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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. **10.040.510**

Solicitud de patente a nombre de **UNIVERSITÉ VICTOR SEGALEN-BORDEAUX 2**

Yo, DILIA MARIA RODRIGUEZ D'ALEMAN, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, obrando como apoderada de la sociedad **UNIVERSITÉ VICTOR SEGALEN-BORDEAUX 2**, solicitante de la patente de la referencia, en respuesta al oficio No. **16568** notificado por fijación en lista el 18 de septiembre de 2012, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial una nueva memoria descriptiva y un nuevo capítulo reivindicatorio, el cual consta de 19 reivindicaciones y reemplaza completamente el capítulo presentado anteriormente.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones planteadas por el examinador en su concepto técnico.

2. Reivindicaciones modificadas

La Reivindicación 1 se ha modificado y se refiere ahora a un medicamento capaz de tratar hemangiomas, dicho medicamento se caracteriza porque comprende un betabloqueador en una dosis que permite una administración de 1 a 5 mg de

dicho betabloqueador por kilogramo de peso corporal por día de un paciente en necesidad del mismo.

Esta reivindicación se encuentra soportada en la descripción PCT presentada originalmente, por ejemplo, en la página 5, líneas 17 a 19, página 7, líneas 9 a 10 y la página 9, líneas 9 a 11.

Alcance de la invención

Como se explicó anteriormente, el grupo de reivindicaciones modificadas se refiere ahora a un medicamento propiamente dicho y ya no a un segundo uso médico o método de tratamiento de una enfermedad. De acuerdo con lo anterior, la materia objeto reivindicada es patentable a la luz de la Decisión 486.

3. Requisitos de patentabilidad

Comentarios relacionados con los documentos citados:

D1: WO2006/015830

Este documento describe el uso de compuestos que tienen un efecto agonista del adrenoreceptor β_3 , y en particular el uso de compuestos que tienen tanto un efecto agonista del adrenoreceptor β_3 como un efecto antagonista del adrenoreceptor β_1/β_2 (página 4, párrafo 3) para el tratamiento de enfermedades arteriales.

Los hemangiomas no se citan en este documento y son enfermedades que no tienen vínculo con las enfermedades arteriales.

Este documento no describe que un betabloqueador sería eficiente como un medicamento para el tratamiento de hemangiomas.

Adicionalmente este documento no describe ni sugiere ningún medicamento contra hemangiomas, ni que dicho medicamento tenga que estar en una forma que permita una administración de 1 a 5 mg de un betabloqueador por kilogramo de peso corporal por día de un paciente en necesidad del mismo con el fin de que sea eficiente.

De acuerdo con lo anterior, la materia objeto reivindicada es claramente novedosa a la luz del documento D1.

Adicionalmente, el documento D1 menciona el propranolol entre los antagonistas de los adrenoreceptores $\beta 2$ (página 14, primer párrafo).

De acuerdo con la enseñanza de D1, el efecto agonista de los adrenoreceptores $\beta 3$ y el efecto antagonista del adrenoreceptor $\beta 1/\beta 2$ estimula la neoangiogenia (véase página 16, párrafo 4).

De acuerdo con lo anterior, el documento D1 está lejos de enseñar al experto en la técnica la posibilidad de un medicamento que comprenda un betabloqueador para el tratamiento de hemangiomas.

Por lo tanto, los expertos en la técnica nunca han tenido en cuenta este documento, cuyo objeto es la estimulación de neoangiogenia.

D2: WO2006122007

Este documento describe un método para tratar a un paciente que tiene un neoplasma que incluye la administración de una pluralidad de agentes. En particular, este documento describe el posible tratamiento de muchas enfermedades en las que ocurren los hemangioblastomas.

El hemangioblastoma es una enfermedad completamente diferente del hemangioma. En efecto, el hemangioblastoma se refiere a los tumores del sistema nervioso central que se originan del sistema vascular por lo general en el transcurso de la enfermedad de cáncer a mediana edad. Esto no tiene ningún vínculo con hemangiomas. Cabe aclarar que todo el mundo sabe eso, sobre todo el experto en la técnica.

Este documento no describe ni sugiere un medicamento contra los hemangiomas, ni un medicamento que comprenda un betabloqueador como se define en el grupo de reivindicaciones adjuntas.

De acuerdo con lo anterior, la materia objeto reivindicada es claramente novedosa a la luz del documento D2.

Adicionalmente, este documento no describe en lo absoluto ni los hemangiomas ni su tratamiento.

De hecho, hasta la fecha todos los especialistas en el tema coinciden en que la fisiopatología del hemangioma aún es poco conocida y, a fortiori, en la fecha de prioridad de la solicitud de patente (véase los documentos adjuntos a este memorial, Ritter et al. 2007; SUNDINE et al., 2007; Weibel, 2009; STORCH y Hoeger, 2010; KHAN et al., 2008). Estos documentos muestran claramente que, a la fecha de prioridad de la solicitud de patente, el origen de los hemangiomas infantiles y los mecanismos que regulan su crecimiento y su involución eran desconocidos y controvertidos (véase Ritter et al., abstract y SUNDINE et al., P. 207, párrafo “Etiology”). De acuerdo con lo anterior, para los expertos en la técnica, los hemangiomas están muy lejos de ser retirados de y no se pueden comparar con, un tumor canceroso como hemangioblastoma.

Adicionalmente, las estirpes neoplásicas están muy lejos de ser retiradas del hemangioma, cuyo origen celular son citoblastos mesenquimales pluripotentes (véase KHAN et al., primero y último párrafo de la descripción). Un hemangioma es un tumor benigno que se observa solamente en bebés y es potencialmente auto-regresivo, lo que para los expertos en la técnica, lo hace estar muy lejos de ser retirado de y no se puede comparar con, un tumor canceroso (véase Ritter et al., Página 4, columna izquierda y página 12, columna izquierda; SUNDINE et al., página 206, columna izquierda, 2º párrafo).. Un hemangioma, por otra parte, se describe como una “enfermedad de angiogenia no neoplásica” por SUNDINE et al. (Véase página 207, párrafo “Etiology”).

De acuerdo con lo anterior, los expertos en la técnica nunca habrían tenido en cuenta este documento, cuya materia objeto es el tratamiento del cáncer tumoral, ya que no hay relación entre el cáncer tumoral y el hemangioma.

Mejora en la técnica de la presente invención:

a) Avance de la invención

En el 2008, no existían medicamentos aprobados por la FDA para el tratamiento de los hemangiomas y otros tratamientos tenían una relación beneficio/riesgo, que era de pobre a aceptable (por ejemplo, altas dosis de corticosteroides, interferón).

El descubrimiento del posible tratamiento de IH mediante propranolol por los presentes inventores en 2008 (Véase documento anexo Léauté-Labrèze et al. 2008) ha sido reconocido internacionalmente por los expertos de los hemangiomas como un gran avance.

En esa ocasión, la señora Léauté-Labrèze recibió el premio ISSVA “Robert Schobinger Award” en reconocimiento al mejor trabajo clínico presentado durante el 17 Taller Internacional sobre anomalías vasculares.

En los tres años siguientes a su descubrimiento por parte de los inventores, más de 140 artículos han replicado el informe inicial usando propranolol u otros beta-bloqueadores en el tratamiento de IH, lo que confirma en particular, el dramático efecto de este tratamiento en situaciones graves y potencialmente letales (afectación visual, visceral y de las vías respiratorias superiores).

Este descubrimiento fue calificado como “casual” por muchos expertos (Véase documentos anexos Itinteang et al. 2011, Frieden et al., 2011, Greenberger y Bischoff, 2011).

Weibel (2009) clasificó los resultados del uso de propranolol en el tratamiento de IH como “resultados sensacionales, en comparación con otros tratamientos”, y que tienen “una gran acogida en todo el mundo”. Weibel señala que un gran número de centros internacionales especializados en el tratamiento de las anomalías vasculares en los niños, han adoptado exitosamente desde el 2008 el propranolol para el tratamiento de los hemangiomas problemáticos.

Storch (2010) calificó el uso de propranolol en el tratamiento de los hemangiomas como de “eficacia impresionante”.

El Dr. Frieden y el Dr. Bischoff, dos expertos de reconocido prestigio en el campo de los hemangiomas han calificado el uso del propranolol como una “terapia innovadora para los hemangiomas infantiles” y “como primera opción de tratamiento para los hemangiomas infantiles complicados desde su descubrimiento en el 2008”.

En un reciente artículo del 2011, Greenberger y Bischoff concluyen, sobre la base del descubrimiento por parte de los inventores del método de tratamiento

reivindicado: “La aparición de propranolol como un nuevo producto terapéutico que es a la vez seguro y eficaz proporciona una terapia alternativa crucial para atrofiar y, en ocasiones, acabar los hemangiomas”.

Antes de su descubrimiento por los inventores en el 2008, ninguna publicación había mencionado nunca la posibilidad de utilizar propranolol para el tratamiento de IH.

b) El uso de propranolol para el tratamiento de hemangiomas claramente proporciona una eficacia superior en muchos casos más que las terapias utilizadas anteriormente

El Propranolol proporciona una eficacia claramente superior en muchos casos, no sólo en detener el crecimiento del hemangioma, sino provocar una disminución del volumen con notable eficiencia más que los corticosteroides u otras terapias utilizadas antes del descubrimiento del tratamiento en el 2008.

Adicionalmente, el propranolol tiene un menor perfil de efectos secundarios que los corticosteroides o todas las otras terapias en el mercado.

Un primer estudio retrospectivo (Véase documento anexo Rössler et al., 2011) mostró que el propranolol es superior a la terapia con corticosteroides. En este estudio se revisaron las historias clínicas de 30 pacientes con 38 IH, tratados con prednisona, así como historias clínicas de 30 pacientes tratados con propranolol con 39 IH. La conclusión es que este “estudio proporciona un fundamento sólido para continuar utilizando propranolol como tratamiento de primera línea en la proliferación de IH”.

Un segundo estudio retrospectivo (Véase documento anexo Bertrand et al., 2011), comparó la eficacia clínica del propranolol oral con la de la prednisona oral en el tratamiento de IH. Este estudio concluye que “el propranolol parece superior a la prednisona oral al inducir mejoría clínica más rápida y en mayor medida” (véase resumen). Los autores señalan que esta “experiencia se correlaciona con los informes iniciales de los Léauté-Labrèze et al.” (Véase p. 652, col. 1, “Discussion”, 1º párrafo).

Un tercer estudio que compara la eficacia del propranolol y la de los corticoides es el de Price et al. (2011) (Véase documento anexo). Los autores concluyen que

“la terapia con propranolol fue clínicamente más eficaz y más rentable que los corticosteroides orales en el tratamiento de IH. También dio lugar a un menor número de intervenciones quirúrgicas y han demostrado una mejor tolerancia, con un mínimo de efectos adversos, en comparación con los corticosteroides orales. Por lo tanto, el propranolol debe ser considerado un agente de primera línea dada su seguridad y eficacia en el tratamiento de IH”.

c) Falta de terapias eficaces para el tratamiento de hemangiomas antes del descubrimiento de la utilización de propranolol

En el 2008, no existían medicamentos aprobados por la FDA para el tratamiento de los hemangiomas infantiles.

Es por eso que en el 2007, la Administración de Fármacos y Alimentos concedió el estado de designación para la vincristina de medicamento sin interés comercial (NCT00555464 - estudio sobre el uso de vincristina vs prednisolona para el tratamiento de los hemangiomas infantiles). El objetivo del programa de otorgamiento de designación de fármaco sin interés comercial de la FDA es apoyar el desarrollo clínico de productos para uso en enfermedades o afecciones raras donde no existe tratamiento actual o cuando el producto propuesto será superior a las terapias existentes. Por lo tanto, la designación de fármaco sin interés comercial para el estudio de vincristina era por sí mismo una buena evidencia de las limitaciones de las otras terapias existentes.

El grupo de expertos a cargo de este estudio fue la HIG (Grupo Investigador del Hemangioma), un grupo de dermatólogos pediatras dedicados a avanzar en el conocimiento con respecto a los hemangiomas infantiles y mejorar los resultados para niños afectados a través de investigación, colaboración, educación y promoción (<http://www.hemangiomaeducation.org/index.html>). Este consorcio agrupa a los principales centros de los Estados Unidos que estudian hemangiomas.

Sin embargo, un año después del momento de inicio de dicho estudio, se publicó el trabajo inicial de los inventores que describen el uso de propranolol en el tratamiento de los hemangiomas infantiles (2008). Teniendo en cuenta la eficacia y la seguridad relativa del propranolol descrito en esa publicación, así como los informes posteriores, pronto quedó claro a las autoridades estadounidenses de que el propranolol es una terapia superior a la prednisolona y a la vincristina, de

tal manera que la vinculación continua de sujetos al estudio con vincristina vs prednisolona ya no podría ser justificado y se suspendió la vinculación de de pacientes para el estudio.

En marcado contraste, la publicación de Jirousek en 1997 no solicita una investigación en el área del tratamiento de IH. Esto demuestra que la persona experta en la técnica no considera las enseñanzas de Jirousek, incluso combinadas con Sozzani y con Duzendorfer, como una publicación relevante en el campo del tratamiento de IH.

d) El uso de propranolol para tratar hemangiomas fue sorprendente e inesperado

A pesar de la ausencia de medicamentos aprobados por la FDA para el tratamiento de hemangiomas y la ausencia de terapias eficaces de buena relación beneficio/riesgo para el tratamiento de hemangiomas, ninguna publicación ha considerado, ni mucho menos sugiere, un posible uso de propranolol en el tratamiento de hemangiomas infantiles antes del descubrimiento de los presentes inventores en el 2008.

A este respecto, llamamos la atención del Examinador en cuanto a la declaración anexa a este memorial, en donde el Dr. Frieden, un reconocido experto en el campo de IH, ha declarado: “(...) en el momento de la publicación inicial de la eficacia del propranolol, no lo habría considerado para el tratamiento de los hemangiomas infantiles. En mi concepto (formado por años de estudio y trabajo en el campo), no había nada en la técnica médica que le hubiera sugerido esta utilidad del propranolol sobre la base de su uso para el tratamiento de hipertensión arterial, cardiopatía isquémica, arritmias, trastornos endocrinos y neurológicos, y trastornos oculares. Mi concepto al respecto no ha cambiado después de leer las referencias que se han aplicado en contra de la solicitud '266 en la Acción Oficial emitida el 15 de abril, 2011. “(Véase declaración adjunta).

Esto se debe a que los efectos del propranolol en IH fueron descubiertos por casualidad (véase Storch et al., 2010). Weibel (2009) califica el tratamiento de IH por los inventores como una “observación fortuita”.

En efecto, el propranolol como tratamiento farmacológico para IH fue reportado por primera vez en el 2008, en dos niños que mostraron regresión rápida de la

afección/enfermedad IH grave cuando fueron tratados con propranolol por síntomas de enfermedades cardiopulmonares (Leaute-Labreze et al. 2008).

Como lo recuerdan muchos expertos, entre ellos el Dr. Frieden y el Dr. Bischoff: (i) el mecanismo de acción del propranolol en el tratamiento de los hemangiomas infantiles es, incluso ahora, claro, y (ii) la patogenia de IH en sí, no se entiende completamente.

Dado que la patogenia del IH, en sí, no se entiende completamente, el experto en la técnica no tenía otra opción que probar cada molécula candidata de una en una para probar su eficacia.

Los inventores sorprendentemente han observado que la administración de propranolol, iniciada en el tratamiento de enfermedades del corazón, tenía un efecto dramático en el hemangioma infantil.

El descubrimiento de los efectos del propranolol en el IH no es resultado de deducciones lógicas basadas en referencias bibliográficas, sino a partir de observaciones clínicas fortuitas y calificadas.

4. Conclusiones

En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones realizadas en el Oficio objeto de este memorial, ya que la presente invención es patentable en vista de la legislación vigente en Colombia y además cumple con los requisitos de novedad y nivel inventivo in vista del estado del arte citado por el Examinador.

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el examinador considera que subsisten razones para objetar la presente invención rogamus expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *"habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión*

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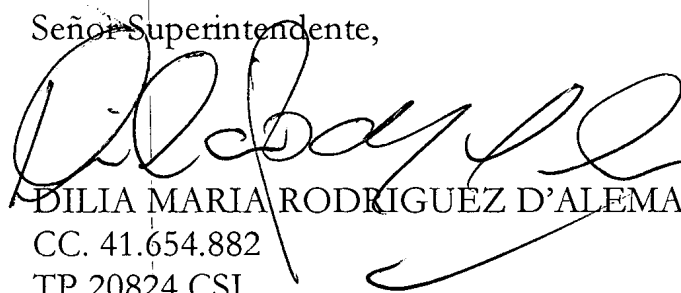
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que será motivada al menos en forma sumaria si afecta a particulares” y “(..) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso”, respectivamente (El subrayado es nuestro).

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

Señor Superintendente,



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REIVINDICACIONES

1. Un medicamento para el tratamiento de los hemangiomas, dicho medicamento capaz de administrar un beta bloqueante a un paciente en una concentración de entre 1 y 5 mg/día por kg del peso corporal de dicho paciente.

2. El medicamento de acuerdo con la reivindicación 1, en donde el beta bloqueante se selecciona del grupo que comprende un beta bloqueante no selectivo, un beta bloqueante selectivo beta 1, una mezcla de antagonistas adrenérgicos alfa-1/beta, un beta bloqueante selectivo beta 2 y una mezcla de dos o más beta bloqueantes.

3. El medicamento de acuerdo con la reivindicación 1, en donde el beta bloqueante es un beta bloqueante no selectivo.

4. El medicamento de acuerdo con la reivindicación 3, en donde el beta bloqueante no selectivo se selecciona del grupo que comprende alprenolol, bucindolol, carteolol, carvedilol, labetalol, levobunolol, medroxalol, mepindolol, metipranolol, nadolol, oxprenolol, penbutolol, pindolol, propafenone, propranolol, sotalol, timolol, sus sales aceptables para uso farmacéutico y sus mezclas.

5. El medicamento de acuerdo con la reivindicación 1 o 2, en donde el beta bloqueante es un beta bloqueante selectivo beta 1.

6. El medicamento de acuerdo con la reivindicación 5, en donde el beta bloqueante selectivo beta 1 se selecciona por ejemplo del grupo que comprende acebutolol, atenolol, betaxolol, bisoprolol, celiprolol, esmolol, metoprolol, nebivolol.

7. El medicamento de acuerdo con la reivindicación 1, en donde el beta bloqueante tiene una actividad simpaticomimética intrínseca.

8. El medicamento de acuerdo con la reivindicación 7, en donde el beta bloqueante se selecciona del grupo que comprende acebutolol, betaxolol, carteolol, carvedilol, labetalol, oxprenolol, penbutolol, pindolol, propranolol

9. El medicamento de acuerdo con la reivindicación 1, en donde dicho beta bloqueante es propranolol o una de sus sales farmacéuticas.

10. El medicamento de acuerdo con cualquiera de las reivindicaciones 1-9, en donde dichos hemangiomas son hemangiomas capilares y/o hemangiomas capilares infantiles.

11. El medicamento de cualquiera de las reivindicaciones 1-10, en forma de jarabe o una solución inyectable.

12. El medicamento de cualquiera de las reivindicaciones 1-10, para administración oral seleccionado del grupo que comprende una formulación líquida, una forma de

dosis oral efervescente, un polvo oral, un sistema de múltiples partículas, una forma de dosis bucodispersable

13. El medicamento de acuerdo con la reivindicación 12, en donde dicho medicamento en formulación líquida está seleccionado del grupo que comprende una solución, un jarabe, una suspensión, una emulsión y gotas orales.

14. El medicamento de cualquiera de las reivindicaciones 1-10, para las vías bucal y sublingual.

15. El medicamento de cualquiera de las reivindicaciones 1-10, para la administración tópica-transdérmica.

16. El medicamento de acuerdo con la reivindicación 15, en donde dicho medicamento para administración tópica se selecciona del grupo que comprende ungüentos, crema, gel, loción, parche, espuma.

17. El medicamento de cualquiera de las reivindicaciones 1-10, para administración nasal.

18. El medicamento de cualquiera de las reivindicaciones 1-10, para administración rectal.

19. El medicamento de cualquiera de las reivindicaciones 1-10, para la administración parenteral.

Hemangiomas: An Overview

Michael J. Sundine, MD, FACS, and Garrett A. Wirth, MD

Introduction

Understanding the behavior of hemangiomas and vascular malformations is of paramount importance to the plastic surgeon. The frequency of these lesions would almost dictate that every plastic surgeon would be involved in the treatment of a patient with some sort of vascular birthmark.

Hemangiomas are vascular lesions that demonstrate a characteristic pattern of rapid postnatal growth followed by slow involution. Vascular malformations are simply collections of excess vascular channels that grow proportionately with the child. Because of the predilection of these vascular birthmarks for the head and neck and the variable growth of these lesions, which may result in grotesque deformity, it is extremely important that plastic surgeons comprehend the biologic behavior of these lesions. It is also important to know when to intervene and treat the patients who have vascular birthmarks.

Vascular birthmarks have been described throughout the history of mankind and many famous figures through history have been known to have vascular birthmarks.¹ Mulliken¹ reviewed in detail the concept of maternal impression, where influences on a mother's emotional state may influence the phenotype of her unborn fetus. He further describes that these birthmarks were often thought related to maternal cravings for various fruits such as currents, strawberries, and raspberries. For the reader who is further interested in this topic, one should review Mulliken's excellent chapter.

Significance of the Problem

Hemangiomas are the most common tumors of infancy² and are also the most common tumors of the head and neck in infancy.³ The incidence of hemangiomas in the first 3 days of life is 1.1% to 2.6%^{4,5} and increases to 8.7% to 12.7% between the ages of 1 month and 1 year of life.⁶⁻⁸ Amir et al⁸ found that the incidence of hemangiomas increased with prematurity. In infants with birth weights of less than 1500 grams, the incidence of hemangiomas was 15.6%, and as the birth weight decreased below 1000 grams, the incidence of hemangiomas increased to 22.9%. Holmdahl⁹ did not observe any difference in the incidence of hemangiomas between premature and mature infants; however, a review of his study shows that the birth weights of the infants were much greater than those of Amir et al.⁸ A review of 830 patients examined by a single pediatric dermatologist during a 3-month period found vascular lesions to be the most common, with 44% of infants having these lesions.¹⁰

Hemangiomas are more frequent in girls than boys, ranging from a 3:1 to 5:1 ratio.¹¹⁻¹³ They are thought to be more common in white patients than in blacks; however, Pratt⁴ noted an 1% incidence of hemangiomas in black infants. Most series show an increased incidence of hemangiomas in the head and neck region, followed by the trunk and then the extremities.^{12,14} The incidence of hemangiomas in infants whose mothers underwent chorionic villus sampling is 10 times greater than those infants whose mothers did not undergo the procedure.¹⁵ Approximately 20% of infants will present with more than 1 lesion.¹⁶

Most hemangiomas occur sporadically; however, a family history of hemangioma has been noted to be present in up to 10% of patients with hemangiomas.¹² Recently, Blei et al¹⁷ were able to identify 6 families that demonstrated autosomal dominant transmission with moderate-to-high penetrance of hemangiomas. The female-male ratio was 2:1,

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rather than the usual 3:1 or 4:1 ratio. Cheung et al¹⁸ performed a study examining the incidence of hemangiomas in monozygotic and dizygotic twins. The concordances between the 2 twin pairs did not reach statistical significance, and they concluded that hereditary factors were not principal causes of hemangioma.

Etiology

At this point, the etiology of hemangiomas remains unknown. According to Cheung et al,¹⁸ there are basically 2 hypotheses to explain the development of hemangiomas. The first theory proposes that the hemangiomas "arise by recrudescence of dormant embryonic angioblasts."¹⁸⁻²⁰ The second theory proposes that hemangiomas are "tumors of neoangiogenesis."^{18,21,22} Folkman²³ believes that hemangiomas are a non-neoplastic disease of angiogenesis.²³ Vasculogenesis refers to the appearance of new blood vessels, whereas, angiogenesis refers to the sprouting of vessels from pre-existing vessels.²³ This angiogenesis is thus dependent on various growth factors that may stimulate or inhibit the angiogenesis process.²⁴ These tumors may then be susceptible to hormonal influences to cause further growth.^{25,26}

Classification

Much of the previous confusion surrounding vascular birthmarks was related to the classification systems used to categorize these lesions. The lesions were classified by the histologic appearance of the vascular or lymphatic channels, or by embryologic classification.²⁷ These classification systems did not, however, predict the biologic behavior of the various vascular birthmarks. In a landmark report, Mulliken and Glowacki²⁸ examined a series of patients with vascular birthmarks from a clinical, histologic, and autoradiographic point of view. They were able to divide vascular birthmarks into the 2 categories of hemangiomas and vascular malformations.

Hemangiomas could be characterized clinically as those lesions that exhibited a period of rapid post-natal growth, followed by a period of slow involution. In the proliferating phase, hemangiomas demonstrated increased endothelial cellularity, with the formation of syncytial masses with and without lumens. They also found alkaline phosphatase to be

localized to the areas of hypercellularity as well as in mature vessels, and factor VIII antigen was present in the endothelial cells of the hemangioma and also in normal adjacent vessels. Autoradiography showed uptake of [³H]thymidine in proliferating lesions. Electron microscopy demonstrated the presence of multilaminated basement membranes under the areas of proliferative endothelium. In the involuting phase, there was noted to be a diminished cellularity of the lesions with areas of fatty deposits, which were intermingled with fibrous tissue. The involuting phase lesions did not show uptake of [³H]thymidine.²⁸

Vascular malformations were characterized as lesions that were present at the birth of the child and grew commensurately with the child. Vascular malformations histologically showed "flat 'mature' endothelium" with alkaline phosphatase and factor VIII antigen localized to the endothelium of the vascular channels. The vascular channels were surrounded by normal reticulin networks, and autoradiography of the vascular malformations did not demonstrate endothelial proliferation.²⁸

From these observations, Mulliken and Glowacki²⁸ defined vascular birthmarks that demonstrated proliferative activity to be hemangiomas, and those vascular birthmarks that did not demonstrate proliferative activity and grew commensurately with the child were defined as vascular malformations. Subsequent application of this classification system has shown its validity.^{28,29}

In 1996, the International Society for the Study of Vascular Anomalies adopted a classification that accounts for the clinical presentation and behavior of these lesions as well as the histologic appearance. Vascular birthmarks are divided into 2 distinct groups: vascular tumors and vascular malformations^{16,30} (Table 1). The vascular malformations can be further classified by flow characteristics and vessel type within the malformation.³¹

Several changes are noted when the old terminology is applied to the current classification system. The strawberry hemangioma, juvenile hemangioma, and the capillary hemangiomas are simply just hemangiomas. The cavernous hemangioma is also just a hemangioma that is deeper in the dermis, fat, or muscle, and therefore has a deeper blue hue.³² The port-wine stain, which is present at birth and will grow commensurately with the child, is now a capillary malformation. The previous lymphangioma and cystic hygroma are now identified as lymphatic malformations.³²

Table 1. Classification of Vascular Anomalies

A. Vascular Tumors
Hemangiomas
Proliferating
Involuting
Kaposiform hemangioma
Tufted angioma (angioblastoma)
Angiosarcoma
Congenital hemangiopericytoma/infantile myofibromatosis
Eccrine angiomatous hamartoma
B. Vascular malformations
High flow
Arterial malformation-aneurysm, ectasia, stenosis, etc.
Arteriovenous fistula
Arteriovenous malformation
Low flow
Capillary malformation-Port-wine stain
Telangiectasias
Venous malformation
Lymphatic malformation
Macrocystic
Microcystic
Complex combined
Slow flow: Klippel-Trenaunay syndrome (capillary lymphatic venous malformation)
Fast flow: Parkes Weber syndrome (capillary arterial venous malformation)

Natural History

Hemangiomas may be present at birth, but most develop in the first few weeks after birth.¹² The hemangiomas may manifest as a pale patch, a telangiectasia surrounded by a pale halo, a bruise-like macule, or an area of simple erythema.^{33,34} Payne et al³³ noted the appearance of telangiectasias in the pale area followed by the development of the irregular pebbly characteristic hemangioma.

The hemangioma grows rapidly in the first 3 to 6 months of life, and then the tumor becomes quiescent and grows at approximately the same rate as the child for the next 6 to 12 months. The maximum size of the hemangioma is usually reached at age 6 to 12 months. Involution generally begins at approximately 18 months of age.¹² Pasyk et al³⁵ have divided the life cycle of hemangiomas into 7 clinical stages, of which the first 4 deal with the growth phase (from birth to approximately 20 months of age), and the next 3 stages are related to phases of involution.

The earliest sign of regression of a hemangioma is a fading of the color of the lesion from a bright red to a dull red or pink color. Next, a gray-white hue develops at the center of the lesion and this spreads



Figure 1. Involuting phase hemangioma showing gray-white discoloration over surface of hemangioma.

to the periphery. The tenseness of the lesion is then reduced, followed by a reduction in the volume of the lesion¹² (Figure 1).

Multiple authors reporting on the natural history of hemangiomas indicate that the rate of involution is relatively consistent. Approximately 50% of hemangiomas will have completely resolved at age 5, with about 70% being completely resolved at age 7. Subsequent improvement may occur in the remaining lesions from age 10 to 12.^{7,11,12,36,37}

In addition to examining the rate of involution of hemangiomas, Bowers et al¹¹ also examined the influence of multiple other factors on the involution of hemangiomas. They found that the size of the lesion did not affect the rate or the completeness of involution. The sex of the infant had no effect on the resolution of the hemangioma, nor did the site of the lesion. They did note, however, that lesions of the lip did tend to resolve more slowly than the others. The presence of multiple lesions was also of no prognostic significance. Interestingly, Bowers et al¹¹ and Lister³⁶ noted that those lesions that did not have a period of rapid growth also did not resolve, thus noting the difference between a vascular malformation and a hemangioma before it was clarified by Mulliken.²⁷

Although almost all hemangiomas develop postnatally, some infants are born with fully developed hemangiomas. Boon et al³⁸ presented a series of 31 infants with these so-called congenital hemangiomas. The infants had a 1:1 female-male ratio, rather than the 3:1 ratio that is usually seen in

hemangiomas that develop postnatally. Most of the lesions were in the craniofacial area or the lower extremities. The hemangiomas presented in 3 different configurations: (1) a raised violaceous tumor with large radial veins, (2) a hemispheric shaped tumor covered with telangiectasias and surrounded by a pale halo, and (3) a pink-to-violaceous tumor, firm to palpation, that was usually located in the lower extremity.

Routine antenatal ultrasound was performed in 23 of the 31 infants; however, the antenatal ultrasound demonstrated the hemangioma in only in 3 infants. Of interest was that the infants with these congenital hemangiomas demonstrated an accelerated rate of involution. Twelve of 24 infants demonstrated involution before 7 months of age, and spontaneous involution occurred by 14 months of age in 24 of the 31 infants who did not require some sort of intervention (corticosteroids or surgery).

Histology

The histologic appearance of hemangiomas fluctuates with the stage of the life cycle of the tumor and essentially can be separated into the proliferative phase or the involuting phase.

The light microscopic appearance of proliferative phase hemangiomas shows a proliferation of endothelial cells. These cells form syncytial masses and may or may not form lumens. A limiting reticulin membrane surrounds these syncytial masses, and periodic acid-Schiff staining demonstrates a thickened basal lamina underneath the endothelial cells. The nuclei show occasional mitotic figures but no pleomorphism. Later in the proliferative phase, the vascular channels become more obvious. Plump endothelial cells line these capillary channels. As the hemangioma matures, the tumor is organized into lobular compartments that are separated by fibrous septa with large feeding and draining vessels.^{27,39}

Proliferative hemangiomas demonstrate uptake of tritiated thymidine into the DNA of replicating endothelial cells. Alkaline phosphatase is found within cytoplasmic granules in the endothelial cells, and the endothelium produces factor VIII, demonstrated by fluorescent antibody and peroxidase techniques. The number of mast cells is increased 30-fold to 40-fold compared with normal skin, vascular malformations, or involuted hemangiomas.^{39,40}

Electron micrographic findings demonstrate a plump endothelium with characteristics of intracellular

activity. These findings include swollen mitochondria, convoluted nuclear membrane, membranes of rough endoplasmic reticulum, and clusters of free ribosomes. The electron microscopic hallmark of the proliferative phase hemangioma is multilamination of the basement membrane.^{27,39}

The involuting phase hemangioma demonstrates diminished cellularity with flattening of the lining endothelial cells. As the endothelium flattens, there is a relative dilation of the vessels supplying the tumor and also progressive deposition of perivascular, intralobular, and interlobular fibrous tissue. The number of vascular channels diminishes as the fibrosis proceeds.^{27,39}

The electron microscopic findings of the involuting hemangioma show endothelial cell discontinuity along with vessel degradation. The lumens of the vessels reveal endothelial cell debris. The basement cell membrane is still multilaminated. Few mast cells are present, and they do not show the cell-to-cell interactions seen in proliferative phase hemangiomas.^{27,39}

Molecular Biology

Much progress has recently taken place in discovering the molecular biology of these lesions.^{16,24,41-44} In addition to the life cycle that is seen clinically and histologically, there is a profile of different immunohistochemical cellular markers that correspond to the life cycle of the hemangioma. The angiogenesis process is the result of a balance between factors that are responsible for endothelial cell proliferation and migration and those that inhibit of blood vessel formation.⁴¹ Growth factors that stimulate angiogenesis include basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), tumor necrosis factor- α (TNF- α), and interleukin-8 (IL-8). Antiangiogenic growth factors include tissue inhibitor metalloproteinase (TIMP-1), transforming growth factor- β (TGF- β), interferon- α , and platelet factor-4.⁴¹

Takahashi et al⁴⁵ examined tissue samples of hemangiomas that were removed at 3 different stages of development: proliferating lesions (defined as those obtained from children 0-12 months old), involuting lesions (12-60 months), and involuted lesions (>61 months). The hemangioma specimens were then tested for the presence of 9 markers that have been implicated in the control of angiogenesis. Proliferating cell nuclear antigen (PCNA), VEGF, and type IV collagenase all preferentially stained those sections from proliferating phase hemangiomas.

Table 2. Gene Marker Profile for Hemangiomas

	Proliferating Phase	Involuting Phase	Involuted Phase
PCNA	Elevated	Decreasing	None
VEGF	Elevated	Decreasing	None
Type IV collagenase	Elevated	Decreasing	Low
bFGF	Elevated	Elevated→decreasing	None
Urokinase	Elevated	Elevated→decreasing	None
TIMP	Elevated	Elevated	Low
CD31	Elevated	Elevated	Decreasing
vWF	Elevated	Elevated	Decreasing
SMC-actin	Elevated	Elevated	Decreasing

Note: PCNA = proliferating cell nuclear antigen; VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor; TIMP = tissue inhibitor metalloproteinase; vWF = von Willebrand factor; SMC = smooth muscle actin.
Source: Adapted from Takahashi et al. Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest.* 1994;93:2357-2364.⁴⁴

Sections from proliferating and involuting phase hemangiomas had higher concentrations of bFGF, urokinase, CD31, and von Willebrand factor (vWF). In the involuting phase, TIMP-1, α -smooth muscle cell actin (SMC-actin), and mast cells were all increased compared with the proliferating and involuted phase. The vessels from various types of malformations showed little to no immunoreactivity to the same set of markers. The authors were able to show that immunohistochemical markers can also be used to separate the 3 clinical stages in the life cycle of hemangiomas (Table 2).

The data of Takahashi et al⁴⁵ are consistent with those of Folkman,²³ who found high levels of bFGF in proliferative phase lesions. This urinary bFGF can be useful in distinguishing vascular malformations from hemangiomas and in monitoring the response of a hemangioma to pharmacologic therapy. Similarly, the Takahashi et al⁴⁵ data are also consistent with those of Chang et al,⁴⁶ who found that cells from proliferative hemangiomas had increased messenger RNA for bFGF and VEGF compared with involuted hemangiomas.

The glycoprotein E-selectin, an endothelial cell adhesion molecule produced by endothelial cells after stimulation with TNF- α and IL-1, is also upregulated in the proliferative phase, as is monocyte chemoattractant protein.^{47,48} The extracellular matrix surrounding hemangiomas also changes.⁴⁹ Jang et al⁴⁹ found extensive deposition of vitronectin in the subendothelial space in proliferating hemangiomas. No vitronectin deposition was found in regressing hemangiomas or venous malformations.

Recent evidence seems to indicate that apoptosis (programmed cell death) is responsible for the

involution that is observed in hemangiomas.^{50,51} Razon et al⁵⁰ found that apoptosis was low in proliferative phase hemangiomas but increased 5-fold in involuting phase hemangiomas. Normal skin specimens demonstrated consistently low apoptosis. Mancini and Smoller⁵¹ found that the *bcl-2* proto-oncogene, which inhibits programmed cell death, progressively decreased with increasing age of the hemangioma. Their findings appear to indicate that the growth of hemangiomas is related to the degree of inhibition of apoptosis as well as to the proliferative activity of the cells.

Diagnosis

The diagnosis of hemangioma is normally made on clinical findings. Foremost in characterizing these lesions is the history. The birthmark generally is not present at birth and grows rapidly in the first few weeks after birth. If the hemangioma is in the upper dermis, it may have a scarlet color that deepens with time. Hemangiomas that are deeper may have a darker hue. Vascular malformations tend to have a persistent vascular hue depending on the components of the malformation. Palpation of a hemangioma will reveal a fleshy tumor that does not empty completely when compressed. Venous malformations are easily compressible and deflate with elevation. Hemangiomas are rarely associated with skeletal deformities.²⁷

Several variations have been noted in the presentation of hemangiomas in addition to the classic cutaneous hemangioma (strawberry hemangioma).^{16,52} Martinez-Perez et al⁵² described 4 unusual presentations for hemangioma: (1) deep hemangioma with

normal overlying skin, previously referred to as cavernous hemangioma, (2) macular hemangioma with an appearance similar to a port-wine stain, (3) bossed hemangioma with telangiectasia, and (4) hemangioma with persistent fast flow. There are also hemangiomas, which are present at birth, so-called congenital hemangiomas that were reviewed previously.

As noted previously, most hemangiomas can be diagnosed on clinical grounds. Occasionally, an imaging study, usually an ultrasound or magnetic resonance imaging (MRI), or rarely, a biopsy specimen may be necessary to rule out other tumors. The differential diagnosis includes tufted angioma (congenital form), neuroblastoma, embryonic rhabdomyosarcoma, fibrosarcoma, hemangiopericytoma, and infantile myofibrosis.⁴¹

Imaging

Imaging studies may be very useful in the assessment of vascular birthmarks. The study may be used to confirm or establish the diagnosis, determine the extent of the lesion, assess other possible associated anomalies, and may also be used therapeutically. Many different modalities are available to evaluate these lesions, with MRI as probably the single best modality to characterize vascular birthmarks. Plain film radiographs of a hemangioma will typically reveal a soft tissue mass but usually does not yield much more information.⁵³

Doppler ultrasonography is considered to be the most cost-effective imaging technique for the evaluation of hemangiomas.⁵⁴ Its added advantage is that it is a noninvasive technique. As with other radiographic techniques, the appearance of hemangioma will differ, depending upon whether it is in the proliferative or involuting phase. A proliferating phase Doppler ultrasound scan will demonstrate nonspecific echogenicity with increased color flow and a high-flow pattern with an arterial and venous component. Numerous vessels are visualized ($>5/\text{cm}^2$) with a high Doppler shift (>2 kHz) and low resistance.^{54,55} Using the criteria of high vessel density ($>5/\text{cm}$) and a high Doppler shift (>2 kHz), Dubois et al⁵⁶ were able to achieve a sensitivity of 84% and a specificity of 98% in the diagnosis of hemangiomas.

The computed tomography (CT) appearance of hemangiomas also fluctuates with the life cycle of the tumor. Proliferating hemangiomas have a homogeneous mass that enhances uniformly after the administration of intravenous contrast material.^{31,55}

Prominent enhancing vessels may be seen on CT with the use of CT angiography. The appearance of phleboliths and calcifications are not features of hemangioma, and a different diagnosis should be considered if they are seen.⁵⁷ Deep seated hemangiomas may cause erosion of adjacent bone, but hemangiomas rarely invade bone or arise in bone.⁵⁷ In the involuting phase, the hemangiomas become heterogeneous masses with fatty infiltration and less intense staining on the administration of intravenous contrast.⁵⁵

Proliferating phase MRIs demonstrate well-defined, lobulated soft-tissue masses. They are isotense-to-hypotense relative to muscle on T1 weighted images, and hypertense relative to muscle on T2 weighted images. Flow voids are seen within and around the lesions on spin echo images. Magnetic resonance angiograms will show high-flow vessels at the center or around the periphery of the hemangioma with an intense and uniform enhancing parenchymal mass on the introduction of intravenous contrast. With involution, there is decreased size of the tumor, diminished vascularity, decreased enhancement, and progressive fibrofatty infiltration of the tumor. This fibrofatty infiltration is demonstrated by high-intensity foci within the tumor on T1 weighted imaging.^{31,55,57,58}

Angiography will demonstrate a well-circumscribed mass with intense straining and lobular architecture.⁵³ Dilated feeding and draining vessels may surround the mass.³¹ Arteriography of hemangiomas should be considered only if endovascular embolization is contemplated.

Complications of Hemangiomas

Although most hemangiomas resolve completely without any sequelae, several potential complications are associated with hemangiomas, most commonly in the proliferative phase of growth of the hemangioma. An estimated 10% to 20% of hemangiomas may be so-called alarming hemangiomas that may threaten life or an important visceral function.⁵⁹ Important complications of hemangiomas include ulceration, bleeding, infection, obstruction (visual axis, auditory canal, airway), congestive heart failure, skeletal distortion, and aesthetic deformity.

Ulceration, a common complication of hemangiomas, occurs in approximately 5%.¹² This is thought to be due to a proliferation of the hemangioma through the epidermal basement membrane and most commonly occurs in the proliferative phase of the hemangioma.²⁷ The most commonly

involved areas are the anogenital area and the lips. The anogenital area is especially problematic owing to the frequent friction associated with diaper changes and also the associated bacterial contamination. These ulcerated areas may then bleed and also may become secondarily infected. The bleeding resulting from these ulcerations is usually minimal and easily controlled with pressure. Deep ulcerations may result in scarring. Moist antimicrobial ointments are used to treat these ulcerated areas. Treatment with the flash-lamp pulsed dye laser has been recommended for an ulcerated hemangioma, especially in the perineal region.^{60,61}

Bleeding from most hemangiomas is inconsequential. It is usually a result of ulceration and is easily controlled with pressure. Kasabach-Merritt syndrome, or bleeding associated with thrombocytopenia and hemangioma, has now been found to be associated with several other vascular tumors but is not associated with hemangioma.

Infection is a less common complication of hemangiomas and is usually associated with ulceration of the hemangioma. Systemic antibiotics are indicated when the area of ulceration is associated with cellulitis.³²

Proliferation of a hemangioma in the periorbital area can be especially problematic. Uncontrolled growth of a hemangioma, especially of the upper eyelid that obstructs the visual axis, can lead to deprivation amblyopia and the lack of development of binocular vision. A review by Hiak et al⁶² found that 80% of patients with eyelid or orbital hemangiomas had complications and 60% of patients had amblyopia. Another series found a 54% complication rate with 41% of patients having amblyopia and 36% having strabismus.⁶³ Refractive errors, both astigmatic and myopic, can be produced by pressure effects from an expanding mass on the cornea.²⁷ Other late effects of periorbital and adnexal hemangiomas include globe proptosis, asymmetric refractive error, blepharoptosis, and optic atrophy.^{27,64}

Hemangiomatous proliferation may also affect any area along the respiratory tract, including the nares, larynx, and subglottic area. Infants are obligate nose breathers for at least the first 3 months of life, and any proliferation that may obstruct the nares may result in significant respiratory compromise.

Subglottic hemangiomas are insidious and can be life threatening. They usually appear in infants between 6 and 12 months of age as biphasic wheezing (inspiratory and expiratory phase) in an afebrile child. The child may have a cough that resembles croup. The respiratory stridor is progressive. Endoscopic findings

reveal a characteristic red or blue lesion in the subglottic area.⁶⁵ Approximately 50% of these subglottic hemangiomas will have associated cutaneous hemangiomas in the "beard distribution."⁶⁶ Many different modalities have been used to treat subglottic hemangiomas, including corticosteroids, interferon, carbon dioxide laser, radiation therapy, cryotherapy, electrocautery, and surgery.⁶⁷ Tracheostomy may be necessary to preserve the child's airway.

Auditory canal obstruction may result from proliferation of a hemangioma in the parotid region and may result in a conductive hearing loss. If the lesion is bilateral and persists beyond a year, when intact hearing is required for speech development, treatment should be considered.³²

Skeletal distortion resulting from hemangiomas is rare. More commonly, skeletal distortion is associated with vascular malformations. There may be erosion or scalloping of the bone adjacent to the hemangioma, which is thought to be due to a pressure effect.

Hemangiomas have been associated with congestive heart failure. This is usually seen in cases of large hepatic hemangiomas or bulky cutaneous hemangiomas without hepatic involvement. The blood flow through the hemangioma creates a hyperdynamic state leading to increased cardiac output, ventricular output, and increased pulmonary vascularization.⁵⁹

Associations With Hemangiomas

A number of associated anomalies or syndromes include hemangiomas. Among the more commonly cited associations is Kasabach-Merritt syndrome. In 1940, Kasabach and Merritt reported a case of thrombocytopenic purpura associated with a giant capillary hemangioma. Kasabach-Merritt syndrome has a reported mortality of 20% to 30%.⁶⁸ Recent evidence has demonstrated that Kasabach-Merritt syndrome is associated with kaposiform hemangioendothelioma and not with the more common hemangioma of infancy.⁶⁸⁻⁷⁰ A number of features clearly distinguish between hemangiomas and Kaposiform hemangioendotheliomas (Table 3).

The association of hemangiomas with posterior fossa brain malformations and other anomalies has been recently described.^{71,72} The acronym PHACE syndrome has been coined to characterize the major features of this association, including posterior fossa malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, and eye abnormalities.⁷²

Table 3. Characteristics of Hemangioma of Infancy Versus Kaposiform Hemangioendothelioma

	Hemangioma	Kaposiform Hemangioendothelioma
Sex ratio (F:M)	3:1	~1:1
Location	Cervicofacial (2/3 of cases)	Predilection for trunk, extremities, and retroperitoneum
Multifocal	~20%	None
Present at birth	Rare	~50%
MRI findings	Well defined, homogenous, enhancing, soft-tissue mass with fast-flow vessels within and around tumor	Poorly defined margin, involving multiple contiguous tissue layers, small feeding/draining vessels relative to tumor size, presence of signal voids without flow-related enhancement

Note: MRI = magnetic resonance imaging.

Source: Adapted from Sarkar et al. Thrombocytopenic coagulopathy (Kasabach-Merritt phenomenon) is associated with Kaposiform hemangioendothelioma and not with common infantile hemangioma. *Plast Reconstr Surg.* 1997;100:1377-1386.⁷⁰

Table 4. Factors Affecting Decision to Treat Hemangiomas

Location of hemangioma
Depth of lesion within the skin
Age of patient
Presence or likelihood of complications
Availability of treatment modality
Expertise of treating physician
Parental preference

Source: From Drolet et al. Hemangiomas in children. *N Engl J Med.* 1999;341:173-181.²

The most common central nervous system anomalies are Dandy-Walker malformation, but cerebellar atrophy and arachnoid cyst with cerebellar hypoplasia have been reported. These patients typically have large facial hemangiomas that may be unilateral or bilateral and appear to be at high risk for developing airway hemangiomas.

Reported vascular anomalies include coarctation of the aorta, persistent trigeminal artery, absence or hypoplasia of the carotid or vertebral arteries, aneurysmal dilatation of the carotid artery, dilated cerebrovascular vessels, and aberrant left subclavian artery. The reported cardiac anomalies have included cor triatriatum with partial anomalous pulmonary venous return, tricuspid and aortic atresia, patent ductus arteriosus, and ventricular septal defect. The ocular findings have included microphthalmia, optic nerve hypoplasia, cataracts, and increased retinal vascularity.⁷²

Hemangiomas of the lumbosacral area have been associated with spine, urogenital, and anorectal anomalies. Albright et al⁷³ reported a series of 7 children with lumbar cutaneous hemangiomas. All had tethered spinal cords, 4 showed intraspinal lipomas, and 2 had tight fila terminalia. Goldberg et al⁷⁴ reported

a series of 5 infants with sacral hemangiomas who also had associated anomalies. Three each had an imperforate anus, an abnormal sacrum, renal anomalies, and lipomeningoceles; 4 had skin tags, and 4 had fistulae. The hemangiomas in these cases are described as superficial, and they cross the midline, often with a central membranous defect. Imaging of the spine is indicated in these cases.²

Orlow et al⁶⁵ have described the association between hemangiomas that occur in a beard distribution (right or left preauricular region, chin, anterior neck, and lower lip) and symptomatic hemangiomas of the upper airway or subglottic region. In a series of 16 patients with cutaneous hemangiomas in a beard distribution, 63% had symptomatic airway involvement, and 40% required a tracheotomy for airway protection. Thus, cutaneous hemangiomas in this distribution should call attention to the possibility of airway compromise.⁷⁵

Management

The management of hemangiomas remains a subject of considerable controversy.^{6,76-82} There are certain clear indications for operative therapy, but these are limited to a small population of lesions. On the other hand, the natural history of hemangiomas is involution after a period of rapid growth, and many physicians emphasize an approach of careful observation with limited intervention.¹² Studies from the 1940s and 1950s demonstrated worse results from excisional treatment than from observation alone, leading many experts to advocate leaving the tumors untreated.² Many factors should be taken into consideration in the decision to treat a hemangioma (Table 4).

Table 5. Goals of Management of Hemangiomas

1. To prevent or reverse any life-threatening complications of hemangiomas
2. To prevent permanent disfigurement left by residual skin changes following involution
3. To minimize the psychosocial distress from the presence of hemangiomas for both patient and family
4. To avoid overly aggressive, potentially scarring procedures or toxic therapies for the treatment of those hemangiomas that are likely to have an excellent prognosis without therapy
5. To prevent or adequately treat ulcerated hemangiomas so that scarring, infection, and pain are minimized

Source: From Frieden IJ. Which hemangiomas to treat and how? *Arch Dermatol.* 1997;133:1593-1595.⁷⁸

Table 6. Tenets of Conservative Management of Hemangiomas

1. Avoidance of overzealous active treatment
2. Careful observation and measurements at frequent intervals to assess rate of growth
3. Accurate description of the lesion: size in 3 dimensions, degree of compressibility, and whether or not blanching can be effected. Photographs should be made of lesions on cosmetically important areas
4. Determination of parents' reaction to the birthmark and use of "before and after" photographs to assure them of the probability of spontaneous involution
5. Observation of the patient for several years to continue counseling

Source: From Margileth AM, Museles M. Cutaneous hemangiomas in children. *JAMA.* 1965;194:523-526.¹²

The tremendous psychosocial consequences of an untreated hemangioma on the affected child and the family should not be underestimated, however. This is especially true with facial hemangiomas that cannot be hidden from view by clothing. Tanner et al⁸³ have reported that reactions of fear, disbelief, and mourning, were common in parents of children with facial hemangiomas greater than 1 cm in diameter, and these reactions were similar to those parents whose children had permanent deformities.² Thus, the physician who recommends a course of nonintervention in a patient with a hemangioma should be prepared to provide active support for the parents and the child. Photographs should be taken at periodic intervals to help the parents see the regression of the lesion over time.⁶⁰

The overall goals in the management of hemangiomas have been outlined by Frieden⁷⁸ (Table 5). Central to these goals are the principles of minimizing the psychosocial distress caused by the lesions while also minimizing any morbidity related to the treatment of the lesions. We would add that an attempt should be made to normalize the child's appearance before entering school.

The principles of "conservative" management of hemangiomas have not changed substantially since they were proposed more than 40 years ago¹² (Table 6). In an update, Margileth⁶⁰ states that in his 37-year experience, only 2% of hemangiomas ever require operative therapy.

A contrary view has been espoused by Waner and Suen.^{60,76} They cite their own experience, along with that of Finn et al,¹⁴ indicating that approximately 50% of all children with hemangiomas will require operative therapy. They also believe that the tremendous psychosocial impact of these disfiguring lesions has been underestimated in the literature. Indeed, Frieden has stated "results considered cosmetically acceptable in 1955 are not necessarily acceptable in 1995."⁶⁰ They are thus prepared to actively treat any "cosmetically significant" lesion. The ultimate goal of this treatment is the normalization of appearance before the child enters school. This approach is similar to that taken in children with cleft and craniofacial anomalies for the similar reason of normalization of appearance before the child begins school.

The treatment course that has been advocated by Waner and Suen⁶⁰ includes a period of early aggressive intervention, followed by a period of benign neglect, and then a period of aggressive intervention after approximately 3½ years of age.

In the early proliferative period when the lesion is flat, Waner and Suen⁶⁰ recommend flashlamp pulsed dye laser treatment at 4- to 6-week intervals until the red (ie, superficial) tissue is removed. For darker blue color underneath the lesion, indicating a deeper subcutaneous component, they begin oral corticosteroid treatment for at least 4 weeks, followed by a tapering over 2 weeks. For this treatment

to be effective, it must be initiated very early before any substantial bulk of the hemangioma occurs.

In the late proliferative period, the lesion has usually acquired enough thickness that the pulsed dye laser will not penetrate deep enough to treat the full thickness of the lesion. Thus, in this period they advocate oral corticosteroids for most lesions, reserving interferon- α -2a for complicated or life-threatening lesions. Hemangiomas in the period of early involution are managed expectantly. Finally, they again advocate active treatment in the phase of late involution. Treatment alternatives include the use of the flashlamp pulsed dye laser for the treatment of telangiectasia, use of carbon dioxide laser resurfacing for epidermal atrophy, and surgical resection for residual skin and fibrofatty tissue.⁶⁰

There are several clear indications for surgical therapy of hemangiomas. Early surgical therapy may be necessary for eyelid or periorbital hemangiomas that may compromise the visual axis, leading to amblyopia, and for large lesions obstructing the larynx, nasal airway, or the ear canal. Surgical therapy may also be considered for gastrointestinal lesions associated with bleeding or in those cases where there is congestive heart failure secondary to large hepatic hemangiomas. Surgery may also be considered early for large deforming lesions of the nose or lips. Late surgical therapy is generally reserved for removal of atrophic skin or fibrofatty tissue that remains after involution of the hemangioma.

In 1967, Zarem and Edgerton⁸⁴ reported their observations on the use of corticosteroids to treat hemangiomas of infancy. This followed the serendipitous observation of accelerated improvement of a large hemangioma in an infant with thrombocytopenia who was given steroids.⁸⁴ There have been numerous other reports on the use of corticosteroids in children with hemangiomas.^{60,76-82,85-92} To date, the exact mechanism of action of corticosteroids causing regression of hemangiomas is unknown. Corticosteroids are not uniformly successful in resolving hemangiomas, however. Enjolras et al⁵⁹ found that there was not a uniform response to corticosteroids in their series of patients, in which approximately 30% of patients were clear responders, approximately 40% of patients were doubtful or equivocal responders, and 30% of patients were clear nonresponders.

Corticosteroids may be administered locally or systemically. Locally administered corticosteroids have typically been used for periorbital and localized

facial lesions. Local injections have normally used a mixture of 40 mg of triamcinolone acetonide (1 mL) and 6 mg of betamethasone acetate (1 mL) that are injected directly into the tumor⁸⁷⁻⁸⁹ with a small diameter needle. Mulliken⁶⁰ advocates compression around the lesion with a finger or the finger ring of a surgical instrument while injecting. No more than 3 to 5 mg/kg of triamcinolone should be injected at a single session. The injections are repeated at intervals of 6 to 8 weeks for a total of 3 to 5 injections. Serious complications have been associated with periorbital steroid injections, including skin atrophy, reversible linear atrophy of the subcutaneous fat, transient eyelid depigmentation, hematomas, temporary cutaneous discoloration caused by deposits of injected material, eyelid necrosis, and occlusion of the central retinal artery with resulting blindness.^{2,67} However, Kushner⁹² believes that this treatment is very safe and effective for the treatment of periorbital hemangiomas.

Most authors use systemic oral corticosteroids rather than local administration for large and potentially disfiguring hemangiomas. Treating hemangiomas with corticosteroids should begin in the proliferative phase of growth. The prescribed daily dosage of prednisone or prednisolone is 2 to 5 mg/kg. However, most authors use daily dosages of 2 to 3 mg/kg, with increased complications noted in those children who received 5 mg/kg per day.⁶⁰ The corticosteroids are typically given as a single morning dose for 4 to 6 weeks. They are then gradually tapered over 2 to 3 months. Many authors recommend a shorter duration of treatment with the corticosteroids; however, rebound growth of the hemangioma is possible in these cases.

Corticosteroids are generally well tolerated for the treatment of hemangiomas. Boon et al⁹¹ performed a comprehensive review of complications due to corticosteroids in a series of 62 children who received a full course of corticosteroids for the treatment of hemangiomas. They found no long-term side effects and only transient side effects in those children that received less than 3 mg/kg per day of corticosteroids for 1 month that was then tapered over 2 to 3 months.

A transient decrease in gain in height was observed in one quarter to one third of patients. This was 4 to 5 times more likely in children in whom corticosteroids were started before 3 months of age. They also found that these children exhibited "catch-up" growth after the steroids were stopped, and the

growth curves returned to normal by 16 months after stopping steroid therapy in all patients treated with 3 mg/kg daily or less of corticosteroids.

Personality changes (depressed mood, euphoria, insomnia, restlessness, irritability) occurred in 29% of their patients. These personality changes occurred within 2 weeks of initiating treatment and then disappeared with stopping the drug. There were gastrointestinal complaints in 21% of their patients. These resolved with antacid therapy in all patients, and no problems with gastrointestinal bleeding were noted. The rate of bacterial infections did not increase in their patients secondary to the steroids, but they did report 4 cases of oral and/or perineal yeast infections and 4 children with viral otitis media. Cushingoid facies were seen in 71% of patients. These facies appeared within 1 to 2 months after starting therapy and resolved spontaneously as the corticosteroids were tapered and stopped.

Interferon- α -2a and interferon- α -2b have gained recent attention for the treatment of hemangiomas.⁹³⁻¹⁰⁵ Interferon- α -2a was initially conceived as an antiviral agent. During the course of clinical trials in patients with AIDS, regression of Kaposi sarcoma lesions was observed in treated patients.⁹³ The use of interferon- α -2a was then extended to patients with pulmonary hemangiomatosis⁹⁴ and then to patients with life-threatening hemangiomas.⁹³ The use of either interferon- α -2a or α -2b appear to be equally effective.¹⁰⁵ The exact mechanism of action of interferon- α -2a on hemangiomas is unclear. Reported potential mechanisms include inhibition of endothelial cell motion and proliferation, inhibition of collagen synthesis, fibroblast proliferation, growth factor release, and smooth muscle proliferation.⁹⁵

Treatment of hemangiomas with interferon- α -2a or α -2b has generally been reserved for those lesions that are believed to be life-threatening or possibly causing function-impairing sequelae or severe cervicofacial disfigurement.⁹³⁻¹⁰⁵ It has been particularly used for those lesions that appear to be resistant to corticosteroids. The usual starting dosage is 1 million units given subcutaneously nightly. This dosage is increased weekly until a target dosage of 3 million units per day. It has been reported that treatment at an early age is an important factor to improve the efficacy of interferon treatment of hemangiomas.¹⁰⁶ Treatment for 9 to 14 months appears to be necessary for optimal results. Rebound growth was noted in lesions where there was earlier withdrawal of the

drug.⁹³ Common side effects of treatment with interferons include flu-like symptoms, fatigue, lethargy, anorexia, and weight loss. Proteinuria has been reported in up to one fifth of patients receiving interferon therapy. Other laboratory abnormalities described in patients receiving interferon include neutropenia and elevation of liver transaminases, which usually disappears after the interferon has been discontinued.⁹⁹ A particularly troublesome complication of interferon- α treatment of hemangiomas has been the reported development of spastic diplegia.^{97,100,102-104} It has been recommended that pretreatment and continual neurologic examinations be obtained in patients who are treated before 1 year of age.¹⁰⁴

There is probably no more controversial area in the treatment of hemangiomas than the role of lasers for the treatment of these lesions. The initial laser used for treatment of hemangiomas was the argon laser.¹⁰⁷⁻¹⁰⁹ This laser had a peak absorption in the blue-green spectrum at 480 to 521 nm.¹⁰⁷ It was not very selective for vascular tissue and penetrates approximately 2 mm into the tissue.¹⁰⁹ Another laser that has been used to treat hemangiomas is the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser.^{106,109-111} This laser has a peak absorption at 1064 nm. Because of the longer wavelength, the Nd:YAG laser is capable of penetrating deeper into the tissue, with a depth of approximately 8 mm.¹⁰⁹ The nonselective laser-tissue interaction of both of these lasers has caused problems with changes in skin texture, pigmentation changes, and scarring.

