Clinical investigation of the novel iron chelating agent, CP94 to enhance topical photodynamic therapy of nodular basal cell carcinoma.

S.M. Campbell1*, C.A. Morton2,
R. Alyahya3, S. Horton1, A. Pye1 and A. Curnow1

1 Cornwall Dermatology Research, Peninsula Medical School, Truro, TR1 3LJ
2 Forth Valley Dermatology Centre, Stirling Royal Infirmary, Stirling. FK8 2AU
3 Department of Maxillo-Facial Surgery, Falkirk Royal Infirmary, Falkirk, FK1 5QE

* Corresponding author

Cornwall Dermatology Research, Peninsula Medical School, Knowledge Spa, Royal Cornwall Hospital, Truro, Cornwall, TR1 3HD, UK. Tel/Fax: 44 (0)1872 256432/97. E-mail:sandra.campbell@rcht.cornwall.nhs.uk
**Background:** Photodynamic therapy (PDT) involves the activation of a photosensitiser by visible light to produce activated oxygen species within target cells, resulting in their destruction. Evidence-based guidelines support the efficacy of PDT using topical 5-aminolaevulinic acid (ALA-PDT) in actinic keratoses, Bowen’s disease and basal cell carcinoma (BCC). Efficacy for nodular BCC appears inferior to that for superficial BCC unless prior debulking or repeat treatments are performed. The aim of this study was to assess the safety and efficacy of adding a novel iron chelating agent, CP94 to topical ALA, to increase the accumulation of the photosensitiser in the tumour.

**Observations:** Enhanced PDT using 40% CP94 resulted in significantly greater clearance rates in nodular BCC than with ALA-PDT alone, in our protocol of single treatment PDT with no lesion preparation.

**Conclusion:** The results of this study demonstrate the safe and effective use of an enhanced ALA-PDT protocol for nodular BCC using CP94, with no adverse reactions to this modification. This is the first time this formulation has been used in patients. This formulation is now the focus of further study.

**Key Words:** Photodynamic therapy; basal cell carcinoma; iron chelator; CP94; hydroxypyridinone.
INTRODUCTION

Photodynamic therapy (PDT) is a sophisticated, selective method of ablating tissue. It involves the activation of a photosensitising drug by visible light to produce activated oxygen species within target cells, resulting in their destruction.¹

Topically active agents are preferable for PDT in dermatology and the most experience to date has been with the photosensitising agent ALA (5-aminolaevulinic acid, ALA) prepared as a cream². This is a precursor in the haem biosynthesis pathway, which occurs in all nucleated cells. Administration of ALA results in the formation of the endogenous photosensitiser, protoporphyrin IX (PpIX). This molecule normally binds with iron to form haem¹. PDT then involves the in situ activation of the pre-administered photosensitiser (in this case ALA-derived PpIX) by a specific wavelength of light (635 nm) in the presence of molecular oxygen resulting in necrosis/apoptosis of the tumour cells¹.

ALA-PDT for the treatment of the most common form of skin cancer, basal cell carcinoma (BCC) has received considerable attention in recent years. There is a substantial subset of BCC which are currently difficult to treat by conventional therapies because of size, site or multiple lesion presentation. Topical PDT may be advantageous in these situations and is associated with excellent cosmesis also making it an attractive option for cosmetically sensitive sites³. Results show that PDT produces good results as long as the disease remains superficial¹. Efficacy for nodular BCC however appears inferior to standard treatment unless prior debulking or repeat treatments are performed⁵,⁶ Clearance rates for ALA-PDT in superficial and nodular BCC are highly variable, with average rates of 87% and 53% respectively for 12
studies treating 826 superficial and 208 nodular BCC\textsuperscript{7}. It is important therefore to try and improve treatment in these thicker lesions, and various adaptations to the standard treatment have been considered.

One proposed method for increasing efficacy is to increase the levels of the photosensitiser, PpIX within cells (which are already being treated with maximal ALA doses), through the addition of an iron chelating agent. \textit{In vitro} studies have shown that by using an iron chelator, the conversion of PpIX to haem is inhibited by removing Fe\textsuperscript{2+} from the cellular system, resulting in an increased temporary accumulation of PpIX, which can then be utilised for PDT (Figure1). In addition to this, the resultant low intracellular iron concentration also inhibits translation of ALA synthase mRNA, which would normally be a major point of regulation for this pathway\textsuperscript{8}.

