Drugs of the Future 2010, 35(1): 11-15

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.1.1439262

MONOGRAPH

PLINABULIN

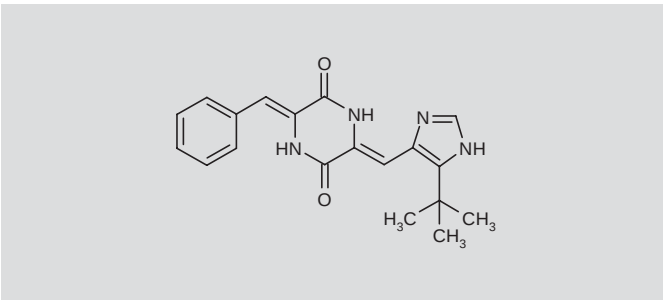
Prop INN; USAN

Tubulin Polymerization Inhibitor
Vascular-Disrupting Agent
Oncolytic

KPU-2
NPI-2358

3(*Z*)-Benzylidene-6(*Z*)-(5-*tert*-butyl-1*H*-imidazol-4-ylmethylene)piperazine-2,5-dione

InChI: 1S/C19H20N4O2/c1-19(2,3)16-13(20-11-21-16)10-15-18(25)22-14(17(24)23-15)9-12-7-5-4-6-8-12/h4-11H,1-3H3,(H,20,21)(H,22,25)(H,23,24)/b14-9-,15-10-



$C_{19}H_{20}N_4O_2$
Mol wt: 336.3877
CAS: 714272-27-2
EN: 340259

SUMMARY

Vascular-disrupting agents (VDAs) are a new class of compounds that target tumor vasculature, causing a rapid and acute collapse of the blood vessel network surrounding the tumor, slowing blood flow and ultimately resulting in tumor necrosis. Plinabulin is a small-molecule VDA that works by selectively binding to the colchicine binding site of endothelial tubulin, leading to disorganization of the endothelial cytoskeleton and inhibition of tumor blood flow. Clinical studies have shown that plinabulin markedly reduces tumor blood flow, providing significant clinical benefit to patients with solid tumors. Further results in patients with non-small cell lung cancer (NSCLC) demonstrated that plinabulin enhanced the antitumor activity of docetaxel, with a favorable safety profile. Plinabulin is currently undergoing phase I clinical studies as monotherapy for the treatment of solid tumors and lymphoma and phase II evaluation in combination with docetaxel for the treatment of NSCLC.

E. Ferrer, J. Bolós and R. Castañer. Thomson Reuters, Provença 388, 08025 Barcelona, Spain.

SYNTHESIS

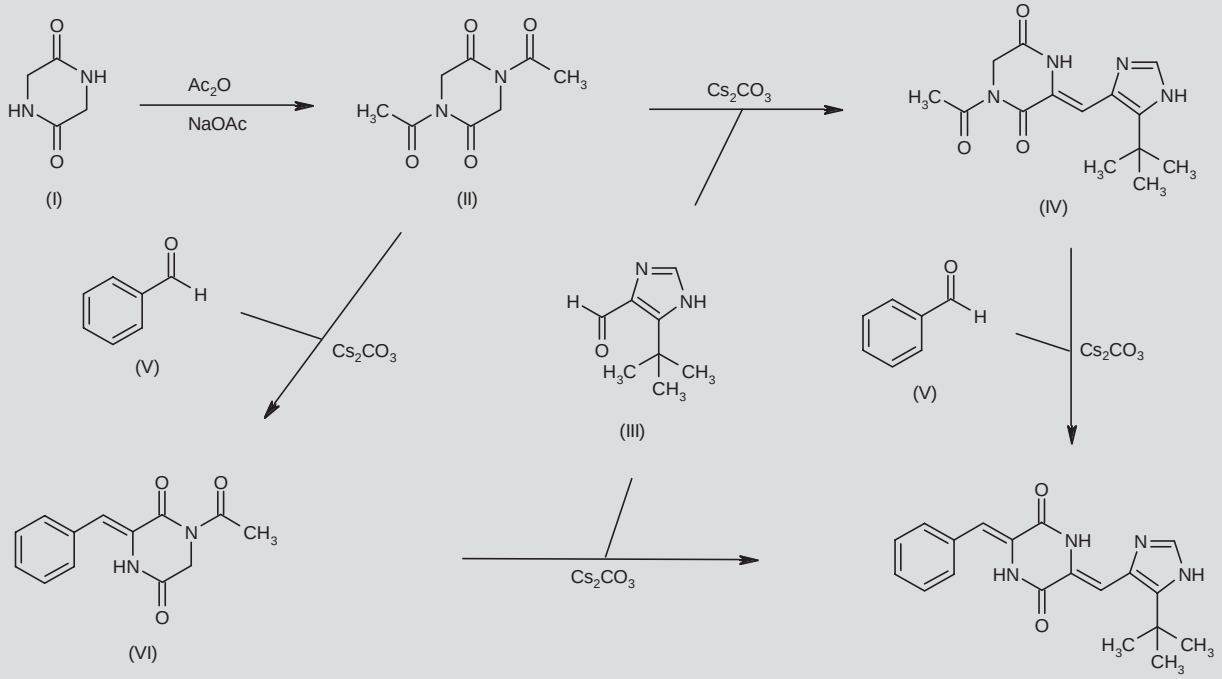
2,5-Piperazinedione (I) is acylated with acetic anhydride at 110 °C in the presence of catalytic NaOAc to afford the diacetyl derivative (II). Claisen condensation of *N,N'*-diacetyl-2,5-piperazinedione (II) with 5-*tert*-butylimidazole-4-carbaldehyde (III) by means of Cs_2CO_3 in DMF at room temperature leads to the monoacetylated (imidazolylmethylene)piperazinedione (IV), which is finally condensed with benzaldehyde (V) in the presence of Cs_2CO_3 in DMF at 80 °C. In a related alternative method, diketopiperazine (II) is first condensed with benzaldehyde (V) in the presence of Cs_2CO_3 in DMF at room temperature, yielding the benzylidene piperazinedione (VI), which is subsequently condensed with the imidazole-aldehyde (III) by means of Cs_2CO_3 in hot DMF (1-3). Scheme 1.

