Perspectives in Pharmacology

Preclinical Predictors of Anticancer Drug Efficacy: Critical Assessment with Emphasis on Whether Nanomolar Potency Should Be Required of Candidate Agents^[S]

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ABSTRACT

In the current paradigm of anticancer drug development, candidate compounds are evaluated by testing their in vitro potency against molecular targets relevant to carcinogenesis, their effect on cultured cancer cells, and their ability to inhibit cancer growth in animal models. We discuss the key assumptions inherent in these approaches. In recent years, great emphasis has been placed on selecting for development compounds with nanomolar in vitro potency, expecting that they will be efficacious and safer based on the assumption that they can be used at lower doses (“the nanomolar rule”). However, this rule ignores critical parameters affecting efficacy and toxicity such as physiochemical and absorption, distribution, metabolism and excretion properties, off-target effects, and multitargeting activities. Thus, uncritical application of the nanomolar rule may reject efficacious compounds or select ineffective or

toxic compounds. We present examples of efficacious chemotherapeutic (alkylating agents, hormonal agents, antimetabolites, thalidomide, and valproic acid) and chemopreventive (aspirin and sulindac) agents having millimolar potency and compounds with nanomolar potency (cyclooxygenase-2 inhibitors) that, nevertheless, failed or proved to be unsafe. The effect of candidate drugs on animal models of cancer is a better predictor of human drug efficacy; particularly useful are tumor xenografts. Given the cost of failure at clinical stages, it is imperative to keep in mind the limitations of the nanomolar rule and use relevant in vivo models early in drug discovery to prioritize candidates. Although in vivo models will continue having a major role in cancer drug development, more robust approaches that combine high predictive ability with simplicity and low cost should be developed.

Introduction

The treatment of cancer represents the greatest medical challenge of our times. After the National Cancer Act of 1971 (The War on Cancer) introduced by President Richard Nixon, there has been an enormous acceleration of efforts to develop effective agents against cancer. There have been notable successes, reflected in improved quality of life and cures of certain types of cancer, including some that were uniformly fatal. Yet much

needs to be accomplished. Cancer still extracts a heavy toll in terms of suffering and human lives lost (Siegel et al., 2011). The need to develop additional agents remains as pressing today as ever, and a worldwide effort is afoot.

During the last 30 years, pharmacology has undergone a radical transformation of its approach to drug development (Keiser et al., 2010). Whereas a generation ago a new compound was seeking a target, now, thanks to high throughput screening (HTS) and combinatorial chemistry, pharmacologists can readily identify agents affecting receptors but they have to seek their potential clinical application. Nevertheless, amid this changing landscape one critical question remains constant: which, if any, among several compounds that may be effective in preclinical models of cancer, will be effective in humans?

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ABBREVIATIONS: HTS, high throughput screening; ADME, absorption, distribution, metabolism, and excretion; COX, cyclooxygenase; FDA, Food and Drug Administration; GEMM, genetically engineered mouse model; HDAC, histone deacetylase; NCI, National Cancer Institute; FAP, familial adenomatous polyposis; GI₅₀, concentration required to achieve 50% growth inhibition; VPA, valproic acid.

Typically, anticancer drug development proceeds through sequential steps that involve testing of the candidate agent in cultured cancer cells, followed by testing in relevant animal tumor models. The key outcomes are efficacy and safety, with metabolism and pharmacokinetics/pharmacodynamics being important parts of this evaluation.

Varied approaches to predicting drug efficacy have been developed, none of them completely satisfactory (Suggitt and Bibby, 2005). Nevertheless, in recent years there has emerged what can be termed the nanomolar rule: during screening of compounds in an *in vitro* (reactivity toward a molecular target) or cell culture system an agent is discarded from further analysis if its potency is not in the nanomolar range.

We believe it is time to assess the validity of this assumption. Here, we evaluate it in the context of preclinical parameters used to predict drug efficacy in its ultimate user, the human patient. We review current knowledge followed by illustrative examples that, in the absence of definitive data, provide an empirical perspective.