The flashlamp-pumped pulsed dye laser has a wavelength of 585 nm. This laser is able to take advantage of the absorption peak of oxyhemoglobin at 577 nm and thus selectively target the vascular tissue. These principles of "selective photothermolysis" have been outlined by Anderson and Parrish.¹¹² The pulsed dye laser has been widely reported for the treatment of hemangiomas.^{60,113-117} Because of the limited depth of penetration of the pulsed dye laser (1 mm), there would appear to be a limited role for the use of this laser in hemangiomas. Potential indications for the use of the pulsed dye laser in hemangiomas have been offered by Garden and Bakus¹¹³ (Table 7). Scheepers and Quaba¹¹⁷ report that the pulsed dye laser is effective in treating red flat lesions, and it also seemed to speed up the healing of ulcerated lesions. They did not find any effect of the laser on the deep component of larger hemangiomas. Other authors have also confirmed this finding. The pulsed dye laser is also useful for the

Table 7. Criteria for Consideration for Use of a Laser for Hemangiomas

1. Potential for functional impairment
2. Risk of ulceration-
Rapid enlargement
Recurrent trauma
Moist area
3. Cosmetic disfigurement-
Highly visible lesion
Extensive surface area

Source: From Garden JM, Bakus AD. Laser treatment of port-wine stains and hemangiomas. *Dermatol Clin.* 1997;15:373-383.¹¹³

treatment of telangiectasias after the hemangioma has involuted.^{60,76}

Another laser that has been reported for the surface treatment of hemangiomas is the copper vapor laser.¹¹⁸ This laser has a wavelength of 587 nm, which corresponds very closely to the absorption peak of oxyhemoglobin at 577 nm. Further reports will be needed before this laser achieves widespread usage for the treatment of hemangiomas.

A recent development in the use of laser technology for the treatment of hemangiomas has been the use of intralesional potassium titanyl phosphate (KTP) or Nd:YAG lasers.¹¹⁹⁻¹²² Achauer et al¹²¹ used the intralesional KTP laser in a series of 12 patients with facial hemangiomas and were able to achieve greater than 50% reduction in the size of the lesion at 3 months. Achauer et al¹²² also reported the use of intralesional KTP or Nd:YAG laser for the treatment of periorbital hemangiomas. They were able to achieve a 50% or more reduction in the size of the lesion within 8 months. Ulceration of the lesion was common in both reports (17%-25%). Perhaps the development of newer technologies such as surface cooling will allow for the laser treatment of thicker hemangiomas without the risk of injury to the epidermis and subsequent scarring.¹²³

Many other modalities have been used for the treatment of hemangiomas, including cryosurgery, chemotherapy agents, embolization, and radiation therapy. Cryosurgery has been used in South America and Europe, but not widely in North America owing to concerns about scarring.² Cremer,¹²⁴ however, disputes this concern and uses cryotherapy for single elevated "classic" hemangiomas with a regular circumference.

Several different chemotherapeutic agents have been used for the treatment of hemangiomas, including cyclophosphamide,¹²⁵ bleomycin,¹²⁶ and

vincristine.¹²⁷ The exact role of chemotherapy in treating hemangiomas remains unclear at this time.

Therapeutic embolization has been reported for the treatment of life-threatening hemangiomas.⁵⁹ Embolization can be useful in the treatment of large liver hemangiomas with an associated hyperdynamic cardiac state. Only trained teams should perform this procedure.

Radiation therapy was one of the first therapeutic modalities for the treatment of hemangiomas. However, concerns of radiation dermatitis, growth retardation, and secondary neoplasms have relegated this treatment to one of historical interest.⁶⁶

The discovery of substances such as endostatin by O'Reilly et al¹²⁸ may make Mulliken's plea for a "biological approach to treatment of hemangiomas of infancy" realized.⁸²

References

1. Mulliken JB. Vascular birthmarks in folklore, history, art, and literature. In Mulliken JB, Young AE, eds. *Vascular Birthmarks: Hemangiomas and Malformations*. Philadelphia, Pa: WB Saunders Company; 1988:3-23.
2. Drolet BA, Esterly NB, Frieden IJ. Hemangiomas in children. *N Engl J Med.* 1999;341:173-181.
3. Robertson RL, Robson CD, Barnes PD, et al. Head and neck vascular anomalies of childhood. *Neuroimaging Clin North Am.* 1999;9:115-132.
4. Pratt AG. Birthmarks in infants. *Arch Dermatol.* 1953;67:302-305.
5. Jacobs AH, Walton RG. The incidence of birthmarks in the neonate. *Pediatrics.* 1976;58:218-222.
6. Bivings L. Spontaneous regression of angiomas in children. *J Pediatr.* 1954;45:643-647.
7. Jacobs AH. Strawberry hemangioma: the natural history of the untreated lesion. *Calif Med.* 1957;86:8-10.
8. Amir J, Metzker A, Krikler R, et al. Strawberry hemangioma in preterm infants. *Pediatr Dermatol.* 1986;3:331-332.
9. Holmdahl K. Cutaneous hemangiomas in premature and mature infants. *Acta Paediatrica.* 1955;44:370-379.
10. Osburn K, Schosser RH, Everett MA. Congenital pigmented and vascular lesions in newborn infants. *J Am Acad Dermatol.* 1987;16:788-791.
11. Bowers RE, Graham EA, Tomlinson KM. The natural history of the strawberry nevus. *Arch Dermatol.* 1969;82:667-680.
12. Margileth AM, Museles M. Cutaneous hemangiomas in children. *JAMA.* 1965;194:523-526.
13. Enjolras O, Mulliken JB. The current management of vascular birthmarks. *Pediatr Dermatol.* 1993;10:311-333.
14. Finn MC, Glowacki J, Mulliken JB. Congenital vascular lesions: clinical application of a new classification. *J Pediatr Surg.* 1983;18:894-900.

15. Burton BK, Schulz CJ, Angle F, et al. An increased incidence of haemangiomas in infants born following chorionic villus sampling (CVS). *Prenat Diagn.* 1995;15:209-214.
16. Mueller BU, Mulliken JB. The infant with a vascular tumor. *Semin Perinatol.* 1999;20:332-340.
17. Blei F, Walter J, Orlow SJ, et al. Familial segregation of hemangiomas and vascular malformations as an autosomal dominant trait. *Arch Dermatol.* 1998;134:718-722.
18. Cheung DS, Warman ML, Mulliken JB. Hemangioma in twins. *Ann Plast Surg.* 1997;38:269-274.
19. Pack GT, Miller TR. Hemangiomas: classification, diagnosis, and treatment. *Angiology.* 1950;1:405-426.
20. Malan, E. *Vascular Malformations (Angiodysplasias)*. Milan, Italy: Carlo Erba Foundation; 1974:38-41.
21. Folkman J, Klagsbrun M. Angiogenic factors. *Science.* 1987;235:442-447.
22. Mulliken JB, Young AE. Diagnosis and natural history of hemangiomas. In: Mulliken JB, Young AE, eds. *Vascular Birthmarks: Hemangiomas and Malformations*. Philadelphia, Pa: WB Saunders Company; 1988:41-46.
23. Folkman J. Clinical applications of research on angiogenesis. *N Engl J Med.* 1995;333:1757-1763.
24. Powell J. Update on hemangiomas and vascular malformations. *Curr Opin Pediatr.* 1999;11:457-463.
25. Sasaki GH, Pang CY, Wittliff JL. Pathogenesis and treatment of infant skin strawberry hemangiomas: clinical and in vitro studies of hormonal effects. *Plast Reconstr Surg.* 1984;73:359-368.
26. Xiao X, Hong L, Sheng M. Promoting effect of estrogen on the proliferation of hemangioma vascular endothelial cells in vitro. *J Pediatr Surg.* 1999;34:1603-1605.
27. Mulliken JB. Cutaneous vascular anomalies. In: McCarthy JG, ed. *Plastic Surgery*. Philadelphia, Pa: WB Saunders Company; 1990:3191-3274.
28. Mulliken JB, Glowacki J. Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg.* 1982;69:412-420.
29. Finn MC, Glowacki J, Mulliken JB. Congenital vascular lesions: clinical application of a new classification. *J Pediatr Surg.* 1983;18:894-900.
30. Enjolras O. Classification and management of the various superficial vascular anomalies: hemangiomas and vascular malformations. *J Dermatol.* 1997;24:701-710.
31. Burrows PE, Laor T, Paltiel H, Robertson RL. Diagnostic imaging in the evaluation of vascular birthmarks. *Derm Clin.* 1998;16:455-488.
32. Kenkel JM, Burns AJ. Vascular anomalies, lasers, and lymphedema (overview). *Select Read Plast Surg.* 1995;8:1-47.
33. Payne MM, Moyer F, Marcks KM, Trevaskis AE. The precursor to the hemangioma. *Plast Reconstr Surg.* 1966;38:64-67.
34. Hidano A, Nakajima S. Earliest features of the strawberry mark in the newborn. *Br J Derm.* 1972;87:138-144.
35. Pasyk KA, Cherry GW, Grabb WC, Sasaki GH. Quantitative evaluation of mast cells in cellularly dynamic and adynamic vascular malformations. *Plast Reconstr Surg.* 1984;73:69-77.
36. Lister WA. The natural history of strawberry nevi. *Lancet.* 1938;1:1429-1434.
37. Simpson JR. Natural history of cavernous haemangiomas. *Lancet.* 1959;2:1057-1059.
38. Boon LM, Enjolras O, Mulliken JB. Congenital hemangioma: evidence of accelerated involution. *J Pediatr.* 1996;128:329-335.
39. Mulliken JB. Pathogenesis of hemangiomas. In: Mulliken JB, Young AE, eds. *Vascular Birthmarks: Hemangiomas and Malformations*. Philadelphia, Pa: WB Saunders Company; 1988:63-76.
40. Glowacki J, Mulliken JB. Mast cells in hemangiomas and vascular malformations. *Pediatrics.* 1981;70:48-51.
41. Enjolras O, Mulliken JB. Vascular tumors and vascular malformations (new issues). *Adv Dermatol.* 1998;13:375-423.
42. Enjolras O. Vascular tumors and vascular malformations: are we at the dawn of a better knowledge? *Pediatr Dermatol.* 1999;16:238-241.
43. Blei F. New clinical observations in hemangiomas. *Sem Cut Med Surg.* 1999;18:187-194.
44. Vikkula M, Boon LM, Mulliken JB, et al. Molecular basis of vascular anomalies. *Trends Cardiovasc Med.* 1998;8:281-292.
45. Takahashi K, Mulliken JB, Kozakewich HPW, et al. Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest.* 1994;93:2357-2364.
46. Chang J, Most D, Bresnick S, et al. Proliferative hemangiomas: analysis of cytokine gene expression and angiogenesis. *Plast Reconstr Surg.* 1999;103:1-9.
47. Kraling BM, Razon MJ, Boon LM, et al. E-selectin is present in proliferating endothelial cells in human hemangiomas. *Am J Path.* 1996;148:1181-1191.
48. Isik FF, Rand RP, Gruss JS, Benjamin D, et al. Monocyte chemoattractant protein-1 mRNA expression in hemangiomas and vascular malformations. *J Surg Res.* 1996;61:71-76.
49. Jang Y-C, Arumugam S, Ferguson M, et al. Changes in matrix composition during the growth and regression of human hemangiomas. *J Surg Res.* 1998;80:9-15.
50. Razon MJ, Kraling BM, Mulliken JB, et al. Increased apoptosis coincides with onset of involution in infantile hemangioma. *Microcirculation.* 1998;5:189-195.
51. Mancini AJ, Smoller BR. Proliferation and apoptosis within juvenile capillary hemangiomas. *Am J Dermatopath.* 1996;18:505-514.
52. Martinez-Perez D, Fein NA, Boon LM, et al. Not all hemangiomas look like strawberries: uncommon presentations of the most common tumor of infancy. *Pediatr Dermatol.* 1995;12:1-6.
53. Burrows PE, Mulliken JB, Fellows KE, et al. Childhood hemangiomas and vascular malformations: angiographic

- differentiation. *AJR Am J Roentgenol.* 1983;141:483-488.
54. Yang WT, Ahuja A, Metreweli C. Sonographic features of head and neck hemangiomas and vascular malformations: review of 23 patients. *J Ultrasound Med.* 1997;16:39-44.
55. Dubois J, Garel L, Grignon A, et al. Imaging of hemangiomas and vascular malformations in children. *Acad Radiol.* 1998;5:390-400.
56. Dubois J, Patriquin HB, Garel L, et al. Soft-tissue hemangiomas in infants and children: diagnosis using doppler sonography. *AJR Am J Roentgenol.* 1998;171:247-252.
57. Robertson RL, Robson CD, Barnes PD, et al. Head and neck vascular anomalies of childhood. *Neuroimaging Clin North Am.* 1999;9:115-132.
58. Meyer JS, Hoffer FA, Barnes PD, et al. Biological classification of soft-tissue vascular anomalies: MR correlation. *AJR Am J Roentgenol.* 1991;157:559-564.
59. Enjolras O, Riche MC, Merland JJ, et al. Management of alarming hemangiomas in infancy: a review of 25 cases. *Pediatrics.* 1990;85:491-498.
60. Management of hemangiomas. *Pediatr Dermatol.* 1997;14:57-83.
61. Garden JM, Bakus AD. Laser treatment of port-wine stains and hemangiomas. *Dermatol Clin.* 1997;15:373-383.
62. Haik GR, Jakobiec FA, Ellsworth RM, et al. Capillary hemangioma of the lids and orbit. An analysis of the clinical features and therapeutic results in 101 cases. *Ophthalmology.* 1979;86:760-789.
63. Stigmar G, Crawford JS, Ward CM, et al. Ophthalmic sequelae of infantile hemangiomas of the eyelids and orbit. *Am J Ophthalmol.* 1978;85:806-813.
64. Goldberg NS, Rosanova MA. Periorbital hemangiomas. *Dermatol Clin.* 1992;10:653-661.
65. Orlow SJ, Isakoff MS, Blei F. Increased risk of symptomatic hemangiomas of the airway in association with cutaneous hemangiomas in a "beard" distribution. *J Pediatr.* 1997;131:643-646.
66. Brown TJ, Friedman J, Levy ML. The diagnosis and treatment of common birthmarks. *Clin Plast Surg.* 1998;25:509-525.
67. Van Den Abbeele T, Triglia JM, Lescanne E, et al. Surgical removal of subglottic hemangiomas in children. *Laryngoscope.* 1999;109:1281-1286.
68. Vin-Christian K, McCalmont TH, Frieden IJ. Kaposiform hemangioendothelioma—an aggressive, locally invasive vascular tumor that can mimic hemangioma of infancy. *Arch Dermatol.* 1997;133:1573-1578.
69. Enjolras O, Wassef M, Mazoyer E, et al. Infants with Kasabach-Merritt syndrome do not have "true" hemangiomas. *J Pediatr.* 1997;130:631-640.
70. Sarkar M, Mulliken JB, Kozakewich HPW, et al. Thrombocytopenic coagulopathy (Kasabach-Merritt phenomenon) is associated with Kaposiform hemangioendothelioma and not with common infantile hemangioma. *Plast Reconstr Surg.* 1997;100:1377-1386.
71. Reese V, Frieden IJ, Paller AS, et al. Association of facial hemangiomas with Dandy-Walker and other posterior fossa malformations. *J Pediatr.* 1993;122:379-383.
72. Frieden IJ, Reese V, Cohen D. PHACE syndrome—the association of posterior fossa brain malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, and eye abnormalities. *Arch Dermatol.* 1996;132:307-311.
73. Albright AL, Gartner JC, Wiener ES. Lumbar cutaneous hemangiomas as indicators of tethered spinal cords. *Pediatrics.* 1988;83:977-980.
74. Goldberg NS, Hebert AA, Esterly NB. Sacral hemangiomas and multiple congenital abnormalities. *Arch Dermatol.* 1986;22:684-687.
75. Ezekowitz RAB. The relationship between facial and airway hemangiomas: does seeing red bode ill? *J Pediatr.* 1997;131:514-515.
76. Pfeiffer N. Guidelines for treating different stages of hemangioma. *J Clin Laser Med Surg.* 1994;12:239-240.
77. Frieden IJ, Eichenfield LF, Esterly NB, et al. Guidelines for care of hemangiomas of infancy. *J Am Acad Dermatol.* 1997;37:631-637.
78. Frieden IJ. Which hemangiomas to treat—and how? *Arch Dermatol.* 1997;133:1593-1595.
79. Achauer BM, Chang C-J, Vander Kam VM. Management of hemangioma of infancy: review of 245 patients. *Plast Reconstr Surg.* 1997;99:1301-1308.
80. Jackson IT, Carreno R, Potparic Z, et al. Hemangiomas, vascular malformations, and lymphovenous malformations: classification and methods of treatment. *Plast Reconstr Surg.* 1993;91:1216-1230.
81. Forte V, Triglia JM, Zalzal G. Hemangioma. *Head Neck.* 1998;20:69-72.
82. Mulliken JB. A plea for a biologic approach to hemangiomas of infancy. *Arch Dermatol.* 1991;127:243-244.
83. Tanner JL, Dechert MP, Frieden IJ. Growing up with a facial hemangioma: parent and child coping with adaptation. *Pediatrics.* 1998;101:466-452.
84. Zarem HA, Edgerton MT. Induced resolution of cavernous hemangiomas following prednisolone therapy. *Plast Reconstr Surg.* 1967;39:76-83.
85. Frost, NC, Esterly NB. Successful treatment of juvenile hemangiomas with prednisone. *J Pediatr.* 1968;72:351-357.
86. Edgerton MT. The treatment of hemangiomas: with special reference to the role of steroid therapy. *Ann Surg.* 1976;183:517-532.
87. Kushner BJ. Intralesional corticosteroid injection for infantile adnexal hemangioma. *Am J Ophthalmol.* 1982;93:496-506.
88. Kushner BJ. The treatment of periorbital infantile hemangioma with intralesional corticosteroid. *Plast Reconstr Surg.* 1985;76:517-524.
89. Sloan GM, Reinisch JF, Nichter LS, et al. Intralesional corticosteroid therapy for infantile hemangiomas. *Plast Reconstr Surg.* 1989;83:459-466.

90. Gangopadhyay AN, Sinha CK, Gopal SC, et al. Role of steroid in childhood hemangioma: a 10 years review. *Int Surg*. 1997;82:49-51.
91. Boon LM, MacDonald DM, Mulliken JB. Complications of systemic corticosteroid therapy for problematic hemangioma. *Plast Reconstr Surg*. 1999;104:1616-1623.
92. Kushner BJ. Hemangiomas in children. *N Engl J Med*. 1999;341:2018.
93. Ezekowitz RAB, Mulliken JB, Folkman J. Interferon alfa-2a therapy for life-threatening hemangiomas of infancy. *N Engl J Med*. 1992;326:1456-1463.
94. White CW, Sondheimer HM, Crouch EC, et al. Treatment of pulmonary hemangiomatosis with recombinant interferon alfa-2a. *N Engl J Med*. 1989;320:1197-1200.
95. Bauman NM, Burke DK, Smith RJH. Treatment of massive or life-threatening hemangiomas with recombinant α_2 -interferon. *Otolaryngol Head Neck Surg*. 1997;117:99-110.
96. Hastings MM, Milot J, Barsoum-Homsy M, et al. Recombinant interferon alfa-2b in the treatment of vision-threatening capillary hemangiomas in childhood. *JAAPOS*. 1997;1:226-230.
97. Egbert JE, Nelson SC. Neurologic toxicity associated with interferon alfa treatment of capillary hemangioma. *JAAPOS*. 1997;1:190.
98. Castello MA, Ragni G, Antini A, et al. Successful management with interferon alpha-2a after prednisone therapy failure in an infant with a giant cavernous hemangioma. *Med Pediatr Oncol*. 1997;28:213-215.
99. Tamayo L, Ortiz DM, Orozco-Covarrubias L, et al. Therapeutic efficacy of interferon alfa-2b in infants with life-threatening giant hemangiomas. *Arch Dermatol*. 1997;133:1567-1571.
100. Barlow CF, Reiebe CJ, Mulliken JB, et al. Spastic diplegia as a complication of interferon alfa-2a treatment of hemangiomas of infancy. *J Pediatr*. 1998;132:527-530.
101. Tryfonas GI, Tsikopoulos G, Liasidou E, et al. Conservative treatment of hemangiomas of infancy and childhood with interferon-alpha 2a. *Pediatr Surg Int*. 1998;13:590-593.
102. Leaute-Labreze C, Taieb A. Caution with regard to the efficacy of interferon alfa-2b in the treatment of giant hemangiomas. *Arch Dermatol*. 1998;134:1297-1298.
103. Enjolras O. Neurotoxicity of interferon alfa in children treated for hemangiomas. *J Am Acad Dermatol*. 1998;36:1037-1038.
104. Greinwald JH, Burke DK, Bonthius DJ, et al. An update on the treatment of hemangiomas in children with interferon alfa-2a. *Arch Otolaryngol Head Neck Surg*. 1999;125:21-27.
105. Chang E, Boyd A, Nelson CC, et al. Successful treatment of infant hemangiomas with interferon α -2b. *J Pediatr Hematol Oncol*. 1997;19:237-244.
106. Rosenfeld H, Sherman R. Treatment of cutaneous and deep vascular lesions with the Nd:YAG laser. *Lasers Surg Med*. 1986;6:20-23.
107. Apfelberg DB, Greene RA, Maser R, et al. Results of argon laser exposure of capillary hemangiomas of infancy-preliminary report. *Plast Reconstr Surg*. 1981;67:188-193.
108. Hobby LW. Further evaluation of the potential of the argon laser in the treatment of strawberry hemangiomas. *Plast Reconstr Surg*. 1983;71:481-485.
109. Achauer BM, Vander Kam VM. Capillary hemangioma (strawberry mark) of infancy: comparison of argon and Nd:YAG laser treatment. *Plast Reconstr Surg*. 1989;84:60-69.
110. Apfelberg DB, Smith T, Lash H. Preliminary report on use of the neodymium-YAG laser in plastic surgery. *Lasers Surg Med*. 1987;7:189-198.
111. Preeyanont P, Nimsakul N. The Nd:YAG laser treatment of hemangioma. *J Clin Laser Med Surg*. 1994;12:225-229.
112. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science*. 1983;220:524-527.
113. Garden JM, Bakus AD. Laser treatment of port-wine stains and hemangiomas. *Dermatol Clin*. 1997;15:373-383.
114. Garden JM, Bakus AD, Paller AS. Treatment of cutaneous hemangiomas by the flashlamp-pumped pulsed dye laser: prospective analysis. *J Pediatr*. 1992;120:555-560.
115. Sherwood KA, Tan OT. The treatment of a capillary hemangioma with the flashlamp pumped-dye laser. *J Am Acad Dermatol*. 1990;22:136-137.
116. Ashinoff R, Geronemus RG. Capillary hemangiomas and treatment with the flash lamp-pulsed dye laser. *Arch Dermatol*. 1991;127:202-205.
117. Scheepers JH, Quaba AA. Does the pulsed tunable dye laser have a role in the management of infantile hemangiomas? Observations based on 3 years experience. *Plast Reconstr Surg*. 1995;95:305-312.
118. Tong MCF, Van Hasselt CA. The 578-nm copper vapor laser in the treatment of cavernous hemangiomas in the oral cavity. *J Clin Laser Med Surg*. 1994;12:109-110.
119. Gregory RO. Treatment of cavernous hemangiomas with intralesional ablation with YAG laser. *Lasers Surg Med*. 1991;11(suppl 3):66.
120. Apfelberg DB. Intralesional laser photocoagulation-steroids as an adjunct to surgery for massive hemangiomas and vascular malformations. *Ann Plast Surg*. 1995;35:144-148.
121. Achauer BM, Celikoz B, Vander Kam VM. Intralesional bare fiber laser treatment of hemangioma of infancy. *Plast Reconstr Surg*. 1998;101:1212-1217.
122. Achauer BM, Chang C-J, Vander Kam VM. Intralesional photocoagulation of periorbital hemangiomas. *Plast Reconstr Surg*. 1999;103:11-16.

123. Chang C-J, Anvari B, Nelson JS. Cryogen spray cooling for spatially selective photocoagulation of hemangiomas: a new methodology with preliminary clinical reports. *Plast Reconstr Surg*. 1998;102:459-463.
124. Cremer H. Cryosurgery for hemangiomas. *Pediatr Dermatol*. 1998;15:410-411.
125. Hurvitz CH, Alkalay AL, Sloninsky L, et al. Cyclophosphamide therapy in life-threatening vascular tumors. *J Pediatr*. 1986;109:360-363.
126. Kullendorf CM. Efficacy of bleomycin treatment for symptomatic hemangiomas in children. *Pediatr Surg Int*. 1997;12:526-528.
127. Perez J, Pardo J, Gomez C. Vincristine-an effective treatment of corticoid-resistant life-threatening infantile hemangiomas. *Acta Oncol*. 2002;41:197-199.
128. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell*. 1997;88:277-285.



Multipotential stem cells recapitulate human infantile hemangioma in immunodeficient mice

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Infantile hemangioma is a benign endothelial tumor composed of disorganized blood vessels. It exhibits a unique life cycle of rapid postnatal growth followed by slow regression to a fibrofatty residuum. Here, we have reported the isolation of multipotential stem cells from hemangioma tissue that give rise to hemangioma-like lesions in immunodeficient mice. Cells were isolated based on expression of the stem cell marker CD133 and expanded from single cells as clonal populations. The CD133-selected cells generated human blood vessels 7 days after implantation in immunodeficient mice. Cell retrieval experiments showed the cells could again form vessels when transplanted into secondary recipients. The human vessels expressed GLUT-1 and merosin, immunodiagnostic markers for infantile hemangioma. Two months after implantation, the number of blood vessels diminished and human adipocytes became evident. Lentiviral expression of GFP was used to confirm that the hemangioma-derived cells formed the blood vessels and adipocytes in the immunodeficient mice. Thus, when transplanted into immunodeficient mice, hemangioma-derived cells recapitulated the unique evolution of infantile hemangioma — the formation of blood vessels followed by involution to fatty tissue. In summary, this study identifies a stem cell as the cellular origin of infantile hemangioma and describes for what we believe is the first time an animal model for this common tumor of infancy.

Introduction

Infantile hemangioma (IH) is a vascular tumor that occurs in 5%–10% of infants of mixed European descent (1). A hallmark of IH is its life cycle, which is divided into 3 stages. The proliferating phase spans the first year of postnatal life and is characterized by cellular masses of endothelium without a defined vascular architecture. The involuting phase begins around 1 year of age and continues for 3–5 years as blood vessels become more obvious and even enlarged. The involuted phase is reached by 5–8 years of age, at which point blood vessels are replaced with a fibrofatty residuum and capillary-sized channels. Intrinsic (2, 3) and extrinsic (4, 5) factors have been hypothesized to contribute to the rapid growth and the slow, yet spontaneous regression. Nevertheless, the cellular origin of IH and the biologic signals for the uncontrolled growth remain elusive.

Histological analyses of IH in the early proliferating phase have generated a number of developmental theories suggesting an embryonic or primitive cell origin of IH. Virchow (6), Pack and Miller (7), and Malan (8) described IH as sequestered embryonic mesoderm and/or activated dormant angioblasts. The presence of CD34-positive cells in the interstitial compartment of IH led Smoller and Apfelberg to postulate a primitive vascular progenitor cell giving rise to both ECs and pericytes in the tumor (9). In

vitro studies have suggested that ECs isolated from IH have an immature fetal phenotype (10). We have shown that hemangioma ECs (HemECs) are clonal (3), indicating that the HemECs might originate from a single stem or progenitor cell. We also used human stem cell (SC) marker CD133 (also known as AC133 and prominin-1) (11) to detect (12) and isolate (13) hemangioma endothelial progenitor cells (HemEPCs). Our demonstration that HemECs and HemEPCs are more similar to normal human cord blood EPCs (cbEPCs) than mature human dermal microvascular ECs (HDMECs) (13) provides further support to the earlier histological studies that first suggested hemangioma is composed of immature vascular progenitor cells.

Herein we report on less-differentiated SCs in hemangioma specimens — referred to as hemangioma-derived SCs (HemSCs). The HemSCs exhibited robust proliferative and clonogenic capacity and differentiated into cells of multiple lineages. Importantly, clonal HemSC populations expanded in vitro from single cells produced human GLUT-1-positive microvessels in vivo. Over time, the human blood vessels diminished and human adipocytes became evident, reminiscent of the involuted phase of IH. In summary, our findings provide evidence for a SC origin of IH, which is in contrast to the long-held view that IH arises from ECs (14).

Results

Isolation and characterization of HemSCs. Anti-CD133-coated magnetic beads have been used to isolate stem/progenitor cells from blood (15) and from human tumors (16, 17). We isolated CD133⁺ cells from proliferating phase IH using this methodology and found that approximately 0.2% of the hemangioma cells were selected with anti-CD133-coated beads ($n = 28$; age 11 ± 1.47 months, CD133⁺ cells, $0.2\% \pm 0.027\%$). The CD133-selected cells, referred

Nonstandard abbreviations used: BM-MSC, BM mesenchymal stromal cell; cbEPC, cord blood EPC; EBM-2, endothelial basal medium 2; EPC, endothelial progenitor cell; GFAP, glial fibrillar acidic protein; HDMEC, human dermal microvascular EC; HemEC, hemangioma EC; HemEPC, hemangioma EPC; HemSC, hemangioma-derived stem cell; IH, infantile hemangioma; MVD, microvessel density; NHDF, normal human dermal fibroblast; SC, stem cell.

Conflict of interest: The authors have declared that no conflict of interest exists.

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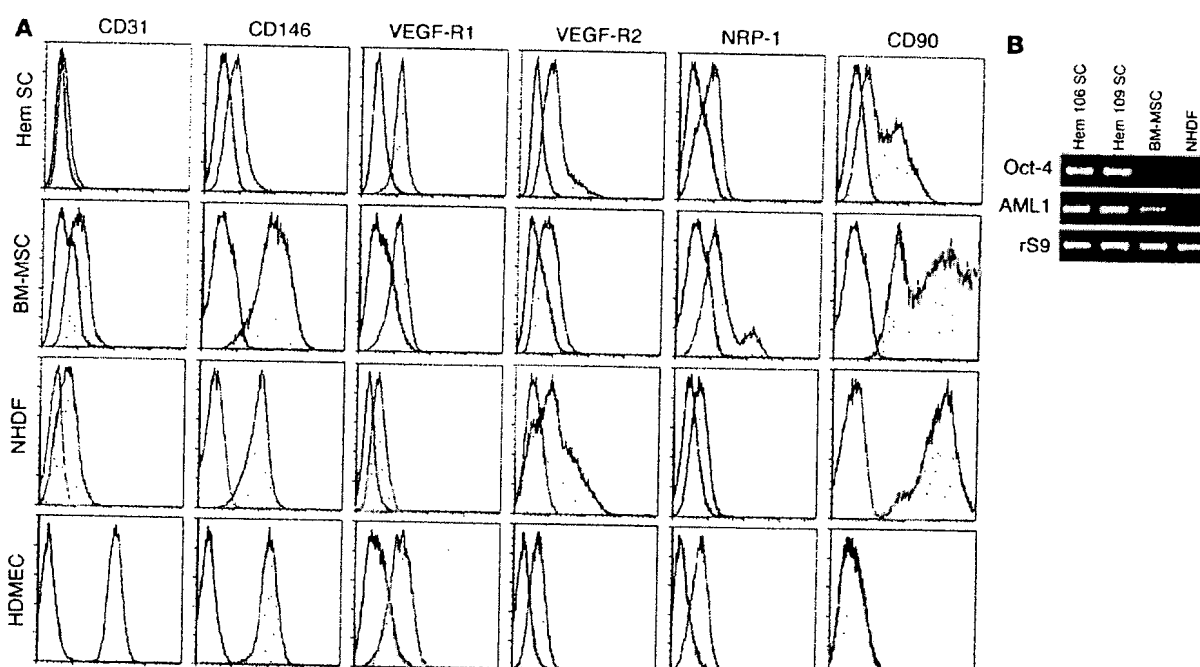


Figure 1

HemSCs express VEGF-Rs and mesenchymal cell marker CD90. (A) Flow cytometric analysis of HemSCs, BM-MSCs, NHDFs, and HDMECs. Each cell type was grown in the EBM-2/20% FBS and assayed at passage 6. Gray histograms show cells labeled with FITC- or PE-conjugated antibodies. Black lines show isotype-matched control FITC- or PE-conjugated antibodies. Incubation with anti-CD31 and anti-VEGF-R2 was carried out following saponin permeabilization of HemSCs, BM-MSCs, and NHDFs. (B) RT-PCR analysis of Oct-4 and AML1 in 2 different HemSCs, Hem 106 and 109, with BM-MSCs and NHDFs shown for comparison. Ribosomal S9 (rS9) served as a control.

to hereafter as HemSCs, were expanded in an endothelial growth medium (endothelial basal medium 2 [EBM-2]/20% FBS). Human umbilical cbEPCs (18), HDMECs, BM-mesenchymal stromal cells (BM-MSCs), and neonatal human dermal fibroblasts (NHDFs) were cultured under identical conditions and used for comparison. Flow cytometric analyses for endothelial and mesenchymal cell-surface markers showed that HemSCs were most similar to BM-MSCs and NHDFs (Figure 1A). HemSCs expressed CD90, a mesenchymal cell marker, and proteins that are displayed on ECs, such as VEGF-R1, VEGF-R2, neuropilin-1 (NRP-1), and CD146, but none of these are exclusive to the endothelial lineage. Hence, the cell-surface markers on HemSCs indicate a mesodermal or mesenchymal cell phenotype. Phase contrast images of HemSCs in culture are shown in Supplemental Figure 1 (supplemental material available online with this article: doi:10.1172/JCI33493DS1).

RT-PCR analyses showed that HemSCs expressed the embryonic SC transcription factor Oct-4 (Figure 1B), which is known to play a pivotal role in SC specification (19). HemSCs were also positive for AML1 (Figure 1B), a transcription factor required for definitive hematopoiesis (20) and expressed in hemogenic endothelium (21). Expression of Oct-4 and AML1 in HemSCs indicates that HemSCs are multipotent.

We next compared the growth parameters of HemSCs with normal mesenchymal cells and ECs. HemSCs grew to 3–4 times greater density than normal control endothelial and mesenchymal cells, which included HDMECs, cbEPCs, NHDFs, and BM-MSCs (Figure 2A). The proliferative response to serum, basic bFGF, and VEGF-A was determined after cells were serum and growth factor

starved for 6 hours (Figure 2B). HemSCs and NHDFs proliferated in response to serum and bFGF, although the response of HemSCs was stronger, as was the basal proliferation. HemSCs, BM-MSCs, and NHDFs did not show increased proliferation in response to VEGF-A, a well-studied endothelial growth and survival factor, while cbEPCs and HDMECs proliferated, as expected.

HemSCs display in vitro clonogenic and multilineage differentiation ability. To study cellular differentiation, we prepared clonal populations from the HemSCs from 3 different proliferating phase I^H specimens. This was done by diluting a single-cell suspension of HemSC to 3 cells/ml and plating 100 μ l in each well of a 96-well plate; thereafter, wells were inspected to verify that only 1 colony was present in the well. Clonally expanded cells were necessary for multilineage assays of differentiation and later for in vivo studies to exclude the possibility that mixed populations of unipotential cells were responsible for the phenotypes of cells observed following the induction. HemSCs showed in vitro clonal expansion, with efficiency ranging from 6%–30% (6% for Hem95, 23% for Hem97, and 30% for Hem106). BM-MSCs and NHDFs failed to produce clonal populations under identical conditions. To induce cell differentiation toward specific lineages, culture conditions reported in the literature were employed. For endothelial differentiation, HemSCs were seeded at high density on fibronectin-coated plates in serum-free medium with VEGF-B (22, 23). Clonal HemSCs differentiated into ECs, shown by expression of the EC markers CD31 (also known as PECAM-1) and VE-cadherin (Figure 2C). Treatment with nerve growth factor and trans retinoic acid (24, 25) induced morphological changes, expression of the glial cell marker

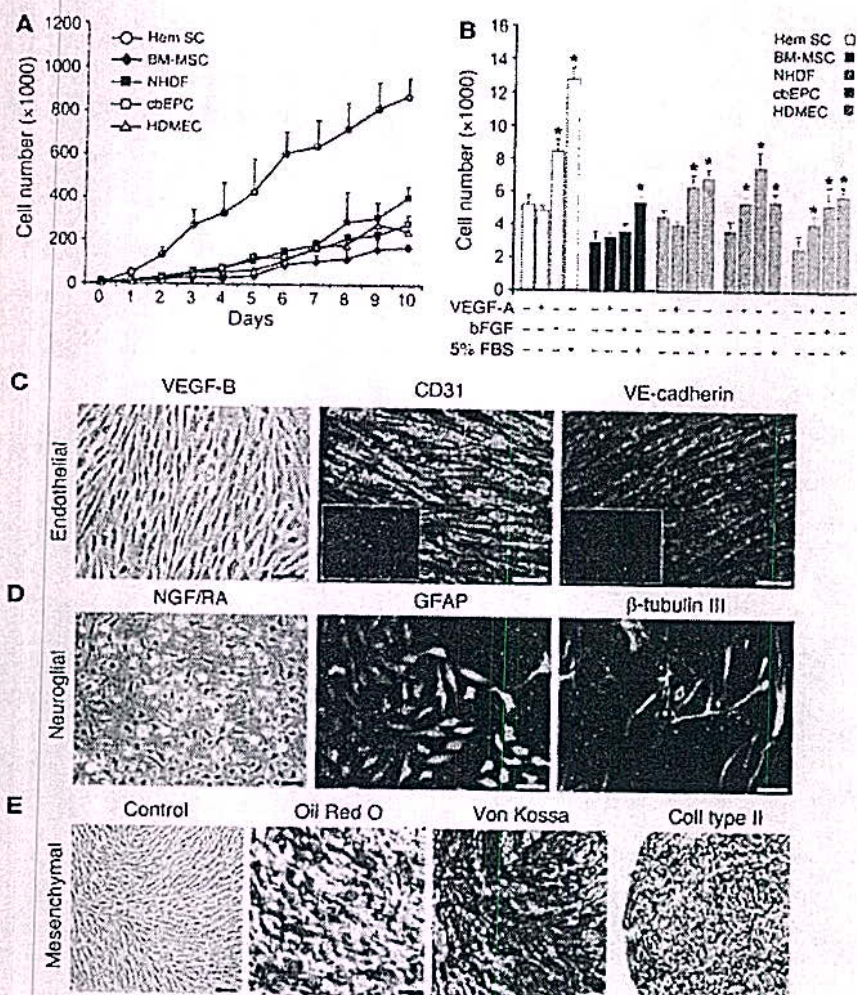


Figure 2

In vitro growth and multilineage differentiation of HemSCs. (A) Proliferation of HemSCs in EBM-2/20% FBS over 10 days compared with normal endothelial and mesenchymal cells. (B) Proliferation in response to VEGF-A, bFGF, or 5% FBS for 24 hours in serum-free, growth factor-free EBM medium. * $P < 0.05$ compared with cells in serum-free, growth factor-free medium. (C–E) Clonal HemSCs differentiated into endothelial (C), neuroglial (D), and mesenchymal (E) cells. Scale bars: 50 μ m. Insets in C show CD31 and VE-cadherin immunostaining of cells induced in the absence of VEGF-B. All experiments were carried out with cells at passages 6–9.

HemSCs or normal control cells were resuspended in Matrigel, a solubilized basement membrane preparation that has been shown to support angiogenesis and vasculogenesis (18, 29, 30). Cells were injected s.c. into 6-week-old mice (1.5×10^6 cells/200 μ l Matrigel/animal). H&E staining of sections showed numerous rbc-filled vascular channels 7 and 14 days after implantation. After 28 and 56 days in vivo, vessels were still present, but large lipid-filled cells became prominent (Figure 3A). Similar results were obtained with nonclonally expanded HemSCs from 4 different hemangioma specimens (data not shown). Blood vessels were not detected when HemECs (3), HemEPCs (13), hemangioma-derived mesenchymal SCs (HemMSCs) (31), BM-MSCs, cbEPCs (18), or NHDFs were

implanted as single-cell types into mice under identical conditions (Supplemental Figure 2).

To verify that the newly formed blood vessels were composed of human ECs, we performed indirect immunofluorescence using an antibody specific for human CD31. Immunostained sections were examined by confocal microscopy. Human CD31 was detected in blood vessels formed from clonal HemSCs, indicating endothelial differentiation and vasculogenesis (Figure 3B). The specificity of the anti-human CD31 was confirmed by lack of staining in mouse tissues (data not shown) and has been demonstrated in other studies (18, 32). We next determined whether the human CD31-positive vessels expressed the GLUT-1-positive immunophenotype, which has been shown to be a specific signature of IH (33). The blood vessels formed from HemSCs in Matrigel were found to coexpress CD31 and GLUT-1 (Figure 3B). High magnification images with DAPI-stained nuclei are shown in Figure 3B to highlight the coexpression of CD31 and GLUT-1. A second marker of the hemangioma phenotype, merosin (34), was also found expressed in the hemangioma lesions formed in mice from HemSCs (Supplemental Figure 3).

To further examine human CD31 expression in the Matrigel model, cells were reisolated from the explants by digestion with a collagenase/dispase solution. Flow cytometry was performed with-

glial fibrillar acidic protein (GFAP), and the neuronal marker β -tubulin III (Figure 2D). Finally, mesenchymal differentiation into adipocytes, osteocytes, and chondrocytes was also seen (Figure 2E) when subjected to well-established protocols (26). BM-MSCs differentiated into fat, bone, and cartilage as expected, whereas NHDFs failed to differentiate into any other cell type in the same inductive medium (data not shown). Interestingly, HemSC-derived ECs exhibited an intracellular localization of CD31 and VE-cadherin (Figure 2C), reminiscent of an earlier study of hemangioma-derived and fetal ECs (10). These findings indicate that other signals or factors may be required to fully differentiate the HemSCs into mature ECs with the expected localization of adhesion molecules at the cell surface. Some IH blood vessels coexpress myeloid markers (27), further illustrating the unique phenotype of IH endothelium. As hematopoietic cells and ECs arise from a common progenitor during development (28), we tested to determine whether HemSCs would produce hematopoietic colonies in vitro. Incubation of clonally expanded HemSCs in MethoCult medium did not yield hematopoietic colonies (data not shown).

HemSCs form hemangioma-like Glut-1⁺ blood vessels in immunodeficient mice. We tested to determine whether HemSCs would form hemangiomas in immunodeficient nu/nu mice. Clonally expanded

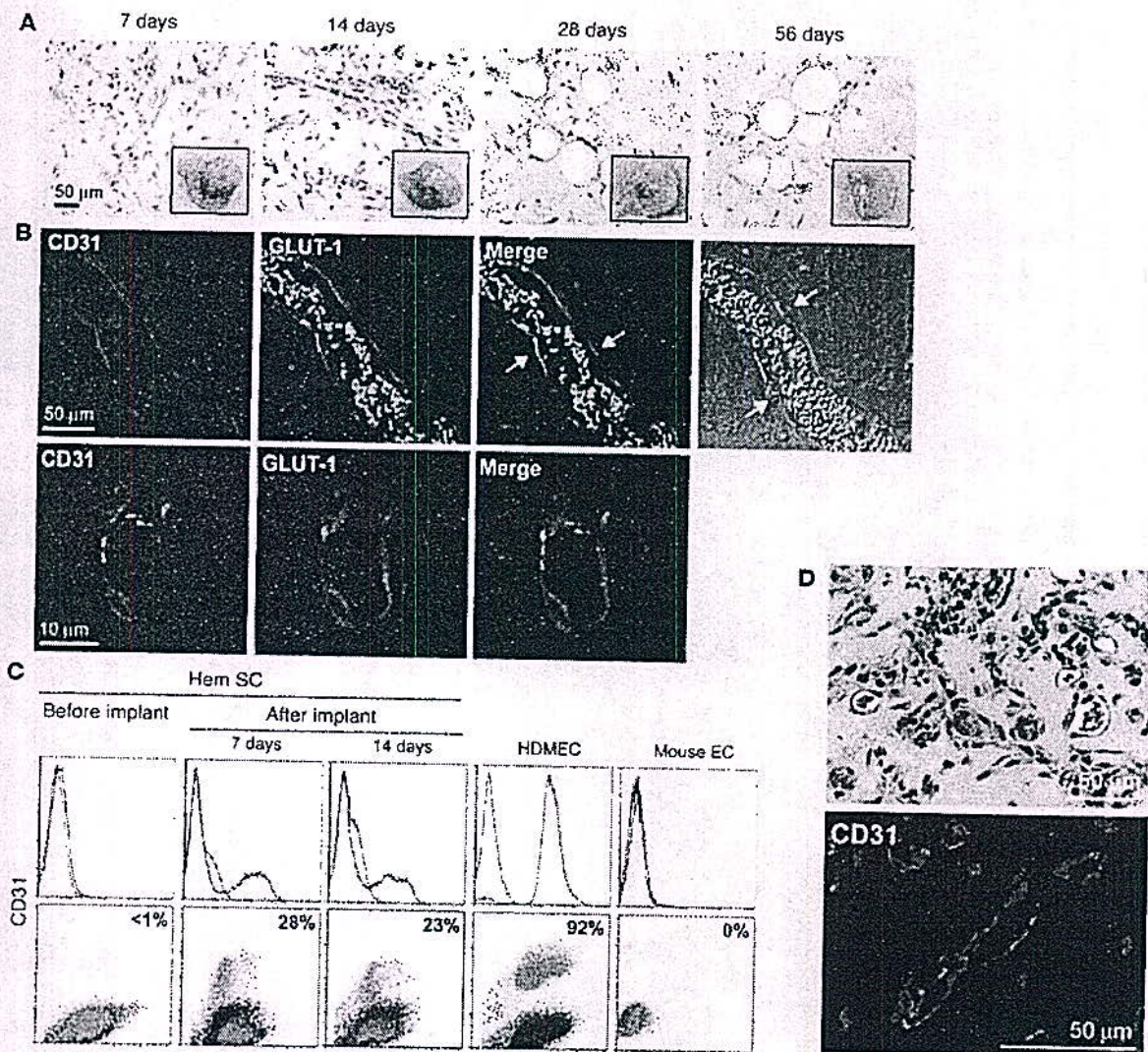


Figure 3

HemSCs form CD31⁺GLUT-1⁺ blood vessels in vivo. (A) Clonal HemSCs were suspended in Matrigel and injected s.c. into nude mice. H&E sections of explants at 7, 14, 28, and 56 days with insets showing explants at the corresponding time points. Scale bar: 50 μ m. Passage 9 clonal HemSCs were used. (B) Immunofluorescent staining of day 7 explants. Human CD31 (red) is shown on the left, followed by GLUT-1 (green), a merged image, and a phase contrast image. In the merged image, arrows point to cells along the lumen of the blood vessel that are double-labeled for human CD31 and GLUT-1. rbc in the lumen are also positive for GLUT-1 (green). In the phase image, arrows point to endothelial nuclei. The bottom row shows higher magnification images. Scale bars: 50 μ m (top row); 10 μ m (bottom row). (C) Flow cytometry of surface-localized CD31. HemSCs before implantation were negative for CD31 (left panels). HDMECs and mouse ECs are shown as positive and negative control cells. (D) Retrieved CD31⁺ cells formed blood vessels in secondary recipient mice. Matrigel implants were removed after 14 days, sectioned, stained with H&E (top panel), and immunostained with anti-human CD31 (bottom panel). Vessel density was 78 ± 35 rbc-filled lumens/mm². Scale bars: 50 μ m.

our permeabilization of the cells with anti-human CD31 (Figure 3C). After 7 and 14 days in vivo, 23%–28% of the retrieved cells were found to express human CD31 on the surface. This is consistent with functional endothelial differentiation, demonstrated by blood vessel formation (Figure 3, A and B), and provides additional evidence for the differentiation of HemSCs into ECs.

To further test the stem/progenitor nature and vasculogenic potential of these cells, the CD31-positive cells retrieved from

the Matrigel were expanded in vitro in EBM-2/20% FBS, verified as human cells by immunostaining for a human nuclear antigen (Supplemental Figure 4), and injected into secondary recipient nude mice. After 14 days, Matrigel explants were removed, sectioned, and analyzed by H&E to quantify rbc-filled lumens and immunostain for human CD31 (Figure 3D). Vessel density was 78 ± 35 vessels/mm², which was greater than that observed in primary recipients. The ability to retrieve HemSCs that had undergone

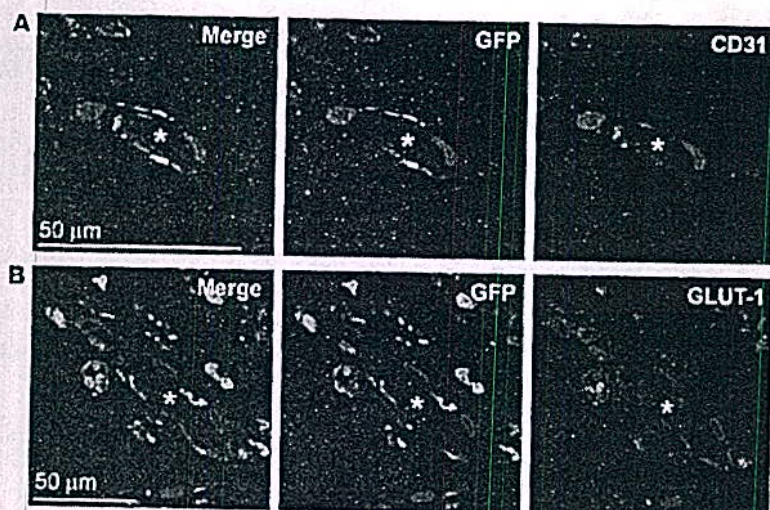


Figure 4

GFP-labeled HemSCs form hemangioma blood vessels in vivo. (A) TurboGFP-expressing HemSCs were injected in Matrigel into mice, explanted at day 14, and localized by staining for TurboGFP (green), CD31 (red), and DAPI (blue). (B) Sections were also stained for TurboGFP (green), GLUT-1 (red), and DAPI (blue). Asterisks mark the lumen of the blood vessels in panels A and B. Scale bars: 50 μm.

endothelial differentiation in vivo and to obtain hemangioma-like blood vessel formation in secondary recipients demonstrates the robust blood vessel-forming potential of the HemSCs.

GFP-labeled HemSCs form blood vessels. To track the cellular fate of the HemSCs in our in vivo model, HemSCs were infected with a lentiviral construct encoding turbo GFP. GFP-positive cells were shown by flow cytometry to be greater than 90% positive for GFP expression (Supplemental Figure 5). The cells were further expanded in vitro for 1 passage and implanted into nude mice under conditions identical to those used in the previous experiments (Figure 3). Matrigel implants were harvested at 2 weeks and analyzed for GFP, human CD31, and GLUT-1. GFP and CD31 were colocalized in luminal structures indicative of blood vessels (Figure 4A). GFP-positive cells lining vessels were also positive for the hemangioma vessel marker GLUT-1 (Figure 4B). Controls to rule on nonspecific staining with

the anti-GFP antibody are shown in Supplemental Figure 6. These data combined with that shown in Figure 3B demonstrate that vessels formed when HemSCs were implanted into nude mice were composed of human ECs; the vessels were not the result of infiltration by murine ECs.

In vivo differentiation of HemSCs to adipocytes. Our next objective was to determine whether HemSCs recapitulated the involuting and involutional phases of IH. After 28 days in vivo, HemSC-formed lesions had diminished microvessel density (MVD), and adipocytes emerged between 28 and 56 days (Figure 5A and Figure 3A). Morphometric quantification showed 25 vessels/mm² at days 7, 14, and 28, which decreased to 15 vessels/mm² at 56 days (Figure 5A), consistent with the reduced number of vascular channels seen during the evolution of IH. It has been reported that Matrigel supplemented with bFGF induces adipogenesis in vivo (35–37). However, no exogenous bFGF is included in our assay. We tested to determine whether the adipocytes in the Matrigel were host derived (murine) or human and had differentiated from the clonal HemSCs. To do this, GFP-HemSCs were implanted, as described in Figure 4, and removed after 2 months in vivo for analysis of GFP and perilipin A (38). Perilipin A encircled the lipid storage droplets in adipocytes, as expected (Figure 5B). GFP and perilipin A colocalization was

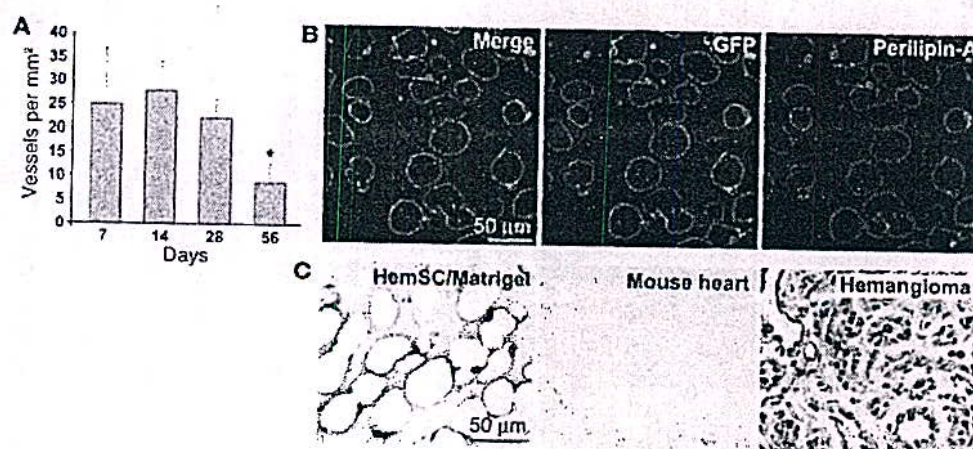


Figure 5

HemSCs form adipocytes in vivo. (A) Clonal HemSCs were suspended in Matrigel and injected s.c. into nude mice for 7, 14, 28, and 56 days, as shown in Figure 3A. Blood vessels, identified in H&E sections as luminal structures containing rbc, were quantified at the 4 time points. **P* < 0.05 compared with other time points. (B) GFP-labeled HemSCs were implanted as described in Figure 4 and analyzed after 56 days for expression of GFP and the adipocyte marker perilipin A. Right panel shows the merged image, middle panel shows GFP (green), and left panel shows perilipin A (red); all panels were stained with DAPI. (C) Sections from the 28-day time point were immunostained with an anti-human nuclear antigen antibody using an HRP-conjugated secondary antibody and a diaminobenzidine substrate. Mouse heart tissue sections showed no immunostaining while nuclei in human hemangioma tissue were uniformly positive, confirming the specificity of anti-human nuclear antigen. Scale bars: 50 μm.

seen in the scant cytoplasm of the lipid-filled adipocytes (Figure 5B). Controls to rule out nonspecific staining with the anti-GFP and anti-perilipin A antibodies are shown in Supplemental Figure 6. As an alternative approach to follow HemSC adipogenesis *in vivo*, sections were immunostained with an anti-human nuclear antigen antibody (Figure 5C). The human nuclear antigen was detected in the nuclei of adipocytes in the HemSC Matrigel implants. Mouse heart tissue sections served as a negative control, and human hemangioma tissue sections served as a positive control (Figure 5C). Some adipocytes negative for the human nuclear antigen were evident, suggesting that recruitment of murine cells into the Matrigel microenvironment may have occurred. These results with tagged, GFP-labeled HemSCs and the anti-human nuclear antigen antibody indicate that HemSCs form adipocytes at time points subsequent to vessel formation. The signals that determine whether and when a HemSC becomes an EC or an adipocyte are unknown.

Discussion

For what we believe is the first time, we have shown that CD133-selected HemSCs recapitulate human IH in a murine *in vivo* model. Clonal HemSCs produced human GLUT-1-positive microvessels at 7–14 days; after 28 days *in vivo*, human adipocytes were also detected. HemSCs that had differentiated into endothelium *in vivo*, as determined by cell-surface CD31 expression, could be retrieved and implanted into secondary recipients, where the cells formed blood vessels once more. This indicates the robust vasculogenic potential of these hemangioma-derived cells. GFP-labeled HemSCs were shown to form vessels and adipocytes at 14- and 56-day time points, respectively, confirming that the vessels and adipocytes were not host derived. Using *in vitro* cellular activity assays, we showed that HemSCs are highly proliferative and able to differentiate into multiple cell types, including adipocytes. These results demonstrate that HemSCs are the cellular precursors of IH.

CD133 has been used to identify tumor-initiating cells from medulloblastomas, glioblastomas, and prostate and colon carcinomas (16, 17, 39–42). Xenografting CD133⁺ tumor cells in immune-compromised mice has also been shown to generate a primary tumor (16, 17). In a similar fashion, we demonstrated that the CD133-selected cells from IH formed GLUT-1-positive blood vessels and, at later time points, differentiated into adipocytes, the prominent cellular compartments of proliferating and involuting phase hemangioma, respectively. For the secondary transplantation experiment (Figure 3, C and D), we reimplanted human CD31-selected cells because (a) these cells had shown vasculogenic activity in the primary implant and (b) the level of CD133 expression in the CD31-positive and CD31-negative populations recovered from the primary implant was very low, which would have made reselecting CD133-positive cells difficult. The human CD31-selected cells showed a robust degree of blood vessel formation (78 vessels/mm²) upon secondary transplantation, a finding that supports the vasculogenic potential of these cells.

To date, there are no animal models that truly reflect the human hemangioma phenotype and life cycle. Although murine hemangioendothelioma cells have been studied (43), these do not phenocopy IH or provide a hemangioma model because the tumors do not regress and are fatal within 3 months. In addition, polyoma middle T antigen-transformed cells used in animal models result in “cavernous hemangioma” lesions, more akin to malignant vascular tumors, which are also fatal within 3–10 months (44). Hem-

angioma tissue fragments xenografted into nude mice have been shown to undergo an initial period of necrosis followed by approximately 2-fold enlargement and then subsequent regression (45), which may provide a model for testing new therapies for IH. Our *in vivo* model is unique in that we are able to produce IH lesions with a small number of purified primary cells suspended in Matrigel but without exogenous growth factors or supplements. These findings implicate a preset genetic or epigenetic program in the HemSCs that directs the cells to recapitulate the IH tumor, including endothelial and adipocytic differentiation. An important future experiment will be to implant hemangioma-derived CD133⁺ cells that have not undergone *in vitro* expansion to determine whether HemSCs lose or gain hemangioma-forming potential during the *in vitro* culture. It is still not understood what triggers the adipogenesis that characterizes the involuting and involuted phases of IH. It is likely that changes in the microenvironment, such as levels of circulating EPCs, immune cells, and growth factors, initiate the regression of the tumor.

