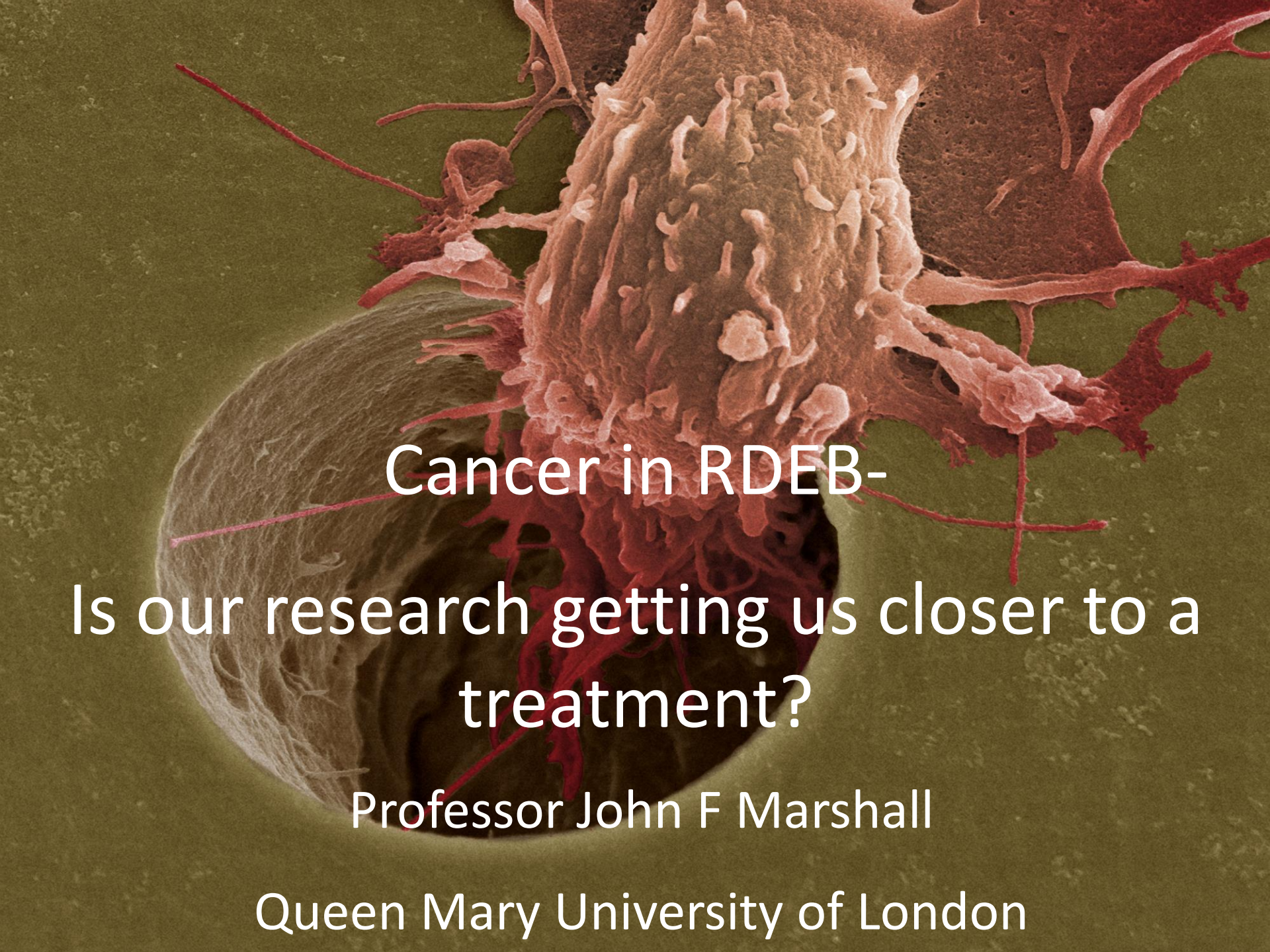


Cancer in RDEB-

Is our research getting us closer to a treatment?

Professor John F Marshall

Queen Mary University of London

15 years ago……..

15 years ago……..what did we know?

Collagen VII is absent/deficient

↓

Blistering at sites of trauma, bony joints

↓

Chronic inflammation and fibrosis

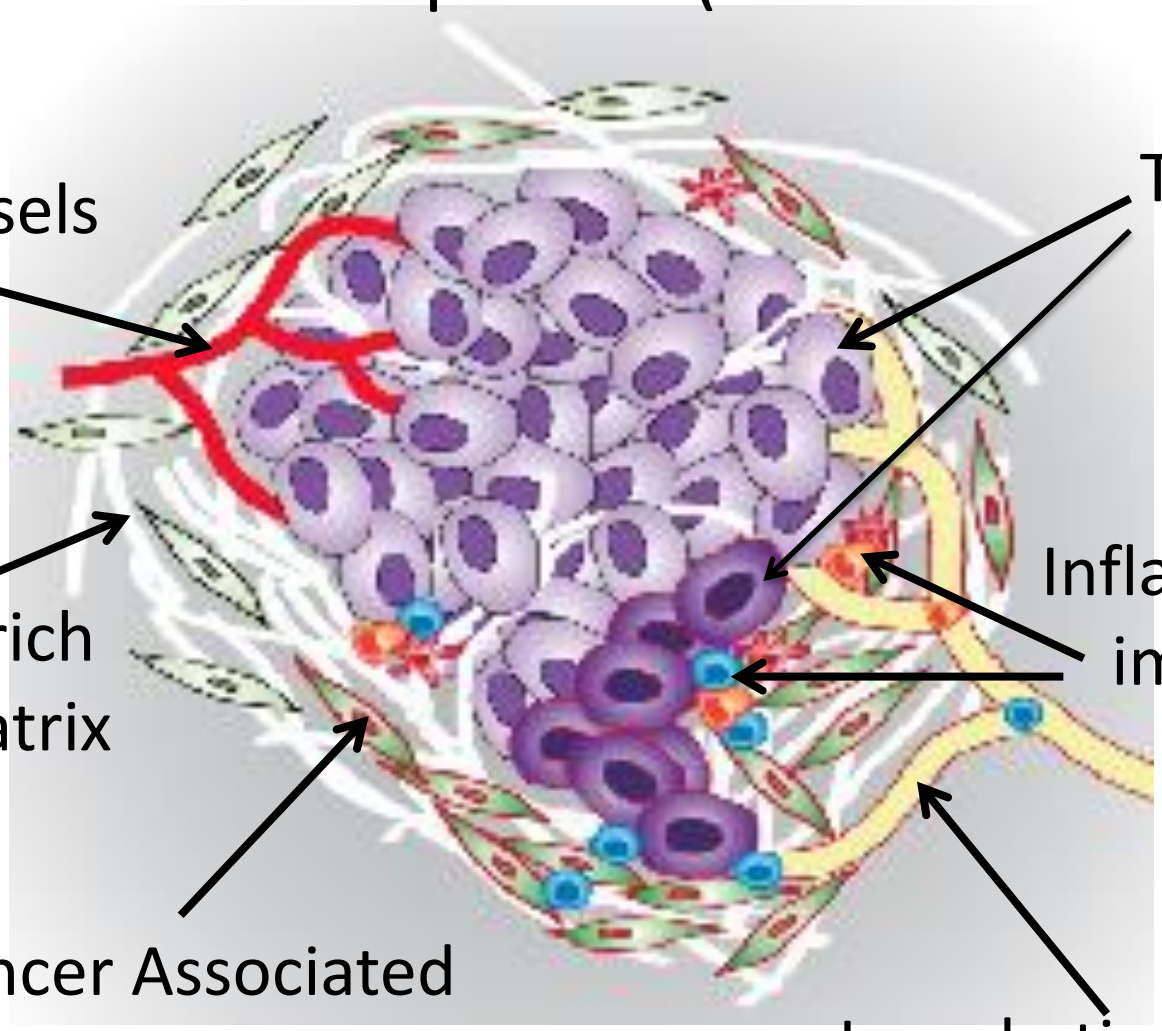
↓

Squamous cell carcinomas developed, often at bony joints

15 years ago……..what didn't know?

