翻译：周迎春。2013年2月

LOTUS TISSUE REPAIR 莲花 组织修复

Systemic Protein Replacement Therapy for Dystrophic Epidermolysis Bullosa (DEB)

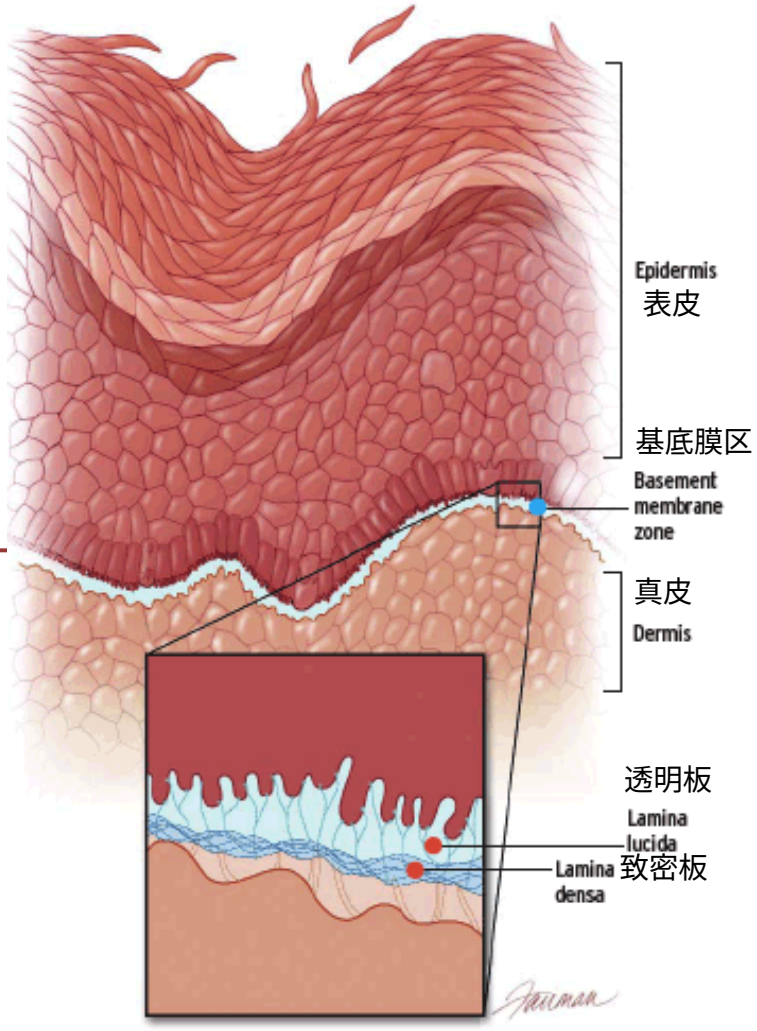
营养不良型大疱性表皮松解症（DEB）的系统性蛋白替代疗法

Mark P. de Souza, PhD

EB-CLINET Conference EB临床大会

Salzburg 萨尔茨堡

October 6, 2012 2012年10月6日



Company Overview 公司简介

LOTUS TISSUE REPAIR

- Founded in 2010 to develop recombinant Collagen Type VII (rC7) as **protein therapy** for Dystrophic Epidermolysis Bullosa (DEB)
  创建于2010年，致力于为营养不良型大疱性表皮松解症（DEB）的蛋白疗法开发重组VII型胶原（rC7）
- **Exclusive license** to USC and Stanford intellectual property on rC7
  南加州大学和斯坦福大学rC7知识产权的独家授权
- Compelling data in mouse and dog models demonstrates **intravenous rC7 reverses the DEB phenotype**
  鼠和狗模型中的有力数据证明静脉注射rC7能逆转DEB的症状
- **Clear path to regulatory approval -** longitudinal severity study in 2012, Phase 1 human clinical trial in 2013 and regulatory approval in DEB in 4-5 years
  在获得审批方面没有障碍 - 2012年纵向严重性研究，2013年一期人体临床试验，4-5年内完成应用于DEB的审批
- **Scientific leaders** in C7 protein therapy and **experienced management team**
  C7蛋白疗法的学界领军人物，经验丰富的管理团队
- Secured **significant financing** in June 2011
  2011年6月落实了大量的资金

USC UNIVERSITY OF SOUTHERN CALIFORNIA 南加州大学

Intradermal rC7 Reverses DEB Phenotype

真皮内rC7逆转了DEB的症状

LOTUS TISSUE REPAIR

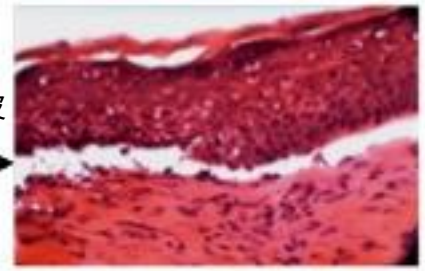
- Drs. Woodley and Chen – pioneers of protein therapy for DEB Woodley和陈博士 - DEB蛋白疗法的先锋
- Recombinant collagen type VII (rC7) purified from a human fibroblast cell line using methods described for a HEK 293 cell line[1] 使用HEK293细胞系的方法，从人纤维原细胞系中提纯了重组VII型胶原（rC7）
- Intradermal rC7 injected into two mouse models of DEB: 在两种小鼠DEB模型中真皮内注射了rC7
  - DEB skin equivalents transplanted onto athymic nude mice[2] 在无胸腺的裸鼠身上移植了DEB皮肤等价物
  - C7 knock out mice[3] C7敲除小鼠

