that bosutinib potently inhibits the anti-proliferative effect of dasatinib on four TNBC cell lines. In a direct measure of cell number, the combination of dasatinib and bosutinib on MDA-MB-231 cells resulted in increased cell number compared to dasatinib alone.

Materials and Methods

Materials. All common reagents such as dimethyl sulfoxide (DMSO) were reagent-grade quality and obtained from Thermo Fisher Scientific (Waltham, MA, USA) or Sigma-Aldrich (St. Louis, MO, USA). Tissue culture media, fetal bovine serum (FBS) and supplements were purchased from Thermo Fisher Scientific. The CellTiter-Glo™ viability assay kit (cat# G7573) was obtained from Promega (Madison, WI, USA). Dasatinib and bosutinib were obtained from LC Laboratories (Woburn, MA, USA) and Selleck Chemicals (Houston, TX, USA), respectively. The manufacturer asserted that this was genuine bosutinib and, in addition, we subjected our lot of bosutinib to NMR analysis and confirmed the structure of the bosutinib used in this report as being genuine bosutinib and not an isomer (34).

Cell culture. All cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured at 37°C in an atmosphere of 5% CO₂ and 95% humidity in their respective media. MDA-MB-231 and Hs578T cells were grown in DMEM-high glucose media supplemented with 10% FBS, 200 mM

L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin and 15 mM HEPES. HCC1143 and HCC1806 were grown in RPMI-1640 supplemented in the same way. Cells used for experiments did not exceed passage 15.

Cell viability assay. Cells were harvested from tissue culture flasks for assay with trypsin/EDTA solution (Versene, Fisher). 80 μ l of cells in normal growth media were added to a 96-well plate such that 2,500 cells/well were plated. After an overnight incubation (16-24 h) in the incubator, cells were treated with compounds. Compound stock solutions were made in 100% DMSO. Serial dilutions of compounds for IC₅₀ determinations were initially performed in 100% DMSO, followed by appropriate dilutions in growth culture media, producing a constant 0.1% DMSO in all wells. Subsequently, 10 μ l of each diluted compound (or 20 μ l of DMSO for controls or 10 μ l diluted DMSO for single-compound treatments) were added to the wells for a final volume of 100 μ l and 0.1% DMSO. After a 72-h exposure to compounds, cell viability was assessed using the CellTiter Glo kit (Promega) per manufacturer's protocol. Luminescence was measured using a BMG Pherastar plate reader. Relative luminescence units (RLU), were normalized to DMSO (100% activity) and "no cells" (0% activity) controls as maximum and minimum signals, respectively. Compound concentration response curves were generated using data points that represent the average of three determinations per concentration. All IC₅₀/EC₅₀ values provided are averages of at least three independent determinations. The IC₅₀/EC₅₀ values were calculated from concentration-response data using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA) employing either a four-parameter or a three-parameter (fixed bottom or top) curve fit.

Cell counting assay. MDA-MB-231 cells were seeded in 6-well plates at 120,000 cells per well and incubated at 37°C and 5% CO₂ for 16-24 h. Cells were treated with the compound as described above. At the time of harvesting, treatment media were removed (and saved); cells were washed briefly in phosphate buffered saline (PBS), trypsinized and complete growth media added. In order to ensure the collection of all cells (living and dead), supernatants, washes, trypsinized cells, and recovery media were pooled and cell count/viability determined using trypan blue and a Vi-CELL XR cell viability analyzer (Beckman Coulter, Brea, CA, USA) following manufacturer's guidelines.

Results

We tested a panel of kinase inhibitor drugs to distinguish the ones that could induce cell death in the presence of the anti-proliferative drug dasatinib, using the TNBC cell line MDA-MB-231. Surprisingly, we discovered that one drug, bosutinib, appeared to inhibit the anti-proliferative activity of dasatinib. In the present report, we have used a panel of 4 dasatinib-sensitive TNBC cell lines and cell viability/proliferation assays to confirm this initial observation. Within the time frame of our 96-well cell viability assay (72 h), these cell lines approximately doubled in number and hence complete inhibition of proliferation resulted in approximately half the signal, representing the originally plated cells. We performed bosutinib concentration response determinations in the