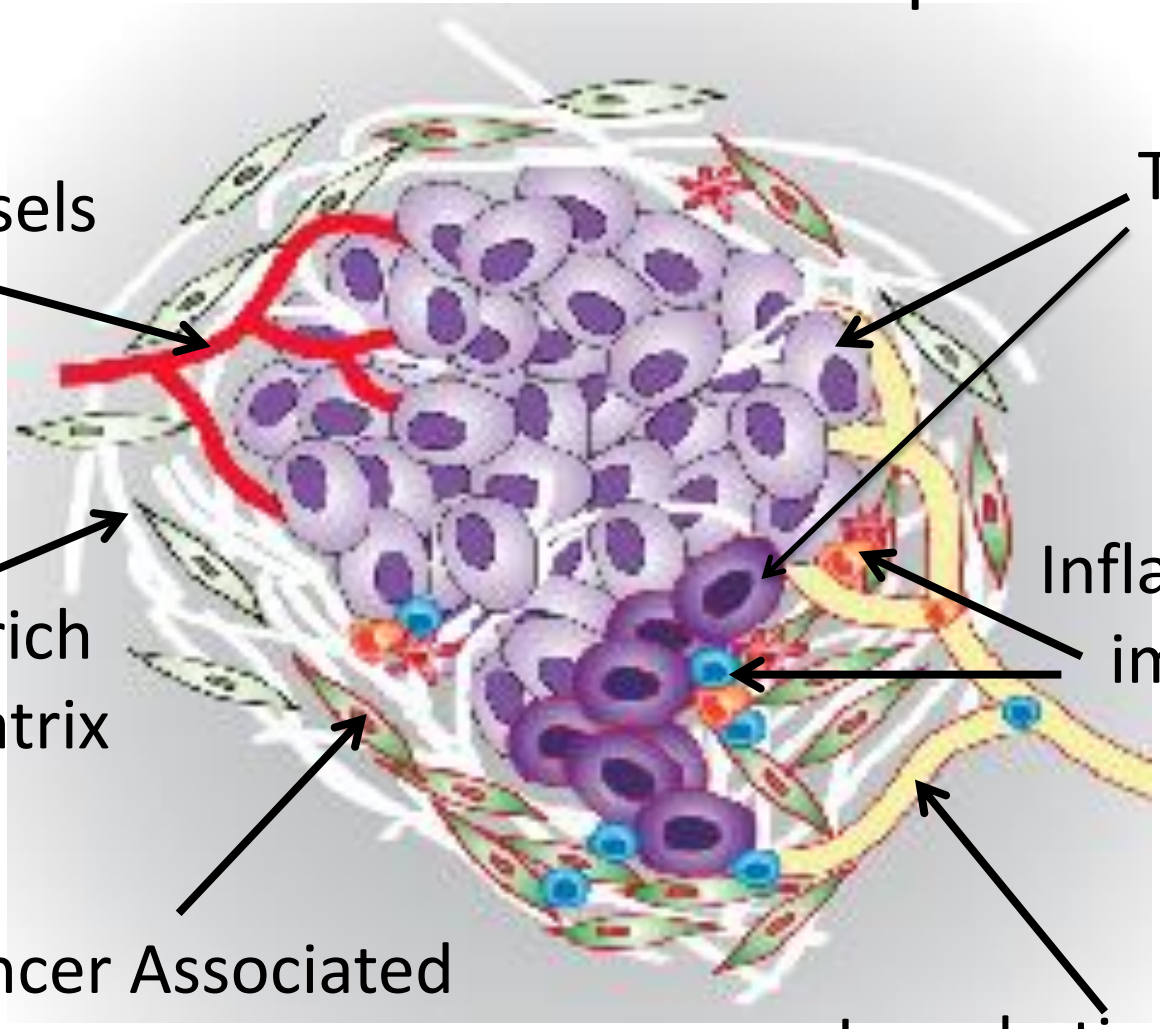
- Does Collagen VII do anything other than anchor the skin cells?
- Can we correct the absence of collagen VII?
- If we correct the absence of collagen VII, can we restore normal cell behaviour?
- Is the chronic inflammation and fibrosis helping to promote the SCC?
- Are the SCC in RDEB patients different to SCC in non-EB patients?

Cartoon of a typical squamous cell carcinoma in an RDEB patient (or non-EB Patient)



Are SCC cancer cells genetically different in RDEB patients and non-RDEB patients?

Cartoon of a typical squamous cell carcinoma in an RDEB patient



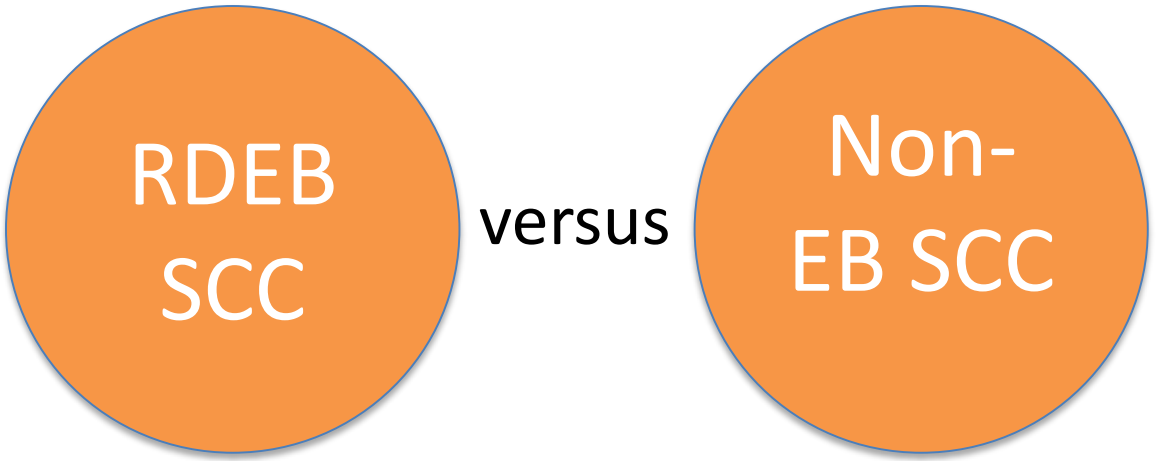
Are SCC cancer cells genetically different in RDEB patients and non-RDEB patients?

Integrative mRNA profiling comparing cultured primary cells with clinical samples reveals PLK1 and C20orf20 as therapeutic targets in cutaneous squamous cell carcinoma

SA Watt[1,8], C Pourreyron[1,8], K Purdie[2], C Hogan[1], CL Cole[1], N Foster[3], N Pratt[3], J-C Bourdon[1], V Appleyard[1], K Murray[1], AM Thompson[1], X Mao[2], C Mein[4], L Bruckner-Tuderman[5], A Evans[6], JA McGrath[7], CM Proby[1], J Foerster[1], IM Leigh[1] and AP South[1]

