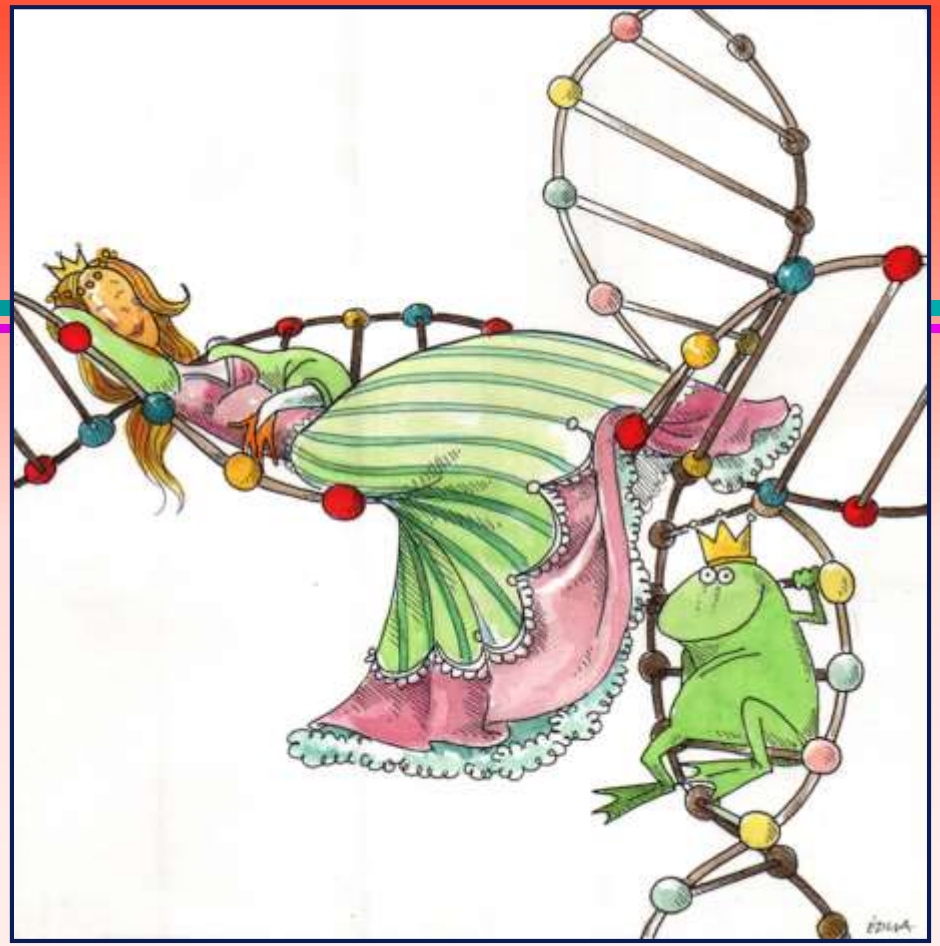
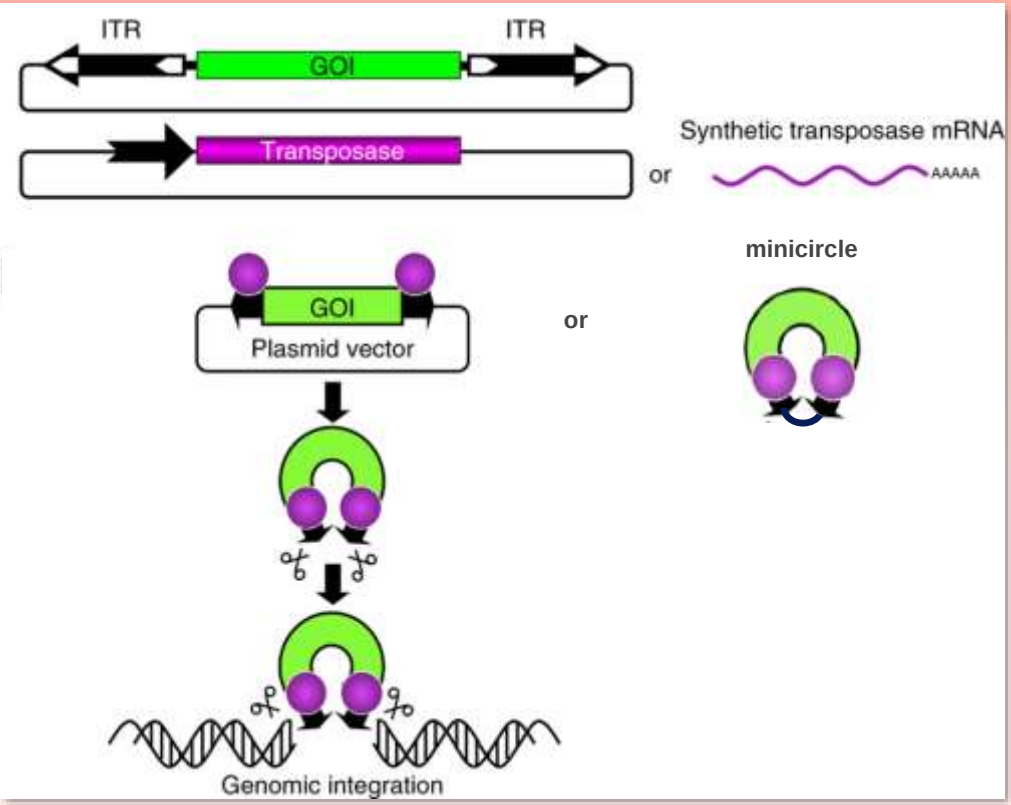
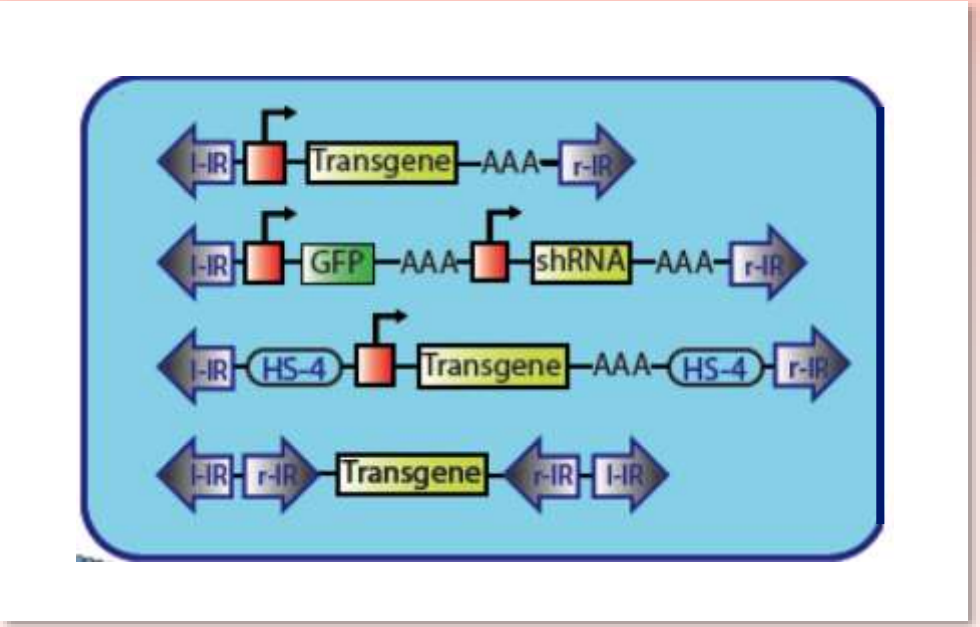


