Supplementary Methods

Ex-vivo lymphocyte stimulation assay

In part 1, samples were collected before dosing and at 2, 3, 4, 6, and 8 hours after dosing on day 1 of cycle 1, as well as on day 8 and day 15. Patients in part 2 receiving 8-hour infusions had samples collected before dosing and 1, 4, 8, 10, and 11 hours after start of the infusion on day 1 of cycle 1, as well as on days 8 and 15. For patients receiving 24-hour infusions, samples were collected pre-dose and 1, 6 to 8, 24, 26, and 30 hours after the start of the first infusion, as well as on days 8 and 15. Whole blood isolated from patients (40 µl) was diluted with RPMI-1640 media (140 µl) and was treated with phytohemagglutinin (PHA;2 µl) to stimulate lymphocyte proliferation. Following a 30-minute exposure to bromodeoxyuridine (BrdU;10 µl), cells were harvested and stained using an FITC-conjugated antibody specific for BrdU, counterstained with propidium iodide/RNase A, and analyzed using a FACSCalibur flow cytometer. Approximately 35% to 40% of the CD45-positive cells in the whole blood incorporate BrdU following PHA stimulation under conditions defined in this assay, indicating DNA synthesis and cell division. Samples with less than 5% BrdU incorporation post-treatment was classified as demonstrating a pharmacodynamic effect. To explore the relationship between exposure and bioactivity of dinaciclib, %BrdU incorporation was correlated with the amount of dinaciclib found in plasma samples taken at the same time.

Immunohistochemistry for Rb, phospho-Rb and other cell cycle targets

Antibodies were as follows:

Antibody	Source	Dilution
Total Rb	BD Biosciences	1:75
Rb [pT356]	Invitrogen	1:800
Rb [pS795]	Cell Signaling Technologies	1:25
Rb [pS249/T252]	Invitrogen	1:500
Rb [S807/811]	Cell Signaling Technologies	1:100
Rb [S780]	Cell Signaling Technologies	1:50
$p27^{Kip1}$	BD Biosciences	1:50
cyclin D1	Neomarkers	1:40
p53	Immunotech	1:500
Ki-67	Vector Labs	1:250

For skin biopsies, immunohistochemical data were quantified using Aperio image analysis; nuclei were scored as 0, 1+, 2+, or 3+ and the percent positive cells was determined. For tumor biopsies, 100-200 cells were scored manually as 0, 1+ or 2+, and the percent positive cells determined. Alternatively, since tumor biopsies obtained from melanoma patients could be complicated by melanin deposits, immuno-histochemistry was also performed using a Texas red secondary antibody (Vulcan Fast Red, Biocare Medical) and quantified using Aperio image analysis, as for the skin biopsies.

Supplementary Table 1

Dose Levels and Dose Limiting Toxicities

a) 2-Hour Infusions

Dose Level (mg/m^2)	No of Subjects Treated	No of Subjects with DLT in Cycle 1	DLT (n)
1.85	1	0	
3.7	1	0	
7.4	1	0	
14.8	1	0	
29.6	6	1	bacterial sepsis (without neutropenia)
41.4	6	1	neutropenic fever
→ 50	15	4	elevated AST (1) hypotension (1) elevated AST and elevated uric acid (1) elevated ALT (1)
58	4	2	pancytopenia and pneumonia (1) neutropenic fever (1)

b) 8-Hour Infusions

Dose Level (mg/m^2)	No of Subjects Treated	No of Subjects with DLT in Cycle 1	DLT (n)
50	2	2	neutropenia (1) neutropenic fever (1)
41.4	2	2	neutropenic fever (1) hypotension (1)
29.6	3	3	syncope (1) neutropenia (1) elevated AST (1)
14.8	3	2	Neutropenia (1) elevated AST (1)
→ 7.4	6	0	

c) 24-Hour Infusions

Dose Level (mg/m^2)	No of Subjects Treated	No of Subjects with DLT in Cycle 1	DLT
7.4	4	0	
→ 10.4	3	0	
14.6	3	2	increased bilirubin (1) delirium (1)

