A platelet biomarker for assessing phosphoinositide 3-kinase inhibition during cancer chemotherapy

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Abstract
Thrombin cleavages of selective proteinase-activated receptors (PAR) as well as PAR-activating peptide ligands can initiate the phosphoinositide 3-kinase (PI3K) signaling cascade in platelets. Downstream to this event, fibrinogen receptors on platelets undergo conformational changes that enhance fibrinogen binding. In our study, we used this phenomenon as a surrogate biomarker for assessing effects on PI3K activity. Our method, using flow cytometric measurement of fluorescent ligand and antibody binding, uncovered a 16- to 45-fold signal window after PAR-induced platelet activation. Pretreatment (in vitro) with the PI3K inhibitors wortmannin and LY294002 resulted in concentration-dependent inhibition at predicted potencies. In addition, platelets taken from mice treated with wortmannin were blocked from PAR-induced ex vivo activation concomitantly with a decrease in phosphorylation of AKT from excised tumor xenografts. This surrogate biomarker assay was successfully tested (in vitro) on blood specimens received from volunteer cancer patients. Our results indicate that measurement of platelet activation could serve as an effective drug activity biomarker during clinical evaluation of putative PI3K inhibitors. [Mol Cancer Ther 2007;6(9):2600–7]

Introduction
The phosphoinositide 3-kinases (PI3K) are a family of enzymes that play a pivotal role in controlling vital cellular functions, including proliferation, motility, glucose metabolism, and survival (1). The structure and function of PI3Ks have been studied extensively and categorized into three main classes (I, II, and III) according to their substrate preference, regulation, and homology of their heterodimeric composition (1–4). The PI3Ks are intimately involved in cellular events of signal transduction. The specific roles of the PI3K isoforms have been extensively studied and reviewed (5–7). Downstream effects of this pathway suggest that aberrant expression or regulation of PI3K may play an important role in the pathogenesis of certain human cancers (5–9). Thus, development of agents that specifically inhibit molecules involved in the PI3K signaling pathway is of increasing interest as a novel targeted approach to cancer therapy (10–12).

With the recent success of several target-directed agents that exhibit lowered off-target toxicity (13), a new and encouraging era for cancer therapy is under way. To assess whether these newer agents “hit” their desired molecular target (in vivo), novel assays must often be developed. These assays are called “drug activity biomarkers.” An ideal drug activity biomarker should measure a pharmacologic effect on a phenotype or activity of the intended molecular target and, ideally, have an association with the disease process. In addition, the selected biomarker should be capable of producing a robust and reproducible signal from an easily obtainable specimen (14). In the clinical setting, blood is an advantageous sample source for biomarker assays due to the ease of obtaining, storing, and transporting the specimen. Blood specimens can provide a disease target tissue (leukemia cells), or in the case of solid tumors, the normal blood components can provide surrogate targets for pharmacologic investigation (15). With the development of less toxic therapies, drug activity biomarkers have become increasingly important for estimating efficacious doses of an experimental agent. Furthermore, these assays are becoming increasingly useful for preclinical measurement of target inhibition, bioavailability, and pharmacodynamic modeling (16).

In the present study, we describe how blood platelets were used as a surrogate target tissue for measuring PI3K activity. Blood platelets are a rich source of PI3K. Prior studies described a clear role of PI3K during the cascade of platelet activation events (17–20). Using blood platelets, known PI3K inhibitors, platelet activators, and fluorescently labeled reagents that selectively bind to activated platelets as the readout, we developed and report here a novel, robust, and easily implemented surrogate biomarker assay that could be useful during preclinical and clinical evaluations of new targeted cancer agents.

Materials and Methods

Blood Specimens

Normal Human. Blood was collected from healthy volunteers into two 4.5 mL Vacutainer tubes containing 3.2% buffered sodium citrate anticoagulant. The first draw tube collected was discarded (to minimize tissue factor–induced activation) and the second tube was used in these experiments.
Cancer Patient. After completion of informed consent documents, blood was similarly collected from volunteer cancer patients diagnosed with solid tumor malignancies meeting the following inclusion criteria: advanced disease (stage IIIb/IV), platelet counts >100,000/μL, and no aspirin, antiplatelet therapy, or chemotherapy for >14 days. All patients had received at least one prior chemotherapy regimen but no more than three.

