Gene therapy

Alessandro Aiuti

AA is the PI of gene therapy clinical trials on ADA-SCID, WAS, MLD, Beta thalassemia sponsored by GSK.

Lisbon, March 19th 2018

Gene and cell therapy

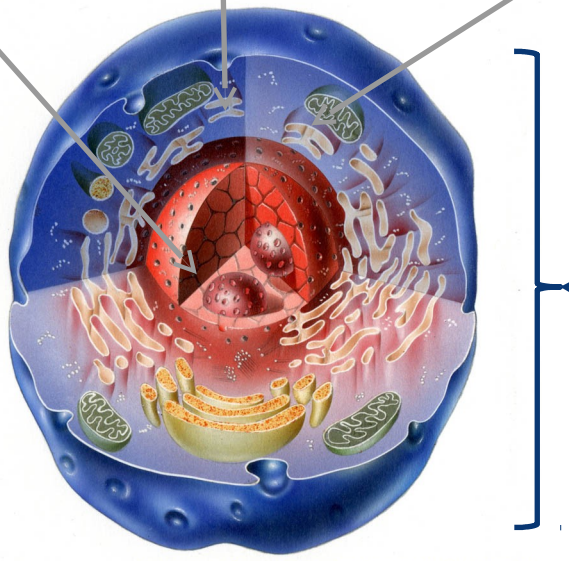
Gene therapy

- Administer the corrected gene (gene addition)
- Turn off/replace/correct the defective gene
- Activate another gene
- Provide a gene with a new function

Enzyme replacement therapy

PHARMACOLOGICAL THERAPY

gene → **protein** → **function**



cell

Cell therapy
Administration of healthy or corrected somatic cells

GENE and CELL THERAPY ADVANCED THERAPIES:

Reg (CE)1394/2007 Definitions

***Article 2* Definitions -**

'Advanced therapy medicinal product' means any of the following medicinal products for human use:

- **a gene therapy medicinal product** as defined in Part IV of Annex I to Directive 2001/83/EC

in Part IV of Annex I to Directive 2001/83/EC

A gene therapy medicinal product means **a biological medicinal product** that has the following characteristics:

- It contains an active substance that contains or consists of a **recombinant nucleic acid** used in or administered to human beings with a view to ***regulating*, *repairing*, *adding* or *deleting* a genetic sequence**
- Its therapeutic, prophylactic or diagnostic **effect relates directly** to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence

Gene therapy viral vectors

		Adenovirus	Adeno-associated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Particle characteristics	Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
	Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
	Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
	Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
	Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
	Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb
							
	Family	*Adenoviridae*	*Parvoviridae*	*Togaviridae*	*Herpesviridae*	*Retroviridae*	*Poxviridae*
Gene Therapy Properties	Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
	Host genome interaction	Non-integrating	Non-integrating*	Non-integrating	Non-integrating	Integrating	Non-integrating
	Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
	Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

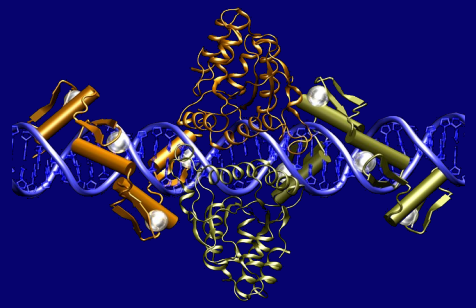
*Figure 2. A comparison of different viral vectors in use for gene therapy: overview of their advantages and disadvantages. * Adeno-associated viruses are able to integrate with low frequency into chromosome 19. Lentiviruses also infect non-dividing cells. You can also download the original image in high resolution as jpg or powerpoint file.*

http://www.genetherapynet.com/viral-vectors.html

Gene therapy platforms

- Gene addition with integrating vectors
- Gene editing
  - Gene correction
  - Inside a gene (downstream of a promoter)
  - In safe harbours

  Technology
  - Zinc fingers (nuclease)
  - TALEN (nuclease)
  - Crispr/Cas9 (RNA/protein)

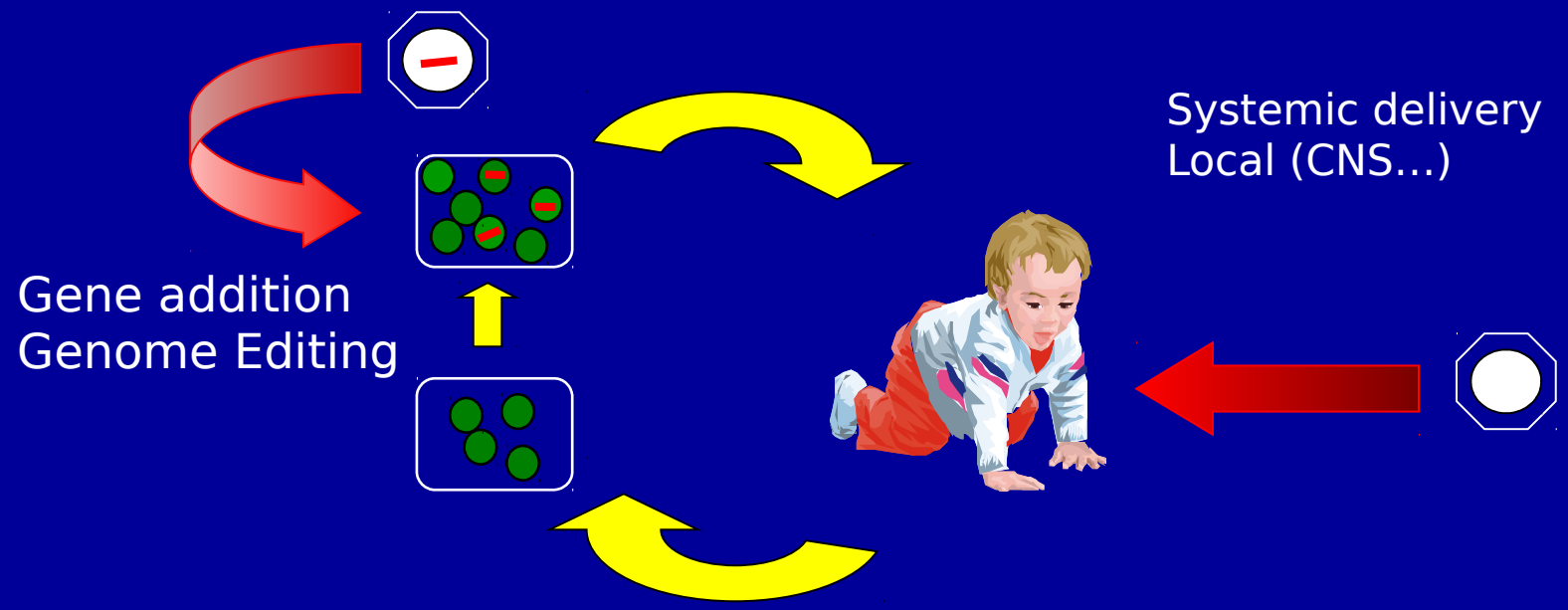


- Gene disruption
- Gene addition with inhibitory activity (shRNA)

Gene therapy approaches for genetic diseases

Ex vivo

In vivo



Primary immunodeficiencies
Thrombocytopenia
Lysosomal storage disorders
Haemoglobinopathies
BM failure

Lysosomal storage disorders
Other metabolic disorders
FIX and FVIII deficiency
Eye disorder
Neuromuscular disorder

Gene therapy based drugs authorised in the world

Name	Company	Disease	Current market area	Positive Opinion
Strimvelis	GSK	ADA-SCID	Europe	**2016**
Zalmoxis	MolMed	add-on treatment in pts with cancer who have received a HSC transplant	Europe	**2016**
Kymriha	Novartis	B cell leukemia	USA	**2017**
Yeskarta	KITE	Non Hodgkin Lymphoma	USA	**2017**
Luxturna	Spark Therapeutics	Leber Amaurosis	USA	**2017**

March 2018

ISTITUTO SAN RAFFAELE TELETHON PER LA TERAPIA GENICA
SR-Tiget

Gene therapies for rare diseases: scientific challenges

- Need to overcome:
  - **Biological barriers to engraftment and regeneration**
  - **Immunological barriers** to transplant of cells or genes
- Limited comprehension of **stem cell biology**
- Knowledge of **disease mechanisms**
- Need of adequate **preclinical models**
- Need of regulated and efficient methods of **gene transfer**
- Overcome **safety isses** (insertional mutagenesis)

