Effect of photodynamic therapy on the Microbiology of Acne

By

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Dedication

To my beloved Dad (late), Mom and Wife

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Abbreviations

α-MSH	α-melanocyte stimulating hormone
5α-R	5α-reductase
ACTH	adrenocorticotropic hormone
ALA	5-aminolaevulinic acid
ANOVA	analysis of variance
AP	activator protein
BPO	benzoylperoxide
CFU	colony-forming unit
CMI	cell-mediated immunity
CoNS	coagulase-negative staphylococci
CRF	case report form
CRH	corticotropin–releasing hormone
CRHBP	corticotropin–releasing hormone binding protein
CRHR	corticotropin-releasing hormone receptor
DC	dendritic cell
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone

DLQI	dermatology life quality index
FDLQI	family dermatology life quality index
FFA	free fatty acid
GP	general practitioner
hBD	human β-defensin
HPA	hypothalamic-pituitary-adrenal
HSP	heat-shock protein
IFN	interferon
IGF-1	insulin-like growth factor-1
IL	interleukin
IL-1Ra	IL-1 receptor antagonist
IMP	investigational medicinal product
IPL	intense pulsed light
LA	linoleic acid
LC	langerhans cell
LRAGS/Leeds	Leeds revised acne grading system
MAL	methyl aminolaevulinate
MAPK	mitogen-activated protein kinase
MC-1R	melanocortin-1 receptor
MHC	major histocompatibility complex

MMP	matrix metalloproteinases
ms	millisecond
NF-κB	nuclear factor-kappaB
PAMP	pathogen-associated molecular pattern
PDT	photodynamic therapy
POMC	proopiomelanocortin
PPAR	peroxisome proliferator-activated receptor
PpIX	protoporphyrin IX
PSF	pilosebaceous follicle
QoL	quality of life
ROS	reactive oxygen species
SER	sebum excretion rate
SP	substance p
TGF	transforming growth factor
TLR	toll-like receptor
TNF	tumour necrosis factor
TRT	thermal relaxation time
UHW	University Hospital of Wales
VAS	visual analogue scale
VCAM	vascular cell adhesion molecule

HLA	human leukocyte antigen

Abstract

Light-based therapies, including photodynamic therapy (PDT), for acne are gaining popularity in dermatology. Based largely upon *in vitro* data, their beneficial outcome in acne is thought to be related to their bactericidal effects on *Propionibacterium acnes*. This randomised controlled study sought to determine the efficacy and tolerability of 610-950 nm IPL (administered as IPL-Placebo) and IPL-assisted methyl aminolaevulinate PDT (IPL-MAL) vs. adapalene 0.1% gel in the treatment of acne and to identify their mode of action, looking specifically at the effect on surface density of *P. acnes*.

Thirty seven patients (31% of target due to slow recruitment) with mild to moderate facial acne were randomly allocated to IPL-MAL treatment, IPL-Placebo or adapalene. Both IPL groups received four treatments to the whole face, 2 weeks apart, while the third group was given adapalene nightly for 12 weeks. Assessments performed at baseline and weeks 8, 11, and 16 included inflamed, noninflamed and total lesion counts, Leeds grading, follicular porphyrin fluorescence, the Family Dermatology Life Quality Index and Dermatology Life Quality Index scores, and patient's perspective of clinical improvement by the visual analogue scale (VAS). Cutaneous microflora was collected from all patients at similar intervals.

Of the 37 patients randomised, only 30 completed the trial (10 in each group) and were included in the final analyses. Adapalene was found to be significantly superior to IPL-MAL and IPL-Placebo in reducing the noninflamed (adapalene 37.6% vs. IPL-MAL 3.4% vs. IPL-Placebo −9.7%) and total lesion counts (adapalene 35.7% vs. IPL-MAL 4.3% vs. IPL-Placebo −8.4%) at week 16. This was accompanied by a significant decrease (52.9%) in the DLQI score in this group ($p = 0.031$). The maximum improvement in inflamed lesion counts from baseline was seen at week 11 in the IPL-MAL (20.7%) and IPL-Placebo (13.4%) groups but occurred at week 16 in the adapalene group (26.5%). Statistical significance, however, was not reached in any group. There was no significant difference within or between the groups in the VAS, Leeds, FDLQI and porphyrin fluorescence results pre- and post- treatment. A significant increase in the density of propionibacteria ($p = 0.021$) and

coagulase-negative staphylococci ($p = 0.039$) was seen in the IPL-Placebo and IPL-MAL groups at week 16 and week 8, respectively; however, there was no significant difference between the groups. All the treatments were well tolerated.

Adapalene remains an effective first line treatment in mild to moderate facial acne. However, the present study has remained indecisive (due to being underpowered) in drawing any firm conclusions regarding the efficacy of IPL and IPL-MAL on inflamed acne lesions. Further research is therefore warranted before their use can be advocated for acne treatment. An alternative mode of action for IPL and IPL-assisted MAL-PDT other than photodynamic destruction of *P. acnes* is suggested from the results of this study.

Chapter 1

BACKGROUND 1: Microbiology of Acne

1. Microbiology of Acne

1.1 Introduction

Acne vulgaris is a multifactorial, pleomorphic skin disease of the pilosebaceous follicles (units) characterised by a variety of noninflamed (open and closed comedones) and inflamed (macules, papules, pustules, and nodules) lesions. It is estimated to affect up to 80% of individuals aged 11-30 [1]. Of the three types of pilosebaceous follicles (PSFs) found on the face (terminal, vellus, and sebaceous), acne only affects the sebaceous follicles which are characterised by widely dilated follicular canals with small inconspicuous vellus hairs and a large sebaceous gland **(Figure 1.1)** [2]. Comedones represent acne-affected follicles filled with horny lamellated material and are typical of, but not peculiar to, acne [2, 3].

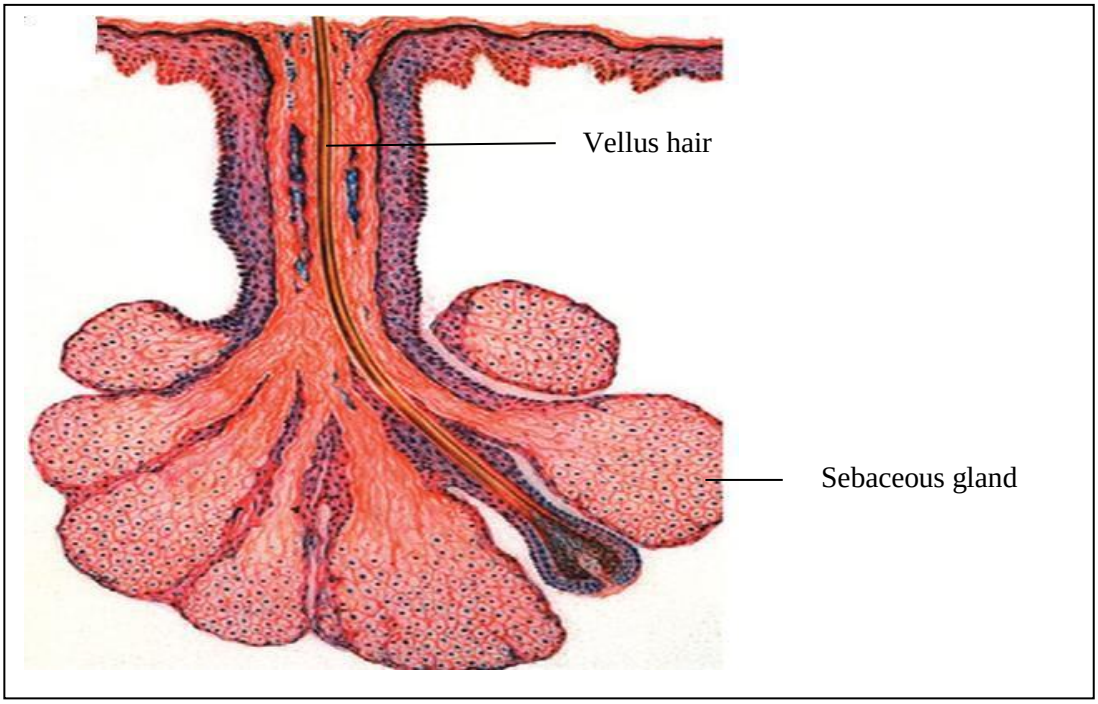


Figure 1.1: Sebaceous follicle. *Adapted from Williams et al. [4]*.

Although a common disease, the aetiology of acne is not yet fully elucidated and is thought to be a multifactorial process. Hormonally influenced excessive sebum production (seborrhoea), comedogenesis (comedone formation), *Propionibacterium acnes* colonisation, genetic susceptibility, as well as inflammation are considered the major contributors to acne pathogenesis [5-8]. Various acne treatments target one or more of these aetiological factors and include topical and systemic antibiotics/retinoids, antimicrobials, and hormonal therapy (e.g. oral contraceptives, antiandrogens) [9]. These are successful in some, but not all, patients. Moreover, a slow onset of action and undesirable effects such as dryness, bleaching of hair and clothing, birth defects and issue of antibiotic resistance result in poor patient compliance [10].

It has long been observed that many patients describe an improvement in their acne following sun exposure [11]. In recent years, a technological explosion has resulted in the development of numerous laser and non-laser light-based treatments which has given us an opportunity to examine the role of light in the treatment of acne. Indeed, visible light has been shown to have a beneficial effect on acne [12]. The possible mechanism of action of these optical therapies in acne has been a subject of debate. It is known that *P. acnes* produces porphyrins [13]. Based largely upon *in vitro* data, the beneficial effect of optical therapies in acne is assumed to be due to the photodynamic eradication of *P. acnes* owing to activation of its endogenous porphyrins [14-16]. However, the role of *P. acnes* in acne has long been a controversial topic and it is still unclear whether this micro-organism has a causal role in the pathogenesis of acne.

1.2 Propionibacterium acnes

1.2.1 *P. acnes*: Causal or bystander?

Propionibacterium acnes, an aerotolerant anaerobic Gram-positive bacillus that produces propionic acid as a metabolic byproduct [17], has been implicated in the pathogenesis of acne since the beginning of the last century. Alexander [18], in 1909, claimed successful treatment of acne with vaccines containing the 'acne bacillus'. However, Lovejoy and Hastings [19] in 1911 isolated the organism from sebaceous secretions of normal skin, casting doubt on its significance in the aetiology of acne. It was not until 1950 that *P. acnes* came to be regarded as a normal and ubiquitous member of the resident cutaneous microflora and its reputation as a pathogen subsequently declined [20]. Interest in the microbial aetiology of acne again developed after the observation that the *P. acnes* population increased greatly at puberty [21]. This was observed to coincide with the onset of acne, and treatment with antibiotics was found to be beneficial in the majority of patients. Moreover, acne patients were observed to carry higher densities of *P. acnes* compared with normal controls [22]. The presence of significantly higher levels of antibodies to *P. acnes* in patients with cystic and pustular acne compared with mild comedopapular acne and persons with healthy skin further strengthened the belief that *P. acnes* is important in the aetiopathogenesis of acne [23]. This also led to the hypothesis that hypersensitivity to *P. acnes* may account for the variation in acne severity [24].

Thus, there is evidence, although largely circumstantial, which suggests that micro-organisms, particularly *P. acnes*, are important in the pathogenesis of acne vulgaris. Despite an abundance of data, it is still unclear whether *P. acnes* is actually a causal

agent in the development of various acne lesions (noninflamed and inflamed), as it is a normal and ubiquitous member of the resident cutaneous microflora [25].

The clinical study in this thesis attempted to evaluate the effect of intense pulsed light (a polychromatic adjustable incoherent light source), used alone and in combination with a photosensitiser, on the cutaneous microflora in acne patients. A review of the literature surrounding the microbiological data on normal and acne-affected skin was conducted, with a view to revisiting the unresolved controversy of the role of *P. acnes* in the initiation of acne. The discussion from this section will act as the basis for next chapter, in which the pathogenesis of acne will be discussed in the context of findings from this review.

1.2.2 Microbial colonisation of normal skin

Various micro-organisms normally reside on human skin. *Propionibacterium acnes* is one of four species of propionibacteria (the other three being *P. granulosum*, *P. avidum*, and *P. propionicum*) which form part of the resident commensal flora in humans [26]. Two further species, formerly known as *P. innocuum* and *P. lymphophilum*, have been reclassified as *Propioniferax innocua* [27] and *Propionimicrobium lymphophilum* [28], respectively. Among these commensal propionibacteria, *P. acnes* is found to be present on the skin in nearly 100% of adults [25]. Although the organism is isolated from the skin surface, its normal habitat is the PSF. *Propionibacterium acnes* shares this habitat with the yeast *Malassezia* (formerly known as *Pityrosporum* [29]), along with the Gram-positive, coagulase-negative cocci, namely staphylococci and micrococci [30].

1.2.2.1 Skin surface microflora

Various techniques have been used over the years to study the cutaneous microflora. The surface scrub technique of Williamson and Kligman [31], which has been extensively used, gives useful information about the surface flora but yields little or no information about the microbial ecology of an individual PSF. This method involves scrubbing the surface of a defined area of skin, using a mild detergent (0.1 % Triton X-100 in 0.075 M phosphate buffer, pH 7.9) and a Teflon rod [31]. By using this technique, it has been shown that propionibacteria colonisation of normal skin shows significant body site and age-related differences [21, 25, 32]. *Propionibacterium acnes* density as well as prevalence is highest in the oily regions of skin such as the face and upper trunk. Body sites with few sebaceous glands such as lower trunk and extremities have a lower prevalence and much lower mean densities [32]. *Propionibacterium acnes* counts on scalp and face can be as high as 10^5 organisms per cm^2 [25]. *Propionibacterium granulosum* is also found in the oily regions but at a lower density and prevalence, while *P. avidum* is normally isolated from wet areas of the body such as the axilla, groin and rectum [25]. It is well recognised that *P. acnes* uses triglycerides in sebum as a carbon and energy source with the resultant liberation of free fatty acids (FFA). The evidence in favour of this process includes the following observations: (i) Marples et al. [33] established that *P. acnes* lipase is responsible for the cleavage of sebaceous triglycerides into FFA; (ii) Rebillo and Hawk [34] demonstrated an inverse correlation between skin surface glycerol levels (an end product of lipolysis of sebaceous triglycerides along with FFA) and the *P. acnes* population, suggesting that glycerol may be a substrate for *P. acnes*; and (iii) results of two studies in which drugs were used to either increase or decrease sebum production resulting in a concomitant increase or decrease in the

numbers of propionibacteria, helped to strengthen this hypothesis further [35, 36]. Thus a high density and prevalence of *P. acnes* at skin sites with large numbers of sebaceous glands is not unexpected.

Likewise, age- related differences have been found in *P. acnes* colonisation, with infants and young children up to the age of five carrying significantly higher numbers than older children up to age 10 [21]. Sebum production by the sebaceous glands is androgen-dependent; at puberty there is an increase in the sebum excretion rate (SER) [37, 38] which is accompanied by a rise in the population density of cutaneous propionibacteria [21]. The population density keeps increasing until the age of 25 years, remaining constant thereafter through adulthood and middle age [21]. A declining trend is seen after age 70 years [21], which is consistent with decreasing sebaceous secretion at that time [38]. After age 20 years, men carry significantly higher numbers of *P. acnes* compared to women [21]. This is in accordance with the observation that normal men over the age of 20 years produce greater quantities of sebum than women [38]. No significant racial difference in the population density of *P. acnes* has been found in healthy black and white individuals [21]. Moreover, the pattern for aerobic bacterial population, particularly cocci, in relation to age, sex and race has been found to be the same as for *P. acnes* [21].

1.2.2.2 Microbial ecology of normal pilosebaceous follicles

The surface scrub technique is useful for removing micro-organisms that are located on or near the skin surface, but it is likely that most of the follicular inhabitants are not removed. As acne is a disease of the PSFs, it is obvious that the microbial ecology of individual follicles is more relevant when dealing with acne-affected skin

and gaining this knowledge could yield more information about the role of micro-organisms in acne vulgaris (**Table 1.1**). Leeming et al. [39] used punch biopsies to separate the epidermis with intact follicles from the dermis after $CaCl_2$ treatment, in order to study the microbiology of normal follicles from the upper back of patients with acne. This technique enables quantification of bacteria from a single PSF but has the drawback that it is dependent on obtaining biopsies of skin for sampling. Of 140 normal follicles isolated from 54 patients, only 12% of the follicles were colonised by propionibacteria (with *P. acnes* the only colonising species), with a mean population density of 2.6×10^5 per follicle [39]. Similarly the incidence of *Staphylococcus* and *Pityrosporum* was 4% and 13% respectively. The geometric mean density of staphylococci was found to be 5.5×10^3, with *S. epidermidis* (formerly known as *S. albus* [40]) being the major colonising species (approximately 50% of all staphylococci) followed by other coagulase-negative staphylococci (CoNS). Colonised follicles, in this study, were described as those containing high densities of bacteria, and PSFs with low numbers were considered to have contaminants [39]. Intriguingly, approximately 34% of the normal follicles were found to be sterile even if the micro-organisms thought to be contaminants were included as colonists [41]. Likewise Till et al. [42], by using the same technique, found propionibacteria (exclusively *P. acnes*) and *Staphylococcus* (exclusively *S. epidermidis*) to colonise only 17% and 10% of 48 normal follicles from the back of patients with persistent and late-onset acne, respectively. They were, however, unable to detect any viable *Malassezia* in these follicles. Moreover, like Leeming et al. [39], they also found 90% of these normal PSFs to be sterile.

Despite the scarcity of data on the microbiology of normal PSFs, based on the findings of Leeming et al. [39] and Till et al. [42] it can be concluded that only a proportion of normal PSFs is colonised by bacteria. Furthermore, Leeming et al. [39] observed the proportion of follicles colonised by micro-organisms to vary widely among patients, which can probably explain individual differences in microbial densities found at the skin surface. The slightly higher prevalence of propionibacteria and staphylococci in the study of Till et al. [42] may be explained by the fact that no attempt was made to differentiate colonised follicles by bacterial density.

Table 1.1: Studies on the microbial ecology of normal pilosebaceous follicles (PSFs)

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Leeming (1984) [39]	14-37	Normal PSFs from the back of patients with acne	140	6	Punch biopsy with micro-dissection of PSFs from unprepared skin	Reinforced clostridial medium supplemented with 0.2% Tween-80/6 days	Propionibacteria (exclusively *P. acnes*) 12 Staphylococci (predominantly *S. epidermidis*) 4 *Pityrosporum* spp. 13 Sterile 34	A proportion of normal PSFs is colonised by three major genera of micro-organisms i.e. *Propionibacterium*, *Staphylococcus*, and *Pityrosporum*. Follicular microenvironment may be responsible for the colonisation of these normal follicles
Till (2000) [42]	Female 26-51 (19)[a] Male 25-50 (17)[a] Female 26-54 (12)[a]	Normal PSFs from the back of patients with acne	48	6	Punch biopsy with micro-dissection of PSFs	Brain-heart infusion agar with furazolidone/ 7 days	Propionibacteria (exclusively *P. acnes*) 17 Staphylococci (exclusively *S. epidermidis*) 10 *Malassezia* 0 Sterile 90	Only a proportion of normal PSFs is colonised by propionibacteria, therefore, validating the results of Leeming et al.

P. acnes, Propionibacterium acnes; S. epidermidis, Staphylococcus epidermidis. [a]Number of patients in each group in parentheses.

1.2.3 Microbiology of acne lesions

In the hope of establishing a microbial aetiology for acne, various investigators have studied the microbiology of noninflamed [41, 43-47] (**Table 1.2**) and inflamed acne lesions [42, 43, 48-51] (**Tables 1.3**). The sampling technique varied with the type of lesion being studied.

1.2.3.1 Microbiology of comedones

1.2.3.1.1 Microbial ecology of extracted comedones

A sampling method involving expression of the comedonal material, with the help of a comedone extractor or stylet, and culturing it for bacteriological examination has been used by various investigators to study the microbial ecology of comedones [43-46]. This method has the drawback that there can be no assurance of complete sampling [52].

Shehadeh and Kligman [43] examined (Gram-stained smears) and cultured a total of 71 comedones (45 open; 26 closed), from over 100 adolescent boys and girls, and found *Corynebacterium acnes* (synonymous with *P. acnes*) and *C. acnes* along with *S. albus* in 96% and 92% of the lesions, respectively. Likewise, *S. albus* was isolated from 96% of the lesions while none of the comedones was found to be sterile. Similarly, Ganor and Sacks [44] compared the microbial flora of acne and senile comedones (seen on the sun-damaged faces of the elderly) and found corynebacteria (synonymous with propionibacteria) and staphylococci in 65% and 51% of the acne comedones respectively. Moreover, yeasts were seen in 67% of these lesions. The investigators failed to culture all the corynebacteria identified microscopically and, therefore, corynebacteria from only 20% of the acne comedones were identified as *C.*

acnes. Similarly staphylococci from only 41% of the comedones were identified as *S. albus* [44]. No significant difference was found in the incidence of staphylococci or *Pityrosporum* spp. between the acne and senile comedones. However, corynebacteria were found less frequently in the senile compared with acne comedones [44]. Both these studies showed that < 100% of the comedones are colonised by propionibacteria or staphylococci but the researchers failed to mention the avoidance of antibiotics (presampling) in their cohort of patients. Therefore, it is difficult to completely rule out the role of antibiotics affecting the bacterial colonisation in these studies.

Subsequently, Marples et al. [45], in an attempt to quantify the microbial population of individual comedones, studied the microflora of open and closed comedones in 15 patients and isolated *C. acnes* in 92% of the lesions at a geometric mean density of 8.2×10^4 per comedone. Furthermore, aerobes (mainly CoNS) were recovered from 85% of all lesions while yeasts were seen in all but one preparation. Similarly, Puhvel and Amirian [46] studied the bacterial ecology of 148 open comedones from the face and back of 38 acne patients and cultured anaerobic diptheroids (synonymous with propionibacteria) and aerobic cocci from 80% and 75% of the lesions, respectively. Seven per cent of the comedones were found to harbour neither of the two micro-organisms. The results of this study were in agreement with the findings of Shehadeh and Kligman, Ganor and Sacks and Marples et al. [43-45] who also showed that comedones are not universally colonised by *P. acnes*, supporting the argument that the presence of *P. acnes* is not a prerequisite for comedogenesis.

1.2.3.1.2 Microbial ecology of follicular casts/comedones isolated by the cyanoacrylate sampling technique

A cyanoacrylate gel technique to isolate and enumerate micro-organisms from individual PSFs (without biopsy) involves the use of cyanoacrylate gel and a sterile glass slide/sampler which is pressed against the skin and then gently peeled away [53]. The procedure extracts the follicular material and its resident bacteria. This method has the disadvantage that incomplete removal of follicular material can occur [52]. Moreover, as the cyanoacrylate gel has been shown to have an antibacterial effect, this may affect the viability of the bacteria to some extent [53].

In an attempt to evaluate the role of *P. acnes* in the initiation of comedogenesis, Lavker et al. [47] investigated the structural organisation and bacteriological profile of follicular casts (isolated by the cyanoacrylate method) and early comedones (sampled by a Schamberg extractor) in prepubertal and early pubertal individuals. Neither follicular casts nor comedones (collected from five children aged 9-11 years with early acne vulgaris) yielded *P. acnes* when cultured. Additionally, in 10 of the 15 prepubertal children, cultures obtained from the forehead and cheek by the surface scrub technique also failed to yield any *P. acnes*. Furthermore, both light and electron microscopy failed to show any bacteria in the prepubertal follicular casts. Prepubertal follicular casts contained all of the abnormalities usually seen in follicular casts and biopsy material from patients with acne, which suggests that these casts represent potential comedones. The authors concluded that as these abnormalities occurred in the complete absence of bacteria, therefore bacteria are not essential for the formation of follicular casts or comedones [47]. Complete absence of *P. acnes* in the follicular casts was validated by various techniques and yielded

compelling evidence against the role of *P. acnes* in the initiation of comedogenesis. However, whether these prepubertal children did develop acne at puberty is unknown. A long-term study investigating the occurrence/prevalence of acne in this group would have been more informative in drawing conclusions as to the role of *P. acnes* in comedogenesis.

1.2.3.1.3 Microbial ecology of comedones isolated by microdissection from skin biopsies

In order to resolve the ongoing controversy of the role of *P. acnes* in the initiation of comedogenesis, Leeming et al. [41] studied the bacteriology of 59 comedones (29 open; 30 closed), isolated by microdissection from skin biopsies, from the upper back of 49 patients with acne. Approximately 55% and 22% of the comedones were found to be colonised by propionibacteria and staphylococci, respectively. Likewise, *Pityrosporum* spp. colonised 74% of these lesions [41]. Comparing these results with the findings of their study on the microbial ecology of normal PSFs, no significant difference was observed in the population density of propionibacteria among normal follicles and comedones. However, micro-organisms (propionibacteria, staphylococci and *Pityrosporum* spp.) were found to colonise significantly more comedones. Moreover, the species of staphylococci and propionibacteria isolated did not differ significantly among normal follicles and comedones. Lastly, compared with 34% of the normal follicles, approximately 11% of closed while 7% of open comedones were found to be sterile [39, 41].

Because of the different sampling and culturing techniques used by various investigators it is difficult to compare all the microbiological data on comedones.

However, one consistent observation that can be made is the fact that there was not universal colonisation of these lesions by a single microbial agent. Secondly, some of the lesions have been found to be sterile [41, 46], again arguing against the role of micro-organisms in the initiation of comedogenesis.

Table 1.2: Studies on the microbial ecology of comedones

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Shehadeh (1963) [43]	Adolescent boys and girls	Comedones	71 (45 open; 26 closed)	NM	Extraction of lesion content by sharp acne stylet or pointed scalpel after wiping surface with 70% isopropyl alcohol. A sopping sponge left on skin for 3 min	Brain-heart infusion blood agar fortified by 1% glucose/5 days under 90% N_2 and 10% CO_2	*C. acnes* 96 *C. acnes* & *S. albus* 92 *S. albus* 96 Sterile 0	Acne flora is a 'stable biad' consisting of *C. acnes* and *S. albus*. These organisms are extension of those colonising normal PSFs
Ganor (1969) [44]	NM	Open comedones/ senile comedones	101 (51 acne comedones; 50 senile comedones)	NM	Comedo extraction by acne stylet after wiping surface with 70% alcohol and an alcohol-soaked gauze left on skin for 3 min	Glucose blood agar/7 days	Acne comedones: Corynebacteria 65 Staphylococci 51 Yeasts 67 Senile comedones: Corynebacteria 36 Staphylococci 50 Yeasts 60	Comedonal microflora is probably of a secondary nature

Table 1.2 Continued

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (Percentage of lesions colonised/sterile)	Conclusion
Marples (1973) [45]	14-23	Comedones	150 (75 open; 75 closed)	3	Comedo extraction by Schamberg extractor after wiping skin with 70% ethanol	Marshall and Kelsey agar/7 days	*C. acnes* 92 Aerobes (mainly coagulase-negative staphylococci) 85 Yeasts 99	Incidence of cocci, *Pityrosporum* and *C. acnes* is nearly 100% in comedones; the absence of a group, in a given comedone, is probably due to technical error
Puhvel (1979) [46]	16-30	Open comedones	148	3	Comedo extraction by a comedone extractor after wiping skin with 70% alcohol	Brain-heart infusion agar supplemented with 1% dextrose/5 days at 37°C under 90% CO_2 and 10% N_2	Anaerobic diptheroids 80 Aerobic cocci 75 No anaerobic diptheroid or aerobic cocci 7	The comedonal microflora is an extension of the normal follicular flora and is unrelated to the event of comedogenesis
Lavker (1981) [47]	5-10 (15)[a] 9-11 (5)[a]	Follicular casts/ comedones	88 follicular casts 28 comedones	NM	Cyanoacrylate /comedo extraction by Schamberg extractor. Information about surface disinfection NM	Brain-heart infusion agar with 0.1% Tween-80/7 days	No *P. acnes* yielded	Bacteria, particularly *P. acnes*, are not involved in the initiation of comedogenesis

Table 1.2 Continued

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Leeming (1985) [41]	13-39	Comedones	59 (29 open; 30 closed)	6	Punch biopsy with microdissection of PSFs from unprepared skin	Reinforced clostridial medium supplemented with 0.2% Tween-80/6 days	Propionibacteria 55 Staphylococci 22 *Pityrosporum* spp. 74 Sterile: closed comedones 11; open comedones 7	The presence of micro-organisms is not essential for the initiation of comedogenesis

P. acnes, Propionibacterium acnes; C. acnes, Corynebacterium acnes; S. albus, Staphylococcus albus; PSFs, pilosebaceous follicles; NM, not mentioned.

[a]Number of patients in each group in parentheses.

1.2.3.2 Microbiology of inflamed acne lesions

Over the years, various researchers have investigated the microbial ecology of inflamed acne lesions, in order to test the hypothesis that the microflora of noninflamed and inflamed acne lesions may be different.

1.2.3.2.1 Inflamed lesions (predominantly pustules)

Shehadeh and Kligman [43] examined a total of 104 inflamed acne lesions (papule, pustule, nodule or cyst) from over 100 adolescent boys and girls, and found *C. acnes* and *C. acnes* along with *S. albus* in 79% and 61% of the lesions respectively. Five per cent of the papules as well as pustules were found to be sterile and were observed to contain very few organisms on Gram-stained smears. Likewise, Marples and Izumi [48], investigated the bacteriology of pustular acne in 109 pustules from 27 acne patients and found *C. acnes* in 73%, Gram-positive cocci in 60% (predominantly *S. epidermidis*), and Gram-negative rods (predominantly *Enterobacter aerogenes*) in 10% of these lesions. Furthermore, 12% of the pustules were found to be sterile, with microscopic examination of the Gram-stained smears substantiating the negative culture results. Brook et al. [49], who also studied the bacteriology of 32 pustular acne lesions, isolated only aerobes/facultative anaerobes (predominantly *S. epidermidis*) and anaerobes (predominantly *Peptostreptococcus* followed by *Propionibacterium* spp.) from 47% and 34% of the lesions, respectively. Moreover, mixed aerobes and anaerobes were seen in 18% of the lesions. The results from the study of Nishijima et al. [50] also yielded propionibacteria (predominantly *P. acnes*) and *S. epidermidis* as the most common (but never 100%) micro-organisms, colonising acne pustules. All these studies were limited by the fact that

the duration of individual lesions was not given and it is possible that the antimicrobial effect of the host immune response could have been responsible for the sterility of a number of these lesions. Furthermore, provision of some essential information, such as avoidance of antibiotics, presampling, in a number of these studies, would have been more informative in drawing conclusions about the bacterial ecology of these lesions.

1.2.3.2.2 Inflamed lesions (papules)

A key shortcoming of the above studies, i.e. not mentioning the duration of individual inflamed lesions, was finally remedied by Leeming et al. [51]. They studied the microbiology of inflammatory papules which have been inflamed for only short periods, and used microdissection from skin biopsies to isolate 52 '1 day' and 19 '3 day' papules from the upper back of patients with acne. They found propionibacteria and staphylococci to have a colonising population (i.e. ≥ 200 organisms per papule) in only 71% and 23% of the papules, respectively. Moreover, '3 day' papules were observed to be colonised more frequently than '1 day' papules, although this difference was not statistically significant [51]. Interestingly, bacterial cultures from 20% and 54% of the papules (including lesions with both high and low microbial densities) were negative for propionibacteria and staphylococci (along with other aerobes), respectively. Furthermore, 10% of '1 day' papules were found not to be colonised by any micro-organism (*Pityrosporum*, *Propionibacterium*, or *Staphylococcus* spp.) while all the '3 day' lesions were found to be colonised. The population density of propionibacteria and staphylococci in '3 day' papules was almost 2 and 2.6 times greater than in '1 day' lesions, respectively. No bacteria were observed on microscopic examination of the papules without associated bacterial

growth, further validating the negative culture results [51]. Comparing these results with the findings of their studies on the microbial ecology of normal PSFs and comedones, propionibacteria, staphylococci and *Pityrosporum* spp. were found to colonise significantly more lesions. However, no significant difference was noted in the bacterial and yeast colonisation rates among the comedones and the two types of papules. Moreover, there was no significant difference amongst the distribution of different species and subspecies colonising normal PSFs, comedones and inflamed lesions. Although a rising trend was seen in the bacterial population densities among the normal PSFs, comedones and inflamed lesions, with the highest being in the '3 day' papules, this was not found to be statistically significant [39, 41, 51]. The authors concluded that micro-organisms found in inflamed lesions are just an extension of those colonising comedones and that their presence is not necessary for the initiation of inflammation in acne.

Similarly Till et al. [42], by using the sampling technique adopted by Leeming et al. [51], also studied the microbiology of acne papules and found propionibacteria (exclusively *P. acnes*) and staphylococci (exclusively *S. epidermidis*) in 60% and 24% of the inflamed lesions, respectively. *Malassezia* serovar A was found in 32% while 10% of the inflamed follicles had no detectable viable micro-organisms. Comparing these findings with the results obtained from the normal PSFs, biopsied from the same cohort of patients, the inflamed lesions were found to have significantly higher density and prevalence of propionibacteria and *Malassezia* [42]. These results also supported the work of Leeming et al. [51] who suggested that the inflammatory response in the follicles is not always initiated by the micro-organisms.

Table 1.3: Studies on the microbial ecology of inflamed acne lesions

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Shehadeh (1963) [43]	Adolescent boys and girls	Papules, pustules, nodules or cysts	104	NM	Extraction of lesion content by sharp acne stylet or pointed scalpel after wiping surface with 70% isopropyl alcohol. A sopping sponge left on skin for 3 min	Brain-heart infusion blood agar fortified by 1% glucose/5 days under 90% N_2 and 10% CO_2	*C. acnes* 79 *C. acnes* & *S. albus* 61 *S. albus* 77 Sterile papules 5 Sterile pustules 5	Acne flora is a 'stable biad' consisting of *C. acnes* and *S. albus*. These organisms are extension of those colonising normal PSFs. After rupture of the comedones, the bacteria aggravate the inflammatory reaction by secondary infection. The antibacterial action of the host immune response is responsible for the sterility of a number of these inflammatory lesions

Table 1.3 Continued

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Marples (1970) [48]	15-24	Pustules	109	Of 27 patients sampled, 21 had not received antibiotics for at least 3 weeks	Skin wiped with 70% ethyl alcohol and pus collected by a sterile blood lancet	Casein yeast extract lactate glucose agar/7 days in N_2 and CO_2	*C. acnes* 73 Gram-positive cocci (predominantly *S. epidermidis*) 60 Gram-negative rods (predominantly *Enterobacter aerogenes)* 10 Lipophilic diptheroids 8 Sterile pustules 12	Normal flora of the face and acne pustules is similar, comprising of *C. acnes* and Gram-positive cocci. The antibacterial action of the host immune response is responsible for the sterility of a number of these pustules
Brook (1995) [49]	12-35	Pustules	32	4	Expression of the lesion content after cleansing surface skin with povidone-iodine followed by alcohol swab	Vitamin K1-enriched Brucella blood agar and thioglycolate broth/5 days on Brucella blood agar and 14 days on thioglycolate broth	Only aerobes and facultative anaerobes (predominantly *S. epidermidis*) 47 Only anaerobes (predominantly *Peptostreptococcus* spp.) 34 Mixed aerobes and anaerobes 18	Bacteria other than *P. acnes* and *Staphylococcus* spp. may contribute to the inflammatory process in acne vulgaris

Table 1.3 Continued

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Nishijima (2000) [50]	13-43	Pustules	24	NM, although none of the patients had any previous oral and/or topical antimicrobial treatment for acne	The contents of pustules squeezed out and collected using a comedonal extractor after cleaning skin with 70% ethanol	Brucella HK agar medium supplemented with 5% defibrinated horse blood/NM	Propionibacteria (predominantly *P. acnes*) 79 *S. epidermidis* 83 *P. acnes* and *S. epidermidis* 58	*S. epidermidis* and *P. acnes* may be the representative bacteria in any acne lesion. Other bacterial species may be contaminants
Leeming (1988) [51]	13-39	Inflammatory papules from the back of patients with acne	‘1 day’ papule 52 ‘3 day’ papule 19	4	Punch biopsy with microdissection of PSFs from unprepared skin	Reinforced clostridial medium supplemented with 0.2% Tween-80/6 days	‘1 day’ papule: Propionibacteria 68 Staphylococci 19 *Pityrosporum* spp. 52 Sterile 10 ‘3 day’ papule: Propionibacteria 79 Staphylococci 32 *Pityrosporum* spp. 68 Sterile 0	Micro-organisms found in papules are an extension of comedonal microflora and their presence is not essential for the initiation of inflammation in acne

Table 1.3 Continued

Study (first author and year)	Age range (years)	Type of lesions sampled	Number of lesions sampled	Abstinence from antibiotics presampling (weeks)	Sampling technique	Culture medium/ incubation period for *P. acnes*	Microbiology (percentage of lesions colonised/sterile)	Conclusion
Till (2000) [42]	Female 26-51 (10)[a] Male 25-50 (10)[a] Female 26-54 (6)[a]	Inflammatory papules (duration unknown)	26	6	Punch biopsy with microdissection of PSFs	Brain-heart infusion agar with furazolidone/7 days	Propionibacteria (exclusively *P. acnes*) 60 Staphylococci (exclusively *S. epidermidis*) 24 *Malassezia* 32 Sterile 10	The presence of micro-organisms is not a prerequisite for the initiation of inflammation in acne. However, micro-organisms may be involved in the inflammatory process at some stage

P. acnes, *Propionibacterium acnes*; *C. acnes*, *Corynebacterium acnes*; *S. albus*, *Staphylococcus albus*; *S. epidermidis*, *Staphylococcus epidermidis*; PSFs, pilosebaceous follicles; NM, not mentioned. [a]Number of patients in each group in parentheses.

1.2.4 Summary of the microbiological data on normal and acne-affected skin

Only a proportion of normal as well as acne-affected PSFs, whether inflamed or noninflamed, is colonised by any specific microbial agent. Further, the microflora of normal or acne-affected PSFs (inflamed and noninflamed) generally consist of three major genera of micro-organisms (*Propionibacterium, Staphylococcus,* and *Malassezia*) with *P. acnes* and *S. epidermidis* being the main colonising species among the propionibacteria and staphylococci, respectively. We propose that bacteria, particularly *P. acnes*, are not a requirement for comedogenesis. The evidence for this is provided by a number of studies which failed to yield *P. acnes* from a proportion of noninflamed acne lesions [41, 43-46]. The findings from the study of Lavker et al. [47], who failed to isolate *P. acnes* from any of the follicular casts or early comedones of prepubertal and early pubertal children respectively, further support this notion. Likewise, *P. acnes* is probably not necessary for the initiation of inflammation, as it is never isolated from 100% of inflamed lesions and various investigators also found a proportion of these lesions to be sterile [42, 43, 48-51].

It has been postulated that the microenvironment of individual follicles, whether normal or acne-affected, is important for colonisation as well as the production of extracellular enzymes by the micro-organisms [54, 55]. Oxygen and carbon dioxide tension, water availability and follicular pH are some of the possible factors that might differ from one PSF to another and may determine colonisation and enzymes production by the micro-organisms [54]. Comparing the available data on the microbiology of normal follicles with the microbiological information obtained from

comedones and inflamed lesions by Leeming et al., it seems that the microenvironment of a proportion of microcomedones (earliest subclinical lesion) is more suitable for microbial growth. This leads to a significantly greater colonisation of these lesions compared with the unaffected follicles [39, 41]. This may explain why acne patients carry higher numbers of *P. acnes* at the skin surface [22]. The fact that Leeming et al. [51] found 10% of the '1 day' papules not to be colonised by any micro-organism while all the '3 day' lesions were found colonised suggest that these early lesions might be free from any microbial colonisation at the start of inflammation. This may partly explain the sterility of a significant proportion of inflamed lesions within acne patients as reported by various investigators. As '3 day' papules were also found to have the highest microbial density (albeit a non-significant difference), it is plausible that an inflamed lesion may provide an enriched environment not only for the colonisation but also for the proliferation of cutaneous micro-organisms. This might explain why Till et al. [42] found a significantly higher density of micro-organisms in the inflamed lesions compared with normal PSFs. Of course, these effects will be only temporary and body will eventually get rid of these micro-organisms as the host defences become ascendant. The results from other studies on the microbial ecology of comedones and inflamed lesions could not be compared because of differences in the sampling techniques used by the researchers.

Antibiotics such as tetracycline have been shown to have anti-inflammatory effects in addition to their antibacterial activity, which may in part explain the improvement delivered by these agents [56]. Indeed, subantimicrobial dose of doxycycline has been demonstrated to improve acne without causing a significant reduction in cutaneous microflora [57]. Similarly, although patients with severe acne produce

more antibodies to *P. acnes* than do normal controls [23, 58], the antibody titres of patients with mild to moderate acne have not been found to differ significantly compared with normal controls [58]. Furthermore, patients with severe acne do not harbour significantly larger numbers of *P. acnes* compared with individuals having less severe form [22, 59]. Thus, it is possible that the increased levels of antibodies to *P. acnes* in severe acne patients is due to an increased exposure of these individuals to the immunogen as a result of their pathological condition. Likewise, cell-mediated immunity (CMI) to *P. acnes* may be a contributing factor in the inflammatory response in acne but this has been found to occur late in the chain of events and does not necessarily initiate inflammation in all patients with acne [60]. Moreover, Gowland et al. [60] found only four of 22 patients with moderate acne and 12 of 22 patients with severe acne to have CMI to *P. acnes*. Additionally, they did not find any evidence of CMI to *P. acnes* in 13- to 14-year-old children with severe acne. This suggests that an exaggerated immune response (humoral or cell-mediated) to *P. acnes* cannot solely be held responsible either for the initiation of inflammation in inflamed acne lesions or for the variation in its severity.

1.3 Conclusion

Propionibacterium acnes is likely to be a bystander and not an active participant in the initiation of noninflamed and inflamed acne lesions. This, however, does not undermine the importance of *P. acnes* in acne pathogenesis. The following chapter will discuss the possible mechanisms leading to the development of a microcomedone and its transformation into an inflamed lesion and how *P. acnes* may be involved in this process.

