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

Cytoluminator Research Pty. Ltd.

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List of Substances and Devices to be Trialled

Photosensitising agent aqueous preparation of hydroxy-aluminium phthalocyanine disulphonate (abbreviated $AlPcS_2$) for intravenous administration.

Illuminator a portable light source that diffusely illuminates the participant from a hand-held light guide, at wavelengths predominantly between $684 - 686$ nm, with variable optical power levels not exceeding 5 W, and with average intensities below the skin pain threshold (around 1 W/cm^2).

Fluorescence imager a video camera with sensitivity to the infrared fluorescence produced by illuminated photosensitising agent ($690 - 780$ nm), 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.

Confidentiality Statement

Contents

1 Photodynamic Therapy

Photodynamic therapy (PDT), also known as photoradiation therapy, photochemotherapy, and by various other names, is the administration of a photosensitising agent followed by irradiation with light having the appropriate physical properties to activate the photosensitiser without heating the tissue [1, 2]. The photosensitiser is chosen to have minimal cytotoxicity when the body is kept in the dark, to be selectively taken up by 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 a diagnostic capability built into the treatment, for any region of increased fluorescence is very likely to contain cancer. This is the basis for Fluorescence Diagnosis (FD).

FD and PDT have been in use worldwide for more than a decade, using a number of approved photosensitisers and illuminators [4, 2, 3, 5]. Unlike chemotherapy or radiotherapy, PDT may be used repeatedly without long-term or cumulative effects [2], and unlike surgery or thermal ablation, there is little damage to underlying structures and the treated site heals normally with an "excellent cosmetic outcome" [1]. Although PDT should be performed early to achieve the best results, it is also ideally suited to palliation [2]. A number of cases have been reported in which PDT achieved complete control of post-salvage disease [1]. A widely accepted consequence of PDT is an immune response to cancer following treatment [10]. In animal models the immunity has been shown to last long after the original tumour was eliminated, since further cancer cell innoculations did not result in fresh tumours. Thus, PDT can be used to produce an in-situ vaccination against the cancer cell lines actually present within the participant. PDT has also been exploited *in vitro* to create inactivated cell vaccines against cancer [11], demonstrating that PDT-induced apoptosis followed by x-ray sterilisation is sufficient to produce the vaccination effect. 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 long-term survival could approach that of similar individuals who had not developed cancer.

Existing procedures within the field of PDT mostly utilise wavelengths of light that have short penetration depths within tissue, limiting their usefulness to surface tumours and bodily fluids treated *ex vivo*, unless specialist equipment and/or surgical implants are used for internal light delivery. The most widely known photosensitisers are porphyrins, which are far more sensitive to ultraviolet light than to their therapeutic wavelengths (around 630 nm, bright red). The skin photosensitivity to sunlight has discouraged the widespread adoption of PDT in Australia. High ambient ultraviolet light levels have previously limited the use of systemic PDT to the most desperate cases, such as the work of researchers at the Royal Melbourne Hospital who achieved $>25\%$ long-term survival of patients with glioblastoma multiforme (a high grade brain cancer), using surgical resection followed by porphyrin PDT [6, 7, 8]. (It is well known that surgery followed by radio- and chemotherapy only achieves a two-year survival of around 5%.)

The choice of illumination wavelength is critical to the success of PDT. Whilst green, blue and ultraviolet light have negligible penetration beyond the skin and into the body, deep red and near infrared light can penetrate a significant distance (see Fig. 1). It is widely recognised that the heme molecule, found in hemoglobin and myoglobin, is responsible for most of the optical absorption of tissue, and that the optimal penetration depth is achieved for light having wavelengths around $700 - 800$ nm. Unfortunately, none of the potential photosensitising agents that might be activated at these wavelengths have yet been found to possess the other necessary properties of a clinically relevant photosensitiser.

2 Summary

This brochure details a novel photosensitising agent for use with a newly developed illuminator in the practise of photodynamic therapy (PDT) of cancer. The photosensitiser is chosen from the class of *phthalocyanine* dyes, hydroxyaluminium phthalocyanine disulphonate, to be activated by light having most of its energy within the therapeutically optimum wavelength interval of 684-686 nm (deep red). This substance is relatively insensitive to ultraviolet light compared to the therapeutic wavelengths, greatly reducing the risk of inadvertent skin damage due to sunlight exposure. A novel nanomolecular formulation further improves the