Overview of Preclinical Screening

Despite recent advances in cancer biology and the development of molecular-targeted therapeutics, the attrition rate of new anticancer drugs in clinical trials is disappointingly high. It is surprising that in the age of combinatorial chemistry and HTS novel oncology drugs undergoing clinical trials have shown low response rates, while offering little therapeutic advantage compared with traditional cytotoxics. Their approval rate by the Food and Drug Administration (FDA) is <5% (Hutchinson and Kirk, 2011). The failure of new drugs at the clinical stage is very costly. Thus, the predictive value of preclinical models assumes critical importance in cancer drug development.

Because of higher throughput and lower costs, *in vitro* screening is the current mainstay for the initial selection of drug leads. The NCI-60 screen, developed in the late 1980s, is comprised of 60 distinct cell lines derived from nine distinct tumor types (Shoemaker, 2006). The primary endpoint of the NCI-60 panel is antiproliferative activity, and the profiles of cell line sensitivity may offer clues to the potential mechanisms of action by using the COMPARE algorithm (Paull et al., 1989). In many ways, NCI-60 is tailored to the selection of conventional cytotoxic drugs. Although the NCI-60 screen identified several cytotoxic molecules, they largely act via known mechanisms. As anticancer drug discovery moves away from traditional cytotoxics to newer, molecular-targeted cytostatic drugs, many emerging new anticancer entities would be considered “inactive” under the NCI-60 screen. As discussed later, many FDA-approved anticancer drugs are not nano-potent cytotoxics *in vitro*. Thus, this assay may have limited value in modern drug discovery, mainly because of its focus on cytotoxicity.

Currently, pharmaceutical companies use single or multiple validated targets to screen millions of potential drug leads in *in vitro* biochemical assays, preceding or parallel to conventional cellular screens. Inhibitors targeting protein kinases, such as imatinib and gefitinib/erlotinib, are molecular-targeted drugs that are efficacious in patients whose tumors depend on these protein kinases. Notwithstanding the validity of the protein targets assessed, in the current

paradigm of HTS, the vigorous selection of the “hit” drug leads plays a critical role in reducing attrition rates at the later stages of drug development. Molecules with nanomolar potency are highly sought after by researchers, based on the extrapolation that such high potency predicts clinical efficacy. Such practice is widespread.

What the liberal application of the nanomolar rule seems to ignore is that *in vitro* potency is only one of numerous factors that contribute to the potential clinical efficacy of a given compound. Physiochemical, pharmacokinetic [absorption, distribution, metabolism, and excretion (ADME)], and toxicological assessments are equally, if not more, important than the absolute potency of the agent in question. Curiously, high *in vitro* potency can often be gained at the expense of physiochemical properties, such as high molecular weight and increased lipophilicity (Hann, 2011). The lack of aqueous solubility, poor bioavailability, and metabolic instability could reduce efficacy in humans, irrespective of a compound's affinity to its targets.

In vitro assays are also inadequate in the evaluation of the potential toxicity of a novel agent arising from unexpected off-target effects. Finally, many drugs have multiple targets that contribute to the overall biological response, which could not be modeled in simple HTS assays. Our drive for ultra-potent molecules, on the basis of *in vitro* assays, may come at the expense of other favorable characteristics of a drug, which contributes to the eventual failure of drug leads.

Compared with *in vitro* assays, *in vivo* tumor models are more reliable indicators of clinical efficacy in the human. Mouse tumor models are well established means of predicting clinical outcomes (Johnson et al., 2001; Sausville and Burger, 2006). In mice, multiple parameters of drug candidates are assessed simultaneously: ADME, efficacy, and the therapeutic index, a critical parameter representing the margin between antitumor activity and toxicity. Here, the relevance of “nanomolar potency” is limited; a more important question is whether sufficient levels of the agent can reach its target sites to realize efficacy, while not causing dose-limiting side effects.

Starting in the 1950s, transplanted murine tumors (L1210 and P-388 leukemia) were used at the NCI to screen anticancer drugs, which contributed to the discovery of drugs such as vinca alkaloids and platinum salts. These murine syngeneic models are less costly, and tumors grow in immunocompetent hosts; however, tumors are of mouse origin. Syngeneic tumors also have rapid growth rates and thus have limited predictive value for the relatively slow-growing solid tumors in humans (Teicher, 2006).