Murine. Mouse blood was collected into (citrated) anticoagulant-coated syringes by cardiac puncture from naïve CD1 nu/nu female mice and also from CD1 nu/nu female mice bearing human glioblastoma (U87MG) or human ovarian (A2780) tumor xenografts. Blood was slowly expelled from syringes into individually labeled polypropylene tubes measuring 12 × 75 mm.

Antibodies and Reagents
Monoclonal (murine) anti-human PAC-1-FITC (specific for the activated conformation of human platelet GPIIb/IIIa), BD FACSTM lysing solution (10× buffered solution containing 30% diethylene glycol and 10% formaldehyde), and BD Cytofix buffer (buffer containing 4% paraformaldehyde) were obtained from Becton Dickinson. Fibrinogen conjugated to Alexa Fluor 488 (Fl*) was obtained from Molecular Probes (Invitrogen Corp.). Anti-human CD41a conjugated to FITC or phycoerythrin (PE), anti-human CD62P-FITC, antimouse CD41a-FITC, antimouse CD41a-PE, and antimouse CD62P-FITC was obtained from BD Pharmingen. The thrombin receptor-activating peptides (TRAP) for proteinase-activated receptor (PAR)-1, SFLRN-PNDKYEPF-NH2, SFLRN-NH2, and SFLRN, and the murine homologue PAR-4 TRAP, AYPGKF-NH2, were obtained from Bachem Biosciences, Inc. Wortmannin, LY294002, Sepharose-2B, and modified Krebs-Henseleit buffer were obtained from Sigma. U0126 mitogen-activated protein/extracellular signal-regulated kinase kinase 1/2 inhibitor was obtained from Cell Signaling Technology, Inc. Isoform-specific inhibitors PI-103 and TGX-221 were both synthesized according to published specifications. Dulbecco’s PBS was obtained from Invitrogen. Albumin bovine fraction V fatty acid-free, low endotoxin (bovine serum albumin) was obtained from MP Biomedical. Matrigel was obtained from Collaborative Biomedical Products. RNase-free pellet pestles were obtained from Kimble Kontes. Detergent-compatible protein assay was obtained from Bio-Rad Laboratories. Total AKT and phosphorylated AKT (pAKT) immunosassays were purchased from Biosource International.

Tumor Cell Line Growth and Xenograft
U87MG and A2780 cells were obtained from the American Type Culture Collection and maintained in American Type Culture Collection—recommended medium. Exponentially growing cells were harvested with light trypsinization (0.25% trypsin) and washed with PBS. Matrigel was used to disperse 5 million (U87MG) or 2 million (A2780) cells and then implanted s.c. in female CD1 nu/nu mice.

Human Assay
Human platelet-rich plasma (PRP) was collected from above the pellet of anticoagulated (sodium citrate) blood after light centrifugation (200 × g for 13 min). Gel-purified platelet (GFP) preparations were prepared using a modified method from Tangen et al. (21). Briefly, PRP was loaded onto Sepharose-2B columns and platelet fractions (1 mL) were eluted with buffer (modified Krebs-Henseleit buffer containing 0.35% bovine serum albumin and 6.25 mmol/L NaHCO3). Aliquots (0.1 mL) of PRP (diluted 1:6) or GFP were added to polypropylene tubes measuring 12 × 75 mm and pretreated with/without PI3K inhibitors for 15 min at 37°C. Antibodies identifying surface membrane proteins were added followed by addition of PAR-1 TRAP (SFLRN-NH2, 5 μmol/L final) and PAR-1-FITC. Samples were incubated for 20 min at 37°C. Cells were then immediately fixed by addition of 4% Cytofix buffer for 10 min at 37°C. Samples were then washed by adding 1 mL of platelet buffer and centrifuging at 2,000 × g for 10 min. Sample supernatants were aspirated and platelets were resuspended in 500 μL of 1% Cytofix buffer (in Dulbecco’s PBS).

Murine Assay
In vivo Drug Treatments. In some experiments, normal female CD1 nu/nu mice were dosed i.p. with either vehicle control (5% DMSO) or wortmannin (4 mg/kg) for 1 h. In other experiments, female CD1 nu/nu mice bearing ~200 mm3 U87MG tumors were dosed i.p. with either vehicle control (5% DMSO) or wortmannin (4 mg/kg) for 1 h.