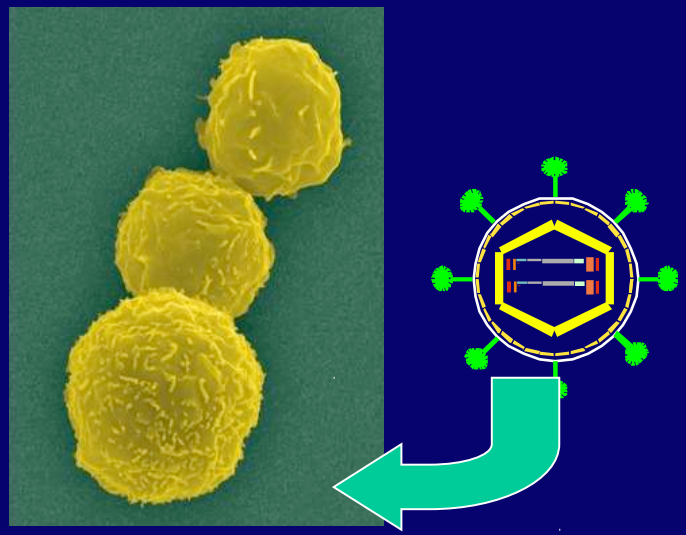
ISTITUTO SAN RAFFAELE TELETHON PER LA TERAPIA GENICA
SR-Tiget

Gene therapies for rare diseases: operational challenges

- **Development**
  - Large scale production and according to regulatory quality standard
- **Clinical trials**
  - Dedicated clinical research unit for ATMP
  - Development and validation of new tests and analitical methods
  - Special needs for infusion/implant
- Specific regulatory needs
- Financial support for research and development
- Industrial alliances to achieve approval and availability of medicinal product to patients
- High costs of the medicinal product

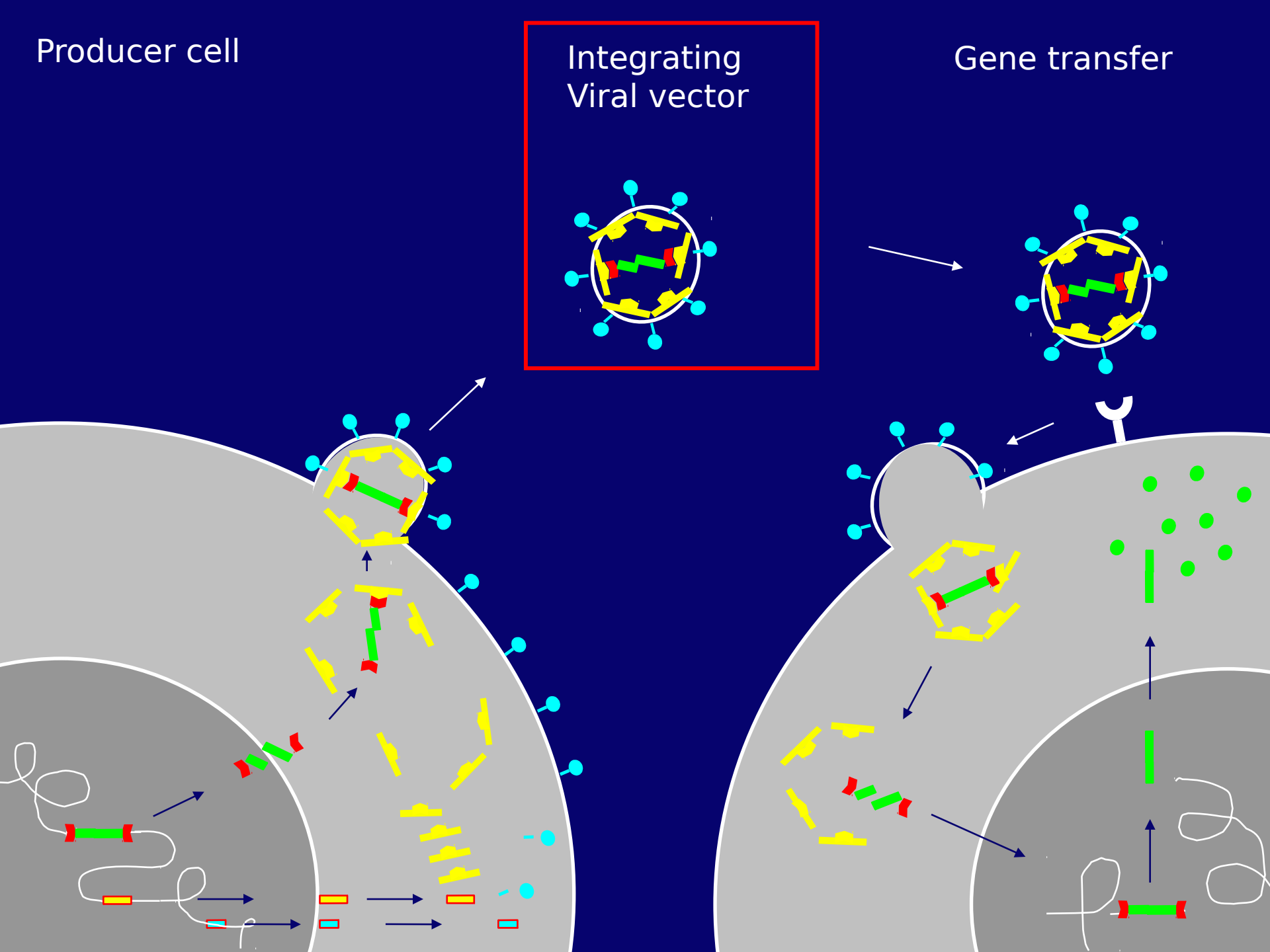
HEMATOPOIETIC STEM CELL TRANSPLANT AND ADVANCED THERAPIES

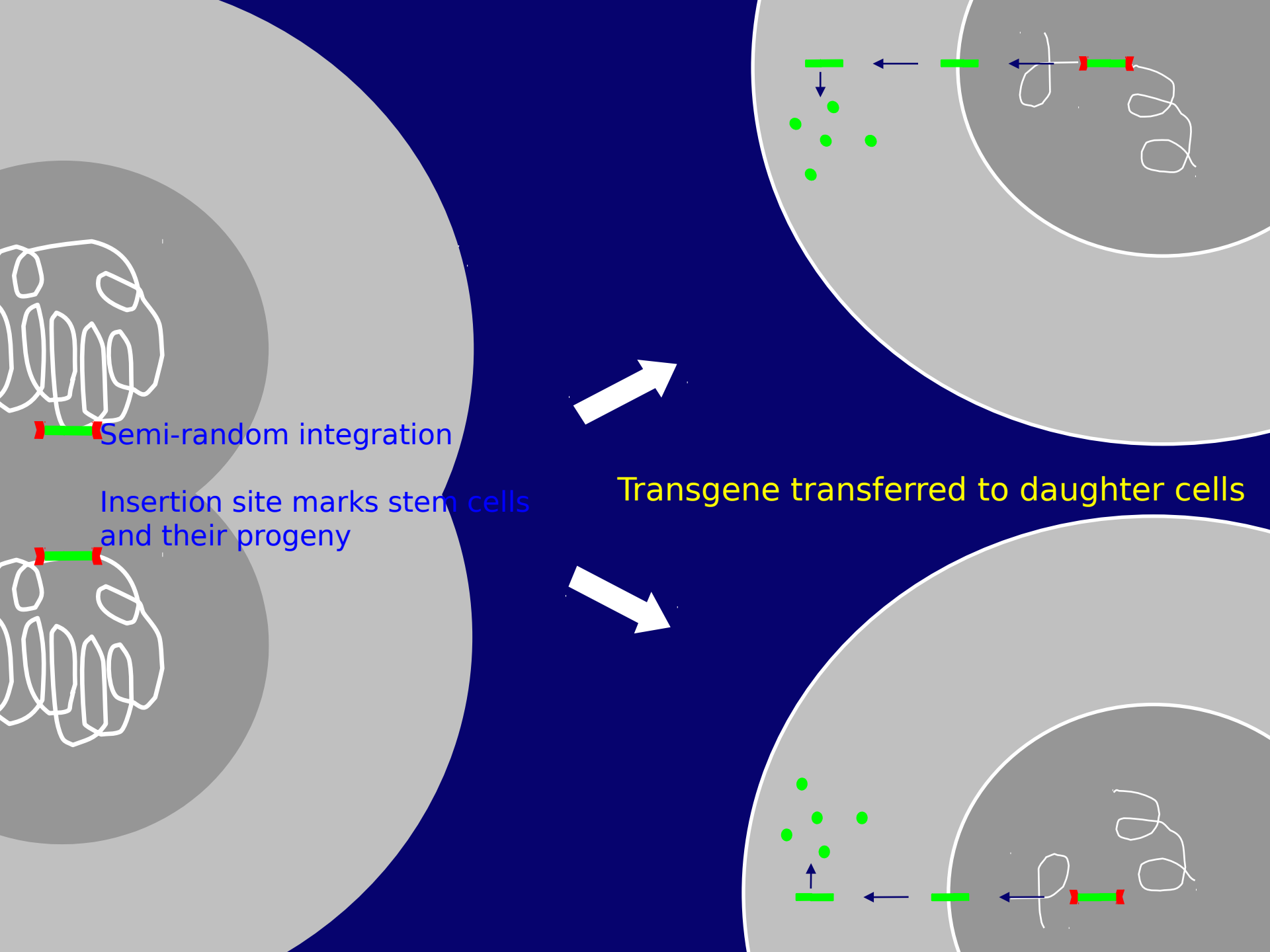
Transplant of normal HSC
from an allogeneic donor