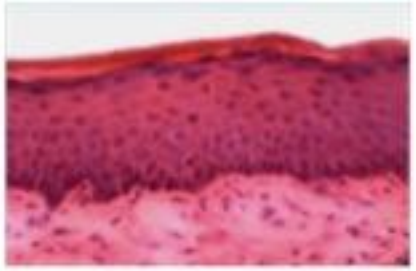
真皮内注射重组C7，在基底膜区出现了锚丝纤维并在两种小鼠模型中逆转了RDEB的症状

- Intradermal injections restored C7 and anchoring fibrils at the basement membrane zone and reversed RDEB disease phenotype in both mouse models

Athymic Nude Mouse Model 无胸腺裸鼠模型

Untreated 未治疗 | rC7 Treated 使用了rC7





Reverses separation of dermal-epidermal junction (DEJ) of RDEB skin equivalents grafted on nude mice

在裸鼠的RDEB皮肤等价物中逆转真皮-表皮交界处的分离

C7 Knock Out Model C7敲除模型

Untreated 未治疗 | 使用了rC7 rC7 Treated

Corrects skin blister formation in C7 knock-out mice

在C7敲除小鼠中纠正了皮肤水疱

1. Chen, M., et al., 2002. J. Biol. Chem. 277:2118–2124
2. Woodley, D.T., et al., 2004. Nat. Med. 10: 693-695
3. Remington, J., et al. 2009. Mol. Ther. 17: 26-33

Development of Intravenous rC7 Protein Therapy

开发静脉注射rC7蛋白疗法

Focus on: 集中于

1) Product Profile/Delivery 产品概况/导入

- What is the best route of delivery for DEB treatment? 什么是治疗DEB最好的用药途径？
- How do we “target” rC7 to skin and tissue with deficient/dysfunctional C7? 怎样把rC7运送到C7缺失或缺陷的皮肤和组织？

2) Manufacturing: 生产

- Can we make rC7 in sufficient quantities? 能否生产出足量的rC7？
- Can we formulate rC7 to be functional and stable? 怎样让rC7稳定的发挥功能？

3) Clinical Development 临床开发

- How to design clinical trials that enable the quickest path to regulatory approval? 怎样设计临床试验，以尽快得到批准？
- How do we identify patients for clinical trials? 怎样筛选临床试验的患者？
- How do we educate regulators and payors about DEB? 怎样给政府部门和投资人介绍DEB？
- What are meaningful clinical endpoints? 什么是有意义的临床试验终点？

Product Profile: Best route of delivery?

产品概况：最好的用药途径

- Lotus explored **multiple ways to deliver** rC7 Louts研究了多种rC7的用药途径
- **Topical** rC7 *reversed disease phenotype* in 2 DEB mouse models without apparent adverse effects. But, it can only be used on open lesions because it does not penetrate intact skin 局部外用：rC7逆转了两只DEB小鼠的疾病症状，没有明显的不良反应。但它只能用在开放的伤口上，因为它无法穿透完整的皮肤。
- **Intradermal** rC7 *reversed disease phenotype* in 2 DEB mouse models and in a dog RDEB model without apparent adverse effects. But, multiple intradermal injections needed for effective treatment 真皮内：rC7逆转了两只DEB小鼠和一只RDEB狗的疾病症状，没有明显不良反应。但需要多处真皮内注射才能有效。
- **Intravenous (**IV) rC7 *reversed disease phenotype* in 3 DEB mouse models and in a dog DEB model, and targets external and internal sites without apparent adverse effects 静脉给药：rC7逆转了3只DEB小鼠和一只DEB狗的疾病症状，同时作用与体表和体内，没有明显的不良反应。
- Potential to **dose** rC7 **once per month** (or less often) as rC7 has a long residence time in skin (~2 months) 可能需要每月使用rC7一次（或频率更低），因为rC7在皮肤中的存活时间较长（~2个月）
- **Only ~30-50% of C7 needs to be restored** for good epidermal-dermal adherence

  只需要恢复~30-50%的C7就能维持良好的表皮-真皮粘附

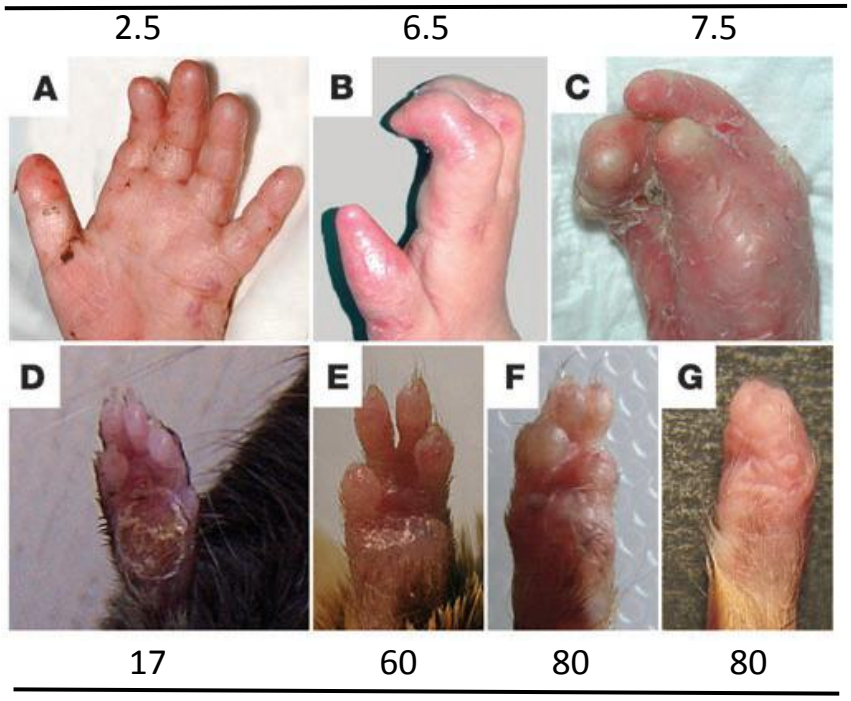
Hypomorphic RDEB Mouse Model - Similar Phenotype to Humans

