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Observations on Enhanced Port Wine Stain Blanching Induced by Combined Pulsed Dye Laser and Rapamycin Administration

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Port wine stain (PWS) is a congenital, progressive vascular malformation of skin involving post-capillary venules that occurs in an estimated 3–4 children per 1,000 live births (1–3). Since most malformations occur on the face and neck, PWS is a clinically significant problem in the majority of patients. PWS are initially flat red macules that can be localized or segmental, but lesions tend to darken progressively, and by middle age, often transform into dark-red or purple plaques with nodularity (4, 5).

The pulsed dye laser (PDL) is the current treatment of choice for PWS. Yellow light is preferentially absorbed by hemoglobin in PWS blood vessels where, after being converted to heat, causes thermal damage and thrombosis. Treatment is often followed by temporary purpura, which resolves in 7–14 days.

Improvements in PDL technology over the past decade, including the use of multiple laser devices through an extended treatment protocol and selective epidermal cooling permitting the use of higher light dosages, have improved PWS therapeutic outcome. However, the degree of lesion blanching seen following PDL treatment remains variable and unpredictable (6). Moreover, PWS can recur after laser therapy due to reformation and reperfusion of blood vessels. In a five-year study by Katugampola and Lanigan (7) involving 640 subjects treated by PDL, less than half of the PWS patients achieved 50% clearance of their lesions after 8–12 treatments. Treatment of 62 patients was discontinued despite incomplete PWS clearance because additional treatments did not result in further PWS lightening. The authors concluded, “A new approach to the laser treatment of PWS birthmarks is urgently needed.”

The increasing availability of Food and Drug Administration (FDA) approved angiogenesis inhibitors and their relatively low side-effects profile have suggested a new focus in therapy designed to prevent reformation and reperfusion of PWS blood vessels after laser photothermolysis (8). Angiogenesis inhibitors are derived from a number of sources, including cleaved proteins, monoclonal antibodies, and natural products, which contain a variety of chemopreventive compounds that can prevent the development of malignancies. One such compound is rapamycin [RPM - Rapamune®, Sirolimus; Wyeth Pharmaceuticals, Collegeville, PA], which is a macrocyclic fermentation product of *Streptomyces*

hygroscopis, an actinomycete, originally isolated from a soil sample in Rapa Nui (Easter Island). RPM is a specific inhibitor of the mammalian target of rapamycin (mTOR), which forms part of the target of rapamycin complex (TORC) whose function is to integrate and transmit signals from a diverse array of growth factors to regulate cell survival and growth through changes in mRNA translation, ribosomal biogenesis, autophagy and metabolism (9). Although initially investigated as an antifungal agent, over the past 15 years RPM has been used for immunosuppression in renal transplantation subjects because of its unique lack of end-organ toxicity and ability to synergize with other agents without overlapping side effects (10). RPM is currently under intensive investigation as an anti-cancer drug through its ability to inhibit tumor cell survival and tumor angiogenesis (11–13). RPM inhibits the proliferation of vascular endothelial cells driven by vascular endothelial growth factor (VEGF) and suppresses the induction of hypoxia-inducible factor 1- α (HIF-1 α), which is a transcriptional regulator of hypoxia-sensitive genes, such as VEGF (14). Therefore, RPM inhibits the growth of vascular endothelial cells and smooth muscle cells, critical elements for new blood vessel formation (15).

After laser photothermolysis, blood supply to the skin is markedly reduced due to vessel photocoagulation resulting in the induction of significant local hypoxia. The skin's normal wound healing response detects hypoxia and initiates appropriate defense mechanisms such as angiogenesis. mTOR signaling is an important component of the cellular response to hypoxia through upregulation of HIF-1 α , which increases the expression of hypoxia responsive genes. Inhibition of mTOR by RPM reduces HIF-1 α nuclear accumulation and potentially suppresses angiogenesis, which may contribute to preventing the reformation and reperfusion of PWS blood vessels disrupted by laser photothermolysis.

RPM has been used for the treatment of a variety of hypervascular anomalies including tuberous sclerosis complex (TSC), angiomyolipomas (16–19) and Kaposi's sarcoma (20) with dramatic results. RPM given in oral dosages of 2–10 mg per day induced remarkable tumor shrinkage and was well tolerated by patients. Subjects were placed on RPM for periods of up to one year and such dosage regimens were found to be safe with minimal adverse effects. RPM is also under study for the treatment of a variety of cutaneous pathologies including psoriasis (21) and cutaneous angiofibromas associated with TSC (22).

In previous animal experiments using the rodent window chamber model (RWCM), the reformation and reperfusion of blood vessels after PDL alone versus combined PDL and RPM administered daily for two weeks after laser exposure were compared. When the RWCM skin was exposed to laser alone, reformation and reperfusion of the photocoagulated blood vessels were always observed. In contrast, blood vessel reformation and reformation were dramatically reduced when RWCM skin was exposed to laser with subsequent daily RPM for 14 days (8, 23).

Preliminary studies on normal human skin demonstrated that combined PDL exposure and RPM administration increased destruction of dermal blood vessels as compared to PDL alone (24). RPM administration abrogated the upregulation of certain "stem cell" antigens, such as nestin, thereby interrupting important wound healing mechanisms by which vascular lesions can repair themselves after PDL exposure.

