Cancer Chemother Pharmacol (2009) 65:191–195
DOI 10.1007/s00280-009-1105-7

SHORT COMMUNICATION

The polyamine analog PG11047 potentiates the antitumor activity of cisplatin and bevacizumab in preclinical models of lung and prostate cancer

K. Dredge · J. A. Kink · R. M. Johnson · I. Bytheway · L. J. Marton

Received: 4 June 2009 / Accepted: 29 July 2009 / Published online: 15 August 2009

Abstract
Purpose PG11047 is a polyamine analog currently in Phase I trials for advanced cancer as a monotherapy and in combination with a number of approved anti-cancer agents. The use of polyamines as a target for antiproliferative therapy is based on findings that cells synthesize polyamines excessively when induced to grow and that polyamine metabolism is frequently dysregulated in cancer. A selective polyamine transport system provides access for PG11047 into rapidly dividing cells to inhibit polyamine biosynthetic enzymes, to induce the polyamine catabolic enzymes spermidine/spermine N^1-acetyltransferase (SSAT) and spermine oxidase (SMO) which could subsequently induce reactive oxygen species that contribute to tumor cell responses to PG11047, and to function as a polyamine with altered function when it binds to natural polyamine binding sites. The objective of the present study was to assess the antitumor effects of PG11047 alone and in combination with approved anti-cancer agents.
Methods The antitumor efficacy of PG11047 as a single agent, and in combination with cisplatin and bevacizumab, was tested in models of lung (A549) and prostate (DU-145) cancer, respectively.
Results PG11047 significantly inhibited tumor development in both lung and prostate cancer models when administered as a single agent. In the lung cancer model, PG11047 potentiated the antitumor effect of cisplatin. Although potent activity was observed with PG11047 and bevacizumab when administered as single agents in the prostate cancer model, the combination arm significantly enhanced antitumor activity compared with either agent alone. In all experiments, PG11047 was well tolerated with no adverse effects on bodyweight gain.
Conclusions The preclinical data support the rationale for the current Phase I trials which are assessing PG11047 as a monotherapy and in combination with a number of approved anti-cancer agents including cisplatin and bevacizumab.

Keywords Polyamine analog · PG11047 · Cisplatin · Bevacizumab · Cancer therapy

K. Dredge (✉) · I. Bytheway
Progen Pharmaceuticals Ltd, PO Box 2403,
Toowong, Brisbane, QLD 4066, Australia
e-mail: keithd@progen-pharma.com

J. A. Kink
Quintessence Biosciences, Madison, WI, USA

R. M. Johnson
KAI Pharmaceuticals, South San Francisco, CA 94080, USA

L. J. Marton
Progen Pharmaceuticals Inc, Redwood City, CA, USA

Introduction

Polyamines are small aminoalkyl polycations, necessary for cell proliferation [1–3] that also influence transcription [4], RNA stabilization, and translational frameshifting [5]. The interest in polyamines as a target for antiproliferative therapy is based on several observations that cells synthesize more polyamines when induced to grow; that the polyamine biosynthetic enzymes are coordinately regulated with growth controls; that polyamine metabolism is frequently dysregulated in cancers; and that polyamines are essential for eukaryotic cell growth [6].

Polyamine analogs represent an emerging approach to inhibition of polyamine biosynthesis in cells [7]. Mechanisms by which polyamine analogs exert their intracellular

effects include the induction of the polyamine catabolic enzyme spermidine/spermine N1-acetyltansferase (SSAT) and inhibition of polyamine biosynthetic enzymes, with subsequent depletion of intracellular polyamine pools, competition with natural polyamines for uptake, and the displacement of natural polyamines from functional sites related to the transcriptional regulation of genes and other functions [6, 8]. Among the classes of synthetic polyamine analogs that have been developed, are symmetrically substituted, asymmetrically substituted, and conformationally restricted analogs. These classes demonstrate that multiple biological activities and improved targeting abilities can be attained with small changes in molecular structure [6].