With the availability of athymic *nu/nu* and severely combined immunodeficient mice, human tumor subcutaneous xenografts have been extensively used in academia and the pharmaceutical industry. The predictive value of subcutaneous human tumor xenograft models is highlighted by the retrospective analysis by the NCI (Johnson et al., 2001), using data from 39 anticancer drugs whose phase II data are available. Of the anticancer drugs (33/39) that were active in one-third or more of the tested xenografts (median = 12 xenografts of various types of cancer), 45% (15/33) possessed significant clinical activity in humans. Conversely, none of the anticancer agents (6/6) with activity in less than one-third of the tested xenografts showed eventual efficacy in humans ($p = 0.04$). Data from the Freiburg xenograft panel

are also compelling (Fiebig et al., 2004). Comparison of drug efficacy in patients and their xenografts established from biopsies revealed that patient-derived xenografts are highly predictive for responsiveness (90%, 19/21) and resistance (97%, 57/59). Such is the predictive value of patient-derived xenografts that it has been proposed that they can be used for the optimization of treatment regimens in personalized chemotherapy (Dong et al., 2010).

The orthotopic xenograft is an alternative model where tumor cells are implanted into the organs from which they are derived; it is more physiologically relevant and is suitable for studying metastasis because subcutaneous tumors rarely metastasize. Orthotopic models, however, often require complex surgical procedures and are time-consuming and costly. Nonetheless, they are useful in confirming the activity of drugs or studying antimetastatic therapy (Bibby, 2004). A limitation of these xenograft models is the immunosuppressed nature of the host, which precludes testing of immunomodulatory agents and assessing the role of the immune system in the drug effect.

Apart from predicting the clinical efficacy of treatment agents, animal models of cancer also have a major role in the discovery of chemopreventive compounds. Classic models typically involve the use of various carcinogens to induce cancer or transplanted xenografts in immunocompromised mice. Murine models of carcinogenesis readily recapitulate initiation, promotion, and progression phases of neoplastic transformation, whereas the relevant *in vitro* models are few. Complementing these xenograft models are newer genetically engineered mouse models (GEMMs). GEMMs closely mimic the complex interaction of tumors with their microenvironment, an aspect of carcinogenesis missing from the xenograft models, and hosts are immunocompetent (Sharpless and Depinho, 2006). However, GEMMs are relatively costly, and there are considerable variations in the rates of tumor development and progression, which prohibit their use in large-scale screening at the present time.

Assumptions Associated with Preclinical Testing

As is always the case in science, in assessing results from any experimental approach, one ought to be cognizant of its limitations. Each of the three approaches for evaluating candidate drugs outlined above (drug effects on target molecules, cultured cancer cells, and animal models) includes a number of assumptions. The most critical of these assumptions are presented below.

The evaluation of the *in vitro* effect of a compound on a given molecule, often a protein transducing a signal vital to carcinogenesis, assumes that the *in vitro* effect will occur *in vivo* as well; this effect will be highly specific to this particular pathway, whose inhibition is expected to kill the cancer cell; and compounds with very low IC_{50} values will have a greater likelihood to be effective *in vivo* and a lower possibility of side effects, because the effective drug dose will be small (assuming toxicity is proportional to dose).

Drug testing in cultured cancer cells assumes that these cells retain or largely reflect the properties of the cancer from which they originate. However, cancers even of the same type could vary considerably among individuals (Shackleton et al., 2009). Moreover, some of the properties of *in situ* tumors are

lost during the process of their adaptation and immortalization in culture. To address these inherent limitations, investigators often resort to the evaluation of multiple cell lines (e.g., NCI-60 panel) that vary genotypically and/or phenotypically; the expectation is that if the results are consistent, they could be generalizable and thus of higher predictive value.

Animal models develop cancers either as a result of mutations (natural or engineered), exposure to carcinogens, or implantation of cancer cells. Implanted cells are often of human origin and thus offer the advantage of the closest possible similarity to their intended therapeutic user. The main assumptions associated with these animal models are: ADME is similar between mice and humans; the murine tumor is similar to the human; and human cancers xenografted into immunocompromised animals closely reflect the human condition (even though the tumor-host interaction is missing).

A shared limitation of all three approaches is that none evaluates humans, whose genotypic and phenotypic complexity is clearly distinct from signaling molecules, cultured cells, or animals. Instead, the best that preclinical testing can offer is the behavior of the candidate agent in lower animal species, most frequently the mouse.