Non-viral, Sleeping Beauty Transposon-based Gene Therapy

Zsuzsanna Izsvák,
Max Delbrück Ctr. For Molecular Medicine

The Two-component SB Transposon-based Non-viral Vector System





- The SB transposon system is flexible and can be combined with variable features

Main Features of the Non-viral *Sleeping Beauty* Transposon System (SB100X)

Mechanism	Cut and paste (no reverse transcription)
Cargo capacity	Transposition is size-sensitive, but no upper limit (BAC)
Immunogenicity	Similar to plasmid-based expression vectors
Tropism	Somatic, germinal, dividing & non-dividing cells
Integration profile	Close to random, can be targeted ~10^7x enrichment
Stable expression	Copy number dependent, non-silenced
Transcriptional act.	Benign promoter/enhancer activity (100x<MLV LTR)
Efficacy	In certain cells comparable to lentivirus (SB100X)

Transposon for Gene Therapy

doi:10.1038/mt.2016.76

Endogenous Transposase Source in Human Cells Mobilizes *piggyBac*

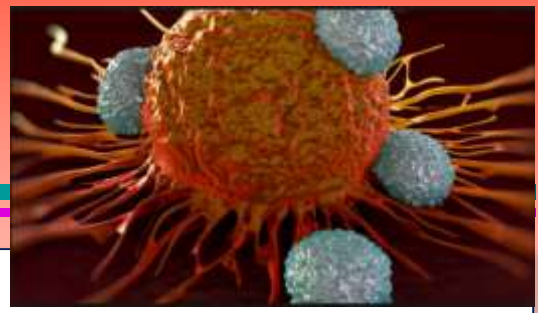
McClintock
Nobel Prize 1983

- T cells (cancer)
- Cytokine-induced killer, CIK (cancer)
- Plasmablast (mucopolysaccharidosis)