It is well established that hemangioma endothelium expresses GLUT-1, merosin, Lewis Y antigen (CD174), and Fcγ receptor II (CD32) in all 3 phases of the life cycle (34). These markers are also expressed by placental microvessels. This immunohistochemical similarity suggested the hypothesis that IH is formed from embolic placental ECs (34). Microarray analyses have supported this link between placenta and hemangioma (46). It is also possible that the progenitor cells reside in fetal and neonatal tissues or come from the BM and express a transcriptional program similar to that of placenta. To date, placenta-derived cells with *in vitro* clonogenic and differentiation abilities similar to HemSCs have not been described.

In conclusion, we have identified the hemangioma-initiating cell. We show that a single multipotential cell (HemSC) produces GLUT-1-positive microvessels and that this lesion undergoes an involutive process by differentiation into adipocytes. This could be a result of a somatic mutation within a stem or progenitor cell and subsequent expansion/differentiation. It is also plausible that a normal SC in the context of the postnatal infantile microenvironment becomes disrupted or delayed in its differentiative program, resulting in an accumulation of early progenitor cells. Such a clonal population could then undergo ill-timed or abnormal differentiation, which subsequently resolves. Understanding the cellular origin of IH provides an avenue for deciphering the mechanisms of EC differentiation and delineating targets for adjuvant therapy. Our *in vitro* and *in vivo* models of IH offer powerful tools to evaluate potential therapeutic modalities and insight into the biology of *de novo* formation of blood vessels.

Methods

Cell isolation and culture. Specimens of proliferating IH were obtained from the Department of Plastic Surgery at Children's Hospital Boston under a human subject protocol that was approved by the Committee on Clinical Investigation. The clinical diagnosis was confirmed in the Department of Pathology at Children's Hospital Boston. Informed consent was provided for the specimens, according to the Declaration of Helsinki. Single-cell suspensions were prepared from the proliferating phase specimens as described previously (13). HemSCs were selected using anti-CD133-coated magnetic beads (Miltenyi Biotec). CD133-selected cells were cultured on fibronectin-coated (1 µg/cm²) plates with EBM-2 (CC-3156; Cambrex). EBM-2 was supplemented with 20% FBS, endothelial growth media-2 SingleQuor (CC-4176; Cambrex), and 1× PSF (100 U/ml penicillin,

100 µg/ml streptomycin, 0.25 µg/ml amphotericin; Invitrogen). Hereafter this supplemented medium is called EBM-2/20% FBS.

Neonatal foreskin and umbilical cord blood were obtained with institutional research board approval from Brigham and Women's Hospital, Boston, Massachusetts, USA. HDMECs were isolated as described (13). Human umbilical cbEPCs were isolated with anti-CD133-coated magnetic beads (Miltenyi Biotec). In addition, BM-MSCs and NIHDFs were obtained commercially (Cambrex) and cultured on fibronectin-coated plates in EBM-2/20% FBS under conditions identical to those used for the HemSCs. For *in vivo* experiments and *in vitro* differentiation studies, individually expanded clones of HemSCs were prepared by a limiting dilution technique in which cells were diluted in EBM-2/20% FBS media to 3 cells/ml and dispensed at 100 µl per well in 96-well dishes. After plating and until colonies were harvested, wells were inspected to insure that only 1 colony per well was present.

To obtain GFP-expressing cells, 1.6×10^4 HemSCs were plated into wells of a 96-well plate. After 24 hours, fresh medium containing 8 µg/ml hexadimethrine bromide (Sigma-Aldrich) was added and cells were then infected with 10 µl lentiviral-based particles containing TurboGFP encoding vector (Mission TurboGFP Control Vector; Sigma-Aldrich). To obtain stable GFP expression, cells were maintained in 2 µg/ml puromycin medium (Sigma-Aldrich).

Flow cytometry. Cells were labeled with FITC-conjugated mouse anti-human CD31 (Ancell), PE-conjugated mouse anti-human CD34 (Miltenyi Biotec), FITC-mouse anti-human CD146 (Millipore), PE-mouse anti-human CD90 (Millipore), and PE-mouse anti-human neuropilin-1 (NRP-1; Miltenyi Biotec). Cells were also incubated with rabbit anti-human VEGF-R1 (Santa Cruz Biotechnology Inc.) and mouse anti-human VEGF-R2 (Clone KDR1; Sigma-Aldrich) antibody followed by FITC-conjugated secondary antibody (Vector Laboratories). VEGF-R1, VEGF-R2, and CD34 labeling were carried out after permeabilization of the cells with 0.5% saponin (Sigma-Aldrich). Flow cytometry was performed on a BD FACScan. Data were analyzed using FlowJo software.

RT-PCR. Total RNA was extracted from cultured cells using RNeasy Mini Kit (QIAGEN). Purity of the RNA samples was assessed by determining the optical density at 260:280 nm. Three micrograms of total RNA was used for cDNA synthesis using iScript cDNA Synthesis Kit (Bio-Rad). Primers for Ocr-4 (47), AML1 (48), and ribosomal S9 (13) were used. All reactions were performed for 30 cycles with the following temperature profiles: 95°C for 3 minutes (initiation); 30 s/cycle thereafter; 55°C for 45 seconds (annealing); and 72°C for 45 seconds (extension). Size of the PCR products was confirmed by running 10 µl of the reaction product on 3% agarose gel.

***In vivo* murine model of IH.** All experiments were carried out with 1.5×10^6 cells per animal. Cells were trypsinized, counted, and resuspended in phenol red-free Matrigel (BD Biosciences). The cell/Matrigel mixture (200 µl/animal) was injected s.c. into the backs of 6-week-old male athymic nu/nu mice ($n = 4$ /group; Massachusetts General Hospital, Boston, Massachusetts, USA). The animals were monitored for weight gain and Matrigel plug size at 7-day intervals throughout the period of the study. Animals were sacrificed and Matrigel plugs harvested at 7, 14, 28, and 56 days. The Matrigel plugs were then either fixed in 10% neutral buffered formalin for immunohistochemistry (described below) or used immediately for cell retrieval experiments. Cell retrieval was performed by incubating the Matrigel explants in DMEM medium supplemented with 2% FBS and 4x PSF for 1 hour. The explants were then digested with 0.2% collagenase A and 2.5 U/ml dispase (BD Biosciences). rbc were lysed by incubating the cell suspension with chilled NH₄Cl (StemCell Technologies). The cells were labeled with FITC-mouse anti-human CD31 antibody as described above. Mouse dermal ECs (kindly provided by Michael Klagsbrun, Children's Hospital Boston) were used to determine the specificity of the CD31 antibody.

Immunohistochemistry. Immunohistochemistry was performed on formalin-fixed/paraffin-embedded Matrigel plugs. The sections were deparaffinized in xylene and hydrated through sequential ethanol gradient. H&E images were captured with AxioPhor II fluorescence microscope (Zeiss) equipped with AxioCam MRC5 (Zeiss). Antigen retrieval was carried out by heating the sections in citrate-EDTA buffer (10 mM citric acid, 2 mM EDTA, 0.05% Tween-20, pH 6.2) or Tris-EDTA buffer (10 mM Trizma-base, 1 mM EDTA, 0.05% Tween-20, pH 9). The sections were blocked for 30 minutes in 5% serum and incubated with rabbit anti-human GLUT-1 antibody (1:50; A3536, Dako) or mouse anti-human GLUT-1 antibody (1:25; ab40084, Abcam), followed by FITC or Texas red-labeled secondary antibody (1:200; Vector Laboratories). The sections were washed and blocked again for 30 minutes. Human-specific CD31 monoclonal antibody (1:50; M0823 clone JC/70A, Dako) followed by Texas red anti-mouse secondary antibody (1:200; Vector Laboratories) was used for detection of human-specific microvessels. Sections were also stained for merosin (1:50; MAB1922 clone 5H2, Millipore), human-specific nuclear antigen (HuNuc, 1:25; MAB 1281 clone 235.1, Millipore), and rabbit anti-human perilipin A (perA, 1:100; P1998, Sigma-Aldrich) or goat anti-human perilipin A (1:200; ab61682, Abcam). The human nuclear antigen staining was performed using an HRP-conjugated secondary antibody followed by incubation with diaminobenzidine solution (Vector Laboratories). GFP expression was detected with anti-TurboGFP antibody (1:2000; AB511, Evrogen). The sections were counterstained with DAPI (Vector Laboratories). All fluorescent images were taken with a Leica TCS SP2 Acousto-Optical Beam Splitter confocal system equipped with DMIRE2 inverted microscope (diode 405 nm, argon 488 nm, HeNe 594 nm; Leica Microsystems).

MVD. For the assessment of MVD, 4 fields from mid-Matrigel H&E sections of each of the 4 animals in the group were used. Microvessels were quantified by counting luminal structures containing rbc. MVD was calculated as vessels/mm².

Assays for *in vitro* cellular activity. HemSCs were subjected to cellular activity assays that measured cell growth, proliferation in response to growth factors, and differentiation. Growth parameters were assessed by seeding the cells at 5000 cells/cm² on fibronectin-coated 48-well plates (4000 cells; growth surface, 0.8 cm²) and culturing in EBM-2/20% FBS. The cells were counted every day using a hemocytometer, and the medium was changed every 2 days. For proliferation experiments, cells were plated as described above. Following attachment (24 hours), plating efficiency was determined, medium was removed, and cells were serum starved for 6 hours. Proliferation was assessed after 24 hours in cells exposed to serum-free medium, 10 ng/ml VEGF-A (R&D Systems), 10 ng/ml VEGF-B (R&D Systems), 5 ng/ml bFGF (Roche Applied Science), or 5% FBS.

To study multilineage differentiation, cells were treated with induction media for 14 days. Mesenchymal differentiation of the cells was carried out with adipogenic media (DMEM/10% FBS, 5 µg/ml insulin, 1 µM dexamethasone, 0.5 mM isobutylmethylxanthine, 60 µM indomethacin, and 1x PSF), osteogenic media (DMEM/10% FBS, 1 µM dexamethasone, 10 mM β-glycerophosphate, 60 µM ascorbic acid-2-phosphate, and 1x PSF), and chondrogenic media (DMEM high glucose, 1x insulin-transferrin-selenium, 1:5000 linoleic acid-albumin, 1 µM dexamethasone, 100 µM ascorbic acid-2-phosphate, 10 ng/ml TGF-β1, and 1x PSF). EC differentiation was carried out in serum-free medium containing VEGF-B (EBM-2, 1x insulin-transferrin-selenium, 1:5000 linoleic acid-albumin, 1 µM dexamethasone, 60 µM ascorbic acid-2-phosphate, 10 ng/ml VEGF-B). For neurogenic differentiation, cells were exposed to initiating medium (DMEM/10% FBS, 1 mM β-mercaptoethanol) for 24 hours. Neurogenic induction medium (DMEM, 0.1 µM dexamethasone, 0.25 µg/ml insulin, 5 µM cAMP, 0.5 mM isobutylmethylxanthine, 5 µM trans-retinoic acid,

0.25 mM L-glutamine, and 25 ng/ml nerve growth factor) was then used for 14 days. Hematopoietic activity was assessed in MethoCult medium (StemCell Technologies) at 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 cells per 35-mm dish. Mesenchymal differentiation was assessed by oil red O (lipid droplets), alkaline phosphatase induction (substrate-based activity) and Von Kossa (mineralization), and collagen type 2 staining. Immunofluorescent analysis of cells was carried out using goat anti-human CD31 (Santa Cruz Biotechnology Inc.), mouse anti-human VE-cadherin (Immunotech), mouse anti-human tubulin β -tubulin III (Millipore), and goat anti-human GFAP (Dako) antibodies. FITC- and PE-conjugated secondary antibodies (Vector Laboratories) were used for antigen detection. Images were taken with a Nikon Eclipse TE300 (Nikon) using SPOT Advanced 3.5.9 software (Diagnostic Instruments Inc.) and a $\times 20/0.45$ objective lens.

Statistics. The data were expressed as means $\pm$ SEM and analyzed by ANOVA followed by Student's two-tailed *t* test where appropriate. Differences were considered significant at $P < 0.05$.

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- Mulliken, J.B., Fishman, S.J., and Burrows, P.E. 2000. Vascular anomalies. *Curr. Probl. Surg.* 37:517-584.
- Bischoff, J. 2002. Monoclonal expansion of endothelial cells in hemangioma: an intrinsic defect with extrinsic consequences? *Trends Cardiovasc. Med.* 12:220-224.
- Boye, E., et al. 2001. Clonality and altered behavior of endothelial cells from hemangiomas. *J. Clin. Invest.* 107:745-752.
- Bielenberg, D.R., et al. 1999. Progressive growth of infantile cutaneous hemangiomas is directly correlated with hyperplasia and angiogenesis of adjacent epidermis and inversely correlated with expression of the endogenous angiogenesis inhibitor, IPN-beta. *Int. J. Oncol.* 14:401-408.
- Berard, M., et al. 1997. Vascular endothelial growth factor confers a growth advantage in vitro and in vivo to stromal cells cultured from neonatal hemangiomas. *Am. J. Pathol.* 150:1315-1326.
- Virchow, R. 1863. *Die Krankhaften Geschwulste*. August Hirschwald, Berlin, Germany. 306 pp.
- Pack, G., and Miller, T.R. 1950. Hemangiomas; classification, diagnosis, and treatment. *Angiology*. 1:405-426.
- Malan, E. 1974. Vascular malformations (angiodysplasias). In *Carlo Erba Foundation*. Milan, Italy. 4.
- Smoller, B.R., and Apfelberg, D.B. 1993. Infantile (juvenile) capillary hemangioma: a tumor of heterogeneous cellular elements. *J. Cutan. Pathol.* 20:330-336.
- Dosanjh, A., et al. 2000. In vitro characteristics of neonatal hemangioma endothelial cells: similarities and differences between normal neonatal and fetal endothelial cells. *J. Cutan. Pathol.* 27:441-450.
- Shmelkov, S.V., St. Clair, R., Lyden, D., and Rafii, S. 2005. AC133/CD133/Prominin-1. *Int. J. Biochem. Cell Biol.* 37:715-719.
- Yu, Y., Flint, A.F., Mulliken, J.B., Wu, J.K., and Bischoff, J. 2004. Endothelial progenitor cells in infantile hemangioma. *Blood*. 103:1373-1375.
- Khan, Z.A., et al. 2006. Endothelial progenitor cells from infantile hemangioma and umbilical cord blood display unique cellular responses to endostatin. *Blood*. 108:915-921.
- Bauland, C.G., et al. 2006. The pathogenesis of hemangiomas: a review. *Plast. Reconstr. Surg.* 117:29e-35e.
- Wu, X., et al. 2004. Tissue-engineered microvessels on three-dimensional biodegradable scaffolds using human endothelial progenitor cells. *Am. J. Physiol. Heart Circ. Physiol.* 287:H480-H487.
- O'Brien, C.A., Pollert, A., Gallinger, S., and Dick, J.E. 2007. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature*. 445:106-110.
- Ricci-Viriani, L., et al. 2007. Identification and expansion of human colon-cancer-initiating cells. *Nature*. 445:111-115.
- Melero-Martin, J.M., et al. 2007. In vivo vasculogenic potential of human blood-derived endothelial progenitor cells. *Blood*. 109:4761-4768.
- Baal, N., et al. 2004. Expression of transcription factor Oct-4 and other embryonic genes in CD133 positive cells from human umbilical cord blood. *Thromb. Haemost.* 92:767-775.
- Okuda, T., van Deursen, J., Hiebert, S.W., Grosfeld, G., and Downing, J.R. 1996. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 84:321-330.
- Li, Z., Chen, M.J., Stacy, T., and Speck, N.A. 2006. Runx1 function in hematopoiesis is required in cells that express Tek. *Blood*. 107:106-110.
- Reyes, M., et al. 2002. Origin of endothelial progenitors in human postnatal bone marrow. *J. Clin. Invest.* 109:337-346.
- Jiang, Y., et al. 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. 418:41-49.
- Egusa, H., Schweizer, H.E., Wang, C.C., Marsuka, Y., and Nishimura, I. 2005. Neuronal differentiation of bone marrow-derived stromal stem cells involves suppression of discordant phenotypes through gene silencing. *J. Biol. Chem.* 280:23691-23697.
- Tondreau, T., et al. 2004. Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. *Differentiation*. 72:319-326.
- Pittenger, M.F., et al. 1999. Multilineage potential of adult human mesenchymal stem cells. *Science*. 284:143-147.
- Ritter, M.R., Reinisch, J., Friedlander, S.F., and Friedlander, M. 2006. Myeloid cells in infantile hemangioma. *Am. J. Pathol.* 168:621-628.
- Choi, K., Kennedy, M., Kazarov, A., Papadimitriou, J.C., and Keller, G. 1998. A common precursor for hematopoietic and endothelial cells. *Development*. 125:725-732.
- Ito, Y., Iwamoto, Y., Tanaka, K., Okuyama, K., and Sugioka, Y. 1996. A quantitative assay using basement membrane extracts to study tumor angiogenesis in vivo. *Int. J. Cancer*. 67:148-152.
- Hoang, M.V., and Senger, D.R. 2005. In vivo and in vitro models of mammalian angiogenesis. *Methods Mol. Biol.* 294:269-285.
- Yu, Y., et al. 2006. Mesenchymal stem cells and adipogenesis in hemangioma involution. *Stem Cells*. 24:1605-1612.
- Parums, D.V., et al. 1990. JC70: a new monoclonal antibody that detects vascular endothelium associated antigen on routinely processed tissue sections. *J. Clin. Pathol.* 43:752-757.
- North, P.E., Waner, M., Mizeracki, A., and Mihm, M.C., Jr. 2000. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum. Pathol.* 31:11-22.
- North, P.E., et al. 2001. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch. Dermatol.* 137:559-570.
- Kawaguchi, N., et al. 1998. De novo adipogenesis in mice at the site of injection of basement membrane and basic fibroblast growth factor. *Proc. Natl. Acad. Sci. U.S.A.* 95:1062-1066.
- Kimura, Y., Ozeki, M., Inamoto, T., and Tabata, Y. 2002. Time course of de novo adipogenesis in matrix by gelatin microspheres incorporating basic fibroblast growth factor. *Tissue Eng.* 8:603-613.
- Tabata, Y., et al. 2000. De novo formation of adipose tissue by controlled release of basic fibroblast growth factor. *Tissue Eng.* 6:279-289.
- Greenberg, A.S., et al. 1991. Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets. *J. Biol. Chem.* 266:11341-11346.
- Singh, S.K., et al. 2003. Identification of a cancer stem cell in human brain tumors. *Cancer Res.* 63:5821-5828.
- Salmaggi, A., et al. 2006. Glioblastoma-derived tumourspheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. *Glia*. 54:850-860.
- Collins, A.T., Berry, P.A., Hyde, C., Stower, M.J., and Mairland, N.J. 2005. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* 65:10946-10951.
- Richardson, G.D., et al. 2004. CD133, a novel marker for human prostatic epithelial stem cells. *J. Cell Sci.* 117:3539-3545.
- Obeso, J., Weber, J., and Auerbach, R. 1990. A hemangioendothelioma-derived cell line: its use as a model for the study of endothelial cell biology. *Lab. Invest.* 63:259-269.
- Bautch, V.L., Toda, S., Hassell, J.A., and Hanahan, D. 1987. Endothelial cell tumors develop in transgenic mice carrying polyoma virus middle T oncogene. *Cell*. 51:529-537.
- Tang, Y., et al. 2007. A novel in vivo model of human hemangioma: xenograft of human hemangioma tissue on nude mice. *Plast. Reconstr. Surg.* 120:869-878.
- Barnes, C.M., et al. 2005. Evidence by molecular profiling for a placental origin of infantile hemangioma. *Proc. Natl. Acad. Sci. U.S.A.* 102:19097-19102.
- Tai, M.H., et al. 2005. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. *Carcinogenesis*. 26:495-502.
- Choi, S.J., et al. 2003. AML-1A and AML-1B regulation of MIP-1alpha expression in multiple myeloma. *Blood*. 101:3778-3783.

Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action

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Summary

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Infantile haemangiomas (IH) are the most common benign tumours of infancy. Although most IH are innocuous and 85–90% regress spontaneously, some may become life- or function-threatening and require immediate treatment. Previous standard therapeutic options include physical measures (laser surgery, cryosurgery) and systemic corticosteroids, in severe cases also vincristine, α -interferon or cyclophosphamide, all bearing the risk of serious side-effects. Oral propranolol is a very recent therapeutic option for complicated IH with impressive efficacy and generally good tolerance. The effects of propranolol on IH were discovered by chance, and very little is known about its mechanisms of action in IH. Here we present a summary of current knowledge of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis. Early, intermediate and long-term effects of propranolol on IH can be attributed to three different pharmacological targets. Early effects (brightening of the haemangioma surface within 1–3 days after start of therapy) are attributable to vasoconstriction due to decreased release of nitric oxide. Intermediate effects are due to the blocking of proangiogenic signals (vascular endothelial growth factor, basic fibroblast growth factor, matrix metalloproteinase 2/9) and result in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells, and result in tumour regression.

Haemangiomas are the most common benign tumours of infancy, affecting 5–10% of all infants, and up to 30% of premature babies.¹ They can be differentiated from vascular malformations by their dynamic growth and spontaneous regression (Fig. 1a). Although 85–90% of all infantile haemangiomas (IH) eventually undergo spontaneous involution, they can still cause disfigurement and serious complications depending on their location (obstruction of airways and vision), size (cardiac insufficiency, hypothyroidism), and speed of regression, which can be associated with painful ulcerations and haemorrhage. Standard treatment options for complicated haemangiomas include oral steroids, laser surgery, cryosurgery, or vincristine, interferon or cyclophosphamide in life-threatening cases. Each of these options has its restrictions and/or side-effects. In 2008, Léauté-Labrèze *et al.*² described their serendipitous observation of an antiproliferative effect of propranolol on IH. Propranolol has since become the first choice of therapy for complicated IH, even though randomized controlled studies have not been finished yet and a generally accepted concept of how propranolol actually works in IH does not exist.

Here we present a summary of current knowledge of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis. Propranolol is a nonselective beta-adrenergic antagonist, which competitively inhibits β_1 - and β_2 -adrenoceptors with the same affinity. Propranolol is a pure antagonist without partial agonistic effects. On account of its lipophilic properties propranolol also exhibits certain membrane-stabilizing characteristics. α_1 -adrenoceptors are not antagonized by propranolol.³

The following pharmacodynamic characteristics are discussed in this review as possible mechanisms of action of propranolol in IH: vasoconstriction, inhibition of angiogenesis and induction of apoptosis.

Vasoconstriction

The vascular tone is controlled by a complex interplay of various endogenous factors. The autonomic nervous system plays a key role in the control of the vascular tone: adrenaline can cause vasoconstriction (by activating α_1 -receptors) as well as

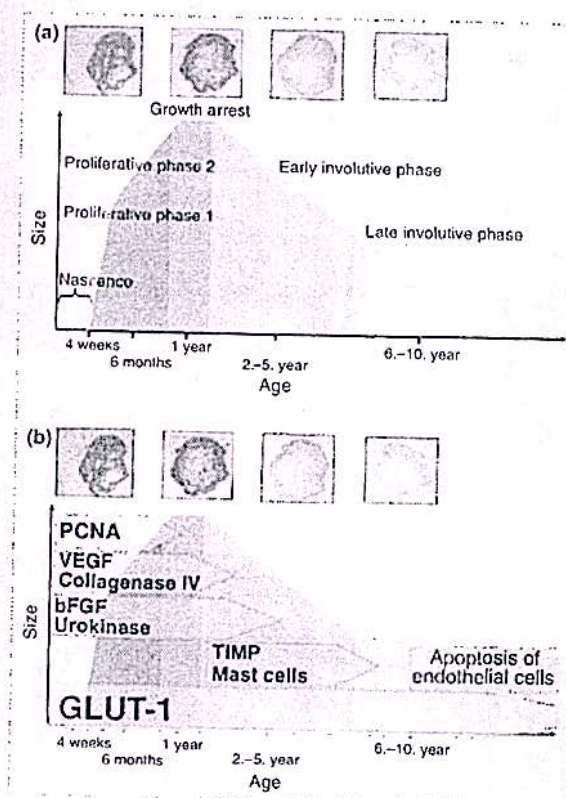


Fig. 1. (a) Growth and regression of infantile haemangiomas (IH). (b) Molecular markers characterizing individual phases of IH. PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TIMP, tissue inhibitor of metalloproteinases; GLUT-1, glucose transporter type 1.

vasodilation (by activating β_2 -receptors). Depending on the partial pressures of oxygen and carbon dioxide, respectively, the vascular tone is either increased or decreased. It is further regulated by transmitters which either lead to vasoconstriction (e.g. endothelin-1, angiotensin II, vasopressin) or to vasodilation [e.g. prostacyclin, nitric oxide (NO), dopamine].

The vasodilatory effect of adrenaline by activating β_2 -adrenoceptors is mediated by a cascade of signals⁴ (Fig. 2): β -adrenoceptors belong to the group of G_s -protein-coupled receptors⁴ and are expressed on endothelial cells.⁵ As soon as an agonist binds to the receptor, adenylate cyclase is activated by the α -subunit of the stimulatory G-protein. The adenylate cyclase converts adenosine triphosphate into cyclic adenosine monophosphate (cAMP), which acts as a second messenger and diffuses into the cytosol where it activates the cAMP-dependent protein kinase A (PKA). PKA can in turn phosphorylate intracellular proteins and thus modulate their activity. In endothelial cells the activation of PKA leads to an activation of the endothelial NO synthase, which results in the formation and release of NO. NO diffuses into the vascular smooth muscle cell, where it leads to an activation of the soluble guanylate cyclase resulting in the formation of cyclic

guanosine monophosphate (cGMP), which in turn activates protein kinase G (PKG, cGMP-dependent protein kinase). This activation of PKG finally induces relaxation of the vascular smooth muscle and vasodilation.

Beta blockers without α -antagonistic effects such as propranolol inhibit the vasodilation mediated by adrenaline via β -receptors and thus lead to vasoconstriction.³ In haemangiomas, vasoconstriction of supplying capillaries caused by propranolol induces a reduction of blood flow within the haemangioma which is associated with a visible change in colour and a palpable softening of the haemangioma. These effects can be observed within 1-3 days after the onset of therapy² (Fig. 3).

Inhibition of angiogenesis

Impact on the expression of proangiogenic growth factors (vascular endothelial growth factor and basic fibroblast growth factor)

In the proliferative phase of IH endothelial cells exhibit increased expression of proliferating cell nuclear antigen, type IV collagenase, and proangiogenic factors, in particular vascular endothelial growth factor (VEGF) more than basic fibroblast growth factor (bFGF)⁶⁻¹¹ (Fig. 1b). VEGF could be detected in various cell types both on the protein level (immunohistochemistry) and at messenger RNA level (by reverse transcription-polymerase chain reaction): endothelial cells (so-called haemangioma-derived endothelial cells, HemECs), pericytes and connective tissue cells. bFGF was also identified in mast cells.¹⁰ Serum levels of VEGF are elevated in infants during the proliferative phase of IH.^{9,12,13} Conversely, expression of VEGF and bFGF is significantly reduced during the involution phase as well as in completely regressed haemangiomas (involved phase).^{7,12,13} Transforming growth factor- β , an inhibitor of endothelial growth,¹⁴ is expressed only at a relatively low level both in the proliferation phase and the involution phase,⁷ and tissue inhibitor of metalloproteinases (TIMP), also an inhibitor of angiogenesis,¹⁴ is expressed only during the involution phase.¹¹ These and other findings are evidence that IH result from a dysregulation of angiogenesis, characterized by an imbalance between pro- and antiangiogenic factors.⁷ The expression of VEGF is regulated by the transcription rate and changes in the stability of the mRNA.¹⁵ Physiologically, hypoxaemia leads to increased expression of VEGF. This effect is mediated by the transcription factor hypoxia-inducible factor (HIF)-1 α : oxygen deficiency leads to an increase of the intracellular concentration of HIF-1 α in its active form. HIF-1 α induces the transcription of the VEGF gene. As a result, VEGF is secreted from the cell, diffuses into the surrounding tissue and induces proliferation of adjacent endothelial cells, which in turn leads to secretion of proteases necessary for reorganization of the extracellular matrix [e.g. matrix metalloproteinases (MMPs); see below], and the coordinated differentiation of vascular cells (endothelial cells, smooth muscle cells, pericytes) into functional

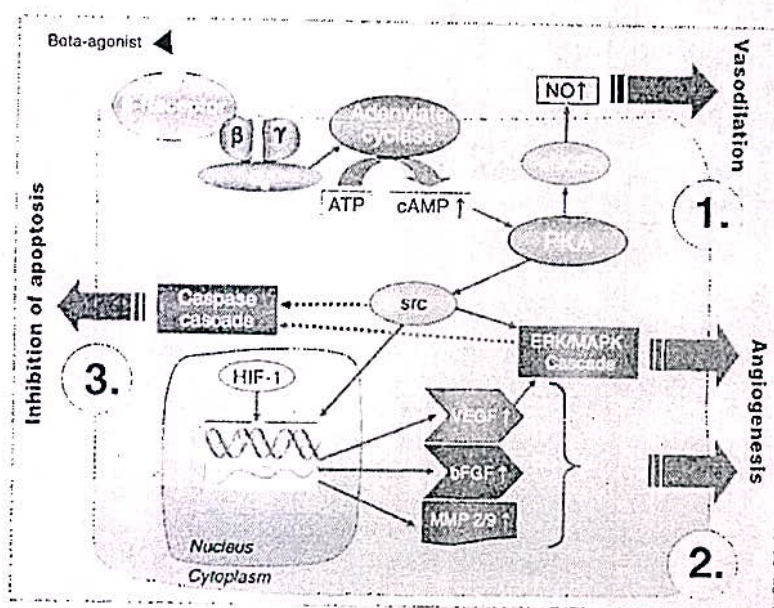


Fig 2. Molecular processes in infantile haemangiomas (IH) that may be affected by propranolol. The figure depicts the pathophysiological situation in IH in the absence of a beta-adrenergic antagonist. (1) Control of vascular tone. Beta-adrenergic agonists (black triangle) lead to vasodilation via release of NO. Conversely, beta-adrenergic antagonists like propranolol lead to vasoconstriction (through inhibition of NO synthesis and NO release). (2) Angiogenesis. Beta-adrenergic agonists (black triangle) stimulate the synthesis of proangiogenic factors [growth factors (VEGF and bFGF) and matrix metalloproteinases (MMP-2 and MMP-9)] and activate proangiogenic cascades (ERK/MAPK cascade) thereby promoting angiogenesis. In contrast, beta blockers like propranolol lead to a downregulation of these proangiogenic proteins and to an inhibition of the ERK/MAPK cascade thus repressing angiogenesis. (3) Apoptosis. Beta-adrenergic agonists (black triangle) inhibit apoptosis via src/MAPK. In contrast, beta blockers induce apoptosis. Continuous arrows indicate activation; dashed arrows indicate inhibition; broad arrows indicate pharmacodynamic effects; ↑ indicates upregulation. β, γ, α, three subunits of the stimulatory G-protein (heterotrimer); ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A (cAMP-dependent protein kinase); ERK, extracellular signal-related kinases; MAPK, mitogen-activated protein kinases; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MMP, matrix metalloproteinase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; HIF-1, hypoxia-inducible factor 1.

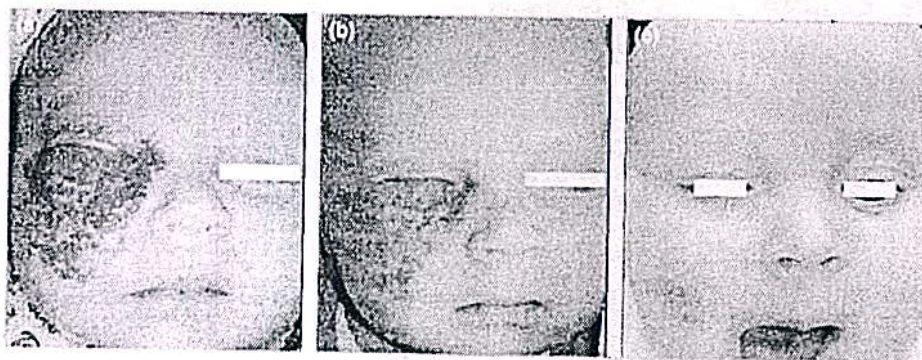


Fig 3. Effect of propranolol on proliferating infantile haemangiomas. (a) Age 8 weeks, before start of therapy; (b) age 9 weeks, 1 week after start of therapy with propranolol 2 mg kg⁻¹ daily; (c) age 8 months, after 6 months of treatment.

vessels (angiogenesis). The newly formed vessels improve local oxygen supply, which in turn leads to decreased intracellular concentration of the active form of HIF-1α and, as a result, reduces the expression of VEGF. Thus, under physiological conditions, angiogenesis is strictly controlled.^{15,16} As perinatal hypoxaemia has been identified as an important aeti-

ological factor in IH,¹⁷ increased production of HIF-1 and consecutively VEGF (as has been shown in proliferating IH¹²) seems a 'logical' consequence.^{9,18,19}

There is evidence from recent publications that the expression of VEGF is not only controlled by the oxygen partial pressure in the tissue (via HIF-1α), but also by adrenergic

stimulation (Fig. 2): as demonstrated in different *in vitro* and *in vivo* models catecholamines such as adrenaline and noradrenaline can induce the expression of VEGF.²⁰⁻²⁴ Src is a mediator downstream to PKA²⁰ which belongs to a family of cytoplasmic tyrosine kinases involved in the control of diverse molecular processes. It activates the extracellular signal-related kinases (ERK)/mitogen-activated protein kinases (MAPK) cascade.²⁰ ERK and MAPK are serine/threonine kinases phosphorylating nuclear transcription factors, thus regulating the expression of multiple genes involved in the control of cell proliferation. VEGF itself exerts its proangiogenic effects, at least partially, by activating the ERK/MAPK cascade.^{21,25} Thus, the proliferation of endothelial cells is stimulated via β_2 -adrenoceptors by two different mechanisms: (i) stimulation of β_2 -adrenoceptors directly leads to an activation of ERK/MAPK (probably via src as mediator²⁰) and (ii) it also induces increased release of VEGF, which itself can activate the ERK/MAPK cascade.^{21,25}

Conversely, beta blockers like propranolol lead to a reduced expression of VEGF and thus to an inhibition of angiogenesis.^{20,22,24,26} Furthermore, it has been demonstrated in animal experiments that carvedilol, also a nonselective beta-antagonist like propranolol, reduces the expression of HIF-1 α .^{27,28} Assuming that dysregulation of endothelial cell proliferation represents an important pathogenetic factor for IH, the anti-VEGF activity of beta blockers would explain their remarkable effect on proliferating haemangiomas. Interestingly, a similar effect has recently been demonstrated for corticosteroids,²⁹ which have been the mainstay of IH therapy until now.

Impact on the expression of matrix metalloproteinases

MMPs are a family of soluble and membrane-anchored proteinases involved in degradation and transformation of extracellular matrix proteins. They play a key role in different physiological and pathophysiological processes, e.g. cell proliferation, migration and adhesion, embryogenesis, wound healing and also in angiogenic processes relevant for tumour growth and metastasis.³⁰ Under physiological conditions the activity of MMPs is strictly regulated on different levels: transcription, activation of inactive precursors (zymogens), interaction with components of the extracellular matrix and inhibition by endogenous inhibitors such as TIMPs.³¹

Elevated concentrations of MMP-2 and MMP-9 were found in tissue samples and in the blood of infants in the proliferative phase of haemangiomas.^{9,32} MMP-9 is important for the migration of endothelial cells and tubulogenesis.³³ It has been shown that inhibition of MMP-9 impedes angiogenesis of human microvascular endothelial cells.³⁴

There is ample evidence that the expression of MMP-9 and MMP-2 is regulated via β -adrenoceptors^{23,24,33,35} (Fig. 2). β -adrenoceptor agonists such as adrenaline or noradrenaline increase the expression of MMP-2 and MMP-9; this effect can be inhibited by propranolol.^{23,24,33,35} Reduced expression of MMP-9 by propranolol leads to an inhibition of tubulogenesis of endothelial cells³³ and thus also provides

a conclusive mechanism for the antiangiogenic effect of propranolol.

Induction of apoptosis in capillary endothelial cells of haemangiomas

Apoptosis is strictly regulated via a complex interplay of caspases, procaspases and proteins of the B-cell lymphoma 2 (bcl-2) family. During the proliferative phase the rate of apoptosis is low in IH. However, during the involution phase it increases by a factor of 5 while the expression of the apoptosis-inhibiting protein bcl-2 decreases in parallel.¹⁶ Only about one-third of the apoptotic cells are of endothelial origin.³⁷ Blockade of β -adrenoceptors by propranolol can induce apoptosis of different cell types (Fig. 2) *in vitro*, e.g. endothelial cells³⁸ or pancreas carcinoma cells,³⁹ respectively. The β_2 -adrenoceptor was identified as probable receptor, as the β_1 -selective beta blocker metoprolol showed significantly lower apoptosis-inducing effects, and the β_2 -selective beta blocker butoxamine exhibited a considerably stronger induction of apoptosis compared with propranolol.³⁹ The authors hypothesize that beta-adrenergic antagonists are capable of disengaging the inhibition of apoptosis caused by beta-adrenergic agonists (mediated by src, MAPK and caspase cascade), resulting in an increased apoptosis rate.³⁹ Induction of apoptosis thus represents another possible mechanism of action of propranolol in the treatment of IH.

Conclusion

The striking effect of propranolol on growing IH can be attributed to three molecular mechanisms: vasoconstriction, inhibition of angiogenesis, and induction of apoptosis. They correspond to early (brightening of haemangioma surface), intermediate (growth arrest), and long-term (regression) clinical observations. Apoptosis is not complete, as partial regrowth of haemangiomas after discontinuation of propranolol has been observed. Propranolol has been used in young infants for a variety of indications (such as hypertension, congestive heart failure, supraventricular tachycardia, long QT-syndrome, thyrotoxicosis), at doses up to 8 mg kg⁻¹ daily.⁴⁰ During 40 years of extensive clinical experience no serious cardiovascular event was recorded for children on chronic beta-blocker therapy.⁴¹ Complications of propranolol treatment of IH reported so far (hypotension, sinusbradycardia, hypoglycaemia)⁴² were not life-threatening, but certainly warrant careful monitoring of all infants with IH before and during propranolol therapy. These effects appear, however, minor compared with the serious side-effects of more 'conventional' antiangiogenic agents such as interferon- α , which is associated with spastic diplegia in up to 25%,⁴³ or the serious side-effects of systemic corticosteroid therapy. Based on case reports and uncontrolled studies the effective dosage for propranolol for IH is 2 mg kg⁻¹ daily in three divided doses, and treatment should be continued until the end of the proliferation phase.^{2,44-46} However, to assess optimal dose and duration of treatment further studies are required.

What's already known about this topic?

- Propranolol is a new therapeutic option with impressive efficacy for life- or function-threatening infantile haemangiomas (IH).

What does this study add?

- This review summarizes the current knowledge on the possible pharmacological targets of propranolol in IH and presents a conclusive model of the different molecular mechanisms of action involved.

Acknowledgments

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References

- 1 Drolet BA, Swanson EA, Frieden IJ et al. Infantile hemangiomas: an emerging health issue linked to an increased rate of low birth weight infants. *J Pediatr* 2008; 153:712–15.
- 2 Léauté-Labrèze C, de la Roque ED, Hubiche T et al. Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008; 358:2649–51.
- 3 Westfall TC, Westfall DP. Adrenergic agonists and antagonists. In: Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (Brunton LL, Lazo JS, Parker KL, eds), 11th edn. New York: McGraw-Hill, 2006; 271–95.
- 4 Westfall TC, Westfall DP. Neurotransmission: the autonomic and somatic motor nervous systems. In: Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (Brunton LL, Lazo JS, Parker KL, eds), 11th edn. New York: McGraw-Hill, 2006; 167.
- 5 Guimarães S, Moura D. Vascular adrenoceptors: an update. *Pharmacol Rev* 2001; 53:319–56.
- 6 Berard M, Sordello S, Ortega N et al. Vascular endothelial growth factor confers a growth advantage in vitro and in vivo to stromal cells cultured from neonatal hemangiomas. *Am J Pathol* 1997; 150:1315–26.
- 7 Chang J, Most D, Bresnick S et al. Proliferative haemangiomas: analysis of cytokine gene expression and angiogenesis. *Plast Reconstr Surg* 1999; 103:1–9.
- 8 Giannoulaki A, Arvanitidou V, Hatzimichael A et al. The HIF-2 α /VEGF pathway activation in cutaneous capillary haemangiomas. *Pathology* 2005; 37:149–51.
- 9 Kleinman ME, Greives MR, Shurgin SS et al. Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with haemangioma. *Arterioscler Thromb Vasc Biol* 2007; 27:2664–70.
- 10 Qu Z, Liebler JM, Powers MR et al. Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol* 1995; 147:564–73.
- 11 Takahashi K, Mulliken JB, Kozakewich HPW et al. Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994; 93:2357–64.
- 12 Przewratil P, Sirkiewicz A, Wyka K et al. Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in children with hemangiomas and vascular malformations – preliminary report. *Pediatr Dermatol* 2009; 26:399–404.
- 13 Przewratil P, Sirkiewicz A, Andrzejewska E. Local serum levels of vascular endothelial growth factor in infantile hemangioma: intriguing mechanisms of endothelial growth. *Cytokine* 2010; 49:141–7.
- 14 Folkman J. Angiogenesis inhibitors generated by tumors. *Mol Med* 1995; 1:120–2.
- 15 Alberts B, Johnson A, Lewis J et al. Blutgefäße und Endothelzellen. In: *Molekularbiologie der Zelle* (Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P, eds; translation by Jaenicke L), 4th edn. Weinheim: Wiley-VCH Verlag, 2004; 1492–7.
- 16 Hickey MM, Simon MC. Regulation of angiogenesis by hypoxia and hypoxia-inducible factors. *Curr Top Dev Biol* 2006; 76:217–57.
- 17 Colonna V, Resta L, Napoli A et al. Placental hypoxia and neonatal haemangioma: clinical and histological observations. *Br J Dermatol* 2010; 162:208–9.
- 18 Boye E, Jimin M, Olsen BR. Infantile hemangioma: challenges, new insights, and therapeutic promise. *J Craniofac Surg* 2009; 20 (Suppl. 1):678–84.
- 19 Léauté-Labrèze C, Taib A. Efficacité des bêtabloquants dans les hémangiomes capillaires infantiles: signification physiopathologique et conséquences thérapeutiques. *Ann Dermatol Venerol* 2008; 135:860–2.
- 20 Fredriksson JM, Lindquist JM, Bronnikov E et al. Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a beta-adrenoceptor/cAMP/protein kinase A pathway involving src but independently of Erk1/2. *J Biol Chem* 2000; 275:13802–11.
- 21 Iaccarino G, Ciccarelli M, Sorrento D et al. Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression – a novel role for the endothelial adrenergic system. *Circ Res* 2005; 97:1182–9.
- 22 Lutgendorf SK, Cole S, Costanzo E et al. Stress-related mediators stimulate vascular endothelial growth factor secretion by two ovarian cancer cell lines. *Clin Cancer Res* 2003; 9:4514–21.
- 23 Thaker PH, Han LY, Kamat AA et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 2006; 12:939–44.
- 24 Yang EV, Sood AK, Chen M et al. Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res* 2006; 66:10357–64.
- 25 Parenti A, Morbidelli L, Cui XL et al. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase 1/2 activation in postcapillary endothelium. *J Biol Chem* 1998; 273:4220–6.
- 26 Al-Wadei HA, Al-Wadei MH, Schuller HM. Prevention of pancreatic cancer by the beta-blocker propranolol. *Anticancer Drugs* 2009; 20:477–82.
- 27 Shyu KG, Liou JY, Wang BW et al. Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1 α and vascular endothelial growth factor in pressure-overloaded rat heart. *J Biomed Sci* 2005; 12:409–20.
- 28 Shyu KG, Lu MJ, Chang H et al. Carvedilol modulates the expression of hypoxia-inducible factor-1 α and vascular endothelial growth factor in a rat model of volume-overload heart failure. *J Card Fail* 2005; 11:152–9.
- 29 Greenberger S, Boscolo E, Adini I et al. Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells. *N Engl J Med* 2010; 362:1005–13.
- 30 Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; 92:827–39.

- 31 Nagase H, Woessner JF. Matrix metalloproteinases (minireview). *J Biol Chem* 1999; 274:21491-4.
- 32 Zhong S, Yang G, Xia C et al. Expression of matrix metalloproteinase and its tissue inhibitor in haemangioma. *J Huazhong Univ Sci Technol Med Sci* 2009; 29:614-19.
- 33 Annabi B, Lachambre MP, Plouffe K et al. Propranolol adrenergic blockade inhibits human brain endothelial cells tubulogenesis and matrix metalloproteinase-9 secretion. *Pharmacol Res* 2009; 60:438-45.
- 34 Jadhav U, Chigurupati S, Lakka SS et al. Inhibition of matrix metalloproteinase-9 reduces in vitro invasion and angiogenesis in human microvascular endothelial cells. *Int J Oncol* 2004; 25:1407-14.
- 35 Sood AK, Bhatti R, Kamat AA et al. Stress hormone-mediated invasion of ovarian cancer cells. *Clin Cancer Res* 2006; 12:369-75.
- 36 Mancini AJ, Smoller B. Proliferation and apoptosis within juvenile capillary hemangiomas. *Am J Dermatopathol* 1996; 18:505-14.
- 37 Razou MJ, Kräling BM, Mulliken JB et al. Increased apoptosis coincides with onset of involution in infantile hemangioma. *Microcirculation* 1998; 5:189-95.
- 38 Sommers Smith SK, Smith DM. Beta blockade induces apoptosis in cultured capillary endothelial cells. *In Vitro Cell Dev Biol Anim* 2002; 38:298-304.
- 39 Zhang D, Ma Q, Shen S et al. Inhibition of pancreatic cancer cell proliferation by propranolol occurs through apoptosis induction. *Pancreas* 2009; 38:94-100.
- 40 Garin EH, Araya CE. Treatment of systemic hypertension in children and adolescents. *Curr Opin Pediatr* 2009; 21:600-4.
- 41 Love JN, Sikka N. Are 1-2 tablets dangerous? Beta-blocker exposure in toddlers. *J Emerg Med* 2004; 26:309-14.
- 42 Lawley LP, Siegfried E, Todd JL. Propranolol treatment for hemangioma of infancy: risks and recommendations. *Pediatr Dermatol* 2009; 26:610-14.
- 43 Michaud AP, Bauman NM, Burke DK et al. Spastic diplegia and other motor disturbances in infants receiving interferon-alpha. *Laryngoscope* 2004; 114:1231-6.
- 44 Buckmiller LM, Munson PD, Dymenahalli U et al. Propranolol for infantile hemangiomas: early experience at a tertiary vascular anomalies center. *Laryngoscope* 2010; 120:676-81.
- 45 Manunza F, Syed S, Laguda B et al. Propranolol for complicated infantile haemangiomas: a case series of 30 infants. *Br J Dermatol* 2010; 162:466-8.
- 46 Sans V, Dumas de la Roque E, Berge J et al. Propranolol for severe infantile hemangiomas: follow-up report. *Pediatrics* 2009; 124:423-31.

Pathogenesis of infantile haemangioma: new molecular and cellular insights

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Sheila F. Friedlander^{1,2,*}

Infantile haemangioma is the most common tumour of infancy, yet the origin of these lesions remains controversial and the predictable life cycle is poorly understood. Much new information on infantile haemangiomas has emerged over the past decade, but experts continue to debate fundamental features, including cell of origin, nonrandom distribution, and mechanisms regulating the sometimes explosive growth and slow involution. The development of useful laboratory models has been difficult, in turn restricting the development of treatment options available to the clinician. Despite this, new research and creative thinking has spawned several hypotheses on the origin of these tumours and their interesting clinical behaviour, including suggestions of an intrinsic defect in local endothelial cells, a contribution of circulating endothelial progenitors or haemangioblasts, embolisation of shed placental cells and developmental field defects. While no single hypothesis seems to describe all features of infantile haemangioma, continued research seeks to integrate these ideas, create a better understanding of these important tumours and bring new treatments to the clinic.

Infantile haemangiomas are vascular tumours that exhibit a generally predictable life cycle consisting of rapid growth in the first months of life followed by a variable period of involution spanning months to years. Occasionally, these tumours can cause significant cosmetic deformity or even induce life-threatening complications. There is no uniformly safe and effective treatment option for these problematic tumours. A better understanding of the pathophysiology of these

lesions, and vascular anomalies in general, is needed in order to develop appropriate therapies. Progress in this field has been hampered by lack of a clear and accurate nomenclature to distinguish between different types of vascular lesions as well as lack of an animal model that faithfully mimics the human condition. Fundamental information such as incidence, commonly estimated at 10%, is based on older studies that may have included lesions

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other than infantile haemangiomas. In 1982, Mulliken and Glowacki proposed a classification scheme that has been generally accepted and also promoted the use of common terminology in this field (Ref. 1). The International Society for the Study of Vascular Anomalies (<http://www.issva.org>) has adopted this nomenclature and has raised awareness within the medical community about the important distinctions between various lesions, as well as advancing our understanding of the clinical and biological characteristics of vascular anomalies (Table 1). Infantile haemangiomas possess interesting and complex characteristics that hold relevance to the fields of vascular biology, stem cell biology, oncology, immunology, dermatology and others.

Clinical characteristics

Infantile haemangiomas are at least three times more likely to develop in females than in males,

and premature infants are at significantly higher risk (Refs 2, 3). Those of Caucasian heritage appear more likely to develop haemangiomas, as are infants whose mothers suffered from placental abnormalities during gestation or delivery (Ref. 4). Haemangiomas vary both in age of onset and clinical characteristics (Fig. 1). The classic presentation is that of a completely normal examination at birth with subsequent development of a red spot on the head or neck during the first few weeks to months of life. However, a small fraction of haemangiomas are 'fully formed' at birth. These congenital lesions have been shown to differ from infantile haemangioma and thus likely represent a distinct vascular tumour. Infantile haemangiomas may initially exhibit a 'precursor lesion', which appears as a blanched area of pallor, sometimes with superimposed telangiectases. A bright red patch subsequently

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Table 1. Classification and features of vascular birthmarks^a

Birthmark	Other terms	Notable features
Vascular tumours		
Infantile haemangioma	Juvenile, strawberry, or capillary haemangioma	Perinatal presentation, growth and involution phases
Kaposiform haemangioendothelioma		Associated with Kasabach-Merritt phenomenon
Tufted angioma		Associated with Kasabach-Merritt phenomenon
Pyogenic granuloma	Lobular capillary haemangioma	Found in all age groups
Haemangioepithelioma		
Vascular malformations^b		
Capillary	Port wine stain	Present at birth
Venous		Present at birth; static or slow growth; thin, distended vessels
Lymphatic	Lymphangioma or cystic hygroma	
Arteriovenous		

^aThe table is adapted from Ref. 108 [Hand, J.L. and Frieden, I.J. (2002) Vascular birthmarks of infancy: resolving nosologic confusion. *Am J Med Genet* 108 (4), 257-264] (Copyright © 2002 Wiley-Liss, Inc.), with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.
^bWell-defined combined malformations: capillary-lymphatic-venous (so-called CLVM – includes most cases of Klippel Trenaunay); capillary-venous (includes many milder cases of Klippel Trenaunay); capillary-venous with arteriovenous shunting and/or fistulae (Parkes-Weber syndrome); cutis marmorata telangiectatica congenita; lymphatic-venous.

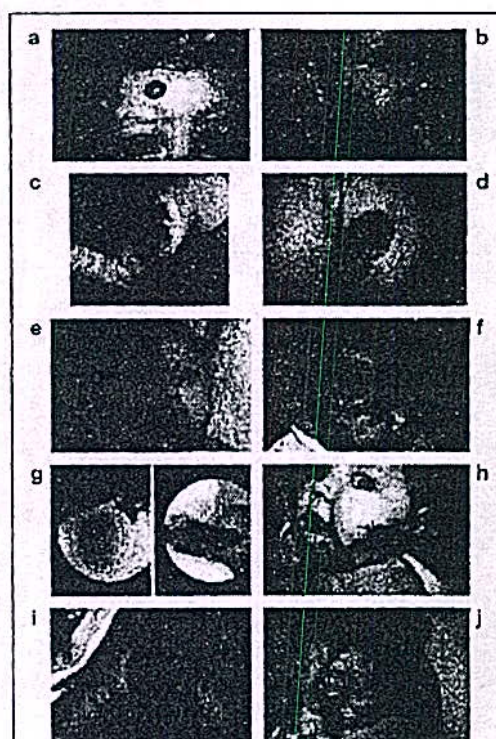
develops within this area, followed by rapid growth of a papular or nodular mass over the next weeks to months.

The clinical significance of haemangiomas varies greatly, and is often linked to their location, size and type (Ref. 5). Although they can present anywhere on the body, they have a predilection for the head and neck regions, where they have the potential to obstruct the visual axis or interfere with breathing and eating. Genital lesions are at higher risk for erosion and pain, as are any lesions that develop in an area where friction may occur. Lesions located in the lumbosacral area may be a marker for spinal dysraphism, and haemangiomas that involve the 'beard' area (neck, chin, pre-

auricular area) may indicate the presence of airway haemangiomas (Ref. 6). In each of these circumstances, appropriate evaluations to rule out such possibilities should be performed.

Haemangiomas are generally categorised based on certain morphological characteristics, and antiquated terms such as 'strawberry' and 'cavernous' have been supplanted by more accurate terms such as 'superficial', 'deep' or 'mixed'. Superficial lesions usually consist of red lobulated papules, plaques or nodules that appear on the surface of the skin. Deep lesions are usually less defined, located beneath the skin in subcutaneous fat, and may possess a blue tint. A 'combined' lesion will possess both deep and superficial qualities. Others have further subdivided lesions as localised, segmental, indeterminate and multifocal (Ref. 7). Segmental haemangiomas are generally larger, plaque-like lesions (Refs 8, 9), and are more likely to be associated with underlying syndromes such as PHACES (Refs 10, 11), a constellation of findings including posterior fossa cranial anomalies, haemangioma, arterial and cardiac defects, eye and sternal anomalies. Segmental morphology is also more likely to portend complications and need for active intervention and therapy.

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Haemangiomas present with varying appearances

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Figure 1. Haemangiomas present with varying appearances.

Figure 1. Haemangiomas present with varying appearances. (a) Superficial focal haemangioma; (b) combined haemangioma. These types of lesions may leave a parchment-like scar but will not cause significant deformity. (c) Congenital haemangioma. This lesion involuted during the first year of life. (d) Ulcerated haemangioma. This location is associated with significant pain and risk of infection. (e) Large ulcerated facial haemangioma obstructing the visual axis. This case is a medical emergency necessitating intervention. (f) Segmental haemangioma associated with PHACES ('posterior fossa cranial anomalies, haemangioma, arterial and cardiac defects, eye and sternal anomalies'). (g) Subglottic haemangioma associated with (h) 'beard-like' haemangioma distribution. (i) Lumbosacral haemangioma associated with tethered cord. (j) Large haemangioma obstructing vision and displacing eyelid and orbit; this requires immediate intervention. Images were obtained from patients being treated at The Children's Hospital and Health Center (San Diego, CA, USA) and were reproduced with consent.

The majority of haemangiomas progress through a sequential life cycle that begins with a rapid proliferative phase lasting approximately 6 months, followed by continued slower growth during the first year of life (Fig. 2). The proliferative phase is followed by what appears clinically to be a more quiescent or 'plateau' phase where cellular proliferation and apoptosis offset each other. When cell death outpaces proliferation, this marks the transition to a longer period of involution that can last months to years: 10% of haemangiomas have completely regressed at 1 year of age, 50% by 5 years of age, 70% by 7 years, and so on. Following involution, some patients are left with an area of redundant cutaneous and/or fibrofatty tissue, while others manifest a central

area of thin, parchment-like tissue, sometimes with superimposed telangiectasia.

Histopathological evaluation of haemangioma tissue reveals findings dependent on the phase of the haemangioma life cycle (Ref. 12). Early proliferating lesions show compact areas of uniform-appearing endothelial cells arranged in cords and nodules. Endothelial-cell-lined lumina, if present, are subtle in early lesions, but become more apparent in lesions at later points in development, often displaying thickened, multilaminated basement membranes. In older, involuting lesions the lumina become narrower and the presence of fibrofatty tissue is often observed.

The treatment of haemangiomas is predicated on the degree of functional compromise, either

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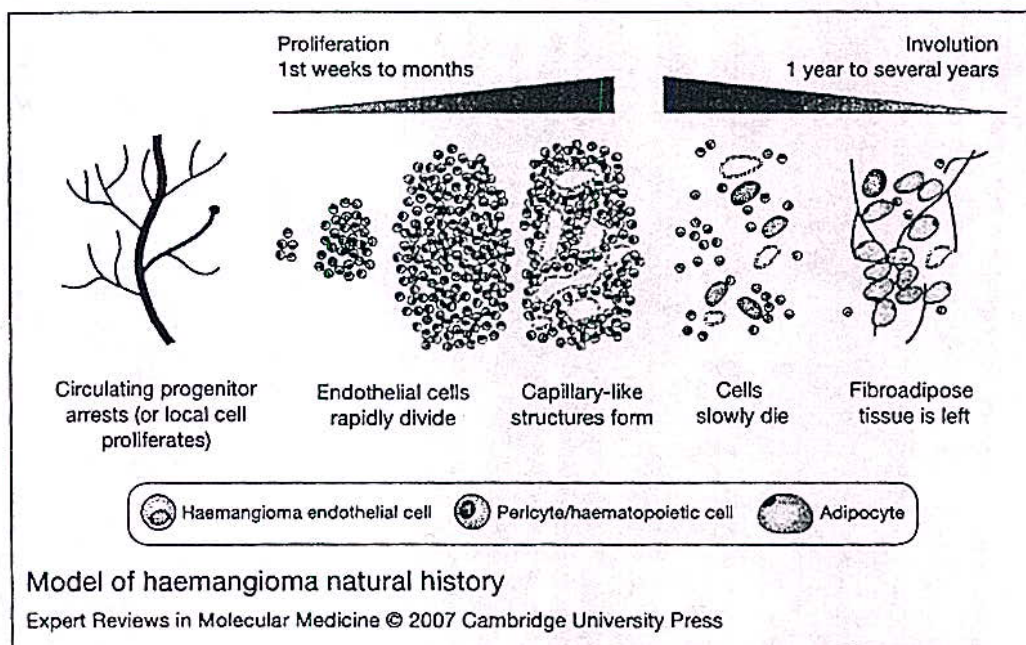


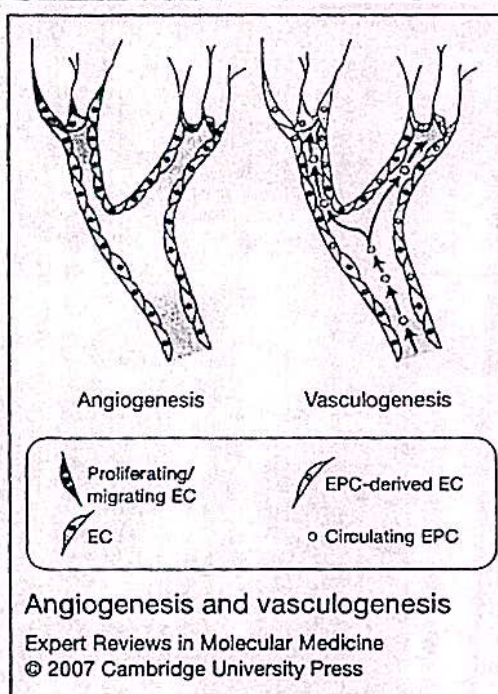
Figure 2. Model of haemangioma natural history. Recent evidence suggests that haemangiomas originate from the clonal expansion of a progenitor cell. The progenitor cells may be of placental origin or blood-borne endothelial progenitor cells. Alternatively, local cells under the influence of unusual conditions may serve as progenitors. The haemangioma progenitor cell may embolise or extravasate and begin dividing under appropriate conditions. The growing tumours comprise haemangioma endothelial cells (red) as well as nonendothelial cells, including haematopoietic cells and pericytes (blue). The early lesion is largely a featureless mass of cells, but sometime later in the proliferative phase, lumenised capillary-like structures are organised. The involution phase is characterised by increased apoptosis and the regression of both the endothelial and nonendothelial compartments. After involution, adipocytes may be found at the site along with a fibrous matrix.

present or predicted. 'Watchful waiting' is appropriate in the majority of cases, when the size, type and location of the lesion indicate that functional impairment or significant cosmetic concerns are unlikely to occur. Ulcerated lesions may require topical antimicrobials and antifungal agents, barrier agents such as zinc oxide formulations, pain relief, and laser treatment if the aforementioned therapy fails to lead to improvement. Oral corticosteroids are the standard of care for lesions that cause functional impairment or significant cosmetic deformity (Ref. 13). Topical and intralesional corticosteroids are occasionally useful, while interferon and vincristine therapy are reserved for refractory, life-threatening lesions. Recently, becaplermin gel [recombinant human platelet-derived growth factor (PDGF)] (Ref. 14) and imiquimod (Refs 15, 16, 17) (see later) have been used to treat haemangiomas.