The examples that follow (some of them summarized in Table 1) underscore the need to keep these assumptions in mind at all times. They also emphasize that the uncritical search for “nanomolar potent” drugs using simplified *in vitro* assays may not be optimal for the selection of drug candidates for the clinic. We briefly review effective compounds that would have been discarded during initial stages of drug discovery simply because they did not exhibit nanomolar potency and compounds with nanomolar potency that failed during clinical testing.

Efficacious Anticancer Agents Lacking Nanomolar Potency

In this section, we describe examples of efficacious anticancer drugs that highlight the disparity between *in vitro* potency and *in vivo* efficacy.

Alkylating Agents, Hormonal Agents, and Antimetabolites

Holbeck et al. (2010) surveyed in a comprehensive manner FDA-approved anticancer drugs. They used the NCI-60 panel data and grouped these clinically used drugs based on their mechanism of action. Their findings reveal that nanomolar cytotoxicity *in vitro* bears only limited relevance to clinical efficacy in humans.

Alkylating agents, among the first anticancer drugs discovered, are still the most commonly used drugs in chemotherapy. Among the 27 alkylating and DNA-damaging agents evaluated, only three possess mean potency (GI_{50}) in the nanomolar range, whereas a majority (18/27) of these agents are only weakly cytotoxic ($GI_{50} > 20 \mu M$). It is noteworthy that actinomycin D, the most potent alkylating agent (GI_{50} 1.4 nM), has limited clinical use because of its extreme toxicity (Rahman et al., 1974).

Hormonal agents are another class of chemotherapeutics that are highly effective in the treatment of hormone-sensitive cancers (e.g., breast and prostate). However, none of the 15 hormonal agents evaluated possesses nanomolar cytotox-

TABLE 1

The in vitro effects and in vivo efficacy of anticancer agents

The numbered references cited here in parentheses are listed in the Supplemental Information.

Drug	In Vitro IC ₅₀	Affinity to Putative Target	Animal Model Efficacy	Human Efficacy
Thalidomide	>100 μ M (1)	Tumor necrosis factor α inhibition, 0.2 mM (2)	Multiple myeloma (3), colon cancer (4, 5), cervical cancer (6), squamous cell carcinoma (7), lung cancer (8, 9), melanoma (10), prostate cancer (11)	Multiple myeloma (12–30), colorectal cancer (31), lung cancer (32), prostate cancer (11, 33), renal cancer (34), Kaposi's sarcoma (35, 36)
Valproic acid	0.5–5 mM (37–41)	HDACs, 0.5–2 mM (42)	Neuroblastoma (43), prostate cancer (44–47), cervical cancer (48), non-small-cell lung cancer (49), sarcoma rhabdomyosarcoma (50), melanoma (51), osteosarcoma (52), colon cancer (53)	Leukemia (54–56), breast cancer (57, 58), prostate cancer, pancreatic cancer (59) and neurological cancers (60, 61)
Sulindac	0.6–1 mM (62–67)	COX-1, >100 μ M	Colon cancer (69, 70), prostate cancer (71), non-small-cell lung cancer (72), head and neck cancer (73), uterine cancer (74), pancreatic cancer (75), colon cancer	Familial adenomatous polyposis (76–80), combination with difluoromethylornithine reduced recurrence of adenomas by 70% and more advanced adenomas by 95% (81)
Aspirin	2–5 mM (82–84)	COX-2, >100 μ M (68) COX-1, 2.3 μ M	Colon cancer (86), fibrosarcoma (87), lung cancer (88–90), cervical cancer (91)	Colorectal polyp recurrence (92, 93).
Rofecoxib		COX-2, 3.6 μ M (85) COX-2, >1 mM	Gastric cancer (95), lung cancer (96)	Marginal efficacy in colon polyps, withdrawn because of adverse cardiovascular effects
Celecoxib	10–75 μ M (97–101)	COX-2, 500 nM (94) COX-1, 15 μ M	Colon cancer (97, 101, 102), liver cancer (98, 103), bladder cancer (98), prostate cancer (99), neuroblastoma (100), breast cancer (104)	Colorectal polyp recurrence, but significantly raises the risk of serious cardiovascular events (105)
		COX-2, 40 nM (94)		

icity. Moreover, in vitro activity in the NCI-60 assay did not seem to predict clinical efficacy of this group of compounds. As an example, anastrozole (GI₅₀ 2500 μ M) had significantly better clinical efficacy than tamoxifen (GI₅₀ 4.6 μ M) in the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial of 9366 postmenopausal women with estrogen receptor-positive breast cancer (Howell et al., 2005).