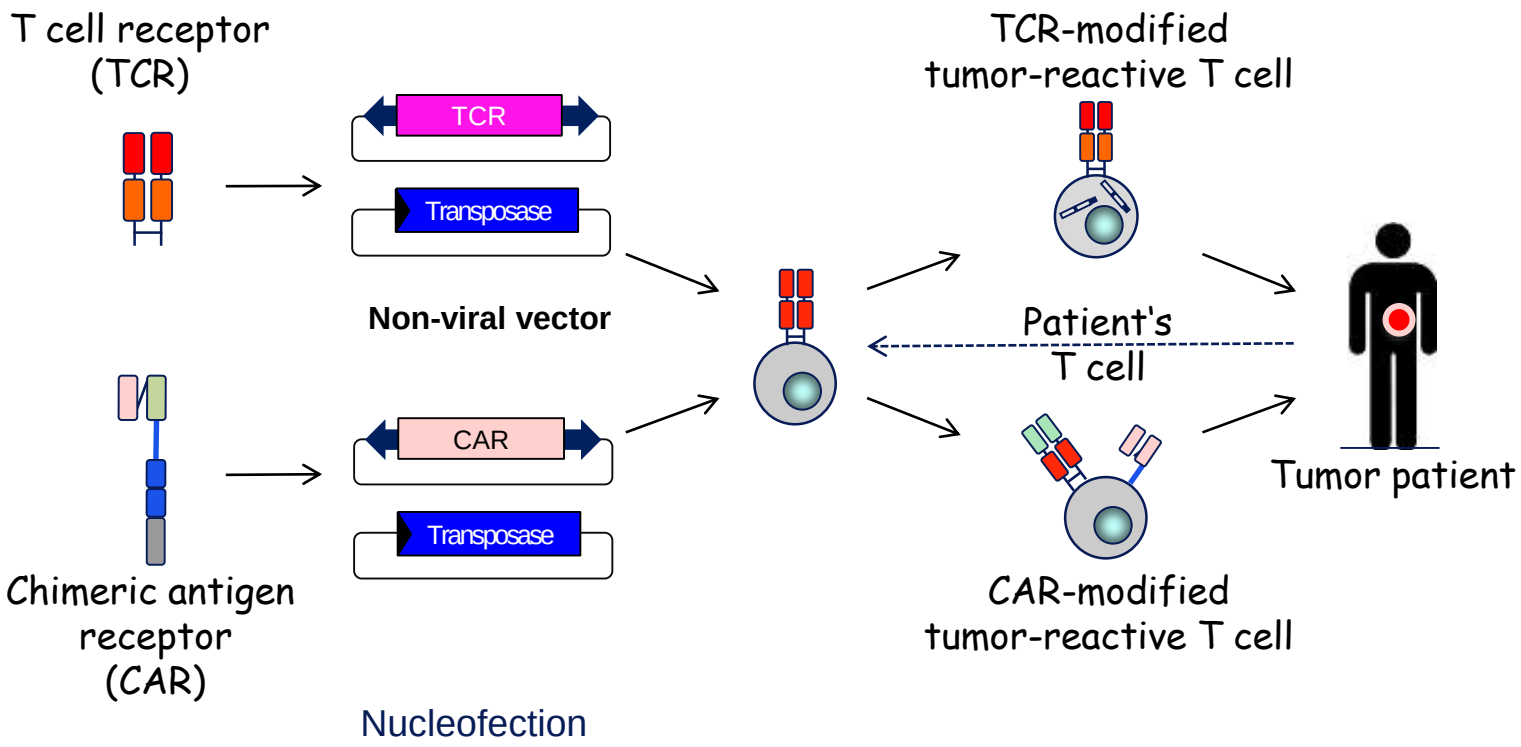
Sleeping Beauty
1997

1st in man clinical trial 2012
N=14 2021, 2 in EU

Adoptive T Cell Therapy Using Non-viral