Published in final edited form as:

Clin Cancer Res. 2013 June 1; 19(11): 2828–2833. doi:10.1158/1078-0432.CCR-13-0043.

A Proposal Regarding Reporting of in Vitro Testing Results

Malcolm A. Smith, MD, PhD¹ and Peter Houghton, PhD²

¹Cancer Therapy Evaluation Program, NCI (Bethesda, MD)

²Nationwide Children's Hospital (Columbus, OH)

Abstract

The high rate of negative clinical trials and failed drug development programs calls into question the utility of preclinical testing as currently practiced.

An important issue for the in vitro testing of agents that have advanced into the clinic is the use of clinically irrelevant concentrations in reports making claims for anticancer activity, as illustrated by publications for sorafenib, vorinostat, and metformin. For sorafenib, high protein binding leads to a dichotomy between concentrations active in the 10% serum conditions commonly used for in vitro testing and concentrations active in plasma. Failure to recognize this distinction leads to inappropriate claims of activity for sorafenib based on the micromolar concentrations commonly used for in vitro testing in low serum conditions. For vorinostat and metformin, results using in vitro concentrations higher than those achievable in patients are reported despite the availability of publications describing human pharmacokinetic data for each agent.

We encourage journal editors and reviewers to pay greater attention to clinically relevant concentrations when considering reports that include in vitro testing of agents for which human pharmacokinetic data are available. Steps taken to more carefully scrutinize activity claims based on in vitro results can help direct researchers away from clinically irrelevant lines of research and toward lines of research that are more likely to lead to positive clinical trials and to improved treatments for cancer patients.

Preclinical and translational research, both from academia and from pharmaceutical companies, is under scrutiny. On the one hand, translational research conducted by academic researchers and evaluated by pharmaceutical companies has been found to often lack reproducibility, and claimed conclusions have not been supported by further independent testing (1, 2). At the same time, preclinical testing conducted by pharmaceutical sponsors has been found lacking, as many agents with preclinical packages considered promising have subsequently failed in definitive clinical trials.

There are surely a number of potential explanations for this discouraging perspective on the utility of preclinical testing. In this commentary we address one specific research setting, that of in vitro testing of agents that are advanced in clinical testing and for which clinically relevant drug exposures are established. This is a common setting for academic investigators who use these in vitro results to help support proposals to funding agencies and pharmaceutical companies for clinical trials of these agents. Similar in vitro data are used to support the “repurposing” of non-oncology drugs for cancer indications.

Corresponding Author: Malcolm A. Smith, MD, PhD Assoc Branch Chief, Pediatrics Cancer Therapy Evaluation Program, NCI 6130 Executive Boulevard Room 7025 Bethesda, MD 20892 Malcolm.Smith@nih.gov.

Conflict of Interest: The authors have no conflicts of interest to disclose.

The example of sorafenib highlights some of the key problems related to in vitro testing of anticancer agents that have advanced into clinical testing, and so we use it to illustrate uses and misuses of in vitro testing data. A cottage industry has developed around studying the in vitro effects of sorafenib at micromolar level concentrations, with scores of papers describing its effects against a variety of cancer cell lines at these concentrations and with no indication that an end to such publications is near. There are advantages to studying sorafenib in vitro at micromolar level concentrations, as at these concentrations it consistently kills cancer cell lines and induces a number of biologically interesting effects on signaling pathways associated with cancer. For example, sorafenib at micromolar concentrations reduces phosphorylation levels of eIF4E (3, 4), reduces levels of Mcl-1 (4-6), induces STAT3 activation (7), promotes autophagy (8-10), and inhibits MAP kinase signaling (11). Unfortunately, the effects of sorafenib at these concentrations have little or no relevance to the clinical setting for the reasons summarized in the paragraphs below.