Autologous transplant of
gene corrected HSC

Advanced therapy (GENE THERAPY)
“personalized therapy”

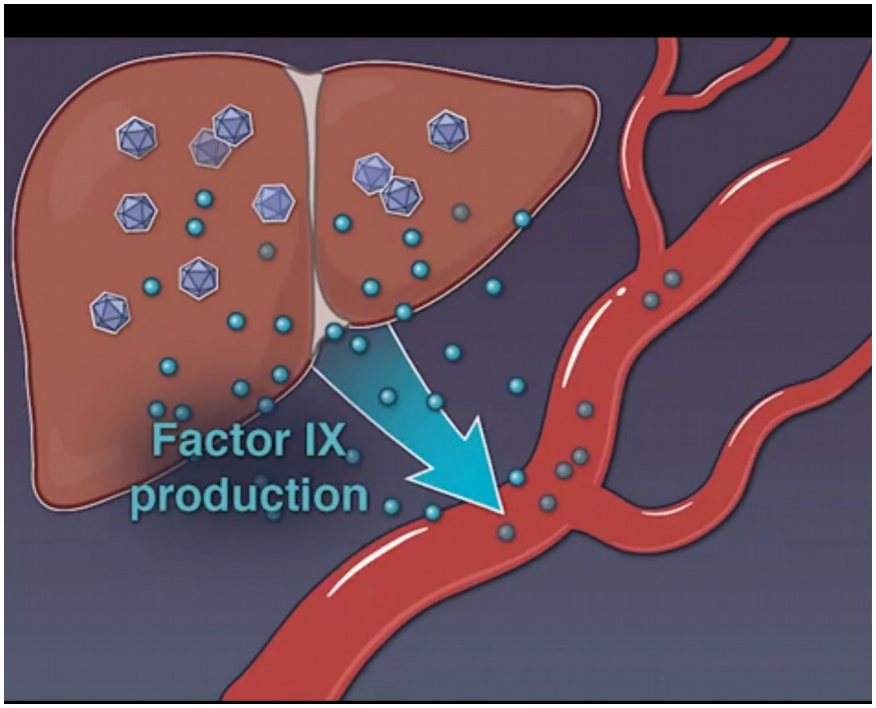

Producer cell
Integrating
Viral vector
Gene transfer

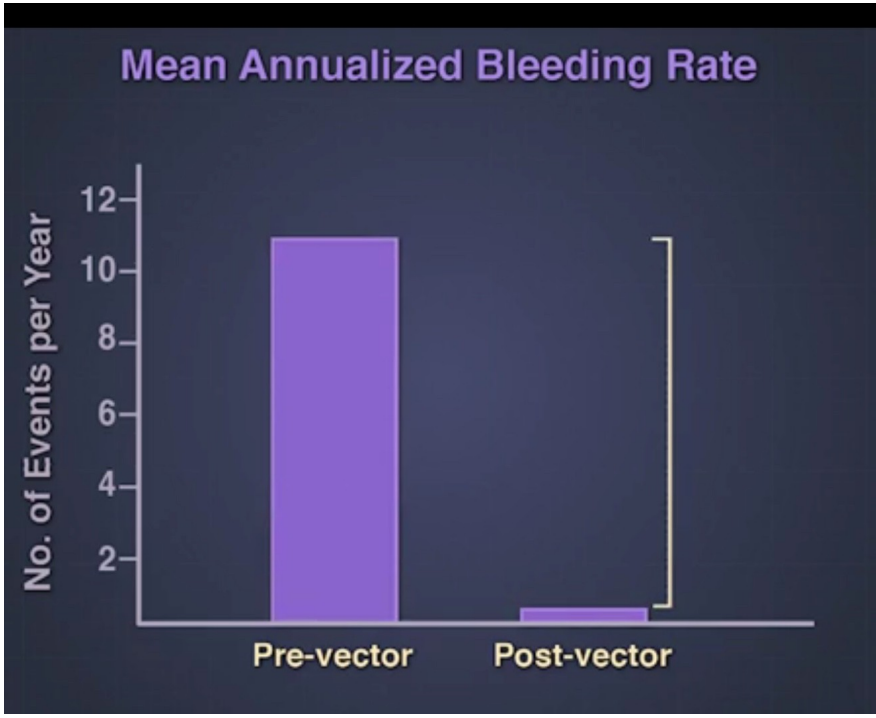

Semi-random integration
Insertion site marks stem cells
and their progeny
Transgene transferred to daughter cells

ORIGINAL ARTICLE FREE PREVIEW

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 Zelenaia, 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.





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

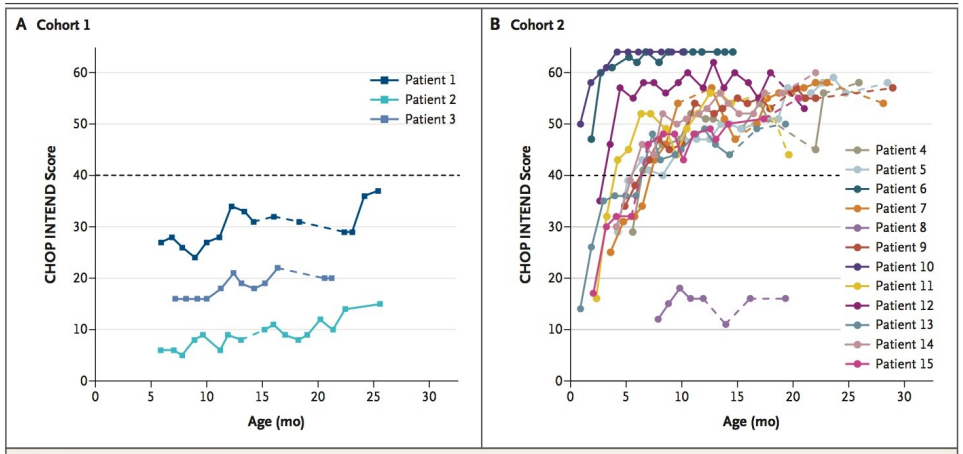


Figure 2. Motor Function after Gene Therapy.

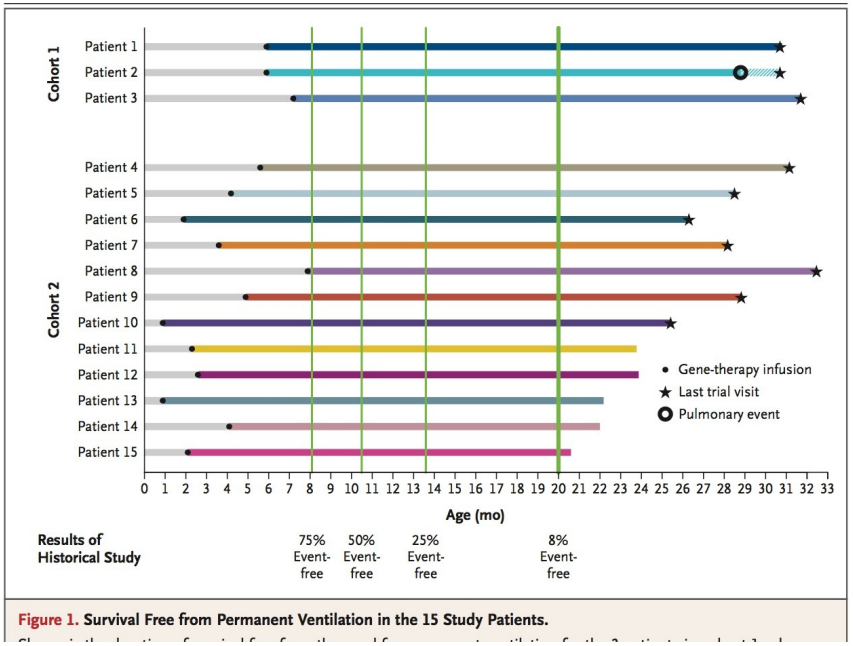
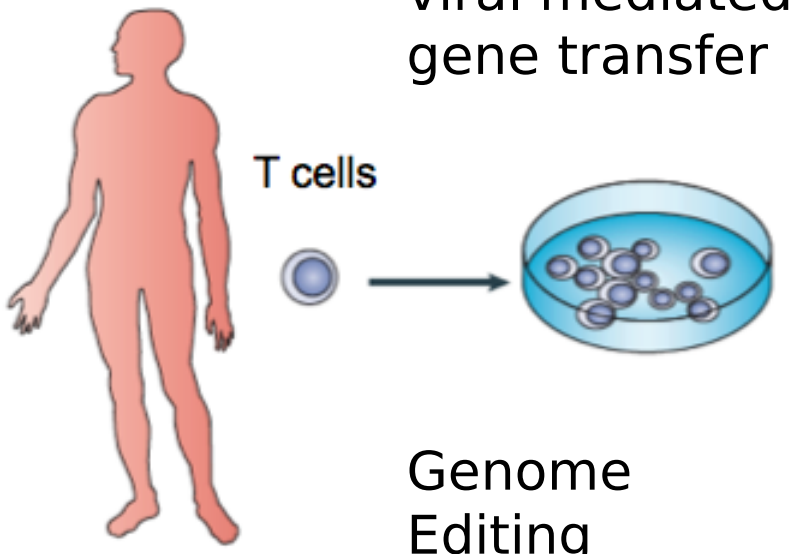