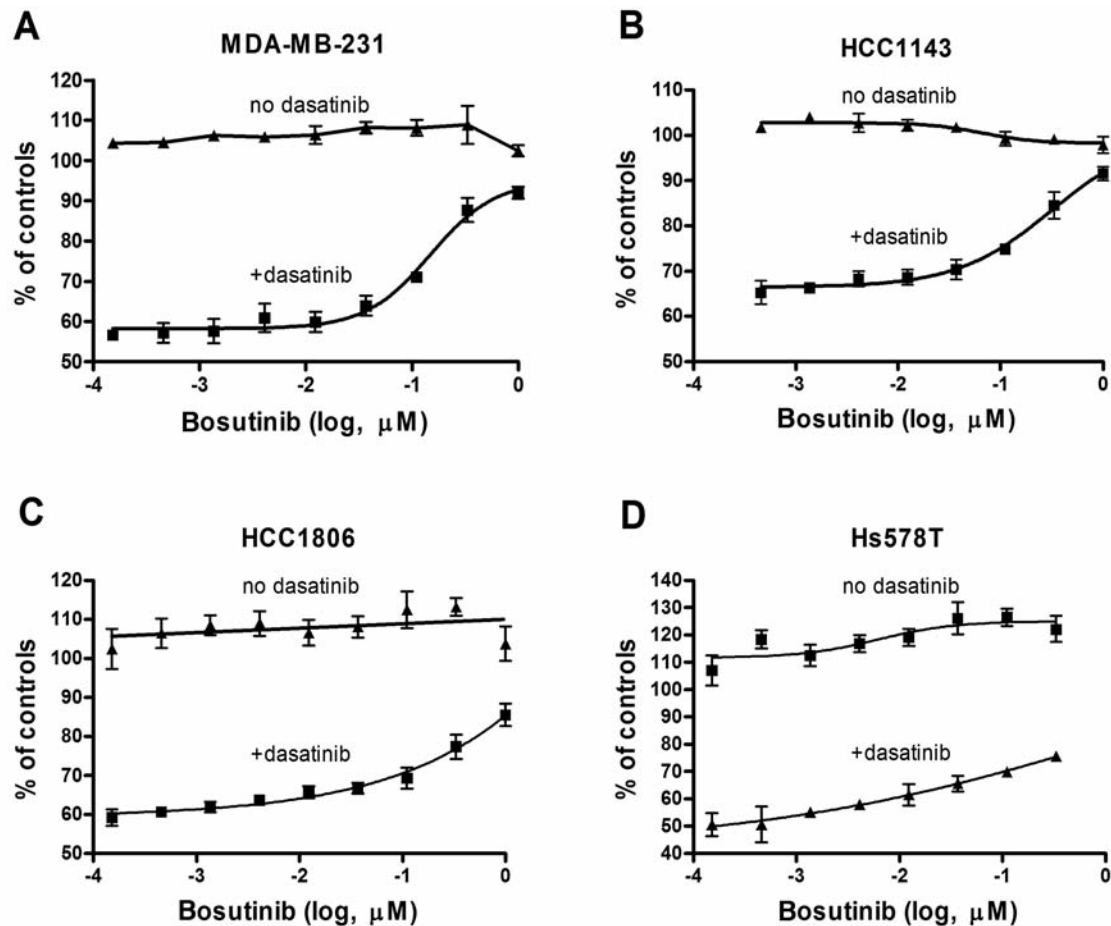


Figure 1. Bosutinib concentration response in cell viability assays with and without dasatinib. Bosutinib concentration response data were generated in the presence and absence of 100 nM dasatinib using the ATP detection cell viability assay. The assay was performed with the cell lines MDA-MB-231 (A), HCC1143 (B), HCC1806 (C) and Hs578T (D). Data were normalized to cells treated with compound vehicle (DMSO) and wells without cells and expressed as a percentage of these controls. Data points represent the average of three determinations per variable and error bars represent standard deviations. Data are representative of three independent experiments.