Sorafenib shows remarkable consistency in killing cancer cell lines at concentrations in the 1 to 10 μM range. This activity is unrelated to histotype and spans a wide range of adult and pediatric cancer cell lines, including cell lines for hepatocellular carcinoma, papillary thyroid cancer with BRAF mutations, breast cancer, lung carcinoma, cholangiocarcinoma, melanoma, neuroblastoma, medulloblastoma, rhabdomyosarcoma, and various types of leukemias (see supplemental materials for references). The uniformity of sorafenib cytotoxic effects at micromolar levels is illustrated by the results of the Pediatric Preclinical Testing Program (PPTP), which showed that 21 of 23 cell lines derived from a range of childhood cancers had IC_{50} values ranging from 3.2 μM to 10 μM (median 4.3 μM), with only a single cell line not achieving IC_{50} by 10 μM . This uniformity creates a sense of unease, as it is the type of response associated with non-specific cytotoxic agents as well as the type of response observed at supra-physiologic concentrations for many kinase inhibitors. This unease increases with the observation that one cell line tested by the PPTP, an AML cell line with an activating KIT mutation, demonstrated an IC_{50} of 20 nM. Other cell lines with certain KIT activating mutations are also inhibited by sorafenib in the 100 nM range (12). These observations support the position that the targeted effects of sorafenib occur in the low nanomolar range, while effects in the micromolar range represent non-specific effects on a range of biological processes that lead to cell death.

There is a body of published data documenting that the micromolar concentrations of sorafenib required to kill most cancer cell lines exceed by 2 to 3 logs the concentrations at which sorafenib inhibits cell lines with activated kinases that are its more specific molecular targets. For example, sorafenib inhibits FLT3-ITD autophosphorylation with IC_{50} values in the low nanomolar range (13, 14), and AML cell lines with FLT3-ITD show low nanomolar IC_{50} values (14, 15). Cell lines with oncogenic RET show IC_{50} values in the low to mid nanomolar range (16), and cell lines driven by selected KIT activating mutations show IC_{50} values < 100 nM (12, 17). Inhibition of VEGFR2 signaling is also observed at sub-micromolar concentrations (11). Cell lines driven by platelet-derived growth factor receptor (PDGFR) signaling have IC_{50} values in the 10 to 100 nM range (15, 18), with concentrations of 100 nM providing virtually complete inhibition of phospho-PDGFR α and phospho-PDGFR β expression (15, 18). This activity of sorafenib at low nanomolar concentrations translates into tumor regressions in xenograft models with activating mutations in sorafenib target gene products, as exemplified by the high level of in vivo activity of sorafenib against FLT3-ITD AML xenografts (14). By contrast, xenografts lacking activating genomic alterations in these target genes show slowing of tumor growth (consistent with inhibition of VEGFR2 signaling), but not tumor regressions (17).

Importantly, the in vitro activity of sorafenib at these nanomolar level concentrations has clear clinical significance as illustrated by the activity of sorafenib against FLT3-ITD AML

(19-22) and its VEGFR2-related anti-angiogenic activity as evidenced by its FDA approval for renal cell carcinoma (a VEGF pathway driven tumor for which VEGFR2-targeted agents show class activity) (23). The rarity of PDGFR-driven leukemias and the effectiveness of imatinib as upfront therapy have limited evaluations of sorafenib against this molecularly defined subset of patients (24, 25), although one patient showed a brief hematologic response prior to outgrowth of a resistant mutation (24).

Conversely, clinical experience with sorafenib supports the lack of positive predictive power for in vitro results obtained at micromolar concentrations. Low to nil objective response rates for sorafenib have been observed for multiple tumor types (most of which have cell lines showing in vitro sensitivity at micromolar concentrations), including: non-small cell lung cancer, breast cancer, melanoma, prostate cancer, squamous cell carcinoma of the head and neck, biliary tract carcinoma, and uterine carcinoma (see supplemental materials for references). Sorafenib response rates for sarcomas were also low, although 5 of 37 patients with angiosarcoma did show responses (26). This tumor type has shown responsiveness to other VEGFR2 targeted agents (27). The paucity of objective responses to sorafenib for most cancers in which the agent has been studied (outside of cancers with the activated kinases described above) despite in vitro cytotoxic activity for sorafenib at micromolar concentrations against cell lines derived from these same cancers supports the lack of clinical relevance for sorafenib in vitro effects observed at micromolar concentrations in 10% serum conditions.

