UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

SERVIZIO SANITARIO REGIONALE
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Policlinico

Beyond the lab: the potential roles of Mesenchymal Stem Cells in the clinical practice and oncology

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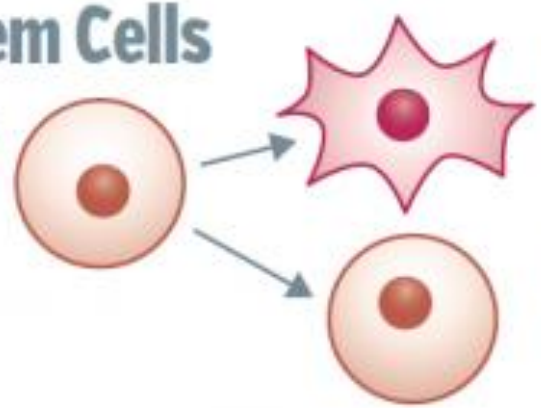
1. Stem cells' definition and biology

- The most widely accepted stem cell definition is an undifferentiated cell with three typical capacities:
  - **i.** **self-renewal** (i.e. the ability to produce unaltered daughter cells by asymmetric celldivision),
  - **ii.** **long-term viability** and
  - **iii.** **potency** (i.e. the possibility to generate different specialised cell types)

Amato B. & Serra R. Int Wound J. 2015

Three Key Facts About Stem Cells

1. The defining characteristic of a stem cell is that it can self-renew or differentiate.
2. Stem cells enable the body to grow, repair and renew.
3. There are three types of stem cells:



Differentiation (Specializing)

Specialized cell
(e.g. muscle cell, nerve cell)

Self-Renewal (Copying)

Stem cell

Tissue Stem Cells

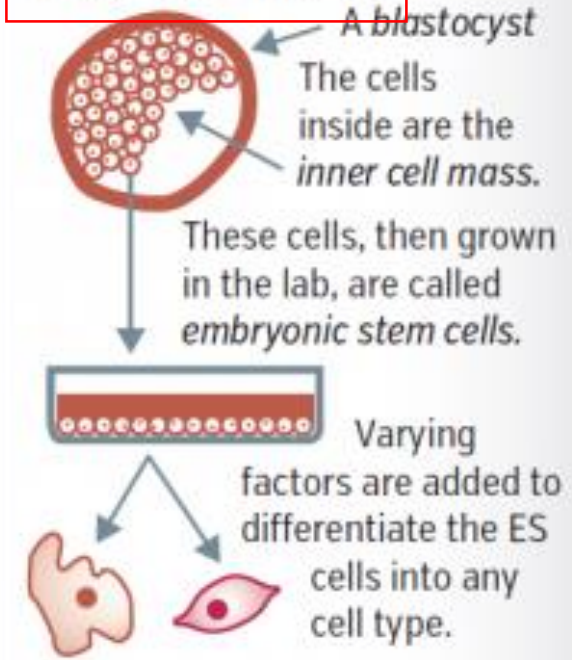
In the fetus, baby and throughout life.

Found throughout the body, each type gives rise to at least one type of more specialized cell.

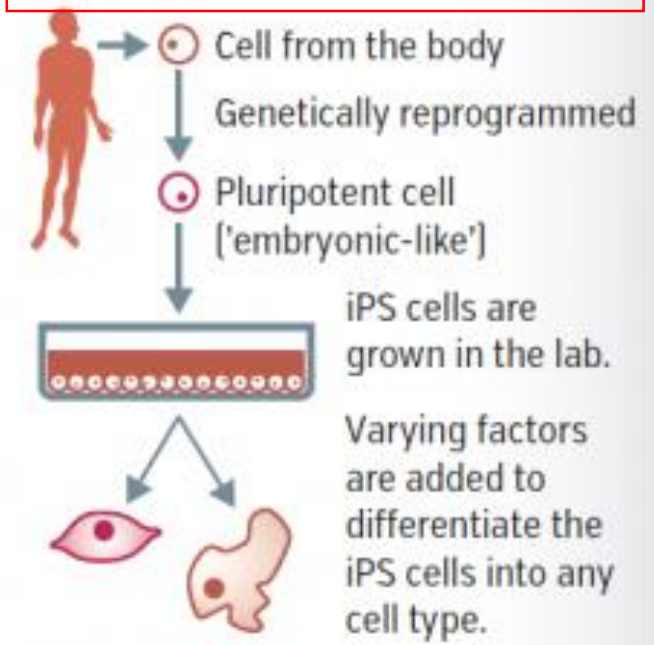
For example, blood stem cells are found in the bone marrow.

Are *multipotent*

Embryonic Stem Cells



Induced Pluripotent Stem Cells (iPS)



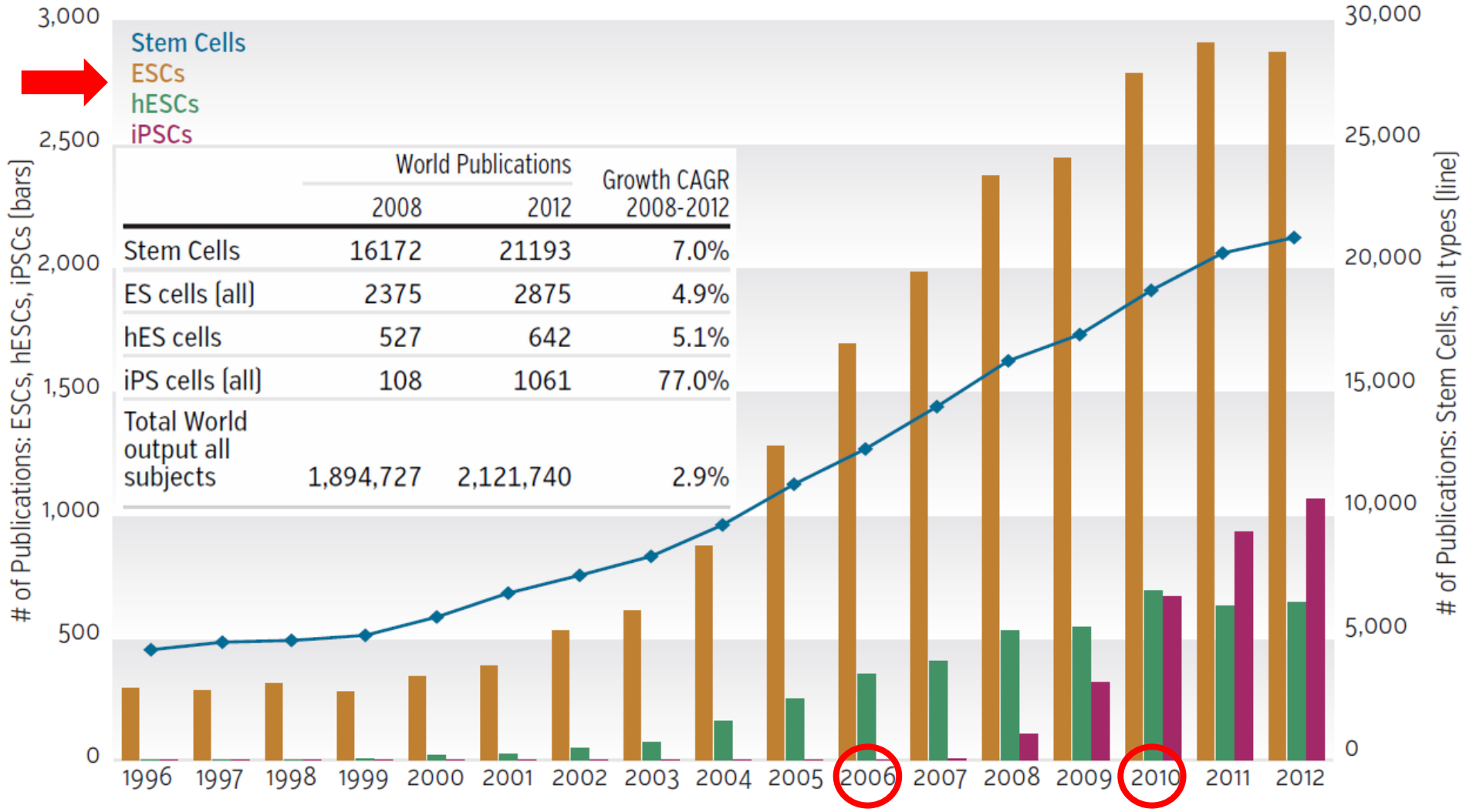
Embryonic stem cells and iPS cells are *pluripotent;* they can generate all the specialized cells of the body.