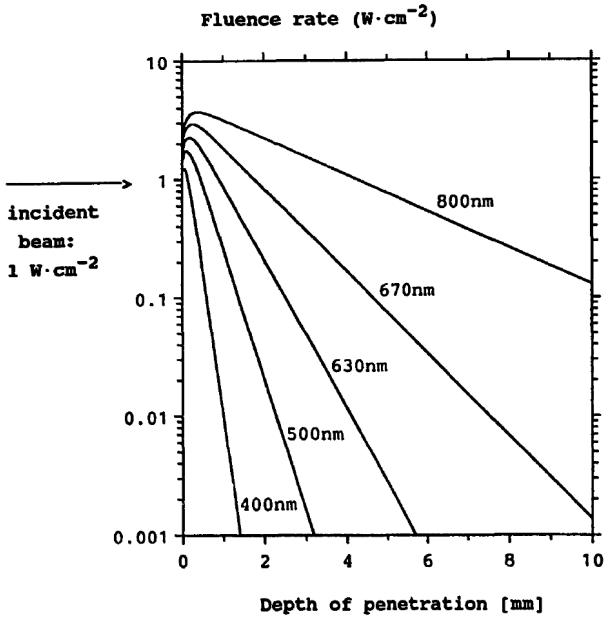

Figure 1: Light penetration into skin versus wavelength. Numerical modelling using the optical parameters of human skin, reproduced from [9].

already favourable pharmacokinetics of the sensitiser, targeting the drug more specifically to tumours and enhancing the clearance from healthy tissue by taking advantage of the leaky vasculature of a tumour and the affinity of cationic molecules for cancer cells.

Infrared fluorescence can be detected upon illumination at the therapeutic wavelength, enabling fluorescence diagnosis and the real-time monitoring of concentrations in healthy tissues and the localisation into tumours. After one to two days from systemic administration, the sensitiser is found to be mostly eliminated from the healthy tissue, enabling the photodynamic cell killing to be performed on the tumour with minimal damage to the surrounding tissue. Tumours have been found to retain the sensitiser for more than a week, so daily irradiation may be performed for a gradual debulking of the tumour masses. This extended protocol is expected to produce enhanced immune responses as compared to the traditional single exposure, especially when the light dose is reduced to a level which produces predominantly apoptosis rather than necrosis.

At the doses of sensitiser required using the nanomolecular formulation, the participant can be expected to return to normal activity within one week of systemic administration.

3 Introduction

It is less well known that deep red light around 685 nm can achieve close to the optimal penetration depth in tissue, and a potent photosensitiser at this wavelenth is the object of the present study. The clinically relevant penetration depth is determined not only by the attenuation of light through tissue, but also by the minimum effective dose (MED) at the cancer and the maximum tolerated dose (MTD) at the skin. Through the use of a novel formulation, the sponsor has improved the tissue localisation of the phthalocyanine sensitiser to greatly reduce the photosensitivity of healthy tissue and thus extend the MTD and permit greater optical power densities without damaging the skin. Tumours may thus be treated at a depth of several cm of tissue without significant pain, heating or damage to healthy structures. Due to excellent penetration of deep red light through bone, the sponsor has also achieved remissions of bony metastases and expects that it may eventually be possible to treat brain tumours using external illumination.

The scope of this therapy is very wide; any cancer is potentially treatable using the proposed sensitiser and sufficient illumination power and duration. It has been found that the present illuminator is sufficient to eradicate most breast tumours, as well as their metastases in the spine and other bones. Remission of multiple breast cancer metastases deep within the lung has also been demonstrated. Partial remission of

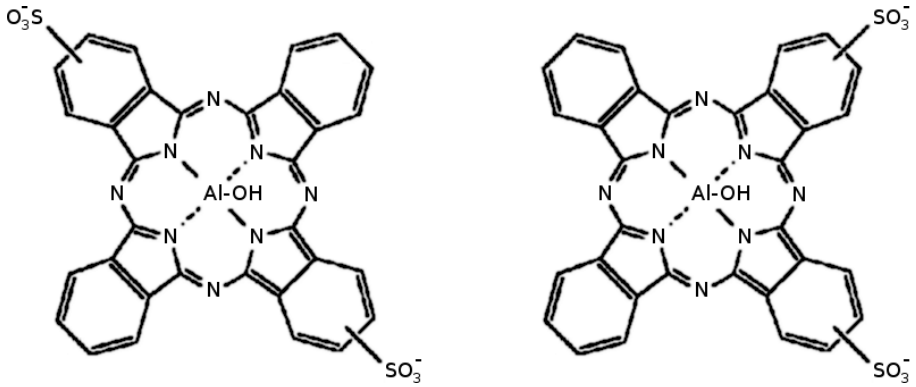

Figure 2: Chemical structures of hydroxy-aluminium phthalocyanine disulphonate. The structural isomers, differing only in the location of the sulphonyl functional groups on the phthalocyanine macrocycle, are prepared in a mixture to reduce intracellular dimerisation.