→ RP2D

Supplementary Table 2

Patients achieving prolonged stable disease for > 6 cycles of dinaciclib

Subject Diagnosis	Dinaciclib Infusion Time	Dinaciclib Dose Level	Response by RECIST	Number of Cycles
Leiomyosarcoma	2-hour	41.4 mg/m^2	Stable Disease	8
Anal cancer		50 mg/m^2	Stable Disease	6
SCC lung		50 mg/m^2	Stable Disease	15
Prostate cancer		50 mg/m^2	Stable Disease	30
Chordoma		58 mg/m^2	Stable Disease	12
Melanoma	8-hour	29.6 mg/m^2	Stable Disease	6
Neuroendocrine carcinoma		41.4 mg/m^2	Stable Disease	22
Liposarcoma		50 mg/m^2	Stable Disease	8
Ovarian carcinoma	2-hour AB sequence	29.6 mg/m^2	Stable Disease	6
Rectal cancer	2-hour BA sequence	29.6 mg/m^2	Stable Disease	6

AB and BA sequences were part of the randomized, 2-way crossover study examining pharmacokinetics of dinaciclib in the absence and presence of aprepitant (Zhang D, Mita M, Shapiro GI, Poon J, Small K, Tzontcheva A, *et al*. Effect of aprepitant on the pharmacokinetics of the cyclin-dependent kinase inhibitor dinaciclib in patients with advanced malignancies. *Cancer Chemother Pharm* 2012; 70:891-8).

Supplementary Figure Legends

Supplementary Figure 1. Biphasic pattern of neutropenia in a representative patient. Absolute neutrophil count followed over time demonstrates an acute fall within hours of dinaciclib administration, with rapid recovery. This is followed by a recurrent decrease at day 8, consistent with a dinaciclib-mediated antiproliferative effect.

Supplementary Figure 2. Mean plasma dinaciclib concentration-time profiles. Profiles are shown after (A) 2-hour, (B) 8-hour, and (C) 24-hour intravenous infusions of dinaciclib at dose levels ranging from 1.85 to 58 mg/m^2.

Supplementary Figure 3. Relationship of maximum reductions in absolute neutrophil count to dinaciclib pharmacokinetic parameters. The maximum decrease from baseline in ANC (expressed as percentage) was determined from each subject and correlated with (A) AUC or (B) C_{max}. A sigmoid maximum effect (Emax) model was selected to describe the data.

Supplementary Figure 4. Effect of dinaciclib on the proliferation of peripheral blood lymphocytes stimulated *ex-vivo* with phytohemagglutinin (PHA). Results from representative patients treated with dinaciclib are shown for 8-hour intravenous infusions. BrdU = bromodeoxyuridine; D = day

Supplementary Figure 5. Effect of dinaciclib on CDK and cell cycle targets in keratinocytes. Results from skin biopsies taken prior to treatment after an 8-hour intravenous infusion are shown. (A) The results confirm the reduction in Rb [pT356] after dinaciclib and absent or modest effects on Rb [pS807/S811], total Rb and Ki-67, similar to results for 2-hour infusions. Effects on Rb [pS249/T252] are more pronounced after 8-hour infusions among this small sample set. (B) As with 2-hour infusions, dinaciclib produces little change in expression of $p27^{Kip1}$, with increased expression of p53.

Supplementary Figure 6. Early metabolic effects of dinaciclib in solid tumor patients. (A) FDG-PET scans obtained pre-treatment and at cycle 1, day 8 in a patient with cholangiocarcinoma treated at 50 mg/m^2 over 2 hours. (B) FDG-PET scans obtained pre-treatment and at cycle 1, day 8 in a patient with liposarcoma, who received 50 mg/m^2 over 8 hours. Abdominal CT obtained after 2 cycles of study treatment demonstrates reduced density of hepatic metastases.

