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# Enhanced port-wine stain lightening achieved with combined treatment of selective photothermolysis and imiquimod

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**Background:** Pulsed dye laser (PDL) is the gold standard for treatment of port-wine stain (PWS) birthmarks but multiple treatments are required and complete resolution is often not achieved. Posttreatment vessel recurrence is thought to be a factor that limits efficacy of PDL treatment of PWS. Imiquimod 5% cream is an immunomodulator with antiangiogenic effects.

**Objective:** We sought to determine if application of imiquimod 5% cream after PDL improves treatment outcome.

**Methods:** Healthy individuals with PWS (n = 24) were treated with PDL and then randomized to apply posttreatment placebo or imiquimod 5% cream for 8 weeks. Chromameter measurements (Commission Internationale de l'Eclairage L\*a\*b\* colorspace) for 57 PWS sites (multiple sites per patient) were taken at baseline and compared with measurements taken 8 weeks posttreatment. The  $\Delta a^*$  (change in erythema) and  $\Delta E$  (difference in color between normal-appearing skin and PWS skin) were measured to quantify treatment outcome.

**Results:** Two patients developed minor skin irritation. Other adverse effects were not noted. Average  $\Delta a^*$  was 0.43 for PDL + placebo sites (n = 25) and 1.27 for PDL + imiquimod sites (n = 32) ( $P$  value = .0294) indicating a greater reduction in erythema with imiquimod. Average  $\Delta E$  was 2.59 for PDL + placebo and 4.08 for PDL + imiquimod ( $P$  value = .0363), again indicating a greater color improvement with imiquimod.

**Limitations:** Effects were evaluated after a single treatment and duration of effect is unknown.

**Conclusion:** Combined selective photothermolysis and antiangiogenic therapy may enhance PWS treatment efficacy. (J Am Acad Dermatol 2012;66:634-41.)

**Key words:** angiogenesis; imiquimod; port-wine stain; pulsed dye laser; selective photothermolysis; vascular malformation.

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A port-wine stain (PWS) is a vascular malformation found in approximately 0.3% of children.<sup>1,2</sup> Light-based therapy utilizing the theory of selective photothermolysis<sup>3</sup> can lighten these birthmarks, although only 10-20% of patients obtain 100% resolution.<sup>4,5</sup> Numerous treatments (15-20) are often required, and incomplete resolution and lesion recurrence are common. Over the last few decades, optimization of light-based protocols designed to treat cutaneous vasculature has focused primarily on improving vascular removal by optimizing therapeutic devices (to allow delivery of higher energies and improve safety with epidermal protection) or exploring alternative methods of removal (eg, photodynamic therapy). However, improving upon the degree of acute vascular destruction may not be adequate to achieve the desired goal of complete, long-term lesion removal.

We postulate that a critical factor limiting PWS treatment efficacy is post-treatment vessel recurrence as a result of angiogenesis.<sup>5</sup> Angiogenesis is a normal process in growth and wound healing, but it is also a contributing factor in a wide range of disease processes.<sup>6</sup> Initial interest in angiogenesis after selective laser injury was generated based on observations, noting that acute vascular destruction does not necessarily result in PWS lightening.<sup>5</sup> Subsequent studies using laser speckle imaging on a rodent dorsal window chamber model demonstrated an initial shutdown in blood flow followed by reperfusion and vascular remodeling.<sup>7</sup> Serial laser speckle imaging monitoring of patients with PWS has also demonstrated the dynamic nature of the posttreatment blood flow response in the clinical setting. Based on the collective data, we hypothesize that the effects of treatment with the pulsed dye laser (PDL) can be enhanced by application of an antiangiogenic agent.

Imiquimod (Graceway Pharmaceuticals, Bristol, TN) is a topically administered immune response modulator approved by the US Food and Drug Administration for treatment of external genital warts, superficial basal cell carcinoma, and actinic keratosis.<sup>8</sup> It has also been used successfully to treat vascular proliferative lesions such as infantile

hemangiomas, pyogenic granulomas, Kaposi sarcoma, and hemangiosarcomas.<sup>9-13</sup> A proposed mechanism of action of imiquimod is inhibition of angiogenesis. Imiquimod affects angiogenesis by: (1) induction of antiangiogenic cytokines including interferon-alpha, interleukin (IL)-10 and IL-12, and tissue inhibitors of metalloproteinases; and (2) inhibition of proangiogenic factors such as matrix metalloproteinases (MMPs).<sup>10,14</sup>

Our objective was to determine if PDL followed by posttreatment application of imiquimod would enhance treatment efficacy.