Based on the results of our animal model and preliminary studies on normal human skin, a clinical study was initiated on PWS patients to determine safety and blanching responses of combined PDL and RPM treated test sites in comparison to those treated with standard PDL therapy alone (FDA Investigational New Drug Application [IND] #103,571). The protocol described herein was approved by the University of California Irvine Institutional Review Board. Our central hypothesis is that combined use of PDL to induce PWS blood vessel

injury, and RPM administration to prevent blood vessel reformation and reperfusion after laser therapy, will improve PWS lesion blanching.

A 37 year-old Caucasian male PWS patient with an extensive lesion involving the left anterior chest was recruited for our RPM study from an outpatient population of subjects with PWS at the Beckman Laser Institute and Medical Clinic, University of California, Irvine. Prior to enrollment, the subject had hematology CBC, chemistry (including liver function and lipid profiles) and urinalysis. After informed consent was obtained, the subject had areas selected on the PWS that appeared optically uniform. Three 3-cm² test sites were selected and assigned a site number (Figure 1A). Those test sites were then treated by PDL alone ($\lambda = 595$ nm, $\tau_p = 1.5$ ms, energy density 7, 8 and 10 J/cm²; 10 mm spot diameter; 30 ms cryogen spurt with a delay of 30 ms) to: 1) ensure that the light dosages do not induce an open wound in the skin (which might not heal well in the presence of RPM therapy); and 2) serve as control.

One month after PDL alone exposure, the subject was given 2 mg RPM per day orally for seven days to ensure that a steady state concentration of RPM had been obtained in the systemic circulation. A blood sample drawn after seven days confirmed a therapeutic RPM trough level (10–20 ng/ml) at which time three additional test sites (assigned site numbers 1 through 3) were subsequently treated by PDL using the same parameters listed above. The subject was instructed to continue taking oral RPM for four weeks following laser exposure of the second set of test sites. A daily RPM dosage regimen will ensure that a steady state concentration of RPM is maintained in the systemic circulation to potentially suppresses angiogenesis. The subject had hematology, chemistry and urinalysis performed at 2 and 4 weeks after the second set of test sites. Other than trace protein noted on urinalysis, all results were within normal limits. Specifically, no leukopenia or hyperlipidemia was noted. Safety was evaluated for each test site by searching for any abnormal wound healing. Blistering, scabbing, erosion, scarring or dyspigmentation was not observed on any test site. After four weeks, oral RPM was discontinued.

The subject was then followed up at intervals of 2–4 weeks over a period of 13 months. At each follow-up visit, photographs of the test sites were taken. Representative photographs of the test sites at 6 weeks, 8 months and 13 months are shown in Figures 1B,C,D. Although some clinical blanching was observed on the PDL alone test site at 6 weeks (Figure 1B), there was almost complete reformation and reperfusion of PWS test sites treated by PDL alone 8–13 months (Figures 1C, 1D). In contrast, there was an enhanced blanching response obtained on the test sites treated with the combined PDL and oral RPM as compared to PDL alone that has now been initiated and maintained long-term for more than 13 months after treatment (Figures 1B, 1C and 1D).

The confounding clinical results achieved after PDL treatment alone of PWS raise the following question: can the wound healing response of human skin after laser therapy be modulated? Preliminary studies have suggested that the combined use of PDL and an angiogenesis inhibitor might suppress the reformation and reperfusion of blood vessels previously disrupted by photothermolysis (8,23,24). The scientific rationale for this approach is that PDL is used to induce PWS blood vessel injury, and then an angiogenesis inhibitor is subsequently administered to prevent PWS blood vessel reformation and reperfusion. Taken together, the proposed combined approach should improve PWS lesion blanching.

In conclusion, these preliminary clinical observations conducted on PWS human skin demonstrate the safety, feasibility and potential of the combined PDL and RPM approach to prevent reformation and reperfusion of PWS blood vessels. If these observations can be

established and expanded by additional clinical studies, the combined approach might potentially offer a novel solution to the long-standing problem of poor PWS lesion blanching responses often observed after current treatment protocols. The laser and anti-angiogenic therapy approach is also expected to result in a reduction in the number of patient visits required to achieve complete PWS removal, which is particularly important for those patients undergoing repeated procedures utilizing general anesthesia. Furthermore, a reduction in the number of laser procedures would result in a lower and more cost effective treatment.

Finally, the development of a topical formulation of RPM should significantly reduce the risks of systemic drug exposure. Reported adverse effects of systemic RPM exposure, such as aphthous oral ulcer, diarrhea, upper respiratory infection, acne, folliculitis, headache, joint pain or fatigue, can result in considerable patient discomfort and morbidity. Most importantly, the systemic risks could preclude the use of RPM in the population of patients expected to obtain the most benefit from the combined approach, namely infants and young children, in whom the effects of systemic RPM exposure has yet to be comprehensively studied. Prospective, comparative and controlled clinical studies conducted by experienced investigators in a multicenter trial against accepted treatment regimens are necessary to better define the role of the combined PDL and RPM approach in the clinical management of PWS patients.

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Figure 1. Laser Only vs. Laser + RPM. Blanching responses by test site number and treatment: A) before treatment; B) 6 weeks after treatment; C) 8 months after treatment; and D) 13 months after treatment. Enhanced blanching of the combined Laser + RPM treated sites has been initiated and maintained. Redarkening of Laser Only test sites was observed.