Aetiology: angiogenesis, vasculogenesis or neither?

Infantile haemangiomas appear, in some respects, to be the product of disorganised and rapid growth of blood vessels. In fact, there are a number of similarities between normal blood vessels and the structures found in these benign infantile tumours. Haemangiomas are primarily composed of endothelial cells possessing components of a basement membrane, such as type IV collagen, and associated pericytes (Ref. 18). During proliferation, both normal endothelial cells and haemangioma endothelial cells express angiogenesis-related receptors E-selectin (Ref. 19) and integrins $\alpha\beta3$ and $\alpha5\beta1$ (Ref. 20). Factors previously associated with normal angiogenesis have also been reported to be expressed in haemangiomas, such as basic fibroblast growth factor (bFGF) (Refs 18, 21, 22). Earlier studies reported expression of vascular endothelial growth factor (VEGF) in proliferating haemangiomas (Refs 22, 23), but more recent investigations show that VEGF levels are relatively low (Refs 20, 24), suggesting a limited role for VEGF in haemangiomas.

Normal blood vessels typically arise from one of two processes: angiogenesis or vasculogenesis (Fig. 3). Angiogenesis is defined as the growth of new blood vessels via extension and remodelling from existing vasculature (Ref. 25), while vasculogenesis involves the development of vessels that



Angiogenesis and vasculogenesis

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Figure 3. Angiogenesis and vasculogenesis. Normal blood vessels grow by one or a combination of these mechanisms. During angiogenesis, existing vessels branch and are extended through the action of local endothelial cells that proliferate and migrate in response to angiogenic factors. Vasculogenesis, in contrast, relies on progenitors, either circulating or in situ, as a source of new endothelium. EC, endothelial cell; EPC, endothelial progenitor cell.

differentiate from precursor cells in situ. While numerous studies have attributed haemangioma pathogenesis to angiogenesis, more recent investigations suggest that vasculogenesis may have an important role in haemangioma development.

The first endothelial structures to form in the embryo are the endocardium and great vessels (Ref. 26); both are examples of vasculogenesis. The cells of these structures originate from mesoderm-derived haemangioblasts, which are believed to function as progenitors to both haematopoietic and endothelial cells (Ref. 27). This is relevant to understanding haemangioma pathophysiology since endothelial cells from haemangiomas coexpress haematopoietic and

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endothelial cell markers (see section 'Progenitor cells in haemangioma'). Angiogenesis is active later in development and occurs in the normal adult under certain conditions such as in the endometrium during the female reproductive cycle and during wound healing. Angiogenesis plays an important role in many pathological conditions, including solid-tumour growth, arthritis and diseases of the eye (Ref. 28). While some aspects of angiogenesis and/or vasculogenesis are likely operational in the growth of haemangiomas, the growth of these tumours differs in several important ways from typical angiogenesis or vasculogenesis.

Haemangioma pathogenesis: assembling the clues

Intrinsic defect hypothesis

Although no universal consensus exists, a number of hypotheses regarding haemangioma aetiology have emerged and some have gained significant support (Refs 29, 30). One hypothesis predicts that the basic defect in haemangiomas is a genetic mutation of the endothelial cell, or its progenitor. Linkage studies have identified six families that appear to have a predisposing genetic mutation and high incidence of haemangioma and/or vascular anomalies (Ref. 31). In these families, haemangiomas segregated as an autosomal dominant trait and a subsequent study established a linkage to chromosome 5q31-33 (Ref. 32), which harbours the genes for FGF receptor 4 (FGFR-4), PDGF receptor beta (PDGF-R β), and FMS-related tyrosine kinase 4 (FLT-4). These genes have known roles in regulating angiogenesis, but it remains to be determined if abnormalities in these genes can contribute to haemangioma pathogenesis. These genetic studies, while important, do not address the common sporadic form, leaving the causative defect in the majority of haemangiomas a mystery.

While previous investigations have presumed that haemangioma growth is the result of uncontrolled, disorganised angiogenesis, other studies support the hypothesis that haemangiomas result from a somatic mutation in a single endothelial (or progenitor) cell (Ref. 33). This hypothesis predicts that haemangiomas should be clonal in nature and suggests that mechanisms other than angiogenesis may be involved. These studies

used the X-linked human androgen receptor gene assay (HUMARA) (Ref. 34), which exploits the fact that, in females, cells generally inactivate one copy of the X chromosome (to maintain appropriate gene dosing) in a random fashion. When a tissue or sample of isolated cells shows a bias towards inactivation of one allele, this is an indication that these cells may have been derived from a single parent cell. The first study used haemangioma endothelial cells isolated from seven heterozygous female patients. Cells grown in culture from all seven patients showed a skewing toward a single allele (Ref. 35). A subsequent study analysed intact tissue with no selection of cells and similarly found some degree of allelic loss in 12 of 14 samples (Ref. 36). Thus, two independent investigations suggest that haemangiomas may arise as a consequence of clonal expansion from a single cell. It remains possible, however, that sampling constraints biased these results. Since it is usually only possible and/or practical to sample a portion of the lesion, it is conceivable that clonality accounts for only portions of the lesion. When considering segmental haemangiomas, it would seem unlikely that this variety could arise from a single cell since the plaque-like nature of these lesions would require large numbers of cells spread over the region to expand simultaneously. Despite these limitations, the evidence of clonality in haemangiomas significantly impacts how we think of these lesions and may help focus future investigative efforts. These and other studies clearly suggest that haemangioma growth does not come about by typical angiogenesis.

Also predicted from the intrinsic defect hypothesis is that cells harbouring a mutation affecting their growth properties should behave abnormally when isolated from the tumour milieu. In one report, it was shown that haemangioma cells had higher expression of the angiopoietin 1 receptor TIE2 and increased response to angiopoietin 1, while angiopoietin 2 mRNA was downregulated in response to serum in endothelial cells from haemangiomas but not in normal endothelial cells (Ref. 24). In another report it was demonstrated that haemangioma endothelial cells differ from normal endothelial cells in their proliferation rates and migration in vitro (Ref. 35). Haemangioma cells proliferated two to three

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times faster and manifested enhanced migration in response to VEGF compared with normal dermal microvascular endothelial cells. In addition, treatment with the angiostatic collagen XVIII fragment endostatin increased migration of haemangioma endothelial cells rather than inhibiting migration as seen with normal cells.

A subsequent study compared endothelial progenitor cells (EPCs) isolated from haemangiomas to similar cells purified from normal cord blood (Ref. 37). (See section 'Progenitor cells in haemangioma' for more on EPCs.) Haemangioma EPCs and cord blood EPCs, like haemangioma endothelial cells, displayed increased adhesion, migration and proliferation in response to endostatin, a protein that typically inhibits these processes in normal adult endothelial cells. Furthermore, it was shown that these two types of cells shared a common profile when 113 cell adhesion genes were analysed. Interestingly, a previous report of differential effects of endostatin suggested that differentiation status may determine the response: migration of mature umbilical-vein-derived endothelial cells was inhibited by endostatin, whereas migration of endothelial cells derived from embryonic stem cells was unaffected (Ref. 38). The fact that haemangioma endothelial cells have altered responses to this factor might reflect the immature nature of these cells rather than the presence of a mutation or intrinsic defect. Could other differences between haemangioma endothelial cells and normal endothelial cells, such as unusual surface marker expression profiles (Ref. 39), be attributed to the fact that they are not fully differentiated or mature cells? Further comparison of haemangioma endothelial cells to immature progenitor-derived endothelial cells should clarify this issue.

The placenta connection

Multiple lines of evidence point to an involvement of the placenta in haemangioma pathogenesis. An increase in haemangioma incidence has been associated with transcervical chorionic villus sampling (CVS), but not with amniocentesis (Refs 40, 41), although a more recent study found no association with CVS (Ref. 4). It has been reasoned that the trauma of CVS could increase the number of placental

cells released into the circulation and thus increase the likelihood that these cells might embolise to fetal vascular sites. Similarly, placental abnormalities, such as placenta praevia and preeclampsia, might also lead to higher risk of shed cells and haemangioma formation. Infants born prematurely have a higher risk of developing haemangiomas, and the incidence increases with lower birth weight (Refs 3, 42). Prematurity can be associated with placental complications as well, strengthening the link to the placenta.

Additional support for the idea of placental origin was provided in a report demonstrating that placental vasculature and haemangioma endothelium share expression of several surface markers (Refs 43, 44). It was shown that both tissues expressed merosin, Lewis Y, FcγRII (CD32) and glucose transporter 1 (GLUT-1, also expressed on endothelium of blood-brain barrier and red blood cells). None of these markers was found in other vascular anomalies or on nonhaemangioma tumour vasculature. Another study assessed whether haemangiomas were derived from trophoblasts, the invasive progenitor cells that mediate implantation into the uterine wall. Twelve haemangioma specimens were uniformly negative for a panel of trophoblast markers including human placental lactogen, placental alkaline phosphatase and cytokeratins 7, 8 and 17 (Ref. 45). While these data might suggest that there is no relationship between placental and haemangioma tissue, the markers used in this study label undifferentiated trophoblasts, leaving open the possibility that haemangiomas may, in fact, be derived from these cells, but have differentiated and down-regulated expression of the markers studied.

More recent support for a placental connection was provided by the results of a study using large-scale genomic analysis (Ref. 46). This experimental approach uses DNA microarrays to measure mRNA levels of nearly the entire human genome, representing a powerful tool for highly detailed comparisons between different tissue types. In this study, intact tissues from placenta, haemangioma, normal skin, lung and brain, pulmonary tumours and a scleroderma specimen were compared, accounting for genes expressed in endothelial cells as well as numerous nonendothelial cells within these samples. Each of the tissues used

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for comparison had at least one feature in common with haemangioma and/or placenta. For example, the lung tumours used have high levels of cell proliferation and are highly vascular. Clustering analysis showed that placenta and haemangioma were as similar as normal lung and lung tumours, raising the interesting possibility of viewing the placenta as 'tumour' and haemangioma as 'metastasis'. When comparisons were restricted to genes with known expression in endothelial cells, the similarities were even higher; haemangiomas were more similar to placental samples than placental samples were to each other. These observations regarding similarities between haemangiomas and placenta are provocative and have generated new ideas about the origin of haemangiomas.

Nonrandom distribution of haemangiomas on the face

Clinical observations have contributed to our understanding of haemangiomas, as exemplified in a study that provided data supporting the long-held belief that sites of haemangioma occurrence on the face are not random (Ref. 8). The authors first described two distinct types of haemangiomas: the more common focal, tumour-like type; and the less common segmental, or plaque-like, type. They found that focal haemangiomas had a nonrandom distribution, and identified 22 sites of occurrence on the face. Interestingly, these sites can be linked to boundaries between the different developmental units of the face, suggesting a rationale for haemangioma distribution. Haemangiomas were found near lines of fusion between mesenchymal growth centres ('placodes'), or between placodes and facial ectoderm (Fig. 4). Perhaps these boundary areas are more likely to sustain haemangioma growth due to the presence of higher secreted levels of particular growth factors or a more favourable extracellular matrix composition. A similar study of segmental haemangiomas demonstrated that while lesions on the lower face conformed to locations previously described as developmental units, those found on the upper face did not (Ref. 9). Thus, when formulating a hypothesis regarding the origin of haemangioma, one must take into account that these lesions are often not randomly



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Figure 4. Nonrandom distribution of infantile haemangioma on the face. Focal sites of occurrence superimposed on the embryological map of facial placodes demonstrating the predilection for haemangioma formation at boundaries between developmental units. Reproduced, with permission, from Ref. 8 [Waner, M. et al. (2003) The nonrandom distribution of facial hemangiomas. *Arch Dermatol* 139, 869-875] (Copyright © 2003 American Medical Association. All Rights reserved.).

distributed, but in fact may be tied to early developmental events that determine where, and perhaps how, they are formed.

Progenitor cells in haemangioma

Recent evidence suggests that several different types of progenitor cells may have roles in haemangioma progression. Normal bone marrow and peripheral blood contain progenitor cells that can differentiate into endothelial cells (Refs 47, 48). New vessel growth in the adult was long thought to depend on sprouting from existing vessels (i.e. angiogenesis), but more recent findings support a role for circulating bone-marrow-derived EPCs in this process. This concept was applied to haemangiomas where an increase of circulating EPCs was identified in patients with these lesions (Ref. 49). The precise identity of

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EPCs has been controversial. Most agree that EPCs cannot be identified by expression of a single marker, but studies have shown that cells expressing both CD34 and AC133 (CD133) can differentiate into endothelial cells in vitro (Ref. 50). Analysis of peripheral blood samples from five haemangioma patients averaging just over 2 years of age revealed a 15-fold average increase in circulating CD34⁺CD133⁺ cells, versus five control subjects with an average age of 38 months.

A second study used samples from four haemangioma specimens to show that proliferating lesions contain mRNA for CD133, while involuting and involuted haemangiomas, haemangioma-derived endothelial cells, normal skin and normal endothelial cells did not contain CD133 mRNA (Ref. 51). Combining antibodies against CD133 and CD34 to identify EPCs using flow cytometry, it was shown that 11 of 12 proliferating haemangiomas contained cells positive for both markers. Positive samples contained 0.1–2.9% CD133⁺CD34⁺ cells with one lesion showing a remarkable 21.4% double-positive cells. Although no EPCs were detected in control samples from venous or lymphatic malformations, the lack of cells coexpressing these markers in one of the haemangioma specimens studied suggests that EPCs may not be important in all haemangiomas. If EPCs could be shown to contribute directly to formation of haemangioma tissue, this would have significance for our understanding of the aetiology of these tumours.

Following haemangioma involution, the tissue at the site of the lesion is often composed of fibroadipose tissue, and a new study indicates that progenitor cells may have a role in the generation of this tissue. Mesenchymal stem cells were observed in haemangiomas more frequently than in normal skin, and their numbers were highest during the proliferative phase (Ref. 52). The cells recovered from haemangiomas were found to have many of the characteristics of mesenchymal stem cells from other tissues, including surface marker expression and multilineage differentiation potential, and it was suggested that these cells may contribute to the generation of adipocytes during haemangioma involution. This study is among the first to shed light on the nature of the residuum left behind after haemangiomas regress.

The involvement of a haematopoietically derived endothelial progenitor would seem consistent with the finding that haemangioma endothelial cells express haematopoietic markers (Ref. 53). Specifically, haemangioma cells were found to express a subset of myeloid-specific markers, which leads to the speculation that perhaps this cell type could serve as a haemangioma progenitor. Supporting this idea, several reports have demonstrated that peripheral myeloid cells (monocytes) are capable of differentiating into cells with endothelial characteristics (Refs 54, 55, 56). Alternatively, coexpression of haematopoietic markers on haemangioma cells could be an indication of a common progenitor between haematopoietic cells and endothelium, such as the haemangioblast. This concept has been discussed above, and may be an indication of the immature nature of the endothelial cells in haemangiomas, as suggested in a recent report showing that haemangioma endothelial cells express lymphatic endothelial hyaluronan receptor 1 (LYVE-1), a marker usually associated with the endothelium of lymphatic vessels (Ref. 57). This, combined with the finding that haemangioma cells also express endothelial markers CD31 and CD34, but not another lymphatic-specific protein, Prox-1, suggest that endothelial cells in haemangiomas are arrested in an early state of differentiation, and display features of both blood vascular and lymphatic endothelium. A follow-up study, however, reported that haemangioma endothelial cells were negative for LYVE-1, showing that LYVE-1 expression is restricted to perivascular cells expressing human leukocyte antigen DR (HLA-DR) with characteristics of dendritic cells (Ref. 58). Such conflicting reports that rely solely on immunohistochemistry leave this issue open and raise the need for further investigation.

If not serving as precursor cells, the myeloid component of haemangiomas may still play a role in either stimulating initial proliferation or subsequent involution of haemangiomas. As discussed below, hypoxia has been hypothesised to have a role in haemangioma pathophysiology (Refs 53, 59, 60), and it is well established that myeloid cells such as macrophages can profoundly promote the growth of new vessels under low oxygen conditions.

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A role for hypoxia in haemangioma development?

While the vast majority of infantile haemangiomas remain insignificant clinically, a fraction of these lesions demonstrate rapid growth in the first months of life. This sometimes alarming rate of growth could represent an exuberant response to one of the mechanisms driving cellular proliferation. Several pieces of evidence, at the molecular, cellular and clinical levels, suggest a possible role for hypoxia in haemangioma pathogenesis.

Hypoxia, or low levels of oxygen, is sensed by cells, which then respond by modifying gene expression. These modifications can enhance the ability of the cell to survive low oxygen conditions as well as induce expression of factors that promote the growth of new blood vessels. These new blood vessels then ultimately increase perfusion and oxygenation of the tissue. Hypoxia is a component of many disease states, but also has an important role in normal development and physiology (Ref. 61).

Hypoxia-inducible factor 1 α (HIF-1 α) is a transcription factor that serves as a major regulator of the cellular response to hypoxia. HIF-1 α is involved in several cellular processes, including regulation of differentiation and myeloid cell function (Ref. 62), regulation of genes involved in angiogenesis, erythropoiesis, energy metabolism, inflammation, and cell survival decisions (Ref. 61). With respect to its role in angiogenesis, HIF-1 α has been shown to regulate the expression of numerous factors including VEGF-A, angiopoietin 2, placental growth factor and PDGFB (Ref. 63). It is interesting to note that GLUT-1 and insulin-like growth factor 2 (IGF-2), two proteins shown to be present in haemangioma at relatively high levels, are both under the control of HIF-1 α and induced by hypoxia (Refs 64, 65), but VEGF is not found at high levels. The mechanisms regulating the high expression of IGF-2 were investigated recently in a study addressing the idea that a disruption in the epigenetic regulation of IGF-2 might be responsible for its high expression (Ref. 66). IGF-2 expression is under the control of imprinting, a mechanism of silencing the maternal copy of a gene under normal conditions to maintain appropriate expression levels (Ref. 67). A loss of imprinting can result in overexpression and contribute to pathogenesis, as has been shown in several

tumours including Wilms tumour where IGF-2 is overexpressed (Ref. 68). In haemangiomas, a normal pattern of imprinting was found in all 15 informative cases, indicating that a loss of imprinting is not involved and leaving open the possibility that an abnormal response to hypoxia could explain the high levels of IGF-2 in these lesions. Alternatively, high IGF-2 levels suggest another parallel to the placenta, where IGF-2 is also expressed (Ref. 69).

Some clinical features of haemangiomas suggest that reduced blood flow could be an important trigger in the growth of these lesions (Ref. 59). During delivery, the infant is likely to experience some degree of hypoxia as a consequence of the birthing process. Systemic hypoxia could alter the levels of circulating factors and trigger or promote growth of cells overly sensitive to high levels of these factors. It is also possible that microenvironmental changes could promote the observed cellular proliferation, alone or in combination with systemic factors. Prior to the rapid growth that characterises haemangiomas, a pale or blanched area of skin may be observed that is subsequently found to define the borders of the lesion that will develop. Although the nature of this phenomenon is not known, it suggests that a local interruption or reduction of blood flow has occurred to the area. This event could represent the growth stimulus for normal or mutated cells residing in the area or for ectopic cells that have embolised to this site from other tissues such as the placenta. Alternatively, this hypoxia could signal circulating progenitor cells to deposit in the area and begin to proliferate, as has been shown in other systems (Ref. 70). The cause of this putative interruption in blood flow would then become important to identify. Perhaps microanatomy exists on the boundaries between developmental units where vascular coverage is incomplete. Such a site could serve as a relatively hypoxic environment where circulating progenitor cells localise and proliferate.

Hypoxia has also been associated with tumours during pregnancy. Chorangioma, a proliferative lesion of the placenta, has some features in common with haemangioma. Both are primarily made up of endothelial cells (Ref. 71), and hypoxia has been reported to have an important role in the pathogenesis of chorangioma (Ref. 72). Another association between these two lesions is the fact that

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pregnancies complicated by the presence of chorangiomas have a higher incidence of infants that develop haemangiomatosis (Ref. 73), a condition characterised by multiple congenital haemangiomas, most often limited to the skin (Ref. 74). Could hypoxia, or a systemic defect in oxygen sensing or response, be at the root of both haemangiomas and chorangiomas?

What is causing involution?

Despite an increase in information relating to the biology of haemangiomas, and new evidence-based hypotheses on their origin, the precise cellular and molecular mechanisms underlying haemangioma pathogenesis remain unclear. Perhaps even more enigmatic than the aetiology of the tumours are the events that stimulate and govern the regression of these lesions. Apoptosis appears to be a major mechanism of cell death in the regressing tumour (Refs 75, 76, 77), but the events that trigger the apoptotic programme remain obscure. Two general routes to haemangioma involution have been proposed. The first might be termed 'passive involution' where inherent properties of the haemangioma endothelial cells place limits on their proliferative lifespan. It is also possible that the microenvironment supporting haemangioma growth/maintenance exists for a limited duration, perhaps being tied to neonatal development. The placenta has a clearly defined life cycle, and is designed to function solely for the period of gestation. Perhaps haemangioma cells also share this programmed lifespan (Refs 78, 79). Another form of passive involution could involve the loss of a critical factor that supports proliferation and/or survival. IGF-2 is highly expressed in proliferating haemangiomas, but not in other vascular anomalies and could represent such a factor (Refs 20, 66). mRNA levels of this factor decrease dramatically as haemangiomas begin to involute, suggesting a role for IGF-2 in haemangioma growth. However, IGF-2 levels appear to decrease as endothelial content decreases, raising the possibility that the observed decrease is related to the loss of the cellular source and may not, in fact, be critical for driving growth (Ref. 66).

The second way of thinking about haemangioma involution involves the action of other cells, and may be thought of as 'active involution'. The immune system would be a

likely candidate to elicit a reaction leading to involution. Haemangioma cells, possessing abnormal surface marker expression, could be targeted by the immune system, which can be exquisitely sensitive to abnormal cells. Large numbers of CD8⁺ T cells have been found within haemangioma tissue (Ref. 80). This subtype of T cell is cytotoxic and capable of inducing apoptosis in target cells. There was no strong correlation, however, with the phase of the haemangioma lesion and the number of CD8⁺ T cells, which might be observed if these cells were, in fact, responsible for mediating involution. This study also described a possible role for indoleamine 2,3-dioxygenase (IDO) in haemangioma and showed, using large-scale expression analysis, that IDO was highly expressed in proliferating haemangiomas (Ref. 80), while a second study similarly described a possible role for IDO (Ref. 81). This enzyme has an important role in preventing maternal rejection of the placenta through inhibition of T-cell function (Ref. 82). IDO catalyses the degradation of the amino acid tryptophan (Ref. 83), an essential compound for optimal T-cell activity (Ref. 84). Is the tumour-killing function of the CD8⁺ T cells in haemangioma suppressed by the action of IDO? Perhaps this would explain the slow regression of these lesions, where it is not until IDO levels fall sufficiently that T cells can function effectively enough to induce apoptosis of haemangioma cells.

T cells are the major effectors of the adaptive immune response, but cells of the innate immune system may also have roles in mediating haemangioma regression. Mast cells are present in haemangioma and may play a role in haemangioma evolution (Refs 85, 86). The increased number of these cells during involution suggests a functional role in regression. Furthermore, haemangioma mast cells stain positively for clusterin/apolipoprotein J (Ref. 87), a secreted protein that may induce apoptosis in other cells. It is known from other work, however, that mast cells act directly and indirectly to promote angiogenesis (Ref. 88), so understanding the precise role for these cells in haemangioma evolution will require further investigation.

Additional support for a role of the immune system in haemangioma regression comes from

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a series of studies examining the effect of the immune-response modifier imiquimod (Refs 15, 16, 17). When combining the results of three reports, topical administration appeared to accelerate involution in 12 of 13 patients treated. Although these are small, uncontrolled studies, it supports the idea that the immune system may be involved in haemangioma involution.

A better understanding of the mechanisms regulating haemangioma involution will provide opportunities to design treatments to accelerate this process. It is also possible that these findings might be applicable to other areas of vascular biology where regression of proliferating blood vessels is desirable.

The future: new models

Potential regulators of haemangioma progression discussed in this article are summarised in Table 2. New information gathered from basic research will support efforts to develop better treatments for haemangiomas. A major impediment to the advancement of new therapies, however, has been the absence of useful models. The availability of an animal model that reproduces the major features of infantile haemangioma would provide a means by which potential therapeutic interventions could be tested, and permit an opportunity to better understand how existing therapies work. In vitro culture models will facilitate the development of high-throughput assays to screen candidate compounds.

There are numerous reports in the literature describing models for 'haemangioma', but most have limited utility. Models of endothelial tumours differing in important ways from infantile haemangioma include a spontaneously arising, immortalised haemangioendothelioma cell line (EOMA) (Ref. 89) and cells carrying polyoma virus middle T antigen (Ref. 90). Tumours generated using these cell lines have more malignant characteristics, are not obligately perinatal and do not spontaneously regress. While potentially useful as tools for investigating some endothelial tumours, caution should be used when extrapolating findings generated in these models to infantile haemangioma.

An ex vivo culture system has been described that has the advantage of using intact human haemangioma tissue and permits biochemical

manipulation (Ref. 91). The drawbacks of this model include loss of cellular features of haemangioma endothelial cells, such as GLUT-1 expression (Ref. 92), and a mechanism of growth involving sprouting. Purified cultures of haemangioma-derived cells have been used to demonstrate their unusual behaviour in terms of growth and migration (Refs 24, 35), but this system, lacking the typical environmental milieu, removes potentially important soluble and insoluble molecules, such as growth factors and various extracellular matrix components, as well as any direct influence from surrounding cells.

It is possible that the complexity and multifactorial nature of haemangiomas may preclude the development of a faithful animal model. Numerous investigators have reported repeated failure at attempts to create models using a wide variety of approaches including the implantation of cells and tissues from haemangioma and placenta (Ref. 93). A recent report combined the hypothesis of an involvement of hormones in haemangioma with an attempt to create a mouse model (Ref. 94). Using nude mice, which have severely compromised adaptive immunity and allow grafting of foreign tissues, fresh haemangioma tissue from a single patient was implanted subcutaneously into 16 mice. Weekly injection of 0.1 ng oestradiol was administered to eight mice and the growth of the implanted tumours was measured over the course of 90 days. Modest growth, or at least maintenance, of the tissue was reported in the animals receiving oestradiol, but none of the control animals. This study is of interest both as a possible indication of a requirement for oestradiol for haemangioma growth, and as a possible long-sought animal model. Follow-up confirmation of this work is needed, but if verified, it is an example of the insight that may be needed to generate a useful model of infantile haemangioma.

Clinical implications/applications

Several therapeutic approaches for haemangiomas have been suggested over the past decade. Interferon α has been used successfully in the treatment of complicated haemangiomas (Refs 95, 96), but its use is associated with the development of spastic diplegia (Ref. 97) and is generally limited to life-threatening cases without other viable options. Initial reports

Table 2. Potential regulators of haemangioma progression

Regulator	Function	Refs
Molecular: proliferation		
Angiopoietin/TIE2	Vessel maturation/stability	24
bFGF	Growth factor	18, 21, 22
E-selectin	Endothelial/leukocyte adhesion	19
IGF-2	Growth factor	20, 66
Integrin $\alpha v \beta 3$	ECM receptor	20, 106
Integrin $\alpha 5 \beta 1$	ECM receptor	20
Mutated VEGFR2/3	Growth factor receptor	36
VEGF	Growth factor	21, 22, 23
Molecular: involution		
Clusterin/ApoJ	Associated with apoptosis	87
Cytochrome b	Mitochondrial protein	107
Interferon β	Angiogenesis inhibitor	21
IDO	Degrades Trp; inhibits T cells	80, 81
TIMP-1	Enzyme inhibitor	22
Cellular		
Apoptosis	Increased during involution	75, 77
Dendritic/myeloid cells	Source of growth factors?	53, 106
Endothelial progenitor cells	Might contribute to haemangioma tissue	37, 49, 51
Hypoxia	Might promote proliferation	53, 60
Mast cells	Contribute to proliferation and/or involution?	85, 86
Mesenchymal stem cells	Possible precursors to adipocytes present in lesion residuum	52
Hormonal		
Oestradiol	Might be related to haemangioma proliferation	94, 103
Oestrogen receptors	Might be related to haemangioma proliferation	104

Abbreviations: ApoJ, apolipoprotein J; ECM, extracellular matrix; bFGF, basic fibroblast growth factor; IDO, indoleamine 2,3-dioxygenase; IGF2, insulin-like growth factor 2; TIMP-1, tissue inhibitor of metalloproteinase 1; Trp, tryptophan; VEGF(R), vascular endothelial growth factor (receptor).

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indicated that the neurological effects were irreversible, but a subsequent study showed that these effects can resolve over time (Ref. 98). Perhaps new therapies would employ angiostatic compounds already in use in other areas of medicine. A concern with this class of drugs is their use in the growing child where new vessel growth may be ongoing, highlighting the need for more targeted options.

Currently, the first-line therapy for troublesome infantile haemangiomas is corticosteroids. These drugs, while efficacious in many patients, carry undesired side effects such as temporary growth retardation, increased risk of infection and psychological changes (Ref. 99). Corticosteroids have known anti-inflammatory activity, but their action in haemangioma is poorly understood. The finding that

proliferating haemangiomas harbour large numbers of haematopoietic cells of the myeloid lineage (Ref. 53) suggests a possible mechanism whereby corticosteroids inhibit the speculated growth-promoting function of these cells. The idea of boosting the adaptive immune response against the tumour is worth exploring and has some limited experimental and clinical support. The discovery of IDO expression in haemangioma (Refs 80, 81) suggests that inhibiting this enzyme and/or supplementing local tryptophan could lead to an increased T-cell response against the tumour. Further exploration of imiquimod or other compounds that modify the immune response is warranted (Ref. 15).

The impact of large haemangiomas has been shown to extend beyond simple mass-related

effects such as encroachment of vital structures and disfigurement. Severe hypothyroidism in children with large haemangiomas was found to result from high type 3 iodothyronine deiodinase activity in the haemangioma tissue (Ref. 100). The finding that large haemangiomas can have far-reaching effects highlights the need to find effective means to limit their growth.

Research in progress and outstanding research questions

The collective efforts of haemangioma researchers have raised numerous questions regarding the mechanisms behind the growth and regression of these tumours. The similarities and differences between haemangioma-derived cells, normal cells and immature normal cells should be further studied while exploring the intrinsic defect hypothesis. The evidence suggesting a connection to the placenta heightens interest in exploring the impact of placental complications such as placenta praevia, preeclampsia and chorangioma on haemangioma incidence and biology. One interesting hypothesis was recently removed from consideration by two studies investigating maternal-fetal chimaerism that showed haemangiomas were exclusively derived from the child, with no evidence of maternal origin (Refs 101, 102). Possible clues into haemangioma pathogenesis include the increased incidence in the head and neck regions and the predilection for the skin. Dermal haemangiomas come in two general forms: the plaque-like segmental form and the tumour-like focal form. This curious dichotomy begs the question of how these two types of lesions differ at the cellular and molecular levels. Speculation regarding the differences in segmental and focal haemangiomas suggests that segmental lesions may be derived from implantation of circulating placental/trophoblastic cells relatively early in development, whereas the focal variety arise from the same cells arriving late in development via the amniotic fluid (Ref. 45). Only rarely are the segmental lesions candidates for surgical excision, leading to a paucity of this tissue for study. In fact, the limited supply of any haemangioma tissue is an ongoing concern. A centralised tissue bank for haemangiomas and other vascular anomalies would be of great value to the research

community, making available these and other rare specimens that have been collected and stored using standardised protocols (Ref. 93). Based on evidence from varying sources, the idea of hypoxia as a trigger and driving force behind haemangioma growth deserves further study. Animal models incorporating hypoxia are available (Ref. 70), and instrumentation is available to measure tissue oxygen levels in the clinic; thus, addressing this question would seem feasible. Another clue before us is the higher rate of haemangioma occurrence in females. Elevated levels of 17 β -oestradiol (Ref. 103) and oestrogen receptor (Ref. 104) have been reported in children with haemangiomas. It was also speculated that corticosteroid treatment may negatively affect oestradiol action (Ref. 103). Higher levels of angiogenic factors have been demonstrated in females, pointing to another possible explanation for the higher incidence in females (Ref. 105). These findings support further investigations into possible hormonal regulation of haemangioma growth and the differences in factor expression between genders.

Despite the perception of some that infantile haemangiomas are merely birthmarks that will quietly regress without major complication, a significant number of children suffer physically and emotionally as a result of living with a haemangioma. For some, these lesions pose life-threatening complications that severely impact on their early years, while for others a permanent disfiguring scar leaves a constant reminder of these usually benign lesions. Further investigations will not only hopefully lead to better treatments, but also teach us more about endothelial biology and the basic processes of blood vessel growth and regression.

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References

- 1 Mulliken, J.B. and Glowacki, J. (1982) Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg* 69, 412-422
- 2 Finn, M.C., Glowacki, J. and Mulliken, J.B. (1983) Congenital vascular lesions: clinical application of a new classification. *J Pediatr Surg* 18, 894-900
- 3 Amir, J. et al. (1986) Strawberry hemangioma in preterm infants. *Pediatr Dermatol* 3, 331-332
- 4 Haggstrom, A.N. et al. (2007) Prospective study of infantile hemangiomas: demographic, prenatal, and perinatal characteristics. *J Pediatr* 150, 291-294
- 5 Haggstrom, A.N. et al. (2006) Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. *Pediatrics* 118, 882-887
- 6 Orlow, S.J., Isakoff, M.S. and Blei, F. (1997) Increased risk of symptomatic hemangiomas of the airway in association with cutaneous hemangiomas in a "beard" distribution. *J Pediatr* 131, 643-646
- 7 Chiller, K.G., Passaro, D. and Frieden, I.J. (2002) Hemangiomas of infancy: clinical characteristics, morphologic subtypes, and their relationship to race, ethnicity, and sex. *Arch Dermatol* 138, 1567-1576
- 8 Waner, M. et al. (2003) The nonrandom distribution of facial hemangiomas. *Arch Dermatol* 139, 869-875
- 9 Haggstrom, A.N. et al. (2006) Patterns of infantile hemangiomas: new clues to hemangioma pathogenesis and embryonic facial development. *Pediatrics* 117, 698-703
- 10 Frieden, I.J., Reese, V. and Cohen, D. (1996) PHACE syndrome. The association of posterior fossa brain malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, and eye abnormalities. *Arch Dermatol* 132, 307-311
- 11 Metry, D.W. et al. (2006) A prospective study of PHACE syndrome in infantile hemangiomas: demographic features, clinical findings, and complications. *Am J Med Genet A* 140, 975-986
- 12 North, P.E. et al. (2006) Vascular tumors of infancy and childhood: beyond capillary hemangioma. *Cardiovasc Pathol* 15, 303-317
- 13 Bennett, M.L. et al. (2001) Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. *Arch Dermatol* 137, 1208-1213
- 14 Metz, B.J. et al. (2004) Response of ulcerated perineal hemangiomas of infancy to becaplermin gel, a recombinant human platelet-derived growth factor. *Arch Dermatol* 140, 867-870
- 15 Martinez, M.I. et al. (2002) Infantile hemangioma: clinical resolution with 5% imiquimod cream. *Arch Dermatol* 138, 881-884
- 16 Welsh, O. et al. (2004) Treatment of infantile hemangiomas with short-term application of imiquimod 5% cream. *J Am Acad Dermatol* 51, 639-642
- 17 Hazen, P.G. et al. (2005) Proliferating hemangioma of infancy: successful treatment with topical 5% imiquimod cream. *Pediatr Dermatol* 22, 254-256
- 18 Tan, S.T. et al. (2000) Cellular and extracellular markers of hemangioma. *Plast Reconstr Surg* 106, 529-538
- 19 Kraling, B.M. et al. (1996) E-selectin is present in proliferating endothelial cells in human hemangiomas. *Am J Pathol* 148, 1181-1191
- 20 Ritter, M.R. et al. (2002) Insulin-like growth factor 2 and potential regulators of hemangioma growth and involution identified by large-scale expression analysis. *Proc Natl Acad Sci U S A* 99, 7455-7460
- 21 Bielenberg, D.R. et al. (1999) Progressive growth of infantile cutaneous hemangiomas is directly correlated with hyperplasia and angiogenesis of adjacent epidermis and inversely correlated with expression of the endogenous angiogenesis inhibitor, IFN-beta. *Int J Oncol* 14, 401-408
- 22 Takahashi, K. et al. (1994) Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 93, 2357-2364
- 23 Chang, J. et al. (1999) Proliferative hemangiomas: analysis of cytokine gene expression and angiogenesis. *Plast Reconstr Surg* 103, 1-9
- 24 Yu, Y. et al. (2001) Increased Tie2 expression, enhanced response to angiopoietin-1, and dysregulated angiopoietin-2 expression in hemangioma-derived endothelial cells. *Am J Pathol* 159, 2271-2280
- 25 Carmeliet, P. (2005) Angiogenesis in life, disease and medicine. *Nature* 438, 932-936
- 26 Drake, C.J. and Fleming, P.A. (2000) Vasculogenesis in the day 6.5 to 9.5 mouse embryo. *Blood* 95, 1671-1679

Pathogenesis of infantile haemangioma:
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- 27 Choi, K. et al. (1998) A common precursor for hematopoietic and endothelial cells. *Development* 125, 725-732
- 28 Ferrara, N. and Kerbel, R.S. (2005) Angiogenesis as a therapeutic target. *Nature* 438, 967-974
- 29 Friedlander, S.F., Ritter, M.R. and Friedlander, M. (2005) Recent progress in our understanding of the pathogenesis of infantile hemangiomas. *Lymphat Res Biol* 3, 219-225
- 30 Bauland, C.G. et al. (2006) The pathogenesis of hemangiomas: a review. *Plast Reconstr Surg* 117, 29e-35e
- 31 Blei, F. et al. (1998) Familial segregation of hemangiomas and vascular malformations as an autosomal dominant trait. *Arch Dermatol* 134, 718-722
- 32 Walter, J.W. et al. (1999) Genetic mapping of a novel familial form of infantile hemangioma. *Am J Med Genet* 82, 77-83
- 33 Bischoff, J. (2002) Monoclonal expansion of endothelial cells in hemangioma: an intrinsic defect with extrinsic consequences? *Trends Cardiovasc Med* 12, 220-224
- 34 Allen, R.C. et al. (1992) Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. *Am J Hum Genet* 51, 1229-1239
- 35 Boye, E. et al. (2001) Clonality and altered behavior of endothelial cells from hemangiomas. *J Clin Invest* 107, 745-752
- 36 Walter, J.W. et al. (2002) Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. *Genes Chromosomes Cancer* 33, 295-303
- 37 Khan, Z.A. et al. (2006) Endothelial progenitor cells from infantile hemangioma and umbilical cord blood display unique cellular responses to endostatin. *Blood* 108, 915-921
- 38 Schmidt, A., Addicks, K. and Bloch, W. (2004) Opposite effects of endostatin on different endothelial cells. *Cancer Biol Ther* 3, 1162-1166; discussion 1167-1168
- 39 Li, Q. et al. (2003) Differential expression of CD146 in tissues and endothelial cells derived from infantile haemangioma and normal human skin. *J Pathol* 201, 296-302
- 40 Kaplan, P. et al. (1990) Malformations and minor anomalies in children whose mothers had prenatal diagnosis: comparison between CVS and amniocentesis. *Am J Med Genet* 37, 366-370
- 41 Burton, B.K. et al. (1995) An increased incidence of haemangiomas in infants born following chorionic villus sampling (CVS). *Prenat Diagn* 15, 209-214
- 42 Powell, T.G. et al. (1987) Epidemiology of strawberry haemangioma in low birthweight infants. *Br J Dermatol* 116, 635-641
- 43 North, P.E. et al. (2001) A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch Dermatol* 137, 559-570
- 44 North, P.E., Waner, M. and Brodsky, M.C. (2002) Are infantile hemangiomas of placental origin? *Ophthalmology* 109, 633-634
- 45 Bree, A.F. et al. (2001) Infantile hemangiomas: speculation on placental trophoblastic origin. *Arch Dermatol* 137, 573-577
- 46 Barnes, C.M. et al. (2005) Evidence by molecular profiling for a placental origin of infantile hemangioma. *Proc Natl Acad Sci U S A* 102, 19097-19102
- 47 Asahara, T. et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275, 964-967
- 48 Yoder, M.C. et al. (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 109, 1801-1809
- 49 Kleinman, M.E. et al. (2003) Increased circulating AC133+ CD34+ endothelial progenitor cells in children with hemangioma. *Lymphat Res Biol* 1, 301-307
- 50 Peichev, M. et al. (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 95, 952-958
- 51 Yu, Y. et al. (2004) Endothelial progenitor cells in infantile hemangioma. *Blood* 103, 1373-1375
- 52 Yu, Y. et al. (2006) Mesenchymal stem cells and adipogenesis in hemangioma involution. *Stem Cells* 24, 1605-1612
- 53 Ritter, M.R. et al. (2006) Myeloid cells in infantile hemangioma. *Am J Pathol* 168, 621-628
- 54 Fernandez Pujol, B. et al. (2000) Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 65, 287-300
- 55 Havemann, K., Pujol, B.F. and Adamkiewicz, J. (2003) In vitro transformation of monocytes and dendritic cells into endothelial like cells. *Adv Exp Med Biol* 522, 47-57
- 56 Bailey, A.S. et al. (2006) Myeloid lineage progenitors give rise to vascular endothelium. *Proc Natl Acad Sci U S A* 103, 13156-13161

Pathogenesis of infantile haemangioma:
new molecular and cellular insights

- 57 Dadras, S.S. et al. (2004) Infantile hemangiomas are arrested in an early developmental vascular differentiation state. *Mod Pathol* 17, 1068-1079
- 58 Nguyen, V.A. et al. (2006) Infantile hemangioma is a proliferation of LYVE-1-negative blood endothelial cells without lymphatic competence. *Mod Pathol* 19, 291-298
- 59 Hamlat, A. et al. (2005) Pathophysiology of capillary haemangioma growth after birth. *Med Hypotheses* 64, 1093-1096
- 60 Kleinman, M.E., Blei, F. and Gurtner, G.C. (2005) Circulating endothelial progenitor cells and vascular anomalies. *Lymphat Res Biol* 3, 234-239
- 61 Semenza, G.L. (1999) Regulation of mammalian O₂ homeostasis by hypoxia-inducible factor 1. *Annu Rev Cell Dev Biol* 15, 551-578
- 62 Cejudo-Martin, P. and Johnson, R.S. (2005) A new notch in the HIF belt: how hypoxia impacts differentiation. *Dev Cell* 9, 575-576
- 63 Manalo, D.J. et al. (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 105, 659-669
- 64 Feldser, D. et al. (1999) Reciprocal positive regulation of hypoxia-inducible factor 1 α and insulin-like growth factor 2. *Cancer Res* 59, 3915-3918
- 65 Loike, J.D. et al. (1992) Hypoxia induces glucose transporter expression in endothelial cells. *Am J Physiol* 263, C326-333
- 66 Yu, Y. et al. (2004) Genomic imprinting of IGF2 is maintained in infantile hemangioma despite its high level of expression. *Mol Med* 10, 117-123
- 67 Burns, J.L., Jackson, D.A. and Hassan, A.B. (2001) A view through the clouds of imprinting. *Faseb J* 15, 1694-1703
- 68 Ogawa, O. et al. (1993) Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. *Nature* 362, 749-751
- 69 Constancia, M. et al. (2002) Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 417, 945-948
- 70 Ceradini, D.J. et al. (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 10, 858-864
- 71 Ogino, S. and Redline, R.W. (2000) Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and chorangiosis. *Hum Pathol* 31, 945-954
- 72 Reshetnikova, O.S. et al. (1996) Increased incidence of placental chorioangioma in high-altitude pregnancies: hypobaric hypoxia as a possible etiologic factor. *Am J Obstet Gynecol* 174, 557-561
- 73 Bakaris, S. et al. (2004) Case of large placental chorioangioma associated with diffuse neonatal hemangiomatosis. *Pediatr Dev Pathol* 7, 258-261
- 74 Metry, D.W. and Hebert, A.A. (2000) Benign cutaneous vascular tumors of infancy: when to worry, what to do. *Arch Dermatol* 136, 905-914
- 75 Iwata, J. et al. (1996) High frequency of apoptosis in infantile capillary haemangioma. *J Pathol* 179, 403-408
- 76 Mancini, A.J. and Smoller, B.R. (1996) Proliferation and apoptosis within juvenile capillary hemangiomas. *Am J Dermatopathol* 18, 505-514
- 77 Razon, M.J. et al. (1998) Increased apoptosis coincides with onset of involution in infantile hemangioma. *Microcirculation* 5, 189-195
- 78 Halperin, R. et al. (2000) Placental apoptosis in normal and abnormal pregnancies. *Gynecol Obstet Invest* 50, 84-87
- 79 Smith, S.C. et al. (2000) Apoptosis is a rare event in first-trimester placental tissue. *Am J Obstet Gynecol* 183, 697-699
- 80 Ritter, M.R. et al. (2003) Identifying potential regulators of infantile hemangioma progression through large-scale expression analysis: a possible role for the immune system and indoleamine 2,3 dioxygenase (IDO) during involution. *Lymphat Res Biol* 1, 291-299
- 81 North, P.E. et al. (2004) High expression of indoleamine 2,3 dioxygenase in infantile hemangioma: evidence for immune tolerance. *Mod Pathol* 17(S1), 97A (Abstract)
- 82 Mellor, A.L. et al. (2001) Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. *Nat Immunol* 2, 64-68
- 83 Takikawa, O. et al. (1986) Tryptophan degradation in mice initiated by indoleamine 2,3-dioxygenase. *J Biol Chem* 261, 3648-3653
- 84 Frumento, G. et al. (2002) Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 196, 459-468
- 85 Glowacki, J. and Mulliken, J.B. (1982) Mast cells in hemangiomas and vascular malformations. *Pediatrics* 70, 48-51

- 86 Tan, S.T. et al. (2004) Mast cells and hemangioma. *Plast Reconstr Surg* 113, 999-1011
- 87 Hasan, Q. et al. (2000) Clusterin/apoJ expression during the development of hemangioma. *Hum Pathol* 31, 691-697
- 88 Meininger, C.J. and Zetter, B.R. (1992) Mast cells and angiogenesis. *Semin Cancer Biol* 3, 73-79
- 89 Obeso, J., Weber, J. and Auerbach, R. (1990) A hemangioendothelioma-derived cell line: its use as a model for the study of endothelial cell biology. *Lab Invest* 63, 259-269
- 90 Bautch, V.L. et al. (1987) Endothelial cell tumors develop in transgenic mice carrying polyoma virus middle T oncogene. *Cell* 51, 529-537
- 91 Tan, S.T. et al. (2000) A novel in vitro human model of hemangioma. *Mod Pathol* 13, 92-99
- 92 North, P.E. et al. (2003) Ex vivo hemangiogenesis in fibrin gels: a morphological and immunohistochemical analysis. *Lab Invest* 83, 18A (Abstract)
- 93 Frieden, I.J. et al. (2005) Infantile hemangiomas: current knowledge, future directions. Proceedings of a research workshop on infantile hemangiomas, April 7-9, 2005, Bethesda, Maryland, USA. *Pediatr Dermatol* 22, 383-406
- 94 Peng, Q. et al. (2005) The establishment of the hemangioma model in nude mouse. *J Pediatr Surg* 40, 1167-1172
- 95 Ezekowitz, R.A., Mulliken, J.B. and Folkman, J. (1992) Interferon alfa-2a therapy for life-threatening hemangiomas of infancy. *N Engl J Med* 326, 1456-1463
- 96 Chang, E. et al. (1997) Successful treatment of infantile hemangiomas with interferon-alpha-2b. *J Pediatr Hematol Oncol* 19, 237-244
- 97 Barlow, C.F. et al. (1998) Spastic diplegia as a complication of interferon Alfa-2a treatment of hemangiomas of infancy. *J Pediatr* 132, 527-530
- 98 Greinwald, J.H., Jr. et al. (1999) An update on the treatment of hemangiomas in children with interferon alfa-2a. *Arch Otolaryngol Head Neck Surg* 125, 21-27
- 99 Boon, L.M., MacDonald, D.M. and Mulliken, J.B. (1999) Complications of systemic corticosteroid therapy for problematic hemangioma. *Plast Reconstr Surg* 104, 1616-1623
- 100 Huang, S.A. et al. (2000) Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. *N Engl J Med* 343, 185-189
- 101 Pittman, K.M. et al. (2006) No evidence for maternal-fetal microchimerism in infantile hemangioma: a molecular genetic investigation. *J Invest Dermatol* 126, 2533-2538
- 102 Regnier, S. et al. (2007) Endothelial cells in infantile haemangiomas originate from the child and not from the mother (a fluorescence in situ hybridization-based study). *Br J Dermatol* 157, 158-160
- 103 Sasaki, G.H., Pang, C.Y. and Witliff, J.L. (1984) Pathogenesis and treatment of infant skin strawberry hemangiomas: clinical and in vitro studies of hormonal effects. *Plast Reconstr Surg* 73, 359-370
- 104 Lui, W. et al. (1997) Sex hormone receptors of hemangiomas in children. *Chin Med J (Engl)* 110, 349-351
- 105 Malamitsi-Puchner, A. et al. (2000) Changes in serum levels of vascular endothelial growth factor in males and females throughout life. *J Soc Gynecol Investig* 7, 309-312
- 106 Nguyen, V.A. et al. (2004) Infantile hemangioma is a proliferation of beta 4-negative endothelial cells adjacent to HLA-DR-positive cells with dendritic cell morphology. *Hum Pathol* 35, 739-744
- 107 Hasan, Q. et al. (2001) Altered mitochondrial cytochrome b gene expression during the regression of hemangioma. *Plast Reconstr Surg* 108, 1471-1476; discussion 1477-1478
- 108 Hand, J.L. and Frieden, I.J. (2002) Vascular birthmarks of infancy: resolving nosologic confusion. *Am J Med Genet* 108, 257-264

Further reading, resources and contacts

Publications

Mulliken, J.B. and Young, A.E. (1988) *Vascular Birthmarks: Haemangiomas and Malformations*, W.B. Saunders Co., Philadelphia, PA, USA

Waner, M. and Suen, J.Y., eds (1999) *Hemangiomas and Vascular Malformations of the Head and Neck*, Wiley-Liss, New York, USA

Shannon, L. and Marshall, C. with Waner, M. (1997) *Birthmarks: A Guide to Hemangiomas and Vascular Malformation*, Women's Health Publishing, Minden, NV, USA

Websites

The International Society for the Study of Vascular Anomalies (ISSVA) is a worldwide organisation of medical specialists with special interest in vascular tumours and congenital vascular malformations:

<http://www.issva.org>

Vascular Birthmarks Foundation is a comprehensive resource network for families affected by vascular birthmarks, including haemangiomas:

<http://www.birthmark.org>

Features associated with this article

Figures

Figure 1. Haemangiomas present with varying appearances.

Figure 2. Model of haemangioma natural history.

Figure 3. Angiogenesis and vasculogenesis.

Figure 4. Nonrandom distribution of infantile haemangioma on the face.

Tables

Table 1. Classification and features of vascular birthmarks.

Table 2. Potential regulators of haemangioma progression.

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Pathogenesis of infantile haemangioma:
new molecular and cellular insights

Propranolol – a new treatment of haemangiomas of infancy

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Translation: Rudolf Schlaepfer, La Chaux-de-Fonds

Introduction

With a prevalence of 10% in the first year of life, haemangiomas are not only the most common vascular tumours, but the most common tumours full stop during infancy¹⁾. Haemangioma growth is characteristic: 1. invisible at birth; 2. proliferation phase from 2-6 weeks old with rapid growth for 3-9 months; 3. plateau phase and 4. spontaneous regression starting from about the second year, for several years²⁾. Despite this favourable spontaneous progression, some infantile haemangiomas can cause considerable functional and aesthetic prejudices. Until very recently, systemic corticosteroids were the first-choice treatment for problem haemangiomas. Other options are topic or intralesional corticosteroids, imiquimod, α -interferon, vincristine, cryotherapy, laser therapy and surgical excision. However, these treatments have limits because of the potential risks and partly owing to the lack of evidence of their efficacy¹⁾.

The discovery of propranolol for treating haemangiomas

On 12 June 2007, Léauté-Labrèze et al., of the Paediatric Clinic of Bordeaux, published for the first time, in the *New England Journal of Medicine*³⁾, their observations concerning the treatment of complex haemangiomas in 11 infants with propranolol, a non-selective β -blocker. It was a fortuitous observation which

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** Swiss Group for Pediatric Dermatology: A-M. Calza, Geneva; S. Christen-Zaech, Lausanne; D. Hohl, Lausanne; J. Izakovic, Basel; K. Kenland, Berne; M. Lacour, Geneva; L. Weibel, Zurich

prompted the authors to test the treatment of haemangiomas with propranolol. A baby had been treated with corticosteroids for a large haemangioma of the nose. The growth of the haemangioma had stabilized, but the child developed an obstructive cardiomyopathy requiring the introduction of the β -blocker propranolol from the age of 4 months. In the days following the beginning of this treatment, a rapid paling and a reduction in the volume of the haemangioma were observed. By the time the propranolol treatment was stopped at the age of 14 months, the haemangioma had virtually disappeared. After having observed the same effect in a second child, the authors treated 9 other children, who had problematic haemangiomas, with propranolol (2 mg/kg/day). In all cases, the haemangiomas turned pale within 24 hours and decreased in volume during the months following the beginning of the treatment.

How can the effect of propranolol be explained?

The mechanism of action of propranolol on haemangiomas of infancy is so far unclear. Haemangiomas are a complex cluster of clonal endothelial cells in combination with pericytes, dendritic cells and mast cells¹⁾. Only very little is known about the regulators of haemangioma proliferation and involution. During the growth phase, angiogenesis is stimulated by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). In this proliferation phase, the histology shows active interstitial and endothelial cell division; during the involution phase, on the other hand, apoptosis of these cell groups is noted¹⁾. The following explanations can be envisaged for the effect of propranolol on haemangiomas of infancy: a) vasoconstriction (the haemangiomas rapidly become pale and soft); b) reduced expression of the VEGF and bFGF genes by down-regulation of the RAF-mitogen-activated protein kinase pathway (which would explain the reduction in the volume of the haemangioma); c) induction of capillary endothelial cell apoptosis³⁾.



Fig. 1: Infant of 3.5 months with a large segmental haemangioma of the right cheek and of the right infraorbital region before treatment with propranolol. Tumefaction of the lower eyelid, leading to shrinking of the ocular fissure and the risk of a limitation of the visual axis, is noted.



Fig. 2: 6 weeks after the beginning of the treatment with propranolol, 2 mg/kg/day, a clear paling and a decrease in volume of the haemangioma, and also a normally opened and symmetrical palpebral fissure, are noted.

(patient of Dr C. Schiestl, Zurich)

What is currently known about the use of propranolol for treating haemangiomas?

The sensational results, compared with the other treatments, had a great response throughout the world. A large number of international centres specializing in the treatment of vascular anomalies in children have since successfully adopted propranolol for the treatment of problematic haemangiomas. Publications are to date still infrequent. In Italy, Bonifazi et al. have recently described 5 children aged 8 to 12 weeks, treated for a haemangioma, and confirm the favourable effect of propranolol⁴⁾. The French group of Bigorre et al. presented, at the *17th International Workshop on Vascular Anomalies* in June 2008 in Boston, 4 children in whom the haemangioma rapidly regressed following treatment with the selective β -blocker acebutolol⁵⁾. In the context of the same conference, Léauté-Labrèze et al. presented 32 children who all experienced a rapid involution of their haemangioma with propranolol treatment⁶⁾. The personal communications of a large number of international experts in paediatric dermatology confirmed these observations; for many centres, propranolol is the first-choice treatment for treating complex

haemangiomas of infancy. Since the publication of these data, several children with problematic haemangiomas have also been successfully treated with propranolol in Switzerland; to date, we are aware of 21 cases (example: figures 1 and 2). Current knowledge is limited to proliferation-phase haemangiomas and the effect of a later use (age > 1 year) is unknown. It is also necessary to know whether any controlled study regarding the use of propranolol exists to date. The publication of further data on the efficacy and tolerance of β -blockers in the treatment of haemangiomas can however be expected.

Is the use of propranolol for treating haemangiomas safe?

The adverse effects mentioned by Léauté-Labrèze et al. at the *17th International Workshop on Vascular Anomalies* were all infrequent, not very serious and temporary. Slight arterial hypotension occurred in one of the 32 children, acrocyanosis occurred in one case, and an obstructive respiration occurred in another⁶⁾. The data listed in Switzerland regarding the side effects of propranolol in infants with haemangiomas are very positive: no treatment has had to be interrupted because of an adverse effect. β -Blockers have been used in infants for more than 40 years without there having been observed any complications endangering the life of the child or potentially dangerous cardiovascular diseases⁷⁾. The possible adverse effects of β -blockers are well known, in particular transient bradycardia and arterial hypotension, which is why close monitoring is indicated at the beginning of treatment. Bronchospasm is in principle observed only in children with pre-existing bronchial hyperreactivity; a full history is therefore important. β -Blockers inhibit lipolysis, glycogenolysis and gluconeogenesis and therefore predispose to hypoglycaemia; their use will consequently be avoided during the first two weeks of life⁸⁾.

Current recommendations for the treatment of problematic haemangiomas with propranolol

The indication for treatment with β -blockers should be set out by a centre specializing in treatments for haemangiomas of infancy, in order to obtain a precise diagnosis and indication for treatment with propranolol. To be excluded are the

differential diagnoses of haemangioma of infancy: vascular malformations (particularly venous, lymphatic and capillary malformations), other vascular tumours in children (kaposiform haemangioendothelioma, tufted angioma, pyogenic granuloma), malignant tumours (rhabdomyosarcoma, fibromyosarcoma), nasal glioma and dermoid cyst²⁾. Congenital haemangiomas should also be differentiated, since they present histological and biological particularities different from haemangiomas of infancy. To date, there is no experience regarding the treatment of congenital haemangioma with propranolol. In the presence of a segmental haemangioma of the head, a PHACES (posterior fossa malformations, haemangioma of the face/head, arterial anomalies, cardiac anomalies, eye anomalies, sternal defects) syndrome should be excluded²⁾.

The mere fact that propranolol is available should not lead to a broadening of the treatment indications. Most haemangiomas of infancy do not pose problems and only about 10% (especially head lesions) require a specific treatment.