Oncogene (2011) 30, 4666–4677

Compared which genes are used in

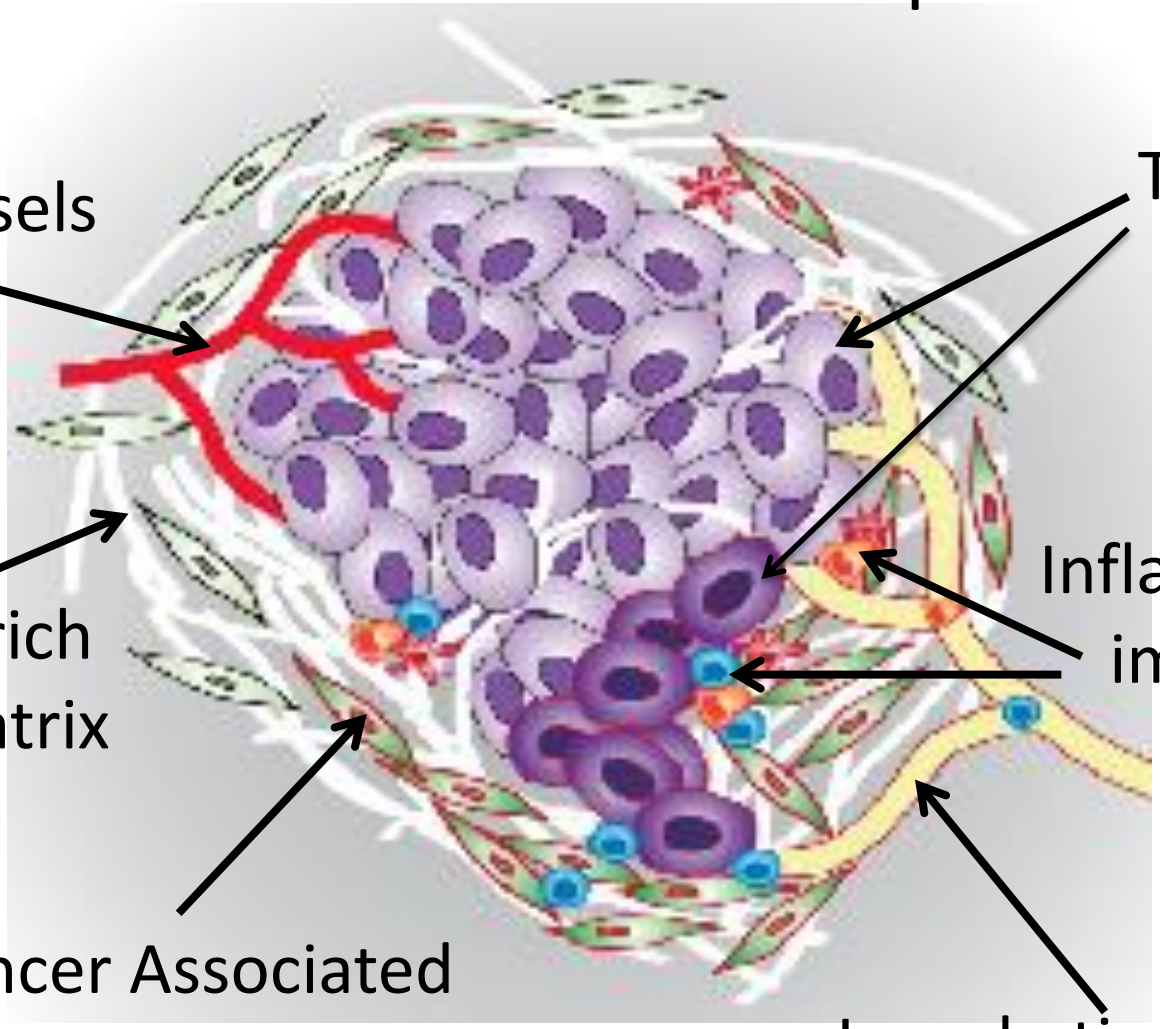


= Significant differences in only 18 genes

Are SCC cancer cells genetically different in RDEB patients and non-RDEB patients?

Not very different at all

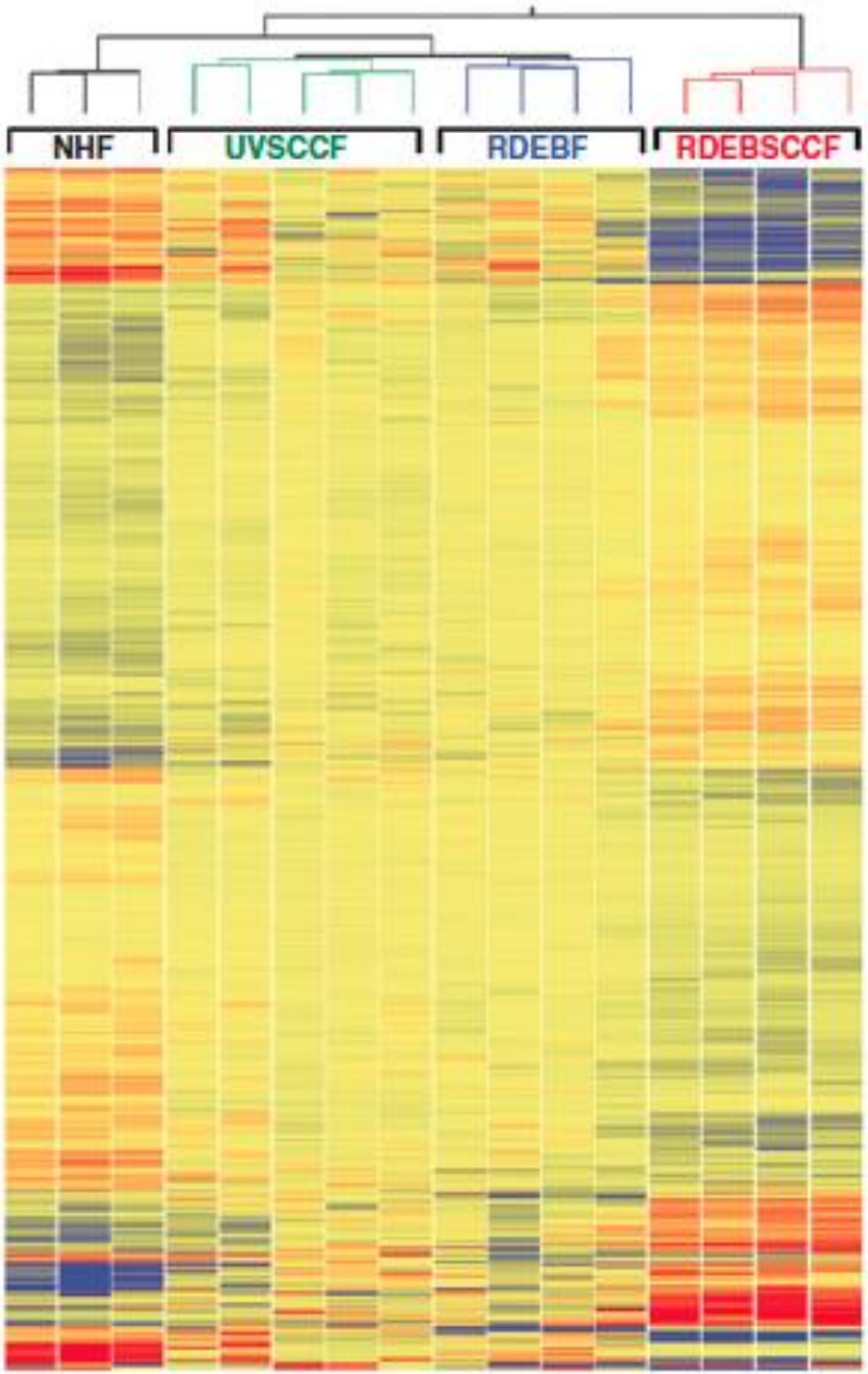
Cartoon of a typical squamous cell carcinoma in an RDEB patient



Are the genes used by fibroblasts different in RDEB patients and non-RDEB patients?

Examined the genes used in fibroblasts from

- Human skin (non-EB) NHF
- Human skin (RDEB patient) RDEBF
- UV light induced SCC UVSCCF
- RDEB SCC RDEBSCCF


NHF
UVSCCF
RDEBF
RDEBSCCF

How can we show if RDEB SCC fibroblasts promote tumour development? Inject SCC cells with different fibroblasts into mice.

Are the genes used by fibroblasts different in RDEB patients and non-RDEB patients?

Yes!!

The data suggest that it is the fibroblasts in the microenvironment surrounding the cancer cells that may be responsible for the aggressive behaviour of RDEB SCC.

They increase the use of genes that code for microenvironmental proteins

Global remodelling of cellular microenvironment due to loss of collagen VII

Victoria Küttner[1,2,3,4], Claudia Mack[3], Kristoffer TG Rigbolt[1,2], Johannes S Kern[3], Oliver Schilling[5,6], Hauke Busch[1,2,5], Leena Bruckner-Tuderman[1,2,3,6,*] and Jörn Dengjel[1,2,6,*]

Molecular Systems Biology 9:657

Examined the proteins released from fibroblasts from non-EB and RDEB patients.

Major findings:

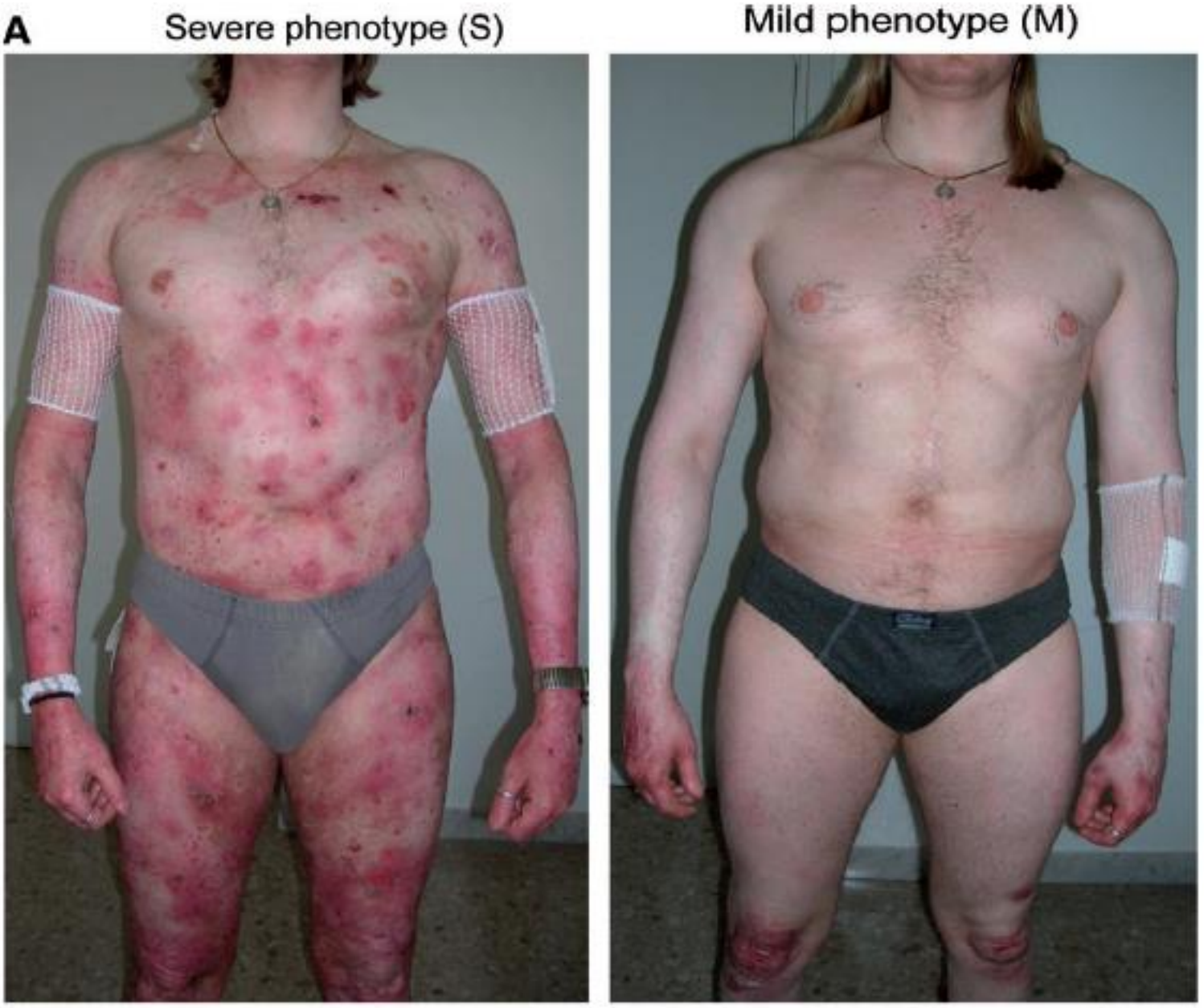
- Basement membrane proteins were decreased
- Many types of collagen and other ECM proteins were increased
- TGFβ (Transforming Growth Factor Beta) was increased