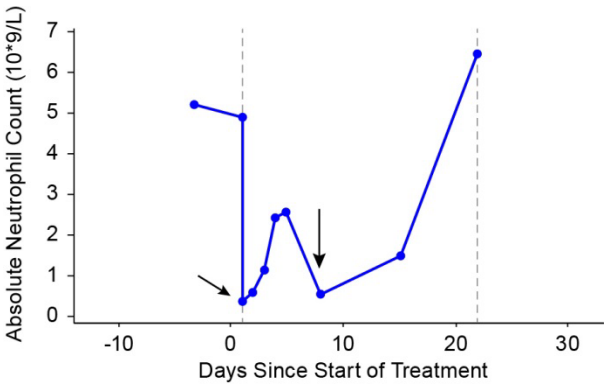
Absolute Neutrophil Count (10*9/L)
7
6
5
4
3
2
1
0
-10
0
10
20
30
Days Since Start of Treatment

Figure S1

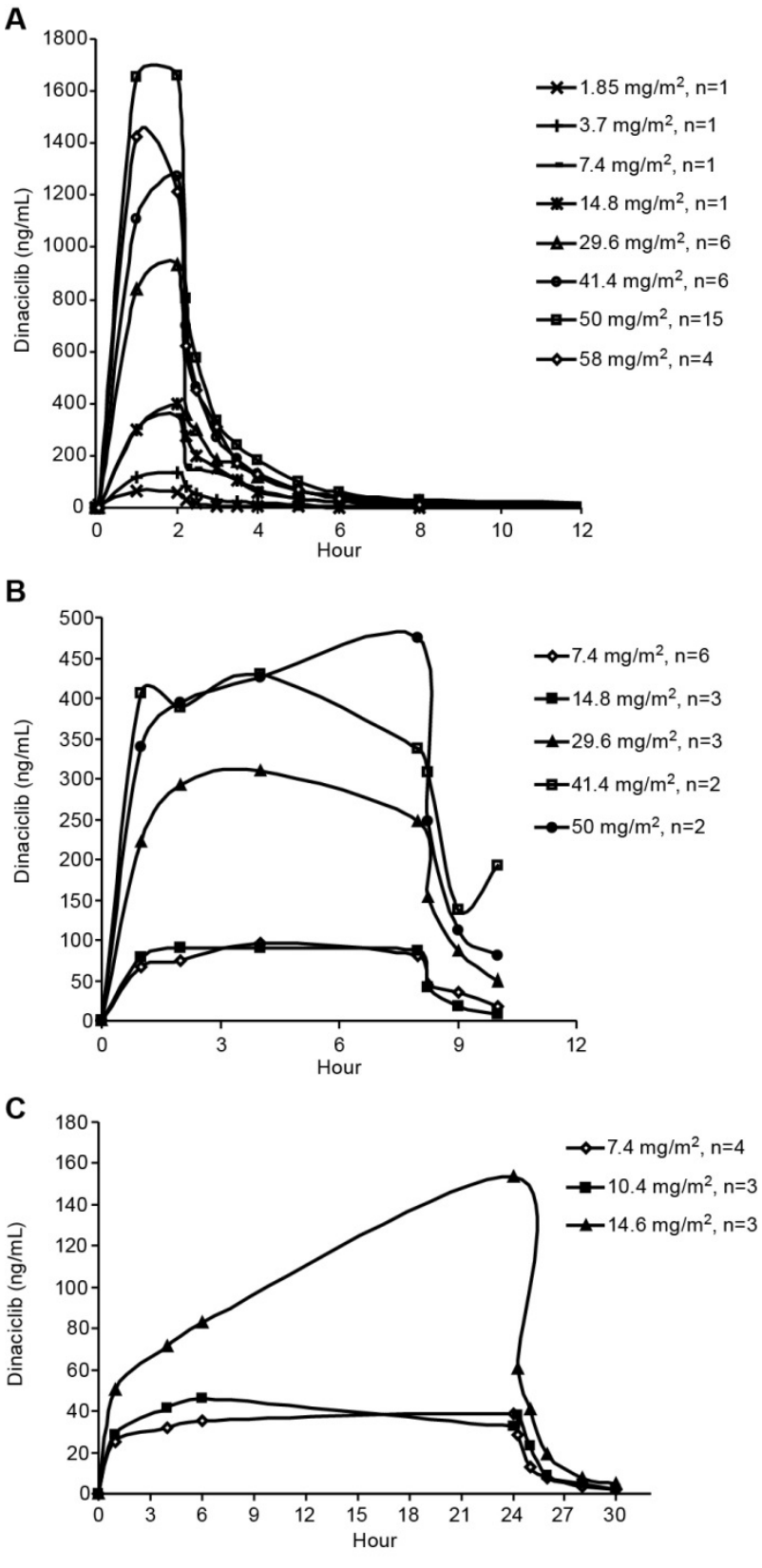
A
Dinaciclib (ng/mL)
Hour
1.85 mg/m2, n=1
3.7 mg/m2, n=1
7.4 mg/m2, n=1
14.8 mg/m2, n=1
29.6 mg/m2, n=6
41.4 mg/m2, n=6
50 mg/m2, n=15
58 mg/m2, n=4
B
Dinaciclib (ng/mL)
Hour
7.4 mg/m2, n=6
14.8 mg/m2, n=3
29.6 mg/m2, n=3
41.4 mg/m2, n=2
50 mg/m2, n=2
C
Dinaciclib (ng/mL)
Hour
7.4 mg/m2, n=4
10.4 mg/m2, n=3
14.6 mg/m2, n=3

