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Review Article

Combretastatin anticancer drugs

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Introduction

We are entering an exciting time in the development of novel therapeutic drugs for treating cancer. As a result of our ever-increasing understanding of the molecular biology of malignancy, potential new targets for drug discovery are emerging and it is hoped that rational drug design will closely follow the advance in molecular biology and translate into therapeutic benefit for cancer patients. In fact some rationally designed agents have already entered clinical study with some success and we await the outcome of existing and forthcoming trials with interest. Of course these advances, although very welcome, do not preclude the development of new agents that interact with older, well-known targets that have already proved to be clinically useful. One such cellular target is the microtubule. The tubulin-binding agents are one of the most effective classes of drugs in clinical use. Microtubules are vital components of the cell. They form the mitotic apparatus and are critical for cell movement, attachment and intracellular transport. Tubulin-binding agents generally exert their effects by microtubule depolymerization or stabilization. The vinca alkaloids, particularly vincristine and vinblastine, have been in clinical use for many years and more recently vinorelbine has found a niche in the treatment of breast cancer (1, 2). The most recent vinca alkaloid to enter clinical development is vinflunine (3). The vinca alkaloids are antimitotic agents known to inhibit mitotic assembly (4). On the other hand, the clinically active drug paclitaxel is known to form stable nonfunctional microtubules (5) so it is clear that interfering with tubulin by drugs with completely different mechanisms of action can be equally useful in treating cancer patients.

These existing agents, although clinically useful, lack the specificity needed to produce cures in patients with advanced cancers without compromising normal tissues so the search for better agents continues. One group of compounds that has received a great deal of attention in the last few years is the combretastatins. These are a family of compounds derived from the African bush willow, *Combretum caffrum* (6, 7) and to date the agent that has progressed furthest in clinical development is the prodrug of combretastatin A4.

Combretastatin A4

Combretastatin A4 [*cis*-1-(3,4,5-trimethoxyphenyl)-2-(3'-hydroxy-4'-methoxyphenyl)ethene] (Fig. 1) is a relatively simple stilbene that has been shown to compete with colchicine for binding sites on tubulin (8, 9). These studies showed combretastatin to be a member of the colchicine-like inhibitors of microtubule assembly rather than the vinca alkaloid type. Alterations in the trimethoxybenzene moiety or in the methoxy and oxy groups of colchicine have been shown to influence antimicrotubule activity (10), and McGown and Fox (9) suggested that the trimethoxybenzene moiety observed in colchicine, podophyllotoxins and combretastatin probably represented a favored binding structure for tubulin. Further studies investigated the structure-activity relationships and indicated the relative importance of the *cis*-configuration over the *trans*-configuration for tubulin activity (11). Combretastatin A4 is only poorly water-soluble but subsequently a water-soluble disodium phosphate prodrug (Fig. 2) was developed (12) allowing much easier *in vivo* administration. The suggestion was that the phosphate group would be cleaved in the plasma releasing the combretastatin A4.

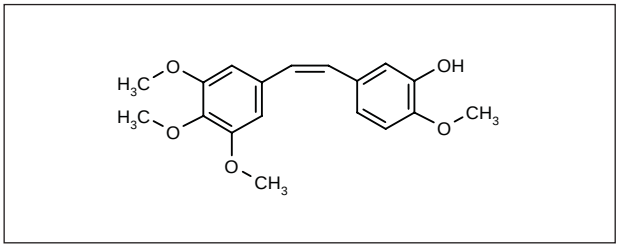

Fig. 1. Structural formula of combretastatin A4.

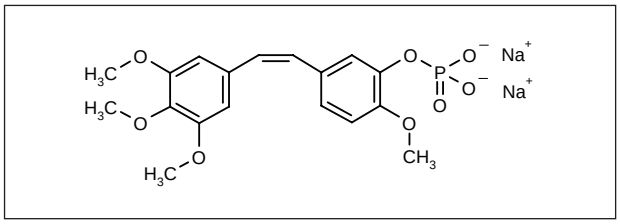

Fig. 2. Structural formula of combretastatin A4 disodium phosphate.

