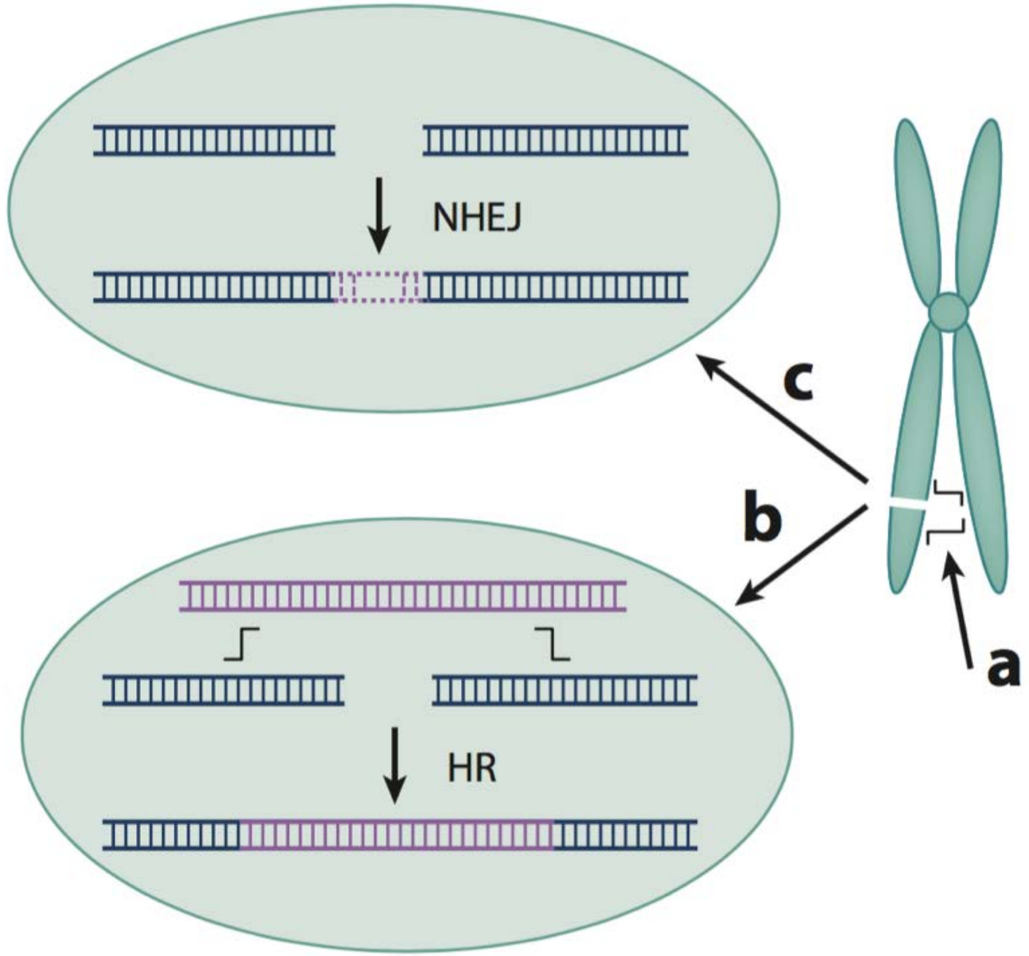
Genome editing: clinical applications

ALTIVS
/ FYODOR URNOV

Double-strand break repair



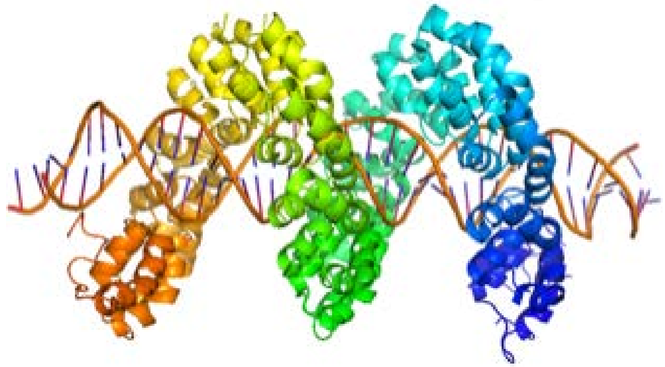
D. Carroll

Genome-Editing Nucleases

Zinc Finger Nucleases (ZFNs)