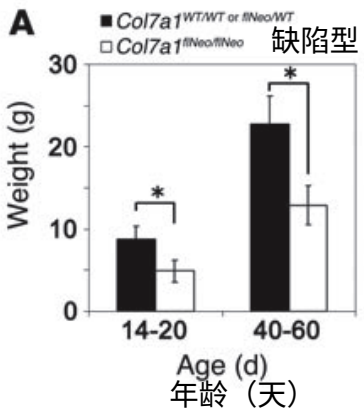
亚等位基因RDEB小鼠模型 - 和人类的症状类似

Fritsch, A., et al., J. Clin. Invest., 2008, 118:1669

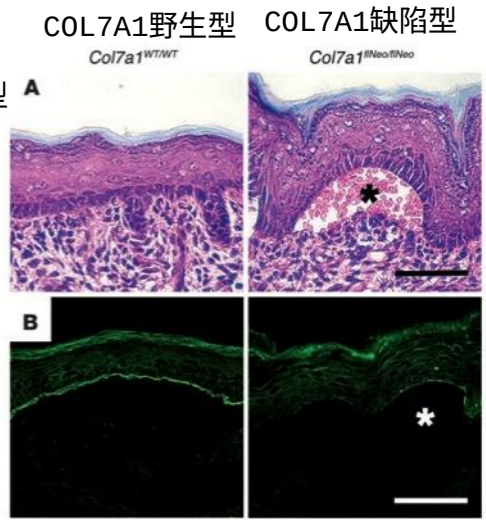
Blistering within 24 hours after birth
出生后24小时内长水疱

Mitten deformity
并指





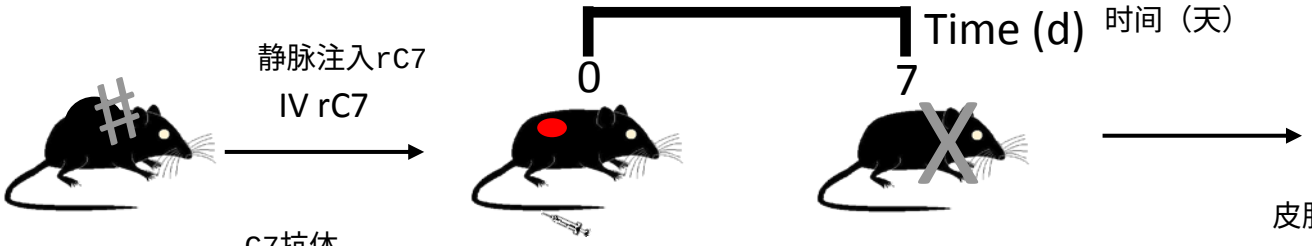
Growth retardation in RDEB mice as a result of malnutrition
RDEB小鼠因营养不良而发育迟缓



(A) Dermal-epidermal separation and (B) absence of C7 at BMZ in 1 day old RDEB mice, compared to wildtype
（A）表皮-真皮分界。
（B）1天大的RDEB小鼠基底膜缺少C7

初期数据：静脉注射rC7迁移到了亚等位基因的小鼠皮肤、舌头、食道上，没有明显的不良反应

Preliminary Data: Intravenous rC7 Homes to the Skin, Tongue, Esophagus of Hypomorphic Mice Without Adverse Effects



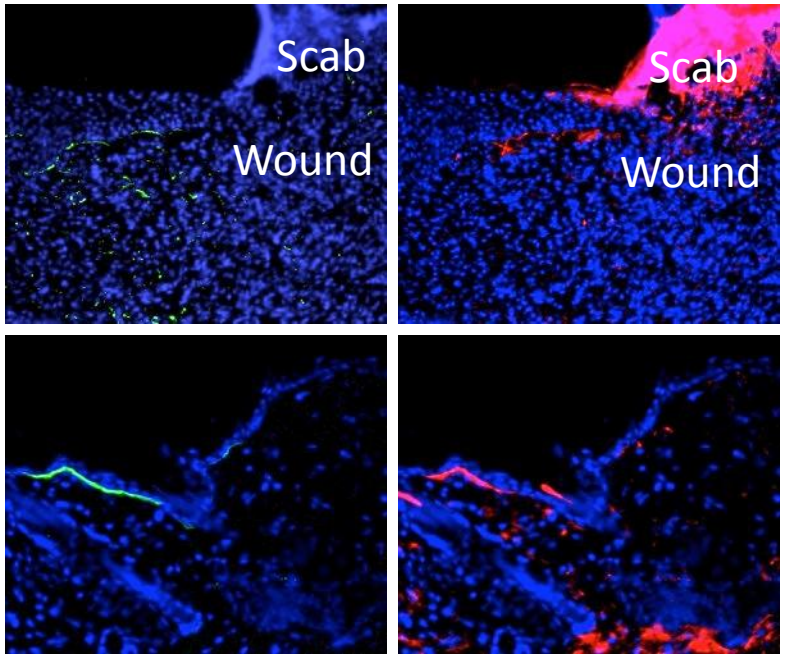
Sectioned and stained skin, wound, and tongue for human C7