presence or absence of 100 nM dasatinib for the TNBC cell lines MDA-MB-231, HCC1143, HCC1806 and Hs578T (Figure 1). Bosutinib (up to 1 μM)-alone had little to no effect on the cell viability assay for all the cell lines tested (Figure 1). These data also indicated that bosutinib does not affect the assay detection method itself (*e.g.* enhancing luciferase activity). At 100 nM, dasatinib almost completely blocked cell proliferation in these cell lines (Figure 2) and is similar to the 90 nM serum concentration achieved in human clinical trials (35). In the presence of a constant 100-nM dasatinib, bosutinib demonstrated a dose-dependent increase in assay signal for all four cell lines (Figure 1). The MDA-MB-231 and HCC1143 breast cancer cell lines showed the most dramatic response to bosutinib, with maximal response at 1 μM bosutinib resulting in signals greater than 90% of control values. Since these are partial curves, we fixed the top of the bosutinib/dasatinib curve

at 100% of controls to obtain relative effective concentration of bosutinib required for a 50% reversal of dasatinib-mediated inhibition (EC_{50}). The calculated average bosutinib EC_{50} values ($n=3$) for MDA-MB-231, HCC1143, HCC1806 and Hs578T were 159 ± 83 nM, 407 ± 287 nM, 823 ± 303 nM and >300 nM (highest bosutinib concentration tested). Thus, bosutinib potentially reduced the dasatinib-mediated inhibition in this cell viability/proliferation assay.

To directly assess the effect of bosutinib on the anti-proliferative activity of dasatinib, we determined viable cell numbers using the trypan blue exclusion and an automated cell counter. We chose the MDA-MB-231 cell line for this assay where we exposed cells for 72 h to DMSO (control), 100 nM dasatinib, 500 nM bosutinib or the combination of dasatinib/bosutinib at these same concentrations (Figure 2). As expected, treatment with dasatinib resulted in approximately

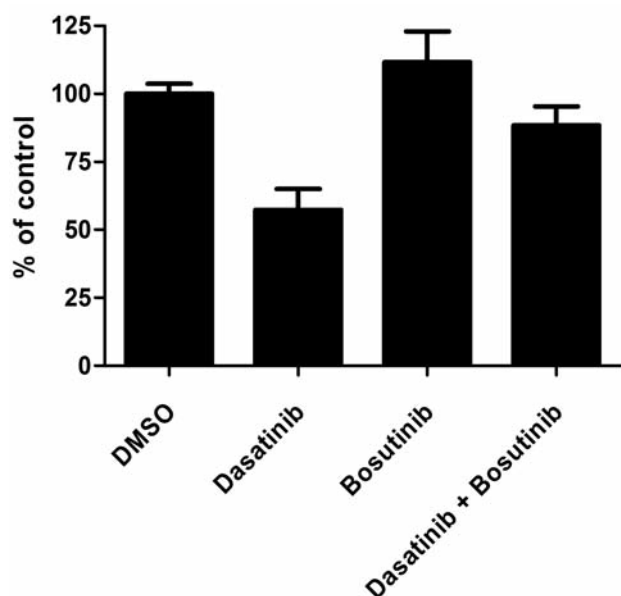


Figure 2. The effect of bosutinib on the proliferation of dasatinib-treated cells measured by automated cell counting. Cells were treated with DMSO, 100 nM dasatinib, 500 nM bosutinib and the combination of dasatinib (100 nM)/bosutinib (500 nM) for 72 h, followed by trypsinization and cell counting in the presence of trypan blue. Non-stained viable cell counts were determined by an automated cell counter and graphed. Data points represent the average of three determinations per condition and error bars represent standard deviations. Data are representative of two independent experiments.

half the number of viable cells compared to cells treated with DMSO (vehicle control). While bosutinib-alone had no significant effect on cell number, bosutinib partially reversed dasatinib-mediated inhibition of cell proliferation.

In order to further characterize the apparent antagonistic effect of bosutinib on dasatinib activity, we performed dasatinib IC_{50} determinations with and without a fixed concentration of bosutinib (Figure 3). Depending on the cell line, bosutinib was fixed at 0.3-1 μ M. The average relative IC_{50} values for all four cell lines ranged from 32-57 nM. In the presence of bosutinib, the relative potency of dasatinib in MDA-MB-231, HCC1806 and Hs578T did not change significantly – less than two-fold (range: 40-90 nM IC_{50} values). However, the maximal efficacy (inhibition) was significantly reduced in these three cell lines, especially dramatic for MDA-MB-231 (see Figure 3). Bosutinib appeared to reduce the maximal efficacy achieved in all three cell lines. For HCC1143, the IC_{50} for dasatinib shifted from an average relative IC_{50} of 32 nM to >3,000 nM in the presence of 1 μ M bosutinib. Consistent with results from Figure 1, MDA-MB-231 and HCC1143 demonstrated the most dramatic reversal of dasatinib's activity by bosutinib.

