資料 1

ヒト体性幹細胞に係る医学・生物学的安全性について

大和雅之

東京女子医科大学先端生命医科学研究所

GLOBAL COE TWMU MERCREM

CSTEC

CSTOF CELL SHEET-BASED TISSUE & ORGAN FACTORY

TWMU FOUNDED IN 1900

TWIns

1990 ■ 温度応答性培養表面の開発

2001 ■ 再生治療事業化のため大学発ベンチャー（株）セルシードを設立

2003 ■ 角膜上皮再生治療の臨床研究開始（阪大眼科）

2007 ■ 心筋再生治療の臨床研究開始（阪大第一外科）

■ 角膜上皮再生治療のフランス治験開始（セルシード）

2008 ■ 食道再生治療の臨床研究開始（東女医大消化器外科）

■ 温度応答性培養皿UpCell®の世界的販売開始（セルシード）

2010 ■ 角膜上皮再生治療フランス治験、経過観察終了（26例）

■ 食道再生治療の臨床研究、計画された10例を完了

2011 ■ 歯周再生治療、ヒト幹細胞臨床研究として承認・開始（東女医大歯科口腔外科）

■ 心筋再生治療の治験確認申請が承認（テルモ）

■ 角膜再生上皮細胞シートの販売承認を欧州医薬品庁に申請（セルシード）

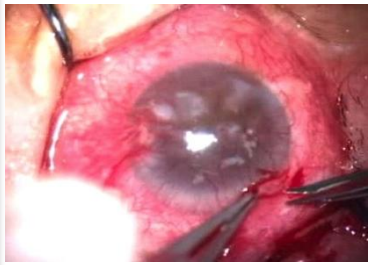
■ 軟骨再生治療、ヒト幹細胞臨床研究として承認・開始（東海大整形外科）

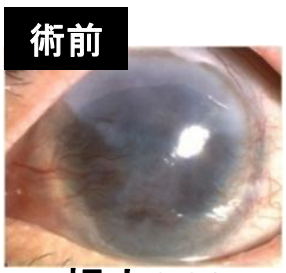
2012 ■ 心筋再生治療の治験開始（テルモ）

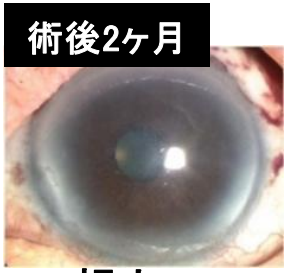
■ 食道再生治療の臨床研究、カロリンスカ研究所で開始

細胞シート再生治療 ①臨床研究・治験

角膜再生治療



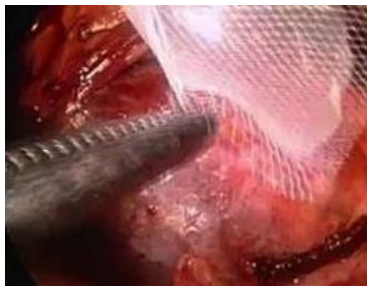


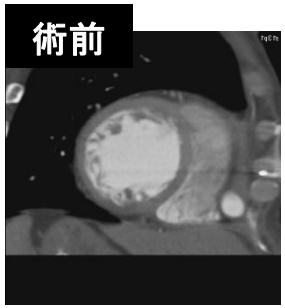


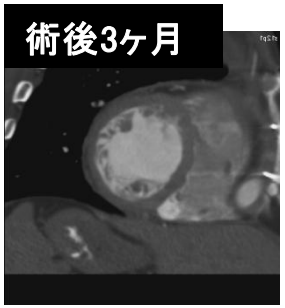
視力0.01　　視力0.7

- 2003年 臨床研究開始（阪大）
- 2007年 フランス治験開始（セルシード）
- 2010年 フランス治験25例経過観察終了
- 2011年 欧州医薬品庁に角膜シートの販売承認を申請（セルシード）