Chapter 2

BACKGROUND 2: Pathogenesis of acne: Pathways to comedogenesis and inflammation

2. Pathogenesis of acne: Pathways to comedogenesis and inflammation

2.1 Introduction

Despite extensive research, the pathogenesis of acne has still not been fully elucidated. The microcomedone is thought to be the precursor lesion that can then develop into a comedone and/or inflamed lesion. However the sequence of events and their possible mechanisms leading to a microcomedone development and its transformation into an inflamed lesion is still a subject of ongoing debate. Considering the microbiological data, which does not support a role for *P. acnes* in the initiation of noninflamed and inflamed acne lesions **(see Chapter 1)**, this chapter details a possible stepwise mechanism to explain this process and will help to identify various key factors that can be therapeutically targeted to improve acne.

2.2 Genetic susceptibility

The risk of acne vulgaris in relatives of patients with acne, as compared with controls, is significantly higher suggesting hereditary influences [61]. A large twin study based on 458 pairs of monozygotic and 1099 pairs of dizygotic twins also suggested a strong genetic basis for acne as 81% of the variance in acne scores was attributable to genetic factors and family history of the disease showed significant familial clustering [62]**.** Furthermore, an autosomal dominant pattern of inheritance for comedones has also been described in thirteen members of a family [63]. Despite all this evidence, strongly suggestive of hereditary influences in the pathogenesis of acne, limited research has been done to date to identify the genes responsible for this common disease.

2.2.1 The androgen receptor gene polymorphism

Response to androgens is mediated through the androgen receptor, a member of the nuclear receptor superfamily [64]. The androgen receptor gene, located on the X chromosome at Xq11-12, contains 8-35 CAG repeats in its first exon that encode a polyglutamine tract in the amino-terminal transactivation domain of the androgen receptor protein [65]. *In vitro*, the transactivation activity of human androgen receptor correlates inversely with the CAG repeat length [66]. Compared with normal controls, a significantly lower mean number of CAG repeats has been demonstrated in male, but not female, Chinese acne patients [67, 68]. Moreover, Pang et al. [68] found Chinese men and women with CAG repeat length < 23 and < 24, respectively, to have a significantly increased risk for acne than those men and women with higher number of repeats. A correlation between CAG repeat length and acne however could not be demonstrated by Sawaya and Shalita [69] in their non-Chinese cohort and may reflect in part the ethnic difference in the pathogenesis of acne.

2.2.2 CYP17 gene polymorphism

A polymorphism in the CYP17 gene (located on chromosome 10q24.3) is another possible contender that has been studied to explain the genetic basis of acne [70]. This gene encodes cytochrome P450c17 enzyme, an enzyme which plays an essential role in the synthesis of dehydroepiandrosterone (DHEA, a weak adrenal androgen and precursor of testosterone) [71]. A polymorphism caused by a single base change from T to C in the 5′- untranslated region of the CYP17 gene (CYP17-34T/C) is thought to up-regulate its transcription, leading to increased levels of

P450c17 enzyme [70], which ultimately may result in increased DHEA synthesis. Compared with normal controls, Chinese male patients with severe acne have been found to have significantly increased frequency of the C allele as well as CC genotype [70]. The authors concluded that CYP17-34C/C homozygosity may increase the risk of severe acne in Chinese men.

2.2.3 Polymorphism of other genes

Polymorphisms in genes encoding cytochrome P450 1A1 (an enzyme involved in retinoids metabolism) [72], tumour necrosis factor (TNF)-α [73, 74], polymorphic epithelial mucin (a glycoprotein secreted from the mammary, gastric, sebaceous, and sweat glands) [75], TNF receptor type 2 [76, 77], interleukin (IL)-1α and IL-1 receptor antagonist (IL-1Ra) [78], and toll-like receptor (TLR)-2 genes [77] are some of the other possible candidates that have been studied for their association with acne and/or its severity. It is possible that acne is a polygenic disorder in which different genes, each of which is not strongly active by itself, may act in combination leading to the development of acne in an individual [2].

2.3 Comedogenesis

Hypercornification of the lower four-fifths (infrainfundibulum) of the pilosebaceous duct **(Figure 2.1)**, leading to mechanical obstruction of the sebum outflow, is an important feature of acne which can be seen histologically as a microcomedone and clinically as a blackhead (open comedone) and whitehead (closed comedone) [2, 79, 80]. Closed comedones have only a microscopic opening which keeps the contents of the distended follicles from escaping **(Figure 2.2)** while open comedones have

wide follicular orifices which allow the contents to escape to the surface **(Figure 2.3)** [3]. Histological features of microcomedones can be seen in 28% of the pilosebaceous units sampled from the clinically normal skin of patients with mild acne [81]. Evaluation of biopsy specimens of papules obtained up to 72 hours after development has demonstrated a microcomedone in 52% of subjects, an open comedone in 27%, and a closed comedone in 9% [82]. This implies that the majority of inflamed lesions originate from microcomedones. Moreover, there is a significant correlation between the severity of acne and the number of microcomedones [81].

Comedogenesis can occur due to hyperproliferation of ductal keratinocytes, inadequate separation of the ductal corneocytes, or a combination of both factors [83]. Hyperproliferation has been confirmed by demonstration of an increase in the Ki-67 (monoclonal antibody reacting with a nuclear antigen expressed by cells in the late G_1, S, M, and G_2 phases of the cell cycle) labelling of ductal keratinocytes [84]. This fact is further substantiated by the presence of keratins 6 and 16 (keratin markers of hyperproliferation) in comedones [85]. The limited data available investigating whether comedogenesis is due to increased intercorneocyte adhesion showed no primary abnormality of ductal desmosomes [86]. Various factors that may be involved in comedogenesis can be explained as follows.

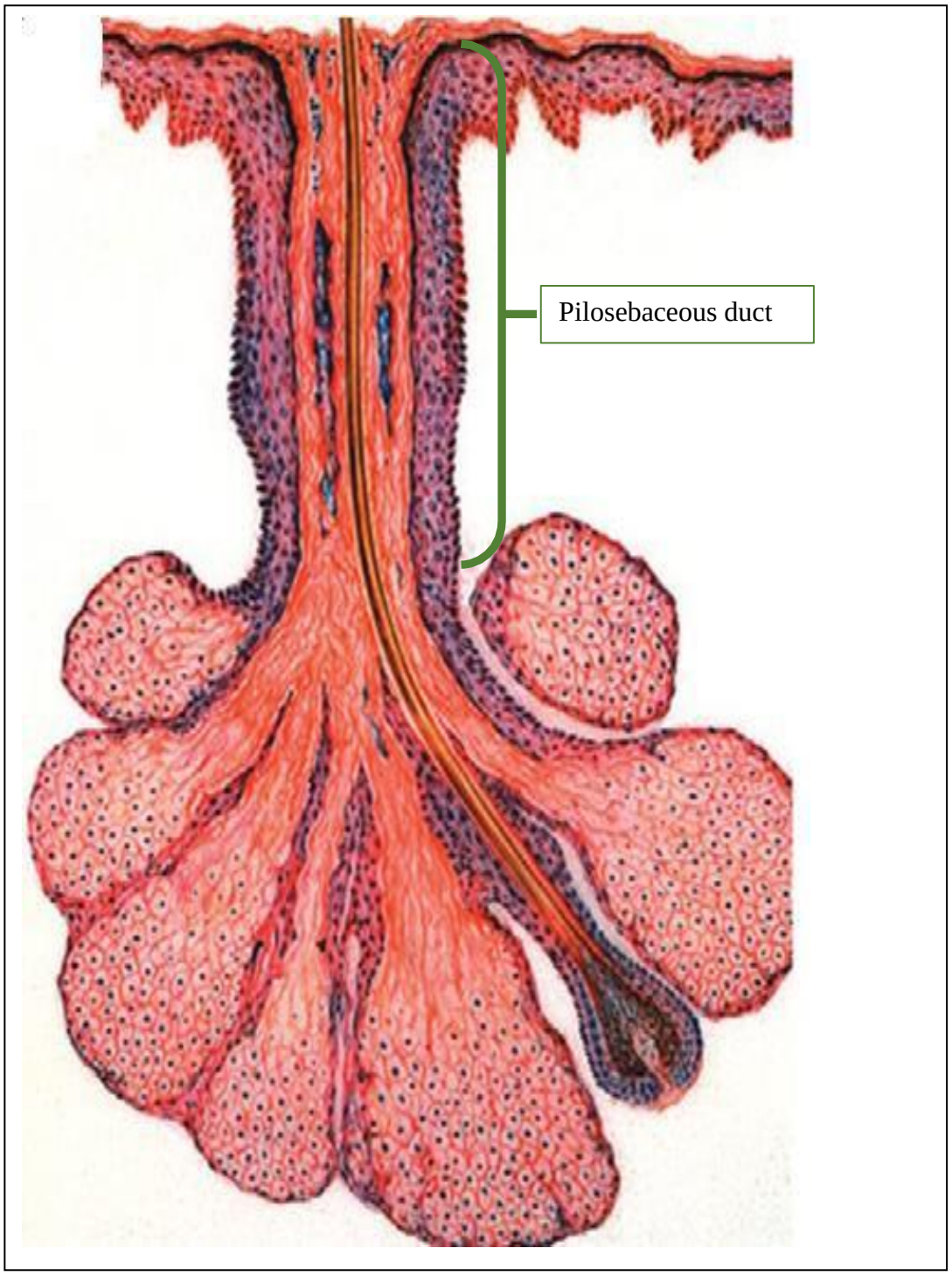


Figure 2.1: Sebaceous follicle with pilosebaceous duct. Comedogenesis begins in the lower four-fifths (infrainfundibulum) of the duct. *Adapted from Williams et al.[4]*.

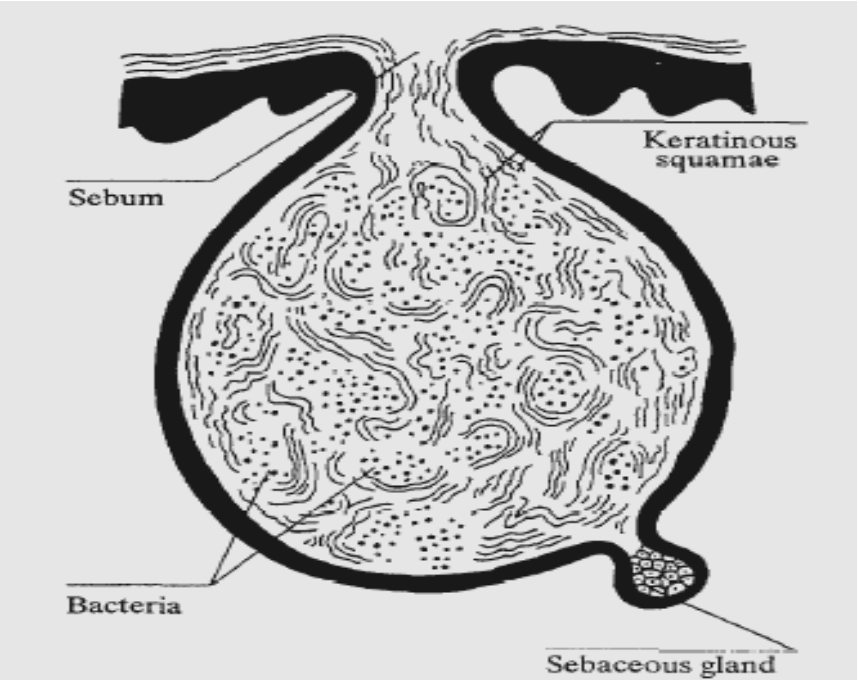


Figure 2.2: Closed comedone. *Adapted from Leyden JJ [87]*.

Figure 2.3: Open comedone. *Adapted from Leyden JJ [87]*.

2.3.1 Androgens

The role of androgens in acne vulgaris and the beneficial effect of antiandrogen therapy are well established. Acne is associated with seborrhoea [37, 38] which is under androgenic control. Male castrates and oophorectomised females produce significantly less sebum compared with matched controls [88]. Moreover, the administration of testosterone to castrated males, children, or postmenopausal women in whom sebum secretion is normally low, results in an increase in sebaceous gland activity [89, 90]. Lastly, antiandrogen therapy reduces SER and improves acne [36]. All these facts support the essential role of androgens in sebum stimulation and acne development.

2.3.1.1 Serum androgens levels in acne

Based upon the above observations it is logical to hypothesise that acne patients may have increased levels of circulating androgens and/or end-organ sensitivity to these hormones, which may account for the seborrhoea seen in acne patients. Thiboutot et al. [91] found mean serum levels of total testosterone, free testosterone, dihydrotestosterone (DHT), and dehydroepiandrosterone sulfate (DHEAS, a sulfated form of DHEA secreted primarily by the adrenal gland and a precursor of testosterone) to be significantly greater (but still within the normal range) in female, but not male, acne patients compared with normal controls. Similarly, Lucky et al. [92] also found higher serum levels of total testosterone, free testosterone, and DHEAS in female acne patients compared with normal controls. The association between female acne and high serum DHEAS levels has been confirmed in a number of other studies [93-95], however, the results for other androgens have differed [94, 95].

2.3.1.2 End-organ sensitivity to androgens in acne

It is likely that high concentrations of adrenal and gonadal androgens, alone or in combination, may lead to the development of seborrhoea and acne vulgaris in an individual. However, there is a considerable overlap of serum androgens levels between normal and female acne patients and higher mean concentrations of serum androgens can not elucidate the development of acne in all individuals [92]. Moreover, it also does not explain the development of acne in male patients who do not have abnormally high androgens levels compared with controls [91, 96]. One possible explanation may be increased end-organ sensitivity of acne patients to the circulating androgens. The enzyme 5α-reductase (5α-R) is responsible for catalysing the conversion of testosterone to its potent metabolite DHT. This enzyme has two isoforms, type 1 and type 2, and the former predominates in the skin [97]. Increased activity of type 1 5α-R enzyme, leading to a higher DHT production, in the skin may be one of the possible ways by which patients with acne may demonstrate increased end-organ sensitivity to the androgens. Indeed, the conversion of testosterone to DHT has been demonstrated to be significantly higher in acne bearing skin compared with normal skin [98]. Furthermore, two subsequent studies also demonstrated the activity of 5α-R to be higher in the infrainfundibular keratinocytes [99] and sebaceous glands [91] of individuals with acne compared with normal controls. However, the difference was not statistically significant. This might be due to the small sample size of the studies.

Similarly, acne patients may differ in their response to the androgens at the receptor level. Androgen receptors have been identified at many sites in the skin including sebaceous glands, pilosebaceous duct keratinocytes, fibroblasts and endothelial cells

[100, 101]. Schmidt et al. [102] demonstrated acne patients to have a significantly higher density of androgen receptors compared with healthy controls. This can be another possible mechanism by which acne patients may have increased end-organ sensitvity to the androgens. It is possible that one or more of the above factors may account for the development of seborrhoea and acne in an individual.

It is prudent to mention here that although androgens have a proliferative effect on cultured human [103] and SZ95 sebocytes (an immortalised human sebocyte cell line) [104], they have only a minimal effect on SZ95 sebocyte differentiation [105, 106]. Peroxisome proliferator-activated receptor (PPAR) ligands, on the other hand, have been shown to be the master regulators of lipid metabolism [105-107]. In addition to the androgen receptors, PPARs are abundantly present in human sebaceous glands [108] and can be important therapeutic targets in acne patients.

2.3.1.3 Mechanism of androgens involvement in comedogenesis

Androgens may lead to comedogenesis not just by causing seborrhoea **(see Section 2.3.2)** but could also increase epithelial turnover in the pilosebaceous duct via their receptors on the ductal keratinocytes [100]. In addition, androgens have been shown to significantly stimulate the proliferation of keratinocytes co-cultured with beard dermal papilla cells via the production of insulin-like growth factor-1 (IGF-1) by the dermal papilla cells [109]. Thus, it is possible that androgens may also influence epithelial turnover in the pilosebaceous duct via the production of IGF-1, which acts as a paracrine growth factor. IGF-1 has also been shown to stimulate lipid production in human SEB-1 sebocytes by an increased expression of sterol response element-binding protein-1, a transcription factor that regulates numerous

genes involved in lipid biosynthesis [110]. This may further aggravate seborrhoea and as a consequence comedogenesis in acne patients.

2.3.2 Sebum

Human sebaceous glands secrete a mixture containing triglycerides, wax esters, squalene, cholesterol esters, and some free cholesterol [111]. As mentioned previously **(Chapter 1, Section 1.2.2.1)** FFA in skin surface lipids are derived from sebaceous triglycerides through the lipolytic action of *P. acnes* lipases [33]. An increase in sebaceous gland activity occurs between ages 7-10 years which corresponds with the time when the adrenal secretion of DHEAS begins to increase [112]. This is reflected by a change in the composition of skin surface lipids towards the adult pattern, i.e. wax esters begin to predominate over cholesterol and cholesterol esters [112]. Interestingly, acne development (predominantly comedonal) also begins at this time [93]. Rate of sebum excretion continues to increases in both sexes during adulthood, reaching a maximum between the ages of 26 and 40. Thereafter, the rate declines in both sexes, particularly in females [113].

FFA have long been implicated in the pathogenesis of acne vulgaris [114]. Acne patients are known to have a low sebaceous linoleic acid (LA, $C_{18}\Delta 9,12$) level [115], which returns to normal with a concomitant decrease in SER, after treatment with antiandrogens [116]. These results indicate that the proportion of LA (an essential fatty acid) in sebum is influenced by SER. In experimental animals, a low linoleate results in scaly skin and decreased epidermal barrier function [117]. A low sebaceous LA concentration has also been proposed to cause follicular hyperkeratosis and decreased barrier function in acne patients [118]. It is, therefore,

understandable that androgen-induced seborrhoea, in acne patients, may lead to a low sebaceous linoleate concentration as a consequence of increased SER, resulting in comedogenesis. Adding more weight to the LA theory is the fact that topical LA has been shown to cause a significant reduction in the size of microcomedones in acne patients [119].

2.3.3 Interleukin-1α

Results from essential fatty acid deficient mice have shown mRNA levels for epidermal IL-1α, IL-1β and TNF to be elevated several-fold over controls [120]. Disruption of the skin permeability barrier and the body's attempt to repair it was postulated to be responsible for this increase. In humans, the major IL-1 species produced by keratinocytes is IL-1α [121]. It is present in many comedones at levels that are likely to be biologically and pathologically relevant [122]. Furthermore, IL-1α has been demonstrated to cause hypercornification of the follicular infundibulum, which can be blocked by IL-1Ra [123]. Therefore, it is possible that low sebaceous LA levels, in acne patients, may cause disruption of the cutaneous permeability barrier of the pilosebaceous duct. This may lead to increased IL-1α production by the ductal keratinocytes which could result in comedogenesis.

In summary, it is highly probable that androgens may play an important role in comedogenesis. They may not only stimulate keratinocytes proliferation in the pilosebaceous duct via their receptors on the ductal keratinocytes but may also lead to seborrhoea by their direct and indirect action (via IGF-1) on the sebocytes. These two androgens mediated effects may, therefore, explain the development of comedones in acne patients. This may also explain the microbiological data which

suggests that comedogenesis can occur in the complete absence of *P. acnes* from the PSFs.

2.3.4 Potentiation of comedogenesis after *P. acnes* colonisation

As explicated in chapter 1, a favourable microenvironment of microcomedones may lead to a significantly greater colonisation of these lesions compared with the unaffected follicles. Moreover, it may also encourage production of extracellular enzymes by the micro-organisms. After colonisation, *P. acnes* can potentiate comedogenesis by various mechanisms. FFA in sebum, produced by the lipolytic action of *P. acnes* lipases on sebaceous triglycerides [33], have been found to be comedogenic in the rabbit ear model (a model for evaluating the comedogenic potential of a substance by its application to the external ear canal of rabbit) and suggests a possible mechanism by which *P. acnes* can influence comedogenesis [114]. However, it is important to note that the rabbit skin is ultrasensitive compared to the comedogenic potential of the human face. Because of this extreme sensitivity, mild or moderate comedogenic substances may have no clinical significance for human skin [124].

Propionibacterium acnes through its production of porphyrins may act as a catalytic agent in squalene oxidation [125]. Oxidised squalene has been found to be comedogenic in the rabbit ear model and this may be another possible mechanism by which the organism can be involved in comedogenesis [126]. It has also been proposed that *P. acnes* biofilm may act as a biological glue causing adhesiveness of keratinocytes, thus aggravating comedogenesis [127].

Propionibacterium acnes-stimulated keratinocytes have been shown to cause significantly higher production of IL-1α compared with unstimulated keratinocytes [128]. IL-1α, as mentioned above, has been shown to cause hypercornification of the follicular infundibulum and may therefore exacerbate comedogenesis in acne patients [123]. Ingham et al. [129], however, failed to demonstrate that cutaneous micro-organisms or their products directly up-regulated IL-1α release, *in vitro*, by the keratinocytes. Hence it is difficult to interpret these findings as supporting a comedogenic role for *P. acnes*.

Both viable and formalin-killed *P. acnes* have been shown to augment sebum production and accumulation by increasing diacylglycerol acyltransferase activity in hamster sebocytes, *in vivo* and *in vitro* [130]. It is, thus, understandable that *P. acnes*, by increasing lipogenesis, can further aggravate LA deficiency in acne patients and as a result may aggravate comedogenesis. Lastly, *P. acnes* has been shown to increase the expression of IGF-1 in the epidermis of human abdominal skin explant [131]. This can then activate IGF-1 receptor, mostly located in the basal layer of the epidermis and also induced by *P. acnes*, leading to proliferation and abnormal differentiation of the keratinocytes through a paracrine pathway [131]. This can potentially be another mechanism by which *P. acnes* can potentiate comedogenesis.

2.4 Inflammation

2.4.1 Subclinical inflammation

As detailed in chapter 1, *P. acnes* is probably not necessary for the initiation of inflammation in inflamed acne lesions. This leaves us with the question what exactly initiates inflammation, if not *P. acnes*? Compared with the control follicles obtained from non-acne patients, clinically normal skin of acne patients has been shown to have increased numbers of $CD4^+$ T helper cells and macrophages in the perifollicular and papillary dermis [132]. However, these cells were not activated and lacked expression of HLA (human leukocyte antigen)-DR. Furthermore, expression of vascular adhesion molecules (E-selectin and vascular cell adhesion molecule-1, VCAM-1) was also found to be up-regulated, with the dermal concentration of IL-1α reported to be three times higher in this clinically normal skin. Similarly, an increase in the concentration of IL-1α in the interfollicular and perifollicular epidermis and down the follicle wall was also found. All these changes occurred in the absence of ductal hyperproliferation and abnormal differentiation in this normal looking skin of acne patients [132]. These data suggest that subclinical inflammation exists in the acne prone areas, even if it appears clinically normal, in people suffering with acne and that these inflammatory changes precede comedogenesis.

Now what factors could be responsible for the subclinical inflammation in the clinically normal skin of acne patients? Based upon the results from essential fatty acid deficient mice model [120] it is possible that chronic barrier perturbation due to LA deficiecy in acne patients may lead to increased production and release of

proinflammatory cytokines i.e. IL-1α and TNF from keratinocytes in the follicular wall. The increased expression of IL-1α will not only contribute to comedogenesis but, along with TNF, may also result in the non-specific subclinical inflammation demonstrated in the normal looking skin of these patients. More recently, upregulation of IL-6 and TNF-α in cultured human sebocytes after addition of DHT has also been demonstrated [133]. As acne patients may have increased levels of circulating androgens and/or end-organ sensitivity to these hormones, the proinflammatory cytokines produced as a result of DHT action on sebocytes could also play an important role in the subclinical inflammation mentioned above.

2.4.2 Clinical inflammation

The transition from subclinical to clinical inflammation might depend on an imbalance between proinflammatory and anti-inflammatory pathways that can be activated as a result of this local stress (**Figure 2.4**).

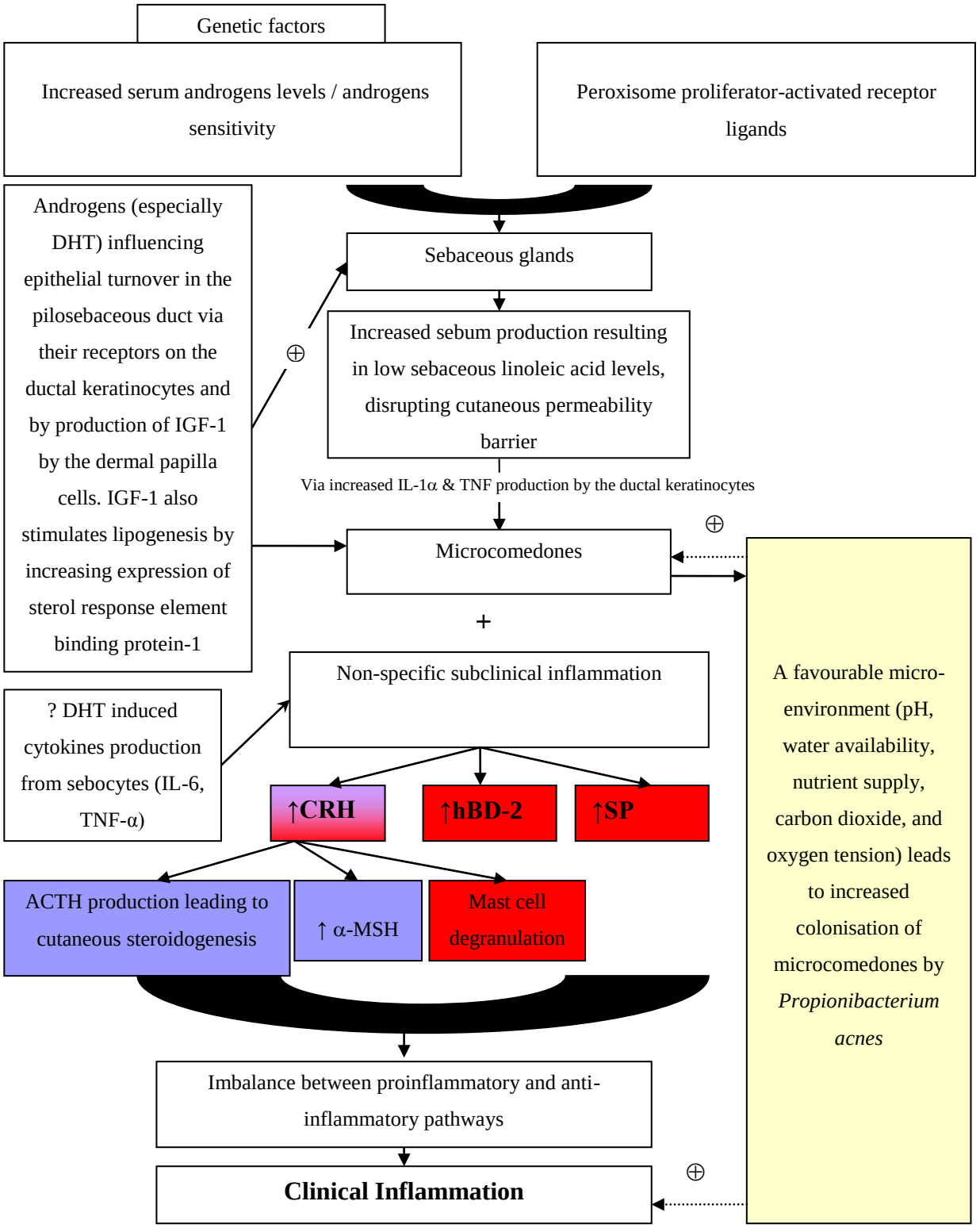


Figure 2.4: Proposed mechanism for the aetiopathogenesis of acne vulgaris and its association with *Propionibacterium acnes*. DHT, dihydrotestosterone; IL, interleukin; CRH, corticotropin-releasing hormone; MSH, melanocyte stimulating hormone; ACTH, adrenocorticotropic hormone; hBD-2, human beta- defensin-2; SP, substance P; TNF, tumour necrosis factor; IGF-1, insulin-like growth factor-1.

2.4.2.1 Activation of the cutaneous equivalent of the central hypothalamic-pituitary-adrenal axis

The hypothalamic-pituitary-adrenal (HPA) axis plays a crucial role in terminating the stress response and buffering tissue damage, in response to systemic stress [134]. This process involves production and release of corticotropin–releasing hormone (CRH) followed by production and secretion of proopiomelanocortin (POMC) derived peptides (adrenocorticotropic hormone, ACTH and α-melanocyte stimulating hormone, α-MSH). ACTH induces production and secretion of the powerful anti-inflammatory protein cortisol, which terminates the stress response and buffers tissue damage. The presence of CRH, its binding protein (CRHBP) and corticotropin-releasing hormone receptors type 1 (CRHR-1) and type 2 (CRHR-2) has been confirmed in human sebaceous glands, *in vivo*, suggesting that a complete CRH system exists in sebocytes [135]. Moreover, a functional CRH-POMC-corticosteroid axis organised similarly to the HPA axis has been demonstrated in epidermal melanocytes [136], dermal fibroblasts [137] and human hair follicles [138].

The CRH expression in the hypothalamic cells can be modulated by various proinflammatory cytokines (TNF-α, IL-1β and IL-6) [139]. These cytokines may also modulate its expression in the skin. Thus, the subclinical inflammation evident in the clinically normal skin of acne patients may lead to increased cutaneous production of CRH. Indeed, CRH expression has been found to be greatly increased in acne-involved skin compared with non-involved and normal skin [135]. As α–MSH peptides, produced locally, as a result of this CRH stimulation of the cutaneous CRH-POMC-corticosteroid axis also modulate the expression of its

receptor (melanocortin-1 receptor, MC-1R) [138], this may explain the increased expression of MC-1R in the sebaceous glands of lesional skin of patients with acne vulgaris [140].

Although CRH can induce cutaneous inflammation by causing mast cell degranulation [141], it might also act as an anti-inflammatory agent by increasing the local production of steroids. Likewise, α-MSH has also been shown to exert anti-inflammatory actions by inhibition of IL-1 mediated IL-8 secretion from SZ95 sebocytes [142] and by suppressing TNF-α and IL-1β gene expression following an ischaemic cerebral event in mice [143]. Furthermore, it has also been demonstrated to induce the production of IL-10 by human peripheral blood monocytes *in vitro* [144]. IL-10 is a regulatory cytokine that acts to harness the release of several proinflammatory cytokines [145].

In brief, activation of the cutaneous CRH-POMC-corticosteroid axis may activate both proinflammatory and anti-inflammatory pathways that when working in conjunction with other proinflammatory pathways (discussed below) may determine the development of a clinically inflamed lesion.

2.4.2.2 Substance P

Substance P (SP), another important proinflammatory neuropeptide [146], has also been found to be over expressed in dermal nerves around the sebaceous glands of acne patients [147]. Recently, IL-1α was found to up-regulate SP expression in the dorsal root neurons from mature rats [148]. This may be another proinflammatory

pathway that can be activated as a result of subclinical inflammation seen in the clinically normal skin of acne patients.

2.4.2.3 Human β-defensins

Defensins are small mammalian antimicrobial peptides that form an integral part of the innate immune system [149]. They have been divided into two main subtypes, α- and β-defensins. In humans, the α-defensins are found in granules of neutrophils and small intestinal Paneth cells whereas β-defensins are mainly expressed in epithelial tissues [149]. To date, six human β-defensins (hBD-1 to -6) have been identified [150-154]. Among them, hBD-1 to -3, are expressed in human skin [150-152]. Interestingly, hBD-1 is synthesised constitutively [155] whereas expression of hBD-2 and hBD-3 can be induced by micro-organisms and proinflammatory cytokines [151, 152].

In acne patients, hBD-2 is more strongly up-regulated than hBD-1 in the lesional skin when compared with healthy controls [156]. hBD-2 does not only possess antimicrobial activities (against Gram-positive and -negative bacteria but not *P. acnes*) [157, 158] but also acts as a chemoattractant for mast cells [159] and can induce histamine release and prostaglandin D_2 production from these cells as well [160]. Moreover, hBD-1 and hBD-2 are also chemotactic for immature dendritic cells (DCs) and memory T cells via the chemokine receptor CCR6 [161]. hBD-2, like its murine counterpart, may further activate these chemoattracted dendritic cells via the TLR-4, leading to the production of proinflammatory cytokines IL-1α, IL-1β, IL-6, and IL-12 [162]. Lastly, hBD-2 can chemoattract TNF-α treated neutrophils via the CCR6 receptor [163]. Proinflammatory cytokines e.g. TNF-α

and IL-1 have been shown to modulate hBD-2 expression and, therefore, may explain its up-regulation in acne lesions [151, 164].

It is evident that various proinflammatory and anti-inflammatory pathways may be activated as a result of subclinical inflammation, seen in the clinically normal skin of acne patients. It is possible that an imbalance between these pathways may lead to the development of clinical inflammation in inflammatory acne. Interestingly, an *in vitro* study has shown that acne patients produce significantly less IL-10 from human peripheral blood monocytes, in response to *P. acnes* stimulation, as compared with healthy controls [165]. It is possible that the production of IL-10 by monocytes, in response to α-MSH, in acne patients may also be impaired: this may be one of the anti-inflammatory pathways that can be defective in these patients.

Our proposed mechanism signifies that the presence of *P. acnes* in PSFs is not necessary for the development of clinical inflammation in acne patients. This may therefore explain the sterility or absence of *P. acnes* from a significant proportion of inflamed lesions within acne patients as reported by various investigators **(see Chapter 1)**.

2.4.3 Potentiation of inflammation by *P. acnes*

As explained above, clinical inflammation in an acne lesion may develop in the complete absence of *P. acnes* from the PSFs. However, micro-organisms (from colonised follicles) may further intensify this inflammation. Spongiosis of the PSF wall has been demonstrated to be an early feature of acne papules [82]. This may alter the permeability of the follicular wall. One outcome of this may be the

diffusion of bacterial products (from colonised follicles) into the dermis, therefore, intensifying the inflammatory response. This may explain why neutrophils are seen predominantly in papules present for 24 hours and not at the initiation of inflammation [82]. *Propionibacterium acnes* and/or its products, after being released into the dermis, may intensify the inflammatory process by its antigenic [23, 58, 166], enzymatic [167], chemoattractant [168, 169], and complement activation [170, 171] activities. Furthermore, *P. acnes'* secretory protein called Christie-Atkins-Munch-Peterson factor and acid sphingomyelinase, which is released from the host cells in the presence of *P. acnes*, are cytotoxic to keratinocytes and macrophages *in vitro* [172]. *Propionibacterium acnes* can also lead to the formation of reactive oxygen species (ROS), especially superoxide anions, by activation of the scavenger receptor CD36 on keratinocytes [173]. These may be other potential mechanisms explaining the involvement of *P. acnes* in inflammatory acne.

2.4.3.1 *P. acnes* and toll-like receptors

TLRs are a family of pattern recognition proteins that recognise pathogen-associated molecular patterns (PAMPs), which represent conserved molecular features of a given microbial class [174]. To date, ten functional TLRs (TLR-1 to -10) have been identified in humans [174]. Upon activation, TLRs mediate their effects by activation of the transcription factor, nuclear factor-kappaB (NF-κB), and mitogen-activated protein kinases (MAPKs) [174]. Activation of NF-κB leads to transcriptional induction of several cytokines/growth factors (e.g. IL-1β, TNF-α, IL-6, IL-10, granulocyte/macrophage colony-stimulating factor), chemokines (e.g. IL-8), adhesion molecules (e.g. VCAM-1), and hBD-2 genes [175-177]. TLRs are expressed on monocytes/macrophages (TLR-1, -2, -4, -5) [178], neutrophils (TLR-1,

-2, -4, -5) [178], DCs (TLR-1 to -5, -7 to -9) [179], and SZ95 sebocytes (TLR-2 and -4) [180]. Moreover, the expression of TLR-2 and TLR-4 in normal human epidermis [181] and TLR-2 expression on macrophages surrounding the PSFs in acne patients [182] has been demonstrated *in vivo*.

The main ligand for TLR-4 is lipopolysaccharide (a component of the outer membrane of Gram-negative bacteria) [183] whereas TLR-2 recognises a broad range of PAMPs including peptidoglycan and lipoteichoic acid from Gram-positive bacteria [184], bacterial lipoprotein/lipopeptide [185], yeast cell wall particle zymosan [186], mycobacterial lipoarabinomannan [187], and glycosylphosphatidylinositol anchors from protozoan parasites [188]. Intriguingly, both these receptors can also recognise various endogenous ligands including heat-shock protein (HSP) 60 and 70 [189, 190].

Compared with normal controls, TLR-2 is more strongly up-regulated than TLR-4 in the inflamed lesions of acne patients [181]. *Propionibacterium acnes* has been shown to up-regulate TLR-2 and TLR-4 expression in cultured keratinocytes, *in vitro*, and may therefore provide one possible explanation for their up-regulation in acne patients [181]. This *in vitro* data, however, does not mean that the up-regulation of these TLRs is caused solely by *P. acnes* as their expression can also be modulated by proinflammatory cytokines e.g. IL-1β, TNF-α and interferon (IFN)-γ [178, 191, 192]. Certain strains of *P. acnes* have been shown to induce the expression of hBD- 2 and IL-8 in cultured keratinocytes via both TLR-2 and TLR-4 activation *in vitro* [193]. Likewise, increased production of IL-12 and IL-8 by TLR-2 activation of primary human monocytes by *P. acnes* has been demonstrated

[182]. Activation of TLRs by various *P. acnes* associated ligands (e.g. peptidoglycan, GroEL (bacterial HSP60) [194], DnaK (bacterial HSP70) [194], lipoteichoic acid) may also explain other reports of increased production of proinflammatory cytokines/chemokines by keratinocytes (IL-1α, TNF-α, and granulocyte/macrophage colony-stimulating factor) [128], peripheral blood mononuclear cells (IL-1β, TNF-α, and IL-8) [195], and SZ95 sebocytes (CXCL8, synonymous with IL-8) [196] after *P. acnes* stimulation. It is therefore plausible that induction and activation of TLRs by *P. acnes* and the resultant production of hBD-2 (an antimicrobial peptide containing proinflammatory properties) and all the other proinflammatory cytokines/chemokines mentioned above, may also intensify inflammation in acne patients.

2.4.4 Potentiation of inflammation by other intrafollicular contents

Antibodies to keratin intermediate filament proteins have been demonstrated in the sera of healthy individuals as well as patients with various cutaneous diseases [197]. It is conceivable that these antibodies may also potentiate inflammation following altered follicular permeability/rupture. After rupture of the duct it is likely that other intrafollicular contents like FFA, keratin and hairs may also contribute to the inflammation.

2.5 Resolution of acne

This can be considered in the context of resolution of individual lesions and the disease as a whole. Comedones are temporary structures [79]. Based upon changes

in the expression of Ki-67 and K16, it has been postulated that comedones undergo cyclical growth [198]. Cunliffe et al. [79] considered this cycling to be important in the development and resolution of comedones. Downie et al. [199], on the other hand, demonstrated that IL-1α and other cytokines (e.g. TNF-α and IFN-γ) can inhibit lipogenesis and induce de-differentiation of human sebocytes into a keratinocyte-like morphology *in vitro*. This implies that IL-1α may not only lead to comedogenesis but can also modulate its resolution-albeit temporarily. Factors associated with acne resolution have been minimally researched and remain obscure. Limited data suggest that the resolution is not related to reduction of sebum production as the SER amongst individuals with a past history of acne remains significantly higher compared with age-matched controls [200].

2.6 Conclusion

Androgens, sebaceous lipid abnormalities, key cytokines such as IL-1α, cutaneous CRH-POMC-corticosteroid axis, SP, and hBDs may play an important role in the aetiopathogenesis of noninflamed and inflamed acne lesions. *Propionibacterium acnes*, by virtue of its various pathogenetic factors, may aggravate both these lesions **(Figure 2.5)**. Targeting *P. acnes* or one or more of the above factors may result in a clinical improvement in acne patients.

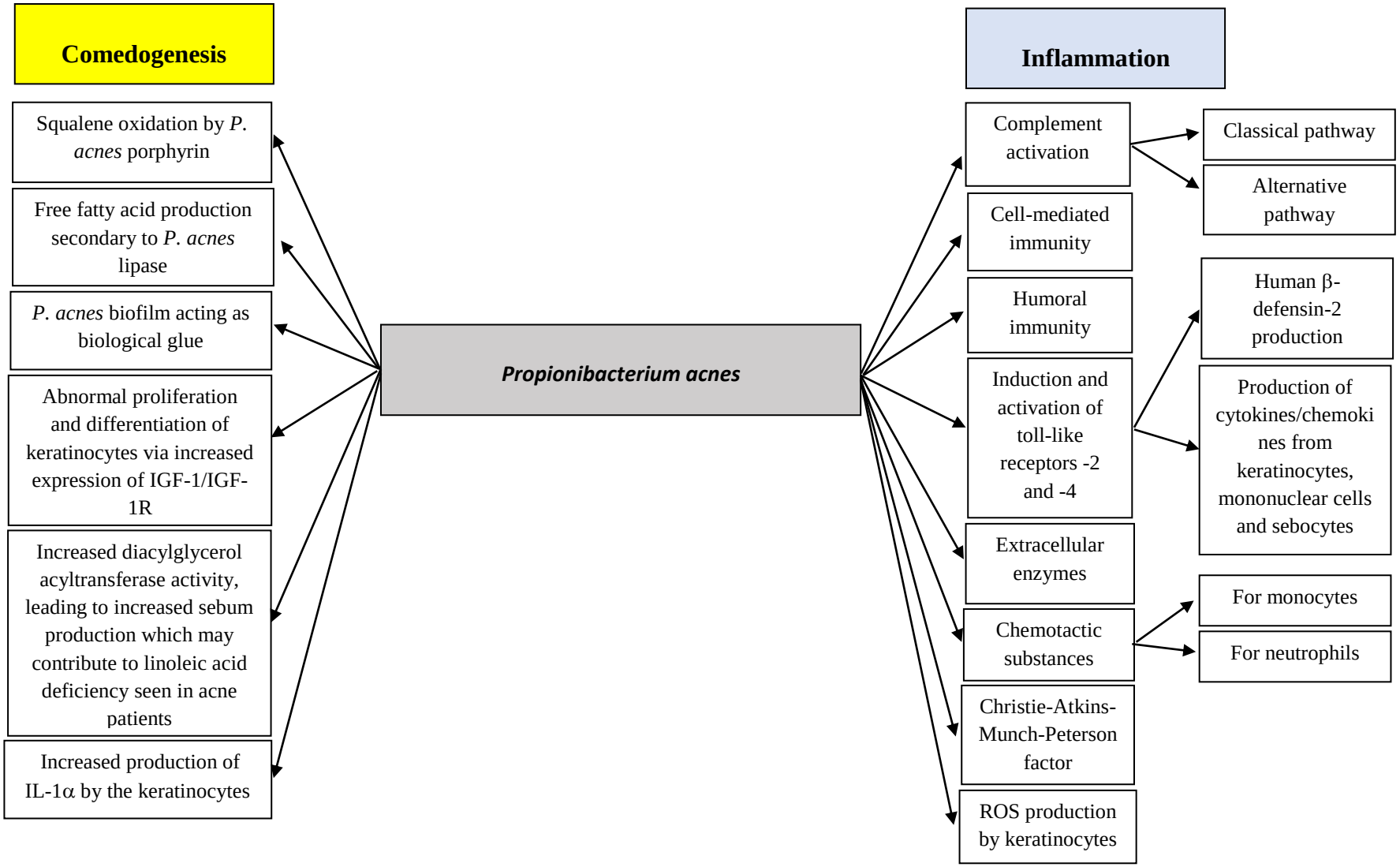


Figure 2.5: Possible ways of involvement of *Propionibacterium acnes* in comedogenesis and inflammation in acne. IL, interleukin; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor.

