Nuove frontiere della ricerca: terapia cellulare adottiva e terapia genica

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Franco Locatelli, MD PhD

Università di Pavia

IRCCS, Ospedale Pediatrico Bambino Gesù Roma, Italy

f.locatelli@opbg.net

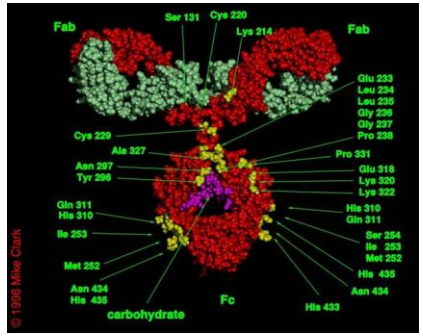
Bambino Gesù
OSPEDALE PEDIATRICO

Increasing the complexity.....

Aspirin

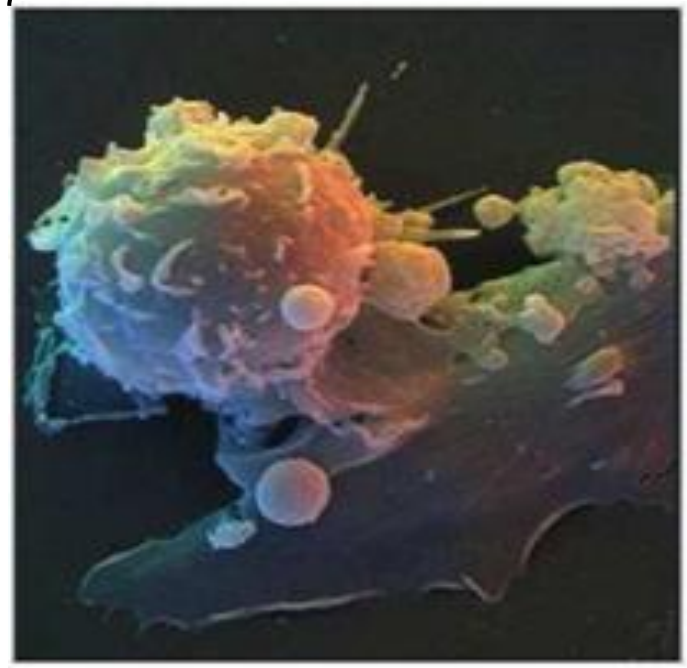
Interferon-α

Biodrugs

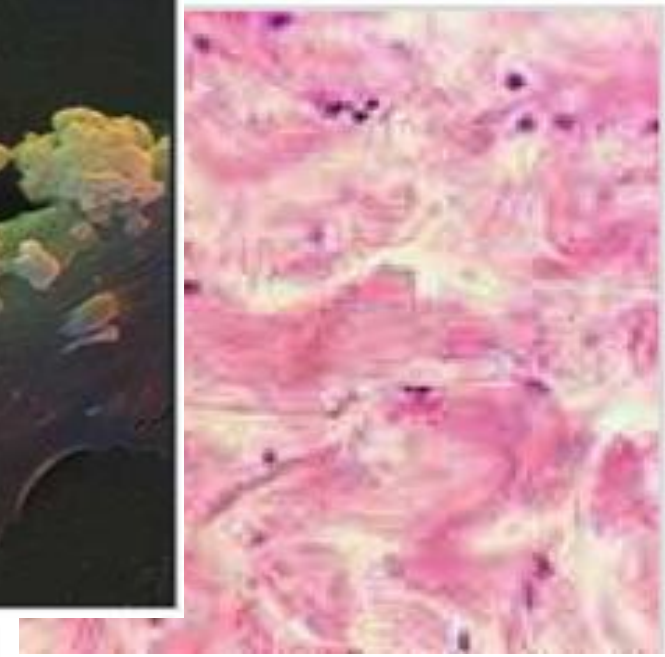


Antibody (IgG)

Advanced Therapies



T lymphocyte



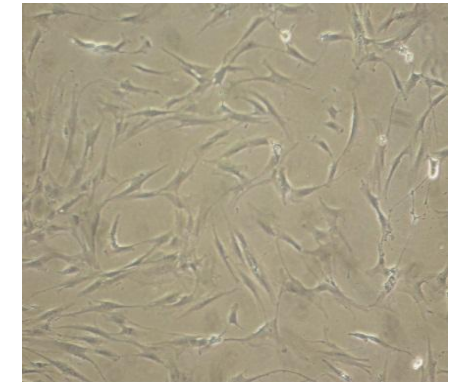
Connective tissue

Gene Therapy

PROPERTIES of MESENCHYMAL STEM CELLS (MSCs)

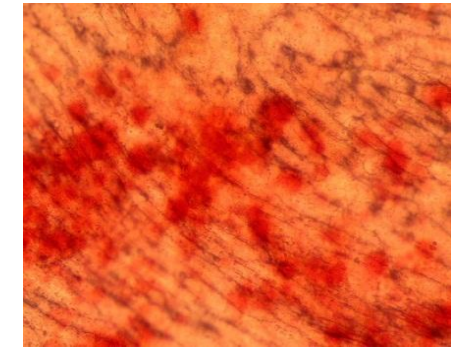
- **Multipotent cells**

  capable of differentiation into several mesenchymal lineages



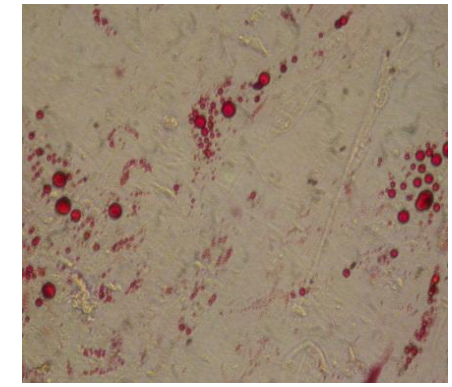
- **Enhancement of hematopoiesis**

- **Immunosuppressive properties**



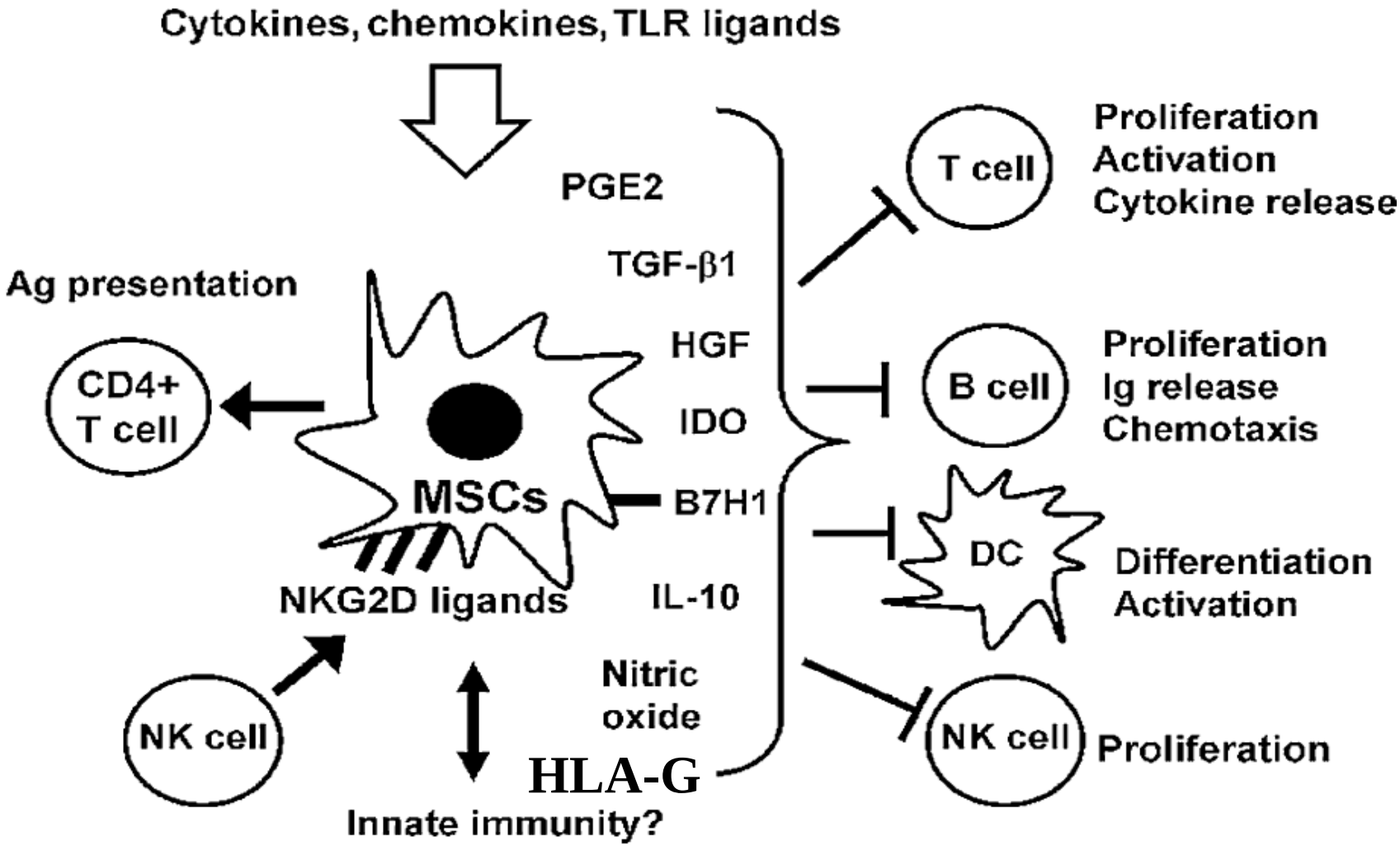
- **Inhibit inflammation (paracrine signaling)**

- **Remarkable expansion after *ex vivo* culture, both with FCS and PLT lysate, with maintenance of genetic stability**



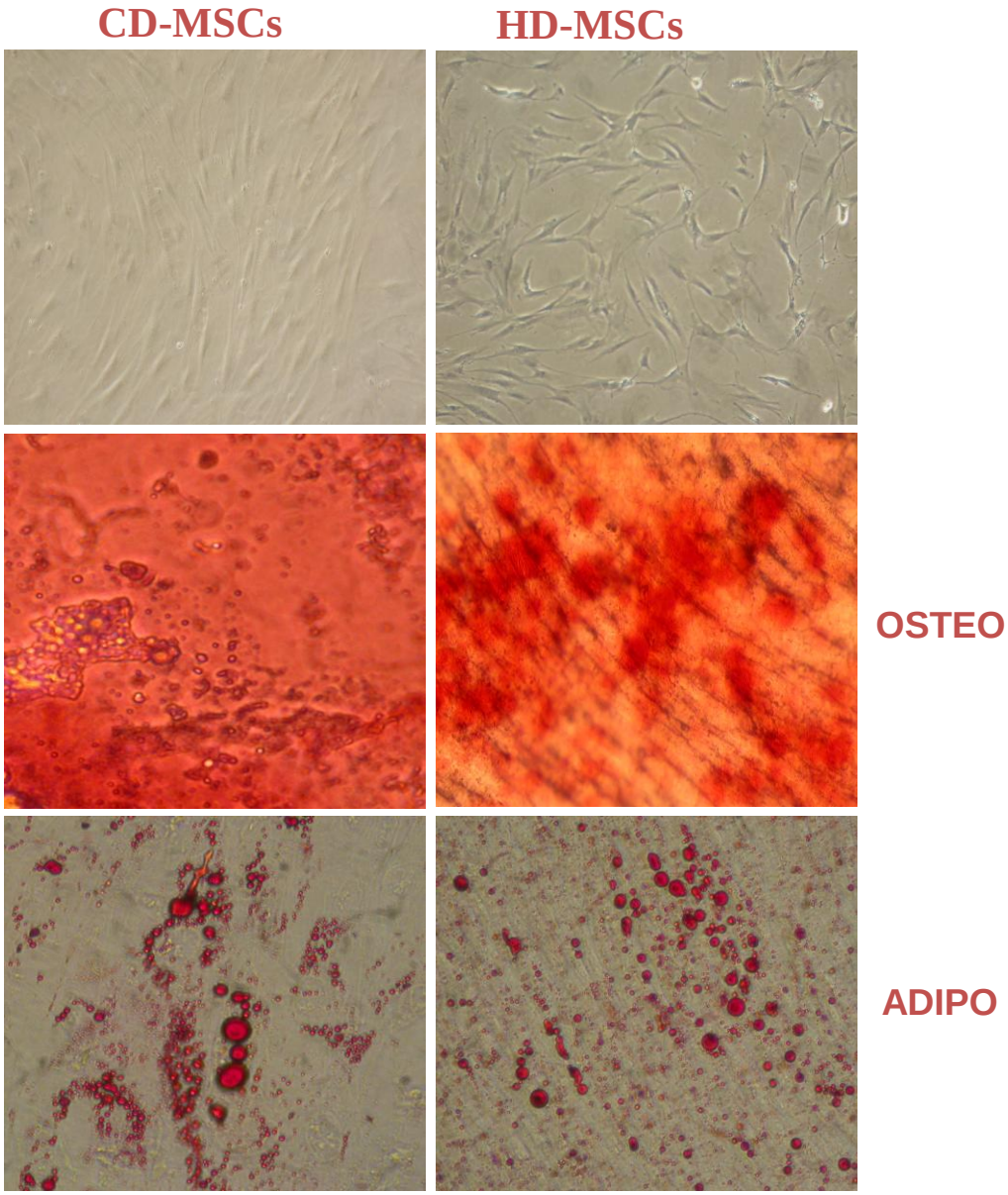
Bernardo et al., Conn Tissue Res 2007
Bernardo et al., J Cell Phys 2007
Bernardo et al., Canc Res 2007