## METHODS

### Study design

To assess the efficacy of combined selective photothermolysis (PDL treatment) and imiquimod, we initiated a single-center, 8-week, blinded, placebo-controlled clinical feasibility study involving patients with PWS. Patients were randomly assigned into two possible

treatments arms: PDL + imiquimod 5% cream or PDL + placebo (vehicle) cream. The study was approved by the Investigational Review Board at University of California, Irvine, and was registered in the clinicaltrials.gov trial register (identifier: NCT00585247). Verbal and written informed consent was obtained for all adult patients and assent was obtained for patients younger than 18 years.

### Patient enrollment

Healthy adults and children with PWS were enrolled. Earlier treatment with PDL was not an exclusion, because PWS generally require multiple treatments. After blinded review of results in 13 patients suggested efficacy, the protocol was amended to allow patients to enroll into the trial on two separate occasions. A total of 5 patients were re-enrolled in this protocol. There was a minimum of a 4-week washout period between end of study and re-enrollment. At the end of the first enrollment, the patient was unblinded by the independent investigator (subinvestigator). During the next enrollment, the patient was placed into the other treatment arm by the independent investigator. The principal investigator and the patient remained blinded until the completion of the trial. Four of the 5 patients had the same PWS area treated during each enrollment

## CAPSULE SUMMARY

- Posttreatment vessel recurrence is thought to be a factor that limits efficacy of pulsed dye laser treatment of port-wine stain birthmarks.
- Combined selective photothermolysis and antiangiogenic therapy may enhance treatment efficacy for port-wine stains.
- Pulsed dye laser followed by application of 5% imiquimod 3 times a week for 8 weeks resulted in measurable improvement in port-wine stain lightening as compared with pulsed dye laser treatment followed by application of placebo.

**Abbreviations used:**

bFGF:	basic fibroblast growth factor
IL:	interleukin
MMP:	matrix metalloproteinases
PDL:	pulsed dye laser
PWS:	port-wine stain
VEGF:	vascular endothelial growth factor

We monitored the change in  $a^*$  ( $\Delta a^*$ ) and  $\Delta E$  to quantify PWS treatment outcome.<sup>10,16-28</sup>  $\Delta a^*$  indicates a change in the erythema of the vascular lesions. The secondary calculation ( $\Delta E$ ),<sup>20</sup> detects all 3 dimensions of colorspace ( $L^*a^*b^*$ ) and represents the difference in color between normal-appearing and PWS skin.  $\Delta E$  is calculated as:

$$\Delta E = \sqrt{(\Delta L^*_{before} - \Delta L^*_{after})^2 + (\Delta a^*_{before} - \Delta a^*_{after})^2 + (\Delta b^*_{before} - \Delta b^*_{after})^2}$$

whereas the fifth patient had another portion of the PWS treated. With each re-enrollment, new baseline measurements were obtained and different measurement spots were used.

**Study laser treatment and medication**

Each patient received a single treatment with the Perfecta 595-nm laser (Candela Corp, Wayland, MA). Settings used included either a 7- or 10-mm spot size, 1.5-millisecond pulse duration, radiant exposure of 6 to 12 J/cm<sup>2</sup>, and cryogen spray cooling (30 milliseconds of cooling with a 30- or 20-millisecond delay).

Beginning on the first day after treatment, patients applied one sachet (250 mg) of either 5% imiquimod or placebo (vehicle) cream to smaller than 25-cm<sup>2</sup> area of the treated PWS, 3 times a week, for 8 weeks.

**Data collection**

The study timeline is outlined in Table I. Patients were assessed for adverse events at 2-week intervals either by office visits or by telephone call. Standardized digital photographs (PowerShot S2 IS, Cannon USA, Lake Success, NY) were taken before and up to 8 weeks after treatment.