Likewise, most clinically effective antimetabolite drugs (14/19), such as 5-fluorouracil (GI₅₀ 18 μ M), also lack nanomolar potency. The only classes of anticancer drugs with nanomolar potency in the NCI-60 panel are the tubulin-disrupting agents and topoisomerase inhibitors.

Aside from this broad survey of chemotherapeutic drugs, there are additional examples of clinically efficacious anticancer agents that lack nanomolar potency. We provide a brief overview of two such compounds: thalidomide and valproic acid (VPA).

Thalidomide

Thalidomide, a glutamic acid derivative, along with dexamethasone, is a standard therapy of multiple myeloma (Fayers et al., 2011) and is under active investigation for the treatment of solid tumors (Kumar et al., 2004).

The exact mechanism of thalidomide's efficacy toward cancer is not known. Thalidomide's diverse biological activities include immunomodulatory, antiangiogenesis, and anti-inflammatory effects (Teo et al., 2005). However, thalidomide is inactive in the NCI-60 screen (GI₅₀ >100 μ M) (Holbeck et al., 2010), and it only slightly reduces the proliferation of multiple myeloma cells (<20%) at high concentrations (>100 μ M) (Hideshima et al., 2000). Moreover, thalidomide did not sig-

nificantly inhibit angiogenesis in vitro in the absence of a microsomal preparation (Dredge et al., 2002). On the contrary, thalidomide significantly inhibited tumor growth in mice bearing xenografts derived from multiple myeloma (Yaccoby et al., 2002; Lentzsch et al., 2003), esophageal squamous cell carcinoma, lung cancer, cervical cancer, and melanoma, consistently demonstrating strong inhibition of angiogenesis in vivo (Table 1). Its efficacy is further enhanced when combined with chemotherapy. The combination of bevacizumab, thalidomide, and docetaxel was highly effective (>70%) in inhibiting prostate xenografts in mice, and such a combination of antiangiogenic and cytotoxic agents also showed remarkable efficacy in a phase II trial in patients with metastatic prostate cancer (Ning et al., 2010).

Thalidomide has considerable clinical efficacy despite an apparent lack of in vitro potency. In vivo, thalidomide undergoes spontaneous degradation and enzymatic metabolism, resulting in more than 20 metabolites. Part of the biological activity of thalidomide, such as the inhibition of angiogenesis, requires metabolic activation in vivo by cytochrome P450 2C19 (Bauer et al., 1998). Perhaps more important is the multifaceted nature of the biological activities of thalidomide, involving multiple aspects of malignant cell growth, which may act synergistically in vivo to produce greater efficacy, as in the case of multiple myeloma (Franks et al., 2004).

Valproic Acid

VPA, a branched short-chain fatty acid, is widely used as an antiepileptic drug. Recently, it has emerged that VPA has promising anticancer properties. VPA is an inhibitor of histone deacetylase (HDAC) (Göttlicher et al., 2001). Phase I/II

trials with VPA, alone or in combination with chemotherapy, have shown clinical efficacy toward various malignancies (Table 1).

In vitro, VPA is a weak inhibitor of HDAC. It inhibits the activities of HDAC isoforms in high micromolar to low millimolar range (0.4–2 mM). Moreover, VPA has low cytotoxic activity against cancer cell lines with IC_{50} values between 0.5 and 6 mM. Thus, it is unlikely that VPA would emerge as a drug lead under the nanomolar rule. In contrast, VPA has shown potent antitumor activity in animal models. VPA strongly suppresses the growth of cancer xenografts (Table 1). Studies in breast xenograft models revealed a potential synergism between VPA and epirubicin (Marchion et al., 2005), a combination that subsequently showed promising efficacy in phase I/II trials (Münster et al., 2007, 2009).

A critical evaluation of the disparate in vitro and in vivo activity of VPA can be appropriately implemented in the context of its pharmacokinetic and toxicity profiles. VPA possesses a high oral bioavailability and is well tolerated by patients. VPA at up to 75 to 120 mg/kg/day was well tolerated (Münster et al., 2007, 2009; Daud et al., 2009), resulting in plasma levels in the range of 1.2 to 1.5 mM. Correspondingly, there is clinical inhibition of HDACs in peripheral blood mononuclear cells (Münster et al., 2007; Daud et al., 2009) and tumor tissues (Münster et al., 2009).