Chapter 3

BACKGROUND 3: Effect of visible light on the microbiology of acne

3. Effect of visible light on the microbiology of acne

3.1 Introduction

Light can work in various skin conditions by its photothermal, photochemical, photomechanical, and/or photoimmunological effects. Photothermal effects denote tissue damage secondary to generation of heat after absorption of photons of appropriate wavelengths by specific molecules, termed chromophores [201]. Absorption profiles of the key skin chromophores (e.g. melanin, haemoglobin, and water) are given in **Figure 3.1**. Selective photothermolysis refers to photothermal destruction of a target without causing damage to the adjacent tissues and depends on the following three variables: (i) a wavelength that is selectively absorbed by the target; (ii) a pulse duration that matches the thermal relaxation time (TRT; time required to lose 50% of the acquired heat) of the target; and (iii) delivery of sufficient energy to damage the target [202]. Photochemical effects occur when the light wavelength matches the absorption characteristics of a chromophore (endogenous or exogenous) within the tissue and produces a chemical response without a noticeable temperature rise. A common example of the use of an exogenous chromophore for photochemical effects is photodynamic therapy (PDT) [201]. Photomechanical effects require a very high power, short duration pulse (e.g. nanoseconds). Sufficiently high power densities remove electrons from the absorbing medium and produce a state of ionised matter known as plasma. The collapse of the plasma produces a shock wave which leads to fragmentation of the absorbing particle. These photomechanical effects are exploited in tattoo dye pigment destruction [201]. Light-induced immunological effects refer to the immunomodulatory consequences of light on cytokines and cells of immune system [203, 204]. An increase in transforming

growth factor (TGF)-β1 mRNA expression after nonablative pulsed-dye laser (PDL) therapy is one example of such effect [205]. Depending on the condition being treated, one or more of these light-tissue effects may contribute towards the success of this treatment modality.

Light-based therapies for acne are becoming increasingly popular in dermatology. A number of laser and non-laser light sources have been used for this purpose. Two systematic reviews of 19 and 25 trials, respectively, concluded that optical treatments can improve acne in the short term, with the most consistent outcomes for PDT [206, 207]. Comparing laser and non-laser light sources, the latter are advantageous in dermatology as they are portable, cheaper to purchase and maintain, and have a large illumination field [208].

The rest of this chapter introduces intense pulsed light (IPL) and 5-aminolaevulinic acid-based PDT followed by a discussion on the possible mechanisms of action of IPL/IPL-assisted PDT in acne, with emphasis predominantly on their anti-*P. acnes* effects.

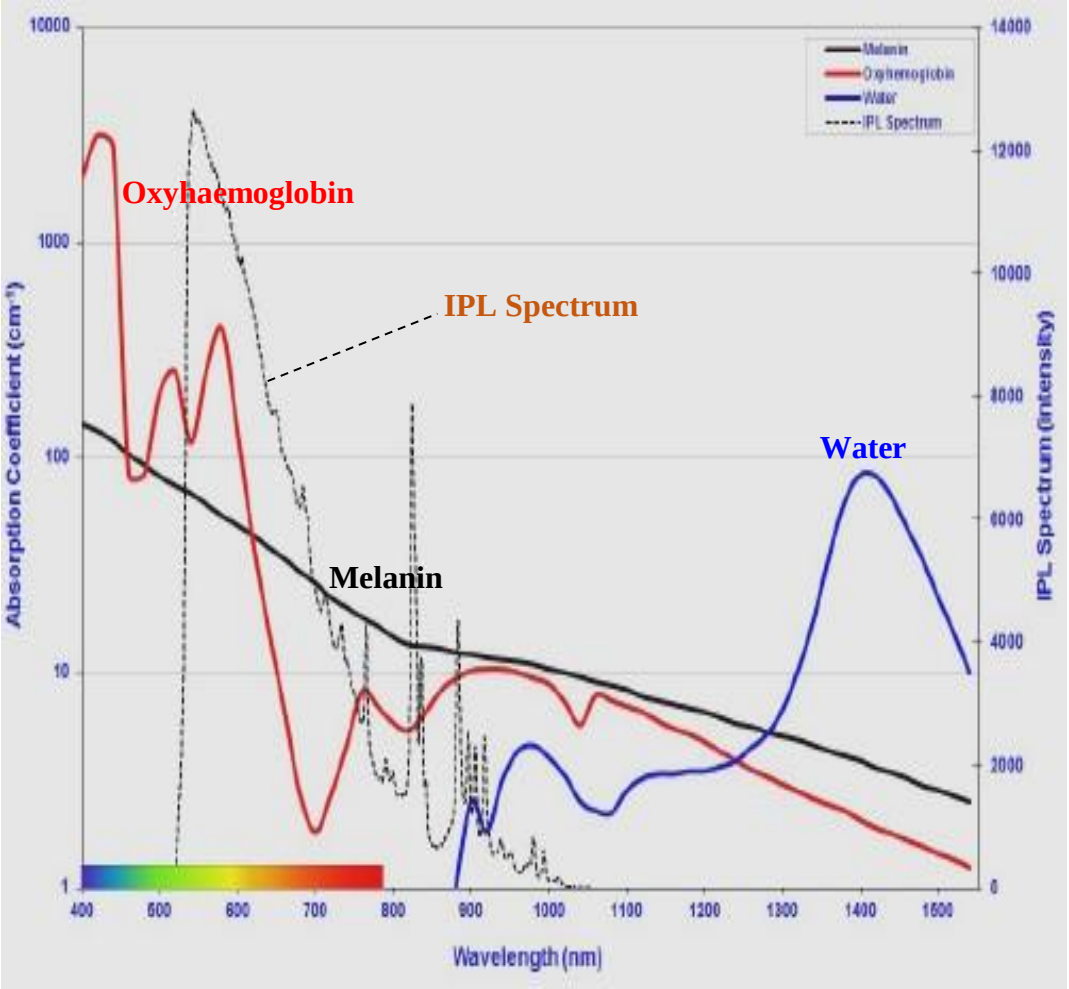


Figure 3.1: Absorption coefficients of melanin, oxyhaemoglobin, and water. The emission spectrum of an IPL device has been overlaid. *Adapted from Ash et al. [209]*.

3.2 Intense pulsed light

IPL is a device that uses flashlamps and computer-controlled capacitor banks to generate pulsed polychromatic (500-1300 nm) high-intensity light [210]. Electrical energy stored in the capacitor bank is passed through xenon gas within a flashlamp leading to emission of bright light [210]. With the aid of different cut-off filters, a wide range of wavelengths are possible for IPL devices. A typical circuit diagram for a flashlamp power supply is given in **Figure 3.2**.

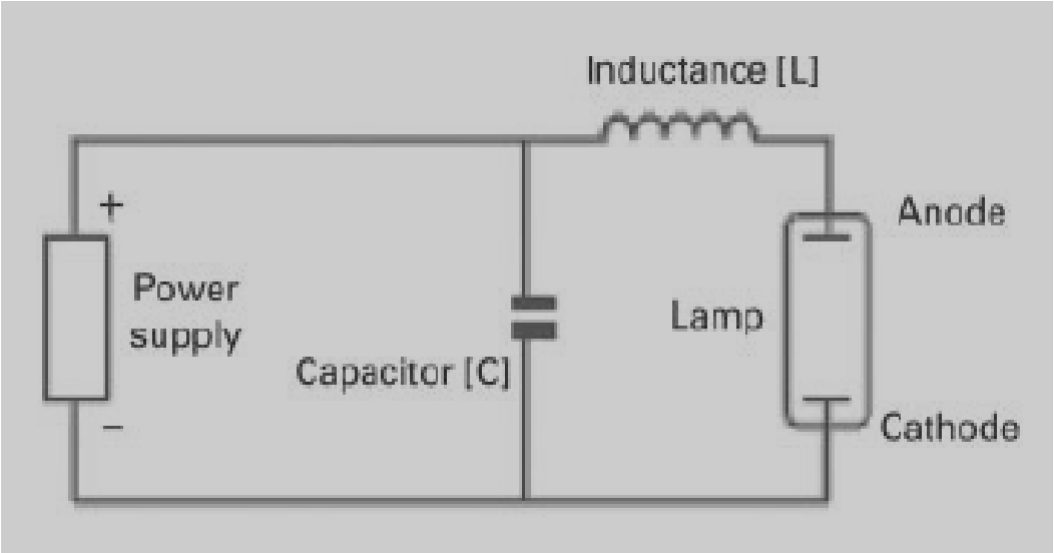


Figure 3.2: Circuit diagram for a flashlamp power supply. *Adapted from Ash et al. [211]*.

Based upon the method used to generate and deliver the energy, IPL devices can be classified into free discharge and constant current systems [211]. A free discharge system produces a rising/falling discharge slope as it applies a large electrical charge to the capacitor which then releases the entire stored energy directly through the flashlamp **(Figure 3.3a)**. Most free discharge systems are unable to generate true long pulse durations to match the TRT of the target structure (e.g. hair follicle with a TRT of 25-55 ms) to cause selective photothermolysis [211]. Ash et al [211] found that in a free discharge system with a measured pulse duration of 15-17 ms, most of the useful energy was concentrated in only 3-4 ms while rest consisted of low energy infrared radiation. In order to resolve this problem, most free discharge IPLs deliver a train of shorter, high energy sub-pulses with variable on and off times to generate an average energy density (synonymous with fluence and defined as energy delivered per unit area measured in joules (J) cm^{-2}) and overall pulse duration in the range of the TRT of the target chromophore **(Figure 3.3b)**. This, however, does not produce stable consistent optical energy and the intensity of the spectral output tails off

towards the end of the set of sub-pulses due to capacitor depletion. This may lead to sub-optimal clinical effects. Moreover, with too short a pulse duration a high proportion of the total energy will be delivered as shorter wavelengths which leads to epidermal heating necessitating use of cooling devices [211].

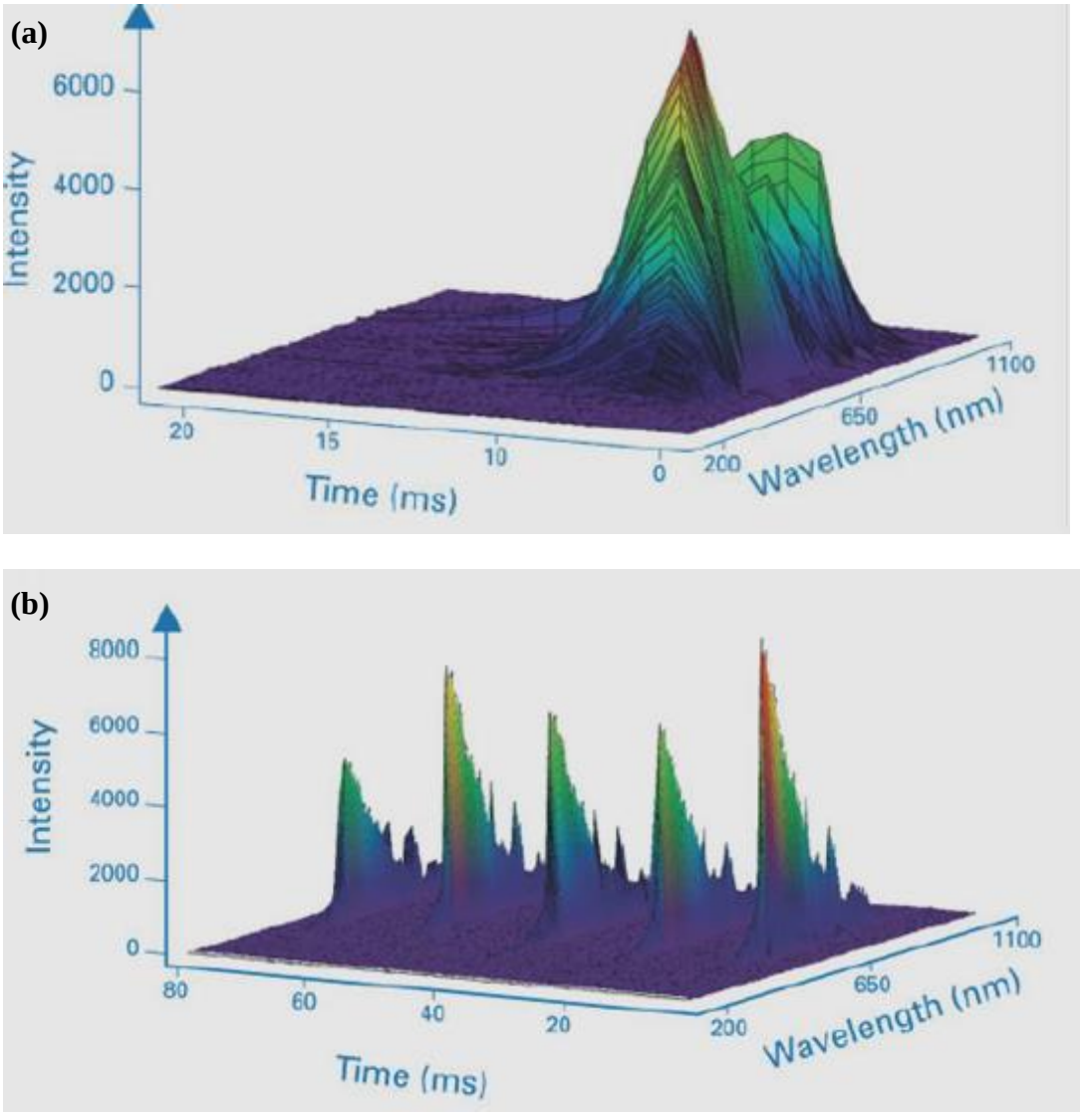


Figure 3.3: Time-resolved spectral output images of a single pulse and multi-pulse free discharge IPL device **(a)** A single pulse IPL device (*Chromolite*, Chromogenex Ltd) spectral image with a measured total pulse duration of 17 ms. Note the bulk of the useful spectral output concentrated into only a few ms **(b)** A multi-pulse free discharge IPL device (*Lumina600*, Lynton Lasers Ltd) spectral image producing long total pulse duration but with considerable spectral output shifts within the pulse train. *Adapted from Ash et al. [211].*

A constant current IPL system differs from the free discharge system in the way in which the electrical energy is delivered from the capacitor to the flashlamp. In this system the electrical energy is delivered to the flashlamp, via a partial discharge capacitor, at a constant level throughout the pulse creating a square pulse discharge profile **(Figure 3.4)**. This means that the optical energy is released throughout the duration of the pulse with only slight attenuation towards the end [211]. Significantly lower energies can be used to obtain equivalent clinical results compared with free discharge systems and if combined with appropriate cut-off filters the need for skin cooling may be obviated [211].

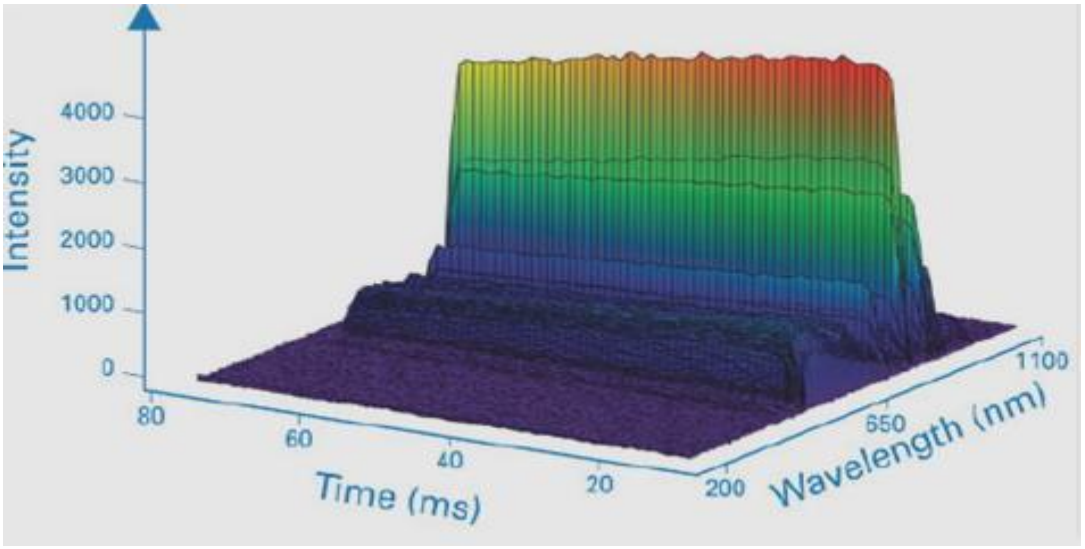


Figure 3.4: Time-resolved spectral output image of a single pulse constant current IPL device (*iPulse*, Cyden Ltd), with a measured total pulse duration of 50 ms, producing a constant optical discharge throughout the pulse. *Adapted from Ash et al. [211]*.

By a combination of different wavelengths and other treatment parameters e.g. number and duration of pulses, delay between pulses, and fluence an IPL device can be adjusted according to the target structure, its depth, and the patient's skin type [210]. This versatility makes IPL an ideal device for a number of indications

including hair removal, rosacea, telengiectasias, pigmented lesions (e.g. melasma, lentigines), and port-wine stains [210]. For similar reasons it can be a useful addition to the therapeutic armamentarium for acne vulgaris.

Side-effects of IPL treatment include pain during irradiation, transient erythema (common, starting immediately after treatment and may last for few hours up to 96 h), oedema (may last up to 5 days), purpura, blistering, postinflammatory hyperpigmentation (PIH), and crusting [212-215]. Scarring occurs rarely and is almost always induced by overfluenced treatment or by crusting with subsequent scratching leading to bacterial infection [210]. Pain during irradiation can be helped by skin cooling [210].

3.3 5-Aminolaevulinic acid-based PDT

PDT (an oxygen-dependent tissue reaction following photosensitisation and subsequent irradiation with light) [216] is also an exciting area of research for the treatment of acne. 5-aminolaevulinic acid (ALA) and methyl aminolaevulinate (MAL) are commonly used topical precursors of porphyrins for PDT in dermatology and will be briefly discussed here. MAL is a methylated ester of ALA that is hydrolysed to ALA before being converted to protoporphyrin IX (PpIX) via the haem metabolic pathway [217]. ALA is not itself a photosensitiser; PpIX is the active photosensitiser in ALA/MAL-PDT [217].

3.3.1 Haem biosynthetic pathway

The rate-limiting step of haem biosynthetic pathway is the conversion of glycine and succinyl-coenzyme A to ALA by the enzyme ALA synthase. This is controlled by haem via a negative feedback mechanism [218]. The final step of this pathway involves the incorporation of iron into PpIX, in the mitochondria, by ferrochelatase enzyme **(Figure 3.5)** [219]. Addition of exogenous ALA, thus bypassing the rate-limiting step, leads to accumulation of PpIX due to the limited capacity of ferrochelatase [219]. PpIX is a highly active photosensitising and fluorescent compound that accumulates selectively in certain tumours [218] as well as in the sebaceous glands, hair follicles, and the epidermis [220]. The body clears PpIX within 24-48 h, minimising the period of photosensitisation [216].

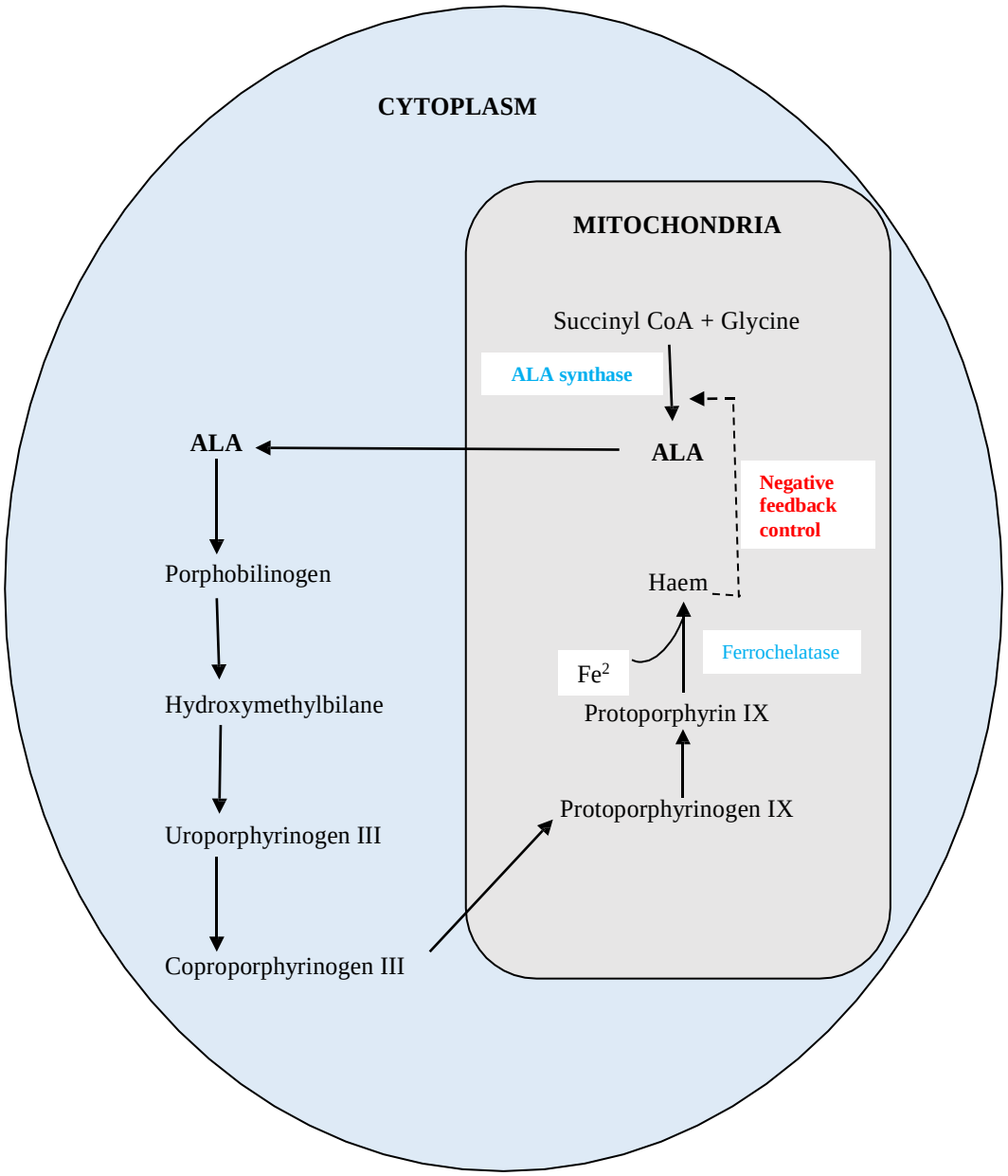


Figure 3.5: The haem metabolic pathway. Exogenous application of 5-aminolaevulinic acid (ALA) overrides the negative feedback control with accumulation of protoporphyrin IX. *Adapted from Peng et al. [219], Sakamoto et al. [221], and Kennedy and Pottier [218].*

3.3.2 Light sources

Following bioconversion of ALA to PpIX, the latter can be activated by light of an appropriate wavelength. Porphyrins are tetrapyrrole molecules, with peak absorption in the blue light spectrum, the Soret band (405-415 nm). Moreover, weaker absorption bands (Q-bands) also exist in the green (~506 nm to ~540 nm), yellow (572-582 nm), and red spectra (628-635 nm) [217]. In the visible light spectrum, scattering and absorption generally decrease with increasing wavelength. Hence, although the strongest porphyrin absorption is in the blue spectrum, blue light penetrates poorly into the dermis [217]. In contrast, wavelengths within the yellow–red range penetrate deeper (230-750 μm) [222] and can be advantageous for PDT in acne vulgaris, as sebaceous glands that are located approximately 0.5-1.0 mm from the cutaneous surface [223] can be effectively targeted.

A range of irradiation sources is available for use in PDT, including laser (e.g. argon laser, Nd:YAG laser, diode laser) and non-laser (e.g. tungsten filament halogen lamp, xenon arc lamp, metal halide lamp, light-emitting diode) light sources [208]. The reasons why incoherent light sources are preferable in dermatology have been mentioned above i.e. large illumination field, portability, and low cost. All these advantages along with the high versatility and short exposure time for IPL makes it a favoured device for PDT.

3.3.3 PDT photochemistry and mechanism of action

The chemical activation energy for PDT is provided when a photosensitiser absorbs a photon of light and is promoted from the ground state to the excited singlet state [224, 225]. The half-life of the singlet excited molecule is extremely short (10^{-6} to

10^{-9} seconds). Therefore, it either returns back to the ground state (resulting in fluorescence) or undergoes a spin conversion, via intersystem crossover, to the more stable and longer lived (10^{-3} seconds) triplet excited state [224]. The excited triplet can undergo type I and/or type II photochemical reactions with the surrounding molecules [224, 225].

Type I pathway involves the direct interaction of an excited triplet photosensitiser with a biomolecule, by electron or hydrogen atom transfer, producing radical forms of the substrate and/or photosensitiser [224]. These intermediates may react with molecular oxygen to form peroxides, superoxide ions, and hydroxyl radicals, which initiate free radical chain reactions [224]. Type II mechanism is mediated by an energy transfer process with spin-matched ground state molecular oxygen, leading to the formation of singlet oxygen ($^1O_2$) and to the return of sensitiser to its ground state [226]. The Type I pathway is highly concentration dependent, and plays a minor role in PDT due to the competition between substrates and oxygen for triplet photosensitisers [224].

$^1O_2$ is considered to be the primary chemical intermediate of PDT [224, 225] that reacts with lipids, proteins, and nucleic acids leading to cellular apoptosis [227, 228], necrosis [229], microvascular compromise [230, 231], increased expression of various proinflammatory cytokines (e.g. IL-1, IL-6, and TNF) [232-234], immunoparesis [235, 236] and hyperimmunity [237, 238].

3.3.4 Variables affecting PDT results and side-effects

Treatment variables that may affect PDT results and side-effects include the choice of photosensitiser and its concentration as well as contact time, temperature of the area being treated, wavelength(s) of light used in the treatment, its fluence and irradiance (the rate at which energy is delivered per unit area measured in W m^{-2} or mW cm^{-2}), and the availability of molecular oxygen at the target site. The importance of molecular oxygen at the target site in PDT is evident from the fact that a reduction in oxygen level from 5% (normal tissue level) to 1% leads to a 50% reduction in PDT effect [239].

Compared with ALA, MAL has been shown to be more lesion specific [240, 241], penetrates deeper [242, 243], and is associated with less severe side-effects [241]. As lower concentrations of photosensitiser have shallower depths of penetration [243] and may not effectively destroy deeper structures, higher concentrations are advantageous in PDT. Moreover, PpIX production is related to the contact time of the ALA/MAL with the skin and higher amounts are formed with long application times [240, 244]. However, comparing long (3 h) [241, 245-247] with short (30-60 min) [248, 249] application times, the latter are associated with milder side-effects and lower patient drop-out rate and may therefore be preferable.

The advantage of using longer wavelengths of light has been discussed above **(Section 3.3.2)**. There is a wide variation in the light dose and irradiance (fluence rate) used in PDT. Light delivery at lower irradiance is preferable as it results in an enhanced photodynamic effect due to tissue re-oxygenation [250, 251]. As mentioned previously, light absorption also leads to heat generation. Generally,

fluence rates above 150 mW cm^{-2} will give hyperthermia [252]. Mild hyperthermia (41.5-42.5 °C) can increase tumour pO_2 by two to threefold and may be useful in PDT [253].

3.3.5 Adverse effects

Adverse effects after ALA/MAL-PDT for acne include varying degree of pain (very common and helped by skin cooling), erythema (common, starting immediately after treatment and may last for 1-2 h up to 20 weeks), oedema (starting immediately after treatment and may last up to 7 days), blistering (rare), acute acneiform eruption (papules, pustules, nodules starting 3-4 days post-treatment, lasting 4 days to 3 weeks, after high fluence red light PDT with 3 h ALA incubation), exfoliation, sterile pustular eruption (starting on the second or third day post-treatment, lasting 3 days, after 3 h MAL/ALA incubation), crusting (developing a few days after treatment), pruritus, and PIH (lasting 4-32 weeks) [241, 245-249, 254-258]. Scarring is very rare and has only been reported once [241].

3.4 Possible mechanisms of action of IPL/IPL-assisted PDT in acne

As mentioned in chapter 1, it is known that *P. acnes* produces porphyrins, particularly coproporphyrin III [13]. Light (of appropriate wavelength) can activate these porphyrins, producing ROS, which may lead to bacterial destruction. There is *in vitro* data to support this hypothesis [14-16]. The addition of ALA increases endogenous porphyrin production by *P. acnes* and *in vitro* data shows that this augments its photodynamic eradication [14, 16]. Based upon these data and the

various possible ways by which *P. acnes* may influence acne **(Chapter 2, Figure 2.4)**, it is believed that this bactericidal effect is responsible for the beneficial outcome of light treatment in acne patients. As the published observations on the specific effect of IPL or IPL-assisted PDT on the microbiology of acne are few, the rest of this chapter describes studies investigating the effect of incoherent light (pulsed and nonpulsed), including PDT, on *P. acnes* colonisation/density vs. clinical efficacy. This will be followed by other possible ways by which IPL or IPL-assisted PDT may improve acne and, again, will be discussed in the context of known effects of pulsed and nonpulsed incoherent light on various pathogenetic factors for acne.

3.4.1 Microbiological effect vs. clinical efficacy of incoherent light (including PDT) in acne

The current literature surrounding the microbiological effect vs. clinical efficacy of incoherent light therapy in acne will be discussed under the various methods that were adopted by various investigators to determine the outcome of light treatment on *P. acnes* colonisation/density **(Table 3.1)**.

3.4.1.1 Studies on the efficacy of incoherent light and assessment of its effect on *P. acnes* colonisation via culture and polymerase chain reaction

Omi et al. [259], in their attempt to assess the efficacy of phototherapy and its effect on cutaneous microflora, carried out bacterial cultures (from acne comedones or cysts) and polymerase chain reaction (from skin surface; no detail given about the sequenced gene) in acne patients, treated with a high-intensity blue light (410-420

nm, irradiance 200 mW cm^{-2}, fluence not stated) twice weekly for 4 weeks. Moreover, ultrastructural changes in the dermis and sebaceous glands were also examined, on skin biopsies, in 8 patients after 4 treatments via electron microscopy. They reported a 64.7% improvement in acne lesions after 8 sessions of light therapy. No change in *P. acnes* colonisation (in comedones/cysts or skin surface) was detected pre- and post-treatment; however, damaged *P. acnes* was observed at the ultrastructural level in one of their eight cases. Although, an interesting study, looking at the density rather than colonisation of *P. acnes* pre- and post-treatment might have yielded more information about the possible mechanism of action of blue light in acne. A significant reduction in the density of *P. acnes* could have partly explained the beneficial effect of this treatment modality in these patients. This shortcoming of the present study was remedied by a number of other investigators who tried to correlate the clinical efficacy of visible light therapy with its effect on the density of *P. acnes*.

3.4.1.2 Studies on the efficacy of incoherent light and indirect assessment of its effect on *P. acnes* density via porphyrin fluorescence

Porphyrins produced by *P. acnes* give rise to orange-red fluorescence seen under Wood's light [260]. The intensity of this fluorescence has been shown to be proportional to the *P. acnes* population [261]. Digital fluorescence photography has been used to evaluate the suppressive effects of anti-acne agents on *P. acnes [262]* and, therefore, various investigators have used this technique to investigate the possible mechanism of action of optical therapies in acne.

Hongcharu et al. [245] found red light-assisted ALA-PDT (550-700 nm, fluence 150 J cm^{-2}) to cause a statistically significant improvement in inflammatory acne for 10 weeks after a single treatment and for at least 20 weeks after multiple treatments. Concomitantly, a significant reduction in porphyrin fluorescence was noticed for 20 weeks in both the groups. The authors concluded that a reduction in *P. acnes* counts may be one of the potential mechanisms by which ALA-PDT can improve acne. Similarly, Tzung et al. [263] found blue light (420 ± 20 nm, fluence 40 J cm^{-2}) to be effective in the management of mild to moderate acne in their Taiwanese cohort; however, therapeutic effectiveness was reported not to be related to the change in fluorescence intensity. It is though prudent to mention here that no information was provided about post-treatment fluorescence intensity in the published study and it is difficult to ascertain whether or not there was any change from the pre-treatment value. The authors concluded that blue light has as yet unknown effects, in addition to *P. acnes* killing, by which it can improve acne. However, no evidence for *P. acnes* killing by the blue light was provided.

Subsequently, Yeung et al. [264] in a single-blind, randomised controlled, split-face study treated patients with moderate inflammatory acne with full-face 0.1% adapalene and half-face IPL (530-750 nm, fluence 7-9 J cm^{-2}) +/- MAL-PDT. Neither MAL-PDT nor IPL was found to significantly improve inflamed acne lesions; however, a significant improvement in noninflamed lesions was seen in the MAL-PDT (38%) and IPL (44%) groups at 12 weeks after the last treatment. Moreover, the investigators did not find a significant difference in porphyrin fluorescence between the three groups. As no statistically significant improvement in inflamed acne lesions was found in the IPL or MAL-PDT groups, it is difficult to

predict whether this lack of efficacy was due to inability of these optical therapies to kill *P. acnes* or insufficient anti-inflammatory/sebostatic effects of the IPL parameters used in this trial.

It is difficult to compare the results of above-mentioned studies because of methodological differences. It can be argued that ALA-PDT using red light at a high fluence (150 J cm^{-2}), as used by Hongcharu et al. [245], may lead to a reduction in porphyrin fluorescence (an indirect assessment of *P. acnes* density) and can improve acne. However, it is important to mention here, that by using bacteriologic culture, *P. acnes* has been shown to repopulate very quickly (within 10 days) after stopping benzoylperoxide (BPO) [262]. The fact that Hongcharu et al. [245] found a significant reduction in porphyrin fluorescence for 20 weeks after ALA-PDT is therefore surprising and casts doubt on the validity of these results. Bacterial counts from cultures would have been more informative in drawing conclusions as to the role of ALA-PDT using red light at the above fluence on *P. acnes* density.

3.4.1.3 Studies on the efficacy of incoherent light and assessment of its effect on *P. acnes* density via propionibacteria culture

The specific quantitative measurement of *P. acnes* on the skin is more likely to be accurate than the indirect assessment by porphyrin fluorescence and has been used by various investigators to elucidate the possible mechanism of action of optical therapies in acne. Shalita et al. [265] treated 10 acne patients (forehead or cheek) with twice weekly visible light in the violet-blue range (407-420 nm, irradiance 90 mW cm^{-2}, fluence not stated). Cultures for *P. acnes* were taken from both the treated and untreated symmetric area at baseline and after the 2^{nd}, 4^{th}, and 6^{th} treatment. Only

patients with a baseline *P. acnes* density of > 10^5 colonies cm^{-2} (6 out of 10 patients) demonstrated a significant reduction in *P. acnes* counts after the 6^{th} treatment. The authors did not correlate this information with the clinical outcome in this cohort, however, reported a 68% mean reduction in inflamed lesion counts after 8 bi-weekly light treatment (407-420 nm, irradiance 90 mW cm^{-2}, fluence not stated) to the face or back of 35 other patients with mild to moderate papulo-pustular acne. Although a frequently cited study (with at least 35 citations), attributing the efficacy of blue light phototherapy to its anti-*P. acnes* effects, the results should be interpreted with caution due to the fact that essential information about methodology (including details about ethical approval, recruitment, inclusion/exclusion criteria, and sampling as well as culturing technique for *P. acnes*) and the statistical tests used was not given. Moreover, information about clinical outcome in patients who showed a reduction in *P. acnes* density vs. those who did not would have been more helpful in determining whether the reported anti-microbial effect of blue light therapy was necessary for its clinical efficacy or not.

Horfelt et al. [256], in his attempt to determine the optimal light dose and mechanism of action of red light-assisted ALA-PDT (600-730 nm, fluences 30, 50, and 70 J cm^{-2}, irradiance 50 mW cm^{-2}) in patients with mild to severe acne demonstrated that the lower light dose is as effective as the higher dose. However, they did not find any significant reduction in the *P. acnes* density over the 10-week follow-up period. It was concluded that the mechanism of action of PDT in acne does not depend upon a reduction in *P. acnes* counts.

Subsequently, Ammad et al. [266] used a single-blind, uncontrolled study design to assess the efficacy of blue light phototherapy (415-425 nm, irradiance 70-90 W cm^{-2}, fluence not stated) and its effect on *P. acnes* density in the treatment of acne vulgaris. They found blue light to be effective in the management of inflammatory acne, however, in accordance with the findings of Horfelt et al. [256] this beneficial effect was not found to be related to a reduction in *P. acnes* density. Similarly, Horfelt et al. [255] found single low dose red light phototherapy (635 nm, fluence 15 J cm^{-2}, irradiance 63 mW cm^{-2}) to be as effective as MAL-PDT in the treatment of moderate to severe facial acne. However, no significant decrease in the *P. acnes* density was observed in any group, thereby, adding to the list of publications favouring the anti-acne effects of visible light therapy to be independent of its influence on the cutaneous microflora.

3.4.1.4 Summary of the microbiological effect vs. clinical efficay of incoherent light (including PDT) in acne

Evidence on the effect of incoherent light, including PDT, on *P. acnes* density in acne patients is contradictory. In fact, except for the studies of Hongcharu et al. [245] and Shalita et al. [265], with their limitations discussed above, none of the other studies showed any significant effect on *P. acnes* colonisation/density (via bacterial culture or porphyrin fluorescence) post-treatment in acne patients. However, as all the studies except one used nonpulsed light sources [245, 255, 256, 259, 263, 265, 266], no definite conclusions can be made regarding the effect of IPL or IPL-assisted PDT on the *P. acnes* density in acne patients. Further studies, such as that presented in this thesis, will be required to clarify this issue. Similarly, as none of the studies used nonpulsed blue light for PDT, its effect on *P .acnes* density in acne patients

remains to be explored. While a reduction in *P. acnes* density in acne patients is desirable and may clinically improve acne, the beneficial effects of light therapy in these patients can be seen independent of this occurrence. It is possible that incoherent light, with or without a photosensitiser, may improve acne by virtue of its immunomodulatory and/or sebostatic effects.