To quantify changes in skin color, we used a tristimulus chromameter (CR-400, Konica Minolta, Osaka, Japan) pretreatment and up to 8 weeks post-treatment. The chromameter provides measurements in the Commission Internationale de l'Eclairage  $L^*a^*b^*$  colorspace. This colorspace was developed in part to provide quantitative values, which correspond to human perception of color.  $L^*$  describes the reflected light intensity and varies from 0 (eg, black) to 100 (eg, white);  $a^*$  describes color saturation and varies from +60 for green to -60 for red; and  $b^*$  also describes color saturation and varies from +60 for blue to -60 for yellow.<sup>15</sup> Multiple sites were measured within each PWS. Lesion tracings with transparency paper were used with the intent to measure identical spots at each visit. Each patient was not an average of multiple sites; rather, each site was an independent data set.

$\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  are the differences in  $L^*$ ,  $a^*$ , and  $b^*$ , respectively, between PWS and normal-appearing skin and subscripts "before" and "after" indicate color values acquired before and 8 weeks after treatment. Starting with patient 6, we collected chromameter data from normal-appearing skin sites to enable calculation of  $\Delta E$  and use a more standardized measure of treatment efficacy. Laser speckle imaging was performed before and after treatment as a method to assess acute posttreatment vascular shutdown.<sup>21</sup>

The final patient had additional analysis carried out. This patient had 3 testing areas: (1) untreated PWS, (2) PWS treated with PDL alone, and (3) PWS treated with PDL + imiquimod 5% cream. Additional testing areas were included to allow comparison of treatment conditions. At week 4, the patient had three 2-mm biopsies performed. Tissue was frozen and sections processed for immunohistochemistry staining of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), MMP-9, and angiopoietin-2 (ANG-2).

Resulting images were blinded and graded by a board-certified dermatopathologist, who evaluated the specimens' degrees of staining for each of the 4 antibodies in the epidermis, dermis, and endothelial cells. The degree of staining was divided by proportion of cells stained into one of 4 categories: 0%, less than 10%, 10% to 50%, or more than 50%. The intensity was graded on a scale of 0 to 3.

**Statistical analyses**

The outcomes of the two treatment groups were analyzed as follows,  $\Delta a^*$  for each treatment site was compared using the Mann-Whitney-Wilcoxon nonparametric test. A  $P$  less than or equal to .05 indicated statistical significance. The  $\Delta E$  for each treatment site was also compared using the Mann-Whitney-Wilcoxon nonparametric test.  $P$  less than or equal to .05 indicated statistical significance.

**RESULTS**

A total of 24 patients were enrolled (Table II). Enrollment consisted of 22 adults (mean age = 37

**Table I.** Study timeline

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Baseline (day 0)

- Informed consent obtained and determined patient eligibility
- Randomized (1:1) into treatment group (PDL + imiquimod 5% cream) or placebo group (PDL + vehicle cream)
- Photographs
- Baseline measurements with chromameter
- Treatment with PDL
- Laser speckle imaging before and after laser treatment
- Study medication dispensed

Day 1

- Patient starts treatment with study cream on 3 times per week schedule

Day 14

- Assess for adverse events
- Study drug accountability

Day 28

- Assess for adverse events
- Study drug accountability

Day 42

- Assess for adverse events
- Study drug accountability

End of study (day 56)

- Assess for adverse events
- Photographs
- Measurements with chromameter
- Study drug accountability and collection

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PDL, Pulsed dye laser.

years) and two children (youngest was 13 years of age). Most patients had previous treatment with the PDL (at least 2 months earlier) but none of the patients had treatment with combination PDL and imiquimod before enrollment. Baseline  $a^*$  values were established through measurement of bloodless in vitro human skin and in vivo normal-appearing and pretreated PWS skin (Table III). For all patients, pretreatment  $a^*$  values were compared with those measured at 8 weeks posttreatment. The  $a^*$  values were measured from 57 independently monitored sites: 25 PDL + placebo and 32 PDL + imiquimod. The first two patients were not included in the analysis because of chromameter malfunction. When comparing the outcomes using the Mann-Whitney-Wilcoxon test the average  $\Delta a^*$  was calculated to be 0.43 (SD = 1.63) for PDL + placebo sites and 1.27 (SD = 1.76) for PDL + imiquimod sites (Fig 1). This statistically significant result ( $P$  value = .0294) suggests that the addition of imiquimod post-PDL improves the reduction of erythema. The  $\Delta E$  was calculated from 49 independently monitored sites: 25 PDL + placebo and 24 PDL +

imiquimod. The average  $\Delta E$  was calculated to be 2.59 (SD = 1.54) for PDL + placebo and 4.08 (SD = 3.39) for PDL + imiquimod sites (Fig 2), again suggesting that imiquimod application improved treatment efficacy ( $P$  value = .0363).