Figure S2

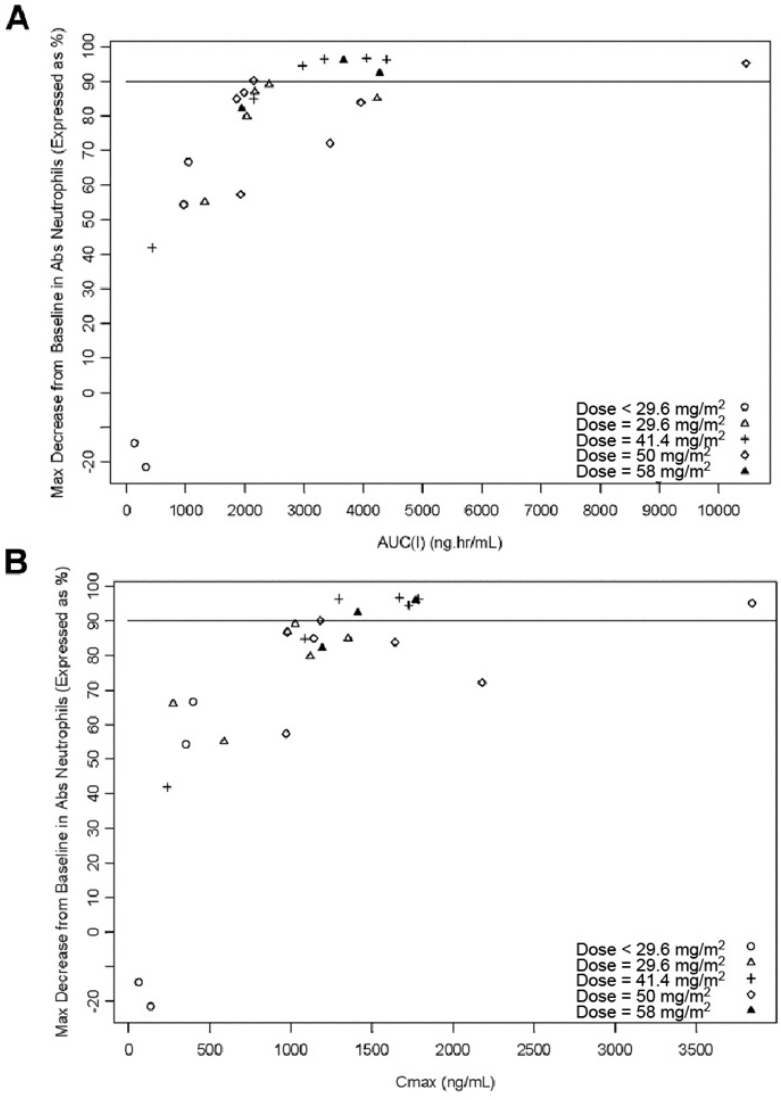
A
Max Decrease from Baseline in Abs Neutrophils (Expressed as %)
AUC(I) (ng.hr/mL)
Dose < 29.6 mg/m2
Dose = 29.6 mg/m2
Dose = 41.4 mg/m2
Dose = 50 mg/m2
Dose = 58 mg/m2
B
Max Decrease from Baseline in Abs Neutrophils (Expressed as %)
Cmax (ng/mL)
Dose < 29.6 mg/m2
Dose = 29.6 mg/m2
Dose = 41.4 mg/m2
Dose = 50 mg/m2
Dose = 58 mg/m2