皮肤、伤口、舌头切片并对人C7染色

C7抗体

Pol C7 Ab

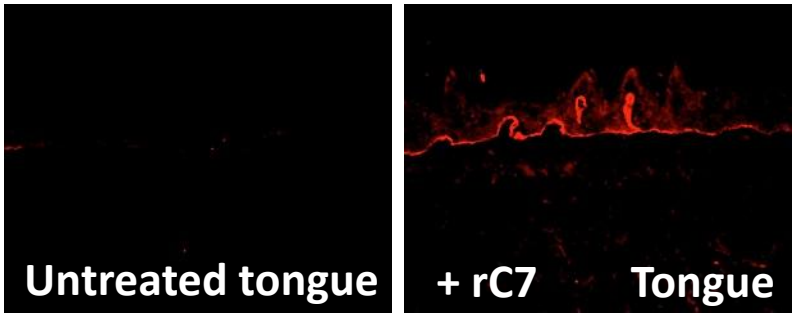
LH7.2

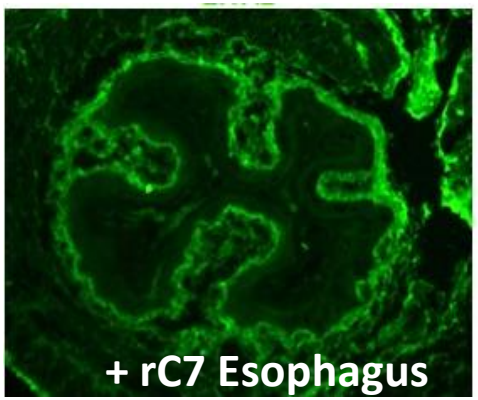


未治疗的舌头

LH7.2

使用了rC7的舌头





使用了rC7的食道

rC7 delivered intravenously via tail vein and retro-orbital injection

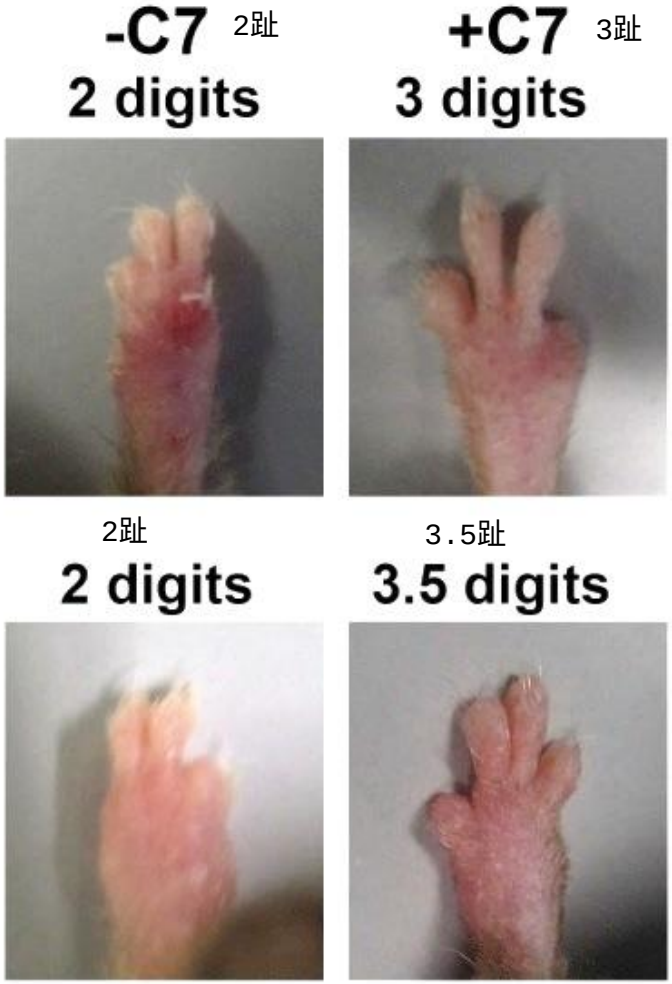
经尾静脉和眼球后注射从静脉导入rC7

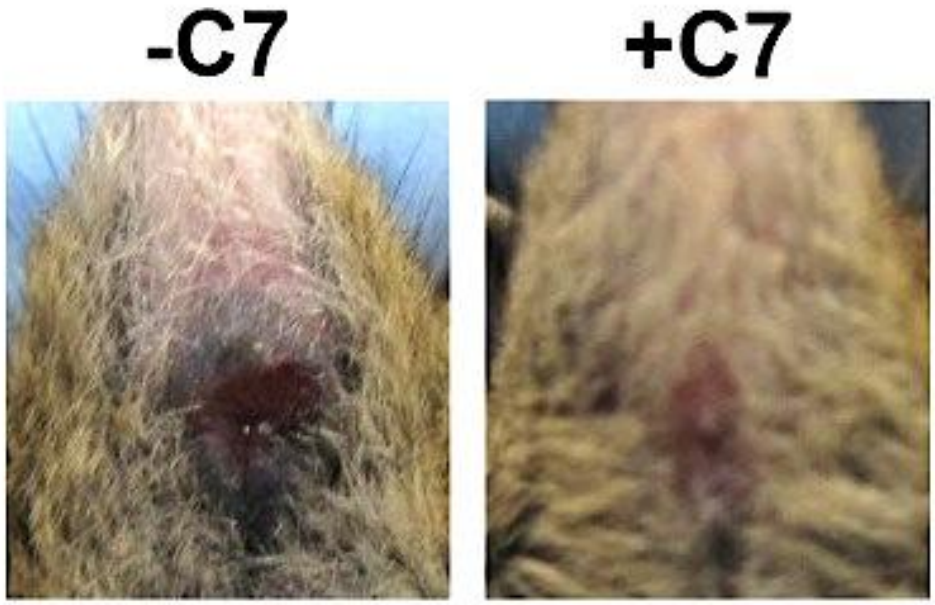
Preliminary Data: Repeated rC7 IV Injections Protect Against Webbing of Forepaws and Anal Blistering in Hypomorphic Mice