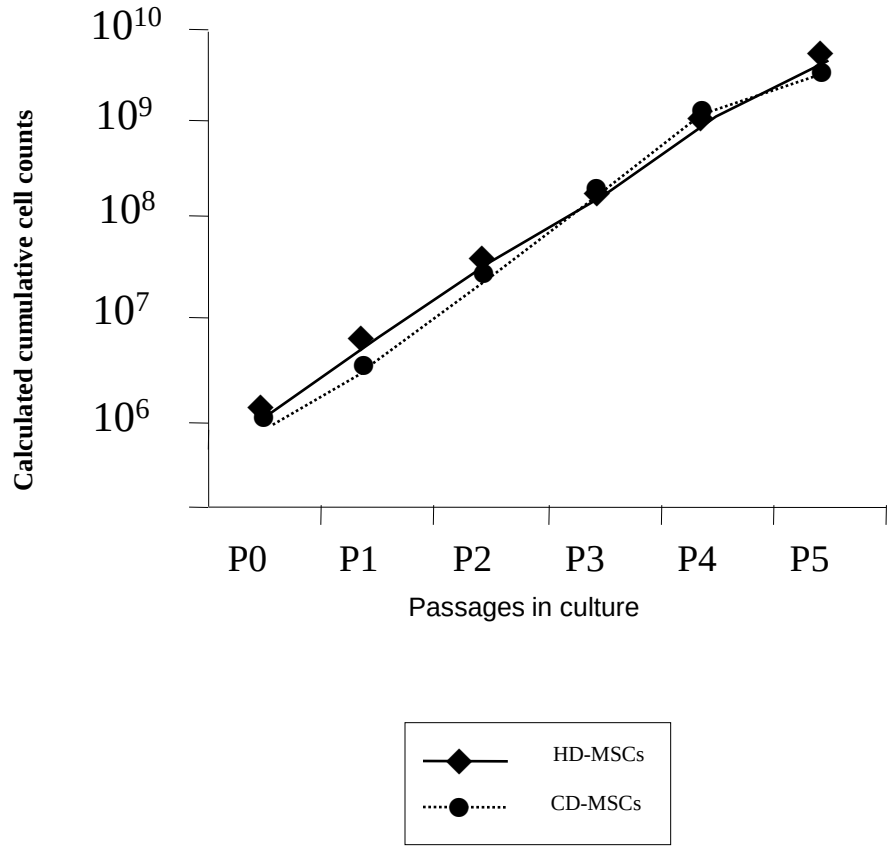
MSCs and immune response: it takes more than two to tango



Locatelli F et al. Haematologica, 2006

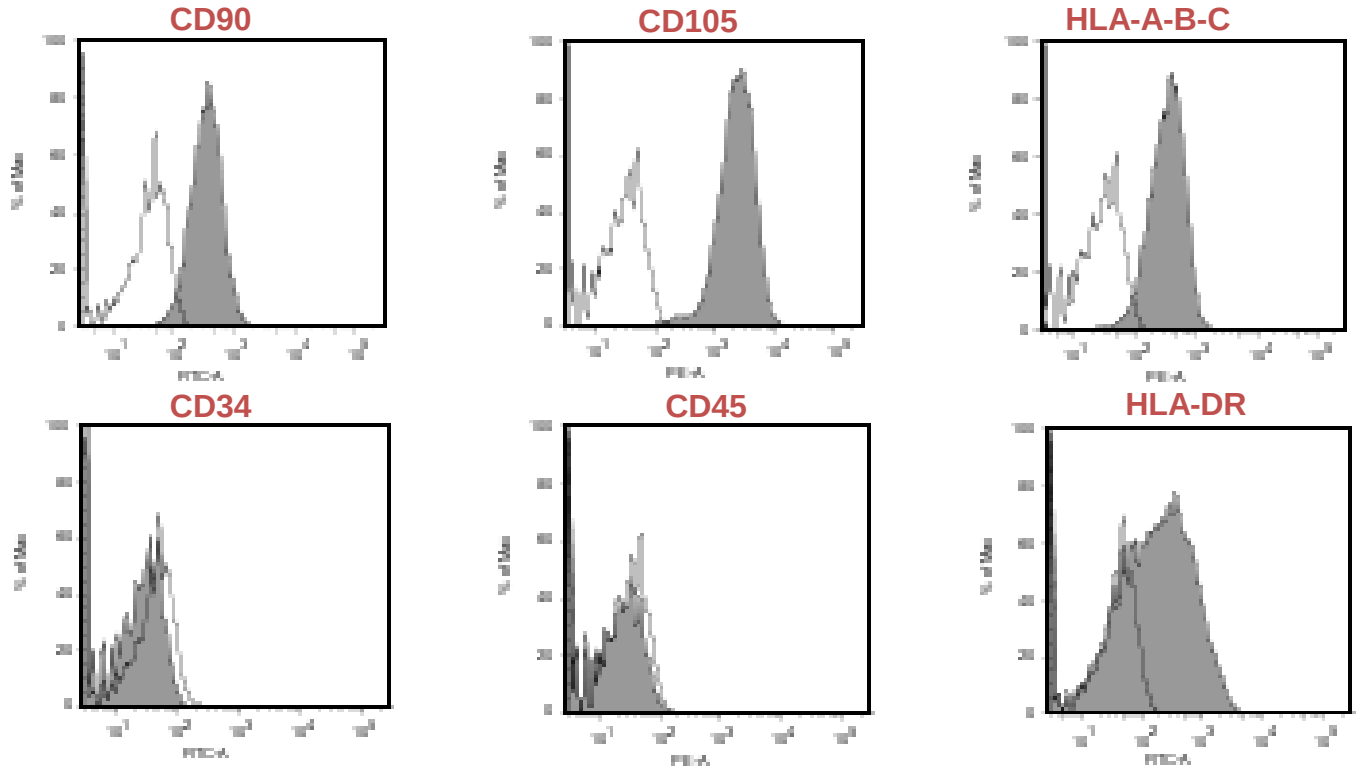
MORPHOLOGY, DIFFERENTIATION and PROLIFERATIVE CAPACITY of CROHN'S DISEASE MSCs





Bernardo et al. Cytotherapy 2009

IMMUNOPHENOTYPICAL CHARACTERIZATION of CD-MSCs



Percentages of HLA-DR expression on *ex-vivo* expanded CD-MSCs

Pt (*n.*)	P1	P2	P3	P4	P5	P6
1	60	45	22	9	2	0
2	30	30	4	2	ND	0
3	20	10	5	0	0	0
4	17	11	0	0	0	ND
5	84	44	7	1	0	0
6	12	0	0	0	ND	ND
7	13	0	0	0	ND	0

ND: not determined

Bernardo et al. Cytotherapy 2009

CD-MSCs show biological characteristics similar to HD-MSCs and can be considered for approaches of anti-inflammatory/reparative cell therapy in patients with refractory CD.

PHASE I-II STUDY in CD PATIENTS:
INTRAFISTULAR INJECTION of AUTOLOGOUS BM-MSCs

12 pts. enrolled to date; **10 pts. evaluable**

intrafistular injection *of MSCs every 4 weeks (median 4 infusions)*

MONITORING: clinical, serological, imaging (magnetic resonance) and endoscopic (rectosigmoidoscopic examination, endoscopy)

PHASE I-II STUDY: INTRAFISTULAR INJECTION of MSCs

MSC intrafistular injection:

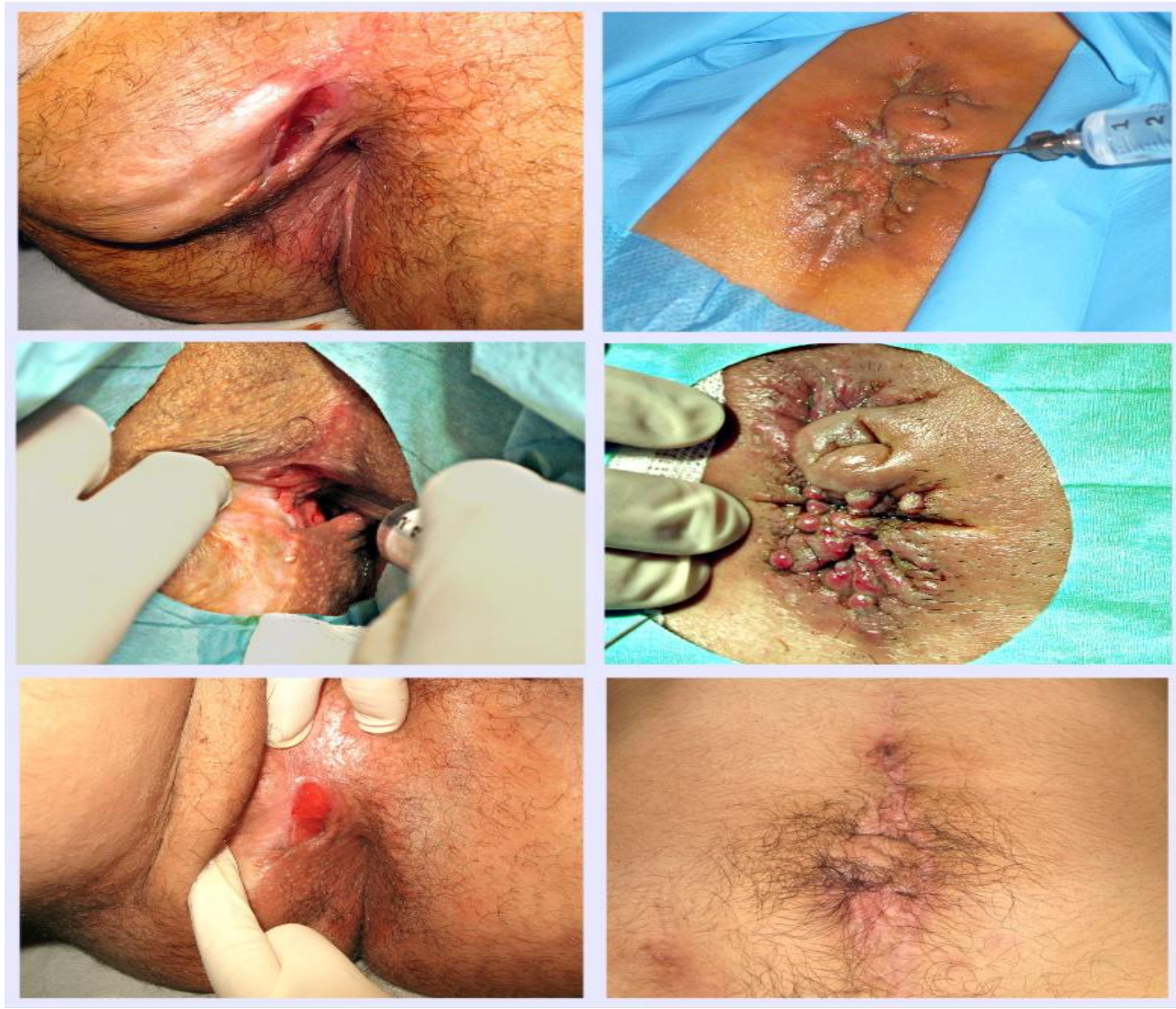
- **safe**; no adverse event up to 12 months after treatment.