Figure S3

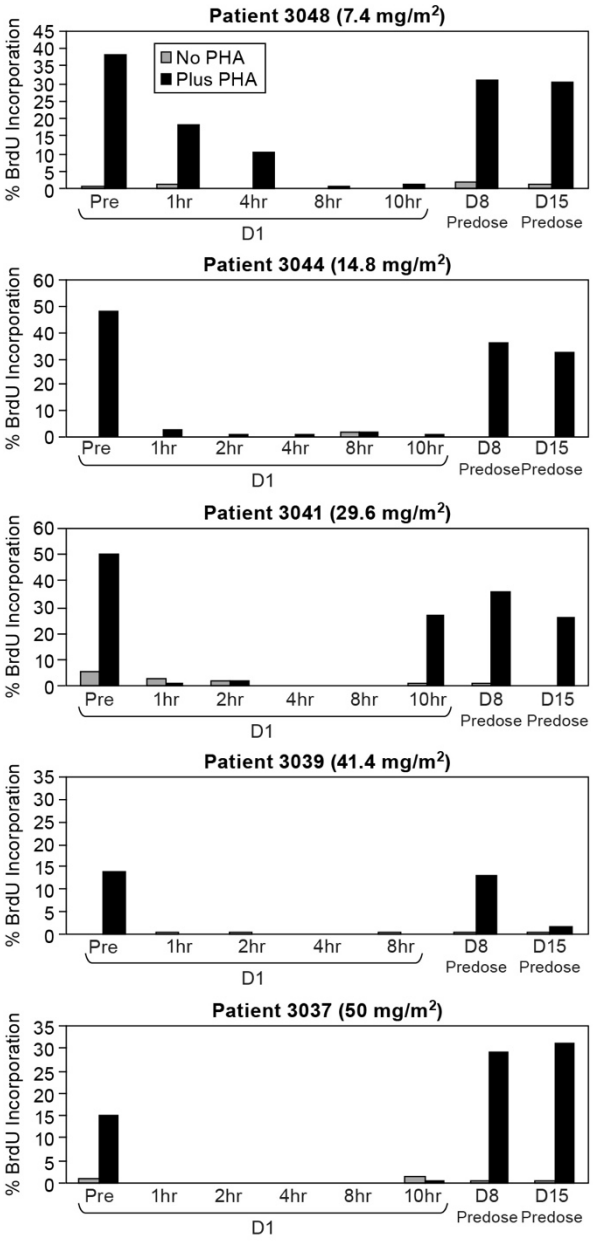
Patient 3048 (7.4 mg/m2)
% BrdU Incorporation
No PHA
Plus PHA
Pre
1hr
4hr
8hr
10hr
D8 Predose
D15 Predose
D1
Patient 3044 (14.8 mg/m2)
% BrdU Incorporation
Pre
1hr
2hr
4hr
8hr
10hr
D8 Predose
D15 Predose
D1
Patient 3041 (29.6 mg/m2)
% BrdU Incorporation
Pre
1hr
2hr
4hr
8hr
10hr
D8 Predose
D15 Predose
D1
Patient 3039 (41.4 mg/m2)
% BrdU Incorporation
Pre
1hr
2hr
4hr
8hr
D8 Predose
D15 Predose
D1
Patient 3037 (50 mg/m2)
% BrdU Incorporation
Pre
1hr
2hr
4hr
8hr
10hr
D8 Predose
D15 Predose
D1

Figure S4

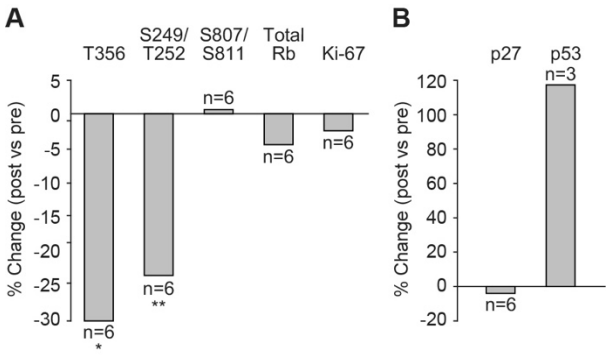
A
T356
S249/
T252
S807/
S811
Total
Rb
Ki-67
% Change (post vs pre)
5
0
-5
-10
-15
-20
-25
-30
n=6
n=6
n=6
n=6
n=6
n=6
*
**
B
p27
p53
n=3
% Change (post vs pre)
120
100
80
60
40
20
0
-20
n=6