Human Molecular Genetics, 2014, Vol. 23, No. 15 **3907–3922**
doi:10.1093/hmg/ddu102
Advance Access published on March 5, 2014

Monozygotic twins discordant for recessive dystrophic epidermolysis bullosa phenotype highlight the role of TGF-β signalling in modifying disease severity

Teresa Odorisio[1], Michela Di Salvio[1], Angela Orecchia[1], Giovanni Di Zenzo[1], Eugenia Piccinni[1], Francesca Cianfarani[1], Antonella Travaglione[2], Paolo Uva[2], Barbara Bellei[3], Andrea Conti[4], Giovanna Zambruno[1] and Daniele Castiglia[1,*]

Identical twins had different clinical appearances of the RDEB. One had a severe form and the other had a mild form.


A
Severe phenotype (S)
Mild phenotype (M)

Human Molecular Genetics, 2014, Vol. 23, No. 15 **3907–3922**
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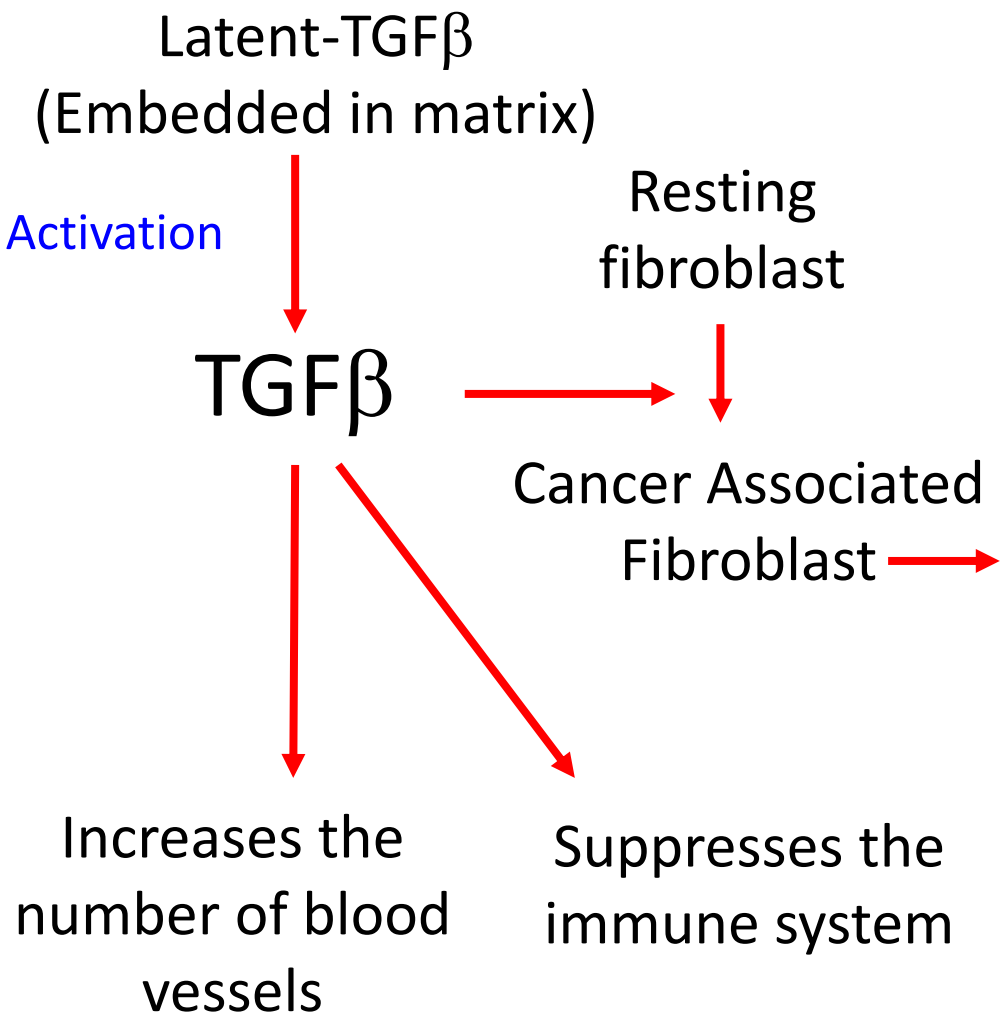
Identical twins had different clinical appearances of the RDEB. One had a severe form and the other had a mild form.

The mild case had fibroblasts that produced a protein, that stops TGFβ working- called DECORIN

Suggests that more TGFβ the more severe the disease

TGFβ can promote fibrosis, cancer growth and spread



- Invade and remodels the tumour microenvironment
- Produces excess collagen – reason for fibrosis
- Contracts the matrix and makes it stiffer
- Releases proteins that help cancer cells grow and spread

Suppression of TGFβ and Angiogenesis by Type VII Collagen in Cutaneous SCC

V. L. Martins*, M. P. Caley*, K. Moore, Z. Szentpetery, S. T. Marsh, D. F. Murrell, M. H. Kim, M. Avari, J. A. McGrath, R. Cerio, A. Kivisaari, V. M. Kähäri, K. Hodivala-Dilke, C. H. Brennan, M. Chen, J. F. Marshall, E. A. O'Toole

JNCI J Natl Cancer Inst (2016) 108(1): djv293

Used a combination of genetic knockdown of collagen VII in SCC cells and adding back Collagen VII protein. Concluded:

- The presence of Collagen VII, bound to the cells via integrin $\alpha 2\beta 1$, stops TGFβ protein appearing and its activity (invasion, angiogenesis)
- Loss of Collagen VII or blockade of $\alpha 2\beta 1$ would increase TGFβ protein and its activities
- The tumour promoting molecules fibronectin and integrin $\alpha v\beta 6$ would also appear in higher levels when collagen VII was missing, in a TGFβ-dependent way.
- Collagen VII seems directly responsible for regulating TGFβ

Summary

- Research from independent laboratories Identify that excess TGFβ is strongly associated with severity of RDEB and with SCC in RDEB
- Natural (DECORIN) blockade of TGFβ can reduce the severity of RDEB
- Can we identify drugs that can also block TGFβ?