- **effective** in inducing sustained closure of fistulas; appearance of regenerative tissue along the tracks

7 pts. (70%): complete and sustained healing of fistula tracks

3 pts. (30%): partial response

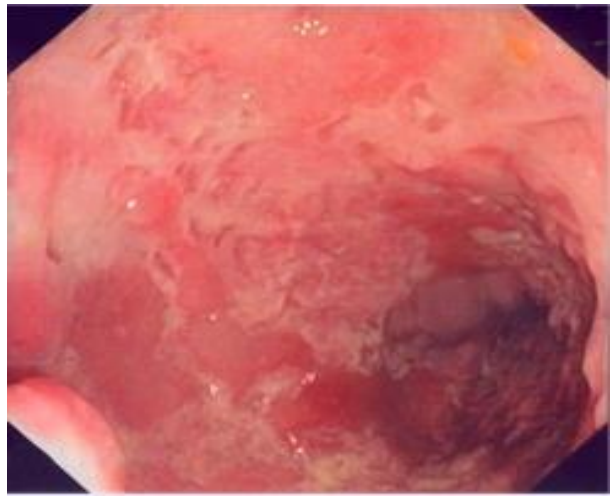
MSCs in IBD



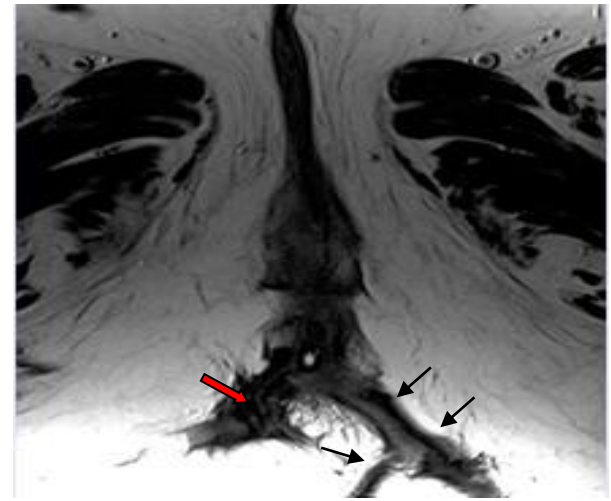
Ciccocioppo R et al. Gut 2011

PT #10 with ACTIVE RECTAL CROHN'S DISEASE BEFORE and AFTER MSC TREATMENT

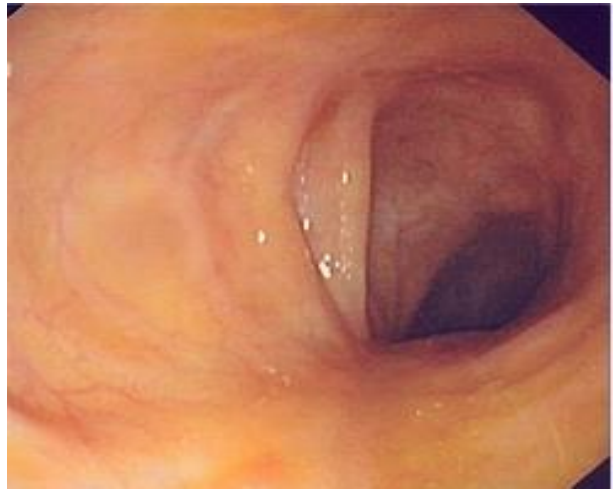
A



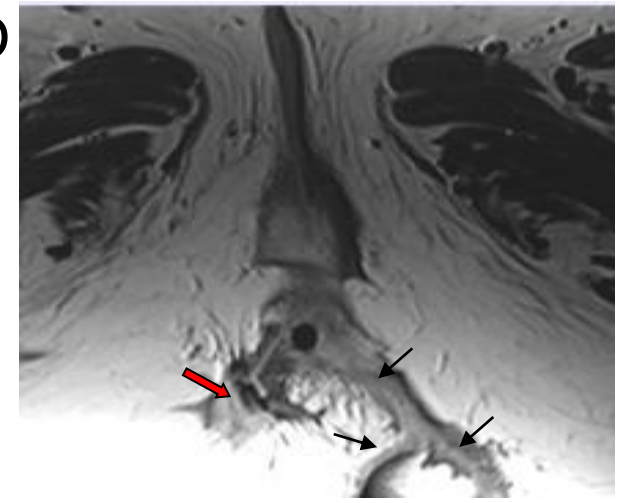
C



B

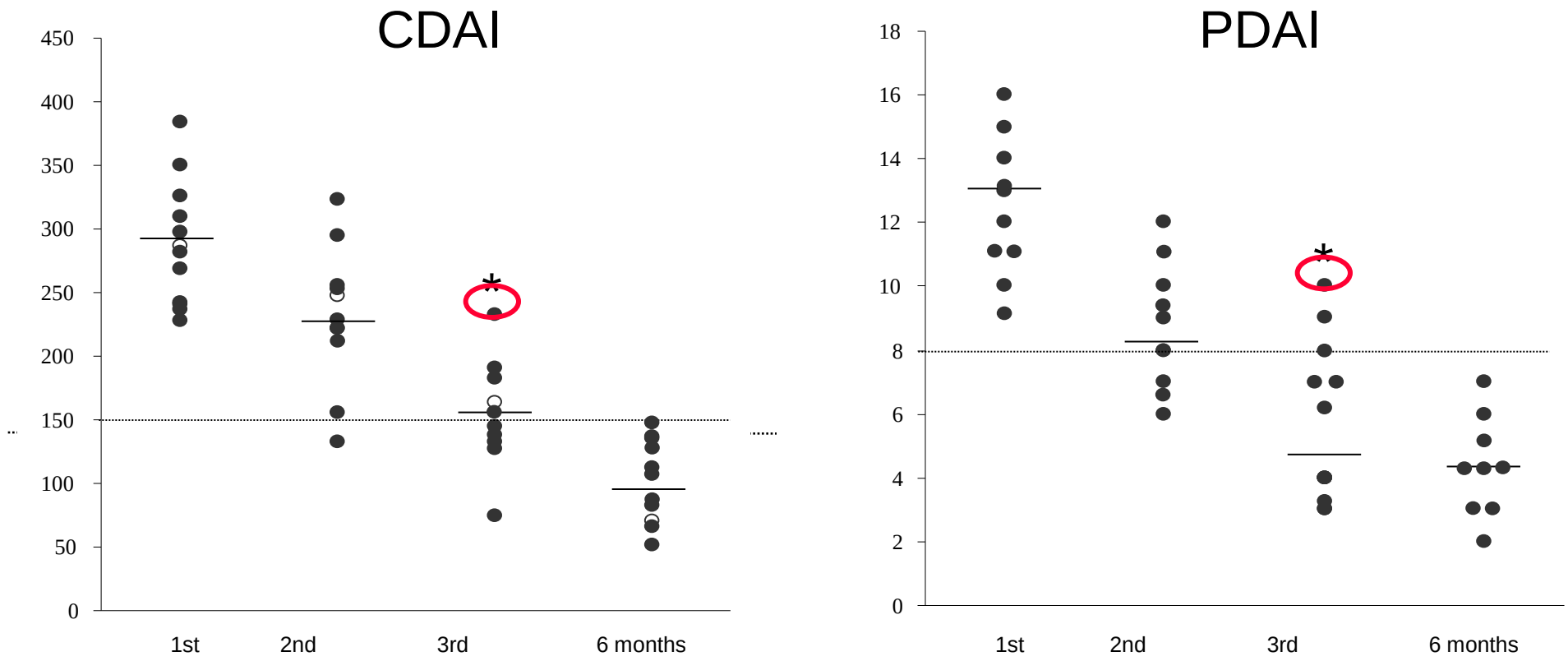


D



(A) Active rectal disease at endoscopy before treatment.
(B) After 12 months, healthy mucosa with ulcer epithelisation.
(C) Complex perianal fistulas at MRI before treatment.
(D) Replacement with regenerative tissue 6 months after the end of treatment.

CDAI / PDAI VALUES BEFORE and AFTER TREATMENT



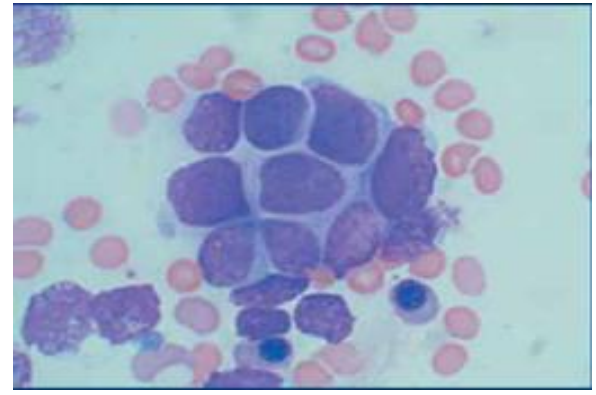
*p<0.001

CDAI / PDAI values at 1st, 2nd, 3rd MSC infusion and after 6 month follow-up

Ciccocioppo R et al. Gut 2011

Neuroblastoma

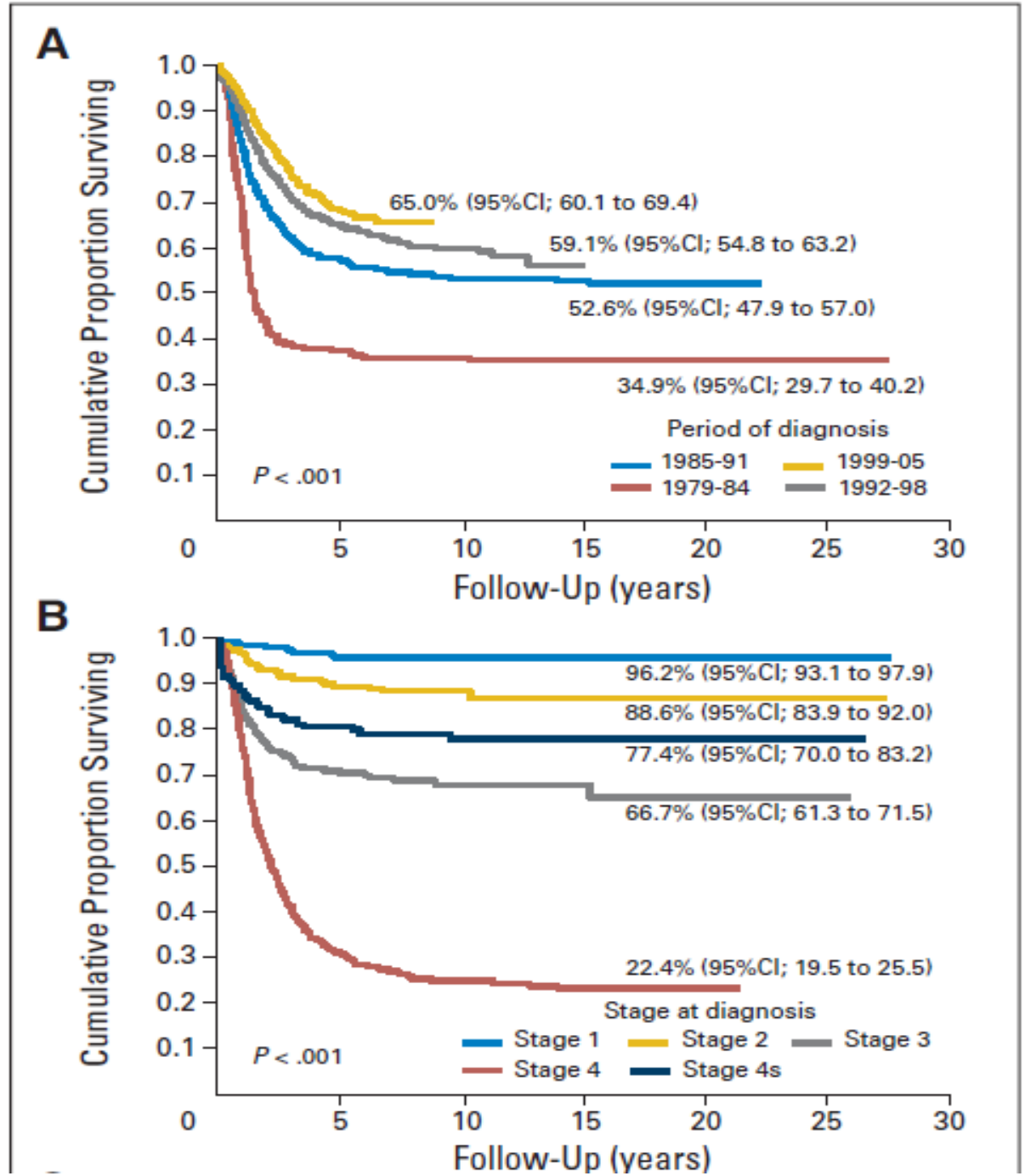
- **Age**
  - 90% < 5 y/o; 50% < 2 y/o
  - Occasional USG detection *in utero*