Murine Platelet Assay. Blood was added to tubes measuring 12 × 75 mm containing anti-CD41a-PE. In some cases, normal mouse blood was pretreated (in vitro) with PI3K inhibitors. Following this, PAR-4 TRAP (AYPGKF-NH2, 60 μmol/L) and Fl* (50 μg/mL) were added. Samples were incubated for 10 min at room temperature. This was followed by red cell lysis and platelet fixation using 1.5 mL of FACSTM lysing solution (1×) for 10 min at room temperature. The samples were then centrifuged (1,800 × g for 10 min), supernatant was discarded and washed again, and platelets were resuspended in 1.0 mL of 1% Cytofix buffer.

Tumor Assay. Measurement of pAKT from excised U87MG tumors was done using a Biosource immunoassay kit. Briefly, mice were euthanized and tumors were excised using dissecting scissors and forceps. Tumors were homogenized by hand using a pellet pestle in ice-cold lysis buffer and clarified by centrifugation at 100,000 × g for 15 min. Remaining supernatant was transferred to a clean microcentrifuge tube. Protein concentrations were determined using a Bio-Rad detergent-compatible protein assay. Appropriate concentrations of diluted tumor homogenates were then used for measurement of total AKT and phosphorylated AKT (pAKT) immunoassays were purchased from BioSource International.

Flow Cytometry. All measurements were made with a Beckman Coulter FC500 flow cytometer. Platelets were differentiated from debris using a light scatter/anti-CD41a+ gate. Listmode data files were analyzed using WinList software (Verity Software House). Single-color controls were used for color compensation and percent positive and the mean fluorescence intensity (MFI) for CD62P-FITC, PAR-1-FITC, or the Fl* signal was computed for each sample.
Pharmacologic Calculations

For agonist effects on platelet fluorescence, the measured MFI signals were normalized to the percentage of maximum fluorescence (% max) using the following formula:

\[
\%\text{max} = 100 \times \left[ \frac{\text{MFI}_{\text{test}} - \text{MFI}_{\text{basal}}}{\text{MFI}_{\text{max}} - \text{MFI}_{\text{basal}}} \right]
\]

For inhibition of binding (% inhibition) by the inhibitors, the following formula was used:

\[
\%\text{inhibition} = 100 \times \left[ 1 - \frac{\text{MFI}_{\text{test}} - \text{MFI}_{\text{basal}}}{\text{MFI}_{\text{max}} - \text{MFI}_{\text{basal}}} \right]
\]

In both equations, MFI_{basal} represents the fluorescence binding by nonactivated platelets, MFI_{test} represents binding induced by various test conditions, and MFI_{max} is the maximum fluorescence achieved in the treatment set.

Concentration response curves (agonist and inhibition) were analyzed using nonlinear (sigmoidal) regression curve fit (GraphPad Prism Software). For xenograft tumor lysates, the levels of pAKT were calculated using normalization to total AKT and a statistical analysis (JMP Discovery Software) with significant inhibition set at \( P \leq 0.05 \).

Results

Human Platelet Detection and Activation

Reactivity with anti-CD41a antibody along with light scatter measurements was used to differentiate human platelets from cell fragments and debris (Fig. 1A, left). Treatment of human PRP with PAR-1–activating peptide resulted in an increase in reactivity and associated fluorescence after reaction with PAC-1-FITC antibody (Fig. 1A, right). The extent of this activation was determined for treatments with several analogues of PAR-1–activating peptides (22, 23), including SFLLRN, and SFLLRN-NH2. Using this approach, SFLLRN-NH2 was determined to be the most potent with an EC50 = 1.4 μmol/L (data not shown).

Mouse Platelet Detection and Activation

Adaptation of the platelet assay for the murine system required attention to blood collection, platelet isolation, agonist selection, and identification of a usable fluorescent probe. A slow draw of a (citrate) precoated collection
syringe provided quality specimens displaying minimal basal platelet activation that was critical for obtaining quality data. Thus, for the murine assay, whole blood was used and platelets were identified in this milieu by their reactivity with anti-CD41a (Fig. 1B, left). Because mouse platelets are not activated by PAR-1 peptides, we used PAR-4–activating sequences (AYPGKF-NH2; refs. 24–26). Finally, PAC-1-FITC was not used as it does not react with activated murine platelet membrane surfaces. Instead, we used a fluorescing (Alexa Fluor 488) fibrinogen derivative (Fb*; ref. 27) and successfully measured PAR-4–induced mouse platelet activation with a robust (30+-fold fluorescence increase) signal (Fig. 1B, right).