We are going to analyse Mesenchymal stem cells (MSCs)

- They have a **mesodermal origin**
- **To date, neither surface nor stemness marker allowing an accurate classification** of these cells have been found, and the exact identity of MSCs in vivo is not yet clear.
- However, to be defined as MSC a cell **must differentiate to osteoblasts, adipocytes and chondroblasts in vitro**.
- **Early research suggested that MSCs could differentiate into many other types of cells but it is now clear that this is not the case**
- Are principally obtained from **the bone marrow and adipose tissue**
- They can also be extracted from **a wide variety of tissues** (e.g. umbilical cord, amniotic fluid, menstrual blood, and dental tissue).

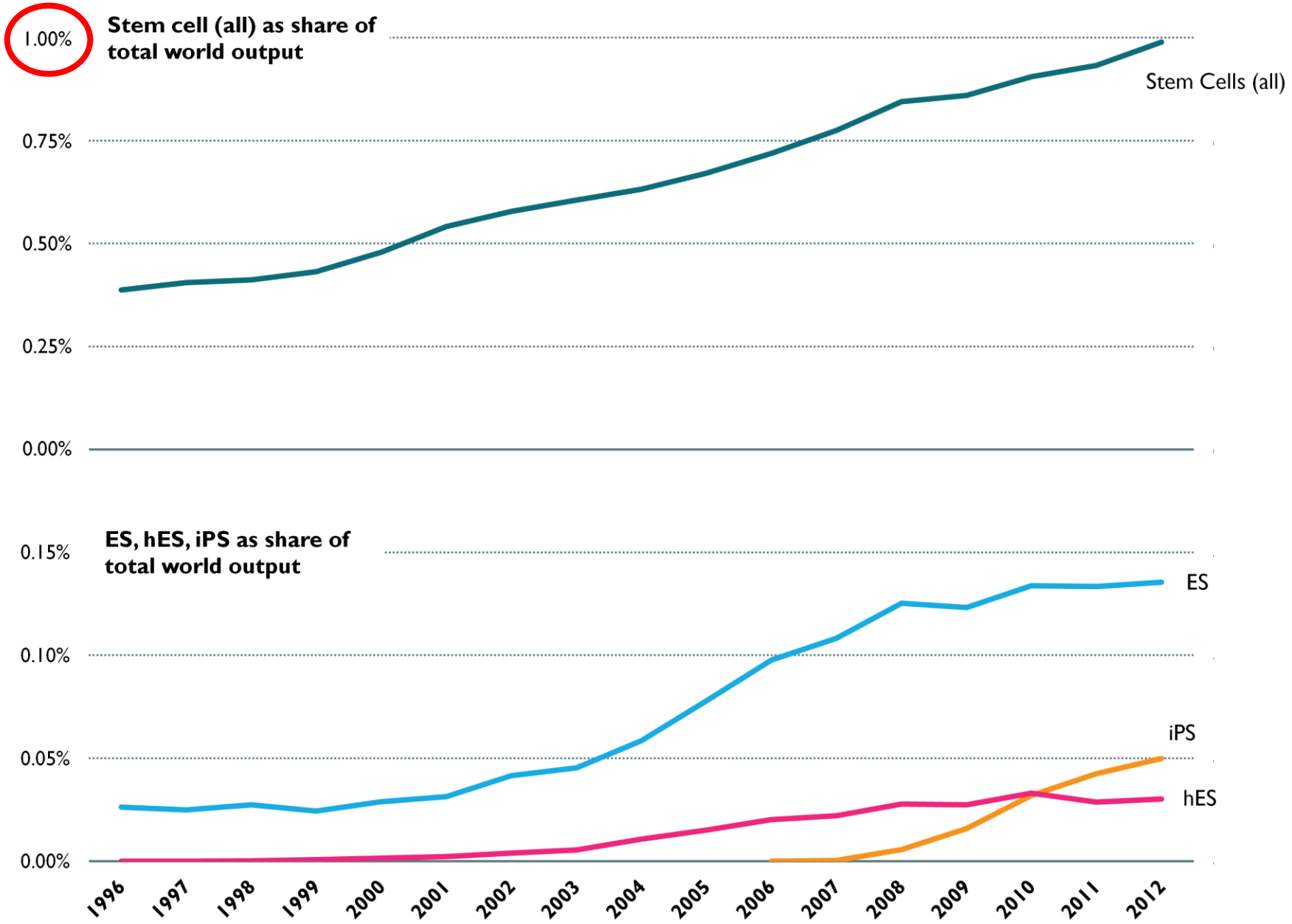
2. Stem cell research: Trends in and perspectives on the evolving international landscape

Global publication output



	World Publications 2008	World Publications 2012	Growth CAGR 2008-2012
Stem Cells	16172	21193	7.0%
ES cells (all)	2375	2875	4.9%
hES cells	527	642	5.1%
iPS cells (all)	108	1061	77.0%
Total World output all subjects	1,894,727	2,121,740	2.9%

Global publication count (1996-2012) and compound annual growth rate (CAGR)(2008-2012) for all stem cells (Stem Cells), ES cells (all organisms; ESCs), hES cells (hESCs), and iPS cells (iPSCs). Source: Scopus.



Global Stem Cell publications (top) and ES, hES and iPS cell publications (bottom), as a share of total world output, from 1996-2012. Source: Scopus