Given the significant clinical activity of sorafenib that can be related to its in vitro activity at nanomolar concentrations against cell lines dependent upon its target kinases and given the otherwise limited clinical activity, what has maintained enthusiasm within the research community for studying sorafenib against cell lines that show no effect until micromolar concentrations are reached? One factor is the clinically achievable sorafenib drug levels, which are indeed in the 10 μM range (28). However, sorafenib is highly protein bound (99.7%) (29), meaning that sorafenib concentrations that are effective in 10% fetal bovine serum (FBS) are ineffective in plasma conditions. For example, FLT3-ITD driven cell lines are inhibited by 50% at 3 nM sorafenib in 10% FBS but require greater than 100-fold higher sorafenib concentrations (approximately 500 nM) in plasma for a comparable level of inhibition (13). Because clinically achievable sorafenib levels are only in the 10 μM range, sorafenib effects observed in vitro in 10% FBS at 1 to 10 μM concentrations lack plausibility for successful clinical translation.

Evaluation of sorafenib at micromolar concentrations is extending into “next generation” in vitro testing. The “CancerCell Line Encyclopedia” project evaluated sorafenib at concentrations ranging from 2.5 nM to 8.0 μM , and 98% of the 504 tested cell lines showed IC_{50} values $> 1 \mu\text{M}$ (30). For the reasons outlined above, these data at higher sorafenib concentrations lack clinical relevance. Analyses that incorporate these data to identify insights into sorafenib mechanism of action or mechanism of synergy against human cancers will produce misleading findings regarding potential clinical applications of sorafenib.

Disconnects between in vitro observations and clinical reality extend beyond the realm of kinase inhibitors. For example, vorinostat inhibits the growth of most cancer cell lines, but with IC_{50} values generally exceeding 1 μM (31-33). Concentrations of vorinostat from 2.5 μM to 10.0 μM are commonly used to demonstrate the in vitro effects of vorinostat on cellular processes related to its enhancement of protein acetylation. However, maximum achievable drug concentrations in humans are in the 1-2 μM range at the standard vorinostat dose of 400 mg (34-37). Because of the short half-life of vorinostat (1.5 to 2.0 hours), concentrations $> 1 \mu\text{M}$ are maintained for at most a few hours of each treatment day with concentrations $< 0.1 \mu\text{M}$ for most of each treatment day (36, 38). Thus, the clinical

relevance of the large body of literature on vorinostat's in vitro effects with prolonged exposure to concentrations exceeding 1-2 μM is highly questionable, and indeed 3 or 6 hour exposure to 1-10 μM vorinostat in vitro fails to elicit significant growth inhibition and exposure for 24 or more hours is required for significant effects (39). This skepticism about the clinical relevance of in vitro results using micromolar concentrations of vorinostat is supported by the disconnect between the broad in vitro cytotoxic activity of vorinostat and its narrow range of therapeutic activity in the clinic (primarily cutaneous T-cell lymphoma) (37). In fairness to vorinostat, other HDAC inhibitors show similar relationships between concentrations that are effective in vitro and those studied in the clinic. Panobinostat, which was described as a "broadly active compound" in a report describing the CancerCell Line Encyclopedia, showed an IC_{50} of approximately 60 nM against the approximately 500 cell lines against which it was tested (30). However, at the commonly used dose and schedule of this agent (20 mg administered orally 2-3 times per week), C_{max} values are only in the 40-70 nM range (40-42), and concentrations above 15 nM are maintained for less than 12 hours out of every 48 hour dosing interval (40). Of note, in vitro exposure to panobinostat for 12 hours or less produces limited in vitro effects (39). The lack of clinical relevance for these nanomolar range IC_{50} values for panobinostat is illustrated by its low single agent activity for patients with multiple myeloma (42), despite myeloma cell lines demonstrating a median IC_{50} value of approximately 15 nM (30).