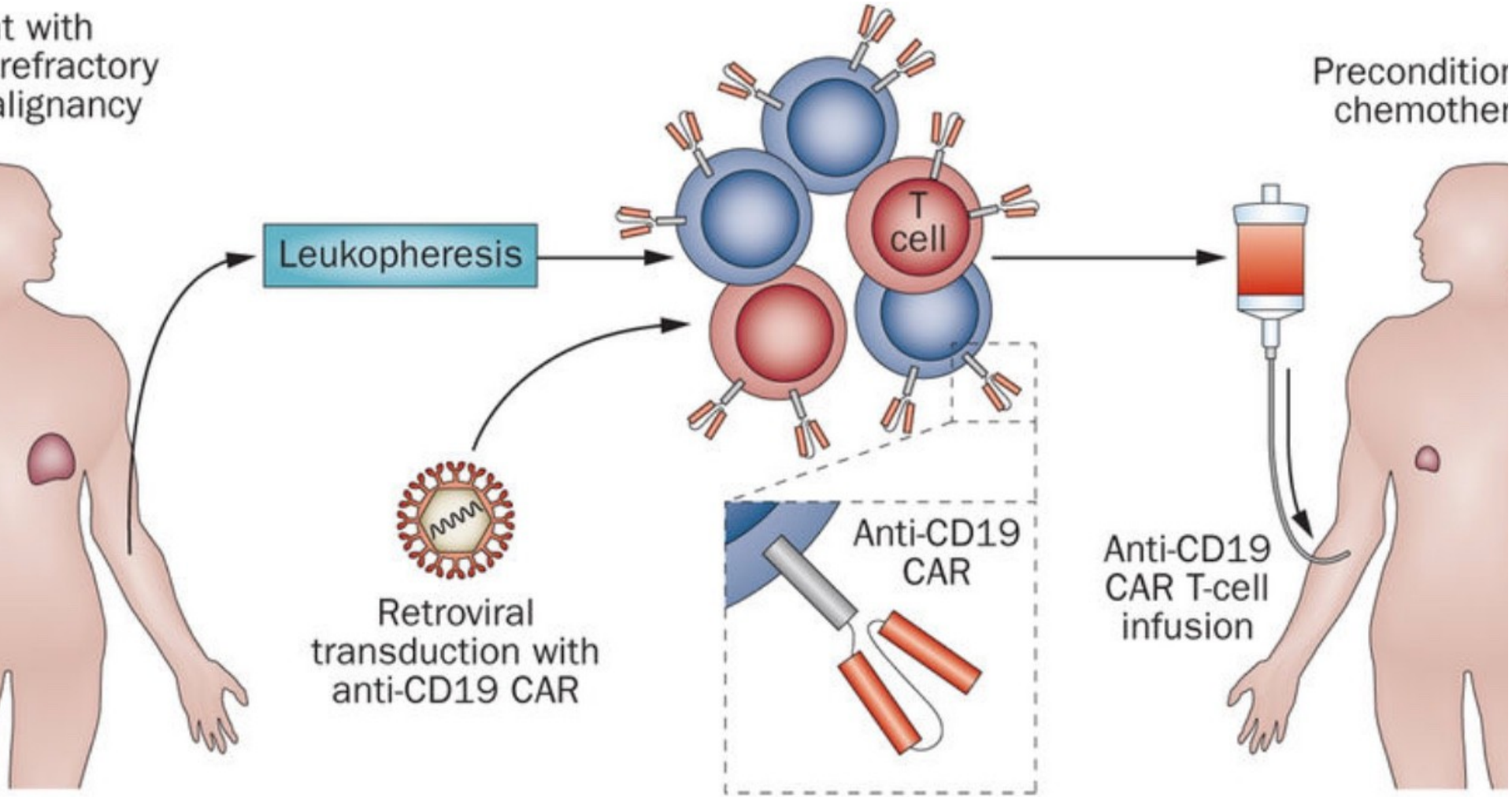
Figure 1. Survival Free from Permanent Ventilation in the 15 Study Patients.

Adoptive T-cell therapy for cancer: The era of genetically engineered cells



- Increasing the safety profile of T cells (suicide genes)
- Redirecting T cell specificity (CAR & TCR)
- Increasing function and persistence of T cells
- Modifying homing of T cells….

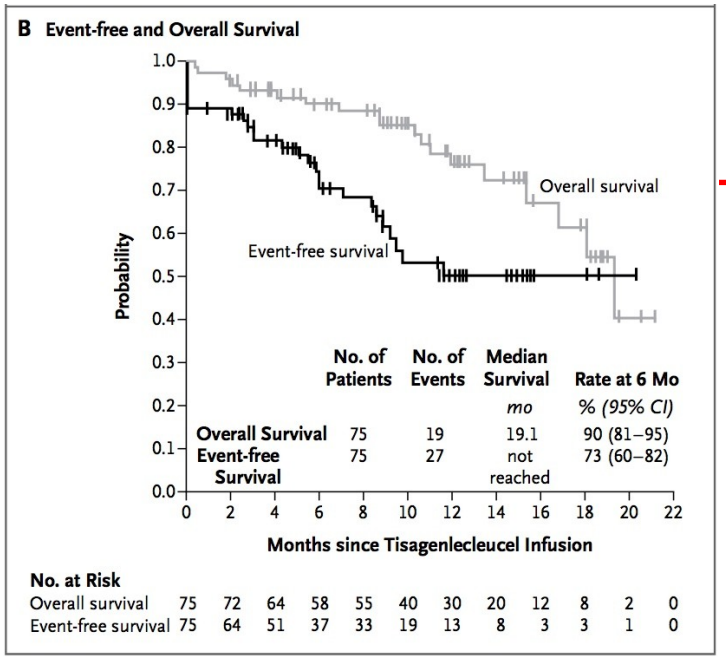
C. Bonini


t with
refractory
alignancy
Leukopheresis
T
cell
Precondition
chemother
Retroviral
transduction with
anti-CD19 CAR
Anti-CD19
CAR
Anti-CD19
CAR T-cell
infusion

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 Santomasso, 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.

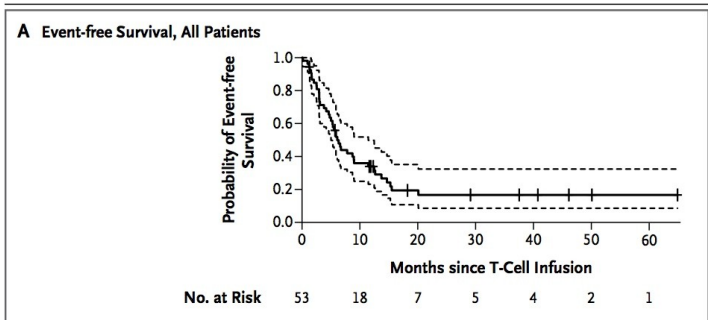


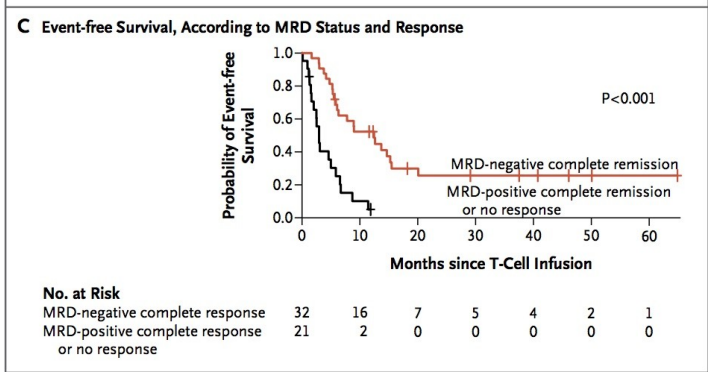
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

