Open Research Online

The Open University's repository of research publications and other research outputs

Photodynamic therapy and diagnosis: Principles and comparative aspects

Journal Item

How to cite:

Dobson, Jane; Fernandes de Queiroz, Genilson and Golding, Jon P. (2018). Photodynamic therapy and diagnosis: Principles and comparative aspects. *Veterinary Journal*, 233 pp. 8–18.

For guidance on citations see [FAQs](#).

© 2017 Elsevier Ltd.

<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Version: Accepted Manuscript

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.1016/j.tvjl.2017.11.012>

Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

oro.open.ac.uk

Photodynamic therapy and diagnosis: Principles and comparative aspects

Jane Dobson^{a*}, Genilson Fernandes de Queiroz^b, Jon P. Golding^c

^a*Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK*

^b*Department of Animal Science, Rural University of Semi-Arid, Av. Francisco Mota, Mossoró 59625-900, Brazil*

^c*School of Life, Health and Chemical Sciences, The Open University, Walton Hall, Milton Keynes MK7 6AA, UK*

*Corresponding author

19 **Abstract**

20 Photodynamic therapy (PDT) is an evolving method of treating superficial tumours that is
21 non-invasive and carries minimal risk of toxicity. PDT combines tumour-selective photosensitiser
22 dyes, tissue oxygen and targeted illumination to generate cytotoxic reactive oxygen species (ROS)
23 within the tumour. In addition to directly acting on tumour cells, PDT damages and restricts tumour
24 microvasculature, and causes a local inflammatory response that stimulates an immune response
25 against the tumour. Unlike surgery or radiotherapy the surrounding extracellular matrix is
26 unaffected by PDT, thus tissue healing is excellent and PDT seldom scars. This, combined with the
27 ease of light application, has made PDT a popular treatment for cancers and pre-cancers in humans.
28 Moreover, because photosensitiser dyes are fluorescent and selectively accumulate in tumour
29 tissues, they can additionally be used to visualise and discriminate tumour from normal tissues,
30 thereby improving the accuracy of tumour surgery.

31

32 In veterinary practice, PDT has been used successfully for treatment of superficial squamous
33 cell carcinoma of the feline nasal planum; urinary tract, bladder and prostate neoplasia in dogs; and
34 for equine sarcoids. The purpose of this article is to make a comparative review of the current
35 literature on PDT in human and veterinary medicine, to provide a basis for future development of
36 PDT in veterinary medicine.

37

38 **Key words**

39 Photodynamic therapy; cancer.
40
41

42 **Introduction**

43 Photodynamic therapy (PDT) involves administration of a photosensitiser drug, or a pro-
44 drug, which selectively accumulates in target cells, followed by local illumination of the lesion with
45 visible light (Luksiene, 2003; Wachowska et al., 2011). It is a minimally invasive therapeutic
46 technique used in the management of various cancerous and pre-malignant diseases. The
47 photosensitiser can also be visualised in tumour cells using an appropriate set of imaging filters to
48 provide a means of tumour detection (Hefti et al., 2010, Mowatt et al., 2011, , Nguyen and Tsien
49 2013, Allison 2016).

50

51 In addition to cancer treatment, PDT has been used for the treatment of microbial infections
52 (Kharkwal et al., 2011, Sharma et al., 2012, Wardlaw et al., 2012), including veterinary applications
53 in dogs (Fabris et al., 2014) and sheep (Sellera et al., 2016). PDT has also been used for light-
54 triggered uptake of pharmaceuticals that would otherwise become entrapped and destroyed within
55 cellular endosomes (photochemical internalisation, PCI; reviewed by Selbo et al., 2015 and Madsen
56 2016). However, the focus of this review will be on the uses of PDT in cancer treatment and
57 diagnosis.

58

59 The origins of PDT can be traced back to ancient Egypt, where photosensitizing plant
60 pigment extracts were applied to the skin and exposed to sunlight, as a treatment for psoriasis
61 (Daniell and Hill 1991). The use of PDT for treatment of various human skin cancers was first
62 investigated in the 1970's by Dougherty et al (1978). Dougherty's use of a haematoporphyrin
63 derivative was based on pioneering work of Policard et al., (1924) who demonstrated that
64 porphyrins were preferentially distributed into malignant rather than normal tissues. The technique
65 was slow to gain acceptance because the 'first generation' photodynamic agents were slow to clear
66 from normal cells with the result that treated human patients had to remain out of bright light

(e.g. sunlight) for several weeks to avoid severe skin reactions. However, the potential for the technique in treating locally advanced carcinomas of the head and neck (Wile et al., 1984), bladder (Misaki et al., 1983), oesophagus and bronchus (Cortese and Kinsey 1984) outweighed this caveat and stimulated further research.

The availability of haematoporphyrin derivatives with faster tissue clearance times stimulated more interest in PDT and numerous human clinical trials have now been published showing encouraging results with photosensitizing dyes administered topically or systemically (orally or intravenously) or instilled into hollow organs (e.g. bladder). A limited number of veterinary studies have been published, also showing promise. A previous review of PDT in veterinary medicine was published in 2013 (Buchholz and Walt, 2013), since then further advances have been made. The purpose of this review is to describe the basic principles of PDT and discuss the clinical application of PDT in humans and animals.

Fundamentals and mechanisms

There are three basic requirements for PDT; (1) a compound with photosensitising properties (photosensitizer, PS), (2) a source of visible light and (3) oxygen. The photosensitizer is a chemical / dye that selectively accumulates in malignant tissues and can be activated by visible light. Energy from the light-excited PS is transferred to oxygen molecules (O_2) to give reactive oxygen species (ROS), notably singlet oxygen (1O_2) and superoxides, that damage biological molecules, initiating a cascade of biochemical events culminating in damage and death of neoplastic cells (Fig. 1) (Dougherty et al., 1998, Juzeniene et al., 2007). Increasing tissue oxygenation can lead to increased ROS formation during PDT and improved outcomes (Maier et al., 2000).

The mechanisms by which different photosensitisers localise selectively in malignant tissues are complex and not fully understood. Physical factors, such as increased vascular permeability and poor lymphatic drainage in tumours, coupled with an affinity for proliferating endothelium likely contribute to their accumulation in tumours (Dougherty et al., 1998).

Three main processes by which ROS contribute to the destruction of tumours by PDT are direct cellular damage, indirect vascular shutdown and activation of immune response against tumour cells (Dougherty et al., 1998, Dolmans et al., 2003, Solban et al., 2006). Direct damage to tumour cells can result in cell death by both programmed (apoptotic) pathways and non-programmed (necrotic) pathways (Oleinick et al., 2002; Igney and Krammer 2002, Allison and Moghissi 2013a). Generally, when the light intensity is low, apoptotic death may be initiated (Agarwal et al., 1991, Allison and Moghissi 2013b). At higher light intensities, tumour cells are rapidly ablated by necrosis due to destruction of cellular and subcellular membranes. This also leads to release of cytokines and lysosomal enzymes (Henderson and Fingar 1987) causing damage to cells nearby, the bystander effect (Dahle et al., 1997, Allison and Moghissi, 2013a). Release of inflammatory mediators from the treated region stimulates activation of leucocytes including neutrophils and macrophages and significant tumour cell death occurs through these activated immune cells (Coutier et al., 1999; Gollnick et al., 2003, Castano et al., 2006). This observation has led to the development of combination therapies of PDT with immunotherapy, by including immunoadjuvants against tumour-specific epitopes (Qiang et al., 2008, Kleinovink et al., 2015).

PDT also mediates a vascular effect within tumours (McMahon et al., 1994, Abels, 2004). Neovascular tumour endothelial cells may accumulate higher levels of PS than normal endothelium (Debefve et al., 2011) and following PDT, microvascular collapse can be observed and can lead to severe and persistent post-PDT tumour hypoxia (Star et al., 1986, Henderson et al., 1987, Chen et

116 al., 2003). PDT may also lead to vessel constriction via inhibition of the production or release of
117 nitric oxide by the endothelium (Gilissen et al., 1993).

118

119 An important clinical consideration is effective analgesia. In humans PDT produces a
120 sensation of stinging or burning during illumination, especially in sensitive areas such the face, and
121 scalp (Halldin et al., 2011, Chaves et al., 2012). Treatment of large skin areas generally produces
122 more pain than smaller areas (Grapenglesser et al., 2002, Hallidin et al., 2011, Chaves et al., 2012).

123

124 **Photosensitizers for PDT**

125 Photosensitising (PS) agents are natural or synthetic chemicals that transfer light energy to
126 neighbouring molecules, importantly to dissolved oxygen (Allison et al., 2004). Most of the
127 photosensitizers used in cancer therapy are based on a tetrapyrrole structure, similar to that of the
128 protoporphyrin contained in haemoglobin. In clinical practice, a successful PS agent is: nontoxic
129 until light activated, hydrophilic for easy systemic application, activated by a clinically useful light
130 wavelength, and reliably generates a photodynamic reaction (PDR). It also concentrates in tumours,
131 clears normal tissue quickly, and is eliminated from the patient relatively rapidly (Allison and
132 Moghissi 2013a).

133

134 The first-generation photosensitizer, haematoporphyrin derivative (HPD) was a mixture of
135 various monomers, dimers, and polymers of haematoporphyrin (Allison and Moghissi 2013a). The
136 commercially available product, porfimer sodium, marketed under the tradename Photofrin was
137 experimentally used in healthy dogs (Tochner et al., 1991; Panjehpour et al., 1993) and a canine
138 glioma model (Whelan et al., 1993). It was approved for treatment of early stage of human lung
139 cancer in 1998 and for Barrett's esophagus in 2003. The clinical application of Photofrin has been
140 limited by two factors: its absorption peak occurs at 630 nm, too short a wavelength to allow deep

141 penetration of light in tissue. Secondly, Photofrin results in cutaneous photosensitivity lasting up to
142 6 weeks (Zhu and Finlay, 2008).

143

144 These limitations stimulated the development of a second generation of photosensitizers
145 with improved efficiency of ROS generation, more rapid clearance, fewer side effects, and
146 absorption peaks at longer wavelengths (>630 nm red light) where the tissue penetration of light is
147 deeper. One such second-generation photosensitizer is 5-aminolevulinic acid (ALA), a naturally
148 occurring pro-photosensitizer and precursor for the biosynthesis of heme. For therapeutic purposes,
149 ALA is administered topically (Morton et al., 2008, 2013), orally (Muller and Wilson, 2006), or
150 intralesionally (Hage et al., 2007; Kim et al., 2012) and enters into all cells; although uptake is
151 potentiated by transporters of beta-amino acids and GABA (Rud et al., 2000), highly expressed on
152 some cancer cells and neurons (Zhang et al., 2013). ALA is then metabolised to the red-fluorescent
153 photosensitizer protoporphyrin IX (PpIX, absorption 635 nm) and finally to non-fluorescent heme
154 (Ajioka et al., 2006, Allison and Moghissi 2013a). This final step relies on ferrochelatase to add
155 Fe^{2+} to PpIX and this rate-limiting enzyme is often deficient in cancer cells (Kemmner et al., 2008).
156 Thus, in the presence of excess ALA, cancer cells that combine high ALA uptake with low PpIX
157 destruction will accumulate PpIX photosensitizer (Collaud et al., 2004). Clinical advantages of
158 ALA treatment include rapid clearance of PpIX from the tissue within 12 hours, resulting in short-
159 lived cutaneous photosensitivity. In human patients ALA has been used for the treatment of T cell
160 lymphoma (Coors et al., 2004), basal cell carcinoma (Kim et al., 2012) squamous cell carcinoma
161 (SCC) and other head and neck cancers (Grant, et al., 1993, Morton et al., 1996). In veterinary
162 medicine, ALA has been used to treat SCC in a cow (Hage et al., 2007) and in cats (Bexfield et al.,
163 2008), sarcoids in horses (Gustafson et al., 2004, Golding et al., 2017) and transitional cell
164 carcinoma in dogs (Lucroy et al., 2003a,b). See Tables 1 and 2.