Preclinical efficacy

There is now a wealth of literature demonstrating that combretastatin A4 is active against preclinical experimental models of cancer. Investigation of the *in vivo* efficacy took on a new dimension through the work of Chaplin and colleagues who demonstrated vascular effects in transplantable tumors in mice (13). Various approaches had already been taken to exploit the vasculature of tumors in experimental models. In fact, colchicine had been demonstrated back in the 1930s to possess antivascular activity but the margin between efficacy and toxicity was very small (14). The Chaplin group went on to show that the prodrug of combretastatin A4 induced vascular shutdown in subcutaneously transplanted experimental tumors at a dose less than one-tenth of the maximum tolerated dose (15). The same study demonstrated that the compound was cytotoxic to rapidly proliferating endothelial cells. It is clear from many studies now that the vascular shutdown caused by combretastatin A4 results in massive hemorrhagic necrosis of subcutaneous tumors (Fig. 3) and although measurable effects on tumor volume have been demonstrated in some studies this is not always the case (15, 16). A viable rim of tumor remains, and this will continue to grow, potentially masking the dramatic effects of the drug. As a result of these observations, a number of studies has addressed the potential for combination therapies with the intention of attacking the viable rim with the second strategy. Investigations from numerous laboratories have demonstrated that combretastatin A4 can significantly enhance experimental tumor response to standard chemotherapeutic agents such as cisplatin, 5-fluorouracil and doxorubicin and to radiation therapy (16-18). More recently, studies have demonstrated enhancement of the antitumor effect of hyperthermia (19) and potentiation of the effects of combretastatin A4 by combination with investigational agents has also been studied, a particularly interesting approach being to combine radioimmunotherapy by the use of [^{131}I]-labeled anticarcinoembryonic antigen (CEA) IgG with combretastatin A4. This latter combination produced cures in CEA expressing human tumor xenografts in nude mice (20).

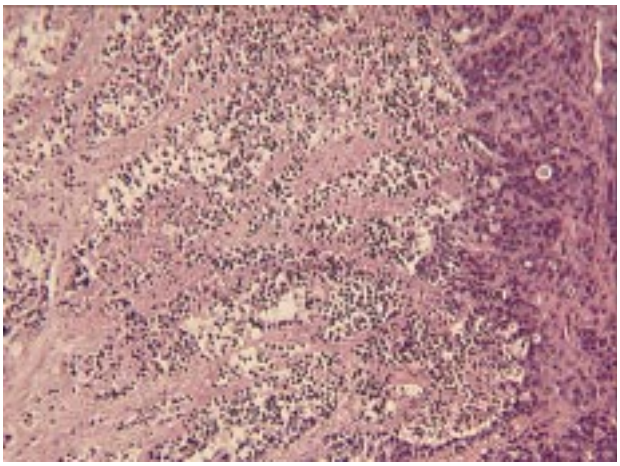

Fig. 3. Hemorrhagic necrosis in HT29 human colon tumor xenograft growing in the cecum of a nude mouse 24 h after treatment with 150 mg/kg combretastatin prodrug (hematoxylin and eosin).