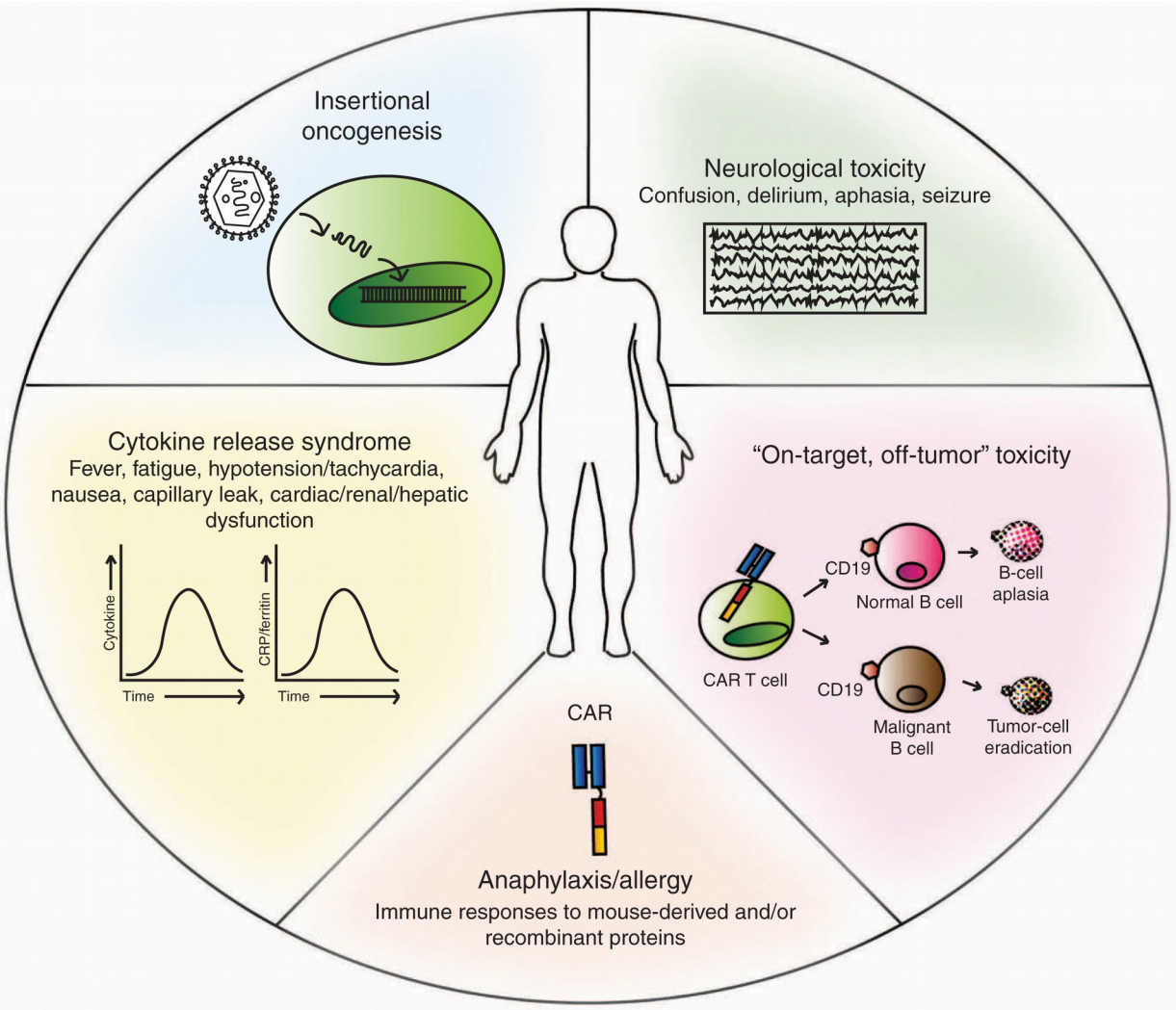
Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

S.L. Maude, T.W. Laetsch, J. Buechner, S. Rives, M. Boyer, H. Bittencourt, P. Bader, M.R. Verneris, H.E. Stefanski, G.D. Myers, M. Qayed, B. De Moerloose, H. Hiramatsu, K. Schlis, K.L. Davis, P.L. Martin, E.R. Nemecek, G.A. Yanik, C. Peters, A. Baruchel, N. Boissel, F. Mechinaud, A. Balduzzi, J. Krueger, C.H. June, B.L. Levine, P. Wood, T. Taran, M. Leung, K.T. Mueller, Y. Zhang, K. Sen, D. Lebwohl, M.A. Pulsipher, and S.A. Grupp





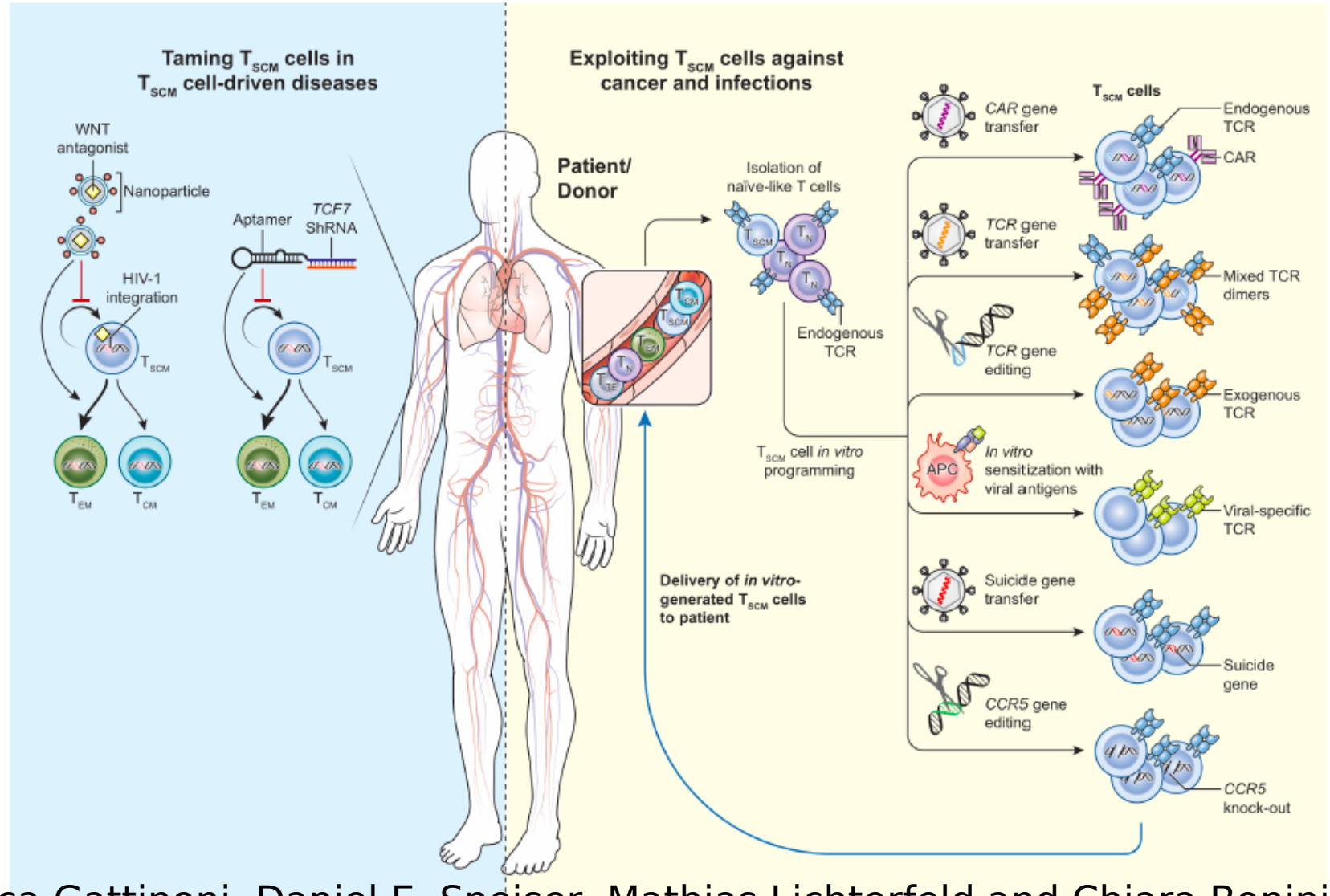
Toxicity and management in CAR T-cell therapy



Challice L Bonifant, Hollie J Jackson, Renier J Brentjens, Kevin J Curran

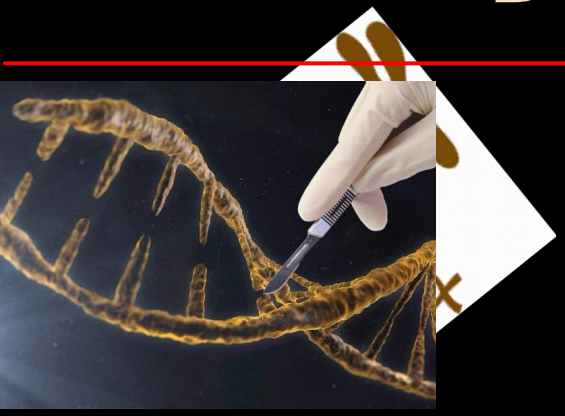
Molecular Therapy - Oncolytics
Volume 3, (January 2016)
DOI: 10.1038/mto.2016.11

T_{SCM}-based therapeutic interventions for human diseases

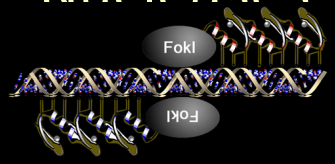


Luca Gattinoni, Daniel E. Speiser, Mathias Lichterfeld and Chiara Bonini*;* *Nature Medicine*

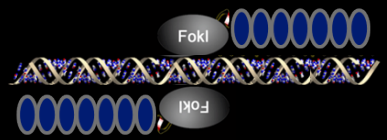
DNA “Nano-Surgery”