Regulation of stem cell research in Italy

The derivation of **ESC** lines is **banned**

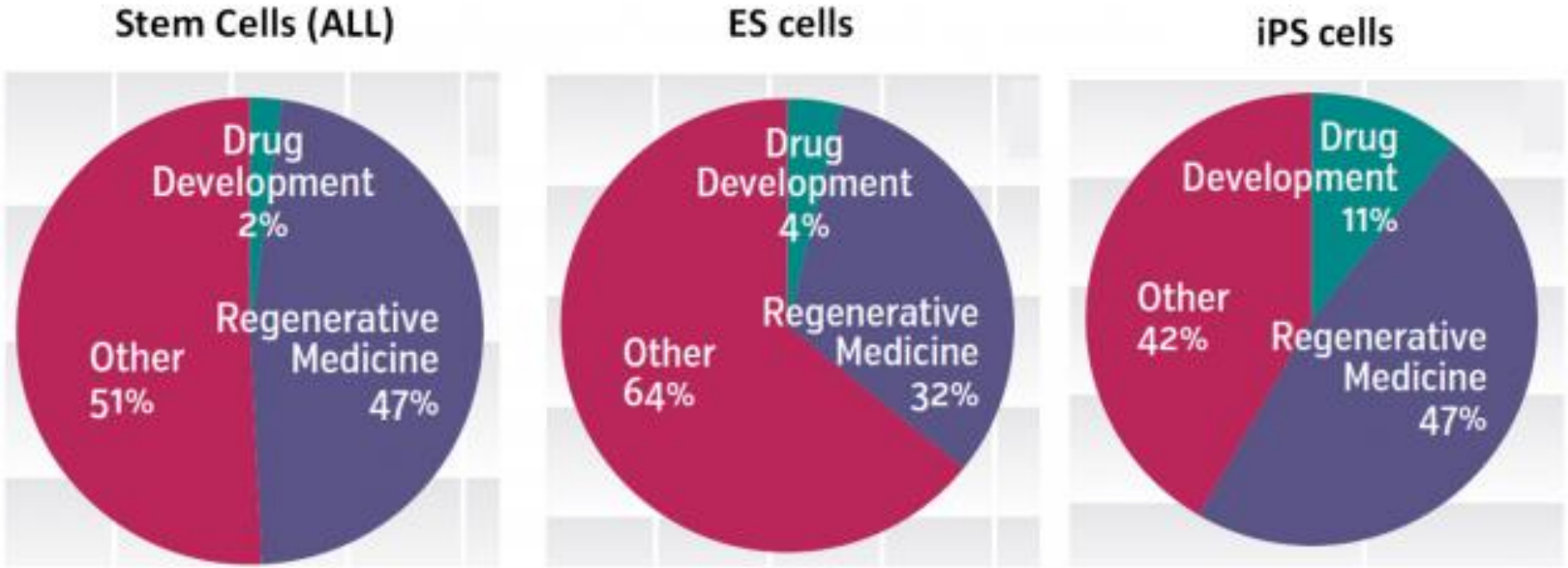
BUT

it is **permitted** to use imported embryonic stem cell lines for research.

ASCs and iPS are **ALLOWED**

3. Current and potential uses of stem cells in Regenerative Medicine and Drug Development

Clinical themes: Regenerative Medicine and Drug Development



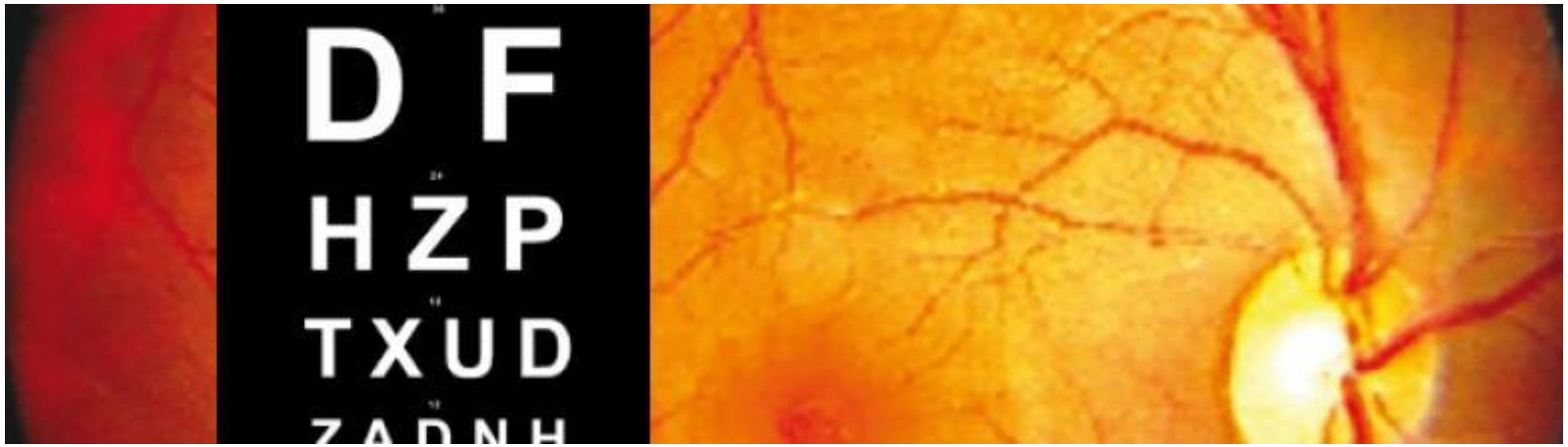
The percentage of stem cell papers published from 2008 to 2012 using keywords related to "drug development," "regenerative medicine," or other by cell type. Source: Scopus

> "I believe the biggest impact to date of iPS cell technology is not regenerative medicine, but in making disease models, drug discovery, and toxicology testing..."
>
> — Shinya Yamanaka, Director Center for iPS Cell Research and Application (CiRA), Kyoto University.