Discussion

We used a cell viability/proliferation assay to characterize the effect of bosutinib on the anti-proliferative activity of dasatinib using a panel of 4 TNBC cell lines. We have discovered a surprising effect of the anti cancer drug bosutinib. By itself, bosutinib at ≤ 1 μ M had little to no inhibitory activity on cell viability/proliferation in these cell lines, while dasatinib alone generated potent IC_{50} s. Treatment of all cell lines with bosutinib showed a dose-dependent increase in signal in the presence of a fixed concentration of dasatinib. We confirmed that bosutinib was inducing cell proliferation in the presence of dasatinib by performing direct cell counting. This partial reversal of the effect of dasatinib was reflected in IC_{50} determinations where the presence of bosutinib shifted the IC_{50} and/or efficacy of dasatinib in all 4 TNBC cell lines. Thus, in these cell lines, bosutinib appeared to antagonize the effect of dasatinib.

There were significant differences in the magnitude of activity that bosutinib had in the cell lines. The MDA-MB-231 and HCC1143 cell lines showed dramatic responses to bosutinib in the presence of dasatinib, especially of HCC1143 which almost lost responsiveness to dasatinib. For HCC1806 and Hs578T, bosutinib reduced the inhibitory activity (efficacy), but to a lesser extent than the other 2 lines. Therefore, the magnitude of inhibiting the effect of dasatinib on cells is clearly cell line dependent.

The mechanism for this bosutinib-induced resistance to dasatinib is unknown. Both dasatinib and bosutinib are frequently referred to as Src or Src family inhibitors. However, both compounds have multiple potential cellular kinase targets as indicated by selectivity studies (32). Kinome re-programming is a term for the activity of certain kinase inhibitors that have been discovered to paradoxically activate cell signaling pathways. For example, MEK inhibitors have been shown to indirectly induce receptor tyrosine kinase (RTK) activation in cells (36). To date, bosutinib has not been reported to trigger kinome re-programming. We postulate that bosutinib induces kinome re-programming of the cell signaling cascades in TNBC cell lines. Bosutinib may activate or enhance alternate cell signaling pathways that circumvent the blockade of cell proliferation caused by dasatinib.

There are several significant aspects to these results. This is the first report to show that bosutinib induces resistance to another drug. Whether this effect is specific to dasatinib or not will require further studies with other anti-proliferative drugs. These data also suggest that TNBC cells may be able to readily-mutate or over-express a pathway component – the same one postulated to be activated by bosutinib – and gain resistance to dasatinib chemotherapy. Of note, use of dasatinib-alone to treat TNBC patients has failed to show efficacy in clinical trials (29). This may be due to rapid selection of cancer cells that activate other pathways to circumvent the dasatinib blockade. Finally, the clinical use