As tubulin is a universal target, it is likely that combretastatin A4 will be effective to varying degrees in all cells, although it is likely that the influence on endothelial cells in the blood vessels of tumors gives it the impressive preclinical activity. It is very clear that tumor vascularization is important for activity against tumors in a subcutaneous model as it has been shown that tumor cells in the same site treated prior to vascularization do not respond (21). Noninvasive monitoring of blood flow has demonstrated changes in blood perfusion associated with treatment (22). Endothelial cells exposed to combretastatin A4 or the prodrug *in vitro* show rapid and dramatic effects on their microtubules (Fig. 4). Although apoptosis has been demonstrated in endothelial cells (23), the observed effects do not occur quickly enough for this to be responsible for the rapid *in vivo* effects on tumor blood flow seen in numerous studies (13, 15, 21, 24). It is possible that the rapid shape change induced in endothelial cells is the primary event in the vascular shutdown demonstrated *in vivo*. Studies from this laboratory showed that the combretastatin A4 prodrug not only caused a rapid effect on microtubules and the actin cytoskeleton in human umbilical vein endothelial cells (HUVEC) but there was also inhibition of HUVEC network formation and inhibition of HUVEC migration through type I collagen (21). In any event, there can be little doubt that the vascular effects caused by combretastatin A4 are significant for its antitumor properties but there is also evidence that the effects are not restricted to tumors but can also occur in neovasculature in nonneoplastic tissue (25). These latter authors described vascular damage in the hyperplastic thyroid.

The majority of the literature referred to in this article thus far relates to *in vitro* observations or investigations in subcutaneously transplanted tumors in rodents. These systems are clearly far from the real target for drug intervention in patients. There are several studies described in the literature (26-28) indicating that, even in experimental studies, tumor site can have an influence on response to therapy as local biochemical and physiological properties will play their part. In the case of combretastatin, attempts

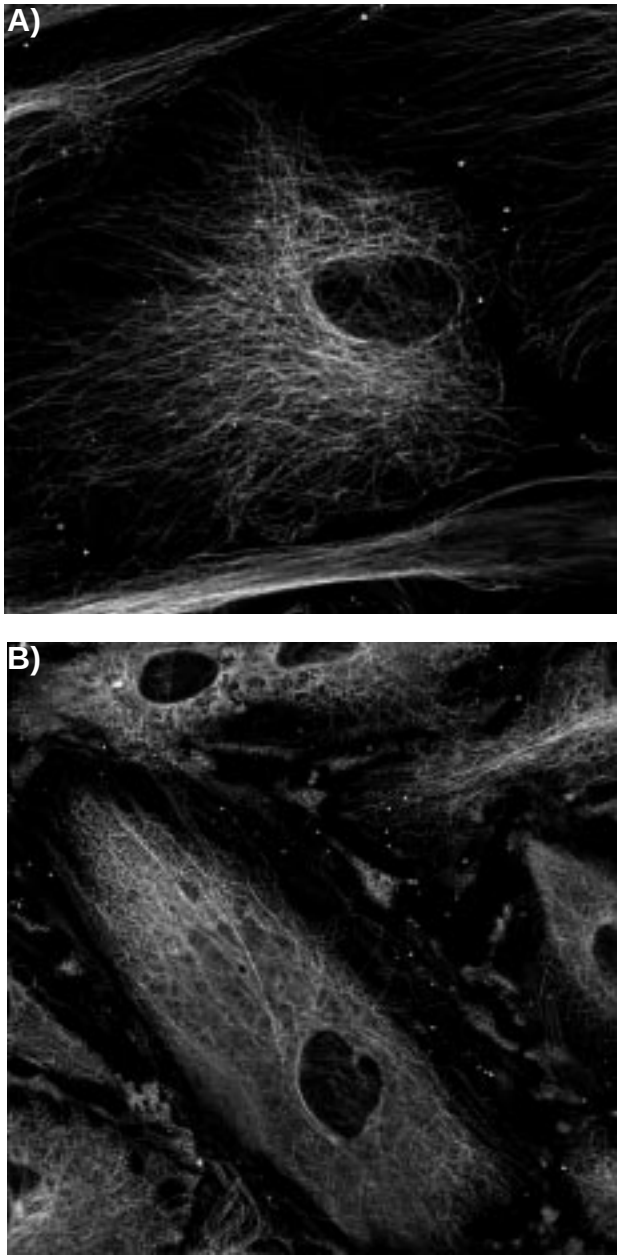

Fig. 4. Confocal microscope appearance of microtubules in human umbilical vein endothelial cells (HUVECs). A) untreated cell, B) disruption of microtubules 30 min after exposure to 0.5 μM combretastatin A4 prodrug (immunostained for α-tubulin).