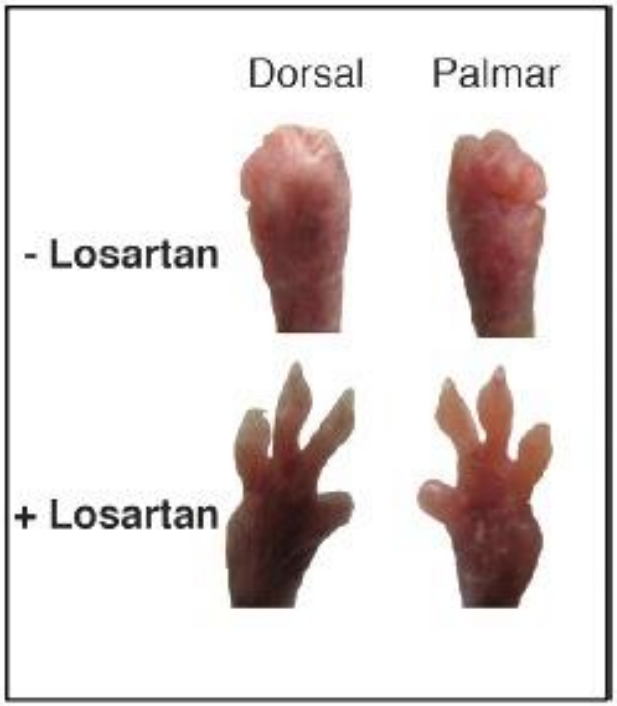
Losartan ameliorates dystrophic epidermolysis bullosa and uncovers new disease mechanisms

Alexander Nyström[1], Kerstin Thriene[1,2,3,†], Venugopal Mittapalli[1,†], Johannes S Kern[1], Dimitra Kiritsi[1], Jörn Dengjel[1,2,3,4] & Leena Bruckner-Tuderman[1,3,*]

EMBO Molecular Medicine Vol 7 | No 9 | 2015

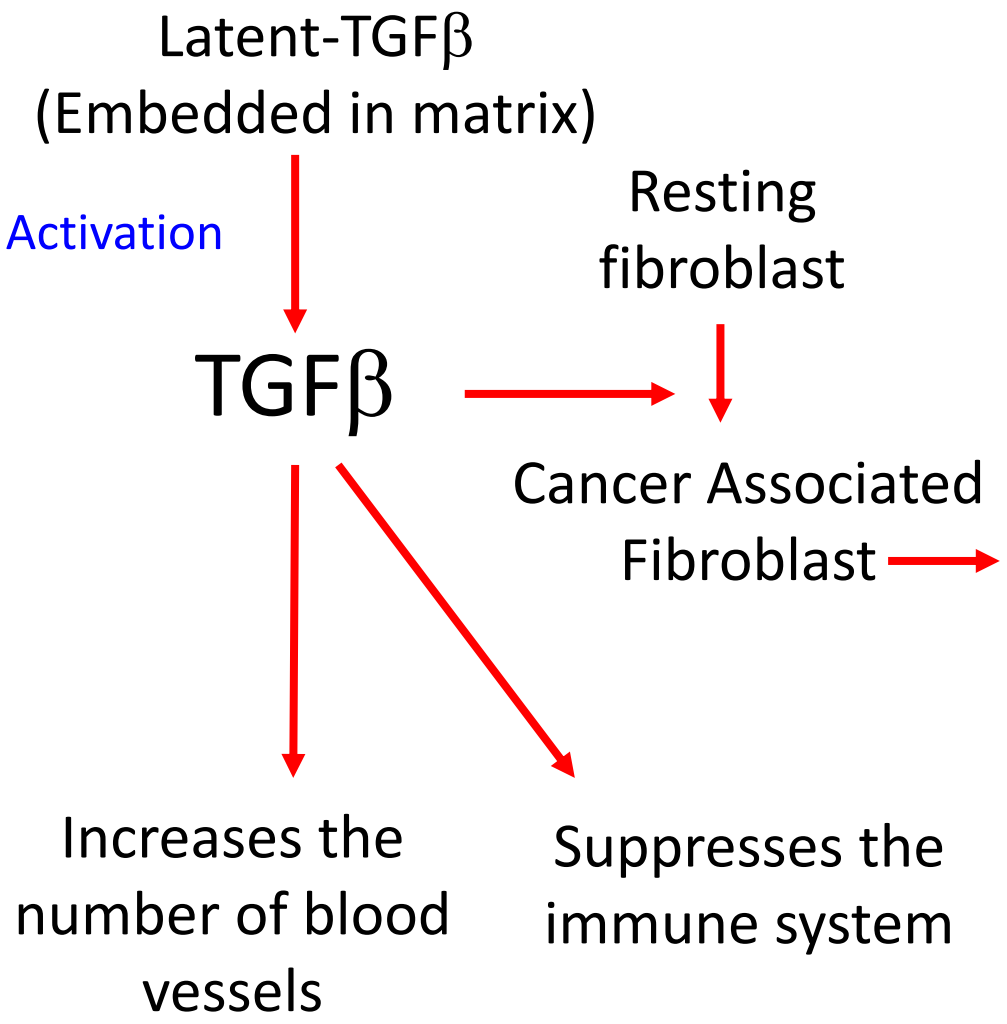
Treated mice genetically engineered to develop RDEB with Losartan, a drug that inhibits TGFβ effects. They found the treatment

- Halted fibrosis in paws
- Stopped fusion of the digits
- Reduced the levels of molecules that promote inflammation
- Reduced inflammation



Clinical trials in RDEB are planned

TGFβ can promote fibrosis, cancer growth and spread



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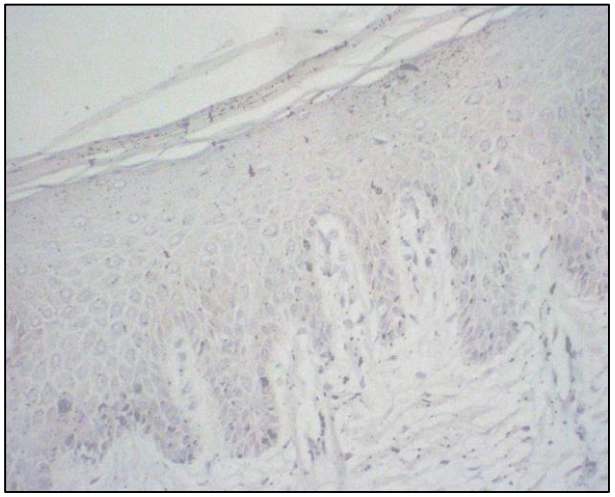
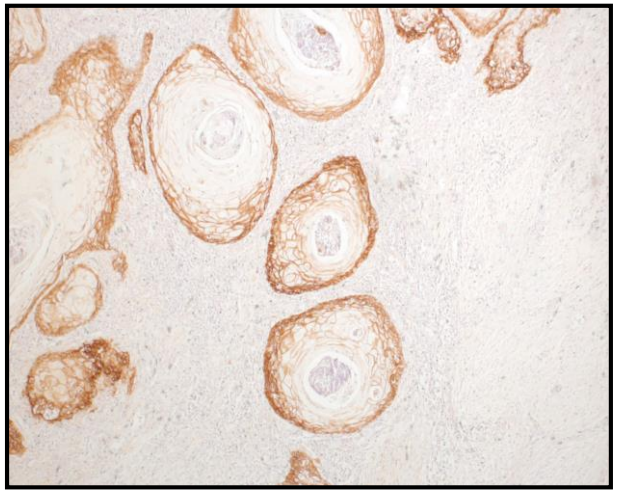
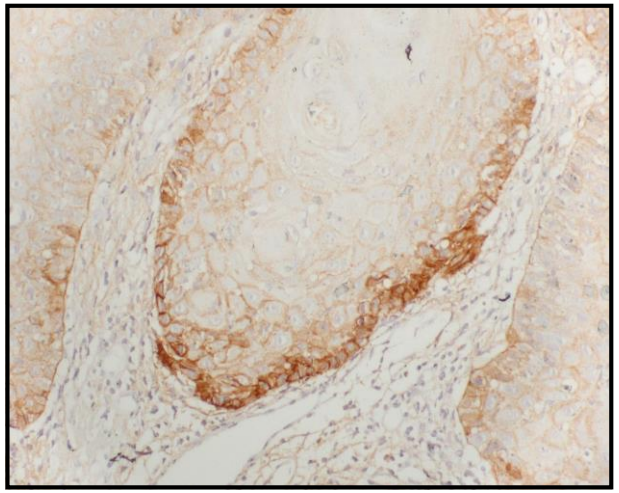
- Contracts the matrix and makes it stiffer
- Releases proteins that help cancer cells grow and spread

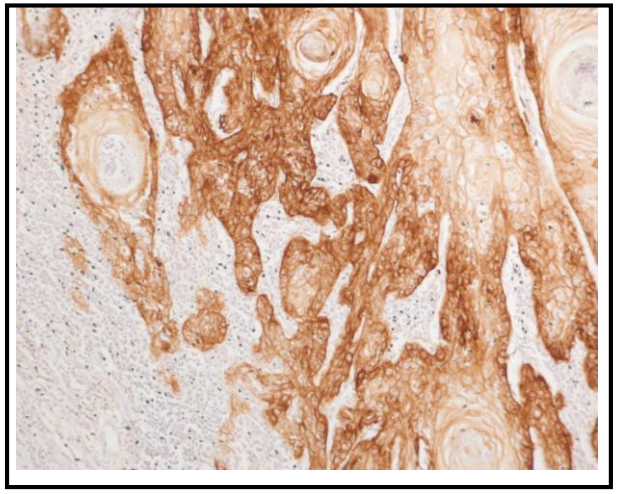
Cell, Vol. 96, 319–328, February 5, 1999, Copyright ©1999 by Cell Press