165

166 The hydrophilic nature of ALA limits its ability to deeply penetrate intact skin and thereby
167 restricts the use of topically applied ALA-PDT to the treatment of superficial diseases, where the
168 tissue structure is disorganised. To overcome this limitation, ALA esters that are less hydrophilic
169 than the parental compound have been developed. The methyl ester of ALA, methyl-
170 aminolevulinate (MAL, Metvix, or Metvixia), was approved by the US Food and Drug
171 Administration for PDT treatment of actinic keratosis in 2004 and has shown good results in
172 treatment of equine sarcoids (Kemp-Symonds 2012, Golding et al., 2017). Hexaminolevulinate, the
173 n-hexyl ester of ALA, (HAL, Hexvix, Cysview) which is converted to PpIX 50–100 times more
174 efficiently than ALA, was licensed in US in 2010 for the detection of human bladder cancer (Furre
175 et al., 2005). Hexaminolevulinate has also been used intra-operatively in a PDT model in dogs with
176 prostate carcinoma (L'Eplattenier et al., 2008).

177

178 Several other second-generation photosensitisers have been, or are in the process of being
179 developed, each with slightly different origins and characteristics. These include m-
180 tetrahydroxophenyl chlorine (m-THPC, Foscan); 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-
181 a (HPPH, Photochlor); palladium bacteriopheophorbide (Padoporfin, TOOKAD) and its more
182 water-soluble monolysotaurine derivative (Padeliporfin, TOOKAD-Soluble); motexafin lutetium
183 (Lu-Tex, Lutrin); and Verteporfin (Visudyne). The advantages and indications for these newer
184 agents are summarised in Table 1.

185

186 **Photosensitisers for diagnosis**

187 Photodynamic diagnosis (PDD) uses the fluorescence of photosensitisers to identify tumour
188 tissue in situ. PDD fits within the broader category of Fluorescence Guided Surgery (Allison 2016).
189 The distinction being that, by increasing the illumination intensity or duration, PDD can become
190 PDT. However, whilst the generation of singlet oxygen by photosensitisers is essential for PDT,
191 these same reactive species can damage the photosensitiser and render it non-fluorescent.

ALA has been trialled for PDD in eleven different human tumour types (Nokes, 2013), and is licensed in humans for intraoperative margin assessment in glioma (Hefti et al., 2010, Stummer et al., 2006) and the n-hexyl derivative for bladder cancer (Kausch et al., 2010, Mowatt et al., 2011). Each of the major surgical microscopy and endoscopy manufacturers (Leica, Olympus, Storz, and Zeiss) have specialized imaging equipment for intraoperative PDD for human surgery. Research versions are available for animal models (e.g. Solaris system, Perkin Elmer). However, relatively little work has been done on translating human PDD to veterinary surgery. Veterinary examples include intraoperative cancer imaging and staging in dogs (Knapp et al., 2007, Cabon et al., 2016, Osaki 2016), and image-guided surgery in cats (Wenk et al., 2013). The next generation of agents for photodiagnosis are generally based on near infra-red dyes, which allow deeper views into tissues, sometimes complexed with tumour-targeting peptides or antibodies (Luo et al., 2011, Wenk et al., 2013).

204

Light sources and delivery systems

The primary requirement when treating lesions with PDT is to ensure that sufficient, homogenous light is delivered to the target tissue. Each PS has an optimal wavelength and intensity (fluence) of light for activation (Sibata et al., 2001). Choice of light source should therefore be based on PS absorption (fluorescence excitation and action spectra), location, size and accessibility of lesions, and tissue characteristics. The clinical efficacy of PDT is dependent on complex dosimetry: total light dose, light exposure time, and light delivery mode (single vs. fractionated or even metronomic). The fluence rate also affects PDT response (Henderson et al., 2006) and as demonstrated in tumour bearing cats by Hahn et al. (1998).

214

The wavelength of light used for PDT is typically in the range between 600–800 nm, the ‘therapeutic window’ (Wilson and Patterson, 1990). In this wavelength range, the energy of each

217 photon is sufficient (1.5 eV) to excite the photosensitizer and yet is low enough to allow the light to
218 penetrate up to 2 cm into the tissue (Zhu and Finlay, 2008).

219

220 The development of light sources and delivery devices with the appropriate dosimetric
221 parameters are key components for the clinical application of PDT. Accurate delivery of the light to
222 the tumour tissue can be accomplished by a variety of light sources and fibre optic delivery devices.
223 Lasers have been one of the main light sources used in PDT. Modern diode lasers are portable and
224 do not require specialized electrical supply or water cooling, providing excellent stability of output
225 power over long periods of time (Mang, 2004). Diode lasers have been approved for use with
226 Photofrin in oesophageal and lung malignancies at 630 nm and at 652 nm for Foscan (Yoon et al.,
227 2013).

228

229 Alternatives to laser technology are non-coherent light sources (Reeds et al., 2004) and light
230 emitting diodes (LEDs), the latter where light is produced by a solid-state process called
231 electroluminescence. LEDs are compact, lightweight and require significantly less energy than
232 lasers. LED systems are capable of output powers up to 150 mW/cm² over a 3 cm x 3 cm area.
233 LEDs have been manufactured with various light output wavelengths, such as 630, 670, and 690
234 nm, which can be used in PDT procedures for flat surface illumination (Mang, 2004 and 2009).
235 Light delivery for treatment of large surface areas, such as treatment of skin diseases, may also be
236 effectively accomplished using broad-spectrum fluorescent lamps (Marcus and McIntyre, 2002).
237 However, LEDs have been shown to be more effective than fluorescent lamps for PDT treatment of
238 squamous cell carcinoma (Novak et al., 2016). One obvious source of light for PDT is the sun, and
239 several recent studies have demonstrated the effectiveness of daylight PDT (reviewed by See et al.,
240 2016). Daylight PDT has obvious potential for veterinary skin cancers, provided the tumour is
241 located where it will be in constant daylight.

242

243 In addition to the light source, delivery devices may be required to provide penetration of
244 light into the target tissue (Star et al., 1992). Fibre-optic devices have been developed for PDT light
245 delivery and dosimetry (Sterenborg et al., 2014). The most widely used fibre-optic device in PDT is
246 a cylindrical diffusing fibre tip available in lengths of 1 - 9 cm depending on the specific
247 application. Two light delivery methods have been developed: intraluminal irradiation using light
248 diffusers for the lung and oesophagus, and interstitial illumination methods to deliver adequate light
249 doses to the target tumour volume in head and neck cancers (Yoon et al., , 2013). Fibre optic
250 delivery of PDT has been used in dogs to treat intramedullary bone tumours (Burch et al., 2009).

251

252 **Photodynamic therapy and diagnosis: clinical uses in humans and animals**

253 In contrast, to its increasing use in human medicine, the use of PDT in veterinary medicine
254 has been relatively limited, and although results from small veterinary clinical studies have been
255 published and despite the fact that the dog and cat have been used as a preclinical model in several
256 studies (Lucroy et al., 1999, 2003b, Griffin et al., 2001, Panjehpour et al., 2002, Tanabe et al.,
257 2004), PDT is not well established as a treatment option for tumour bearing animals to date. The
258 main indication currently is in treatment of in situ carcinoma/SCC in cats. Other possible
259 indications are urinary tract neoplasia and glioma in dogs and SCC and sarcoids in horses
260 (Buchholz and Walt, 2013). The following is a comparative review of the clinical experience of
261 application of PDT in human and veterinary medicine to provide a basis for future development and
262 application of the technique in veterinary medicine.

263

264 **Cutaneous tumours**

265 *Carcinoma in situ / Squamous cell carcinoma (SCC)*

266 ALA-PDT is mainly used to treat dermatological cancers in humans and several reviews of
267 current guidelines have been published (Morton et al., 2008, 2013; Wan and Lin, 2014). The results
268 of ALA-PDT in the treatment of human Bowen's disease (squamous cell carcinoma *in situ*) have

269 been promising; randomized, controlled trials comparing ALA-PDT or MAL-PDT to cryotherapy
270 (Morton et al., 1996) or 5-fluorouracil (5-FU) cream (Salim et al., 2003) reveal complete response
271 rates of 82-100% for PDT vs 67-100% for cryotherapy or 79-94% for 5-FU at 12-24 months. The
272 efficacy of topical ALA-PDT in the management of primary cutaneous invasive SCC is variable,
273 with response rates of 54 – 100% reported for superficial lesions and recurrence rates ranging from
274 0 – 69%, but with reduced efficacy in more nodular lesions (Wolf et al., 1993; Morton et al 2002).
275 Current evidence supports the potential of topical ALA-PDT for superficial, micro-invasive SCC
276 but in view of its metastatic potential topical PDT cannot be recommended for invasive SCC
277 (Morton et al., 2008, 2013).

278

279 Cutaneous in situ-carcinoma/SCC in the cat represents the main application for PDT in
280 veterinary medicine to date (Fig. 2). A number of studies have reported response rates from 60 –
281 80+% and disease-free intervals of over 68 weeks, for topical and systemic PDT in cats using a
282 variety of photosensitisers (as detailed in Table 2). As is the case in human patients, the smaller and
283 less invasive tumours respond best to PDT (Magne et al., 1997). PDT has also been used to treat
284 SCC in dogs (McCaw et al., 2000), horses (Giuliano 2008), a cow (Hage et al., 2007), snakes
285 (Roberts WG et al., 1991) and a Great Hornbill (Suedmeyer et al., 2001).

286

287 *Basal cell Carcinoma*

288 PDT has been successfully employed for treatment of basal cell carcinoma (BCC) in human
289 patients as a sole agent or in neoadjuvant setting (Berroeta et al., 2007, Rhodes et al., 2007). A 92%
290 complete response rate was reported with topical ALA-PDT in 330 patients with superficial BCC,
291 but the response rate dropped to 71% in patients with nodular BCC (Zeitouni et al., 2001) , and
292 when topical PDT (with ALA or MAL) is compared to surgery for BCC, PDT consistently shows
293 an increased recurrence rate for both superficial and nodular BCC (Basset-Seguin et al., 2008). This
294 may be due to insufficient penetration of the photosensitizer to deeply located tumour cells when

295 the PS is applied topically. To overcome this problem, the PS may be injected intralesionally.
296 Twenty patients with nodular BCC were treated with ALA in 1% saline solution at estimated dose
297 of 1 mL/cm² injected into the base of tumour. PDT resulted in tumour necrosis, followed by
298 complete re-epithelization after 4-6 weeks with good cosmetic results, no histological evidence of
299 BCC after 3 months and no recurrence during follow-up of 19.5 months (Rodríguez-Prieto et al.,
300 2012).

