Genetic Therapy of Cells

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- Principle of integrating viral vectors
- Principles of non-integrating viral vectors
- Nonviral alternatives for genetic therapies

Genetic Therapies

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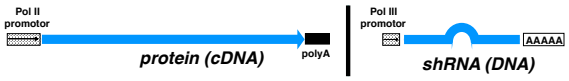

Therapeutic agent:	protein	⟷	RNAi
Expression:	permanent	⟷	transient
genetic manipulation:	*ex vivo*	⟷	*in vivo*
target cells:	dividing	⟷	nondividing/ post-mitotic
target organ structure:	hierachical	⟷	heterachical

Hierachy in the Hematopoietic System

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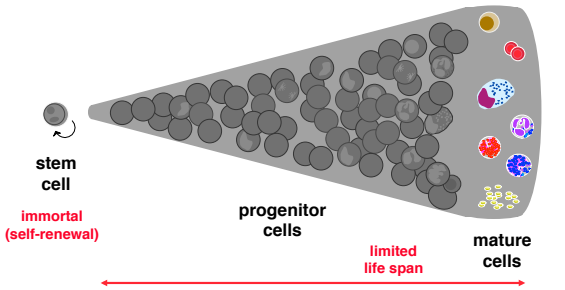
after JCM van der Loo



Hematopoietic Stem Cell Transplantation

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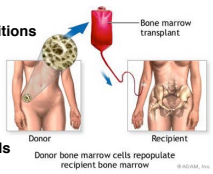
- Pioneered from 1950-70 at the Fred Hutchingson Cancer Research Center, Seattle, by E. Donnall Thomas, MD & colleagues
- The first 200 patients died („Rainer Storb")
- *Indications* are cancers and nonmalignant conditions
- In allogeneic transplantation, host and patient should be *HLA identical/matched*
- Host/patient needs to be *conditioned* with myeloablative regimens (chemo ± radiotherapy) to ensure long-term engraftment of the stem cells



Effects for nonmalignant genetic disorders

- Healthy donor stem cells engraft and repopulated the entire hematopoietic blood/immune system with normal progeny for the life-time of the stem cell

Gene Transfer into Hematopoietic Cells

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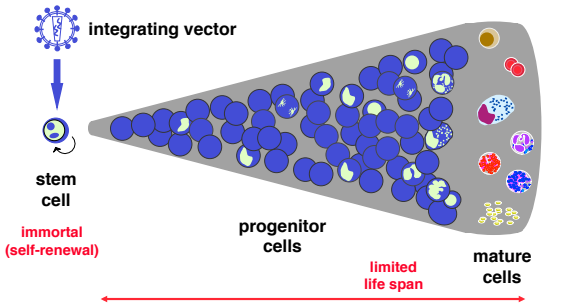

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Three Principles of Stem Cell Gene Therapy

- The integrated vector as part of the genome will also be present after devision in each daughter cell.
- If a stem cell was the target for the integrating vector, all its progeny (= all blood & immune cells) will be genetically modified - for the life of the stem cell.
- If a selective advantage for corrected over deficient cells exists, the corrected stem cells & their progeny will repopulate the entire hematopoietic system.

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Does Stem Cell Gene Therapy Exists in nature?

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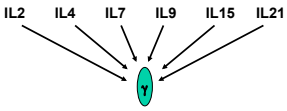
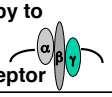
X-SCID (γ_c)



- X-linked severe combined immunodeficiency (SCID) without T or NK cells, normal B-cells
- deficiency in the common γ-chain (γ_c) of the IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptor
- lethal within the first year due to severe infections
- stem cell transplantation or genetic therapy to replace the deficient lymphoid system



IL-2 receptor

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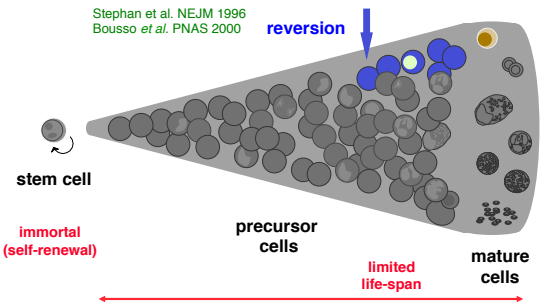
Reversions in X-linked SCID Patients