have been made to tackle this problem experimentally by the use of orthotopically transplanted tumors or metastatic models. These systems were utilized with the view that these clinically relevant sites might be more predictive of effects in patients. The study by Grosios *et al*. (21) was the first to demonstrate activity of combretastatin A4 against orthotopically transplanted colon tumors and their metastases but activity has since been confirmed for metastatic deposits of the Lewis lung carcinoma (29) and a murine model of liver metastasis in colorectal cancer (30).

In addition to studies on solid cancers, there is also preclinical evidence of activity in lymphoma (31), although *in vivo* studies still suggest a vascular component in the mechanism of action (32).

Clinical studies

As a result of the exciting preclinical activity of combretastatin A4, the compound was selected for clinical evaluation in both the U.K. and the U.S. Although the phase I studies are not published in detail yet, a small number of meeting abstracts has described the clinical pharmacokinetics and patient tolerance and, importantly, there is evidence of effects on tumor vasculature in patients (33-36). These data provide proof of principle and indicate that combination studies with other therapies such as standard cytotoxic drugs or radiation may be useful but they also suggest that more active molecules with antivascular activity should be sought.

Other analogues

A large number of analogues of combretastatin are now available but to date few of these have gone on to detailed *in vivo* evaluation, so an extensive list has not been included here. That is not to say that from the molecules being synthesized and evaluated at present there may be agents with better pharmacological properties than the lead agent combretastatin A4 prodrug.

AC-7700 (Fig. 5) is another combretastatin derivative that has been demonstrated to have preclinical *in vivo* activity. This is a water-soluble compound synthesized in Japan (37-39). It has been suggested that this compound has stronger antitumor affects against C26 than combretastatin A4 prodrug (40) and tumor vascular shutdown has been demonstrated (41). Other studies have indicated that this compound is also active against methylcholanthrene-induced autochthonous primary tumors (42) and orthotopically transplanted tumors (43).

One analogue that we have evaluated in our laboratory is combretastatin A1 prodrug (Fig. 6) (44). This is a water-soluble phosphate that is highly active *in vivo*.

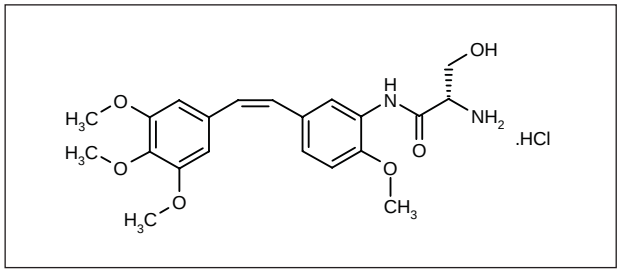

Fig. 5. Structural formula of AC-7700.

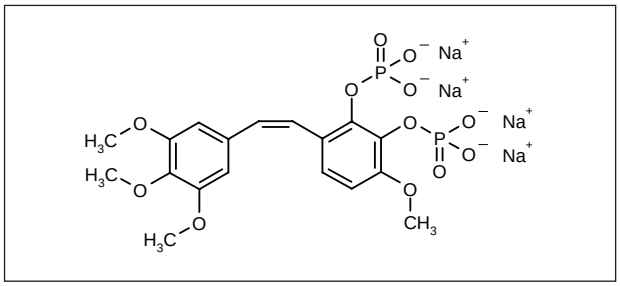

Fig. 6. Structural formula of combretastatin A1 phosphate.