Iron chelators such as desferrioxamine (DFO) and ethylenediamine tetraacetic acid (EDTA) have been investigated as potential enhancers of ALA-PDT. Combinations of EDTA (a nonspecific chelator and antioxidant) and dimethylsulphoxide (DMSO, principally a penetration enhancer) have been used during ALA-PDT to try and increase treatment effect\textsuperscript{9,10}. The combination of EDTA and DMSO with ALA, in comparison with ALA alone, indicates improved penetration of ALA and PpIX accumulation in BCC\textsuperscript{10} but there was no measurable increase in PpIX levels when EDTA was the only additive employed\textsuperscript{10,11}. DFO, on the other hand, is both specific for iron and can penetrate into the cellular cytosol\textsuperscript{12} resulting in an increased enhancement of ALA-PDT\textsuperscript{12}. 
Relatively little research has been conducted previously on the effects of the novel iron chelator, 1,2-diethyl-3- hydroxypyridin-4-one hydrochloride (CP94) (Figure 2) on ALA induced PDT. The hydroxypyridinones are a relatively new series of iron-chelating agents. They are of lower molecular weight and greater lipophilicity than previously studied iron chelators, eg. DFO, facilitating absorption directly into the skin and they enter the intracellular iron pools rapidly, being neutrally charged\textsuperscript{13}. They were originally developed to supersede DFO for the treatment of thalassaemia and other disorders of iron overload. They have greater affinity for iron than DFO being bidentate (rather than hexadentate), binding to iron in the ratio of 3:1, compared to 1:1 with DFO\textsuperscript{14}.

\textit{In vivo} studies have shown that CP94 enhances porphyrin fluorescence and photosensitivity in all cell lines studied\textsuperscript{15}. Recent studies by Pye and Curnow have shown that CP94 is superior to DFO at increasing PpIX fluorescence in cultured human cells\textsuperscript{16}.

Animal studies have shown that the outcome of ALA-PDT can be significantly enhanced by the addition of CP94. Preliminary studies in a rat colonic tumour model have shown that CP94 can double the depth of effect produced by ALA alone and increase the volume of necrosis 7-fold\textsuperscript{17}. A topical pilot study in normal rat skin showed that ALA-PDT + CP94 tripled the effect of ALA alone\textsuperscript{18}. These findings however have not been applied clinically previously and the investigation reported here is the first to do so. Previous research has shown that CP94 can be administered orally to patients with iron-overload without significant toxicity to produce rapid and effective iron mobilization suggesting its safety in topical use as employed here\textsuperscript{19}. It is
unlikely that CP94 will be absorbed to any extent into the plasma when applied topically to the skin and any systemic circulation will be rapidly cleared by first pass metabolism (glucoronidation) in the liver.

The aim of this study, therefore, was to assess the safety and determine an indication of efficacy, of a simple pharmacological modification to single visit ALA-PDT for the treatment of nodular BCC (without prior debulking), by incorporating the novel iron chelating agent, CP94, into the ALA cream, to temporarily further enhance the accumulation of the active photosensitiser. By slowly increasing the concentration of CP94 the safety profile could be monitored in a controlled manner.
MATERIALS and METHODS

A parallel, open, dose-escalating, pilot study was conducted by two centres: Royal Cornwall Hospital, Truro and Forth Valley Dermatology Centre (Stirling and Falkirk Royal Infirmary).

Full ethical approval was obtained for the study and the Declaration of Helsinki protocols were followed. The patients were recruited from the standard outpatient clinic. Thirty six patients (23 males and 13 females) participated in the study, with a mean age of 72 years (range: 32-88 years). This number was chosen to establish the safety of this modified formulation.