Current clinical uses

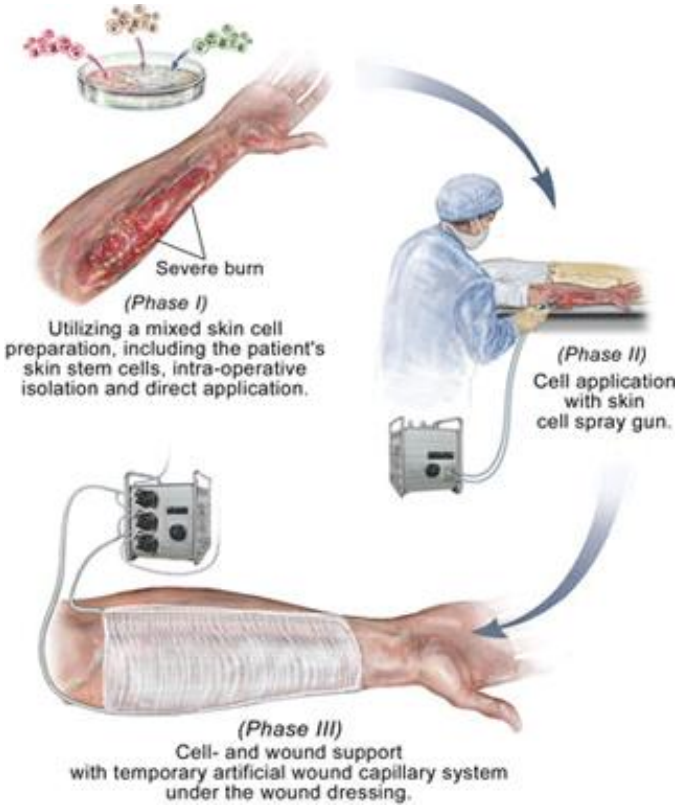
Embryonic stem cells

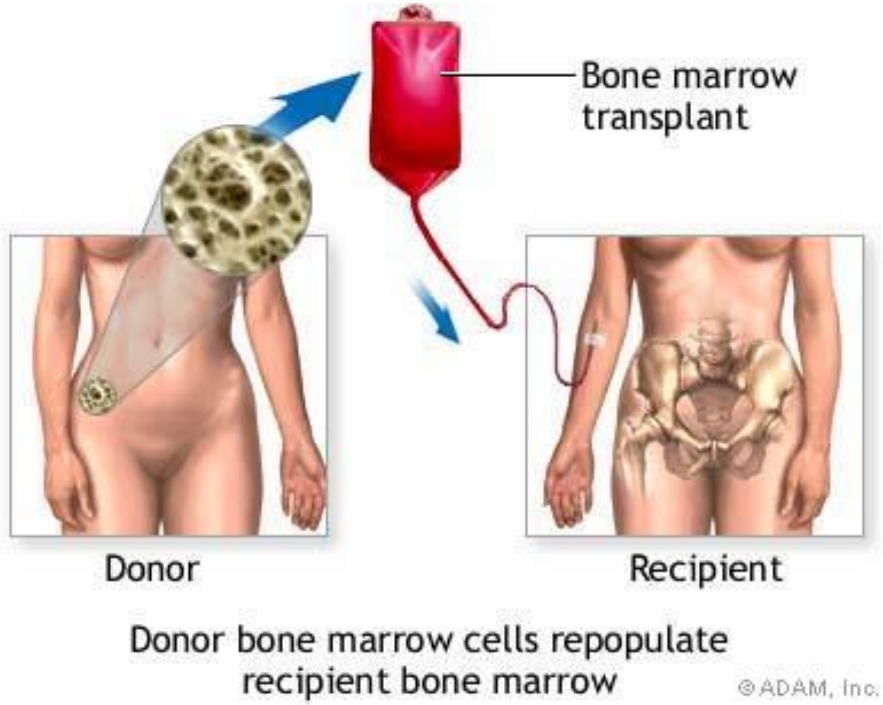
- Have been **approved for use in a very small number of early clinical trials**.
- One example is a clinical trial carried out by **The London Project to Cure Blindness**, using ESCs to produce a particular type of eye cell for treatment of patients with **age-related macular degeneration**.



Blood and skin stem cells: therapy pioneers

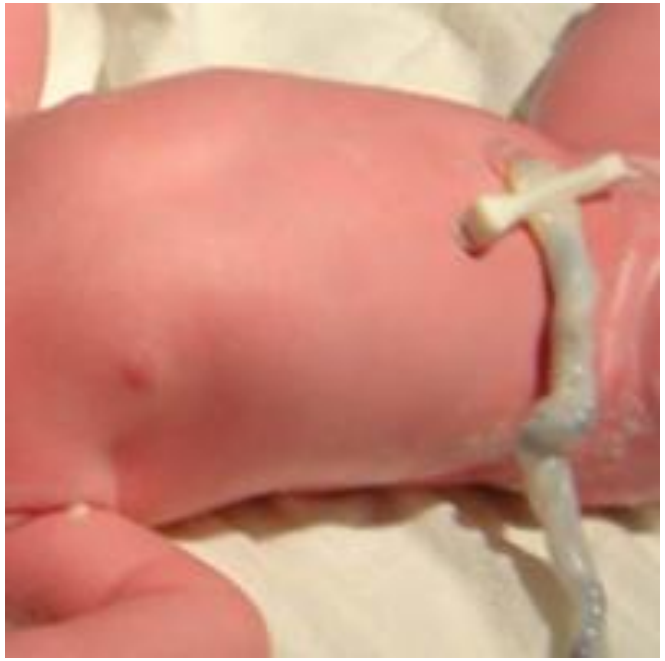
- **Routine** use since the **1970s**!
  - **Bone marrow transplants**
  - **Skin stem cells … but no appendages!**





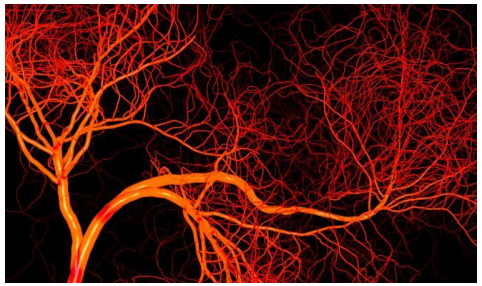
Cord blood stem cells

- Currently used to treat **children with cancerous blood disorders** such as **leukaemia** and **Fanconi anaemia**.
- Why these? **Lower probability of GVHD** + can be frozen (‘**cryopreserved**’) in cell banks



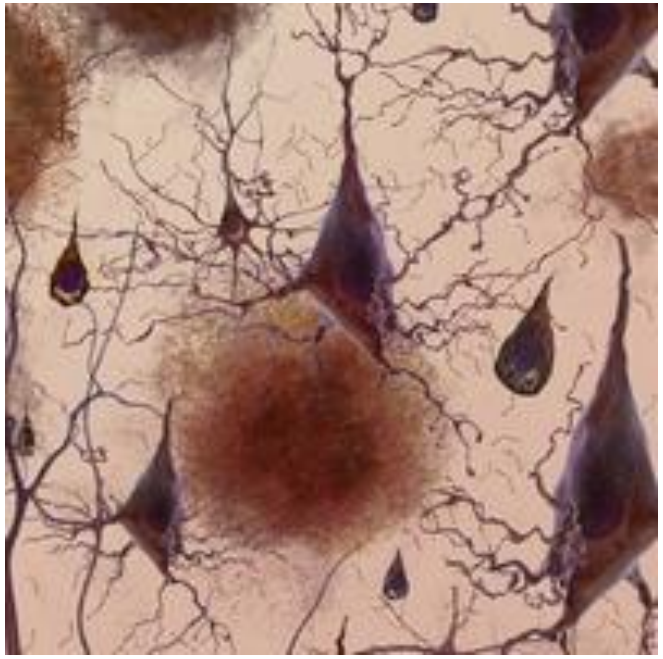
Mesenchymal stem cells

- Are responsible for **bone** and **cartilage repair**. They also produce **fat** cells.
- **Some clinical trials (phase III) are investigating** the safety and effectiveness of MSC treatments for **repairing bone, cartilage**
- Also investigated for **blood vessel damage linked to heart attacks or diseases such as critical limb ischaemia.**
- **MSCs do not themselves produce blood vessel cells but might support other cells to repair damage**. Indeed MSCs appear to play a crucial role in supporting blood stem cells.



iPSs

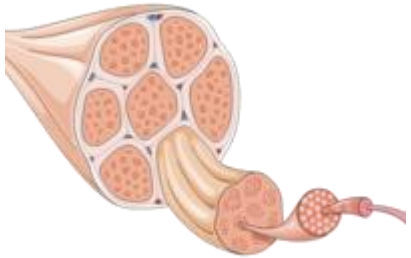
- Huge implications for **disease research** and **drug development**
  - e.g. **Parkinson's disease**:
- **Can we treat diseases now? No, we cannot**. The technology is very new and the reprogramming process is not yet well understood.



And what about these diseases?

Diabetes

- Now we can transplant, but problems with the need of immunosuppression and few donors
- There are currently **no proven treatments for diabetes using stem cells**
- **BUT** we are trying to male new beta cells from pluripotent (ESCs and iPS) stem cells

