

遺伝子治療の本格的幕開け



小澤 敬也

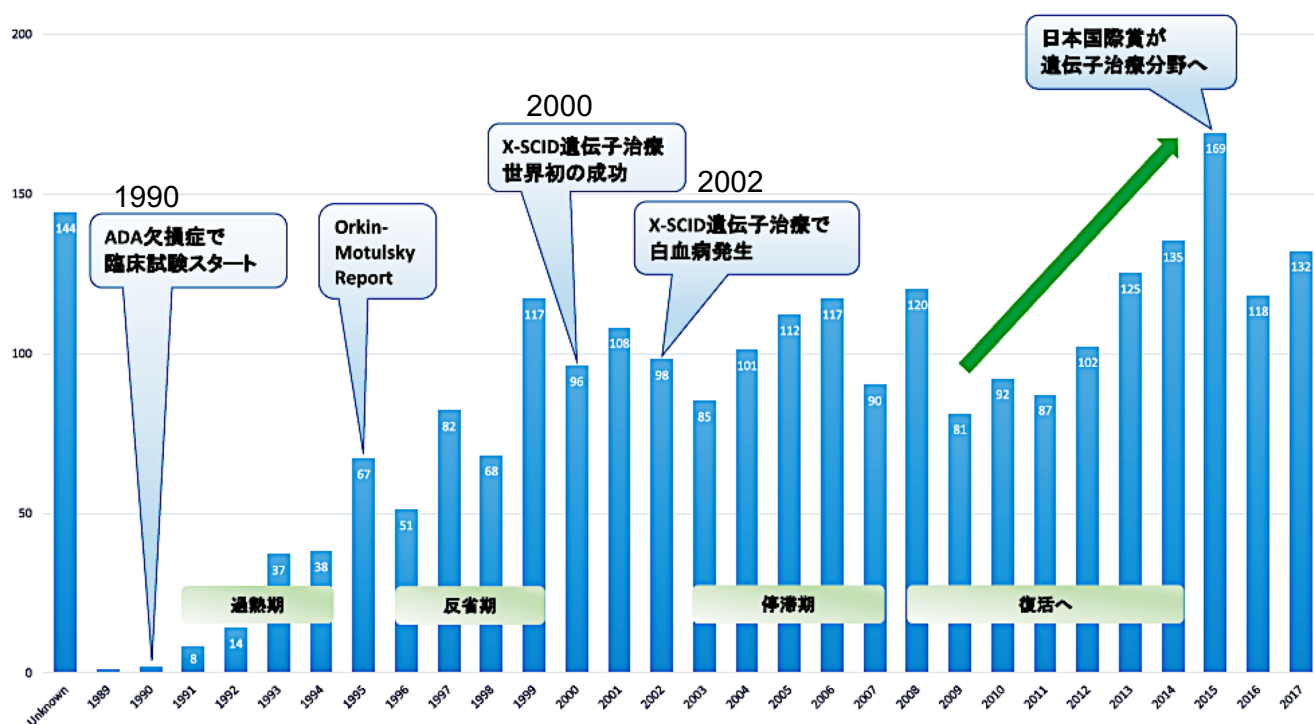
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免疫遺伝子細胞治療学(タカラバイオ)講座
遺伝子治療研究センター(CGTR)
シニアアドバイザー

講演内容

1. 遺伝子治療の動向
2. AAVベクターを用いた遺伝子治療
3. がんに対する遺伝子治療
—CAR-T細胞療法を中心に—
4. ゲノム編集治療
5. まとめ

遺伝子治療の動向

遺伝子治療臨床研究のプロトコル数（全世界1989-2017）



（資料）The Journal of Gene Medicine, © 2017 John Wiley and Sons Ltd (www.abedia.com/wiley/years.php) より作成

遺伝子治療の最近の動向：主な成功例

造血幹細胞遺伝子治療

X-SCID（初期に白血病発生 5 例-死亡 1 例；その後は順調）
ADA欠損症（全例生存、無病生存率80%、**Strimvelis**が欧州で2016年に承認）
Wiskott-Aldrich症候群（初期に白血病発生；その後は順調）
β-サラセミア（**Zynteglo**が欧州で2019年に承認）
Adrenoleukodystrophy（ALD：副腎白質ジストロフィー）
Metachromatic leukodystrophy（MLD：異染性白質ジストロフィー）
（レトロウイルスベクターからレンチウイルスベクターへ）

AAVベクター遺伝子治療

リポタンパク質リパーゼ欠損症
（2012年に**Glybera**が欧州で初めて承認；2017年終了）
レーバー先天性黒内障（2017. 12 **Luxturna** FDA承認）
コロイデレミア（網脈絡膜変性疾患）
パーキンソン病（日本では6例実施、その後 2015年再開）
AADC欠損症（台湾でスタート；その後、日本では 2015年から実施）
血友病 A, B（静脈注射で効果）
脊髄性筋萎縮症（SMA）（静脈注射で効果、2019. 5 **Zolgensma** FDA承認）

キメラ抗原受容体(CAR)を用いたがん遺伝子治療

B細胞性腫瘍（ALL、CLL、悪性リンパ腫）（1000例以上）

2017年
FDA承認 **Kymriah**
Yescarta

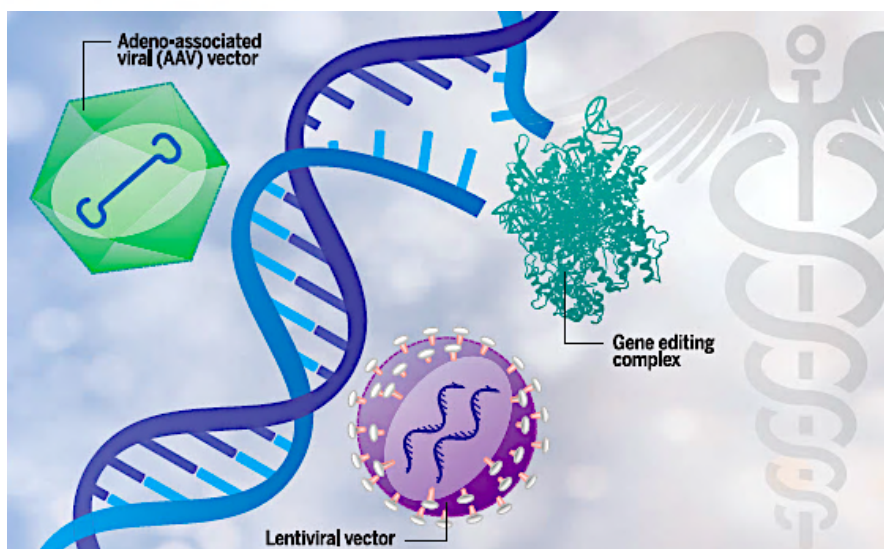
REVIEW

Science 359, eaan4672 (2018) 12 January 2018

MEDICINE

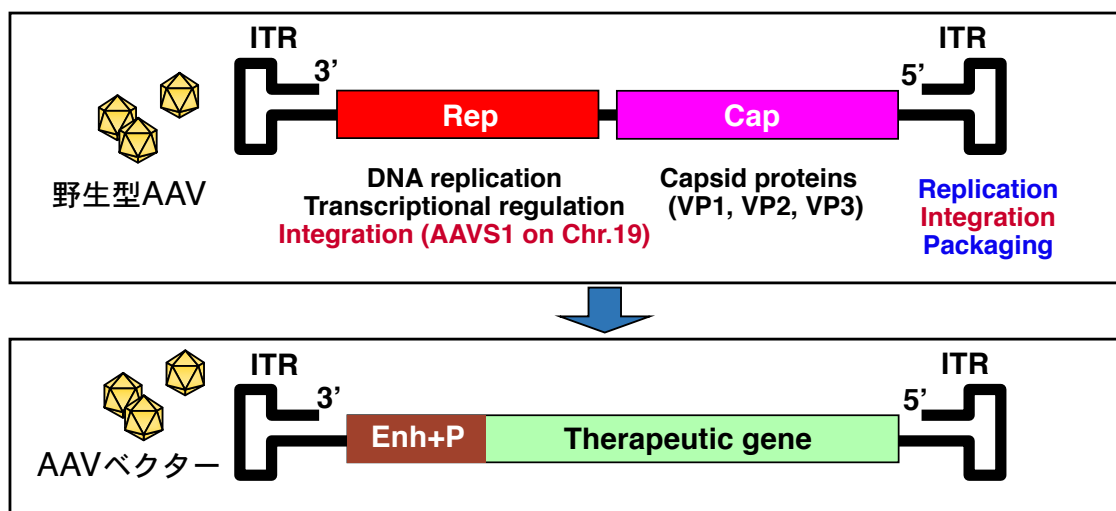
Gene therapy comes of age

Cynthia E. Dunbar,^{1*} Katherine A. High,² J. Keith Joung,³ Donald B. Kohn,⁴
Kei-ya Ozawa,⁵ Michel Sadelain^{6*}