初期数据：重复静脉注射rC7使亚等位基因小鼠免于前爪并指和肛门水疱

After 13 weeks of rC7 injections

经过13周rC7注射





Dog Model: Recapitulates the RDEB Phenotype

狗模型：反映了RDEB的症状

Heterozygous for C7 mutation
C7突变杂合（不发病）

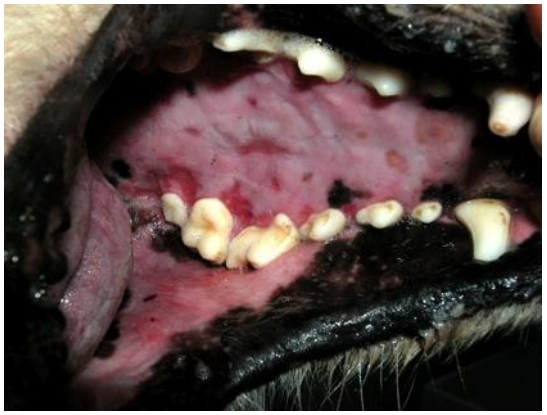
Homozygous for C7 mutation
C7突变纯合

Growth retardation due to poor nutrition in RDEB puppy who had **esophageal lesions** at 4 months. The RDEB puppy is homozygous for the C7 mutation and is shown with his heterozygous sister.