Although combretastatin A1 had been isolated earlier (6), its development as a potential anticancer agent was hampered owing to the instability (oxidation to the 1,2-quinone) of the 2,3-dihydroxy unit (45, 46). In a recent head-to-head comparison in a murine colon tumor model, antitumor activity was shown to be superior to combretastatin A4 prodrug (47). Further studies are ongoing to confirm these observations and to identify the reasons for the seemingly superior activity.

Potent orally active heterocycle-based combretastatin A4 analogues have recently been described in a preliminary communication from Abbott Laboratories (48). 3-Amino-4-methoxyphenyl and 5-*N*-methylindole were suggested as the best replacements for the 3-hydroxy-4-methoxyphenyl in combretastatin A4. 4,5-Disubstituted imidazole was found to be the best replacement for the *Z*-double bond in combretastatin A4. Activity has been demonstrated in the M5046 tumor in mice, although mechanistic details have not been published.

Conclusions

There are numerous ongoing approaches to develop molecules that will target the blood supply to human tumors. Many of these strategies are attempting to target the process of angiogenesis itself with a view to interrupting or slowing down the process, thus producing dormancy or cytostasis in the tumor. It is likely that chronic treatment strategies will be necessary for these approaches as specific inhibitors of steps in the pathways of the angiogenic cascade are identified. On the other hand, if an antivascular strategy is employed, *i.e.,* targeting the existing vasculature supplying the tumor, the treatment is likely to be more analogous to conventional chemotherapy and rapid effects on the tumor should occur. The data that are accruing on the combretastatins indicate that they fall into the category of antivascular agents. Some existing clinically useful tubulin-interactive agents possess a vascular component in their mechanism of action against murine solid cancer models (49, 50) but the relevance of these observations to clinical responses needs to be determined. In preclinical terms, the combretastatins seem to have advantages over many existing tubulin agents because the observed effects on tumor vasculature occur well below the maximum tolerated dose. Publication in detail of clinical studies is awaited with interest, but preliminary data suggest that this is an extremely interesting group of compounds that is worthy of further investigation. Very interesting preclinical results also indicate that molecules other than the combretastatins that bind tubulin at the colchicine binding site may be useful anticancer agents. The AstraZeneca investigational prodrug ZD-6126 (*N*-acetylcolchicinol-*O*-phosphate) has been shown to be active *in vivo* (51) and to cause vascular damage in experimental tumors (52). Much of the experimental work on this compound is available in abstract form only but the compound has progressed to phase I clinical study (53).

Studies to date with combretastatins and similar molecules demonstrate that tubulin still represents a useful target for drug development for the treatment of cancer, and well-tolerated molecules that bind at or near to the colchicine binding site should still be sought.

References

1. Gasparini, G., Caffo, O., Barni, S. et al. *Vinorelbine is an active antiproliferative agent in pretreated advanced breast cancer patients – a phase II study.* J Clin Oncol 1994, 12: 2094-101.

2. Bruno, S., Puerto, V.L., Mickiewicz, E. et al. *Phase II trial of weekly iv vinorelbine as a single agent in first line advanced breast cancer chemotherapy – the Latin-American experience.* Am J Clin Oncol-Cancer Clin Trials 1995, 18: 392-6.

3. Fahy, J., Duflos, A., Ribet, J.P. et al. *Vinca alkaloids in superacid media: A method for creating a new family of antitumor derivatives.* J Am Chem Soc 1997, 119: 8576-7.

4. Jordan, M.A., Wilson, L. *Kinetic analysis of tubulin exchange at microtubule ends at low vinblastine concentration*. Biochemistry 1990, 29: 2730-9.

5. Schiff, P.B., Fant, J., Horwitz, S.B. *Promotion of microtubule assembly in vitro by Taxol*. Nature 1979, 277: 665-7.

6. Pettit, G.R., Singh, S.B., Niven, M.L., Hamel, E., Schmidt, S.M. *Isolation, structure and synthesis of combretastatins A1 and B1, potent new inhibitors of microtubule assembly, derived from Combretum caffrum.* J Nat Prod 1987, 50: 119-31.