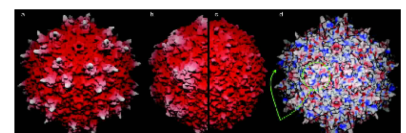
AAVベクターを用いた 遺伝子治療

AAV (adeno-associated virus) ベクター

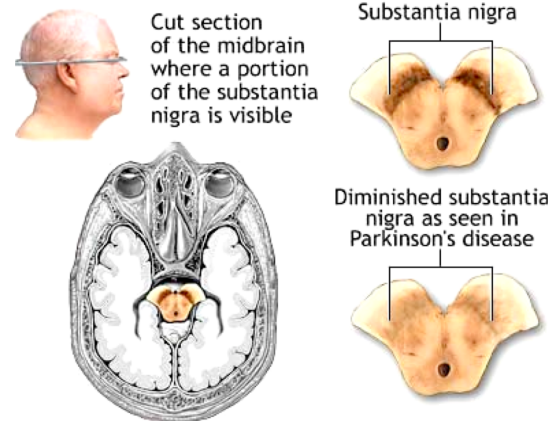
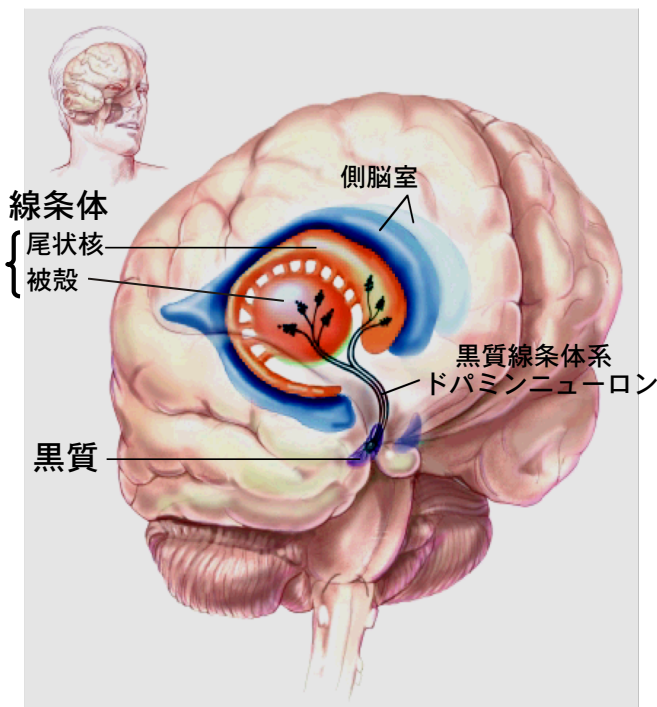


- 非病原性のアデノ随伴ウイルスに由来
- 非分裂細胞（神経細胞、網膜細胞、筋細胞、肝細胞など）へ遺伝子導入可能
- 非分裂細胞では遺伝子発現が長期間持続する
- 標的細胞のゲノムへの組込みが起こらない
- 様々な血清型が存在
- 搭載できる遺伝子のサイズが限定される
- 重複感染可能（複数の遺伝子を別々のベクターで）

↑
20~30 nm
↓



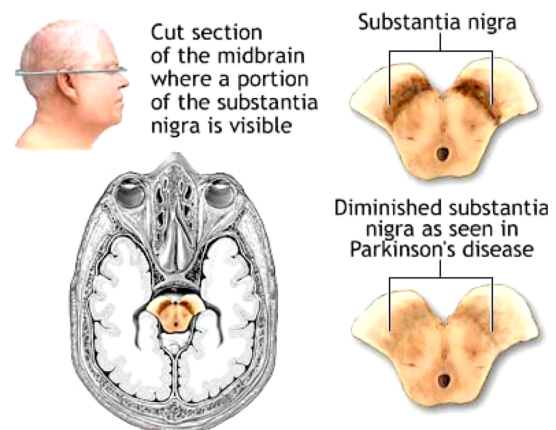
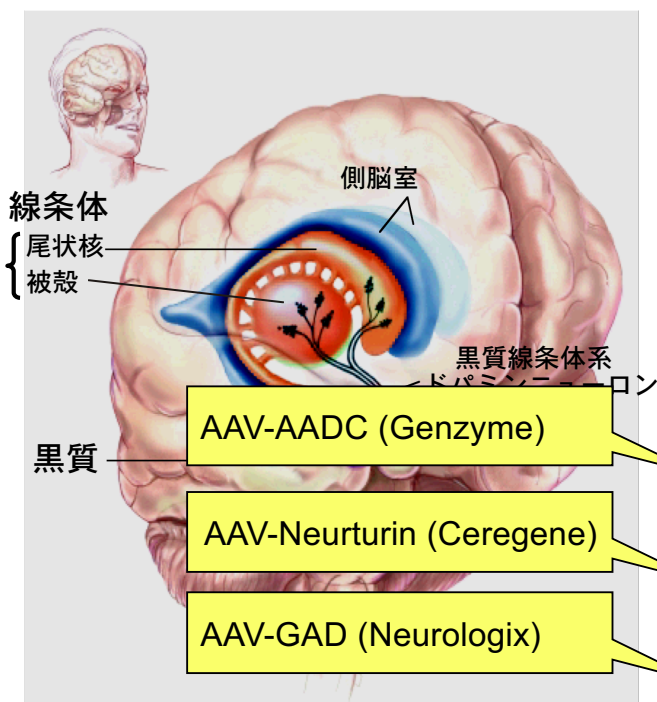
パーキンソン病 黒質線条体系ドパミンニューロンの選択的変性



遺伝子治療のストラテジー

- **ドパミン合成酵素遺伝子治療**
(ドパミン補充)
- **GDNF遺伝子治療**
(神経細胞死防御：病態進行阻止)
- **GAD遺伝子治療**
(視床下核神経細胞の活動を抑制)

パーキンソン病 黒質線条体系ドパミンニューロンの選択的変性



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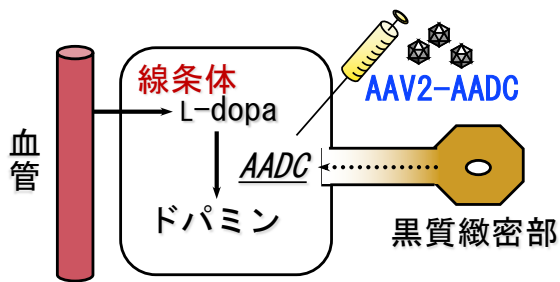
パーキンソン病に対する遺伝子治療の臨床研究