Each patient had a solitary, previously untreated, nodular BCC. Twenty lesions were located on the face or neck, seven on the limbs and nine on the trunk and back. Each lesion had a minimum diameter of 5 mm and a maximum diameter of 20 mm. Patients under the age of 18 and pregnant women were excluded from the study. Patients within the child-bearing years were only included if they were able to use adequate contraceptive measures. Patients taking photoactive drugs were excluded. After written informed consent was obtained, a 3 mm punch biopsy was taken from the area within the lesion which appeared clinically to be the thickest. A 5.0 Vicryl rapide suture was used to close the biopsy site. Histology was performed on each specimen including Breslow thickness.

Six patients were treated in each of the control groups (ALA alone and CP94 alone) (control groups) and 6 patients were treated with each escalating dose of CP94, 3 per centre in parallel.
Two weeks following the biopsy, the patients were allocated into one of the following groups in strict order of recruitment at each centre:

1) 20% ALA alone (0% CP94)
2) 20% ALA + 5% CP94
3) 20% ALA + 10% CP94
4) 20% ALA + 20% CP94
5) 20% ALA + 40% CP94
6) 40% CP94 alone (0% ALA)

The lesions were digitally photographed and measured using skin landmarks to help isolate the exact area for future excision. Each lesion received one application of topical ALA (20% ALA w/w in Unguentum Merck cream base, Crawfords Pharmaceuticals, UK) +/- the various doses of the iron chelator CP94 w/w depending on the treatment group. The various concentrations of ALA with CP94 were made up in the pharmacy of each hospital under strict protocol, no more than 1 hour prior to application to the lesion. No prior lesion preparation was performed and approximately 2 mm thickness of the cream was applied to the whole lesion and a 5 mm rim of surrounding normal skin, using a wooden spatula. The lesion was then covered with an occlusive dressing (Tegaderm®, 3M, Loughborough, UK) followed by a light occlusive Mepore® dressing as per the standard protocol for ALA-PDT. Patients were asked to comment on any adverse reactions such as irritation during the time of cream application and an observation of the treated area was made to assess any contact skin reaction such as erythema prior to irradiation.
Six hours following application of the cream, the lesion was illuminated by a filtered narrow-band (630 nm +/-15 nm) red xenon light source (Photo Therapeutics Limited, UK, 100 J/cm², <130 mW/cm²). Six hours is the standard preillumination interval for ALA-PDT for BCC up to 2 mm thick. This activating red light was delivered, in a continuous manner to the entire lesion and to at least a 5 mm margin of adjacent skin. The treatment regime employed, apart from the incorporation of the CP94, was therefore the same as our standard dermatological ALA-PDT protocol. Pain levels during treatment were assessed by the patient on a 0-10 visual analogue scale. The patients were asked to grade their pain during illumination by drawing a line through a scale where 0 represented no pain and 10 represented intolerable pain. The treatment area was also assessed clinically, post irradiation to check for phototoxic reactions such as erythema. In all cases, at 6 weeks following the single PDT treatment, clinical response rate was assessed and surgical excision of the whole treatment site was undertaken for histology. Previous photographs, skin landmarks and precise measurements were used to ensure exact excision margins. Histological examination was carried out on each specimen by the one dermatopathologist at each centre, using transverse bread slice histological examination and maximum Breslow thickness of the tumour was measured. The dermatopathologists were blinded in that they received the specimens without knowledge of the treatments received. Blood samples were taken for liver function tests prior to and 1 week following application of the iron chelator, to assess any adverse effect on liver function.
RESULTS

Immediate clinical observations Whilst the cream was in contact with the skin, there was no report of any irritation or discomfort, in any of the patients. After removal of the cream at 6 hours there was no clinical evidence of increased erythema at the treatment site or surrounding area in those patients treated with ALA+CP94 over ALA alone and no other adverse reactions. Following illumination there were no adverse events, beyond the expected post therapy inflammatory response (erythema and oedema) normally observed with ALA-PDT. There was no increased erythematous response either lesional or paralesional in those patients receiving CP94-enhanced therapy.