The use of propranolol for haemangiomas is still an off-label treatment, which requires appropriate information. In accordance with the published data, the consensus of the International Society for the Study of Vascular Anomalies (ISSVA) and the recommendations of paediatric cardiologists, we currently propose the following guidelines for the treatment of haemangiomas of infancy in Switzerland^{3), 4), 6), 9)}.

1. Indications

- Large haemangiomas of the face
- Haemangiomas with problematic localization (eyes, nose, lips, ears, genital region)
- Large segmental haemangiomas of the thorax and of the extremities

2. Beginning of treatment

- As soon as possible during the proliferation phase

3. Contraindications

- Premature babies before the age corresponding to the calculated full term
- Newborns during the first 2 weeks of life
- Congenital heart malformations with β -blocker contraindication

- Infants with episodes of obstructive bronchitis
 - Central nervous system diseases
 - Reduced renal function
4. **Propranolol dosage**
- On the first day, 1 mg/kg/day in 3 doses
 - From the second day, 2 mg/kg/day in 3 doses
5. **Monitoring**
- Monitoring in hospital for the first 48 hours
 - Before treatment: measurement of heart rate and of arterial blood pressure in the four limbs, 12-lead ECG and echocardiography, renal functions and blood glucose
 - During the first 2 days: ECG monitoring during sleep and 1 hour after administration of propranolol, arterial blood pressure 3 ×/day, blood glucose tests if additional risk factors
 - Before leaving hospital: 12-lead ECG
 - After leaving: heart rate and arterial blood pressure after 1 and 4 weeks, then every 8 weeks
6. **Duration of treatment**
- According to the age of the child and the 4-8 month progression. A gradual reduction in dose is not necessary.

These guidelines are directed towards the current data, which are still limited, concerning the use of propranolol for treating haemangiomas of infancy. Further data will require these guidelines to be adapted.

References:

See German text.

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negative predictive value.¹ It should be noted that plasma levels of imatinib in patients given high doses of the drug exceed those of the no-effect level in the study of rat fertility. We also disagree with the specific reservations expressed by the correspondents. The time relationship was reasonable, with oligomenorrhea occurring a few months after the increase in the dose of imatinib. There were no other exposures, and no alternative causes were found on routine investigation of amenorrhea. Finally, the hypothesis of an etiologic link between imatinib and ovarian insufficiency is biologically plausible, since pathways involving kinases targeted by imatinib appear to play critical roles in the survival and maturation of follicles and oocytes.²⁻⁴

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1. García-Cortés M, Lucena MI, Pachkoria K, Borraz Y, Hidalgo R, Andrade RJ. Evaluation of Naranjo Adverse Drug Reactions Probability Scale in causality assessment of drug-induced liver injury. *Aliment Pharmacol Ther* 2008;27:780-9.
2. Hutt KJ, McLaughlin EA, Holland MK. Kit ligand and c-Kit have diverse roles during mammalian oogenesis and folliculogenesis. *Mol Hum Reprod* 2006;12:61-9.
3. Nilsson EE, Detzel C, Skinner MK. Platelet-derived growth factor modulates the primordial to primary follicle transition. *Reproduction* 2006;131:1007-15.
4. Carlsson IB, Laitinen MP, Scott JE, et al. Kit ligand and c-Kit are expressed during early human ovarian follicular development and their interaction is required for the survival of follicles in long-term culture. *Reproduction* 2006;131:641-9.

Propranolol for Severe Hemangiomas of Infancy

TO THE EDITOR: Despite their self-limited course, infantile capillary hemangiomas can impair vital or sensory functions or cause disfigurement. Corticosteroids are the first line of treatment for problematic infantile capillary hemangiomas^{1,2}; other options include interferon alfa³ and vincristine.⁴ We have observed that propranolol can inhibit the growth of these hemangiomas. Our preliminary data from 11 children are summarized in Table 1 in the Supplementary Appendix, available with the full text of this letter at www.nejm.org.

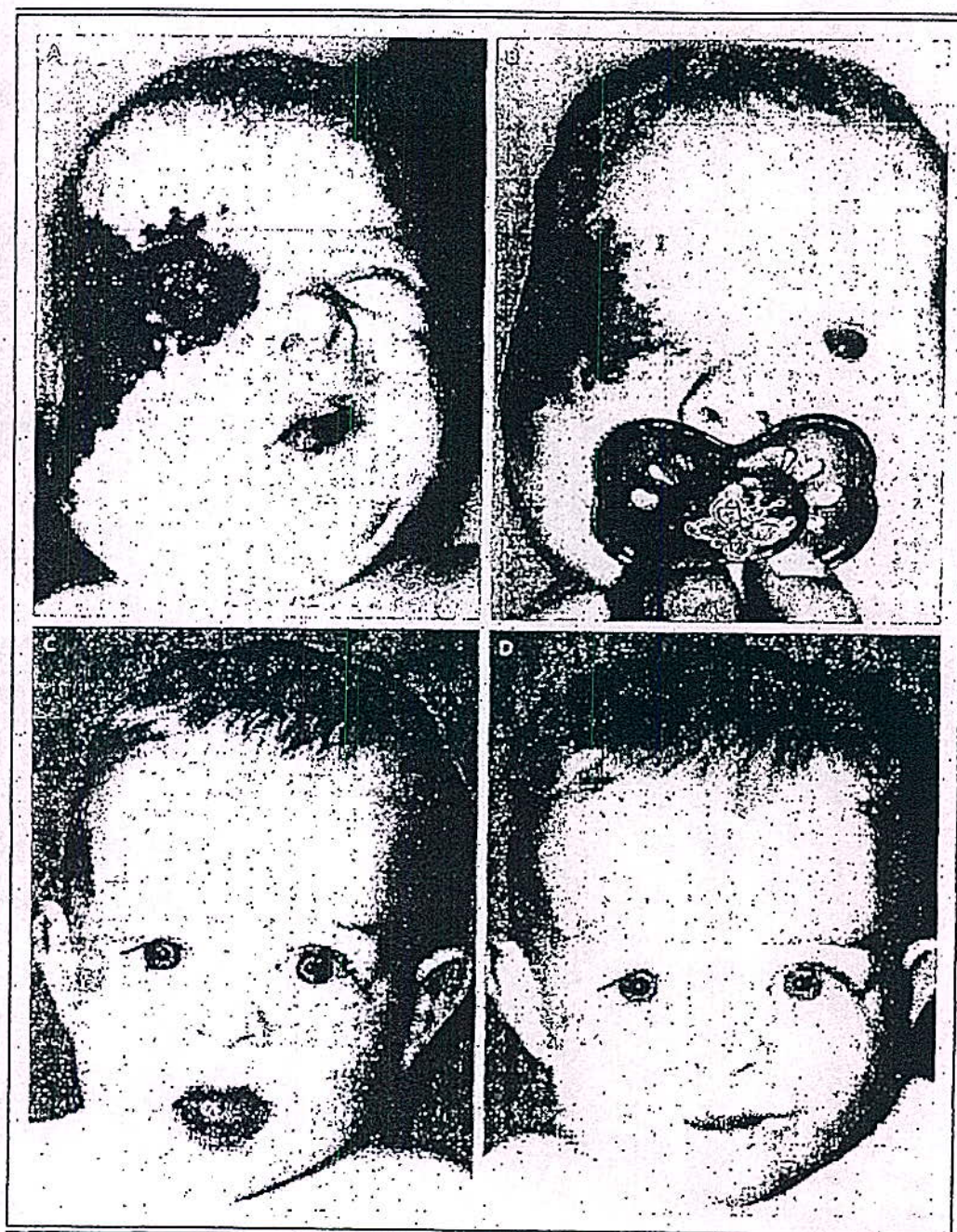
The first child had a nasal capillary hemangioma. Despite corticosteroid treatment, the lesion was stabilized but obstructive hypertrophic myocardiopathy developed, so the patient was treated with propranolol. The day after the initiation of treatment, the hemangioma changed from intense red to purple, and it softened. The corticosteroids were tapered, but the hemangioma continued to improve. When the corticosteroids were discontinued, no regrowth of the hemangioma was noted. When the child was 14 months of age, the hemangioma was completely flat.

The second child had a plaque-like infantile capillary hemangioma involving the entire right upper limb and part of the face (Fig. 1). At 1 month of age, a subcutaneous component developed, and despite corticosteroid treatment, the hemangioma continued to enlarge. Magnetic resonance imaging revealed intraconal and extra-

conal orbital involvement, as well as an intracervical mass causing compression and tracheal and esophageal deviation (see the Supplementary Appendix). Ultrasonography showed increased cardiac output, and treatment with propranolol, at a dose of 2 mg per kilogram of body weight per day, was initiated. Seven days later, the child was able to open his eye spontaneously, and the mass near the parotid gland was considerably reduced in size. Prednisolone was discontinued at 4 months of age, without any regrowth of the hemangioma; at 9 months of age, the eye opening was satisfactory, and no major visual impairment was noted.

After written informed consent had been obtained from the parents, propranolol was given to nine additional children who had severe or disfiguring infantile capillary hemangiomas (see Table 1 in the Supplementary Appendix). In all patients, 24 hours after the initiation of treatment, we observed a change in the hemangioma from intense red to purple; this change was associated with a palpable softening of the lesion. After these initial changes, the hemangiomas continued to improve until they were nearly flat, with residual skin telangiectasias. Ultrasound examinations in five patients showed an objective regression in thickness associated with an increase in the resistive index of vascularization of the hemangioma (Table 1 in the Supplementary Appendix).

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Infantile capillary hemangiomas are composed of a complex mixture of clonal endothelial cells associated with pericytes, dendritic cells, and mast cells.¹ Regulators of hemangioma growth and involution are poorly understood. During the growth phase, two major proangiogenic factors

are involved: basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF);² histologic studies have shown that both endothelial and interstitial cells are actively dividing in this phase. During the involution phase, apoptosis has been shown.³ Potential explanations for

CORRESPONDENCE

Figure 1 (facing page). Photographs of Patient 2 before and after Treatment with Propranolol.

Panel A shows the patient at 9 weeks of age, before treatment with propranolol, after 4 weeks of receiving systemic corticosteroids (at a dose of 3 mg per kilogram of body weight per day for 2 weeks and at a dose of 5 mg per kilogram per day for 2 weeks). Panel B shows the patient at 10 weeks of age, 7 days after the initiation of propranolol treatment at a dose of 2 mg per kilogram per day while prednisolone treatment was tapered to 3 mg per kilogram per day. Spontaneous opening of the eye was possible because of a reduction in the size of the subcutaneous component of the hemangioma. Panel C shows the patient at 6 months of age, while he was still receiving 2 mg of propranolol per kilogram per day. Systemic corticosteroids had been discontinued at 2 months of age. No subcutaneous component of the hemangioma was noted, and the cutaneous component had considerably faded. The child had no visual impairment. Panel D shows the child at 9 months of age. The hemangioma had continued to improve, and the propranolol treatment was discontinued.

the therapeutic effect of propranolol — a nonselective beta-blocker — on infantile capillary hemangiomas include vasoconstriction, which is immediately visible as a change in color, associated with a palpable softening of the hemangioma; decreased expression of VEGF and bFGF genes through the down-regulation of the RAF-mitogen-activated protein kinase pathway⁴ (which explains the progressive improvement of the hemangioma); and the triggering of apoptosis of capillary endothelial cells.⁵

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The authors report applying for a patent for the use of beta-blockers in infantile capillary hemangiomas. No other potential conflict of interest relevant to this letter was reported.

1. Frieden IJ, Haggstrom AM, Drolet BA, Mancini AJ, Friedlander SF, Boon L. Infantile hemangiomas: current knowledge, future directions: proceedings of a research workshop on infantile hemangiomas, April 7-9, 2005, Bethesda, Maryland, USA. *Pediatr Dermatol* 2005;22:383-406.
2. Bennett ML, Fleischer AB Jr, Chamlin SL, Frieden IJ. Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. *Arch Dermatol* 2001;137:1208-13.
3. Ezekowitz RAB, Phil CBD, Mulliken JB, Folkman J. Interferon alfa-2a therapy for life-threatening hemangiomas of infancy. *N Engl J Med* 1992;326:1456-63. (Errata, *N Engl J Med* 1994;330:300, 1995;333:595-6.)
4. D'Angelo G, Lee H, Weiner RI. cAMP-dependent protein kinase inhibits the mitogenic action of vascular endothelial growth factor and fibroblast growth factor in capillary endothelial cells by blocking Raf activation. *J Cell Biochem* 1997;67:353-66.
5. Sommers Smith SK, Smith DM. Beta blockade induces apoptosis in cultured capillary endothelial cells. *In Vitro Cell Dev Biol Anim* 2002;38:298-304.

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Table 1. Characteristics and Treatment of the 11 Patients in the Study.*

Variable	Patient No.										
Characteristic	1	2	3	4	5	6	7	8	9	10	11
Sex	M	M	F	F	F	F	M	M	F	F	F
Birth date	4/5/2006	9/21/2006	1/22/2006	9/12/2006	11/6/2006	11/28/2006	5/24/2007	2/9/2007	4/26/2007	7/9/2007	7/29/2007
Location of IH	None	Face (periorbital area and preauricular gland) and upper limb	Face (periorbital area, preauricular gland, and lip)	Face (periorbital area and preauricular gland)	Periorbital area	Periorbital area	Face (infraorbital cystic, duct, and lip)	Periorbital area and forehead (two distinct areas)	Forehead	Forearm	Face (glabella)
Treatment											
Previous treatment with prednisone											
Dosage (mg/kg/day)	3	3, then 5	2	2-3	None	None	None	None	None	None	None
Effect on IH	Stabilization	Continued growth	Stabilization	Stabilization but relapse of dysplasia at each attempt to stop treatment							
Age at discontinuation (mo)	3.5	4	4	7							
Outcome	No response	No response	No response	No response							
Prognosis											
Age at initiation (mo)	4	2	2	6	2	6	2	4	4	2	2
Reason for treatment	Hypertrophic myocardopathy	Increased cardiac output									
Dosage (mg/kg/day)	3	2	2	2	2	2	2	2	2	2	2
Regression of IH											
Visual assessment	Yes	In volume and color	Yes	Yes	Yes	In color and thickness	In color and thickness	In color and thickness	In color and thickness	In color and thickness	In color and thickness
Ultrasound findings					At D7 of treatment, decrease in size from 11 mm to 3.2 mm in diameter and 6 mm to 1.2 mm in thickness; at 60 days, no detectable component (V1/B6)	150 days after initiation of treatment, decrease in thickness (10 mm vs 1.4 mm) and relative blood flow (0.8-0.7 vs 0.5-0.6)				60 days after initiation of treatment, decrease in thickness (6 mm vs 9 mm) and relative index (0.6-0.7 vs 0.5)	60 days after initiation of treatment, decrease in thickness (4 mm vs 10 mm)
Other effects		Spontaneous eye reopening without amblyopia	Eye reopening	Improvement of dysplasia disappearance of atrophic hemangioma on nasal (dermatology)							
Age at discontinuation (mo)	14	9	12	14	9	11	6	8	7	8	Ongoing
IH status at discontinuation	Stable	Mild recolonization, no response	Stable (mild recolonization and response when propranolol stopped at 9 mo)	Mild recolonization and response	Mild recolonization	No response	Stable	No response	Mild response	Stable	Ongoing
Age at last follow-up (mo)	18	18	14	15	16	11	10	8	7	8	8
IH status at last follow-up	Regression in color, no response	Regression in color, no response	Regression in color	Stable	Regression in color	Regression in color and thickness	Regression in color	Stabilization	Stabilization	Regression in color and thickness	Regression in color and thickness

* Stabilization was defined as the absence of clinical growth in the hemangioma and the absence of significant regression. The relative index = peak systolic velocity - diastolic flow + peak systolic velocity. During the growth phase of a hemangioma, the relative index is low, and it increases during the involuting phase. IH denotes infantile hemangioma, and NA not available. Ultrasound findings were not available for all patients.

Primitive erythropoiesis in infantile haemangioma

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Conflicts of interest

None declared.

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Background Infantile haemangioma (IH) is a tumour of the microvasculature composed predominantly of proliferating endothelial cells. It expresses markers associated with endothelial, haematopoietic and mesenchymal lineages. We have previously shown that the cells forming the capillary endothelium of proliferating IH express cell surface markers and transcription factors consistent with it being a haemogenic endothelium.

Objectives We wished to determine whether the expression of transcription factors associated with the erythroid lineage was of physiological relevance and sufficient for IH tissue cultured *in vitro* to undergo erythropoiesis.

Methods Immunohistochemical staining of paraffin-embedded sections of proliferating IHs was undertaken and expression of the embryonically associated haemoglobin ζ (HBZ) chain and the erythropoietin receptor (EPO-R) was determined. Relative expression of mRNA encoding these proteins was determined by quantitative reverse transcription-polymerase chain reaction using snap-frozen biopsy samples. Differentiation towards erythrocytes was investigated using freshly resected tissue cultured as explants in Matrigel.

Results The endothelium of the microvessels, but not the pericyte layer, was strongly immunoreactive for the EPO-R and the embryonically associated HBZ chain. Abundant expression of transcripts encoding these proteins was also detected, corroborating the immunohistochemical staining. When tissue was grown in culture the cells emanating from IH explants were able to generate enucleated erythrocytes *in vitro*. The erythrocytes were immunoreactive for the erythrocyte-specific marker glycophorin A.

Conclusions The microvessels in IH are a functional haemogenic endothelium that expresses the embryonically associated HBZ chain and is able to form erythrocytes *in vitro*. IH thus represents a possible extramedullary site for tumour-associated primitive erythropoiesis.

Infantile haemangioma (IH) is a tumour of the microvasculature characterized by excessive angiogenesis during infancy. It affects about 10% of white infants with predilection for female, white and premature infants.^{1,2} It typically undergoes rapid postnatal growth (proliferative phase) followed by spontaneous involution over a period of up to 10 years, leaving a fibrofatty residuum with a few mature capillaries.¹⁻⁴

The emerging evidence for the role of primitive mesoderm⁵ and its haemogenic endothelium derivative⁶ in proliferating IH has led us to investigate the ability of IH to form cells of the haematopoietic lineage.

Erythropoiesis is the process through which multipotential haematopoietic stem cells differentiate to form enucleated

erythrocytes.⁷ Primitive erythropoiesis originates from primitive mesodermal cells that develop into a haemogenic endothelium, which sequentially give rise to blast colony forming cells, erythroblasts, reticulocytes and definitive enucleated erythrocytes as development progresses.⁸⁻¹⁰ The stages of *in utero* erythropoiesis have been appropriately termed, in chronological order, mesoblastic, hepatic and myeloid stages corresponding with each subsequent major site of haematopoiesis.¹¹ Haemoglobin production also involves three corresponding developmental switches. The initial embryonic ($\zeta\epsilon\epsilon 2$) haemoglobin switches to fetal ($\alpha 2\gamma 2$) haemoglobin from about 6-7 weeks gestation, and to predominantly adult ($\alpha 2\beta 2$) haemoglobin at birth.¹²

Here we report that IH expresses mRNA and protein for erythropoietin receptor (EPO-R) and embryonic haemoglobin ζ (HBZ), proteins associated with primitive erythropoiesis, and that cells that emanate from IH explants⁴ are able to generate erythrocytes *in vitro*. IH thus represents a possible extra-medullary site for tumour-associated primitive erythropoiesis.

Materials and methods

Immunolabelling

Four-micrometre paraffin-embedded sections of IH biopsies were obtained from 12 patients aged 3–10 months (mean 6), according to a protocol approved by the Wellington Regional Ethics Committee. Immunohistochemistry and immunocytochemistry were performed as previously described.^{5,6,13} All primary antibodies were obtained from Abcam (Cambridge, MA, U.S.A.), and were used at the following dilutions: glycophorin A (1 : 200), CD34 (1 : 300), smooth muscle actin (SMA; 1 : 400), EPO-R (1 : 200) and HBZ (1 : 200).

Negative controls included staining of paraffin sections of tissue (human uterine fibroid) not expected to be immunoreactive with the same series of antibodies, and also by omission of the primary antibodies when staining proliferating IHs excised from three patients. These controls showed the expected staining pattern consistent with the previous reported specificity for the antibodies used^{5,6} (data not shown).

Quantitative reverse transcription–polymerase chain reaction

RNA was extracted from fresh proliferating IH biopsies of five patients aged 3–8 months (mean 5), and quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was performed using primers specific for EPO-R and HBZ. Expression relative to that for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is reported as Δ Ct value. Primers for HBZ and GAPDH have been previously reported.¹⁴ Primers for EPO-R were: forward CTCATCCTCGTGGTCATCCT, reverse GCTGGAAGT-TACCCCTGTGG. Amplification was achieved using an iQ qPCR system (Bio-Rad Laboratories, Hercules, CA, U.S.A.) with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s and extension at 72 °C for 20 s. Data were collected during the extension step and melt-curve analysis and agarose gel electrophoresis performed to confirm the specificity of amplification (data not shown).

Cell culture

Fresh proliferating IH biopsies from five patients aged 3–8 months (mean 5), and two patients with involuted IH, aged 8 and 10 years, were cultured using an *in vitro* explant model as previously described by Tan *et al.*⁴ For all experiments fibrin was replaced with Matrigel (Becton Dickinson, Auckland, New Zealand). Erythrocytes formed in culture were

imaged using an Olympus IX51 inverted microscope (upgraded to IX71) fitted with phase contrast objectives and a Colour View 1 camera (Olympus, Tokyo, Japan). Peripheral blood from each patient was also cultured in parallel as a control. Erythrocytes were collected and processed for immunocytochemistry as previously described.⁵

Results

The capillary endothelium of haemangioma expresses erythropoietin receptor and haemoglobin ζ

The production of erythrocytes depends on the interaction between EPO-R and its ligand, erythropoietin.¹⁵ The process of erythropoiesis is intricately linked to haemoglobin production and the expression of HBZ is associated with embryonic haemoglobin production during the first trimester of pregnancy.¹⁶ Figure 1a shows that immature capillaries of proliferating IH consist of an inner endothelial layer that expresses CD34 (green) and an outer concentric pericyte layer that expresses SMA (red). Figure 1b shows that the cells of the capillary endothelium express EPO-R (green), whereas the cells of the outer pericyte layer that express SMA (red) do not. Figure 1c shows that the cells of the endothelium are immunoreactive for both EPO-R (green) and HBZ (red). qRT-PCR of mRNA isolated from tissue confirmed the abundant expression of both HBZ and EPO-R transcripts relative to that for the control reference gene GAPDH (mean $\pm$ SEM: HBZ Δ Ct = 4.5 $\pm$ 0.8; EPO-R Δ Ct = 10.2 $\pm$ 4).

Proliferating haemangioma explants generate erythrocytes *in vitro*

The functional capacity of proliferating IH to generate erythrocytes was revealed following *in vitro* growth as explants. On initial culturing of explants there were no cells visible within the Matrigel (Fig. S1a; see Supporting Information) and following 2 weeks in culture outgrowths could be seen in most of the explants of proliferating IH (Fig. S1b; see Supporting Information). Consistent with observations of Tan *et al.*,⁴ no visible outgrowths were seen in the involuted IH explants (data not shown). We have previously shown that the cells that form the explant outgrowths express haematopoietic-associated markers,⁵ and here we report that following 1 month in culture blast-like cells can be seen arising from these outgrowths (Fig. S1c; see Supporting Information), that then go on to form blast colonies after 3 months in culture (Fig. S1d; see Supporting Information). Following extended periods of culture (5 months) biconcave cells resembling erythrocytes were visible within the Matrigel culture (Fig. 1d, e, arrows) of proliferating IH lesions. The biconcave cells were present only in regions of Matrigel that had previously contained regions of outgrowth. These biconcave cells were confirmed to be erythrocytes by the expression of the erythrocyte-specific marker, glycophorin A (Fig. 1e, bottom, green). Peripheral blood obtained from the same patient

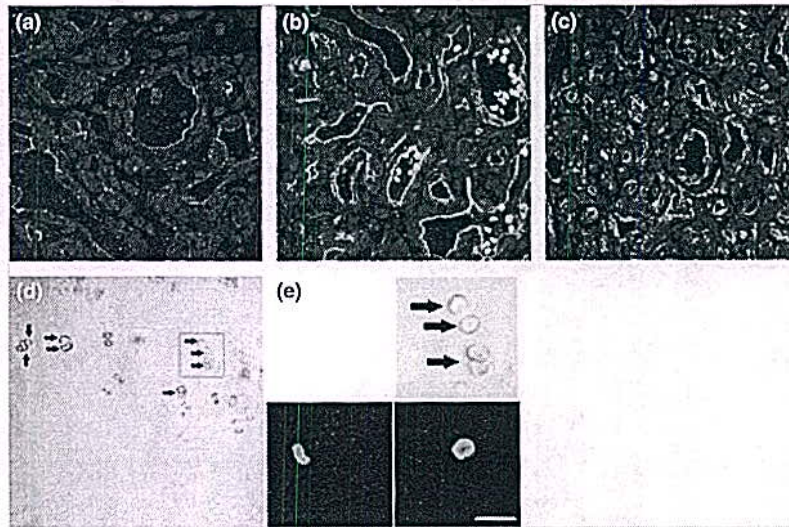


Fig 1. Erythropoiesis in proliferating infantile haemangioma (IH). (a–c) Representative immunohistochemical staining of proliferating IH. (a) The immature capillaries of IH consist of a CD34+ endothelium (green) surrounded by a concentric layer of pericyte-like cells that are immunoreactive for smooth muscle actin (SMA; red). (b) The endothelium is immunoreactive for erythropoietin receptor (EPO-R; green) but the SMA+ pericyte layer (red) is not. (c) The EPO-R+ cells (green) of the endothelium are also immunoreactive for haemoglobin ζ (red). (d) After 5 months in culture biconcave cells morphologically similar to erythrocytes are observed (black arrows). (e) The boxed region containing biconcave cells presented in (d) is shown enlarged; the cells are enucleated and are immunoreactive for the erythrocyte-specific protein glycophorin A (green; bottom). (a–c, e) Counterstained with 4',6-diamidino-2-phenylindole (blue) to identify cell nuclei. Scale bar (e), 10 μ m.

cultured under identical conditions showed no surviving erythrocytes after 3 weeks (data not shown), confirming that erythrocytes that stained positively for glycophorin A were formed *de novo* during culture of proliferating IH lesions rather than originating from carry-over of peripheral blood contaminating the tissue piece.

Discussion

The capillaries of IH consist of an inner endothelial layer, characterized by the expression of endothelial markers, and an outer concentric layer, characterized by the expression of SMA.^{5,6,13} We have recently shown that this endothelial layer of the capillaries reflects a primitive mesodermal phenotype, with the expression of brachyury,⁵ and with potential haemogenic capacity, through the expression of TAL-1, GATA-2, angiotensin-converting enzyme (ACE), CD133 and CD34.⁶

The concept of haematopoiesis originating from an endothelial phenotype forms the basis of the concept of the haemogenic endothelium.^{17–19} Here we demonstrate the expression of erythropoietic-associated proteins by the endothelium of the capillaries of proliferating IH. It is intriguing that the expression of EPO-R, a receptor for the ligand erythropoietin,¹⁵ which is the major haematopoietic associated growth factor responsible for the proliferation, survival and differentiation of erythroid progenitor cells,²⁰ is present on the endothelium of proliferating IH. Reports have confirmed the expression of EPO-R in extra-embryonic yolk sac blood islands,²⁰ the site of primitive haematopoiesis during the first trimester of pregnancy where HBZ is also expressed.^{16,21}

The morphological appearance of blast colony forming units, the precursor to erythroblasts, after 3 months in culture of IH explants, provides further evidence of the presence of another crucial lineage in the overall process of erythropoiesis. We have previously isolated and confirmed the formation of this blast phenotype from IH, by the expression of CD34, vascular endothelial growth factor receptor-2, CD133 and ACE,¹³ and here we show the functional ability of cultured IH explants to form enucleated erythrocytes *in vitro*. To the best of our knowledge, our report is the first to show that IH is a potential site of extramedullary erythropoiesis. We have also previously speculated that it is the cells of the endothelial layer in proliferating IH that form the cells in the explant outgrowths. This suggestion is consistent with the expression of primitive mesoderm-associated proteins by the endothelium and the cells of the outgrowths, but not the cells of the pericyte layer.⁵ We have now demonstrated that these cells have the capacity to undergo functional extramedullary erythropoiesis, although the molecular mechanism and pathway involved require further investigation. Similarly, whether the observed extramedullary erythropoiesis observed in culture takes place *in vivo* within proliferating IH, and if it contributes to the patient's circulating erythrocytes, are yet to be determined.

We infer that the haemogenic endothelium of proliferating IH has the capacity to form cells of the haematopoietic lineage. Given the evidence for formation of erythrocytes and myeloid cells during primitive blood island formation,²² it is also exciting to speculate that cells of the myeloid lineage, e.g. mast cells, found in abundance within IH²³ may be

downstream progenitors of the process of primitive haematopoietic differentiation.

This paper contributes to an emerging body of evidence pointing to IH as a tumour involving the proliferation and eventual differentiation of primitive mesodermal cells. This finding gives new insights into the pathogenesis of IH, as a possible extramedullary site of haematopoiesis, and may form the basis for a novel model for investigating the process of human haematopoietic ontogeny.

What's already known about this topic?

- Cells of the endothelial, haematopoietic and mesenchymal lineages have been identified in infantile haemangioma (IH).
- The capillary endothelium of IH expresses transcription factors associated with haematopoiesis.

What does this study add?

- We demonstrate that the endothelium of IH expresses the embryonically associated haemoglobin ζ chain and the erythropoietin receptor.
- When cultured *in vitro* explanted IH tissue can be differentiated to form enucleated erythrocytes.

References

- 1 Tan ST, Itinteang T, Leadbitter P. Low-dose propranolol for infantile haemangioma. *J Plast Reconstr Aesthet Surg* 2011; 64:292–9.
- 2 Takahashi K, Mulliken JB, Kozakewich HP *et al.* Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994; 93:2357–64.
- 3 Frieden IJ, Eichenfield LF, Esterly NB *et al.* Guidelines of care for hemangiomas of infancy. American Academy of Dermatology Guidelines/Outcomes Committee. *J Am Acad Dermatol* 1997; 37:631–7.
- 4 Tan ST, Hasan Q, Velickovic M *et al.* A novel *in vitro* human model of hemangioma. *Mod Pathol* 2000; 13:92–9.
- 5 Itinteang T, Tan ST, Brasch H, Day DJ. Primitive mesodermal cells with a neural crest stem cell phenotype predominate proliferating infantile haemangioma. *J Clin Pathol* 2010; 63:771–6.
- 6 Itinteang T, Tan ST, Brasch H, Day DJ. Haemogenic endothelium in infantile haemangioma. *J Clin Pathol* 2010; 63:982–6.
- 7 Richmond TD, Chohan M, Barber DL. Turning cells red: signal transduction mediated by erythropoietin. *Trends Cell Biol* 2005; 15:146–55.
- 8 Dieterlen-Lièvre F, Jaffredo T. Decoding the hemogenic endothelium in mammals. *Cell Stem Cell* 2009; 4:189–90.
- 9 Ena M, Yokomizo T, Wakamatsu A *et al.* Primitive erythropoiesis from mesodermal precursors expressing VE-cadherin, PECAM-1, Tie2, endoglin, and CD34 in the mouse embryo. *Blood* 2006; 108:4018–24.
- 10 Gregory C, Eaves A. Three stages of erythropoietic progenitor cell differentiation distinguished by a number of physical and biologic properties. *Blood* 1978; 51:527–37.
- 11 Al-Mufti R, Hambley H, Farzaneh F, Nicolaides K. Fetal and embryonic hemoglobins in erythroblasts from fetal blood and fetal cells enriched from maternal blood in fetal anemia. *Haematologica* 2001; 86:1270–6.
- 12 Peschle C, Mavilio F, Carè A *et al.* Haemoglobin switching in human embryos: asynchrony of zeta-alpha and epsilon-gamma-globin switches in primitive and definitive erythropoietic lineage. *Nature* 1985; 313:235–8.
- 13 Itinteang T, Brasch HD, Tan ST, Day DJ. Expression of components of the renin-angiotensin system in proliferating infantile haemangioma may account for the propranolol-induced accelerated involution. *J Plast Reconstr Aesthet Surg* doi: 10.1016/j.bjps.2010.08.039.
- 14 Hogg AM, Hviid TVF, Christensen B *et al.* zeta-, epsilon-, and gamma-Globin mRNA in blood samples and CD71(+) cell fractions from fetuses and from pregnant and nonpregnant women, with special attention to identification of fetal erythroblasts. *Clin Chem* 2001; 47:645–53.
- 15 Moritz KM, Lim GB, Wintour EM. Developmental regulation of erythropoietin and erythropoiesis. *Am J Physiol Regul Integr Comp Physiol* 1997; 273:R1829–44.
- 16 Schechter AN. Hemoglobin research and the origins of molecular medicine. *Blood* 2008; 112:3927–38.
- 17 Jaffredo T, Gautier R, Eichmann A, Dieterlen-Lièvre F. Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny. *Development* 1998; 125:4575–83.
- 18 Eilken HM, Nishikawa S-I, Schroeder T. Continuous single-cell imaging of blood generation from haemogenic endothelium. *Nature* 2009; 457:896–900.
- 19 Zovein AC, Hofmann JJ, Lynch M *et al.* Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell* 2008; 3:625–36.
- 20 Kertesz N, Wu J, Chen THP *et al.* The role of erythropoietin in regulating angiogenesis. *Dev Biol* 2004; 276:101–10.
- 21 Qiu C, Olivier EN, Velho M, Bouhassira EE. Globin switches in yolk sac-like primitive and fetal-like definitive red blood cells produced from human embryonic stem cells. *Blood* 2008; 111:2400–8.
- 22 Kaufman DS, Hanson ET, Lewis RL *et al.* Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 2001; 98:10716–21.
- 23 Tan ST, Wallis RA, He Y, Davis PF. Mast cells and hemangioma. *Plast Reconstr Surg* 2004; 113:999–1011.

Supporting Information

Additional Supporting Information may be found in the online version of the article:

Fig S1. (a–d) A representative proliferating infantile haemangioma (IH) explant culture. (a) No cells are visible in the Matrigel at the edge of the IH explant culture at day 0. (b) After 2 weeks in culture cellular outgrowths are clearly visible emanating from the IH explant. (c) Following 1 month in culture blast-like cells are visible arising from the cellular outgrowths. (d) After 3 months in culture multiple blast colonies are clearly visible.

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Infantile Hemangioma—Mechanism(s) of Drug Action on a Vascular Tumor

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Infantile hemangioma (IH), a benign vascular tumor, is the most common tumor of infancy, with an incidence of 5%–10% at the end of the first year. The tumor displays a distinctive life cycle consisting of a proliferating phase, occurring in the first months of life, followed by an involuting phase. Thus, IH represents a unique model of postnatal vasculogenesis, angiogenesis, and vessel regression. Traditionally, corticosteroids were the drug of choice when treatment of IH was indicated. In recent years, beta-blockers, most specifically propranolol, have serendipitously been shown to be an effective pharmacological treatment. This article will focus on the mechanism of action of these two drugs, the old and the new treatments, in slowing the growth and accelerating involution of IH.

Infantile hemangioma (IH), a benign vascular tumor, is the most common tumor of infancy, with an incidence of 5%–10% at the end of the first year. There is increased risk in premature neonates under the weight of 1500 g (Amir et al. 1986), in females and in Caucasians (Haggstrom et al. 2007). The tumor displays a distinctive life cycle that can be separated, both clinically and histologically, into three phases (Enjolras and Mulliken 1993; Frieden et al. 2005). The proliferating phase starts within a few weeks from birth and ends within the first year of life, with the most growth occurring during the first 4–6 months of life (Fig. 1) (Chang et al. 2008). In the proliferating phase the tumor is composed of densely packed cells that express endothelial and pericytic markers,

with features of immature vessels such as plump endothelium and high nuclei/cytoplasmic ratio. Multipotent progenitor-like cells reside outside of the vessels (Khan et al. 2008; Boscolo and Bischoff 2009). The involuting phase typically begins at 1 yr of age. Clinically, the infantile hemangioma shrinks centrifugally from the center of the lesion, changes color to less red, and softens. The vascular channels appear more mature, with flattened endothelial cells, an organized perivascular layer, and basement membrane. There is a prominent apoptosis, some occurring in endothelial cells (Iwata et al. 1996; Razon et al. 1998; Dosanjh et al. 2000), and an increase in the number of mast cells. Finally, at the involuted phase, tumor growth has stopped and the tumor has

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Figure 1. Proliferating, involuting, and involuted phases of infantile hemangioma. Hematoxylin and eosin stain (H&E) stained sections from each phase. (A) Proliferating phase is highly cellular with immature vessels, (B) involuting phase contains well-formed blood vessels with endothelial and perivascular layers, and (C) involuted phase contains adipocytes, fibrous deposits, and few remaining vessels. Scale bar, 100 μ m.



regressed. The regression is either complete or, more often, leaves scar tissue, telangiectasia, or redundant or anetodermic skin (Jackson 1998). When viewed in histological sections, fat, fibroblasts, and connective tissue replace the vascular tissue at this stage, with few large feeding and draining vessels evident.

IH is a benign tumor, and in most patients no specific treatment is required. However, in 10% of cases IH is problematic or even endangering to the child, owing to its location or owing to excessive growth. In these circumstances that carry the risk of irreversible disfigurement, airway obstruction, or decreased vision, treatment is indicated. Corticosteroids have been the first-line treatment for IH for more than 40 years (Zarem and Edgerton 1967; Cohen and Wang 1972). In recent years, beta-blockers, most specifically propranolol, have serendipitously been shown to be an effective pharmacological treatment of proliferating IH. Other, less common medications are interferon-2 α and vincristine (Maguiness and Frieden 2010). This article will focus on the mechanism of action of corticosteroids and propranolol, the old and the new treatments, in slowing the growth and accelerating involution of IH. For each drug, a general mechanism of action will be reviewed, then its specific mechanism against IH, and finally, broader mechanisms in antiangiogenic therapy.

CORTICOSTEROIDS

General Mechanism of Action

Glucocorticoids, natural or synthetic, belong to a class of steroid hormones that bind to the glucocorticoid receptor (GR). Unbound GR resides within the cytoplasm in an inactive state, as an oligomeric complex with regulatory proteins such as the HSP90 (heat shock protein-90 KD) (Dalman et al. 1989), HSP70 (heat shock protein-70 KD), and the p59 immunophilin. On diffusion of the glucocorticoid ligand into the cytoplasm and its binding to high-affinity GR, the receptor is activated, and a nuclear localization signal is unmasked (Pelaia et al. 2003). The GR then translocates to the nucleus

where it binds to a glucocorticoid response element (GRE) within the controlling region for glucocorticoid responsive target genes (Adcock et al. 2006). Once bound to DNA, GR can elicit either transactivation or transrepression of gene expression. Transactivation occurs through the recruitment of complexes containing basal transcription factors, coactivators, chromatin modifiers, and RNA polymerase II, which together induce histone modifications and chromatin remodeling that lead to increased production of messenger RNAs (mRNAs) (Adcock et al. 2006). As for transrepression, GR can bind to a GRE that overlaps the DNA-binding site for a transcription factor or the start site of transcription, thus preventing gene expression. Importantly for IH, GR can repress AP-1/NF- κ B-mediated gene expression. Furthermore, it can interact with corepressors such as NCoR (nuclear receptor corepressor receptor) or dephosphorylate RNA polymerase II (Schaaf and Cidlowski 2002; Schoneveld et al. 2004). Finally, GR can induce rapid nongenomic effects through a membrane-associated receptor (Norman et al. 2004) or by modulating the activity of kinases such as ERK, Akt, or phosphatidylinositol 3-kinase and protein kinase Akt.

Mechanism of Action in Infantile IH

When studying the effect of a drug on a tumoral process, one has to consider the activity on the different cellular compartments as well as on the inter-relationships between the cells. Thus, a brief description of the major cell types in IH is provided. Endothelial cells constitute about 20% of the cell population within the tumor (Yu et al. 2004). These hemangioma-derived endothelial cells have been shown to be clonal, have immature morphology, and display increased growth and migratory properties compared to normal dermal endothelial cells (Dosanjh et al. 2000; Boye et al. 2001; Yu et al. 2001; Dadras et al. 2004). Pericytes, or mural cells, are arranged around the endothelial cell layer in a proliferating IH tissue section (Li et al. 2003; Boscolo and Bischoff 2009). Several cell types have been identified in the tumor's interstitium. Among them are mast cells, which



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increase in number during involution (Qu et al. 1995; Tan et al. 2004), and myeloid cells (CD45⁺/CD14⁺), which are present during the proliferative phase. Hemangioma progenitor cells or hemangioma-derived stem cells (HemSCs) have been identified and isolated by us based on the expression of the human stem/progenitor cell marker CD133 (Shmelkov et al. 2005). These cells have self-renewal capacity and display multipotential activity, being able to differentiate in vivo into endothelial cells and adipocytes (Khan et al. 2008). When injected subcutaneously into nude mice, HemSC form human blood vessels that express GLUT-1, a specific IH marker. Hence—the HemSC are vasculogenic—able to form blood vessels de novo. Furthermore, 2 mo after injection the vessels are replaced by fat tissue, indicating the HemSCs can recapitulate key features of the life cycle of IH.

Evidence from In Vitro and In Vivo Studies of IH

Blocking of the Vasculogenic Potential of HemSC

To study the effect of corticosteroids on vasculogenic processes that occur when HemSC are implanted in vivo, we used our animal model. HemSC were injected subcutaneously into nude mice, and dexamethasone (a synthetic glucocorticoid) was injected intraperitoneally thereafter for 7 d. Dexamethasone inhibited the formation of blood vessels in a dose-dependent manner (Greenberger et al. 2010b). Furthermore, by pretreating the HemSC and endothelial cells with dexamethasone, each separately in vitro and before implantation, we were able to show that HemSCs, and not the endothelial cells, are the target of corticosteroids. This indicated that steroids might work by inhibiting the vasculogenesis that occurs in IH.

Down-Regulation of Vascular Endothelial Growth Factor A

Vascular endothelial growth factor A (VEGF-A), a master regulator of angiogenesis and vasculogenesis, has been shown by us and by others

to be present at higher levels in IH tumors in the proliferating phase compared to the involuting phase (Takahashi et al. 1994; Chang et al. 1999; Kleinman et al. 2007; Greenberger et al. 2010a,b). It has also been shown that corticosteroids suppress the expression of VEGF-A in a variety of cell types (Bandi and Kompella 2001; Kompella et al. 2003; Kim et al. 2008). We found that the corticosteroids dexamethasone, prednisone, prednisolone, methylprednisolone, and hydrocortisone were each able to dramatically down-regulate VEGF-A secretion by HemSC. The suppression was reversed by RU-486, a synthetic steroid hormone antagonist, indicating a GR-dependent mechanism (Greenberger et al. 2010b). These findings are consistent with reports that show decreased levels of VEGF-A in the serum of IH patients following systemic steroid therapy (Zhang et al. 2005). Silencing the expression of VEGF-A in HemSC by short hairpin RNA (shRNA) was sufficient to block blood vessel formation in vivo (Greenberger et al. 2010b). The effect of the steroids was not mediated by the induction of apoptosis as dexamethasone did not lead to reduced viability of HemSC or endothelial cells, even at concentrations 1000 times higher than those that led to VEGF suppression.

Modification of Proangiogenic Profile

The direct target genes of corticosteroids in vivo are still largely unknown and might be tissue or even cell specific (Phuc Le et al. 2005; van Batenburg et al. 2010). Examining HemSC and endothelial cells in vitro revealed that several corticosteroid-modified genes with relevance for angiogenesis, in addition to VEGF-A, were expressed (Greenberger et al. 2010b). Monocyte chemoattractant protein-1 (MCP-1), which has been reported to be overexpressed in proliferating versus involuting infantile hemangioma (Isik et al. 1996), was suppressed by dexamethasone. Interleukin-6, which was detected in in vitro hemangioma models (Hasan et al. 2003), was also suppressed, as were matrix metalloproteinase-1 (MMP-1) and urokinase-type plasminogen activator receptor (uPAR). These target genes, MCP-1,



uPAR, and IL-6, were found to be differentially expressed in proliferating versus involuting IH tissue by quantitative real-time polymerase chain reaction (PCR) measurements (Greenberger et al. 2010b). In sum, corticosteroids seem to affect key molecules in the angiogenesis process when analyzed in HemSC and hemangioma-derived endothelial cells cultured in vitro.

Suppression of Nuclear Factor κ -Light-Chain-Enhancer of Activated B Cells Activity

Corticosteroids are known to be regulators of nuclear factor κ -light-chain enhancer of activated B cells (NF- κ B) activity, through several distinct mechanisms (Tao et al. 2001), for example, (1) physical interaction between NF- κ B subunits and GR (Caldenhoven et al. 1995), (2) increased transcription of the NF- κ B inhibitor I κ B α (Auphan et al. 1995), and (3) competition for interactions with coactivators (Kamei et al. 1996; Lee et al. 1998). In HemSC, corticosteroids suppress NF- κ B activity. This suppression in turn leads to down-regulation of VEGF-A, as well as other proangiogenic cytokines MCP-1, uPAR, MMP-1, and IL-6 (Greenberger et al. 2010a). As VEGF-A is critical for hemangiogenesis, this finding suggests that the effect of corticosteroids is to interfere with NF- κ B regulation. In addition, suppression of NF- κ B may also have direct antiendothelial effects, as NF- κ B has been shown to contribute to embryonic carcinoma (EC) survival in vitro, and NF- κ B blockade in vivo sensitizes ECs to stress-induced apoptosis (Kisseleva et al. 2006).

Antagonizing Estrogen's Effects

Corticosteroids have been shown to antagonize estrogen responses (Bever et al. 1956; Rhen et al. 2003), including the prosurvival effects of estrogen (Zhou et al. 1989) and its stimulatory effects on insulin growth factor-1 (IGF-1) (Sahlin 1995). This inhibition might occur through corticosteroid induction of the expression and activity of estrogen sulfotransferase, an enzyme that deactivates estrogens (Gong et al. 2008). In

infants with proliferating IH, serum levels of estrogen (estradiol 17 β) have been shown to be increased compared to normal infants. (Sasaki et al. 1984; Liu et al. 1999). Furthermore, estrogen receptor (ER) expression and binding activity were reported to be higher in patients with proliferating IH (Sasaki et al. 1984; Kleinman et al. 2007) and to decrease following corticosteroid treatment (Sasaki et al. 1984). As estrogen has been shown to be essential for neoangiogenesis (Das et al. 2009) and vasculogenesis (Masuda et al. 2007) in the reproductive system, it might then be the case that corticosteroids exert an additional effect on IH—that of antagonizing the activity of estrogen.

PROPRANOLOL

Propranolol as a pharmacological treatment of IH was first reported in 2008, in two children who showed rapid regression of disease when treated with propranolol for cardiopulmonary indications (Leaute-Labreze et al. 2008). Since then, propranolol treatment for IH has become popular worldwide and positive results with its use are reported by many groups (Cheng et al. 2010; Mazereeuw-Hautier et al. 2010; Rosbe et al. 2010). Propranolol is a noncardioselective β -adrenergic receptor blocker used traditionally for other indications such as hypertension, angina pectoris, myocardial infarction, migraines, anxiety disorders, and tremor. The mechanism of action of propranolol on IH remains elusive. To date there are no studies on the effects of propranolol using IH animal models or cultured IH-derived cells. Therefore, we will summarize here the general mechanism of action of propranolol and suggest possible mechanisms based on data derived from studies performed using angiogenesis assays and endothelial cells.

General Mechanism of Action

When catecholamines, adrenaline and noradrenaline, interact with the β -adrenergic receptors β 1 or β 2, an agonist-promoted binding of the receptor to the heterotrimeric guanosine



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triphosphate-binding protein Gs occurs, causing dissociation of G α -GTP and G $\beta\gamma$ subunits. This in turn leads to activation of adenylate cyclase and production of cyclic adenosine monophosphate (cAMP). Downstream effectors of cAMP include cAMP-dependent protein kinase (PKA) as well as cAMP-gated ion channels (Benovic 2002). Propranolol is an orthosteric antagonist of both β 1- and β 2-adrenergic receptors. In addition, it has been shown to function as central serotonin 5-HT receptor antagonist, inhibitor of noradrenaline reuptake and indirect agonist of α -adrenergic receptors (Young and Glennon 2009). The drug exists as a pair of optical isomers: *S*(-)-propranolol and *R*(+)-propranolol. The enantiomers bind with relatively large differences in affinity to the β -adrenoceptors (Young and Glennon 2009). Most commercial preparations contain a mix of the two isomers.

Hemodynamic Effects

Following the administration of propranolol, a rapid change in the tumor is typically noticed, including decreased redness and softening (Sans et al. 2009; Rosbe et al. 2010). This raises the question of whether propranolol exerts its effect via vasoconstriction of the high-flow blood vessels feeding the IH tumor. Propranolol has been shown to decrease blood flow to many tissues following single administration (Nies et al. 1973; McSorley and Warren 1978; Vandenburg et al. 1981). Particularly in the skin, adrenaline-induced vasoconstriction has been shown to be increased by oral propranolol (Doshi et al. 1984). However, as prolonged treatment probably does not affect vascular resistance in subcutaneous tissues (Jensen et al. 1983), it is reasonable to assume that there are additional mechanisms that account for the prolonged effects of propranolol on the course of IH.

VEGF-A

VEGF-A is a critical factor for the growth of IH. Thus, suppression of VEGF-A by propranolol is a plausible mechanism for its activity. Noradrenaline has been shown to enhance VEGF-A

production by several cell types, both normal and cancerous (Fredriksson et al. 2000; Lutgen-dorf et al. 2003; Park et al. 2011). Furthermore, in an ovarian carcinoma animal model, catecholamines up-regulated tumoral VEGF and increased angiogenesis (Thaker et al. 2006). These effects of the catecholamines are mediated by the β 1 and β 2 adrenergic receptors and are blocked by propranolol (Fredriksson et al. 2000; Park et al. 2011). In addition to VEGF, noradrenaline up-regulates HIF-1 α protein (Park et al. 2011). The signal transduction pathway leading to HIF-1 α and VEGF-A up-regulation is mediated through cAMP and PKA (Fredriksson 2000). In some cell lines, Src tyrosine kinase, a downstream effector of PKA, is involved, however, not through activation of the mitogen-activated protein (MAP) kinases Erk1/2 (Fredriksson et al. 2000). Importantly, propranolol does not affect baseline level of VEGF-A production by the cells, but rather opposes catecholamine stimulation (Fredriksson et al. 2000; Thaker et al. 2006). Thus, if VEGF suppression is a proposed mechanism of action, one must assume increased noradrenaline stimulation or increased sensitivity to noradrenaline in the tumor. Studies testing these assumptions in vitro and in vivo on IH models are needed to begin to gain some insight into how propranolol exerts its effects on IH. In our laboratory, we examined the effect of propranolol on VEGF-A production in hemangioma-derived stem cells and endothelial cells, but found no evidence that propranolol affected VEGF-A under basal cell culture conditions (S Greenberger et al., unpubl.).

Down-Regulation of Other Proangiogenic Cytokines

Angiogenesis is a process in which a coordinated set of events leads to the formation of new blood vessels by sprouting from pre-existing vessels. Among those events is the proteolysis of components of the extracellular matrix allowing the endothelial cells to migrate and sprout (Aznavorian et al. 1993; Bergers et al. 2000). Accordingly, matrix metalloproteinases (MMPs), secreted both by tumor cells



and by tumor-associated macrophages (TAMs) have been shown to be critical for angiogenesis (Bergers et al. 2000; Fang et al. 2000; Huang et al. 2002). In a vascular tumor model, using endothelioma cells, a synthetic inhibitor of MMPs inhibited in vivo tumor growth (Tarabozletti et al. 1995). Noradrenaline increases the expression of MMP-2 and MMP-9 by tumor cells and by macrophages, and these effects could be inhibited by the beta-blocker, propranolol (Lutgendorf et al. 2003; Guo et al. 2009). Propranolol has also been reported to block the up-regulation of MMP-7 in a gastric tumor (Shi et al. 2010). In brain vascular endothelial cells, propranolol blocked the induction of MMP-9 by PMA (a phorbol ester that causes an extremely wide range of effects in cells (Blumberg 1981; Annabi et al. 2009). Thus, it is possible that propranolol's effect is mediated, at least in part, by MMPs regulation.

In addition to MMP down-regulation, propranolol might inhibit hemangiogenesis by the control of another proangiogenic cytokine, IL-6. Recent reports have shown IL-6 is up-regulated by catecholamines, both in the transcriptional and in the translational levels, and that the up-regulation is blocked by propranolol. In-depth analysis of this signaling pathway also suggests a cAMP and Src kinase-dependent mechanism (Nilsson et al. 2007). It is of interest that both corticosteroids and propranolol suppress VEGF-A, MMPs, and IL-6. This overlap in activity may provide a clue to the roles of these proteins in the pathogenesis of IH.

Effects on Differentiation

IH is a tumor in which new blood vessels form, presumably de novo, from immature progenitor (stem) cells. Thus, it is possible that propranolol prevents the hemangioma stem cells from differentiating into endothelial cells or pericytes (Frieden and Drolet 2009). Another possibility is that propranolol hastens the differentiation of the progenitor cells into adipocytes, which would in effect convert the tumor stage from "proliferating" to "involuting." Although support for this hypothesis has not yet been

provided by experimentation with in vitro or in vivo models of IH, effects have been reported on mesenchymal stem cells. For example, noradrenaline has been shown to push mesenchymal progenitors within white adipose tissue toward a brown adipocytic phenotype (Vegiopoulos et al. 2010). Also, an effect of noradrenaline on mesenchymal stem cell adipogenesis has been shown in vitro (Li et al. 2010).

CONCLUDING REMARKS

In recent years, our understanding of the pathological vasculogenic process leading to IH proliferation has expanded with the identification of hemangioma stem cells and the corticosteroid-sensitive targets. The emergence of propranolol as a new therapeutic option that is both safe and effective provides a crucial alternative therapy for disfiguring, and at times, life-endangering hemangiomas. A major challenge for the future years will be to elucidate the mechanism of action of propranolol on proliferating IH. Insights gained from such studies will enable the development of target-specific drugs, not only for IH but also for malignant vascular tumors and tumor angiogenesis.

REFERENCES

- Adcock IM, Caramori G, Ito K. 2006. New insights into the molecular mechanisms of corticosteroids actions. *Curr Drug Targets* 7: 649–660.
- Amir J, Metzker A, Krikler R, Reisner SH. 1986. Strawberry hemangioma in preterm infants. *Pediatric Dermatol* 3: 331–332.
- Annabi B, Lachambre MP, Plouffe K, Moumdjian R, Beliveau R. 2009. Propranolol adrenergic blockade inhibits human brain endothelial cells tubulogenesis and matrix metalloproteinase-9 secretion. *Pharmacol Res* 60: 438–445.
- Auphan N, DiDonato JA, Rosette C, Helmsberg A, Karin M. 1995. Immunosuppression by glucocorticoids: Inhibition of NF- κ B activity through induction of I κ B synthesis. *Science* 270: 286–290.
- Aznavorian S, Murphy AN, Stetler-Stevenson WG, Liotta LA. 1993. Molecular aspects of tumor cell invasion and metastasis. *Cancer* 71: 1368–1383.
- Bandi N, Kompella UB. 2001. Budesonide reduces vascular endothelial growth factor secretion and expression in airway (Calu-1) and alveolar (A549) epithelial cells. *Eur J Pharmacol* 425: 109–116.
- Benovic JL. 2002. Novel β 2-adrenergic receptor signaling pathways. *J Allergy Clin Immunol* 110: S229–S235.



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- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, et al. 2000. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2: 737–744.
- Bever AT, Hisaw FL, Velardo JT. 1956. Inhibitory action of desoxycorticosterone acetate, cortisone acetate, and testosterone on uterine growth induced by estradiol-17 β . *Endocrinology* 59: 165–169.
- Blumberg PM. 1981. In vitro studies on the mode of action of the phorbol esters, potent tumor promoters, part 2. *Crit Rev Toxicol* 8: 199–234.
- Boscolo E, Bischoff J. 2009. Vasculogenesis in infantile hemangioma. *Angiogenesis* 12: 197–207.
- Boye E, Yu Y, Paranya G, Mulliken JB, Olsen BR, Bischoff J. 2001. Clonality and altered behavior of endothelial cells from hemangiomas. *J Clin Invest* 107: 745–752.
- Caldenhoven E, Liden J, Wissink S, Van de Stolpe A, Raaijmakers J, Koenderman L, Okret S, Gustafsson JA, Van der Saag PT. 1995. Negative cross-talk between RelA and the glucocorticoid receptor: A possible mechanism for the antiinflammatory action of glucocorticoids. *Mol Endocrinol* 9: 401–412.
- Chang J, Most D, Bresnick S, Mehrara B, Steinbrech DS, Reinisch J, Longaker MT, Turk AE. 1999. Proliferative hemangiomas: Analysis of cytokine gene expression and angiogenesis. *Plast Reconstr Surg* 103: 1–9.
- Chang LC, Haggstrom AN, Drolet BA, Baselga E, Chamlin SL, Garzon MC, Horii KA, Lucky AW, Mancini AJ, Metry DW, et al. 2008. Growth characteristics of infantile hemangiomas: Implications for management. *Pediatrics* 122: 360–367.
- Cheng JF, Gole GA, Sullivan TJ. 2010. Propranolol in the management of periorbital infantile haemangioma. *Clin Experiment Ophthalmol* 38: 547–553.
- Cohen SR, Wang CI. 1972. Steroid treatment of hemangioma of the head and neck in children. *Ann Otol Rhinol Laryngol* 81: 584–590.
- Dadras SS, North PE, Bertoni J, Mihm MC, Detmar M. 2004. Infantile hemangiomas are arrested in an early developmental vascular differentiation state. *Mod Pathol* 17: 1068–1079.
- Dalman FC, Bresnick EH, Patel PD, Perdew GH, Watson SJ Jr, Pratt WB. 1989. Direct evidence that the glucocorticoid receptor binds to hsp90 at or near the termination of receptor translation in vitro. *J Biol Chem* 264: 19815–19821.
- Das A, Mantena SR, Kannan A, Evans DB, Bagchi MK, Bagchi IC. 2009. De novo synthesis of estrogen in pregnant uterus is critical for stromal decidualization and angiogenesis. *Proc Natl Acad Sci* 106: 12542–12547.
- Dosanjh A, Chang J, Bresnick S, Zhou L, Reinisch J, Longaker M, Karasek M. 2000. In vitro characteristics of neonatal hemangioma endothelial cells: Similarities and differences between normal neonatal and fetal endothelial cells. *J Cutan Pathol* 27: 441–450.
- Doshi BS, Kulkarni RD, Dattani KK, Anand MP. 1984. Effect of labetalol and propranolol on human cutaneous vasoconstrictor response to adrenaline. *Int J Clin Pharmacol Res* 4: 25–28.
- Enjolras O, Mulliken JB. 1993. The current management of vascular birthmarks. *Pediatr Dermatol* 10: 311–313.
- Fang J, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G, Moses MA. 2000. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proc Natl Acad Sci* 97: 3884–3889.
- Fredriksson JM, Lindquist JM, Bronnikov GE, Nedergaard J. 2000. Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a β -adrenoreceptor/cAMP/protein kinase A pathway involving Src but independently of Erk1/2. *J Biol Chem* 275: 13802–13811.
- Frieden IJ, Drolet BA. 2009. Propranolol for infantile hemangiomas: Promise, peril, pathogenesis. *Pediatr Dermatol* 26: 642–644.
- Frieden IJ, Haggstrom AN, Drolet BA, Mancini AJ, Friedlander SF, Boon L, Chamlin SL, Baselga E, Garzon MC, Nopper AJ, et al. 2005. Infantile hemangiomas: Current knowledge, future directions. In *Proceedings of a research workshop on infantile hemangiomas*, April 7–9, 2005, Bethesda, Maryland. *Pediatr Dermatol* 22: 383–406.
- Gong H, Jarzynka MJ, Cole TJ, Lee JH, Wada T, Zhang B, Gao J, Song WC, DeFranco DB, Cheng SY, et al. 2008. Glucocorticoids antagonize estrogens by glucocorticoid receptor-mediated activation of estrogen sulfotransferase. *Cancer Res* 68: 7386–7393.
- Greenberger S, Adini I, Boscolo E, Mulliken JB, Bischoff J. 2010a. Targeting NF- κ B in infantile hemangioma-derived stem cells reduces VEGF-A expression. *Angiogenesis* 13: 327–335.
- Greenberger S, Boscolo E, Adini I, Mulliken JB, Bischoff J. 2010b. Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells. *New Engl J Med* 362: 1005–1013.
- Guo K, Ma Q, Wang L, Hu H, Li J, Zhang D, Zhang M. 2009. Norepinephrine-induced invasion by pancreatic cancer cells is inhibited by propranolol. *Oncol Rep* 22: 825–830.
- Haggstrom AN, Drolet BA, Baselga E, Chamlin SL, Garzon MC, Horii KA, Lucky AW, Mancini AJ, Metry DW, Newell B, et al. 2007. Prospective study of infantile hemangiomas: Demographic, prenatal, and perinatal characteristics. *J Pediatr* 150: 291–294.
- Hasan Q, Tan ST, Xu B, Davis PF. 2003. Effects of five commonly used glucocorticoids on haemangioma in vitro. *Clin Exp Pharmacol Physiol* 30: 140–144.
- Huang S, Van Arsdall M, Tedjarati S, McCarty M, Wu W, Langley R, Fidler IJ. 2002. Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. *J Natl Cancer Inst* 94: 1134–1142.
- Isik FF, Rand RP, Gruss JS, Benjamin D, Alpers CE. 1996. Monocyte chemoattractant protein-1 mRNA expression in hemangiomas and vascular malformations. *J Surg Res* 61: 71–76.
- Iwata J, Sonobe H, Furihata M, Ido E, Ohtsuki Y. 1996. High frequency of apoptosis in infantile capillary haemangioma. *J Pathol* 179: 403–408.
- Jackson R. 1998. The natural history of strawberry naevi. *J Cutan Med Surg* 2: 187–189.
- Jensen K, Skagen K, Henriksen O, Kjoller E. 1983. Local blood flow regulation in normotensive man during prolonged treatment with propranolol. *Eur J Clin Pharmacol* 24: 723–726.