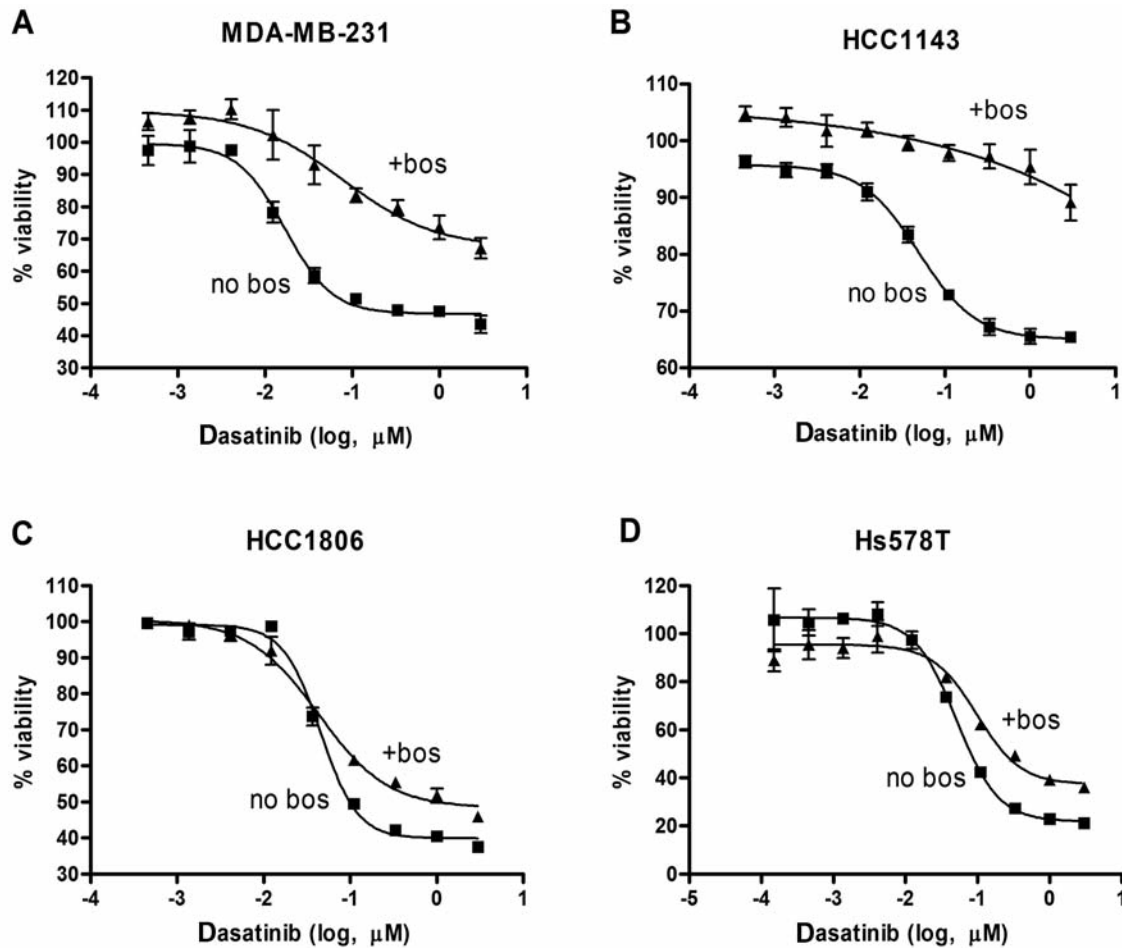


Figure 3. Dasatinib concentration response in cell viability assays with and without bosutinib. Dasatinib concentration response data were generated in the presence and absence of 0.3-1 μM bosutinib (bos) using the ATP detection cell viability assay. The assay was performed with MDA-MB-231 (A), HCC1143 (B), HCC1806 (C) and Hs578T (D). The data were normalized to cells treated with compound vehicle (DMSO) and wells without cells and expressed as a percentage of these controls. Data points represent the average of three determinations per variable and error bars represent standard deviation. Data are representative of three independent experiments.

of the combination of dasatinib and bosutinib for treatment of TNBC patients, and perhaps other cancers, should be avoided without further studies.

Acknowledgements

This work was supported by National Institutes of Health (NIH) grant U54CA156735 (JES and GLJ) and in part by a grant from the Golden LEAF Foundation (JES) and funds from the State of North Carolina (JES).

References

- Griffiths CL and Olin JL: Triple negative breast cancer: a brief review of its characteristics and treatment options. *Journal of pharmacy practice* 25: 319-323, 2012.
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO and Botstein D: Molecular portraits of human breast tumours. *Nature* 406: 747-752, 2000.
- Dawson SJ, Provenzano E and Caldas C: Triple negative breast cancers: clinical and prognostic implications. *Eur J Cancer* 45(Suppl 1): 27-40, 2009.
- Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG and Halmos B: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 352: 786-792, 2005.
- Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG and Varmus H: Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS medicine* 2: e73, 2005.