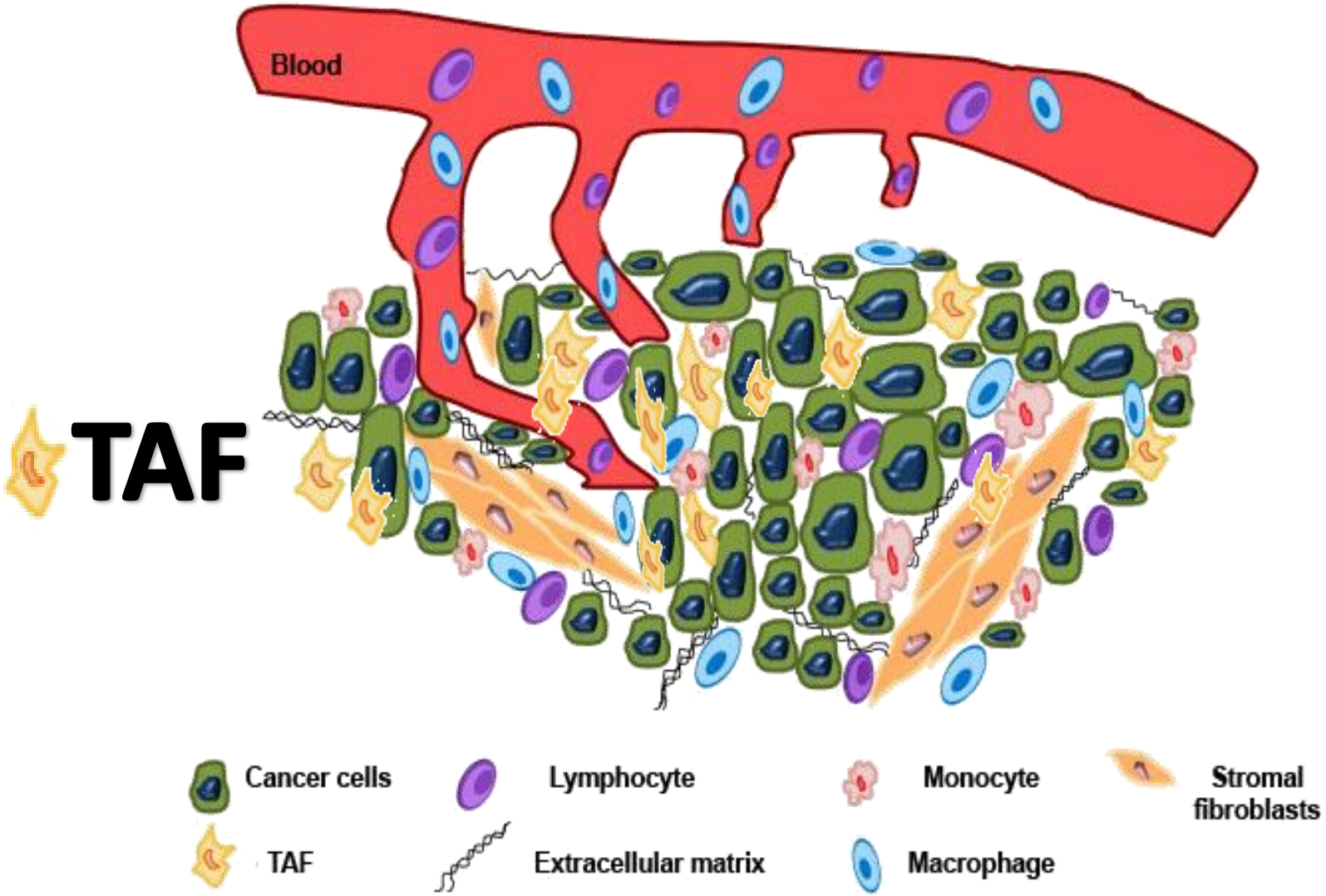


Muscular dystrophy

- Currently there is no definitive cure for DMD
- Main stem-cell-based approaches **currently being investigated**
  1. **Producing healthy muscle fibres** (correcting the genetic defect, see below)
  2. **Reducing inflammation** (tem cells may release chemicals that reduce inflammation)

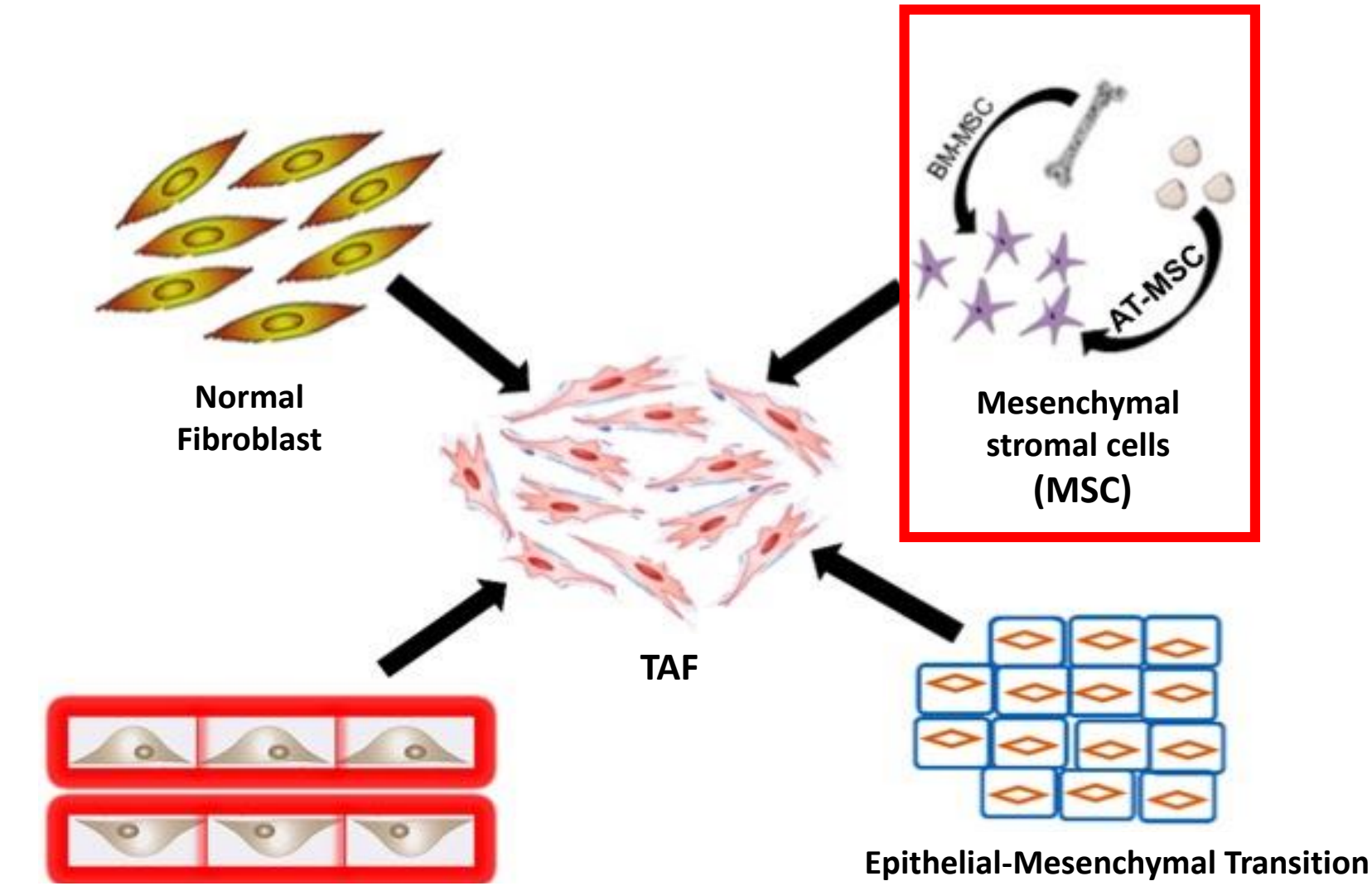
4. Actual roles of Mesenchymal Stem Cells in cancer therapy and Ewing sarcoma

The tumor micro-environment



Grisendi G. et al. Am J Cancer Research, 2011

TAF: where do they come from?