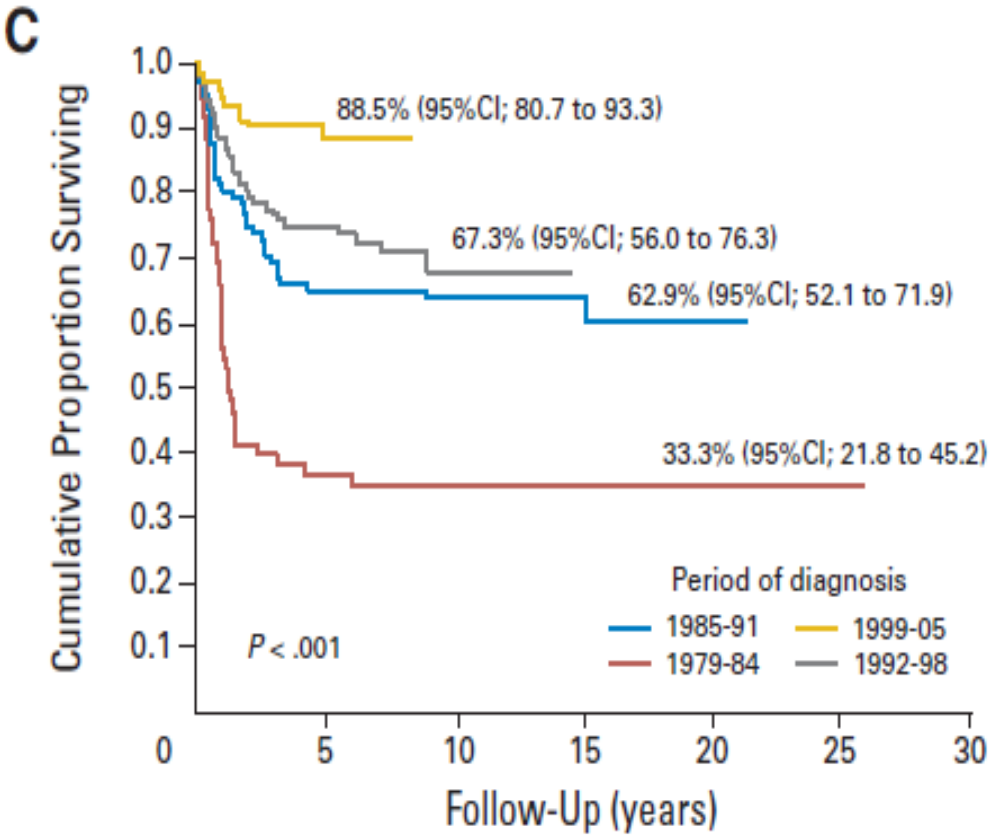


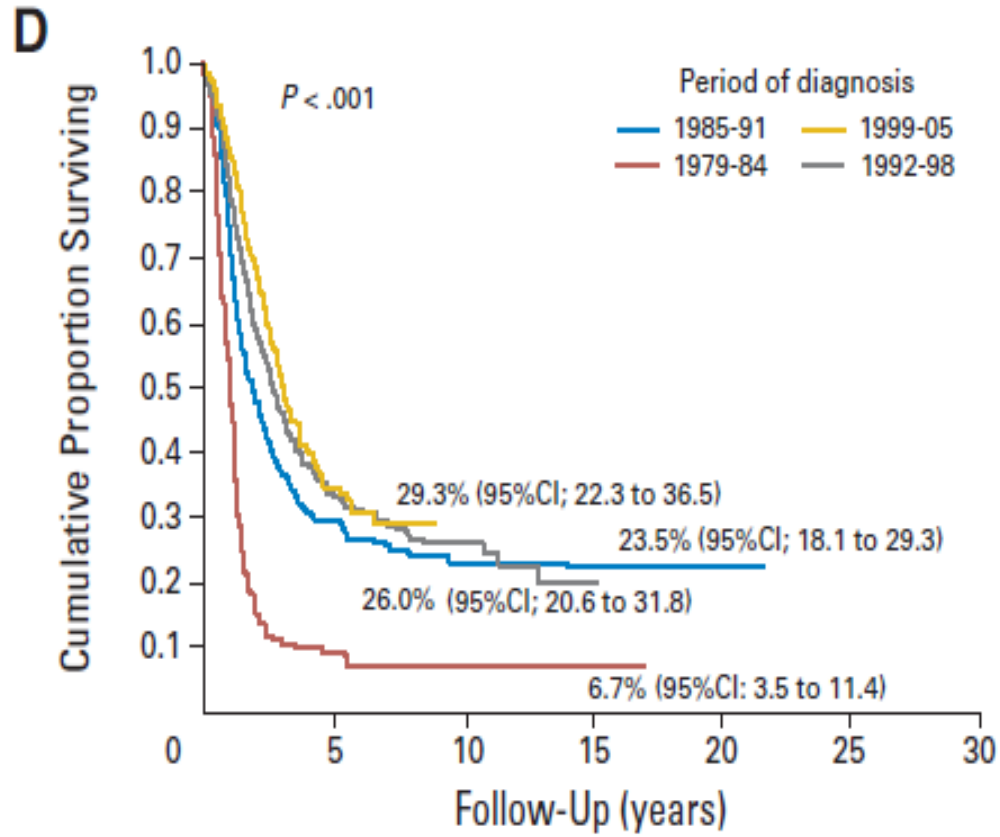
- **Location:** any neural crest tissue
  - Adrenal
  - Paraspinal sympathetic tissue
    - Cervical, Thoracic, Pelvic

- **Often metastatic at diagnosis**
  - Bone and/or bone marrow
    - > 1 y/o: 70%

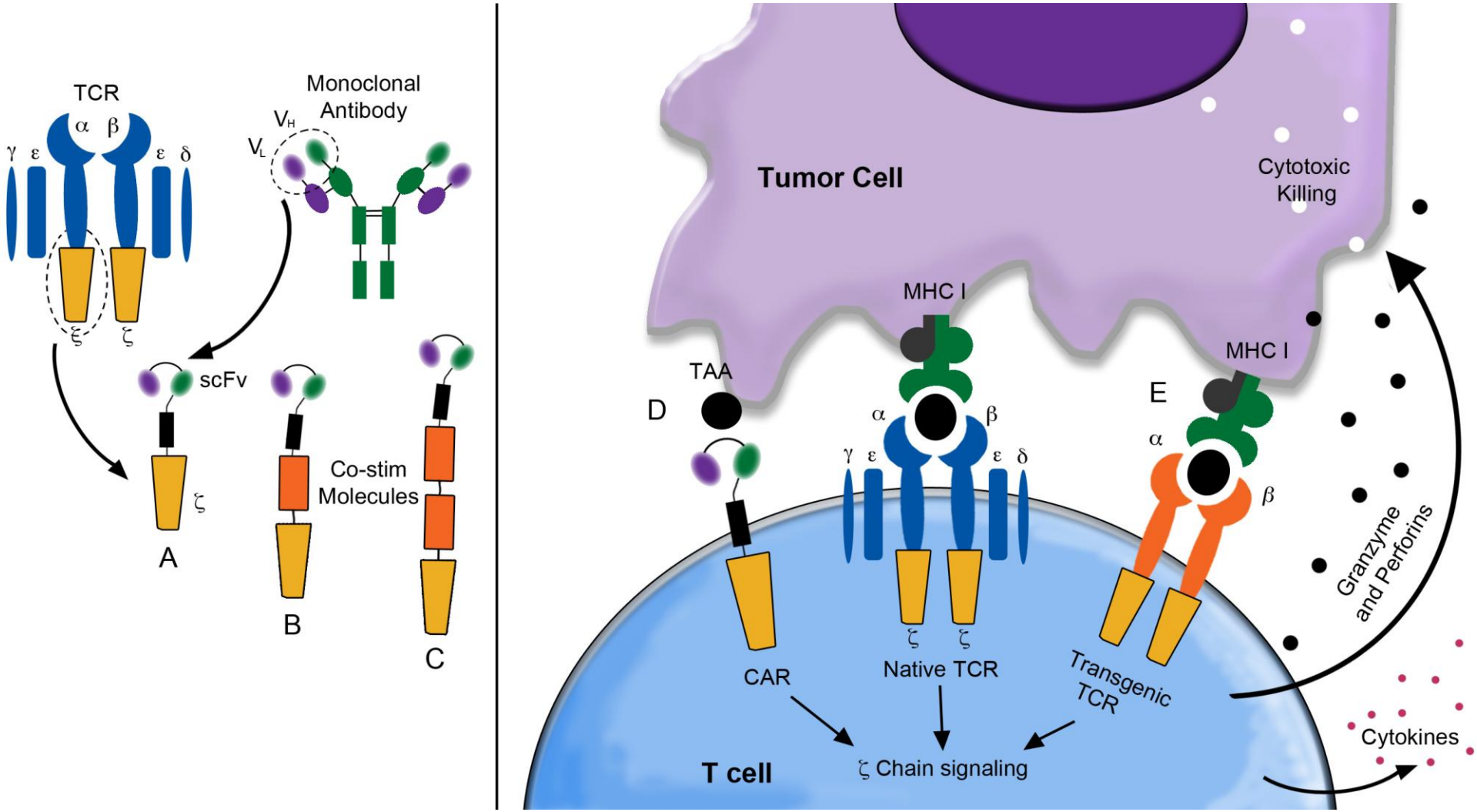
- **GD2 disialogangloside is highly expressed on NBL cells**