Table 3.1: Studies on the microbiological effects vs. clinical efficacy of incoherent light (including PDT) in acne

Reference (first author and year)	Study size (n)	Study design	Interventions	Photosensitiser (contact time)	Light source	Irradiation values[b]	No. of treatments and interval	Clinical efficacy	Porphyrin fluorescence {Assessment interval}	Microbiology {Assessment interval}
Hongcharu (2000) [245]	23	RCT, single-blind	ALA-PDT, ALA alone, light alone, untreated control	20% ALA (3h)	Red light, 550-700 nm	150 J cm^{-2}	2 groups: 1 treatment and 4 treatments 1 week apart	Statistically significant improvement in inflammatory acne for 10 weeks after a single treatment and for at least 20 weeks after multiple treatments with ALA-PDT. Multiple ALA-PDT treatment sites showed more improvement than single treatment ($p < 0.001$)	Statistically significant reduction in porphyrin fluorescence for 20 weeks after a single treatment and multiple treatments with ALA-PDT. No significant difference between the two groups {Baseline, 2, 3, 10, and 20 weeks after the last treatment}	ND
Shalita (2001) [265]	10	Controlled trial (blinding and randomisation details not available)	Violet-blue light, untreated control	NA	Violet-blue light (CureLight Ltd.), 407-420 nm	Treated for 20 min at an irradiance of 90 mW cm^{-2} Fluence NM	6, twice a week	No clinical evaluation performed on this cohort of 10 patients. However, a 68% reduction in inflamed lesion counts was reported for another group of 35 acne patients after 8 bi-weekly treatment with the same light source. No statistical analysis given for these 35 patients	ND	A significant ↓ (> 1 log, $p < 0.05$) in the *P. acnes* density was seen in 6 out of 10 patients after 6 treatments. These patients had a baseline *P. acnes* density of > 10^5 colonies cm^{-2} {Baseline, and after the 2^{nd}, 4^{th}, and 6^{th} treatment}

Table 3.1 Continued

Reference (first author and year)	Study size (n)	Study design	Interventions	Photosensitiser (contact time)	Light source	Irradiation values[b]	No. of treatments and interval	Clinical efficacy	Porphyrin fluorescence {Assessment interval}	Microbiology {Assessment interval}
Omi (2004) [259]	28	Open, uncontrolled	Blue light only	NA	Blue light (ClearLight™), 410-420 nm	Treated for 15 min at an irradiance of 200 mW cm^{-2} Fluence NM	8, twice a week	A 64.7% ↓ in acne lesions after 8 treatments ($p < 0.01$). Improvement sustained for 2 months after the last treatment in 6 out of 9 patients	ND	No change in *P. acnes* colonisation detected pre- and post-treatment via bacterial culture or polymerase chain reaction {Baseline and after 8 treatments}
Tzung (2004) [263]	31	RCT, split-face, single-blind	Blue light, untreated control	NA	Blue light (F-36 W/Blue V, Waldmann), 420 ± 20 nm	40 J cm^{-2}	8, twice a week	After 8 treatments: significant improvement in global acne severity score with blue light vs. control (52% vs.12%, $p < 0.001$). Improvement sustained for at least 1 month after the last treatment	Therapeutic effectiveness was not found to be related to fluorescence intensity change ($p = 0.812$) {Before and at the end of the treatment}	ND
Horfelt (2007) [256]	15	Open, uncontrolled	Various fluences of red light (30, 50 and 70 J cm^{-2}) compared with each other	20% ALA (3 h)	Red light (Waldman PDT 1200 lamp), 600-730 nm	30, 50, and 70 J cm^{-2} 50 mW cm^{-2}	1	No statistical analysis. Acne response same for 30 and 50 J cm^{-2}. 7 out of 9 patients' facial acne improved by global severity score of at least one (out of four) at week 10 after the treatment	ND	No significant ↓ in *P. acnes* density for up to 10 weeks after treatment with 50 J cm^{-2} {Baseline, 1, 2, 3, and 10 weeks after treatment}

Table 3.1 Continued

Reference (first author and year)	Study size (n)	Study design	Interventions	Photosensitiser (contact time)	Light source	Irradiation values[b]	No. of treatments and interval	Clinical efficacy	Porphyrin fluorescence {Assessment interval}	Microbiology {Assessment interval}
Yeung (2007) [264]	30	RCT, split-face, single-blind	All participants used 0.1% adapalene gel and were randomised to two split-face treatment groups; IPL assisted MAL-PDT vs. IPL alone; and IPL vs. control (adapalene-only group)	16% MAL (30 min)	IPL (Ellipse Flex), 530-750 nm	7-9 J cm^{-2}	4, 3 weeks apart	Inflamed lesions: Significant ↓ in the adapalene-only group at 4 weeks (72%, p = 0.01) and 12 weeks (88%, p = 0.01) after the last treatment. No significant ↓ in the MAL-PDT or IPL groups Noninflamed lesions: Significant ↓ in the MAL-PDT (38%, p = 0.05) and IPL (44%, p = 0.01) groups at 12 weeks after the last treatment. No significant change in the adapalene-only group	Porphyrin reduction > 50% at 4 weeks after the last treatment: MAL-PDT (27%) vs. IPL (17%) vs. adapalene-only (0%). No significant difference between the three groups {Baseline, before each treatment and 4 and 12 weeks after the last treatment}[a]	ND

Table 3.1 Continued

Reference (first author and year)	Study size (n)	Study design	Interventions	Photosensitiser (contact time)	Light source	Irradiation values[b]	No. of treatments and interval	Clinical efficacy	Porphyrin fluorescence {Assessment interval}	Microbiology {Assessment interval}
Ammad (2008) [266]	21	Single-blind, uncontrolled	Blue light only	NA	Blue light (ClearLight™), 415-425 nm	Treated for 14 min at an irradiance of 70-90 W cm^{-2} Fluence NM	8, twice a week	After the 8th treatment: ↓ inflamed lesions 13.87%, ↓ noninflamed lesions 10.23%. $p < 0.05$ for inflamed lesions only	ND	No significant ↓ in *P. acnes* density after the 8th treatment ($p = 0.66$) {Baseline and after the 8th treatment}
Horfelt (2009) [255]	23	Open, nonrandomised, split-face controlled study	MAL-PDT, light alone	MAL cream 160mg/g (3h)	Red light (Aktilite® CL 128 lamp), 635 nm	15 J cm^{-2} 63 mW cm^{-2}	1	Global acne severity score, papules/pustules and noninflamed lesions showed a statistically significant ↓ in both groups at 10 and 20 weeks follow up ($p < 0.05$). No significant difference between the groups for any of these parameters	Fluorescence imaging only used for the assessment of photobleaching after irradiation. No data for the follow-up visits reported	No significant ↓ in *P. acnes* density for up to 20 weeks after treatment at any site {Baseline, 1, 10, and 20 weeks after treatment}

n, number of participants at the start of trial; NA, not applicable; ND, not done; NM, not mentioned; PDT, photodynamic therapy; RCT, randomised controlled trial; ALA, 5-aminolaevulinic acid; MAL, methyl aminolaevulinate; IPL, intense pulsed light; *P. acnes, Propionibacterium acnes*; ↓, reduction. [a]Results only reported for week 4 after the last treatment. [b]Fluence (J cm^{-2}) and irradiance (mW cm^{-2}) where stated.

3.4.2 Other potential mechanisms of action of incoherent light (including PDT) in acne

3.4.2.1 Photoimmunological effects

Light may be beneficial in acne because of its immunomodulatory properties. Shnitkind et al. [267] demonstrated narrow-band blue light (420 nm) to inhibit IFN-γ and TNF-α induced production of the proinflammatory cytokine IL-1α in two immortalised keratinocyte cell lines. Moreover, the expression of intercellular adhesion molecule-1 was also reduced. Ultraviolet B synergistically increased the effect of blue light.

IPL (570 nm cut-off filter, triple pulses of 7 ms with a pulse interval of 70 ms, fluence 75 J cm^{-2}) has been demonstrated to up-regulate TGF-β1 expression in human skin fibroblasts, *in vitro* [268]. Similarly, IPL-assisted ALA-PDT (555-950 nm, fluence 8 J cm^{-2}, ALA contact time 6 h) has been shown to up-regulate TGF-β1 expression in cultured HaCaT cells [269]. TGF-β is an immuno-regulatory cytokine that can lead to inhibition of T lymphocytes proliferation and differentiation. Moreover, it also inhibits activation of macrophages and their production of proinflammatory cytokines, and can prevent maturation of dendritic cells [270]. In addition, it is also a potent inhibitor of keratinocytes proliferation [271]. Therefore, increased expression of TGF-β can be one of the mechanisms by which IPL/IPL-assisted PDT may improve acne.

Byun et al. [269] showed increased expression of IL-10 in cultured HaCaT cells after treatment with IPL (555-950 nm, fluence 8 J cm^{-2}). IL-10 induction has also been demonstrated in skin explants of PDT-treated (630 nm) mice [233]. As previously

explained **(Chapter 2, Section 2.4.3.1)**, TLRs mediate their effects by activation of the transcription factor, NF-κB, and MAPKs [174]. MAPKs subsequently potentiate the activity of another transcription factor, activator protein (AP)-1 [272]. Moreover, TLR-2 and TLR-4 are up-regulated in inflamed lesions of acne patients [181]. In line with these observations, AP-1 and NF-κB activation has been found to be markedly increased in inflamed acne lesions, compared with normal skin, and was accompanied by significant increases in mRNA levels for genes regulated by NF-κB (IL-8, IL-1β, IL-10, TNF-α) and AP-1 (matrix metalloproteinases (MMP)-1, -3, -9) [273]. IL-10 has been shown to inhibit activation of NF-κB, *in vitro* [274, 275], and its induction in acne-affected skin may suppress TLR-mediated signalling and could improve acne. IL-10 likewise inhibits MHC (major histocompatibility complex) class II and co-stimulatory molecule B7-1/B7-2 expression on monocytes and macrophages, resulting in suppression of their antigen-presenting function [145]. Moreover, it also negatively regulates costimulatory signals on Langerhans cells (LCs) [276]. Up-regulation of IL-10 may therefore represent another possible mechanism by which IPL/IPL-assisted PDT may positively impact acne.

Comparable to UVB reactions in skin, red light-assisted ALA-PDT (630 $\pm$ 3 nm) has been shown to decrease the number of epidermal LCs in a murine model [277]. This local immunosuppression caused by reduced epidermal LCs could also potentially improve acne.

3.4.2.2 Effect on sebaceous glands

As mentioned earlier, IPL has been successfully used to treat telengiectasias and port-wine stain [210]. The mechanism, as in lasers, involves selective absorption of

light by haemoglobin in the blood vessels. The absorbed light is converted to heat leading to coagulation of the abnormal vessels [278]. Thus IPL, like pulsed-dye laser which has been used to treat sebaceous gland hyperplasia [279], can potentially damage sebaceous gland as a result of photocoagulation of its blood supply. Similarly, the fact that PpIX accumulates selectively in sebaceous glands after topical ALA application has been successfully used to treat sebaceous gland hyperplasia with ALA-PDT irradiated with IPL (500-1200 nm with a 550 nm cut-off filter, ALA contact time 30-60 min, fluence 32 J cm^{-2}, 3.5/3.5 ms pulse duration, 20 ms delay between pulses) [280]. In terms of acne, a decrease in sebaceous glands size along with reduction in SER has been demonstrated for up to 20 weeks after treatment with red light-assisted ALA-PDT (550-700 nm, fluence 150 J cm^{-2}) [245]. Reduction in SER, due to sebaceous gland damage, may therefore represent other possible ways by which IPL-assisted PDT/IPL may improve acne

3.4.3 Summary of the therapeutic targets for IPL/IPL-assisted PDT

There is insufficient evidence to draw definite conclusions regarding the effect of IPL/IPL-assisted PDT on *P. acnes* density in acne patients. Further studies are needed to clarify this issue. IPL/IPL-assisted PDT may also work in acne via their photoimmunological, and/or sebostatic (as a result of photothermal/photodynamic damage to the sebaceous glands) properties. However, *in vivo*, the simultaneous activation of all these three mechanisms may not be necessary for the clinical efficacy of these treatments.

The clinical and laboratory arms of our study were designed to assess the clinical efficacy of IPL/IPL-assisted MAL-PDT vs. adapalene 0.1% gel (conventional acne treatment) in mild to moderate acne patients and to determine their effect on the counts of *P. acnes.* ALA-PDT has also been shown to cause photodynamic eradication of other cutaneous micro-organisms (e.g. *S. aureus* and CoNS specifically *S. epidermidis*), *in vitro* [281], and therefore our laboratory arm also evaluated the effect of above treatments on *S. aureus* and CoNS, *in vivo*. These micro-organisms do not normally produce porphyrins; however excessive production and accumulation can be seen after incubation with ALA [281].

3.5 Hypothesis and Aims

As mentioned earlier, two systematic reviews have concluded that optical therapies, including PDT, can improve acne at least temporarily [206, 207]. This has led us to a hypothesis that IPL and IPL-assisted MAL-PDT are effective treatments for acne and that their beneficial effect is due to their anti-*P. acnes* property by virtue of its porphyrin producing capability. Thus, a prospective, RCT was designed to determine the efficacy of IPL (administered as IPL-Placebo) and IPL-assisted MAL-PDT (IPL-MAL) vs. adapalene 0.1% gel in the treatment of acne and to identify their mode of action, looking specifically at the effect on surface density of *P. acnes*. Moreover, as mentioned above, the laboratory arm was further extended to evaluate the effect of these treatments on the surface densities of *S. aureus* and CoNS as well.

3.5.1 Clinical study aims

To evaluate the clinical efficacy of IPL-Placebo and IPL-MAL and adapalene 0.1% gel on their ability to reduce:

1) Inflamed lesion counts

2) Noninflamed lesion counts

3) Total lesion counts

4) Leeds revised acne grading system score

5) Visual analogue scale score

6) Dermatology Life Quality Index score

7) Family Dermatology Life Quality Index score

8) Porphyrin fluorescence

3.5.2 Laboratory study aims

To determine whether IPL-Placebo and IPL-MAL exert the following effects on cutaneous micro-organisms:

1) Reduce the density of *P. acnes*

2) Change the density of CoNS and *S. aureus*

Chapter 4

Methods and Materials

4. Methods and Materials

4.1 The clinical study

4.1.1 Rationale

In accordance with previous studies assessing the clinical efficacy of optical therapies [247, 266, 282, 283], a global acne severity grade (the Leeds revised acne grading system) and lesion counts were used to assess the clinical outcome of the three treatments (IPL-Placebo, IPL-MAL, adapalene 0.1% gel) used in this trial. Likewise, a subjective global acne severity scale (the visual analogue scale) was used to account for the patient's impression about the effectiveness of the received treatment [266]. The Dermatology Life Quality Index and Family Dermatology Life Quality Index questionnaires were used to assess the outcome of the three treatments on the quality of life (QoL) of the patients and their family members/partners, respectively. In keeping with the study of Yeung et al. [264], standardised clinical and fluorescent photographs were taken via the Canfield VISIA® Complexion Analysis system (Canfield Scientific Inc., USA). The clinical and fluorescent photographs were used for the assessment of Leeds revised acne grading system score and follicular porphyrin fluorescence pre- and post-treatment, respectively. As mentioned in chapter 3 **(Section 3.4.1.2)**, *P. acnes* produced porphyrins give rise to orange-red follicular fluorescence under Wood's light examination. As the intensity of this fluorescence has been shown to be proportional to the *P. acnes* population, the digital fluorescent photographs were used as an indirect way of evaluating the suppressive effects of above three treatments on this micro-organism.

Based upon the results of two split-face studies in which improvement was also seen in untreated half of the face, raising the possibility of a systemic effect of light treatment, we opted for a parallel group study design [282, 284]. The Energist ULTRA VPL^{TM} (Energist Ltd. Swansea, UK), a CE-marked IPL device currently being used in our department, was used as the light source in the trial. As elucidated in chapter 3 **(Section 3.3.4)**, MAL was preferred over ALA in this trial because of its greater lesion specificity, deeper penetration, and a better side-effect profile.

4.1.1.1 Lesion counts

Lesion counting involves recording the number of noninflamed and inflamed acne lesions and is an objective way of determining the response to a specific treatment [285]. Although time consuming, it is more accurate than acne grading. Moreover, lesion counting does not only distinguish small differences in therapeutic response but the effect of treatment on individual lesions can also be estimated [285]. It was for all these reasons lesion counting was chosen to evaluate the response to the various treatments used in this trial.

4.1.1.2 Leeds revised acne grading system

An acne grading system is a quick and subjective method of determining the severity of acne [285]. However, it does not distinguish small differences in therapeutic response [285]. In clinical trials, an acne grading system can be used along with lesion counts to get an overall picture of a treatment's efficacy. Moreover, it can also be used to select the patients with the correct level of acne for a treatment under investigation [286].

Several systems for grading the severity of acne currently exist [285]. The Leeds revised acne grading system (LRAGS/Leeds) [286] is a rapid and reproducible grading scale for inflammatory acne that relies on assessment of acne severity using photographic standards for 3 sites: the face, back, and chest. The criteria used to assess severity are extent of inflammation, range and size of inflamed lesions, and associated erythema. The grading system displays a series of colour photographs of facial and truncal (back and chest) acne producing 12 colour pictorial grades (1-12) for facial acne and 8 grades (1-8) for acne affecting the back or chest. In the grading system, grade 1 represents mild acne while grades 12 and 8 signify severe facial and chest or back acne, respectively. As the LRAGS allows for retrospective evaluation, via comparison of the patients' photographs taken during the trial period with the photographic standards, this grading system was chosen for the study.

4.1.1.3 Visual analogue scale

Visual analogue scales (VAS) can be used to measure subjective experience and archetypally consist of a 10 cm line, with words descriptive of the maximal and minimal extremes of the parameter being measured, fixed at both ends [287]. They are user-friendly, require little motivation for completion by the patients, and allow the use of numerical values suitable for statistical analysis [287].

In order to get patient's perspective regarding the treatment's efficacy, a 10 cm VAS was used in this trial, where 0 represents 'no acne, the best it could possibly be' and 10 'very bad acne, the worst it could possibly be'. Similarly, subjective evaluation of pain after IPL-Placebo and IPL-MAL was again measured by a 10 cm VAS, where 0 represents 'no pain' and 10 'the worst ever pain'.

4.1.1.4 Quality of Life

Skin disease can have a major impact on the QoL of patients [288] and their families [289]. Over the past two decades various generic scales e.g. Dermatology-specific Quality of Life instrument [290], Skindex [291], Dermatology Life Quality Index (DLQI) [292], Dermatology Quality of Life Scales [293], and Impact of Chronic Skin Disease on Daily Life [294] have been described to measure the impact of skin disease on patients' QoL. Similarly, the Family Dermatology Life Quality Index (FDLQI) [295, 296] is a validated dermatology-specific instrument that has been described to measure the secondary impact of patients' skin disease on the QoL of family members.

The DLQI is a self-administered and user-friendly questionnaire with a completion time of 1-3 min [292]. It was the first dermatology-specific QoL instrument [297] and remains the most commonly used health-related QoL outcome measure to date [298]. It has been validated for dermatology patients aged 16 years and above, and has been successfully used in various studies to demonstrate change in the patients' QoL before and after treatment [298]. It was for all these reasons that prompted the use of this QoL tool in this trial.

The DLQI consists of 10 items encompassing the impact of skin disease on different areas of patients' life such as symptoms/feelings, daily activities, leisure, work/school, personal relationships, and the side-effects of treatment over the past 1 week [292]. Each item is scored on a four-point scale; 0, not at all/not relevant; 1, a little; 2, a lot; 3, very much. Scores of each item (0-3) are summed to yield the total score, ranging from 0 (no impairment of life quality) to 30 (maximum impairment).

The FDLQI is the only generic dermatology-specific measure explicitly designed to measure the family impact of any type of skin disease [296]. It consists of 10 items encompassing the impact of patients' skin disease on various aspects of QoL (e.g. emotional, physical well-being, relationships, peoples' reaction, social life, leisure activities, burden of care, job/study, housework, and expenditure) of their family members/partners over the last 1 month [295]. Each item is scored on a four-point scale; 0, not at all/not relevant; 1, a little; 2, quite a lot; 3, very much. Scores of each item (0-3) are added to give a total score that ranges from 0 to 30; a higher score indicates greater impairment of QoL.

4.1.1.5 Energist ULTRA VPL™ as an IPL source

The Energist ULTRA VPL™ **(Figure 4.1)** is a variable pulsed IPL device equipped with a 530 nm and 610 nm interchangeable applicators. Both the applicators remove infrared wavelengths above 950 nm, thereby reducing non-specific epidermal heating. Moreover, the 530 nm and 610 nm applicators filter wavelengths below 530 nm and 610 nm, respectively **(Figure 4.2)**. This ensures delivery of the optimal wavelengths to the target chromophore. Wavelengths in the yellow-red range may be more effective, as a virtue of their deeper penetration into the skin, than blue light in the photodynamic eradication of *P. acnes* whose habitat is the PSF. Additionally, they may also damage the sebaceous glands which lie deep in the dermis.

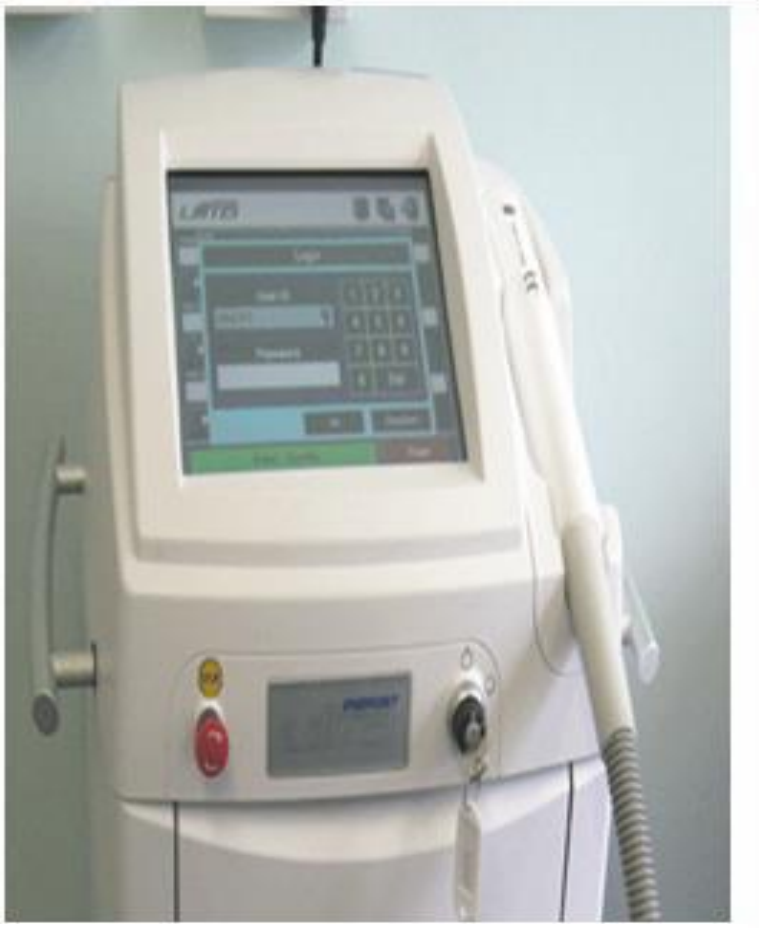


Figure 4.1: The Energist ULTRA VPL^{TM} device with its touch-screen interface.

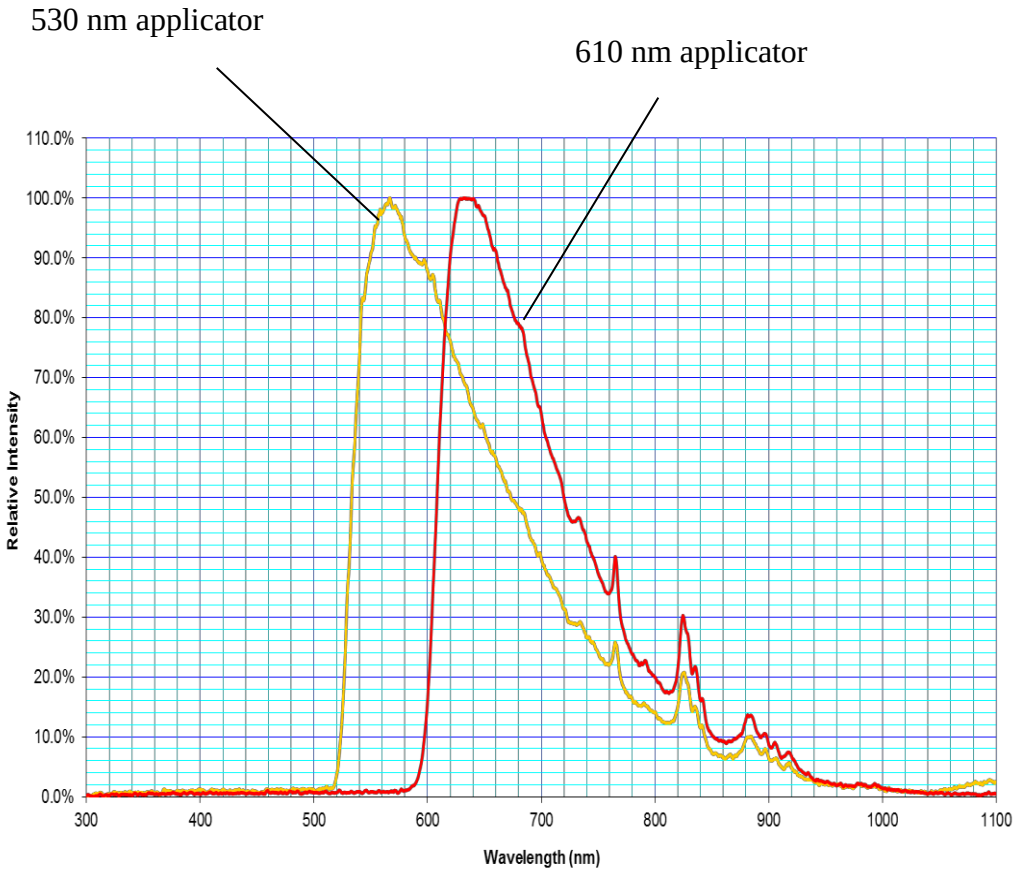


Figure 4.2: Spectral output of 530 & 610 nm applicators. *Courtsey of Darren Thomas, Energist Ltd. Swansea, UK, © 2014.*

4.1.2 Clinical methods

Ethical approval for this study was obtained from the South East Wales Research Ethics committee (reference number: 09/WSE03/40) and Research & Development approval was granted by the Cardiff and Vale NHS Trust (reference number: 07/CMC/4136E). The study was also approved by the Medicines and Healthcare Products Regulatory Agency (reference number: 21323/0026/001-0001) and was conducted in accordance with the International Conference on Harmonisation's Good Clinical Practice guidelines.

4.1.2.1 Subjects and recruitment

Male and female volunteers aged 18-45 years with mild to moderate facial acne were eligible for the study. Patients were invited to enter the study through newspaper advertising, Cardiff University's online notice board announcements and by poster advertisements at the halls of residence. Patients attending the dermatology department, University Hospital of Wales (UHW), for the treatment of acne were offered information about the study and the general practitioners (GPs) within the Cardiff locale were asked to directly refer appropriate patients. Posters about the study were also displayed in the GP surgeries so that interested subjects may also contact the research team directly. The trial period spanned from March 2010 to October 2011 with the first patient being enrolled into the study on March 9, 2010 and the last patient completed follow-up on October 26, 2011.

A standard screening questionnaire was used to assess general suitability of the patients. This was available online, for those who had access to the Cardiff University intranet, with the results being available to the investigators on an Excel datasheet. The same questionnaire was also used for any telephone enquiries. Patients whose suitability for the trial could not be ascertained on the basis of the screening questionnaire were given the option to meet the investigator. All prospective patients were given an information document detailing the study's objectives and overall requirements. After they had been given sufficient time (at least 48 h) to read and understand the information document, all interested patients were asked to come for a baseline visit where conformity with the eligibility criteria was again ensured and a written informed consent was obtained. All the assessments

and treatments were carried out in the dermatology department at Glamorgan House, UHW.

Patients recruited to the trial were sequentially allocated a patient number and their data were collected on case report forms (CRFs). The patients' anonymity was maintained, as patients were identified on CRFs by their initials and the allocated patient number. For any emergency a record of patient's number, name and address was also maintained and kept separate from the CRFs. The data from each patient were eventually stored on an Excel data sheet for further analysis.

During the course of the study, the patient could be discontinued for the following reasons: voluntary withdrawal, serious adverse event, suspected unexpected serious adverse event and noncompliance with the study protocol. The inclusion and exclusion criteria as detailed in the study protocol are listed below.

4.1.2.1.1 Inclusion criteria

1. Mild to moderate facial acne (face = area from hairline to jawline) with at least 15 inflamed and/or noninflamed lesions, but no more than 3 nodulocystic lesions, thus, not exceeding Leeds grade 7 (for the face)
2. Patients willing to have only their face treated
3. Fitzpatrick skin phototypes I-IV
4. Patients willing and able to provide written informed consent
5. Patients who agree to avoid the use of sunbeds or undergo any ultraviolet light treatment for 4 weeks prior to entering the study and are willing to

minimise the amount of exposure to direct sunlight for the duration of the study

6. Access to an active email account and willing to reveal their email account details to the study team in order to receive weekly reminders
7. Patients considered to be reliable and expected to be compliant with the investigational products and protocol requirements

4.1.2.1.2 Exclusion criteria

1. Unable to give written informed consent
2. Severe acne or scarring
3. Pregnant women or those contemplating pregnancy
4. Lactating females
5. Use of anti-androgen containing contraceptives
6. keloids or a tendency to heal with keloids
7. Facial treatment in the previous year with collagen, dermabrasion and laser resurfacing
8. Facial microdermabrasion within the past 3 months
9. Treatment with alpha-hydroxyl acids within the past 3 months
10. History of hypersensitivity to any of the study drugs or their excipients i.e. adapalene, Metvix ™ (e.g. peanut oil, soya oil), Unguentum M®
11. Photosensitivity disorders e.g. solar urticaria, porphyrias, systemic lupus erythematosus
12. Allergy to porphyrins
13. Epilepsy
14. Oral photosensitisers within the past 4 weeks

15. Oral retinoid use within the past 12 months
16. Use of vitamin A supplements > 2000 IU/day
17. Use of systemic medications such as steroids, immunosuppressants, statins and preparations containing St. John's wort[a]
18. Oral antibiotics and topical retinoids use within the past 4 weeks
19. Treatment with IPL or lasers to the face within the last 12 months
20. Psoriasis, rosacea, bacterial, viral, fungal, or other diseases of the facial skin[a]
21. Mild to moderate acne patients who never had any over the counter treatment
22. Severe systemic diseases such as: impaired renal or liver function; regional enteritis or ulcerative colitis; a history of antibiotic-associated colitis; severe cardiovascular, neurological or any other disease that may interfere with the evaluation of the study medications
23. Unavailability at any time during the course of the study i.e. 16 weeks
24. Involvement in another clinical trial
25. Patients living with anyone that is also taking part in this study

[a]Patients temporarily ineligible. Could be enrolled into the study after a wash-out period of 4 weeks or after resolution of their illness.

4.1.2.2 Randomisation

The randomisation was performed by the St. Mary's Pharmaceutical Unit, Cardiff. The patients were randomly assigned by a computer-generated allocation sequence with a block size of 30 to three main groups: IPL-MAL group, IPL-Placebo group and adapalene group. The randomisation list was held within the pharmacy department of the UHW and unveiled at the end of the study. All the patients were randomised and treated within 30 days of the baseline visit **(Figure 4.3)**.

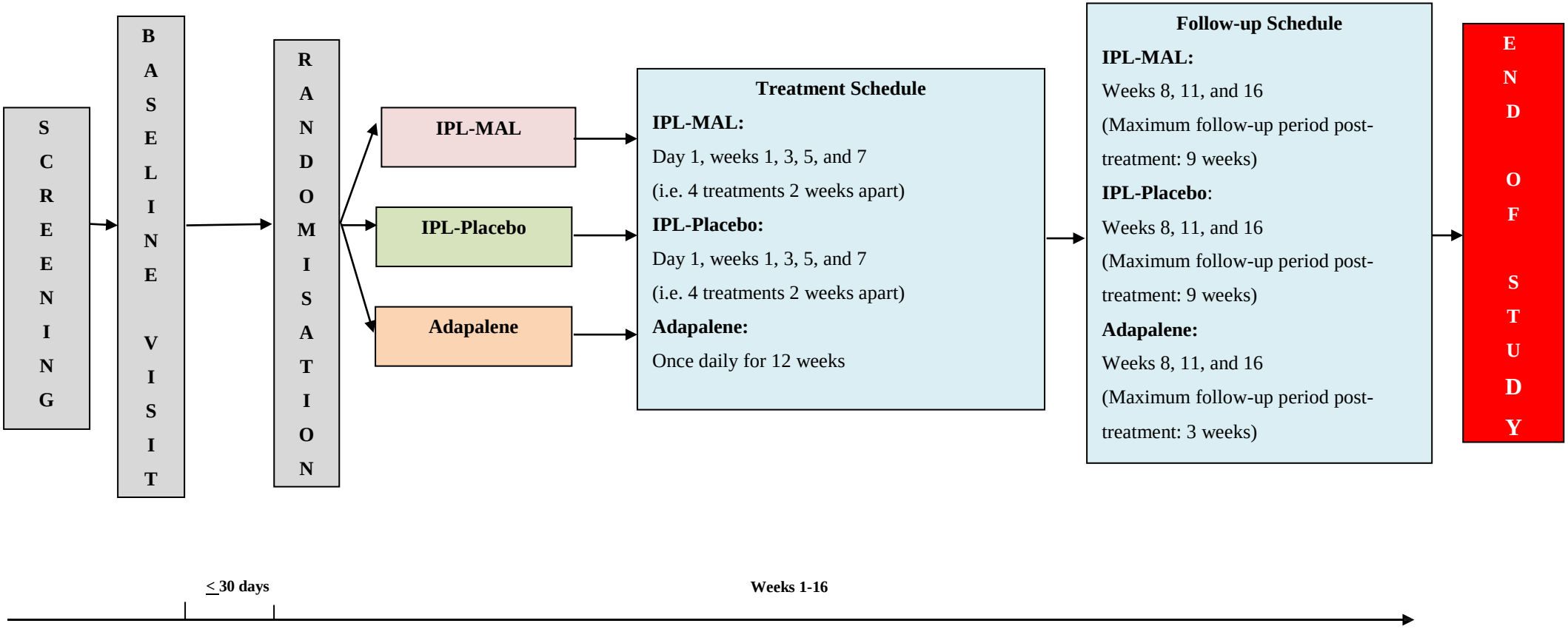


Figure 4.3: Study schema. IPL, intense pulsed light; MAL, Methyl aminolaevulinate.

4.1.2.3 Blinding and handling of trial medications

Both patients and investigators remained blinded to the IPL-MAL and IPL-Placebo treatment allocation from the time of randomisation until database lock. However, the patients as well as investigators were aware of the treatment allocation in the adapalene group. This is contradictory to the main title of the trial which suggests it to be a completely double blind trial. Laboratory staff, however, remained blinded to the IPL-MAL, IPL-Placebo and adapalene treatment allocation throughout the trial.

An unblinded trial nurse delivered the trial specific prescription to the pharmacy department of the UHW, where the treatment allocated to the patient was identified from the randomisation list and supplied to the unblinded nurse. Treatment in the IPL-MAL and IPL-Placebo groups was applied by the unblinded trial nurse throughout the trial and the treatment allocation was not discussed, at any time, with the patients or investigators. Emergency unblinding was possible through the pharmacy department, if deemed necessary, for reasons of patient safety.

All the investigational medicinal products (IMP) used in the trial were supplied directly to the pharmacy department of the UHW. All the IMP were stored in the recommended storage conditions and appropriate temperature monitoring was performed especially for the MAL cream which is supposed to be stored at 2–8°C (in a refrigerator).

4.1.2.4 Treatment

The IPL-MAL group received 16% MAL cream (Metvix®; Galderma UK Ltd, Watford) while Unguentum M® cream (Almirall Ltd, Uxbridge, UK) was used, as a placebo, in the IPL-Placebo group. MAL as well as Unguentum M® creams were applied as a 1-mm thin film to the face (avoiding the nose, lips, moustache area and areas immediately around the eyes) and covered with an occlusive dressing (3M Tegaderm; 3M Company, Bracknell, UK) for 60 min. All the patients sat in a darkened room during this time period. As explained in chapter 3 **(Section 3.3.4)**, this contact time was chosen to minimise PDT-related side-effects as investigations using 3 hour incubation period [241, 245-247] have been associated with frequent and severe side-effects with a significant patient dropout rate compared with contact times of 30-60 min [248, 249]. Prior to the illumination, the patients washed their faces using a gentle cleanser (E45® Emollient Wash Cream; Forum Health Products Limited, Surrey, UK) followed by pat drying.

The irradiated area corresponded to the facial area where MAL or Unguentum M® creams were applied under occlusion. Illumination was performed whilst patient relaxed on a couch with the backrest adjusted to an angle of +45°. The following irradiation parameters were used after application of a thin layer of ultrasound gel on the skin (for optical coupling): 610-950 nm cut-off filter, 50×10 mm spot size, 5-15 pulses, 5 ms pulse duration, 20 ms delay between pulses, 20-40 J cm^{-2} fluence and two passes in conjunction with cold air cooling (SmartCool™; Cynosure, USA) **(Figure 4.4)**. No topical anaesthetic agent was applied and non-overlapping pulses were delivered. The eyes were protected with goggles. Both IPL groups received 4 treatments to the face with an interval of 2 weeks between each session **(Figure 4.3)**.

Marked discrepancies have been demonstrated between the measured IPL devices outputs (e.g. fluence, pulse duration, pulse shape, spectral output) and those claimed by the manufacturers [299]. Therefore parameters claimed to be successful in one device might not demonstrate similar efficacy in another. Moreover, as limited information was available on the use of IPL for acne treatment in Fitzpatrick skin types I/II, the above settings were roughly based on those used by Babilas and colleagues [300]. They also used the Energist ULTRA VPL™ device in Fitzpatrick skin types II/III (610-950 nm cut-off filter, 15 pulses, 5 ms pulse duration, 20 ms delay between pulses, two passes, fluence 40 J cm^{-2}) during MAL-PDT for actinic keratosis. The investigators reported similar efficacy but reduced pain with this device compared to a light-emitting diode (LED).

Patients in the adapelene group applied a thin film of adapalene gel (Differin® Gel 0.1% w/w; Galderma UK Ltd, Watford) to their faces, after washing it, nightly, avoiding the areas just around the eyes, nostrils and lips. Treatment in this arm lasted for 12 weeks **(Figure 4.3)**. All the patients in this group were sent weekly emails to remind them regarding the daily application of adapalene. Compliance in this group was further ensured by weighing the adapalene tubes at follow-up visits.

All the patients participating in the trial were provided a sunblock lotion (Delph® lotion; SPF 30; Fenton Pharmaceuticals Ltd, London, UK) to be used daily on the face for the entire duration of the study. Patients in the PDT arms were also advised to avoid sun exposure as much as possible for the first 48 hours after treatment. Moreover, an emollient (Diprobase® ointment; Merck Sharp & Dohme Ltd,

Hertfordshire, UK), to be used twice a day for a total of 5 days, was given to all patients in the PDT groups post-treatment.

Figure 4.4: SmartCool^{TM} cooling device.

4.1.2.5 Assessments

All patients were evaluated at baseline, week 8, week 11, and week 16 of the trial. This amounted to a follow-up period, post-treatment, of 1 week, 4 weeks and 9 weeks in the PDT groups whilst the maximum follow-up period, post-treatment, in the adapalene group was 3 weeks **(Figure 4.3)**. Demographic data including each patient's age, medical and medication history (including a detailed acne history), Fitzpatrick skin type, height, weight, history of smoking, and pregnancy test (females only) were recorded for all patients at baseline.

At baseline and subsequent visits the numbers of inflamed and noninflamed facial acne lesions were counted. Moreover, standardised clinical and fluorescent photographs were also performed. Leeds assessments were performed by 2 dermatologists, blinded to all 3 arms of treatment and not involved in the study, by using the photographs taken at baseline and subsequent visits.

As mentioned in section 4.1.1.3, a 10 cm VAS was used to assess patients' own impression about their acne at baseline and follow-up visits. The impact of acne pre- and post-treatment on the quality of life of the patients was assessed, at each visit, using the DLQI questionnaire. Furthermore, the FDLQI questionnaire was used to assess the impact of acne, at baseline and week 16, on the quality of life of family members/partner of patients.

Patients in the PDT groups had side-effects evaluated before the next treatment and at each follow-up visit. As mentioned previously (section 4.1.1.3), a 10 cm VAS was used to evaluate treatment-related pain in these groups. Side-effects in the adapalene group were assessed at week 8, week 11, and week 16.

4.1.2.5.1 Acne lesion counts

The facial area used for lesion counting extended from the hair to the jaw line (excluding the nose, lips, moustache area and areas immediately around the eyes). The numbers of different acne lesions (comedones, papules, pustules, nodules and cysts) were counted separately from the right and left forehead, both cheeks and the chin above the jaw line. Lesion counting was done, by gentle palpation of the non-stretched facial skin, whilst patient relaxed on a couch with the backrest adjusted to

an angle of +45°. A bright light source (Brandon Optica 288 Magnifier lamp; Brandon Medical Company Ltd, Leeds, England) containing a 22W fluorescent bulb, placed not more than 30cm from the patient, was also used during this assessment **(Figure 4.5)**. Only lesions that could be palpated, whether inflamed or noninflamed, were included in the final count.

Figure 4.5: Couch and light source used during assessment.

4.1.2.5.2 Photography

The Canfield VISIA® Complexion Analysis system **(Figure 4.6)** has a booth, chin cup and headrest to facilitate proper placement of the patient's face, allowing for consistency throughout the study. Moreover, the system also uses an overlay feature to help align images, for consistency, at follow-up visits.

Prior to photography, all the patients washed their face using a gentle cleanser (E45® Emollient Wash Cream) followed by pat drying. With the patient's eyes closed, three standard close-up photographs (right, left, and central) were taken using white and ultraviolet (365 nm) light.

The manual masking feature of VISIA® was used to draw a boundary ("analysis mask") on the photographs to delineate areas (forehead, right, and left cheeks) where follicular (porphyrin) fluorescence was counted. The mask that was created at the first session was used for analysis of pictures at all later sessions ensuring consistency throughout the study. VISIA® software was used to store all the images and to count the number of follicular fluorescence in the above-mentioned delineated areas.

4.1.2.6 Outcome measures

The primary outcome parameter was reduction in lesion counts (inflamed and noninflamed) evaluated at weeks 8, 11, and 16. Secondary outcome parameters were reduction in total lesion counts, porphyrin fluorescence, microbial densities (discussed below), VAS, Leeds, DLQI, and FDLQI scores.