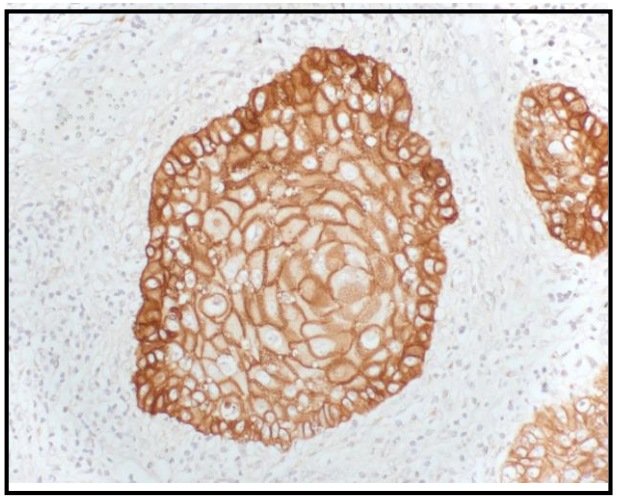
The Integrin αvβ6 Binds and Activates Latent TGFβ1: A Mechanism for Regulating Pulmonary Inflammation and Fibrosis





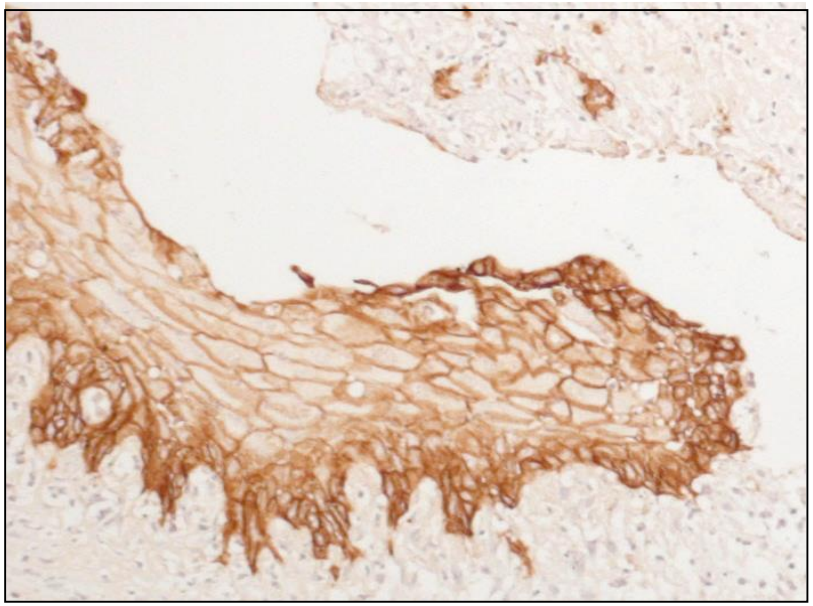




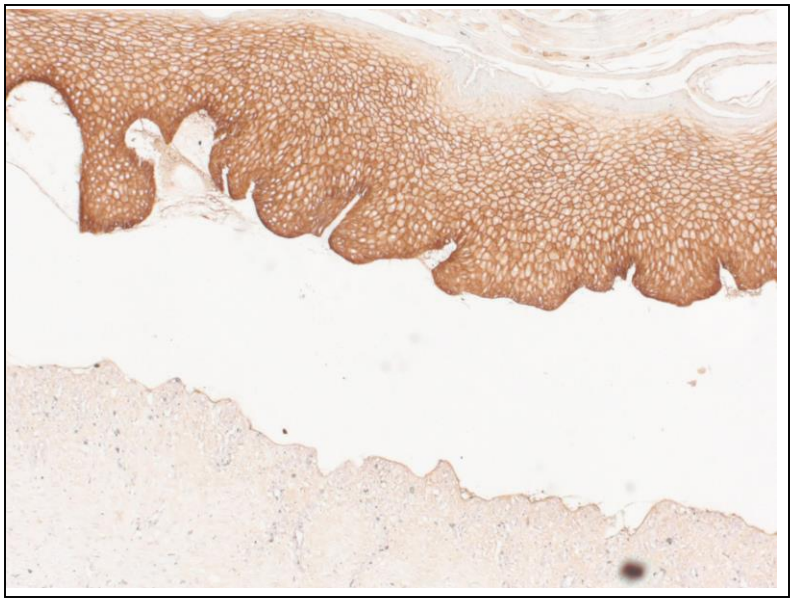


αvβ6 is on 86% of RDEB SCC tested

αvβ6 is upregulated significantly on RDEB healing wounds



Wound edge

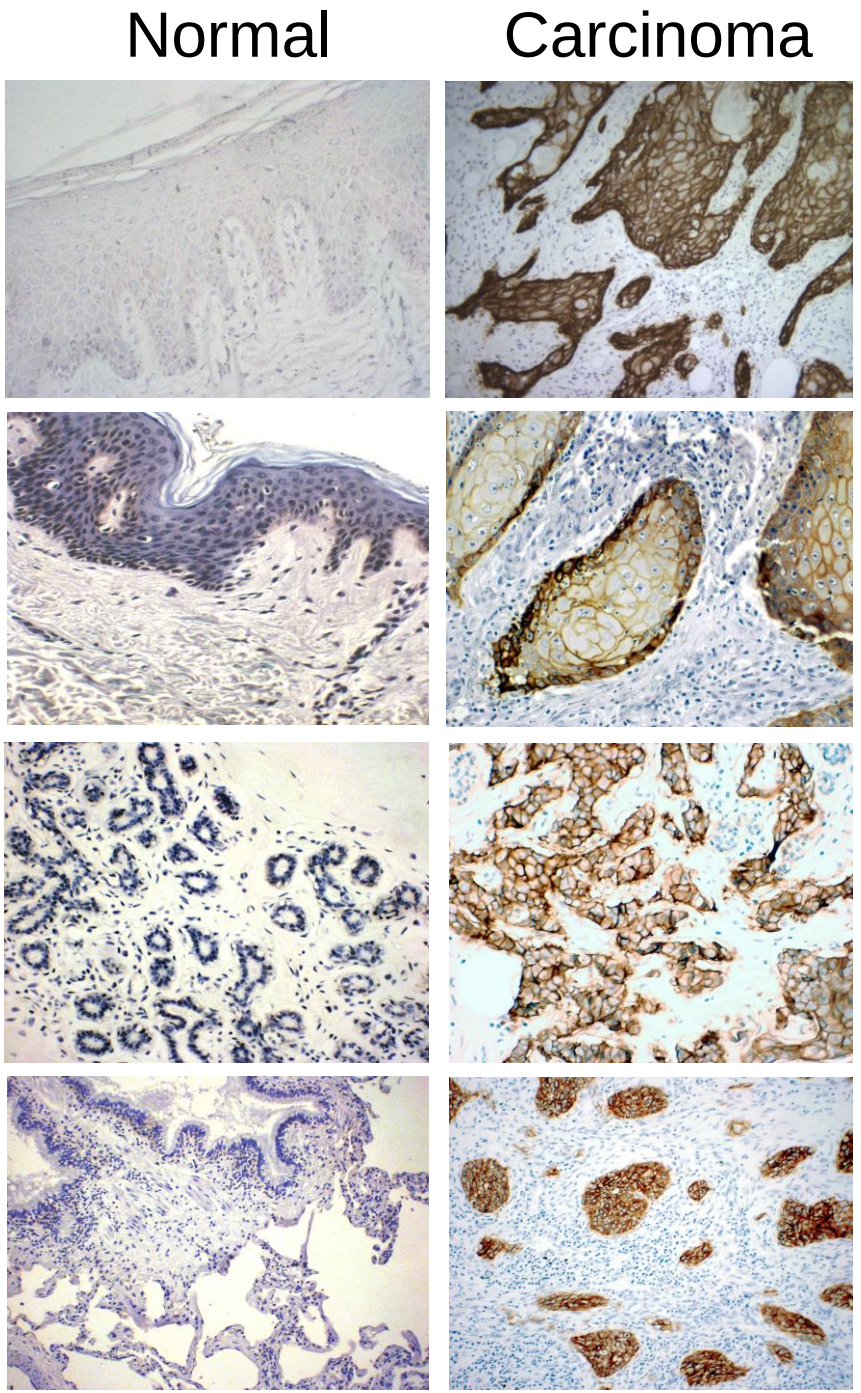


Blister

Over the last 15 years we have learned that integrin αvβ6 ?