PG11047 is a conformationally restricted polyamine analog, with a structure based on the symmetrically alkylated analog N1, N11-bis (ethyl) norspermine (BENSpm), modified by the introduction of a double bond into the central 4-carbon methylene chain [9, 10]. Antiproliferative activity of PG11047 has been reported against human breast cancer cells and both small cell and non-small cell lung cancer cell lines [11, 12]. PG11047 significantly delayed the development of A549 xenografts in nude nu/nu mice [12]. The known biochemical mechanisms of the antitumor effects of PG11047 include the cell type specific downregulation of orthinine decarboxylase (ODC), the induction of SSAT and spermine oxidase (SMO) activities, and the antizyme-mediated feedback inhibition of polyamine uptake [6, 11, 13]. The generation of reactive oxygen species, particularly hydrogen peroxide, has been implicated in polyamine analog-induced, SSAT-associated cytotoxicity [14], and many of the same analogs that induce SSAT also induce SMO [15–17]. Thus, both SSAT and SMO could be responsible for producing reactive oxygen species that contribute to analog activity [6].

PG11047 is currently being tested in a Phase I clinical trial in patients with relapsed or refractory cancers as a single agent, and in a Phase Ib trial in combination with bevacizumab (Avastin; Genentech), erlotintib (Tarceva; OSI Pharmaceuticals), docetaxel (Taxotere; Sanofi-Aventis), gemcitabine (Gemzar; Eli Lilly), 5FU/Leucovorin, cisplatin, or sunitinib (Sutent; Pfizer). The aim of the present study was to determine the effect of PG11047 in a non-small cell lung cancer model alone and in combination with cisplatin, and in a prostate cancer model, alone and in combination with bevacizumab. The effects observed here are consistent with earlier reports of synergy or enhanced antitumor activity when inhibitors of polyamines are used in conjunction with established cytotoxic or antiangiogenic agents. Synergistic activity was apparent when N1, N11-bis-ethylnorspermine (BE-3-3-3), and cisplatin were used together against murine cell lines L1210 leukemia and B16F1 melanoma both in vitro and in vivo [18]. Enhanced antitumor effects have also been noted when bevacizumab was used in combination with the irreversible inhibitor of ODC, alpha-difluoromethylornithine (DFMO) [19]. These data support the concept that polyamine analogs may enhance the antitumor effects of agents such as cisplatin and bevacizumab.

Materials and methods

Chemicals and drugs

The test article PG11047, formerly known as CGC-11047 (Lot AV773), was dissolved in 0.9% saline and filter sterilized through a 0.2 μM membrane. Cisplatin (cis-Diammineplatinum(II) dichloride; Lot # 014K0993) was obtained from Sigma, St Louis, MO. Bevacizumab (Avastin; Lot#M46866) was provided as a gift by Genentech, CA.

Cell culture

Non-small cell lung cancer tumor cell line A549 obtained from ATCC was grown in F12K media supplemented with 10% fetal calf serum. Prostate cancer tumor cell line DU-145 obtained from ATCC was grown in DMEM media supplemented with 10% fetal calf serum.

Animals

Male nude nu/nu Fox chase mice aged 4–5 weeks and weighing 25–35 g were used in all studies (Source of Animals Harlan, Madison, WI). The animals were housed in micro isolator barrier cages with filtered top lids (Allentown Caging) in a dedicated Animal Room at Quintessence Biosciences throughout the study. The housing complied with the Guide for the Care and Use of Laboratory Animals, DHHS, (NIH) No. 85-23, revised 1985 and the Animal Welfare Act (9 CFR 3). Temperature and humidity were recorded daily. The room was equipped with an automatic timer and the animals received 12 h of light and dark each day. Temperatures were maintained from 68–74°F and humidity was maintained from 20–80%. Animals were quarantined one to two weeks prior to the treatment. Animals were fed ad libitum with 16% protein rodent diet (2016S; Harlan/Teklad). The water used for this study (Madison municipal water) was provided ad libitum.