301

302 Experience of intralesional injection of PS is very limited in animals. One study reported
303 PDT in a cow with ocular SCC using intratumoural injection of ALA. A complete response was
304 observed after 3 months and no relapse 12 months after the treatment (Hage et al., 2007). PDT has
305 also been used for treatment of periocular SCC in horses. A pilot study was conducted using
306 surgical resection plus PDT for periocular SCC in horses by infiltrating wound beds with HPPH
307 prior to illumination. This combination yielded disease-free intervals of 25–68 months. The overall
308 recurrence rate was 22% (2 of 9 horses) and for those horses where local PDT was the first and only
309 treatment modality used, the recurrence rate was 0% (Giuliano et al., 2008).

310

311 *Equine sarcoids*

312 Although of fibroblastic rather than of basal cell origin, equine occult and nodular sarcoids
313 form dermal nodules or plaques and as such bear some physical resemblance to the human nodular
314 BCC. Currently there is no ‘gold standard’ treatment for equine sarcoids, however, PDT has shown
315 promise in the treatment of these common and frustrating lesions. Several small studies have
316 reported encouraging response rates using topical or locally injected ALA or MAL in equine occult
317 and nodular sarcoids. For instance, Gustafson et al., (2004) found a 72% treatment response using
318 ALA-PDT, with recurrence in 39% of lesions after 2 years ($n=18$). Due to their fibroblastic and
319 bulky nature, cytoreductive surgery may significantly improve response for larger lesions. In one
320 study, CO₂ laser excision with adjunctive MAL-PDT was reported to achieve a 93% one-year

321 disease-free rate (Kemp-Symonds 2012). Most recently, a single application of topical ALA-PDT
322 followed by glycolysis inhibition has been shown to successfully treat equine sarcoids up to 5 mm
323 thick with a 93% response rate ($n=27$) after 1 month, compared with a 14% response rate using
324 ALA-PDT only ($n=7$). Treated sarcoids became scabby with desquamation for 2-4 weeks before
325 healing (Golding et al., 2017) (Fig. 3).

326

327 **Prostate cancer**

328 In humans definitive management of early stage prostate cancer with either surgery or
329 ionizing radiation therapy is associated with significant associated morbidities due to the proximity
330 of normal structures such as nerves, bladder and rectum. By contrast, PDT has the potential to
331 selectively treat the prostate while sparing the surrounding normal tissues because light can be
332 delivered to the entire prostate gland using interstitial cylindrically diffusing optical fibres. Prostate
333 cancer is therefore an attractive target for PDT (Agostinis et al., 2011, Ahmed et al, 2012).

334

335 Vascular-targeted PDT using Padeliporfin mediated PDT and a short drug-to-light interval
336 was shown to carry minimal toxicity in a phase I trial, of prostatic carcinoma patients ($n = 24$) with
337 local failure following radiotherapy (Weersink et al., 2005; Trachtenberg et al., 2007). In a follow-
338 up phase II study, patients were treated with increasing light doses. At 6 months all patients where
339 $>60\%$ of the prostate was determined to be avascular by post-PDT magnetic resonance imaging,
340 had negative biopsies, however, 2 patients (of 28) developed urethrorectal fistulae (Trachtenberg et
341 al., 2008). Following refinement of the technique, a recent phase III randomised controlled study of
342 padeliporfin vascular-targeted PDT (versus active surveillance) has shown this to be a safe and
343 effective treatment for low risk localized prostate cancer (Azzouzi et al., 2017).

344

345 The normal canine prostate has served as a useful preclinical model for evaluating responses
346 to PDT in vivo, since its size and general anatomical structure are similar to those of the human

347 prostate (Waters and Bostwick, 1997). An experimental study was conducted assessing padeliporfin
348 PDT on canine prostate pre-treated with ionizing radiation. All dogs presented normal spontaneous
349 urination upon recovery from the procedure, with no signs of incontinence or significant
350 macroscopic hematuria (Huang et al., 2004). Vascular-targeted photodynamic therapy with WST11
351 (TOOKAD Soluble) has been investigated in a dog model of benign prostatic hyperplasia and was
352 uneventful in all except one dog, which experienced urinary retention. Prostatic urethral width
353 increased as early as 6 weeks after treatment, while prostatic volume decreased, reaching 25% by
354 18 to 26 weeks, this response lasted up to 1 year (Chevalier et al., 2013). Unfortunately canine
355 prostatic carcinoma is not usually detected until symptomatic at which point the disease is in late
356 stage, often with metastatic disease, so it is unlikely that PDT would be beneficial in such patients.

357

358 **Bladder cancer**

359 Photodiagnosis is used in management of human bladder cancers (Mowatt et al, 2011), and
360 bladder cancer is also a potential target for PDT. Human bladder cancers are often superficial and
361 multifocal and can be assessed and debulked endoscopically. Furthermore, the geometry of the
362 bladder allows for homogeneous light delivery via diffusing fibres. In general, early response rates
363 (2 to 3 months) to PDT have been about 50% to 80% of patients with longer-term (1 to 2 years)
364 durable responses in 20% to 60% of patients. It should be noted that many of the patients treated in
365 these studies had recurrent disease that developed after standard therapies such as Bacillus
366 Calmette-Guerin (BCG) (Agostinis et al., 2011). Treatment of superficial bladder cancer with PDT
367 is generally well tolerated, with dysuria, hematuria, and skin photosensitivity being the most
368 common acute toxicities. Bladder wall fibrosis/diminished bladder capacity can be a problem in
369 some patients (Prout et al., 1987; Uchibayashi et al., 1995). Studies of locally applied (intravesical)
370 ALA demonstrate that comparable complete response rates of 52-60% at 2-3 years can be achieved
371 for patients with treatment refractory bladder carcinoma *in situ* without the prolonged skin
372 photosensitivity experienced using systemic Photofrin (Berger et al., 2003; Waidelich et al., 2003).

373 Despite these promising results, PDT for bladder cancer remains largely investigational with limited
374 use (Agostinis et al., 2011).

375

376 Canine transitional cell carcinoma (TCC) is most commonly located in the trigone region of
377 the bladder precluding complete surgical resection and palliative medical management is often the
378 only treatment available (Fulkerson and Knapp, 2015). PDT could represent a promising option for
379 dogs with TCC. However, canine TCC is often diagnosed late and is more invasive than human
380 bladder cancers, making comparisons with human studies difficult (Fulkerson and Knapp, 2015). In
381 vitro-studies have shown, that ALA-PDT destroys canine TCC cells (Ridgway and Lucroy, 2003).
382 When studied in vivo, 70% of dogs vomited after oral administration of ALA, but this did not
383 appear to have a negative impact on pharmacokinetics and the active metabolite (PpIX) was shown
384 to accumulate in the bladder mucosa, compared to the muscularis and serosa. Five dogs with TCC
385 of the urinary bladder treated with ALA-PDT and a laser fibre delivery system, showed transient
386 improvement of clinical symptoms with tumour progression free intervals ranging from 4 to 34
387 weeks (Lucroy et al., 2003a,b). The application of PDT for canine TCC clearly warrants further
388 investigation.

389

390 **Brain tumours / glioma**

391 Experimental and clinical studies have demonstrated that PDT can complement current
392 standard therapies (surgical resection, radiation therapy and chemotherapy) in the treatment of brain
393 tumours (Muller and Wilson, 1995, 1996). PDT may be particularly useful as an adjunct to surgery
394 as it can non-invasively target tumour cells infiltrating normal brain. Initial trials provided
395 encouraging results using various formulations of hematoporphyrin derivatives (HPD, Photofrin),
396 ALA as well as mTHPC with light sources including lamps, dye lasers and diode lasers (Agostinis
397 et al., 2011). One of the main indications for ALA in management of glioma is in fluorescence
398 guided surgery (FGS). ALA based FGS has been shown to provide longer survival times than

399 conventional surgery in patients with suspected malignant gliomas ($n=322$), 16.7 versus 11.8
400 months respectively (Stummer et al., 2006).

401

402 In a canine glioma model, dogs were given 0.75 mg/kg Photofrin-II intravenously, followed
403 24 h later by PDT, delivered using a fiberoptic catheter directly to the tumour via a burr hole in the
404 skull (Whelan et al., 1993). This destroyed the tumour without significant brain-stem injury.

405

406 The new classes of PSs, the better understanding of dosimetry and further improvements in
407 technology may significantly change the currently achieved clinical outcome for glioma and other
408 brain tumours both in human and veterinary patients. Pre-clinical data indicating that protracted
409 light delivery may increase the therapeutic index of PDT in the brain combined with newer
410 technologies such as implantable, LED-based light delivery systems could lead to significant
411 improvements in treatment outcomes (Kostron, 2010).

412

413 **Future perspectives**

414 Photodynamic therapy offers great potential due to its selective targeting of tumour cells and
415 minimal normal tissue toxicity. Several innovative strategies have been used to improve PS
416 penetration into tumour cells, including: using an electric current to draw PS deeper into the skin
417 (Lopez et al., 2003), intratumoural PS injection (Hage et al., 2007; Rodríguez-Prieto et al., 2012)
418 and pretreatment with chemical penetration enhancers (Malik et al., 1995; De Rosa et al., 2000;
419 Golding et al., 2017), liposomal formulations and nanoemulsions (Buchholz et al., 2005, 2007).
420 The efficacy of PDT may also be improved by overcoming the antioxidant defences of cancer cells.
421 Antioxidant defences that remove excess ROS are upregulated in many cancers (Tracootham et al.
422 2009), undermining the full potential of PDT. Combination of glycolysis inhibitors with PDT has
423 been shown to deplete cellular antioxidants and significantly improve PDT cytotoxicity against
424 human cancer cells in vitro (Golding et al., 2013) and this combination has proved effective in

425 treatment of equine sarcoids (Golding et al 2017). Other ways in which efficacy of PDT may be
426 improved clinically include: Metronomic PDT (mPDT) to delivery both the drug and light at very
427 low dose rates over an extended period (hours-days) (Lilge et al., 2000), and through use of
428 nanoparticles for PS delivery (Bechet et al., 2008). If the potential for use of PDT in veterinary
429 medicine could be realized this could make a significant contribution to the overall development of
430 the technique.

431

432 **Conclusions**

433 PDT is a safe and effective therapy for many cancers and pre-cancers that can be accessed
434 externally or endoscopically. Small, localised lesions can achieve long-term clearance with
435 negligible scarring or damage to adjacent structures.

436 The science of PDT has seen enormous progress within the past 30 years. For instance: the
437 development of improved photosensitisers, light sources (including endoscopic delivery and
438 daylight PDT), improved understanding of how PDT works, and an expansion of the uses of
439 photosensitisers to allow intraoperative detection of tumour margins. Although PDT has hitherto
440 been used as a monotherapy, the future of the technique undoubtedly lies in combining it with other
441 drugs and approaches as part of a synergistic multimodal treatment.