Sangamo / Biogen

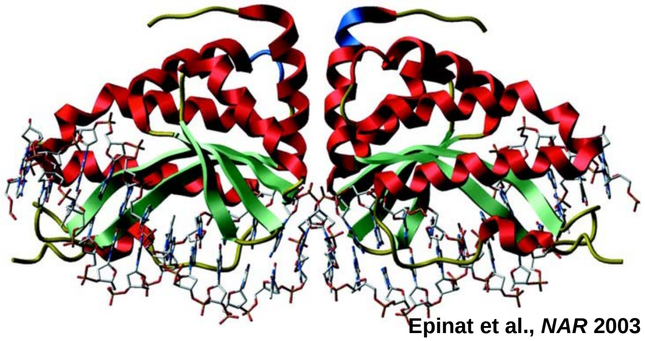
TALE Nucleases (TALENs)



Cellectis / Pfizer

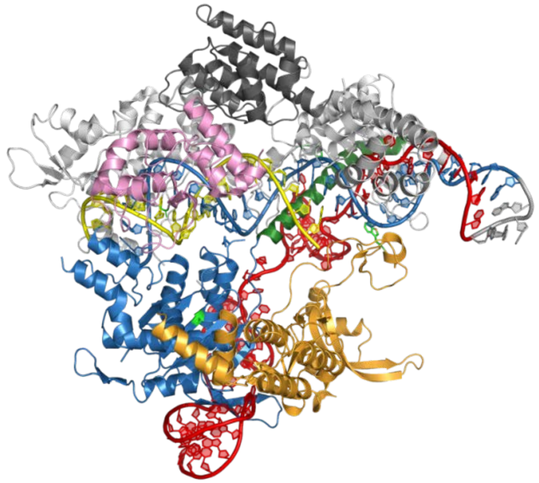
Meganucleases

Bluebird



Epinat et al., *NAR* 2003

CRISPR RNA-Guided Nucleases



Intellia / Novartis
Editas
CRISPR / Bayer

Slide courtesy of J. Keith Joung, Mass General / Harvard

Genome editing

nuclease → break → edited allele

1. indel – KO
2. SNP – correction of mutation
3. transgene – targeted integration

Basic science origins of editing

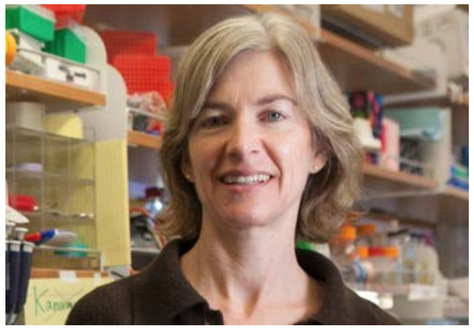
Maria Jasin – I-SceI

Aaron Klug and Carl Pabo – zinc finger proteins

Dana Carroll – zinc finger nucleases

Ulla Bonas – TAL effector

Jennifer Doudna and Emmanuelle Charpentier – RNA-guided nature of Cas9



Genome editing: clinical record (Oct 2016)

Target -> IND -> clinic:

Case study 1: CCR5 / HIV / T cells

Target validation: human genetics; Berlin patient
Nuclease: ZFN (Sangamo)
Preclinical efficacy:
- No adverse effect on T cell in vitro post-editing
- Mouse model efficacy

Preclinical safety:
- Patient cell dose NSG mouse tumorigenicity study

CMC:
- GMP ex vivo Ad5/35 or ex vivo mRNA electroporation

Clinical record (2009 -)
- 80 subjects treated
- Treatment well-tolerated to date
- Edited cells persist >4 yrs
- Stable increase in CD4 T cell count ~150 cells / ul
- Decrease in viral reservoir
- T cells home to the GALT and retain function
- >60% subjects in recent cohort control virus in absence of ART