Tumor model and drug treatment

Tumor cells were harvested by centrifugation at 1,200 RPM for 10 min at 4°C. One hundred microliters of A549 cells (2.8×10^6 cells) or DU-145 cells

(1.80×10^6 cells) were injected s.c. in the right rear dorsal flank of each mouse using a 27 gauge syringe needle. Tumors were allowed to reach approximately 75 mm^3 in volume before the start of treatment. A sufficient number of mice were implanted with cells so that tumors in a weight range as narrow as possible could be selected for the trial on the day of treatment initiation (day 15 after tumor implantation). Those animals selected with tumors in the proper size range were randomized and divided into the various treatment groups so that the average tumor sizes for each group were approximately equivalent. Tumors were allowed to develop for a minimum of 60 days after tumor implantation, except where the tumor on any animal reached 2,000 mm^3 in size or ulcerated. In this case, mice were sacrificed prior to study termination.

The experiments were initiated to evaluate the efficacy of PG11047 in combination with either cisplatin in nude nu/nu Fox chase mice implanted with the human non-small cell lung cancer tumor (A549) or with bevacizumab in nude nu/nu Fox chase mice implanted with the human prostate cancer tumor (DU-145). All treatments were initiated when all mice had established tumors approximately 75 mm^3 in size. Test agents were administered intraperitoneally (i.p.) as a bolus dose. The drugs were administered once a week according to the following schedule:

- Cisplatin study: Days 15, 22, 29, 36, 43, 50, and 57 after implantation at doses of PG11047 (25 mg/kg/wk) and cisplatin (1.5 mg/kg/wk).
- Bevacizumab study: Days 23, 30, 37, 44, 51, and 59 after implantation at doses of PG11047 (25 mg/kg/wk); bevacizumab was administered twice a week (days 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 59, and 62) at 5.0 mg/kg.

The same doses were administered in the combination groups. In these groups the test agents were administered separately, the polyamine analog was administered first, immediately followed by cisplatin or bevacizumab. The control group was treated with a vehicle (sterile saline) using the same treatment schedule. The animals were observed for adverse clinical signs after each dose and once daily thereafter throughout the course of the study. Body weights and tumor volumes were obtained and recorded prior to each dose and additional measurements were taken once a week, typically 3–4 days after dosing. Morbidity and mortality were monitored once daily following the initial dose and continued throughout the course of the study. The experiment was terminated 60 days after tumor implantation, but not before.

The subcutaneous tumors were measured by caliper measurements (mm) and the volume calculated using the formula for an ellipsoid sphere: $V\ (mm^3) = L \times W^2/2$ where L and W refer to the length and width dimensions collected at each measurement. Animals were euthanized by CO_2 asphyxiation according to Guide for the Care and Use of Laboratory Animals, DHHS, (NIH) No. 86-23 and the Animal Welfare Act (9 CFR 3). The antitumor activity was assessed by the comparison of the median tumor volume of the treated groups to the median tumor volume of the control group and expressed as the Tumor Growth Inhibition (TGI) using the equation:

$$TGI = \left(1 - \left(\frac{V_{\text{endTA}} - V_{\text{startTA}}}{V_{\text{endControl}} - V_{\text{startControl}}}\right)\right) \times 100\%$$

V_{endTA} = Ending Tumor Volume of Test Agent treated animal; V_{startTA} = Starting Tumor Volume of Test Agent treated animal; $V_{\text{endControl}}$ = Ending Tumor Volume of Control animal; $V_{\text{startControl}}$ = Starting Tumor Volume of Control animal.

Statistics

The Mann–Whitney U Test is a statistical formula to determine statistical significance between two data sets. This method was used to calculate the significant effect between the treatment groups with respect to tumor volume and body weight.

Results

Clinical observations

No adverse clinical signs were observed in either treatment group. There were no mortalities due to drug treatment during the study.