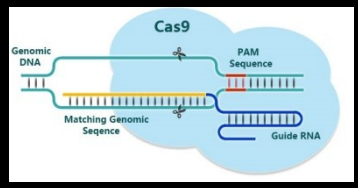
Zinc Finger Nucleases

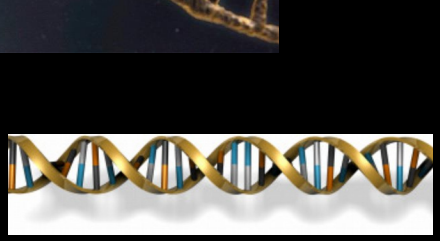


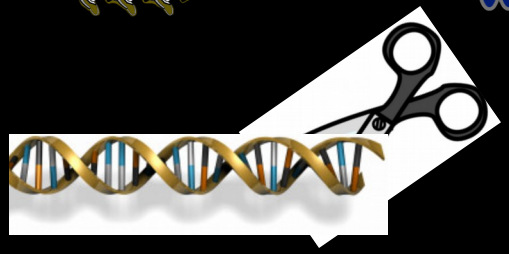
TALENs



CRISPR/Cas9





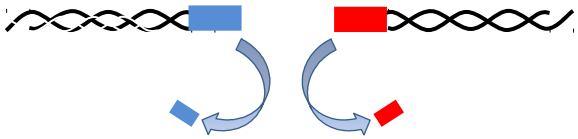


Repair by Non Homologous End Joining

↓

Loss of Function

Genomic DNA



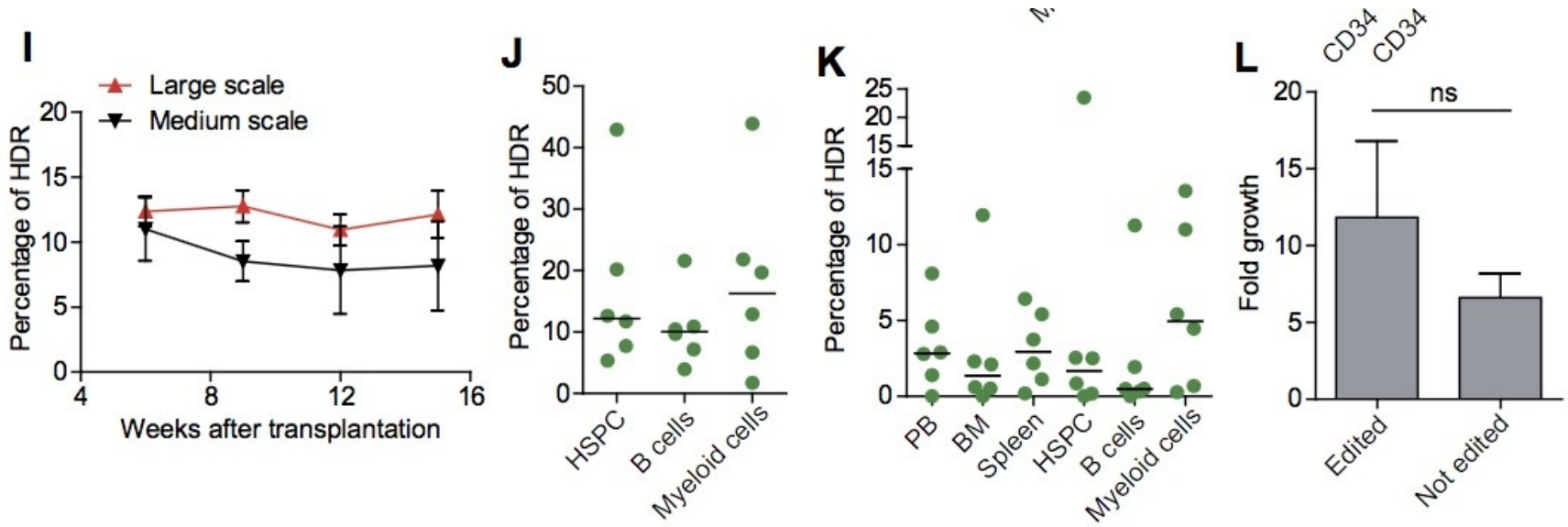
Loss of nucleotides

Gene Knock Out

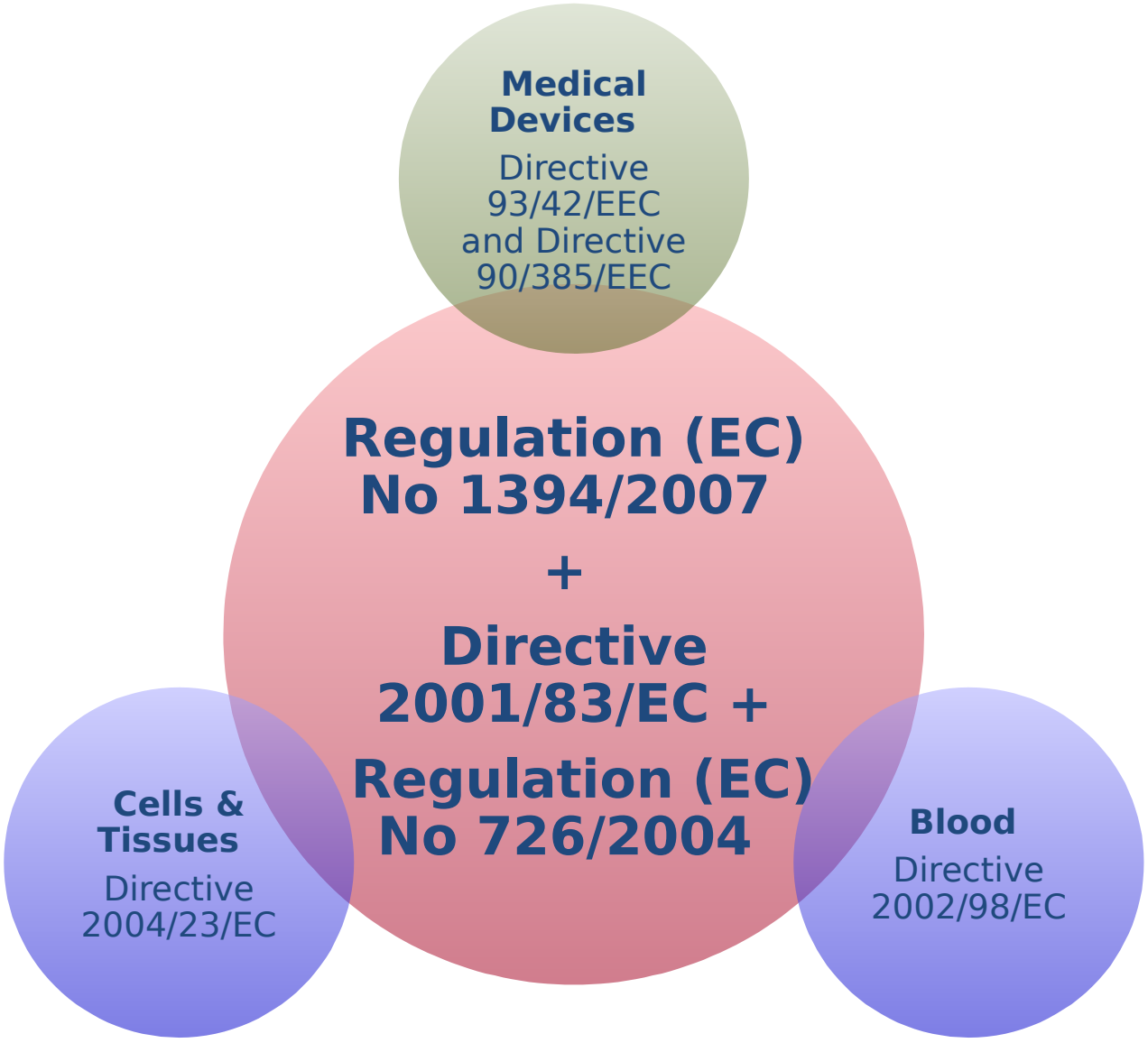
GENE THERAPY

Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1

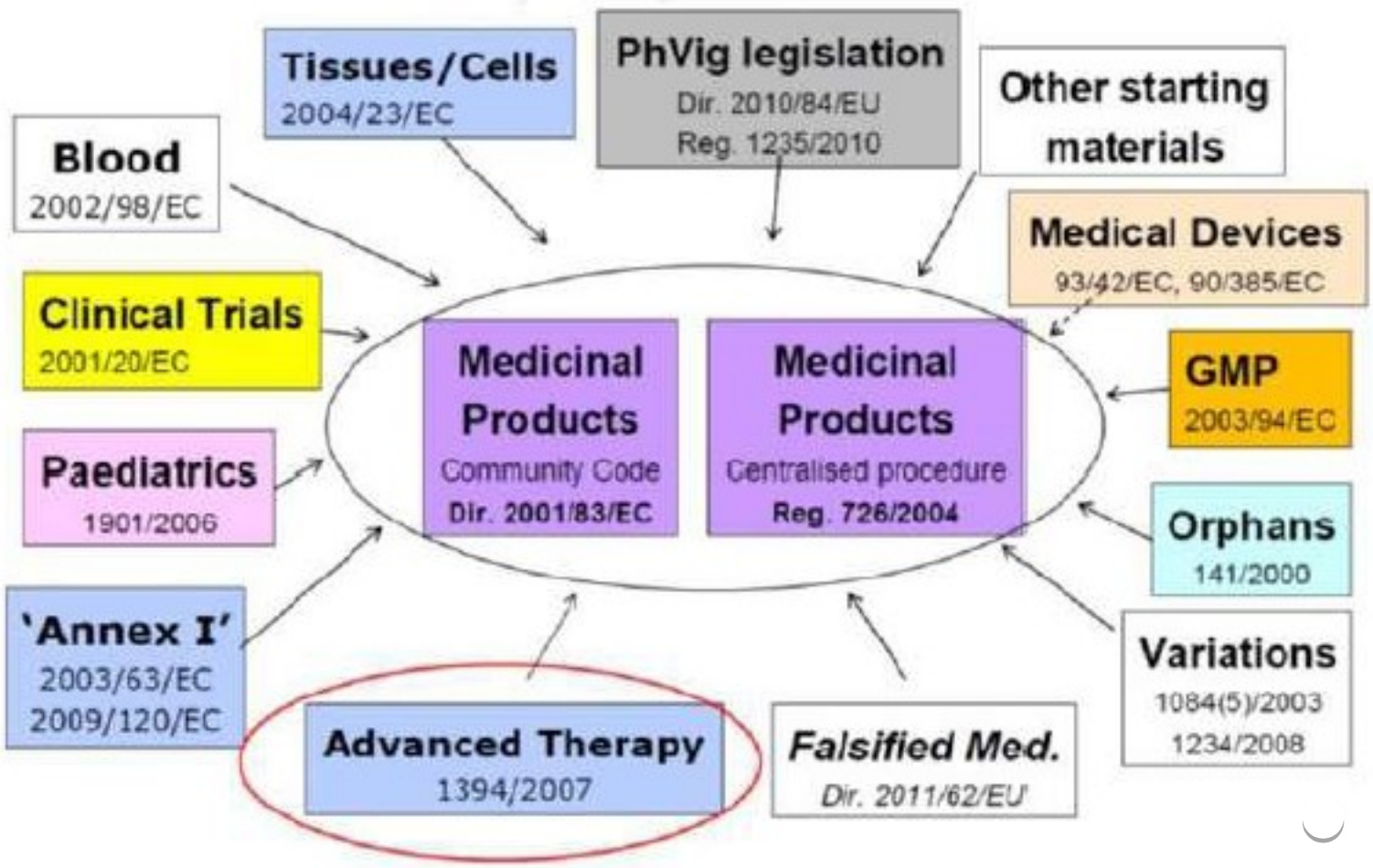