- 6 Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y, Mano H, and Group ALKLCs: EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N Engl J Med* 363: 1734-1739, 2010.
- 7 Katayama R, Shaw AT, Khan TM, Mino-Kenudson M, Solomon BJ, Halmos B, Jessop NA, Wain JC, Yeo AT, Benes C, Drew L, Saeh JC, Crosby K, Sequist LV, Iafrate AJ and Engelman JA: Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers. *Sci Transl Med* 4: 120ra117, 2012.
- 8 Ernst T, La Rosee P, Muller MC and Hochhaus A: BCR-ABL mutations in chronic myeloid leukemia. *Hematology/oncology clinics of North America* 25: 997-1008, v-vi, 2011.
- 9 Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC and Janne PA: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316: 1039-1043, 2007.
- 10 Takezawa K, Pirazzoli V, Arcila ME, Nebhan CA, Song X, de Stanchina E, Ohashi K, Janjigian YY, Spitzler PJ, Melnick MA, Riely GJ, Kris MG, Miller VA, Ladanyi M, Politi K and Pao W: HER2 amplification: a potential mechanism of acquired resistance to EGFR inhibition in EGFR-mutant lung cancers that lack the second-site EGFR T790M mutation. *Cancer discovery* 2: 922-933, 2012.
- 11 Wu CP, Hsieh CH and Wu YS: The emergence of drug transporter-mediated multidrug resistance to cancer chemotherapy. *Mol Pharm* 8: 1996-2011, 2011.
- 12 Olivieri A and Manzione L: Dasatinib: a new step in molecular target therapy. *Ann Oncol* 18(Suppl 6): vi42-46, 2007.
- 13 Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Corneliussen LA, Das J, Doweiko AM, Fairchild C, Hunt JT, Inigo I, Johnston K, Kamath A, Kan D, Klei H, Marathe P, Pang S, Peterson R, Pitt S, Schieven GL, Schmidt RJ, Tokarski J, Wen ML, Wityak J and Borzilleri RM: Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem* 47: 6658-6661, 2004.
- 14 Finn RS, Dering J, Ginther C, Wilson CA, Gaspy P, Tchekmedyian N and Slamon DJ: Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing *in vitro*. *Breast Cancer Res Treat* 105: 319-326, 2007.
- 15 Okamoto W, Okamoto I, Yoshida T, Okamoto K, Takezawa K, Hatashita E, Yamada Y, Kuwata K, Arao T, Yanagihara K, Fukuoka M, Nishio K and Nakagawa K: Identification of c-Src as a potential therapeutic target for gastric cancer and of MET activation as a cause of resistance to c-Src inhibition. *Mol Cancer Ther* 9: 1188-1197, 2010.
- 16 Nagaraj NS, Smith JJ, Revetta F, Washington MK and Merchant NB: Targeted inhibition of SRC kinase signaling attenuates pancreatic tumorigenesis. *Mol Cancer Ther* 9: 2322-2332, 2010.
- 17 Johnson FM, Saigal B, Talpaz M and Donato NJ: Dasatinib (BMS-354825) tyrosine kinase inhibitor suppresses invasion and induces cell cycle arrest and apoptosis of head and neck squamous cell carcinoma and non-small cell lung cancer cells. *Clin Cancer Res* 11: 6924-6932, 2005.
- 18 Konecny GE, Glas R, Dering J, Manivong K, Qi J, Finn RS, Yang GR, Hong KL, Ginther C, Winterhoff B, Gao G, Brugge J and Slamon DJ: Activity of the multikinase inhibitor dasatinib against ovarian cancer cells. *Br J Cancer* 101: 1699-1708, 2009.
- 19 Nam S, Kim D, Cheng JQ, Zhang S, Lee JH, Buettner R, Mirosevich J, Lee FY and Jove R: Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* 65: 9185-9189, 2005.
- 20 Huang F, Reeves K, Han X, Fairchild C, Platero S, Wong TW, Lee F, Shaw P and Clark E: Identification of candidate molecular markers predicting sensitivity in solid tumors to dasatinib: rationale for patient selection. *Cancer Res* 67: 2226-2238, 2007.
- 21 Montero JC, Seoane S, Ocana A and Pandiella A: Inhibition of SRC family kinases and receptor tyrosine kinases by dasatinib: possible combinations in solid tumors. *Clin Cancer Res* 17: 5546-5552, 2011.
- 22 Beauchamp EM, Woods BA, Dulak AM, Tan L, Xu C, Gray NS, Bass AJ, Wong KK, Meyerson M and Hammerman PS: Acquired resistance to dasatinib in lung cancer cell lines conferred by DDR2 gatekeeper mutation and NF1 loss. *Mol Cancer Ther* 2013.
- 23 Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W, Brace LE, Woods BA, Lin W, Zhang J, Deng X, Lim SM, Heynck S, Peifer M, Simard JR, Lawrence MS, Onofrio RC, Salvesen HB, Seidel D, Zander T, Heuckmann JM, Soltermann A, Moch H, Koker M, Leenders F, Gabler F, Querings S, Ansen S, Brambilla E, Brambilla C, Lorimier P, Brustugun OT, Helland A, Petersen I, Clement JH, Groen H, Timens W, Sietsma H, Stoelben E, Wolf J, Beer DG, Tsao MS, Hanna M, Hatton C, Eck MJ, Janne PA, Johnson BE, Winckler W, Greulich H, Bass AJ, Cho J, Rauh D, Gray NS, Wong KK, Haura EB, Thomas RK and Meyerson M: Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer discovery* 1: 78-89, 2011.
- 24 Park SI, Zhang J, Phillips KA, Araujo JC, Najjar AM, Volgin AY, Gelovani JG, Kim SJ, Wang Z and Gallick GE: Targeting SRC family kinases inhibits growth and lymph node metastases of prostate cancer in an orthotopic nude mouse model. *Cancer Res* 68: 3323-3333, 2008.
- 25 Trevino JG, Summy JM, Lesslie DP, Parikh NU, Hong DS, Lee FY, Donato NJ, Abbruzzese JL, Baker CH and Gallick GE: Inhibition of SRC expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am J Pathol* 168: 962-972, 2006.
- 26 Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y and Pietenpol JA: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 121: 2750-2767, 2011.
- 27 Nautiyal J, Majumder P, Patel BB, Lee FY, and Majumdar AP: Src inhibitor dasatinib inhibits growth of breast cancer cells by modulating EGFR signaling. *Cancer Lett* 283: 143-151, 2009.
- 28 Pichot CS, Hartig SM, Xia L, Arvanitis C, Monisvais D, Lee FY, Frost JA and Corey SJ: Dasatinib synergizes with doxorubicin to block growth, migration, and invasion of breast cancer cells. *Br J Cancer* 101: 38-47, 2009.
- 29 Finn RS, Bengala C, Ibrahim N, Roche H, Sparano J, Strauss LC, Fairchild J, Sy O and Goldstein LJ: Dasatinib as a single agent in triple-negative breast cancer: results of an open-label phase 2 study. *Clin Cancer Res* 17: 6905-6913, 2011.