7. Pettit, G.R., Singh, S.B., Hamel, E., Lin, C.M., Alberts, D.S., Garia-Kendall, D. *Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A4*. Experientia 1989, 45: 209-11.

8. Hamel, H., Lin, C.M. *Interactions of combretastatin, a new plant derived antimitotic agent with tubulin*. Biochem Pharmacol 1983, 32: 3864-7.

9. McGown, A.T., Fox, B.W. *Structural and biochemical comparison of the anti-mitotic agents colchicine, combretastatin A4 and amphethinile.* Anti-Cancer Drug Des 1989, 3: 249-54.

10. Ray, K., Bhattacharyga, B., Biswas, B.B. *Role of B-ring of colchicine in its binding to tubulin.* J Biol Chem 1981, 256: 6241-4.

11. Woods, J.A., Hadfield, J.A., Pettit, G.R., Fox, B.W., McGown, A.T. *The interaction with tubulin of a series of stilbenes based on combretastatin A-4.* Br J Cancer 1995, 71: 705-11.

12. Pettit, G.R., Temple, C., Narayanan, V.L. et al. *Antineoplastic agents 322. Synthesis of combretastatin A4 prodrugs.* Anti-Cancer Drug Des 1995, 10: 299-309.

13. Chaplin, D.J., Pettit, G.R., Parkins, C.S., Hill S.A. *Anti-vascular approaches to solid tumor therapy: Evaluation of tubulin binding agents.* Br J Cancer 1996, 74(Suppl. 27): S86-8.

14. Clearkin, P.A. *The effect of colchicine on normal and neoplastic tissue in mice.* J Pathol Bact 1937, 44: 469-80.

15. Dark, G.G., Hill, S.A., Prise, V.E., Tozer, G.M., Pettit, G.R., Chaplin, D.J. *Combretastatin A4, an agent that displays potent and selective toxicity towards tumour vasculature.* Cancer Res 1997, 57: 1829-34.

16. Grosios, K., Loadman, P.M., Swaine, D.J., Pettit, G.R., Bibby, M.C. *Combination chemotherapy with combretastatin A4 phosphate and 5-fluorouracil in an experimental murine colon adenocarcinoma.* Anticancer Res 2000, 20: 229-33.

17. Chaplin, D.J., Pettit, G.R., Hill, S.A. *Anti-vascular approaches to solid tumour therapy: Evaluation of combretastatin A4 phosphate.* Anticancer Res 1999, 19: 189-96.

18. Li, L.Y., Rojiani, A., Siemann, D.W. *Targeting the tumor vasculature with combretastatin A4 disodium phosphate: Effects on radiation therapy.* Int J Rad Oncol Biol Phys 1998, 42: 899-903.

19. Murata, R., Overgaard, J., Horsman, M.R. *Combretastatin A-4 disodium phosphate: A vascular targeting agent that improves the anti-tumor effects of hyperthermia, radiation, and mild thermoradiotherapy.* Int J Rad Oncol Biol Phys 2001, 51: 1018-24.

20. Pedley, R.B., Hill, S.A., Boxer, G. M.et al. *Eradication of colorectal xenografts by combined radioimmunotherapy and combretastatin A-4 3-0-phosphate.* Cancer Res 2001, 61: 4716-22.

21. Grosios, K., Holwell, S.E., McGown, A.T., Pettit, G.R., Bibby, M.C. *In vivo and in vitro evaluation of combretastatin A4 and its sodium phosphate prodrug.* Br J Cancer 1999, 81: 1318-27.

22. Jesberger, J.A., Rafie, N., Duerk, J.L., Remick, S., Lewin J.S. *Non invasive in vivo assessment of tumor perfusion after antiangiogenic therapy with combretastatin A4 with dynamic contrast-enhanced MRI.* Radiology 2000, 217(Suppl.) Abst 625.

