Investigators' Brochure for the Clinical Trial of Photodynamic Therapy of Advanced Cancer

Cytoluminator Research Pty. Ltd.

DRAFT, 10 October 2008

List of Substances and Devices to be Trialled

Photosensitising agent aqueous solution (slightly basic) of aluminium phthalocyanine disulphonate (abbreviated $AlPcS_2$), for intravenous administration.

Illuminator a solid-state laser producing diffuse illumination from a hand-held light guide, at wavelengths predominantly between 684-686 nm, with variable power levels not exceeding 6W, and with average intensities below the skin pain threshold (around $1W/cm^2$).

Fluorescence imager a video camera with sensitivity to the infrared fluorescence produced by illuminated photosensitising agent, but insensitive to the nonfluorescent and ambient light. An illumination accessory is incorporated for projecting light from the illuminator onto the subject with a uniform intensity distribution centred on the imager field of view.

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1 Summary

2 Introduction

Ref. [1, 2].

Photodynamic therapy (PDT), also known as photochemotherapy and under various other names, is the administration of a photosensitising agent followed by irradiation with light having the appropriate physical properties to activate the photosensitiser. The photosensitiser is chosen to have minimal cytotoxicity when the body is kept in the dark, to be selectively taken up by the cancer cells and cleared from healthy tissue, and then to exhibit a potent cytotoxic effect under appropriate illumination. Due to the bimodal nature of the treatment, the practitioner is given increased flexibility and control as compared with traditional chemotherapy. For example, the concentration and localisation of sensitiser may be evaluated based on its fluorescence in real-time, and only once it has been adequately cleared from healthy tissue will the sensitiser be activated. There is an obvious diagnostic capability built into the treatment, for any region of increased fluorescence is very likely to contain cancer.

PDT has been in use worldwide for more than a decade, using a number of approved photosensitisers and illuminators. They mostly suffer from drawbacks that limit their usefulness to surface tumours, as well as side-effects that discourage the widespread adoption of PDT. It is the object of this clinical trial to demonstrate the safety and efficacy of the photosensitisers and illuminators under development by Cytoluminator Research Pty. Ltd., an Australian Company. Specifically, the trial is using a photosensitiser chosen from the class of *phthalocyanine* dyes, aluminium phthalocyanine disulphonate, to be activated by light having most of its energy within the wavelength interval of 684-686 nm (deep red, not quite infrared but having poor visibility). This is technically only feasible using a laser, although the illumination should be diffuse rather than tightly focussed.

Preliminary results obtained by the sponsor have achieved remissions in tumours several cm below the surface of the body. This is primarily achieved by using a wavelength of light that penetrate deep into the body (as well as *through bone*), in combination with a sensitiser that is very selective to cancer and has rapid clearance from healthy tissue (around one day for treatment, and one week for limited sun exposure).

A widely accepted consequence of PDT, especially with phthalocyanines, is an immune response to cancer following treatment [3]. In animal models this has been shown to last long after the original tumour was killed, since the animals were no longer susceptible to the original cancer cell innoculation. PDT has been exploited *in vitro* to create vaccines against cancer [4]. Preliminary investigation by the sponsor has shown a strong immunological response, which may have contributed to the remission of metastases that were deeper than the presumed depth of light penetration. It is hoped that once the body is cleared of detectable tumours, the immune response will completely eradicate microscopic remnants and provide a true "cure", meaning future relapse by the same cancer is very unlikely. To date, no approved treatment for cancer can give this assurance, because a single cancer cell can remain dormant for many years, only to aggressively return in an advanced (metastatic) form. Even with surgical removal, there is no way of knowing whether metastasis has begun.

The scope of this trial is very wide; any cancer is potentially treatable using the proposed sensitiser and sufficient illumination power and duration. However, technical limitations in the present laser system will result in a limited depth of penetration, which depends on the optical properties of the intervening tissue. It has been found that the present laser is sufficient to eradicate most breast tumours, as well as their metastases in the spine and other bones. Remission of multiple breast cancer metastases on the lung has also been demonstrated. Remission of prostate cancer has been demonstrated, and a partial remission has been achieved in a deep brain tumour. An area of present concern is tissue that is rich in blood, such as the liver and bone marrow. It is unclear how far the light will penetrate, if at all. However, if a tumour is accessible to the illuminator, then the immune response may control the disease progression in those inaccessible regions of the body. In the case of leukemias, it may be possible to reduce the disease burden and stimulate an immune response, however no studies have been performed by the sponsors at the time of writing.