Giulia Schiroli,[1,2] Samuele Ferrari,[1,2] Anthony Conway,[3] Aurelien Jacob,[1] Valentina Capo,[1] Luisa Albano,[1] Tiziana Plati,[1] Maria C. Castiello,[1] Francesca Sanvito,[4] Andrew R. Gennery,[5] Chiara Bovolenta,[6] Rahul Palchaudhuri,[7,8] David T. Scadden,[8] Michael C. Holmes,[3] Anna Villa,[1,9] Giovanni Sitia,[10] Angelo Lombardo,[1,2] Pietro Genovese,[1*†] Luigi Naldini[1,2*†]



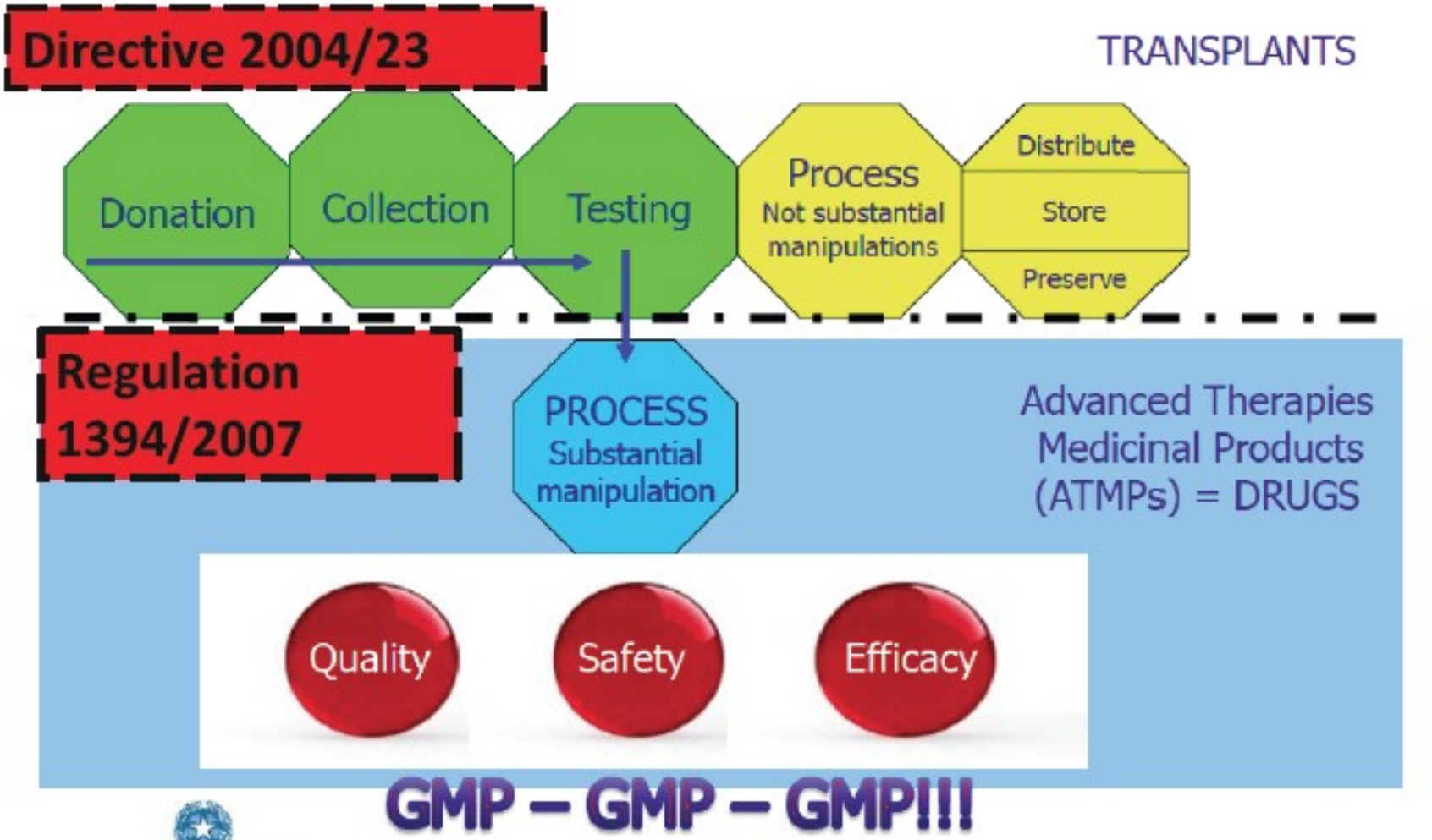
Core Legal Framework for ATMPs in the EU



EU Legal/Regulatory Framework for Pharmaceuticals



Regulatory Framework



Source: Montesoro, AIFA

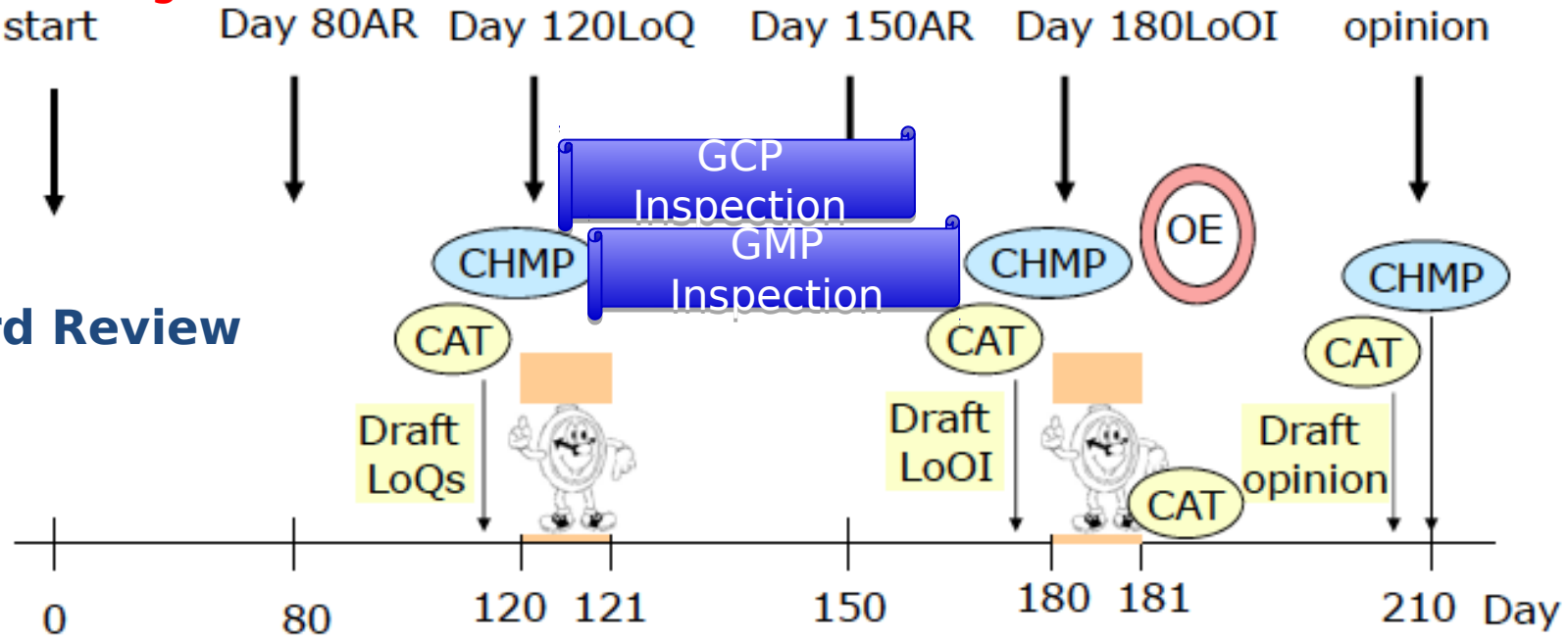
Applicability of various EU regulations and directives to some example ATMPs