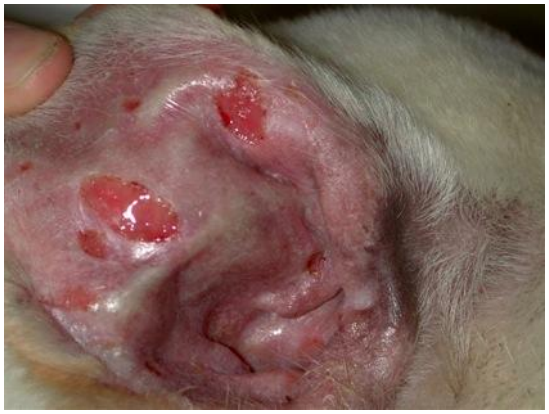
有食道溃疡的RDEB小狗因营养不良而发育迟缓

Palazzi, X., et al., J. Invest. Dermatol., 2000, 115:135

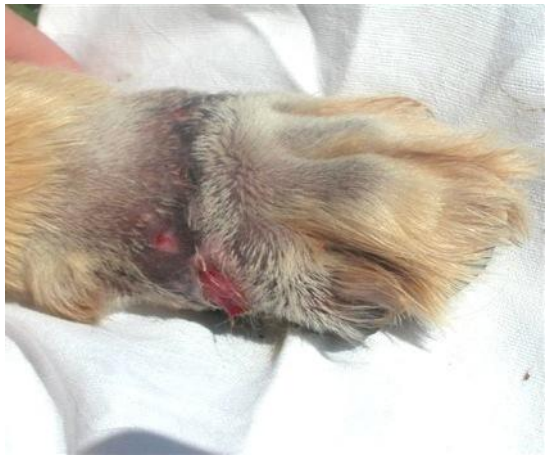
RDEB小狗是C7突变纯合，和他杂合的姐妹并列



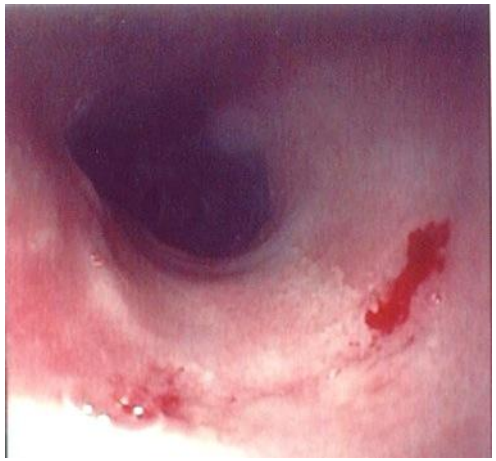
Ulcers on the hard palate
硬腭溃疡



Ulcers inside the ear
耳朵内侧水疱



Ulcerated atrophic scar of the foot
脚上的溃疡和萎缩性疤痕

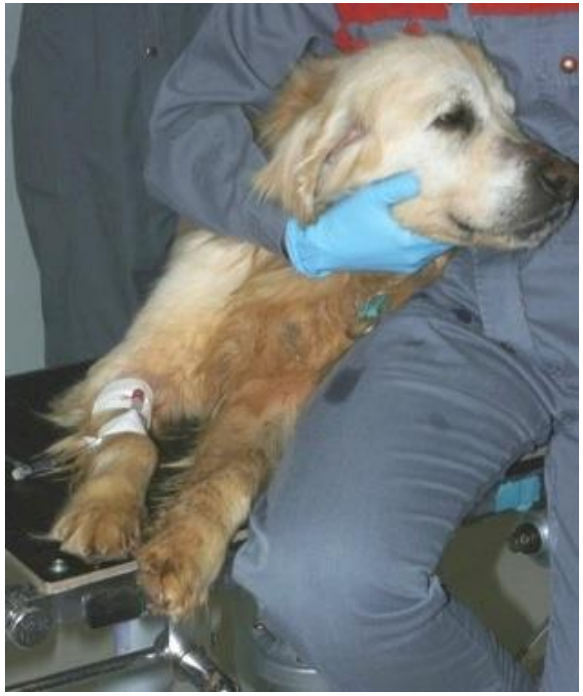


Ulcers of the esophagus
食道溃疡

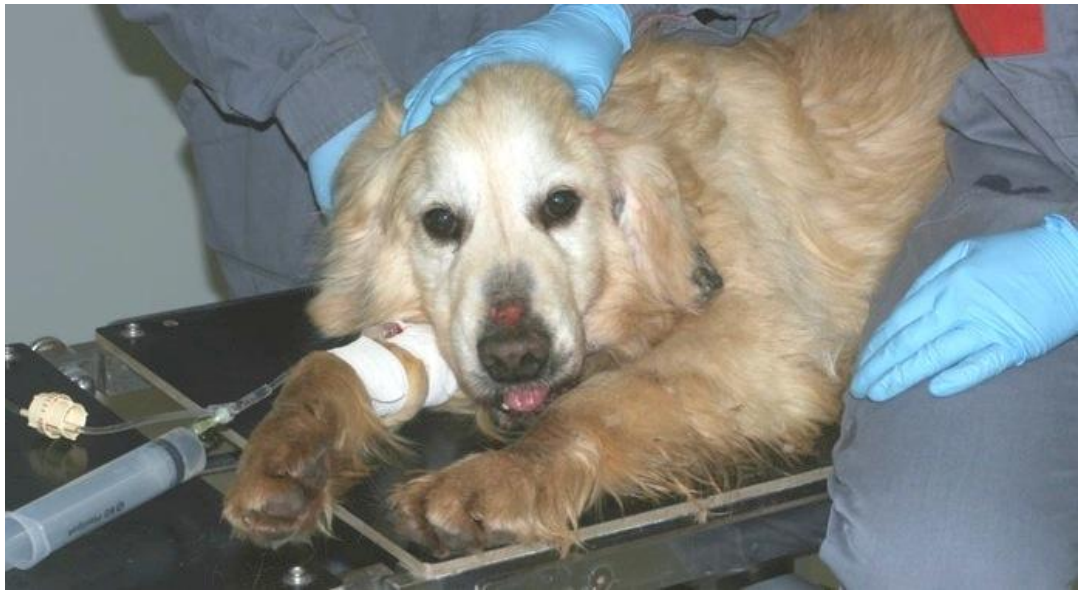
Delivery: No Adverse Effects from IV Injection of rC7

给药：静脉注射rC7没有明显的不良反应

- Dogs received a **single IV injection** into cephalic vein of forearm 狗从前臂头静脉做了单次注射
- Injection was done **without anesthesia** 注射时没有麻醉
- **No adverse effects** seen in either dog (no change in breathing or behavior) 两只狗都没有看到不良反应（呼吸和行为没有变化）
- **Targets wounded and unwounded skin** 作用于创伤和无伤的皮肤
- Dogs had **less erythema and blistering** after 4 weeks 4周后狗的红斑和水疱减少



Dog 1



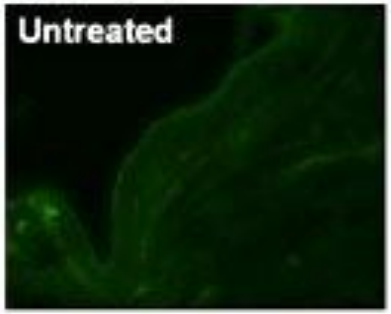
Dog 2

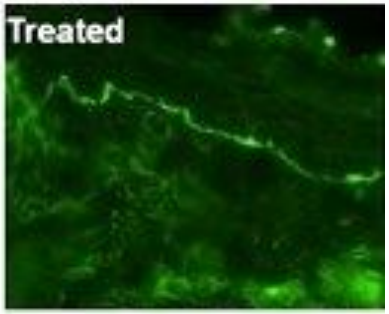
IV rC7 Incorporates Into BMZ and Reverses Epidermal-Dermal Separation