Stephan et al. NEJM 1996
Bousso *et al.* PNAS 2000



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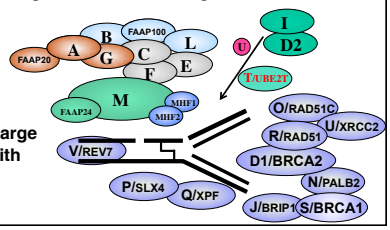
Fanconi Anemia (FA)

Inherited DNA repair disorder

- clinical trias of > congenital abnormalities
  > progressive BM failure
  > high incidence of malignancies
- germ-line defects in ≥22 DNA repair genes (*FANCA-W*)
- FA proteins form large complexes also with other DNA repair proteins



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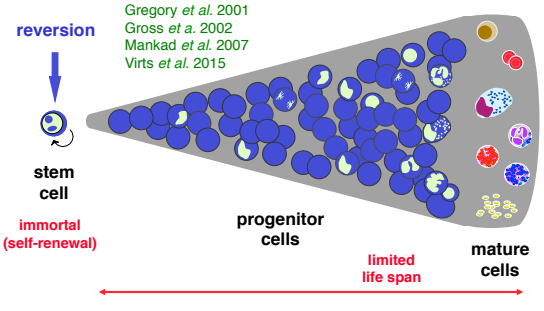
Reversions in Fanconi Anemia Patients

Waiszfisz *et al.* 1999
Gregory *et al.* 2001
Gross *et a.* 2002
Mankad *et al.* 2007
Virts *et al.* 2015



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Stem Cell Gene Therapy

Disorders with *in vivo* selective advantage for corrected cells
- Fanconi Anemia => (stem cells & all progeny)
- X-SCID (γc) => (progenitor/precursor cells)

Disorders without selective advantage for corrected cells
- ADA-SCID
- WAS
- Thalassaemia
- Leukodystrophies
- Chronic Granulomatosis (CGD)
- Sickle cell disease

Disorders with *in vivo* selection advantage for genetically modified stem cells & their progeny, if chemotherapy is used
- MGMT gene therapy for Glioblastoma

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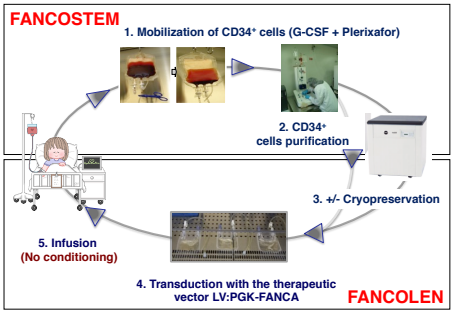
Approved Clinical Trial for FA Gene Therapy



courtesy of Juan Bueren & Paula Rio

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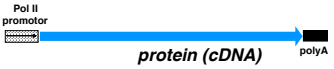
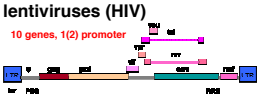
Viral Vectors to Introduce Therapeutic DNA

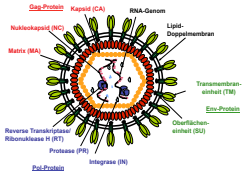


Retroviral vectors derived from wildtype retroviruses are evolutionary optimized to stably & efficiently introduce foreign DNA into the genome of mammalian cells

lentiviruses (HIV)
10 genes, 1(2) promoter



murine retroviruses
3 genes, 1(2) promoter



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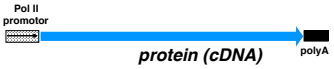
Viral Vectors to Introduce Therapeutic DNA