In vitro testing results are also being used to support "repurposing" of non-oncology drugs for anticancer indications. Since the observation that the anti-diabetic agent metformin could reduce the overall incidence of cancer in diabetics and reduce the risk of pancreatic and other cancers (43-45), a plethora of reports have appeared demonstrating the biochemical effects of metformin, and effects on inhibition of proliferation, survival, invasion, motility, DNA repair, and synergy with cytotoxic agents in vitro. A review of recent publications through PubMed reveals that the vast majority of these studies used metformin at 1 to 20 mM (usually > 5mM) (see supplemental materials for references). The concern about the value of such in vitro studies is not because metformin binds to plasma proteins (it has very low or negligible binding), but that accumulation in cells is mediated by organic ion transporters (OCTs) SLC22A1, SLC22A2 or SLC22A3) (46, 47). These transporters are highly restricted to expression in liver and kidney, and are dramatically reduced during malignant transformation, even in hepatocellular carcinoma (48). Population pharmacokinetics modeling in humans shows plasma levels of metformin are low with median C_{max} of approximately 10 μM (CI 95% 2.8-21.6 μM) following an oral dose of 663 mg (49). Some biochemical effects of metformin have been demonstrated at 50 – 100 μM , under conditions of physiologic glucose, in vitro, however these effects are relatively small (50), and even these exposures to metformin exceed those attainable in patients. While it is clear that metformin can retard progression of some cancers in rodents, it is unlikely that the mechanism of action is a consequence of direct effects on tumor cell metabolism. The antitumor effects are more likely via indirect effects on gluconeogenesis or secretion of insulin-like growth factors from liver (51). Thus, biochemical changes, or physiological changes (proliferation/survival) observed in vitro at metformin concentrations in the millimolar range appear irrelevant for understanding the in vivo activity of metformin.

A basic challenge in interpreting in vitro testing results is the difficulty in assessing therapeutic window. The obvious point is that 96 and 386 well plates don't develop mucositis, neutropenia, or diarrhea, making it virtually impossible to assess whether the concentration of a novel agent showing in vitro activity is clinically relevant. Uniform cytotoxic activity, as shown for sorafenib at micromolar concentrations, can imply that the agent at these concentrations is extraordinarily promising or alternatively that it is a non-discriminant cytotoxic agent at these concentrations with no greater therapeutic relevance than Clorox. Attempts at using non-transformed cell lines as a surrogate for the host have

not been effective at assessing therapeutic window. The one consistent in vitro predictive factor for clinical success for a candidate oncologic agent is wide variation in sensitivity across cell lines, with most cell lines requiring high concentrations and with a select few cell lines with distinctive molecular characteristics being sensitive at much lower concentrations. Examples of the successful application of this principle include small molecule kinase inhibitors that show specificity for BRAF V600E mutated cell lines, or ALK-translocated cell lines, or EGFR mutated cell lines, to name a few.

There are costs to publishing results claiming anticancer activity based on clinically unachievable exposures, the most important being the potential for misallocation of clinical research resources and patients to clinical trials with little or no chance of success. Referring back to sorafenib again, over 500 clinical trials for sorafenib are listed in “ClinicalTrials.gov”, and nearly 200 clinical trials are listed as currently active across a wide range of cancer types. While many of these sorafenib trials are for either renal cell carcinoma or hepatocellular carcinoma (cancers for which sorafenib is licensed by regulatory agencies) or for cancers for which an antiangiogenic effect is targeted, other trials are for cancers for which the justification is based in part on sorafenib’s micromolar level in vitro activity. Similarly, approximately 50 cancer-related metformin clinical trials are listed. Would these trials have been initiated if there had been greater scrutiny of the clinical relevance of the drug concentrations used in the in vitro testing experiments that contributed to the rationale for these clinical trials?