Fig 3 provides images taken from a patient in the PDL + imiquimod group. Because of the large area of PWS skin, we evaluated not only the intended test condition (PDL + imiquimod) but also additional test and control conditions. Site 1 received imiquimod alone; site 2 received PDL + imiquimod; site 3 received PDL alone; and site 4 was an untreated PWS site. The  $\Delta a^*$  for each site was: site 1 (imiquimod alone) = 1.41; site 2 (PDL + imiquimod) = 2.68; site 3 (PDL alone) = 2.37; and site 4 (untreated PWS) = -1.63. Positive numbers indicate a reduction of erythema; a greater positive number indicated enhanced treatment response. The corresponding  $\Delta E$  value was 1.09, 11.47, 9.97, and 4.83, for sites 1 to 4, respectively. Enhanced lightening in the PDL + imiquimod site is particularly impressive as this demonstrates augmentation of an already dramatic PDL response (a result that is not often achieved with a single PDL treatment).

Fig 4 summarizes the data, from a single patient, evaluating the immunohistochemical assessment of angiogenesis mediators of an untreated PWS compared with PWS treated with PDL alone and also PDL + imiquimod. As expected, the degree of staining of the mediators (VEGF, bFGF, MMP-9, ANG-2) found in the epidermis was lower compared with dermal staining; this was especially true for VEGF. VEGF staining was decreased in both endothelial cells and the dermis with PDL + imiquimod compared with PDL alone. bFGF was found in all 3 structures (epidermis, dermis, endothelial cells) and it was found in lower levels with the PDL alone compared with PDL + imiquimod. With MMP-9 there were low levels of staining in an untreated PWS and no change was seen with treatment of the PDL. The MMP-9 increased in the PDL + imiquimod sample. ANG-2 levels decreased with PDL, although there was a variable response seen with PDL + imiquimod.

### Adverse events

The treatment was well tolerated by all patients. Two patients receiving PDL + imiquimod required a (1- and 4-week) rest period beginning at the second week post-PDL therapy, as a result of mild erythema and crusting. Both of these patients (ages 14 and 21 years) demonstrated only mild sun damage on clinical examination. After the rest period, imiquimod dosing was resumed without incident. No other adverse effects were reported.

**Table II.** Patient demographics

Patient no.	Sex	Race	Age, y	Treatment site	Second enrollment
1	Female	Caucasian	71	Right hand	
2	Male	Caucasian	44	Left arm	
3	Female	Caucasian	31	Face	
4	Female	Hispanic	21	Right leg	
5	Female	Asian	35	Left hand	
6	Female	Caucasian	55	Left thigh	
7	Female	Caucasian	22	Back	
8	Female	Caucasian	13	Face	
9	Female	Hispanic	47	Right thigh	
10	Female	Caucasian	38	Left leg	
11	Male	Caucasian	51	Left arm/chest	
12	Male	Caucasian	14	Face/left ear	
13	Male	Caucasian	51	Left arm/chest	Yes
14	Male	Caucasian	14	Face/left ear	Yes
15	Female	Caucasian	14	Face	Yes
16	Male	Asian	23	Face	
17	Female	Caucasian	23	Left leg	
18	Male	Asian	19	Face	
19	Male	Asian	23	Face	Yes
20	Male	Hispanic	45	Face	
21	Male	Caucasian	35	Face	
22	Male	Asian	20	Face	Yes
23	Male	Caucasian	27	Face	
24	Male	Caucasian	62	Face	