課題名：AADC発現AAVベクター線条体内投与による
重症パーキンソン病遺伝子治療の臨床研究
総括責任者：故中野今治 ⇨ 村松慎一（自治医科大学）

第1段階 AAV2-AADCの被殻への注入とL-dopa内服療法の併用

対象患者：L-dopaの効果が減弱してきた患者

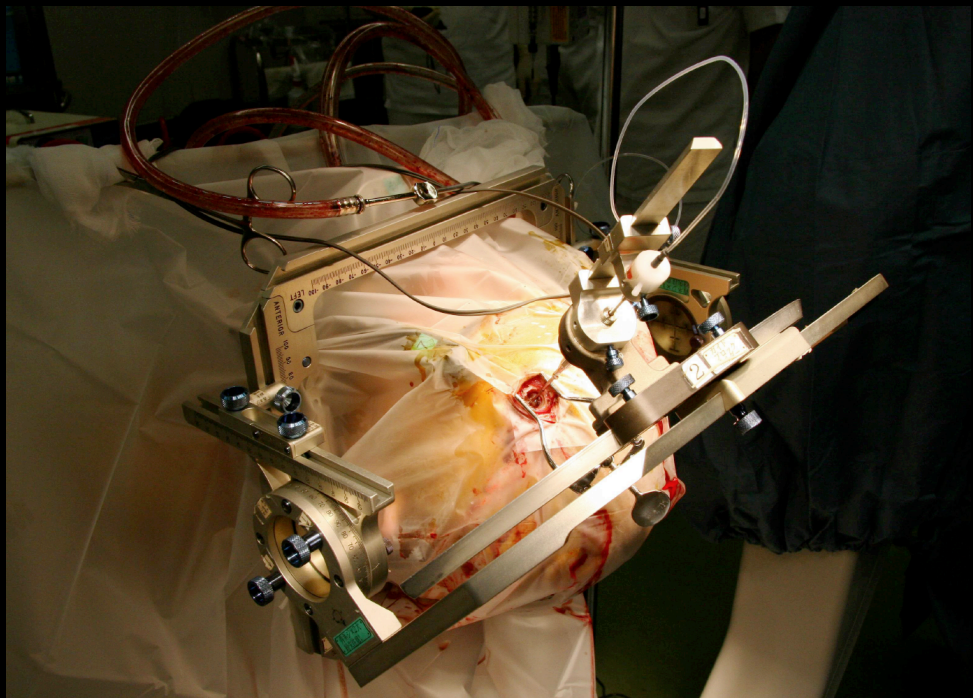
利点：ドパミン産生量をL-dopaの投与量で調節可能，安全性が高い



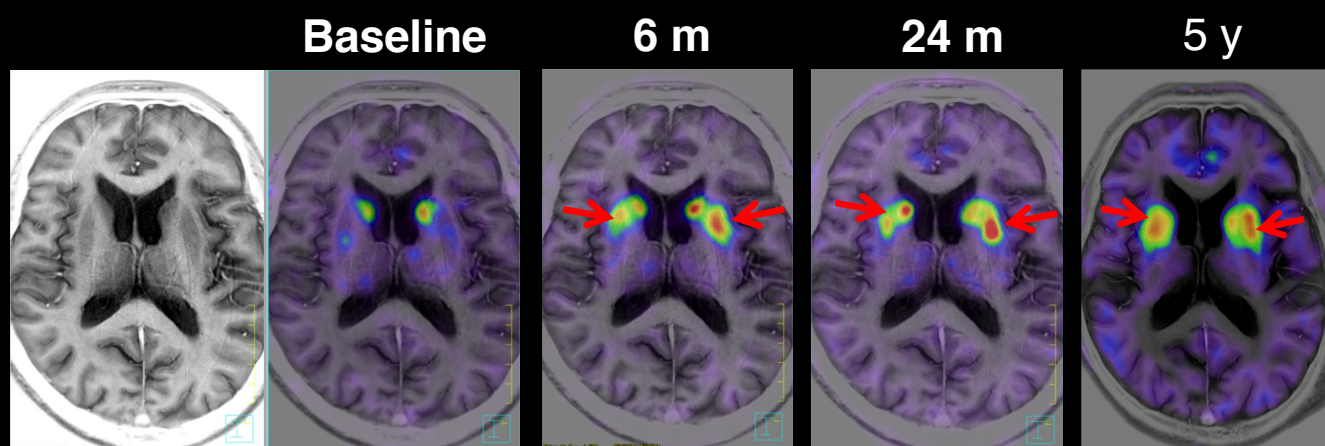
- ・パーキンソン病モデルサルにおいて
運動障害改善，副作用（－）
- ・2004年12月：UCSFで臨床研究開始
- ・2005年 5月：自治医大倫理委員会承認
- ・2006年10月：厚生労働省/環境省承認
- ・2007年 5月：臨床研究開始

AADC発現2型AAVベクターの線条体内投与による パーキンソン病に対する遺伝子治療

定位脳手術で線条体にAAVベクターを注入



FMT-PET Patient A-2



FMT ([¹⁸F]6-fluoro-m-tyrosine): Substrate of AADC

AADC欠損症に対する遺伝子治療

<Patients>

- About 140 in the world
- About 40 in Taiwan
- 8 patients in Japan

<2009 – 2014 >

16 patients (2 to 8 years of age)
Most of the patients can sit, and
some patients can walk with support.

Hwu WL, et al. Sci Transl Med. 2012



Before Gene Tx
(4Y 3M old)



28M after Gene Tx
(6Y 7M)

Japanese patients with AADC deficiency

- Patient 1) 15-year-old male
 - Patient 2) 12-year-old female
 - Patient 3) 5-year-old female
 - Patient 4) 19-year-old male
 - Patient 6) 4-year-old male
- siblings

Takanori Yamagata
Shin-ichi Muramatsu
(Jichi Medical University)

Gene therapy:

- Patient 1 on June 29, 2015
- Patient 2 on July 27, 2015
- Patient 3 on Nov 16, 2015
- Patient 4 on Jan 25, 2016
- Patient 6 on July 10, 2017

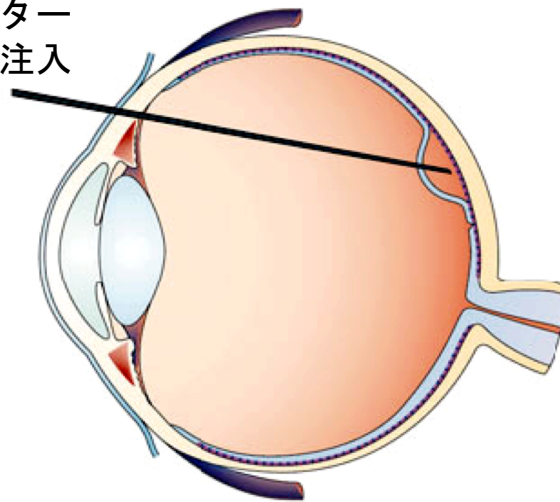
レーバー先天性黒内障に対する遺伝子治療

Lancet. 2009 Nov 7;374(9701):1597-605.

Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial.

Maguire AM, High KA, Auricchio A, Wright JF, Pierce EA, Testa F, Mingozzi F, Bennicelli JL, Ying GS, Rossi S, Fulton A, Marshall KA, Banfi S, Chung DC, Morgan JJ, Hauck B, Zelenia O, Zhu X, Raffini L, Coppieters F, De Baere E, Shindler KS, Volpe NJ, Surace EM, Acerra C, Lyubarsky A, Redmond TM, Stone E, Sun J, McDonnell JW, Leroy BP, Simonelli F, Bennett J.

治療遺伝子搭載AAVベクター
(AAV2-RPE65) の網膜下注入



The NEW ENGLAND
JOURNAL of MEDICINE

ORIGINAL ARTICLE

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

Lindsey A. George, M.D., Spencer K. Sullivan, M.D., Adam Giermasz, M.D., Ph.D., John E.J. Rasko, M.B., B.S., Ph.D., Benjamin J. Samelson-Jones, M.D., Ph.D., Jonathan Ducore, M.D., M.P.H., Adam Cuker, M.D., Lisa M. Sullivan, M.D., Suvankar Majumdar, M.D., Jerome Teitel, M.D., Catherine E. McGuinn, M.D., Margaret V. Ragni, M.D., M.P.H., Alvin Y. Luk, Ph.D., Daniel Hui, Ph.D., J. Fraser Wright, Ph.D., Yifeng Chen, M.D., Yun Liu, Ph.D., Katie Wachtel, M.S., Angela Winters, M.P.H., Stefan Tiefenbacher, Ph.D., Valder R. Arruda, M.D., Ph.D., Johannes C.M. van der Loo, Ph.D., Olga Zelenia, Ph.D., Daniel Takefman, Ph.D., Marcus E. Carr, M.D., Ph.D., Linda B. Couto, Ph.D., Xavier M. Anguela, Ph.D., and Katherine A. High, M.D.

N Engl J Med 2017; 377:2215-2227

December 7, 2017

DOI: 10.1056/NEJMoa1708538



脊髄性筋萎縮症 (SMA: Spinal Muscular Atrophy) に対する遺伝子治療



- 6.7×10^{13} vg/kg of AVXS-101 delivered one-time through a venous catheter inserted into a peripheral vein (n=3)
- 2.0×10^{14} vg/kg of AVXS-101 delivered one-time through a venous catheter inserted into a peripheral vein (n=12)

Self-complementary AAV9 carrying the SMN gene under the control of a hybrid CMV enhancer/chicken- β -actin promoter

N Engl J Med 2017; 377:1713–1722

Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy

J.R. Mendell, S. Al-Zaidy, R. Shell, W.D. Arnold, L.R. Rodino-Klapac, T.W. Prior, L. Lowes, L. Alfano, K. Berry, K. Church, J.T. Kissel, S. Nagendran, J. L'Italien, D.M. Sproule, C. Wells, J.A. Cardenas, M.D. Heitzer, A. Kaspar, S. Corcoran, L. Braun, S. Likhite, C. Miranda, K. Meyer, K.D. Foust, A.H.M. Burghes, and B.K. Kaspar

《参考》

平成30年3月27日

AVXS-101 (AveXis, Inc.)