Transposon-based Delivery

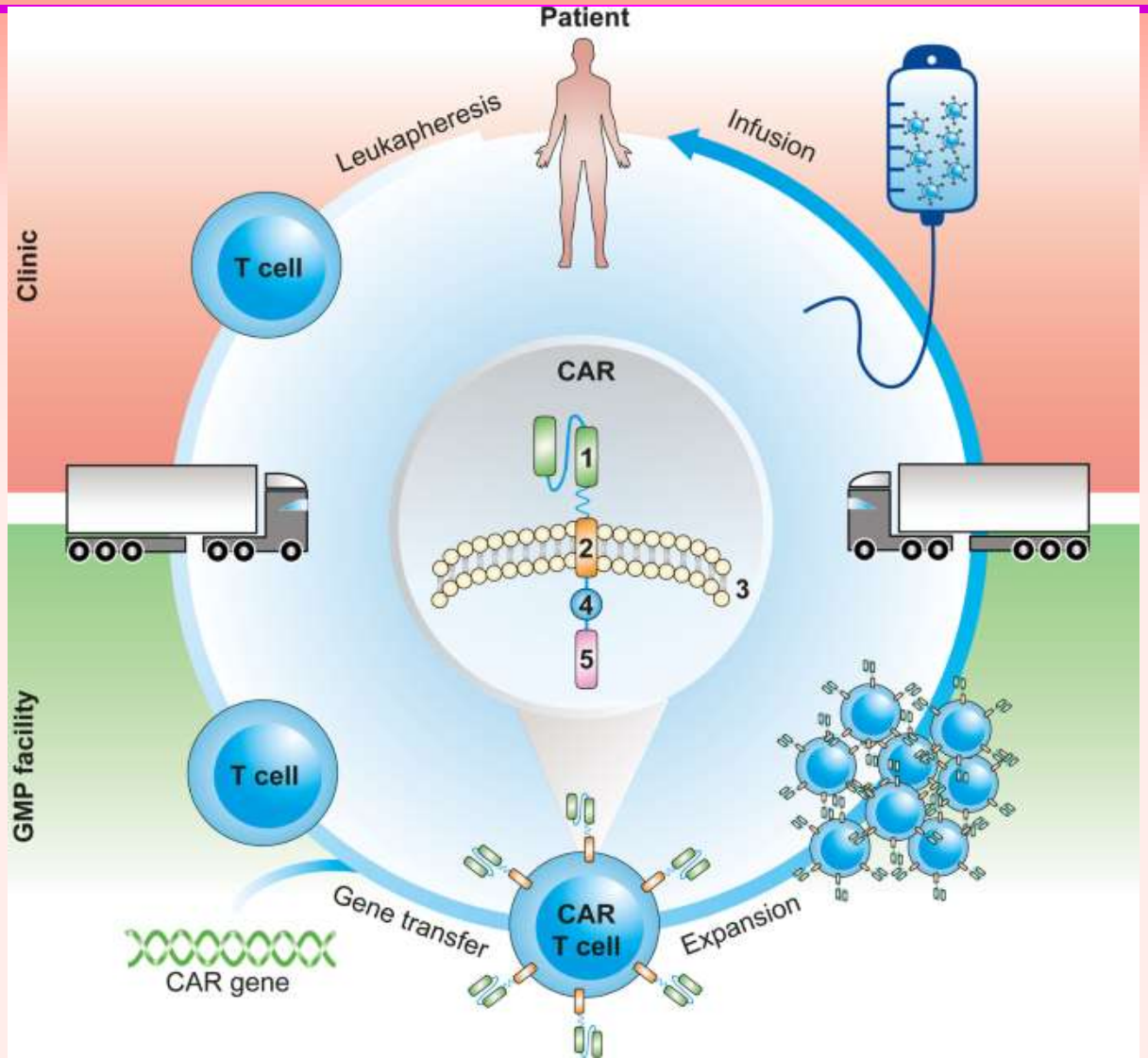


TCR gene therapy



CAR gene therapy

Ex vivo manufacturing pipeline (CAR-T cell)