Response to treatment

In the non-small cell lung cancer model PG11047 and cisplatin significantly inhibited tumor development when administered as single agents, exhibiting TGI values of 58 and 56%, respectively. When combined with cisplatin in this model, these agents produced a significant additive effect (Fig. 1a), increasing the TGI value to 86%, an increase of 30% compared to cisplatin alone. No body weight loss was noted in any of the treatment groups (Fig. 1b).

In the prostate cancer model, a significant additive effect was also observed between PG11047 and bevacizumab in combination (Fig. 2a). Upon completion of the treatment (day 65), TGI values were 76 and 95% for PG11047 and bevacizumab, respectively. It is not known whether such strong efficacy signals from the single agents masked the optimal signal from the combination modality. Nonetheless, the significant additive effect of the combination

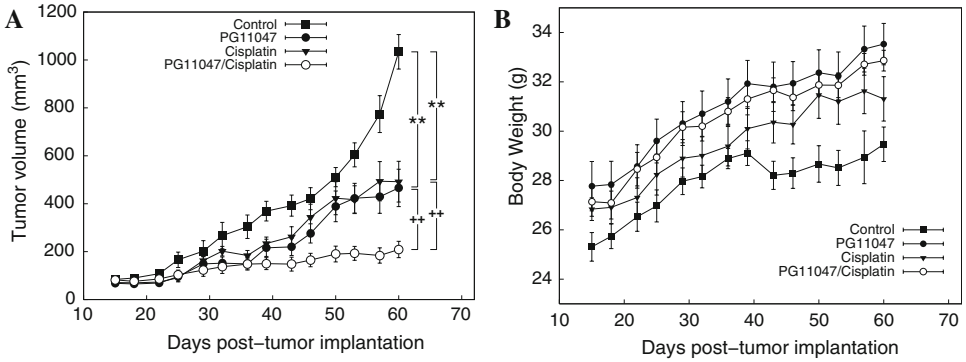

Fig. 1 A significant additive effect is observed when PG11047 is combined with cisplatin in the A549 non-small cell lung cancer tumor model (**a**). Body weight is not affected following treatment PG11047 and/or cisplatin (**b**). $^{**}P < 0.01$ versus vehicle control, $^{++}P < 0.01$ versus PG11047 plus cisplatin

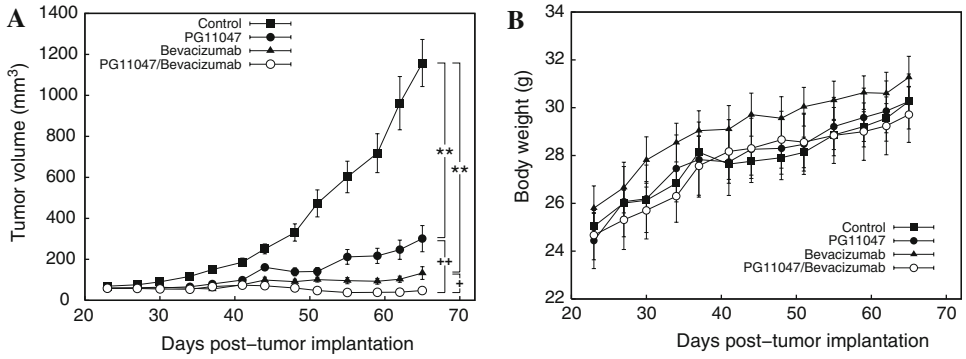

Fig. 2 A significant additive effect is observed when PG11047 is combined with bevacizumab in the DU-145 prostate cancer tumor model (**a**). Body weight is not affected following treatment PG11047 and/or cisplatin (**b**). $^{**}P < 0.01$ versus vehicle control, $^{+}P < 0.05$ versus PG11047 plus bevacizumab, $^{++}P < 0.01$ versus PG11047 plus bevacizumab

group produced a TGI value of 103%, an increase of 8% compared to bevacizumab alone. No body weight loss was noted in any of the treatment groups (Fig. 2b).