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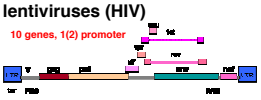
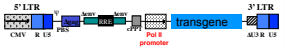
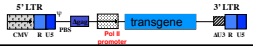


murine retroviruses
3 genes, 1(2) promoter

lentiviral vector
1 promoter, 1 therapeutic gene, 0 viral genes



retroviral vector



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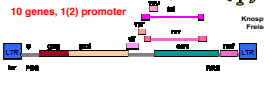
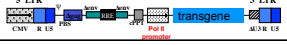
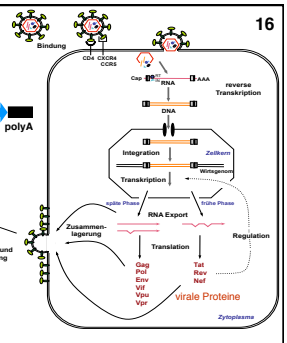
Lentiviral Vector

lentiviruses (HIV)
10 genes, 1(2) promoter



lentiviral vector
1 promoter, 1 therapeutic gene, 0 viral genes





Lentiviral Life Cycle

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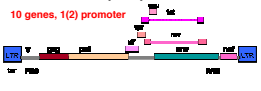
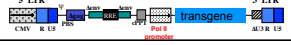
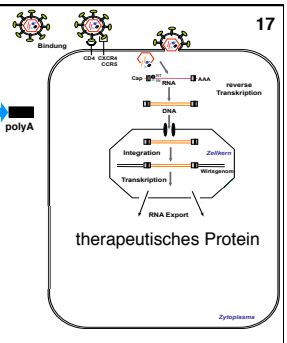
Lentiviral Vector

lentiviruses (HIV)
10 genes, 1(2) promoter



lentiviral vector
1 promoter, 1 therapeutic gene, 0 viral genes





Vector Fate

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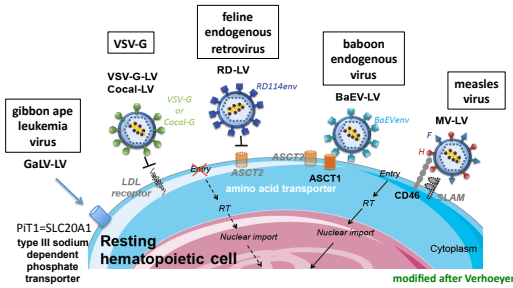
Cellular Proteins used for Viral Entry

all highly suitable for use on lentiviral vectors



modified after Verhoeyen *et al.* 2017

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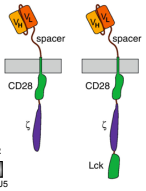
Lentiviral Vectors For

- ***in vitro/ex vivo*** **transduction of proliferating (autologous) cells that are infused back to the patients**
- **Hematopoietic stem cell gene therapy**
- **CAR T-cell therapy**
- **CAR NK-cell therapy**
- **Aim in clinical studies: 1-3 lentiviral copies (integrations) per host cell genome**



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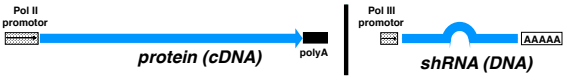
Genetic Therapies



Therapeutic agent:	**protein**	⟷	RNAi
Expression:	permanent	⟷	**transient**
genetic manipulation:	*ex vivo*	⟷	***in vivo***
target cells:	dividing	⟷	**nondividing/ post-mitotic**
target organ structure:	hierachical	⟷	**heterachical**

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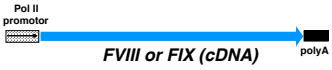
Genetic Therapies for Hemophilia A/B



- **x-chromosomal recessive with mutations in *FVIII* or *FIX* genes**
- **11,000 patients in Germany: 4,000 severe with activities ≤1%**
- **factor replacement as standard-of-care therapy**
- **treatment costs: appr. 150,000 Euro/patient/year**
- **factor production in the liver/hepatocytes (dividing 1-2x/year)**

=> *in vivo* gene delivery with vectors for 'transient' expression

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Choice of Vector System

Haemophilia gene therapy: Progress and challenges

Elsa Lheriteau [a,b], Andrew M. Davidoff [d], Amit C. Nathwani [a,b,c,*]

[a] Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free NHS Foundation Trust, UK
[b] Department of Haematology, UCL Cancer Institute, UK
[c] National Health Services Blood and Transplant, UK
[d] Department of Surgery, St. Jude Children's Research Hospital, Memphis, TN, USA

Blood Reviews 29 (2015) 321–328

Table 1
Vector properties.