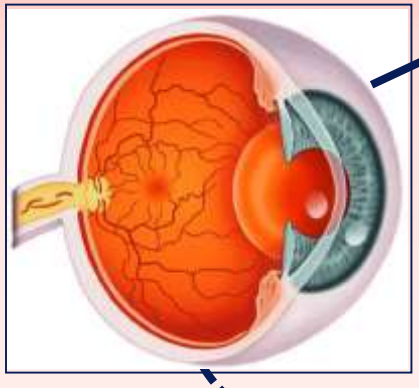


Non-viral vectors significantly facilitate the generation of engineered T cell products

Advantage	Viral vectors	Non-viral vectors (Sleeping Beauty transposons)
More flexible	Based on retrovirus/lentivirus	Based on plasmid DNA
Faster	GMP-grade virus 6-12 months	Plasmid DNA 1-2 months
Cheaper	GMP-grade virus Costs per dose: 1,000 $	Plasmid DNA Costs per dose: 4 $
Safer	Cell engineering requires S2-conditions	Cell engineering requires S1-conditions

Ex vivo strategy to treat Age-dependent Macula Degeneration (AMD)

(https://www.targetamd.eu/)



Cell isolation

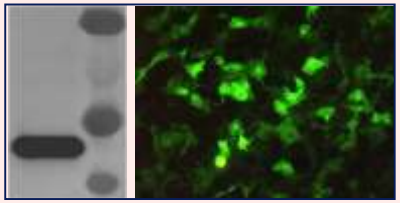
Iris pigment epithelial cells

Nucleofection

Transposon-based therapeutic vector

Subretinal Transplantation

rPEDF



Proof of Principle

Efficient (~75%)

Stabile ~2 year

- The efficient transfection of freshly isolated cells allows for a one step cell-based gene therapy for the treatment of neovascular AMD in 45-60 minutes session.

Further directions in gene therapy: *in vivo* application

GENE THERAPY

Comment on Richter et al, page 2206

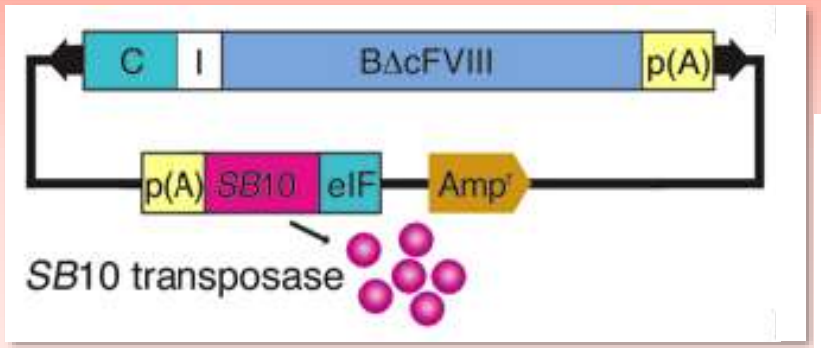
Gene therapy simplified

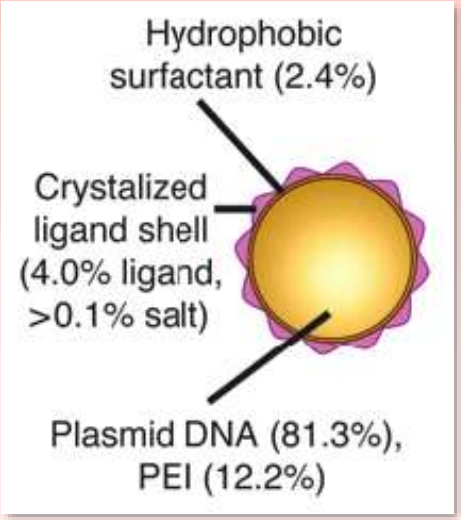
Jiaqiang Ren and David F. Stroncek NATIONAL INSTITUTES OF HEALTH CLINICAL CENTER