心筋再生治療



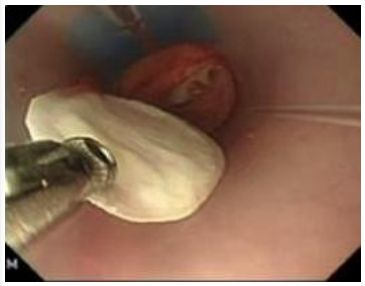


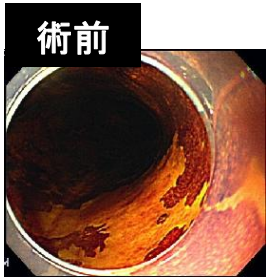


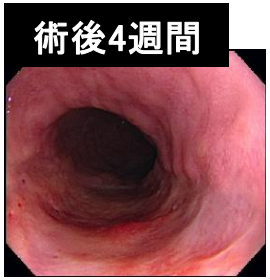
駆出率37%　　駆出率49%

- 2007年 臨床研究開始（阪大）
- 2012年 治験開始（テルモ）

食道再生治療

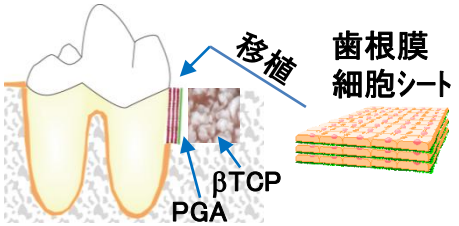


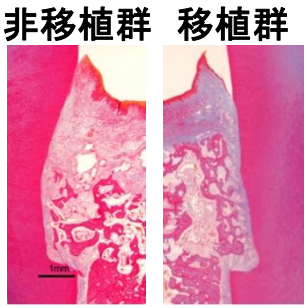




- 2008–2010年 臨床研究実施（10例，東女医大）
- 2010年 カロリンスカ医科大学との共同研究開始

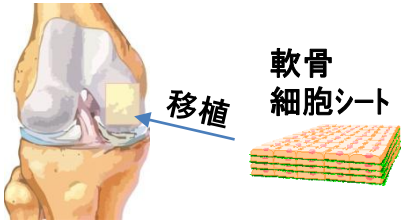
歯周再生治療

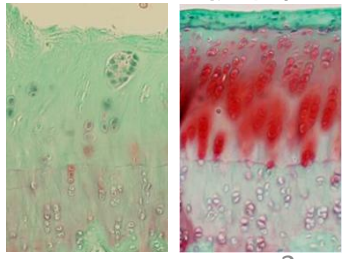




- 2011年 臨床研究開始（東女医大）

軟骨再生治療

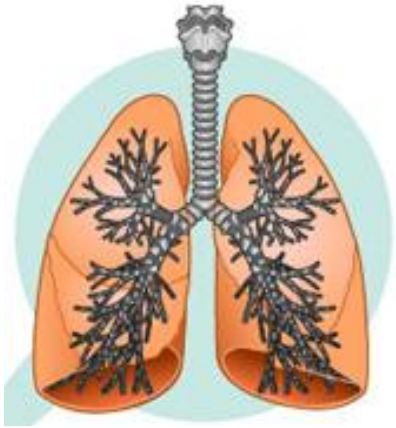




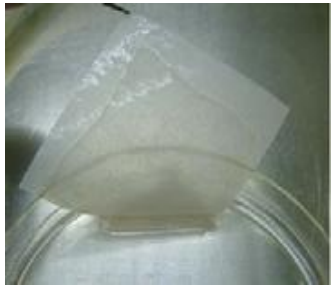
- 2011年 臨床研究開始（東海大）

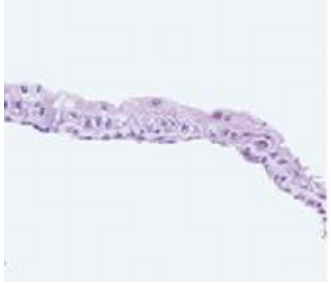
細胞シート再生治療 ②臨床研究準備中

肺
（東京女子医大胸部外科）

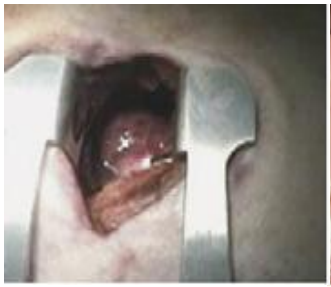


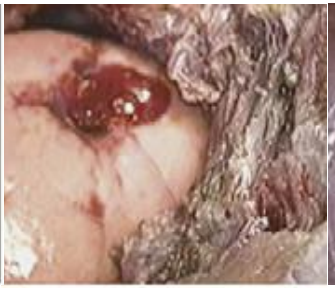
線維芽細胞シート

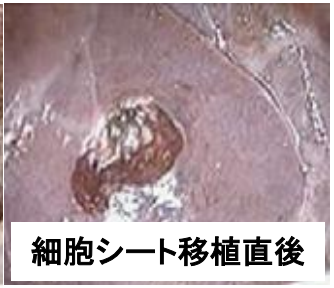


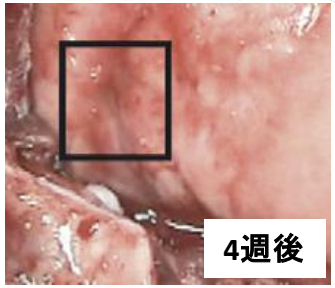


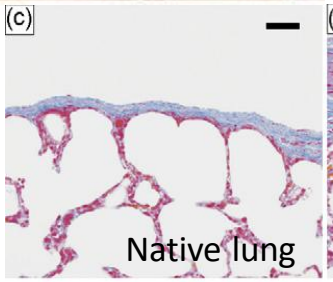
ブタ気胸モデル

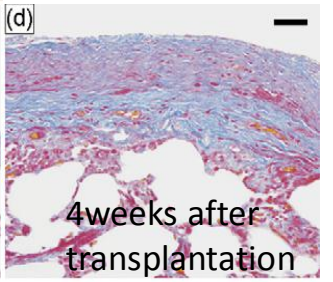