Ninety one percent knockout in primary T cells

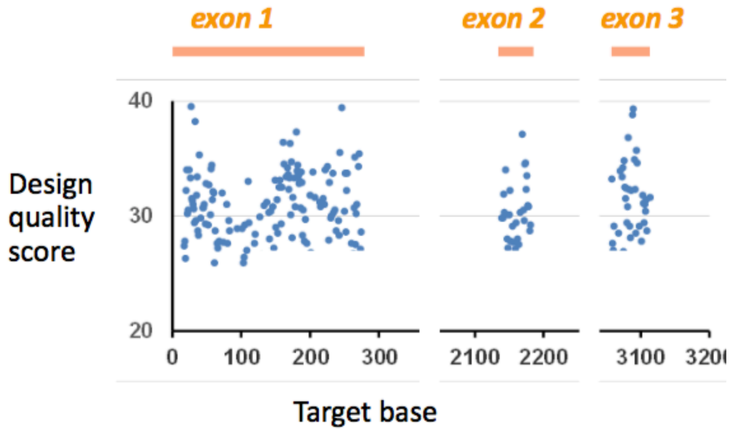
ZFNs Targeting TCRα Gene Are Highly Active and Well-Tolerated in Transformed and Primary Human Cells

Example: TCRα constant region

- **3 exons / 374 bp**
- **374 potential locations for cleavage**

→ **218 locations are targetable with ZFNs (58%)**

→ **One ZFN pair per every 1.7 bp**



K562 cells

ZFN pair #	Modification level (% indels) 250 ng RNA	500 ng RNA
1	77 ± 1.3	88 ± 1
2	91 ± 1.5	94 ± 1.7
3	82 ± 0.6	92 ± 1.3
4	95 ± 1.8	96 ± 1.2
5	88 ± 1.3	93 ± 1
6	85 ± 1.4	89 ± 0.4
7	77 ± 1.7	87 ± 1.1
8	54 ± 3.2	75 ± 1.3
9	90 ± 1.6	90 ± 1.5
10	77 ± 0.8	84 ± 2.2
GFP	0	0

T cells (10 days post transfection)

ZFN pair #	% TCR negative cells
1	78.4%
2	88.1%
3	85.3%
4	85.2%
5	56.1%
6	91.4%
7	69.4%
8	81.7%
9	90.9%
10	85.6%
mock	1.8%

Genome-edited T cells in the clinic: general lessons

Targeted knockout ex vivo – single and multiple loci – clinical scale efficiency and specificity.