23. Iyer, S., Chaplin, D.J., Rosenthal, D.S., Boulares, A.H., Li, L.Y., Smulson M.E. *Induction of apoptosis in proliferating human endothelial cells by the tumor-specific antiangiogenesis agent combretastatin A-4.* Cancer Res 1998, 58: 4510-4.

24. Tozer, G.M., Prise, V.E., Wilson, J. et al. *Combretastatin A-4 phosphate as a tumor vascular-targeting agent: Early effects in normal tissues.* Cancer Res 1999, 59: 1626-34.

25. Griggs, J., Hesketh, R., Smith, G.A. et al. *Combretastatin-A4 disrupts neovascular development in non-neoplastic tissue.* Br J Cancer 2001, 84: 832-5.

26. Smith, K.A., Begg, A.C., Denekamp, J. *Differences in chemosensitivity between subcutaneous and pulmonary tumours.* Eur J Cancer Clin Oncol 1985, 21: 249-56.

27. Fidler, I.J., Wilmanns, C., Staroselsky, A. et al. *Modulation of the tumor-cell response to chemotherapy by the organ environment.* Cancer Met Rev 1984, 13: 209-22.

28. Cowen, S.E., Bibby, M.C., Double, J.A. *Characterization of the vasculature within a murine adenocarcinoma growing in different sites to evaluate the potential of vascular therapies.* Acta Oncologica 1995, 34: 357-60.

29. Griggs, J., Brindle, K.M., Metcalfe, J.C. et al. *Potent antimetastatic activity of combretastatin-A4.* Int J Oncology 2001, 19: 821-5.

30. Malcontenti-Wilson, C., Muralidharan, V., Skinner, S., Christophi, C., Sherris, D., O'Brien, P.E. *Combretastatin A4 prodrug study of the effect on the growth and the microvasculature of colorectal liver metastases in a murine model.* Clin Cancer Res 2001, 7: 1052-60.

31. Nabha, S.M., Wall, N.R., Mohammad, R.M., Pettit, G.R., Al-Katib, A.M. *Effects of combretastatin A-4 prodrug against a panel of malignant human B-lymphoid cell lines.* Anti-Cancer Drugs 2000, 11: 385-92.

32. Nabha, S.M., Mohammad, R.M., Wall, N.R., Pettit, G.R., Al-Katib, A.M. *Evaluation of combretastatin A-4 prodrug in a non-Hodgkin's lymphoma xenograft model: Preclinical efficacy.* Anti-Cancer Dugs 2001, 12: 57-63.

33. Stevenson, J.P., Gallagher, M., Sun, W. et al. *Phase I/pharmacokinetic trial of the endothelial toxin combretastatin A4P (CA4P) administered as an IV bolus on a daily x 5 schedule every 21 days.* Proc Am Assoc Cancer Res 2000, 41: 54.

34. Stratford, M., Folkes, L., Galbraith, S. et al. *Phase I pharmacokinetic and toxicity study of weekly intravenous combretastatin A4 phosphate (CA4P).* Clin Cancer Res 2000, 6(Suppl.): Abst 280.

35. Galbraith, S.M., Taylor, N.J., Lodge, M. et al. *Combretastatin A4 phosphate targets vasculature in animal and human tumours.* Br J Cancer 2000, 83(Suppl. 1): 12.

36. Remick, S., Robertson, K., Dowlati, A. et al. *A phase I pharmacokinetic (PK) study of single dose intravenous (IV) combretastatin A4 phosphate (CA4P) in patients with advanced cancer: Final report.* Clin Cancer Res 2001, 7(Suppl.): Abst 393.

37. Ohsumi, K., Nakagawa, R., Hatanaka, T. et al. *Novel combretastatin analogues effective against murine solid tumors: Design and structure-activity relationships.* J Med Chem 1998, 41: 3022-33.