「先駆け審査指定制度」の対象品目に指定

AAVベクターを用いた遺伝子治療

<局所投与>

- ❖ パーキンソン病、AADC欠損症
- ❖ 網膜疾患

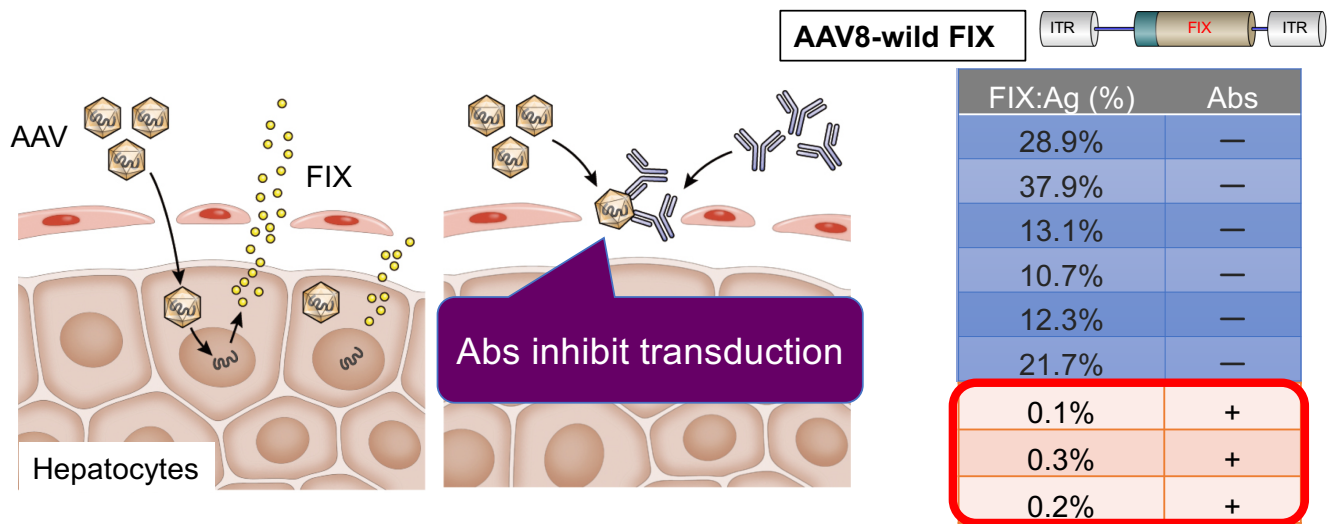
<静脈注射、（門脈投与、髄注）>

- ❖ 血友病
- ❖ 脊髄性筋萎縮症（SMA）

但し、静脈注射の場合、

- 1) 莫大な量のAAVベクターが必要
- 2) 中和抗体陽性の患者では効果なし

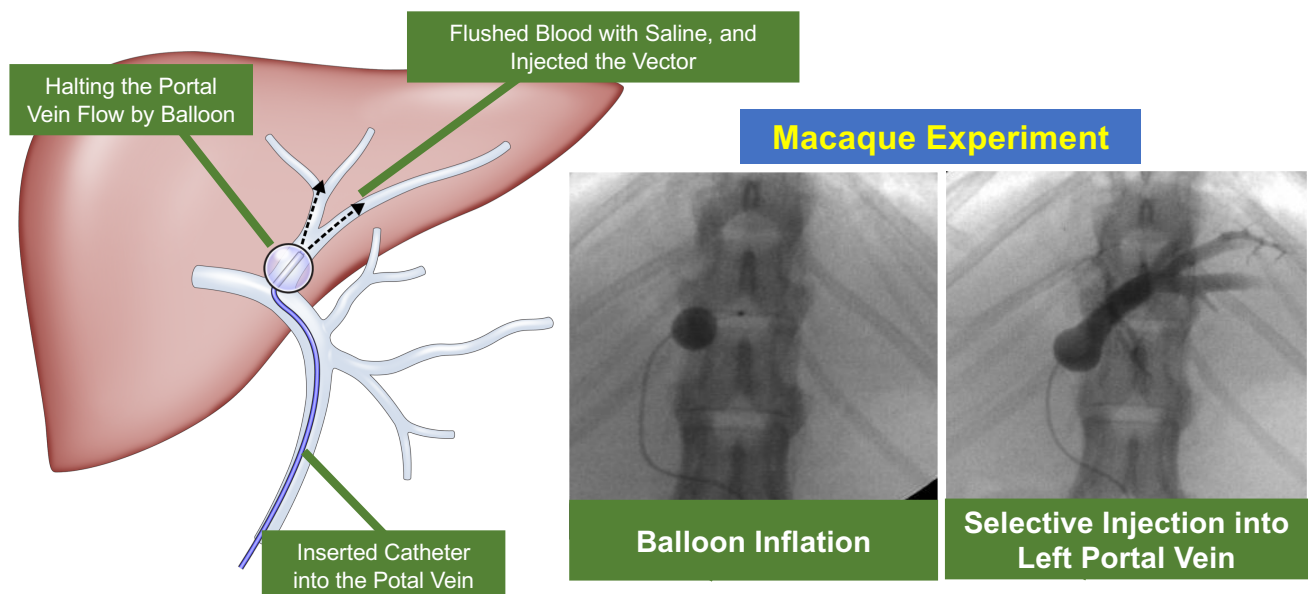
Neutralizing Antibody Prevents Vector Transduction in Macaques



(Mimuro J, Mizukami H, et al. Mol Ther, 2013)

大森 司 博士 (自治医大) 原図

Technique to Minimize the Effect of AAV NAb



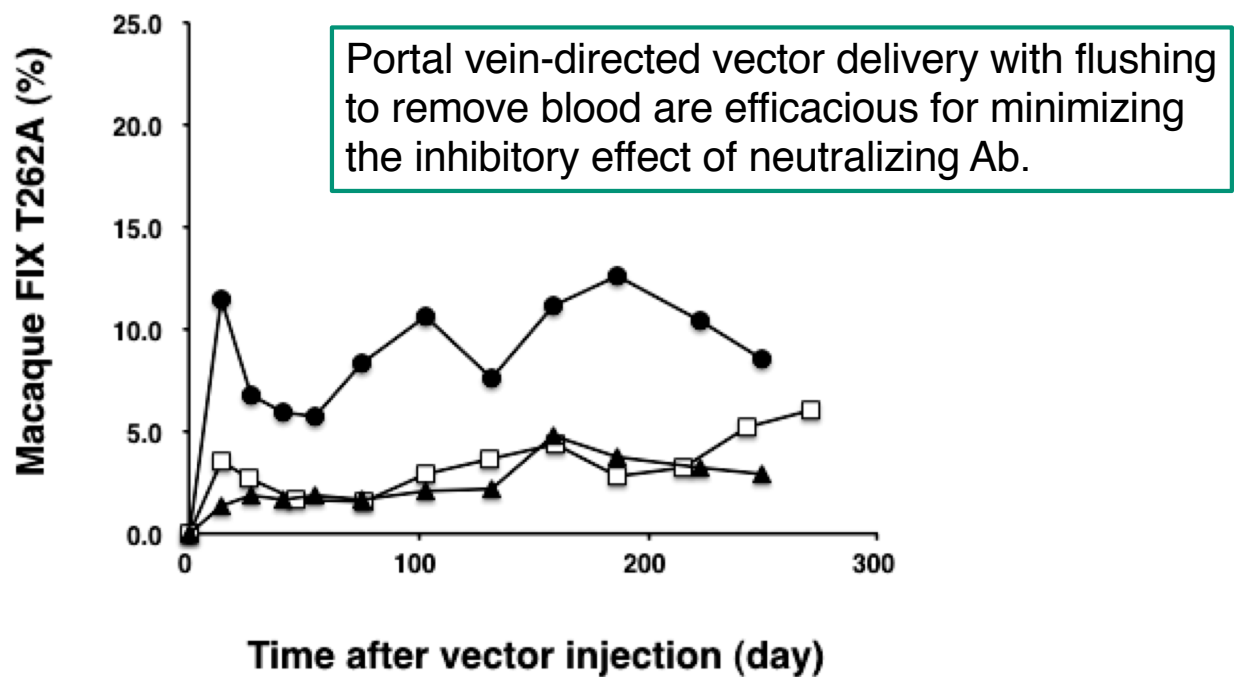
Molecular Therapy (2013); **21** 2, 318–323.

Minimizing the Inhibitory Effect of Neutralizing Antibody for Efficient Gene Expression in the Liver With Adeno-associated Virus 8 Vectors

Jun Mimuro¹, Hiroaki Mizukami², Shuji Hishikawa³, Tomokazu Ikemoto⁴, Akira Ishiwata¹, Asuka Sakata¹, Tsukasa Ohmori¹, Seiji Madoiwa¹, Fumiko Ono⁵, Keiichi Ozawa² and Yoichi Sakata¹

大森 司 博士 (自治医大) 原図

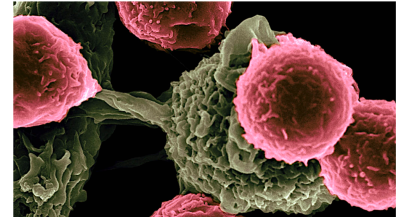
Expression of FIX in macaques following balloon catheter-guided vector injection into portal veins after saline flushing