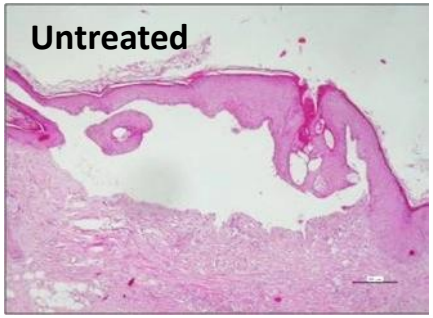
静脉rC7整合到基底膜并逆转了真皮-表皮的分离

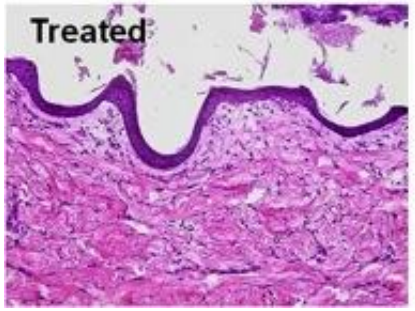
未治疗 治疗后 未治疗 治疗后

Week 1 Dorsal Skin
第一周 背上皮肤





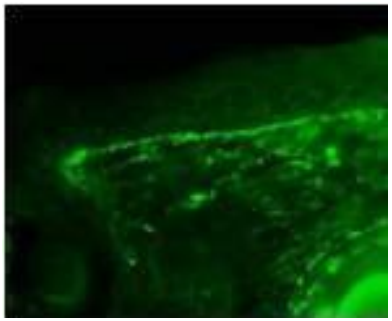


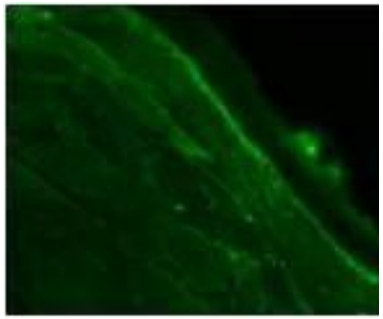


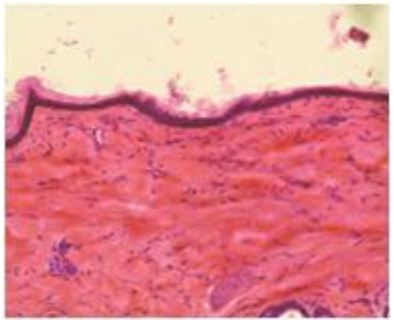
诱导出创伤 无创伤 诱导出创伤 无创伤

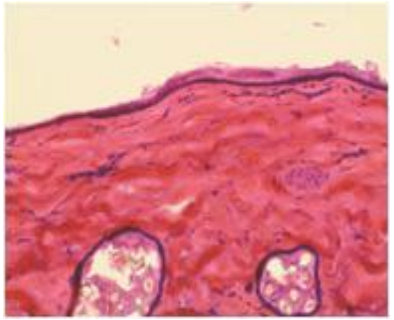
Induced wound Unwounded Induced wound Unwounded

第四周 背上皮肤
Week 4 Dorsal Skin

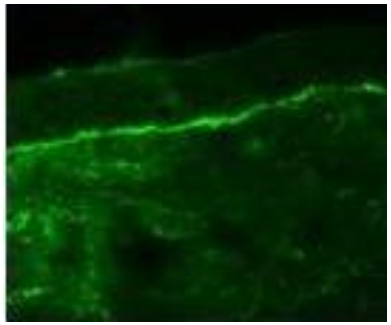


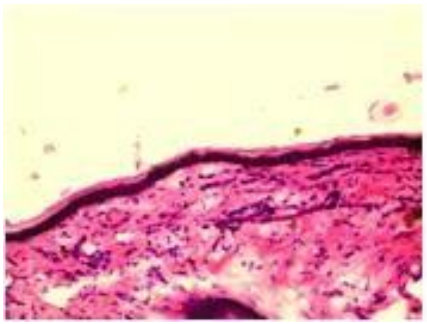






Week 9 Lip Induced wound
第9周 嘴唇诱导出创伤





Product Profile: Intravenous Delivery Preferred

产品简介：优选静脉导入

- **IV delivery** of rC7 **preferable** to intradermal or topical delivery because:

  静脉导入rC7比真皮内注射或局部外用好，因为：

  - It **reversed DEB phenotype** in 3 mouse models and 1 dog model of DEB **without** apparent **adverse effects**

    它在3只小鼠和一只狗身上逆转了DEB的症状，没有明显不良反应

  - It incorporates into skin and **forms anchoring fibrils**

    它整合进皮肤中，形成了锚丝纤维

  - In DEB models, it appears to **target wounded and unwounded skin** and areas normally expressing C7 but **not to other organs**

    在DEB模型中，它似乎瞄准了创伤和无伤的皮肤，以及其它应该表达C7的组织，但没有到其它器官中

  - It offers **systemic therapy to treat both skin and internal sites** (oral, esophageal, anal, urethral)

    它能系统性的治疗皮肤和体内器官（口腔、食道、肛门、尿道）

  - Most **protein therapies** for rare diseases are **delivered IV** (patient convenience and acceptance)

    多数罕见病的蛋白疗法是静脉注射的（患者舒适度和接受程度）

- **Additional** pharmacology **experiments planned** in mouse and dog models of RDEB

  计划在小鼠和狗模型中进行更多药理实验

Manufacturing: Making sufficient quantities

生产：制造足够的量

- Making rC7 in Chinese Hamster Ovary **(CHO) cells**
  使用中国仓鼠卵巢细胞（CHO）制造rC7
- CHO is **well validated** with FDA/regulators for expression of biologic drugs
  CHO用于表达生物制剂，已经经过FDA/监管机构验证
- CHO offers a **scalable** manufacturing process (up to 20,000 liter bioreactors)
  CHO有批量制造的工艺流程（大到20,000升的生物反应器）
- **Licensed technology** from Stanford University to increase stability of rC7
  斯坦福大学的授权技术，用于提高rC7的稳定性
- Manufacturing process being developed at Laureate Biopharmaceutical Services, CMO with proven late stage clinical and commercial supply capabilities
  Laureate Biopharmaceutical Services研发了制造流程，CMO用于后期临床和商业供应的能力已得到验证

Initial Process with adherent fibroblasts: 0.015L/plate

初期用粘附性的纤维原细胞工艺：0.015升/盘

CHO cells are not adherent, so **process is scalable**

CHO细胞没有粘附性，所以工艺可以扩张

0.25L

2L

12L

250L

1000L

Clinical Development with Intravenous rC7

静脉rC7的临床开发

What is the **quickest path to regulatory approval** for IV rC7 in DEB?

监管机构批准DEB使用静脉rC7的最快路径是什么？

- Conduct **dose range finding** studies and **IND-enabling toxicology** studies in mice and dogs 在小鼠和狗身上进行确定使用剂量的研究和申请IND资格需要的毒性研究

IND：研究性新药

- Conduct a **longitudinal severity study** in DEB (natural history study) to determine best clinical endpoints

  进行DEB患者的纵向严重性研究（自然病史研究），确定最佳的临床试验终点

- Conduct a **Phase 1 trial** in small number of (6-9) DEB patients

  在少数DEB患者（6-9）中进行一期临床试验

- Approval based on **small pivotal trial** (30-40 patients) may be possible, subject to statistical considerations and regulatory feedback

  根据统计学上的考虑或监管机构的反馈，有可能基于小规模关键试验（30-40患者）获得批准

- **USC's** NIH-sponsored clinical trial with **intradermal rC7** will provide supporting information for Lotus' intravenous development program (Lotus has exclusive license to the results from this trial)

  南加州大学的NIH资助的真皮内rC7临床试验可以为Lotus的静脉项目提供支持信息。

  （Lotus有使用这次试验结果的排他性授权）

What are Meaningful Clinical Endpoints?