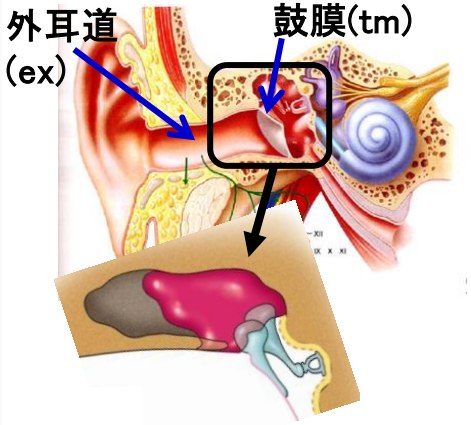




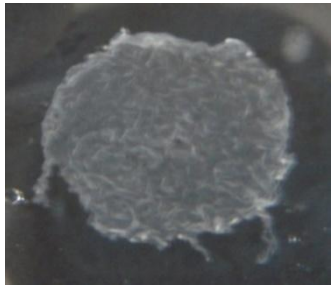


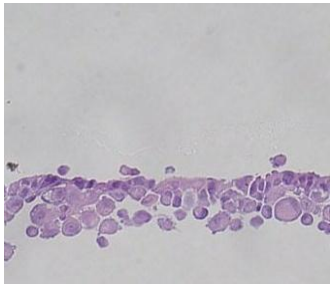


中耳
（慈恵医大耳鼻科）

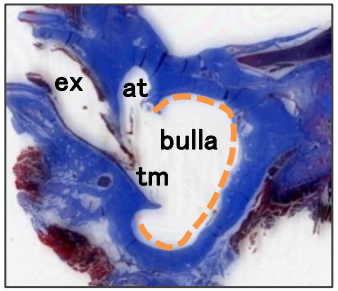


鼻粘膜上皮シート

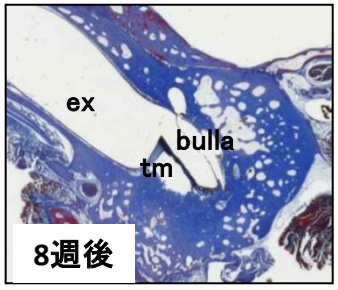




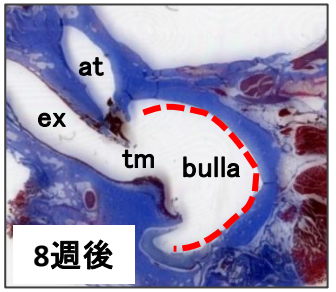
正常中耳骨胞



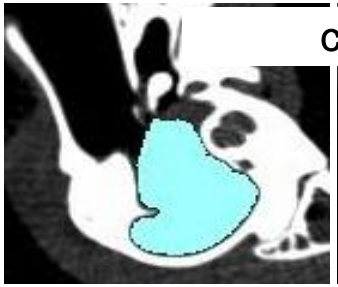
粘膜除去

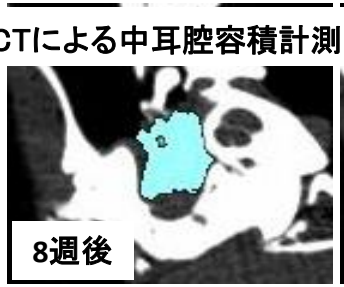


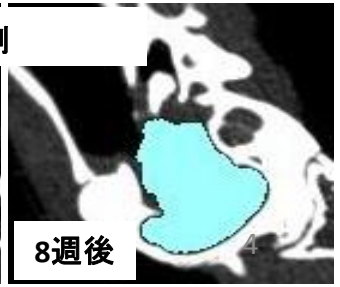
細胞シート移植



CTによる中耳腔容積計測





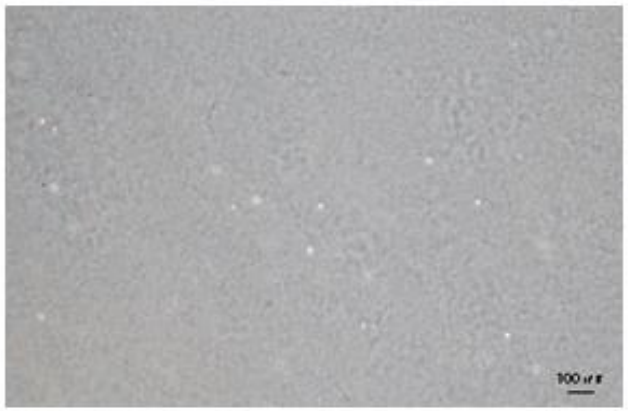


現行の造腫瘍性試験に意味があるのか？

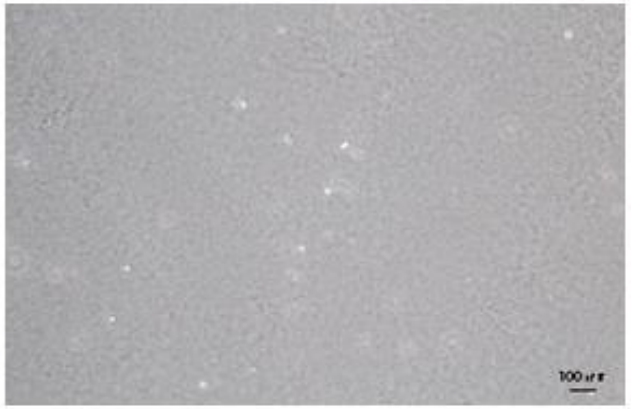
軟寒天培地培養（足場依存性増殖能）
免疫不全動物への移植
　nu/nu, SCID, NOG...
核型解析
CGH
抗がん遺伝子のメチル化
など

Tumorigenicity test (soft agar)

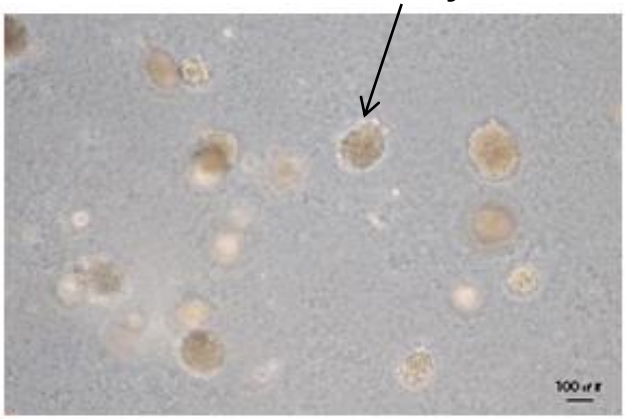
9000 cells/well were cultured in 0.5% agar for 7 days.



Negative control (hFibroblast)



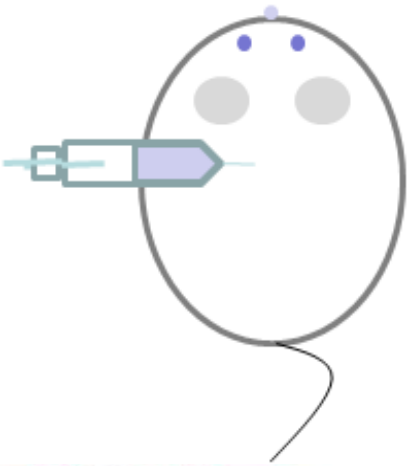
hPDL cells

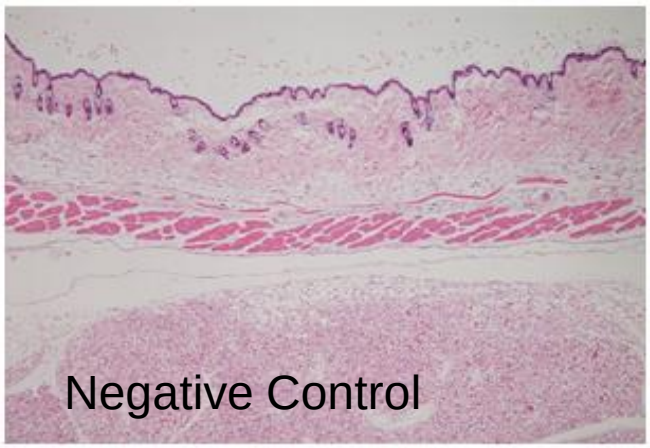


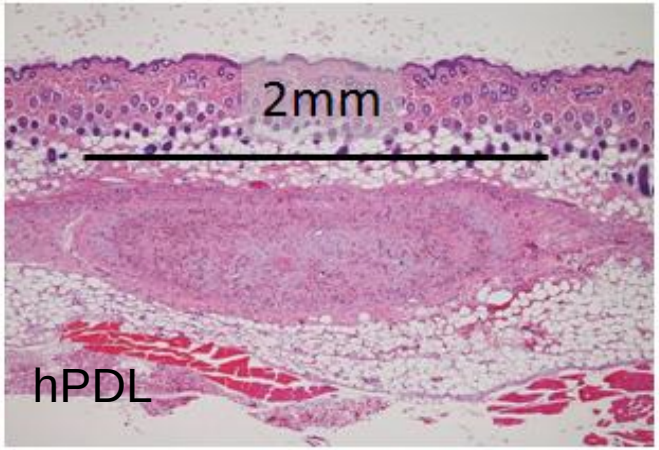
Positive control (HeLa)