Chemopreventive Agents Lacking Nanomolar Potency

The recent definitive documentation that aspirin is a chemopreventive agent against colon cancer has energized this nascent field, providing the impetus to develop effective and safe chemopreventive agents. Cancer chemoprevention, in many ways akin to the pharmacological prevention of cardiovascular diseases, is preferable to chemotherapy, because it minimizes human suffering, saves lives, and reduces health-care costs.

From a drug development point of view, chemopreventive agents present different challenges compared with chemotherapeutic agents. It is noteworthy that chemopreventive agents target tumor biology at an earlier stage, when the complexity of the neoplastic cell is lower compared with full-blown cancer requiring chemotherapy. On the other hand, agent safety is far more critical: chemoprevention agents are administered to otherwise healthy subjects for the rest of their lives, whereas chemotherapeutic agents are given to a patient at imminent risk to his life, when less safe agents are acceptable. Thus, in selecting chemopreventive agents, animal data that incorporate ADME and safety parameters generate highly consequential information.

Below, we discuss two prototypical chemopreventive agents, aspirin and sulindac; both would have been discarded if the nanomolar rule had been applied to them.

Aspirin

Epidemiological evidence has suggested that long-term aspirin use reduces the risk of colorectal neoplasia (Arber, 2000; Elwood et al., 2009). Randomized clinical trials showed that low-dose aspirin prevented the development of polyps (Baron et al., 2003), whereas high-dose aspirin suppressed recurrent adenomas in patients who previously had colorectal cancer (Sandler et al., 2003).

Aspirin and salicylic acid, its major metabolite, exhibit very weak cytotoxicity toward cancer cell lines (IC_{50} 2–5 mM). Aspirin is only a weak inhibitor of cyclooxygenase (COX)-2, the COX isoform implicated in carcinogenesis. Nonetheless, aspirin is effective in animal models of cancer, including chemically induced lung and colon cancer, human cancer xenografts, as well as *Apc^{Min}* mice (Table 1).

Despite poor in vitro potency, aspirin has shown considerable efficacy in humans. Similar to valproic acid, the activity of aspirin should be evaluated in the context of its pharmacokinetic properties, which can be assessed comprehensively only in vivo. Plasma salicylate levels can reach up to 1 mM with high-dose aspirin (325 mg), which is sufficient to inhibit the in vivo production of prostaglandin E_2 , a tumor promoter. Clinical trials with aspirin are ongoing, because of the mounting evidence that aspirin can reduce the risk of various cancers.

Sulindac

Sulindac is a nonsteroidal anti-inflammatory drug used for the treatment of arthritis. Early clinical trials found that sulindac regressed polyps in patients with familial adenomatous polyposis (FAP) (Labayle et al., 1994). A clinical trial showed that a combination of sulindac and difluoromethylornithine reduced recurrence of adenomas by 70% and advanced adenomas by 95% (Meyskens et al., 2008).

Sulindac inhibits carcinogenesis through COX-dependent and -independent mechanisms (Kashfi and Rigas, 2005). Sulindac is a prodrug that is reduced to sulindac sulfide, a COX inhibitor, or oxidized to sulindac sulfone. Neither of these compounds has potent cytotoxicity in vitro. Sulindac sulfide is the most potent, with IC_{50} values ~ 100 μ M, whereas sulindac and sulindac sulfone are weakly cytotoxic, with IC_{50} values between 0.3 and 5 mM. The binding affinities of sulindac and its metabolites to their targets are also moderate. On the basis of in vitro data, the anticancer potential of sulindac seems unimpressive. In vivo models, however, suggest another story. Sulindac was highly active (>75% inhibition) in arresting the growth of human colon, prostate, head, and neck cancer xenografts (Table 1). Sulindac also significantly arrested tumor growth in *Apc^{Min}* mice, a model of FAP. Synergy between sulindac and difluoromethylornithine was also reproduced in *Apc^{Min}* mice (Ignatenko et al., 2008). The lack of satisfactory in vitro/in vivo correlation of the antitumor activity of sulindac may be attributed to the multitargeted nature of this drug.