Pain response During light treatment, all patients tolerated therapy well without the need for local anaesthesia. There was no need for interruption to treatment in any of the cases. There was no correlation between pain and increasing concentrations of CP94 and this was confirmed by the patient’s pain perception scores from the visual analogue scale. The score on the visual analogue scale ranged from 2-5 in both ALA only and ALA+CP94 groups and with no increasing trend with addition of the iron chelator. Little or no pain was experienced by the patients receiving 40% CP94 alone (data not shown).

Blood results Liver function tests were not altered in any of the patients following treatment.

Histological results The clinical and histological response to all 36 treatment sites, 6 weeks following the ALA-PDT treatment is shown in Table 1. In the CP94 control
group there was no clinical response to the PDT treatment at 6 weeks. This was confirmed using digital photography. All other lesions showed some clinical response, with the clinical impression matching the histological response in all cases. With ALA-PDT alone, 2 out of 6 histological specimens showed an actual increase in depth, or thickness, from the pre-treatment biopsy. Similarly four out of the six ALA + 5% CP94 treated nodular BCC group showed an increased thickness in the post treatment excision specimens. The reason for this could be twofold. Firstly any remaining BCC could have continued to grow in the 8-week period between biopsy and excision and secondly, the biopsy may not have been taken from the thickest part of the tumour even though it appeared to be so clinically. There was no significant improvement in response (i.e. reduction in tumour thickness) with the addition of 5% CP94 compared with the control groups (ALA alone or CP94 alone) (p < 0.172 and p < 0.79 respectively, unpaired student’s t-test). With increasing concentrations of CP94 (10, 20 and 40%), the thickness of any tumour remaining in the excised specimens decreased in all cases (Figure 3). The addition of 40% CP94 showed a significant improvement in tumour response (i.e. reduction in tumour thickness after treatment) compared with both ALA and CP94 alone controls (p < 0.001 and p < 0.001 respectively, unpaired student’s t-test). Some complete tumour responses were observed following PDT treatment (Figure 4), with 5 out of 6 showing complete or partial (greater than 50%) histological response in the ALA + 40% CP94 group. The BCC lesion in this group which showed no response was a thicker tumour with a thickness of 3 mm prior to treatment and it was noted that in general, in all groups, the tumours greater than 3 mm prior to treatment seemed to respond less well than tumours less than 3 mm thick pre-treatment. Figures 3 and 4 demonstrate this increased trend towards complete clearance with increased concentrations of CP94,
both clinically and histologically. Overall there was a significant improvement in (histological) tumour response with the addition of the CP94 iron chelator ($p < 0.001$ ANOVA).
DISCUSSION

This safety study was designed to assess the clinical feasibility and potential advantage of the addition of CP94 to routine dermatological ALA-PDT in this first clinical investigation of this novel pharmacological modification.

The results have shown that the addition of CP94 to ALA-PDT would appear to be safe, with no dose-limiting adverse events noted in any of the 30 patients treated with CP94 during this investigation. The CP94 mixed well with the 20% ALA with no apparent separation and was used immediately following preparation. Further studies to assess stability will be required in the future to increase convenience. There were no increased side effects such as erythema noted before or after illumination with increased concentrations of CP94, over that observed with the ALA only controls. This is in contrast to a study carried out applying different concentrations of ALA via iontophoresis, to normal skin previously treated with DFO, where there was a significant increase in erythema using ALA+ DFO compared with ALA alone\textsuperscript{21}.

There was also no apparent increase in pain noted during illumination, when increasing concentrations of CP94 were employed. Pain during irradiation is one of the most frequent side effects that can be observed during dermatological PpIX-induced PDT, so this finding was reassuring. Irradiation-induced pain is thought to be associated with an effective PDT treatment taking place. Previous studies however have shown that pain scores do not correlate with the intensity of PpIX fluorescence either during ALA-PDT of tape stripped normal skin\textsuperscript{20} or during ALA-PDT of skin cancer\textsuperscript{22}. This suggests that pain associated with ALA-PDT is not simply due to the
activation of PpIX alone. Additionally as ALA is transported into cells by gamma-aminobutyric acid transporters which are prevalent in peripheral nerve endings, the cellular location of the PpIX on irradiation as well as the quantity is likely to be important. As only PpIX levels produced from endogenous ALA were present in the CP94 only control group, this may explain the lower pain scores obtained from these patients.