Under the terms of the trial, the treatment protocol will be in accordance with the following summary. In a darkened room, a systemic administration of photosensitising agent shall be performed using an intravenous infusion, with the measured dose of sensitiser injected through a 0.2μm filter (or equivalent) into a bag of saline solution just prior to infusion. The rate at which the dilute sensitiser is infused will be limited by the practitioner so as to manage any discomfort and to allow for the fluorescence imager to highlight the localisation of the sensitiser into any tumour masses within its depth of observation. The amount of sensitiser present within the healthy tissue

(the "background") shall be measured from the intensity of the soft palate or the inner ear, at a known laser power output and distance between the imager and the patient. This shall be used as a reference for comparison with the same measurement taken over the following days, to a) verify the correct clearance from the healthy tissue before commencement of tumour irradiation, and b) determine when it is safe for the patient to begin an escalation of sun exposure to normal levels. The patient shall be made aware of the requirement that they avoid sun exposure until the practitioner has given them approval. As noted above, this is expected to be around one week from treatment.

The photosensitiser is expected to remain within tumours for extended periods (days or weeks) until they have been successfully killed by the illuminator. This allows the course of irradiation to be extended over several days without further administration of sensitiser. In fact, this may be the key to achieving complete remission even when the tumour appears to be completely necrotised. Viable cancer cells could remain, due to insufficient light penetration through the tumour mass, inadequate oxygen perfusion during the treatment, or inhomogeneous sensitiser uptake. Since the sensitiser is redistributed after tumour death, the remaining cells are likely to be killed by a subsequent irradiation. In addition, a more powerful immune response could result from ongoing exposure of dying cancer cells to the immune system, when compared to simultaneous destruction of the entire tumour. This will be a topic of further investigation for the trial.

It has been found by the sponsor, that irradiation is painless and only a mild warmth is felt, however a sharp pain response is usually felt after a duration of tumour irradiation, probably due to release of sensitiser into nearby tissue. This occurs after around 10–15 min of illumination at 3W laser power onto a tumour within intact breast tissue or axillary lymph nodes. After this time, the region of the tumour is very sensitive to light and it is obvious that a change has occurred. Further irradiation would not be possible without anaesthesia, nor does it appear beneficial. When the same region is observed on the following day, the tumours that have been irradiated no longer fluoresce above the background level. It is therefore straightforward to detect any tumours that have been missed and illuminate them again. Once there are no discernable bright regions on the fluorescence image, the practitioner must be guided by other sources of information to illuminate the deeper tumours, such as MRI, CT or PET scans, in the hopes that these may be treated with the present level of illumination (and a longer duration of exposure). It is expected that a surface tumour, such as 2cm below the skin or in the spine, will require at most 15 s per cm^2 of surface area, with the present 6W illuminator. It is unknown how the dose must be scaled for very deep tumours – if the practitioner is willing to perform a few hours of illumination, it is possible to greatly reduce the tumour burden of even widespread metastatic cancer for which patient death is imminent. For that reason, use in palliative care of post-salvage patients is strongly encouraged.

3 Physical, Chemical, Pharmaceutical Properties and Formulation

The sensitising agent to be used, as prepared by the sponsor, is an aqueous solution of hydroxy-aluminium phthalocyanine disulphonate (abbreviated $AlPcS_2$), with charges neutralised by sodium hydroxide. It is translucent and cyan (blue-green) in colour, due to the absorption of red light. The solution is slightly basic, as under acidic conditions the $AlPcS_2$ molecules will tend to dimerise and lose their cytotoxic effect [5]. The chemical structure of $AlPcS_2$ is depicted in Fig. 1. $AlPcS_2$ is structurally similar to other phthalocyanines, and is expected to exhibit similar physical and chemical properties. In particular, pure AlPc is known to be thermally stable up to its boiling point *in vacuo* [6], and the sponsor has found its sulphonated derivatives to be similarly stable when dried at 250 deg C.