442 Despite the scientific advances, the clinical practice of PDT is still limited to a small number of
443 individual practitioners or centres of excellence; partly due to a vicious cycle of high photosensitiser
444 costs due to limited demand. With pun intended, veterinary PDT needs to come out of the shadows
445 and into the light. This will only happen if PDT becomes a standard part of the training syllabus and
446 existing PDT practitioners provide internships for the next generation of veterinary surgeons. The
447 referral system for PDT is also in need of improvement.

448

449 **Conflict of Interest**

450 None of the authors of this paper have a financial or personal relationship with other people or
451 organisations that could inappropriately influence or bias the content of the paper.

452

453 **Acknowledgements**

454 Genilson Fernandes de Queiroz received post-doctoral funding from CAPES (The Coordination of
455 Improvement of Higher Education Personnel – Brazil)

456

457 **References**

458 Abels, C., 2004 Targeting of the vascular system of solid tumours by photodynamic therapy (PDT).
459 Photochemical and Photobiological Sciences 3, 765-671.

460

461 Agarwal, M.L., Clay, M.E., Harvey, E.J., Evans, H.H., Antunez, A.R., Oleinick, N.L., 1991
462 Photodynamic therapy induces rapid cell death by apoptosis in L5178Y mouse lymphoma cells.
463 Cancer Research 51, 5993-5996.

464

465 Agostinis, P., Berg, K., Cengel, K.A., Foster, T.H., Girotti, A.W., Gollnick, S.O., Hahn, S.M.,
466 Hamblin, M.R., Juzeniene, A., Kessel, D., et al., 2011 Photodynamic therapy of cancer: An update.
467 CA: a Cancer Journal for Clinicians 61, 250–281.

468

469 Ahmed, H.U., Hindley, R.G., Dickinson, L., Freeman, A., Kirkham, A.P., Sahu, M., Scott, R.,
470 Allen, C., Van der Meulen, J., Emberton, M., 2012 Focal therapy for localized unifocal and
471 multifocal prostate cancer: a prospective development study. Lancet Oncology 13, 622–632.

472

473 Ajioka, R.S., Phillips, J.D., Kushner, J.P., 2006 Biosynthesis of heme in mammals. Biochimica et
474 Biophysica Acta 1763, 723–736.

475

476 Allison, R.R., Downie, G.H., Cuenca, R., Hu, X.H., Childs, C.J., Sibata, C.H., 2004 Photosensitizers
477 in clinical PDT. Photodiagnosis and Photodynamic Therapy 1, 27-42.

478

479 Allison, R.R., Moghissi, K., 2013a Photodynamic Therapy (PDT): PDT Mechanisms. Clinical
480 Endoscopy 46, 24-29.

481

482 Allison, R.R., Moghissi, K., 2013b Oncologic photodynamic therapy: Clinical strategies that
483 modulate mechanisms of action. Photodiagnosis and Photodynamic Therapy 10, 331-341.

484

485 Allison, R.R., 2016 Fluorescence guided resection (FGR): A primer for oncology. Photodiagnosis
486 and Photodynamic Therapy 13, 73-80.

487

488 Azzouzi, A.R., Vincendeau, S., Barret, E., Cicco, A., Kleinclauss, F., van der Poel, H.G., Stief,
489 C.G., Rassweller, J., Salomon, G., Solsona, E., et al., 2017 Padeliporfin vascular-targeted
490 photodynamic therapy versus active surveillance in men with low-risk prostate cancer (CLIN1001
491 PCM301): an open-label, phase 3, randomised controlled trial. Lancet Oncology 18, 181-191.

492

493 Basset-Seguín, N., Ibbotson, S.H., Emtestam, L., Tarstedt, M., Morton, C., Maroti, M., Calzavara-
494 Pinton, P., Varma, S., Roelandts, R., Wolf, P., 2008 Topical methyl aminolaevulinate
495 photodynamic therapy versus cryotherapy for superficial basal cell carcinoma: a 5 year randomized
496 trial. *European Journal of Dermatology* 18, 547–553.

497
498 Bechet, D., Couleaud, P., Frochot, C., Viriot, M-L., Guillemin, F., Barberi-Heyob, M., 2008
499 Nanoparticles as vehicles for delivery of photodynamic therapy agents. *Trends in Biotechnology* 26,
500 612-621.

501
502 Berger, A.P., Steiner, H., Stenzl, A., Akkad, T., Bartsch, G., Holtl, L., 2003 Photodynamic therapy
503 with intravesical instillation of 5-aminolevulinic acid for patients with recurrent superficial bladder
504 cancer: a single-center study. *Urology* 61, 338–341.

505
506 Berroeta, L., Clark, C., Dawe, R.S., Ibbotson, S.H., Fleming, C.J., 2007 A randomized study of
507 minimal curettage followed by topical photodynamic therapy compared with surgical excision for
508 low-risk nodular basal cell carcinoma. *British Journal of Dermatology* 157, 401-403.

509
510 Bexfield, N.H., Stell, A.J., Gear, R.N., Dobson, J.M., 2008 Photodynamic therapy of superficial
511 nasal planum squamous cell carcinomas in cats: 55 cases. *Journal of Veterinary Internal Medicine*
512 22, 1385-1389.

513
514 Biel, M.A., 2006 Advances in photodynamic therapy for the treatment of head and neck cancers.
515 *Lasers in Surgery and Medicine* 38, 349–355.

516
517 Buchholz, J., Kaser-Hotz, B., Khan, T., Rohrer Bley, C., Meizer, K., Schwendener, R.A., Roos, M.,
518 Walt, H., 2005 Optimizing photodynamic therapy: in vivo pharmacokinetics of liposomal meta-
519 (tetrahydroxyphenyl)chlorine in feline squamous cell carcinoma. *Clinical Cancer Research* 11,
520 7538-7544.

521
522 Buchholz, J., Wergin, M., Walt, H., Gräfe, S., Bley, C.R., Kaser-Hotz, B., 2007 Photodynamic
523 therapy of feline cutaneous squamous cell carcinoma using a newly developed liposomal
524 photosensitizer: Preliminary results concerning drug safety and efficacy. *Journal of Veterinary*
525 *Internal Medicine* 21, 770-775.

526
527 Buchholz, J., Walt, H., 2013 Veterinary photodynamic therapy: A review. *Photodiagnosis and*
528 *Photodynamic Therapy* 10, 342-347.

529
530 Burch, S., London, C., Seguin, B., Rodriguez, C., Wilson, B.C., Bisland, S.K., 2009 Treatment of
531 canine osseous tumors with photodynamic therapy: a pilot study. *Clinical Orthopaedics and Related*
532 *Research* 467,1028-1034.

533
534 Cabon, Q., Sayag, D., Texier, I., Navarro, F., Boisgard, R., Virieux-Watrelet, D., Ponce, F.,
535 Carozzo, C., 2016 Evaluation of intraoperative fluorescence imaging-guided surgery in cancer-
536 bearing dogs: A prospective proof-of-concept phase II study in 9 cases. *Translational Research* 170,
537 73-88.

538
539 Castano, A.P., Mroz, P., Hamblin, M.R., 2006 Photodynamic therapy and anti-tumour immunity.
540 *Nature Reviews of Cancer* 6, 535–545.

541
542 Chaves, Y.N., Torezan, L.A., Niwa, A.B., Sanches Junior, J.A., Festa Neto, C., 2012 Pain in
543 photodynamic therapy: Mechanism of action and management strategies. *Anais Brasileiros de*
544 *Dermatologia*. 87, 521-526.

545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596

- Chen, B., Pogue, B.W., Goodwin, I.A., O'Hara, J.A., Wilmot, C.M., Hutchins, J.E., Hoopes, P.J., Hasan, T., 2003 Blood flow dynamics after photodynamic therapy with verteporfin in the RIF-1 tumor. *Radiation Research* 160, 452-459.
- Chevalier, S., Cury, F.L., Scarlata, E., El-Zayat, E., Hamel, L., Rocha, J., Zouanat. F.Z., Moussa, S., Scherz, A., Elhilali, M., et al., 2013 Endoscopic vascular targeted photodynamic therapy with the photosensitizer WST11 for benign prostatic hyperplasia in the preclinical dog model. *Journal of Urology* 190, 1946-1953.
- Collaud, S., Juzeniene, A., Moan, J., Lange, N., 2004 On the selectivity of 5-aminolevulinic acid induced protoporphyrin IX formation. *Current Medicinal Chemistry – Anti-Cancer Agents* 4, 301-316.
- Coors, E.A., von den Driesch, P., 2004 Topical photodynamic therapy for patients with therapy resistant lesions of cutaneous T-cell lymphoma. *Journal of the American Academy of Dermatology* 50, 363-367.
- Cortese, D.A., Kinsey, J.H., 1984 Hematoporphyrin-derivative phototherapy for local treatment of bronchogenic carcinoma. *Chest* 86, 8-13.
- Coutier, S., Bezdetnaya, L., Marchal, S., Melnikova, V., Belitchenko, I., Merlin, J.L., Guillemin, F., 1999 Foscan (mTHPC) photosensitized macrophage activation: enhancement of phagocytosis, nitric oxide release and tumour necrosis factor-alpha-mediated cytolytic activity. *British Journal of Cancer* 81, 37-42.
- Dahle, J., Kaalhus, O., Moan, J., Steen, H.B., 1997 Cooperative effects of photodynamic treatment of cells in microcolonies. *Proceedings of the National Academy of Sciences of the United States of America* 94, 1773-1778.
- Daniell, M.D, Hill, J.S., 1991 A history of photodynamic therapy. *ANZ Journal of Surgery* 61, 340-348.
- De Rosa, F.S., Marchetti, J.M., Thomazini, J.A., Tedesco, A.C., Bentley, M.V., 2000 A vehicle for photodynamic therapy of skin cancer: influence of dimethylsulphoxide on 5-aminolevulinic acid in vitro cutaneous permeation and in vivo protoporphyrin IX accumulation determined by confocal microscopy. *Journal of Controlled Release* 65, 359-366.
- Debefve, E., Mithieux, F., Perentes, J.Y., Wang, Y., Cheng, C., Schaefer, S.C., Ruffieux, C., Ballini, J.P., Gonzalez, M., van den Bergh, H., et al., 2011 Leukocyte-endothelial cell interaction is necessary for photodynamic therapy induced vascular permeabilization. *Lasers in Surgery and Medicine* 43, 696-704.
- Dolmans, D.E., Fukumura, D., Jain, R.K., 2003 Photodynamic therapy for cancer. *Nature Reviews of Cancer* 3, 380–387.
- Dougherty, T.J., Kaufman, J.E., Goldfarb, A., Weishaupt, K.R., Boyle, D., Mittleman, A., 1978 Photoradiation therapy for the treatment of malignant tumors. *Cancer Research* 38, 2628-2635.
- Dougherty, T.J., Gomer, C.J., Henderson, B.W, Jori, G., Kessel, D., Korblik, M., Moan, J., Peng, Q., 1998 Photodynamic therapy. *Journal of the National Cancer Institute* 90, 889-905.

597 Dougherty, T.J., Pandey, R., Nava, H.R., Smith, J.L., Douglass, H.O., Edge, S.B., Bellnier, D.A.,
598 O'Malley, L., Cooper, M., 2000 Preliminary clinical data on a new photodynamic therapy
599 photosensitizer: 2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH) for treatment of
600 obstructive esophageal cancer. In: Optical methods for tumor treatment and detection: Mechanisms
601 and techniques in photodynamic therapy IX. SPIE Proceedings 3909.