prostate cancer has been demonstrated). An area of present concern is tissue that is rich in blood, such as the liver and bone marrow, and it is unclear how far the light will penetrate. However, if a tumour is at least partially accessible to the illuminator, then the immune response may control the disease progression in those inaccessible regions of the body. (It is also possible to combine PDT with surgery to illuminate deeply buried tumours, with surgical debulking used where necessary.) In the case of leukemias, it may be possible to reduce the disease burden and stimulate an immune response, however no human studies have been performed by the sponsors at the time of writing. In a ~ 50 kg dog with an unknown lymphoma occupying > 100 cc of total lymphatic tumour volume, $<$50% of the tumour volume remained after three < 10 min treatments on three consecutive days, suggesting that human lymphoma tumours will be susceptible to the present treatment.

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 filtered through a 0.2 μm filter (or equivalent) and injected into a bag of normal saline prior to use. The rate at which the dilute sensitiser is infused will be limited by the practitioner so as to manage any discomfort and to permit monitoring for changes in blood sugar levels. It has been hypothesised that the photosensitiser reduces the bio-availability of blood sugar and additional sugars may need to be given to overcome a transient of mild hypoglycemia (depending on the dose). Over the course of the infusion, the fluorescence imager can be used 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 optical 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 verify the correct clearance from the healthy tissue before commencement of tumour irradiation, and to 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. This is expected to be around one week from treatment.

4 Physical, Chemical, Pharmaceutical Properties and Formulation

The photosensitising agent to be used, as prepared by the sponsor, is an aqueous solution of the sodium salt of hydroxy-aluminium phthalocyanine disulphonate, $\mathrm{Al(OH)Pc\left(SO_3^-Na^+\right)_2}$ (abbreviated $\mathrm{AlPcS_2}$). The pH is raised by NaOH to minimise aggregation of the molecules, as under acidic or neutral conditions the $\mathrm{AlPcS_2}$ molecules will tend to dimerise and lose their photodynamic activity [12, 13]. The chemical structures of the two isomers of $\mathrm{AlPcS_2}$ are depicted in Fig. 2. The preparation to be trialled is a mixture of the two, which has been shown to exhibit greatly reduced dimerisation over the use of one isomer or another [12], both in the as-supplied concentrated solution and in the intracellular compartment (where concentrations are much higher than in the serum).

$\mathrm{AlPcS_2}$ is structurally similar to the other phthalocyanines, and exhibits similar physical and chemical prop-

erties. The solution is translucent and cyan (blue-green) in colour, due to the strong absorption of red light. It is relatively photostable. When illuminated with UV-A or red light, the sensitiser absorbs light energy and fluoresces in the deep red part of the spectrum, with a peak emission wavelength around $670 - 690$ nm [13] as well as a significant amount of near-IR emission extending to 780 nm. It also efficiently promotes O_2 molecules to a singlet excited state [13], which contains excess energy and is highly reactive. Singlet oxygen, and the reactive oxygen species (ROS) it produces, are highly cytotoxic but have a very short lifetime and a correspondingly short range of diffusion (< 0.02 μm). For this reason, the photodynamic cytotoxicity is entirely confined to the cells in which the sensitiser is localised at the time of illumination. The sensitiser is not consumed in the process.

Pure AlPc is known to be thermally stable up to its boiling point *in vacuo* [14], and the sponsor has found its sulphonated derivatives to be similarly stable when dried at 250 °C. 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 AlPc and its monosulphonate derivative ($AlPcS_1$) will accumulate in the skin and eyes for extended periods, due to lipophilicity (poor water solubility). The tetrasulphonate ($AlPcS_4$) largely remains in the interstitial compartment, due to its hydrophilicity. These substances are detrimental to the light penetration and increase the phototoxicity to healthy tissue. The disulphonate ($AlPcS_2$) and trisulphonate ($AlPcS_3$) are taken up specifically by cancer cells and immune cells, facilitated by endocytosis of low-density lipoproteins which contain bound sensitiser. $AlPcS_2$ exhibits the greatest therapeutic effect by relocalising to the mitochondrial membrane upon illumination, inactivating many proteins anchored there. The main therapeutic target has been identified as the anti-apoptotic proteins Bcl-2 and its relatives, depletion of which triggers apoptosis through loss of mitochondrial membrane potential and release of cytochrome-c from the mitochondria. (Bcl-2 is part of the signalling pathway used by cytotoxic T cells and natural killer cells to induce apoptosis in cancer and virally infected cells.) Even cells that are unable to complete the apoptotic process will die due to loss of mitochondrial function. $AlPcS_3$ may prove beneficial in subsequent sensitiser mixtures, possibly due to reduction of intracellular aggregation, which will be investigated further.