Aluminium phthalocyanines are photostable, and the sensitiser is not directly consumed as part of the treatment. The addition of sulphonyl functional groups results in water soluble forms, with pharmacokinetic properties dependent on the degree of sulphonation and thus the net ionic charge and hydrophilicity or lipophilicity of the molecule. It is known that aluminium phthalocyanine and its monosulphonate derivative will accumulate in the skin and eyes for extended periods, due to lipophilicity (and poor water solubility). The tetrasulphonate remains in the serum, due to its hydrophilicity. These substances are detrimental to the light penetration and increase the phototoxicity to healthy tissue. The disulphonate and trisulphonate are taken up specifically by cancer cells and immune cells, localising on the outer cellular membranes. The disulphonate exhibits the greatest therapeutic effect by relocalising to the mitochondrial membrane upon illumination, destroying many proteins anchored there. The primary target has been identified as the anti-apoptotic proteins Bcl-2 and Bcl-xL, which dooms the cell and triggers apoptosis if the cell is competent. The trisulphonate has a lower selectivity for cancer versus immune cells, and has other uses in treating autoimmune disorders.

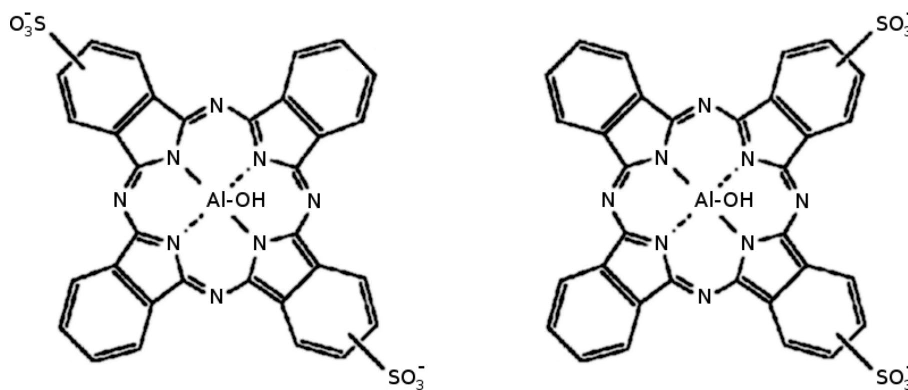

Figure 1: Chemical structures of hydroxy-aluminium phthalocyanine disulphonate. The structural isomers, differing only in the location of the sulphonyl functional groups on the phthalocyanine macrocycle, are prepared in a mixture to reduce intracellular dimerisation.

The preparation to be trialled is a mixture of the structural isomers of hydroxy-aluminium phthalocyanine disulphonate, depicted in Fig. 1. This mixture has been shown to exhibit greatly reduced dimerisation over the use of one isomer or another [5], both in the as-supplied concentrated solution and in the intracellular compartment (where concentrations are much higher than in the serum).

It is anticipated that the highest administered dose will be 1.0mg/ml of sensitiser per body mass. The sensitiser will be supplied in a sterile, pre-filtered solution at a concentration of 1.0mg/ml, packaged in a glass bottle with a rubber septum for withdrawal into a syringe. The solution should be stored in a cool, dark place (preferably in a refrigerator), although it is not known whether this is necessary. Due to the possibility of aggregation and settling (which has not been ruled out), the bottle shall be shaken vigorously prior to withdrawal, to ensure the correct dose is obtained. It shall be injected into saline (0.9% NaCl) via an in-line 0.2μm filter. As the concentrated solution is not isotonic, is is not suitable for direct injection, and must be diluted in saline before administration. Only saline shall be used, as other solutions may promote dimerisation or aggregation after filtration.

4 Nonclinical Studies

4.1 Nonclinical pharmacology

Ref. [7, 8, 9, 10, 11, 12, 13, 14, 1].

4.2 Pharmacokinetics and product metabolism in animals

Ref. [15, 16, 17, 18, 19, 20, 21, 22, 4, 23]. (For animal models of bone metastases treated using PDT, see Ref. [24].)

For the correct wavelengh see Ref. [25].

4.3 Toxicology

4.3.1 Single dose

4.3.2 Repeated dose

Ref. [26, 27].

4.3.3 Carcinogenicity

None reported.

4.3.4 Special studies

N/A

4.3.5 Reproductive toxicity

No studies have been performed.

4.3.6 Genotoxicity

No studies have been performed.

5 Effects in humans

5.1 Pharmacokinetics and product metabolism in humans

Ref. [1].

5.2 Safety and efficacy

Ref. [28, 29].

5.3 Marketing experience

The sponsor has no marketing experience with this formulation.