602

603 Etienne, J., Dorme, N., Bourg-Heckly, G., Raimbert, P., Flejou J.F., 2004 Photodynamic therapy
604 with green light and m-tetrahydroxyphenyl chlorin for intramucosal adenocarcinoma and high-
605 grade dysplasia in Barrett's esophagus. *Gastrointestinal Endoscopy* 59, 880-889.

606

607 Fabris, C., Soncin, M., Camerin, M., Corsi, F., Cattin, I., Cardin, F., Guidolin, L., Jori, G.,
608 Coppellotti, O., 2014 Photodynamic therapy: a never promising approach for the treatment of
609 spontaneous microbial infections in pet animals. In: *Photodynamic therapy: From theory to*
610 *application*. Springer, New York, NY, USA, pp 255-268.

611

612 Ferreira, I., Rahal, S.C., Rocha, N.S., Gouveia, A.H., Corrêa, T.P., Carvalho, Y.K., Bagnato, V.S.,
613 2009 Hematoporphyrin-based photodynamic therapy for cutaneous squamous cell carcinoma in
614 cats. *Veterinary Dermatology* 20, 174-178.

615

616 Friedberg, J.S., Mick, R., Stevenson, J., Metz, J., Zhu, T., Buyske, J., Sterman, D.H., Pass, H.I.,
617 Glatstein, E., Hahn, S.M., 2003 A phase I study of Foscan-mediated photodynamic therapy and
618 surgery in patients with mesothelioma. *Annals of Thoracic Surgery* 75, 952-959.

619

620 Fulkerson, C.M., Knapp, D.W., 2015 Management of transitional cell carcinoma of the urinary
621 bladder in dogs: A review. *Veterinary Journal* 205, 217-25.

622

623 Furre, I.E., Shahzidi, S., Luksiene, Z., Moller, M.T.N., Borgen, E., Morgan, J., Tkacz-Stachowska,
624 K., Nesland, J.M., Peng, Q., 2005 Targeting PBR by hexaminolevulinate-mediated photodynamic
625 therapy induces apoptosis through translocation of apoptosis-inducing factor in human leukemia
626 cells. *Cancer Research* 65, 11051-11060.

627

628 Gilissen, M.J., van de Merbel-de Wit, L.E., Star, W.M., Koster, J.F., Sluiter, W., 1993 Effect of
629 photodynamic therapy on the endothelium-dependent relaxation of isolated rat aortas. *Cancer*
630 *Research* 53, 2548-2552.

631

632 Giuliano, E.A., MacDonald, I., McCaw, D.L., Dougherty, T.J., Klauss, G., Ota, J., Pearce, J.W.,
633 Johnson, P.J., 2008 Photodynamic therapy for the treatment of periocular squamous cell carcinoma
634 in horses: A pilot study. *Veterinary Ophthalmology* 11, 27-34.

635

636 Giuliano, E.A., Johnson, P.J., Delgado, C., Pearce, J.W., Moore, C.P., 2014 Local photodynamic
637 therapy delays recurrence of equine periocular squamous cell carcinoma compared to cryotherapy.
638 *Veterinary Ophthalmology* 17, 37-45.

639

640 Golding, J.P., Wardhaugh, T., Patrick, L., Turner, M., Phillips, J.B., Bruce, J.I., Kimani, S.G., 2013
641 Targeting tumour energy metabolism potentiates the cytotoxicity of 5-aminolevulinic acid
642 photodynamic therapy. *British Journal of Cancer* 109, 976-982.

643

644 Golding, J.P., Kemp-Symonds, J.G., Dobson, J.M., 2017 Glycolysis inhibition improves
645 photodynamic therapy response rates for equine sarcoids. *Veterinary and Comparative Oncology*
646 DOI: 10.1111/vco.12299

647

648 Gollnick, S.O., Evans, S.S., Baumann, H., Owczarczak, B., Maier, P., Vaughan, L., Wang, W.C.,
649 Unger, E., Henderson, B.W., 2003 Role of cytokines in photodynamic therapy-induced local and
650 systemic inflammation. *British Journal of Cancer* 88, 1772-1779.

651
652 Grant, W.E., Hopper, C., MacRobert, A.J., Speight, P.M., Bown, S.G., 1993 Photodynamic therapy
653 of oral cancer: Photosensitisation with systemic aminolaevulinic acid. *Lancet* 342, 147-148.

654
655 Grapenglosser, S., Ericson, M., Gudmundsson, F., Larko, O., Rosen, A., Wennberg, A.M., 2002
656 Pain caused by photodynamic therapy of skin cancer. *Clinical and Experimental Dermatology* 27,
657 493-497.

658
659 Griffin, G M., Zhu, T., Solonenko, M., Del Piero, F., Kapakin, A., Busch, T.M., Yodh, A., Polin,
660 G., Bauer, T., Fraker, D., et al., 2001 Preclinical evaluation of Motexafin Lutetium-mediated.
661 intraperitoneal photodynamic therapy in a canine model. *Clinical Cancer Research* 7, 374–381.

662
663 Gustafson, S.B., Engelking, K., Jacques, S.L., Bildfell, R., 2004 Clinical results of photodynamic
664 therapy for the treatment of sarcoid tumors in equids. In: *Lasers in surgery: Advanced*
665 *characterization, therapeutics, and systems XIV*. SPIE Proceedings 5312.

666
667 Hage, R., Mancilha, G., Zângaro, R.A., Munin, E., Plapler, H., 2007 Photodynamic Therapy (PDT)
668 using intratumoral injection of the 5- aminolevulinic acid (5-ALA) for the treatment of eye cancer
669 in cattle. In: *Optical methods for tumor treatment and detection: Mechanisms and techniques in*
670 *photodynamic therapy XVI*. SPIE Proceedings 6427.

671
672 Hahn, K.A., Panjehpour, M., Legendre, A.M., 1998 Photodynamic therapy response in cats with
673 cutaneous squamous cell carcinoma as a function of fluence. *Veterinary Dermatology* 9, 3-7.

674
675 Halldin, C.B., Gillstedt, M., Paoli, J. Wennberg, A-M., Gonzalez, H., 2011 Predictors of pain
676 associated with photodynamic therapy: a retrospective study of 658 treatments. *Acta Dermato*
677 *Venereologica* 91, 545-551.

678
679 Hefti, M., Mehdorn, H.M., Albert, I., Dorner, L., 2010 Fluorescence-guided surgery for malignant
680 glioma: a review on aminolevulinic acid induced protoporphyrin IX photodynamic diagnostic in
681 brain tumors. *Current Medical Imaging Reviews* 6, 254-258.

682
683 Henderson, B.W., Fingar, V.H., 1987 Relationship of tumor hypoxia and response to photodynamic
684 treatment in an experimental mouse tumor. *Cancer Research* 47, 3110- 3114.

685
686 Henderson, B.W., Busch, T.M., Snyder, J.W., 2006 Fluence rate as a modulator of PDT
687 mechanisms. *Lasers in Surgery and Medicine* 38, 489-493.

688
689 Huang, Z., Chen, Q., Trncic, N., LaRue, S.M., Brun, P.H., Wilson, B.C., Shapiro, H., Hetzel, F.W.,
690 2004 Effects of Pd-bacteriopheophorbide (TOOKAD)-mediated photodynamic therapy on canine
691 prostate pretreated with ionizing radiation. *Radiation Research* 161, 723–731.

692
693 Huang, Z., Chen, Q., Luck, D., Beckers, J., Wilson, B.C., Trncic, N., LaRue, S.M., Blanc, D.,
694 Hetzel, F.W., 2005 Studies of a vascular-acting photosensitizer, Pd-bacteriopheophorbide (Tookad),
695 in normal canine prostate and spontaneous canine prostate cancer. *Lasers in Surgery and Medicine*
696 36, 390–397.

697
698 Igney, F.H., Krammer, P.H., 2002 Death and anti-death: tumour resistance to apoptosis. *Nature*
699 *Reviews of Cancer* 2, 277-288.

700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751

Ishigaki, K., Nariai, K., Izumi, M., Teshima, K., Seki, M., Edamura, K., Takahashi, T., Asano, K., 2017 Endoscopic photodynamic therapy using talaporfin sodium for recurrent intranasal carcinomas after radiotherapy in three dogs. *Journal of Small Animal Practice* DOI: 10.1111/jsap.12667.

Juzeniene, A., Peng, Q., Moan, J., 2007 Milestones in the development of photodynamic therapy and fluorescence diagnosis. *Photochemical and Photobiological Sciences* 6, 1234-1245.

Kausch, I., Sommerauer, M., Montorsi, F., Stenzi, A., Jacqmin, D., Jichlinski, P., Jocham, D., Ziegler, A., Vonthein, R., 2010 Photodynamic diagnosis in non-muscle invasive bladder cancer: A systematic review and cumulative analysis of prospective studies. *European Urology* 57, 595-606.

Kemmner, W., Wan, K., Ruttinger, S., Ebert, B., Macdonald, R., Klamm, U., Moesta, K.T., 2008 Silencing of human ferrochelatase causes abundant protoporphyrin-IX accumulation in colon cancer. *FASEB Journal* 22, 500–509.

Kemp-Symonds, J.G., 2012 Carbon dioxide laser surgery with adjunctive photodynamic therapy as a treatment for equine peri-ocular sarcoid: outcome and complications in 21 patients. *Equine Veterinary Journal* 44, 2.

Kharkwal, G.B., Sharma, S.K., Huang, Y-Y., Dai, T., Hamblin, M.R., 2011 Photodynamic therapy for infections: Clinical applications. *Lasers in Surgery and Medicine* 43, 755-767.

Kim, S.K., Shin, J., Kim, Y.C., 2012 Nodular basal cell carcinoma successfully treated with intralesional 5-aminolevulinic acid injection-photodynamic therapy. *Annals of Dermatology* 24, 486-487.

Kleinovink, J.W., van Driel, P.B., Snoeks, T.J.A., Prokopi, N., Fransen, M.F., Cruz, L.J., Mezzanotte, L., Chan, A., Lowick, C.W.G.M., Ossendorp, F., 2015 Combination of photodynamic therapy and specific immunotherapy efficiently eradicates established tumors. *Clinical Cancer Research* 22, 1459-1468.

Knapp, D.W., Adams, L.G., DeGrand, A.M., 2007 Sentinel lymph node mapping of invasive urinary bladder cancer in animal models using invisible light. *European Urology* 52, 1700-1709.

Kostron, H., 2010 Photodynamic diagnosis and therapy and the brain. *Methods in Molecular Biology* 635, 261–280.

L'Eplattenier, H.F., Klem, B., Teske, E., van Sluijs, F.J., van Nimwegen, S.A., Kirpensteijn, J., 2008 Preliminary results of intraoperative photodynamic therapy with 5-aminolevulinic acid in dogs with prostate carcinoma. *The Veterinary Journal* 178, 202–207.

Lilge, L., Portnoy, M., Wilson, B.C., 2000 Apoptosis induced in vivo by photodynamic therapy in normal brain and intracranial tumour tissue. *British Journal of Cancer* 83, 1110–1117.