- Epithelial-specific, RGD-directed integrin
- Weak or undetectable in most normal adult tissues
- Presence may increase during tissue remodelling e.g. wound healing, chronic inflammation
- Main function of αvβ6 is to activate TGFβ via binding to Latent-TGFβ.
- Presence is increased in many types of carcinoma including oral and skin SCC, colon, breast, cervical, ovarian, lung, oesophageal

Expression of integrin αvβ6 in normal and cancer tissues



The problem with developing an experimental drug in only EB is its difficult to develop the clinical trial

We don't have sufficient patient numbers to correlate protein presence and survival

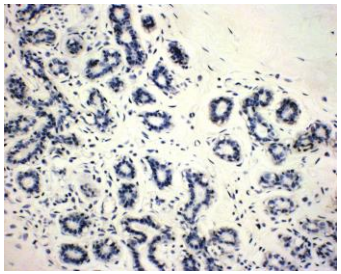
We cannot easily get the patient numbers to run the Phase I trial

Solution:
Develop the drug in another condition where these factors are less restrictive, then use the drug in EB once approved.

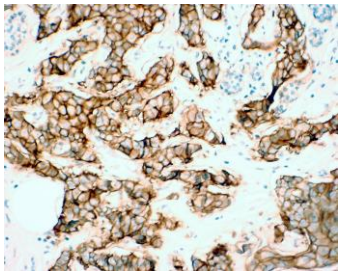
HER2, αvβ6 & breast cancer

- Stained breast cancer tissue microarrays for αvβ6 expression (London & Nottingham cohorts, 2000 cases).
- We have shown over 40% of breast cancers express moderate to strong levels of αvβ6.

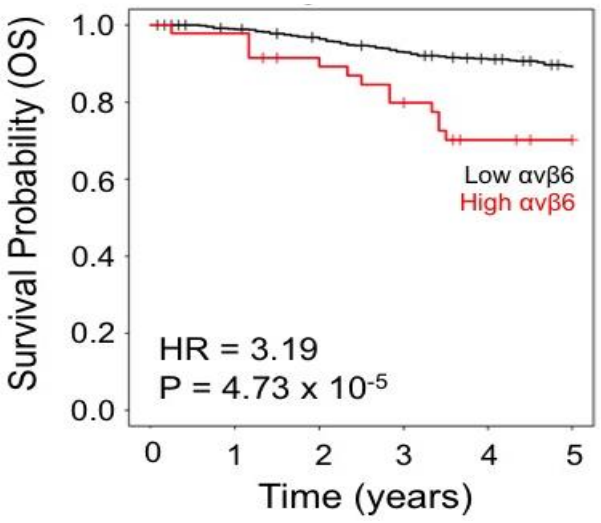
A Normal breast



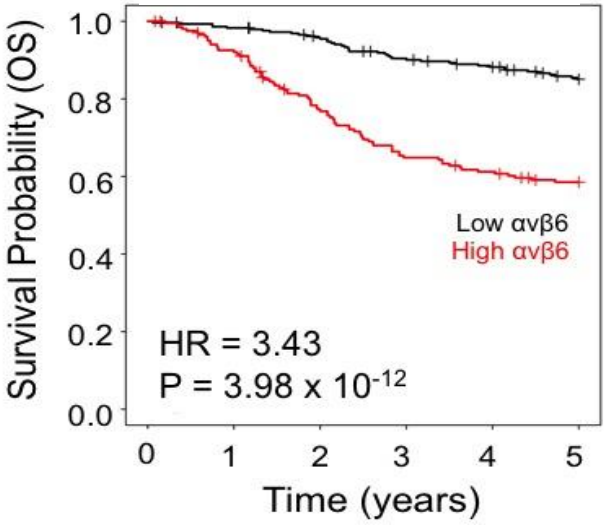
Grade 3 Breast cancer



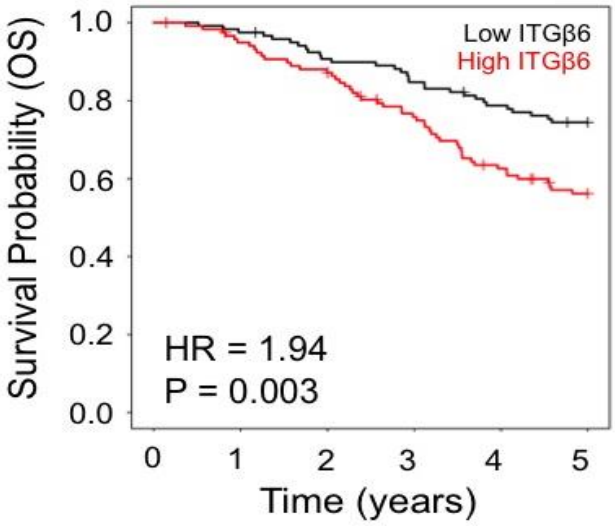
Nottingham Cohort: αvβ6



Lond/Nott: HER2+/αvβ6



METABRIC: HER2+

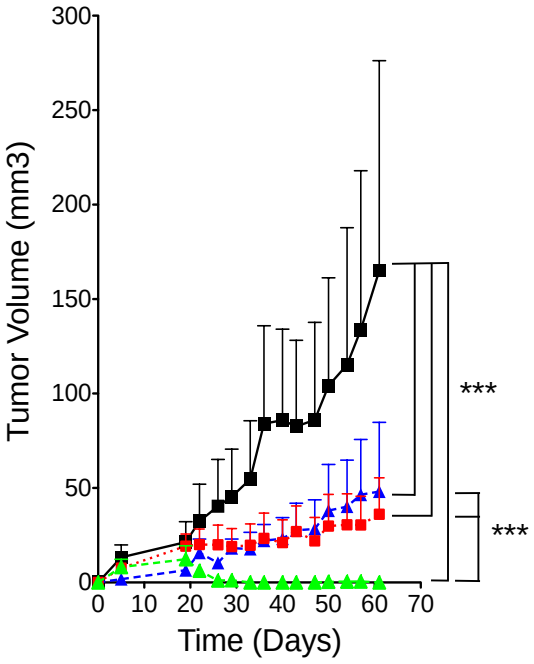


Moore *et al*, 2014. *JNCI* 106 (8)

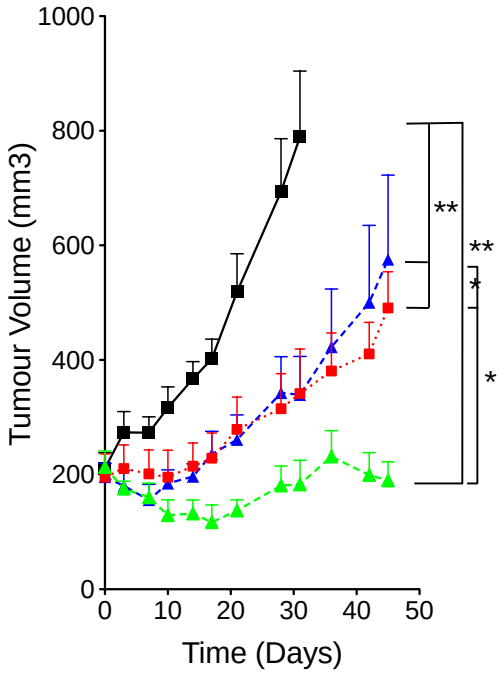
Targeting αvβ6 & HER2 in breast cancer *in vivo*

IgG	264RAD	TRA	264RAD+TRA

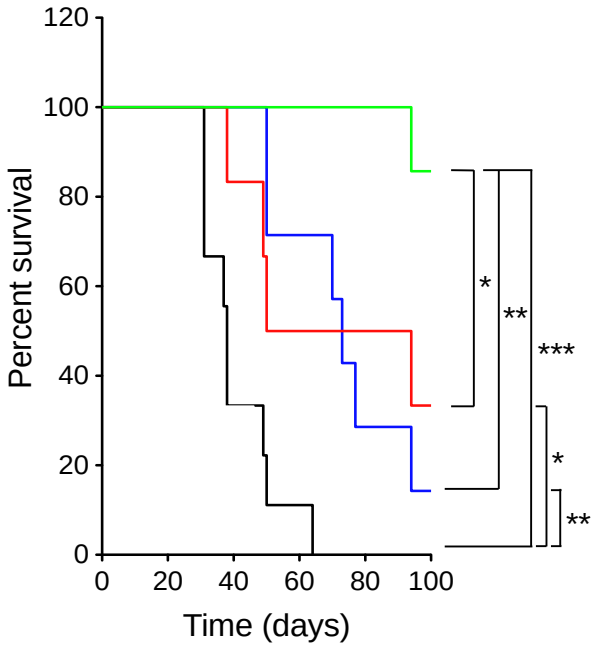
A Palpable tumours



B 200mm^3 tumours



C Survival Post Rx



αvβ6 antibody shrank tumours & increased survival & restored trastuzumab-sensitivity

Funding from CRUK

Barts Cancer Institute
Queen Mary University of London

November 26th 2014, CRUK and Medimmune signed an agreement for a Phase I trial of 264RAD in pancreatic and breast cancer.