The intermediate 5-*tert*-butylimidazole-4-carbaldehyde (III) is prepared by reaction of ethyl pivaloyl acetate (VII) with sulfuryl chloride in chloroform to provide the 2-chloro derivative (VIII), which is cyclized with formamide (IX) in the presence of a trace of water in a sealed tube at 150 °C, affording ethyl 5-*tert*-butylimidazole-4-carboxylate (X). Reduction of ester (X) with $LiAlH_4$ in THF gives alcohol (XI), which is finally oxidized to the target carbaldehyde (III) using MnO_2 in acetone (1-3). Scheme 2.

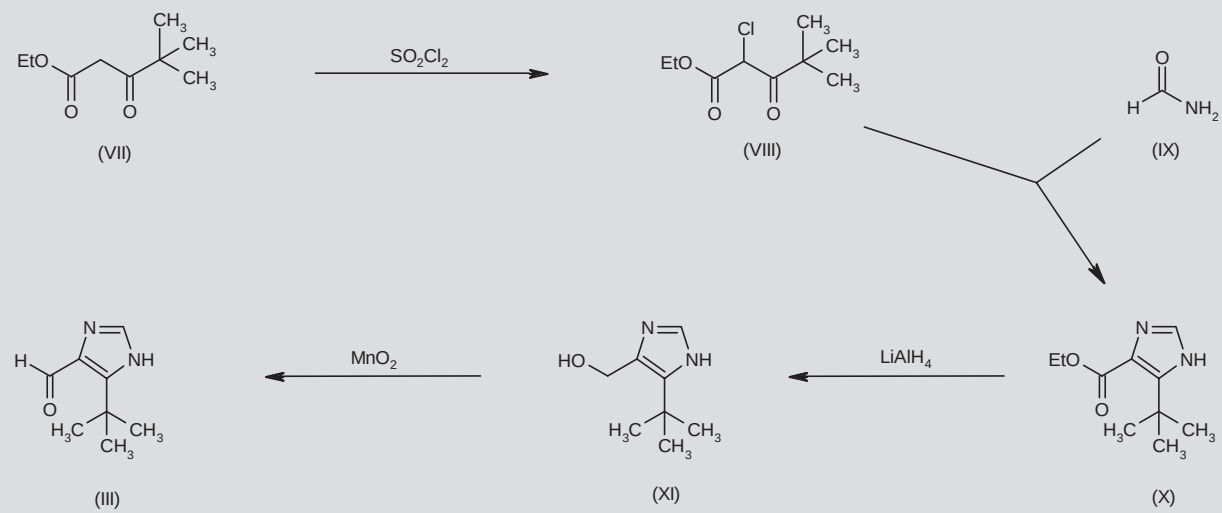
BACKGROUND

Cancer is the leading cause of death in men and women younger than 85 years of age, with 474,808 deaths reported in the United States in 2006, compared with 394,257 deaths from heart disease (4). Recent investigations have evidenced a growing incidence of cancer cases and deaths, with a total of 1,479,350 new cases and 562,340 deaths projected to occur in the U.S. in 2009 (4), with an expected increase of approximately 45% in the number of cases diagnosed over the next two decades (5). Lung cancer is the second most frequently diagnosed cancer, accounting for about 15% of all new cases in both genders, and it remains the main cause of cancer death in both men and women, with expected mortality rates in the U.S. during 2009 of 30% and 26%, respectively (4). The two main forms of lung cancer are small cell lung cancer (SCLC) and the slower-growing and less invasive non-small cell lung cancer (NSCLC);

Scheme 1. Synthesis of Plinabulin



Scheme 2. Synthesis of Intermediate (III)



the latter accounts for approximately 85% of all lung cancer cases (6). In addition, hematological malignancies, i.e., leukemia, lymphoma and myeloma, are expected to affect over 100,000 persons in 2009, with non-Hodgkin's lymphoma the most frequently diagnosed type (4).

Tumor vasculature emerged as a promising therapeutic target in cancer therapy after the observation that the formation of new blood vessels, or angiogenesis, is crucial for tumor growth and the development of metastases. While antiangiogenic drugs block new blood vessel sprouting, vascular-disrupting agents (VDAs) target the already established blood vessel network that supplies oxygen and nutrients to the tumor. Unlike normal vasculature, tumor blood vessels are structurally disorganized, with increased rates of endothelial cell proliferation and highly permeable thinned-wall vessels, result-

ing in high resistance to blood flow. In particular, VDAs act selectively on tumor vascular endothelial cells by interfering with adhesion junctions and the tubulin cytoskeleton, which are essential to endothelial cell motility, attachment and proliferation. As a consequence of the activity of VDA on endothelium, protein permeability and interstitial pressure increase, resulting in plasma leakage, decreased vessel diameter and higher blood viscosity. As a result, blood flow is further slowed and nutrient and oxygen supply interrupted, thereby triggering tumor necrosis (7).