Lopez, R.F., Bentley, M.V., Begona Delgado-Charro, M., Guy, R.H., 2003 Optimization of aminolevulinic acid delivery by iontophoresis. *Journal of Controlled Release* 14, 65-70.

Lovat, L.B., Jamieson, N.F., Novelli, M.R., Mosse, C.A., Selvasekar, C., Mackenzie, G.D., Thorpe, S.M. and Bown, S.G., 2005 Photodynamic therapy with m-tetrahydroxyphenyl chlorin for high-grade dysplasia and early cancer in Barrett's columnar lined esophagus. *Gastrointestinal Endoscopy* 62, 617–623.

752

753 Lucroy, M.D., Edwards, B., Peavy, G.M., Krasieva, T.B., Griffey, S.M., Stiles, J.B., Madewell,
754 B.R., 1999 Preclinical study in cats of the pro-photosensitizer 5-aminolevulinic acid. *American*
755 *Journal of Veterinary Research* 60, 1364-1370.

756

757 Lucroy, M.D., Bowles, M.H., Higbee, R.G., Blaik, M.A., Ritchey, J.W., Ridgway, T.D., 2003a
758 Photodynamic therapy for prostatic carcinoma in a dog. *Journal of Veterinary Internal Medicine* 17,
759 235- 237.

760

761 Lucroy, M.D., Ridgway, T.D., Peavy, G.M., 2003b Preclinical evaluation of 5-aminolevulinic acid-
762 based photodynamic therapy for canine transitional cell carcinoma. *Veterinary and Comparative*
763 *Oncology* 1, 76-85.

764

765 Luksiene, Z., 2003 Photodynamic therapy: Mechanism of action and ways to improve the efficiency
766 of treatment. *Medicina*. 39, 1137-1150.

767

768 Luo, S., Zhang, E., Su, Y., Cheng, T., Shi, C., 2011 A review of NIR dyes in cancer targeting and
769 imaging. *Biomaterials* 32, 7127-7139.

770

771 Madsen, S. 2016 Photochemical internalisation for solid malignancies. *Lancet Oncology*. 17, 1173-
772 1174.

773

774 Magne, M.L., Rodriguez, C.O., Autry, S.A., Edwards, B.F., Theon, A.P., Madewell, B.R., 1997
775 Photodynamic therapy of facial squamous cell carcinoma in cats using a new photosensitizer. *Lasers*
776 *in Surgery and Medicine* 20, 202-209.

777

778 Maier, A., Anegg, U., Tomaselli, F., Rehak, P., Sankin, O., Fell, B., Renner, H., Pinter, H., Smolie-
779 Juttner, F.M., Friehs, G.B., 2000 Does hyperbaric oxygen enhance the effect of photodynamic therapy
780 in patients with advanced esophageal carcinoma? A clinical pilot study. *Endoscopy* 32, 42-48.

781

782 Malik, Z., Kostenich, G., Roitman, L., Ehrenberg, B., Orenstein, A., 1995 Topical application of 5-
783 aminolevulinic acid, DMSO and EDTA: Protoporphyrin IX accumulation in skin and tumours of
784 mice. *Journal of Photochemistry and Photobiology B: Biology* 28, 213-218.

785

786 Mang, T.S., 2004. Lasers and light sources for PDT: Past, present and future. *Photodiagnosis and*
787 *Photodynamic Therapy* 1, 43-48.

788

789 Mang, T.S., 2009 Light sources and delivery devices for photodynamic therapy. *Photodiagnosis and*
790 *Photodynamic Therapy* 6, 147.

791

792 Marcus, S.L., McIntyre, W.R., 2002 Photodynamic therapy systems and applications. *Expert*
793 *Opinion on Emerging Drugs* 7, 321-334.

794

795 McCaw, D.L., Pope, E.R., Payne, J.T., West, M.K., Tompson, R.V., Tate, D., 2000 Treatment of
796 canine oral squamous cell carcinomas with photodynamic therapy. *British Journal of Cancer* 82,
797 1297-1299.

798

799 McMahon, K.S., Wieman, T.J., Moore, P.H., Fingar, V.H., 1994 Effects of photodynamic therapy
800 using mono-L-aspartyl chlorin e6 on vessel constriction, vessel leakage, and tumor response.
801 *Cancer Research* 54, 5374-5379.

802

803 Misaki, T., Hisazumi, H., Miyoshi, N., 1983 Photoradiation therapy of bladder tumors. *Journal of*
804 *Urology* 130, 685-687.

805

806 Moore, C.M., Nathan, T.R., Lees, W.R., Mosse, C.A., Freeman, A., Emberton, M., Bown, S.G,
807 2006 Photodynamic therapy using meso tetra hydroxy phenyl chlorin mTHPC in early prostate
808 cancer. *Lasers in Surgery and Medicine* 38, 356–363.

809

810 Morton, C.A., Whitehurst, C., Moseley, H., McColl, J.H., Moore, J.V., Mackie, R.M., 1996
811 Comparison of photodynamic therapy with cryotherapy in the treatment of Bowen’s disease. *British*
812 *Journal of Dermatology* 135, 766–771.

813

814 Morton, C.A., Brown, S.B., Collins, S., Ibbotson, S., Jenkinson, H., Kurwa, H., Langmack, K.,
815 McKenna, K., Moseley, H., Pearse, A.D., et al., 2002 Guidelines for topical photodynamic therapy:
816 Report of a workshop of the British Photodermatology Group. *British Journal of Dermatology* 146,
817 552–67.

818

819 Morton, C.A., McKenna, K.E., Rhodes, L.E., 2008 Guidelines for topical photodynamic therapy:
820 update. *British Journal of Dermatology*. 159, 1245–1266.

821

822 Morton, C.A., Szeimies, R.M., Sidoroff, A., Braathen, L.R., 2013 European guidelines for topical
823 photodynamic therapy part 1: Treatment delivery and current indications – actinic keratosis,
824 Bowen’s disease, basal cell carcinoma. *Journal of the European Academy of Dermatology and*
825 *Venerology* 27, 536-544.

826

827 Mowatt, G., N’Dow, J., Vale, L., Nabl, G., Boachie, C., Cook, J.A., Fraser, C., Griffiths, T.R.,
828 Aberdeen Technology Assessment Review (TAR) Group, 2011 Photodynamic diagnosis of bladder
829 cancer compared with white light cystoscopy: Systematic review and meta-analysis. *International*
830 *Journal of Technology Assessment in Health Care* 27, 3-10.

831

832 Muller, P.J., Wilson, B.C., 1995 Photodynamic therapy for recurrent supratentorial gliomas.
833 *Seminars in Surgical Oncology* 11, 346–354.

834

835 Muller, P.J., Wilson, B.C., 1996 Photodynamic therapy for malignant newly diagnosed
836 supratentorial gliomas. *Journal of Clinical Laser Medicine and Surgery* 14, 263–270.

837

838 Muller, P.J., Wilson, B.C., 2006 Photodynamic therapy of brain tumors--a work in progress. *Lasers*
839 *in Surgery and Medicine* 38, 384–389.

840

841 Nathan, T.R., Whitelaw, D.E., Chang, S.C., Lees, W.R., Ripley, P.M., Payne, H., Jones, L.,
842 Parkinson, M.C., Emberton, M., Gillams, A.R., et al., 2002 Photodynamic therapy for prostate
843 cancer recurrence after radiotherapy: A phase I study. *Journal of Urology* 168, 1427–1432.

844

845 Nguyen, Q.T., Tsien, R.Y., 2013 Fluorescence-guided surgery with live molecular navigation – a
846 new cutting edge. *Nature Reviews of Cancer*. 13, 653-662.

847

848 Nokes, B., Apel, M., Jones, C., Brown, G., Lang, J.E., 2013 Aminolevulinic acid (ALA):
849 Photodynamic detection and potential therapeutic applications. *Journal of Surgical Research* 181,
850 262-271.

851

852 Nomura, T., Mimata, H., 2012 Focal therapy in the management of prostate cancer: An emerging
853 approach for localized prostate cancer. *Advances in Urology* 2012, 1-8.

854

855 Novak, B, Peteja, M., Brueck, T., Luebbert, H., 2016 Potency of different red light sources in
856 photodynamic induction of cell death in a squamous cell carcinoma cell line. *Photodiagnosis and*
857 *Photodynamic Therapy* 14, 128-130.

858

859 Oleinick, N., Morris, R.L., Belichenko, I., 2002 The role of apoptosis in response to photodynamic
860 therapy: What, where, why, and how. *Photochemical and Photobiological Sciences* 1, 1-21.

861

862 Osaki, T., Yokoe, I., Ogura, S., Takahashi, K., Murakami, K., Inoue, K., Ishizuka, M., Tanaka, T.,
863 Li, L., Suglyama, A., et al., 2016 Photodynamic detection of canine mammary gland tumours after
864 oral administration of 5-aminolevulinic acid. *Veterinary and Comparative Oncology*. DOI:
865 10.1111/vco.12213.

866

867 Panjehpour, M., Overholt, B.F., DeNovo, R.C., Petersen, M.G., Sneed, R.E., 1993 Comparative
868 study between pulsed and continuous wave lasers for Photofrin photodynamic therapy. *Lasers in*
869 *Surgery and Medicine* 13, 296-304.

870

871 Panjehpour, M., DeNovo, R.C., Petersen, M.G., Overholt, B.F., Bower, R., Rubinchik, V., Kelly,
872 B., 2002 Photodynamic therapy using Verteporfin (benzoporphyrin derivative monoacid ring A,
873 BPD-MA) and 630 nm laser light in canine esophagus. *Lasers in Surgery and Medicine* 30, 26–30.

874

875 Patel, H., Mick, R., Finlay, J., Zhu, T.C., Rickter, E., Cengel, K.A., Malkowicz, S.B., Hahn, S.M.,
876 Busch, T.M., 2008 Motexafin Lutetium-photodynamic therapy of prostate cancer: Short- and long-
877 term effects on prostate-specific antigen. *Clinical Cancer Research* 4869, 4869-4876.

878

879 Pereira, S.P., Ayaru, L., Rogowska, A., Moose, A., Hatfield, A.R., Bown, S.G., 2007 Photodynamic
880 therapy of malignant biliary strictures using mesotetrahydroxyphenylchlorin. *European Journal of*
881 *Gastroenterology and Hepatology* 19, 479–485.

882

883 Policard, A., 1924 Etude sur les aspects offerts par des tumeurs experimentales examinees a la
884 lumiere de Wood. *Comptes Rendues Hebdomadaires des Seances Memories la Societe Biologie ses*
885 *Filiales* 91, 1423–1424.

886

887 Prout, G.R. Jr., Lin, C.W., Benson, R. Jr., Nseyo, U.O., Daly, J.J., Griffin, P.P, Kinsey, J., Tian,
888 M.E., Lao, Y.H., Mian, Y.Z., et al., 1987 Photodynamic therapy with hematoporphyrin derivative in
889 the treatment of superficial transitional-cell carcinoma of the bladder. *New England Journal of*
890 *Medicine* 317, 1251–1255.

891

892 Qiang, Y-G., Yow, C.M.N., Huang, Z., 2008 Combination of photodynamic therapy and
893 immunomodulation – current status and future trends. *Medicinal Research Reviews* 28, 632-644.