Figure S5

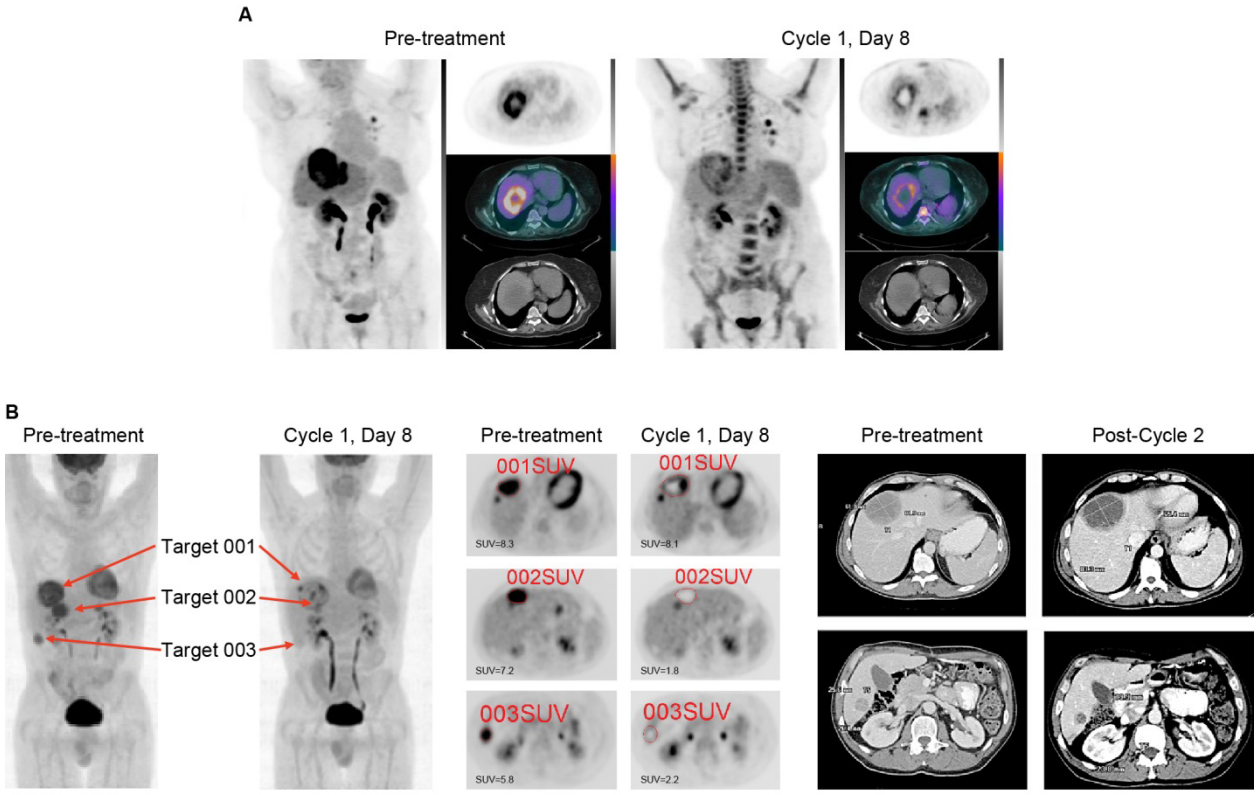
A
Pre-treatment
Cycle 1, Day 8
B
Pre-treatment
Cycle 1, Day 8
Target 001
Target 002
Target 003
Pre-treatment
Cycle 1, Day 8
001SUV
SUV=8.3
001SUV
SUV=8.1
002SUV
SUV=7.2
002SUV
SUV=1.8
003SUV
SUV=5.8
003SUV
SUV=2.2
Pre-treatment
Post-Cycle 2

Figure S6