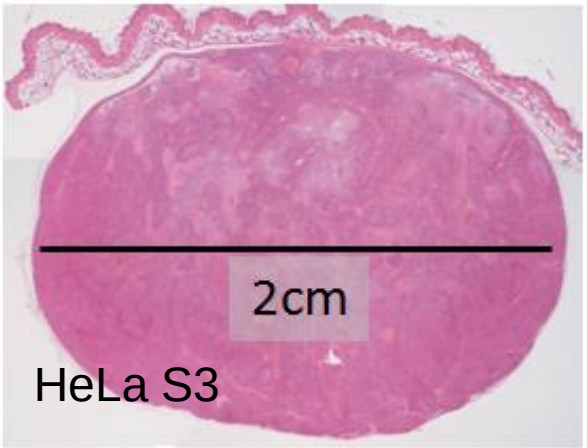
Tumorigenicity test (injection)

- $1x10^7$ cells/200 µL
  Positive control: HeLa S3
  Negative control: medium only









No tumor formation was observed after 4 and 12 weeks

Tumorigenicity test (injection)

hPDL injected mouse

There was no mass.

HeLa S3 injected mouse

Tumor mass was observed on the mice back.

そもそも…

ヒト培養細胞の無限寿命獲得は ほとんど生じない

Published March 1, 1988

Normal Keratinization in a Spontaneously Immortalized Aneuploid Human Keratinocyte Cell Line

Petra Boukamp, Rule T. Petrussevska, Dirk Breitkreutz, Jürgen Hornung, Alex Markham,* and Norbert E. Fusenig

Division of Differentiation and Carcinogenesis in Vitro, Institute of Biochemistry, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany; and *ICI Diagnostics, Gadbrook Park, Rudheath, Northwich Cheshire, England

The Journal of Cell Biology, Volume 106, March 1988 761–771

Table I. Adaptation of the HaCaT Cell Line to Autonomous Growth during In Vitro Propagation

Culture passages	Culture temperature	Cloning efficiency on plastic	Population doubling time	Cloning efficiency in soft agar	Tumorigenicity
n	°C	%	*h*	%*	
5	38.5	7.5	26 (0.2 mM Ca^{++})		
	37	0.9	50	ND	–
7	38.5	ND	38	ND	–
	37		39		
11	37	7.8	ND	ND	ND
15	37	10.8	22	–	ND
18	37	ND	ND	0.24	–
29	37	14.2	ND	0.27	–
37	37	13.3	23	0.27	–
48	37	13.2	23	0.36	–
81	37	ND	21	ND	–

* As tested in high Ca^{++} 4× MEM (1.4 mM) unless specified.
ND, not done.

Table II. Chromosomal Changes of HaCaT Cells during Adaptation to Autonomous Growth In Vitro

Passage no.	Numerical distribution (percent of metaphases)			Marker chromosomes* (percent of metaphases)				
	Diploid (46)	Hypodiploid (38–45)	Hypotetraploid (72–88)	M1	M2	M3‡	M4‡	M5-M8§
2	10	90	0	100	100	100	0	0
5	0	67	33	100	100	77	23	5
11	0	58	42	100	100	70	30	8
17	0	0	100	100	100	0	100	25
33	0	0	100	100	100	0	100	100‖
50	0	0	100	100	100	0	96	100¶

* M1 t(3,4)(qter → q11:q11 → qter), M2 i(9)(qter → q11:q11 → qter), M3 del(4)(q28 → qter), M4 (4,18)(pter → p11:q11 → qter).
‡ Metaphases contained only either M3 or M4 alternatively.
§ M5: dup(1)(q 23.1 → 25.3); M6: t(15,22); M7: dup(6)(p22); M8: dup(17)(q23.1 → q25.3).
‖ Present in 47, 30, 30, and 15% and
¶ Present in 50, 30, 25, and 25% of the metaphases, respectively.

PERMANENT COVERAGE OF LARGE BURN WOUNDS WITH AUTOLOGOUS CULTURED HUMAN EPITHELIUM

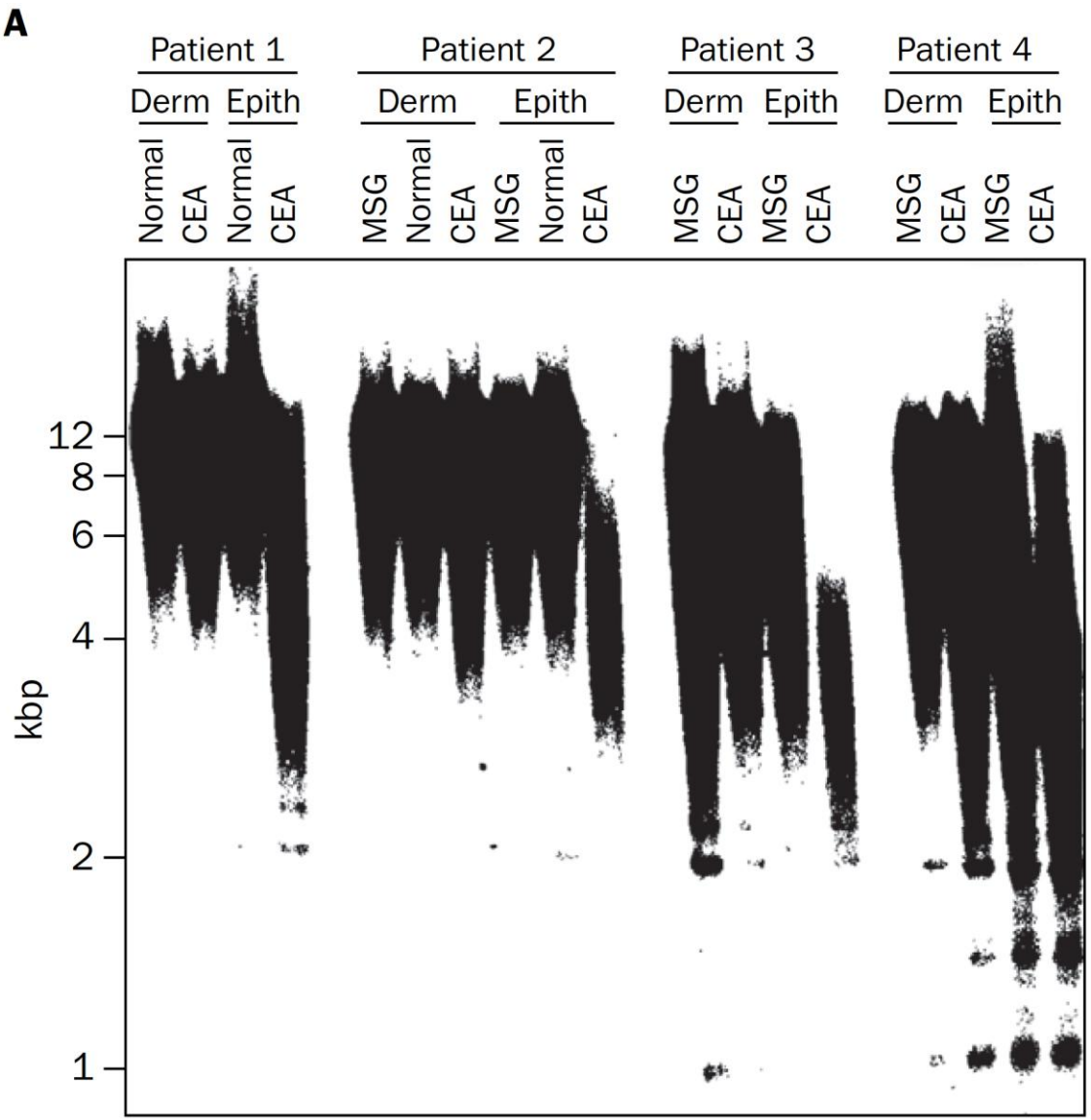
G. GREGORY GALLICO, III, M.D.,
NICHOLAS E. O'CONNOR, M.D.,
CAROLYN C. COMPTON, M.D.,
OLANIYI KEHINDE, B.A., AND HOWARD GREEN, M.D.