There are two types of VDAs: ligand-directed VDAs, composed of a targeting part (antibody, peptide or growth factor) and an effector moiety that kills tumor cells through a variety of mechanisms; and small-molecule VDAs, which are further subdivided into synthetic flavonoids, which stimulate local cytokine production, and tubulin-binding agents, such as plinabulin (formerly NPI-2358). Plinabulin is a synthetic derivative of the low-molecular-weight cyclic dipeptide of marine origin (*Aspergillus* sp.), phenylahistin, or halimide, which blocks microtubule assembly by selectively interacting at the colchicine binding site of endothelial tubulin, disrupting the endothelial cytoskeleton and inhibiting tumor blood flow, while sparing normal vasculature (8, 9).

Nereus Pharmaceuticals is currently conducting a phase II clinical trial evaluating plinabulin in combination with docetaxel for the treatment of NSCLC (ClinicalTrials.gov Identifier NCT00630110). Plinabulin is also undergoing phase I evaluation as monotherapy for the treatment of solid tumors and lymphoma (ClinicalTrials.gov Identifier NCT00322608).

PRECLINICAL PHARMACOLOGY

Initial structure–activity relationship studies evaluating over 110 analogues identified plinabulin as a halimide derivative with potent cytotoxic activity against HT-29 human colon adenocarcinoma cells in vitro (IC_{50} = 15 nM) (9, 10). Unlike paclitaxel, plinabulin maintained equivalent cytotoxicity against multidrug-resistant tumor cells (11), overcoming the P-glycoprotein-mediated mechanism of resistance (12).

In addition to targeting tumor vasculature, plinabulin also exerts a direct apoptotic effect on tumor cells. Fluorescence binding and competition assays demonstrated that plinabulin binds faster than colchicine to tubulin, but that this association is of lower affinity, suggesting a less toxic profile. Plinabulin's antiproliferative effect was mainly mediated by mitotic block, as evidenced by similar IC_{50} values for inhibiting MCF7 human breast cancer cell growth (3 nM) and causing half-maximal mitotic block at prometaphase (1 nM). At the antimitotic IC_{50}, plinabulin suppressed mitotic spindle formation and chromosome alignment in MCF7 breast cancer cells, resulting in cell cycle arrest at prometaphase. The results of this study pointed to tubulin sequestering as the mechanism by which plinabulin blocks spindle and interphase microtubule polymerization and, consequently, tumor cell proliferation (13, 14).