894

895 Rauschnig, W., Tan, I.B., Dolivet, G., 2004 Photodynamic therapy (PDT) with mTHPC in the
896 palliation of advanced head and neck cancer in patients who have failed prior therapies and are
897 unsuitable for radiotherapy, surgery or systemic chemotherapy. *Journal of Clinical Oncology* 22,
898 5596.

899

900 Reeds, K.B., Ridgway, T.D., Higbee, R.G., Lucroy, M.D., 2004 Non-coherent light for
901 photodynamic therapy of superficial tumours in animals. *Veterinary and Comparative Oncology* 2,
902 157-163.

903

904 Rhodes, L.E., de Rie, M.A., Leifsdottir, R., Yu, R.C., Bachmann, I., Goulden, V., Wong, G.A.E.,
905 Richard, M-A., Anstey, A., Wolf, P., 2007 Five-year follow-up of a randomized, prospective trial of

906 topical methyl aminolevulinate photodynamic therapy vs surgery for nodular basal cell carcinoma.
 907 Archives of Dermatology 143, 1131–1136.
 908

909 Ridgway, T.D., Lucroy, M.D., 2003 Phototoxic effects of 635-nm light on canine transitional cell
 910 carcinoma cells incubated with 5-aminolevulinic acid. American Journal of Veterinary Research 64,
 911 131-136.
 912

913 Roberts, W.G., Klein, M.K., Loomis, M., Weldy, S., Berns, M.W., 1991 Photodynamic therapy of
 914 spontaneous cancers in felines, canines, and snakes with chloro-aluminium sulfonated
 915 phthalocyanine. Journal of the National Cancer Institute 83, 18-23.
 916

917 Rodríguez-Prieto, M.Á., González-Sixto, B., Pérez-Bustillo, A., Alonso-Alonso, T., Ortega-Valín,
 918 L., Martínez-Valderrábano, V., González-Morán, A., Doval, I.G., 2012 Photodynamic therapy with
 919 intralesional photosensitizer and laser beam application: An alternative treatment for nodular basal
 920 cell carcinoma. Journal of the American Academy of Dermatology 67, 134-136.
 921

922 Ross, H.M., Smelstoys, J.A., Davis, G.J., Kapatkin, A.S., Del Piero, F., Reineke, E., Wang, H., Zhu,
 923 T.C., Busch, T.M., Yodh, A.G., et al., 2006 Photodynamic therapy with Motexafin Lutetium for rectal
 924 cancer: A preclinical model in the dog. Journal of Surgical Research 135, 323–330.
 925

926 Rud, E., Gederaas, O., Hogset, A., Berg, K., 2000 5-aminolevulinic acid, but not 5-aminolevulinic
 927 acid esters, is transported into adenocarcinoma cells by system BETA transporters. Photochemistry
 928 and Photobiology 71, 640–647.
 929

930 Salim, A., Leman, J.A., McColl, J.H., Chapman, R., Morton, C.A., 2003 Randomized comparison
 931 of photodynamic therapy with topical 5-fluorouracil in Bowen's disease. British Journal of
 932 Dermatology. 148, 539–543.
 933

934 Scott, L.J., Goa, K.L., 2000 Verteporfin. Drugs and Aging 16, 139-148.
 935

936 See, J-A., Shumack, S., Murrell, D.F., Rubel, D.M, Fernandez-Penas, P., Salmon, R., Hewitt, D.,
 937 Foley, P., Spelman, L., 2016 Consensus recommendations on the use of daylight photodynamic
 938 therapy with methyl aminolevulinate cream for actinic keratosis in Australia. Australasian Journal
 939 of Dermatology 57, 167-174.
 940

941 Sellera, F.P., Gargano, R.G., Libera, A.M., Benesi, F.J., Azedo, M.R., de Sa, L.R., Ribeiro, M.S., da
 942 Silva Baptista, M., Pogliani, F.C., 2016 Antimicrobial photodynamic therapy for caseous
 943 lymphadenitis abscesses in sheep: Report of ten cases. Photodiagnosis and Photodynamic Therapy.
 944 13, 120-122.
 945

946 Selbo, P.K., Bostad, M., Olsen, C.E., Edwards, V.T., Hogset, A., Weyergang, A., Berg, K., 2015
 947 Photochemical internalisation, a minimally invasive strategy for light-controlled endosomal escape
 948 of cancer stem cell-targeting therapeutics. Photochemical and Photobiological Sciences 14, 1433-
 949 1450.
 950

951 Sharma, S.K., Mroz, P., Dai, T., Huang, Y-Y., St Denis, T.G., Hamblin, M.R., 2012 Photodynamic
 952 therapy for cancer and for infections: What is the difference? Israel Journal of Chemistry 52, 691-
 953 705.
 954

955 Sibata, C.H., Colussi, V.C., Oleinick, N.L., Kinsella, T.J., 2001 Photodynamic therapy in oncology.
 956 Expert Opinion on Pharmacotherapy 2, 917-927.
 957

- 958 Solban, N., Rizvi, I., Hasan, T., 2006 Targeted photodynamic therapy. *Lasers in Surgery and*
959 *Medicine* 38, 522–531.
- 960
- 961 Star, W.M., Marijnissen, H.P., van den Berg-Blok, A.E., Versteeg, J.A., Franken, K.A., Reinhold,
962 H.S., 1986 Destruction of rat mammary tumor and normal tissue microcirculation by
963 hematoporphyrin derivative photoradiation observed in vivo in sandwich observation chambers.
964 *Cancer Research* 46, 2532-2540.
- 965
- 966 Star, W.M., Wilson, B.C., Patterson, M.S., 1992 Light delivery and optical dosimetry in
967 photodynamic therapy of solid tumors. In: *Photodynamic therapy: Basic principles and clinical*
968 *applications*. CRC Press, Boca Raton, FL, USA. pp. 335-368.
- 969
- 970 Stell, A.J., Dobson, J.M., Langmack, K., 2001 Photodynamic therapy of feline superficial squamous
971 cell carcinoma using topical 5-aminolaevulinic acid. *Journal of Small Animal Practice* 42, 164-169.
- 972
- 973 Sterenberg, H.J.C.M., van Veen, R.L.P., Aans, J-B., Amelink, A., Robinson, D.J., 2014 Light
974 Dosimetry for Photodynamic Therapy: Basic Concepts. In: *Handbook of Photomedicine*, Taylor
975 and Francis, Oxford, UK, pp. 281-291.
- 976
- 977 Stummer, W., Pichlmeier, U., Meinel, T., Wiestler, O.D., Zanella, F., Reulen, H.J., 2006
978 Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: A
979 randomised controlled multicentre phase III trial. *Lancet Oncology* 7, 392–401.
- 980
- 981 Suedmeyer, W.K., McCaw, D., Turnquist, S., 2001 Attempted photodynamic therapy of squamous
982 cell carcinoma in the casque of a Great Hornbill (*Buceros bicornis*). *Journal of Avian Medicine and*
983 *Surgery* 15, 44-49.
- 984
- 985 Tanabe, S., Yamaguchi, M., Iijima, M., Nakajima, S., Sakata, I., Miyaki, S., Takemura, T., Furuoka,
986 H., Kobayashi, Y., Matsui, T., et al., 2004 Fluorescence detection of a new photosensitizer, PAD-
987 S31, in tumour tissues and its use as a photodynamic treatment for skin tumours in dogs and a cat:
988 A preliminary report. *The Veterinary Journal* 167, 286-293.
- 989
- 990 Tochner, Z., Mitchell, J.B., Hoekstra, H.J., Smith, P., DeLuca, A.M., Barnes, M., Harrington, F.,
991 Manyak, M., Russo, D., Russo, A., et al., 1991 Photodynamic therapy of the canine peritoneum:
992 Normal tissue response to intraperitoneal and intravenous photofrin followed by 630 nm light.
993 *Lasers in Surgery and Medicine* 11, 158-64.
- 994
- 995 Tracootham, D., Alexandre, J., Huang, P., 2009 Targeting cancer cells by ROS-mediated
996 mechanisms: A radical therapeutic approach? *Nature Reviews Drug Discovery* 8, 579-591.
- 997
- 998 Trachtenberg, J., Bogaards, A., Weersink, R.A., Haider, M.A., Evans, A., McCluskey, S.A., Scherz,
999 A., Gertner, M.R., Yue, C., Appu, S., et al., 2007 Vascular targeted photodynamic therapy with
1000 palladium-bacteriopheophorbide photosensitizer for recurrent prostate cancer following definitive
1001 radiation therapy: Assessment of safety and treatment response. *Journal of Urology* 178, 1974-
1002 1979.
- 1003
- 1004 Trachtenberg, J., Weersink, R.A., Davidson, S.R., Haider, M.A., Bogaards, A., Gertner, M.R.,
1005 Evans, A., Scherz, A., Savard, J., Chin, J.L., et al., 2008 Vascular-targeted photodynamic therapy
1006 (padoporfin, WST09) for recurrent prostate cancer after failure of external beam radiotherapy: A
1007 study of escalating light doses. *BJU International* 102, 556–562.
- 1008

1009 Triesscheijn, M., Ruevekamp, M., Antonini, N., Neering, H., Stewart, F.A. Baas, P., 2006
 1010 Optimizing meso-tetra-hydroxyphenyl-chlorin-mediated photodynamic therapy for basal cell
 1011 carcinoma, *Photochemistry and Photobiology* 82, 1686–1690.

1012
 1013 Uchibayashi, T., Koshida, K., Kunimi, K., Hisazumi, H., 1995 Whole bladder wall photodynamic
 1014 therapy for refractory carcinoma in situ of the bladder. *British Journal of Cancer* 71, 625–628.

1015
 1016 Usuda, J., Tsutsui, H., Honda, H., Ichinose, S., Ishizumi, T., Hirata, T., Inoue, T., Ohtani, K.,
 1017 Maehara, S., Imai, K., et al., 2007 Photodynamic therapy for lung cancers based on novel
 1018 photodynamic diagnosis using talaporfin sodium (NPe6) and autofluorescence bronchoscopy. *Lung*
 1019 *Cancer* 58, 317-323.

1020
 1021 Wachowska, M., Muchowicz, A., Firczuk, M., Gabrysiak, M., Winiarska, M., Wańczyk, M.,
 1022 Bojarczuk, K., Golab, J., 2011 Aminolevulinic acid (ALA) as a prodrug in photodynamic therapy of
 1023 cancer. *Molecules* 16, 4140-4164.

1024
 1025 Waidelich, R., Beyer, W., Knuchel, R., Stepp, H., Baumgartner, R., Schroder, J., Hofstetter, A.,
 1026 Kriegmair, M., 2003 Whole bladder photodynamic therapy with 5-aminolevulinic acid using a
 1027 white light source. *Urology* 61, 332–337.

1028
 1029 Wan, M.T., Lin, J.Y., 2014 Current evidence and applications of photodynamic therapy in
 1030 dermatology. *Clinical, Cosmetic and Investigational Dermatology* 7, 145-163.

1031
 1032 Wardlaw, J.L., Sullivan, T.J., Lux, C.N., Austin, F.W., 2012 Photodynamic therapy against
 1033 common bacteria causing wound and skin infections. *Veterinary Journal* 192, 374-377.