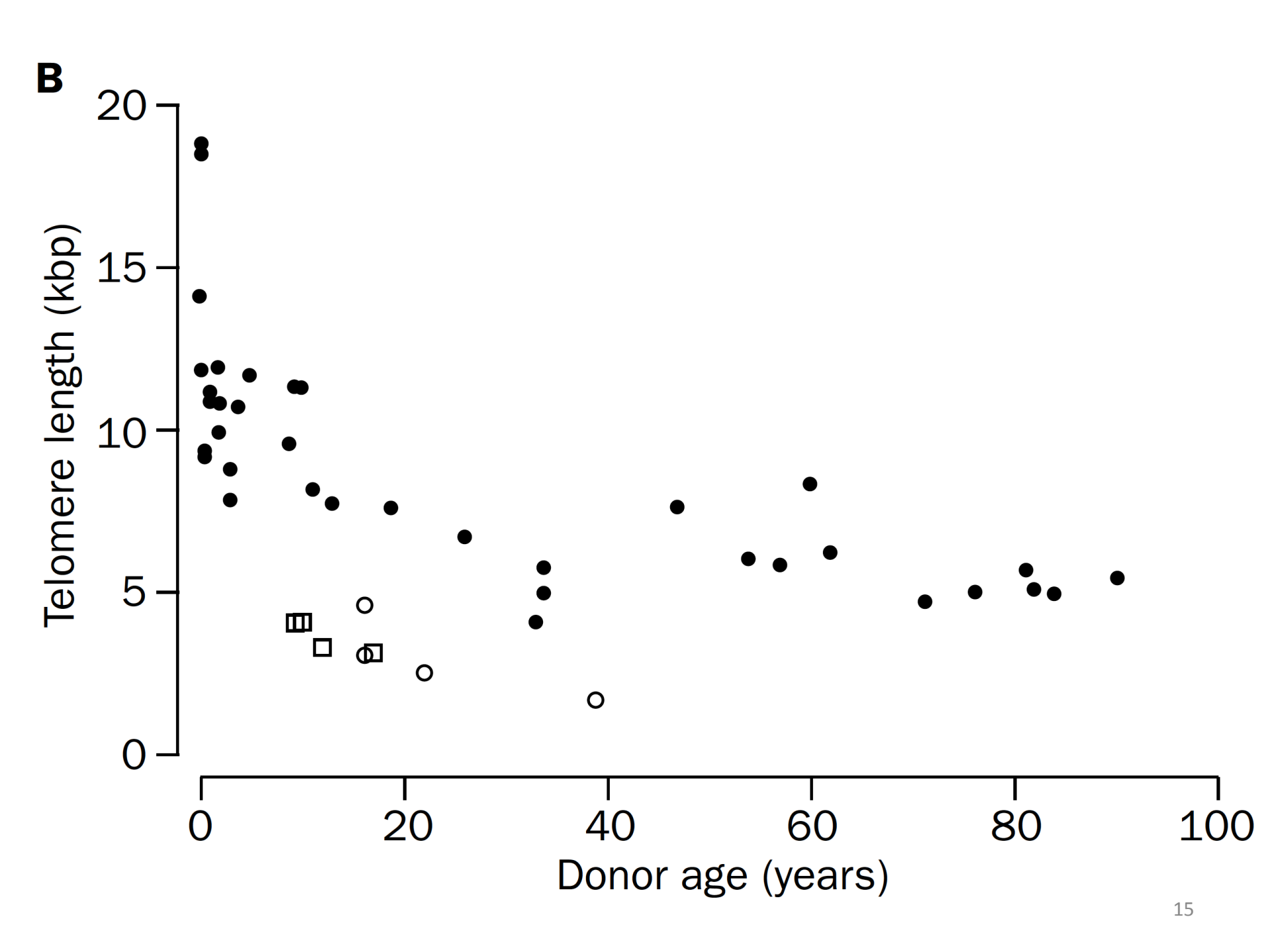
WHEN burns are so extensive that skin grafts obtainable from remaining donor sites are insufficient to provide wound coverage, a new source of autograft must be found. Human epidermal cells from a

Telomere shortening in cultured autografts of patients with burns

Christopher M Counter, William Press, Carolyn C Compton

Lancet 2003; **361:** 1345–46




B
Telomere length (kbp)
0
5
10
15
20
0
20
40
60
80
100
Donor age (years)

しかも、

無限寿命化はがん化の第一歩

がん化には無限寿命獲得後に多数の変異が必要

さらに、がんの悪性化にはさらなる変異が必要

ヒト間葉系幹細胞が
培養の間に自然にがん化！！

Priority Reports

Spontaneous Human Adult Stem Cell Transformation

Daniel Rubio,[1] Javier Garcia-Castro,[1,2] María C. Martín,[3] Ricardo de la Fuente,[1]
Juan C. Cigudosa,[3] Alison C. Lloyd,[4] and Antonio Bernad[1]