6 Summary of data and guidance for the investigator

Revsion History

DRAFT 10 Oct 2008 Initial version by Darren Freeman

References

[1] Janine D Miller, Elma D Baron, Heather Scull, Andrew Hsia, Jeffrey C Berlin, Thomas McCormick, Valdir Colussi, Malcolm E Kenney, Kevin D Cooper, Nancy L Oleinick. Phtodynamic therapy with the phthalocyanine photosensitizer Pc 4: The case experience with preclinical mechanistic and early clinical-translational studies. *Toxicology and Appl Pharmacology*, 224:290–299, Feb 2007.

[2] Thomas J Dougherty, Charles J Gomer, Barbara W Henderson, Giulio Jori, David Kesseland Mladen Korbelik, Johan Moan, Qian Peng. Photodynamic therapy. *J Natl Cancer Inst*, 90(12):889–905, Jun 1998.

[3] Frederieke H van Duijnhoven, Remco I J M Aalbers, Jeroen P Rovers, Onno T Terpstra, Peter J K Kuppen. The immunological consequences of photodynamic treatment of cancer, a literature review. *Immunobiol*, 207:105–113, 2003.

[4] M Korbelik, B Stott, J Sun. Photodynamic therapy-generated vaccines: relevance of tumor cell death expression. *British J Cancer*, 97:1381–1387, 2007.

[5] R Edrei, V Gottfried, J E Van Lier, S Kimel. Sulfonated phthalocyanines: photophysical properties, in vitro cell uptake and structure-activity relationships. *J Porphyrins Phthalocyanines*, 2:191–199, 1998.

[6] M E Azim-Araghi, A Krier. The influence of ammonia, chlorine and nitrogen dioxide on chloro-aluminium phthalocyanine thin films. *Appl Surf Sci*, 119(3-4):260–266, Oct 1997.

[7] G M Malham, R J Thomsen, G J Finlay, B C Baguley. Subcellular distribution and photocytoxicity of aluminium phthalocyanines and haematoporphyrin derivative in cultured human meningioma cells. *British J Neurosurgery*, 10(1):51–57, Feb 1996.

[8] Yu Luo, David Kessel. Initiation of apoptosis versus necrosis by photodynamic therapy with chloroaluminium phthalocyanine. *Photochem Photobiol*, 66(4):479–483, Oct 1997.

[9] Hyeong-Reh Choi Kim, Yu Luo, Gangyong Li, David Kessel. Enhanced apoptotic response to photodynamic therapy after bcl-2 transfection. *Cancer Res*, 59(14):3429–3432, Jul 1999.

[10] J Usuda, Song-mao Chiu, Erin S Murphy, Minh Lam, Anna-Liisa Nieminen. Domain-dependent photodamage to Bcl-2: a membrane-anchorage region is needed to form the target of phthalocyanine photosensitization. *J Bio Chem*, 278(3):2021–2029, Stycze/n 2003.

[11] Liang-yan Xue, Song-mao Chiu, Aline Fiebig, David W Andrews, Nancy L Oleinick. Photodamage to multiple Bcl-xL isoforms by photodynamic therapy with the phthalocyanine photosensitizer Pc 4. *Oncogene*, 22(58):9197–9204, Dec 2003.

[12] S-M Chiu, L-Y Xue, K Azizuddin, N L Oleinick. Photodynamic therapy-induced death of HCT 116 cells: apoptosis with or without Bax expression. *Apoptosis*, 10(6):1357–1368, Dec 2005.

[13] Hui-Fang Huang, Yuan-Zhong Chen, Yong Wu. $ZnPcS_2P_2$-based photodynamic therapy induces mitochondria-dependent apoptosis in K562 cells. *Acta Biochimica et Biophysica Sinica*, 37(7):488–494, 2005.

[14] Liang-yan Xue, Song-mao Chiu, Nancy L Oleinick. Differential responses of Mcl-1 in photosensitized epithelial vs lymphoid-derived human cancer cells. *Oncogene*, 24(46):6987–6992, Oct 2005.

[15] Natalia Kazachkina, Natalia Zharkova, Galina Fomina, Raisa Yakubovskaya, Victor Sokolov, Eugeniji Luk'yanetz. Pharmacokinetical study of Al- and Zn-sulphonated phthalocyanines. *Proc SPIE*, 2924:233–242, Sep 1996.

[16] W-S Chan, N Brasseur, C La Madeleine, R Ouellet, J E van Lier. Efficacy and mechanism of aluminium phthalocyanine and its sulphonated derivatives mediated photodynamic therapy on murine tumours. *European J Cancer*, 33(11):1855–1859, 1997.