CMC GMP path established

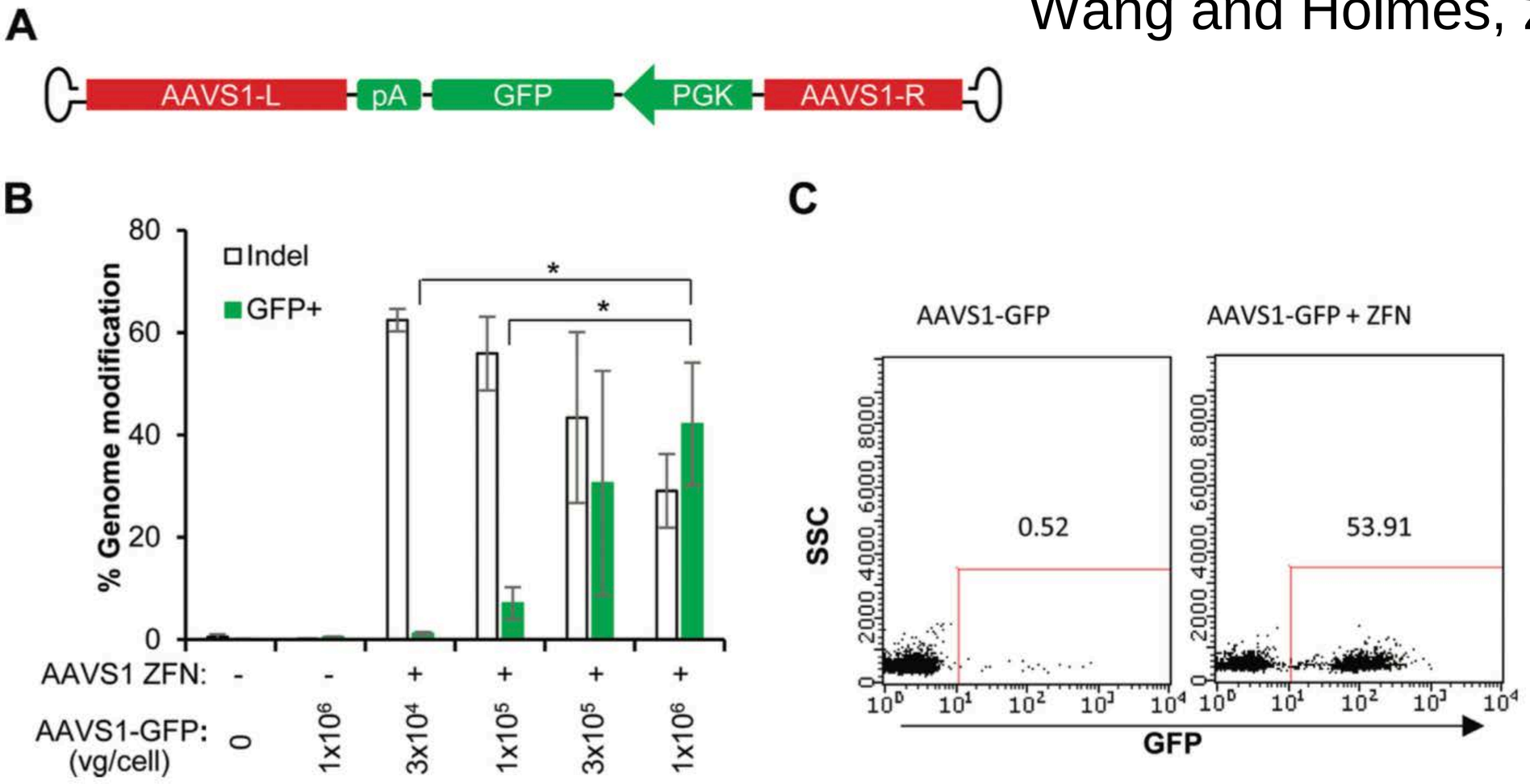
Preclinical pharm-tox path established

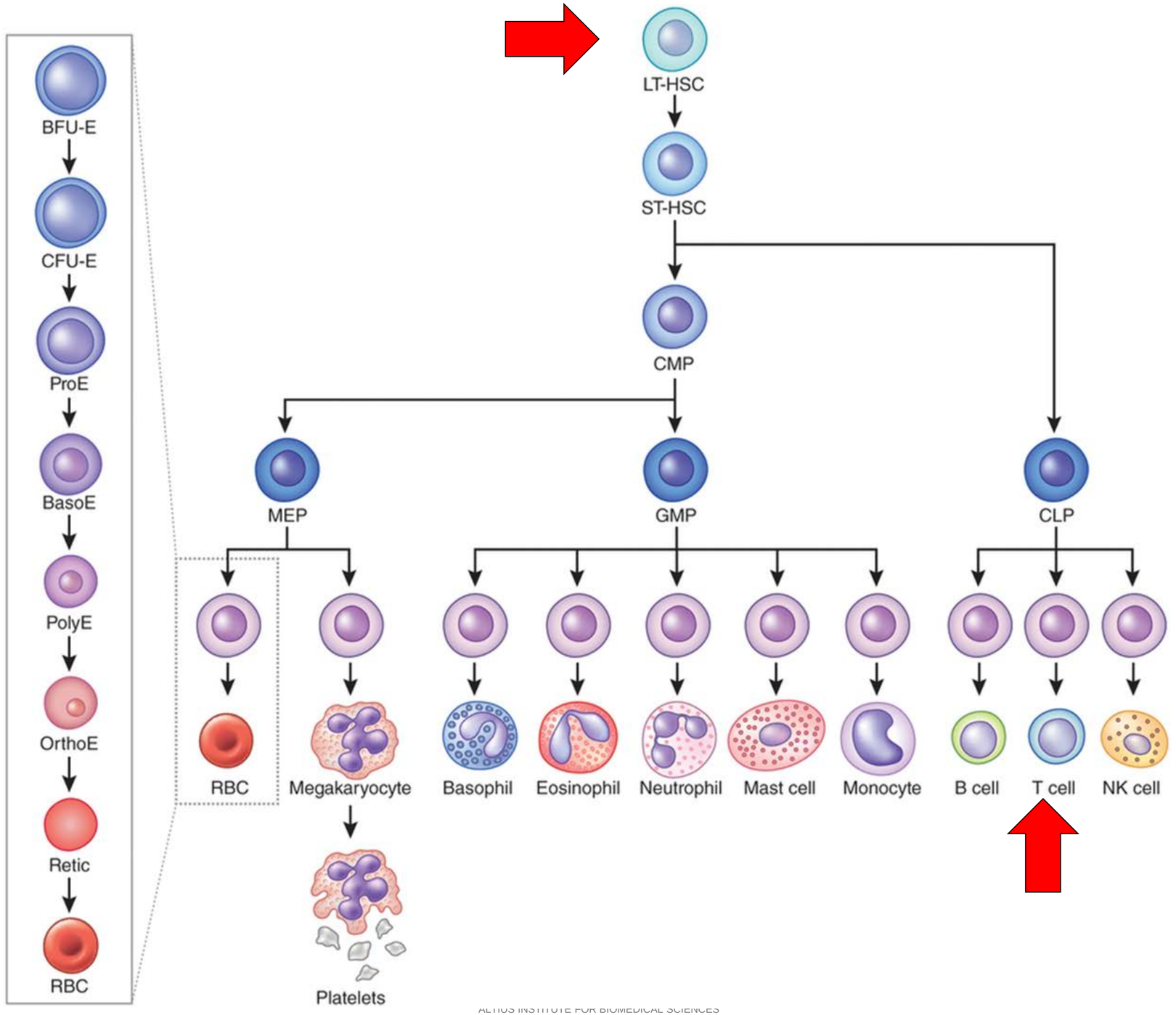
Regulatory framework (OCGCT CBER FDA) in place

Avenues for improvement
1. cell processing
2. allogeneic ”off the shelf” rather than autologous product

Efficient targeted addition in primary human T cells using mixed mode delivery

Wang and Holmes, 2015




LT-HSC
ST-HSC
CMP
MEP
GMP
CLP
BFU-E
CFU-E
ProE
BasoE
PolyE
OrthoE
Retic
RBC
RBC
Megakaryocyte
Platelets
Basophil
Eosinophil
Neutrophil
Mast cell
Monocyte
B cell
T cell
NK cell

Target -> IND -> clinic:
Case study 2: CCR5 / HIV / HSPCs

Target validation: human genetics; Berlin patient

Nuclease: ZFN (Sangamo)

Preclinical efficacy:

- No adverse effect on CD34 cell in vitro post-editing
- Mouse model efficacy

Preclinical safety:

- Patient cell dose NSG mouse tumorigenicity study

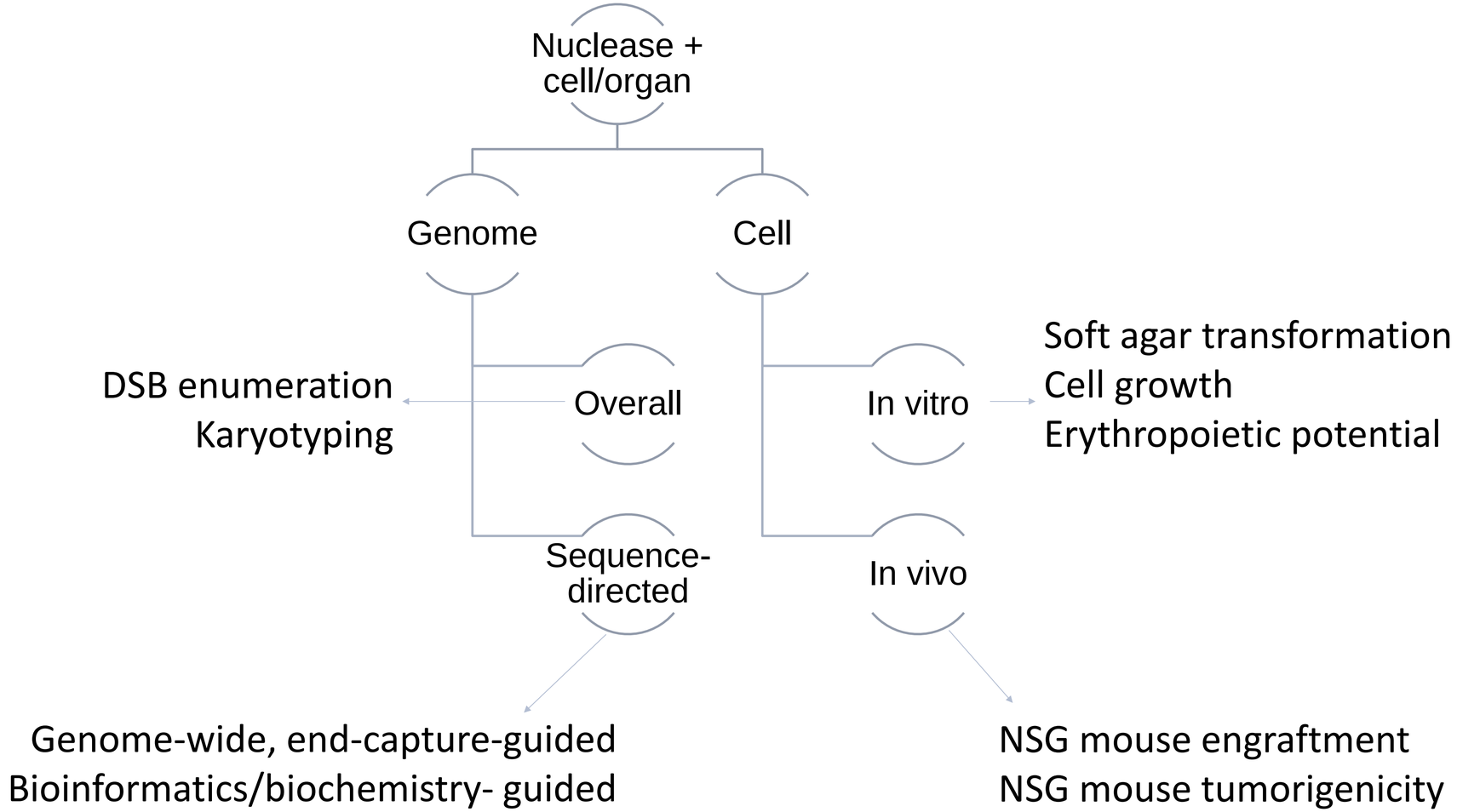
CMC:

- mRNA electroporation

Clinical record

- Clinical trial open

Readouts of Genome Editing Safety: Charted Path Based on Two Open INDs For ZFN-Driven HSPC Editing



Genome-edited CD34+ cells in the clinic: general lessons

Targeted knockout ex vivo – single loci – clinical scale efficiency and specificity.