[1]Department of Immunology and Oncology, Centro Nacional de Biotecnología/Consejo Superior de Investigaciones Cien
UAM Campus de Cantoblanco; [2]Oncology Department, Hospital Universitario del Niño Jesús; [3]Cytogenetics Unit,
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Cancer Res 2005; 65: (8). April 15, 2005

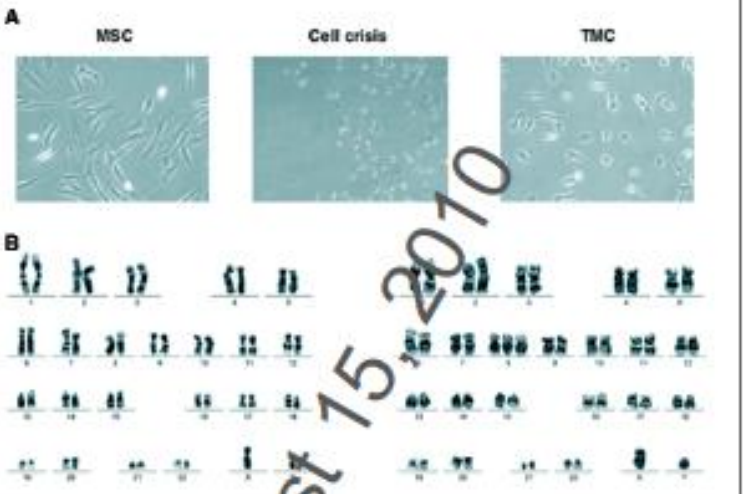


Figure 2. TMC characterization. *A*, evolution of MSC morphology during *in vitro* culture (*left* to *right*): MSC, cell crisis phase, and TMC. *B*, G-banded karyotype of a normal MSC line, 46,XY (*left*) and of a preclosis MSC line, 47,XY ((8) (*right*). *C*, G-banded karyotype of a TMC line, 45,XO, t(2;2)(p12;q37),del(5)(5;5)(p15;?),i(8)(q10), der(11)(3;11)(?;q25) (*left*); right, spectral karyotyping of the TMC line as in (*B*). Chromosome abnormalities (*small numbers at right of each chromosome*). *D*, characteristics associated with long-term MSC culture, including MSC-to-TMC transition and karyotypes. *ND*, not determined.

D

Sample	MSC-TMC transition	Post-senescence MSC	TMC karyotype
14	YES	46,XY, del(5)[5;5](p15;?), del(11)[3;11](?;q25)	81,XXYYYY, del(5)[5;5](p15;?), del(11)[3;11](?;q25)
14 EGFP	YES	ND	45,XO, del(5)[5;5](p15;?), del(11)[3;11](?;q25)
14 EGFP2	YES	47,XY ((8)	45,X0,t(2;2)(p12;q37), del(5)[5;5](p15;?), ((8)(q10), del(11)[3;11](?;q25)
16	NO	47,XY ((8)	—
16 EGFP	YES	47,XY ((8)	45,XO, del(5)[5;5](p15;?), del(11)[3;11](?;q25)
16 EGFP2	YES	ND	45,XY, del(5)[5;5](p15;?), del(11)[3;11](?;q25)
21	NO	46,XY	—
23	NO	46,XY	—
31	NO	ND	—
32	NO	ND	—
C57/BL6	YES	ND	ND
CD1	YES	ND	ND

ヒトがん細胞の
コンタミネーションだった！！！

Retraction

Retraction: Spontaneous Human Adult Stem Cell Transformation

The authors retract the article titled "Spontaneous Human Adult Stem Cell Transformation," which was published in the April 15, 2005, issue of *Cancer Research* (1). Upon review of the data published in this article, the authors have been unable to reproduce some of the reported spontaneous transformation events and suspect the phenomenon is due to a cross-contamination artifact. Five of the seven authors have agreed to the retraction of this paper.