This study has also shown encouraging results with regard to complete clearance of nodular BCC using this novel iron chelator, with no prior lesion preparation and only one cycle of enhanced PDT treatment (Figure 5). We would not normally expect a single standard ALA-PDT treatment to clear these lesions. Standard routine therapy with methyl aminolaevulinic acid (MAL), involves two treatments, 7 days apart plus careful debulking of lesions before treatment, so the treatment modification presented here is of great interest. Lesions treated with ALA alone and with the lower concentrations of CP94 (<10%) showed no complete clearances but with increasing concentrations of CP94 we were beginning to see some increased response including some complete clearance of the tumours. Some of the thicker tumours were in the “ALA” alone group, which may have explained the much lower response rate in this group, but in general the average tumour thickness among the groups receiving the combined treatment was fairly similar, This problem is inherent in a study where patients are allocated to the various treatment groups in strict order rather than stratified by tumour thickness. The histological findings matched the clinical findings in all cases and clearly showed a higher PDT efficacy as shown by cytotoxicity, with increasing doses of CP94.
These findings are in contrast to studies using other iron chelating agents particularly DFO, where the addition of DFO to the higher ALA dose being employed in clinical treatment did not significantly enhance PpIX levels, when examined in matched lesions in the same patient\textsuperscript{21}. CP94 however is of lower molecular weight and higher lipophilicity than DFO. It is also neutrally charged in both iron free and iron complexed forms enabling it to move freely in and out of cells by simple diffusion\textsuperscript{23}. CP94 consequently enters the intracellular iron pools more readily and it is this factor which is most likely to account for the increased effect of CP94 over DFO. Studies support the efficacy of CP94 in the enhancement of ALA-PDT of normal colon and bladder tissue in animal models\textsuperscript{18,24} and in normal and malignant cell lines\textsuperscript{15,16}. Studies using digital imaging fluorescence microscopy have revealed inter- and intratumour fluorescence variability and heterogeneity in BCC treated with ALA for the standard 6 hours application time\textsuperscript{26}. A relatively stable level of fluorescence existed from the epidermis to some depth, after which a quantifiable gradient of decreasing PpIX fluorescence intensity versus depth was seen\textsuperscript{26}. The ability of CP94 to increase PpIX fluorescence particularly in cells where the concentration of ALA is low may explain the increased effect in deeper nodular tumours\textsuperscript{16}. The addition of CP94 would enable the production of clinically useful amounts of PpIX deeper in the tumour cells, where otherwise PpIX production would be at sub-lethal levels.

Further studies are now underway to explore and confirm these observations and to determine the optimal concentration of CP94 to achieve maximum effect. It may be expected that there will be a limit to the addition of the iron chelator to the mixture both physically and also beyond which there would be insufficient ALA present as a substrate, to further increase the levels of PpIX to produce greater enhancement of
treatment effect. Studies are also underway to measure the time taken to achieve maximum PpIX concentration with increasing doses of CP94 as it may be possible to allow shorter drug-light intervals if PpIX formation is optimised sooner particularly when treating superficial disease.

The combined use of ALA+CP94 in conjunction with penetration enhancers such as DMSO, tape stripping or iontophoresis, or the use of pressure techniques such as oxygen pressure injection (OPI)\textsuperscript{27} may further increase the proportion of nBCC completely responding to PDT treatment.

This study therefore has indicated that this enhanced therapy with CP94 provides a simple and safe pharmacological modification to dermatological ALA-PDT which may increase the proportion of BCC that can be treated by this therapy. This may be a real advance in improving the response rate in these nodular tumours without the need for repeat treatment cycles, although this will require confirmation in a large randomised controlled trial.
ACKNOWLEDGMENTS

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References:


16. Pye A, Curnow A. Direct comparison of d-aminolevulinic-acid and methyl-aminolevulinate-derived-protoporphyrin IX accumulation potentiated by desferrioxamine or the novel hydroxypyridinone iron chelator CP94 in cultured human cells. Accepted Photochemistry & Photobiology 2007


**Table 1.**

Clinical and histological response in each patient following PDT with 20% ALA and increasing concentrations of CP94.