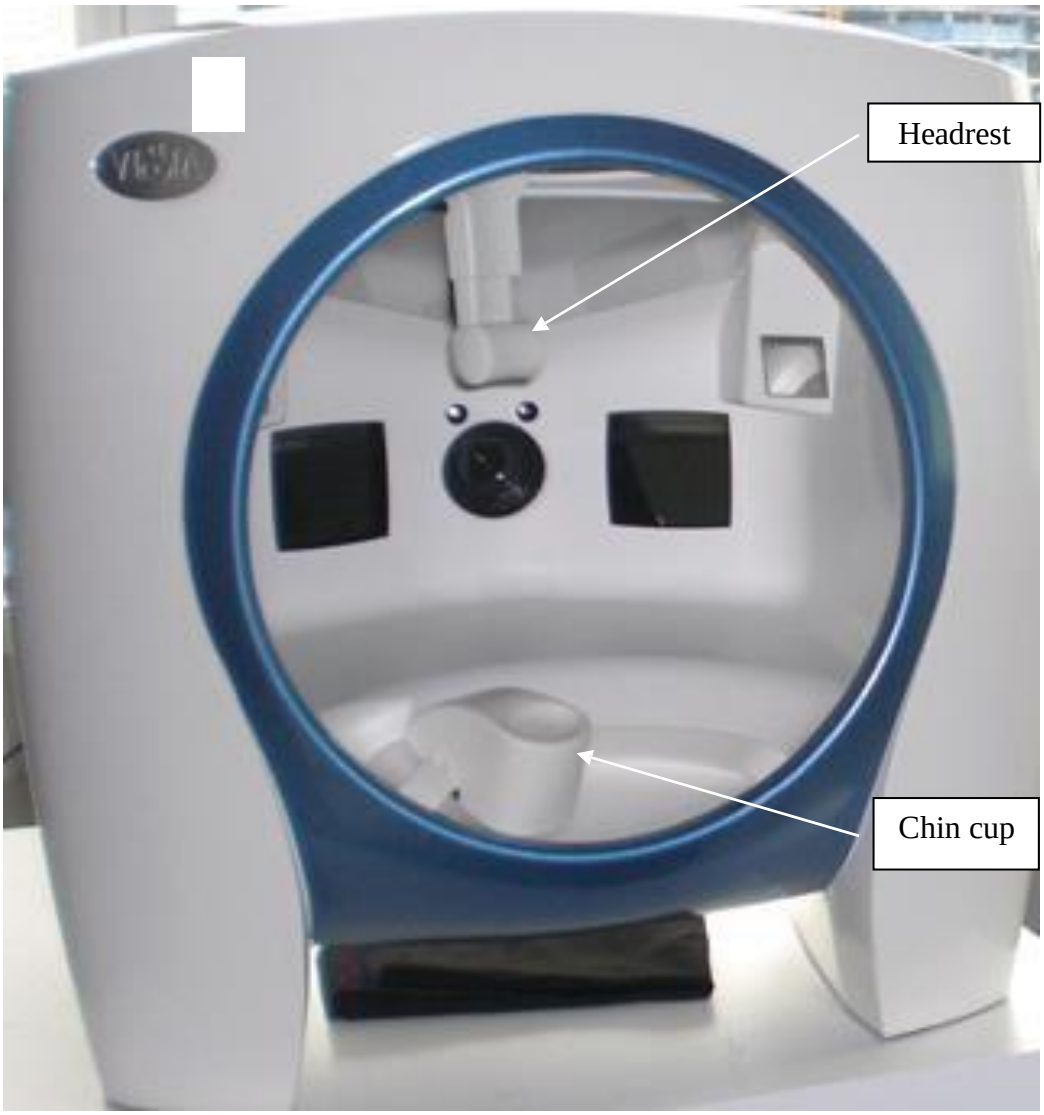


Figure 4.6: The Canfield VISIA® Complexion Analysis system consisting of a booth, chin cup and headrest.

4.2 The laboratory study

4.2.1 Rationale

In accordance with previous studies [257, 266], the surface scrub technique of Williamson and Kligman [31] was used to investigate the effect of various treatments used in this trial on the cutaneous microflora of acne patients. As detailed in chapter 2, *P. acnes* may aggravate inflammation and comedogenesis in acne patients and a reduction in its density due to irradiation with IPL (with or without a topical photosensitiser) may lead to an improvement in acne. As mentioned previously **(Section 3.4.3)**, the effect of our treatments on *S. aureus* and CoNS densities was also investigated as *in vitro* data has shown a reduction in the densities of these micro- organisms following ALA-PDT [281].

4.2.2 Bacterial sampling from skin surface

4.2.2.1 Sample collection

The surface cutaneous microflora was sampled from a defined area (6.1cm^2) demarcated by a sterile stainless steel ring on the skin **(Figure 4.7)**. All the samples were taken from the right cheek about 4 cm lateral to the alar rim. One milliliter of sterile 0.1% Triton X-100 in 0.075 M phosphate buffer (pH 7.9) was pipetted into the steel ring which was held with firm pressure against the skin to create a water-tight seal. The skin was gently rubbed for 1 min using a sterile Teflon rod, after which the wash fluid was removed to a sterile sample bottle (amber). The sampling procedure was repeated at the same site and the two 1 ml samples combined in the same sample bottle (neat sample). Samples were sent to the microbiology laboratory, UHW for immediate processing.

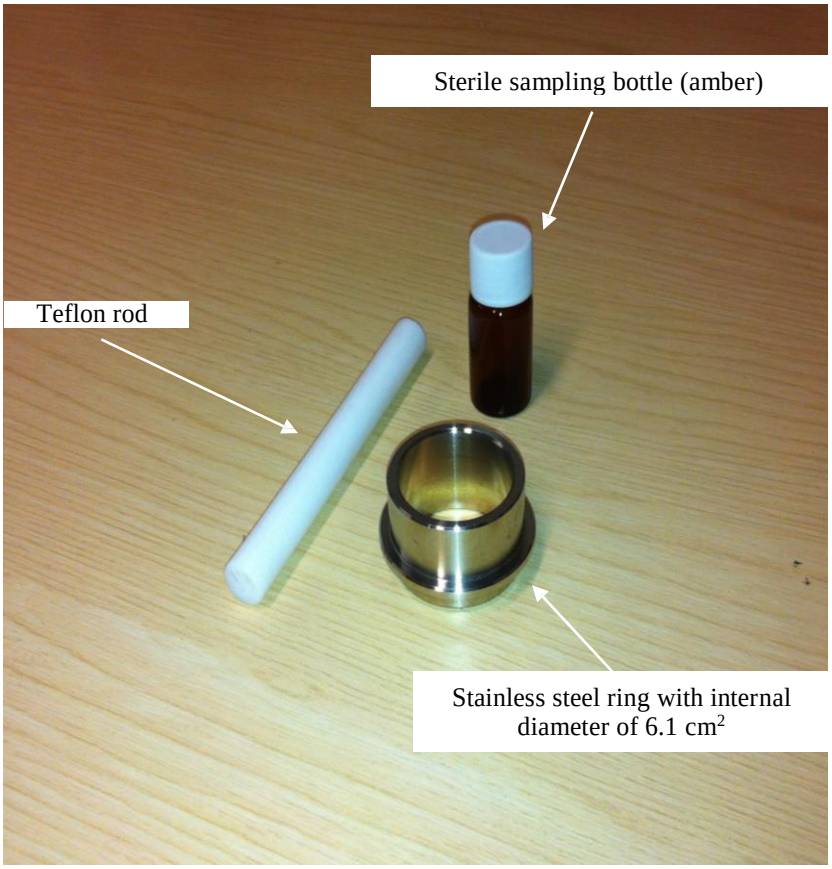


Figure 4.7: Instruments used for sampling cutaneous microflora using the surface scrub technique of Williamson and Kligman.

4.2.2.2 Sample processing

Samples were diluted with sterile saline to give a 100 and 1000-fold dilution. Fifty microliters of the undiluted, 100 and 1000-fold dilutions were then spiral plated using Whitley Automated Spiral Plater (Don Whitley Scientific Ltd., Shipley, UK). Counts are expressed as colony-forming units (CFU) cm^{-2} of skin.

Enumeration of propionibacteria was performed by plating the sample on to reinforced clostridial agar (CM0151; Oxoid Ltd., Basingstoke, UK) containing 6 µg mL^{-1} furazolidone to inhibit the growth of staphylococci. Cultures were incubated at 37°C for 7 days in an anaerobic chamber (Concept Plus; Ruskinn Technology Ltd., Bridgend, UK) under an atmosphere of 80% N_2, 10% H_2, and 10% CO_2.

Mannitol salt agar (PP0660; E&O Laboratories Ltd., Bonnybridge, Scotland) was used for the isolation of staphylococci and was incubated in air at 37°C for 48 hours. Moreover, samples were also plated on to Columbia agar with 5% defibrinated horse blood (PP0120; E&O Laboratories Ltd., Bonnybridge, Scotland) and incubated in air at 37°C for 48 hours. This is a general purpose medium suitable for the isolation of most organisms including many fastidious anaerobes.

After appropriate incubation differential counts were made according to colony type, Gram stain and cell morphology. Differentiation of *S. aureus* from CoNS was done using a latex agglutination test (Staphaurex®; Remel, Lenexa, Kansas). This test uses coated (human fibrinogen and specific IgG) latex particles to identify *S. aureus* by the simultaneous detection of clumping factor and protein A. Biochemical tests (indole, catalase production) were used for the basic identification of *P. acnes.*

Propionibacteria are generally catalase-positive with indole being produced by *P. acnes* but not *P. granulosum* or *P. avidum* [25]. 16S rRNA gene sequencing, a powerful method for investigating the phylogenetic relationship between bacteria [301], was used to identify propionibacteria at the species level where the micro-organisms were catalase-positive but indole-negative. Indole production by the propionibacteria was detected by smearing the growth from an actively growing pure culture onto a paper strip containing 1-2 drops of spot indole reagent (R21245; Oxoid Ltd., Basingstoke, UK). A colour change to blue within 30 seconds indicated a positive reaction. Similarly the presence of catalase in propionibacteria was detected by reacting colonies with 1-2 drops of hydrogen peroxide (289132; Sigma-Aldrich Ltd., Dorset, UK). A positive reaction resulted in formation of bubbles due to the release of oxygen.

For the 16S rRNA gene sequencing of propionibacteria, bacterial genomic DNA was prepared by suspending a few colonies in 20% w/v Chelex-100 suspension (Cat no: 142-2822; Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) and heating it to 95°C for 10 min. The suspension was then cooled briefly at room temperature before centrifugation (Centrifuge 5424; Eppendorf UK Ltd., Stevenage, UK) at 13,000 revolutions per minute for 10 min.1ul of the resulting supernatant, containing genomic DNA, was used as a template for amplification of the 16S rRNA gene by using the universal primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) [302]. Samples were initially heated at 94°C for 2 min, followed by 30 cycles at 95°C for 45s, 55°C for 1 min, 72°C for 30s, followed by a final extension at 72°C for 5 min. PCR products were visualized by electrophoresis on 1% (w/v) agarose gels (Cat no: 16500-500; UltraPure^{TM} Agarose;

Invitrogen Life Technologies Ltd., Paisley, UK) and then purified with a PureLink® quick gel extraction kit (K2100-25; Invitrogen Life Technologies Ltd., Paisley, UK). The resulting DNA was sent to the Central Biotechnology Services (Cardiff University, School of Medicine, Heath Park, Cardiff, UK) for sequencing. The returned sequences were loaded into BioNumerics v5.10 database (Applied Maths, Sint-Martens-Latem, Belgium) and were screened using the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST) to determine the most probable propionibacterium species.

4.3 Statistical analysis

Based upon the results from the study of Yeung et al. [264] it was calculated (by a statistician) that 30 subjects per treatment group to assess the improvement of inflamed lesion counts by 76.7±28.9%, 24.9±31.1%, and 49.2±27.4% for IPL-MAL, IPL-Placebo, and adapalene groups, respectively will give power of > 99% for comparing IPL-MAL vs. IPL-Placebo and 95% for IPL-MAL vs. adapalene. Similarly, 30 persons per group to assess the improvement of noninflamed lesion counts by 44.0±32.5%, 21.8±27.7%, and −9.8±47.1% for IPL-MAL, IPL-Placebo, and adapalene groups, respectively will give power of 80% for comparing IPL-MAL vs. IPL-Placebo and 90% for IPL-MAL vs. adapalene. The number of persons needed per treatment group was increased to 40 to account for possible dropouts. Statistical advice was also sought for all the final analyses. Statistical analyses were done using SPSS version 20 and $p < 0.05$ was considered statistically significant.

Treatment groups at baseline were compared with the Fisher's exact test for sex, skin type, and antibiotics and retinoids use in the past. Where the Fisher's exact test was

significant (topical antibiotics use in the past), column proportions were compared using the *z*-test. Quantitative variables were tested whether they come from a normal distribution and have equal variances. The variables propionibacteria and CoNS density as well as porphyrin fluorescence at baseline were log-transformed to achieve a normal distribution. As there were zero values for CoNS, the log-transformation was performed after adding 1 to the original values. There were minor deviations from the normal distribution, however, as ANOVA (analysis of variance) is relatively robust to violations of the normality assumption, especially when samples are of equal sizes, a one-way ANOVA was performed for all variables except for *S. aureus*, DLQI, FDLQI, and Leeds grading where the Kruskal-Wallis test was performed. For porphyrin fluorescence and CoNS density, the Welch's test was performed as the assumption of homogeneity of variances was not met.

For each treatment group, intragroup comparisons with baseline were done for all the follow-up visits. For variables with a normal distribution of the paired differences (inflamed lesions, noninflamed lesions, VAS, DLQI), the paired-samples t-test was performed. Where a normal distribution of the paired differences could not be assumed (total lesion counts, porphyrin fluorescence, FDLQI), the Wilcoxon signed-rank test was performed. Where neither a normal nor a symmetric distribution of the paired differences could be assumed (CoNS, propionibacteria, and *S. aureus* densities, Leeds grading), the exact sign test was performed.

For all variables (where applicable), week 8, 11, and 16 values were substracted from the baseline data (BL) and compared between the treatment groups. A one-way ANOVA was performed for inflamed lesions (BL−week 8, 11, and 16), noninflamed

lesions (BL−week 8, 11, and 16), porphyrin fluorescence (BL−week 8, 11, and 16), VAS (BL−week 8, 11, and 16), and DLQI (BL−week 16). The Kruskal-Wallis and Mann-Whitney tests were performed for total lesion counts (BL−week 8, 11, and 16), propionibacteria density (BL−week 8, 11, and 16), FDLQI (BL−week 16), Leeds grading (BL−week 8, 11, and 16), and CoNS density (BL−week 8, 11, and 16). For the DLQI (BL−week 8 and 11), the Welch's test was performed as the assumption of homogeneity of variances was not met. The least significant difference post-hoc tests were also performed. For pain scores, the mean of week 1, week 3, week 5, and week 7 was calculated and compared with an independent- samples t-test. No correction for multiple testing was done.

Chapter 5

Results

5. Results

5.1 The clinical effects

Of the 512 patients screened, 37 were randomised to the study **(Figure 5.1)**. This was lower than our target of 120 patients as the identification of suitable patients was much slower than anticipated, and recruitment was therefore stopped after 19 months. In brief, two main factors contributed to the poor recruitment including: (i) duration of the trial (16 weeks), number of visits (8 and 5 visits for patients in the light groups and adapalene group, respectively) and length of baseline and follow-up visits (approximately 3 hours). Our target population was mostly young individuals who were either studying or working full time and found it difficult to commit to the abovementioned requirements because of their busy schedule; and (ii) reluctance of young acne patients to minimise direct sunlight exposure for the duration of study. Throughout the study, a variety of techniques were used in order to boost recruitment, with varying degrees of success **(Table 5.1)**.

Of the 37 patients randomised, 13 patients were allocated to IPL-MAL, 11 to IPL-Placebo and 13 to adapalene. Two patients in the IPL-MAL group left the study before any treatment whilst 1 patient in the IPL-Placebo group was lost to follow-up after the third treatment. Three patients in the adapalene group did not show up for any of the follow-up visits whilst one patient in the IPL-MAL group was lost to follow-up after week 11. Thirty patients completed the study and were analysed for the primary outcome **(Figure 5.1)**.

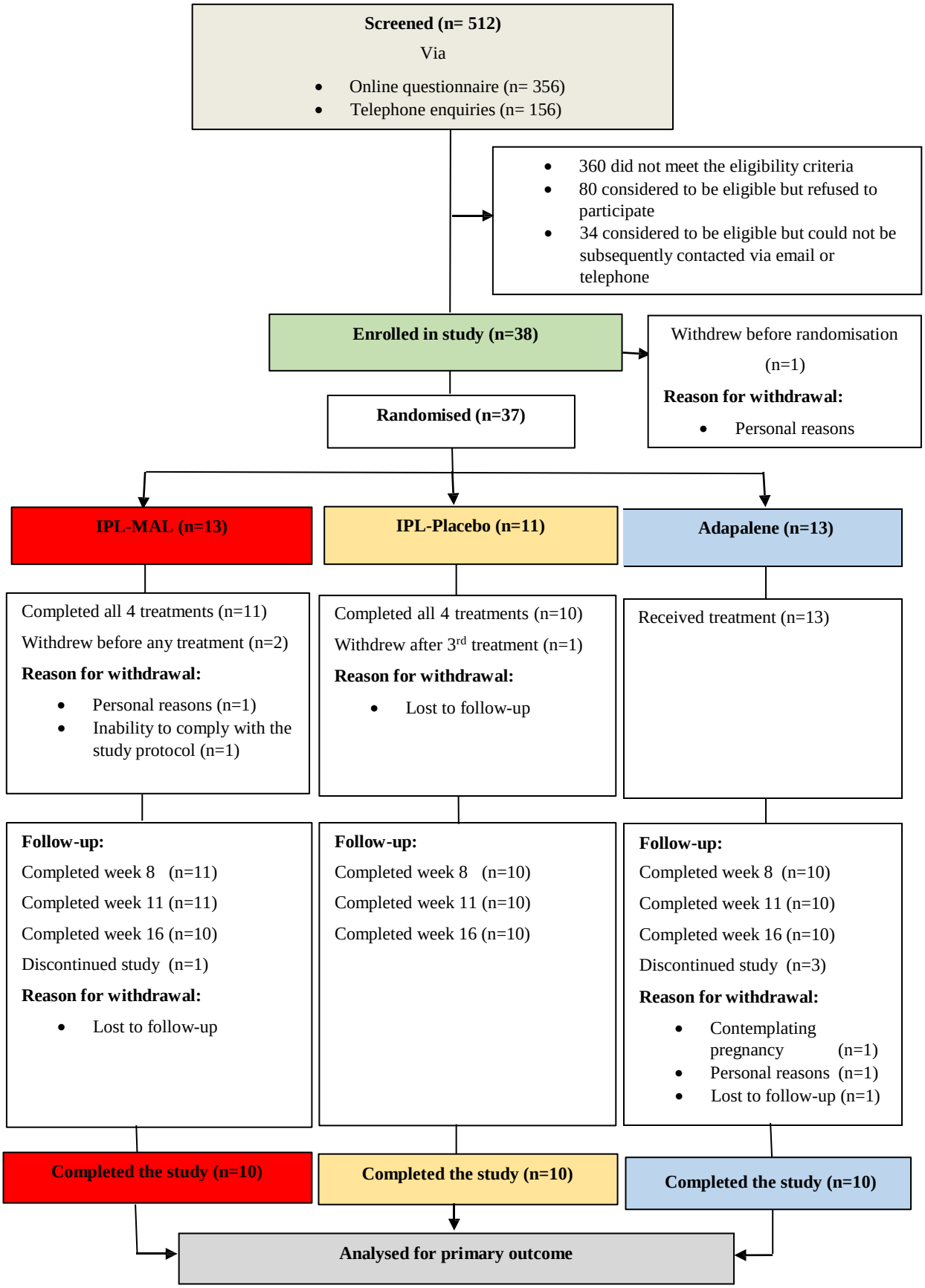


Figure 5.1: Flow chart. IPL, intense pulsed light; MAL, Methyl aminolaevulinate.

Table 5.1: Strategies employed to boost recruitment

Strategies with good impact	
Amend protocol/procedure	Some success
Advertise in local papers	Some success, but limited by lack of funds
Regular emails regarding the trial to all the Cardiff University students and staff	Some success
Weekly announcements on Cardiff University's online notice board	Some success
Strategies with little impact/unsure of impact	
Regular reminders to local general practitioners	Unsure
Regular reminders to clinicians at the University Hospital of Wales and nearby hospitals	Unsure
Distribution of pamphlets amongst the Cardiff University students during Freshers' week	Unsure
Setting up a stall, periodically, in the University Hospital of Wales concourse	Unsure
Display of posters about the trial within Cardiff University and Cardiff Metropolitan University campuses	Unsure
Display of posters about the trial in the local General practitioners' surgeries	Unsure

5.1.1 Demographic and baseline characteristics

Except for prior antibiotic use, the three treatment groups were similar with respect to the demographic and baseline characteristics **(Table 5.2)**. The IPL-Placebo group had used topical antibiotics significantly more often than the adapalene group. This was a coincidence as the patients were randomly assigned to the three groups.

Table 5.2: Baseline demographic and disease characteristics

Variable	Adapalene (n = 10)	IPL-MAL (n = 10)	IPL-Placebo (n = 10)†	P-value
Age (years), mean ± SD (range)	24.0 ± 5.9 (19.0 - 39.0)	23.0 ± 3.2 (20.0 - 29.0)	24.0 ± 5.0 (19.0 - 33.0)	0.868
Sex, F/M (n)	6/4	7/3	5/5	0.893
Height (cm), mean ± SD (range)	170.9 ± 8.5 (156.0 - 183.0)	169.7 ± 6.0 (161.5 - 182.0)	173.5 ± 7.8 (161.0 - 187.0)	0.528
Weight (kg), mean ± SD (range)	68.1 ± 12.9 (51.0 - 92.0)	65.8 ± 9.7 (52.0 - 78.0)	72.0 ± 14.8 (55.0 - 96.0)	0.548
Skin type, n (%)				
I	0 (0)	2 (20)	0 (0)	
II	5 (50)	3 (30)	4 (40)	
III	4 (40)	3 (30)	6 (60)	0.387
IV	1 (10)	2 (20)	0 (0)	
Current or ex-smokers, n (%)	2 (20)	1 (10)	5 (50)	0.192
Duration of acne (years), mean ± SD (range)	10.4 ± 6.1 (4.0 - 26.0)	9.2 ± 2.9 (5.0 - 13.0)	7.9 ± 4.2 (3.0 - 15.0)	0.486
Prior topical therapy, n (%)				
Antibiotics	1 (10)	5 (50)	7 (70)	0.035
Retinoids	4 (40)	0 (0)	2 (20)	0.122
Prior systemic therapy, n (%)				
Antibiotics	5 (50)	6 (60)	8 (80)	0.510
Retinoids	1 (10)	0 (0)	1 (10)	1.000

Table 5.2 Continued

Variable	Adapalene (n = 10)	IPL-MAL (n = 10)	IPL-Placebo (n = 10)†	P-value
Acne lesions, mean ± SD (range)				
Inflamed lesions (papules, pustules, nodules, cysts)	38.5 ± 20.6 (9.0 - 66.0)	26.6 ± 12.6 (8.0 - 45.0)	34.3 ± 18.3 (15.0 - 63.0)	0.319
Noninflamed lesions (open and closed comedones)	189.2 ± 114.6 (48.0 - 419.0)	100.1 ± 66.2 (25.0 - 242.0)	140.5 ± 82.0 (51.0 - 303.0)	0.104
Total lesion count (inflamed and non inflamed lesions)	227.7 ± 117.7 (72.0 - 432.0)	126.7 ± 72.0 (33.0 - 286.0)	174.8 ± 90.1 (66.0 - 332.0)	0.077
VAS score (cm), mean ± SD (range)	3.8 ± 2.0 (1.0 - 7.0)	4.0 ± 1.5 (2.0 - 6.0)	5.2 ± 1.9 (3.0 - 8.0)	0.195
Leeds grade, median (range)	1.5 (1.0 - 2.5)	1.0 (1.0 - 2.5)	1.3 (1.0 - 3.5)	0.430
DLQI score, mean ± SD (range)	5.1 ± 3.2 (2.0 - 11.0)	7.6 ± 5.7 (1.0 - 18.0)	7.1 ± 6.2 (1.0 - 17.0)	0.727
FDLQI score, mean ± SD (range)	1.7 ± 1.7 (0.0 - 5.0)	4.2 ± 6.2 (0.0 - 21.0)	1.7 ± 1.9 (0.0 - 4.0)	0.551
Porphyrin fluorescence, median (range)	539.5 (221.0 - 2550.0)	717.5 (27.0 - 3688.0)	548.0 (263.0 - 2383.0)	0.854

†For the FDLQI score in the IPL-Placebo group n=9. IPL, intense pulsed light; MAL, methyl aminolaevulinate; VAS, visual analogue scale; DLQI, Dermatology Life Quality Index; FDLQI, Family Dermatology Life Quality Index.

5.1.2 Effect on noninflamed lesions

Mean noninflamed lesion counts were reduced significantly in the adapalene group from 189.2 at baseline to 118.0 at week 16 (p = 0.007). No significant improvement was seen in the other two groups at any time **(Table 5.3, Figure 5.2)**.

For the change in noninflamed lesion counts from baseline, a statistically significant reduction of 37.6% was found in the adapalene group vs. 3.4% decrease in the IPL-MAL (p = 0.009) and 9.7% increase in the IPL-Placebo group (p = 0.001) at week 16 **(Table 5.4, Figure 5.3)**. There was no significant difference in the change in noninflamed lesion counts from baseline between the IPL-MAL and IPL-Placebo groups at week 16 (p = 0.197).

	Noninflamed lesion counts					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	189.2 ± 114.6 (48.0 - 419.0)		100.1 ± 66.2 (25.0 - 242.0)		140.5 ± 82.0 (51.0 - 303.0)	
Week 8	156.1 ± 106.4 (38.0 - 382.0)	0.076	87.7 ± 46.9 (28.0 - 196.0)	0.138	152.9 ± 77.5 (76.0 - 274.0)	0.531
Week 11	154.2 ± 110.1 (27.0 - 402.0)	0.063	105.1 ± 60.4 (25.0 - 253.0)	0.645	149.1 ± 87.4 (63.0 - 298.0)	0.639
Week 16	118.0 ± 74.4 (38.0 - 267.0)	0.007	96.7 ± 48.8 (29.0 - 204.0)	0.736	154.1 ± 88.6 (60.0 - 319.0)	0.478

Table 5.3: Mean noninflamed lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

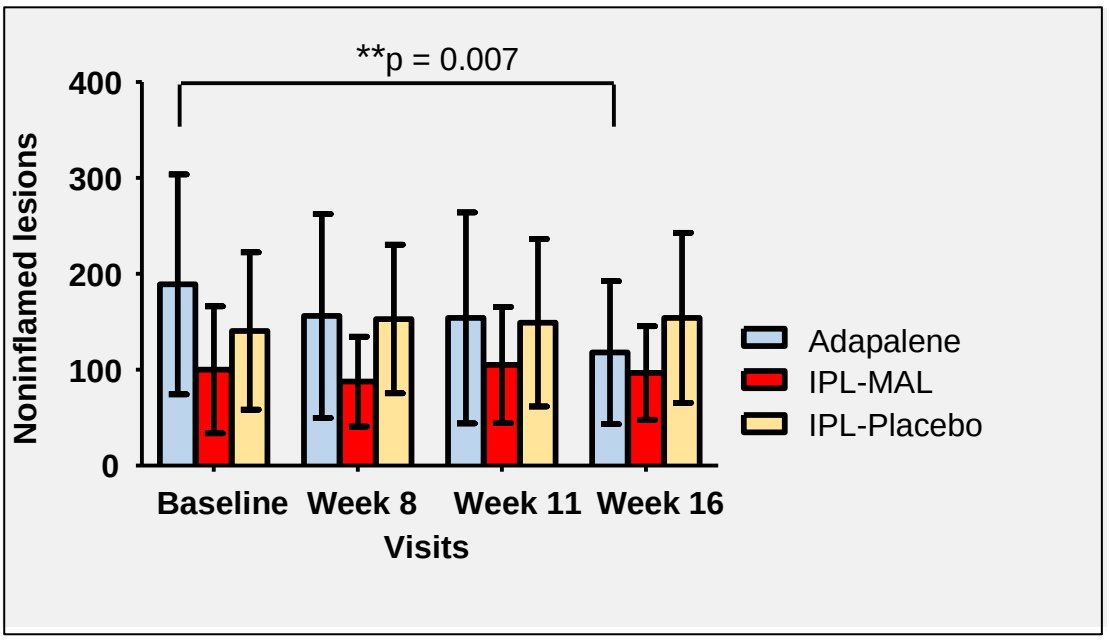


Figure 5.2: Mean noninflamed lesion counts at baseline, week 8, week 11, and week 16. Bar chart shows mean and standard deviation. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ Noninflamed lesion counts			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Mean (range)	33.1 (−21.0 - 140.0)	12.4 (−28.0 - 46.0)	−12.4 (−120.0 - 98.0)	0.125
Δ Week 11 Mean (range)	35.0 (−36.0 - 146.0)	−5.0 (−74.0 - 31.0)	−8.6 (−118.0 - 89.0)	0.099
Δ Week 16 Mean (range)	71.2 (−23.0 - 172.0)	3.4 (−54.0 - 38.0)	−13.6 (−97.0 - 115.0)	0.004

Table 5.4: Mean difference from baseline in noninflamed lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

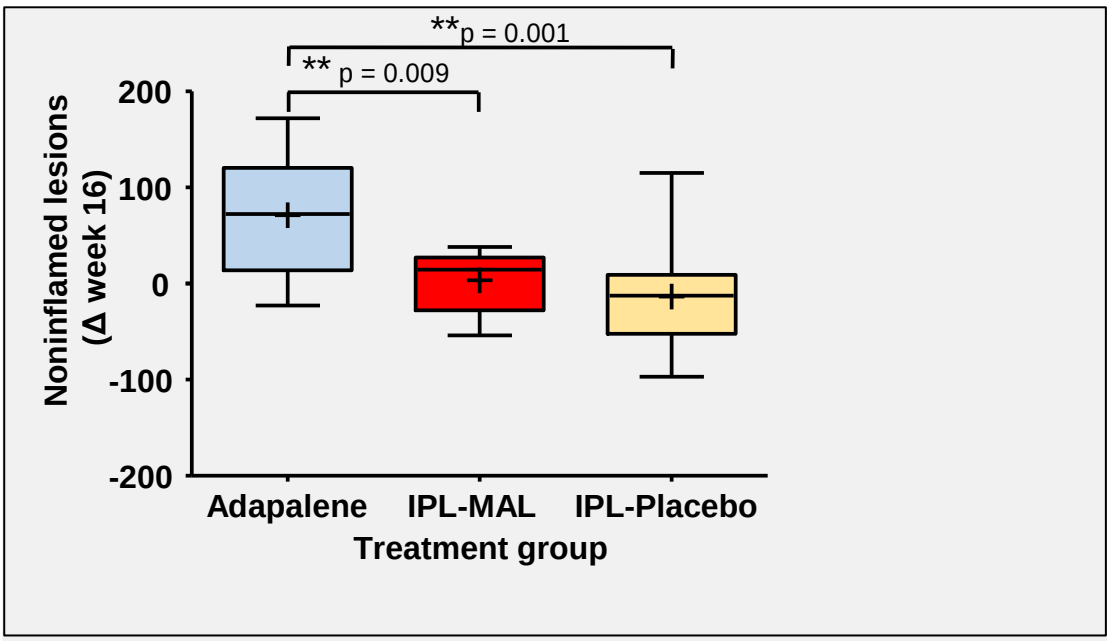


Figure 5.3: Difference from baseline in noninflamed lesion counts at week 16. Boxplot shows minimum, maximum, 25th and 75th percentiles, and median. + indicates the mean. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.3 Effect on inflamed lesions

Mean baseline inflamed lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups were 38.5, 26.6 and 34.3, respectively **(Table 5.5, Figure 5.4)**. In the IPL-MAL and IPL-Placebo groups, maximum improvement was seen at week 11 with mean lesion counts of 21.1 and 29.7 giving a mean percentage decrease from baseline of 20.7% and 13.4%, respectively. On the contrary, maximum improvement in the adapalene group was seen at week 16 with a mean value of 28.3. These results, however, did not reach statistical significance. Likewise, no significant difference in the change in inflamed lesion counts from baseline was found between the three treatment groups at any time **(Table 5.6)**.

	Inflamed lesion counts					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	38.5 ± 20.6 (9.0 - 66.0)		26.6 ± 12.6 (8.0 - 45.0)		34.3 ± 18.3 (15.0 - 63.0)	
Week 8	37.7 ± 33.1 (1.0 - 123.0)	0.924	24.4 ± 10.7 (10.0 - 44.0)	0.549	35.9 ± 25.1 (20.0 - 104.0)	0.793
Week 11	33.4 ± 31.0 (5.0 - 110.0)	0.506	21.1 ± 14.8 (8.0 - 54.0)	0.211	29.7 ± 26.3 (10.0 - 102.0)	0.511
Week 16	28.3 ± 20.2 (6.0 - 77.0)	0.137	24.6 ± 18.1 (8.0 - 62.0)	0.706	35.3 ± 21.5 (17.0 - 89.0)	0.823

Table 5.5: Mean inflamed lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

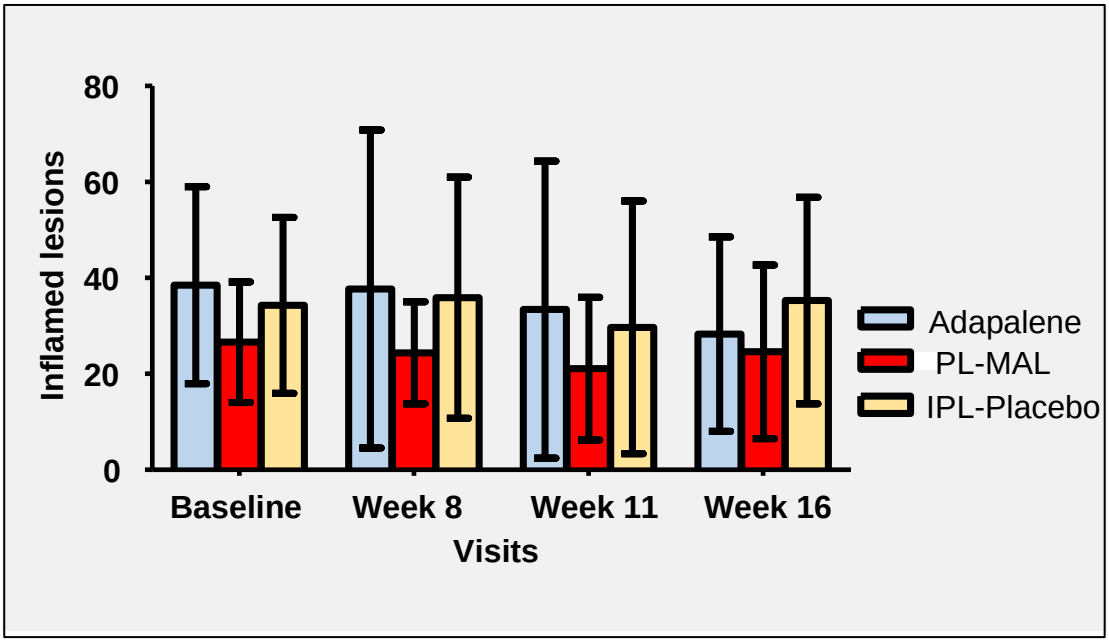


Figure 5.4: Mean inflamed lesion counts at baseline, week 8, week 11, and week 16. Bar chart shows mean and standard deviation. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ Inflamed lesion counts			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Mean (range)	0.8 (−60.0 - 25.0)	2.2 (−10.0 - 25.0)	−1.6 (−41.0 - 29.0)	0.908
Δ Week 11 Mean (range)	5.1 (−47.0 - 45.0)	5.5 (−9.0 - 33.0)	4.6 (−39.0 - 40.0)	0.995
Δ Week 16 Mean (range)	10.2 (−15.0 - 48.0)	2.0 (−26.0 - 35.0)	−1.0 (−26.0 - 23.0)	0.318

Table 5.6: Mean difference from baseline in inflamed lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.4 Effect on total lesion counts

In the adapalene group, mean total lesion counts were reduced significantly from 227.7 at baseline to 193.8 (p = 0.010), 187.6 (p = 0.014) and 146.3 (p = 0.006) at weeks 8, 11, and 16, respectively **(Table 5.7, Figure 5.5)**. No significant improvement was seen in the other two groups.

For the change in total lesion counts from baseline, a statistically significant reduction of 35.7% was found in the adapalene group vs. 4.3% decrease in the IPL-MAL (p = 0.011) and 8.4% increase in the IPL-Placebo group (p = 0.005) at week 16 **(Table 5.8, Figure 5.6)**. There was no significant difference in the change in total lesion counts from baseline between the IPL-MAL and IPL-Placebo groups at week 16 (p = 0.210).

	Total lesion counts					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	227.7 ± 117.7 (72.0 - 432.0)		126.7 ± 72.0 (33.0 - 286.0)		174.8 ± 90.1 (66.0 - 332.0)	
Week 8	193.8 ± 114.1 (64.0 - 414.0)	0.010	112.1 ± 47.6 (38.0 - 215.0)	0.250	188.8 ± 91.4 (97.0 - 378.0)	0.570
Week 11	187.6 ± 118.7 (52.0 - 422.0)	0.014	126.2 ± 60.3 (33.0 - 264.0)	0.629	178.8 ± 100.3 (73.0 - 333.0)	0.770
Week 16	146.3 ± 82.5 (54.0 - 295.0)	0.006	121.3 ± 47.4 (37.0 - 213.0)	0.695	189.4 ± 96.7 (81.0 - 347.0)	0.264

Table 5.7: Mean total lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16**.** IPL, intense pulsed light; MAL, methyl aminolaevulinate.

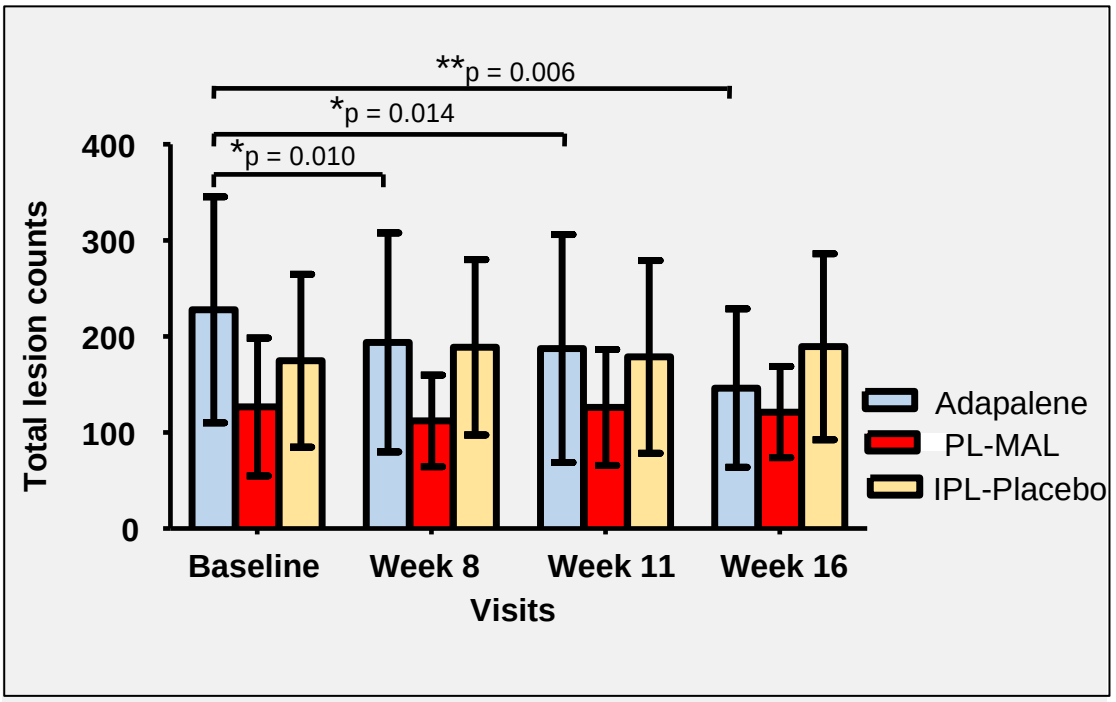


Figure 5.5: Mean total lesion counts at baseline, week 8, week 11, and week 16. Bar chart shows mean and standard deviation. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ Total lesion counts			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Mean (range)	33.9 (−5.0 - 156.0)	14.6 (−29.0 - 71.0)	−14.0 (−161.0 - 127.0)	0.092
Δ Week 11 Mean (range)	40.1 (−32.0 - 167.0)	0.5 (−82.0 - 36.0)	−4.0 (−122.0 - 129.0)	0.178
Δ Week 16 Mean (range)	81.4 (−18.0 - 185.0)	5.4 (−80.0 - 73.0)	−14.6 (−92.0 - 138.0)	0.006

Table 5.8: Mean difference from baseline in total lesion counts in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11 and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

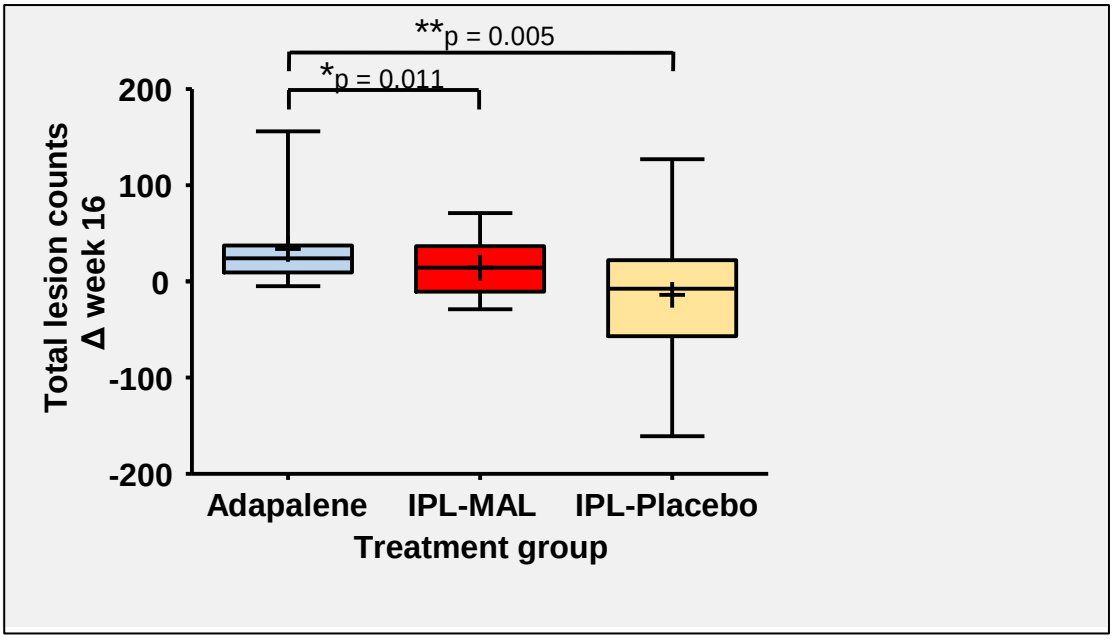


Figure 5.6: Difference from baseline in total lesion counts at week 16. Boxplot shows minimum, maximum, 25th and 75th percentiles, and median. + indicates the mean. IPL, intense pulsed light, MAL, methyl aminolaevulinate.