What can be done to address the issues described above? One step that journals can take is to reject without review manuscripts that make activity claims for sorafenib at concentrations exceeding 1 μM , metformin at concentrations exceeding 50 μM , and vorinostat at concentrations exceeding 1 μM . More general steps that journal editors and reviewers can take include:

- Requiring that manuscripts describing the in vitro activity of agents that have advanced into clinical evaluation specifically address and provide convincing rationale with appropriate reference to the published literature for the potential clinical relevance of the concentrations explored.
- Rejecting manuscripts that make activity claims for targeted agents at concentrations that substantially exceed (e.g., 10-fold) the concentrations at which the agents are effective against cell lines expressing the agents’ molecular targets.
- Rejecting manuscripts that make activity claims for agents based on “clinically achievable drug levels”, when the concentrations used in vitro either exceed those observed in humans or else are present for only a short period of each treatment day.
- Requiring that manuscripts describing the in vitro testing of agents with high protein binding adjust for low serum versus plasma protein concentrations whenever comparisons to clinically achievable concentrations are made.

We recognize that exceptions to the above “rules” will be justifiable in some cases (e.g., for agents that have active metabolites that achieve sufficiently high levels in patients), but authors proposing exceptions should provide clear rationale to justify their position. Importantly, we believe that these proposals, if consistently applied, can help direct investigators away from lines of research that lack clinical relevance and toward lines of research that are more likely to lead to positive clinical trials and to improved treatments for patients with cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Begley CG, Ellis LM. Drug development: Raise standards for preclinical cancer research. *Nature*. 2012; 483:531–3. [PubMed: 22460880]
2. Prinz F, Schlange T, Asadullah K. Believe it or not: how much can we rely on published data on potential drug targets? *Nat Rev Drug Discov*. 2011; 10:712. [PubMed: 21892149]
3. Rahmani M, Nguyen TK, Dent P, Grant S. The multikinase inhibitor sorafenib induces apoptosis in highly imatinib mesylate-resistant bcr/abl+ human leukemia cells in association with signal transducer and activator of transcription 5 inhibition and myeloid cell leukemia-1 down-regulation. *Molecular pharmacology*. 2007; 72:788–95. [PubMed: 17595328]
4. Rahmani M, Davis EM, Bauer C, Dent P, Grant S. Apoptosis induced by the kinase inhibitor BAY 43-9006 in human leukemia cells involves down-regulation of Mcl-1 through inhibition of translation. *The Journal of biological chemistry*. 2005; 280:35217–27. [PubMed: 16109713]