It took till February 2016 to finalise contracts.

Antibody production started July 2016.

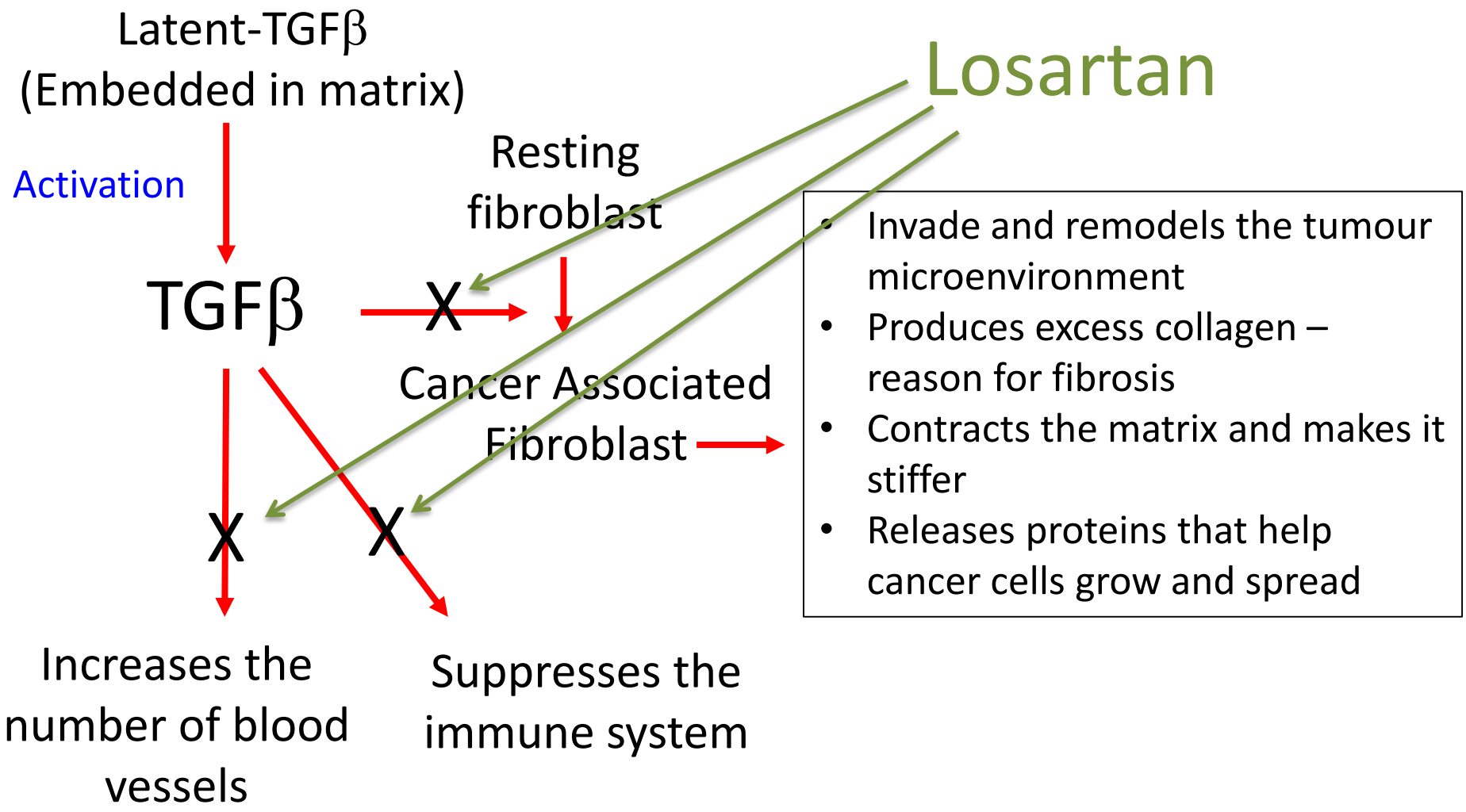
Will take till December 2017 to make sufficient 264RAD for the trials

Once confirmed as safe 264RAD will be available for testing in EB patients with SCC

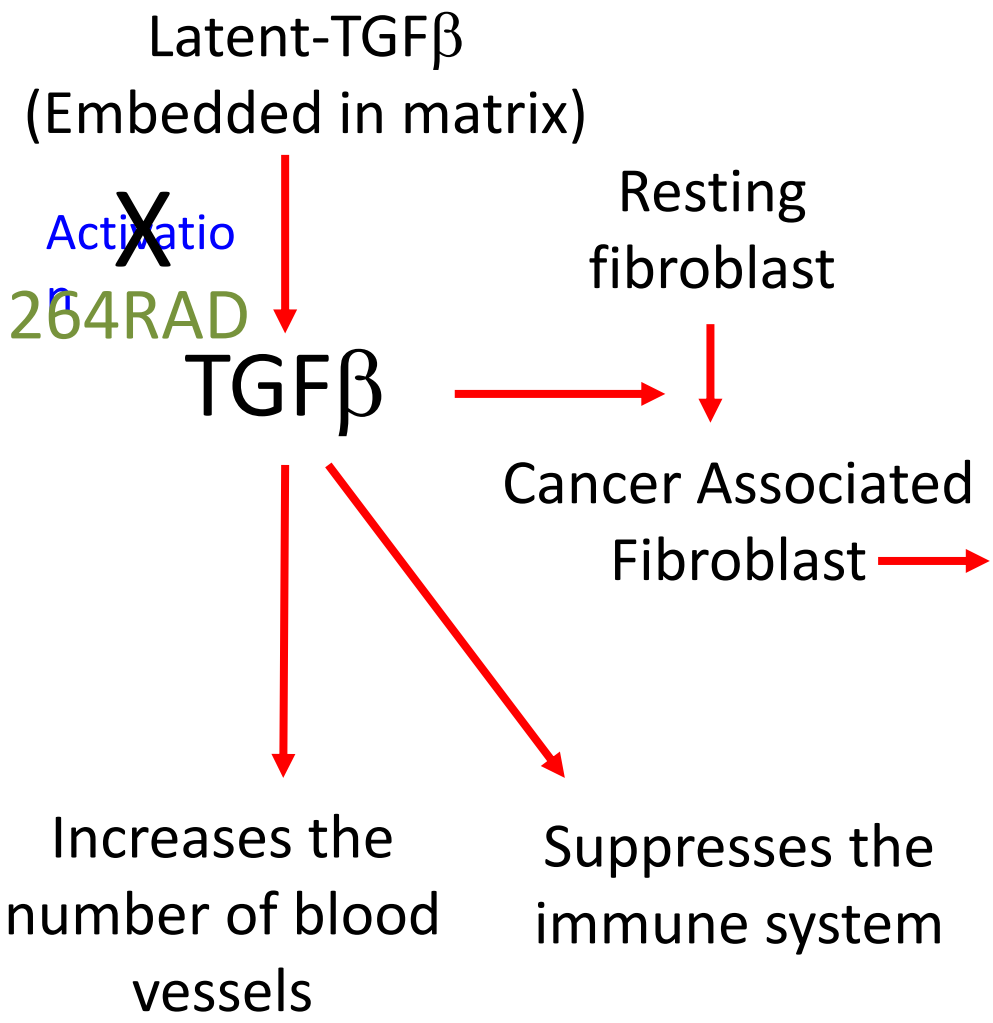
15 years ago…….what didn't know?

- Does Collagen VII do anything other than anchor the skin cells? YES!
- Can we correct the absence of collagen VII? YES!
- If we correct the absence of collagen VII, can we restore normal cell behaviour? YES!
- Is the chronic inflammation and fibrosis helping to promote the SCC? Very likely
- Are the SCC in RDEB patients different to SCC in non-EB patients? Yes but it's the microenvironment more than the cancer cells

The Future?


Latent-TGFβ
(Embedded in matrix)
Activation
TGFβ
Resting
fibroblast
X
Cancer Associated
Fibroblast
X
X
Losartan
Increases the
number of blood
vessels
Suppresses the
immune system
• Invade and remodels the tumour microenvironment
• Produces excess collagen – reason for fibrosis
• Contracts the matrix and makes it stiffer
• Releases proteins that help cancer cells grow and spread

The Future ?



- Invade and remodels the tumour microenvironment
- Produces excess collagen – reason for fibrosis
- Contracts the matrix and makes it stiffer
- Releases proteins that help cancer cells grow and spread

Acknowledgements

Andrew South (USA)
Edel O'Toole (London, UK)
Leen Bruckner-Tudermann (Frieburg, Germany)

Simon Barry (AstraZeneca-Medimmune)
Kate Moore (Barts Cancer Institute)

RDEB patients

ALL OF YOU FOR YOUR ATTENTION