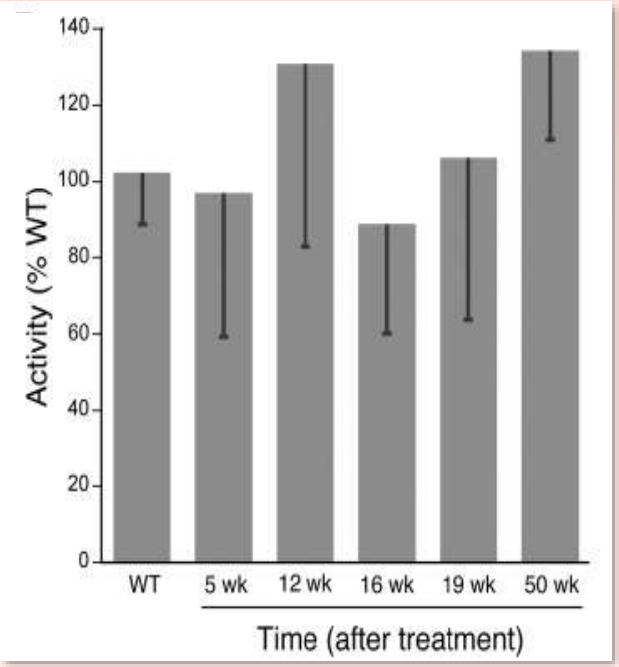
In this issue of *Blood*, Richter et al report their work on an in vivo gene transduction system using the adenovirus-based vector and hyperactive SB transposase (SB100×) system. Their results show that this system is effective and safe and performs without needing ex vivo expansion and transduction of hematopoietic stem cells (HSCs).[1] This system may overcome some of the difficulties associated with cell collection and manufacturing and provide technical advances for the field of gene therapy.

Richter et al., *Blood* (2016): Ivics et al. (2017) *Trends in Genet*. Li, Mol Ther.MCD (2018), Wang, unpub

In vivo Nanocapsule-delivered *Sleeping Beauty* Mediates Factor VIII expression in Liver Sinusoidal Endothelial Cells





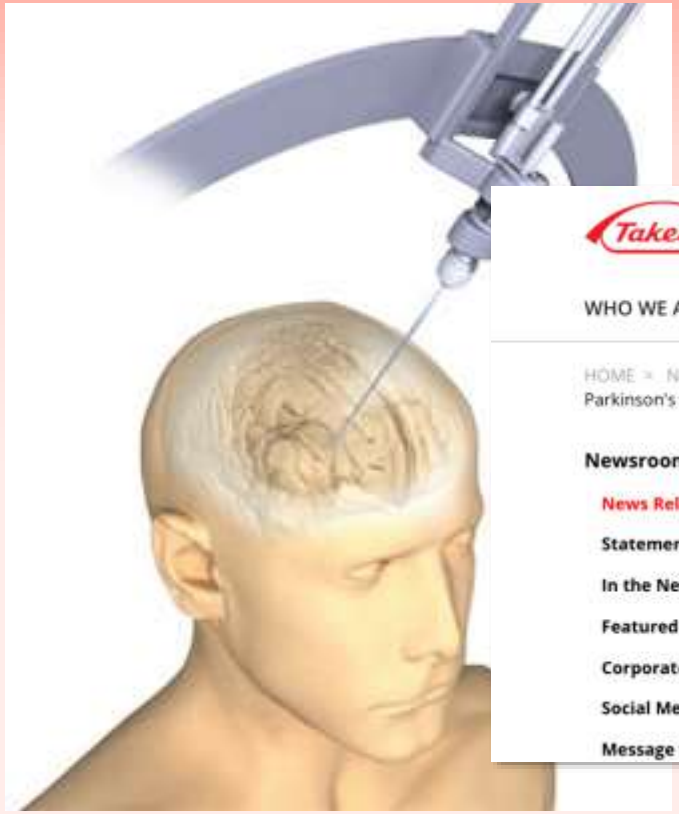


- B domain-deleted canine FVIII
- Nanocapsule <50 nm
- Specific targeting to hepatocyte by natural ligands
- No apparent antibody formation