In combination with standard chemotherapeutic drugs, plinabulin markedly enhanced tumor growth inhibition in three xenograft models in the nude (*nu/nu*) mouse (15). In the presence of plinabulin (7.5 mg/kg i.p.) the tumor growth inhibition rate of irinotecan (100 mg/kg i.p.) increased from 33.1% to 74.9% in the HT-29 xenograft model. Similarly, tumor growth-inhibitory rates for docetaxel and paclitaxel were significantly increased from 44.8% to 75.1% and from 47.4% to 70.9%, respectively, in DU 145 human prostate tumor and MDA-MB-231 human breast tumor xenografts. These results can likely be explained by the combination of mechanistically distinct approaches, targeting tumor vasculature and inducing cell apoptosis.

In vitro studies have shown the ability of plinabulin (20 nM) to induce tubulin depolymerization in human umbilical vein endothelial cells (HUVEC) and concentration-dependently increase HUVEC monolayer permeability at lower concentrations compared with colchicine and vincristine (12). Using intravital microscopy of tumor spheroids in a dorsal skinfold mouse model, plinabulin was associated with rapid vasculature collapse in established N202 breast tumors, resulting in central tumor necrosis and decreased tumor size (12, 14).

Similar results were obtained in the rat P22 carcinosarcoma model, in which plinabulin reduced blood flow; this reduction was highly selective for tumors compared with organs (brain, heart, ileum) and resulted in tumor necrosis (16).

Studies in the human MV522 NSCLC subcutaneous xenograft mouse model demonstrated significant reductions in tumor size, with an overall response rate of 75% and two complete tumor regressions following combined docetaxel (15 mg/kg i.v. on days 1, 3 and 5) and plinabulin (3.75 mg/kg i.p. on days 1, 3, 5, 8 and 11) treatment. These effects were observed both in small (100 mm^3) and large (1700 mm^3) tumor models. Interestingly, the addition of plinabulin prevented the severe weight loss noted in mice administered docetaxel alone (17). Moreover, administration of plinabulin 1 h after docetaxel at the aforementioned doses was the optimal dosing schedule to potentiate the antitumor activity of docetaxel.

The time- and dose-dependency of plinabulin-induced vascular effects was confirmed in further investigations using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), which measures changes in tumor blood flow, in the mouse C3H mammary carcinoma xenograft model. Compared with pretreatment values, a single administration of plinabulin (7.5 mg/kg i.p.) significantly and time-dependently reduced the initial area under the contrast concentration–time curve 90 s after contrast injection ($IAUC_{90}$) by 64% at 1 h, 50% at 3 h and 62% at 6 h. A persistent, although non-significant, reduction in tumor blood flow was still observed at 24 h (87%) (18).

CLINICAL STUDIES

The safety, pharmacokinetics and pharmacodynamics of plinabulin were assessed in a first-in-human single-agent study in patients (N = 38) with advanced solid tumors or lymphoma (19). This open-label study was designed as a dynamic accelerated dose titration, in which the dose of plinabulin was escalated in cohorts from 2 mg/m^2 to a recommended phase II dose (RP2D). The cohorts comprised one to three patients, depending on observed adverse events, and were further extended to six patients if a dose-limiting toxicity (DLT) was reported in the prior cohort. Patients received a weekly i.v. infusion of plinabulin for 3 weeks in 4-week cycles. Intensive cardiac monitoring and standard safety assessments (electrocardiograms, troponin I and natriuretic peptide B determination, blood pressure and echocardiography) were run. Additional DCE-MRI measurements taken at different time points provided a pharmacodynamic measure

of tumor blood flow. Based on safety and pharmacodynamic results, the RP2D was established at 30 mg/m^2. At this dose, adverse events included nausea, vomiting, fatigue, fever, tumor pain and transient blood pressure elevation. A decrease in the DCE-MRI parameter K_{trans} (a measure of blood flow) was noted at the biologically effective dose (BED; 13.5 mg/m^2) and at 30 mg/m^2 reductions in K_{trans} ranged from 16% to 82%. Stable disease for two or more cycles was observed in 30% of patients with the following tumor types: pancreatic, colorectal, anal and adrenocortical carcinoma, melanoma, leiomyosarcoma, gastrointestinal stromal tumor and hemangiopericytoma. Pharmacokinetic analysis showed dose-dependent increases in mean peak plasma concentration (C_{max}) and the AUC from 34.4 to 527 ng/mL and from 101 to 3319 ng.h/mL, respectively, from day 1 to day 15, without evidence of drug accumulation. Plinabulin had an elimination half-life of 6.35 h, a clearance rate of 31 L/h and a volume of distribution of 208 L.