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- Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, et al. 1996. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 85: 403–414.
- Khan ZA, Boscolo E, Picard A, Psutka S, Melero-Martin JM, Barch TC, Mulliken JB, Bischoff J. 2008. Multipotential stem cells recapitulate human infantile hemangioma in immunodeficient mice. *J Clin Invest* 118: 2592–2599.
- Kim H, Lee JM, Park JS, Jo SA, Kim YO, Kim CW, Jo I. 2008. Dexamethasone coordinately regulates angiopoietin-1 and VEGF: A mechanism of glucocorticoid-induced stabilization of blood-brain barrier. *Biochem Biophys Res Commun* 372: 243–248.
- Kisseleva T, Song L, Vorontchikhina M, Feirt N, Kitajewski J, Schindler C. 2006. NF- κ B regulation of endothelial cell function during LPS-induced toxemia and cancer. *J Clin Invest* 116: 2955–2963.
- Kleinman ME, Greives MR, Churgin SS, Blechman KM, Chang EI, Ceradini DJ, Tepper OM, Gurtner GC. 2007. Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma. *Arterioscler Thromb Vasc Biol* 27: 2664–2670.
- Kompella UB, Bandi N, Ayalasomayajula SP. 2003. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest Ophthalmol Vis Sci* 44: 1192–1201.
- Leaute-Labreze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taieb A. 2008. Propranolol for severe hemangiomas of infancy. *New Engl J Med* 358: 2649–2651.
- Lee SK, Kim HJ, Na SY, Kim TS, Choi HS, Im SY, Lee JW. 1998. Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. *J Biol Chem* 273: 16651–16654.
- Li Q, Yu Y, Bischoff J, Mulliken JB, Olsen BR. 2003. Differential expression of CD146 in tissues and endothelial cells derived from infantile haemangioma and normal human skin. *J Pathol* 201: 296–302.
- Li H, Fong C, Chen Y, Cai G, Yang M. 2010. β -Adrenergic signals regulate adipogenesis of mouse mesenchymal stem cells via cAMP/PKA pathway. *Mol Cell Endocrinol* 323: 201–207.
- Liu W, Zhang S, Hu T, Jiang X, Hu X, Feng J. 1999. Sex hormone receptor of hemangioma and vascular malformation in children. *Zhonghua Wai Ke Za Zhi* 37: 295–297.
- Lutgendorf SK, Cole S, Costanzo E, Bradley S, Coffin J, Jabbari S, Rainwater K, Ritchie JM, Yang M, Sood AK. 2003. Stress-related mediators stimulate vascular endothelial growth factor secretion by two ovarian cancer cell lines. *Clin Cancer Res* 9: 4514–4521.
- Maguiness SM, Frieden IJ. 2010. Current management of infantile hemangiomas. *Semin Cutan Med Surg* 29: 106–114.
- Masuda H, Kalka C, Takahashi T, Yoshida M, Wada M, Kobori M, Itoh R, Iwaguro H, Eguchi M, Iwami Y, et al. 2007. Estrogen-mediated endothelial progenitor cell biology and kinetics for physiological postnatal vasculogenesis. *Circ Res* 101: 598–606.
- Mazereeuw-Hautier J, Hoeger PH, Benlahrech S, Ammour A, Broue P, Vial J, Ohanessian G, Leaute-Labreze C, Labenne M, Vabres P, et al. 2010. Efficacy of propranolol in hepatic infantile hemangiomas with diffuse neonatal hemangiomatosis. *J Pediatr* 157: 340–342.
- McSorley PD, Warren DJ. 1978. Effects of propranolol and metoprolol on the peripheral circulation. *Brit Med J* 2: 1598–1600.
- Nies AS, Evans GH, Shand DG. 1973. Regional hemodynamic effects of β -adrenergic blockade with propranolol in the unanesthetized primate. *Am Heart J* 85: 97–102.
- Nilsson MB, Armaiz-Pena G, Takahashi R, Lin YG, Trevino J, Li Y, Jennings N, Arevalo J, Lutgendorf SK, Gallick GE, et al. 2007. Stress hormones regulate interleukin-6 expression by human ovarian carcinoma cells through a Src-dependent mechanism. *J Biol Chem* 282: 29919–29926.
- Norman AW, Mizwicki MT, Norman DP. 2004. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nat Rev Drug Discov* 3: 27–41.
- Park SY, Kang JH, Jeong KJ, Lee J, Han JW, Choi WS, Kim YK, Kang J, Park CG, Lee HY. 2011. Norepinephrine induces VEGF expression and angiogenesis by a hypoxia-inducible factor-1 α protein-dependent mechanism. *Int J Cancer* 128: 2306–2316.
- Pelaia G, Vatrella A, Cuda G, Maselli R, Marsico SA. 2003. Molecular mechanisms of corticosteroid actions in chronic inflammatory airway diseases. *Life Sci* 72: 1549–1561.
- Phuc Le P, Friedman JR, Schug J, Brestelli JE, Parker JB, Bochkis IM, Kaestner KH. 2005. Glucocorticoid receptor-dependent gene regulatory networks. *PLoS Genet* 1: e16. doi: 10.1371/journal.pgen.0010016.
- Qu Z, Liebler JM, Powers MR, Galey T, Ahmadi P, Huang XN, Ansel JC, Butterfield JH, Planck SR, Rosenbaum JT. 1995. Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol* 147: 564–573.
- Razon MJ, Kraling BM, Mulliken JB, Bischoff J. 1998. Increased apoptosis coincides with onset of involution in infantile hemangioma. *Microcirculation* 5: 189–195.
- Rhen T, Grissom S, Afshari C, Cidlowski JA. 2003. Dexamethasone blocks the rapid biological effects of 17 β -estradiol in the rat uterus without antagonizing its global genomic actions. *FASEB J* 17: 1849–1870.
- Rosbe KW, Suh KY, Meyer AK, Maguiness SM, Frieden IJ. 2010. Propranolol in the management of airway infantile hemangiomas. *Arch Otolaryngol Head Neck Surg* 136: 658–665.
- Sahlin L. 1995. Dexamethasone attenuates the estradiol-induced increase of IGF-I mRNA in the rat uterus. *J Steroid Biochem Mol Biol* 55: 9–15.
- Sans V, de la Roque ED, Berge J, Grenier N, Boralevi F, Mazereeuw-Hautier J, Lipsker D, Dupuis E, Ezzedine K, Vergnes P, et al. 2009. Propranolol for severe infantile hemangiomas: Follow-up report. *Pediatrics* 124: e423–e431.
- Sasaki GH, Pang CY, Wittliff JL. 1984. Pathogenesis and treatment of infant skin strawberry hemangiomas: Clinical and in vitro studies of hormonal effects. *Plast Reconstr Surg* 73: 359–370.



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- Schaaf MJ, Cidlowski JA. 2002. Molecular mechanisms of glucocorticoid action and resistance. *J Steroid Biochem Mol Biol* 83: 37–48.
- Schoneveld OJ, Gaemers IC, Lamers WH. 2004. Mechanisms of glucocorticoid signalling. *Biochim Biophys Acta* 1680: 114–128.
- Shi M, Liu D, Duan H, Han C, Wei B, Qian L, Chen C, Guo L, Hu M, Yu M, et al. Catecholamine up-regulates MMP-7 expression by activating AP-1 and STAT3 in gastric cancer. *Molecular Cancer* 9: 269.
- Shmelkov SV, Meeus S, Moussazadeh N, Kermani P, Rashbaum WK, Rabbany SY, Hanson MA, Lane WJ, St Clair R, Walsh KA, et al. 2005. Cytokine preconditioning promotes codifferentiation of human fetal liver CD133⁺ stem cells into angiomyogenic tissue. *Circulation* 111: 1175–1183.
- Takahashi K, Mulliken JB, Kozakewich HP, Rogers RA, Folkman J, Ezekowitz RA. 1994. Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 93: 2357–2364.
- Tan ST, Wallis RA, He Y, Davis PE. 2004. Mast cells and hemangioma. *Plast Reconstr Surg* 113: 999–1011.
- Tao Y, Williams-Skipp C, Scheinman RI. 2001. Mapping of glucocorticoid receptor DNA binding domain surfaces contributing to transrepression of NF- κ B and induction of apoptosis. *J Biol Chem* 276: 2329–2332.
- Taraboletti G, Garofalo A, Belotti D, Drudis T, Borsotti P, Scanziani E, Brown PD, Giavazzi R. 1995. Inhibition of angiogenesis and murine hemangioma growth by batimastat, a synthetic inhibitor of matrix metalloproteinases. *J Natl Cancer Inst* 87: 293–298.
- Thaker PH, Han LX, Kamat AA, Arevalo JM, Takahashi R, Lu C, Jennings NB, Armaiz-Pena G, Bankson JA, Ravoori M, et al. 2006. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 12: 939–944.
- van Batenburg ME, Li H, Polman JA, Lachize S, Datson NA, Bussemaker HJ, Meijer OC. 2010. Paired hormone response elements predict caveolin-1 as a glucocorticoid target gene. *PLoS One* 5: e8839, doi: 10.1371/journal.pone.0008839.
- Vandenburg MJ, Conlon C, Ledingham JM. 1981. A comparison of the effects of propranolol and oxprenolol on forearm blood flow and skin temperature. *Br J Clin Pharmacol* 11: 485–490.
- Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Berriel Diaz M, Rozman J, Hrabec de Angelis M, Nusing RM, et al. 2010. COX-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. *Science* 328: 1158–1161.
- Young R, Glennon RA. 2009. S(-)-Propranolol as a discriminative stimulus and its comparison to the stimulus effects of cocaine in rats. *Psychopharmacology* 203: 369–382.
- Yu Y, Varughese J, Brown LE, Mulliken JB, Bischoff J. 2001. Increased Tie2 expression, enhanced response to angiopoietin-1, and dysregulated angiopoietin-2 expression in hemangioma-derived endothelial cells. *Am J Pathol* 159: 2271–2280.
- Yu Y, Flint AE, Mulliken JB, Wu JK, Bischoff J. 2004. Endothelial progenitor cells in infantile hemangioma. *Blood* 103: 1373–1375.
- Zarem HA, Edgerton MT. 1967. Induced resolution of cavernous hemangiomas following prednisolone therapy. *Plast Reconstr Surg* 39: 76–83.
- Zhang L, Lin X, Wang W, Zhuang X, Dong J, Qi Z, Hu Q. 2005. Circulating level of vascular endothelial growth factor in differentiating hemangioma from vascular malformation patients. *Plast Reconstr Surg* 116: 200–204.
- Zhou F, Bouillard B, Pharaboz-Joly MO, Andre J. 1989. Non-classical antiestrogenic actions of dexamethasone in variant MCF-7 human breast cancer cells in culture. *Molec Cell Endocrinol* 66: 189–197.

to RAF inhibitors; thus, it will be worthwhile to examine changes in Axl expression in cell line-based models of resistance to RAF inhibitors, as well as in samples from relapsed patients. In summary, Gas6-Axl signaling may be a signaling "axis of evil" in melanoma, and it should be investigated further as a "druggable" pathway.

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

- Goodall J, Carreira S, Denat L *et al.* (2008) Brn-2 represses microphthalmia-associated transcription factor expression and marks a distinct subpopulation of microphthalmia-associated transcription factor-negative melanoma cells. *Cancer Res* 68:7788–94
- Hoek KS, Eichhoff OM, Schlegel NC *et al.* (2008) In vivo switching of human melanoma cells between proliferative and invasive states. *Cancer Res* 68:650–6
- Holland SJ, Pan A, Franci C *et al.* (2010) R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. *Cancer Res* 70:1544–54
- Johannessen CM, Boehm JS, Kim SY *et al.* (2010) COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 468:968–72
- Liu E, Hjelle B, Bishop JM (1988) Transforming genes in chronic myelogenous leukemia. *Proc Natl Acad Sci USA* 85:1952–6
- O'Bryan JP, Frye RA, Cogswell PC *et al.* (1991) Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol* 11:5016–31
- Sensi M, Catani M, Castellano G *et al.* (2011) Human cutaneous melanomas lacking MITF and melanocyte differentiation antigens express a functional Axl receptor kinase. *J Invest Dermatol* 131:2448–57
- Tworowski K, Singhal G, Szpakowski S *et al.* (2011) Phosphoproteomic screen identifies potential therapeutic targets in melanoma. *Mol Cancer Res* 9:801–12
- Villanueva J, Vultur A, Lee JT *et al.* (2010) Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. *Cancer Cell* 18:683–95
- Widlund HR, Fisher DE (2003) Microphthalmia-associated transcription factor: a critical regulator of pigment cell development and survival. *Oncogene* 22:3035–41

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Infantile Hemangioma Research: Looking Backward and Forward

Ilona J. Frieden¹

This is a remarkable time to be a student of infantile hemangiomas (IHs). IH is a common tumor, estimated to occur in approximately 4% of infants. Studied for many decades, the acquisition of knowledge and pace of IH research are accelerating. The article by Greenberger *et al.* in this issue is a welcome addition to the literature. It examines rapamycin as a possible treatment for IH that could potentially be curative because suppression of self-renewal of stem cells might deplete hemangiomas of the stem cells from which they originate. However, before we get too enthusiastic about using rapamycin for IHs, it is important to reflect on lessons learned from previous hemangioma therapies.

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Infantile hemangioma research in the 20th century

Although there was evidence as far back as the late 1930s that

infantile hemangioma (IH) involuted spontaneously, studies describing the natural history of IHs published in the 1960s more clearly delineated the

characteristic natural history of rapid growth followed by gradual involution. X-irradiation therapy, an effective treatment for hemangiomas, was used widely from 1930 to 1950 (Mulliken and Young, 1988). Starting in the 1950s, prominent physicians—among them the founders of the subspecialty of pediatric dermatology, Alvin Jacobs and Sidney Hurwitz—decried the use of X-irradiation and attempts at preemptive surgical removal, advocating a hands-off approach. Why, after all, would you treat something that would go away on its own? This was sage advice, but it had its limitations. Although most hemangiomas involute without leaving major sequelae, a significant minority are problematic.

An important therapeutic breakthrough came in the mid-1960s, when systemic corticosteroids were found to be an effective treatment. Despite their many potential side effects, prednisolone and other corticosteroids became a mainstay of treatment for those hemangiomas severe enough to require systemic therapy. Treatment of IH causing medical morbidities (e.g., amblyopia or airway disease) was clearly necessary. Less clear, however, was the approach for hemangiomas that are not medically endangering but are arguably life-altering—leaving scarring or permanent distortion of important anatomic structures of the face, such as the nose and lips.

In 1982, Mulliken and Glowacki proposed a biologic classification of vascular birthmarks into "hemangiomas" and "vascular malformations." This was a major conceptual breakthrough that helped to clearly delineate IHs as an entity distinct from other vascular birthmarks (particularly venous malformation, with which it was often lumped, under the diagnosis of "cavernous hemangioma") (Mulliken and Glowacki, 1982). Like many other important ideas, this one seems obvious in retrospect. At the time, however, nosologic confusion was a major obstacle to understanding the biology of IH; this confusion hampered efforts to define optimal treatments. A chronic lack of funding also hindered research. Without funding from the National Institutes

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COMMENTARY

of Health or pharmaceutical interest, research continued to consist mainly of case reports and small case series.

By the late 1980s and early 1990s, multidisciplinary vascular anomalies centers were being established both in the United States and internationally. These centers began to concentrate referral patients and increase collective expertise regarding hemangiomas as well as other vascular anomalies. Interested physicians and scientists began to hold informal workshops, and in 1992, the International Society for the Study of Vascular Anomalies was established. Other developments that spurred interest in hemangiomas included the trend toward increasing patient self-advocacy, which led many parents of infants with IHs to question the standard advice "not to worry" and instead look for other answers. Patient-support organizations were formed.

Advances in laser therapy, particularly the pulsed dye laser (PDL), which became commercially available in 1989, added another potential treatment for IHs. This advance, in turn, led many physicians to reconsider whether there might be better therapies (including PDL) to treat hemangiomas. In 1991, Ezekowitz *et al.* reported the use of interferon (IFN)- α as a novel hemangioma therapy; in 1992 they published a case series of 20 patients, most of whom had failed systemic corticosteroid therapy. Recognition of the need for better therapies is attested to by more than a dozen articles published within five years after the initial reports of IFN therapy (Ezekowitz *et al.*, 1992). Unfortunately, another lesson was learned from the IFN experience: in the late 1990s, severe neurotoxicity was recognized as a side effect, occurring in up to 20% of patients.

IH research in the 21st century

The pace of hemangioma research has increased significantly in the 21st century (Figure 1). In January 2000, North *et al.* published their seminal observation that, unlike other vascular tumors or vascular malformations, IHs stain with the immunohistochemical marker Glut-1 (North *et al.*, 2000). This and the many observations that followed revolutionized the way we

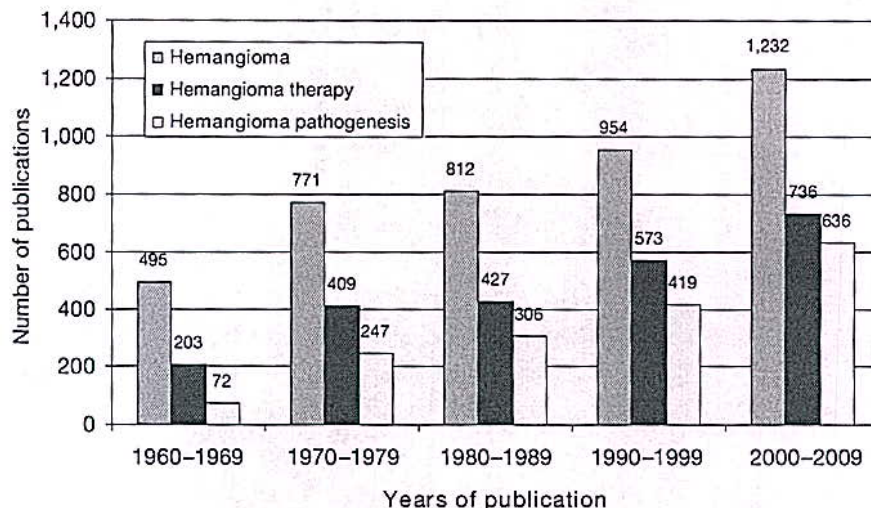


Figure 1. Results of a PubMed search on 1 August 2011. Search terms: hemangioma[majr:noexp] OR (hemangioendothelioma[majr:noexp]) OR (hemangioma, capillary[majr:noexp]) OR (hemangioma, cavernous[majr:noexp]). Limits: All infant: birth-23 months. Subsearches were with "therapy" as a subheading and with "physiopathology," "pathology," and "physiology" and keyword "pathogene*" as subheadings.

think about hemangiomas. We now know that IH is not a tumor of ordinary cutaneous blood vessels that have "run amok" but, rather, a growth with a unique vascular phenotype. The demonstration of the remarkable similarities between IH and placental blood vessels led several investigators to look more closely at the cells of origin of IH. We now recognize that IH may be not solely a disorder of angiogenesis (i.e., the sprouting of vessels from existing ones) but in fact due—at least in part—to a disorder of vasculogenesis (i.e., *de novo* formation of new blood vessels from circulating stem cells) (Boscolo and Bischoff, 2009; Dadras *et al.*, 2004). The progenitor cells of IH have many pluripotent qualities, with features of myeloid, monocytic, endothelial, and even neural crest differentiation (Itinteang *et al.*, 2010; Kleinman *et al.*, 2007). Therefore, IHs may represent a growth of primitive mesenchymal cells in a state of arrested development. Attempts to characterize the nature of the progenitor cells of hemangiomas continue. The development of sustainable *in vitro* and *ex vivo* models was another major step. In 2000, Tan *et al.* reported such a model, with tissue explants of IH embedded in fibrin gel (Tan *et al.*, 2000). In 2008, Khan *et al.* reported the creation of a sustainable *ex vivo* model in nude

mice. In this model, used in the current study, isolation of multipotential stem cells from surgically removed IH tissue gave rise to hemangioma-like lesions, retaining phenotype and eventually involuting with decreased blood vessels and increased adipocytes, mimicking the natural history of IHs (Khan *et al.*, 2008).

Over the past decade, many studies have increased our understanding of the clinical characteristics of IHs. We now know that hemangiomas are not randomly distributed. We have also moved beyond the traditional morphologic descriptions of IH as superficial, deep, and mixed to recognize two other important patterns of hemangiomas: those that are spatially confined, or "localized," and those with a territorial distribution, or "segmental" (Chiller *et al.*, 2002; Waner *et al.*, 2003). The patterns of segmental hemangiomas of the face were found to correspond to embryologic prominences (such as the maxillary and mandibular placodes) that arise early in embryologic development (Haggstrom *et al.*, 2006b; Waner *et al.*, 2003). Like their smaller, more localized counterparts, segmental IHs have all the immunohistochemical and molecular features of other IHs, including rapid growth and spontaneous involution, but the recognition of these patterns of distribution

and the increased appreciation that IHs can have associated structural malformations (e.g., PHACE syndrome) provided further clues that segmental IHs arise via an early error in embryologic development that is permissive for hemangioma progenitor cell growth.

A study of early hemangioma growth by the Hemangioma Investigator Group (HIG) added yet another clue to the developmental nature of hemangiomas. Both localized and segmental hemangiomas, rather than having a lateral growth phase (as one might expect from a conventional tumor), “mark out their territory” and grow volumetrically (Chang *et al.*, 2008). Other HIG studies have helped to identify the clinical characteristics that best predict the greatest risk for complications. A prospective cohort study of more than 1,000 infants demonstrated that segmental hemangiomas have a higher risk of complications, need for treatment, and associated structural anomalies (Haggstrom *et al.*, 2006a). Current HIG studies include the creation of a disease-severity score and the creation of an instrument for measuring the impact on parental quality of life.

This history would not be complete without mention of one of the most remarkable recent developments: the serendipitous observation that propranolol is an effective treatment for IHs. In the three years since June 2008 when Leaute-Labreze *et al.* first reported their findings in a letter to the *New England Journal of Medicine*, more than 140 articles regarding β -blocker therapy for IHs have been published, nearly all reporting positive results (Leaute-Labreze *et al.*, 2008).

Other β -blockers, such as acebutolol and nadolol, may also be effective, and preliminary reports of a topical β -blocker, timolol, suggest that it may be helpful for smaller, more superficial hemangiomas. Experience thus far with propranolol for IHs again points to caution when treating young infants with hemangiomas—one might have expected bradycardia or hypotension as possible side effects, but, instead, hypoglycemia has emerged as a rare but potentially serious side effect.

Future therapies

Greenberger and colleagues’ study (2011, this issue) regarding the effects of rapamycin on an *in vitro* model of IH is a welcome step in this journey. Rapamycin appears to target HemSC via a mechanism distinct from corticosteroids, suggesting that the drugs used together (perhaps at lower doses) could enhance their effects. Interestingly, this report does not mention whether propranolol or other β -blockers have similar effects—something that would be of great interest because propranolol is becoming a first-line therapy in many centers. The finding of a loss of stem cell properties should be of particular interest because such an effect might conceivably offer a cure without the rebound growth that can be a problem, for a significant minority of patients, in both steroid and β -blocker use. The target of rapamycin (mTOR) is a kinase of the phosphoinositide 3-kinase signaling pathway. It is one of the most important intracellular mediators of the activity of growth factor receptors, including vascular endothelial growth factor (VEGF). Although preliminary evidence suggests that rapamycin, given systemically or

applied topically, is beneficial under other conditions with vascular overgrowth (such as angiofibromas in tuberous sclerosis), its activity would not necessarily be anticipated in IH. In addition to its possible therapeutic implications, the finding of its effects in a laboratory model of IH should certainly be taken as a clue to looking closely at its effects on VEGF and VEGF receptors, which have already been shown to play a role in hemangioma growth (Jinnin *et al.*, 2008).

The idea of yet another drug that might be useful in treating IHs is exciting, but the history of unanticipated problems with previous hemangioma therapies makes it important to inject a cautionary note. We have known for decades that not all hemangiomas need treatment; enthusiasm for a new therapy should never let us lose sight of this important fact. Given the tremendous heterogeneity in size, location, and growth potential, the development of validated disease-severity scores should help to ensure that we compare hemangiomas of similar severity rather than lumping together disparate IHs. Equally important is the recognition that the target population for hemangioma therapy is infants who both are undergoing rapid somatic growth and have rapidly maturing organs—in particular, the central nervous system. Infants may thus be particularly vulnerable to side effects that are either unanticipated (e.g., spastic diplegia with IFN) or rare in older patients (e.g., hypoglycemia with propranolol). Rapamycin (sirolimus), a calcineurin inhibitor, has many potential side effects and has boxed warnings regarding risk of immunosuppression and risk of developing lymphoma and other malignancies. Experience regarding its toxicities in infants is limited (Blatt *et al.*, 2010). It is generally not used in combination with corticosteroids because of the potential for additive immunosuppressive effects. Do these concerns mean we should not consider rapamycin a potential treatment? Not necessarily—but we should hold ourselves to a higher standard than in decades past and study this and future hemangioma therapies in an evidence-based manner.

Clinical Implications

- Multipotential stem cells from surgically removed infantile hemangioma (IH) tissue give rise to hemangioma-like lesions that mimic the natural history of IH.
- Based on research reported in this article, rapamycin may benefit infants with IH.
- Even if rapamycin does benefit some infants with IH, its potential for toxicity appears to be greater than that of propranolol.
- There is a severe lack of high-quality randomized controlled trials that assess the effects of treatment for IH.

COMMENTARY

A recent Cochrane analysis highlights the deficiencies of previous hemangioma treatment. More than four decades after corticosteroids were found to be effective in treating IHs and two decades after the introduction of PDL for IHs, the authors of this meta-analysis were able to find only four randomized controlled trials (RCTs), involving only 271 patients, to analyze (Leonardi-Bee *et al.*, 2011). They found insufficient evidence to support existing interventions, such as corticosteroids and laser, and concluded that there is "a need for further high-quality RCTs to validate the findings from these studies, and RCTs to assess the effect of other treatments, in particular relating to propranolol." Fortunately, at least 10 treatment studies for IH are currently listed at ClinicalTrials.gov, including a large international RCT comparing propranolol and placebo, and it is hoped that more will follow. We owe it to infants with IHs and the scientific community to study this disease and its potential therapies rigorously.

CONFLICT OF INTEREST

The author has consulted for Pierre-Fabre Dermatology.

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REFERENCES

- Blatt J, Stavas J, Moats-Staats B *et al.* (2010) Treatment of childhood kaposiform hemangioendothelioma with sirolimus. *Pediatr Blood Cancer* 55:1396–8
- Boscolo E, Bischoff J (2009) Vasculogenesis in infantile hemangioma. *Angiogenesis* 12:197–207
- Chang LC, Haggstrom AN, Drolet BA *et al.* (2008) Growth characteristics of infantile hemangiomas: implications for management. *Pediatrics* 122:360–7
- Chiller KG, Passaro D, Frieden IJ (2002) Hemangiomas of infancy: clinical characteristics, morphologic subtypes, and their relationship to race, ethnicity, and sex. *Arch Dermatol* 138:1567–76
- Dadras SS, North PE, Bertoni J *et al.* (2004) Infantile hemangiomas are arrested in an early developmental vascular differentiation state. *Mod Pathol* 17:1068–79
- Ezekowitz RA, Mulliken JB, Folkman J (1992) Interferon alfa-2a therapy for life-threatening hemangiomas of infancy. *N Engl J Med* 326:1456–63
- Greenberger S, Yuan S, Walsh LA *et al.* (2011) Rapamycin suppresses self-renewal and vasculogenic potential of stem cells isolated from infantile hemangioma. *J Invest Dermatol* 131:2467–76
- Haggstrom AN, Drolet BA, Baselga E *et al.* (2006a) Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. *Pediatrics* 118:882–7
- Haggstrom AN, Lammer EJ, Schneider RA *et al.* (2006b) Patterns of infantile hemangiomas: new clues to hemangioma pathogenesis and embryonic facial development. *Pediatrics* 117:698–703
- Itinteang T, Tan ST, Brasch H *et al.* (2010) Haemogenic endothelium in infantile haemangioma. *J Clin Pathol* 63:982–6
- Jinnin M, Medici D, Park L *et al.* (2008) Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma. *Nat Med* 14:1236–46
- Khan ZA, Boscolo E, Picard A *et al.* (2008) Multipotential stem cells recapitulate human infantile hemangioma in immunodeficient mice. *J Clin Invest* 118:2592–9
- Kleinman ME, Greives MR, Churgin SS *et al.* (2007) Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma. *Arterioscler Thromb Vasc Biol* 27:2664–70
- Leaute-Labreze C, Dumas De La Roque E, Hubiche T *et al.* (2008) Propranolol for severe hemangiomas of infancy. *N Engl J Med* 358:2649–51
- Leonardi-Bee J, Batta K, O'Brien C *et al.* (2011) Interventions for infantile haemangiomas (strawberry birthmarks) of the skin. *Cochrane Database Syst Rev* 5:CD006545
- Mulliken JB, Glowacki J (1982) Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg* 69:412–22
- Mulliken JB, Young AE (1988) Treatment of hemangiomas. In: McAllister L (ed) *Vascular Birthmarks: Hemangiomas and Malformations*. WB Saunders: Philadelphia, PA. pp77–103
- North PE, Waner M, Mizeracki A *et al.* (2000) GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol* 31:11–22
- Tan ST, Hasan Q, Velickovic M *et al.* (2000) A novel in vitro human model of hemangioma. *Mod Pathol* 13:92–9
- Waner M, North PE, Scherer KA *et al.* (2003) The nonrandom distribution of facial hemangiomas. *Arch Dermatol* 139:869–75

Propranolol versus Prednisone in the Treatment of Infantile Hemangiomas: A Retrospective Comparative Study

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Abstract: The goal of the current study was to compare the clinical effectiveness of oral propranolol with that of oral prednisone in the treatment of infantile hemangiomas (IH). Patients treated for IH with oral propranolol were retrospectively matched with patients treated with oral prednisone according to type, location, and size of the IH and age at start of treatment. Response to treatment was evaluated by rating serial medical photographs taken 1, 2, and 6 months after initiation of treatment. Degree of clinical improvement in overall appearance (including color and size) was rated as follows: worse or stable (0), slight (<25%), moderate (25–50%), good (50–75%), or excellent (>75%). A second assessment was done using a 100-mm visual analog scale to rate improvement at 6 months. Pre and post-treatment imaging was available for several patients. Twelve pairs of infants with IH were analyzed. At 1 month, clinical improvement in the propranolol group was moderate to good in all patients. In the prednisone group, only one patient had moderate improvement, with others showing slight (7/12) or no improvement or stabilization (3/12) from baseline and one case worsening. At 6 months, the propranolol group showed good to excellent response in all cases, whereas nine in the prednisone group showed slight to moderate response. Doppler ultrasound and magnetic resonance imaging correlated with the clinical improvement in the cases in which it was performed. No major side effects were observed in either group. Propranolol appears superior to oral prednisone in inducing more-rapid and greater clinical improvement in this study. A larger prospective study comparing these two treatment modalities is warranted.

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Infantile hemangiomas (IH) are the most common tumor of infancy, occurring in 4% to 10% of infants, mostly female (1). Most IH have a characteristic dynamic natural history of growth early in infancy followed by spontaneous involution, although a minority can be problematic and even life threatening. Size, location, and subtype (segmental or localized) of IH are factors predicting possible complications, including ulceration, functional impairment, and cardiac compromise (2). Finally, IH of the face can lead to permanent disfigurement.

Oral corticosteroids have been the gold standard in the treatment of problematic IH (1,3). Prospective data on corticosteroid therapy for IH are lacking, and no consensus exists as to the optimal treatment regimen and response rate (2,3). Effectiveness, defined as stabilization or decrease in size, has been reported in up to 75% of cases with doses of 2–3 mg/kg/day (3), but optimal dose and regimen for tapering remain unknown (3). Other therapeutic modalities for complicated IH include interferon and vincristine (4,5). A significant risk of neurologic and hematologic toxicity is associated with these modalities, limiting their use (4). As reported by Léauté-Labreze et al (6), propranolol at 2–3 mg/kg/day has been reported to be efficient at controlling the proliferation of IH. Improvement appears to be more rapid and greater with propranolol than with systemic steroids (7). Multiple reports have confirmed the efficacy of propranolol, and most feel that it is superior to oral corticosteroid, but no objective data exist to support this clinical impression.

We sought to retrospectively compare the clinical effectiveness of oral propranolol with that of oral prednisone in the treatment of IH.

METHODS

This study was conducted in the dermatology and vascular anomalies clinic in a pediatric academic referral center. Patients treated with oral propranolol were retrospectively matched with patients treated with oral prednisone from our vascular anomalies database and matched according to type, location, and size of IH and age at start of treatment. Serial medical photography, by professional medical photographers, is part of our regular follow-up of patients with IH.

Response to treatment was primarily evaluated by rating of serial medical photographs by two independent evaluators using two methods of assessment. Two registered nurses involved in the general dermatology clinic, but not in vascular anomalies, were asked to evaluate the overall appearance (defined as the general aspect of the lesion, including color and size). Photographs taken 1, 2,

TABLE 1. Rating of Improvement (Method 1)

Degree of improvement	Rate
Stable or worse (0%)	0
Slight improvement (< 25%)	1
Moderate (25–50%)	2
Good (50–75%)	3
Excellent (> 75%)	4

and 6 months after initiation of treatment were rated according to percentage of improvement (Table 1). A 100-mm visual analog scale (VAS) was used at 6 months for the same purpose. Serial photographs were randomly given to the evaluators without matching pairs. They were evaluated independently, and evaluators were blinded to treatment.

A VAS is a measurement instrument used to evaluate a characteristic that cannot be easily measured. A horizontal 100-mm line with a range from –100 to 100 was used. Doubling in size was recorded as –100, no change as 0, and complete resolution as 100 (8). Our methods of evaluating efficacy of treatment, including the VAS, were previously used in other studies on the treatment of IH (8,9). Intraclass correlation coefficients (ICC) were used to quantify correlation between evaluators and the *t*-test was used to establish statistically significant differences in efficacy of both treatments. Using the Wilcoxon analysis, we calculated that the mean age at initiation of treatment was similar for the two groups. Retrospective chart review was performed for all patients.

RESULTS

Twelve selected pairs of infants were analyzed. Locations of treated IH were eyelid (*n* = 3), cheek (*n* = 3), nose (*n* = 2), forehead or glabella (*n* = 2), parotid gland (*n* = 1), and lip (*n* = 1). Types of IH were mixed (*n* = 6), deep (*n* = 4), and superficial (*n* = 2). Of these, two pairs of segmental facial IH were included; they did not have cerebrovascular anomalies and did not fulfill the diagnostic criteria for PHACE syndrome.

Mean age at initiation of treatment was similar in both groups (3.8 months [range 1–9 months] for prednisone and 3.7 months [range 1.5–8.7 months] for propranolol (*p* = 0.97). Mean dose was 2.8 mg/kg/day (range 2.0–4.0) for patients treated with prednisone and 2.7 mg/kg/day (range 2.5–3.5) for propranolol.

After 1 month of treatment, clinical improvement was moderate to good in all patients treated with propranolol. In the prednisone group, only one patient had moderate improvement, and others showed slight (7/12) or no improvement or stabilization (3/12) from

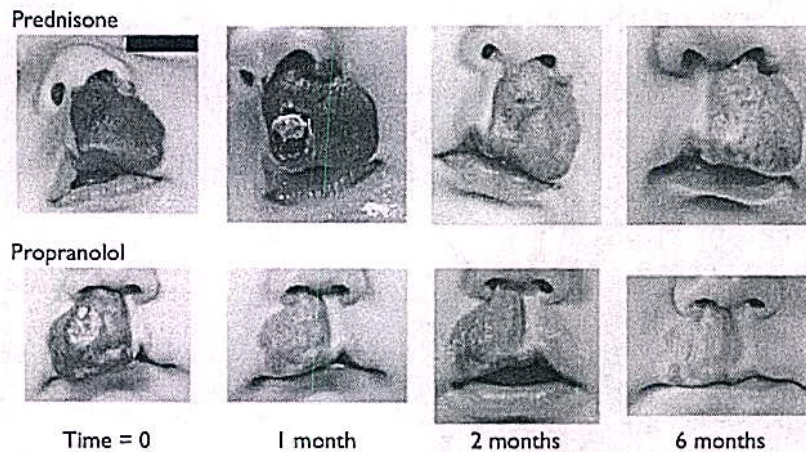


Figure 1. Right upper lip infantile hemangioma: comparative medical photographs taken before treatment (time = 0) and after 1, 2, and 6 months of treatment (upper row: prednisone, lower row: propranolol). Age at start of treatment: 3.7 months (prednisone) and 4.5 months (propranolol).

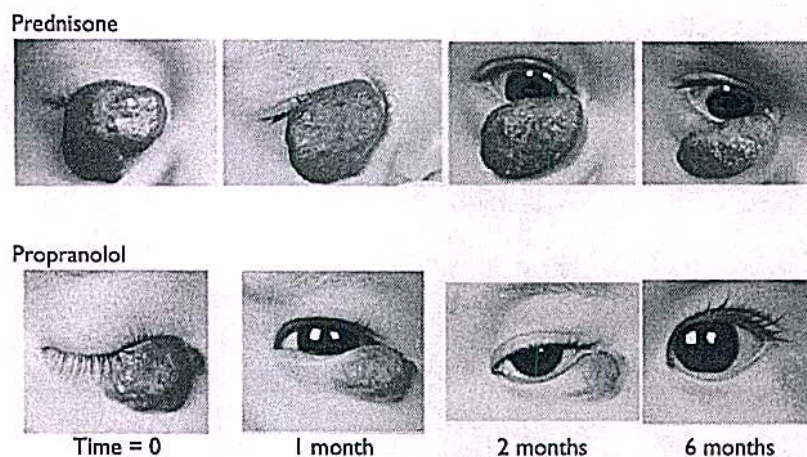


Figure 2. Lower eyelid infantile hemangioma: comparative medical photographs taken before treatment (time = 0) and after 1, 2, and 6 months of treatment (upper row: prednisone, lower row: propranolol). Age at start of treatment: 2.5 months (prednisone) and 3.3 months (propranolol).

baseline photographs, with one case that had worsened. All patients treated with propranolol had good to excellent improvement at 6 months, whereas all of those treated with prednisone had slight to moderate improvement. We calculated the mean rating of improvement by the two investigators at each time point for both treatments. Prednisone was rated as 1.1 at 1 month, 1.3 at 2 months, and 1.8 at 6 months, versus propranolol, which was rated as 2.2 at 1 month, 2.9 at 2 months, and 3.7 at 6 months (Figs. 1–4). After 1, 2, and 6 months of continuous treatment, propranolol was significantly superior to prednisone according to mean rating of improvement ($p = 0.007$, $p = 0.002$, $p < 0.001$, respectively). ICC was between 0.762 and 0.862 for both treatments, at each point in time ($p = 0.001$ – 0.01) (Fig. 4).

Using the VAS, mean improvement after 6 months of treatment was 44.8% with prednisone, versus 78.7% with propranolol (ICC 0.833, $p < 0.001$; Table 2).

No serious adverse effects were observed. Based on chart review, reported events in patients treated with prednisone were irritability ($n = 2$), oral thrush ($n = 2$), insomnia ($n = 1$), hypertension ($n = 1$), and growth failure ($n = 1$). In patients treated with propranolol, adverse effects were sleep disturbances ($n = 6$), transient asymptomatic hypotension ($n = 1$), and vomiting ($n = 1$).

Mean duration of treatment was 12.7 months (range 5–28 months) for prednisone and 10.6 months (range 7–13 months) for patients treated with propranolol; five patients were still being treated with propranolol at the time of chart review.

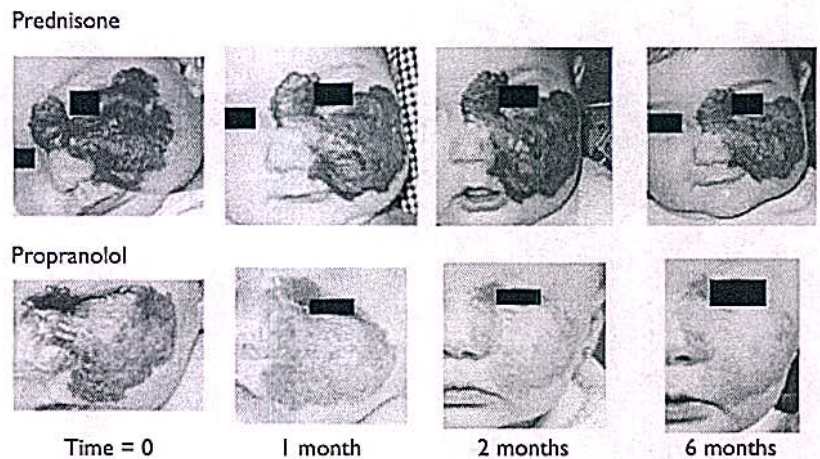


Figure 3. Segmental facial infantile hemangioma (IH): comparative medical photographs taken before treatment (time = 0) and after 1, 2, and 6 months of treatment (upper row: prednisone, lower row: propranolol). Age at start of treatment: 2.5 months (prednisone) and 1.8 months (propranolol). These segmental IH were not associated with cerebrovascular anomalies or PHACE syndrome.

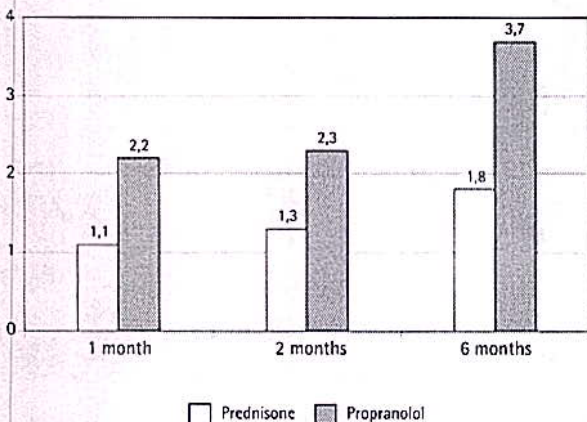


Figure 4. Clinical Improvement: Overall Appearance of Serial Photographs. Prednisone group: 1 month: Intraclass coefficient (ICC) = 0.843, $p = 0.002$; 2 months, ICC = 0.778, $p = 0.01$; 6 months, ICC = 0.862, $p = 0.001$. Propranolol group: 1 month: ICC = 0.762, $p = 0.01$; 2 months, ICC = 0.850, $p = 0.002$; 6 months, ICC = 0.804, $p = 0.006$.

TABLE 2. Clinical Improvement: Visual Analog Scale (6 Months)

	Evaluator 1	Evaluator 2	Mean
Prednisone	47.5	42.1	44.82* (SD: 12.21)
Propranolol	82.9	75.6	78.73* (SD: 22.47)

*Intraclass coefficient (ICC) = 0.833, 95%, $p < 0.001$ (t -test).

DISCUSSION

Propranolol was significantly superior to oral prednisone in inducing more-rapid and greater clinical improvement according to evaluation of serial clinical photographs. Our experience correlates with the initial reports by

Leauté-Labréze et al (6,10). The degree of improvement with propranolol was almost twice that of prednisone at all time points (Fig. 4).

This study's retrospective nature and the small number of subjects limited it, and therefore, we could not come to a conclusion on a relationship between location or type of IH and response to treatment. Also, follow-up imaging was not standardized. Moreover, side effects were obtained by review of patients' charts. Reporting of side effects was not standardized, but growth in weight and height was recorded for all patients.

The pathogenesis of IH is incompletely understood (6). Angiogenesis is characterized by a tightly regulated cascade of events (11). IH result from dysregulation of angiogenesis with imbalance in proangiogenic (vascular endothelial growth factor (VEGF), basic fibroblast growth factor bFGF) and antiangiogenic factors (tissue inhibitor of metalloproteinases). Overexpression of VEGF occurs through hypoxemia-induced secretion of hypoxia-inducible factor-1 alpha (HIF-1 α) and adrenergic stimulation (11).

Beta-adrenergic receptors are found on endothelial cells. With propranolol, a nonselective beta-adrenergic antagonist, clinical improvement of IH is observed in the first hours or days of treatment, probably by inhibition of the vasodilatory effect of adrenaline on vascular smooth muscle cells (11). Propranolol also seems to control the proliferative phase of IH and induce its involutive phase through reduced expression of VEGF, inhibition of adrenergic stimulation, and reduction of HIF-1 α . In addition, propranolol reduces expression of metalloproteinase-9, which inhibits tubulogenesis of endothelial cells and induces apoptosis (12–15).

Propranolol should be used with caution in patients with bronchospastic disease and heart conditions such as heart failure and heart block. Other known adverse effects include acrocyanosis, nightmares, insomnia, and hypoglycemia (16,17). Propranolol can predispose patients to hypoglycemia and blunt-related epinephrine-related signs and symptoms (16). This risk is well recognized in the neonatal period. Holland et al identified 21 cases of hypoglycemia occurring with oral propranolol; 17 cases occurred after an overnight to 24-hour fast. Physicians should be aware of this risk and instruct parents regarding signs and symptoms of hypoglycemia, temporarily withhold propranolol if the child has diminished oral intake, and avoid use of other medications known to induce hypoglycemia (17). Long-term evaluation of IH after discontinuation of propranolol is lacking, but rebound has been observed, although it has not been as striking or refractory as the rebound seen at times when systemic prednisone is stopped.

The use of propranolol in PHACE syndrome with cerebrovascular anomalies is controversial. Two of our patients with segmental facial IH (S2 segment) were treated with propranolol; they did not have cerebral vascular abnormalities on magnetic resonance imaging or angiography (MRI/MRA), nor did they fulfill the diagnostic criteria for PHACE syndrome. It is generally recommended that MRI/MRA should be performed in patients with large segmental facial IH to exclude significant cerebral arteriopathy that could theoretically represent a relative contraindication to propranolol treatment. In cases with cerebrovascular anomalies, propranolol should be used with caution because its safety has not been established.

CONCLUSION

Despite its retrospective nature, our study supports previous impressions that propranolol is superior to prednisone in controlling the growth phase in complicated IH. No serious adverse side effects were reported in either group; the overall safety profile of propranolol appears positive, but long-term data are lacking.

Propranolol should be a first-line therapy for problematic IH, but optimal dosage, modalities of administration, duration of treatment, and safety have yet to be established. Until results of ongoing prospective randomized trials are available, careful selection and monitoring of patients treated with propranolol is mandatory. Systemic corticosteroids remain a therapeutic option in selected cases.

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REFERENCES

1. Boye E, Jinnin M, Olsen BR. Infantile hemangioma: challenges, new insights, and therapeutic promise. *J Craniofac Surg* 2009;20(Suppl 1):678-684.
2. Haggstrom AN, Drolet BA, Baselga E et al. Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. *Pediatrics* 2006;118:882-887.
3. Bennett ML, Fleischer AB Jr, Chamlin SL et al. Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. *Arch Dermatol* 2001;137:1208-1213.
4. Frieden IJ, Haggstrom AN, Drolet BA et al. Infantile hemangiomas: current knowledge, future directions. Proceedings of a research workshop on infantile hemangiomas, April 7-9, 2005, Bethesda, Maryland, USA. *Pediatr Dermatol* 2005;22:383-406.
5. Enjolras O, Brevière GM, Roger G et al. Vincristine treatment for function- and life-threatening infantile hemangioma. *Arch Pediatr* 2004;11:99-107.
6. Léauté-Labrèze C, Dumas de la Roque E, Hubiche T et al. Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008;358:2649-2651.
7. Manunza F, Syed S, Laguda B et al. Propranolol for complicated infantile haemangiomas: a case series of 30 infants. *Br J Dermatol* 2010;162:466-468.
8. Ho NT, Lansang P, Pope E. Topical imiquimod in the treatment of infantile hemangiomas: a retrospective study. *J Am Acad Dermatol* 2007;56:63-68.
9. Pope E, Krafchik BR, Macarthur C et al. Oral versus high-dose pulse corticosteroids for problematic infantile hemangiomas: a randomized, controlled trial. *Pediatrics* 2007;119:e1239-e1247.
10. Sans V, de la Roque ED, Berge J et al. Propranolol for severe infantile hemangiomas: follow-up report. *Pediatrics* 2009;124:e423-e431.
11. Storch CH, Hoeger PH. Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action. *Br J Dermatol* 2010;163:269-274.
12. D'Angelo G, Lee H, Weiner RI. cAMP-dependent protein kinase inhibits the mitogenic action of vascular endothelial growth factor and fibroblast growth factor in capillary endothelial cells by blocking Raf activation. *J Cell Biochem* 1997;67:353-366.
13. Sommers Smith SK, Smith DM. Beta blockade induces apoptosis in cultured capillary endothelial cells. *In Vitro Cell Dev Biol Anim* 2002;38:298-304.
14. Annabi B, Lachambre MP, Plouffe K et al. Propranolol adrenergic blockade inhibits human brain endothelial cells

- tubulogenesis and matrix metalloproteinase-9 secretion. *Pharmacol Res* 2009;60:438-445.
15. Léauté-Labrèze C, Taïeb A. Efficacité des bêtabloquants dans les hémangiomes capillaires infantiles: signification physiopathologique et conséquences thérapeutiques. *Ann Dermatol Venereol* 2008;135:860-862.
16. Siegfried EC, Keenan WJ, Al-Juraidini S. More on propranolol for hemangiomas of infancy. *N Engl J Med* 2008;359:2846.
17. Holland KE, Frieden IJ, Frommelt PC et al. Hypoglycemia in children taking propranolol for the treatment of infantile hemangioma. *Arch Dermatol* 2010;146:775-778.

ONLINE FIRST

Propranolol vs Corticosteroids for Infantile Hemangiomas

A Multicenter Retrospective Analysis

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Objective: To determine whether propranolol therapy is safe and effective and superior to oral corticosteroids for treating infantile hemangiomas (IHs).

Design: Multicenter retrospective chart review.

Setting: University of Miami and Miami Children's Hospital, Miami, Florida.

Patients: The study included 110 patients with IHs.

Main Outcome Measures: The percentage of clearance was quantified by documented serial global photography and clinical examinations (length, height, and width) to segregate patients into 2 groups: patients who had clearance of 75% or more and patients who had less than 75% clearance.

Results: The mean duration of treatment was 7.9 months for propranolol and 5.2 months for oral corticosteroids. Fifty-six of 68 patients (82%) who were receiving propranolol achieved clearance of 75% or more compared with 12 of 42 patients (29%) who were receiving oral corticosteroids ($P < .01$). Adverse effects were minimal in

the propranolol group: 1 patient had hypoglycemia and 2 patients had a nonspecific skin eruption that was not associated with propranolol therapy. All 42 patients in the corticosteroid group had 1 or more adverse effects ($P < .01$). Relapse after discontinuation of propranolol therapy occurred in 2 of the 68 patients; however, both patients responded to propranolol therapy on reinitiation of treatment. Surgical referrals after treatment were required in 8 patients (12%) in the propranolol group and 12 patients (29%) in the oral corticosteroid group ($P < .01$).

Conclusions: Propranolol therapy was more clinically effective and more cost-effective than oral corticosteroids in treating IHs. It also resulted in fewer surgical interventions and demonstrated better tolerance, with minimal adverse effects, compared with oral corticosteroids. Therefore, propranolol should be considered a first-line agent given its safety and efficacy in the treatment of IHs.

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INFANTILE HEMANGIOMAS (IHs) ARE common vascular tumors with a characteristic natural history of rapid growth during the first 3 to 12 months of age, followed by slow and spontaneous involution from 3 to 7 years of age.¹ However, spontaneous regression is no guarantee of a satisfactory cosmetic result. Also, ulceration, scarring, recurrent bleeding, and obstruction of vital structures with important functions such as vision, respiration, hearing, feeding, and defecation may complicate untreated IHs.¹ Larger and/or multiple cutaneous IHs may be associated with high-output cardiac failure, substantial structural abnormalities, cosmetic disfigurement, and psychosocial morbidity in both the child and the family. Therefore, IHs often require systemic, surgical, and/or laser treatment to avoid these adverse effects.

Until recently, the mainstay of treatment for IHs has been corticosteroids in various forms, including topical, intralesional, and oral formulations, with the most common being oral prednisolone.² Only in complicated or refractory IH cases have other treatment modalities, such as interferon alfa-2a, imiquimod, vincristine, cyclophosphamide, pulsed-dye laser, and, most recently, propranolol, been considered. Since 2008, several reports have demonstrated the effectiveness of propranolol (a nonselective β -blocker that is used to treat hypertension, supraventricular tachycardia, congestive heart failure, and thyrotoxicosis) for the treatment of IH.³⁻⁶ Propranolol's presumed mechanisms of action on IHs are attributable to vasoconstriction by decreasing the release of nitric oxide, inhibition of proangiogenic signals such as vascular endo-

thelial growth factor, basic fibroblast growth factor, and matrix metalloproteinase 2/9, and induction of apoptosis in proliferating endothelial cells.⁷

Thus far, to our knowledge, no large retrospective study has been published to address several unanswered questions regarding propranolol's efficacy for treating IHs in a larger pediatric population without underlying cardiac abnormalities. Also, data are lacking on the specific therapeutic parameters of propranolol therapy for IHs, including the duration of treatment required to achieve clearance, the adverse effect profile, and the IH relapse rate after discontinuation of treatment. To date, the efficacy and adverse effect profile of propranolol therapy has yet to be compared with that of systemic corticosteroid therapy.

METHODS

STUDY DESIGN

Two major centers (the University of Miami and the Miami Children's Hospital, Miami, Florida) participated in the study. After receiving institutional review board approval, we conducted a retrospective chart review of 139 patients with IHs between February 2005 and October of 2010. The review comprised 2 parts.

PART 1

Data were collected from 139 IH patient charts, which included race, sex, mother's pregnancy history, age at initiation of therapy, location, presence of PHACES syndrome and other comorbidities, presence or absence of ulceration during treatment, dosage of propranolol or corticosteroid therapy, age at discontinuation of therapy, age at last follow-up visit, status of IH at last follow-up visit, presence or absence of rebound growth after discontinuation of therapy, imaging data, adverse effect profile, and approximate cost of therapy per patient.

PART 2

Twenty-nine of 139 charts were excluded: 22 patients had other treatments (eg, topical or intralesional corticosteroids, topical imiquimod, pulsed-dye laser), and 7 patients were unavailable for follow-up. A total of 110 patients remained for treatment analysis. As a measure of clinical outcome, the percentage of clearance was quantified by serial global photography and documented by clinical examinations (using length, height, and width to calculate volume). The patients were placed into 2 groups: those who had clearance of 75% or more (defined by a correlating percentage of decrease in volume, a cosmetically acceptable result by physician and/or parent, and a lack of need for further treatment) and those who did not (ie, <75% clearance).

For the propranolol group, the treatment protocol used in the 2 centers participating in the study included an outpatient pretherapeutic pediatric cardiology consultation as well as blood glucose and blood pressure monitoring during the first 48 hours of treatment. Hospital admission was necessary in 2 of the premature patients in the neonatal period for close monitoring. In patients with facial segmental IHs who were at risk for PHACES syndrome, magnetic resonance images of the brain were obtained to assess the vasculature before the initiation of propranolol therapy and to avoid any potential consequence of medication-induced hypotension, which could potentially precipitate a vaso-occlusive event given the risk of malformed vasculature.

The propranolol treatment regimen started at 0.5 mg/kg/d divided into 2 doses for 3 days, increased to 1.0 mg/kg/d divided

into 2 doses for 3 days, and finally reached the target dose of 2.0 mg/kg/d divided into 2 doses. Vitals were checked after the first dose was administered and after each additional increased dose thereafter. At the end of the treatment period, the propranolol therapy was tapered over a 2-week period to minimize the risk of a hyperadrenergic withdrawal response.⁸ For the oral corticosteroid group, the dosage of therapy ranged from 2 to 4 mg/kg/d. However, most patients were treated at the dose of 4 mg/kg/d.

STATISTICAL ANALYSIS

The data were analyzed using SPSS version 18.0 software (SPSS Inc, Chicago, Illinois). Mann-Whitney tests were used to compare the nonparametrically distributed outcomes of clearance between the propranolol and the oral corticosteroid groups, adverse effects, ulcerations during treatment, clearance between patients with periocular involvement in the propranolol and corticosteroid groups, and clearance between patients who were previously treated with corticosteroids followed by propranolol and those who received only propranolol. $P < .05$ was considered statistically significant.

RESULTS

PART 1

A total of 139 charts were reviewed. We captured patient demographics and the characteristics of their IHs (**Table**). Our patients were predominantly female (male:female ratio, 107:32). Seventy-five patients (54%) were white, 62 (45%) were Hispanic, and 2 (1%) were African American. These demographics are similar to the national IH data.⁹ However, there is a greater representation of Hispanics living in Miami, Florida, where our research was conducted. Hispanic ethnicity was not categorized by race in our retrospective study, so we may have missed a subcategory of white Hispanics.

While most of the IHs (78%) were located on the head and neck (59% on the face and 19% on the scalp), the rest were distributed on the trunk (7%), extremities (10%), and genitalia (5%). Twenty-four patients (17%) had a history of prematurity (defined as <37 weeks' gestational age), and 11 (8%) were products of multiple gestations. Fourteen of the patients' mothers (10%) had pregnancy complications: 2 placental abnormalities (1 placenta previa, 1 placental insufficiency) and 2 gestational diabetes resulting in macrosomia. Nine patients required neonatal intensive care unit hospitalizations, and 1 patient had evidence of a subarachnoid bleed. The mean age at treatment initiation was 4.5 months.