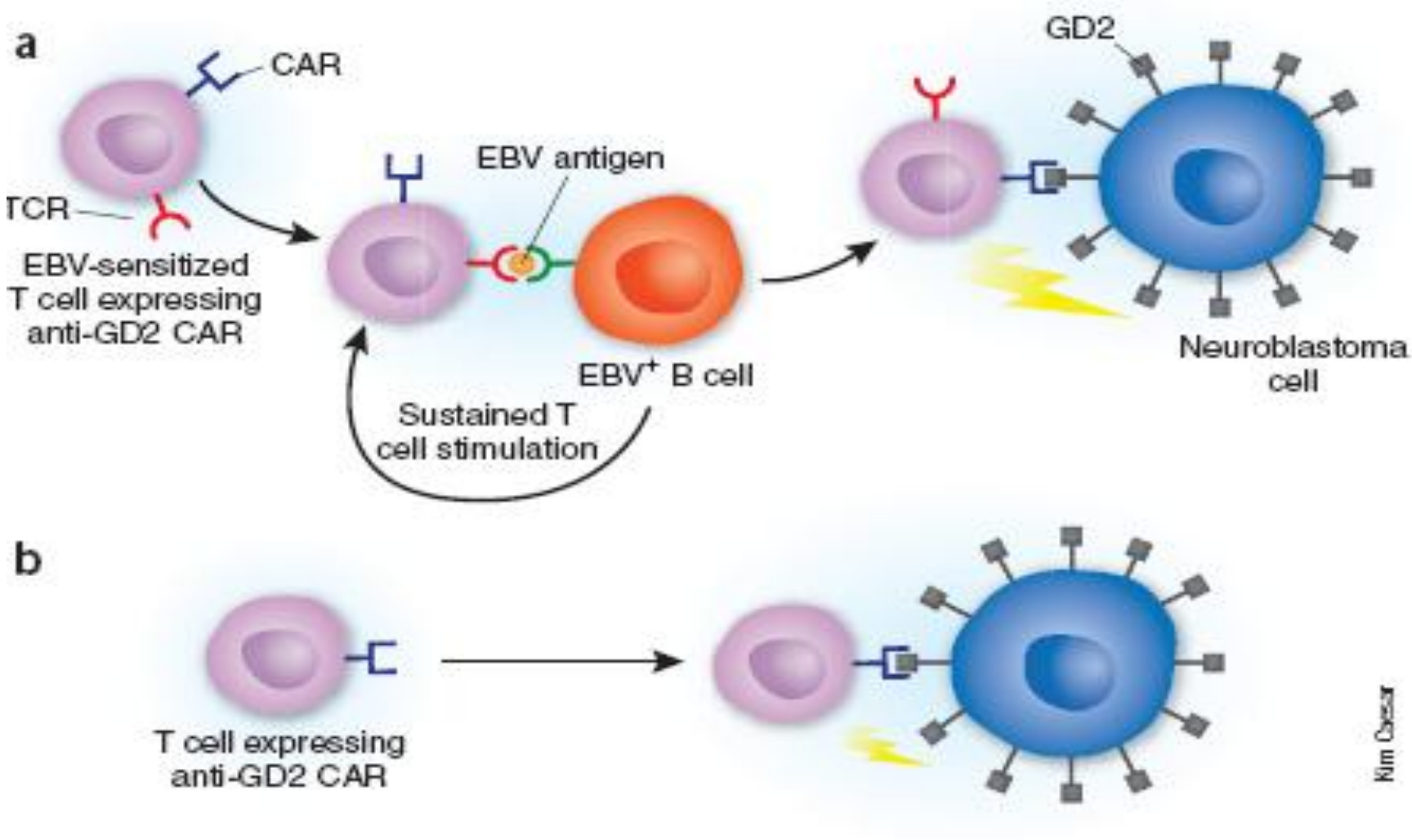

A
Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
Follow-Up (years)
65.0% (95%CI; 60.1 to 69.4)
59.1% (95%CI; 54.8 to 63.2)
52.6% (95%CI; 47.9 to 57.0)
34.9% (95%CI; 29.7 to 40.2)
Period of diagnosis
1985-91
1999-05
1979-84
1992-98
P < .001
B
Cumulative Proportion Surviving
Follow-Up (years)
96.2% (95%CI; 93.1 to 97.9)
88.6% (95%CI; 83.9 to 92.0)
77.4% (95%CI; 70.0 to 83.2)
66.7% (95%CI; 61.3 to 71.5)
22.4% (95%CI; 19.5 to 25.5)
Stage at diagnosis
Stage 1
Stage 2
Stage 3
Stage 4
Stage 4s
P < .001

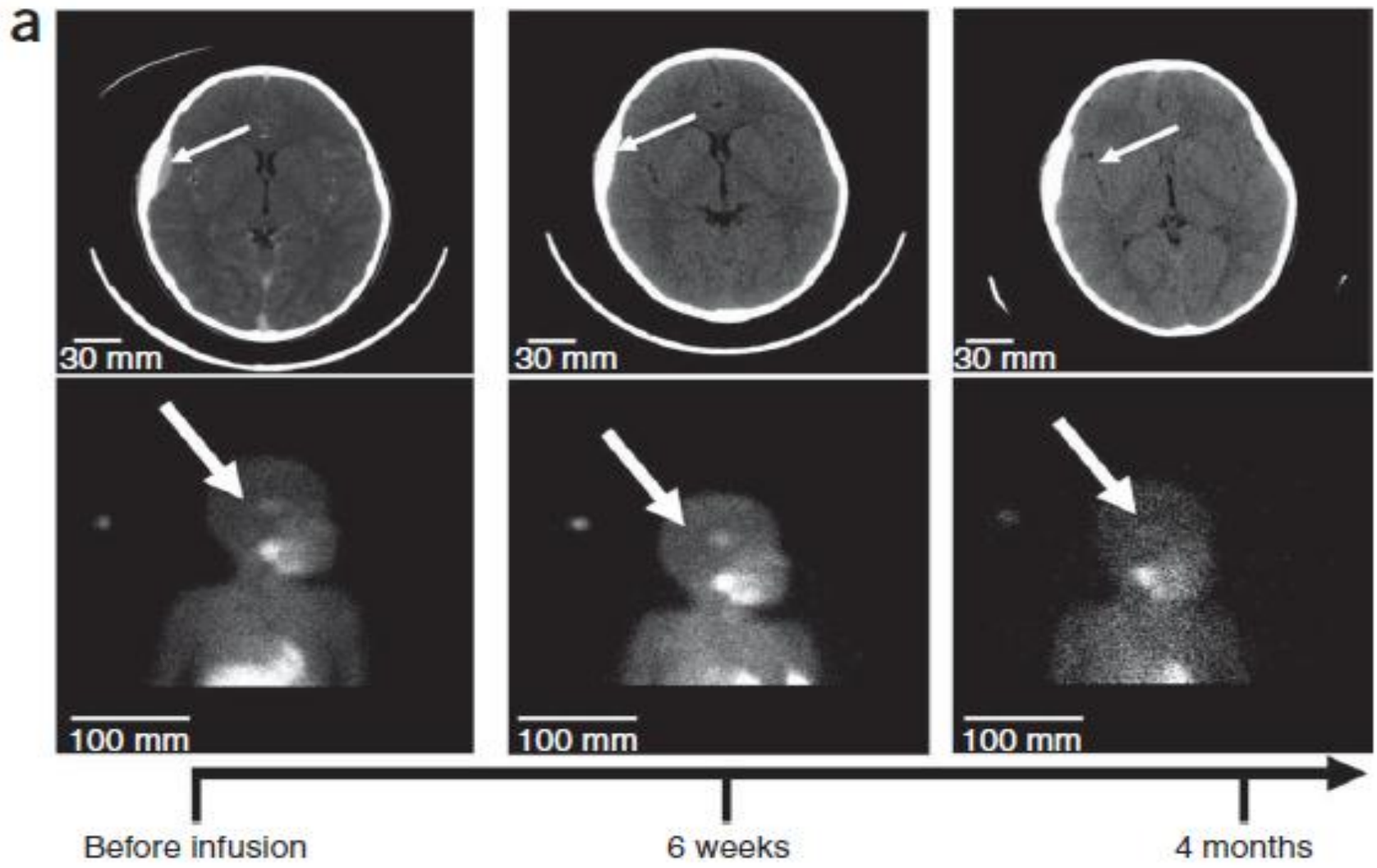

C
Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
Follow-Up (years)
88.5% (95%CI; 80.7 to 93.3)
67.3% (95%CI; 56.0 to 76.3)
62.9% (95%CI; 52.1 to 71.9)
33.3% (95%CI; 21.8 to 45.2)
P < .001
Period of diagnosis
1985-91
1999-05
1979-84
1992-98


D
Cumulative Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25
30
Follow-Up (years)
P < .001
Period of diagnosis
1985-91
1999-05
1979-84
1992-98
29.3% (95%CI; 22.3 to 36.5)
23.5% (95%CI; 18.1 to 29.3)
26.0% (95%CI; 20.6 to 31.8)
6.7% (95%CI: 3.5 to 11.4)

Chimeric Antigen Receptor (CAR)-transduced T cells

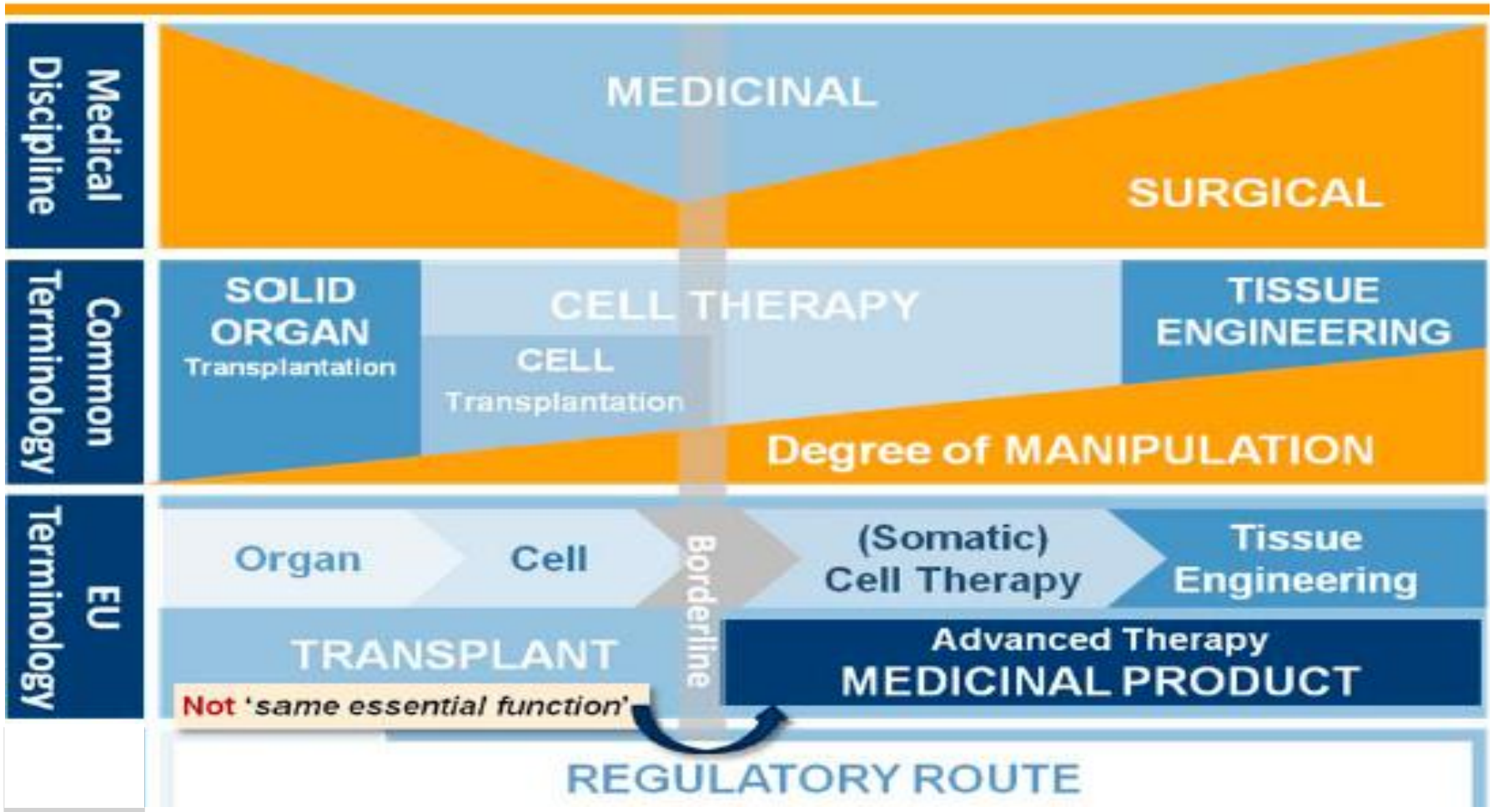



a
CAR
TCR
EBV-sensitized
T cell expressing
anti-GD2 CAR
EBV antigen
EBV+ B cell
Sustained T
cell stimulation
GD2
Neuroblastoma
cell
b
T cell expressing
anti-GD2 CAR
Kim Caesar