**Assay Optimization Issues**

**Human.** Using SFLLRN-NH2, we did titration experiments to determine the optimal agonist concentration for subsequent inhibitor studies. The resultant data, shown in Fig. 2A, summarize three separate experiments using different donors. The EC_{50} calculated for SFLLRN-NH2 in PRP (1.89 μmol/L) in these experiments was approximately half that obtained in GPP (0.925 μmol/L). Treatment of platelets with 5.0 μmol/L SFLLRN-NH2 produced a signal window of 14- and 7-fold for PRP and GPP, respectively. We then conducted time course experiments using human PRP to optimize assay timing. We measured the induction and stability of the PAC-1-FITC signal using 5.0 μmol/L SFLLRN-NH2 induction for 0, 1, 5, 10, 15, 20, or 30 min at either 37°C or room temperature. The resulting data (data not shown) uncovered a robust (16-fold) signal after 5-min stimulation at 37°C that was maintained for 20 min. At room temperature, samples displayed less activation (13-fold) at 5 min but were stable for 30 min. For subsequent inhibition studies, human specimens were activated with 5.0 μmol/L SFLLRN-NH2 for 20 min at 37°C.

We conducted experiments to address concerns over sample stability and assay reproducibility. Our results (data not shown) indicate that specimens stored for 24 h at

**Figure 2.** A, TRAP activation curve for human platelets. Dilutions of SFLLRN-NH2 (TRAP) were incubated with human PRP or GPP followed by anti-PAC-1-FITC and analyzed using flow cytometry. Points, TRAP response curves for three donors normalized for % max fluorescence; bars, SD. B, PAR-4 peptide activation curve for murine platelets. Dilutions of AYPGKF-NH2 (PAR-4) were added to mouse whole blood followed by treatment with Fb* and analyzed using flow cytometry. Points, data from three experiments normalized to % max MFI; bars, SD.

**Figure 3.** A and B, wortmannin and LY294002 effect on SFLLRN-NH2–induced activation of human platelets. Dilutions of wortmannin or LY294002 were preincubated (15 min at room temperature) with human PRP and GPP preparations followed by treatment with SFLLRN-NH2 (5 μmol/L) and anti-PAC-1 FITC. Points, dose response was determined for three human donors and the percent inhibition of MFI was determined; bars, SD. C, wortmannin effect on AYPGKF-NH2–induced activation of mouse platelets. Dilutions of wortmannin were preincubated with mouse whole blood (30 min at room temperature) followed by treatment with AYPGKF-NH2 (60 μmol/L) and addition of Fb*. Points, dose response was determined for three mouse samples in separate experiments and the percent inhibition of MFI was determined; bars, SD.
room temperature performed comparably with those assayed immediately. However, cold storage at 4°C dramatically decreased the PAR-induced PAC-1 signal window (data not shown). In other studies, assay reproducibility was assessed by testing platelets from three normal volunteers once weekly during a 3-week period. Inhibition of PAR-induced platelet PAC-1 with 2 μmol/L wortmannin treatment showed consistent inhibition (94–99%) for all three donors over this period (data not shown).

**Murine.** Figure 2B summarizes three replicate experiments for titrating activation of murine platelets by AYPGKF-NH2. From these data, the EC50 for AYPGKF-NH2 was determined to be 63.9 μmol/L. We also conducted time course studies at both 37°C and room temperature. In contrast to the human assay, incubation at room temperature was shown to be optimal for Fb* binding to mouse platelets. Incubation at 37°C resulted in a dramatic loss of the Fb* signals presumably due to ligand endocytosis (data not shown). The optimal activation time for the murine assay was determined to be 10 min (data not shown).

**Effect of PI3K Inhibitors on Human and Murine Platelet Activation.** Inhibition of PAR peptide-induced platelet activation was determined by pretreatment with the well-established PI3K inhibitors wortmannin (28, 29) and LY294002 (30). Untreated platelets served as the basal response control and treatments lacking PI3K inhibitors as the maximal response controls for intra-assay normalization. The SFLRN-NH2–induced increase in PAC-1FITC fluorescence was inhibited by pretreatment with wortmannin or LY294002 in a concentration-dependent manner (Fig. 3). The IC50 for wortmannin and LY294002 was 13.4 nmol/L and 4.1 μmol/L (respectively) using GPP and 123 nmol/L and 10.3 μmol/L for platelets in plasma. These values are consistent with IC50 ranges previously reported for these inhibitors (28–30) in other assays. The approximately 3- to 9-fold decrease in potency of the compounds in plasma suggests that the reduction in activity may be due to either protein binding or partial inactivation by plasma components.