Discussion

The relevance of polyamines in cancer is becoming increasingly recognized as targets of this pathway are identified. These targets include the rate-limiting enzymes S-adenosylmethionine decarboxylase (AdoMetDC) and ODC; and the catabolic enzymes such as SSAT and SMO, as well as the polyamine transport system [6]. The chemical structures of polyamine analogs exploit structural similarity with the natural polyamines, so as to allow their recognition and subsequent uptake by the polyamine transporter, while the structural differences result in the inhibition of other important and related protein targets. This confers the added benefit of preventing the induction of compensatory changes in metabolism such as those seen with DFMO [20]. PG11047 is thought to downregulate ODC expression, induce SSAT and SMO activities and modulate the antizyme-mediated feedback inhibition of polyamine uptake [6, 10, 13]. Whichever mechanism takes precedence, the data reveal that PG11047, at a dose well below its MTD, significantly reduces tumor growth in models of lung and prostate cancer. Importantly, significant additive activity has been identified when PG11047 is used in combination with cisplatin and bevacizumab.

The antitumor activity was assessed by the comparison of the median tumor volume of the treated groups to the median tumor volume of the control group. No adverse clinical signs or mortalities were observed in any treatment group. No body weight loss was noted in any of the treatment groups during the study. At day 60, the TGI for PG11047 treatment alone was 58% and cisplatin alone was 56%, whereas combination therapy increased TGI to 86%, an increase of 30%. Thus, the data indicate there is a significant additive effect with both test agents administered in combination, compared to the individual compounds alone.

The additive activity for the PG11047/bevacizumab combination in the DU-145 model was significant but perhaps not optimal, due to single agent activity being highly potent with TGI values of 76% for PG11047 alone and 95% bevacizumab alone. The impact of bevacizumab, which only inhibits the tumor-derived vascular endothelial growth factor (VEGF) and not mouse stromal VEGF [21], was greater than expected in the DU-145 model. This is possibly due to a low level of stromal VEGF in this particular model, making the tumor-derived VEGF the main driver for tumor angiogenesis. Nonetheless, a TGI of 103%

demonstrated that enhanced activity could still be achieved when PG11047 was combined with bevacizumab. Further studies may be warranted to fully appreciate the potential for these two agents when administered in a combination setting.

Encouraging preclinical data for polyamine analogs alone and in combination with cytotoxic drugs support their continued evaluation [22]. A number of chemotherapeutic agents including platinum drugs such as cisplatin and oxaliplatin or 5-fluorouracil have been identified as compounds that exert a concerted effect on the polyamine pathway, up-regulating the catabolic pathway enzyme SSAT [22, 23]. The polyamine analog PG11047 induces SSAT [6], thus providing a rationale for its combination with cisplatin. As polyamines are essential for proliferation of endothelial cells [24], it is envisaged that the combination of a polyamine analog with an angiogenesis inhibitor could potently inhibit tumor angiogenesis. In support of this hypothesis, CGC-11093, a polyamine analog structurally related to PG11047, increased the anti-angiogenic properties of bortezomib [25]. The combination effects noted here, namely PG11047 with either cisplatin or bevacizumab, are currently being explored in the clinic in a Phase 1b trial in advanced cancer patients. Other agents under evaluation in the trial include gemcitabine, erlotinib, sunitinib, 5-flurouracil, and docetaxel. These preclinical data support the current clinical strategy to examine the utility of PG11047 in combination with cisplatin and bevacizumab in advanced cancer.