5.1.5 Effect on visual analogue scale

Mean VAS scores did not differ significantly from baseline in the three treatment groups at any time **(Table 5.9)**. Moreover, there was no significant difference in the change in VAS scores from baseline between the three groups **(Table 5.10)**.

	VAS score (cm)					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	3.8 ± 2.0 (1.0 - 7.0)		4.0 ± 1.5 (2.0 - 6.0)		5.2 ± 1.9 (3.0 - 8.0)	
Week 8	3.9 ± 2.4 (1.0 - 8.0)	0.931	3.7 ± 2.0 (0.5 - 6.0)	0.242	4.6 ± 2.3 (2.0 - 8.0)	0.193
Week 11	4.7 ± 2.7 (1.0 - 7.5)	0.273	3.9 ± 2.0 (0.0 - 7.0)	0.888	4.1 ± 2.7 (0.5 - 8.0)	0.074
Week 16	3.1 ± 1.9 (0.0 - 6.0)	0.143	3.8 ± 2.7 (0.0 - 8.0)	0.794	4.4 ± 2.8 (0.0 - 8.0)	0.223

Table 5.9: Mean VAS (visual analogue scale) scores in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ VAS score (cm)			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Mean (range)	−0.1 (−2.0 - 3.0)	0.3 (−1.0 - 1.5)	0.6 (−1.0 - 2.0)	0.577
Δ Week 11 Mean (range)	−0.9 (−5.0 - 3.0)	0.1 (−4.0 - 2.0)	1.1 (−1.0 - 3.5)	0.123
Δ Week 16 Mean (range)	0.7 (−2.0 - 3.0)	0.2 (−5.0 - 3.0)	0.8 (−1.0 - 4.0)	0.750

Table 5.10: Mean difference from baseline in VAS (visual analogue scale) scores in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.6 Effect on Leeds score

As an example, photographs of three patients, one from each treatment arm, that were used for Leeds grading are given in **Figures 5.7-5.9**. Leeds scores did not differ significantly from baseline in the three treatment groups at any time **(Table 5.11)**. Moreover, there was no significant difference in the change in Leeds grades from baseline between the three groups **(Table 5.12)**.

	Leeds grade					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Median (range)	P-value	**Median (range)**	P-value	**Median (range)**	P-value
Baseline	1.5 (1.0 - 2.5)		1.0 (1.0 - 2.5)		1.3 (1.0 - 3.5)	
Week 8	1.0 (1.0 - 4.5)	0.453	1.0 (1.0 - 3.5)	0.625	1.8 (1.0 - 4.0)	0.125
Week 11	1.3 (1.0 - 3.5)	0.625	1.3 (1.0 - 3.5)	0.375	1.0 (1.0 - 4.0)	1.000
Week 16	1.0 (1.0 - 3.0)	0.219	1.0 (1.0 - 5.5)	0.625	1.0 (1.0 - 5.0)	1.000

Table 5.11: Median Leeds scores in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ Leeds grade			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Median (range)	0.25 (−2.0 - 0.5)	0.0 (−1.5 - 0.5)	−0.5 (−1.5 - 1.0)	0.167
Δ Week 11 Median (range)	0.0 (−1.0 - 0.5)	0.0 (−1.5 - 0.5)	0.0 (−1.0 - 0.5)	0.254
Δ Week 16 Median (range)	0.25 (−0.5 - 1.5)	0.0 (−3.5 - 0.5)	0.0 (−1.5 - 1.0)	0.144

Table 5.12: Median difference from baseline in the Leeds grades in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

Figure 5.7: Clinical photographs of a patient treated with adapalene (front and right/left views): (a-c) before treatment, (d-f) at week 8, (g-i) week 11 and (j-l) week 16 visit.

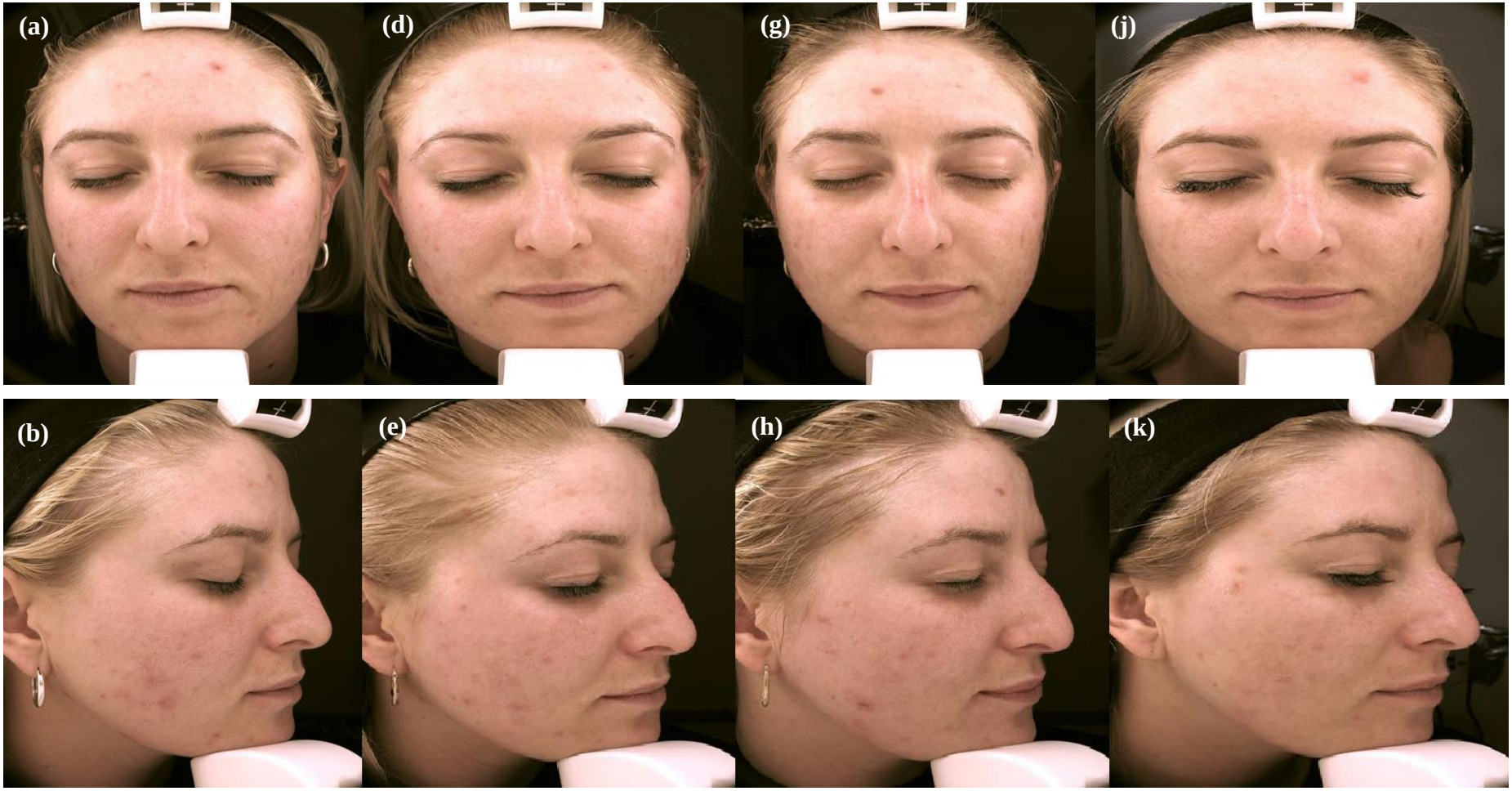


Figure 5.7 Continued

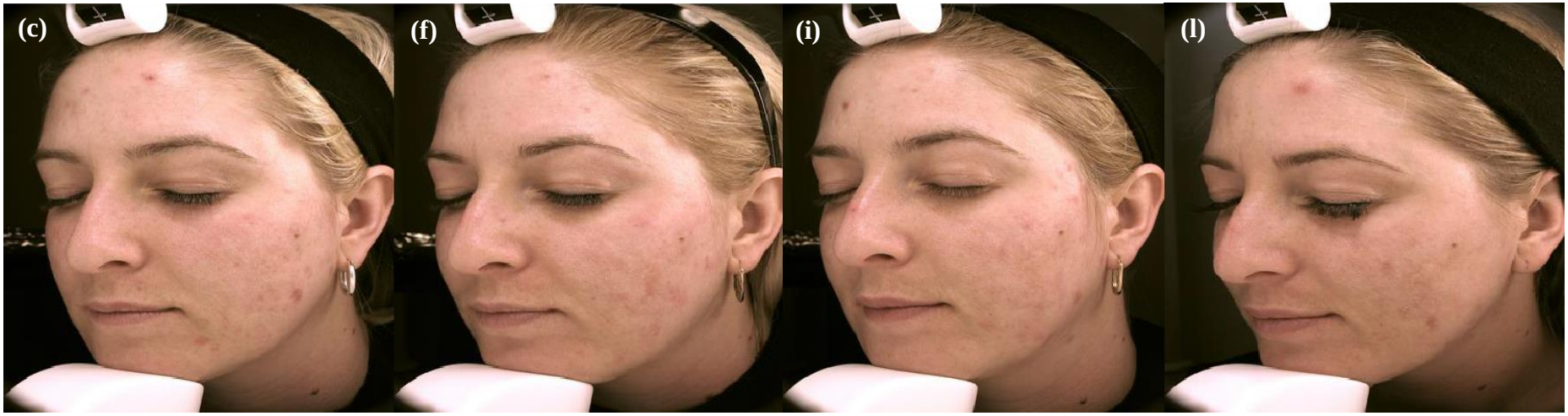


Figure 5.8: Clinical photographs of a patient treated with IPL-MAL (front and right/left views): (a-c) before treatment, (d-f) at week 8, (g-i) week 11 and (j-l) week 16 visit. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

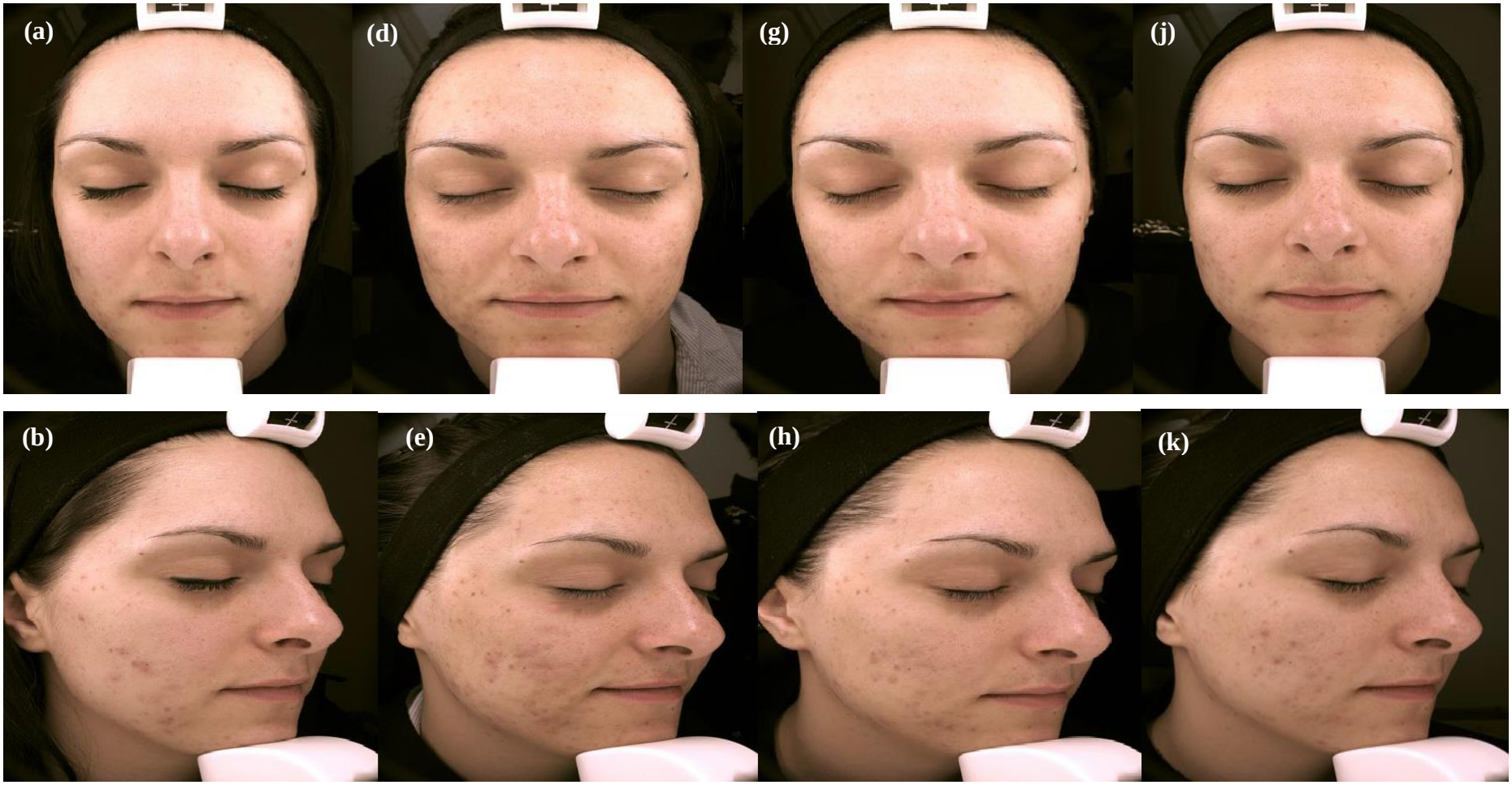


Figure 5.8 Continued

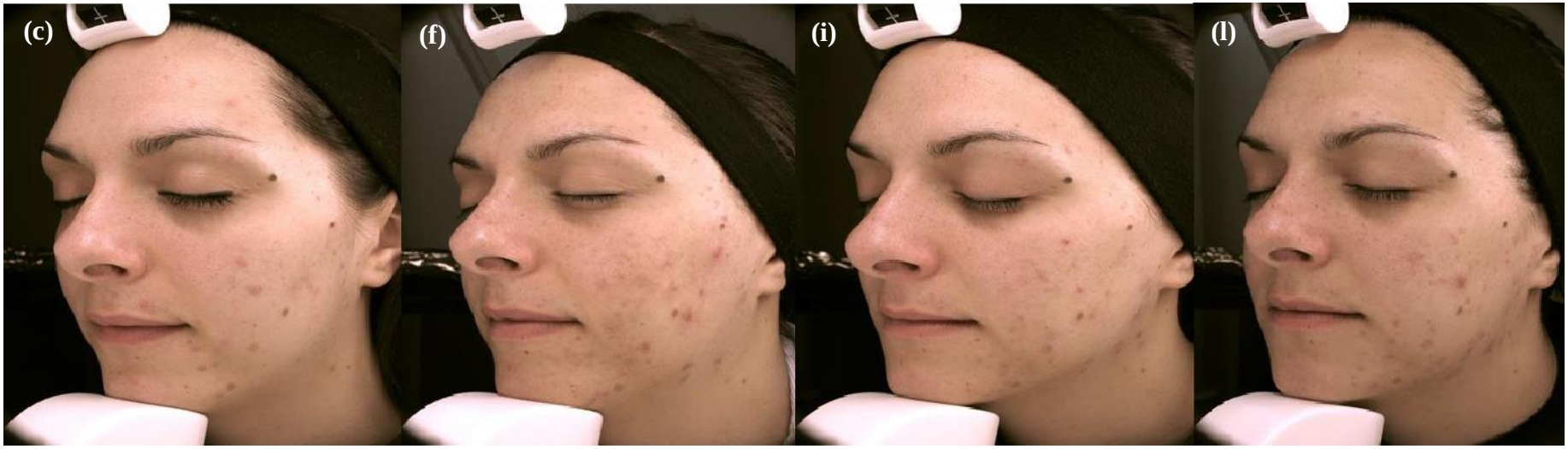


Figure 5.9: Clinical photographs of a patient treated with IPL-Placebo (front and right/left views): (a-c) before treatment, (d-f) at week 8, (g-i) week 11 and (j-l) week 16 visit. IPL, intense pulsed light.

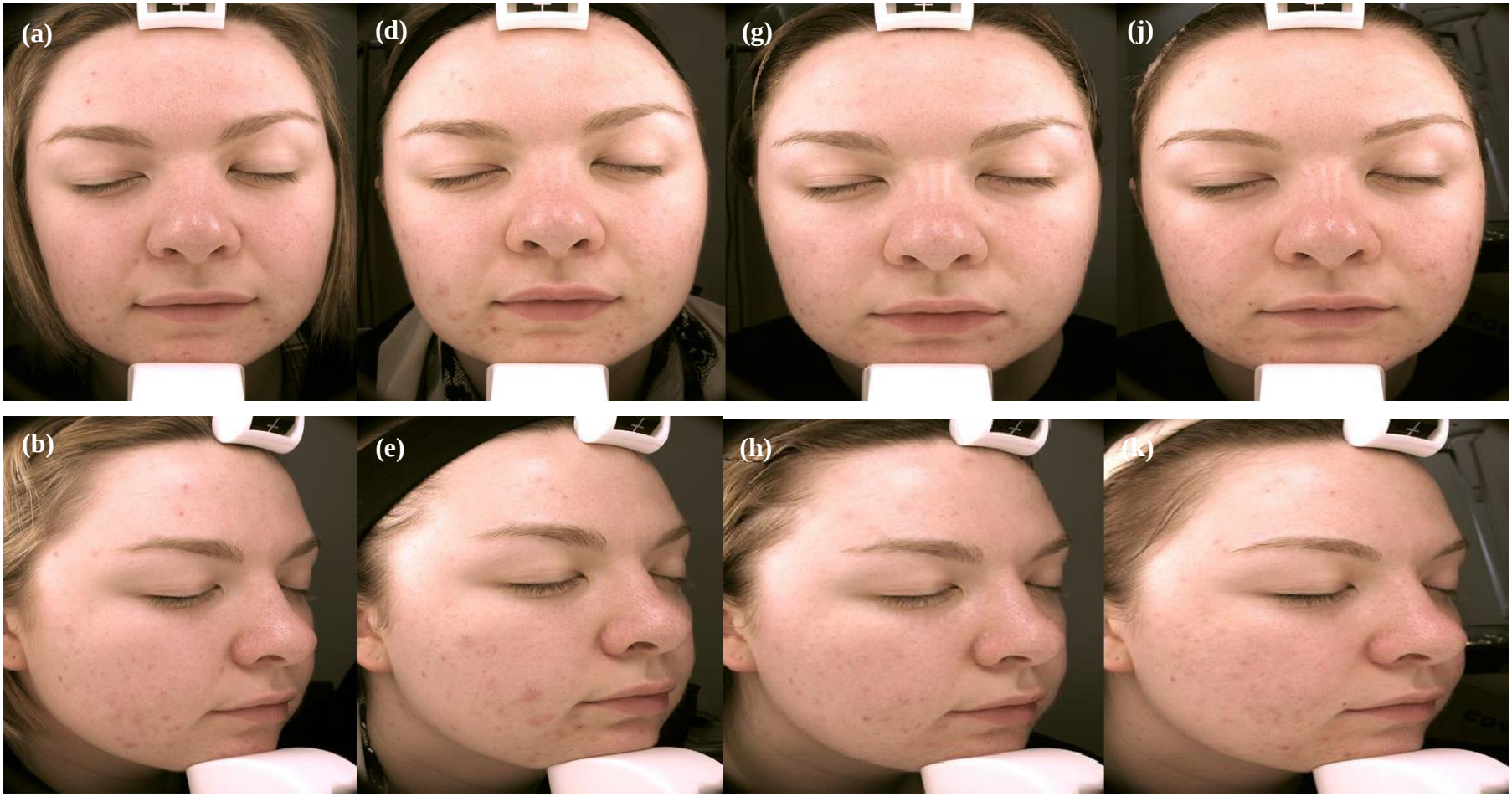
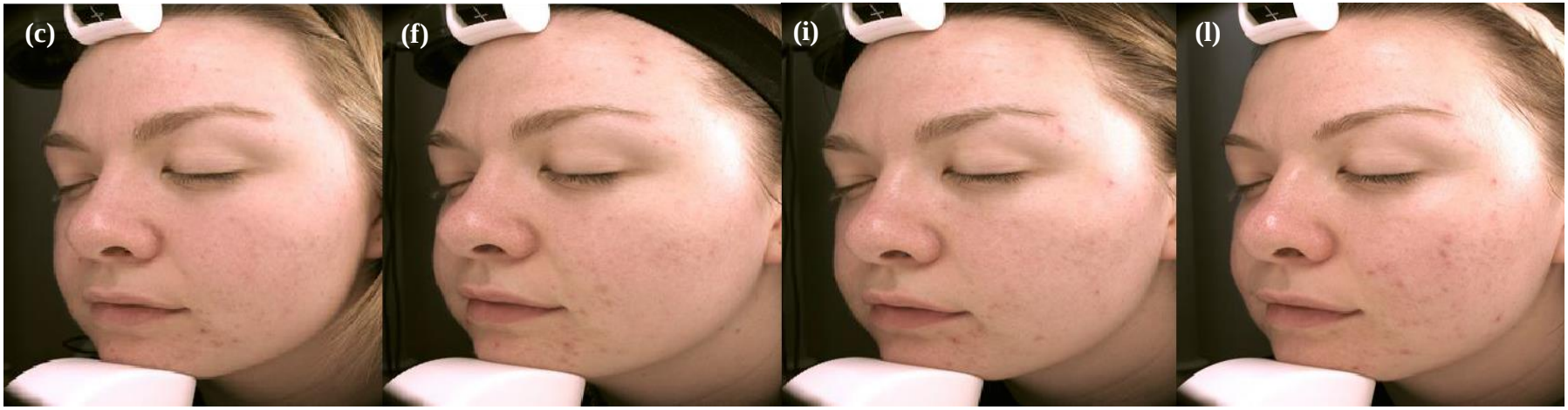


Figure 5.9 Continued



5.1.7 Effect on the Dermatology Life Quality Index

In the adapalene group, the mean DLQI score fell significantly from 5.1 at baseline to 2.4 at week 16 (p = 0.031) **(Table 5.13, Figure 5.10)**. Although there was a reduction in the DLQI scores in the IPL-MAL and IPL-Placebo groups at all the follow-up visits, this did not reach statistical significance. There was no significant difference in the change in DLQI scores from baseline between the three treatment groups at any time **(Table 5.14)**.

	DLQI score					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	5.1 ± 3.2 (2.0 - 11.0)		7.6 ± 5.7 (1.0 - 18.0)		7.1 ± 6.2 (1.0 - 17.0)	
Week 8	3.9 ± 4.2 (0.0 - 12.0)	0.181	6.4 ± 4.7 (0.0 - 16.0)	0.460	5.9 ± 5.9 (0.0 - 17.0)	0.089
Week 11	4.7 ± 4.7 (1.0 - 12.0)	0.637	5.4 ± 5.5 (0.0 - 18.0)	0.237	6.3 ± 6.7 (0.0 - 20.0)	0.366
Week 16	2.4 ± 3.1 (0.0 - 11.0)	0.031	5.7 ± 5.2 (1.0 - 16.0)	0.364	6.7 ± 7.0 (0.0 - 20.0)	0.613

Table 5.13: Mean DLQI (Dermatology Life Quality Index) scores in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

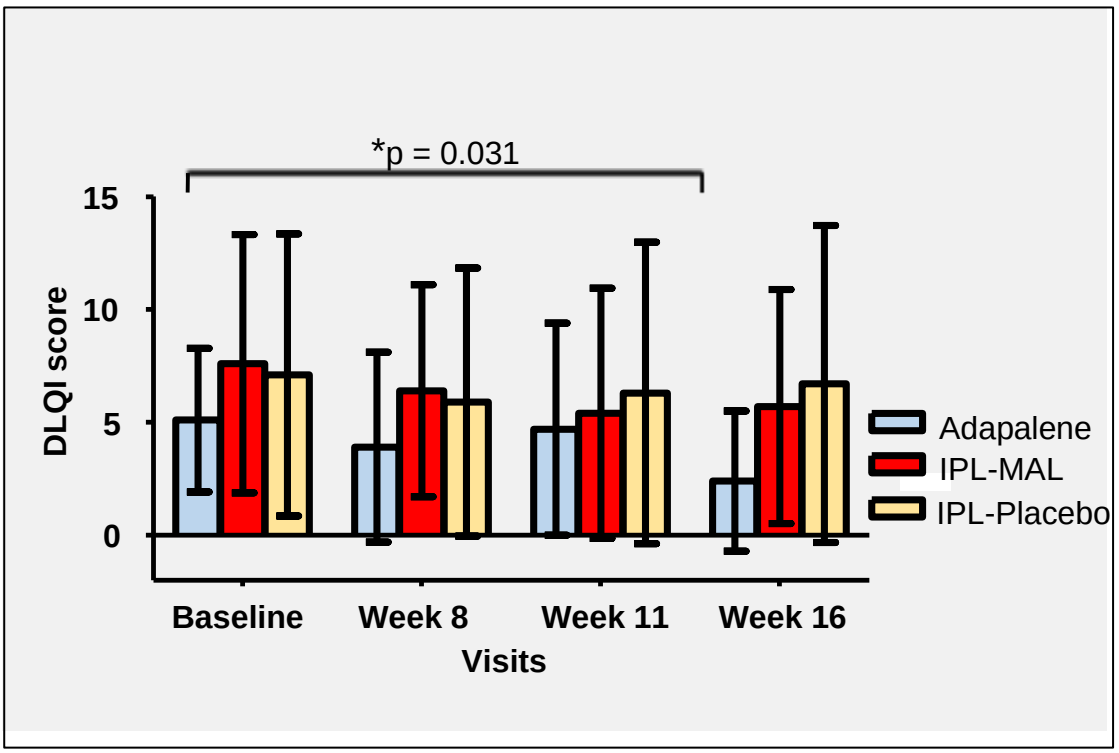


Figure 5.10: Mean DLQI (Dermatology Life Quality Index) scores at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ DLQI score			
	Adapalene (n = 10)	**IPL-MAL (n=10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Mean (range)	1.2 (−3.0 - 7.0)	1.2 (−5.0 - 10.0)	1.2 (−1.0 - 6.0)	1.000
Δ Week 11 Mean (range)	0.4 (−4.0 - 4.0)	2.2 (−6.0 - 12.0)	0.8 (−3.0 - 6.0)	0.661
Δ Week 16 Mean (range)	2.7 (−2.0 - 9.0)	1.9 (−8.0 - 15.0)	0.4 (−4.0 - 4.0)	0.493

Table 5.14: Mean difference from baseline in the DLQI (Dermatology Life Quality Index) scores in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.8 Effect on the Family Dermatology Life Quality Index

Mean FDLQI scores did not differ significantly from baseline in the three treatment groups at week 16 **(Table 5.15)**. Moreover, there was no significant difference in the change in FDLQI scores from baseline between the three groups **(Table 5.16)**.

	FDLQI score					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 9)**	
	Mean ± SD (range)	P-value	**Mean ± SD (range)**	P-value	**Mean ± SD (range)**	P-value
Baseline	1.7 ± 1.7 (0.0 - 5.0)		4.2 ± 6.2 (0.0 - 21.0)		1.7 ± 1.9 (0.0 - 4.0)	
Week 16	2.0 ± 1.8 (0.0 - 5.0)	0.750	4.2 ± 4.8 (0.0 - 16.0)	1.000	2.1 ± 4.0 (0.0 - 12.0)	1.000

Table 5.15: Mean FDLQI (Family Dermatology Life Quality Index) scores in the adapalene, IPL-MAL, and IPL-Placebo groups at baseline and week 16. A last value carried forward approach was used for the missing values in the three treatment groups at week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ FDLQI score			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 9)**	P-value
Δ Week 16 Mean (range)	−0.3 (−3.0 - 1.0)	0.0 (−5.0 - 5.0)	−0.4 (−8.0 - 4.0)	0.898

Table 5.16: Mean difference from baseline in the FDLQI (Family Dermatology Life Quality Index) scores in the adapalene, IPL-MAL, and IPL-Placebo groups at week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.9 Effect on porphyrin fluorescence

Median porphyrin fluorescence did not differ significantly from baseline in the three treatment groups at any time **(Table 5.17)**. There was also no significant difference in the change in porphyrin fluorescence from baseline between the groups **(Table 5.18)**. As an example, fluorescent photographs of a patient treated with adapalene are given in **Figure 5.11**.

	Porphyrin fluorescence					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Median (range)	P-value	**Median (range)**	P-value	**Median (range)**	P-value
Baseline	539.5 (221.0 - 2550.0)		717.5 (27.0 - 3688.0)		548.0 (263.0 - 2383.0)	
Week 8	443.5 (194.0 - 1286.0)	0.922	490.0 (62.0 - 4551.0)	0.846	401.0 (114.0 - 1946.0)	0.922
Week 11	574.0 (179.0 - 1666.0)	0.922	564.5 (94.0 - 4259.0)	1.000	777.0 (241.0 - 1854.0)	0.695
Week 16	574.0 (215.0 - 2075.0)	0.496	557.5 (56.0 - 4600.0)	0.770	448.0 (159.0 - 2289.0)	0.695

Table 5.17: Median porphyrin fluorescence in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

	Δ Porphyrin fluorescence			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Median (range)	−77.5 (−267.0 - 2314.0)	−12.5 (−1143.0 - 1999.0)	−27.0 (−676.0 - 1607.0)	0.848
Δ Week 11 Median (range)	−51.5 (−654.0 - 1978.0)	−110.5 (−851.0 - 1554.0)	80.5 (−521.0 - 529.0)	0.907
Δ Week 16 Median (range)	11.5 (−236.0 - 694.0)	−23.5 (−1192.0 - 1567.0)	−8.0 (−713.0 - 1946.0)	0.996

Table 5.18: Median difference from baseline in porphyrin fluorescence in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

Figure 5.11: Fluorescent photographs of a patient treated with adapalene (1) Enlarged view to show "analysis mask" and follicular fluorescence (2) front and right/left views: (a-c) before treatment, (d-f) at week 8, (g-i) week 11 and (j-l) week 16 visit.

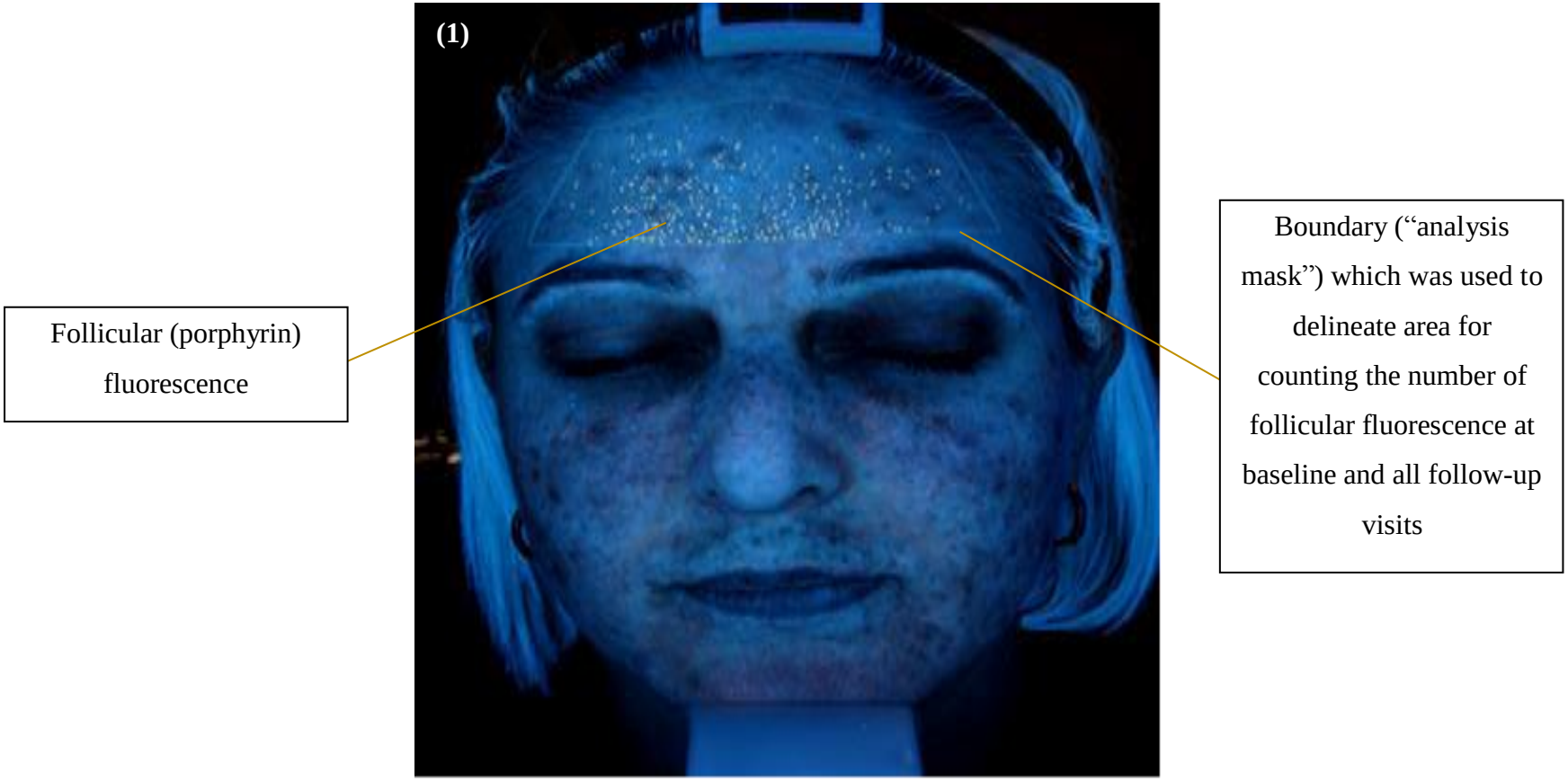


Figure 5.11 Continued

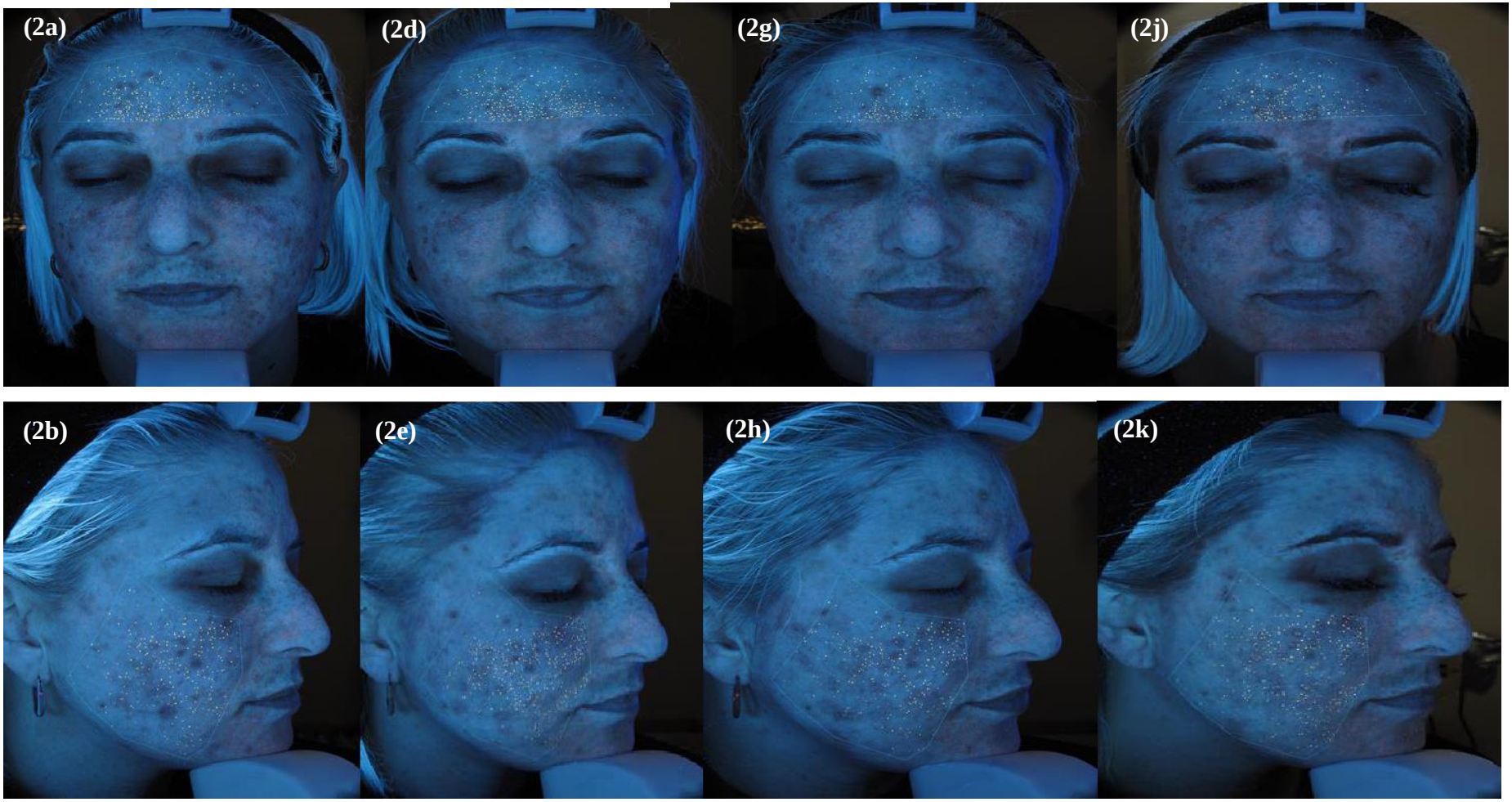
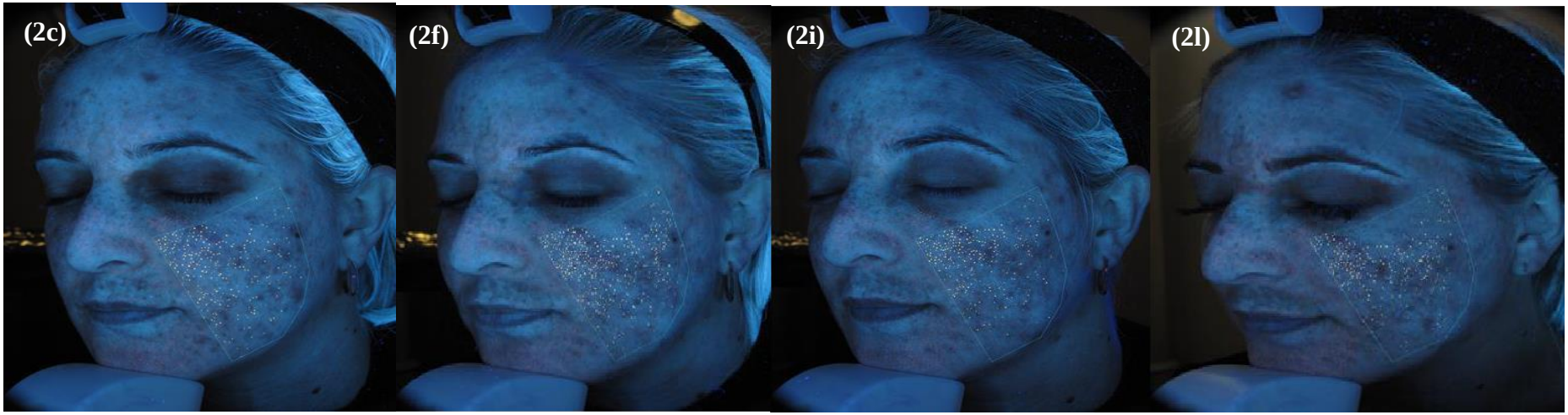


Figure 5.11 Continued



5.1.10 Adverse events

In the adapalene group, majority of the side-effects were reported at week 8 which improved, with the continued use of adapalene, at week 11 **(Table 5.19)**. At week 8, skin dryness was the commonest reported side-effect (9/10) followed by exfoliation (4/10), erythema (3/10), worsening of acne (3/10, lasting 1-6 weeks) and skin tenderness (2/10), respectively.

All the patients described a burning pricking pain during illumination in the IPL-Placebo and IPL-MAL groups. Mean VAS scores for pain were 2.74 (range 0.25-6.50) and 2.60 (range 0.25-5.50) in the IPL-MAL and IPL-Placebo groups, respectively. There was no significant difference in the mean pain scores between the two groups ($p = 0.868$). In the IPL-Placebo group, majority of the side-effects were reported after the second treatment (week 5) with oedema (2/10, lasting 1-2 days) being the commonest adverse event **(Table 5.20)**. Cold sore and patchy alopecia in the beard area were reported by a patient each at weeks 5 and 11, respectively. Similarly, worsening of acne (starting few days after treatment and lasting 15-25 days) was reported by a patient each at weeks 5 and 7. In the IPL-MAL group, equal number of side-effects were reported after the second and third treatments (weeks 5 and 7) with two patients reporting oedema (lasting 2-3 days) and exfoliation (lasting 2 days) at each visit **(Table 5.21)**. Moreover, worsening of acne was reported by two patients (lasting 4-17 days) at week 5 and one patient (lasting 17 days) at week 7. One patient also reported blistering at week 7, which resolved without any sequelae after 5 days.