5. Ding Q, Huo L, Yang JY, Xia W, Wei Y, Liao Y, et al. Down-regulation of myeloid cell leukemia-1 through inhibiting Erk/Pin 1 pathway by sorafenib facilitates chemosensitization in breast cancer. *Cancer research*. 2008; 68:6109–17. [PubMed: 18676833]
6. Yu C, Bruzek LM, Meng XW, Gores GJ, Carter CA, Kaufmann SH, et al. The role of Mcl-1 downregulation in the proapoptotic activity of the multikinase inhibitor BAY 43-9006. *Oncogene*. 2005; 24:6861–9. [PubMed: 16007148]
7. Yang F, Van Meter TE, Buettner R, Hedvat M, Liang W, Kowolik CM, et al. Sorafenib inhibits signal transducer and activator of transcription 3 signaling associated with growth arrest and apoptosis of medulloblastomas. *Molecular cancer therapeutics*. 2008; 7:3519–26. [PubMed: 19001435]
8. Bareford MD, Park MA, Yacoub A, Hamed HA, Tang Y, Cruickshanks N, et al. Sorafenib enhances pemetrexed cytotoxicity through an autophagy-dependent mechanism in cancer cells. *Cancer research*. 2011; 71:4955–67. [PubMed: 21622715]
9. Park MA, Zhang G, Martin AP, Hamed H, Mitchell C, Hylemon PB, et al. Vorinostat and sorafenib increase ER stress, autophagy and apoptosis via ceramide-dependent CD95 and PERK activation. *Cancer biology & therapy*. 2008; 7
10. Shi YH, Ding ZB, Zhou J, Hui B, Shi GM, Ke AW, et al. Targeting autophagy enhances sorafenib lethality for hepatocellular carcinoma via ER stress-related apoptosis. *Autophagy*. 2011; 7:1159–72. [PubMed: 21691147]
11. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer research*. 2004; 64:7099–109. [PubMed: 15466206]
12. Guida T, Anaganti S, Provitera L, Gedrich R, Sullivan E, Wilhelm SM, et al. Sorafenib inhibits imatinib-resistant KIT and platelet-derived growth factor receptor beta gatekeeper mutants. *Clin Cancer Res*. 2007; 13:3363–9. [PubMed: 17545544]
13. Pratz KW, Sato T, Murphy KM, Stine A, Rajkhowa T, Levis M. FLT3-mutant allelic burden and clinical status are predictive of response to FLT3 inhibitors in AML. *Blood*. 2010; 115:1425–32. [PubMed: 20007803]
14. Auclair D, Miller D, Yatsula V, Pickett W, Carter C, Chang Y, et al. Antitumor activity of sorafenib in FLT3-driven leukemic cells. *Leukemia*. 2007; 21:439–45. [PubMed: 17205056]
15. Lierman E, Lahortiga I, Van Mieghroet H, Mentens N, Marynen P, Cools J. The ability of sorafenib to inhibit oncogenic PDGFRbeta and FLT3 mutants and overcome resistance to other small molecule inhibitors. *Haematologica*. 2007; 92:27–34. [PubMed: 17229632]
16. Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, et al. BAY 43-9006 inhibition of oncogenic RET mutants. *Journal of the National Cancer Institute*. 2006; 98:326–34. [PubMed: 16507829]
17. Keir ST, Maris JM, Lock R, Kolb EA, Gorlick R, Carol H, et al. Initial testing (stage 1) of the multi-targeted kinase inhibitor sorafenib by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2010; 55:1126–33. [PubMed: 20672370]