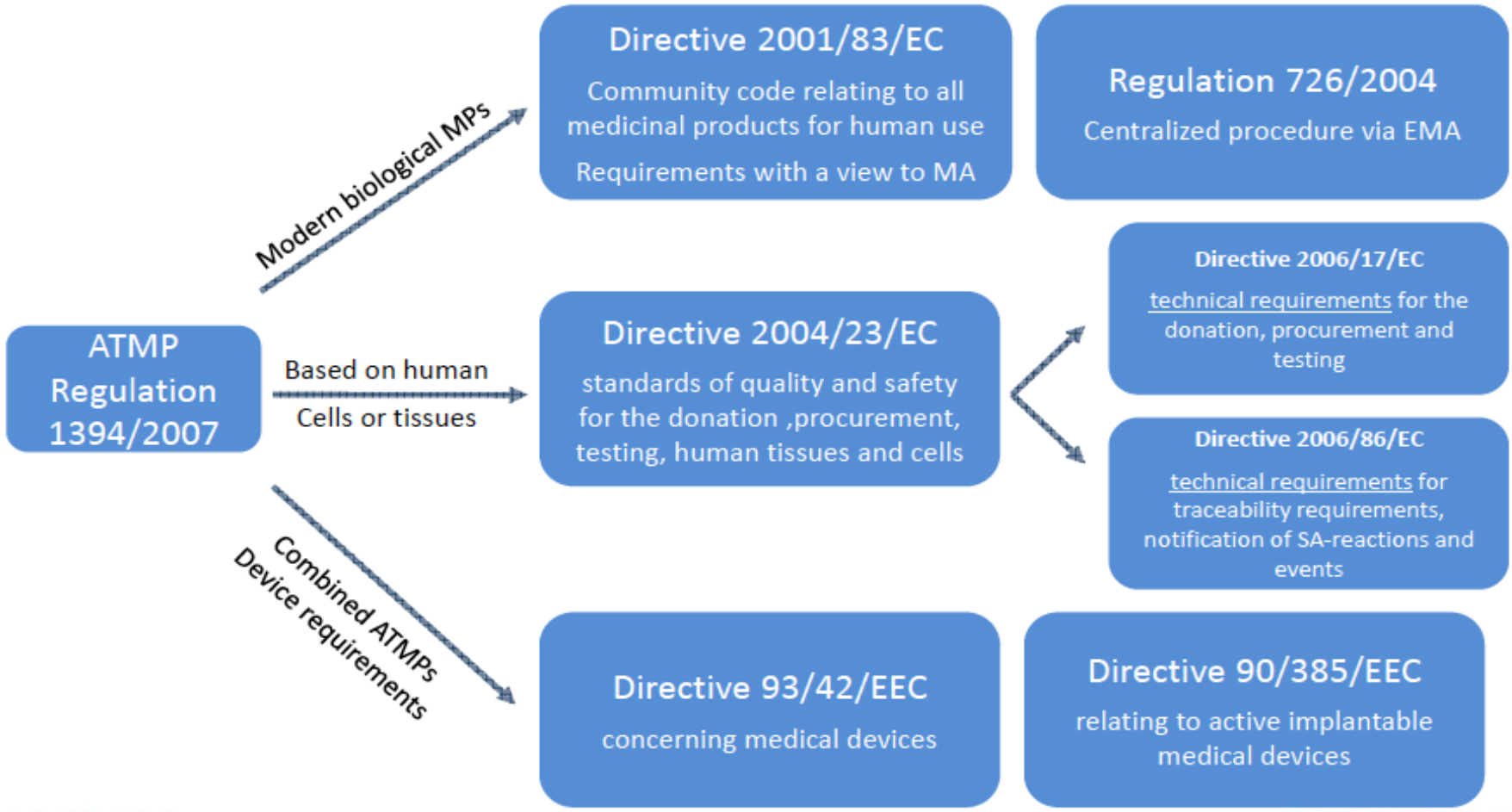
Pulle M, Dotti G, Savoldo B et al. Nature Medicine 2008

EU Definitions



Courtesy of Prof. A. Aiuti

The regulatory framework for cell therapy-based ATMP



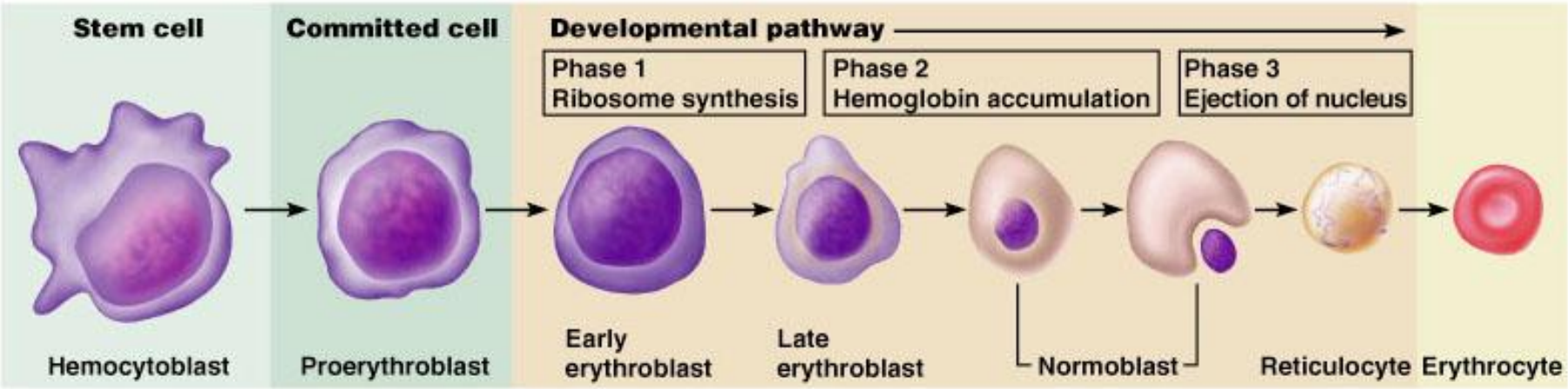
Patient-/Bio-safety tests

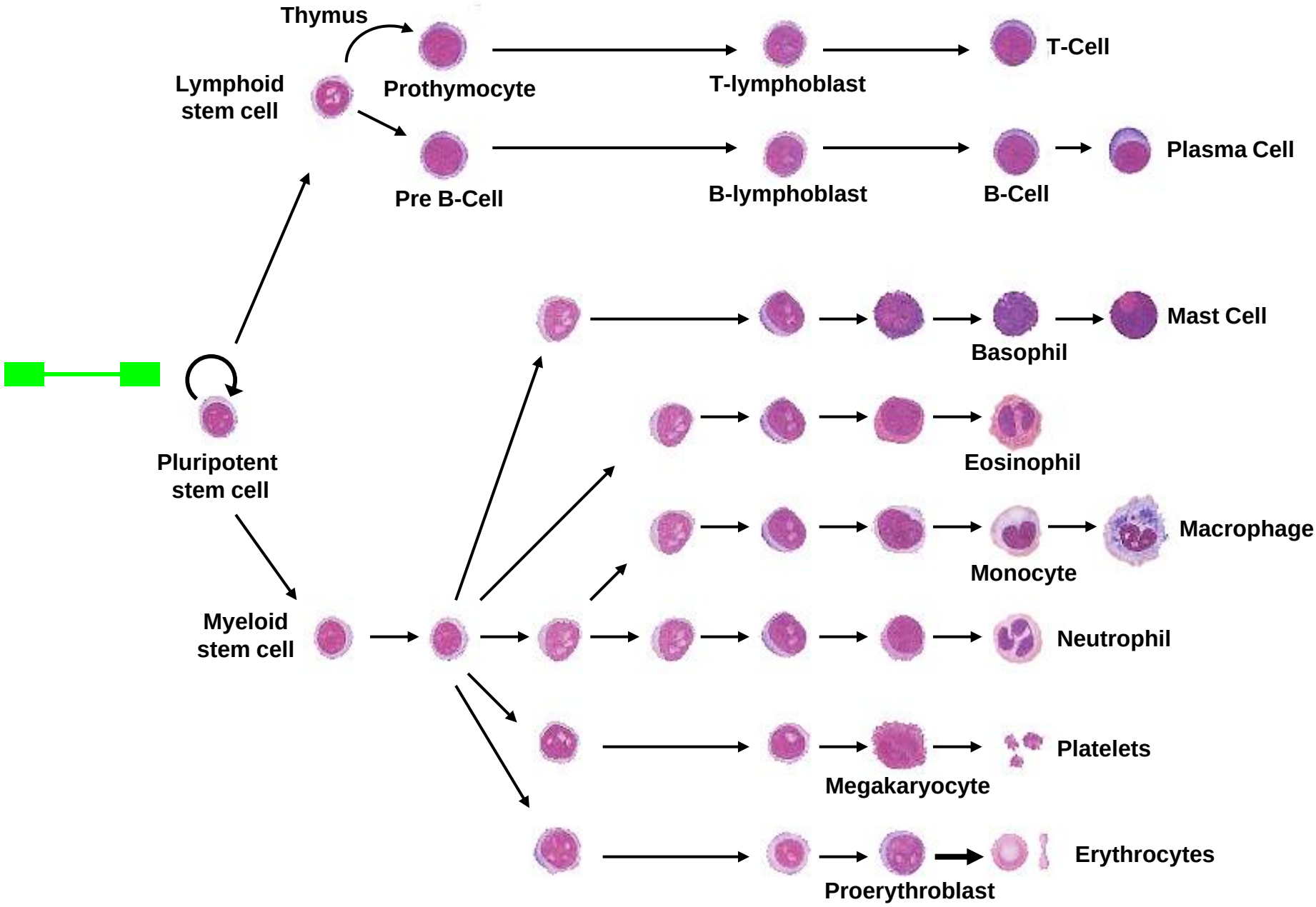
- ✓ **Sterility**
- ✓ **Identity of the cell product**
- ✓ **Functional features**
- ✓ **Vitality and cell number**
- ✓ **Phenotype of the cell product**
- ✓ **Differentiation capacity**
- ✓ **Genetic stability**
- ✓ **Medium in which the cells are grown**