D'souza et al. Adv Biochem Eng Biotechnol. 2012

MSC and homing capacity in the tumor

TUMOR	MSC SOURCE	REFERENCES
PANCREATIC CANCER	**BM-MSC**	Ishii et al. Biochem Biophys Res Commun 2003 Direkze et al. Cancer Res. 2004
BREAST CANCER	**BM-MSC; AD-MSC**	Karnoub AE et al. Nature 2007 Goldstein RH et al. Cancer Res 2010 Liu S et al. Cancer Res 2011 Kidd S et al. PLoS One 2012
GLIOMA	**BM-MSC; AD-MSC; UC-MSC**	Nakamura, K. et al. Gene Ther 2004 Nakamizo A et al. Cancer Res 2005 Lee DH et al. Clin Cancer Res 2009 Menon, L. G. et al. Stem Cell 2009
COLON CANCER	**BM-MSC**	Hung S-C et al. Clin Cancer Res 2005
OVARIAN	**BM-MSC; AD-MSC**	Mader EK et al. Clin Cancer Res 2009 Komarova S et al. J Ovarian Res 2010
SARCOMA	**BM-MSC; AD-MSC**	Khakoo, A. Y. et al. J Exp Med 2006 Lee HJ et al. Ann Surg 2013
MELANOMA	**BM-MSC; AD-MSC**	Studeny, M. et al. Cancer Res 2002 Ren C, at al. Stem Cell 2008 Kucerova L et al. J Gene Ther 2014
LUNG CANCER	**BM-MSC; AD-MSC**	Loebinger MR et al. Cancer Res 2009 Matuskova M et al; J Exp Clin Cancer Res 2015
HEPATOCELLULAR CARCINOMA	**BM-MSC**	Niess H, et al. Ann Surg 2011

What do MSCs actually do to the tumor

FAVORISCONO LA CRESCITA TUMORALE

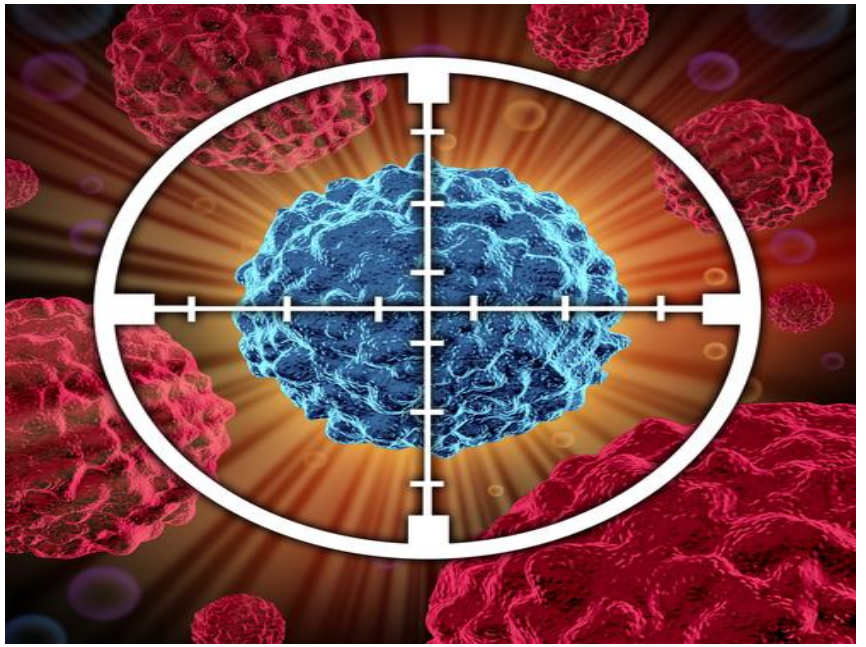
MSC SOURCE	TUMOR MODEL	FINDINGS	AUTHORS
BM-MSC	Breast	Increased tumor size and increased metastasis	Karnoub et al., Nature 2007.
BM-MSC	Colon cancer	Increased incidence	Zhu et al., Exp. Mol. Pathol. 2006.
BM-MSC	Breast	Increased tumor size	Mishra et al., Cancer Res. 2008.
MSC	Colon cancer	Increased tumor size and metastasis	Shinagawa et al., Int. J. Cancer 2010.
MSC	Ovarian cancer	Increased tumor size	Spaeth et al., Plos ONE 2009.
AD-MSC	Breast	Increased tumor size	Muehlberg et. al, Carcinogenesis 2009.
AD-MSC	Lung and Glioma	Increased tumor size	Yu et al., Stem Cell Dev. 2008.
AD-MSC	Kaposi's sarcoma	Increased tumor size	Zhang et al., Cancer Res. 2009.
AD-MSC	Prostate	Increased incidence and tumor size	Lin et al., Prostate 2010.
AD-MSC	Melanoma	Decreased latency and increased size	Kucerova et al., Mol Cancer 2010.
AD-MSC	Prostate	Increased tumor size	Pranlt et al., Prostate 2010.