Kren *et al.* 2009 J Clin invest

Encapsulated Cell (NGC2011- SB delivered) Therapy for Neurogenerative Diseases

nsgene



- Implanted in six patients with

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Takeda and NsGene Partner to Perform Research in Cell Encapsulation Therapies for Parkinson's Disease

January 8, 2016

Osaka, Japan, January 8, 2016, and Providence, RI; January 7, 2016 – Takeda Pharmaceutical Company Limited **(TSE: 4502)** and NsGene, Inc. today announced a research agreement to develop encapsulated cell therapies for the potential treatment of Parkinson's disease. The partnership will focus on the delivery of recombinant Glial Cell Line-Derived Neurotrophic Factor (GDNF) to affected brain regions by way of implanted, encapsulated cell therapy devices.

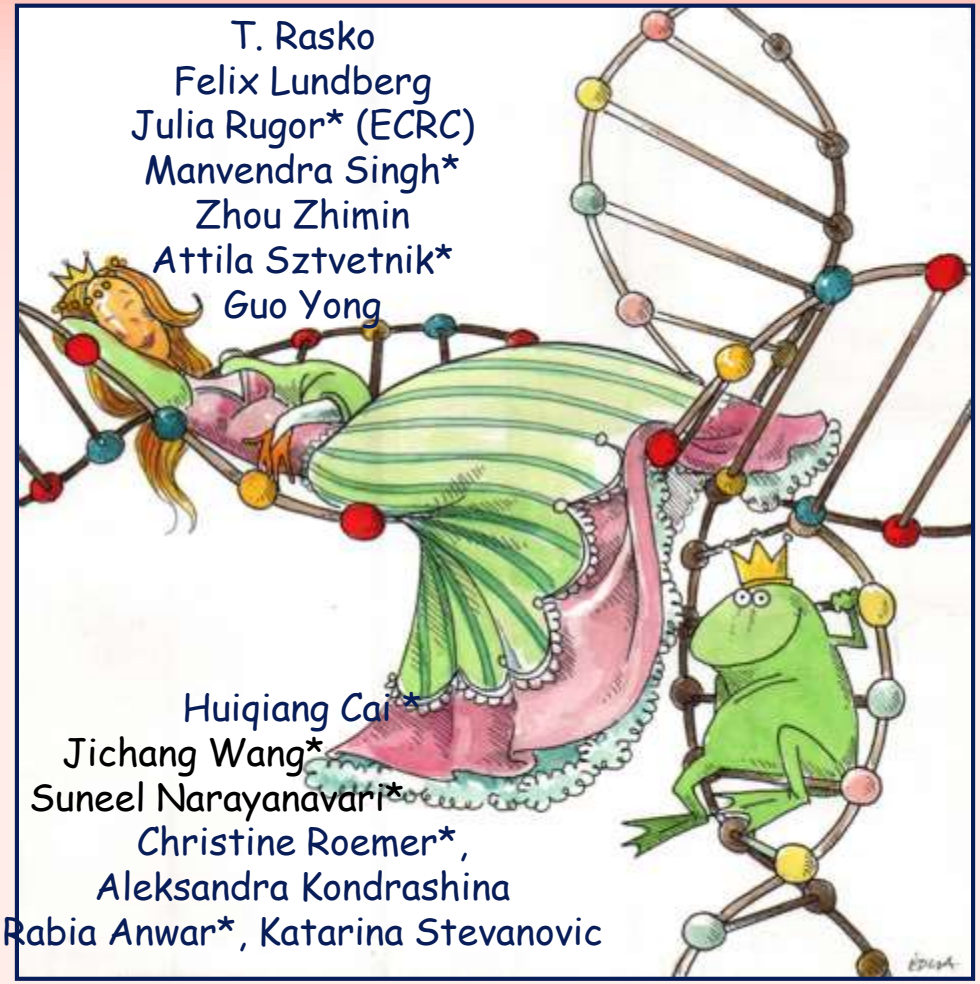
- Encapsulated Cell (EC) biodelivery™ system
  NGC0211 – Nerve Growth Factor expressed from SB
  Preclinical studies are successful (in rat, mini pig) → Phase I

Thanks to...

Max Delbrück Center for Molecular Medicine, Berlin, Germany

MDCell Helmholtz Innovation Lab

Collaborators



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