**NR:** Nil Response: Less than 50% reduction in tumour depth compared with pre-treatment biopsy.

**PR:** Partial Response: Greater than 50% reduction in tumour depth compared with pre-treatment biopsy.

**CR:** Complete Response: No residual cells seen.

- * ulcerated
- ** residual superficial BCC- central fibrosis
- *** small focus of BCC at 1.4mm
- **** virtually clear-one focus of BCC on one section only
Figure Legends

**Figure 1.** Schematic diagram of haem biosynthesis indicating the difference in PpIX and haem production with and without the iron chelating agent being present.

**Figure 2.** Molecular structure of the iron chelator CP94 (1,2-diethyl-3-hydroxypyridin-4-one hydrochloride).

**Figure 3.** Tumour thickness before and after PDT treatment of each group of nBCC lesions (n=6) with increasing concentrations of CP94 incorporated.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Graph Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% ALA + 40% CP94</td>
<td>Grey</td>
</tr>
<tr>
<td>20% ALA + 0% CP94</td>
<td>Blue</td>
</tr>
<tr>
<td>20% ALA + 5% CP94</td>
<td>Orange</td>
</tr>
<tr>
<td>20% ALA + 10% CP94</td>
<td>Green</td>
</tr>
<tr>
<td>20% ALA + 20% CP94</td>
<td>Purple</td>
</tr>
<tr>
<td>20% ALA + 40% CP94</td>
<td>Red</td>
</tr>
</tbody>
</table>

**Figure 4.** Scatter diagram indicating percentage reduction or increase in tumour thickness of individual nBCC lesions produced using ALA-PDT in combination with increasing concentrations of the iron chelator, CP94.