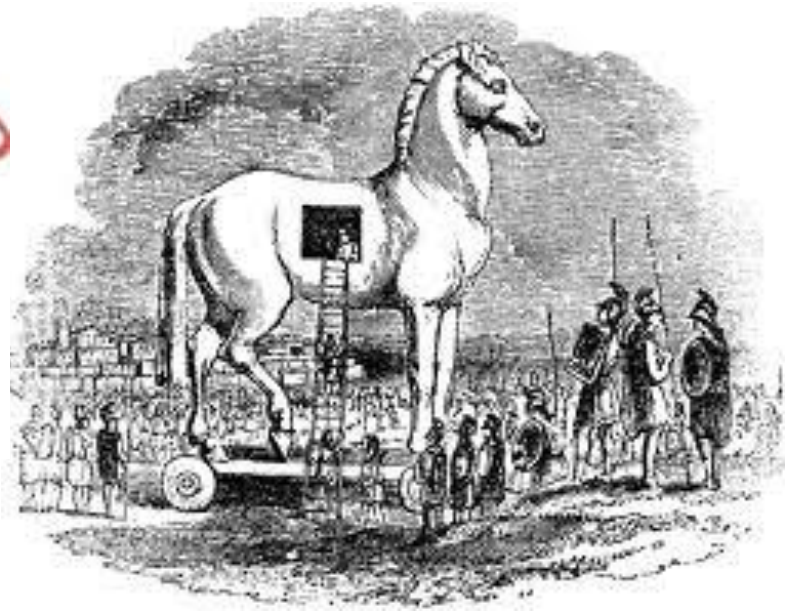
INIBISCONO LA CRESCITA TUMORALE

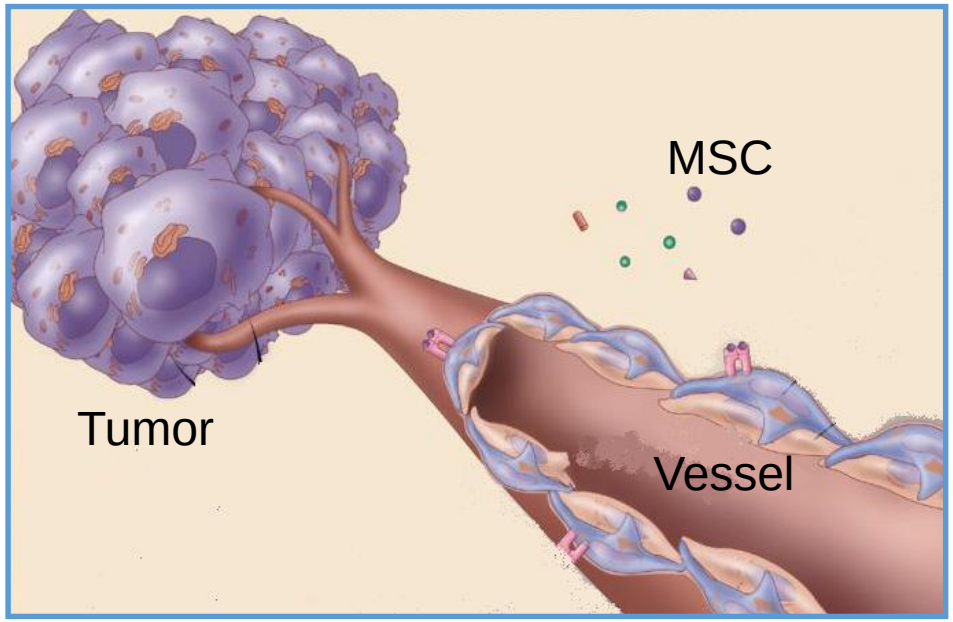
MSC SOURCE	TUMOR MODEL	FINDINGS	AUTHORS
BM-MSC	Kaposi's sarcoma	Tumor size smaller	Khakoo et al., J. Exp. Med. 2006.
Human fetal skin	Human hepatoma cell line	Tumor size smaller	Qiao et al., Cell. Res.. 2008.
Human fetal skin	Breast	Increased latency, reduced tumor size and metastasis	Qiao et al., Cancer Lett., 2008.
AD-MSC	Human myelogenous leukaemia	Proliferation inhibited	Zhu et al., Leukaemia 2009.
AD-MSC	Pancreatic cancer cell	Tumor size smaller	Cousin et al., Plos ONE 2009.
BM-MSC	Lung and melanoma	Tumor size smaller and decreased metastasis	Maestroni et al., Cell. Mol. Life Sci. 1999.
Umbilical cord blood derived	Glioma	Increased tumor size	Yu et al., Stem Cell Dev. 2008.
BM-MSC	Non-Hodgkin's lymphoma	Decreased tumor burden and increased survival	Secchiero et al., Plos ONE 2010.
AD-MSC	Glioblastoma	Decreased tumor incidence	Kucerova et al., Mol. Cancer 2010.

So, can we use MSC to selectively destroy the tumor?

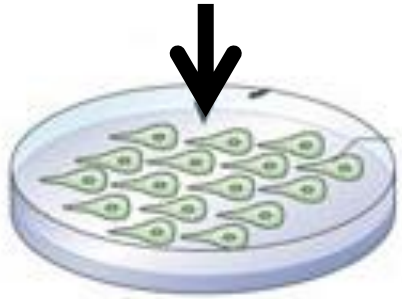
The «Trojan Horse» Strategy







Why should we use MSC and not other cells?



EASY TO ISOLATE

FAST GROWING IN VITRO

DIFFERENTIATING CAPACITY

IMMUNOMODULATORY CAPACITY

LOW IMMUNOGENICITY

ATTRACTED BY THE TUMOR

EASY TO GENETICALLY MANIPULATE

CELL THERAPY

REGENERATIVE MEDICINE

ANTITUMORAL MEDICINE

How can we arm our MESENKILLERS?

Table 1 Mesenchymal stem cells as cellular vehicles for targeting cancer

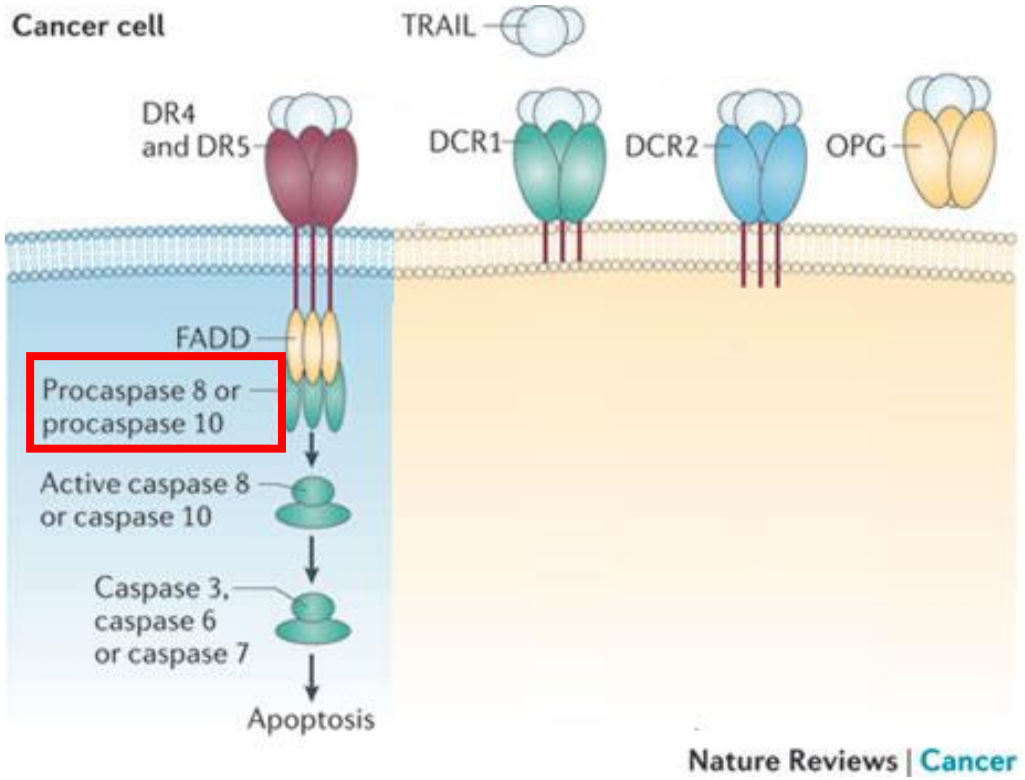
Anticancer agent	Anticancer mechanism	Tumor model	Route of MSC admi-nistration	Species: MSC/tumor/host	Ref.
CX3CL1	Immunostimulatory	Lung	iv	Mouse/mouse/mouse	[40]
CD	Prodrug converting	Prostate	sc/iv	Human/human/mouse	[41]
		Colon	sc/iv	Human/human/mouse	[14]
HSV-tk		Glioma	it	Rat/rat/rat	[42]
		Pancreas	iv	Mouse/mouse/mouse	[28]
IFNα	Immunostimulatory and apoptosis indcing	Melanoma	iv	Mouse/mouse/mouse	[43]
		Glioma	it/ic	Mouse/mouse/mouse	[44]
IFNβ		Breast	sc/iv	Human/human/mouse	[29,45]
		Pancreas	ip	Human/human/mouse	[27]
IL2		Glioma	it/ic	Rat/rat/rat	[17]
IL7	Immunostimulatory	Glioma	it	Rat/rat/rat	[46]
IL12	Activates cytotoxic lymphocyte and NK cells	Melanoma	iv	Mouse/mouse/mouse	[47]
		Hepatoma	iv	Mouse/mouse/mouse	[47]
		Breast	iv	Mouse/mouse/mouse	[47]
IL18	Immunostimulatory	Glioma	it	Rat/rat/rat	[48]
NK4	Inhibits angiogenesis	Colon	iv	Mouse/mouse/mouse	[49]
TRAIL	Induces apoptosis	Glioma	it	Human/human/mouse	[20]
		Glioma	ic	Human/human/mouse	[50]
		Glioma	iv	Human/human/mouse	[22,51]
		Lung	iv	Human/human/mouse	[52]
		Breast, lung	sc/iv	Human/human/mouse	[53]
		Colon	sc	Human/human/mouse	[54,55]
		Pancreas	iv	Human/human/mouse	[56]

CD: Cytosine deaminase; CX3CL1: Chemokine fractalkine; HSV-tk: Herpes simplex virus-thymidine kinase; ic: Intracerebral; IFN: Interferon; IL: Interleukin; ip: Intraperitoneal; it: Intratumoral; iv: Intravenous; NK: Natural killer; sc: Subcutaneous; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand.