がんに対する遺伝子治療
—遺伝子改変T細胞療法—

がんに対する遺伝子治療

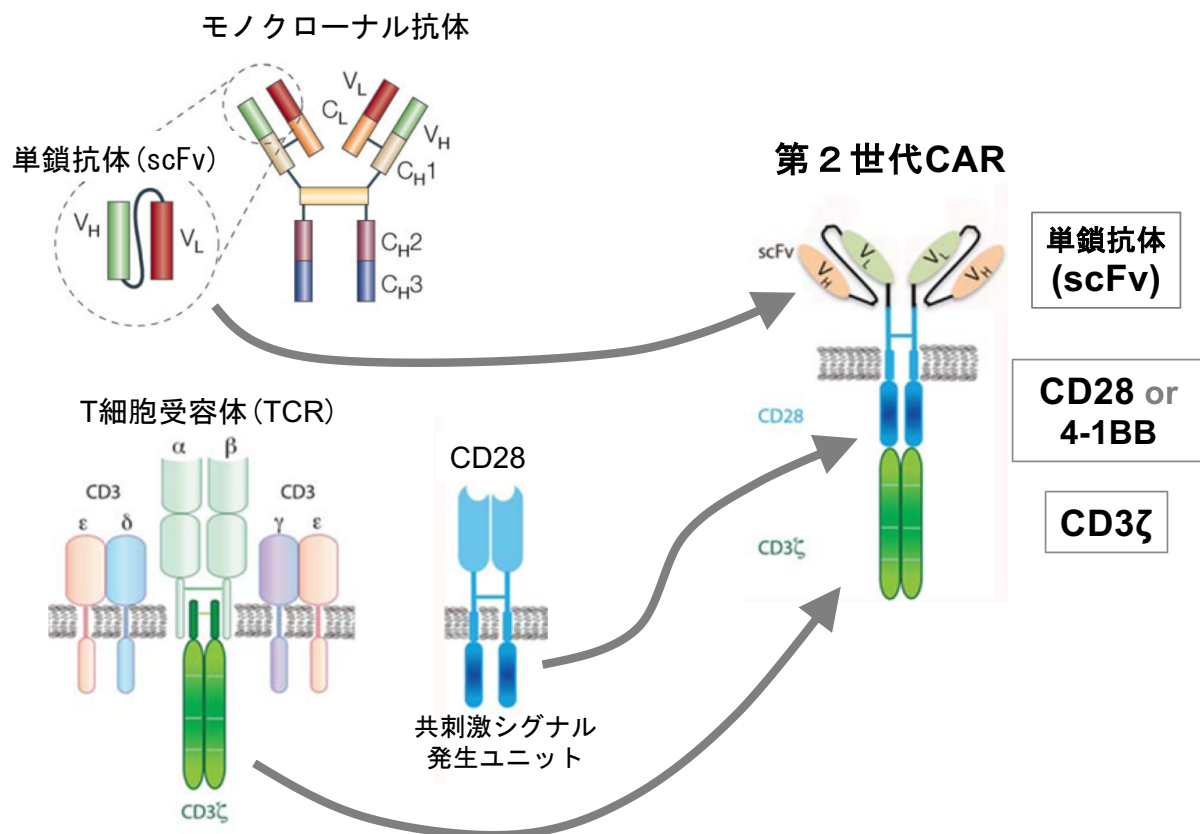
遺伝子改変T細胞療法 (Gene-modified T cell therapy)



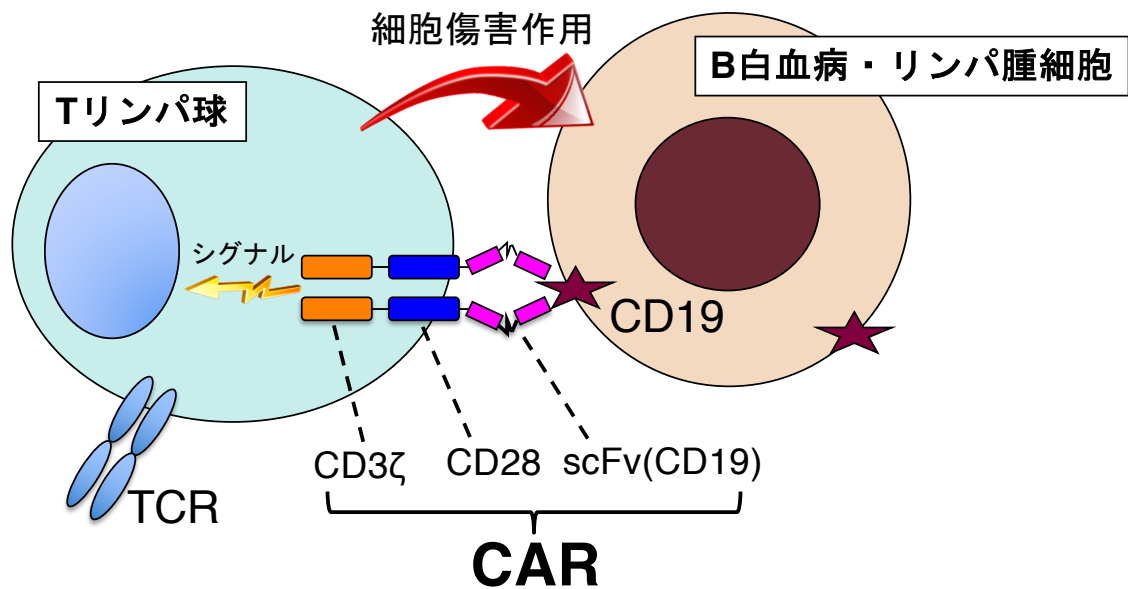
➡ **TCR** (T cell receptor)-T細胞療法

➡ **CAR** (chimeric antigen receptor)-T細胞療法

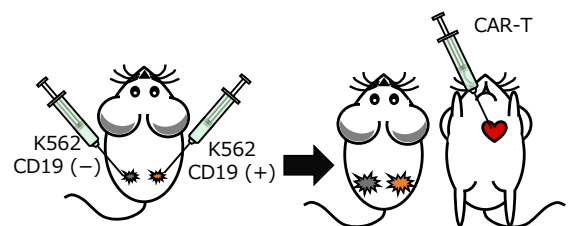
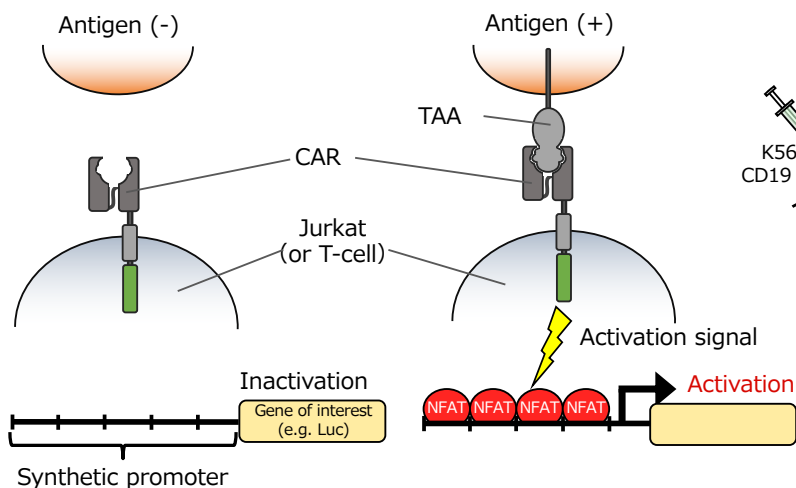
キメラ抗原受容体 (CAR: Chimeric Antigen Receptor) の構造



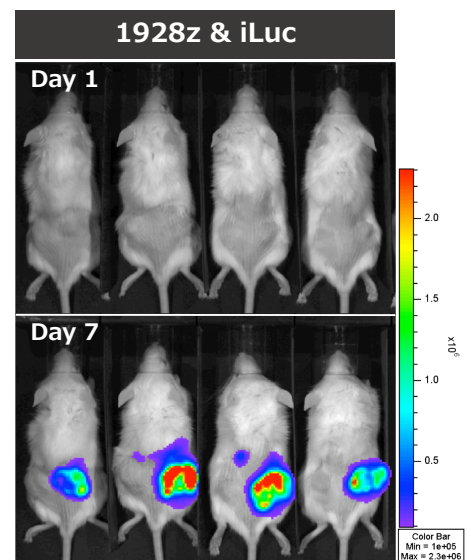
CD19抗原を標的としたCAR-T細胞による B細胞性白血病・リンパ腫細胞の破壊



CAR-T細胞の抗原特異的活性化

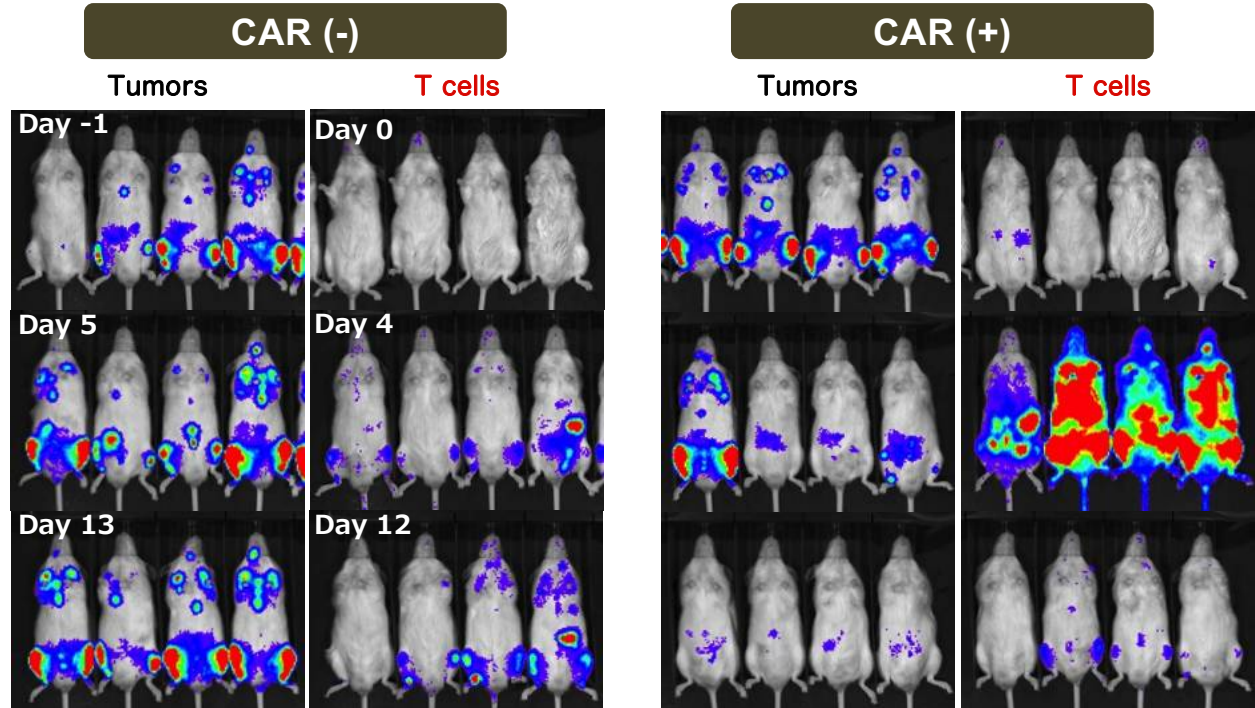


	Antigen (-)		Antigen (+)	
	w/o target	K562	K562/CD19	Raji
CAR (-)				
CAR (+)				