- 30 Rassi FE and Khoury HJ: Bosutinib: a SRC-ABL tyrosine kinase inhibitor for treatment of chronic myeloid leukemia. *Pharmacogenomics and personalized medicine* 6: 57-62, 2013.
- 31 Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, Frost P, Ye F, Boschelli DH and Boschelli F: SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. *Cancer Res* 63: 375-381, 2003.
- 32 Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, Muller A, Baumgartner C, Valent P, Augustin M, Till JH and Superti-Furga G: Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. *Leukemia: official journal of the Leukemia Society of America, Leukemia Research Fund, UK* 23: 477-485, 2009.
- 33 Li J, Rix U, Fang B, Bai Y, Edwards A, Colinge J, Bennett KL, Gao J, Song L, Eschrich S, Superti-Furga G, Koomen J and Haura EB: A chemical and phosphoproteomic characterization of dasatinib action in lung cancer. *Nat Chem Biol* 6: 291-299, 2010.
- 34 Levinson NM and Boxer SG: Structural and spectroscopic analysis of the kinase inhibitor bosutinib and an isomer of bosutinib binding to the Abl tyrosine kinase domain. *PLoS One* 7: e29828, 2012.
- 35 Padmanabhan S, Ravella S, Curiel T and Giles F: Current status of therapy for chronic myeloid leukemia: a review of drug development. *Future Oncol* 4: 359-377, 2008.
- 36 Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, Johnson NL, Granger DA, Jordan NV, Darr DB, Usary J, Kuan PF, Smalley DM, Major B, He X, Hoadley KA, Zhou B, Sharpless NE, Perou CM, Kim WY, Gomez SM, Chen X, Jin J, Frye SV, Earp HS, Graves LM and Johnson GL: Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. *Cell* 149: 307-321, 2012.

Received January 23, 2014

Revised February 10, 2014

Accepted February 12, 2014