什么是有意义的临床终点？

LOTUS TISSUE REPAIR

Conduct longitudinal severity study (natural history study, no drug)

进行纵向严重性研究（自然病史研究，不用药物）

Objectives 目标

- Investigate the variability in potential efficacy endpoints over time
  调查可能的有效性终点随时间的变化
- Gain experience with instruments used to measure these endpoints
  获得测量这些终点的工具的经验
- Prepare for regulatory submission of clinical trial data
  为临床试验数据提交给监管机构做准备

Design 设计

- Prospective, longitudinal study over 12 months with IRB/EC approval
  未来获得IRB/EC批准后为期12个月的纵向研究
- Multi-center study with sites in North America and Europe (3-4 sites, at least 1 in EU)
  多中心研究，包括北美和欧洲（3-4个点，至少一个在欧盟）
- Up to 50 DEB patients (consecutive RDEB or DDEB patients who visit study sites)
  多达50名DEB患者（到研究单位就诊的连续的RDEB或DDEB患者）

Measures 测量

- Physical exam and photos to quantify blistering 体检或照片以定量水疱
- Questionnaires: Quality of Life, EB severity scores, instruments for pain, itching, disability 问卷：生活质量，EB严重程度打分，疼痛、瘙痒、残疾评估

Patient Registry Will Help Clinical Development

患者登记会帮助临床开发

- How do we identify patients for clinical trials? 怎样确认临床试验的患者？
- How do we educate regulators and payers about DEB?
  怎样向监管机构和出资人介绍DEB？

www.EBCare.org

Launched the EB patient registry with patient-reported data

启动了EB患者注册，患者填写数据

- **First** international, on-line, patient-reported registry launched on Feb 29, 2012 by DEBRA
  第一个国际性的，在线，患者填写的登记项目，由DebRA在2012年2月29日启动
- Data owned by EBCare, LLC with a board of managers comprised of DEBRA, Lotus, KOLs
  数据属于EBCare，这是一个由DEBRA，Lotus，KOLs组成董事会管理的有限责任公司
- Requires patient consent and is **compliant with privacy laws**
  需要患者同意，遵从隐私法律
- Will help determine patient **numbers**, **severity** of disease, **costs** of current care
  将帮助确定患者数量，严重程度，当前护理开支
- Will help **identify** patients for clinical trials, **educate** regulators, and support **reimbursement**
  将帮助确定临床试验的患者，教育监管机构，支持资助
- Access provided to all qualified researchers. Email coordinator@ebcare.org with research proposals/requests for data
  访问权开放给所有有资质的研究人员

M. de Souza and V. Rangel-Miller (2012) Significance of Patient Registries for Dermatological Disorders. *Journal of Investigative Dermatology* 132:1749–1752

Registry Will Help Determine Prevalence of EB 登记有助于确定EB的患病率

Physician Registries Suggest RDEB Prevalence Ex-US 2-4x Higher

美国之外的登记，显示RDEB的发病率比美国高2-4倍

Country 国家	Population 人口	KOL/Source 数据源	Actuals 患病人口	Prevalence 患病率
USA 美国	307M	Fine et al., 1999	282	0.92:1000K
Austria 奥地利	8M	Pohla-Gubo, 2011	17	1:470K
England, Wales 英国、威尔士	55M	Browne et al., 2011	130	1:423K
Germany 德国	82M	L. Tuderman, 2011	157	1:522K
France 法国	65M	Bodemer, Lacour, 2011	192	1:338K
Spain 西班牙	46M	DEBRA Spain, 2011	130	1:353K
Italy 意大利	60M	G. Zambruno, 2011	120	1:500K
Chile 智利	16M	Palisson, 2011	64	1:265K
Ireland (Republic and N. Ireland) 爱尔兰	6M	O'Donnell et al., 2012	24	1:250K

这些数据可能代表了中度和重度患者，只是患病率的一个下限

- These numbers likely represent the moderate to severe patients and establish a lower limit for prevalence
- **Please email me (mark@lotustr.com) if you would like to provide me with your patient numbers**
- I will compile the data and present it at EB2012
- Does not include RDEB patients without a definitive diagnosis. Double-counting an issue

Summary 总结

- Lotus is founded on the opportunity to **treat DEB** with rC7 protein replacement therapy Lotus的建立是为了开发DEB的rC7蛋白替代疗法
- Compelling pre-clinical data in hand suggest the possibility that **intravenous rC7 can target areas deficient in C7, and reverse the DEB phenotype without apparent adverse effects,** comparable to other protein replacement therapies
  有力的前临床数据说明静脉注射rC7能作用于缺少C7的位置，逆转DEB症状，没有明显不良反应。与其它蛋白替代疗法类似。
- **Exclusive license** to University of Southern California and Stanford **intellectual property** on methods of making and using rC7 as a treatment for DEB
  南加州大学和斯坦福大学关于制造和使用rC7治疗DEB知识产权的独家授权
- **Clear path to** first human clinical trial with IV rC7 in 1 year and **regulatory approval** in 4-5 years 1年内开始首次人体静脉rC7临床试验，4-5年内获得监管机构批准没有障碍
- **EB patients and families can help by participating** in the EBCare registry (www.ebcare.org) EB患者和家庭能通过在EBCare登记提供帮助
- **Email the EBCare Registry Coordinator** (coordinator@ebcare.org) if you are interested in accessing the de-identified data 想使用无身份信息的数据，可以发邮件
- **Please email me your DEB patient numbers** (RDEB, DDEB, DEB – unknown) to help our prevalence efforts 请把你的DEB患者数量发给我，帮助我们确定患病率

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