	Non-viral vectors
Packaging capacity	Unlimited
Ease of production	+++
Integration into host genome	Rarely
Duration of expression	Usually transient
Transduction of post-mitotic cells	++
Pre-existing host immunity	None
Safety concerns	None
Germ-line transmission	None

Direct intravenous injection of plasmid DNA

- *'hydrodynamic'* injection of plasmid DNA in **mice (30g)**: 5-25µg DNA in volume (8-12% of body weight) i.v. in ≤30sec
  => 40% gene transfer in hepatocytes but also high mortality
- **human (75kg)**: 12-62 mg DNA in 7.5L infusion i.v. in ≤30sec
- injection of plasmid DNA complexed with **liver-targeting polycathions** (anti-ASGPR) e.g. **JetPEI-hepatocyte™**
  => no gene transfer into hepatocyte reported/observed

Liu *et al.* Gene Ther 1999, Ehrhardt *et al.* Hum Gene Ther 2003

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Blood Reviews 29 (2015) 321–328

Table 1
Vector properties.

	Non-viral vectors	Retroviral vectors	Adenoviral vectors	AAV vectors
Packaging capacity	Unlimited	8.0 kb	30.0 kb	4.6 kb
Ease of production	+++	Lentiviral vectors −− No reliable producer cell lines Oncoretroviral vectors +/−	+/−	Cumbersome
Integration into host genome	Rarely	Yes	No	Rarely
Duration of expression	Usually transient	Long term	Transient	Long term in post mitotic cells
Transduction of post-mitotic cells	++	Lentiviral vectors ++ Oncoretroviral vectors −	+++	++
Pre-existing host immunity	None	None	+++	++
Safety concerns	None	Insertional mutagenesis	Inflammatory response	None
Germ-line transmission	None	+/−	None	+/−

strong T- & B-cell responses

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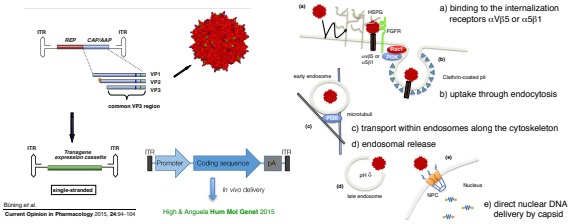
AAV Nanoparticles as Vectors



- Adeno-Associated Viruses are single-stranded non-enveloped DNA parvoviruses
- genome size is a single 4.7 kB DNA with multifunctional Rep and Capsid proteins
- different AAV serotypes with sizes of ~25 nm have different tissue preferences
- AAV1, 2, 6, 8, 9 serotypes used in >100 clinical trials
- no clear/proven association of the wild-type viruses with diseases in mammals

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AAV Nanoparticles as Vectors

Adeno-associated virus vector as a platform for gene therapy delivery

Nat Rev Drug Discov. Author manuscript; available in PMC 2019 February 01.
Published in final edited form as: Nat Rev Drug Discov. 2018 August; 17(8): 541–555. doi:10.1038/nrd.2018.110
NATURE REVIEWS | DRUG DISCOVERY
February 2019

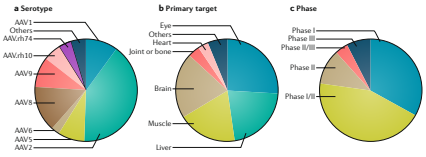

Fig. 4 | **Overview of rAAV interventional gene therapy clinical trials.** The data set is from ClinicalTrials.gov, accessed on 13 November 2018. The 145 registered trials are categorized on the basis of adeno-associated virus (AAV) capsid serotype (part **a**), primary tissue target for gene delivery (part **b**) and clinical trial phase (part **c**). rAAV, recombinant AAV.