Radiological abnormalities were observed in 19 of the 139 patients (14%). Two of the 19 patients had multifocal hemangiomatosis with diffuse liver involvement on ultrasonography. Nine patients had abnormal findings on magnetic resonance imaging of the brain, with aberrant external carotid artery connections feeding the IH that was located in the head and neck area. Two patients had an IH that was located in the brain (posterior pons, left frontal lobe), and 1 patient had an IH that was located in the right anterior chamber of the eye. One patient had dilation of the superior ophthalmic vein with frontal bone remodeling. Another patient had a hypoplastic posterior cerebral artery (PCA); 1 patient had a prominent left PCA;

Table. Characteristics of Study Patients

Characteristic	Propranolol (n=75)	Oral Corticosteroids (n=42)	Other Treatments (n=22)	Total No. (%) of Patients (N=139)
Mean age at initiation of treatment, mo	4.9	4.5	7.6	139 (100)
Prior treatment, ^a %	53	16	0	69 (50)
Male	20	8	4	32 (23)
Female	56	34	17	107 (77)
History of prematurity	15	6	3	24 (17)
Placental abnormalities	2	0	0	2 (1)
Products of multiple gestations, twin gestation	9	1	1	11 (8)
PHACES syndrome	0	1	0	1 (1)
Radiological abnormalities ^b	9	7	2	18 (14)
Ophthalmologic abnormalities	8	5	0	13 (9)

^aTopical and intralesional corticosteroids, topical imiquimod, and pulsed-dye laser.

^bTwo patients had multifocal hemangiomatosis with diffuse liver involvement on ultrasonograms; 9 patients had abnormal findings on magnetic resonance images of the brain, with aberrant external carotid artery connections; 2 patients had an infantile hemangioma that was located in the brain (posterior pons, left frontal lobe); 1 patient had an infantile hemangioma that was located in the right anterior chamber of the eye; 1 patient had dilation of the superior ophthalmic vein with frontal bone remodeling; 1 patient had a hypoplastic posterior cerebral artery (PCA); 1 patient had a prominent left PCA, and 1 patient had absence of the right PCA.

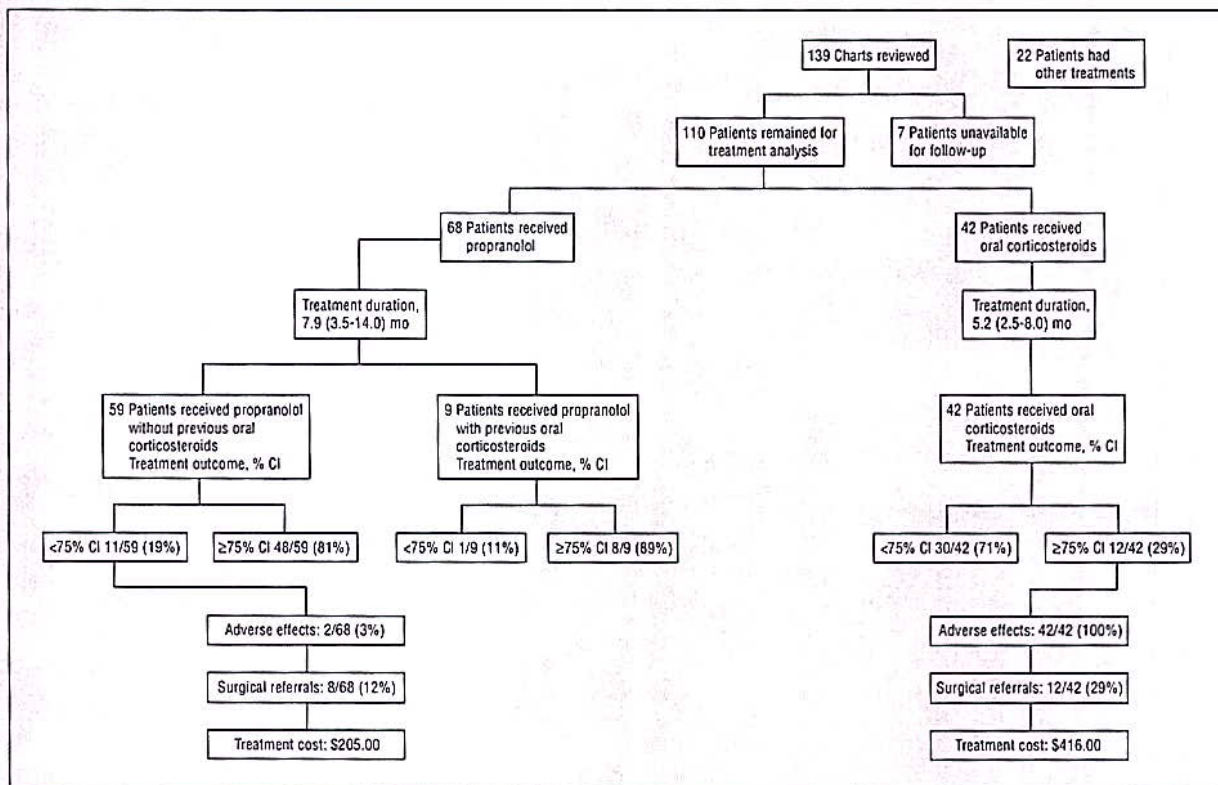


Figure 1. Study flowchart. CI indicates clearance.

and 1 patient had an absence of the right PCA. Also, 1 patient met the criteria for PHACES syndrome (Dandy-Walker malformation and a segmental facial IH).

PART 2

We compared the outcomes of IHs treated with propranolol vs oral corticosteroids. We matched both groups based on patient age and location, size, and type of lesion. This comparison was possible because it has been shown that IHs occur in a nonrandom distribution, along the embry-

onic lines of cleavage.¹⁰ Finally, we compared the overall clinical outcomes of these 2 groups using the percentage of clearance (**Figure 1**). Overall, 56 of 68 patients (82%) achieved clearance of 75% or more in the propranolol group compared with 12 of 42 patients (29%) in the corticosteroid group ($P < .01$). The number of adverse effects observed in the propranolol group was 1 of 68 (1%). This number was significantly less than that observed in the corticosteroid group (42 of 42 [100%]) ($P < .01$). The number of surgical referrals in the propranolol group (8 of 68 [12%]) was significantly less than the number of surgical

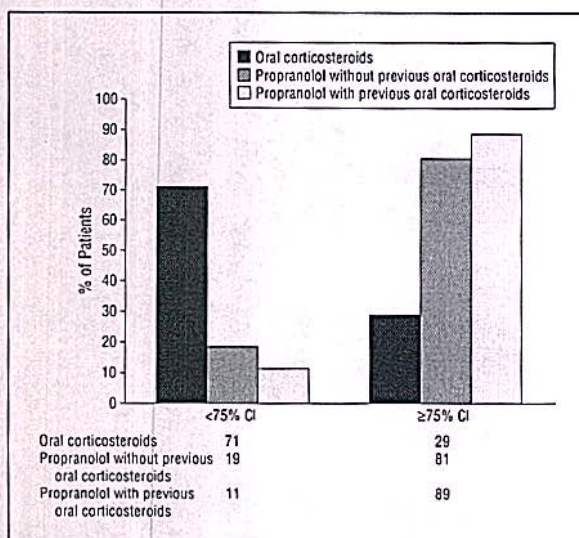


Figure 2. Treatment outcome graph. CI indicates clearance.

referrals in the corticosteroid group (12 of 42 [29%]) ($P = .03$). Furthermore, the number of ulcerations was significantly lower in the propranolol group (4 of 68 [6%]) than in the corticosteroid group, in which 11 of the 42 IHs (26%) ulcerated ($P < .01$).

PROPRANOLOL GROUP

The propranolol group was further divided into 2 groups. The first group comprised patients who had been treated only with propranolol without previous oral corticosteroids. In this group, 48 of 59 patients (81%) had clearance of 75% or more and 11 of 59 patients (19%) had clearance of less than 75%. The second group consisted of those patients who were receiving propranolol and who also had undergone previous treatment with oral corticosteroids. In this group, 8 of 9 patients (89%) had clearance of 75% or more and 1 of 9 patients (11%) had clearance of less than 75%. The difference in clearance between these 2 groups was not statistically significant ($P = .52$).

One of 68 patients (1%) had hypoglycemia, and 2 (3%) had a nonspecific skin eruption. Propranolol therapy was discontinued in the latter 2 patients and then restarted at a later date, without recurrence. Therefore, their adverse effects were determined unlikely to be related to propranolol therapy. Eight of 68 patients (12%) were referred for surgery after propranolol treatment. The average duration of treatment was 7.9 months, and the total average treatment cost for propranolol therapy alone was \$205.32 per patient.

ORAL CORTICOSTEROID GROUP

In the oral corticosteroid group, 12 of 42 patients (29%) had clearance of 75% or more, and 30 patients (71%) had clearance of less than 75%. Forty-two patients (100%) patients had adverse effects. All patients exhibited Cushingoid features. Four patients had gastroesophageal reflux, 2 had hypertension, and 1 experienced a life-threatening arterial bleed as a result of an ulceration that

eroded through the external carotid artery and required emergency surgery to achieve hemostasis. Also, 1 patient developed hirsutism, 1 had failure to thrive and poor weight gain, 1 had hypercholesterolemia, 1 had scarring and lip contraction leading to functional impairment, 1 received trimethoprim-sulfamethoxazole (Bactrim) for *Pneumocystis carinii* pneumonia prophylaxis, and 2 received respiratory syncytial vaccination series with palivizumab (Synagis).

Twelve patients (29%) were referred for surgery after oral corticosteroid treatment. The average duration of treatment was 5.2 months, and the total average treatment cost for oral prednisolone was \$416.00 per patient.

COMMENT

TREATMENT OUTCOMES

This retrospective study of 110 patients confirmed that a daily cumulative dose of 2 mg/kg of propranolol is highly effective in the treatment of IHs. Furthermore, it demonstrated that propranolol therapy is superior to oral corticosteroid treatment, the former criterion standard therapy for IHs (Figure 2).

Our study revealed that of the patients who received systemic corticosteroids before propranolol, 89% achieved lesion clearance of 75% or more compared with the 81% who achieved lesion clearance of 75% or more with propranolol alone. The difference in the treatment outcome may indicate that a combination treatment of a short course of systemic corticosteroids in conjunction with propranolol may be more effective than treatment with propranolol alone; however, these results were not statistically significant. Nevertheless, they indicate that the outcome in the propranolol group did not seem to be greatly affected by previous oral corticosteroid treatment.

It has been believed that therapy must be administered during the proliferative phase of IHs to arrest the progressive growth of the vascular lesion.¹¹ However, in this study, we found similar outcomes in patients treated with propranolol before and after 1 year of age (7 of 68 patients were started on propranolol therapy after the age of 12 months, and all had $\geq 75\%$ clearance). This finding indicates that propranolol's long-term effect is not solely related to its antiproliferative effect on the vasculature but is also due to its apoptosis and vasoconstriction.

Seven of the 68 patients in the propranolol group had periorbital involvement of their IHs. One of the 7 was unavailable for follow-up, 3 had clearance of 75% or more, and 3 had clearance of less than 75%. In the oral corticosteroid group, 10 of the 42 patients had periorbital involvement of their IHs. Six of the 10 had clearance of less than 75%, and 4 had clearance of 75% or more. This number of patients was not sufficient to draw a definitive conclusion as to whether propranolol therapy is superior to oral corticosteroid treatment for IHs with periorbital involvement. Also, the difference between the 2 groups that achieved clearance of 75% or more was not statistically significant ($P = .71$). Unfortunately, we could not obtain any follow-up data regarding the improvement of amblyopia or astigmatism.

Eleven of the 42 IHs (26%) ulcerated during oral corticosteroid treatment; however, only 4 of 68 (6%) ulcerated during propranolol treatment ($P < .01$). These results indicate that IHs treated with oral corticosteroids ulcerate at a greater rate than those treated with propranolol, which could be related to their presumed mechanism of action.

ADVERSE EFFECTS OF PROPRANOLOL THERAPY

The known adverse effects of propranolol therapy include bradycardia, hypotension, hypoglycemia, bronchospasm, congestive heart failure, nausea, vomiting, abdominal cramping, sleep disturbance, night terrors, and depression. Several recent reports have also associated hyperkalemia with early initiation of propranolol therapy, requiring treatment with furosemide, fluids, and nebulized albuterol.¹²

In our study, only 1 of 68 patients had hypoglycemia. Although 2 of the 68 patients experienced nonspecific skin eruptions, neither of the eruptions had a clear correlation to propranolol therapy, and neither recurred on re-treatment. The low incidence of adverse effects in our study patients may be partially related to our detailed pretherapeutic protocol and to the protective measures taken during intercurrent illnesses. For example, the treatment was interrupted in 6 of the 68 patients: 2 developed fever, 2 developed rash, 1 developed tachycardia, and 1 developed viral upper respiratory infection with reactive airway disease. It should be mentioned that several cases of hypoglycemia have been reported in the literature among patients with IHs who were treated with propranolol.^{13,14} However, these cases have mainly been associated with concurrent illnesses or poor oral intake for various reasons. Therefore, propranolol should not be administered on days with diminished oral intake, and parents should be educated about the warning signs of hypoglycemia, such as fussiness, poor feeding, apnea, loss of consciousness, seizures, and hypothermia.

Although further prospective studies are needed to determine the precise incidence of the adverse effects of propranolol therapy in patients with IHs, we encountered only 1 serious adverse effect in our retrospective study. Taken together, propranolol therapy had a much safer adverse effect profile when compared with oral corticosteroid therapy.

RELAPSE AND RESISTANCE

Relapse of individual IHs was observed in 2 of the 68 patients (3%) who were receiving propranolol: one with diffuse neonatal hemangiomas, and the other with periorcular involvement. Both patients started the treatment at 3 months of age and discontinued therapy at 10 months of age. These 2 patients may have ended therapy prematurely, because when propranolol was reintroduced, both IHs again regressed. A similar observation was made in the 6 patients whose propranolol therapy had to be temporarily discontinued owing to intercurrent illnesses (fever, rash, tachycardia, and viral upper respiratory illness or reactive airway disease). Likewise, our results agree with those of a previous study in which 2 of 32 patients

had rebound growth in their IHs after cessation of treatment before 12 months of age.¹⁵ In both of these patients, re-treatment with propranolol was effective as well. Moreover, relapse after discontinuation of propranolol therapy in patients older than 12 to 14 months has also been described.⁶ Because of these experiences, most of our patients who received propranolol underwent extended treatment for up to 1 year of age to prevent relapses of their IHs. On the other hand, in the oral corticosteroid group, the treatment duration was limited owing to the adverse effects associated with prolonged treatment. Therefore, it is possible that longer treatment with propranolol may have influenced the final clinical outcome that was observed in our patients. However, as has been described previously, improvement with propranolol therapy is seen relatively quickly and not solely as a result of prolonged treatment.³⁻⁶

SURGICAL REFERRALS

Propranolol reduced the need for surgery, with overall better cosmetic outcomes. In our study, 8 of 68 IHs (12%) treated with propranolol required surgery after treatment, whereas 12 of 42 IHs (29%) treated with oral corticosteroids required surgery. All of these lesions were considered refractory to treatment or were deemed cosmetically unacceptable owing to critical locations such as the nasal tip or the periorbital region.

TREATMENT COST

The average cost of oral corticosteroids per IH treated was \$416.00, which was calculated based on an average treatment duration of 5.2 months. This amount does not include the additional costs incurred for the prophylactic treatment of oral corticosteroid-related adverse effects, such as increased rates of infection owing to immunosuppression and gastroesophageal reflux. On the other hand, the average cost of propranolol per IH treated was \$205.32, which was based on an average treatment duration of 7.9 months. However, this amount does not include a pediatric cardiology consultation for cardiac clearance to initiate therapy.

In conclusion, our study showed that propranolol therapy was more effective in lesion clearance, required fewer surgical referrals after treatment, and demonstrated superior tolerance, with minimal adverse effects. Propranolol proved to be safe in treating IH in our patients as no major adverse effects occurred. Also, propranolol therapy was more cost-effective, with a cost reduction of more than 50% per patient. Therefore, based on the results of this study, we believe that propranolol therapy is superior to traditional first-line oral corticosteroids in the treatment of IH, and we propose that propranolol be considered a first-line agent given its safety and efficacy for the treatment of IH.

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Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Price and Alvarez Connelly. **Acquisition of data:** Price, Lattouf, and Baum. **Analysis and interpretation of data:** Price, Lattouf, Baum, McLeod, Schachner, Duarte, and Alvarez Connelly. **Drafting of the manuscript:** Price, Lattouf, Baum, and McLeod. **Critical revision of the manuscript for important intellectual content:** Price, Schachner, Duarte, and Alvarez Connelly. **Statistical analysis:** McLeod. **Administrative, technical, and material support:** Lattouf and Duarte. **Study supervision:** Price, Schachner, and Alvarez Connelly.

Financial Disclosure: None reported.

Additional Contributions: Whitney Valins, BS, organized the data for this study.

REFERENCES

1. Maguiness SM, Frieden IJ. Vascular birthmarks: tumors and malformations. In: Schachner LA, Hansen RC, eds. *Pediatric Dermatology*. Vol 2. 4th ed. Philadelphia, PA: Mosby Elsevier; 2011:1135-1153.
2. Bennett ML, Fleischer AB Jr, Chamlin SL, Frieden IJ. Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. *Arch Dermatol*. 2001;137(9):1208-1213.
3. Léauté-Labrèze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taïeb A. Propranolol for severe hemangiomas of infancy. *N Engl J Med*. 2008;358(24):2649-2651.
4. Theletsane T, Redfern A, Raynham O, Harris T, Prose NS, Khumalo NP. Life-threatening infantile haemangioma: a dramatic response to propranolol. *J Eur Acad Dermatol Venereol*. 2009;23(12):1465-1466.
5. Mazereeuw-Hautier J, Hoeger PH, Benlahrech S, et al. Efficacy of propranolol in hepatic infantile hemangiomas with diffuse neonatal hemangiomatosis. *J Pediatr*. 2010;157(2):340-342.
6. Schiessel C, Neuhaus K, Zoller S, et al. Efficacy and safety of propranolol as first-line treatment for infantile hemangiomas. *Eur J Pediatr*. 2011;170(4):493-501.
7. Storch CH, Hoeger PH. Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action. *Br J Dermatol*. 2010;163(2):269-274.
8. Lawley LP, Siegfried E, Todd JL. Propranolol treatment for hemangioma of infancy: risks and recommendations. *Pediatr Dermatol*. 2009;26(5):610-614.
9. Haggstrom AN, Drolet BA, Baselga E, et al; Hemangioma Investigator Group. Prospective study of infantile hemangiomas: demographic, prenatal, and perinatal characteristics. *J Pediatr*. 2007;150(3):291-294.
10. Waner M, North PE, Scherer KA, Frieden IJ, Waner A, Mihm MC Jr. The nonrandom distribution of facial hemangiomas. *Arch Dermatol*. 2003;139(7):869-875.
11. Frieden IJ. Which hemangiomas to treat—and how? *Arch Dermatol*. 1997;133(12):1593-1595.
12. Pavlakovic H, Kletz S, Lauerer P, Zutt M, Lakomek M. Hyperkalemia complicating propranolol treatment of an infantile hemangioma. *Pediatrics*. 2010;126(6):e1589-e1593.
13. Bonifazi E, Acquafredda A, Milano A, Montagna O, Laforgia N. Severe hypoglycemia during successful treatment of diffuse hemangiomatosis with propranolol. *Pediatr Dermatol*. 2010;27(2):195-196.
14. Holland KE, Frieden IJ, Frommelt PC, Mancini AJ, Wyatt D, Drolet BA. Hypoglycemia in children taking propranolol for the treatment of infantile hemangioma. *Arch Dermatol*. 2010;146(7):775-778.
15. Sans V, de la Roque ED, Berge J, et al. Propranolol for severe infantile hemangiomas: follow-up report. *Pediatrics*. 2009;124(3):e423-e431.

Archives Web Quiz Winner

Congratulations to the winner of our September quiz, Vijay Zawar, MD, DNB, DVD, Skin Diseases Center, Nashik, India. The correct answer to our September challenge was *trichodysplasia spinulosa*. For a complete discussion of this case, see the Off-Center Fold section in the October *Archives* (Burns A, Arnason T, Fraser R, Murray S, Walsh N. Keratotic "spiny" papules in an immunosuppressed child. *Arch Dermatol*. 2011; 147[10]:1215-1220).

Be sure to visit the *Archives of Dermatology* Web site (<http://www.archdermatol.com>) to try your hand at the interactive quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month's print edition of the *Archives*. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of *The Art of JAMA II*.

Docket No.: 4384-000140/US/NP
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:

Christine LÉAUTÉ-LABRÈZE et al.

Application No.: 12/599,266

Confirmation No.: 8098

Filed: May 11, 2010

Art Unit: 1627

For: USE OF A BETA BLOCKER FOR THE
MANUFACTURE OF A MEDICAMENT FOR
THE TREATMENT OF HEMANGIOMAS

Examiner: S. Pihonak

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132 OF ILONA J. FRIEDEN, M.D.

I, Dr. Ilona J. Frieden, declare the following.

1. I am currently Professor of Dermatology and Pediatrics; Chief, Division of Pediatric Dermatology and Vice-Chair, Department of Dermatology, at the University of California, San Francisco. I have an M.D. from the University of California, San Francisco, and a B.A. from Boston University.

2. My Curriculum Vitae is attached as Attachment A. In pertinent part, I have studied, conducted research, and practiced medicine in the field of children's skin

diseases for more than 30 years. I have the following Board Certifications: the American Board of Pediatrics; the American Board of Dermatology; the American Board of Dermatology, Pediatric Dermatology Subspecialty Certification; and the American Board of Dermatology, Dermatology Recertification. I am currently Editor-in-Chief of the journal *Pediatric Dermatology*, Co-Editor of the textbook *Neonatal Dermatology*, have served as President of the Society of Pediatric Dermatology and President of the American Board of Dermatology, and currently serve as a member of the Board of Directors of the American Academy of Dermatology. I am also a founder of the Hemangioma Investigator Group, which is a multi-center group of researchers which was founded in 2001 and has conducted collaborative research on hemangiomas resulting in over 40 articles published or in-press.

3. I am fully trained in and familiar with the subject matter of the invention disclosed in the subject U.S. Patent Application 12/599,266 (the '266 Application). In particular, I have conducted extensive research regarding infantile hemangiomas and published more than 85 articles and more than a dozen book chapters on this topic.

4. I have previously served as a consultant to Pierre Fabre Dermatologie, helping them to create a study design for their randomized controlled trial, HEMANGIOL, for which I received financial compensation. I understand Pierre Fabre Dermatologie has a license to the '266 Application. I have not received any compensation from Pierre Fabre in over one year.

5. I am currently chair of the Independent Data Monitoring Board (IDMC) for the HEMANGIOL study, which is investigating the use of propranolol in the treatment of infantile hemangioma (the subject matter of the '266 Application) sponsored by Pierre Fabre Dermatologie. The aim of the IDMC is to safeguard the interests of the study participants, investigators and the sponsor; to assess the appropriateness of the required study sample size; to assess the safety and efficacy of the study's interventions; to select none, one or two regimen(s) of propranolol for further study in stage two of the design; to monitor the overall conduct of the study; and to protect its validity and credibility. I am being compensated for this work by Cardinal Systems, which is the clinical research organization in charge of the study.

6. I have been asked by the Applicants of the '266 Application to comment on the invention, its medical significance, and the comments that have been made by the Examiner. Before I offer more specific comments about the invention, however, I think that it is important to put the invention into context and explain the importance thereof from a medical perspective; I will do so in the paragraphs to follow.

A Brief Discussion Regarding Infantile Hemangiomas

A. Pathogenesis

7. Infantile hemangiomas are among the most common birthmarks in children and the most common benign growth in infancy. Infantile hemangiomas (IH) occur exclusively in infants – never in older children or adults. Hemangiomas are collections of blood vessels that have increased cell division and growth. The appearance of a hemangioma depends on whether it is superficial (just in the top few layers of the skin) or deep (involving more layers of the skin). Superficial hemangiomas are bright red and elevated (“strawberry” marks). Deep hemangiomas are blue or skin-colored. Some hemangiomas have both superficial and deep components. More than half of hemangiomas are on the head and neck. Most hemangiomas are solitary, but some infants - particularly premature infants - have multiple hemangiomas.

8. Many hemangiomas are small and physiologically harmless, although they can have a psychological impact on children as they grow older. Other hemangiomas require treatment because they interfere with vital functions or are potentially permanently disfiguring. For example, infantile hemangiomas may impinge on vital structures, ulcerate, bleed, cause high-output cardiac failure, or create significant structural abnormalities or result in permanent disfigurement.

9. Until the past decade, the pathogenesis of infantile hemangiomas was very poorly understood. Previous to that time it was assumed that IH were the result of aberrant angiogenesis (i.e., the sprouting of new blood vessels from pre-existing ones) in a manner similar to many other tumors. The seminal observation published in 2000 by North et al., that hemangiomas have a unique vascular phenotype, led to a steep rise in research in this area and a critical look at previous assumptions. (North, *Hum Pathol.*) Infantile hemangiomas are now known to express Glut-1, merosin, and other markers

which are virtually identical to placental blood vessels, and studies have suggested a genomic identity to placental blood vessels. (North, *Arch Derm*; Barnes.) In addition to the findings of a unique relationship with placental vessels, there is other evidence that IH may not be solely a disorder of angiogenesis and not just an endothelial tumor, but may actually be due to *vasculogenesis*, i.e. the *de novo* formation of new blood vessels from circulating stem cells. (Dadras; Boscolo.) In addition to understanding the unique vascular phenotype of IH – which is not shared by any other vascular tumors or malformations – there is emerging evidence that IH arise from progenitor cells which have features of myeloid, monocytic, endothelial and even neural crest differentiation. (Kleinman; Yu, Bischoff, *J Craniofac Surg*; Itinteang, *J Clin Pathol* 2010 Sep and Nov and 2011; Itinteang, *Br J Dermatol*.) Recent studies have provided compelling evidence that IH may represent a growth of primitive mesenchymal cells which are most likely in a state of arrested development. These observations suggest the possibility that IH may be more similar to a hamartoma rather than a conventional tumor. This is further supported by the *in vitro* model of Yu et al. which has shown that mesenchymal stem cells of hemangiomas transform into adipocytes similar to the fibrofatty residua seen in many hemangiomas after involution. (Yu et al.) Other investigators have focused particularly on the role of VEGF and VEGF-mediated pathways. (Calicchio et al; Jinnin, *Nature Medicine*.) Recent reviews of pathogenesis further summarize some of the many advances made in the past 10 years. (Drolet and Frieden; Jinnin; Bischoff *J Craniofac*; Boye; Lo; Bauland.)

10. Although some research has suggested that clonality is present in hemangiomas (Walters et al.), this has not been replicated by other laboratories and thus remains unproven. The patterns of segmental hemangiomas suggests that at least a subset of hemangiomas may *not* arise through clonal mutations since their anatomic distribution and patterns correspond to neuroectodermal patterned embryologic prominences (such as the maxillary and mandibular placodes which arise early in embryologic development at age four to six weeks of gestations. (Haggstrom et al.) These hemangiomas – like their smaller, more localized counterparts – have all of the immunohistochemical and molecular features of other IH including rapid growth and spontaneous involution. They suggest that an early error in development is permissive for hemangioma progenitor cell

growth limited to a specific embryologic distribution, rather than represent a clonal tumor per se. Another clinical feature which supports this concept is the observation that IH do not have a lateral growth phase, similar to other tumors. Instead they "mark out their territory" (presumably in utero) and grow volumetrically, rather than invading adjacent anatomic territory as a tumor would be expected to do. (Chang et al.)

B. Known Treatments Prior to 2008

11. Standard treatment options for infantile hemangiomas include oral steroids, laser treatment, and surgical removal. In serious cases, vincristine may be used, and interferon α -2a has been used in life-threatening situations. All of these treatments are associated with side effects which can be significant, particularly in infants. A recent Cochrane meta-analysis clearly demonstrates the lack of good evidence-based therapies for IH. (Leonardi-Bee et al.)

12. Since the 1960s, corticosteroids (such as prednisolone) have been considered the treatment of choice for infantile hemangiomas. Corticosteroids may help reduce the frequency of ulceration and may decrease lesion size. Systemic corticosteroids appear to be effective in causing cessation of growth in the large majority of hemangiomas but result in actual shrinkage only in approximately one third of infants treated. (Bennett et al.) Potential side effects include irritability, gastrointestinal upset, immunosuppression, hypertension, and growth retardation. The duration of treatment ranges from a few weeks to many months, depending on the age of the child, the indications for treatment, and the growth characteristics of the hemangioma, since rapid tapering of the dose of corticosteroids while the tumor is still in the proliferative phase may result in rebound growth.

13. Lasers have been used for treating infantile hemangiomas. However, because of the limited depth of penetration, lasers are typically effective for superficial hemangiomas and less effective for those that are thicker or destined to be both superficial and deep. They also can result in scarring, particularly during the proliferative phase.

14. Interferon α -2a, an inhibitor of angiogenesis, has been used in the treatment of hemangiomas that fail to respond to corticosteroid therapy. The interval between the administration of interferon α -2a and the response to treatment ranges from a few weeks to several months. Side effects include irritability, neutropenia, and abnormalities of liver enzymes. Interferon α -2a is now reserved for infants with life-threatening hemangiomas because of the significant risk of neurotoxicity, particularly spastic diplegia, in approximately 20% of patients treated with interferon for IH.

15. Vincristine has been used in some serious infantile hemangiomas that do not respond to corticosteroid treatment. Vincristine interferes with mitotic spindle microtubules and induces apoptosis in tumor cells in vitro. However, as with interferon α -2a, side effects can be significant, such as peripheral neuropathy, constipation, jaw pain, rare hematologic toxicity, syndrome of inappropriate secretion of antidiuretic hormone. Furthermore, vincristine treatment is complicated due to the need for a central line or PICC line for administration.

The Importance of the Invention

16. Propranolol has become the first choice of therapy for infantile hemangiomas. In my opinion, as well as in the opinions of many others in the field, propranolol is a breakthrough therapy for infantile hemangiomas, and is now the first-line therapy for this disorder. Remarkably, in the three years since this observation was published as a letter to editor in the *New England Journal*, more than 140 articles have been published citing the use of propranolol and other beta-blockers in treating IH, and the vast majority of these attest to its efficacy and superiority over previous treatments.

17. The clinical superiority of propranolol relative to corticosteroids and other infantile hemangiomas treatments is two-fold. First, it provides clearly superior efficacy in many cases, not only in stopping hemangioma growth, but in causing decreased tumor volume with more regularity than corticosteroids. Secondly, propranolol has a lower side-effect profile than corticosteroids or other therapies, with a well characterized history of use in infants as a non-selective beta-blocker for a variety of indications, (e.g., treatment of hypertension, supraventricular tachycardia, long Q-T syndrome, congestive heart failure, and thyrotoxicosis).

18. Evidence of the lack of effective therapies for treating hemangiomas prior to this report in 2008 is provided by a study launched in 2007 to compare systemic corticosteroids to vincristine in the treatment of severe hemangiomas. Briefly, in 2007, the Food and Drug Administration (FDA) granted orphan drug designation for studying the use vincristine vs. prednisolone for infantile hemangiomas (NCT00555464). The goal of the FDA's orphan drug designation grant program is to support the clinical development of products for use in rare diseases or conditions where no current therapy exists or where the proposed product will be superior to the existing therapy. Thus, the orphan drug designation for the vincristine study was, itself, evidence of the limitations in other therapies that were then known. Together with Dr. Beth Drolet of the Medical College of Wisconsin, I was a co-investigator on this study and helped her to design it. We were excited to get a grant from the FDA Office of Orphan Product Development to support the study as we knew that we needed to move beyond anecdotes and case series into real evidenced-based observations including this randomized controlled trial. As a background, we noted that infants with large hemangiomas are often treated systemically with oral steroids (Prednisolone) to prevent complications, that the best treatment for hemangiomas is not known and there are no medications approved by the FDA for treatment of hemangiomas. Within a year of the time of the initiation of the study, however, the initial work describing the use of propranolol in treating infantile hemangiomas was published in 2008. (Léauté-Labrèze C et al., *NEJM*.) Given the efficacy and relative safety of propranolol described in that publication as well as subsequent reports (Sans et al., *Pediatrics*), it soon became evident that propranolol was likely a superior therapy to both prednisolone and vincristine, such that continued enrollment of subjects in this study could not be justified and the study recruitment was suspended.

19. The mechanism of action of propranolol in treating infantile hemangiomas is unclear. It has been hypothesized that propranolol may cause vasoconstriction, resulting in color change and palpable softening of the hemangioma. It has also been hypothesized that propranolol might down-regulate growth factors, such as vascular endothelial growth factor, and up-regulate cellular apoptosis, or block the renin-angiotensin system either at the level of the kidney (renin production) or peripherally.

However, since the pathogenesis of these hemangioma is, itself, incompletely understood, there is little current basis to articulate the mechanism of action of propranolol or of other, previously used therapies for hemangiomas. (Itinteang, *J Plast Reconstr Aesthet Surg*. 2011 and *Plast Recon Surg* 2011.) Moreover, at the time of the initial publication of propranolol's efficacy, I would not have considered it for treatment of infantile hemangiomas. In my opinion (informed by years of study and work in the field), there was nothing in the medical art that would have suggested this utility of propranolol based on its use for treating hypertension, ischemic heart disease, arrhythmias, endocrine and neurologic disorders, and eye disorders. My opinion in this regard is unchanged after reading the references that have been applied against the '266 Application in the Office Action issued April 15, 2011.

20. In particular, I have reviewed the two references that I understand have formed the basis of a rejection of the claims in the '266 Application: PCT Application Publication WO 97/40830, Jirousek et al., published November 6, 1997; and Sozzani, et al, "Propranolol, a Phosphatidate Phosphohydrolase Inhibitor, Also Inhibits Protein Kinase C*," *J. Biological Chemistry*, 267:20481 (1992). I have also studied the Office Action issued April 15, 2011. I note, in part, that the rejection under 35 U.S.C. 103(a) is summarized on page 8 by the statement,

It would have been prima facie obvious to one of ordinary skill in the art, at the time of the invention, to treat a hemangioma comprising administration of a formulation comprising propranolol in a therapeutically effective amount, because Jirousek et. al. teaches that protein kinase C inhibitors are successfully used to treat neoplastic conditions such as capillary hemangiomas and hemangiomas of infancy, and Sozzani et al. teaches that propranolol is an effective protein kinase C inhibitor which is less toxic than other known protein kinase C inhibitors.

21. In my opinion, the efficacy of propranolol in treating infantile hemangiomas is not suggested by reading the Jirousek and Sozzani references. Jirousek describes a wide variety of neoplastic diseases to be treated by a specific class of inhibitors of the β -isozyme of protein kinase C (PKC). As discussed above, there is no clear evidence that infantile hemangiomas are truly neoplastic. Moreover, there is no substantiated evidence that infantile hemangiomas are associated with PKC. Thus, there is little basis in Jirousek for the otherwise unsubstantiated statement that the anti-

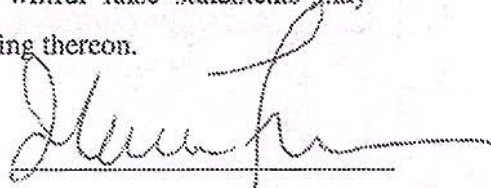
neoplastic compounds described therein would be useful in treating infantile hemangiomas, much less that any other PKC inhibitor would necessarily also be useful in treating infantile hemangiomas.

22. Further, while Sozanni describes the PKC inhibition activity by propranolol, the ultimate utility of propranolol in treating any disorder mediated by PKC is highly uncertain. Many potential drugs targeting various PKC isozymes have proven to be ineffective in treating tumors, however. See, Mackay et al., "Targeting the Protein Kinase C Family: Are We There Yet?," *Nature Reviews*, 7:554 (2007). Even assuming that propranolol can inhibit PKC, there is little basis to predict that it would actually be effective in treating any given disorder mediated by PKC.

23. Accordingly, in my opinion, the teachings of Jirousek and Sozanni did not and do not provide any motivation to attempt the treatment of infantile hemangiomas with propranolol. Nor would I have expected that such a treatment would have had a reasonable prospect for success based on these references. I note that there was no attempt to use propranolol to treat hemangiomas following the publication of Jirousek. Indeed, in my opinion, the discovery of the use of propranolol to treat hemangiomas, published 11 years after Jirousek reference, was surprising and unexpected.

24. I further declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: September 8, 2011

By: 

Ilona J. Frieden, M.D.

SELECTED REFERENCES:

North PE, Waner M, Mizeracki A, Mihm MC Jr. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol.* 2000 Jan;31(1):11-22.

Paula E. North, Milton Waner, Adam Mizeracki, Robert E. Mrak, Richard Nicholas, Jay Kincannon, James Y. Suen, and Martin C. Mihm, Jr. A Unique Microvascular Phenotype Shared by Juvenile Hemangiomas and Human Placenta *Arch Dermatol*, May 2001; 137: 559 - 570.

Barnés CM, Huang S, Kaipainen A, Sanoudou D, Chen EJ, Eichler GS, Guo Y, Yu Y, Ingber DE, Mulliken JB, Beggs AH, Folkman J, Fishman SJ. Evidence by molecular profiling for a placental origin of infantile hemangioma. *Proc Natl Acad Sci U S A.* 2005 Dec 27;102(52):19097-102.

Dadras SS, North PE, Bertoncini J, Mihm MC, Detmar M. Infantile hemangiomas are arrested in an early developmental vascular differentiation state. *Mod Pathol.* 2004 Sep;17(9):1068-79

Yu Y, Flint AF, Mulliken JB, Wu JK, and Bischoff J. Endothelial progenitor cells in infantile hemangioma *Blood*, Feb 2004; 103: 1373 - 1375.

Kleinman ME, Greives MR, Churgin SS, Blechman KM, Chang EI, Ceradini JF, Tepper OM and Gurtner GC. Hypoxia-Induced Mediators of Stem/Progenitor Cell Trafficking Are Increased in Children With Hemangioma. *Arterioscler Thromb Vasc Biol*, Dec 2007; 27: 2664 - 2670.

Bischoff J. Progenitor cells in infantile hemangioma. *J Craniofac Surg.* 2009;20(suppl 1):695-697.

Itinteang T, Withers AH, Leadbitter P, Day DJ, Tan ST. Pharmacologic therapies for infantile hemangioma: is there a rational basis? *Plast Reconstr Surg.* 2011 Aug;128(2):499-507. PubMed PMID: 21788841.

Itinteang T, Tan ST, Brasch HD, Vishvanath A, Day DJ. Primitive erythropoiesis in infantile haemangioma. *Br J Dermatol.* 2011 May;164(5):1097-100. doi:10.1111/j.1365-2133.2010.10187.x. PubMed PMID: 21518328.

Itinteang T, Vishvanath A, Day DJ, Tan ST. Mesenchymal stem cells in infantile haemangioma. *J Clin Pathol.* 2011 Mar;64(3):232-6. Epub 2011 Jan 17. PubMed PMID: 21242328.

Itinteang T, Tan ST, Brasch H, Day DJ. Haemogenic endothelium in infantile haemangioma. *J Clin Pathol.* 2010 Nov;63(11):982-6. Epub 2010 Oct 5. PubMed PMID: 20924092.

Itinteang T, Brasch HD, Tan ST, Day DJ. Expression of components of the renin-angiotensin system in proliferating infantile haemangioma may account for the propranolol-induced accelerated involution. *J Plast Reconstr Aesthet Surg*. 2011 Jun;64(6):759-65. Epub 2010 Sep 26. PubMed PMID: 20870476.

Itinteang T, Tan ST, Brasch H, Day DJ. Primitive mesodermal cells with a neural crest stem cell phenotype predominate proliferating infantile haemangioma. *J Clin Pathol*. 2010 Sep;63(9):771-6. Epub 2010 Aug 9. PubMed PMID: 20696686.

Haggstrom AN, Lammer EJ, Schneider RA, Marcucio R, and Frieden IJ. Patterns of Infantile Hemangiomas: New Clues to Hemangioma Pathogenesis and Embryonic Facial Development. *Pediatrics*, Mar 2006; 117: 698 - 703.

Drolet BA, Frieden IJ. Characteristics of infantile hemangiomas as clues to pathogenesis: does hypoxia connect the dots? *Arch Dermatol*. 2010 Nov;146(11):1295-9. PubMed PMID: 21079070.

Jinnin M, Ishihara T, Boye E, Olsen BR. Recent progress in studies of infantile hemangioma. *J Dermatol*. 2010;37(4):283-298.

Lo K, Mihm M, Fay A. Current theories on the pathogenesis of infantile hemangioma. *Semin Ophthalmol*. 2009;24(3):172-177.

Bauland CG, van Steensel MA, Steijlen PM, Rieu PN, Spauwen PH. The pathogenesis of hemangiomas: a review. *Plast Reconstr Surg*. 2006;117(2):29e-35e.

Walter JW, North PE, Waner M, Mizeracki A, Blei F, Walker JW, Reinisch JF, Marchuk DA. Somatic mutation of vascular endothelial growth factor receptors in juvenile hemangioma. *Genes Chromosomes Cancer*. 2002 Mar;33(3):295-303.

Calicchio ML, Collins T, Kozakewich HP, Identification of Signaling Systems in Proliferating and Involuting Phase Infantile Hemangiomas by Genome-Wide Transcriptional Profiling. *Am J Pathol*, 2009 May;174(5):1638-49. E Pub 2009 Apr 6.

Boye E, Masatoshi Jinnin, Bjorn R. Olsen, Infantile Hemangioma, *Journal of Craniofacial Surgery*, 2009, 20, Suppl 1, 678.

Masatoshi Jinnin, Damian Medici, Lucy Park, Nisha Limaye, Yanqiu Liu, Elisa Boscolo, Joyce Bischoff, Miikka Vikkula, Eileen Boye, Bjorn R Olsen, Suppressed NFAT-dependent VEGFR1 expression and constitutive VEGFR2 signaling in infantile hemangioma, *Nature Medicine*, 2008, 14, 11, 1236.

Ying Yu, Jasmin Fuhr, Eileen Boye, Steve Gyorffy, Shay Soker, Anthony Atala, John B. Mulliken, Joyce Bischoff, Mesenchymal Stem Cells and Adipogenesis in Hemangioma Involution, *STEM CELLS*, 2006, 24, 6.

Leonardi-Bee J, Batta K, O'Brien C, Bath-Hextall FJ. Interventions for infantile haemangiomas (strawberry birthmarks) of the skin. Cochrane Database Syst Rev. 2011 May 11;5:CD006545. Review. PubMed PMID: 21563155.

Bennett ML, Fleischer AB Jr, Chamlin SL, Frieden IJ. Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. Arch Dermatol. 2001 Sep;137(9):1208-13. PubMed PMID: 11559219.

Léauté-Labrèze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taïeb A. Propranolol for severe hemangiomas of infancy. N Engl J Med. 2008 Jun 12;358(24):2649-51. PubMed PMID: 18550886.

Sans V, de la Roque ED, Berge J, Grenier N, Boralevi F, Mazereeuw-Hautier J, Lipsker D, Dupuis E, Ezzedine K, Vergnes P, Taïeb A, Léauté-Labrèze C. Propranolol for severe infantile hemangiomas: follow-up report. Pediatrics. 2009 Sep;124(3):e423-31. Epub 2009 Aug 10. PubMed PMID: 19706583.

Chang LC, Haggstrom AN, Drolet BA, Baselga E, Chamlin SL, Garzon MC, Horii KA, Lucky AW, Mancini AJ, Metry DW, Nopper AJ, Frieden IJ; Hemangioma Investigator Group. Growth characteristics of infantile hemangiomas: implications for management. Pediatrics. 2008 Aug;122(2):360-7.

Boscolo E, Bischoff J. Vasculogenesis in infantile hemangioma. Angiogenesis. 2009;12(2):197-207. Epub 2009 May 10. Review. PubMed PMID: 19430954; PubMed Central PMCID: PMC2810616.

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BOARD CERTIFIED:

1983	American Board of Pediatrics
1983	American Board of Dermatology
2004	American Board of Dermatology, Pediatric Dermatology Subspecialty Certification
2005	American Board of Dermatology, Dermatology Recertification

EDUCATION AND POSITIONS HELD:

1973	B.A. Boston University
1977	M.D. University of California, San Francisco
1977-1980	Pediatric Intern and Resident, University of California, San Francisco
1980-1982	Dermatology Resident, University of California, San Francisco
1983	Pediatric Dermatology Training Elective, Children's Memorial Hospital, Chicago
1983-1985	Clinical Instructor Pediatrics and Dermatology, University of California, San Francisco
1983-1988	Staff Dermatologist, Kaiser Permanente Medical Group, Oakland, California
1983-	Director, Pediatric Dermatology Clinic, San Francisco General Hospital
1985-1988	Chief of Dermatology Department, Kaiser Permanente Medical Group, Oakland
1985-1992	Assistant Clinical Professor, Pediatrics and Dermatology, University of California, San Francisco

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1989- 2004	Chief, Pediatric Dermatology Clinics, University of California, San Francisco
1989- 2001	Assistant Director, Pediatric Dermatology, University of California, San Francisco
1992-1997	Associate Clinical Professor, Pediatrics and Dermatology, University of California, San Francisco
1997- 2006	Clinical Professor, Pediatrics and Dermatology, University of California, San Francisco
2002-	Chief, Division of Pediatric Dermatology, University of California, San Francisco
2003-	Consultant, Oakland Children's Hospital, Tuberous Sclerosis clinic
2006 -	Professor, Pediatrics and Dermatology, University of California, San Francisco

PROFESSIONAL SOCIETIES:

1983-	Society for Pediatric Dermatology
1983-	San Francisco Dermatologic Society
1984-	American Academy of Dermatology
1985-	Women's Dermatologic Society
1989-	American Academy of Pediatrics
1993-	Mentoring Comm, Women's Derm Soc
1995-2003	American Society Laser Medicine & Surgery
1996-	International Society for the Study of Vascular Anomalies
2004-	America Dermatologic Association

PROFESSIONAL ACTIVITIES:

1985-1988	Member, Task Force on Pediatric Dermatology of the American Academy of Dermatology
1989-1991	Member, Awards & Goals Committee, Society for Pediatric Dermatology
1989-	Member, Section of Dermatology, American Academy of Pediatrics
1990-1993	Society for Pediatric Dermatology, Board of Directors
1992-	Consultant, Drug Evaluation for the American Medical Association, Division of Drugs and Toxicology
1993-1994	Member, Task Force on Pediatric Dermatology of the American Academy of Dermatology
1993-2000	Current Problems in Dermatology Editorial Board
1994-1999	Executive Committee, Section of Dermatology, American Academy of Pediatrics
1994-	Member, Task Force on Youth Education, American Academy of Pediatrics
1995-	American Board of Dermatology Recertification Committee, Pediatric Dermatology subsection

- 1995 Chairman, American Academy Dermatology Task Force on Guidelines for Care: Hemangiomas
- 1994-1996 Nominating Committee, Society for Pediatric Dermatology
- 1995-1997 Chair, Mentorship Committee, Women's Dermatologic Society
- 1996-1997 Chair, Nominating Committee, Society for Pediatric Dermatology
- 1996- Active Member, International Society for the Study of Vascular Anomalies
- 1998-2006 Editorial Board, Pediatric Dermatology
- 1999-2007 Board of Directors, American Board of Dermatology
- 1999-2003 Editorial Board, Journal of the American Academy of Dermatology
- 1999-2003 Board of Directors, Women's Dermatologic Society
- 1999 President-Elect, Society of Pediatric Dermatology
- 2000-2005 Editorial Board, Archives of Dermatology
- 2001-2002 President, Society of Pediatric Dermatology
- 2001- Co-Editor, Textbook of Neonatal Dermatology
- 2003-2006 Member, American Academy of Dermatology's Intersociety Liaison Committee
- 2003-2006 Chair, meetings committee society of Pediatric Dermatology
- 2004- Member, American Dermatologic Association
- 2005-2006 President-Elect, Board of Directors, American Board of Dermatology
- 2005- Designated Mentor, American Academy of Dermatology Academic Dermatology Leadership Program.
- 2005- Executive Committee, American Board of Dermatology
- 2005- Medical Advisory Panel, National Organization of Vascular Anomalies
- 2005-2006 Member, CME Advisory Board, Elsevier Office
- 2006- Editor-in-Chief, Pediatric Dermatology
- 2006-2007 President, Board of Directors, American Board of Dermatology
- 2007- Medical Advisory Board, Birthmarks.com
- 2008- Steering Committee, Leadership Institute, American Academy of Dermatology
- 2010- Board of Directors, American Academy of Dermatology
- 2010- Secretary, International Society for the Study of Vascular Anomalies (ISSVA)
- 2011- Scientific Assemblies Committee (SAC) member, AAD

HONORS:

- 1998 Who's Who of American Women
- 1998- Best Doctors in America®
- 2000 Teacher of the Year Award, UCSF Dermatology Department
- 2000 Honorary member, Sociedad Argentina de Dermatologia
- 2000 Pommerening Lecturer, Washington Dermatological Society
- 2000 The Nancy B. Esterly, MD Lectureship, Wisconsin Dermatological Society
- 2001- America's Top Doctors (Castle Connolly Guide)
- 2001 Woman of Distinction at UCSF, Celebrating Women Faculty, UCSF, (Inaugural Event)
- 2001 Founders Lecturer, Society for Pediatric Dermatology
- 2002 Honoree, UCSF Chancellor's Committee Status of Women "Celebrating Women Faculty 2002"
- 2003 Harold O. Perry Lecturer, Mayo Clinic Department of Dermatology O'Leary Meeting

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2004-	American Dermatologic Association Membership
2005	Mentor of the year, Women's Dermatologic Society
2007	American Academy of Pediatrics Walter Tunnessen Lectureship, University of Hawaai Kapiolani Medical Center, April 9-11, 2007
2007	William Moores Lecturer, Indiana State Dermatologic Society, May 2, 2007
2007	Lecturer, Circulo Dermatologico del Peru First National Meeting on Clinical and Therapeutics June 27-30, 2007
2008	Visiting Professor Montagna Lectureship, Department of Dermatology, Oregon Health & Science University November 12-13, 2008
2008	BMA (British Medical Association) Medical Book Competition Awards First Prize – Dermatology for Neonatal Dermatology, 2 nd Edition.
2010	Louis A. Brunsting Lecturer, The Mayo Clinic, November 11, 2010
2011	AAD Presidential Citation

UNIVERSITY SERVICE:

1983-2003	Faculty Attending, Pediatric Dermatology Clinic, San Francisco General Hospital
1985-1999	Advisory Board, Department of Dermatology, University of California, San Francisco
1989-	Executive Committee, Department of Dermatology, University of California, San Francisco
1989-	Faculty Practice, Department of Dermatology, University of California, San Francisco
1989-	Dermatology Resident Education Committee, University of California, San Francisco,
1989-	Dermatology Resident Selection Committee, University of California, San Francisco,
1989-1990	Cost Awareness Committee, University of California, San Francisco
1991-	Director, Multidisciplinary Vascular Anomalies Clinic University of California, San Francisco
1992-2004	Faculty, Ectodermal Dysplasia Clinic, Craniofacial Anomalies Center University of California, San Francisco
1993-	Medical Student Advisor, University of California, San Francisco
1995-1999	Quality Assurance Representative, Dept of Dermatology University of California, San Francisco
1998-	Grand Rounds Scheduling, Department of Dermatology University of California, San Francisco
1999-	Executive Committee, Department of Dermatology University of California, San Francisco
2000-	Fellowship director, Pediatric Dermatology Fellowship University of California, San Francisco
2002-2004	Search Committee, Dermatopathologist, Department of Dermatology University of California, San Francisco
2002-2004	Search Committee, Dermatologic Surgeon, Department of Dermatology University of California, San Francisco

2001-2005 Search Committee, Tenured Research Faculty, Department of Dermatology
University of California, San Francisco
2004-2005 Search Committee, Tenured Research Faculty, Department of Dermatology
University of California, San Francisco
2005 Chair, Search Committee, Assistant Clinical Professors Dermatology (4 positions)
2005-2008 Library Committee, University of California, San Francisco
2005- Search Committee, Ophthalmology
2007-2009 Search Committee, Dermatopathology faculty
2007- UCSF Junior Faculty Mentoring program: Co-Director Dermatology Department
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2009 Search Committee, Dermatology junior faculty

BIBLIOGRAPHY

ORIGINAL ARTICLES:

- 1) Frieden IJ, Esterly NB. Selected genodermatoses in infants and children. *Clinics in Dermatology* 1985;3:14-32.
- 2) Frieden IJ. Aplasia cutis congenita: a clinical review and proposal for classification. *J Am Acad Dermatol* 1986;14:646-60.
- 3) Detlefs R, Frieden IJ, Berger TG, Westrom D. Eosinophil fluorescence: a cause of false positive slide tests for herpes simplex virus. *Pediatr Dermatol* 1987;4:129-33.
- 4) Frieden IJ. Diagnosis and management of tinea capitis. *Pediatric Annals* 1987;16:39-48.
- 5) LeBoit PE, Beckstead JH, Bond B, Epstein WL, Frieden IJ, Parslow TG. Granulomatous slack skin: clonal rearrangement of the T-cell receptor beta gene is evidence for the lymphoproliferative nature of a cutaneous elastolytic disorder. *J Invest Derm* 1987;89:183-6.
- 6) Stein DH, Paller AS, Frieden IJ, Rasmussen JE. Society for Pediatric Dermatology, 12th Annual Meeting. *J Am Acad Dermatol* 1988;19:1142-5.
- 7) Frieden IJ, Prose N, Fletcher V, Turner MC. Granulomatous perioral dermatitis in children. *Arch Dermatol* 1989;125:369-73.
- 8) Frieden IJ. Blisters and pustules in the newborn. *Curr Probl Pediatr* 1989;19:551-614.
- 9) Frieden IJ. Blistering dactylitis caused by group B streptococci. *Pediatr Dermatol* 1989;6:300-2.
- 10) Becker B, Frieden IJ, Odom R, Berger TG. Atypical plaque-like staphylococcal folliculitis in human immunodeficiency virus-infected persons. *J Am Acad Dermatol* 1989;21:1024-6.
- 11) Frieden IJ. Tinea infections in adolescents. *Adolesc Med* 1990;1:333-44.

- 12) Frieden IJ, Prose NS, Fletcher V, Turner ML. Granulomatous perioral dermatitis or sarcoid? *Arch Dermatol* 1990;126:1237-8.
- 13) Waldman B, Frieden IJ. Osteomyelitis caused by nail biting. *Pediatr Dermatol* 1990;7:189-90.
- 14) Jahan V, LeBoit PE, Frieden IJ. Inflammatory nodule on the scalp *Pediatr Dermatol* 1990;7:152-4.
- 15) Shpall S, Frieden IJ. Mahogany discoloration of the skin due to the defensive secretion of a millipede. *Pediatr Dermatol* 1991;8:25-7.
- 16) Shpall S, Frieden IJ, Chesney M, Newman T. Risk of malignant transformation of congenital melanocytic nevi in blacks. *Pediatr Dermatol* 1991;11:204-8.
- 17) Kadonaga JN, Frieden IJ. Neurocutaneous melanosis: definition and review of the literature. *J Am Acad Dermatol* 1991;24:747-55.
- 18) Frieden IJ, Resnick S. Childhood exanthems. Old and new. *Ped Cl N Amer* 1991;38:859-88.
- 19) Frieden IJ. Blisters and pustules in the newborn. *Current Concepts in Skin Disorders* 1991;12:5-17.
- 20) Barnett BO, Frieden IJ: Streptococcal skin disease in children. *Semin Dermatol* 1992;11:3-10.
- 21) Frieden IJ, Garbin GS, Grekin RC. Anesthesia and/or sedation for pulsed dye laser therapy. *Pediatr Dermatol* 1992;9:134-5.
- 22) Kadonaga JN, Edwards M, Frieden IJ. Neurocutaneous melanosis in association with the Dandy-Walker complex. *Pediatr Dermatol* 1992;9:37-43.
- 23) Scully MC, Frieden IJ. Toxic epidermal necrolysis in early infancy. *J Am Acad Derm* 1992;27:340-4.
- 24) Frieden IJ. The dermatologist in the newborn nursery: aproach to the neonate with blisters, pustules, erosions and ulcerations. *Curr Probl Dermatol* 1992;4:123-68.
- 25) Frieden IJ, Esterly NB. Pyogenic granulomas of infancy masquerading as strawberry hemangiomas. *Pediatr* 1992;90:989-91.
- 26) Kanegaye JN, Frieden IJ. Lichen striatus: simultaneous occurrence in siblings. *Pediatrics* 1992;90:104-6.
- 27) Grekin RC, Shelton RM, Geisse JK, Frieden IJ. 510-nm Pigmented Lesion Dye Laser. *J Dermatol Surg Oncol* 1993;19:380-7.

- 28) Reese V, Frieden IJ, Paller AS, Esterly NB, Ferriero D, Levy ML, Lucky AE, Gellis SE, Siegfried EC. Association of facial hemangiomas with Dandy-Walker and other posterior fossa malformations. *J Pediatr* 1993;122:379-84.
- 29) Baker L, Dillon WP, Hieshima GB, Dowd CF, Frieden IJ. Hemangiomas and vascular malformations of the head and neck: MR characterization. *AJNR Am J Neuroradiol* 1993;14:307-14.
- 30) Frieden IJ, Esterly NB. Comments and opinions: you say potato, we say potatoe. *Arch Dermatol* 1994;130:114-5.
- 31) Frieden IJ. Acyclovir to treat varicella. *Western J Med* 1994;160:361-2.
- 32) Barkovich AJ, Frieden IJ, Williams M. MR of neurocutaneous melanosis. *AJNR Am J Neuroradiol* 1994;15:859-67.
- 33) Frieden IJ, Williams M. Familial site-specific congenital melanocytic nevus: report of two families. *Arch Dermatol* 1994;130:1075-6.
- 34) Frieden IJ, Williams M, Barkovich AJ. Giant congenital melanocytic nevi: brain magnetic resonance findings in neurologically asymptomatic children. *J Amer Acad Dermatol* 1994;31:423-9.
- 35) Frieden IJ, Howard R. Tinea capitis: epidemiology, diagnosis, treatment, and control. *J Amer Acad Dermatol* 1994;31:S42-6.
- 36) Sphall S, Newman T, Chesney M, Frieden IJ. Risk of malignant transformation of congenital melanocytic nevi in blacks. *Pediatr Dermatol* 1994;3:204-8.
- 37) Longaker M, Frieden IJ, LeBoit PE, Sherertz EF. Congenital "self-healing" Langherans' cell histiocytosis: the need for long-term follow-up *J Am Acad Dermatol* 1994;31:910-16.
- 38) Bircher AJ, Koo JYM, Frieden IJ, Berger TG. Angiodysplastic syndrome with capillary and venous malformation associated with soft tissue hypotrophy. *Dermatology* 1994;189:292-6.
- 39) Singalavanija S, Frieden IJ. Diaper dermatitis. *Pediatr Review* 1995;16:142-7.
- 40) Howard R, Frieden IJ. Tinea capitis: new perspectives on an old disease. *Semin Dermatol* 1995;14:2-8.
- 41) Frieden IJ. Childhood exanthems. *Current Opinion in Pediatrics* 1995;7:411-14.
- 42) Salmon JK, Frieden IJ. Congenital and genetic disorders of hyperpigmentation. *Curr Probl Dermatol* 1995;7:143-98.
- 43) Fisher CA, LeBoit PE, Frieden IJ. Linear porokeratosis presenting as erosions in the newborn period. *Pediatr Dermatol* 1995;12:318-22.