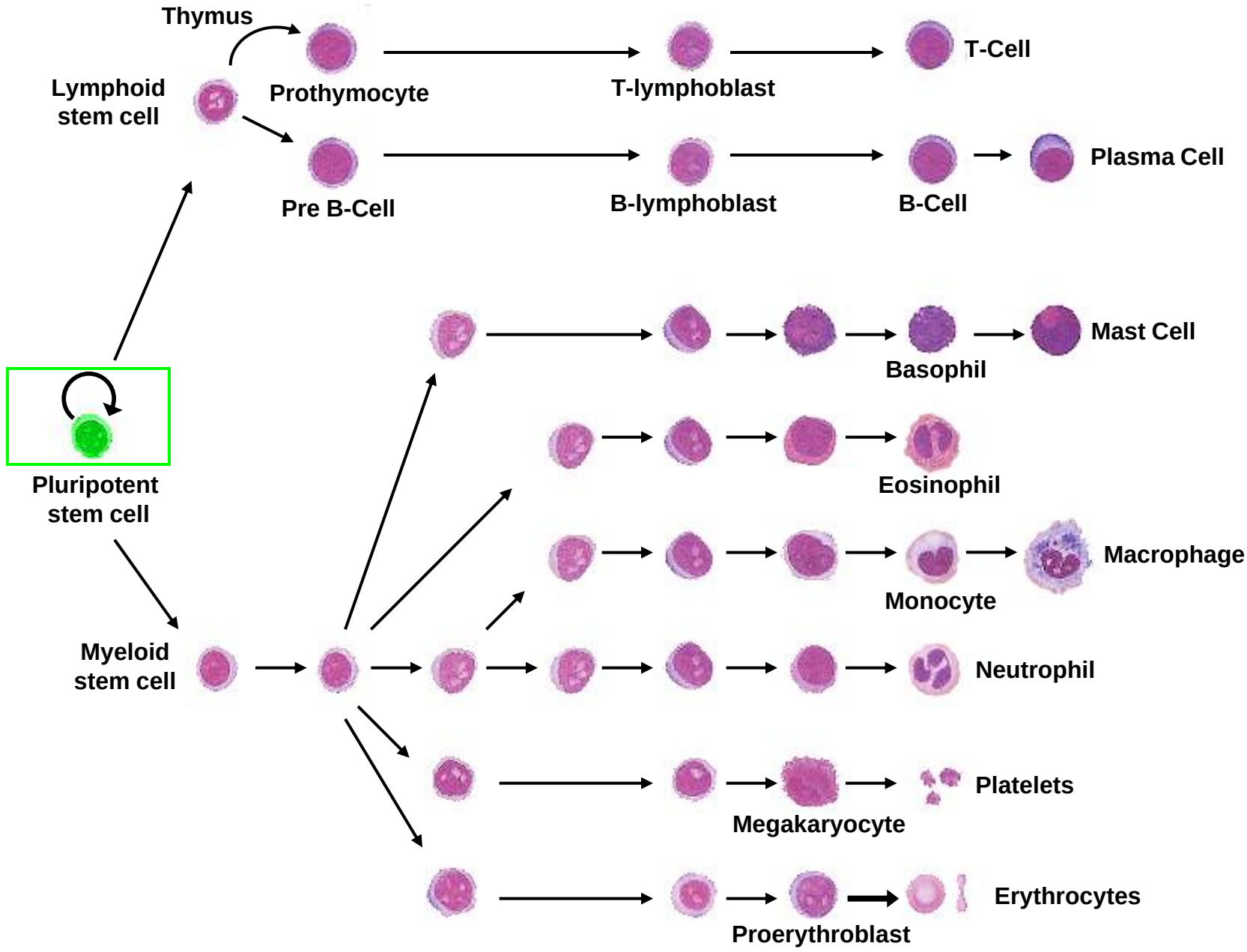
Requirements for globin vector expression

- Lineage-restricted, erythroid-specific
- Differentiation stage-specific
- Elevated
  - therapeutic (anemia)
  - safe (1-2 vector copies per cell)
- Sustained over time

Targeting gene expression to late-stage erythropoiesis




Thymus
Lymphoid stem cell
Prothymocyte
T-lymphoblast
T-Cell
Pre B-Cell
B-lymphoblast
B-Cell
Plasma Cell
Pluripotent stem cell
Basophil
Mast Cell
Eosinophil
Monocyte
Macrophage
Myeloid stem cell
Neutrophil
Megakaryocyte
Platelets
Proerythroblast
Erythrocytes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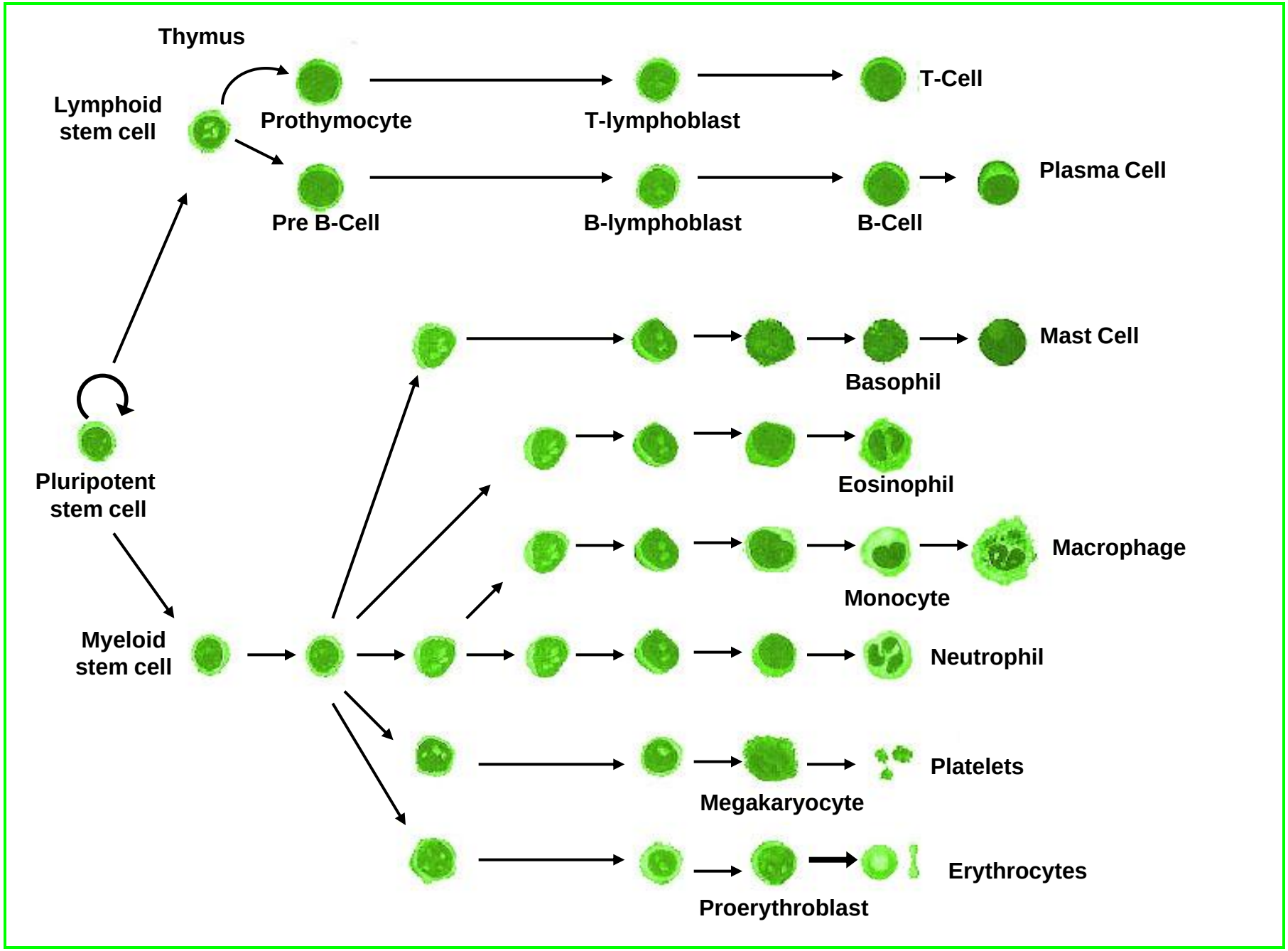
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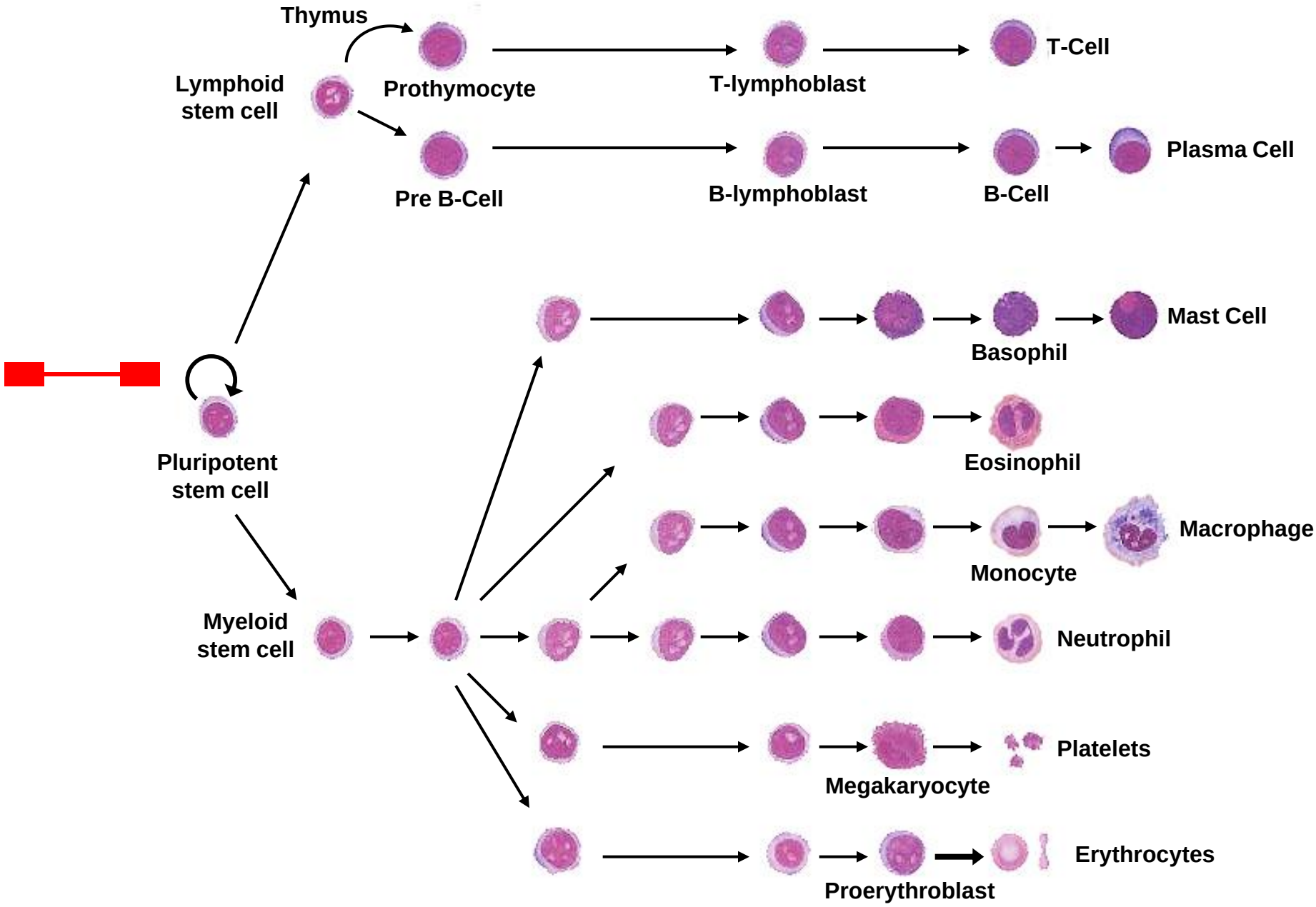
Erythrocytes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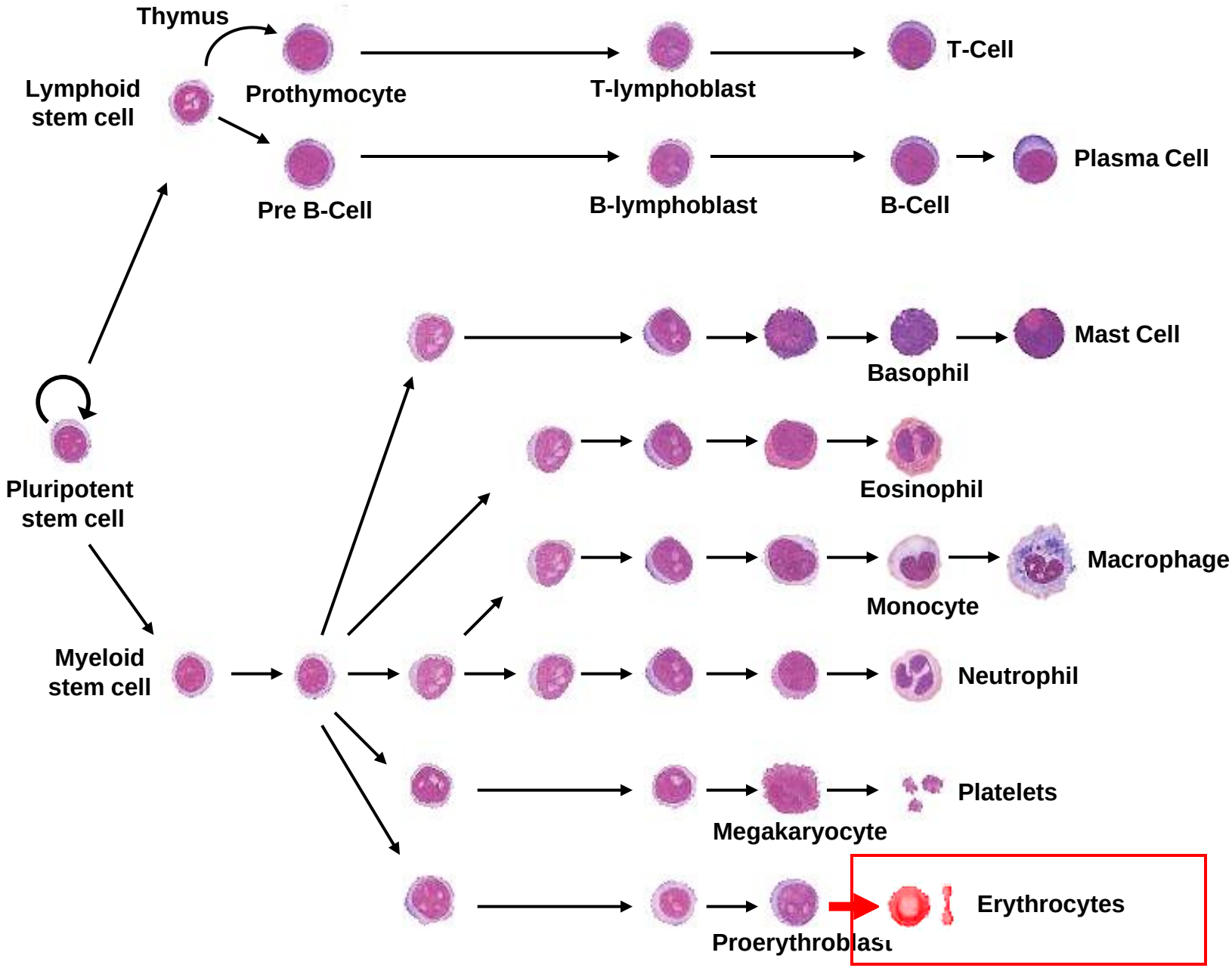
T-Cell
Pre B-Cell
B-lymphoblast
B-Cell
Plasma Cell
Pluripotent stem cell
Basophil
Mast Cell
Eosinophil
Monocyte
Macrophage
Myeloid stem cell
Neutrophil
Megakaryocyte
Platelets
Proerythroblast
Erythrocytes