AAV DNA Delivery Platform

suited for postmitotic cells

- **gene replacement**
- **gene silencing**
- **gene addition**

Key Challenges

- **Large-scale vector manufactoring and costs Glybera® (liver) => US$ 1.2 million for one shot LuxturnaR (eye) => US$ 425,000 for one eye**
- **hemophilia A: "*likely to be cost-saving/-effective compared with FVIII prophylaxis*" at US$ 1 million**

Machin, Ragni *et* Smith. **Blood Advances** Nov 2018

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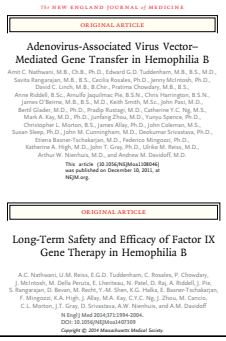
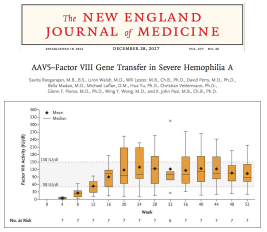
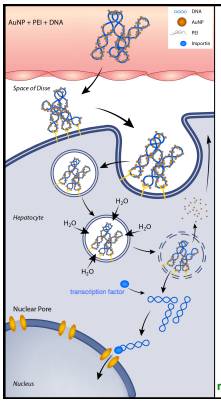
Seminal AAV Studies for *FIX* and *FVIII* Deficiency





FVIII activity in the high-dose cohort (6×10^{13} vector genomes/kg)

- **FVIII and FIX activities >5% for years**
- **10-1000 vector genomes per hepatocyte**
- **strong immunity against AAV capsid**



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Nonviral Genetic Therapy For Hemophilia

- **DNA for delivery of the genetic information**
- **nonviral system for transport of DNA to liver cells**
- **uptake of the delivery system by endocytosis**
- **controlled release/escape of the DNA from endosomes into the cytoplasma**
- **transport of the DNA into nucleus**
- **persistance of the DNA without integration**
- **RNA transcription from nonsilenced promotors**

modified after Li *et al.* J Hepathol 2016

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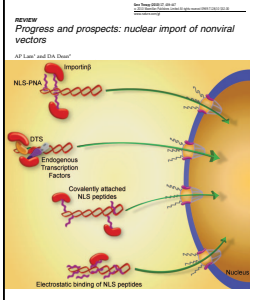
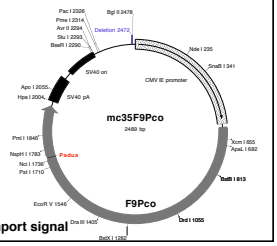
Construction of a disease-specific vector



Minicircle Vector Construction for FIX

- **inclusion of the SV40 origin DNA for binding to transcription factors for nuclear import**
- **removal of all bacterial DNA sequences in the plasmid backbone**



Minicircle Vector for FIX with nuclear import signal

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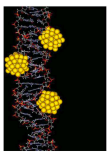
Nanoparticle-based delivery of cargo

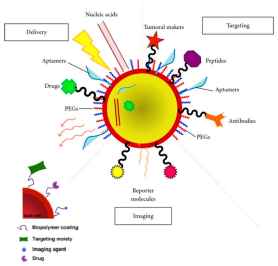
Gold Nanoparticles (AuNPs)

- **cell-free particles (2-250 nM)**
- **generated by laser or chemical reaction**
- **animal toxicity data is favourable**
- **no immunity after repeated applications**
- ***in vivo* tissue distribution depends on particle size, ligand & application route**
- **industrial production is well established**



- **toxicity of AuNP occurred at a size of ≤1.4nm, as the nanoparticles can bind to the major groove of the DNA**

Semmler-Behnke *et al.* **small** 2008



Sun *et al.* Angew Chem Intern Ed 2014
Kim *et al.* Acta Pharmacol Sinica 2011
Silva *et al.* Chapter 4 Intech 2014

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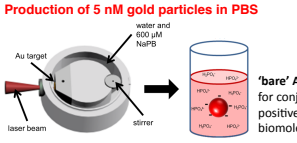
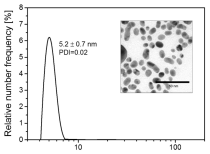
Production of '*Naked*'of Gold Nanoparticles

Production of 5 nM gold particles in PBS



Pulsed laser ablation in liquid

'bare' AuNP:
for conjugation of
positively charged
biomolecules



evenly distributed particles

Conjugation to cargo: => cDNA for *GFP*, *FVIII* or *FIX* expression
=> binding of DNA with polyethylenimine (PEI)
=> other targeting moieties for tissues