References

1. Heby O (1981) Role of polyamines in the control of cell proliferation and differentiation. Differentiation 19:1–20
2. Janne J, Alhonen L, Leinonen P (1991) Polyamines: from molecular biology to clinical applications. Ann Med 23:241–259
3. Tabor CW, Tabor H (1984) Polyamines. Annu Rev Biochem 53:749–790
4. Igarashi K, Kashiwagi K (2000) Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 271:559–564
5. Matsufuji S, Matsufuji T, Miyazaki Y et al (1995) Autoregulatory frameshifting in decoding mammalian ornithine decarboxylase antizyme. Cell 80:51–60
6. Casero RA Jr, Marton LJ (2007) Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nat Rev Drug Discov 6:373–390
7. Seiler N (2005) Pharmacological aspects of cytotoxic polyamine analogs and derivatives for cancer therapy. Pharmacol Ther 107:99–119
8. Reddy VK, Valasinas A, Sarkar A et al (1998) Conformationally restricted analogues of 1N, 12N-bisethylspermine: synthesis and growth inhibitory effects on human tumor cell lines. J Med Chem 41:4723–4732
9. Valasinas A, Sarkar A, Reddy VK et al (2001) Conformationally restricted analogues of 1N, 14N-bisethylhomospermine (BE-4–4-4): synthesis and growth inhibitory effects on human prostate cancer cells. J Med Chem 44:390–403
10. Huang Y, Pledgie A, Casero RA et al (2005) Molecular mechanisms of polyamine analogs in cancer cells. Anticancer Drugs 16:229–241
11. Holst CM, Frydman B, Marton LJ, Oredsson SM (2006) Differential polyamine analogue effects in four human breast cancer cell lines. Toxicology 223:71–81
12. Hacker A, Marton LJ, Sobolewski M, Casero RA Jr (2008) In vitro and in vivo effects of the conformationally restricted polyamine analogue CGC-11047 on small cell and non-small cell lung cancer cells. Cancer Chemother Pharmacol 63(1):45–53
13. Mitchell JL, Thane TK, Sequeira JM et al (2007) Antizyme and antizyme inhibitor activities influence cellular responses to polyamine analogs. Amino Acids 33:291–297
14. Ha HC, Woster PM, Yager JD et al (1997) The role of polyamine catabolism in polyamine analogue-induced programmed cell death. Proc Natl Acad Sci USA 94:11557–11662
15. Wang Y, Devereux W, Woster PM (2001) Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analogue exposure. Cancer Res 61:5370–5373
16. Wang Y, Murray-Stewart T, Devereux W (2003) Properties of purified recombinant human polyamine oxidase PAOh/SMO. Biochem Biophys Res Commun 304:605–611
17. Devereux W, Wang Y, Stewart TM (2003) Induction of the PAOh1/SMO polyamine oxidase by polyamine analogues in human lung carcinoma cells. Cancer Chemother Pharmacol 52(5):383–390
18. Hawthorn TR, Austin JK Jr (1996) Synergism of the polyamine analogue, N1, N11-bisethylnorspermine with cis-diaminedichloroplatinum (II) against murine neoplastic cell lines in vitro and in vivo. Cancer Lett 99(1):99–107
19. Svensson KJ, Welch JE, Kucharzewska P (2008) Hypoxia-mediated induction of the polyamine system provides opportunities for tumor growth inhibition by combined targeting of vascular endothelial growth factor and ornithine decarboxylase. Cancer Res 68(22):9291–9301
20. Wallace HM, Fraser AV, Hughes A (2003) A perspective of polyamine metabolism. Biochem J 376:1–14
21. Liang WC, Wu X, Peale FV et al (2006) Cross-species vascular endothelial growth factor (VEGF)-blocking antibodies completely inhibit the growth of human tumor xenografts and measure the contribution of stromal VEGF. J Biol Chem 281(2):951–961
22. Allen WL, McLean EG, Boyer J et al (2007) The role of spemidine/spermine N1-acetyltransferase in determining response to chemotherapeutic agents in colorectal cancer cells. Mol Cancer Ther 6(1):128–137
23. Varma R, Hector S, Greco WR et al (2007) Platinum drug effects on the expression of genes in the polyamine pathway: time-course and concentration-effect analysis based on Affymetrix gene expression profiling of A2780 ovarian carcinoma cells. Cancer Chemother Pharmacol 59:711–723
24. Morrison RF, Seidel ER (1995) Vascular endothelial cell proliferation: regulation of cellular polyamines. Cardiovasc Res 29:841–847
25. Carew JS, Nawrocki ST, Reddy VK et al (2008) The novel polyamine analogue CGC-11093 enhances the antimyeloma activity of bortezomib. Cancer Res 68:4783–4790