	Gene Therapy Medicinal Product (not using human derived cell lines)	Autologous Cell Therapy (derived from human blood) genetically modified using a viral vector	Autologous Somatic Cell Therapy (not blood derived)	Allogeneic Cell Therapy (blood derived)
Medicinal Products Regulation (2001/83/EC)[2]	✓	✓	✓	✓
ATMP Regulation ((EC) 1394/2007)[1]	✓	✓	✓	✓
Blood Directive (and national laws) (2002/98/EC)[7]	✗	✓	✗	✓
Cells and Tissue Directive (and national laws) (2004/23/EC)[8]	✗	✗	✓	✗
Technical requirements for the donation, procurement and testing of human tissues and cells (2006/17/EC)[9]	✗	✗	✓	✗
Traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells (2006/86/EC)[10]	✗	✗	✓	✗
Amending Directive 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells (2012/39/EU)[11]	✗	✗	✓	✗

Reg (CE)1394/20 EU Centralised Procedure

- Often **ODD** => MAA reviewed by **CHMP**&**CAT** + **COMP** (re-evaluation at the time of MAA)
- ***APPROVAL****: Standard*, Conditional, Under Exceptional Circustancies
- *Accelerated* vs *standard* review timelines for possible for Life Threatening MP

Standard Review Process



Other topics of discussion:

- **duration** of the post-approval **registry**: 15 year up vs. lifelong follow up
- inclusion of **specific safety monitoring** in the registry

SR-Tiget CLINICAL RESEARCH UNIT AND KEY COLLABORATOR

Clinical Pediatric Research
A. Aiuti
E Bernardo-MP Cicalese (c

Clinical Haematology Research Unit
F. Ciceri
S. Marktel (coord)

faele Stem Cell Program
(Head: F. Ciceri)

GET clinical trial office (TCTO)

ancan (coordinator)
astagnaro (QA)
asiraghi G. Antonioli
arin
acchini S. Locatelli
Bergami A. Corti
ossati E. Albertazzi
Hossary
omaselli

ucano, A. Cazzato "come a casa"

IGET clinical lab (TCL)

Zancan
Castagnaro (QA)
Albertini
Brigida S. Scaramuzza
Giannelli F. Dionisio
Sartirana F. Salerio
Acquati D. Redaelli
Attanasio C. Rossi
Mezzanotte A. Corti
Tommasoni

Pediatric Immunohematology

A. Aiuti (Head)
ME Bernardo (RUF BMT Unit)
MP Cicalese (RUF Ped DH)
F. Ferrua
V. Calbi
A. Assanelli
M. Migliavacca
F. Tucci F. Barzaghi
M. Doglio G. Prunotto
F. Ciotti /M. Fraschini
M. Sarzana

F. Calzatini
D. Canarutto
G. Consiglieri
R. Pajno
S. Recupero

Adult BMT and hematolo

F. Ciceri (Head)
S. Marktel
B. Gentner
F. Giglio
C. Soliman/A. Biella (head nurse)
M. Coppola R. Milani
L. Santoleri
S. Gattillo
Other staff

Pediatric Neurology

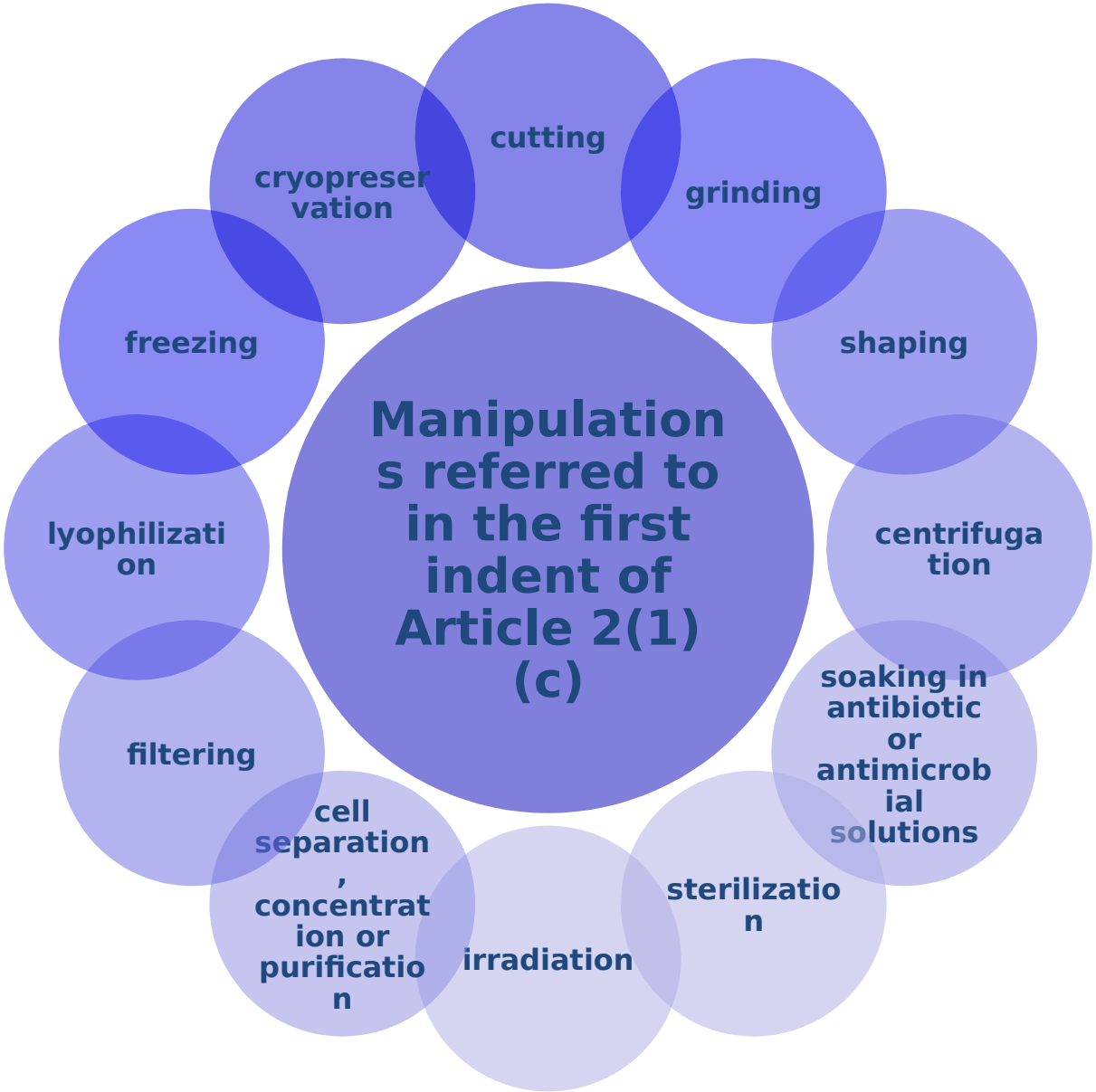
MG Natali Sora
F. Fumagalli
A. Zambon

MolMEd (GMP CMO)

M. Gabaldo
(Head Alliance Management &Reg Affairs Manager)
G. Farinelli

External labs and collaborators

Examples of processes NOT considered "Substantial Manipulation" (Reg 1394/2007/EC ANNEX I)



Reg (CE)1394/2007 Incentives

ATMP CLASSIFICATION

ATMP **CERTIFICATION**: Quality & N-clinical data (*SME only*)

ATMP Incentives

SCIENTIFIC ADVICE

EMA **FEE REDUCTION**