- 44) Hwang ST, Williams ML, McCalmont TH, Frieden IJ. Sweet's syndrome leading to acquired cutis laxa (Marshall's Syndrome) in an infant with alpha-1-antitrypsin deficiency. *Arch Dermatol* 1995;31:1175-8.
- 45) Kaufman AJ, Grekin RC, Geisse JK, Frieden IJ. Treatment of adenoma sebaceum with the co2 vapor laser. *J Amer Acad Dermatol* 1995;33:770-4.
- 46) Huttenlocher A, Frieden IJ, Emery H. Neonatal onset multisystem inflammatory disease. *J Rheumatol* 1995;22:1171-3.
- 47) Frieden IJ, Wu M, Lee I. Suture ligation of supernumerary digits and 'tags': an outmoded practice? *Arch Pediatr Adol Med* 1995;149:1284.
- 48) Cunningham ET, Demetrius R, Frieden IJ, Emery HM, Irvine AR, Good WV. Vogt-Koyanagi-Horada syndrome in a 4 year old child. *Am J Ophth* 1995;120:675-7.
- 49) Chang MW, Frieden IJ, Good W. The risk of intraocular juvenile xanthogranuloma: survey of current practices and assessment of risk. *J Am Acad Dermatol* 1996;34:445-9.
- 50) Frieden IJ, Reese V, Cohen D. PH.A.C.E. Syndrome: The association of posterior fossa brain malformations, hemangiomas, arterial anomalies, coarctation of the aorta & cardiac defects, and eye abnormalities. *Arch Dermatol* 1996;132:307-11.
- 51) Prizant T, Lucky AW, Frieden IJ, Burton PS, Suarez AM. Spontaneous atrophic patches in extremely premature infants. Anetoderma of prematurity. *Arch Dermatol* 1996;132:671-4.
- 52) Howard R, Frieden IJ. Papular urticaria in children. *Pediatr Dermatol* 1996;13:246-9.
- 53) Wilmington MR, Aly R, Frieden IJ. Trichophyton tonsurans tinea capitis in the San Francisco Bay area: increased infection demonstrated in a 20-year survey of fungal infections from 1974 to 1994. *J Med & Vet Mycol* 1996;34:285-7.
- 54) Williams ML, Frieden IJ. The management of congenital melanocytic nevi: a decade later. *Pediatr Dermatol* 1996;13:321-4.
- 55) Fung MA, Frieden IJ, LeBoit PE, Berger TG, Epstein E, Kay D, Van SL, Williams ML. Confluent and reticulate papillomatosis: successful treatment with minocycline. *Arch Dermatol* 1997;132:1400-1.
- 56) Mirowski GW, Frieden IJ, Miller C. Iatrogenic scald burn: a consequence of institutional infection control Measures. *Pediatrics* 1996;98:963-5.
- 57) Frieden IJ, Enjolras O., Mulliken JB et al. Special symposium: management of hemangiomas. *Pediatr Dermatol* 1997;14:57-83.

- 58) Liang M, Frieden IJ. Perineal and lip ulcerations as the presenting manifestation of hemangioma of infancy. *Pediatrics* 1997;99:256-9.
- 59) Lobato MN, Vugia DJ, Frieden IJ. Tinea capitis in California children: a population-based study of a growing epidemic. *Pediatrics* 1997;99:551-4.
- 60) Enjolras O, Wassef M, Mazoyer E, Frieden IJ, Rieu PN, Drouet L, Taïeb A, Stalder JF, Escande JP. Infants with Kasabach-Merritt syndrome do not have "true" hemangiomas. *J Pediatr* 1997;130:631-40.
- 61) Vin-Christian K, McCalmont T, Frieden IJ. Kaposiform hemangioendothelioma: An aggressive, locally invasive vascular tumor which can mimic hemangioma of infancy. *Arch Dermatol* 1997;133:1573-78.
- 62) Frieden IJ, Eichenfield LF, Esterly NB, Geronemus R, Mallory SB. Guidelines of care for hemangiomas of infancy. American Academy of Dermatology Guidelines/Outcomes Committee. *J Am Acad Dermatol* 1997;37:631-7.
- 63) Robinson JC, Lozada-Nur F, Frieden IJ. Oral pemphigus vulgaris: a review of the literature and a report on the management of 12 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;84:349-55.
- 64) Howard RM, Frieden IJ, Crawford D, McCalmont T, Levy ML, Rosenblatt DS, Sweetman L, Goodman SI, Ohnstad C, Hart K, Berrios M, Packman S. Methylmalonic acidemia, cobalamin C Type, presenting with cutaneous manifestations. *Arch Dermatol* 1997;133:1563-6.
- 65) Frieden IJ. Which hemangiomas to treat ... and how? *Arch Dermatol* 1997;133:1593-95.
- 66) Tanner JL, Dechert MP, Frieden IJ. Growing up with a facial hemangioma: parent and child coping and adaptation. *Pediatrics* 1998;101:446-452.
- 67) Crowley E, Frieden IJ. Neonatal lupus erythematosus: an unusual congenital presentation with cutaneous atrophy, erosions, alopecia and pancytopenia. *Pediatr Dermatol* 1998;15:38-42.
- 68) Murakawa GJ, McCalmont TH, Frieden IJ. Chronic plaques in a patient with ataxia telangiectasia. Cutaneous granulomatous lesions in a patient with AT. *Arch Dermatol* 1998;134:1145-8.
- 69) Slavin KA, Frieden IJ. Picture of the month. Hand-foot-and-mouth disease. *Arch Pediatr Adolesc Med* 1998;152:505-6.
- 70) Mancini AJ, Frieden IJ, Paller AS. Infantile acropustulosis revisited: history of scabies and response to topical corticosteroids. *Pediatr Dermatol* 1998;15:337-41.
- 71) Frieden IJ. Guidelines of care: hemangiomas of infancy reply (Letter)". *J Am Acad Dermatol* 1998;39:662.

- 72) Le, KVT, Shahidullah, H, Frieden IJ. Review of modern techniques in detecting port-wine stain response to laser therapy. *Dermatol Surg* 1999;25:127-32.
- 73) Shahidullah H, Frieden IJ. Eczema as a complication of pulsed dye laser therapy. *Arch Dermatol* 1999;135:215-6.
- 74) Giam Y, Williams ML, Leboit PE, Orlow SJ, Eichenfield, LF, Frieden IJ. Neonatal erosions and ulcerations in giant congenital melanocytic nevi. *Pediatr Dermatol* 1999;16:354-8.
- 75) Drolet B, Esterly N, Frieden IJ. Hemangiomas in children. *N Engl J Med* 1999;341:173-81.
- 76) Frieden IJ. Tinea capitis: asymptomatic carriage of infection. *Pediatr Infect Dis J* 1999;18:186-90.
- 77) Kwong PC, O'Marcaigh AS, Howard R, Cowan MJ, Frieden IJ. Oral and genital ulceration: a unique presentation of immunodeficiency in Athabaskan-speaking American Indian children with severe combined immunodeficiency. *Arch Dermatol* 1999;135:927-31.
- 78) Drolet B, Esterly N, Frieden IJ. Hemangiomas in children (Letter) *N Engl J Med*. 1999;341:173-81.
- 79) Howard RM, Frieden IJ. Dermatophyte infections in children. *Adv Pediatr Infect Dis*. 1999;14:73-107.
- 80) Eastlack J, Howard R, Frieden IJ. Congenital midline cervical cleft: case report and review of the English literature. *Pediatr Dermatol* 2000;17(2):118-22.
- 81) Enjolras O, Mulliken JB, Wassef M, Frieden IJ, Rieu PN, Burrows PE, Salhi A, Leaute-Labreze C, Kozakewich HP Residual lesions after Kasabach-Merritt phenomenon in 41 patients. *J Am Acad Dermatol*. 2000;42:225-35.
- 82) Garzon MC, Enjolras O, Frieden IJ. Vascular tumors and vascular malformations: evidence for an association. *J Am Acad Dermatol*. 2000;42:275-9.
- 83) Frieden IJ. Commentary on viral exanthemas. Mosby, St Louis. *Year Book of Pediatrics* 2000;57-8.
- 84) Frieden IJ, Garzon MC. Hemangiomas: when to worry. *Pediatric Annals* 2000;29:58-67.
- 85) Forney VR, Frieden IJ. Multidisciplinary approach to vascular anomalies. *Scope on Phlebology and Lymphology* 2000;2:44-7.
- 86) O'Hare P, Frieden IJ. Virginal breast hypertrophy. *Pediatr Dermatol* 2000;17:277-81.
- 87) Kim H-J, Colombo M, Frieden IJ. Ulcerated hemangiomas: clinical characteristics and response to therapy. *J Am Acad Dermatol* 2001;44:962-72.

- 88) Foster R, Williams ML, Barkovich AJ, Hoffman WY, Mathes, SJ, Frieden IJ. Giant congenital melanocytic nevi: the significant of neurocutaneous melanosis in neurologically asymptomatic children. *Plast Reconstr Surg* 2001;107:933-41.
- 89) El Shabrawi-Caelen L, White WL, Soyer HP, Kim BS, Frieden IJ, McCalmont TH. Rudimentary meningocele: remnant of a neural tube defect? *Arch Dermatol* 2001;137:45-50.
- 90) Liu T, Howard RM, Mancini AJ, Weston WL, Paller AS, Drolet BA, Esterly NB, Levy ML, Schachner L, Frieden IJ. Kwashiorkor in the United States: fad diets, perceived and true milk allergy, and nutritional ignorance. *Arch Dermatol* 2001;137:630-6.
- 91) Hong S, Frieden IJ. Diagnosing eruptive vellus hair cysts. *Pediatr Dermatol* 2001;18:258-9.
- 92) Metry D, Barkovich AJ, Dowd C, Frieden IJ. The many faces of PHACE syndrome. *J Pediatr* 2001;139:117-23.
- 93) Bennett ML, Fleischer AB, Chamlin SL, Frieden IJ. Oral corticosteroid use is effective for cutaneous hemangiomas: an evidence-based evaluation. *Arch Dermatol* 2001;137:1208-13.
- 94) Sugarman, JL, Mauro, TM, Frieden, IJ. Treatment of an ulcerated hemangioma with recombinant platelet-derived growth factor. *Arch Dermatol* 2002;138:314-6.
- 95) Hand J, Frieden IJ. Vascular birthmarks of infancy: resolving nosologic confusion. *Am J Med Genet* 2002;108:257-64.
- 96) Chamlin SL, Frieden IJ, Fowler A, Williams M, Kao J, Sheu M, Elias PM. Ceramide-dominant, barrier-repair lipids improve childhood atopic dermatitis. *Arch Dermatol* 2001;137:1110-2.
- 97) Haisley-Royster C, Enjolras O, Frieden IF, Garzon M, Oranje A, Gonzalez F, Frangoul H, LeMoine P, Prose NS, Adams D. Kasabach-merritt phenomenon: a retrospective study of treatment with vincristine. *Am J Pediatr Hematol Oncol* 2002;4:459-62.
- 98) Sharma V, Silverberg N, Howard R, Tran CT, Frieden IJ. Do hair practices affect the acquisition of tinea capitis? A case-control study. *Arch Pediatr Adolesc Med* 2001;155:818-21.
- 99) Denianke KS, Frieden IJ, Cowan MJ, Williams ML, McCalmont TH. Cutaneous manifestations of maternal engraftment in patients with severe combined immunodeficiency: a clinicopathologic study. *Bone Marrow Transplantation* 2001;28:227-33.
- 100) Liao PB, Robinson R, Howard R, Sanchez, G, Frieden IJ. Annular pustular psoriasis – the most common form of pustular psoriasis in children: report of three cases and review of the literature. *Pediatric Dermatology* 2002;19:19-25.
- 101) Crowder J, Frieden I, Price V. Alopecia areata in infants and newborns. *Pediatric Dermatology* 2002;19:155 -8.

- 102) Liang MG, Frieden IJ. Perineal ulcerations as the presenting manifestation of hemangioma. *Arch Dermatol* 2002;138:126-7.
- 103) Chamlin S, Kao J, Frieden IJ, Sheu, MY, Fowler AJ, Fluhr JW, Williams ML, Elias PM. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol* 2002;47:198-208.
- 104) North PE, Waner M, James CA, Mizeracki A, Frieden IJ, Mihm MC. Congenital nonprogressive hemangioma: a distinct clinicopathological entity unlike infantile hemangioma. *Arch Dermatol* 137:1607-20.
- 105) Evans-Ramsey T, Frieden IJ. Arcuate plantar plaques as the initial sign of chronic cutaneous lupus in a child. *J Am Acad Dermatol* 2003;49:S270-1.
- 106) Makkar HP, Frieden IJ. Congenital melanocytic nevi: an update for the pediatrician. *Current Opinion in Pediatrics* 2002;14:397-403.
- 107) Chiller, KG, Passaro, D, Frieden, IJ. Hemangiomas of infancy: clinical characteristics, morphologic subtypes, and their relationship to race, ethnicity, and sex. *Arch Dermatol* 2002;138:1567-76.
- 108) Chamlin SL, McCalmont TH, Cunningham BB, Esterly N, Lai CH, Mallory, SB, Mancini AJ, Tamburro J, Frieden IJ. Cutaneous manifestations of hyper-IgE syndrome in infants and children. *J Pediatr* 2002;141:572-5.
- 109) Kundu, RV, Frieden, IJ. Presence of vascular anomalies with congenital hemihypertrophy and Wilms' tumor: an evidence-based evaluation. *Pediatr Dermatol* 2003;20:199-206.
- 110) Aly R, Hafeez ZH, Rodwell C, Frieden IJ, Abrams B. Rapid response of Trichophyton tonsurans-induced onychomycosis after treatment with terbinafine. *Int J Dermatol* 2002;41:357-9.
- 111) Liu T, Frieden IJ. Rice dream nondairy beverages (Letter). *Arch Dermatol* 2002;138:838.
- 112) Sugarman JL, McCalmont TH, Frieden IJ, Dover J, Arndt K. Gigantic metameric seborrheic keratosis". *Plast Reconstr Surg* 2002;111:1775-6.
- 113) Bruckner AL, Frieden IJ. Hemangiomas of infancy. *J Am Acad Dermatol* 2003;48:477-93.
- 114) Waner M, North PE, Scherer KA, Frieden IJ, Wanter A, Mihm Jr MC. The nonrandom distribution of facial hemangiomas. *Arch Dermatol* 2003;139:869-75.
- 115) Urbatsch A, Frieden IJ, Williams ML, Elewski BE, Mancini AJ, Paller AS. Extrafacial and generalized granulomatous 'Periorificial' dermatitis. *Arch Dermatol* 2002; 138:1354-8.
- 116) Bastian BC, Xiong J, Frieden IJ, Williams, ML, Chou P, Busam K, Pinkel D, LeBoit PE. Genetic changes in neoplasms arising in congenital melanocytic nevi: differences between nodular proliferations and melanomas. *American J Pathol* 2002;161:1163-9.

- 117) Honig PJ, Frieden IJ, Kim HJ, Yan AC. Streptococcal intertrigo: an underrecognized condition in children. *Pediatrics* 2003;112:1427-9.
- 118) Jan F, Segal J, Dyer J, LeBoit PE, Siegfried E, Frieden IJ. Nephrogenic fibrosing dermopathy: two pediatric cases. *J Pediatr* 2003;143:678-81.
- 119) Siegel DH, Ashton GHS, Penagos HG, Lee JV, Feiler HS, Wilhelmsen KC, South AP, Smith FJ, Prescott AR, Wessagowit V, Oyama N, Akiyama M, Al Aboud D, Al Aboud K, Al Githami A, Al Hawsawi K, Al Ismaily A, Al-Suwaid R, Atherton DJ, Caputo R, Fine JD, Frieden IJ, Fuchs E, Haber RM, Harada T, Kitajima Y, Mallory SB, Ogawa H, Sahin S, Shimizu H, Suga Y, Tadini G, Tsuchiya K, Wiebe CB, Wojnarowska F, Zaghoul AB, Hamada T, Mallipeddi R, Eady RAJ, McLean WHI, McGrath JA, and Epstein, Jr. EH. Loss of kindlin-1, a human homolog of the *Caenorhabditis elegans* actin-extracellular-matrix linker protein UNC-112, causes Kindler syndrome. *Am J Human Genetics* 2003;73:174-87.
- 120) Metry DW, Dowd CF, Barkovich AJ, Frieden IJ. Facial hemangioma and cerebral corticovascular dysplasia: a syndrome associated with epilepsy. (Letter) *Neurology* 2003;61:1461.
- 121) Makkar HP, Frieden IJ. Images in clinical medicine. Transillumination of a cystic lymphatic malformation. *N Engl J Med* 2003;349:e18.
- 122) Frieden, IJ. Risk stratification in hemangiomas of infancy. *Lymph Res Biol* 2003;4:313-6.
- 123) Chiller KG, Frieden IJ, Arbiser JL. Molecular pathogenesis of Vascular Anomalies: Classification into three categories based upon clinical and biochemical characteristics. *Lymph Res Biol* 2003;4:267-81.
- 124) Frieden IJ, Haggstrom AN, McCulloch C, Drolet B, Esterly NB, Garzon MC, Lucky AW, Baselga E, Friedlander SF. Combined antiretroviral therapy during pregnancy and risk of congenital malformations. (Letter) *JAMA* 2004;291:1961-2.
- 125) Metry DW, Hawrot A, Frieden IJ. Association of solitary, segmental hemangiomas of the skin with visceral hemangiomatosis. *Arch Derm* 2004;140:591-596.
- 126) North PE, Kahn T, Cordisco MR, Dadras SS, Detmar M, Frieden IJ. Multifocal lymphangoendotheliomatosis with thrombocytopenia: a newly recognized clinicopathological entity. *Arch Derm* 2004;140:599-606.
- 127) Makkar HP, Frieden IJ. Neurocutaneous Melanosis. *Semin Cutan Med Surg* 2004;23:135-144.
- 128) Haggstrom AN, Frieden IJ. Hemangiomas: past, present, and future. *J Am Acad Dermatol*.2004;51:S50-52.
- 129) Penagos H, Jaen M, Sancho M, Fallas V, Frieden I. Kindler syndrome in native Americans from Panama: report of 26 cases. *Arch Derm* 2004;140:939-44.

- 130) Mathes EFD, Haggstrom AN, Dowd C, Hoffman W, Frieden IJ. Clinical characteristics and management of vascular anomalies: findings of a multidisciplinary vascular anomalies clinic. *Arch Derm* 2004;140:979-83.
- 131) Chamlin SL, Frieden IJ, Williams ML, Chren MM. Effects of atopic dermatitis on young American children and their families. *Pediatrics* 2004;114:607-11.
- 132) Maari C, Frieden IJ. Klipel-Trenaunay syndrome: the importance of 'geographic stains' in identifying disease and risk of complications. *J Am Acad Dermatol* 2004;51:391-8.
- 133) Acosta F, Binder D, Barkovich AJ, Frieden IJ, Gupta N. Neurocutaneous melanosis presenting with hydrocephalus: case report and review of the literature. *J Neurosurgery*. 2005 Jan;102(1 Suppl):96-100.
- 134) Garzon MC, Lucky AW, Hawrot A, Frieden IJ. Ultrapotent topical corticosteroid treatment of hemangiomas of infancy. *J Am Acad Dermatol*. 2005 Feb;52(2):281-6.
- 135) Shinkai K, Kilcline C, Connelly K, Frieden, IJ. The pyrin family of fever genes: unmasking genetic determinants of autoinflammatory disease. *Arch Dermatol*. 2005 Feb;141(2):242-7.
- 136) Kilcline C, Shinkai K, Bree A, Modica R, Scheven EV, Frieden IJ. Neonatal Onset Multisystem Inflammatory Disorder (NOMID): The emerging role of pyrin genes in autoinflammatory diseases. *Arch Dermatol*. 2005 Feb;141(2):248-53.
- 137) Miller T, Frieden IJ. Hemangiomas: new insights and classification. *Pediatric Annals*. 2005 Mar; 34(3):179-87.
- 138) Gruman A, Liang MG, Mulliken JB, Fishman SJ, Burrows PE, Kozakewich HPW, Blei F, Frieden IJ. Kaposiform hemangioendothelioma without Kasabach-Merritt phenomenon. *J Am Acad Dermatol*. 2005 Apr; 52(4):616-22
- 139) Frieden IJ, Drolet BA. Twin Nevus? *Pediatr Dermatol*. 2005 May-Jun;22(3):276.
- 140) Gilliam AE, Bruckner AL, Howard RM, Lee BP, Wu S, Frieden IJ. Bullous "cellulites" with eosinophilia: case report and review of Wells' syndrome in childhood. *Pediatrics*. 2005 Jul; 116(1): e149-55.
- 141) Chamlin SL, Mattson CL, Frieden IJ, Williams ML, Mancini AJ, Cella D, Chren M. The price of pruritus: sleep disturbance and co-sleeping in atopic dermatitis. *Arch Pediatric Adol Med*. 2005; 159:745-750.
- 142) Frieden, IJ, Haggstrom AN, Drolet BA, Mancini AJ, Friedlander SF, Boon L, Chamlin SL, Baselga E, Garzon MC, Nopper AJ, Siegel DH, Mathes EW, Goddard DS, Bischoff J, North PE, Esterly NB. Infantile hemangiomas: current knowledge, future directions. Proceedings of a research workshop

on infantile hemangiomas, April 7-9, 2005, Bethesda, Maryland, USA. *Pediatric Dermatol.* 2005 Sep-Oct; 22(5):383-406.

- 143) Ranchod TR, Frieden IJ, Frederick DR. Corticosteroid treatment of periorbital hemangioma of infancy: A review of evidence. *Br J Ophthalmol.* 2005 Sep;89(9):1134-8.
- 144) Frieden IJ, Dolev J. Medical Analogies: Their role in teaching dermatology to both patients and professionals. *J Am Acad Dermatol.* 2005 Nov;53(5):863-6.
- 145) Chamlin SL, Cella D, Frieden IJ, Williams ML, Mancini AJ, Lai JS, Chren MM. Development of the childhood atopic dermatitis impact scale: initial validation of a quality-of-life measure for young children with atopic dermatitis and their families. *J Invest Dermatol.* 2005 Dec; 125(6):1106-11.
- 146) Witman PM, Wagner AM, Scherer K, Waner M, Frieden IJ. Complications following pulsed dye laser treatment of superficial hemangiomas. *Lasers Surg Med.* 2006 Feb;38(2):116-23.
- 147) Cabrera AR, Gilliam AE, Frieden IJ. Papules overlying the finger joints. *Arch Dermatol.* 2006 Feb;142(2):235-40
- 148) Haggstrom AN, Lammer EJ, Schneider RA, Marcucio Ralph, Frieden IJ. Patterns of infantile hemangiomas: new clues to hemangioma pathogenesis and embryonic facial development. *Pediatrics.* 2006 Mar;117(3):698-703.
- 149) Tsang MW, Garzon MC, Frieden IJ. How to measure a growing hemangioma and assess response to therapy. *Pediatr Dermatol.* 2006 Mar-Apr;23(2):187-90.
- 150) Berger T, Duvic M, Van Voorhes A, Frieden I. The use of topical calcineurin inhibitors in dermatology: safety concerns. Report of the American Academy of Dermatology Association Task Force. *J Am Acad Dermatol.* 2006 May; 54(5):818-23.
- 151) Metry DW, Haggstrom AN, Drolet BA, Baselga E, Chamlin S, Garzon M, Horii K, Lucky A, Mancini AJ, Newell B, Nopper A, Heyer G, Frieden IJ. A prospective study of PHACE syndrome in infantile hemangiomas: demographic features, clinical findings, and complications. *Am J Med Genet A.* 2006 May 1;140(9):975-86.
- 152) Mathes E, Koerper M, Frieden I. A 6-month-old boy with an enlarging bruise on his back. *Pediatric Annals.* 2006 June; 35(6): 441-443.
- 153) Browning J, Frieden IJ, Baselga E, Wagner A, Metry D. Congenital, self – regressing tufted angioma. *Arch Dermatol.* 2006 Jun;142(6):749-51.
- 154) Frieden, I. Is it a hemangioma or could it be cancer? *Pediatric Dermatology.* 2006 Jul-Aug;23(4):402-3.

- 155) Haggstrom A, Drolet B, Baselga E, Chamlin S, Esterly N, Garzon M, Horii K, Lucky A, Mancini A, Metry D, Newell B, Nopper A, Frieden I. Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. *Pediatrics*. 2006 Sep;118(3):882-7.
- 156) Bruckner A, Frieden IJ. Infantile hemangiomas – periodic synopsis. *J Am Acad Dermatol*. 2006 Oct; 55(4):671-82.
- 157) Siegel, DH, Holland, K, Phillips, RJ, Drolet, BA, Esterly, N and Frieden, IJ. Erosive Pustular Dermatitis of the Scalp after Perinatal Scalp Injury. *Pediatr Dermatol*. 2007 Nov-Dec;23(6):533-6.
- 158) Huang JT, Koerper MA, Frieden IJ, Chamlin SL. Treatment of vaginal bleeding from a low-flow pelvic vascular malformation with Desmopressin. *Journal of Ped Hematol Oncol*. 2006 Dec;28(12):845-6.
- 159) Marqueling A, Frieden, IJ, et al. Keratosis Pilaris Rubra: a common but under-recognized condition. *Arch Dermat*. 2006 Dec;142(12):1611-6.
- 160) Haggstrom A, Drolet B, Baselga E, Chamlin S, Esterly N, Garzon M, Horii K, Lucky A, Mancini A, Metry D, Newell B, Nopper A, Frieden I. Prospective study of infantile hemangiomas: demographic, prenatal, and perinatal characteristics. *J Pediatr* 2007 Mar;150(3):291-4.
- 161) Garzon MC, Huang JT, Enjolras O, Frieden IJ. Vascular malformations: Part I. *J Am Acad Dermatol*. 2007 Mar;56(3):353-70; quiz 371-4. Review.
- 162) Garzon MC, Huang JT, Enjolras O, Frieden IJ. Vascular malformations. Part II: associated syndromes. *J Am Acad Dermatol*. 2007 Apr;56(4):541-64.
- 163) Nyquist GG, Mumm C, Grau R, Crowson AN, Shurman DL, Benedetto P, Allen P, Lovelace K, Smith DW, Frieden I, Hybarger CP, Richard G. Malignant proliferating pilar tumors arising in KID syndrome: a report of two patients. *Am J Med Genet A*. 2007 Apr 1;143(7):734-41.
- 164) Poindexter G, Metry DW, Barkovich AJ, Frieden IJ. PHACE syndrome with intracerebral hemangiomas, heterotopia, and endocrine dysfunction. *Pediatr Neurol*. 2007 Jun;36(6):402-6.
- 165) Chen T, Frieden IJ. Development of extensive flat warts after pulsed dye laser treatment of a port-wine stain. *Dermatol Surg*. 2007 Jun;33(6):734-5.
- 166) Chamlin SL, Lai JS, Cella D, Frieden IJ, Williams ML, Mancini AJ, Chren MM. Childhood Atopic Dermatitis Impact Scale: reliability, discriminative and concurrent validity, and responsiveness. *Arch Dermatol*. 2007 Jun;143(6):768-72.
- 167) Minzer-Conzetti K, Garzon MC, Haggstrom AN, Horii KA, Mancini AJ, Morel KD, Newell B, Nopper AJ, Frieden IJ. Information about infantile hemangiomas on the Internet: How accurate is it? *J Am Acad Dermatol*. 2007 Dec;57(6):998-1006. Epub 2007 Aug 8.

- 168) Chamlin SL, Haggstrom AN, Drolet BA, Baselga E, Frieden IJ, Garzon MC, Horii KA, Lucky AW, Metry DW, Newell B, Nopper AJ, Mancini AJ. Multicenter Prospective Study of Ulcerated Hemangiomas. *J Pediatr*. 2007 Dec;151(6):684-689.e1. Epub 2007 Aug 24.
- 169) Metry DW, Siegel DH, Cordisco MR, Pope E, Prendiville J, Drolet BA, Horii KA, Stein SL, Frieden IJ. A comparison of disease severity among affected male versus female patients with PHACE syndrome. *J Am Acad Dermatol*. 2008 Jan;58(1):81-7. Epub 2007 Oct 29.
- 170) Dekoltz C, Frieden I, Picture of the month quiz case. Vulvar ulcerations resulting from acute Epstein-Barr virus infection. *Arch Pediatr Adolesc Med*. 2008 Jan;162(1):86-7.
- 171) Wu P, Mancini A, Marghoob A, Frieden I. Simultaneous occurrence of infantile hemangioma and congenital melanocytic nevus: Coincidence or real association? *J A Acad Dermatol*. 2008 Feb, 58(2 Suppl):S16-22.
- 172) Haggstrom AN, Frieden IJ. Segmental Hemangioma: An important clinical term. *Am J Genet A*. 2008 Mar 1;146(5):670-1.
- 173) Ladrigan MK, LeBoit, PE, Frieden IJ. Neonatal Eosinophilic Pustulosis: in a 2-month Old. *Pediatr Dermatol*. 2008 January/February 25(1):52-55.
- 174) Kilcline C., Frieden, IJ. Infantile hemangiomas: how common are they? A systematic review of the medical literature. *Pediatr Dermatol*. 2008 March/April;25(2):168-173.
- 175) Revencu N, Boon LM, Mulliken JB, Enjolras O, Cordisco MR, Burrows PE, Clapuyt P, Hammer F, Dubois J, Baselga E, Brancati F, Carder R, Quintal JM, Dallapiccola B, Fischer G, Frieden IJ, Garzon M, Harper J, Johnson-Patel J, Labrèze C, Martorell L, Paltiel HJ, Pohl A, Prendiville J, Quere I, Siegel DH, Valente EM, Van Hagen A, Van Hest L, Vaux KK, Vicente A, Weibel L, Chitayat D, Vikkula M. Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat*. 2008 Jul;29(7):959-65.
- 176) Chang, L, Haggstrom A, Drolet, B, Baselga, E, Chamlin, S, Garzon, M, Horii, K, Lucky, A, Mancini, A, Metry, D, Nopper, A, Frieden, I. The Growth Characteristics of Infantile Hemangiomas: Implications for Management. *Pediatrics*. 2008 Aug;122(2):360-7.
- 177) Garzon MC, Drolet BA, Baselga E, Chamlin SL, Haggstrom AN, Horii K, Lucky AW, Mancini AJ, Metry DW, Newell B, Nopper AJ, Frieden IJ; Hemangioma Investigator Group. Comparison of infantile hemangiomas in preterm and term infants: a prospective study. *Arch Dermatol*. 2008 Sep;144(9):1231-2.
- 178) Liu T, McCalmont TH, Frieden IJ, Williams ML, Connolly MK, Gilliam AE. The stiff skin syndrome: case series, differential diagnosis of the stiff skin phenotype, and review of the literature. *Arch Dermatol*. 2008 Oct;144(10):1351-9.

- 179) Drolet BA, Swanson EA, Frieden IJ; Hemangioma Investigator Group. Infantile hemangiomas: an emerging health issue linked to an increased rate of low birth weight infants. *J Pediatr*. 2008 Nov;153(5):712-5, 715.e1.
- 180) Camarillo D, McCalmont TH, Frieden IJ, Gilliam AE. Two pediatric cases of nonbullous histiocytoid neutrophilic dermatitis presenting as a cutaneous manifestation of lupus erythematosus. *Arch Dermatol*. 2008 Nov;144(11):1495-8
- 181) Yan AC, Frieden IJ, Eichenfield LF. Pediatric Dermatology. Announcing a new section. *Pediatr Dermatol*. 2008 Nov-Dec;25(6):585-6.
- 182) Frieden IJ. Addendum: Commentary on becaplermin gel (Regranex) for hemangiomas. *Pediatr Dermatol*. 2008 Nov-Dec;25(6):590.
- 183) Brandling-Bennett HA, Metry DW, Baselga, E, Cordisco MR, Lucky AW, Frieden, IJ. Infantile hemangiomas with unusually prolonged growth phase. A case series and review of the literature. *Arch Dermatol*. 2008 Dec;144(12):1632-7.
- 184) Cordoro KM, Speetzen LS, Koerper MA, Frieden IJ. Physiologic changes in vascular birthmarks during early infancy: Mechanisms and clinical implications. *J Am Acad Dermatol*. 2009 Apr;60(4):669-75. Epub 2009 Jan 23.
- 185) Good WV, Hou C, Frieden IJ, Norcia AM. Evidence for Visual Compromise in Preverbal Children with Orbital Vascular Birthmarks. *Am J Ophthalmol*. 2009 Apr;147(4):679-682.e1. Epub 2009 Feb. 4.
- 186) Mirmirani P, Willey A, Chamlin S, Frieden IJ, Price VH. Tinea capitis mimicking cicatricial alopecia: What host and dermatophyte factors lead to this unusual clinical presentation? *J Am Acad Dermatol*. 2009 Mar;60(3):490-5.
- 187) Wright DR, Frieden J, Orlow S, Shin H, Chalmin S, Schaffer J, Paller, A. The Misnomer "Macrocephaly-Cutis Marmorata Telangiectatica Congenita Syndrome (M-CMTC)": Report of 12 New Cases and Support for Revising the Name to Macrocephaly-Capillary Malformations (M-CM). *Archives of Dermatol*. 2009 Mar;145(3):287-93.
- 188) Frieden IJ, Rogers M, Garzon MC. Conditions Masquerading as Infantile Haemangioma. Part I. *Australasian J Dermatol*. 2009 May;50(2):77-97; quiz 98.
- 189) Frieden IJ, Rogers M, Garzon MC. Conditions Masquerading as Infantile Haemangioma. Part II. *Australasian J Dermatol*. 2009 Aug;50(2):153-68; quiz 169-70.
- 190) Goddard DS, Rogers M, Frieden IJ, Krol AL, White CR Jr, Jayaraman AG, Robinson-Bostom L, Bruckner AL, Ruben BS. Widespread porokeratotic adnexal ostial nevus: Clinical features and proposal of a new name unifying porokeratotic eccrine ostial and dermal duct nevus and

porokeratotic eccrine and hair follicle nevus. *J Am Acad Dermatol*. 2009 Dec;61(6):1060e1-14. Epub 2009 Aug 6.

- 191) Metry DW, Garzon MC, Drolet BA, Frommelt P, Haggstrom A, Hall J, Hess CP, Heyer GL, Siegel D, Baselga E, Katowitz W, Levy ML, Mancini A, Maronn ML, Phung T, Pope E, Sun G, Frieden IJ. PHACE syndrome: current knowledge, future directions. *Pediatr Dermatol*. 2009 Jul-Aug;26(4):381-98.
- 192) Frieden IJ, Drolet BA, Propranolol for Infantile Hemangiomas: Promise, Peril, Pathogenesis. *Pediatr Dermatol*. 2009 Sep-Oct;26(5):642-4.
- 193) Metry D, Heyer G, Hess C, Garzon M, Haggstrom A, Frommelt P, Adams D, Siegel D, Hall K, Powell J, Frieden I, Drolet B. Consensus Statement on Diagnostic Criteria for PHACE Syndrome. *Pediatrics*. 2009 Nov;124(5):1447-56.
- 194) Maguiness S, Koerper M, Frieden I. Relevance of D-dimer testing in patients with venous malformations. *Arch Dermatol*. 2009 Nov; 145(11):1321-4.
- 195) Feramisco JD, Zedek DC, Hogeling M, Frieden IJ. Confluent scaly erythematous plaques on the trunk of a 16-year-old boy—quiz case. *Arch Dermatol*. 2009 Nov;145(11):1325-30.
- 196) Chen, TS, Goldyne, ME, Mathes, EFD, Frieden, IJ, Gilliam AE. Pediatric Tele dermatology: Observations Based on 429 Consults. *J Am Acad Dermatol*. 2009 Nov 17. [Epub ahead of print].
- 197) Maguiness, SM, Frieden, IJ. Infantile Hemangiomas: A Look Back and Future Directions. *Actas Dermosifiliogr*. 2009 Nov;100:Supl.1:73-6.
- 198) Chen TS, Goldyne ME, Mathes EF, Frieden IJ, Gilliam AE. Pediatric tele dermatology: observations based on 429 consults. *J Am Acad Dermatol*. 2010 Jan;62(1):61-6. Epub 2009 Nov 18.
- 199) Horri KA, Drolet BA, Basela E, Frieden IJ, Metry DW, Morel KD, Newell BD, Nopper AJ, Garzon MC; for the Hemangioma Investigator Group. Risk of Hepatic Hemangiomas in Infants with Large Hemangiomas. *Arch Dermatol*. 2010 Feb;146(2):201-3.
- 200) Hogeling M, Frieden IJ. Segmental Pigmentation Disorder. *Br J Dermatol*. 2010 June;162(6):1337-1341. Epub 2010 Feb 15 ahead of print.
- 201) Mathes EF, Frieden IJ. Treatment of molluscum contagiosum with cantharidin: a practical approach. *Pediatr Ann*. 2010 Mar;39(3):124-8.
- 202) Frieden IJ. Commentary: Early laser treatment of periorbital infantile hemangiomas may work, but is it really the best treatment option? *Dermatol Surg*. 2010 May;36(5):598-601. Epub 2010 Apr 2. Review.
- 203) Cordoro KM, Frieden IJ. Pulsed dye laser for port wine stains. *J Am Acad Dermatol*. 2010 Jun;62(6):1065-6.

- 204) Suh KY, Rosbe KW, Meyer AK, Frieden IJ. Extensive Airway Hemangiomas in Two Patients Without Beard Hemangiomas. *Pediatr Dermatol*. 2010 Jun 16. [Epub ahead of print]
- 205) Maguiness SM, Frieden IJ. Current management of infantile hemangiomas. *Semin Cutan Med Surg*. 2010 Jun;29(2):106-14.
- 206) Iacobas I, Burrows PE, Frieden IJ, Liang MG, Mulliken JB, Mancini AJ, Kramer D, Paller AS, Silverman R, Wagner AM, Metry DW. LUMBAR: Association between Cutaneous Infantile Hemangiomas of the Lower Body and Regional Congenital Anomalies. *J Pediatr*. 2010 Jun 30. [Epub ahead of print]
- 207) Haggstrom AN, Garzon MC, Baselga E, Chamlin SL, Frieden IJ, Holland K, Maguiness S, Mancini AJ, McCuaig C, Metry DW, Morel K, Powell J, Perkins SM, Siegel D, Drolet BA. Risk for PHACE Syndrome in Infants With Large Facial Hemangiomas. *Pediatrics*. 2010 Aug;126(2):e418-26. [Epub ahead of print 2010 Jul 19.]
- 208) Holland KE, Frieden IJ, Frommelt PC, Mancini AJ, Wyatt D, Drolet BA. Hypoglycemia in children taking propranolol for the treatment of infantile hemangioma. *Arch Dermatol*. 2010 Jul;146(7):775-8.
- 209) Rosbe KW, Suh KY, Meyer AK, Maguiness SM, Frieden IJ. Propranolol in the management of airway infantile hemangiomas. *Arch Otolaryngol Head Neck Surg*. 2010 Jul;136(7):658-65.
- 210) Juern AM, Glick ZR, Drolet BA, Frieden IJ. Nevus simplex: A reconsideration of nomenclature, sites of involvement, and disease associations. *J Am Acad Dermatol*. 2010 Aug 20 [Epub ahead of print].
- 211) Suh KY, Frieden IJ. Infantile hemangiomas with minimal or arrested growth: a retrospective case series. *Arch Dermatol*. 2010 Sep;146(9):971-6.
- 212) Drolet BA, Chamlin SL, Garzon MC, Adams D, Baselga E, Haggstrom AN, Holland KE, Horii KA, Juern A, Lucky AW, Mancini AJ, McCuaig C, Metry DW, Morel KD, Newell BD, Nopper AJ, Powell J, Frieden IJ. Prospective Study of Spinal Anomalies in Children with Infantile Hemangiomas of the Lumbosacral Skin. *J Pediatr*. 2010 Sep 7. [Epub ahead of print].
- 213) Drolet BA, Frieden IJ. Characteristics of infantile hemangiomas as clues to pathogenesis: does hypoxia connect the dots? *Arch Dermatol*. 2010 Nov;146(11):1295-9. PubMed PMID: 21079070.
- 214) Juern AM, Glick ZR, Drolet BA, Frieden IJ. Nevus simplex: a reconsideration of nomenclature, sites of involvement, and disease associations. *J Am Acad Dermatol*. 2010 Nov;63(5):805-14. Epub 2010 Aug 21.
- 215) Maguiness SM, Hoffman WY, McCalmont TH, Frieden IJ. Early white discoloration of infantile hemangioma: a sign of impending ulceration. *Arch Dermatol*. 2010 Nov;146(11):1235-9. PubMed PMID: 21079059.

- 216) Siegel DH, McKenzie J, Frieden IJ, Rauen KA. Dermatologic Findings in 61 Mutation-Positive Individuals with Cardio-facio-cutaneous Syndrome. *Br J Dermatol*. 2010 Nov 9. doi: 10.1111/j.1365-2133.2010.10122.x. [Epub ahead of print]
- 217) Rosbe KW, Hess CP, Dowd CF, Frieden IJ. Masseteric venous malformations: Diagnosis, treatment, and outcomes. *Otolaryngol Head Neck Surg*. 2010 Dec;143(6):778-83. Epub 2010 Oct 25.
- 218) Goujon E, Cordoro KM, Barat M, Rousseau T, Brouillard P, Vikkula M, Frieden IJ, Vabres P. Congenital Plaque-Type Glomuvenous Malformations Associated with Fetal Pleural Effusion and Ascites. *Pediatr Dermatol*. 2010 Dec 7. doi: 10.1111/j.1525-1470.2010.01216.x. [Epub ahead of print]
- 219) Hogeling M, Nakano T, Dvorak CC, Maguiness S, Frieden IJ. Severe Neonatal Congenital Erythropoietic Porphyria. *Pediatr Dermatol*. 2011 Mar 1. doi: 10.1111/j.1525-1470.2010.01376.x. [Epub ahead of print] PubMed PMID: 21362030.
- 220) Zvulunov A, McCuaig C, Frieden IJ, Mancini AJ, Puttgen KB, Dohil M, Fischer G, Powell J, Cohen B, Amitai DB. Oral Propranolol Therapy for Infantile Hemangiomas Beyond the Proliferation Phase: A Multicenter Retrospective Study. *Pediatr Dermatol*. 2011 Mar;28(2):94-98. doi: 10.1111/j.1525-1470.2010.01379.x. Epub 2011 Mar 1. PubMed PMID: 21362031.
- 221) Nabatian AS, Milgraum SS, Hess CP, Mancini AJ, Krol A, Frieden IJ. PHACE without Face? Infantile Hemangiomas of the Upper Body Region with Minimal or Absent Facial Hemangiomas and Associated Structural Malformations. *Pediatr Dermatol*. 2011 Mar 31. doi: 10.1111/j.1525-1470.2011.01407.x. [Epub ahead of print] PubMed PMID: 21453307.
- 222) Sugarman JL, Hersh AL, Okamura T, Howard R, Frieden IJ. A Retrospective Review of Streptococcal Infections in Pediatric Atopic Dermatitis. *Pediatr Dermatol*. 2011 Apr 26. doi: 10.1111/j.1525-1470.2010.01377.x. [Epub ahead of print] PubMed PMID: 21517950
- 223) Horii KA, Drolet BA, Frieden IJ, Baselga E, Chamlin SL, Haggstrom AN, Holland KE, Mancini AJ, McCuaig CC, Metry DW, Morel KD, Newell BD, Nopper AJ, Powell J, Garzon MC; for the Hemangioma Investigator Group. Prospective Study of the Frequency of Hepatic Hemangiomas in Infants with Multiple Cutaneous Infantile Hemangiomas. *Pediatr Dermatol*. 2011 Apr 26. doi: 10.1111/j.1525-1470.2011.01420.x. [Epub ahead of print] PubMed PMID: 21517952.
- 224) Yeh I, Bruckner AL, Sanchez R, Jeng MR, Newell BD, Frieden IJ. Diffuse Infantile Hepatic Hemangiomas: A Report of Four Cases Successfully Managed with Medical Therapy. *Pediatr Dermatol*. 2011 Apr 26. doi: 10.1111/j.1525-1470.2011.01421.x. [Epub ahead of print] PubMed PMID: 21517953.

BOOKS:

- 1) Eichenfield LF, Frieden IJ, Esterly NB Eds, Textbook of Neonatal Dermatology, W.B. Saunders. 2001.
- 2) Eichenfield LF, Frieden IJ, Esterly NB Eds, Textbook of Neonatal Dermatology, 2nd edition. Elsevier. 2008.

CHAPTERS:

- 1) Frieden IJ and Price VH. Androgenetic Alopecia. In Pathogenesis of Skin Disease (Thiers BH and Dobson RL, eds.) New York, Churchill Livingstone, 41-55, 1986.
- 2) Frieden IJ. Pediatric skin diseases without systemic illness. In Emergency Therapy (Callaham M ed.) Toronto, B.C. Decker, 987-990, 1987.
- 3) Frieden IJ, Penneys N. Viral Infections. In Pediatric Dermatology (Schachner L and Hansen R, eds.) New York, Churchill Livingstone, 2nd Edition 27:1371-1413, 1988.
- 4) Frieden IJ. Viral exanthems. In Principles and Practice of Dermatology (Sams WM, Lynch PJ eds.) New York, Churchill Livingstone, 113-118, 1990.
- 5) Williams ML, Frieden IJ. Dermatologic Disorders. In Pediatric Emergencies (Grossman M, Dieckmann R eds.) Philadelphia, JB Lipincott, 474-483, 1990.
- 6) Frieden IJ. Genetic hair disorders. In Genodermatoses (Alper J ed.) Chicago, Year Book Publishers, 209-220, 1991.
- 7) Williams ML, Frieden IJ, Prose NS. The skin. In Pediatrics (Rudolph A, ed.) Connecticut, Apleton-Century-Crofts, 19th Edition, 875-922, 1991.
- 8) Frieden IJ. Common pediatric rashes without systemic illness. In Current Practice of Emergency Medicine (Callaham ML, ed.) Philadelphia, B.C. Decker, 2nd Edition, 1295-1297, 1991.
- 9) Frieden IJ. Sturge-Weber syndrome. In Clinical Dermatology (Dermis DJ, Dobson RL, et. al. eds.) Philadelphia, Harper & Row, Unit 7-64,1-6, 1991.
- 10) Frieden IJ. Maffucci's syndrome. In Clinical Dermatology (Dermis DJ, Dobson RL, et. al. eds.) Philadelphia, Harper & Row, Unit 7-65,1-3, 1992.
- 11) Frieden IJ. Cobb syndrome. In Clinical Dermatology (Dermis DJ, Dobson RL, et. al. eds.) Philadelphia, Harper & Row, Unit 7-66A,1-3, 1992.
- 12) Paller AS, Abel E, Frieden IJ. Dermatologic problems. In Comprehensive Adolescent Health Care (Friedman, Fisher, Schonberg, eds.) St. Louis, MO, Quality Medical Publishing Inc, Section 11, Ch 69,584-602, 1992.

- 13) Kadonaga JN, Frieden IJ. Congenital and genetic diseases of hyperpigmentation. In Pigmentation and Pigmentary Disorders (Levine N, Lynch P eds.) Boca Raton, FL, CRC Press, Section 2, Ch7, 149-207, 1993.
- 14) Frieden IJ. Update on Childhood Exanthems. In Current Opinion in Dermatology (Dahl MV, Lynch P eds.) Philadelphia, PA, Current Science, 285-289, 1993.
- 15) Frieden IJ. Aplasia Cutis Congenita. In Clinical Dermatology (Demis DJ, ed.) Philadelphia, JB Lipincott Company. Vol. 1,4-34, 1993.
- 16) Frieden IJ, Nigro J. Port-Wine Stains and Other Vascular Malformations. In Clinical Dermatology (Demis DJ, ed.) Philadelphia, JB Lipincott Company. Vol. 2, Unit 7-63A, 1-7, 1994.
- 17) Frieden IJ, Nigro J. Hemangiomas. In Clinical Dermatology (Demis DJ, ed.) Philadelphia, JB Lipincott Company. Vol. 2, Unit 7-63, 1-8, 1995.
- 18) Frieden IJ, Calkin JM. New aspects of neurocutaneous syndromes associated with large facial hemangiomas and giant melanocytic nevi. In Pediatric Dermatology. The World's Reality in the Children's Skin (Pierini A-M, Pierini RG-D, Bustamante RE, eds.) Elsevier Science B.V.,173-176, 1995.
- 19) Frieden IJ. Viral Exanthems. In Principles and Practice of Dermatology, 2nd Edition. (Sams WM, Lynch PJ, Eds.) Churchill Livingstone, New York, 117-26, 1996,.
- 20) Frieden IJ, Pennys NS. Viral Infections. In Pediatric Dermatology, 2nd Edition. (Schachner LA, Hansen RC Eds.) Churchill Livingstone, New York, 1257-1294, 1996.
- 21) Frieden IJ. Blistering and Pustular Disorders of the Neonate. In Cutaneous Medicine and Surgery: An Integrated Program in Dermatology. (Arndt KA, LeBoit PE, Robinson JK, Wintroub BU Eds.) WB Saunders, Philadelphia, 712-22, 1996.
- 22) Howard R, Frieden IJ. Viral Exanthems. In Cutaneous Medicine and Surgery: An Integrated Program in Dermatology. (Arndt KA, LeBoit PE, Robinson JK, Wintroub BU Eds.) WB Saunders, Philadelphia, 1123-1136, 1996.
- 23) Nigro J, Swerlick RA, Sep NT. Geronemus RG, LeBoit PE, Frieden IJ. Angiogenesis, Vascular Malformations and Proliferations. In Cutaneous Medicine and Surgery: An Integrated Program in Dermatology. (Arndt KA, LeBoit PE, Robinson JK, Wintroub BU Eds.) WB Saunders, Philadelphia, 1492-1521,1996.
- 24) Frieden IJ. Focal Dermal Hypoplasia and Aplasia Cutis Congenita. In Cutaneous Medicine and Surgery: An Integrated Program in Dermatology. (Arndt KA, LeBoit PE, Robinson JK, Wintroub BU Eds.) WB Saunders, Philadelphia, 1780-1786, 1996.

- 25) Williams ML, Frieden IJ, Prose NS. The skin. In Rudolph's Pediatrics, 20th Edition. (Rudolph AM, Hoffman J, Rudolph CD eds.) Apleton-Century-Crofts, Connecticut, Ch 12, 879-938, 1996.
- 26) Howard R, Frieden IJ. Viral exanthems. In Principles and Practice of Dermatology, 2nd Edition, (Sams WM, Lynch PJ eds.) Churchill Livingstone, New York Ch 10, 117-125, 1996.
- 27) Frieden IJ. Tinea Capitis. In Cutaneous Infection and Therapy. (Aly R, Beutner KR, Maibach H Eds.) Marcel Dekker, Inc., New York, 169-181, 1997.
- 28) Paller AS, Abel EA, Frieden IJ. Dermatologic Problems. In Comprehensive Adolescent Health Care, 2nd Edition, (Friedman SB, Fisher M, Schonberg SK, Alderman EM eds.) CV Mosby, St. Louis, 608-627, 1997.
- 29) Frieden IJ. Viral Exanthems. In. Atlas of Infections of the Skin, (Aly R, Maibach H Eds.) Churchill Livingstone, Philadelphia, 231-243, 1999.
- 30) Frieden IJ. Perioral Dermatitis. In Textbook of Pediatric Dermatology, Vol 1, (Harper J, Oranje A, Prose N eds.) Blackwell Science, Oxford, 262-264, 2000.
- 31) Chamlin SL, Frieden IJ. Cystic Hygroma. In Essence of Office Pediatrics, (Stockman JA, Lohr JA eds.) W.B. Saunders Company, Philadelphia, Pennsylvania, 100, 2001.
- 32) Chamlin SL, Frieden IJ. Human Papillomavirus Infections, Nongenital. In Essence of Office Pediatrics, (Stockman JA, Lohr JA eds.) W.B. Saunders Company, Philadelphia, Pennsylvania, 203, 2001.
- 33) Frieden IJ, Howard R. Vesiculobullous eruptions. In Textbook of Neonatal Dermatology, (Eichenfield LF, Frieden IJ, Esterly NB eds.) W.B. Saunders, Philadelphia, 137-178, 2001
- 34) Frieden IJ, Enjolras O, Esterly N. Vascular Birthmarks and other Abnormalities of Blood Vessels and Lymphatics". Pediatric Dermatology. 3rd Edition. Churchill Livingstone, New York, 833-862, 2003.
- 35) Frieden IJ, Pauporte MC. Bologna et al. Vesiculobullous and Erosive Diseases in the Newborn. Dermatology, Vol 1, Mosby, St Louis, 509-522, 2003.
- 36) Sugarman J, Frieden IJ. Epidermal Nevus Syndromes. In Neurocutaneous Disorders. (Roach ES, Miller VS Eds.) Cambridge University Press, Cambridge, 88-104, 2004.
- 37) Frieden IJ. Vascular tumors and malformations. In Rudolph's Pediatrics, 21th Edition, (Rudolphs AM Ed.) Apleton-Century-Crofts, Connecticut, 1204-1208, 2003.
- 38) Bruckner A, Frieden IJ, Esterly, N. Hemangiomas of infancy and other vascular tumors. In Harper J, Oranje A, Prose N eds. Textbook of Pediatric Dermatology, 2nd Ed, Wiley & Sons, 2007.

- 39) Gilliam AG, Frieden IJ. Transplacentally Acquired Dermatoses of the Newborn. In Zaoutis L and Chiang V, eds. Comprehensive Pediatric Hospital Medicine. Mosby Inc, Philadelphia. 1032-1036, 2007.
- 40) Gilliam AG, Pauporte MC, Frieden IJ. Bologna et al., Vesiculobullous and Erosive Diseases in the Newborn Bologna. In Jorizzo, JL, Rapini, RR eds. Dermatology, 2nd Edition, Eds. Mosby Inc., Philadelphia. 2008.
- 41) Frieden IJ, Howard R. Vesicles, Pustules, Bullae, Erosions, and Ulcerations. In Eichenfield LF, Frieden IJ, Esterly NB eds, Textbook of Neonatal Dermatology, W.B. Saunders, Philadelphia. 131-158, 2008.
- 42) Frieden IJ, Greco MF. Enfermedades vesicoampollares del recién nacido. In Larralde M, Abad E, Luna P eds, Dermatología pediátrica, 2nd edición, Ediciones Journal, Argentina. 29-37, 2010.
- 43) Frieden IJ. Aplasia cutis congenita. In Pueyo de Casabé S, Valverde R eds. Dermatología Neonatal, Talleres Buschi, Buenos Aires. (In Press 2003).
- 44) Frieden IJ. Epidemiology Pathology and Pathogenesis of Hemangiomas and Hemangiomas and Associated Structural Defects. In Cordisco MR, Vascular Anomalies in Infancy. (In Preparation 2003).

ABSTRACTS:

- 1) Foster R, Williams ML, Hoffman WY, Barkovich AJ, Frieden IJ. "Neurocutaneous Melanosis: Further Observations and Follow-up of Central Nervous System MR in Neurologically Asymptomatic Children".
- 2) Ming Michael E., Frieden IJ, Ho C, Hoffman WY, McCalmont TH. "The Histopathologic Appearance of 'Scarring' Involving Hemangiomas". Presented at the International society of Dermatopathology, New Orleans, Louisiana, March 17-18, 1999.
- 3) Maari, C, Frieden, IJ. "Klippel Trenaunay Syndrome". Presented at the International Society for the Study of Vascular Anomalies, 14th International Workshop on Vascular Anomalies, Nijmegen, Netherlands. June 27-29, 2002.
- 4) Frieden, IJ. "PHACE and Other Syndromes in Hemangioma of Infancy". Presented at the World Congress of Dermatology, Paris, France. July 4, 2002.
- 5) Dowd CF, Cullen SP, Haggstrom AN, Hoffman WY, Frieden IJ. "Radiological Misdiagnosis Confounds Evaluation of Vascular Anomalies". Presented at the International Society for the Study of Vascular Anomalies, 15th International Workshop on Vascular Anomalies, Wellington, New Zealand. February 23-25, 2004.

- 6) Kim HJ, Colombo M, Frieden IJ. "Ulcerated hemangiomas: clinical characteristics and response to therapy. J Am Acad Dermatol. 2001;44:962-72.
- 7) Adams D, Haisley-Royster C, Enjolras O, Frieden IJ. "Treatment of Kasabach-Merritt Syndrome with Vincristine: A Retrospective Study of 15 Patients". Presented at the International Society for the Study of Vascular Anomalies, 13th International Workshop on Vascular Anomalies, Montreal, Canada. May 10-13, 2000.
- 8) Forney R, Frieden IJ. "Multidisciplinary Vascular Anomalies Clinic: Patient and Physician Perspectives". Presented at the International Society for the Study of Vascular Anomalies, 13th International Workshop on Vascular Anomalies, Montreal, Canada. May 10-13, 2000.
- 9) Chamlin S, Koerper M, Frieden IJ. "Nasal Desmopressin to Control Vaginal Bleeding Secondary to a Pelvic Vascular Malformation". Presented at the International Society for the Study of Vascular Anomalies, 13th International Workshop on Vascular Anomalies, Montreal, Canada. May 10-13, 2000.
- 10) Garzon MC, Drolet BA, Haggstrom A, Frieden IJ. "Incidence and Clinical Associations of Multiple Hemangiomas of Infancy". Presented at the International Society for the Study of Vascular Anomalies, 15th International Workshop on Vascular Anomalies, Wellington, New Zealand. February 22-25, 2004.
- 11) Haggstrom A., Drolet BA, Beth A, Frieden IJ. "Hemangioma Subtype Characterization, A Cohort Prospective Study". Presented at the International Society for the Study of Vascular Anomalies, 15th International Workshop on Vascular Anomalies, Wellington, New Zealand. February 22-25, 2004.
- 12) Dowd CF, Cullen SP, Hoffman WY, Frieden IJ. "Radiological Misdiagnosis Confounds Evaluation of Vascular Anomalies". Presented at the International Society for the Study of Vascular Anomalies, 15th International Workshop on Vascular Anomalies, Wellington, New Zealand. February 22-25, 2004.
- 13) Bhardwaj S, Tope WD, Frieden IJ. "Long Pulsed Nd:Yag Laser Treatment of Venous Malformations". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.
- 14) Garzon MD, Drolet BA, Frieden IJ, Hemangioma Investigator Group. "Clinical Characteristics of Infantile Hemangiomas in Premature Infants". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.
- 15) Drolet B, Swanson E, Frieden I. "Perinatal Characteristics of Infants with Hemangiomas". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.

- 16) Chang LC, Frieden IJ, Hemangioma Investigator Group. "Infantile Hemangiomas: Growth Characteristics, Clinical Features, and Implications for Referral". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.
- 17) Makkar HS, Frieden IJ, Hemangioma Investigator Group. "Abortive Hemangiomas: A Unique Morphologic Variant of Infantile Hemangiomas". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.
- 18) Kuri M, Dosanjh A, Gupta A, Lipshutz G, Frieden I, Boudreau N, Hansen S, Hoffman W. "Homeobox Gene Expression in Infantile Hemangiomas: Evidence for Passive Involution". Presented at the International Society for the Study of Vascular Anomalies, 16th International Workshop on Vascular Anomalies, Milan, Italy. June 14-17, 2006.
- 19) Haggstrom A, Frieden I, Garzon M, Drolet B. "Large facial hemangiomas: what is the risk of PHACE(S)?" Presented at the International Investigative Dermatology Conference, Kyoto, Japan. May 14-17, 2008.
- 20) Chen TS, Goldyne ME, Mathes FD, Frieden IJ, Gilliam AE. "Pediatric Teledermatology: Observations Based on 429 Consults". Presented at the American Telemedicine Association 2009 ATA Mid-Year Meeting, Palm Springs, CA. September 24-25, 2009.
- 21) Vogel S, Frieden IJ. "Venous malformations: Age of presentation and tissue type involvement". Presented at American Academy of Dermatology 69th Annual Meeting, New Orleans, Louisiana, February 4-8, 2011.