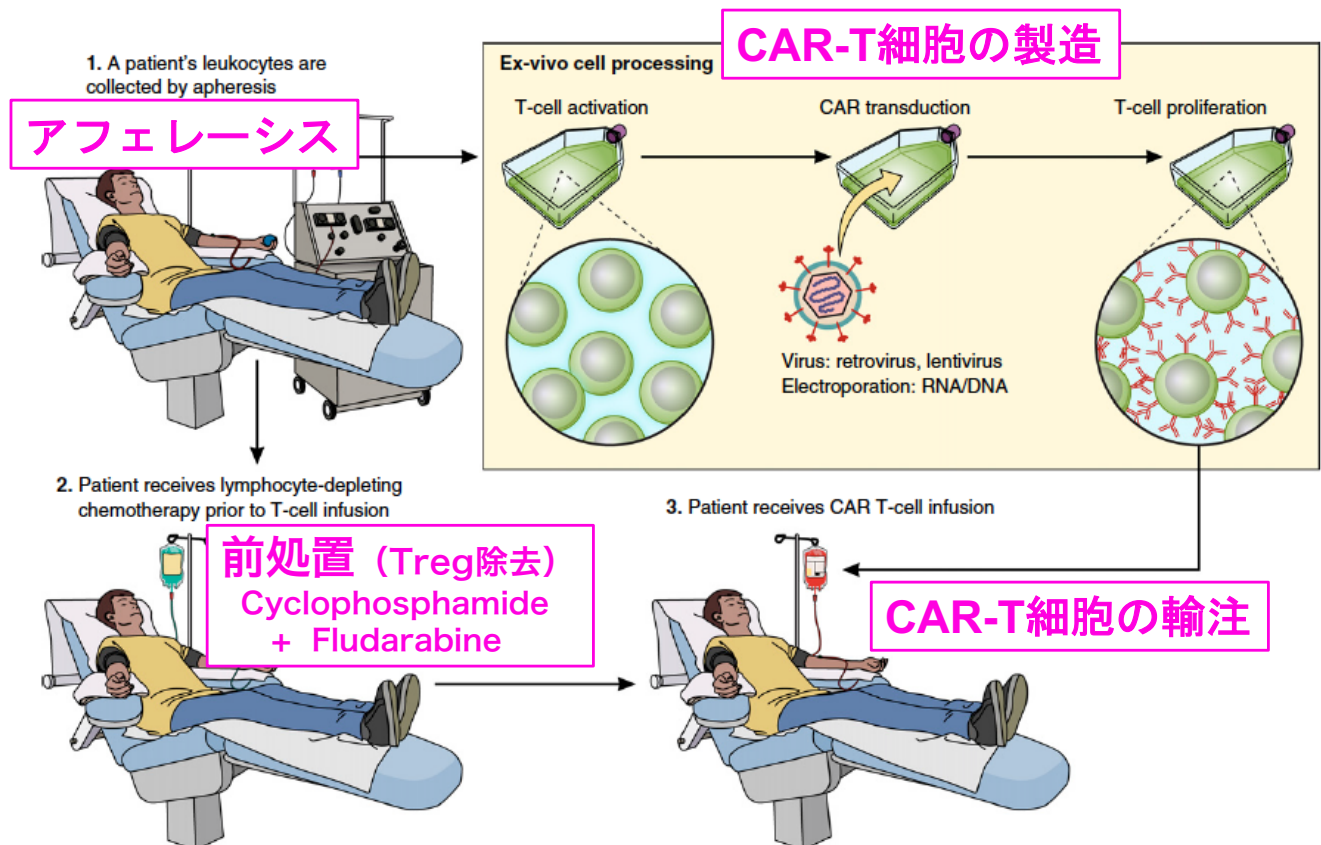
R. Uchibori et al. (Jichi Medical Univ)

活性化T細胞の生体イメージング



R. Uchibori et al. (Jichi Medical Univ)

CAR-T細胞療法の流れ



Mikkilineni L and Kochenderfer JN: Blood 130: 2594, 2017

B細胞性ALLに対するCD19-CAR-T 細胞療法の臨床試験

Institution	CAR design	Patient population	Outcome	Toxicities	Reference
MSKCC	CD28, CD3ζ	• n=32 adults • R/R B-ALL	91% CR	• B-cell aplasia • CRS	NCT01044069 (REF. 13)
UPenn/CHOP	4-1BB, CD3ζ	• n=30 children and young adults • B-ALL	90% CR	• B-cell aplasia • CRS	NCT01626495 (REF. 15)
NCI	CD28, CD3ζ	• n=20 children and young adults • B-ALL	70% CR	• B-cell aplasia • CRS	NCT01593696 (REF. 17)
Fred Hutchinson	4-1BB, CD3ζ	• n=20 adults • B-ALL	83% CR	CRS	NCT01865617 (REF. 18)

Jackson HJ, Rafiq S, and Brentjens RJ.

Driving CAR T-cells forward. Nature Reviews 13:370-383, 2016.

CD19-CAR遺伝子治療における主な毒性

【早期】

👉 サイトカイン放出症候群

(Cytokine Release Syndrome: CRS)

Tリンパ球やMφの活性化に伴う高サイトカイン血症によるもの
トシリズマブが有効

👉 神経毒性

Anakinra (IL-1 receptor antagonist) ?

👉 腫瘍崩壊症候群 (Tumor Lysis Syndrome: TLS)

【後期】

👉 正常B細胞の減少 (血清免疫グロブリン減少 ← 補充療法)

On-target, Off-tumor 反応

今後の検討課題

- ☞ 長期成績：CARの構築の改良など
- ☞ 治癒を目指す？あるいは、移植までのブリッジ役か？
- ☞ 移植後のCAR-T療法
- ☞ 再投与／繰り返し投与
- ☞ CD19抗原以外の標的（特に、固形がん）
- ☞ ゲノム編集技術の応用
 - アロ（ユニバーサル）CAR-Tの開発 --- TCR遺伝子の破壊
 - 免疫チェックポイント阻害（局所） --- PD-1遺伝子の破壊
- ☞ 医療経済的視点：超高額医療

February 1, 2018 N Engl J Med 2018; 378:449-459

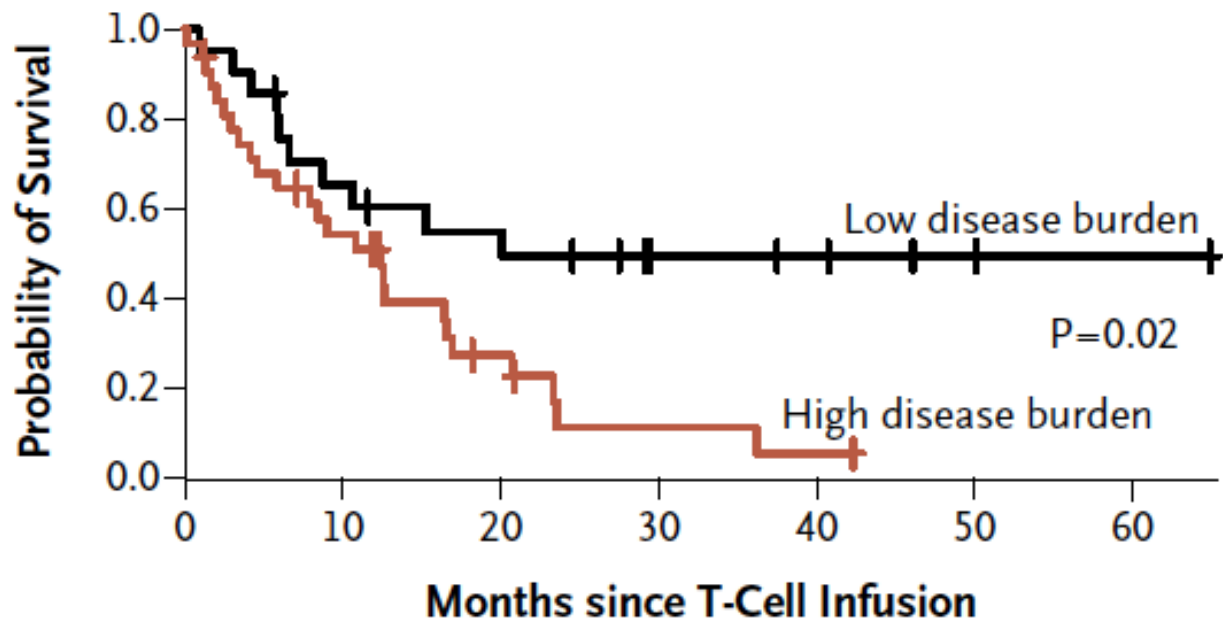
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia

Jae H. Park, M.D., Isabelle Rivière, Ph.D., Mithat Gonen, Ph.D.,
Xiuyan Wang, Ph.D., Brigitte Sénéchal, Ph.D., Kevin J. Curran, M.D.,
Craig Sauter, M.D., Yongzeng Wang, Ph.D., Bianca Santomaso, M.D., Ph.D.,
Elena Mead, M.D., Mikhail Roshal, M.D., Peter Maslak, M.D.,
Marco Davila, M.D., Ph.D., Renier J. Brentjens, M.D., Ph.D.,
and Michel Sadelain, M.D., Ph.D.