ヒトとマウスの細胞はまったく違う

DNA複製時のエラーを修正する酵素
　マウスでは、生殖細胞のみで発現
　ヒトでは、ほとんどすべての体細胞で発現

マウスは1年以上の飼育でほぼ全個体でがんを認める
ヒト小児がんは決して多くない

多くの分子生物学者はマウス細胞を利用
　継代の間に高頻度に無限寿命化し
　細胞株ができる

一方、正常（二倍体）ヒト細胞の培養は
これまで老化研究で用いられてきた
　ヘイフリックの限界（細胞老化）

ヘイフリックの限界はテロメア、テロメレースにより分子的に説明される

正常ヒト細胞の無限寿命化はきわめて例外的

ヒト細胞の無限寿命化にはSV40遺伝子の導入や、化学的刺激などが必要

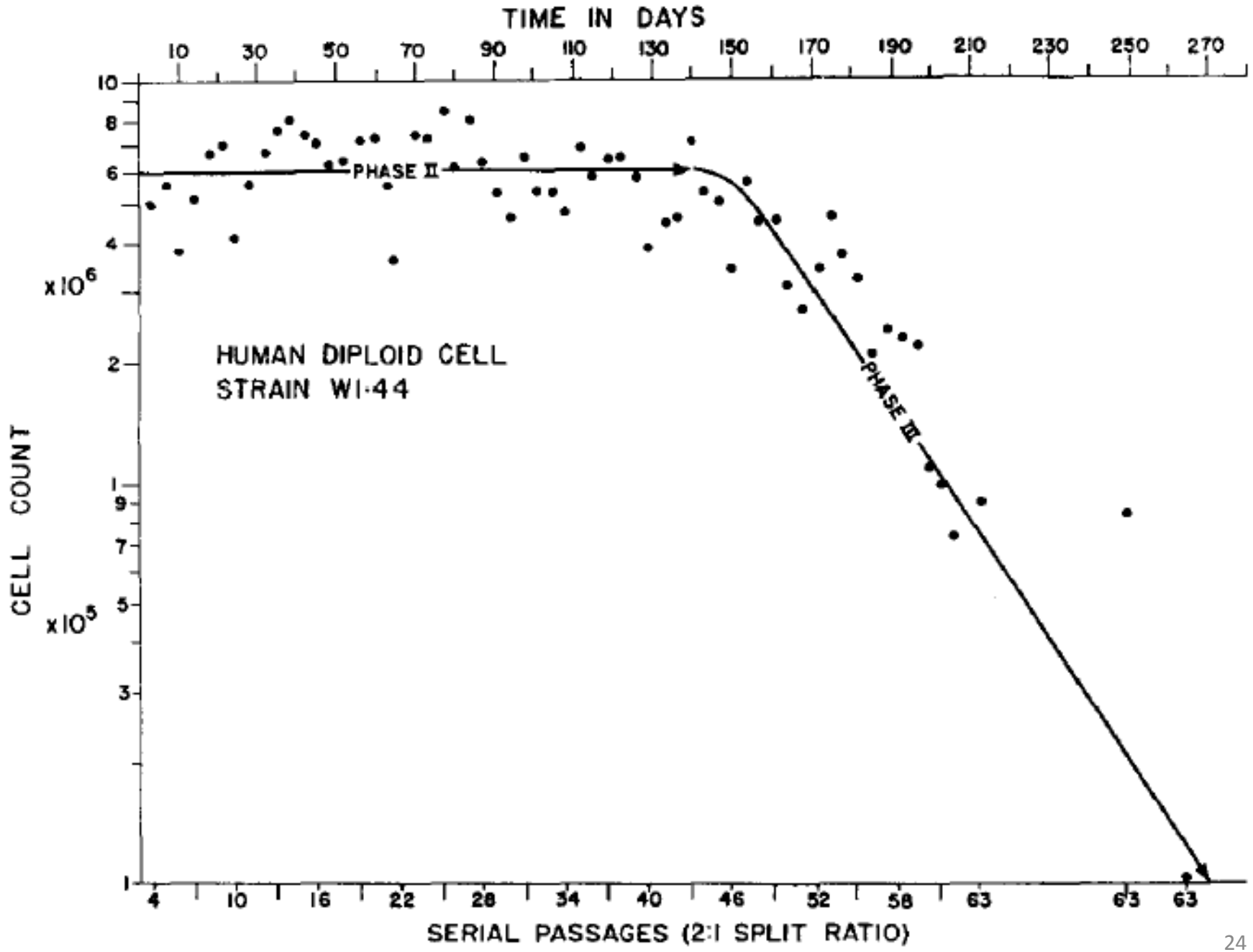
Experimental Cell Research **37**, *614–636 (1965)*

THE LIMITED *IN VITRO* LIFETIME OF HUMAN DIPLOID CELL STRAINS[1,2]

L. HAYFLICK

The Wistar Institute of Anatomy and Biology, Philadelphia, Pa., U.S.A.

Received May 4, 1964


TIME IN DAYS
10 30 50 70 90 110 130 150 170 190 210 230 250 270
PHASE II
PHASE III
HUMAN DIPLOID CELL
STRAIN WI-44
CELL COUNT
x10^6
x10^5
10 8 6 4 2 1 9 7 5 3 1
4 10 16 22 28 34 40 46 52 58 63 63 63
SERIAL PASSAGES (2:1 SPLIT RATIO)

Hayflick limit

From Wikipedia, the free encyclopedia

The **Hayflick limit** (or Hayflick Phenomenon) is the number of times a normal cell population will divide before it stops, presumably because the telomeres shorten to a critical length.[1][2]

The Hayflick limit was discovered by Leonard Hayflick in 1961,[1] at the Wistar Institute, Philadelphia, when Hayflick demonstrated that a population of normal human fetal cells in a cell culture divide between 40 and 60 times. It then enters a senescence phase (refuting the contention by Alexis Carrel that normal cells are immortal). Each mitosis shortens the telomeres on the DNA of the cell. Telomere shortening in humans eventually makes cell division impossible, and it is presumed to correlate with aging. Maintenance of the length of the telomeric region appears to prevent genomic instability and the development of cancer.

一方、ヒト生体内ではがん化は起きる

ヒト一個体中には60兆個の細胞
（生涯では数百兆個）

現在の日本人の寿命は約80年

日本人口の1/2でがんを発症

実際には40歳代で
ほぼ全員ががん細胞を有する
　→ドーマント

生体内でがん化が生じる理由

血液細胞は一日に1250億個作られる

皮膚表皮細胞は4週間でターンオーバー

便1gから検査に十分な数の細胞を採取可能
　→消化管上皮の旺盛なターンオーバー

UV、タバコ、アルコールなど多数のストレス
　→培養系には存在しない

細胞移植で腫瘍化が観察された症例は非常に少ない

OPEN ACCESS Freely available online

PLoS MEDICINE

Donor-Derived Brain Tumor Following Neural Stem Cell Transplantation in an Ataxia Telangiectasia Patient

Ninette Amariglio[1,2], Abraham Hirshberg[3], Bernd W. Scheithauer[4], Yoram Cohen[1], Ron Loewenthal[5], Luba Trakhtenbrot[2], Nurit Paz[1], Maya Koren-Michowitz[2], Dalia Waldman[6], Leonor Leider-Trejo[7], Amos Toren[6], Shlomi Constantini[8], Gideon Rechavi[1,6*]

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ロシアで中絶胎児の神経幹細胞を移植
ヒトでも成体細胞以外は慎重にすべき？

Published online 22 June 2010 | *Nature* **465**, 997 (2010) | doi:10.1038/465997a

News

Strange lesions after stem-cell therapy

Unproven treatment results in mysterious masses.

David Cyranoski

In a stark reminder that stem-cell therapy is uncharted territory, a stem-cell transplant given to a patient in Thailand who had kidney disease resulted in the development of cellular masses not previously reported. The lesions, described in a paper published online on 17 June in the *Journal of the American Society of Nephrology*, were not directly linked to the patient's subsequent death (D. Thirabanjasak *et al. J. Am. Soc. Nephrol.* **doi:10.1681/ASN.2009111156;** 2010).

With hundreds of poorly regulated clinics that offer unproven stem-cell therapies now running, notably in China and Thailand, the episode is a warning to patients who may be considering such treatment.

CLINICAL RESEARCH www.jasn.org

Angiomyeloproliferative Lesions Following Autologous Stem Cell Therapy

Duangpen Thirabanjasak,* Kavirach Tantiwongse,† and Paul Scott Thorner*‡§

Departments of *Pathology and †Surgery, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; ‡Division of Pathology, Hospital for Sick Children, Toronto, Canada; and §Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

ABSTRACT

Some reports suggest that autologous hematopoietic stem cell transplantation holds potential for treatment of renal diseases such as lupus nephritis, but the safety of delivering various stem cell types (hematopoietic, mesenchymal, and endothelial precursors) is not well established. Here, we report a case of lupus nephritis treated by direct renal injection of autologous stem cells recovered from peripheral blood. The patient developed masses at the sites of injection and hematuria. We suspected transitional cell carcinoma but nephrectomy revealed that the masses were angiomyeloproliferative lesions. We believe that this previously undescribed pathologic entity is stem cell–derived or –induced. The biologic potential, including the neoplastic potential, of this lesion is unknown. This case illustrates that the development of angiomyeloproliferative lesions is a possible complication of stem cell therapy.