No scarring or pigmentary complications were seen in any patient treated with light. Most of the side-effects in the three treatment groups were mild to moderate and resolved completely by week 16.

Adapalene (n = 10)			
Adverse event	**Week 8 n (severity)**	**Week 11 n (severity)**	**Week 16 n (severity)**
Erythema	3 (mild)	1 (mild)	0
Dryness	9 (7 mild, 2 moderate)	8 (7 mild, 1 moderate)	0
Pruritus	1 (mild)	0	0
Exfoliation	4 (2 mild, 2 moderate)	1 (mild)	0
Stinging	1 (moderate)	0	0
Skin tenderness	2 (1 mild, 1 moderate)	1 (mild)	0
Worsening of acne	3 (2 moderate)	1 (moderate)	0

Table 5.19: Adapalene side-effects.

IPL-Placebo (n = 10)						
Adverse event	**Week 3 n (severity)**	**Week 5 n (severity)**	**Week 7 n (severity)**	**Week 8 n (severity)**	**Week 11 n (severity)**	**Week 16 n (severity)**
Dryness	0	1 (moderate)	0	1 (mild)	0	0
Oedema	1 (mild)	2 (mild)	0	0	0	0
Exfoliation	0	1 (mild)	0	0	0	0
Cold sore	0	1 (mild)	0	0	0	0
Alopecia	0	0	0	0	1 (mild)	0
Lip tenderness	0	0	1 (mild)	0	0	0
Worsening of acne	0	1 (severe)	1	0	0	0

Table 5.20: IPL-Placebo side-effects. IPL, intense pulsed light.

IPL-MAL (n = 10)						
Adverse event	**Week 3 n (severity)**	**Week 5 n (severity)**	**Week 7 n (severity)**	**Week 8 n (severity)**	**Week 11 n (severity)**	**Week 16 n (severity)**
Persistent erythema (> 3 days)	1 (mild)	0	0	1 (mild)	0	0
Dryness	0	1 (mild)	1 (mild)	1 (mild)	0	0
Oedema	1 (mild)	2 (mild)	2 (mild)	1 (mild)	0	0
Exfoliation	2 (mild)	2 (1 mild, 1 moderate)	2 (1 mild, 1 moderate)	3 (1 mild, 1 moderate, 1 severe)	0	0
Post-treatment pain	1 (mild)	1 (moderate)	1 (mild)	0	0	0
Migraine	1 (moderate)	0	0	0	1 (moderate)	0
Scab formation	0	1 (mild)	1 (moderate)	0	0	0
Pruritus	0	1 (moderate)	1 (moderate)	1 (mild)	0	0
Blisters	0	0	1 (moderate)	0	0	0
Post-treatment burning	0	0	0	1 (severe)	0	0
Worsening of acne	2 (1 mild, 1 severe)	2 (1 mild, 1 moderate)	1 (moderate)	0	0	0

Table 5.21: IPL-MAL side-effects. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

5.1.11 Summary of the clinical findings

1. Adapalene reduced the noninflamed lesion counts by 37.6% at week 16, which was statistically significant when compared with the 3.4% decrease in the IPL-MAL ($p = 0.009$) and 9.7% increase in the IPL-Placebo group ($p = 0.001$).

2. The maximum improvement in the inflamed lesion counts of 20.7% and 13.4% in the IPL-MAL ($p = 0.211$) and IPL-Placebo ($p = 0.511$) groups at week 11, respectively, was not statistically significant. In the adapalene group, a non-significant improvement of 13.2% ($p = 0.506$) and 26.5% ($p = 0.137$) was seen at weeks 11 and 16, respectively.

3. Adapalene reduced the total lesion counts by 35.7% at week 16, which was statistically significant when compared with the 4.3% decrease in the IPL-MAL ($p = 0.011$) and 8.4% increase in the IPL-Placebo group ($p = 0.005$).

4. Adapalene reduced the DLQI score by 52.9% at week 16, which was significant at $p = 0.031$. However, this difference was not significant when compared with the 25% decrease in the IPL-MAL and 5.6% decrease in the IPL-Placebo group.

5. There was no significant difference within or between the groups in the VAS, Leeds, and FDLQI scores pre- and post-treatment.

6. There was no significant difference within or between the groups in the porphyrin fluorescence pre- and post-treatment.

5.2 Effect on cutaneous microflora

5.2.1 Baseline characteristics

The prevalence of propionibacteria, CoNS, and *S. aureus* at baseline in the three treatment groups are given in **Table 5.22**. At baseline, *P. acnes* was isolated from all the patients in the three treatment groups whilst *P. granulosum* was cultured from 10% and 20% of the patients in the IPL-MAL and IPL-Placebo groups, respectively. Similarly, except for two patients in the IPL-MAL group, CoNS were ubiquitous in the three treatment groups. Conversely, *S. aureus* was isolated from 20% of the patients in the adapalene group only. Because of technical problems the laboratory staff were unable to separately count *P. acnes* and *P. granulosum* colonies and therefore only total propionibacteria count is given in the trial. The three treatment groups were similar with respect to the population densities of propionibacteria, CoNS, and *S. aureus* **(Table 5.23)**.

Variable	Adapalene (n = 10)	IPL-MAL (n = 10)	IPL-Placebo (n = 10)
Propionibacterium acnes, n (%)	10 (100)	10 (100)	10 (100)
Propionibacterium granulosum, n (%)	0 (0)	1 (10)	2 (20)
Coagulase-negative staphylococci, n (%)	10 (100)	8 (80)	10 (100)
Staphylococcus aureus, n (%)	2 (20)	0 (0)	0 (0)

Table 5.22: Baseline prevalence of various micro-organisms in the adapalene, IPL-MAL, and IPL-Placebo groups. IPL, intense pulsed light; MAL, methyl aminolaevulinate.

Variable	Adapalene (n = 10)	IPL-MAL (n = 10)	IPL-Placebo (n = 10)	P-value
Propionibacteria, median CFU cm^{-2} (range)	2.6×10^4 (9.8×10^3 - 2.3×10^6)	1.8×10^5 (3.7×10^2 - 1.0×10^7)	2.7×10^4 (3.0×10^3 - 4.1×10^6)	0.881
CoNS, median CFU cm^{-2} (range)	1.8×10^4 (2.7×10^3 - 1.2×10^5)	6.4×10^3 (0.0×10^0 - 1.6×10^6)	1.3×10^4 (1.5×10^1 - 7.3×10^4)	0.369
Staphylococcus aureus, median CFU cm^{-2} (range)	0.0 (0.0×10^0 - 3.3×10^3)	a	a	0.126

Table 5.23: Baseline densities of various micro-organisms in the adapalene, IPL-MAL, and IPL-Placebo groups. [a]No *Staphylococcus aureus* was isolated and therefore its value is constant (=0). IPL, intense pulsed light; MAL, methyl aminolaevulinate; CFU, colony-forming units; CoNS, coagulase-negative staphylococci.

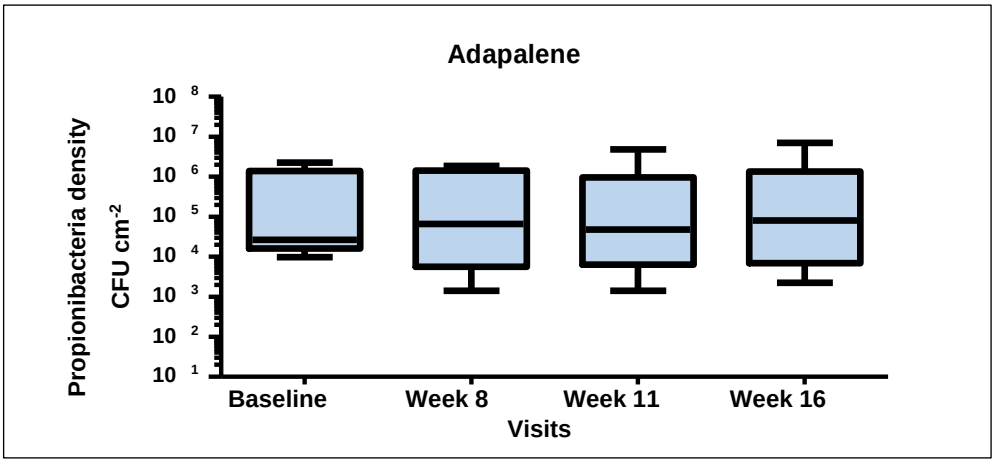
5.2.2 Effect on propionibacteria density

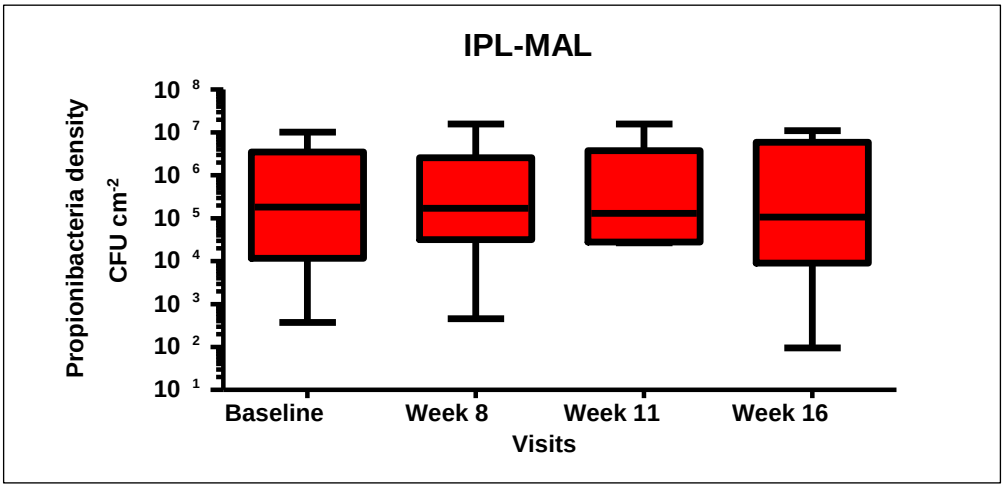
A significant increase in the median density of propionibacteria from baseline was observed in the IPL-Placebo group at week 16 (p = 0.021) **(Table 5.24, Figure 5.12)**. A non-significant increase was also seen in the adapalene group whilst a reduction occurred in the IPL-MAL group at all the follow-up visits. There was no significant

difference in the change in propionibacteria densities from baseline between the three groups **(Table 5.25)**.

	Propionibacteria density (CFU cm^{-2})					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Median (range)	P-value	**Median (range)**	P-value	**Median (range)**	P-value
Baseline	2.6×10^4 (9.8×10^3 - 2.3×10^6)		1.8×10^5 (3.7×10^2 - 1.0×10^7)		2.7×10^4 (3.0×10^3 - 4.1×10^6)	
Week 8	6.5×10^4 (1.4×10^3 - 1.9×10^6)	0.344	1.7×10^5 (4.6×10^2 - 1.6×10^7)	0.344	2.9×10^5 (1.2×10^4 - 2.1×10^6)	0.344
Week 11	4.8×10^4 (1.4×10^3 - 4.9×10^6)	0.344	1.3×10^5 (2.7×10^4 - 1.6×10^7)	0.344	4.7×10^5 (1.1×10^4 - 7.1×10^6)	0.344
Week 16	8.1×10^4 (2.2×10^3 - 7.0×10^6)	0.754	1.1×10^5 (9.5×10^1 - 1.1×10^7)	1.000	1.6×10^5 (9.1×10^3 - 1.7×10^7)	0.021

Table 5.24: Median propionibacteria densities in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.





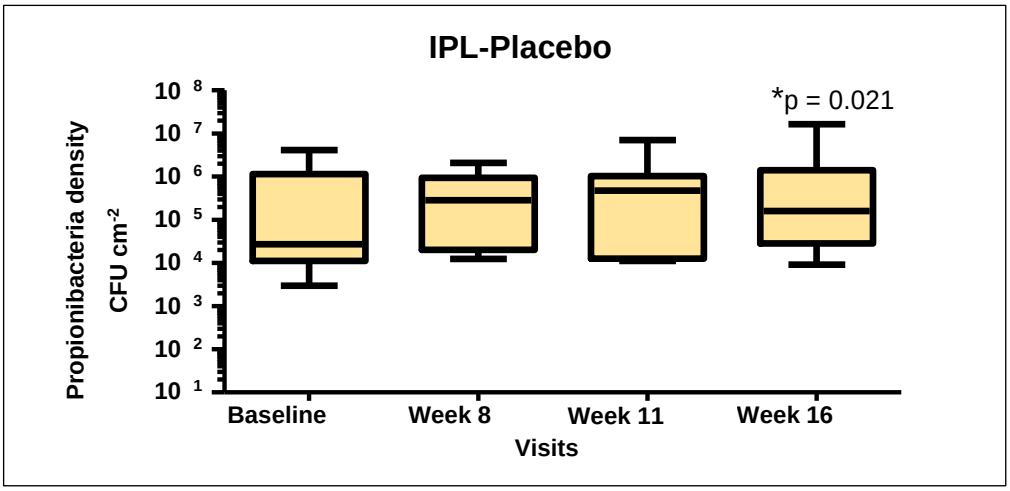


Figure 5.12. Propionibacteria densities at baseline, week 8, week 11, and week 16. Boxplot shows minimum, maximum, 25th and 75th percentiles, and median. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

	Δ Propionibacteria density (CFU cm^{-2})			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Median (range)	8.3×10^3 (-4.9×10^5 - 5.3×10^5)	-7.6×10^3 (-5.5×10^6 - 1.8×10^6)	-1.3×10^4 (-9.3×10^5 - 2.4×10^6)	0.468
Δ Week 11 Median (range)	8.8×10^3 (-2.6×10^6 - 5.9×10^5)	-2.6×10^4 (-5.6×10^6 - 1.0×10^6)	-8.6×10^4 (-2.9×10^6 - 1.5×10^6)	0.362
Δ Week 16 Median (range)	-2.2×10^4 (-4.8×10^6 - 9.4×10^5)	8.2×10^2 (-6.9×10^6 - 2.5×10^6)	-4.6×10^4 (-1.4×10^7 - 1.4×10^6)	0.658

Table 5.25: Median difference from baseline in the propionibacteria densities in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

5.2.3 Effect on coagulase-negative staphylococci density

A significant increase in the median CoNS density from baseline was observed in the IPL-MAL group at week 8 (p = 0.039) **(Table 5.26, Figure 5.13)**. A non-significant increase was also seen in the IPL-Placebo group whilst a reduction occurred in the adapalene group at all the follow-up visits. There was no significant difference in the change in CoNS densities from baseline between the three treatment groups **(Table 5.27)**.

	Coagulase-negative staphylococci density (CFU cm^{-2})					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Median (range)	P-value	**Median (range)**	P-value	**Median (range)**	P-value
Baseline	1.8×10^4 (2.7×10^3 - 1.2×10^5)		6.4×10^3 (0.0×10^0 - 1.6×10^6)		1.3×10^4 (1.5×10^1 - 7.3×10^4)	
Week 8	1.4×10^4 (0.0×10^0 - 1.1×10^5)	0.344	2.7×10^4 (0.0×10^0 - 5.6×10^5)	0.039	1.8×10^4 (9.6×10^2 - 9.0×10^4)	0.344
Week 11	1.2×10^4 (0.0×10^0 - 1.3×10^5)	0.344	1.4×10^4 (0.0×10^0 - 9.1×10^4)	1.000	2.2×10^4 (1.7×10^3 - 1.5×10^5)	0.754
Week 16	1.4×10^4 (0.0×10^0 - 2.7×10^5)	0.344	2.6×10^4 (0.0×10^0 - 1.0×10^5)	1.000	1.5×10^4 (7.3×10^2 - 7.8×10^4)	0.754

Table 5.26: Median coagulase-negative staphylococci densities in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

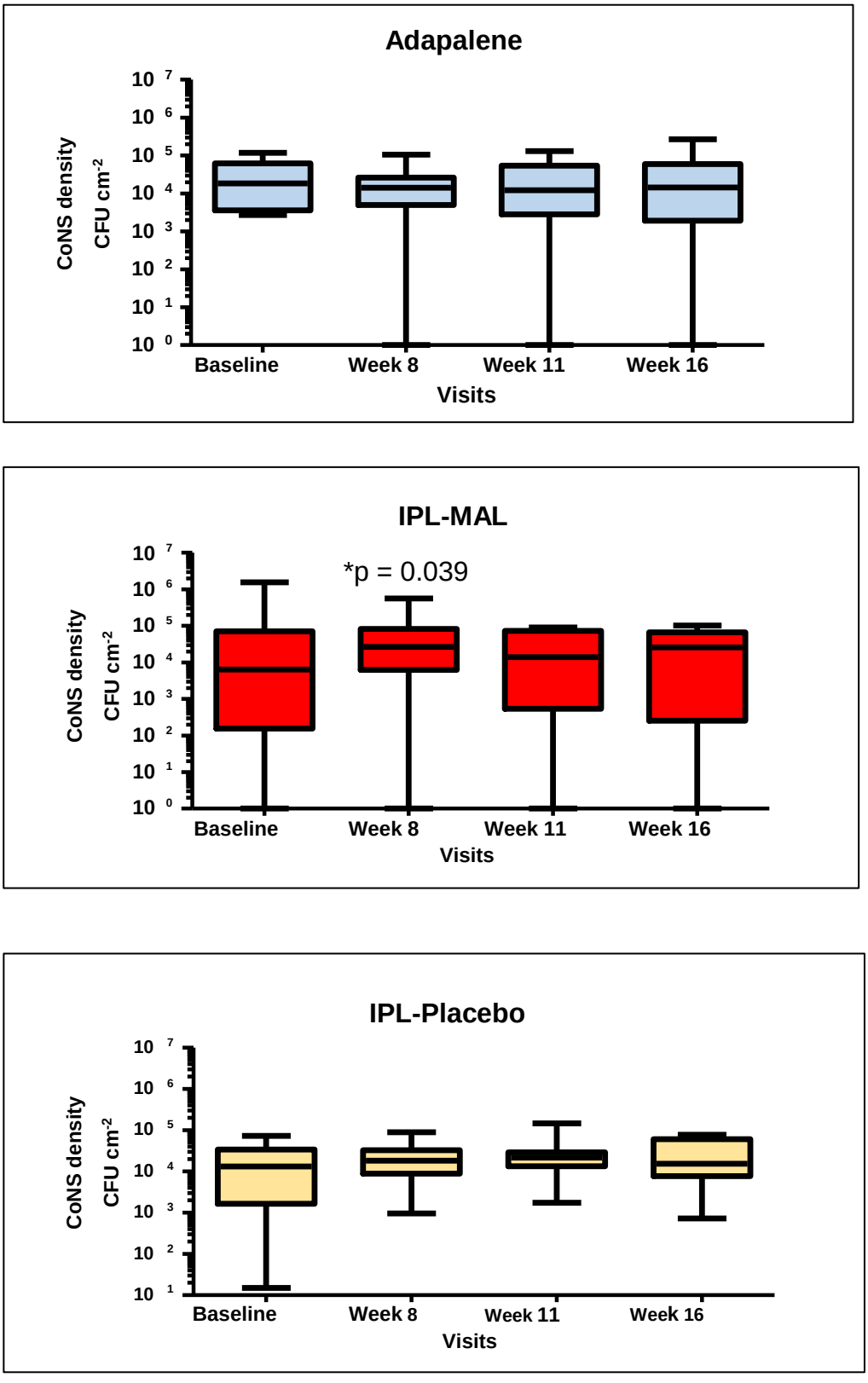


Figure 5.13: Coagulase-negative staphylococci (CoNS) densities at baseline, week 8, week 11, and week 16. Boxplot shows minimum, maximum, 25th and 75th percentiles, and median. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

	Δ Coagulase-negative staphylococci density (CFU cm^{-2})			
	Adapalene (n = 10)	**IPL-MAL (n = 10)**	**IPL-Placebo (n = 10)**	P-value
Δ Week 8 Median (range)	5.4×10^3 (-10.0×10^3 - 7.2×10^4)	-4.5×10^3 (-4.4×10^4 - 1.0×10^6)	-1.2×10^4 (-2.5×10^4 - 5.9×10^4)	0.059
Δ Week 11 Median (range)	5.3×10^3 (-1.0×10^5 - 4.8×10^4)	5.1×10^2 (-5.1×10^4 - 1.5×10^6)	-2.9×10^3 (-7.5×10^4 - 3.1×10^4)	0.294
Δ Week 16 Median (range)	3.2×10^3 (-1.5×10^5 - 6.9×10^4)	-5.3×10^2 (-5.7×10^4 - 1.5×10^6)	-3.2×10^3 (-5.9×10^4 - 1.2×10^4)	0.860

Table 5.27: Median difference from baseline in coagulase-negative staphylococci densities in the adapalene, IPL-MAL and IPL-Placebo groups at week 8, week 11, and week 16. IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

5.2.4 Effect on *staphylococcus aureus* density

In the adapalene group, the median *S. aureus* density remained 0 at baseline and all the follow-up visits **(Table 5.28)**. *Staphylococcus aureus* was not isolated from the IPL-Placebo group at any time point whilst a transient colonisation in the IPL-MAL group (median density 0) was seen at week 8 only. As there were no apparent differences in the median *S. aureus* densities between the groups, no intergroup comparison was done.

	***Staphylococcus aureus* density (CFU cm^{-2})**					
	Adapalene (n = 10)		**IPL-MAL (n = 10)**		**IPL-Placebo (n = 10)**	
	Median (range)	P-value	**Median (range)**	P-value	**Median (range)**	P-value
Baseline	0.0 (0.0×10^0 - 3.3×10^3)		a		a	
Week 8	0.0 (0.0×10^0 - 6.1×10^2)	1.000	0.0 (0.0×10^0 - 2.9×10^5)	0.250	a	1.000
Week 11	0.0 (0.0×10^0 - 1.8×10^3)	1.000	a	1.000	a	1.000
Week 16	0.0 (0.0×10^0 - 1.4×10^3)	1.000	a	1.000	a	1.000

Table 5.28: Median *Staphylococcus aureus* densities in the adapalene, IPL-MAL and IPL-Placebo groups at baseline, week 8, week 11, and week 16. [a]No *Staphylococcus aureus* was isolated and therefore its value is constant (=0). IPL, intense pulsed light; CFU, colony-forming units; MAL, methyl aminolaevulinate.

5.2.5 Summary of the effects on cutaneous microflora

1. IPL-Placebo caused a significant increase in the density of propionibacteria at week 16 ($p = 0.021$). There was no significant difference from baseline in the density of propionibacteria in the IPL-MAL or adapalene groups. Moreover, there was no significant difference in the change in propionibacteria densities from baseline between the three groups at any time.

2. IPL-MAL caused a significant increase in the density of CoNS at week 8 ($p = 0.039$). There was no significant difference from baseline in the density of CoNS in the IPL-Placebo or adapalene groups. Moreover, the difference in the change in CoNS densities between the groups was not significant at any time.

3. The median *S. aureus* density remained 0 in the adapalene group at all times. Moreover, it was only transiently isolated from the IPL-MAL group at week 8 but was never cultured from the IPL-Placebo group.

Chapter 6

Discussion

6. Discussion

6.1 Lesion counts

The results from this study demonstrated that adapalene was superior to IPL-Placebo and IPL-MAL in reducing the noninflamed (adapalene 37.6% vs. IPL-MAL 3.4% vs. IPL-Placebo −9.7%) and total lesion counts (adapalene 35.7% vs. IPL-MAL 4.3% vs. IPL-Placebo −8.4%) at week 16. A maximum improvement of 20.7% and 13.4% in the inflamed lesion counts was observed in the IPL-MAL and IPL-Placebo groups at week 11, respectively. However, this improvement was transient, statistically not significant, and clinically not relevant when compared with the 13.2% change in the adapalene group. Contrary to the IPL-Placebo and IPL-MAL groups, the maximum improvement in inflamed lesion counts in the adapalene group (26.5%) was observed at week 16, however, statistical significance could not be achieved. Efficacy rates of 46-74% [303-305] and 49-54% [303, 305] for noninflamed and total lesion counts, respectively, have been reported for adapalene and the results from this trial compare favourably with these.

No clinically relevant effect on noninflamed lesions and a maximum improvement of 13.4% in the inflamed lesion counts in the IPL-Placebo group in this trial is inferior to some of the other studies which have assessed the efficacy of IPL in the treatment of acne [264, 306, 307]. Choi et al. [306], in a randomised comparative single-blind split-face study, treated 20 patients with facial acne (skin phototypes III-V) with 4 sessions of 585 nm PDL on one side of the face and IPL (Ellipse Flex System, Horsholm, Denmark; 530-750 nm, triple pulses, 2.5 ms pulse duration, 9 ms delay between pulses, two passes, fluence 7.5-8.3 J cm^{-2}) on the contralateral side at fortnightly intervals. They reported a maximum improvement in inflamed and

noninflamed lesion counts of 66% and 43% at 4 and 8 weeks after the last IPL treatment, respectively ($p < 0.05$ for both). Similarly, El-latif et al. [307] in a randomised comparative open study, treated 50 patients (skin phototype IV) with mild to severe facial acne with either IPL or BPO 5% gel. The patients in the IPL group (530 nm filter, single pulse, 35 ms pulse duration, fluence 35 J cm^{-2}) received weekly sessions for 5 weeks. The maximum improvement reported here in the inflamed lesion counts for the IPL group was 61.56%. Finally, as mentioned in chapter 3 **(Section 3.4.1.2)**, the split-face study of Yeung et al. [264] in an Asian cohort of 30 patients showed a 44% decrease ($p = 0.01$) in noninflamed lesions following 4 treatments of IPL (Ellipse Flex System, Horsholm, Denmark; 530-750 nm, 10 × 48 mm spot size, double pulses, 2.5 ms pulse duration, 10 ms delay between pulses, single pass, fluence 7-9 J cm^{-2}), 3 weeks apart. However no significant improvement in inflamed lesion counts could be demonstrated.

There are two main factors that might influence the therapeutic effectiveness of light-based treatments including PDT; (i) light source (e.g. lasers, pulsed and non-pulsed broad spectrum light) and its dosimetry (ii) photosensitiser (e.g. ALA or MAL) and its concentration along with contact time [308]. Altering any of these variables might yield different therapeutic results. The difference in IPL parameters might explain why we were unable to duplicate the results seen in the above studies. This might also explain the mixed results on inflamed lesion counts in the three trials mentioned above [264, 306, 307].

Similarly, a maximum improvement of 20.7% in inflamed lesion counts and the lack of a clinically relevant effect on noninflamed lesions in the IPL-MAL group of this

study is less than that reported by other investigators [264, 309]. Hong et al. [309] conducted a randomised investigator blinded split-face study to treat 20 patients with facial acne (skin phototypes IV-V). The patients received three MAL-PDT sessions (3 h MAL) 2 weeks apart and were irradiated with red light on one-half of the face and IPL (Ellipse Flex System, Horsholm, Denmark; 530-750 nm, 10 × 48 mm spot size, double pulses, 2.5 ms pulse duration, 10 ms delay between pulses, single pass, fluence 8-10 J cm^{-2}) on the other half. The authors reported a 72% and 46.3% statistically significant reduction in the inflamed and noninflamed lesion counts on the IPL side, respectively. Similarly, Yeung et al. [264] found a significant improvement in noninflamed lesion counts (38%) in the IPL-assisted MAL-PDT group (Ellipse Flex System, Horsholm, Denmark; 530-750 nm, 10 × 48 mm spot size, double pulses, 2.5 ms pulse duration, 10 ms delay between pulses, single pass, fluence 7-9 J cm^{-2}; 30 min MAL); however, no significant improvement in inflamed lesions could be established. Again, the difference in IPL parameters between this study and those mentioned above may explain the variability of results.

As explained in chapter 4 **(Section 4.1.2.4)**, marked discrepancies have been demonstrated between the measured IPL devices outputs and those claimed by the manufacturers [299]. Therefore, parameters claimed to be successful in one device might not demonstrate similar efficacy in another. Moreover, as limited information was available on the use of IPL for acne treatment in Fitzpatrick skin types I/II the parameters used were based on those used successfully by Babilas et al. [300] who also utilised the Energist ULTRA VPL™ system (IPL device used in this trial) in the treatment of actinic keratosis. However, even arguing the fact that this study was underpowered, therefore not giving statistically significant results, a maximum

improvement of 20.7% and 13.4% in inflamed lesion counts in the IPL-MAL and IPL-Placebo groups, respectively, is clearly less than the benefit reported in above studies [264, 306, 307, 309]. It is prudent to mention though that even if this study was adequately powered, the possibility of above results failing to achieve statistical significance cannot be ruled out. Being underpowered may also be argued as a possible explanation of why a 26.5% improvement in the inflamed lesion counts in the adapalene group at week 16 did not reach statistical significance.

6.2 Leeds and VAS scores

Evaluation by the Leeds and VAS scores did not reveal a clinically relevant significant improvement in acne severity in any of the treatment groups. Leeds grading is influenced by changes in the inflamed rather than noninflamed lesions [286]. Similarly, by default, VAS will be more influenced by the inflamed than the noninflamed lesions (which are better palpated than seen). As mentioned previously, an acne grading system does not distinguish small differences in therapeutic response and the same may also hold true for VAS [285]. The treatment groups in this study only showed modest improvement in inflamed lesion counts (albeit non-significant) and may therefore explain why a clinically relevant and statistically significant change could not be seen in acne severity in any of these groups.

6.3 DLQI and FDLQI scores

This study was also aimed at comparing the impact of adapalene, IPL-Placebo and IPL-MAL on the quality of life of acne patients and their families. A 52.9% statistically significant improvement in the DLQI score was observed in the adapalene group at week 16, however there was no significant difference between the

three groups. Without a significant change in acne severity, a decrease in the DLQI score in the adapalene group is quite interesting and might be explained by a better tolerability of the drug and/or its positive impact on patient's symptoms. On the contrary, there was no significant improvement in the FDLQI scores in any group. This corresponds rather well with the fact that no clinically relevant and statistically significant change in acne severity was seen in any treatment group.

6.4 Effect on cutaneous microflora

The 100% and 93% prevalence of *P. acnes* and CoNS, respectively, in this trial at baseline is in agreement with the ubiquitous presence of these micro-organisms on normal human skin [25, 310]. Similarly, the 7% colonisation rate of *S. aureus* can be explained by its prevalence (< 10%) on normal human skin [311]. However, *P. granulosum* prevalence of 10% in this study is less than its previously reported value of 42.8% in acne patients [22]. It is important to mention here that Leyden et al. [22] used bacteriophage susceptibility to differentiate *P. acnes* from *P. granulosum.* However, as per the original study of Marples and McGinley [312], they did not attempt to further subdivide phage-resistant propionibacteria into *P. granulosum* and *P. avidum* and labelled all such strains as *P. granulosum.* It is possible that some of these phage-resistant strains were *P. avidum.* Moreover, in contrast to the present study where bacterial samples were taken from the cheeks only, they obtained samples from two different sites; forehead and cheek. Regional variation in *P. granulosum* colonisation rates exist (with the highest prevalence on the alae nasi followed by ear, scalp, and forehead respectively) [25] and this along with the failure to differentiate *P. granulosum* and *P. avidum* might explain the higher prevalence of the former in their study.

This study found no significant change in the density of propionibacteria in the adapalene and IPL-MAL groups, however a significant increase was seen in the IPL-Placebo group at week 16. On the contrary, no significant change in the porphyrin fluorescence was found in any group. This implies that follicular porphyrin fluorescence as a sole method for assessing the change in propionibacteria density post-treatment may not be 100% reliable. This fact has been highlighted by Burkhart [313] who surmised that acne treatments may destroy/change the chemical structure of porphyrins, therefore changing its absorption and emission spectrum, and/or impair its production by *P. acnes* via altering some intracellular signalling pathway. This may affect follicular porphyrin fluorescence without a change in *P. acnes* density. While this hypothesis does not explain the lack of change in the porphyrin fluorescence in the context of a significant increase in the propionibacteria density in the IPL-Placebo group of this study, it emphasises (in the light of results from this study) the importance of using bacterial culture for determining the anti-propionibacteria activity of acne treatments.

The CoNS densities did not differ significantly in the IPL-Placebo and adapalene groups whilst a significant increase was observed in the IPL-MAL group at week 8 only. As *S. aureus*, at baseline, was only isolated from 2 patients in the adapalene group (median density 0) but not from the other two groups, no conclusions can be made regarding the effect of these treatments on this micro-organism.

The results from this study are in agreement with those obtained by Horfelt et al. [255, 256] who did not find a significant difference in the *P. acnes* counts after MAL-PDT or ALA-PDT. Ammad et al. [266], did not find a significant difference in

the density of *P. acnes* after treatment with blue light and increased propionibacteria counts in the IPL-Placebo group of this study differed from these results. Similarly, increased CoNS density at week 8 in the IPL-MAL group was unexpected as ALA-PDT has been shown to significantly decrease *S. epidermidis* density *in vitro* [281]. The results from this study indicate that *in vivo* IPL/IPL-MAL treatment does not directly lead to the destruction of micro-organisms. Moreover, the observed unexpected findings may be explained by the follicular microenvironment theory elucidated in chapter 1 **(Section 1.2.4)**. It is possible that IPL/IPL-MAL treatment (at the settings used in this trial) selectively changed the microenvironment of individual follicles making them more suitable to the growth of a specific micro-organism. Differential change in the follicular pH, by the variable anti-inflammatory properties of the two treatments **(see Chapter 3, Section 3.4.2.1)**, might be one example of such alteration [55]. This might explain the selective increase in the densities of proprionibacteria and CoNS in the IPL-Placebo and IPL-MAL groups at weeks 16 and 8, respectively, in this study. As no clinically relevant change in the inflamed lesion counts was seen in the IPL-MAL group at week 16, it is unlikely that an increase in the propionibacteria density in the IPL-Placebo group had any impact on its efficacy. Similarly, CoNS are not implicated in the pathogenesis of acne and a significant increase in the density in the IPL-MAL group at week 8 is unlikely to have influenced the efficacy of this treatment.

6.5 Safety data

This study showed no significant difference in the pain scores, during illumination, between the IPL-MAL and IPL-Placebo groups. This is contrary to the study conducted by Yeung et al. [264] in which 25% of the patients in the IPL-MAL group

withdrew from the trial because of significant stinging, burning and erythema after the treatment; however treatment with IPL was well tolerated. Similarly, in the study of Hong et al. [309], MAL-PDT with IPL was significantly more painful than red light after the second treatment. Light absorption leads to heat generation which contributes to PDT pain [244]. IPL-MAL treatment may have been well tolerated by the patients in this trial due to the cold air cooling that was used to prevent excessive heating of tissues, therefore, minimising PDT pain. Moreover, as previously mentioned **(Chapter 4, Section 4.1.2.4)**, our IPL parameters were carefully chosen from a previous study [300] in which these settings were found to be less painful than a LED for MAL-PDT.

Very few adverse events were reported by the patients in the IPL-MAL and IPL-Placebo groups with most of the side-effects being mild to moderate and resolved completely without any sequelae. Moreover, none of the patients developed PIH or scarring. This is in contrast to the side-effects reported by Yeung et al. [264] in which 2 patients in the IPL group whilst one in the IPL-MAL group developed PIH. PIH is more common in darker skin types [314] and its increased incidence in the study of Yeung et al. [264] (despite using a lower fluence) can be explained by the fact that they treated facial acne in patients with skin types IV-V whilst the cohort in this study included patients with skin types I-IV. Moreover, the use of cold air cooling in this trial prevented an excessive rise in epidermal temperature, which also helped to further minimise the complications [315]. In terms of adapalene side-effects, all but one experienced skin dryness followed by exfoliation, erythema and temporary worsening of acne being reported by almost one-third of the patients.

Again, all these adverse events were mild to moderate and resolved completely at the end of the trial.

6.6 Summary

Adapalene 0.1% gel proved to be more effective than IPL-Placebo and IPL-MAL in reducing noninflamed and total lesion counts at week 16. Moreover, it was also associated with a significant increase in the quality of life of patients. This study also found a modest improvement (albeit non-significant) in inflamed acne lesions in both the IPL-Placebo and IPL-MAL groups at week 11; however, ruling this out as a chance finding needs an adequately powered study. Treatment with IPL-Placebo and IPL-MAL was associated with a significant increase in the densities of propionibacteria and CoNS at weeks 16 and 8, respectively, which might be due to the induction of selective changes in the microenvironment of PSFs promoting the growth of a specific micro-organism. This suggests that, if a significant improvement in inflamed lesion counts can be confirmed in an adequately powered study, IPL-Placebo and IPL-MAL have anti-inflammatory and/or sebostatic effects rather than bactericidal properties. Recently, other investigators from our department have shown IPL to up-regulate TGF-β1 expression and activity (via nuclear translocation of its signalling transducer, Smad3) *in vivo* [316]. As explained in chapter 3 **(Section 3.4.2.1)**, TGF-β is an immuno-regulatory cytokine and its up-regulation may be one of the anti-inflammatory pathways by which IPL/IPL-MAL may improve acne. Similarly, IPL and IPL-assisted ALA-PDT have been demonstrated to significantly decrease SER in acne patients [317]. In a separate study on the cohort of this trial, the effect of IPL and IPL-MAL on the SER was also investigated and the results

when available might show the influence of these light-based treatments (at the parameters used in this trial) on sebaceous glands.

6.7 Study critique

1. Awareness of the treatment allocation in the adapalene group by the patients as well as investigators could have been a source of ascertainment bias in this study. Blinding of the treatment allocation in this group, by a placebo control, would have been preferable. However, this would have further increased the sample size of the trial, making useful interpretation of the results more difficult due to the recruitment difficulties we faced.

2. This study was underpowered and therefore increased the probability of a type II error (failure to reject the null hypothesis when it is false). Hence, the results which are clinically relevant but statistically not significant should be interpreted with caution.

3. The data from this study was evaluated by the per-protocol analysis rather than intention-to-treat (ITT) analysis and therefore included only those patients who completed the study without any major protocol violations. While an ITT analysis is considered ideal, the estimate of treatment efficacy using this approach is generally conservative because of dilution due to noncompliance. Therefore, to show the maximum possible improvement with the IPL parameters used in this trial a per-protocol analysis was chosen. This could be a potential source of bias in this trial [318].

4. The follow-up period in this trial was short (9 weeks and 3 weeks for the light groups and adapalene group, respectively) and ideally should have been longer to assess the durability of response in the three treatment groups. In fact, a 44 weeks follow-up study was designed in conjunction with this project, but due to its length my participation was limited to that described in this thesis.

5. The current study speaks only to the efficacy of a specific IPL treatment protocol and better results may be achieved by optimisation of the treatment parameters.

6. The use of ultrasound gel on the skin to be treated can reduce reflection of the incident rays and enhance the delivery of energy to the targeted chromophore. However, heating of the gel, due to inadequate time between discharges, can create small bubbles in it which can impede light passage by light scattering [299]. This may potentially reduce the efficacy of light treatment. However, ultrasound gel was used by Choi et al. [306] and Babilas et al. [300] in their trials which demonstrated IPL and IPL-assisted MAL-PDT to be an effective treatment modality for acne and actinic keratosis, respectively. This means that a loss of efficacy due to the bubbling phenomenon, if any, is normally minimal.

7. Discrepancies have been demonstrated between the measured IPL device outputs and the values displayed on the system or claimed by the manufacturers [299]. Our IPL machine was calibrated and serviced before

the trial, thereby reducing machine error as a cause of lack of significant clinical efficacy in our trial.

8. One of the aims of this trial was to assess the effect of IPL and IPL-assisted PDT on the *P. acnes* density. However, as mentioned in chapter 5 **(Section 5.2.1)**, because of technical problems, the laboratory staff were unable to separately count *P. acnes* and *P. granulosum* colonies and therefore only total propionibacteria count was given in the trial. We believe that this did not influence our interpretation of the results as there was no significant reduction in the density of propionibacteria and *P. granulosum* was only isolated from 10% of the patients (IPL-MAL = 1, IPL-Placebo = 2).

9. Disease-specific quality of life instruments have greater power to detect change by focusing on aspects of functioning that are most affected by the disease and tend to be of greatest importance to patients [319]. Using such an instrument (e.g. the Acne-Specific Quality of Life questionnaire) alongside the generic DLQI instrument could have been advantageous in the trial.

6.8 Future studies

Although, due to the underpowered nature of this study, no definite conclusions could be made regarding the efficacy of IPL and IPL-assisted MAL-PDT on inflamed lesion counts, further research on the use of these treatment modalities in acne is still warranted. This is largely due to reports from other investigators

demonstrating these treatment modalities to be effective in acne management. Moreover, any future studies should aim to clarify the questions raised by this project, especially with respect to the possible mechanism of action of these treatment modalities in acne. We suggest the following changes for future studies:

1. The treatment parameters should be further optimised to improve the clinical efficacy of these light-based treatments. Delivering smaller fluences in a train of 2-3 sub-pulses over a shorter time period, by decreasing the delay between pulses, could yield better results [306, 309].

2. Using a disease-specific quality of life instrument (e.g. the Acne-Specific Quality of Life questionnaire) alongside the generic DLQI instrument would give a more accurate estimate of the effect of these light-based treatments on the quality of life of acne patients.

3. With the caveat of increasing the sample size, a placebo group would reduce the ascertainment bias in the adapalene arm.

4. As shown in this study, enumeration of *P. acnes* by culture is more accurate than porphyrin follicular fluorescence for the evaluation of suppressive effect of anti-acne treatments on this micro-organism. Hence, either both or the former technique alone should be used in any future trials.

5. Keeping in mind the recruitment difficulties faced during this trial, a multicentre study might be more appropriate for any future attempt at comparing these light-based therapies with a conventional anti-acne treatment.

6. The possibility of sebostatic properties of IPL-assisted PDT/IPL has been discussed in the thesis **(Section 3.4.2.2)** and any further study should also include quantitative sebum analysis which is possible using a SM 810® sebumeter (Courage + Khazaka Electronic, Köln, Germany) [108].