CAR-T療法後の全生存率：治療前の腫瘍量による違い



Jae H. Park, et al., N Engl J Med 378: 449-489, 2018

Long-term efficacy of CAR-T therapy is unsatisfactory

➤ Tumor burden

Tumor burden should be reduced by chemotherapy before CAR-T therapy.

➤ Inadequate persistence of CAR-T ➡ Relapse

Structures of CAR molecules should be optimized.

Culture conditions should be optimized.

Culture with PI3 kinase inhibitor, bb007, to enrich

for memory-like T cells

➤ Antigen escape ➡ Relapse

Complete and permanent CD19 antigen loss (~30%)

Genomic mutations, Shifts in splicing

CD22-CAR-T therapy is effective

CAR targets for the treatment of solid malignancies

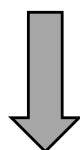
Target	CAR structure	Malignancy	Institution
PSMA	CD3ζ and CD28	Prostate cancer	MSKCC Roger Williams
Mesothelin	CD3 and 4-1BB	Malignant pleural mesothelioma	UPenn
		Pancreatic cancer	UPenn
		Metastatic pancreatic (ductal) adenocarcinoma, epithelial ovarian cancer and malignant epithelial pleural mesothelioma	UPenn
	CD3ζ and CD28	Mesothelioma and malignant pleural disease	MSKCC
	CD3ζ, CD28 and 4-1BB	Mesothelioma, pancreatic and ovarian cancer	NCI
FAP	CD3ζ and CD28	Mesothelioma	University of Zurich (Switzerland)
EGFRvIII	CD3ζ and 4-1BB	Glioma	UPenn
	CD3ζ, CD28 and 4-1BB	Glioma	NCI
EGFR	Unknown	Malignant glioma	Renji Hospital (China)
Target	CAR structure	Malignancy	Institution
CEA	CD3ζ and CD28	Liver metastases	Roger Williams
	Unknown	Lung, colorectal, gastric, breast and pancreatic cancer	Southwest Hospital (China)
CD171	CD3ζ and 4-1BB or CD3ζ, CD28 and 4-1BB	Neuroblastoma	Seattle Children's
GD2	CD3ζ, OX40, CD28	Neuroblastoma, osteosarcoma and melanoma	NCI
		Neuroblastoma	Baylor
		Sarcoma	Baylor
Glypican-3	CD3ζ, CD28 and 4-1BB	Advanced-stage hepatocellular carcinoma	Renji Hospital (China)
HER2	CD3ζ and CD28 virus specific	Sarcoma	Baylor
	CD3ζ and CD28	Glioblastoma	Baylor
		Glioblastoma multiforme	Baylor
IL-13	RαCD3ζ and 4-1BB	Glioma	City of Hope

Jackson HJ, Rafiq S, and Brentjens RJ.

Driving CAR T-cells forward Nature Reviews 13:370-383, 2016.

固形がんに対するCAR-T細胞療法の戦略

- 特異性の高い標的分子
 - 不均一性
- 腫瘍形成
 - T細胞の集積が不十分
- 間質系組織の存在
 - T細胞の侵入の阻害
- がん微小環境への対策
 - 免疫抑制状態



- CD4⁺ CD25^{hi} FoxP3⁺ Tregs
- MDSCs (myeloid-derived suppressor cells)
- TAMs (tumor-associated macrophages)
- Expression of inhibitory ligands by tumor (PD-L1)
- Tumor secretion of T-cell suppressive cytokines (TGF-β and IL-10)

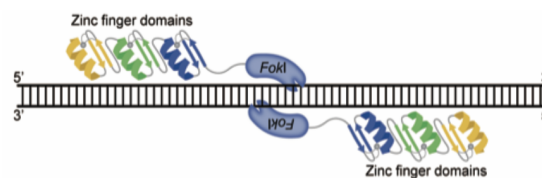
複合療法

ゲノム編集治療

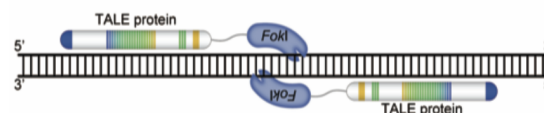
Genome Editing Technologies

ZFN (Zinc finger nuclease)

 Sangamo BioSciences

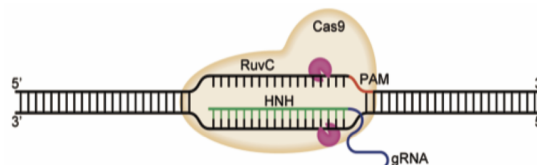


Talen (Transcription Activator-Like Effector Nuclease)



CRISPR/Cas9

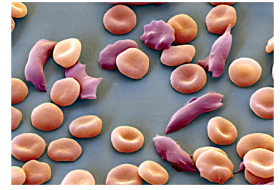
(Clustered Regularly Interspaced Short Palindromic Repeats)



Erasing sickle-cell disease

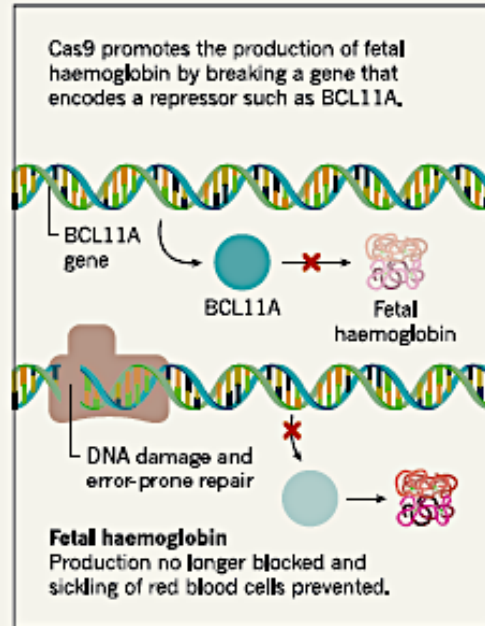
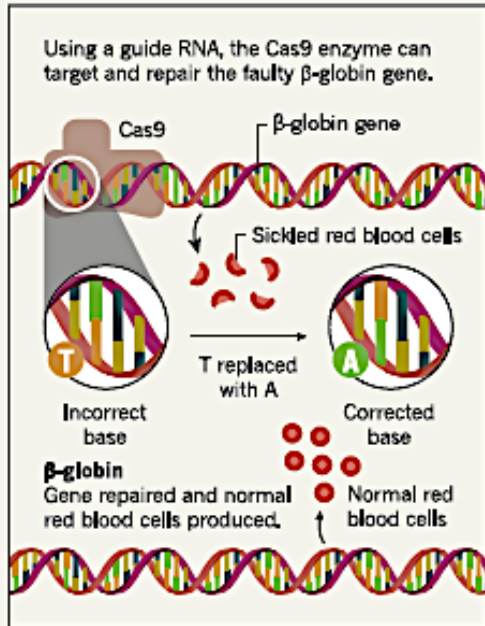
Clinical trials may soon test whether gene editing can cure a group of debilitating haemoglobin disorders.

28 SEPTEMBER 2017 | VOL 549 | NATURE | S29



GENE EDITING WITH CRISPR

CRISPR-Cas9 gene editing is helping to tackle sickle-cell disease in two ways.



AAVベクターを利用した *in vivo* ゲノム編集治療

Sangamo
THERAPEUTICS

Sangamo Announces Treatment of First Patient in Landmark Phase 1/2 Clinical Trial Evaluating In Vivo Genome Editing for MPS II

RICHMOND, Calif., Nov. 15, 2017 /PRNewswire/ – Sangamo Therapeutics, Inc. (Nasdaq: SGMO) today announced treatment of the first patient in the Phase 1/2 clinical trial ("the CHAMPIONS study") evaluating SB-913, an investigational *in vivo* genome editing therapy for people with mucopolysaccharidosis type II (MPS II), also known as Hunter syndrome.