J Am Soc Nephrol 21: 1218–1222, 2010. doi: 10.1681/ASN.2009111156

J Am Soc Nephrol 21: 1218–1222, 2010. doi: 10.1681/ASN.2009111156

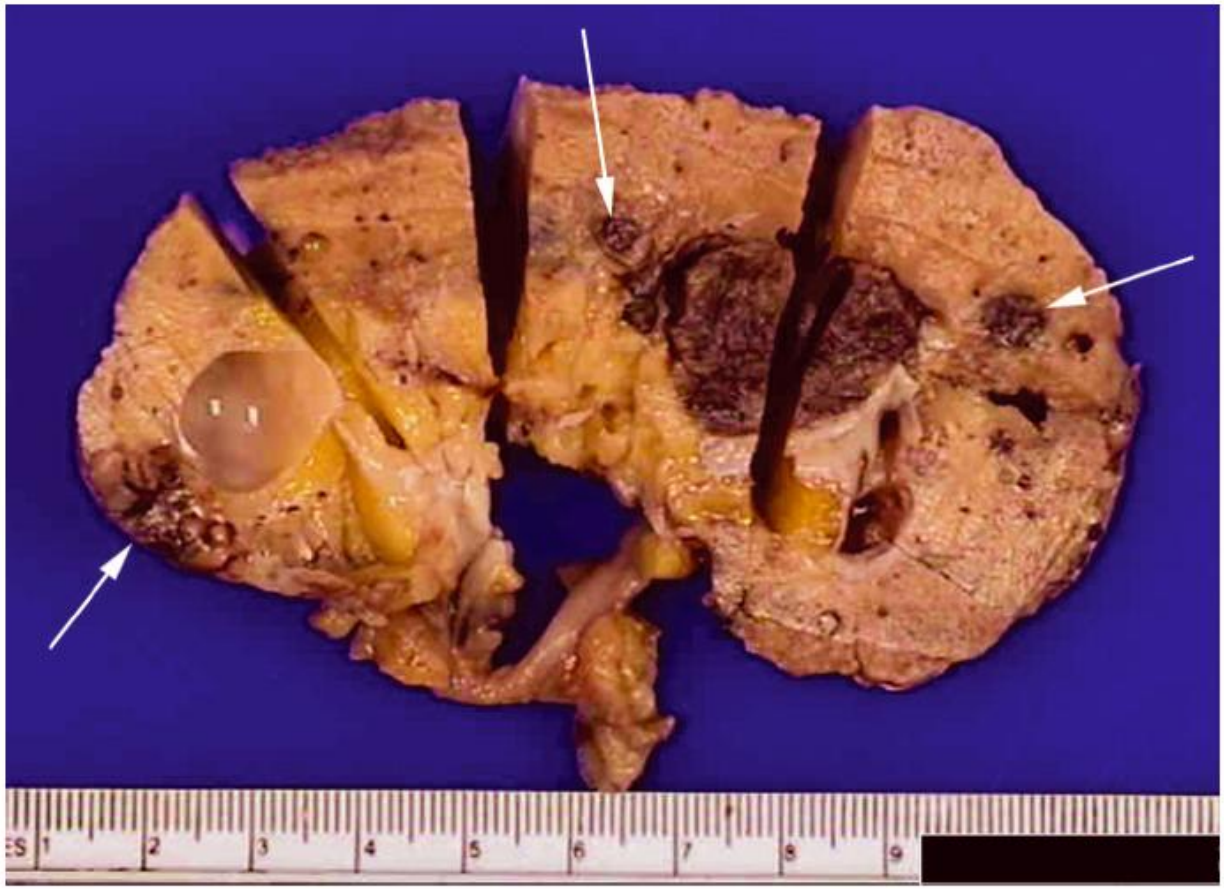

Figure 1. Macroscopic appearance of resected kidney. A solid hemorrhagic mass is present in the renal sinus, external to which is atrophic renal parenchyma. In addition, three similar smaller lesions are present (arrows), separate from the main lesion.

類似の見解を示す学者は他にもいます

editorial

doi:10.1038/mt.2010.99

Molecular Therapy

Defining the Probability that a Cell Therapy Will Produce a Malignancy

Darwin J Prockop
Associate Editor

Molecular Therapy vol. 18 no. 7 july 2010

欧米で承認済みのヒト体細胞加工製品の造腫瘍性試験
（それぞれの審査概要から抽出・整理）

	製品名	細胞／足場材料	適　用	造腫瘍性試験			核型分析	免疫不全動物を用いた他の試験（動物）	備考
				in vivo（動物）	軟寒天コロニー形成試験	細胞増殖特性解析			
USA	Carticel	自己軟骨細胞	軟骨損傷						
	Provenge	自己樹状細胞（PAP抗原提示）	転移性前立腺がん						「自己由来製品なので」非臨床安全性試験なし
	laViv (azficel-T)	自己線維芽細胞	ほうれい線解消（美容整形）						「ヒトでの経験が豊富なので」非臨床試験なし、なお臨床試験中に腫瘍形成1例
	HemaCord（HPC−C）	同種臍帯血造血前駆細胞	造血幹細胞移植			○			Colony forming unit測定
	Epicel	自己角化細胞／マウス細胞層	熱傷	○（ヌードマウス）	○		○	○（ヌードマウス）	ヌードマウス・軟寒天ともに陰性
	Apligraf (Grafskin)	同種角化細胞＋同種線維芽細胞／ウシ由来コラーゲン	皮膚潰瘍				○	○（hu-SCIDマウス）	
	TransCyte (Dermagraft-TC)	同種線維芽細胞／ナイロン基材	熱傷		○			○（ヌードマウス）	軟寒天で陰性
	Dermagraft	同種線維芽細胞／ポリグラクチンメッシュ	皮膚潰瘍	○（ヌードマウス）			○	○（ヌードマウス）	ヌードマウスで陰性
	OrCel	同種角化細胞＋同種線維芽細胞／ウシ由来コラーゲン	熱傷 表皮水疱症					○（SCIDマウス，ヌードマウス）	
EU	ChondroCelect	自己軟骨細胞	軟骨損傷			○		○（ヌードマウス）	既定期間を越えた培養で細胞老化確認