**Table III.** Average  $a^*$  values in control tissues

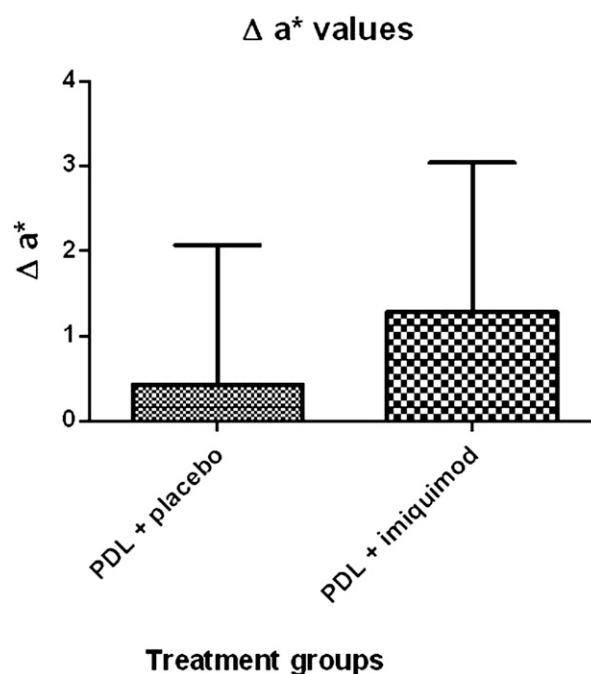
Description	Average $a^*$ Indicative of erythema color
Bloodless tissue (cadaver skin)	0.92
Normal-appearing skin	9.28
PWS skin	15.50

PWS, Port-wine stain.

## DISCUSSION

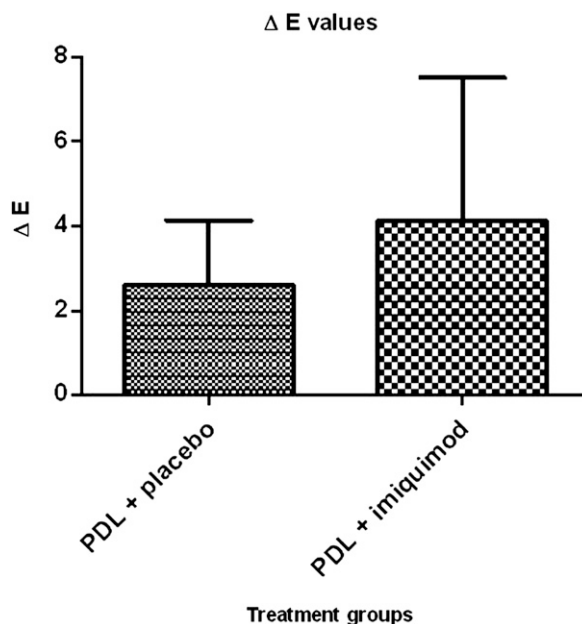
Our preliminary findings offer important considerations for therapeutic applications of light and may indicate an important paradigm shift for this field. Based on the average  $\Delta a^*$  values, the addition of imiquimod post-PDL improved the reduction of erythema. Average  $\Delta E$  values suggest improved efficacy with PDL + imiquimod compared with PDL alone. Imiquimod appears to minimize post-laser treatment angiogenesis. The addition of imiquimod was safe; few minor adverse events were reported during the study.

Only two patients reported irritation. Application of the imiquimod more than 3 times a week may have resulted in more patients with irritation. We chose the 3 times a week application because this regimen specifically has been reported to have antiangiogenic effect.<sup>10</sup>



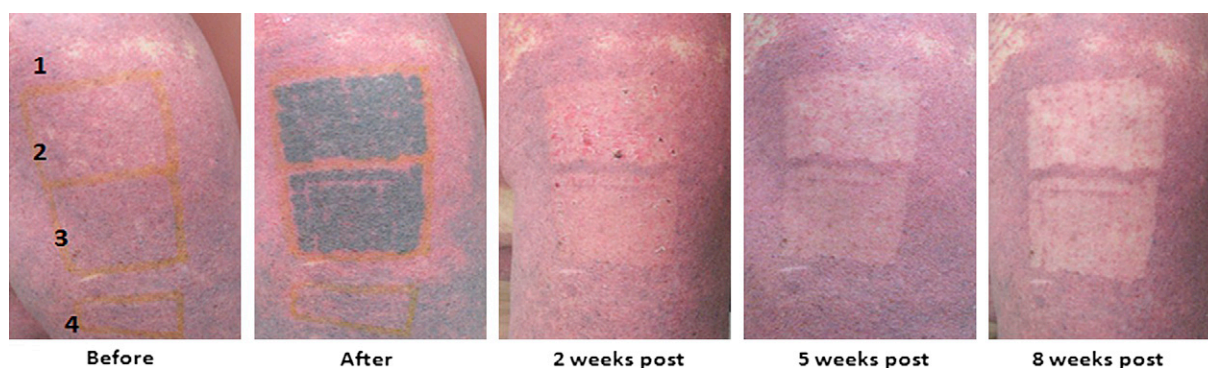
**Fig 1.** Change in  $a^*$  values between baseline and 8-week measurements.  $\Delta a^*$  indicates change in erythema of vascular lesions.  $P = .0294$ . PDL, Pulsed dye laser.