An open-label phase I study assessed escalating doses (from the BED to the RP2D) of plinabulin in combination with docetaxel in patients with advanced NSCLC (n = 10) or other malignancies (n = 3) in whom docetaxel use was indicated. The combination of both agents at full dose (plinabulin 30 mg/m^2 given on days 1 and 8 on 21-day cycles and docetaxel 75 mg/m^2 given on day 1) was safe, with an adverse event profile similar to that of both agents given alone. Only one DLT (nausea, vomiting, dehydration and neutropenia) was noted with plinabulin 30 mg/m^2. Eight of 10 patients with NSCLC had measurable disease, of whom 2 demonstrated a partial response and 4 had regressions of lesser magnitude. These data appear more favorable than the response rate for docetaxel alone (5-10%) reported in this patient population. Plinabulin demonstrated stationary, linear, non-dose-dependent pharmacokinetics, which were not affected by docetaxel. No drug–drug interactions were observed. Further clinical studies will investigate the combination of these two agents with an RP2D of plinabulin 30 mg/m^2 and docetaxel 75 mg/m^2 (20, 21).

After encouraging preclinical data demonstrating the efficacy of plinabulin in combination with docetaxel and positive results from the phase I study, a randomized phase II study was initiated. The ADVANCE (Assessment of Docetaxel and Vascular Disruption in Non-Small Cell Lung Cancer) trial will evaluate plinabulin in combination with docetaxel compared with docetaxel alone in patients with NSCLC who previously failed at least one chemotherapy regimen. This study will enroll approximately 150 participants at clinical sites in the U.S., Australia, India and South America, with overall survival as the primary endpoint and progression-free survival and tumor response rates as secondary endpoints (ClinicalTrials.gov Identifier NCT00630110) (22).

SOURCE

Nereus Pharmaceuticals, Inc. (US).

DISCLOSURES

The authors have no potential conflicts of interest to disclose.