1034
 1035 Waters, D.J., Bostwick, D.G., 1997 The canine prostate is a spontaneous model of intraepithelial
 1036 neoplasia and prostate cancer progression. *Anticancer Research* 17, 1467–1470.

1037
 1038 Weersink, R.A., Forbes, J., Bisland, S., Trachtenberg, J., Eihilali, M., Brun, P.H., Wilson, B.C.,
 1039 2005 Assessment of cutaneous photosensitivity of TOOKAD (WST09) in preclinical animal models
 1040 and in patients. *Photochemistry and Photobiology* 81, 106–113.

1041
 1042 Wenk C.H., Ponce, F., Guillermet, S., Tenaud, C., Boturyn, D., Dumy, P., Watrelot-Virieux, D.,
 1043 Carozzo, C., Josserand, V, Coll, J.L., 2013 Near-infrared optical guided surgery of highly
 1044 infiltrative fibrosarcomas in cats using an anti- $\alpha v \beta 3$ integrin molecular probe. *Cancer Letters* 334,
 1045 188-195.

1046
 1047 Whelan, H.T., Schmidt, M.H., Segura, A.D., McAuliffe, T.L., Bajic, D.M., Murray, K.J., Moulder,
 1048 J.E., Strother, D.R., Thomas, J.P., Meyer, G.A., 1993 The role of photodynamic therapy in posterior
 1049 fossa brain tumors: A preclinical study in a canine glioma model. *Journal of Neurosurgery* 79, 562-
 1050 568.

1051
 1052 Wile, A.G., Novotny, J., Passy, V., Berns, M.W., 1984 Photoradiation therapy of head and neck
 1053 cancer. In: *Porphyrin localization and treatment of tumors*. Alan R.Liss, New York, NY, USA. pp.
 1054 681-691

1055
 1056 Wilson, B.C., Patterson, M.S., 1990 The determination of light fluence distributions in
 1057 photodynamic therapy. In: *Photodynamic therapy of neoplastic disease, volume 1*. CRC Press, Boca
 1058 Raton, FL, USA. pp. 129–146.

1059

1060 Wolf, P., Rieger, E., Kerl, H., 1993 An alternative treatment modality for solar keratoses,
1061 superficial squamous cell carcinomas and basal cell carcinomas? Journal of the American Academy
1062 of Dermatology 28, 17–21.
1063
1064 Yano, T., Kasai, H., Horimatsu, T., Yoshimura, K., Teramukai, S., Morita, S., Tada, H., Yamamoto,
1065 Y., Kataoka, H., Kakushima, N., et al., 2017 A multicentre phase II study of salvage photodynamic
1066 therapy using talaporfin sodium (ME2906) and a diode laser (PNL6405EPG) for local failure after
1067 chemoradiotherapy or radiotherapy for esophageal cancer. Oncotarget 8, 22135-22144.
1068
1069 Yoon, I., Li, J.Z., Shim, Y.K., 2013 Advance in photosensitizers and light delivery for
1070 photodynamic therapy. Clinical Endoscopy 46, 7-23.
1071
1072 Zeitouni, N.C., Shieh, S., Oseroff, A.R., 2001 Laser and photodynamic therapy in the management
1073 of cutaneous malignancies. Clinical Dermatology 19, 328–338.
1074
1075 Zhang, X., Zhang, R., Zheng, Y., Shen, J., Xiao, D., Li, J., Shi, X., Huang, L., Tang, H., Liu, J., et
1076 al., 2013 Expression of gamma-aminobutyric acid receptors on neoplastic growth and prediction of
1077 prognosis in non-small cell lung cancer. Journal of Translational Medicine 11, 102.
1078
1079 Zhu, T.C., Finlay, J.C., 2008 The role of photodynamic therapy (PDT) physics. Medical Physics 35,
1080 3127-3136.

1081
1082

Table 1.
Summary of characteristics and application of selected second generation photosensitizers.

Agent (synonyms)/ manufacturer	Activation wavelength (nm)	Advantages	Reported tumour applications (human unless stated)	References
Foscan (m-tetrahydroxophenyl chlorine (mTHPC), temoporphin)/ Biolitec Pharma.	525 - 660	-Short duration of skin photosensitivity (15 days) -High quantum yield for singlet oxygen -Depth of tumour necrosis (10 mm)	Pleural mesothelioma Head and neck cancers Oesophagus Prostate Pancreas Skin tumours Skin tumours (cats)	Friedberg et al., 2003. Rauschnig et al., 2004; Biel et al., 2006. Lovat et al., 2005; Etienne et al. 2004. Nathan et al., 2002; Moore et al., 2006. Pereira et al., 2007. Triesscheijn et al., 2006. Buchholz et al., 2007.
Photochlor (2-(1- hexyloxyethyl)-2-devinyl pyropheophorbide (HPPH))/ AdooQ Bioscience.	665 - 680	Extremely hydrophobic, increasing penetration into tissue	Obstructive oesophageal cancer oral squamous cell carcinomas (dogs) facial squamous cell carcinoma (cats) squamous cell carcinoma (horses)	Dougherty et al., 2000. McCaw et al., 2000. Magne et al., 1997. Giuliano et al., 2008.
TOOKAD (WST-09, padoporfin, palladium bacteriopheophorbide)/ Steba Biotech.	760	New generation photosensitiser with greater stability and short half-life	Prostate (dogs)	Nomura and Mimata, 2012. Huang et al., 2005.
Padeliporfin (TOOKAD Soluble, WST-11, palladium bacteriopheophorbide	760	Vascular-targeted PDT	Prostate Prostate (dogs)	Azzouzi et al., 2017. Chevalier et al., 2013.

monolysotaurine)/ Steba Biotech.				
Lu-TeX (Motexafin lutetium, lutetium texaphyrin/ Pharmacyclics Inc.	730	Water soluble. Selectively retained in tumour. Only 24 – 48 h skin photosensitivity	Prostate Rectal (dogs)	Patel et al., 2008. Ross et al., 2006.
Talaporphin sodium (aspartyl chlorin, Laserphyrin, Aptocine)/ Meiji Seika Pharma.	664 - 667	Retained in tumour for 50 h	Lung. Esophageal. Intranasal (dogs)	Usuda et al., 2007. Yano et al., 2017. Ishigaki et al., 2017.
ALA (5-aminolevulinic acid)/ various. Methyl-ALA (MAL, Metvix)/ Galderma. Hexyl-ALA (HAL Hexvix)/ Ipsen.	Pro-drugs. Each metabolized to protoporphyrin IX (414, 635)	Short loading 3 h; short skin photosensitivity 12 h Epithelial penetration improves in sequence ALA>MAL>HAL	ALA: at least 11 different human tumours. Equine sarcoids MAL: basal cell carcinoma. Equine sarcoids. HAL: prostate photodynamic detection. Prostate (dogs)	Nokes et al., 2013. Golding et al., 2017. Morton et al., 2008, 2013. Kemp-Symonds, 2012, Golding et al., 2017. Furre et al., 2005. L'Eplattenier et al., 2008.
Verteporfin (Visudyne)/ Novartis	689 - 693	Binds low density lipoprotein receptors on abnormal blood vessels and tumours	Wet macular degeneration Esophagus (dogs) Squamous cell carcinoma (horses)	Scott and Goa, 2000. Panjehpour et al., 2002. Giuliano et al., 2014.

1084 **Table 2.**
1085 Clinical Reports of photodynamic therapy (PDT) for superficial squamous cell carcinoma (SCC) or
1086 SCC *in situ* in cats

Cases / tumour location	PDT agent	PDT method	Response rate / outcome / side effects	Reference
51 cats Cutaneous SCC facial skin	HPPH-23 Pyropheophorbid- alpha-hexyl-ether	Intravenous administration Argon-pumped dye laser	Overall 61% response rate at 1 year. 100% T1a tumours, 56% T1b and 18% T2b. No toxicity, but some morbidity.	Magne et al., 1997
4 dogs and 4 cats Superficial carcinoma	HPPH	Intravenous administration LED (100 J/cm ² , 33 min)	8/9 CR >50% PFS > 68 weeks. No cutaneous photosensitivity	Reeds et al., 2004
13 lesions / cats 10 nasal planum, 2 pinna 1 eyelid	ALA (Cream)	Topical application LED 635 nm 12 J/cm ²	85% CR rate But with 64% local recurrence, median 21 weeks. Cats attempt to scratch lesion after treatment. Local analgesia required.	Stell et al., 2001
18 cats with 20 cutaneous SCC	Liposomal formulation of Foscan (m-THPC)	Intravenous administration 625 nm diode laser	100% CR rate Overall 1 year control rate 75% 20% recurrence, 172 days. Mild erythema/ edema in 15% of cats.	Buchholz et al, 2007
55 cats Superficial SCC nasal planum	ALA (Cream)	Topical application LED 635 nm 12 J/cm ²	85% CR rate, 11% PR rate But with 51% recurrence; median 157 days. Transient, mild, local adverse effects.	Bexfield et al., 2008
12 cats Cutaneous SCC (7 pinna, 2 nasal planum)	Haematoporphyrin derivative (Photogem)	Intravenous administration LEDs (300 J/cm ² 30 min)	No response in invasive tumours or pinna. Small non- infiltrative lesions of nasal planum (n=3) showed CR/PR. One cat developed nasal oedema and died.	Ferreira et al., 2009

1087 Abbreviations: LED (light-emitting diode), CR (complete response), PR (partial response), PFS
1088 (progression-free survival).

1089 **Figure legends**

1090

1091 **Figure 1.** Fundamentals of photodynamic therapy.

1092 A) Visible and near infra-red light spectrum showing the wavelengths (in nanometres) of maximum
1093 tissue penetration by light (above) and absorbance maxima of selected photosensitisers (below). B-
1094 D) Chemical structures of selected photosensitisers. E) Schematic of photosensitiser mechanism of
1095 action. Photosensitiser (PS) becomes activated (PS*) by light (hν). PS* can undergo two types of
1096 reaction. In Type I reactions, biological material (BM) interacts directly with PS* forming ion
1097 radicals of both species (PS⁻ and BM⁺). BM radical interacts with oxygen and becomes oxidised.
1098 PS radical is either destroyed or reacts with oxygen to regenerate PS and make a superoxide anion
1099 (O₂⁻) that can react with BM to oxidise it. In Type II reactions, PS* interacts with oxygen to
1100 regenerate PS and make singlet oxygen (¹O₂), which reacts with BM to oxidise it.

1101

1102 **Figure 2.** Feline nasal squamous cell carcinoma (SCC)

1103 A) An early SCC on the right nasal planum in a Domestic Short-haired cat. B) Application of
1104 photodynamic therapy (PDT) using a high intensity light-emitting diode (LED). C) Complete
1105 resolution of the lesion at 6 weeks, with minimal scar formation.

1106

1107 **Figure 3.** Treatment of equine sarcoids.

1108 A) Painting 5-aminolevulinic acid (ALA) onto sarcoid. B) Application of photodynamic therapy
1109 (PDT). C) Appearance of sarcoid at time of PDT treatment. D) Appearance of sarcoid 1 month after
1110 PDT.