18. Lierman E, Folens C, Stover EH, Mentens N, Van Mieghroet H, Scheers W, et al. Sorafenib is a potent inhibitor of FIP1L1-PDGFRalpha and the imatinib-resistant FIP1L1-PDGFRalpha T674I mutant. *Blood*. 2006; 108:1374–6. [PubMed: 16645167]
19. Sora F, Chiusolo P, Metafuni E, Bellesi S, Giammarco S, Laurenti L, et al. Sorafenib for refractory FMS-like tyrosine kinase receptor-3 (FLT3/ITD+) acute myeloid leukemia after allogeneic stem cell transplantation. *Leukemia research*. 2011; 35:422–3. [PubMed: 21093053]
20. Winkler J, Rech D, Kallert S, Rech J, Meidenbauer N, Roesler W, et al. Sorafenib induces sustained molecular remission in FLT3-ITD positive AML with relapse after second allogeneic stem cell transplantation without exacerbation of acute GVHD: a case report. *Leukemia research*. 2010; 34:e270–2. [PubMed: 20627386]
21. Safaian NN, Czibere A, Bruns I, Fenk R, Reinecke P, Dienst A, et al. Sorafenib (Nexavar) induces molecular remission and regression of extramedullary disease in a patient with FLT3-ITD+ acute myeloid leukemia. *Leukemia research*. 2009; 33:348–50. [PubMed: 18573526]
22. Metzelder S, Wang Y, Wollmer E, Wanzel M, Teichler S, Chaturvedi A, et al. Compassionate use of sorafenib in FLT3-ITD-positive acute myeloid leukemia: sustained regression before and after allogeneic stem cell transplantation. *Blood*. 2009; 113:6567–71. [PubMed: 19389879]
23. Kane RC, Farrell AT, Saber H, Tang S, Williams G, Jee JM, et al. Sorafenib for the treatment of advanced renal cell carcinoma. *Clin Cancer Res*. 2006; 12:7271–8. [PubMed: 17189398]
24. Lierman E, Michaux L, Beullens E, Pierre P, Marynen P, Cools J, et al. FIP1L1-PDGFRalpha D842V, a novel panresistant mutant, emerging after treatment of FIP1L1-PDGFRalpha T674I eosinophilic leukemia with single agent sorafenib. *Leukemia*. 2009; 23:845–51. [PubMed: 19212337]
25. Metzgeroth G, Erben P, Martin H, Mousset S, Teichmann M, Walz C, et al. Limited clinical activity of nilotinib and sorafenib in FIP1L1-PDGFRalpha positive chronic eosinophilic leukemia with imatinib-resistant T674I mutation. *Leukemia*. 2012; 26:162–4. [PubMed: 21818111]
26. Maki RG, D'Adamo DR, Keohan ML, Saulle M, Schuetze SM, Undevia SD, et al. Phase II study of sorafenib in patients with metastatic or recurrent sarcomas. *J Clin Oncol*. 2009; 27:3133–40. [PubMed: 19451436]
27. Park MS, Ravi V, Araujo DM. Inhibiting the VEGF-VEGFR pathway in angiosarcoma, epithelioid hemangioendothelioma, and hemangiopericytoma/solitary fibrous tumor. *Current opinion in oncology*. 2010; 22:351–5. [PubMed: 20485168]
28. Strumberg D, Clark JW, Awada A, Moore MJ, Richly H, Hendlisch A, et al. Safety, pharmacokinetics, and preliminary antitumor activity of sorafenib: a review of four phase I trials in patients with advanced refractory solid tumors. *The oncologist*. 2007; 12:426–37. [PubMed: 17470685]
29. Villarroel MC, Pratz KW, Xu L, Wright JJ, Smith BD, Rudek MA. Plasma protein binding of sorafenib, a multi kinase inhibitor: in vitro and in cancer patients. *Investigational new drugs*. 2012; 30:2096–102. [PubMed: 22089297]
30. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature*. 2012; 483:603–7. [PubMed: 22460905]
31. Butler LM, Agus DB, Scher HI, Higgins B, Rose A, Cordon-Cardo C, et al. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. *Cancer research*. 2000; 60:5165–70. [PubMed: 11016644]
32. Yin D, Ong JM, Hu J, Desmond JC, Kawamata N, Konda BM, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor: effects on gene expression and growth of glioma cells in vitro and in vivo. *Clin Cancer Res*. 2007; 13:1045–52. [PubMed: 17289901]
33. Luong QT, O'Kelly J, Braunstein GD, Hershman JM, Koeffler HP. Antitumor activity of suberoylanilide hydroxamic acid against thyroid cancer cell lines in vitro and in vivo. *Clin Cancer Res*. 2006; 12:5570–7. [PubMed: 17000694]
34. Badros A, Burger AM, Philip S, Niesvizky R, Kolla SS, Goloubeva O, et al. Phase I study of vorinostat in combination with bortezomib for relapsed and refractory multiple myeloma. *Clin Cancer Res*. 2009; 15:5250–7. [PubMed: 19671864]