It is anticipated that the highest administered dose will be 1.0 mg/kg (of body mass). The sensitiser will be supplied in a sterile, pre-filtered solution at a concentration of 1.0 mg/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. The primary reason for storage at low temperature is to prevent the growth of any microbial contamination that might be introduced inadvertently. Due to the possibility of aggregation and settling (which has not been ruled out), the bottle shall be shaken vigorously prior to use, to ensure the correct dose is obtained. It shall be injected into isotonic saline via an in-line 0.2 μm filter. As the concentrated solution is not isotonic, is is not suitable for direct injection and must be diluted into saline before administration. Only saline shall be used, as other solutions may promote dimerisation or aggregation after filtration.

5 Nonclinical Studies

5.1 Nonclinical pharmacology

As this document is a preliminary draft, this section has yet to be completed. See Refs. [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 5].

5.2 Pharmacokinetics and product metabolism in animals

As this document is a preliminary draft, this section has yet to be completed. See Refs. [25, 26, 27, 28, 15, 29, 30, 31, 32, 33, 34, 35, 36, 37, 11, 38]. (For animal models of bone metastases treated using PDT, see Ref. [39].) For the optimum therapeutic wavelengh see Refs. [40, 41].

5.3 Toxicology

5.3.1 Single dose

5.3.2 Repeated dose

Ref. [42, 43].

5.3.3 Carcinogenicity

None reported.

5.3.4 Special studies

N/A

5.3.5 Reproductive toxicity

No studies have been performed.

5.3.6 Genotoxicity

No studies have been performed.

6 Effects in humans

6.1 Pharmacokinetics and product metabolism in humans

A functionalised silicon phthalocyanine, referred to as Pc4, has been investigated by researchers from Case Western Reserve University for more than a decade, see Ref. [5]. It has entered early clinical trials.

6.2 Safety and efficacy

Mixtures of sulphonated aluminium phthalocyanines have been used to treat more than 100 cases of metastatic breast cancer in a Phase 3 clinical trial in Russia, see Refs. [44, 45]. This provides strong support for the safety of the present study, and the sponsor believes the Australian-made sensitiser to be of higher purity than the Russian version, which is poorly defined. The cited trial made use of the clinically ineffective wavelength of 673 nm at a much higher fluence for skin metastases, while resorting to implantation of interstitial fibre-optics to illuminate deep tumours.

6.3 Marketing experience

The sponsor has no marketing experience with this formulation.

7 Summary of data and guidance for the investigator

Preliminary investigation by the sponsor has shown a strong immunological response, which may have contributed to the remission of remote metastases that were deeper than the presumed depth of light penetration.

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 eradicated. Viable cancer cells could remain, due to insufficient light penetration through the tumour mass, inadequate oxygen perfusion during the treatment, or inhomogeneous sensitiser uptake. 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 investigation for the trial, along with the use of immune modulating adjuvants such as: granulocyte colony stimulating factor (G-CSF, marketed as Filgrastim), a cytokine which boosts the blood neutrophil count and level of activity; or granulocyte-monocyte colony stimulaing factor (GM-CSF), a cytokine which boosts the neutrophil and macrophage counts and level of activity.

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 an excessive duration of tumour irradiation, probably due to release of sensitiser into nearby tissue by necrotic cells. This occurs after around $10 - 15$ min of illumination at 3 W optical 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 from the illuminator, and it is obvious that a change has occurred. Further irradiation would not be possible without anaesthesia, nor does it appear beneficial since apoptotic cell death occurs at a small fraction of this dose. 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 just below the skin, will require at most 2 s/cm^2 (of skin surface area), to achieve apoptotic cell death with the present 5 W illuminator. It is unknown how the dose must be scaled for very deep tumours as this is a topic of further research – 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 disease, for which patient death is imminent. For that reason, use in palliative care of post-salvage patients is strongly encouraged. In one case of widely metastatic breast cancer in which multiple treatments were performed along with immune modulating adjuvants, near complete remission was achieved over a period of less than one year of continuous improvement.

Revsion History

DRAFT 3 Feb 2009 Initial version by Darren Freeman

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