To help us understand the selectivity of our assay for PI3K signaling, we conducted human PRP experiments using a mitogen-activated protein/extracellular signal-regulated kinase 1/2 inhibitor (U0126) that is selective for the mitogen-activated protein kinase signaling pathway. Results from our tests revealed (data not shown) that this agent could not block TRAP-induced PAC-1 binding at concentrations up to 40 μmol/L. We also tested two additional PI3K inhibitors, PI-103 and TGX-221, reported to be more selective for PI3K α or β isoforms, respectively (31–33). Wortmannin was included in these experiments as a positive control. Inhibition of 50% at 10 μmol/L and 75% at 40 μmol/L was observed using both isoform-selective agents (data not shown).

Inhibition of murine platelet activation was investigated in an analogous manner using wortmannin, AYPGKF-NH2, and Fb* fluorescence. The concentration response curve from three separate experiments for wortmannin in mouse whole blood is shown in Fig. 3, bottom (IC50 = 163 nm).

**Effects of In vivo Treatment with Wortmannin in Mice.** Blood was collected from female CD1 nu/nu mice (three per group) that had either received no treatment or dosed i.p. with either vehicle (5% DMSO) or wortmannin (4 mg/kg) for 1 h. Blood was then collected and assayed for their platelet response to PAR activation. A robust (~30-fold) increase in Fb* binding was noted in the untreated and vehicle groups, whereas the platelet response of the wortmannin-dosed mice was inhibited by 78% (P ≤ 0.001; data not shown). To determine the sensitivity of the assay, we conducted a dose response experiment using the same experimental design as described above with four mice per group and doses of wortmannin at 0.5, 1, 2, and 4 mg/kg. Resulting data showed an ~40-fold increase in Fb* binding in vehicle-treated group with dose-dependent inhibition shown in wortmannin-treated groups (Fig. 4). In addition, xenograft tumor-bearing mice (eight per group, 14 days after implant, ~200 mm3) were dosed i.p. with either vehicle (5% DMSO) or wortmannin (4 mg/kg) and blood and tumors were collected 1 h after dose. In parallel to the platelet assay, excised tumors were homogenized and measured for pAKT levels. Platelets from the vehicle-treated mice showed a 45-fold induction of Fb* binding after ex vivo stimulation. The signal was reduced by 84%.
(P ≤ 0.0001) in the wortmannin-treated mice (Fig. 5A). This result correlated well (r² = 0.9457; Fig. 5C) with the 87% inhibition (P ≤ 0.0001) of pAKT from excised tumors from the same mice (Fig. 5B). We conducted an experiment in an analogous manner using mice bearing A2780 human ovarian carcinoma to test the assay performance in an additional tumor model. Resulting data were comparable with U87MG glioblastoma experiment with 25-fold increase in Fb⁺ binding and significant 98% (P ≤ 0.0001) inhibition of fibrinogen binding in wortmannin-treated group (data not shown).

**Use of Platelets from Cancer Patients.** Blood specimens from patients diagnosed with late-stage solid tumors were tested for suitability in the platelet assay. Blood specimens from 10 cancer patients were collected over a 2-week period and assayed on the day of collection. Blood was also collected from 10 healthy volunteers to serve as normal controls. For each of the patient and normal donor samples, the following data were collected: complete blood count, platelet counts from processed samples of PRP and GPP, and platelet CD41a and CD62P. All specimens were treated (in vitro) with 2 μmol/L wortmannin final concentration and assayed as described previously. Data obtained from these studies are summarized in Table 1. Routine hematology variables (WBC, RBC, and platelets) of the cancer patient specimens were similar to that obtained from the normal donor samples. We observed slightly lower SFLLRN-NH₂–induced activation in the cancer patient samples (16.2-fold) compared with that of the normal donors (24.7-fold). Wortmannin produced similar inhibition (>93% at 2 μmol/L) of normal donor and cancer patient platelet activation. Of note, a single cancer patient specimen displayed weak SFLLRN-NH₂–induced activation (1.5-fold) that remained weak in a second specimen drawn 5 weeks later. The reason for this poor response is unexplained.