GENE THERAPY

In vivo genome editing of the albumin locus as a platform for protein replacement therapy

Rajiv Sharma,^{1,*} Xavier M. Anguela,^{1,2,*} Yannick Doyon,^{3,*} Thomas Wechsler,³ Russell C. DeKor,³ David E. Paschon,³ Jeffrey C. Miller,³ Robert J. Davidson,¹ David Shivak,³ Shangzhen Zhou,¹ Ju Philip D. Gregory,³ Michael C. Holmes,³ Edward J. Rebar,³ and Katherine A. High^{1,2}

Blood 126: 1777-1784, 2015

Key Points

- AAV- and ZFN-mediated targeting of the albumin locus corrects disease phenotype in mouse models of hemophilia A and B.
- Robust expression from the albumin locus provides a versatile platform for liver-directed protein replacement therapy.

Universal CAR-T Cells Produced Using Genome Editing Technology



CANCER IMMUNOTHERAPY

Baby's leukemia recedes after novel cell therapy

Gene editing used to create "off-the-shelf" T cells

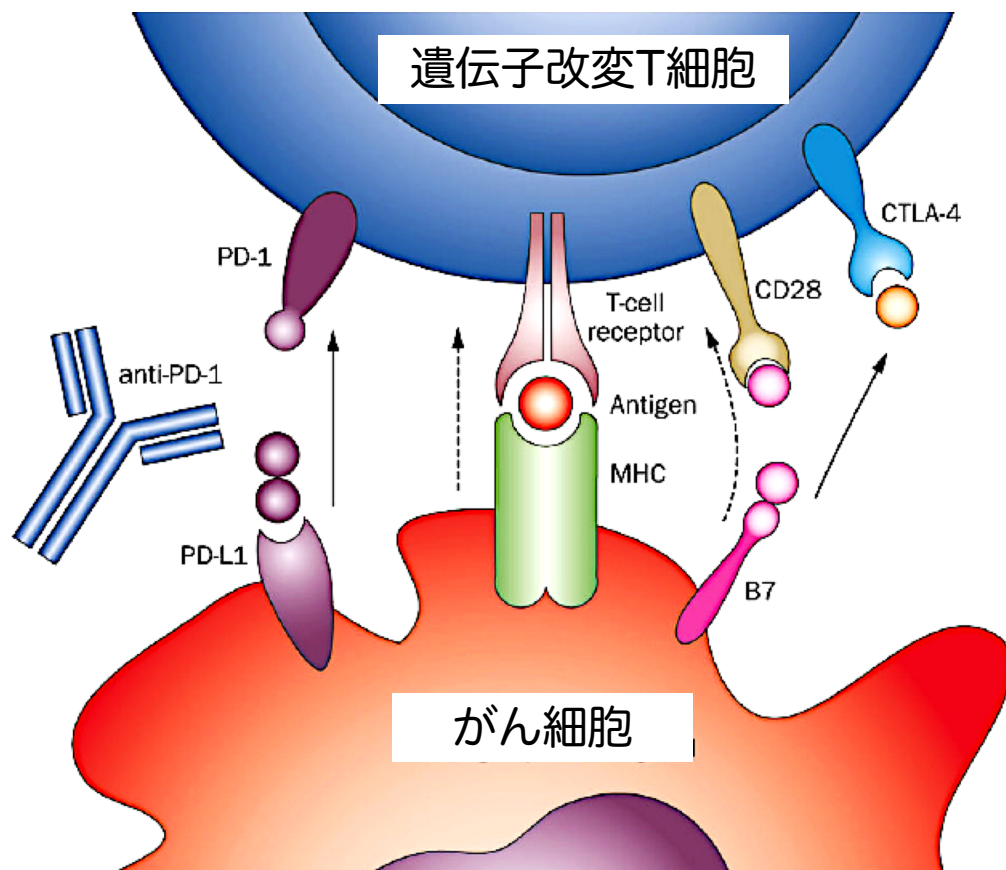
*Science 13 November 2015:
Vol. 350 no. 6262 p. 731*

The 11-month-old girl had already run through every treatment. Her immune system was barely functioning, and oncologists couldn't collect T cells from her for personalized therapy.

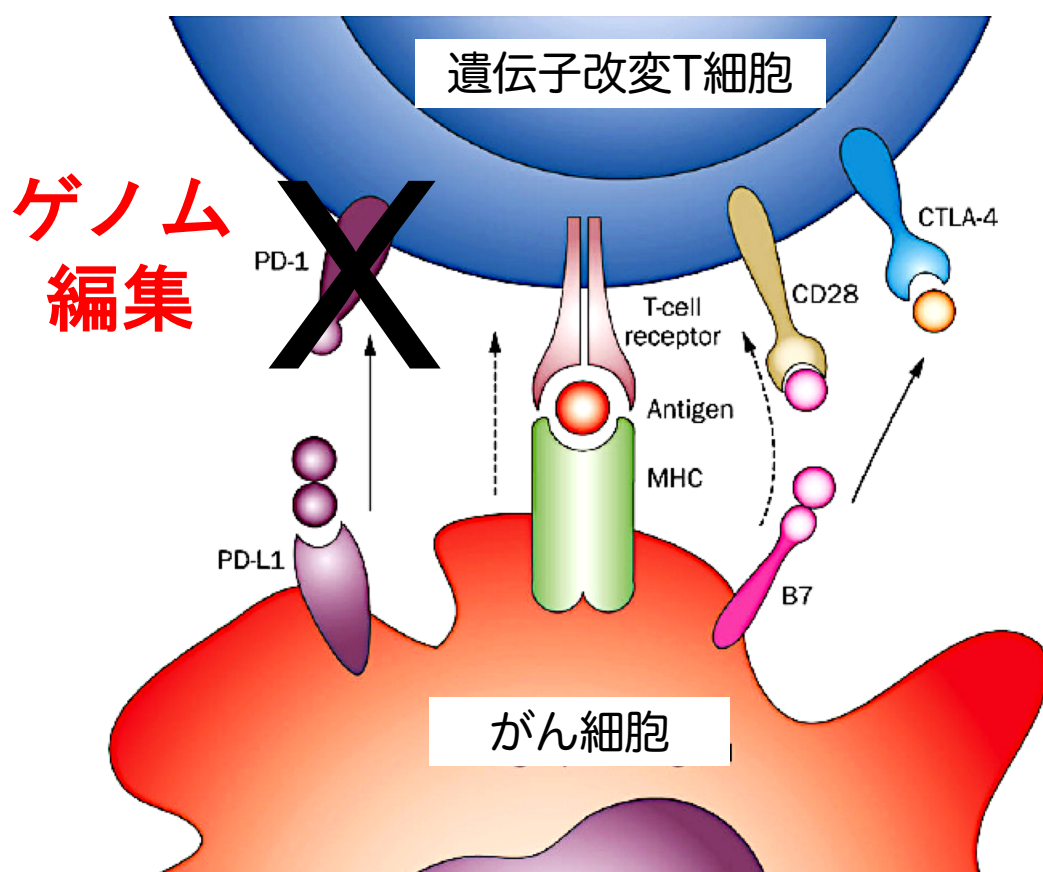
The **Collectis** cells had been genetically engineered to avoid two major pitfalls. Scientists used a gene-editing technique called transcription activator-like effector nucleases (**TALENs**) to cut out the **T cell receptor gene**. Without it, the cells can't recognize the recipient's body as foreign. The cells were also designed to survive the intense therapy: an antibody called Campath, intended to protect the donor T cells from attack by wiping out the child's own immune system. Campath targets an immune-cell marker called CD52, so the company used TALENs to remove **CD52** from its donor cells—ensuring that Campath wouldn't attack them, too. Finally, just as doctors have done with a patient's own T cells, the researchers made DNA modifications to the foreign cells so they would home in on leukemia.

Allogenic (Universal) CAR-T

- **Off-the-shelf, Constant quality**
 - appropriate for medical products (business point)
- **Methods to prevent GVHD (KO of TCR gene)**
- **Methods to prevent rejection (**Bridge to SCT**)**
- **Inadequate expansion of T-cells *in vitro***
 - **iPSC-derived T-cells ?**



免疫チェックポイント阻害



免疫チェックポイント阻害

Immune checkpoint blockade

- Anti-PD-1 Ab vs **PD-1 gene KO**
(genome editing)

The latter can avoid systemic side effects.



遺伝子治療の現状と今後の展望

- 従来の治療法とは全く異なるアプローチ（遺伝子操作技術）
- 生理的にはあり得ないことが可能（アイデア次第で画期的治療法！）
- 幹細胞にはレンチウイルスベクター
- 終末分化細胞にはAAVベクター（またはレンチウイルスベクター）
- 対象は単一遺伝子病から多因子性疾患へ
- 遺伝子改変T細胞（CAR-T, TCR-T）で画期的がん免疫療法
- ゲノム編集治療も急速に進展
- 世界の動きは速い（日本が取り残されつつある）
- 医療経済的視点も重要（超高額医療）



Jichi Medical University