Clinically Failed Compounds with Nanomolar Potency

The widely publicized withdrawal of COX-2-specific inhibitors after their clinical testing for cancer provides an instructive example of compounds with remarkably high (nanomolar) potency in vitro that did not prove to be either efficacious or safe enough to be clinically useful.

The observations that COX-2 is largely responsible for the production of prostaglandins in inflammation and cancer, in which it is overexpressed (Fournier and Gordon, 2000), have led to the notion that specific inhibition of COX-2 could prevent or treat cancer. The COX-2-selective inhibitors rofecoxib, celecoxib, and valdecoxib inhibited COX-2 activity with nanomolar potency (IC_{50} 5–500 nM), while being less active

toward COX-1 (Gierse et al., 2005). It was hoped that, compared with nonsteroidal anti-inflammatory drugs, they would offer higher efficacy and improved gastrointestinal safety because they spared COX-1 (Warner et al., 1999). Coxibs were assessed for both their chemopreventive and chemotherapeutic applications to cancer. However, they failed to meet the dual expectation of efficacy and lower toxicity in clinical trials for both applications.

Treatment of patients with FAP with celecoxib for 6 months reduced the number of colorectal polyps by 28% (Steinbach et al., 2000); rofecoxib had a marginal effect (6.8% reduction) (Higuchi et al., 2003). Rofecoxib was also ineffective in improving overall survival in the adjuvant setting of colorectal cancer (Midgley et al., 2010). These results are in stark contrast to the high efficacy of sulindac. Other disappointing results were the clinical failures of celecoxib and rofecoxib to provide clinical benefits when combined with chemotherapy/epidermal growth factor receptor inhibitors in treating non-small-cell lung cancer (Gridelli et al., 2007; O'Byrne et al., 2007; Edelman et al., 2008; Fidler et al., 2008). Consistent with these findings, celecoxib has only a moderate inhibitory effect in human colon xenograft models, whereas neither rofecoxib nor etoricoxib possess significant antitumor activity in vivo (Schiffmann et al., 2008).

The chemopreventive efficacy of rofecoxib was also evaluated in the Adenomatous Polyp Prevention on VIOXX (APPROVe) trial (Baron et al., 2006). Rofecoxib treatment was associated with a modest reduction (24%) in the risk of colorectal adenomas. However, it caused a 1.92-fold increase in serious thrombotic cardiovascular events (Bresalier et al., 2005). This led to the high-profile withdrawal of rofecoxib and valdecoxib from the market and limitations in the clinical use of celecoxib. The Achilles heel of the coxibs lies in their potency toward COX-2, leading to selective suppression of the COX-2-dependent cardioprotective prostacyclin (Fries and Grosser, 2005). Chronic intake of coxibs therefore heightens the risk of thrombosis. The limited efficacy and adverse cardiovascular effects of coxibs have cast major doubts on the feasibility of COX-2 inhibition as a strategy for cancer prevention and treatment.

Mechanistic work on coxibs underscores another limitation of the nanomolar rule. There is now evidence that targets other than COX-2 contribute to the anticancer effects of celecoxib, such as nuclear factor- κ B, phosphodiesterases, and mitogen-activated protein kinases (Kashfi and Rigas, 2005).

Conclusions

Prediction of anticancer efficacy using preclinical models remains difficult and requires further development. Investigators evaluating candidate drugs should be constantly aware of the limitations of preclinical approaches. In recent years, potency in in vitro assays has become a predominant influence on screening and selection of drug leads. Although nanomolar potency remains desirable, it has no predictive value in complex diseases such as cancer. We believe that a more holistic approach that strives to achieve a judicious balance between potency, solubility, bioavailability, metabolic stability, and safety of candidate drugs would facilitate the discovery of novel therapeutics for cancer.

Key to this multiparameter evaluation is the use of appropriate in vivo models. In vivo models, such as subcutaneous and orthotopic xenografts in mice, when properly used, are

more reliable indicators of clinical efficacy. Indeed, none of the clinically approved agents has lacked activity in the mouse xenograft models. Patient-derived xenografts are highly predictive of drug response and have great potential in individualized medicine. Until more reliable approaches are developed, we foresee that because of their predictive capabilities, in vivo models, imperfect as they are, will continue to play a major role in the development of novel drugs. Every effort should be made to develop new approaches that combine high predictive ability with simplicity and low cost.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Wong, Cheng, and Rigas.

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