[17] Gianfranco Canti, Angelo Nicolin, Rinaldo Cubeddu, Paola Taroni, Gaetano Bandieramonte, Gianluca Valentini. Antitumor efficacy of the combination of photodynamic therapy and chemotherapy in murine tumors. *Cancer Lett*, 125(1-2):39–44, 1998.

[18] Merrill J Egorin, Eleanor G Zuhowski, Dorothy L Sentz, Jason M Dobson, Patrick S Callery, Julie L Eiseman. Plasma pharmacokinetics and tissue distribution in CD_2F_1 mice of Pc4 (NSC 676418), a silicone phthalocyanine photodynamic sensitizing agent. *Cancer Chemother Pharmacol*, 44(4):283–294, Oct 1999.

[19] Katrin Kalka, Nihal Ahmad, Tracy Criswell, David Boothman, Hasan Mukhtar. Up-regulation of clusterin during phthalocyanine 4 photodynamic therapy-mediated apoptosis of tumor cells and ablation of mouse skin tumors. *Cancer Res*, 60:5984–5987, 2000.

[20] Cecilia M Whitacre, Taroh H Satoh, Liang yan Xue, Nahida H Gordon, Nancy L Oleinick. Photodynamic therapy of human breast cancer xenografts lacking caspase-3. *Cancer Lett*, 179(1):43–49, May 2002.

[21] John E George, Yusra Ahmad, Davood Varghai, Xiaolin Li, Jeffrey Berlin, David Jackowe, Marc Jungermann, Michael S Wolfe, Lothar Lilge, Ali Totonchi, Rachel L Morris, Allyn Peterson, W David Lust, Malcolm E Kenney, Charles L Hoppel, Jiayang Sun, Nancy L Oleinick, David Dean. Pc 4 photodynamic therapy of U87-derived human glioma in the nude rat. *Lasers Surg Med*, 36(5):383–389, Jun 2005.

[22] Antonella Borgatti-Jeffreys, Stephen B Hooser, Margaret A Miller, Michael D Lucroy. Phase I clinical trial of the use of zinc phthalocyanine tetrasulfonate as a photosensitizer for photodynamic therapy in dogs. *Am J Vet Res*, 68(4):399–404, Apr 2007.

[23] Yan Huang, Guoxing Xu, Yiru Peng, Hong Lin, Xuedong Zheng, Maosong Xie. Zinc phthalocyanine tetrasulfonate ($ZnPcS_4$): a new photosensitizer for photodynamic therapy in choroidal neovascularization. *J Ocular Pharmacol Therapeutics*, 23(4):377–386, 2007.

[24] S Burch, S K Bisland, J Siewerdsen, A Bogaards, D Moseley, A Yee, J Finkelstein, B Wilson. Photodynamic therapy for the treatment of metastatic lesions in bone: Studies in rat and porcine models. *Proc SPIE*, 5315:76–87, Jan 2004.

[25] Claes af Klinteberg, Antonio Pifferi, Stefan Andersson-Engels, Rinaldo Cubeddu, Sune Svanberg. In vivo absorption spectroscopy of tumor sensitizers with femtosecond white light. *Appl Opt*, 44(11):2213–2220, Apr 2005.

[26] Wei Liu, Naisheng Chen, Hongtao Jin, Jinling Huang, Jinfeng Wei, Jie Bao, Chenghe Li, Yan Liu, Xueyong Li, Aiping Wang. Intravenous repeated-dose toxicity study of $ZnPcS_2P_2$-based-photodynamic therapy in beagle dogs. 47(3):221–231, Apr 2007.

[27] Zibo Zhang, Hongtao Jin, Jie Bao, Fang Fang, Jinfeng Wei, Aiping Wang. Intravenous repeated-dose toxicity study of $ZnPcS_2P_2$-based-photodynamic therapy in Wistar rats. 5(11):1006–1017, Nov 2006.

[28] Elena G Vakulovskaya, Victor V Shental, Yury V Buidenok, Georgui I Solomakho, Tatiana M Nadezhdina. Photodynamic therapy of breast cancer with photosense. *Proc SPIE*, 4248:46–48, Jan 2001.

[29] Elena G Vakulovskaya, Victor P Letyagin, Loubov V Umnova, Georgiu N Vorozhcsov, Victor Philinov. Photodynamic therapy and fluorescent diagnostics of breast cancer. *Proc SPIE*, 5315:152–156, Jan 2004.