Gamrad *et al.* J Phys Chem C 2014

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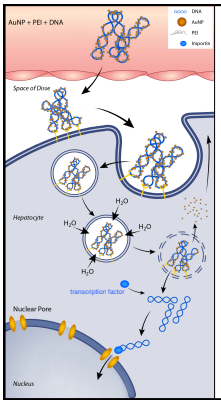
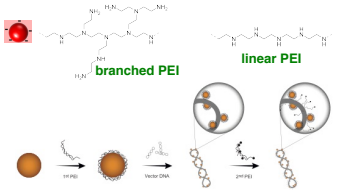
PEI-mediated binding of DNA to Gold NPs for endosomal escape



DNA and Gold nanoparticles are both negatively charged
=> complex formation achieved by polyethylenimine (PEI)
=> highly hydroscopic => influx of H_2O into endosomes
=> bursting of the endosome before fusion with lysosome



2nd PEI layer for specific targeting,
e.g. JetPEI Hepatocyte or PEI-PEG

sandwich formation

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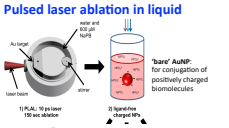
Laser-Derived vs. Chemically Synthesized AuNPs

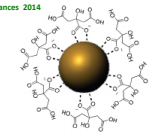
Gamrad *et al.* J Phys Chem C 2014

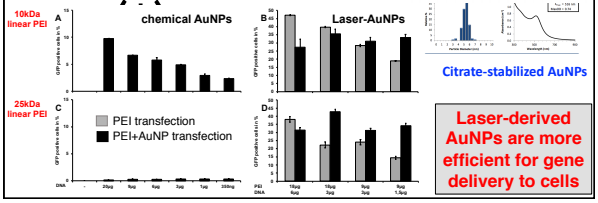
Pulsed laser ablation in liquid



- HLF liver cell line
- 5nm AuNPs with PEI + DNA
- GFP as readout by FACS

Guo *et al.* RSC Advances 2014





Citrate-stabilized AuNPs

Laser-derived AuNPs are more efficient for gene delivery to cells

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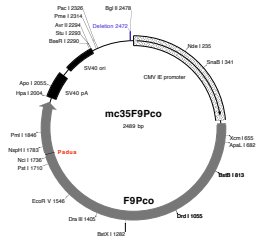
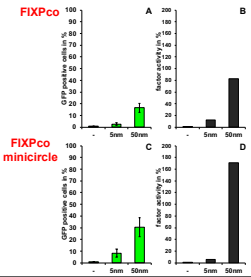
Gene Transfer achieved with 5 vs. 50nm Gold NPs

Minicircle Vector Construction for FIX

- inclusion of the SV40 origin DNA for binding to transcription factors for nuclear import
- removal of all bacterial DNA sequences in the plasmid backbone



- Transfection of FIXPadua cDNA with 5nm or 50nm AuNP in primary rat hepatocytes
- GFP cDNA as transfection control



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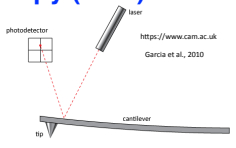
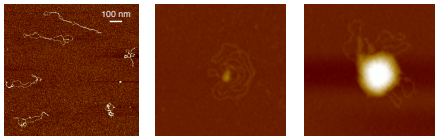
Atomic Force Microscopy (AFM)

- images by topography of the sample
- samples are dried on a carbon mica plate
- raster scanning mode (x-y grid)
- force measurement between probe and sample (stiffness, adhesion strength)





Plasmid DNA | 5nm Gold NP + DNA | 50nm Gold NP + DNA

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Content

- Introduction
- Principle of integrating viral vectors
- Principles of non-integrating viral vectors
- Nonviral alternatives for genetic therapies
  - transport of the DNA into hepatocytes
  - expression of factor in liver cells
  - repeated applications possible => application via peripheral veins
  - excellent safety & toxicity
  - platform suitable for other liver disorders
  - industrial production of the formulation