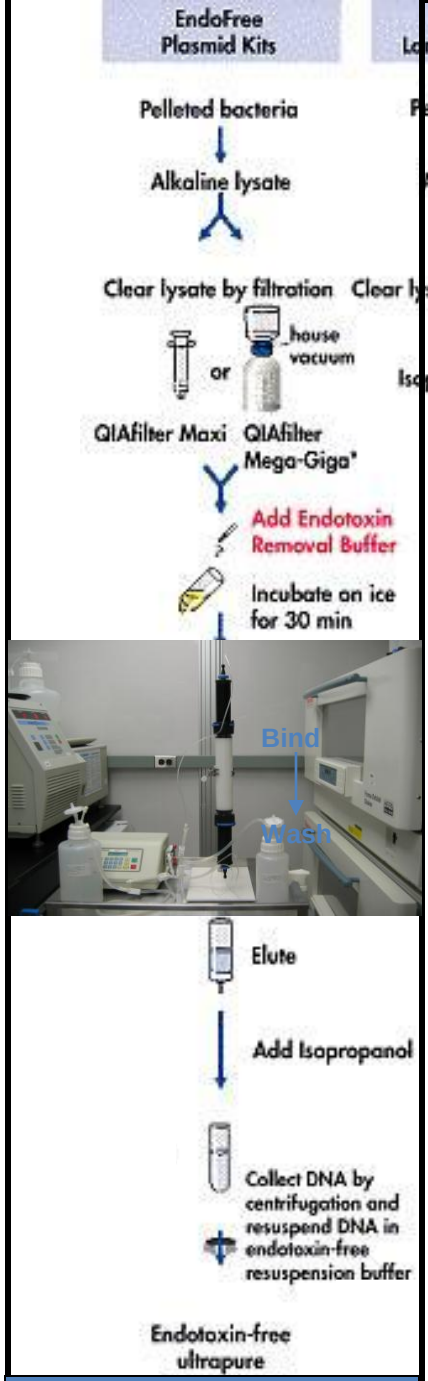
TNS9.3.55 is lineage restricted, erythroid specific, differentiation stage specific



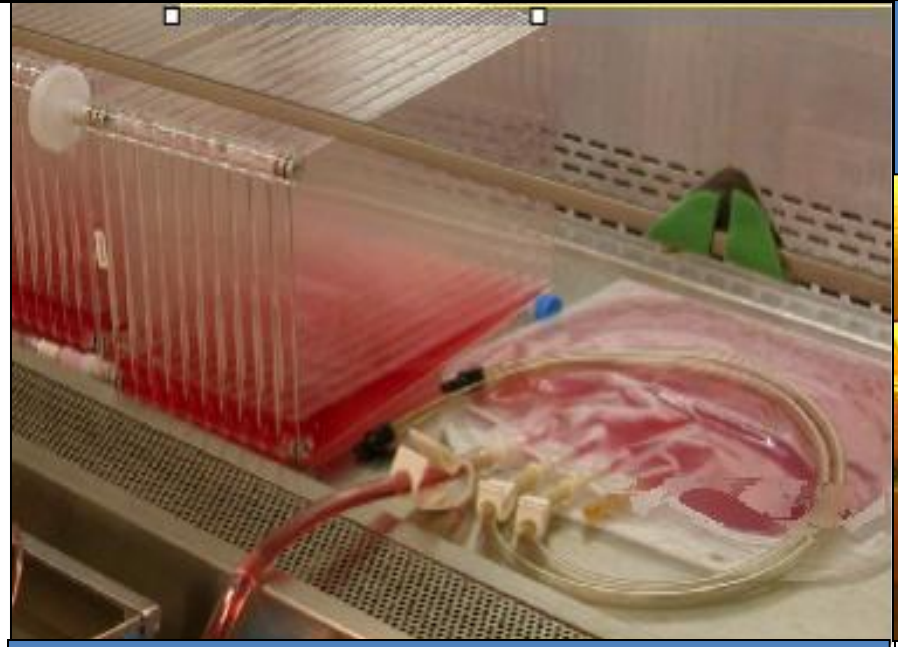
Objective 1

Transduce patient CD34+ cells in 0.5-2.0 VCN range

(Upper limit of 2.0 is mandated by FDA)



Plasmid DNA



Lentiviral vector

Patient cell transduction

Patient cell expansion - Wave bio

Patient cell selection

Objective 2

Improving the titer and manufacturing of TNS9.3

(without altering the globin transcription unit)

Large Scale Lentiviral Production Flow Chart

Thaw out cells from MCB

Expand to 10-layer Cell Factory

Transfection

Media Change

Harvest

Concentration

Benzonase Treatment

Concentration
Diafiltration/IEX chromatography

Sterilisation

Vialing

10 to 25 liter

Increasing Purity

50-150 ml

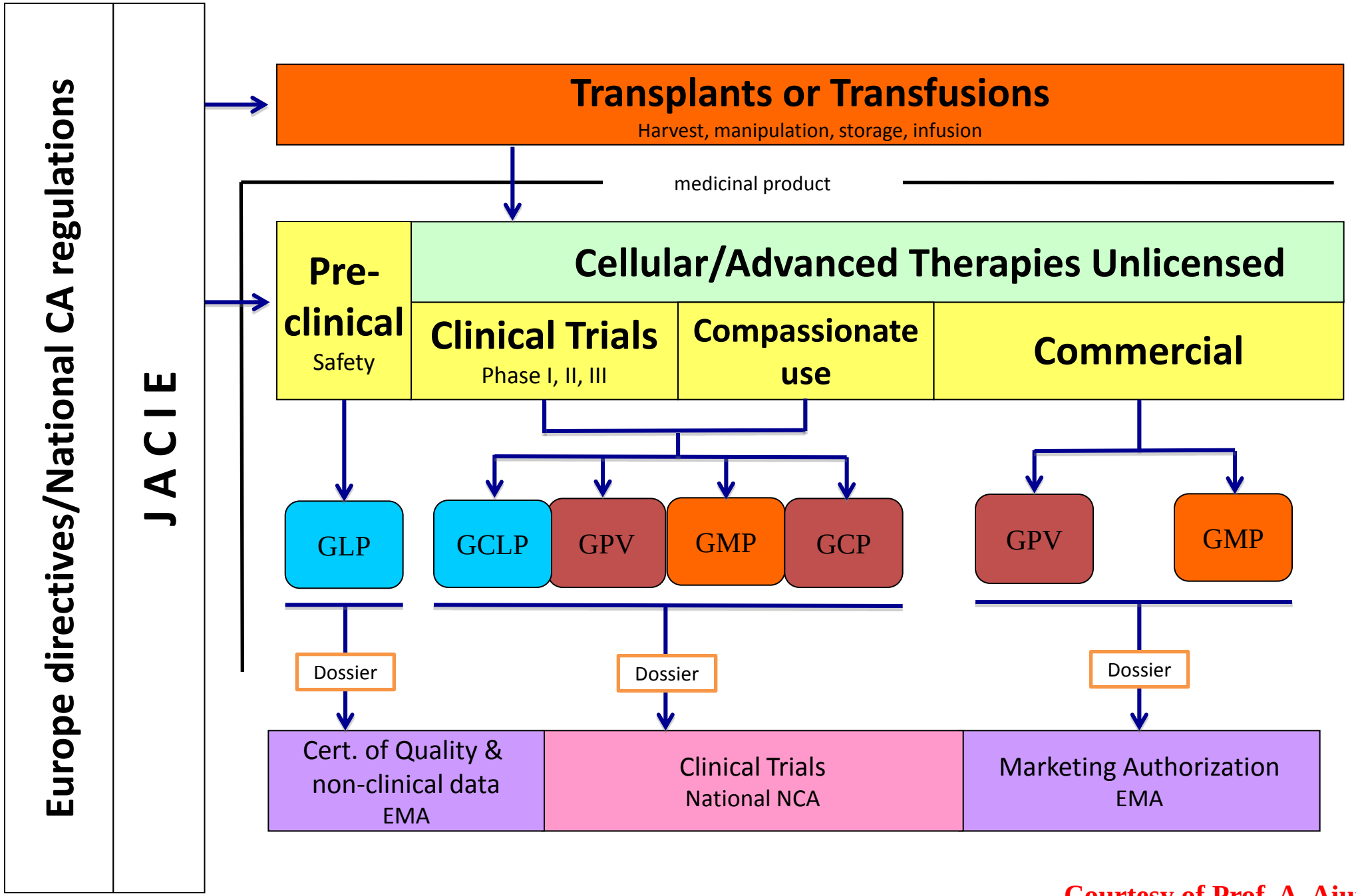
Preclinical issues in cell therapy

Animal models

- Species specificity on a molecular, cellular and tissue level
- Animal model suitable & predictive of safety
- Homologous model/disease model (efficacy/proof of concept)
- Non-homologous model (immunocompromised?) to test human products
- Biologically relevant animal model available (concomitant treatment immunogenicity, delivery) ?
- Large animal model necessary?

Biodistribution

Tumorigenicity & chromosomal stability



Courtesy of Prof. A. Aiuti

Now, this is not the end. It is not even the beginning of the end, but it is, perhaps, the end of the beginning…….