7. The anti-inflammatory properties of light-based treatments have been discussed **(Section 3.4.2.1)** and should also be investigated concurrently in any future studies. Reverse transcription-polymerase chain reaction can be used to assess the effect of IPL/IPL-assisted PDT on various cytokines e.g. TGF-β, IL-1, TNF-α, IL-10 [273]. Similarly, immunohistochemistry can be used to assess the effect of these light-based treatments on other proinflammatory and anti-inflammatory molecules thought to be involved in acne pathogenesis e.g. SP, CRH, and hBD-2 [135, 147, 156].

6.9 Future of IPL and IPL-assisted PDT in acne management

Despite the availability of numerous medical therapies for acne, issues of safety, compliance, and less than ideal efficacy help drive the search for alternative treatments for this common clinical problem. In light of these issues, IPL and IPL-assisted MAL-PDT were proposed as possible therapeutic alternatives and were

compared with adapalene to find a place for these light-based treatments on the acne therapeutic ladder. Moreover, the effect of these treatment modalities was hypothesised to be due to their anti-*P. acnes* properties. In this thesis, the microbiology assessments suggest that *in vivo* IPL and IPL-MAL (at the treatment parameters used in this trial) do not have any bactericidal effects on *P. acnes*. Therefore any future attempt at elucidating the mechanism of action of these treatments should assess their effect on SER, various cytokines e.g. TGF-β, IL-1, TNF-α, IL-10, and other proinflammatory and anti-inflammatory molecules e.g. CRH, SP, and hBD-2 **(see Chapter 2, Section 2.4.2)** concurrently with microbiological assessment.

Regarding the practical implications of the present study, it can be concluded that adapalene remains an effective first line treatment in mild to moderate facial acne. The present study has remained inconclusive (due to being underpowered) regarding the efficacy of IPL and IPL-MAL on inflamed acne lesions. However, the modest improvement seen with these treatments, as monotherapy, in this trial suggest that they are only marginally effective for inflammatory acne. This, in combination with the cost and time needed for these treatments, is likely to lead to patient dissatisfaction. All these facts should, therefore, be critically evaluated before embarking on any further research in this field.

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Appendices

Appendix 1

- South East Wales Research Ethics Committee Approval
- Patient Information Sheet
- Consent Form
- Advertisements
    1. Poster Advertisement
    2. Advertisement in English and Welsh for Cardiff University Online Notice Board
- Questions in Online Questionnaire
- Screening Questions for Telephone Enquiries
- The Family Dermatology Life Quality Index
- Dermatology Life Quality Index
- The Leeds Revised Acne Grading System

South East Wales Research Ethics committee Approval

GIG CYMRU NHS WALES | Canolfan Gwasanaethau Busnes / Business Services Centre

South East Wales Research Ethics Committee Panel C
Direct Line: 02920 376823/376822
Telephone: 02920 376823
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Email: Carl.phillips@bsc.wales.nhs.uk

Dr C A Suthananthan
Dept. of Dermatology
CardiffUniversity
3rd Floor Glamorgan House
Heath Park, Cardiff
CF14 4XN

23 October 2009

Dear Dr Suthananthan

Study Title:	**Randomised, controlled, double-blind, parallel group clinical trial evaluating the efficacies and safety of methyl-aminolevulinate photodynamic therapy and intense pulsed light, administered as placebo-photodynamic therapy, compared with the efficacy of adapalene 0.1% gel in the treatment of adults with mild to moderate acne vulgaris.**
REC reference number:	**09/WSE03/40**
Protocol number:	**1.9**
EudraCT number:	**2008-000475-25**

Thank you for your letter of the 19 October 2009, responding to the Committee's request for further information on the above research, and for submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/WSE03/40	**Please quote this number on all correspondence**

Yours sincerely

Mrs J Jenkins
Chair, Panel C
South East Wales Research Ethics Committees

Enclosures: "After ethical review – guidance for researchers" SL-AR1

Copy to: Clinical Trials Unit MHRA

R&D office for Cardiff University

R&D office for Cardiff & Vale University Health Board

Patient Information Sheet

A comparison of the clinical efficacies of intense pulsed light, photodynamic therapy and adapalene in the treatment of mild to moderate acne vulgaris

Dear Sir or Madam,

You are being invited to take part in a research study to see how well intense pulsed light (IPL) clears the spots (called 'acne') on your face. This light may be given alone or after using a cream called 'methyl - aminolaevulinic acid' (MAL).

Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others like your family members or family doctor if you wish.

Please do not hesitate to ask us any questions or to request more information as necessary. You may contact Dr. Suthananthan, Dr Shaheen or Sister Anne Thomas who are all working on this study. Their phone numbers and email addresses are on the last page of this leaflet.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of this study.

Thank you for taking the time to read this.

PATIENT INFORMATION SHEET- PART 1

What is Intense Pulsed Light (IPL)?

The visible or 'white' light that we see consists of all the colours of the rainbow. Laser machines produce only one colour of light. Intense Pulsed Light (**IPL**) is NOT a laser machine. Its light is made up of different colours or shades of the same colour of light. This is referred to as being 'broad-spectrum'. This light is produced in short bursts or pulses hence the term "intense *pulsed* light''.

IPL acts on (i.e. 'targets') specific things in the skin like pigments, chemicals and collagen. Depending on the machine settings, IPL can target the dark pigment found in hair, and hence it is useful in hair removal. It can also target the red pigment in blood and therefore can help to reduce the appearance of red spots and veins on the skin.

IPL machines can give out blue, green, yellow and red light. The machine's settings can be changed to produce only the colours that are most beneficial to you. In acne, blue light acts on chemicals called porphyrins. These porphyrins are made by the bacteria that cause acne. Through the action of blue light on these porphyrins, the

bacteria are either weakened or killed. Red light can go deeper in the skin than blue light, and in some studies it has been shown to reduce the number and size of the oil-producing glands. These oil-glands are also involved in causing acne.
It is important to note that unlike sunlight, IPL machines have filters that remove ultraviolet (UV) light. Therefore the bad effects of UV light that cause skin wrinkling and cancer are removed.

What is the purpose of the study?

Over the past few years, doctors, mostly in Europe and the United States, have said that light therapy, with and without MAL cream, is helpful in treating acne. Nonetheless, the research that has been done in this area has been relatively little. Though useful, it has not properly addressed how well light works on its own or in combination with creams like MAL. Also, these studies do not say how well light treatments work compared to acne treatments that are already available through your doctor, like adapalene (Differin™).

Why have I been chosen?

You have been chosen because you have been identified by your doctor as having acne on your face or because you responded to one of our advertisements.

Do I have to take part in the study?

Participation in this study is entirely up to you. If you decide to take part, you will be given this information sheet to keep and asked to sign a consent form. If for any reason, you would like to withdraw from the study, after agreeing to join, you are free to do so at any time. Withdrawal will not affect your standard of care or ability to receive treatment from any hospital or your GP.

Reports from this study will not contain any personal identifiable information about you.

What will happen to me during this study?

There will be three treatment groups:

1. IPL and MAL cream together
2. IPL and placebo (or 'dummy') cream
3. Adapalene only

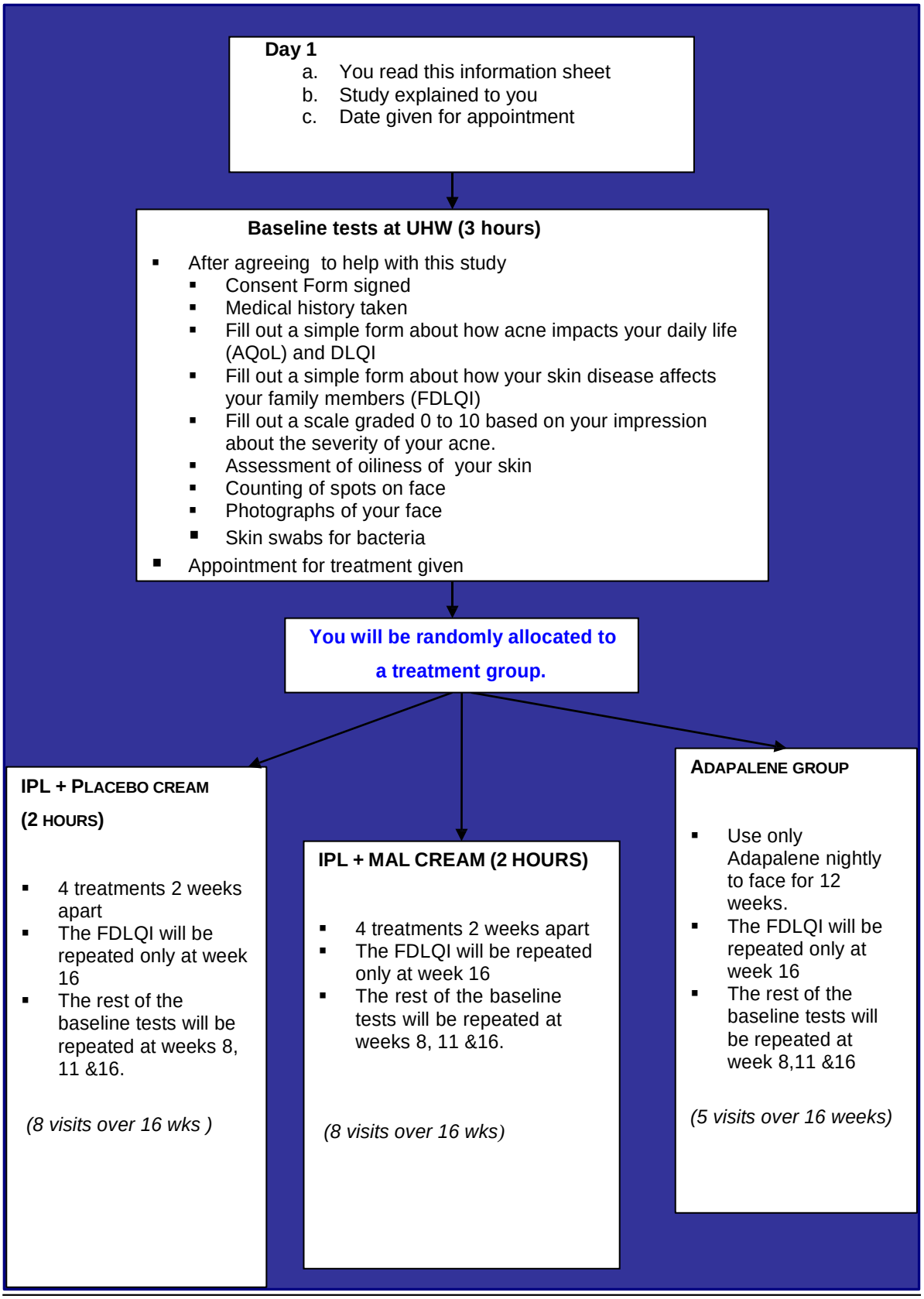
All of these treatments have been used to treat acne. Therefore no matter which group you are placed in, you will receive real treatment. These groups are selected by a process that has no information about the persons involved; therefore your assignment will be purely by chance.

If you are in either group 1 or group 2: A cream (which could be placebo or active treatment) will be put on your face for 60 minutes. You will be asked to wash it off after 60 minutes. Prior to the treatment with IPL, you will be required to wear goggles to protect your eyes. Then, a cooling gel will be put on your face. IPL will

then be administered onto your face through the cooling gel. IPL may cause a stinging or burning sensation. Some say that it feels similar to the sensation of an elastic band being snapped against the skin. The cool gel should help to reduce any discomfort you may experience. Only your face will be treated.

The treatment and clinical review times vary between the treatment groups. Phase 1 of the study lasts for 16 weeks. In phase 1, Patients in the IPL groups will have a total of 8 visits over this 16 week period whereas patients in the adapalene group will only attend 5 visits over the 16 week period. At the end of 16 weeks, we will ask patients in the IPL groups to keep an acne diary (how their acne is doing) for the next 44 weeks. No treatment will be given during this phase of the study. These patients from the IPL groups will be reviewed at 2 follow up appointments with the department during the subsequent 44 week period (Phase 2).

Below is a simple diagram and notes that help us explain to you what will happen.


Day 1
a. You read this information sheet
b. Study explained to you
c. Date given for appointment
Baseline tests at UHW (3 hours)
After agreeing to help with this study
Consent Form signed
Medical history taken
Fill out a simple form about how acne impacts your daily life (AQoL) and DLQI
Fill out a simple form about how your skin disease affects your family members (FDLQI)
Fill out a scale graded 0 to 10 based on your impression about the severity of your acne.
Assessment of oiliness of your skin
Counting of spots on face
Photographs of your face
Skin swabs for bacteria
Appointment for treatment given
You will be randomly allocated to a treatment group.
IPL + PLACEBO CREAM (2 HOURS)
4 treatments 2 weeks apart
The FDLQI will be repeated only at week 16
The rest of the baseline tests will be repeated at weeks 8, 11 &16.
(8 visits over 16 wks)
IPL + MAL CREAM (2 HOURS)
4 treatments 2 weeks apart
The FDLQI will be repeated only at week 16
The rest of the baseline tests will be repeated at weeks 8, 11 &16.
(8 visits over 16 wks)
ADAPALENE GROUP
Use only Adapalene nightly to face for 12 weeks.
The FDLQI will be repeated only at week 16
The rest of the baseline tests will be repeated at week 8,11 &16
(5 visits over 16 weeks)

What do I have to do?

A. Please protect your face from getting tanned for the time that you are involved in this study.

Being in the sun or on a sunbed causes your skin to produce more pigment, which we call a 'tan'. Also, the pigments in sun-tanning lotions leave extra pigment in your skin. Darker skin types have more pigment too. As said before, IPL works by 'targeting' pigments in and on the skin. Hence, IPL treatment of your skin while you have a deep tan *may* result in much worse side-effects. For example, a bad 'sun-burn' effect, blisters, scarring, darkening or lightening of the skin that has been treated can happen. These side-effects are much less likely if you do not have a tan or use sun-tanning lotions. Therefore, please do not sunbathe while you are helping us in this study, even if you are wearing a sun protection cream.

You can do this easily by staying out of direct sunlight during the course of this study. If you have to go outside, then wear a wide-brimmed hat and sun protection with a minimum sun protection factor (SPF) 30 on your face. We will provide you with Delph Sunblock Lotion that should be used for your face only. Please do not use any other sunblock on your face except for the one provided by the department. An emollient (to be applied twice a day for a total of 5 days) will be given to all patients in the IPL groups post treatment.

As a result, before every treatment, please tell us if you may have recently had prolonged exposure to sunlight or have a tan.

B. If you are placed in any of the **IPL groups**, you will have to be especially careful about protecting yourself from the sun for the **first 2 days after treatment**. This is because you may have been given the MAL cream. MAL cream may make the sunlight act even more strongly on the skin for up to 48 hours after washing the cream off the skin.

In these 2 days:

1. From 11 am to 3 pm the sun's rays are most damaging. Please avoid being in direct sunlight especially during these times. Staying indoors as much as possible is a good way to do this.
2. Wear your sun cream, paying special attention to your face and neck. Re-apply it every 2 to 4 hours, because most of its protective effect wears off after this. Wearing the wide-brimmed hat as well helps.

C. Certain medications may cause you to be more sensitive to light. If you take any herbal supplements or medications before or after starting this project, please let us know.

Otherwise, you should be able to work, travel and socialise as normal.

What are the alternatives for treatment?

Many treatments are now available from your local pharmacy or through your doctor. If you are using creams such as benzoyl peroxide, azelaic acid or nicotinamide on your face OR if you are on tablets for your acne e.g. minocycline, we will ask you to wait a little while until these have worn out of your system before entering you into this study.

Please do not use any medication or medicated soaps, unless we have prescribed it for you. If you need other treatment for your skin e.g. steroids or antibiotics to be taken by mouth, please tell us about it as soon as possible. If you can, tell us before you start any treatment. Doing this helps us decide if it affects your IPL treatments. We may be able to suggest a suitable alternative for that medication while you are participating in this study.

More information about acne and its treatment is available on the British Skin Foundation's website at:
http://www.britishskinfoundation.org.uk/standard.aspx?id=208

What are the possible benefits of taking part?

Your acne may get better. IPL is also used to help sun-damaged skin appear younger and more even-toned, with a smoother texture. Therefore, you may receive some of this benefit. Also, it is hoped that results from this study will help us to better understand the benefits (or otherwise) of this type of treatment. Hence, in the future, you would have helped doctors and patients decide if this is a good treatment option for them based on our findings.

You will not pay for the special investigations required for this study. Your treatments will be provided at no cost to you throughout the period of the study. Transportation costs incurred through participation in this trial will be reimbursed at a fixed rate. This money will be paid to you at the end of your involvement in this study.

What are the side-effects of any treatment received when taking part?

Side effects of IPL: Looking at the studies done with IPL in the treatment of acne, many patients experienced little more than warmth, burning or tingling during treatment. Other effects include mild to moderate pain, redness and mild swelling of

the area for up to a week. Uncommonly, this area can become infected. Especially in darker-skinned individuals, temporary darkening or lightening of the treated area may occur. Blisters,crusting and scarring have been reported but are quite uncommon in all skin types. There may be temporary, long term patchy hair loss affecting the beard area in men.

Side effects of IPL + MAL: First, we will give a brief explanation about how IPL + MAL works.

Our red blood cells contain a red pigment called 'haemoglobin' that carries the oxygen around our bodies. Aminolaevulinic acid (ALA) is one of the first ingredients that our body uses to make the haemoglobin. This ALA can be sprayed or rubbed onto the skin and is allowed to absorb, making the skin more sensitive to light. Once light, such as IPL, is shone onto the skin, the areas that have absorbed the ALA will become inflamed and are destroyed. Thereafter, new healthy cells will grow to replace the destroyed ones. This whole process is called 'photodynamic therapy' or PDT. This technique is very useful in curing certain early and late skin cancers or even cancers *inside* the body when the patient takes the ALA by mouth. PDT is also used to improve the appearance of aged skin. **MAL** is very much like ALA and behaves similarly when used on the skin.

Since PDT works by causing inflammation and destruction of the cells that have absorbed the ALA, patients often experience a burning sensation while getting the light treatment that may last for a few hours and then resolve. Pain, redness, swelling, stripping of the skin, scab formation and temporary worsening of your acne commonly occur and may last for 1 to 2 weeks or sometimes longer. There is a small risk of scarring and darker skin types may experience discolouration of their skin, which often gets better with skin lightening treatment. We will use MAL for only 60 minutes on the skin, which has been shown to cause milder side-effects.

Other side effects that have been reported include: eczema (dry skin patches that may itch) where MAL is applied, wheals (hives), skin irritation, prolonged sensitivity to the sun, bleeding (if there is a wound), nausea, eye swelling, eye pain, headaches, tiredness and sensations of tingling and numbness.

Side-effects of Adapalene: Usually patients have no problems with adapalene, but you may experience some burning, warmth, stinging, tingling, itching, redness, dryness, peeling, or irritation while you are using it. Let us know immediately if these side effects are excessive, so that we can reduce your adapalene to every other day or a few days per week. These side effects should decrease after the first few weeks of treatment.
Contact names and numbers are listed below if you are concerned in any way or have an emergency related to this study.

What if I get pregnant or is there anything else I should know?

The cream, adapalene, belongs to a group of drugs that are similar to vitamin A called 'retinoids'. Retinoids may cause damage to an unborn child. Therefore, pregnant women and women contemplating pregnancy will not be asked to join this

study. Therefore, if you are a female of childbearing age, you will be asked to do a pregnancy test before taking part to exclude the possibility of pregnancy. IPL treatments on the other hand, are unlikely to be harmful to the unborn child or have any negative effect on a man's sperm causing damage to a foetus.

Women who can become pregnant must consistently use effective birth control during the course of their treatment. Please use methods such as condoms rather than hormonal methods such as the pill or implants beneath the skin, since the hormones in them can affect the results of this study.

If you become pregnant while in this study, please inform us as soon as possible using the contact information below.

It is not known whether adapalene is secreted in human milk, therefore its use in breast feeding women should be avoided. Hence women who are breast feeding will not be asked to join this study.

Due to the non-invasive nature of any of these treatments, it should not affect your life or private medical insurance. However, you should check with your provider to make sure that assisting us with this study does not affect your coverage.

If we discover any other condition of which you were previously unaware, we will inform your GP and re-assess your ability to participate in this study.

What happens when the research study stops?

Unfortunately, IPL treatments for acne are not yet available on the NHS; therefore they are not routinely available to public patients. If you require further help for your acne after the end of the study, please return to your usual GP or dermatologist.

During the follow-up phase of the study 44 weeks after the study, we will arrange for you to have 2 follow-up appointments in the Dermatology Department at the University Hospital of Wales.

This completes Part 1 of the Information Sheet. If the Information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PATIENT INFORMATION SHEET- PART 2

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment being studied. If this affects the study, your research doctor will tell you about it and discuss with you whether you can or would like to continue in the study. If you decide to continue in the study, you will be asked to sign an updated consent form.

What will happen if I don't want to carry on with the study?

You may stop your participation in the study at any time. This will not affect your standard of care or ability to receive treatment from any hospital or your GP.
Your doctor may stop your participation in the study without your consent if you experience a serious adverse event, are found not to be eligible to participate in the study, need additional medication or do not follow study procedures.
You can withdraw from treatment but keep in contact with us to let us know your progress. Information collected may still be used. Any identifiable samples that can still be identified as yours will be destroyed if you wish.

What if something goes wrong?

If you are harmed by taking part in this project, there are no special compensation arrangements. If you are harmed by someone's negligence, then you may have grounds for legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Will my taking part in this study be kept confidential?

All the information that is collected about you during the course of this research will be kept strictly confidential. Only staff members directly involved in your care will have access to it and these will be secured in a locked room within the department.

Your file will be identified only by your study number and gender so that you cannot be recognised from it. Your GP would probably like to know about your participation in this study, but we will inform them only if you give us your permission to do so and provide your GP's contact information.

What about my photographs?
These will be stored on a secure laptop owned by Cardiff University. It will be kept in the Dermatology Department and be accessible only to the members of the study team. This data will be encrypted. Back up copies will be held on the University Hospital of Wales' FotoWeb site in a password protected area – this means that only select members of the study team will have a password allowing them to access this site. The photographs taken by the photography unit in the Dermatology Department will be stored on a secure server that is password protected.

After completion of the study, the data will be removed from the laptop and secured electronically on data disks for 15 years. After 15 years, the photographs will be destroyed via the confidential waste system.

What will happen to the results of this study?

The results of this study will be published in reputable medical journals and in an academic thesis. The results will not contain any personal identifiable information about you.

Due to the nature of this study, we may use your photographs to illustrate the outcomes of your treatment in these publications and for teaching purposes. We will not use them without your permission. Therefore, you will be asked to give your consent for this separately.

Who is organising and funding the research?

Cardiff University, through the Department of Dermatology, is sponsoring and funding this study. Your doctor is not being paid for including you in this study.

Who has reviewed the study?

To ensure your safety and that the highest research standards are being met, the Cardiff & Vale R&D Committee, South East Wales Research Ethics Committee and the Medicines & Healthcare Products Regulatory Agency have reviewed this study's protocol and are satisfied that it is in accordance with the latest version of the ethical principles for human research.

Contacts for Further Information

If you have any queries, or experience injuries or adverse events related to this study please contact any of the persons below during work hours:

Dr. Maria Gonzalez (Principal Investigator & Academic Supervisor)
Work: (0)29 2074 4398 Email: gonzalezml@cardiff.ac.uk

Sister Anne Thomas (Research Sister)
Work: (0)29 2074 2672 Email: thomasag1@cardiff.ac.uk

Dr. Chantal Suthananthan (Study Coordinator)
Work: (0)29 2074 5875 Email: SuthananthanCA@cardiff.ac.uk

Dr B. Shaheen (Study Coordinator)
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3rd Floor Glamorgan House
School of Medicine, Heath Park
Cardiff University, CF14 4XN
Fax: (0)29 2074 4312

This is your copy. Thank you for agreeing to help with this study.

Consent Form

CONSENT FORM

CANDIDATE NUMBER											

PATIENT D.O.B (dd/mmm/yyyy)

Title of Project: A comparison of the clinical efficacies of intense pulsed light, photodynamic therapy and adapalene in the treatment of mild to moderate acne vulgaris

Name of Researchers: Dr. Maria Gonzalez, Dr C A Suthananthan, Dr B Shaheen, Dr. Marisa Taylor, Dr. A. A. Atwan

Please initial box

1. I confirm that I have read and understood the information sheet dated November 2nd 2010, Version 6 for the above study and have had the opportunity to ask questions. ☐

2. I agree to attend all clinics applicable to me as outlined in my information sheet. (IPL Groups: 8 visits in Phase 1 and 2 visits in Phase 2; Adapalene Group: 5 visits in Phase 1) ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason. My legal rights and medical care will not be affected. ☐

4. I understand that sections of any of my medical notes may be looked at by those named above or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

5. I agree to my GP or family doctor being informed that I am taking part in this study. ☐

6. I agree for my photographs to be taken and stored as outlined in this

patient information sheet

a. For use in this research thesis and my case notes ☐

b. For teaching purposes at the University Hospital of Wales ☐

c. For use in peer-reviewed medical publications ☐

7. I agree to take part in the above study ☐

_____	_____	
Name of Patient	Signature	Date
_____	_____	
Name of Person taking consent (if different from researcher)	Signature	Date
_____	_____	
Researcher	Signature	Date

FURTHER CONSENT FOR ANALYSIS OF BACTERIAL SAMPLES

Please initial box

1. I consent to the taking of bacterial samples for the purpose of this research study. ☐

2. I consent to the tests listed here being done on my bacterial samples. I understand that the results will be used mainly for the purpose of the current study, but my doctors may use these results in another study. ☐

    a. P. acnes counts & P. acnes typing. ☐

    b. P. acnes drug sensitivities ☐

    c. Culturing of any other pertinent bacterial species ☐

3. I agree to my sample being used for more unforeseeable tests as required in the future for this or other research. This may depend on the results of my culture results e.g. additional sensitivity testing may be required. ☐

    a. I would like to be contacted before further tests as mentioned above are done on the stored sample for research purposes. ☐

    b. I consent to further tests being done on the sample without being contacted. ☐

_____	_____	
Name of Patient	Signature	Date
_____	_____	
Name of Person taking consent (if different from researcher)	Signature	Date
_____	_____	
Researcher	Signature	Date

Advertisements

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DO YOU SUFFER FROM ACNE?

Can you say **'YES'** to these questions:

- Do you have problems with facial acne? (also known as spots)
- Are you between 18 and 45 years of age?
- Are you willing to be treated for your acne?

If so, you may wish to take part in a research study which aims to compare an established acne treatment with a new available treatment.

This research project will take place at the Department of Dermatology, University Hospital of Wales.

For further details, please call:

Sister Anne Thomas
(02920 742672)
Or
Dr C A Suthananthan
(02920 745875)

Department of Dermatology
3rd Floor Glamorgan House
University Hospital of Wales
Cardiff University
CF14 4 XW

(Travel reimbursements up to £100 will be paid)

- ***Advertisement in English and Welsh for Cardiff University Online Notice Board***

DO YOU SUFFER FROM ACNE?

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This research project will take place at the Department of Dermatology, University Hospital of Wales.

To find out if you are suitable for this study, please answer a short questionnaire linked below. Once you have completed it, you will be contacted about your eligibility. Thank you very much for your interest in the study.

Click HERE

A YDYCH YN DIODDEF O'R ACNE?

A ydych yn gallu ateb '**YDWYF**' i'r cwestiynau hyn:

- Ydych chi'n cael trafferth gydag acne (plorod neu smotiau yw'r enw gan rai) ar eich wyneb?
- A ydych rhwng 18 a 45 mlwydd oed?
- A ydych yn fodlon cael triniaeth am acne?

Os felly, efallai y byddech yn dymuno cymryd rhan mewn astudiaeth ymchwil sy'n anelu at gymharu'r dull sefydlog o drin acne â thriniaeth newydd sydd ar gael.

Bydd y prosiect ymchwil hwn yn digwydd yn Adran Dermatoleg, Ysbyty Prifysgol Cymru.

I weld a ydych yn addas ar gyfer yr astudiaeth, atebwch holiadur byr a ddolennir isod. Ar ôl i chi ei gwblhau, cysylltir â chi ynglŷn â'ch addasrwydd. Diolch yn fawr iawn am eich diddordeb yn yr astudiaeth.

Cliciwch YMA

Questions in Online Questionnaire

1. What is your email address? …….(Answer)
2. What is your postcode?.....(Answer)
3. What is your telephone number?.....(Answer)
4. How old are you?.....(Answer)
5. What is your first name?.....(Answer)
6. What is your last name?.....(Answer)
7. Do you have facial acne?.....(Y/N)
8. Have you read the Patient Information sheet (PIS)?.....(Y/N)
9. What happens when you are exposed to the sun?

A: Always burns, does not tan
B: Burns easily, tans poorly
C: Tans after initial burn
D: Burns minimally, tans easily
E: Rarely burns, tans darkly easily
F: Never burns, always tans darkly

10. Are you allergic to the sun i.e. itchy rash, blister formation etc ?.....(Y/N)
11. Have you had IPL before?.....(Y/N)
12. When were you last treated with IPL?.....(Answer)
13. Have you had treatment for your facial acne?.....(Y/N)
14. What have you had for your facial acne?.....(Answer)
15. When were you last treated for your facial acne?.....(Answer)
16. Do you have any skin problems affecting your face? Eg: psoriasis, eczema (Y/N)
17. Do you have other medical problems?.....(Answer)

Screening Questions for Telephone Enquiries

Title: A comparison of the clinical efficacies of intense pulsed light, photodynamic therapy and adapalene in the treatment of mild to moderate acne vulgaris

DATE: _____

SURNAME	
FIRST NAME	
HOME PHONE NUMBER	
MOBILE	
MAILING ADDRESS	
GENDER(M/F)	
AGE	
EMAIL:	

QUESTIONS **(Please Tick)**	YES	NO	Comment
Do you have facial acne?			
Have you read the Patient Information sheet (PIS)?			
What happens when you are exposed to the sun? A: Always burns, does not tan B: Burns easily, tans poorly C: Tans after initial			

burn D: Burns minimally, tans easily E: Rarely burns, tans darkly easily F: Never burns, always tans darkly			
Are you allergic to the sun- i.e. itchy rash, blister formation etc?			
Have you had IPL before?			
When were you last treated with IPL?			
Have you had treatment for your facial acne?			
What have you had for your facial acne?			
When were you last treated for your facial acne?			
Do you have any skin problems affecting your face? Eg: psoriasis, eczema			
Do you have other medical problems?			

The Family Dermatology Life Quality Index

The Family Dermatology Life Quality Index (FDLQI)

Name: …… FDLQI Score
Relationship with patient: . ...……..
Patient's diagnosis (if known): …...…… Date:
...……

- The questions relate to the impact of your relative/partner's skin disease on your quality of life over the last month.
- Please read the questions carefully and tick one box for each.

1. Over the last month how much emotional distress have you experienced due to your relative/partner's skin disease (e.g. worry, depression, embarrassment, frustration)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

2. Over the last month how much has your relative/partner's skin disease affected your physical well-being (e.g. tiredness, exhaustion, contribution to poor health, sleep/rest disturbance)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

3. Over the last month how much has your relative/partner's skin disease affected your personal relationships with him/her or with other people?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

4. Over the last month how much have you been having problems with other peoples' reactions due to your relative/partner's skin disease (e.g. bullying, staring, need to explain to others about his/her skin problem)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

5. Over the last month how much has your relative/partner's skin disease affected your social life (e.g. going out, visiting or inviting people, attending social gatherings)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

(*Please turn over*)

6. Over the last month how much has your relative/partner's skin disease affected your recreation/leisure activities (e.g. holidays, personal hobbies, gym, sports, swimming, watching TV)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

7. Over the last month how much time have you spent on looking after your relative/partner (e.g. putting on creams, giving medicines or looking after their skin)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

8. Over the last month how much extra house-work have you had to do because of your relative/partner's skin disease (e.g. cleaning, vacuuming, washing, cooking)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

9. Over the last month how much has your relative/partner's skin disease affected your job/study (e.g. need to take time off, not able to work, decrease in the number of hours worked, having problems with people at work)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

10. Over the last month how much has your relative/partner's skin disease increased your routine household expenditure (e.g. travel costs, buying special products, creams, cosmetics)?

Not at all/Not relevant ☐ A little ☐ Quite a lot ☐ Very much ☐

Thank you for completing the questionnaire.

Dermatology Life Quality Index

DERMATOLOGY LIFE QUALITY INDEX

DLQI

Hospital No: Date:
Name: Score:
Address: Diagnosis:

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick one box for each question.

1.	Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	
2.	Over the last week, how **embarrassed** or **self conscious** have you been because of your skin?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	
3.	Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
4.	Over the last week, how much has your skin influenced the **clothes** you wear?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
5.	Over the last week, how much has your skin affected any **social** or **leisure** activities?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
6.	Over the last week, how much has your skin made it difficult for you to do any **sport**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
7.	Over the last week, has your skin prevented you from **working** or **studying**?	Yes No	❒ ❒	Not relevant ❒
	If "No", over the last week how much has your skin been a problem at **work** or **studying**?	A lot A little Not at all	❒ ❒ ❒	
8.	Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
9.	Over the last week, how much has your skin caused any **sexual difficulties**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒
10.	Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant ❒

Please check you have answered EVERY question. Thank you.

The Leeds Revised Acne Grading System

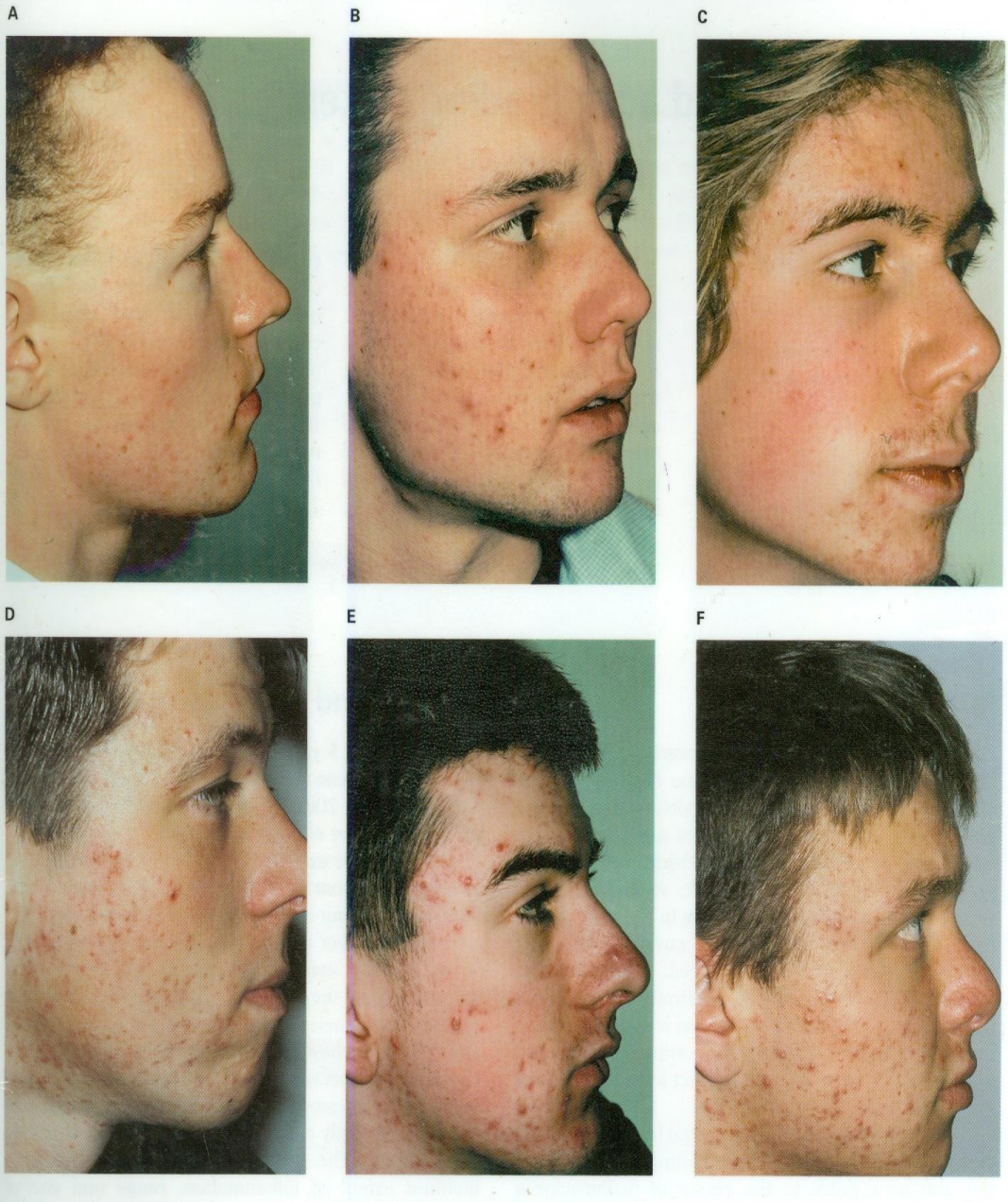

Figure 1
Grades of facial acne: (a) grade 1, (b) grade 2, (c) grade 3, (d) grade 4, (e) grade 5, (f) grade 6, (g) grade 7, (h) grade 8, (i) grade 9, (j) grade 10, (k) grade 11, (l) grade 12, (m) nodulocystic acne.

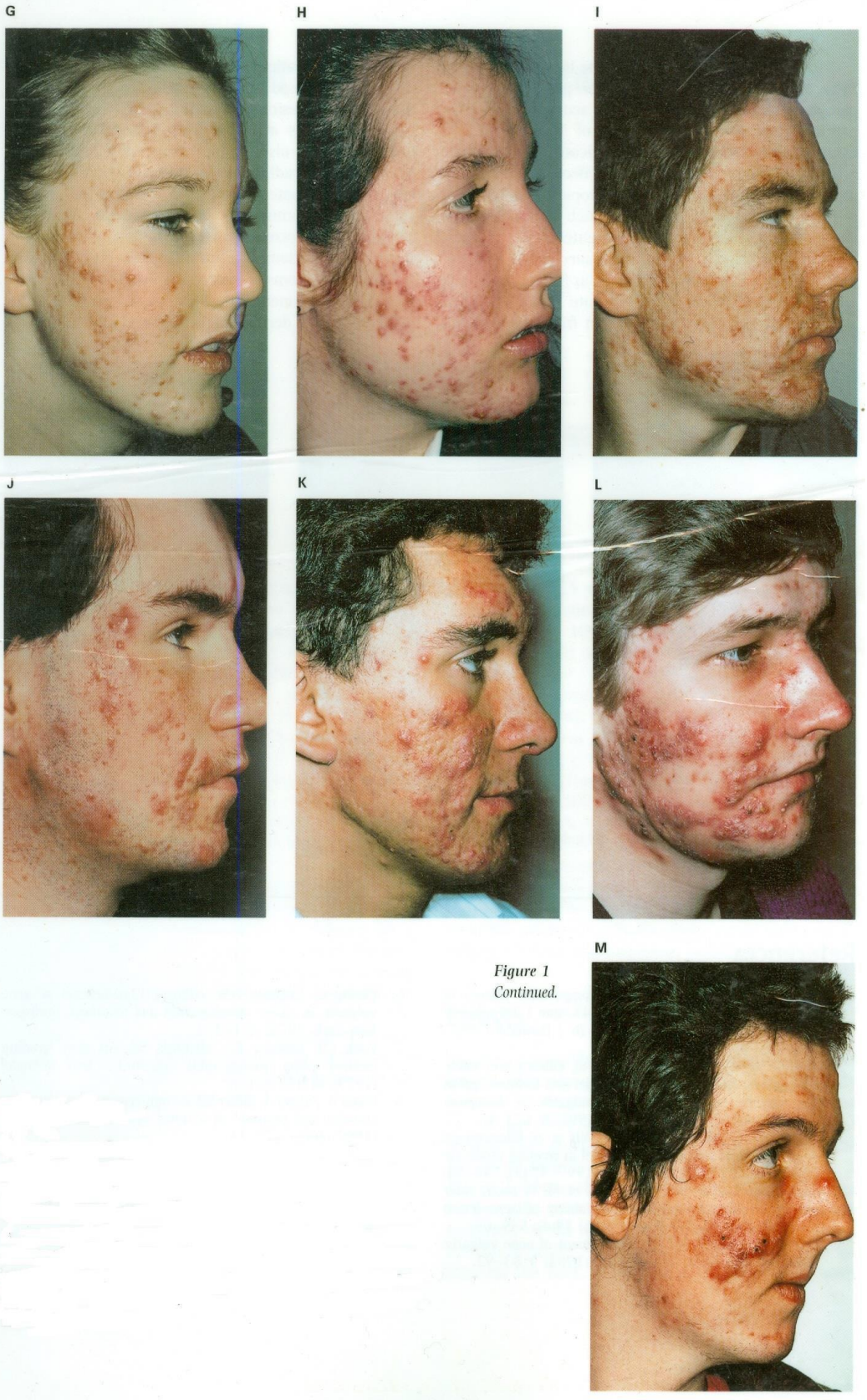

Figure 1
Continued.

Appendix 2

Publications & Presentations

Published Articles

- Shaheen B, Gonzalez M. A microbial aetiology of acne: what is the evidence? *Br J Dermatol* 2011; **165**: 474-85.

- Shaheen B, Gonzalez M. Acne sans *P. acnes*. *J Eur Acad Dermatol Venereol* 2013; **27**: 1-10.

Published Abstracts

- Shaheen B, Gonzalez M. Randomized, controlled, double-blind, clinical trial evaluating the mechanism of action, efficacies, and safety of methylaminolaevulinate photodynamic therapy (PDT) and intense pulsed light, administered as placebo-PDT, compared with adapalene 0.1% gel in the treatment of adults with mild to moderate acne vulgaris (Abstract). *Br J Dermatol* 2011; **165**: pp93-114.

Manuscript in progress

- Shaheen B, Porter RM, Gonzalez M. Effect of light treatment on the microbiology of acne.