38. Ohsumi, K., Hatanaka, T., Fujita K. et al. *Syntheses and antitumor activity of cis-restricted combretastatins: 5-Membered heterocyclic analogues.* Bioorg Med Chem Lett 1998, 8: 3153-8.

39. Hatanaka, T., Fujita, K., Ohsumi, K. et al. *Novel B-ring modified combretastatin analogues: Syntheses and antineoplastic activity.* Bioorg Med Chem Lett 1998, 8: 3371-4.

40. Nihei, Y., Suzuki, M., Okano, A. et al. *Evaluation of antivascular and antimitotic effects of tubulin binding agents in solid tumor therapy.* Jpn J Cancer Res 1999, 90: 1387-95.

41. Hori, K., Saito, S., Nihei, Y., Suzuki, M., Sato, Y. *Antitumor effects due to irreversible stoppage of tumor tissue blood flow: Evaluation of a novel combretastatin A-4 derivative AC7700.* Jpn J Cancer Res 1999, 90: 1026-38.

42. Hori, K., Saito, S., Sato, Y., Kuboto, K. *Stoppage of blood flow in 3-methylcholanthrene-induced autochthonous primary tumor due to a novel combretastatin A-4 derivative, AC7700, and its antitumor effect.* Med Sci Monit 2001, 7: 26-33.

43. Nihei, Y., Suga, Y., Morinaga, Y. et al. *A novel combretastatin A-4 derivative, AC-7700, shows marked antitumor activity against advanced solid tumors and orthotopically transplanted tumors.* Jpn J Cancer Res 1999, 90: 1016-25.

44. Pettit, G.R., LippertIII, J.W. *Antineoplastic agents 429. Syntheses of the combretastatin A-1 and combretastatin B-1 prodrugs.* Anti-Cancer Drug Des 2000, 15: 203-16.

45. Haines, A.H. (Ed.). Methods for the Oxidation of Organic Compounds. Academic Press: New York 1988, 2: 305, 438.

46. Sackett, D.L. *Podophyllotoxin, steganacin and combretastatin: Natural products that bind at the colchicine binding site of tubulin.* Pharmacol Ther 1993, 59: 163-228.

47. Holwell, S.E., Cooper, P.A., Grosios, G. et al. *Combretastatin A-1 phosphate a novel tubulin binding agent with in vivo antivascular effects in experimental tumors*. Anticancer Res 2002, 22: in press.

48. Hill, S.A., Lonergan, S.J., Denekamp, J., Chaplin, D.J. *Vinca alkaloids: Anti-vascular effects in a murine tumour.* Eur J Cancer 1993, 29A: 1320-4.

49. Holwell, S.E., Hill, B.T., Bibby, M.C. *Anti-vascular effects of vinflunine in the MAC15A transplantable adenocarcinoma model.* Br J Cancer 2001, 84: 290-5.

50. Wang, L., Woods, K.W., Li, Q. et al. *Potent, orally active heterocycle-based analogs: Synthesis, structure-activity relationship, pharmacokinetics and in vivo antitumor activity evaluation.* 221st Meet ACS 2001, MEDI 140.

51. Blakey, D.C., Ashton, S.E., Douglas, S., Westwood, F.R., Curry, B. *Anti-tumour activity of the novel vascular targeting agent ZD6126 in a human lung tumour xenograft model.* Clin Cancer Res 2000, 6(Suppl.): Abst 283.

52. Westwood, F.R., Hughes, G.D., Ryan, A.J., Ashton, S.E., Blakey, D. *ZD6126, a novel vascular targeting agent induces rapid changes to tumor blood vessel endothelial cells in an Hras5 nude mouse xenograft.* Clin Cancer Res 2001, 7(Suppl.): Abst 61.

53. Lorusso, P.M., Gadgeel, S. Wozniak, A. et al. *A phase I dose-escalation trial of ZD6126, a novel vascular targeting agent, in patients with cancer refractory to other treatments.* Clin Cancer Res 2001, 7(Suppl.): Abst 36.