Our study size was small and the treatment areas were not uniform between patients, but each measurement site was independently monitored. The



**Fig 2.** Change in E between baseline and 8-week measurements.  $P = .0363$ .  $\Delta E$  detects all 3 dimensions of colorspace ( $L^*a^*b^*$ ) and represents difference in color between normal-appearing and port-wine stain skin. *PDL*, Pulsed dye laser.  $\Delta E$  is calculated as:

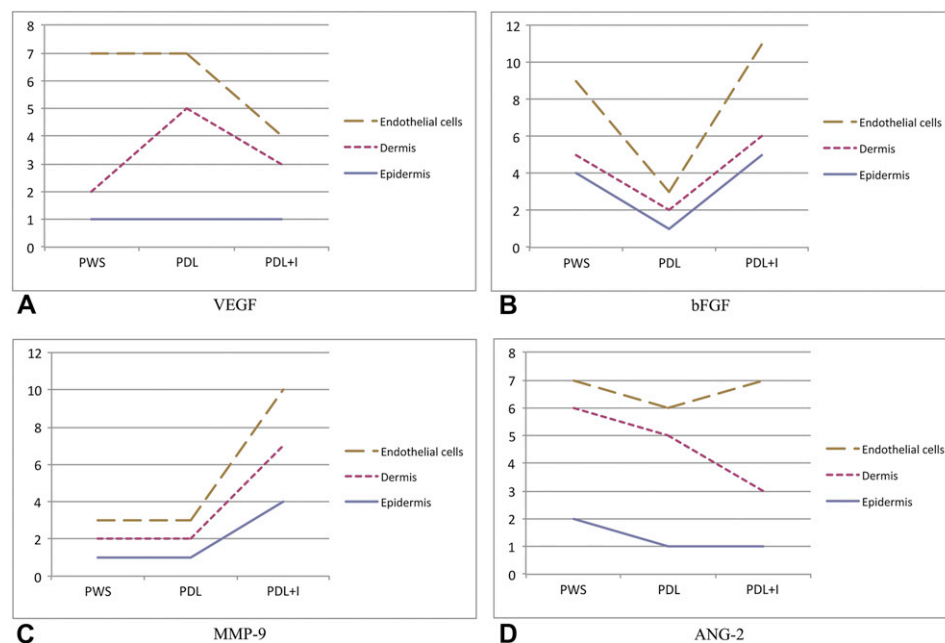
$$\Delta E = \sqrt{(\Delta L^*_{before} - \Delta L^*_{after})^2 + (\Delta a^*_{before} - \Delta a^*_{after})^2 + (\Delta b^*_{before} - \Delta b^*_{after})^2}$$



**Fig 3.** Port-wine stain (*PWS*) treated in pulsed dye laser (*PDL*) + imiquimod (*I*) group. Site 1 received *I* alone; site 2 received *PDL* + *I*; site 3 received *PDL* alone; and site 4 is untreated *PWS* site.  $\Delta a^*$  for each site was: site 1 (*I* alone) = 1.41; site 2 (*PDL* + *I*) = 2.68; site 3 (*PDL* alone) = 2.37; and site 4 (untreated *PWS*) = -1.63.

laser settings were variable but the calculation of  $\Delta a$  and  $\Delta E$  allowed for each site to be measured independently. Finally, the duration of effect of our results is not known as the study was only 8 weeks long. Further experiments are required, but the initial results are intriguing and suggest that treatment optimization should focus on both initial vascular destruction and modulation of the biological repair processes.