**Discussion**

With increasing knowledge of the central role of PI3K in regulation of cell growth and survival, molecular agents that selectively inhibit PI3K signal transduction are being developed and investigated as potential cancer therapies. Our study was designed to quantitatively measure PI3K inhibition on intact cells during animal and human testing. The assay described here uses human and mouse platelets as a source of PI3K activity. After treatment with known PI3K inhibitors, platelet specimens are stimulated via PAR activation and downstream conformational changes in the GPIIb/IIIa fibrinogen receptor are used as the readout. In this report, we show concentration-dependent inhibition of this response after in vitro (human and mouse) and in vivo (mouse) treatments with the well-recognized PI3K

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<th>Table 1. Platelet evaluation in normal donors and cancer patients</th>
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*Routine hematology variables WBC, RBC, platelet count, and platelet activation results for 10 normal donors and 10 cancer patients were obtained as described in Materials and Methods.
† Ratio of PAC-1-FITC signal in TRAP-stimulated sample divided by signal in unstimulated sample.
‡ 100 × [1 – (treated MFI – unstimulated MFI) / (max stimulated MFI – unstimulated MFI)].
inhibitors wortmannin and LY294002. The importance of developing drug activity biomarker assays has been detailed by other investigators eager to obtain early measures of activity before determinations of therapeutic effect (15, 34–37). Flow cytometric analysis of cells in clinical blood and bone marrow specimens seems particularly useful for tracking signal transduction and target inhibition in diseased and surrogate target cells (38–44).

Initially, we attempted to develop a PI3K biomarker that measured pAKT induction in human leukocytes using the method of Chow et al. (37). This seemed appropriate as the pAKT is a direct downstream measure of PI3K activity (45). Unfortunately, all of our studies using various human leukocyte subpopulations stimulated by a variety of signal transduction agonists provided only minimal increases in the pAKT signal (<2-fold). Based on our previous experience, a drug activity biomarker assay must be capable of producing a robust signal window to sensitively monitor drug inhibition. The weak pAKT signaling in human leukocytes proved inadequate and forced us to try an alternative approach. Based on reviews by Rittenhouse (18) and Jackson et al. (19) supporting PI3K involvement in platelet signaling as well as recent reports of PI3K/AKT linkage to platelet function (33, 46, 47), we investigated use of platelets as a surrogate target for measuring PI3K activity. First, we investigated intracellular pAKT induction in human platelets using PAR-activating peptides. Data from these experiments (data not shown) revealed another weak 2-fold pAKT increase that could be blocked with wortmannin (50 nmol/L). To obtain a more robust response, we looked downstream for an amplified activity. We chose the well-documented PAR activation of GPⅡb/Ⅲa (fibrinogen receptor) as our readout. After establishing optimal conditions for PAR-induced platelet activation, we then added known PI3K inhibitors (wortmannin and LY294002) and obtained inhibitory activity that was in agreement with published reports for these compounds in other cell-based assays. The classic dose response curves generated were in part due to the robust signal window elicited in our system. The assay was optimized for use with human PRP and GPP specimens. We accurately generated were in part due to the robust signal window to sensitively monitor drug inhibition. We then systematically modified the human assay for use in preclinical mouse studies of in vivo target validation and pharmacodynamic modeling. We conducted in vivo wortmannin treatment experiments in normal and U87MG (glioblastoma) tumor-bearing mice and described data showing significant ($P \leq 0.0001$) inhibition of the platelet response from wortmannin-treated mice in two studies. The glioblastoma tumor model was selected due to recent reports indicating proliferative arrest of glioma cells by PI3K-selective agents (32, 48). These platelet effects were paralleled by a concomitant decrease in pAKT from excised tumors of the wortmannin-treated animals. The linkage of these two activities in a tumor model in which PI3K inhibitors show promise as effective therapeutic approach (31, 32, 48) suggests that use of the platelet assay could provide a shorter and simpler approach for in vivo evaluation of putative novel PI3K inhibitors.

Finally, we surveyed a small sampling of clinical specimens from 10 volunteer cancer patients to determine if the platelet assay could perform adequately during clinical evaluations of investigational drugs. Concerns of assay performance using cancer patient platelets included possible preactivation, inadequate platelet counts, and poor response to PAR activation. Data from our study showed that specimens from all but one patient performed adequately in this assay.

In summary, we showed that platelet specimens can be used as a readily accessible surrogate target tissue for measuring the effects of investigational PI3K inhibitors. This effect of PAR-induced activation of the fibrinogen receptor is robust and subject to sensitive inhibition by known PI3K inhibitors in pharmacologically relevant doses both in vitro and in vivo. Use of this approach could provide valuable information for both preclinical animal studies and early-stage clinical trials.

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