- Nil Response
- Partial Response
- Complete Response

**Figure 5.**

a) Nodular BCC right side of nose before treatment with ALA-PDT.
b) Nodular BCC 6 weeks after PDT treatment prior to excision using 20% ALA+ 40% CP94 showing almost complete clinical clearance and excellent cosmesis.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (mm)</th>
<th>Site</th>
<th>Initial tumour thickness (mm)</th>
<th>Tumour thickness on excision (mm)</th>
<th>Clinical Outcome</th>
<th>Histological Outcome &amp; % reduction/increase from biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% CP94</td>
<td>20x12</td>
<td>Limb</td>
<td>1.2</td>
<td>1.2</td>
<td>NR</td>
<td>NR 0%</td>
</tr>
<tr>
<td>40% CP94</td>
<td>18x9</td>
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<td>1.8</td>
<td>1.6</td>
<td>NR</td>
<td>NR -11%</td>
</tr>
<tr>
<td>40% CP94</td>
<td>20x13</td>
<td>Limb</td>
<td>1.2</td>
<td>1.0</td>
<td>NR</td>
<td>NR -17%</td>
</tr>
<tr>
<td>40% CP94</td>
<td>20x13</td>
<td>Trunk</td>
<td>1.3</td>
<td>3.3*</td>
<td>NR</td>
<td>NR + &gt;100%</td>
</tr>
<tr>
<td>40% CP94</td>
<td>18x16</td>
<td>Back</td>
<td>0.7</td>
<td>0.9</td>
<td>NR</td>
<td>NR +29%</td>
</tr>
<tr>
<td>40% CP94</td>
<td>19x12</td>
<td>Trunk</td>
<td>0.9</td>
<td>1.1</td>
<td>NR</td>
<td>NR +22%</td>
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<td>19x10</td>
<td>Trunk</td>
<td>3.2</td>
<td>2.6</td>
<td>PR</td>
<td>NR -18%</td>
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<td>2.3</td>
<td>1.5</td>
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<td>NR -34%</td>
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<td>12x12</td>
<td>Face</td>
<td>2.3</td>
<td>1.5</td>
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<td>NR -34%</td>
</tr>
<tr>
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<td>Limb</td>
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<td>1.8</td>
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<td>Face</td>
<td>1.0</td>
<td>1.7</td>
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<td>PR</td>
<td>PR -73%</td>
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<td>0.1</td>
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<td>PR</td>
<td>NR + &gt;100%</td>
</tr>
<tr>
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<td>Neck</td>
<td>1.8</td>
<td>1.6</td>
<td>PR</td>
<td>NR -11%</td>
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<td>3.0</td>
<td>PR</td>
<td>NR +50%</td>
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<td>Face</td>
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<td>3.0</td>
<td>PR</td>
<td>NR +36%</td>
</tr>
<tr>
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<td>12x10</td>
<td>Face</td>
<td>1.2</td>
<td>0</td>
<td>CR</td>
<td>CR - 100%</td>
</tr>
<tr>
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<td>10x10</td>
<td>Limb</td>
<td>1.9</td>
<td>1.4</td>
<td>PR</td>
<td>NR - 26%</td>
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<td>17x11</td>
<td>Face</td>
<td>2.2</td>
<td>1.7</td>
<td>PR</td>
<td>NR - 23%</td>
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<td>15x15</td>
<td>Limb</td>
<td>0.3</td>
<td>0.1**</td>
<td>PR</td>
<td>PR -67%</td>
</tr>
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<td>ALA+10%CP94</td>
<td>10x10</td>
<td>Face</td>
<td>2.0</td>
<td>0.3</td>
<td>PR</td>
<td>PR - 85%</td>
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<tr>
<td>ALA+10%CP94</td>
<td>12x10</td>
<td>Face</td>
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<td>1.0</td>
<td>PR</td>
<td>NR - 37%</td>
</tr>
<tr>
<td>ALA+20%CP94</td>
<td>10x10</td>
<td>Face</td>
<td>0.7</td>
<td>0</td>
<td>CR</td>
<td>CR -100%</td>
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<tr>
<td>ALA+20%CP94</td>
<td>10x11</td>
<td>Face</td>
<td>3.2</td>
<td>3.0</td>
<td>PR</td>
<td>NR - 6%</td>
</tr>
<tr>
<td>ALA+20%CP94</td>
<td>20x18</td>
<td>Back</td>
<td>2.0</td>
<td>1.7</td>
<td>PR</td>
<td>NR - 5%</td>
</tr>
<tr>
<td>ALA+20%CP94</td>
<td>12x9</td>
<td>Face</td>
<td>3.0</td>
<td>1.4***</td>
<td>PR</td>
<td>PR -53%</td>
</tr>
<tr>
<td>ALA+20%CP94</td>
<td>9x7</td>
<td>Face</td>
<td>2.0</td>
<td>0.4****</td>
<td>PR</td>
<td>PR -80%</td>
</tr>
<tr>
<td>ALA+20%CP94</td>
<td>10x7</td>
<td>Face</td>
<td>1.4</td>
<td>0</td>
<td>CR</td>
<td>CR -100%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>12x13</td>
<td>Face</td>
<td>2.0</td>
<td>0.4</td>
<td>PR</td>
<td>PR -80%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>15x16</td>
<td>Trunk</td>
<td>3.0</td>
<td>2.0</td>
<td>PR</td>
<td>NR -33%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>9x10</td>
<td>Neck</td>
<td>1.25</td>
<td>0</td>
<td>CR</td>
<td>CR -100%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>6x7</td>
<td>Limb</td>
<td>1.5</td>
<td>0.12</td>
<td>PR</td>
<td>PR - 92%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>10x12</td>
<td>Trunk</td>
<td>2.0</td>
<td>0</td>
<td>CR</td>
<td>CR -100%</td>
</tr>
<tr>
<td>ALA+40%CP94</td>
<td>6x6</td>
<td>Face</td>
<td>2.0</td>
<td>0.8</td>
<td>PR</td>
<td>PR - 60%</td>
</tr>
</tbody>
</table>

Table 1
Fig. 1

5 Aminolaevulinic Acid

↓

↓

↓

Protoporphyrin IX

Fe$^{2+}$

Ferrochelatase

Haem

5 Aminolaevulinic Acid

↓

↓

↓

Protoporphyrin IX

Fe$^{2+}$

Ferrochelatase

Haem

Iron Chelator
Fig. 2
Fig. 3
Fig. 4
Fig. 5a