The antiangiogenic effect of imiquimod occurs through the activation of toll-like receptor 7. Toll-like receptor 7 induces antiangiogenic cytokines (interferon-alpha, IL-10, IL-12, IL-18), reduces angiogenic stimulators (MMP-9 and bFGF), and locally up-regulates endogenous inhibitors of MMP (tissue inhibitors of metalloproteinases). In addition, imiquimod induces endothelial cell apoptosis. This cascade of events has the potential to halt the



**Fig 4.** Immunohistochemistry of angiogenic mediators. *ANG-2*, Angiopoietin-2; *bFGF*, basic fibroblast growth factor; *MMP*, matrix metalloproteinases; *VEGF*, vascular endothelial growth factor.

postlaser treatment vessel recurrence seen in PWS birthmarks. Imiquimod has been used successfully as a single-agent treatment for hemangiomas, thus one might consider whether imiquimod alone would be effective in treating PWS.<sup>14</sup> PWS are stable vascular lesions consisting of dermal, dilated, capillary-like vessels with no abnormal endothelial proliferation.<sup>22</sup> In contrast, hemangiomas or other benign vascular tumors are characterized by rapid vascular proliferation that may be followed by involution.<sup>23-26</sup> As a result of the slowly proliferating nature of PWS vasculature, it is likely the use of an antiangiogenic agent alone would have limited effect on PWS vessels. Addition of an antiangiogenic agent is more useful as an adjunct to PDL-induced selective photothermolysis, which is effective for acute destruction of PWS vasculature, but limited by vessel repair during the wound-healing phase.

Other antiangiogenic agents may have use in treatment of cutaneous vascular lesions. Imiquimod was chosen for this study because of its commercial availability, ease of topical administration, and good safety record. Our results are statistically significant but we do think even more impressive results may be obtained as studies reveal which angiogenesis mediators are stimulated by laser therapy and thus, should be targeted for reduction.

Recently there has been research directed toward the antiangiogenic effect of rapamycin (Pfizer, New York, NY), an immunosuppressive medication with

inhibitory action against the mammalian target of rapamycin.<sup>22,27-29</sup> Using an in vivo window chamber model (rodent and hamster), investigators have demonstrated significant decrease in revascularization with laser (PDL or neodymium:yttrium-aluminum-garnet) and topical rapamycin compared with laser alone.<sup>22,28</sup> When topical rapamycin was applied to normal-appearing human skin in situ, similarly there was suppression of reformation and reperfusion of vessels in the area treated.<sup>29</sup> Other macrolides inhibiting the mammalian target of rapamycin pathway have been evaluated including tacrolimus (Astellas Pharma, Deerfield, IL) and temsirolimus (Pfizer). There is emerging evidence that temsirolimus may have increased solubility and thus have superior efficacy compared with rapamycin.<sup>30</sup> Further evaluation of this family of angiogenesis inhibitors is needed.

In this protocol, the immunohistochemical analysis of angiogenesis mediators was limited in that only a single patient was evaluated. The initial trends confirm that known angiogenesis mediators are present in the dermis and endothelial cells of untreated PWS and are modified by PDL and imiquimod. Further evaluation of angiogenesis promoters and inhibitors in unmanipulated and posttreatment tissue is necessary. Additional research may indicate potential therapeutic targets for a wide range of dermatologic diseases including benign vascular tumors (hemangiomas, angiofibromas), malignant vascular tumors

(Kaposi sarcoma, angiosarcoma), and inflammatory conditions with a prominent vascular component (rosacea, psoriasis). Successful therapies targeting angiogenesis have been developed and are now a standard-of-care treatment in oncology and ophthalmology. Use of antiangiogenic agents for treatment of skin conditions has been limited, but this is now changing and it is likely that angiogenesis targeting therapies will play an increasing role in dermatology.

Other methods of vascular destruction (including other lasers or photodynamic therapy) could also be combined with posttreatment antiangiogenic therapy in an effort to enhance results. Safety and efficacy of other combined approaches should also be studied in the future.

In summary, we provide preliminary data that treatment efficacy of selective photothermolysis of PWS may be enhanced by posttreatment application of imiquimod, an immunomodulating agent with antiangiogenic activity. This combined therapy may have significant impact in the fields of biomedical optics and dermatology.

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