35. Kelly WK, O'Connor OA, Krug LM, Chiao JH, Heaney M, Curley T, et al. Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol*. 2005; 23:3923–31. [PubMed: 15897550]
36. Ramalingam SS, Parise RA, Ramanathan RK, Lagattuta TF, Musguire LA, Stoller RG, et al. Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. *Clin Cancer Res*. 2007; 13:3605–10. [PubMed: 17510206]
37. Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *The oncologist*. 2007; 12:1247–52. [PubMed: 17962618]
38. Rubin EH, Agrawal NG, Friedman EJ, Scott P, Mazina KE, Sun L, et al. A study to determine the effects of food and multiple dosing on the pharmacokinetics of vorinostat given orally to patients with advanced cancer. *Clin Cancer Res*. 2006; 12:7039–45. [PubMed: 17145826]
39. Wilson PM, Labonte MJ, Martin SC, Kuwahara ST, El-Khoueiry A, Lenz HJ, et al. Sustained inhibition of deacetylases is required for the antitumor activity of the histone deacetylase inhibitors panobinostat and vorinostat in models of colorectal cancer. *Investigational new drugs*. 2013
40. Shapiro GI, Frank R, Dandamudi UB, Hengelage T, Zhao L, Gazi L, et al. The effect of food on the bioavailability of panobinostat, an orally active pan-histone deacetylase inhibitor, in patients with advanced cancer. *Cancer chemotherapy and pharmacology*. 2012; 69:555–62. [PubMed: 22057852]
41. Rathkopf D, Wong BY, Ross RW, Anand A, Tanaka E, Woo MM, et al. A phase I study of oral panobinostat alone and in combination with docetaxel in patients with castration-resistant prostate cancer. *Cancer chemotherapy and pharmacology*. 2010; 66:181–9. [PubMed: 20217089]
42. Wolf JL, Siegel D, Goldschmidt H, Hazell K, Bourquelot PM, Bengoudifa BR, et al. Phase II trial of the pan-deacetylase inhibitor panobinostat as a single agent in advanced relapsed/refractory multiple myeloma. *Leukemia & lymphoma*. 2012; 53:1820–3. [PubMed: 22288662]
43. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ*. 2005; 330:1304–5. [PubMed: 15849206]
44. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology*. 2009; 137:482–8. [PubMed: 19375425]
45. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. *Cancer Prev Res (Phila)*. 2010; 3:1451–61. [PubMed: 20947488]
46. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *The Journal of pharmacology and experimental therapeutics*. 2002; 302:510–5. [PubMed: 12130709]
47. Kimura N, Masuda S, Tanihara Y, Ueo H, Okuda M, Katsura T, et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug metabolism and pharmacokinetics*. 2005; 20:379–86. [PubMed: 16272756]
48. Heise M, Lautem A, Knapstein J, Schattenberg JM, Hoppe-Lotichius M, Foltys D, et al. Downregulation of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) in human hepatocellular carcinoma and their prognostic significance. *BMC cancer*. 2012; 12:109. [PubMed: 22439694]
49. Bardin C, Nobecourt E, Larger E, Chast F, Treluyer JM, Urien S. Population pharmacokinetics of metformin in obese and non-obese patients with type 2 diabetes mellitus. *European journal of clinical pharmacology*. 2012; 68:961–8. [PubMed: 22274405]
50. Sinnott-Smith J, Kisfalvi K, Kui R, Rozengurt E. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: Dependence on glucose concentration and role of AMPK. *Biochemical and biophysical research communications*. 2012
51. Memmott RM, Mercado JR, Maier CR, Kawabata S, Fox SD, Dennis PA. Metformin prevents tobacco carcinogen--induced lung tumorigenesis. *Cancer Prev Res (Phila)*. 2010; 3:1066–76. [PubMed: 20810672]

Statement of translational relevance

Preclinical and translational cancer research is under scrutiny because of high rates of negative clinical trials and failed drug development programs. Regarding the in vitro testing of agents that have advanced past phase 1 testing, an important issue is the use of clinically irrelevant concentrations to support lines of clinical research, as illustrated by publications describing preclinical testing results for sorafenib, vorinostat, and metformin. To the extent that these publications provide rationale for clinical trials with an inherently low likelihood of success, policies limiting such publications can contribute by directing investigators away from clinically irrelevant lines of research and toward lines of research that are more likely to lead to positive clinical trials and to improved treatments for patients with cancer.