REFERENCES

1. Hayashi, Y., Grodberg, J., Palladino, M. (Nereus Pharmaceuticals, Inc). *Dehydrophenylahistins and analogs thereof and the synthesis of dehydrophenylahistins and analogs thereof.* EP 1529044, JP 2006511534, WO 2004054498.
2. Palladino, M.A., Lloyd, G.K., Hayashi, Y., Nicholson, B. (Nereus Pharmaceuticals, Inc). *Dehydrophenylahistins and analogs thereof and the synthesis of dehydrophenylahistins and analogs thereof.* EP 1711487, JP 2007520565, WO 2005077940.
3. Palladino, M., Lloyd, G.K., Hayashi, Y. (Nereus Pharmaceuticals, Inc). *Analogs of dehydrophenylahistins and their therapeutic use.* EP 1926724, US 2007078138, WO 2007035841.
4. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Thun, M.J. *Cancer statistics, 2009.* CA Cancer J Clin 2009, 59(4): 225-49.
5. Smith, B.D., Smith, G.L., Hurria, A., Hortobagyi, G.N., Buchholz, T.A. *Future of cancer incidence in the United States: Burdens upon an aging, changing nation.* J Clin Oncol 2009, 27(17): 2758-65.
6. Molina, J.R., Yang, P., Cassivi, S.D., Schild, S.E., Adjei, A.A. *Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship.* Mayo Clin Proc 2008, 83(5): 584-94.
7. Gridelli, C., Rossi, A., Maione, P., Rossi, E., Castaldo, V., Sacco, P.C., Colantuoni, G. *Vascular disrupting agents: A novel mechanism of action in the battle against non-small cell lung cancer.* Oncologist 2009, 14(6): 612-20.
8. Hayashi, Y., Nicholson, B., Tanaka, K., Oda, A., Lloyd, G.K., Palladino, M.A., Kiso, Y. *Discovery of new tubulin inhibitors KPU-2 (NPI-2358) and its derivatives based on diketopiperazine structure and their anti-tumor activity.* 23rd Med Chem Symp (Nov 24-26, Tsukuba) 2004, Abst 2P-04.
9. Hayashi, Y., Yamazaki, Y., Kiso, Y. et al. *Development of vascular disrupting agents based on anti-microtubule diketopiperazine - Synthesis of a photoaffinity probe for tubulin recognition.* 25th Med Chem Symp (Nov 29-Dec 1, Nagoya) 2006, Abst 2P-20.
10. Mori, Y., Yamazaki, Y., Yoshida, T. et al. *Synthesis and structure-activity relationship of diketopiperazine-type vascular disrupting agents.* 27th Med Chem Symp (Nov 26-28, Osaka) 2008, Abst 2P-42.
11. Palladino, M.A. Jr., Borgstrom, P., Deisseroth, A., Nicholson, B., Bahjat, R., Neuteboom, S., Lloyd, G.K. *The halimides, a novel family of diketopiperazines with vascular targeting properties.* 15th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 17-21, Boston) 2003, Abst B20.
12. Nicholson, B., Bahjat, F.R., Borgstrom, P., Neuteboom, S.T.C., Palladino, M.A. Jr., Lloyd, G.K. *NPI-2358, a novel diketopiperazine, induces tubulin depolymerization in vitro and tumor vascular collapse in vivo.* Proc Am Assoc Cancer Res (AACR) 2004, 45: Abst 5419.
13. Bishop, J., Nicholson, B., Lloyd, G.K., Jordan, M.A., Wilson, L. *The antiproliferative mechanism of action of novel microtubule-targeted phenylahistins NPI-2350 and NPI-2358 as compared with colchicine.* 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst B235.
14. Nicholson, B., Bishop, J., Hayashi, Y. et al. *NPI-2358, a novel tumor vascular disrupting agent.* Proc Am Assoc Cancer Res (AACR) 2005, 46: Abst 3428.
15. Lloyd, G.K., Nicholson, B., Neuteboom, S., Marty, J., Mangold, G., Palladino, M.A. Jr. *NPI-2358 and NPI-2386: Two new vascular/tubulin modifying agents greatly potentiate standard chemotherapy in xenograft models.* 15th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 17-21, Boston) 2003, Abst B59.
16. Lloyd, G.K., Wilson, L., Bishop, J. et al. *Preclinical profile of NPI-2358, a tumor vascular disrupting agent.* 17th AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther (Nov 14-18, Philadelphia) 2005, Abst B4.
17. Neuteboom, S., Medina, E., Palladino, M.A., Spear, M.A., Lloyd, G.K., Nawrocki, S. *NPI-2358, a novel tumor vascular disrupting agent potentiates the anti-tumor activity of docetaxel in the non small cell lung cancer*

model MV522. Eur J Cancer Suppl [20th EORTC-NCI-AACR Symp Mol Targets Cancer Ther (Oct 21-24, Geneva) 2008] 2008, 6(12): Abst 450.

18. Lloyd, G.K., Shen, Y.Y., Bertelsen, L.B., Stodkilde-Jorgensen, H., Nielsen, T., Horsman, M. *Utilizing DCE-MRI to monitor the vascular changes induced in a murine cancer model by the novel vascular disrupting drug NPI-2358.* Proc Am Assoc Cancer Res (AACR) 2009, 50: Abst 5641.
19. Pilat, M.J., LoRusso, P., Spear, M.A. et al. *Phase 1 trial of NPI-2358 (a novel vascular disrupting agent) in patients with solid tumors or lymphomas.* Proc Am Assoc Cancer Res (AACR) 2009, 50: Abst 3598.
20. Millward, M., Mita, A., Spear, M.A. et al. *Phase I trial of NPI-2358 (a novel vascular disrupting agent) plus docetaxel.* J Clin Oncol [45th Annu Meet Am Soc Clin Oncol (ASCO) (May 29-June 2, Orlando) 2009] 2009, 27(15, Suppl.): Abst 3571.
21. Millward, M., Mainwaring, P., Spear, M.A. et al. *Safety and pharmacokinetics of the vascular disrupting agent (VDA) NPI-2358 combined with docetaxel for the treatment of non-small cell lung cancer (NSCLC).* J Thorac Oncol [13th World Conf Lung Cancer (July 31-Aug 4, San Francisco) 2009] 2009, 4(9, Suppl. 1): Abst D10.5.
22. Spear, M.A. *NPI-2358 (a novel vascular disrupting agent).* 11th Int Symp Anti-Angiogenic Agents: Recent Adv Future Directions Basic Clin Cancer Res (Feb 5-7, San Diego) 2009, Abst.