CMC GMP path established

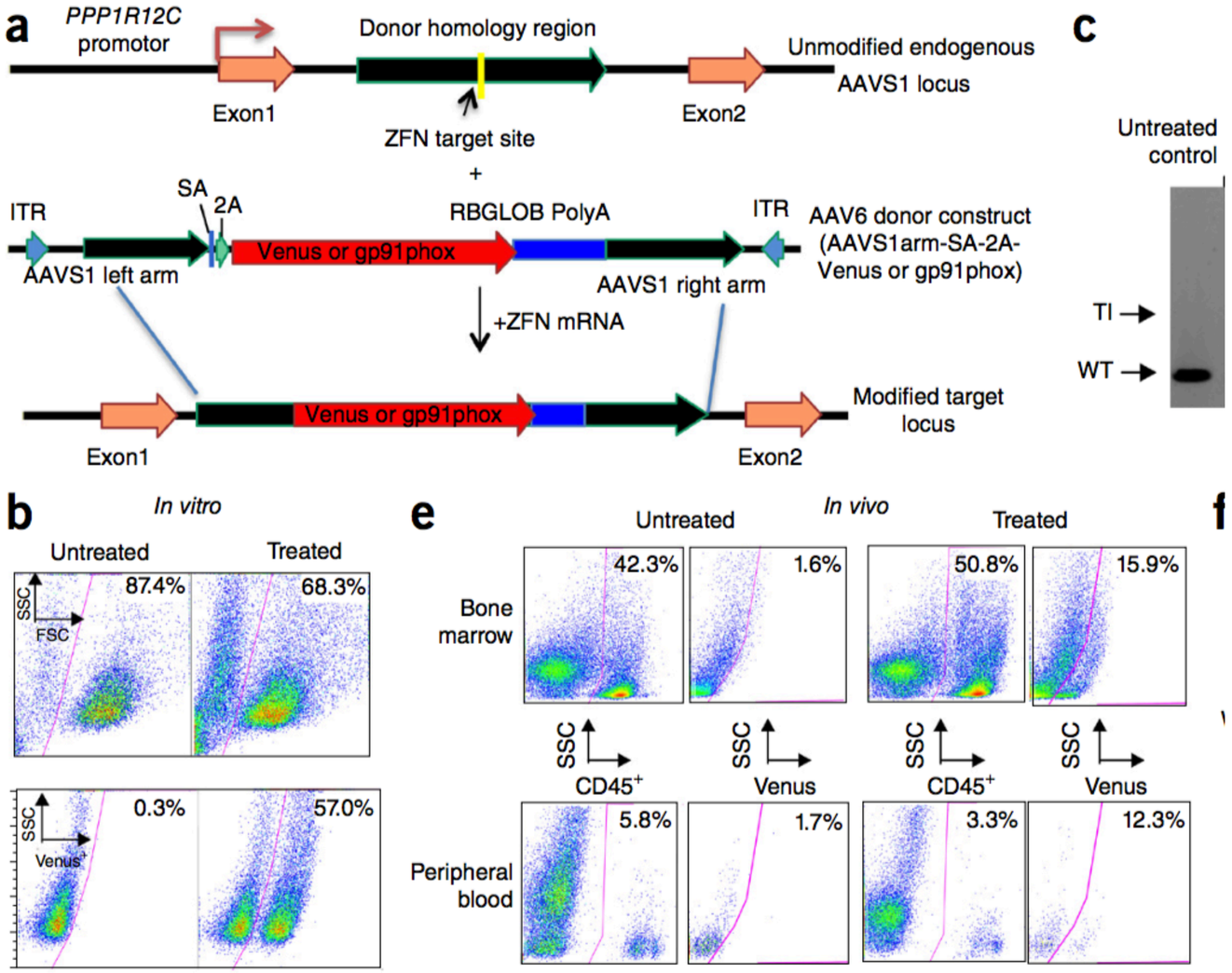
Preclinical pharm-tox path established

Regulatory framework (OCGCT CBER FDA) in place

Avenues for improvement
1. cell harvest (“sufficient numbers”)
2. targeted correction/addition

High efficiency targeted integration in human HSPCs using mixed-mode delivery

DeRavin
NBT 2016



Near- and longer-term challenges

T cells (oncology): cell weaponized beyond a CAR/TCR
T cells (oncology): allogeneic (off the shelf) product

T cells (beyond oncology and HIV): editing for improved potency – targets!

HSPCs: harvest and expansion (the iPSC route?)
HSPCs: higher efficiency of targeted integration
HSPCs: rare diseases of the blood

Next-generation challenge

In vivo delivery (note three open INDs for in vivo editing in the liver – F9 deficiency, MPS I, MPDs II, and multiple ongoing clinical trials by Alnylam and Ionis on RNAi and antisense).