Sun X et al. World J Stem Cells, 2011

TRAIL mechanism

- TRAIL induces **apoptosis** in tumor cells
- TRAIL is **physiologically expressed and produced by immune cells** (tumor surveillance)
- TRAIL can be **trans membrane** or **soluble**
- TRAIL receptors are 5.
  - **DR-4 and DR-5** induce apoptosis in the expressing cells.
  - **DcR-1, DcR-2 and OPG** are decoy receptors



TRAIL sensibile tumor

- COLON CANCER (Jalving M. et al. 2006)
- LUNG CANCER (Jin H. et al. 2004)
- OVARIAN CANCER (Pukac L. et al. 2003)
- BREAST CANCER (Rahman M. et al. 2009)
- CERVICAL CARCINOMAS (Sheridan JP. et al. 1997)
- GLIOBLASTOMA (Pollack IF. et al. 2001)
- PANCREATIC CANCER (Halpern W. et al. 2004)
- PROSTATE CANCER (Yu R. et al. 2000)
- THYROID CANCER (Mitsiades N. et al. 2000)
- LYMPHOMAS (Daniel D. et al. 2007)
- MULTIPLE MYELOMA (Mitsiades CS. et al. 2001)
- SARCOMAS (Petak I. et al. 2001)

rhTRAIL limits in the clinical practise

✓Bad pharm
(multiple infu

✓ High toxic

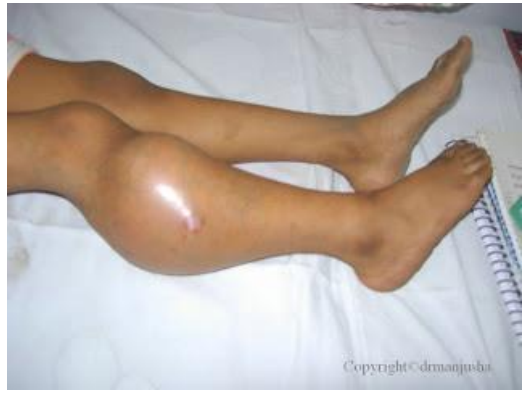
✓ Expensive

minutes

… but we can try using MSCs to produce TRAIL directly into the tumor!

Ashkenazi A et al. JCO 2008

What about TRAIL- MESENKILLERS and Ewing sarcoma?



- **Definition** (Ewing sarcoma family of tumors)
- **Epidemiology** 1 case per 1 million per year in the United States, 10-20 yo
- **Prognosis**
  - **Metastatic** (long term survival: <30%) vs **limited** (80% cases; cure: 75%)
  - **Distal** vs **pelvic**
  - **Youngers** do better
- **Data in line with what we found in Modena** (Oncoematology, Unit of Pediatrics directed by Prof. Iughetti L.)

Actual treatment

National Comprehensive Cancer Network®

NCCN Guidelines Version 2.2016
Ewing's Sarcoma Family of Tumors

PRESENTATION[a,b,c]

Ewing's sarcoma family of tumors →

WORKUP

- History and physical
- MRI ± CT of primary site
- Chest CT
- PET scan and/or bone scan
- Bone marrow biopsy and/or screening MRI of spine and pelvis[d]
- Cytogenetics and/or molecular studies[e] (may require re-biopsy)
- LDH
- Fertility consultation should be considered

PRIMARY TREATMENT

Multiagent chemotherapy[f] (category 1) for at least 12 weeks prior to local therapy[h]

RESTAGE

For patients with localized disease
Restage with:
- Chest imaging
- Imaging of primary site
- Consider PET scan or bone scan[g]

For patients with metastatic disease
Restage with:
- Chest imaging
- of
- site
- er PET
- bone
- ther
- l studies

LOCAL CONTROL THERAPY

Wide excision[b] → Positive margins
Wide excision[b] → Negative margins[i]

or

Definitive RT[l] and chemotherapy[f,j]

or

Amputation[b] in selected cases

Stable/improved disease following response to primary treatment

Progre
followi
treatm

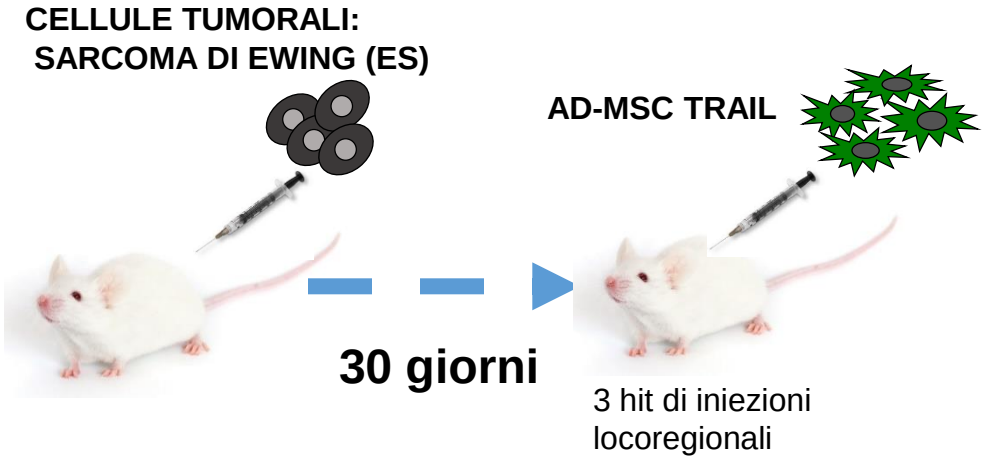
ADJUVANT TREATMENT/ ADDITIONAL THERAPY

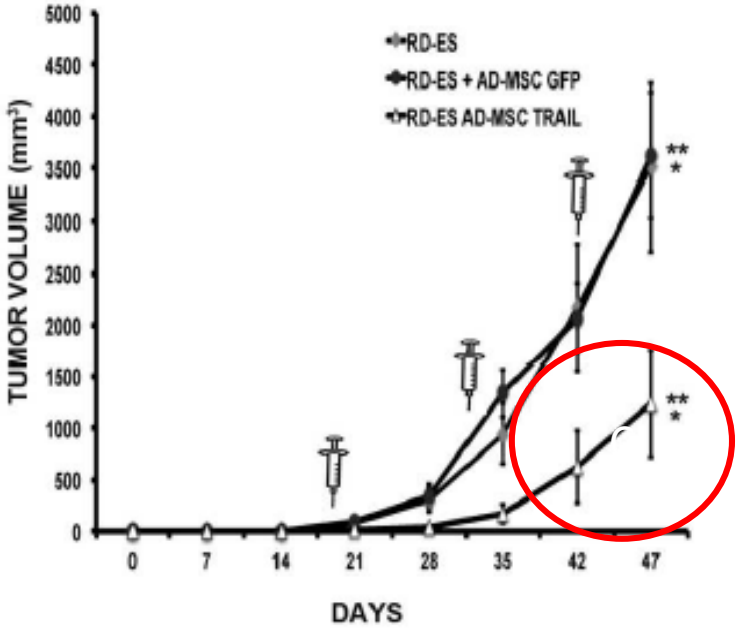
Positive margins → Continue chemotherapy[f,j] (category 1) followed by RT[l] or RT[l] and chemotherapy[f,j] (category 1, for chemotherapy)

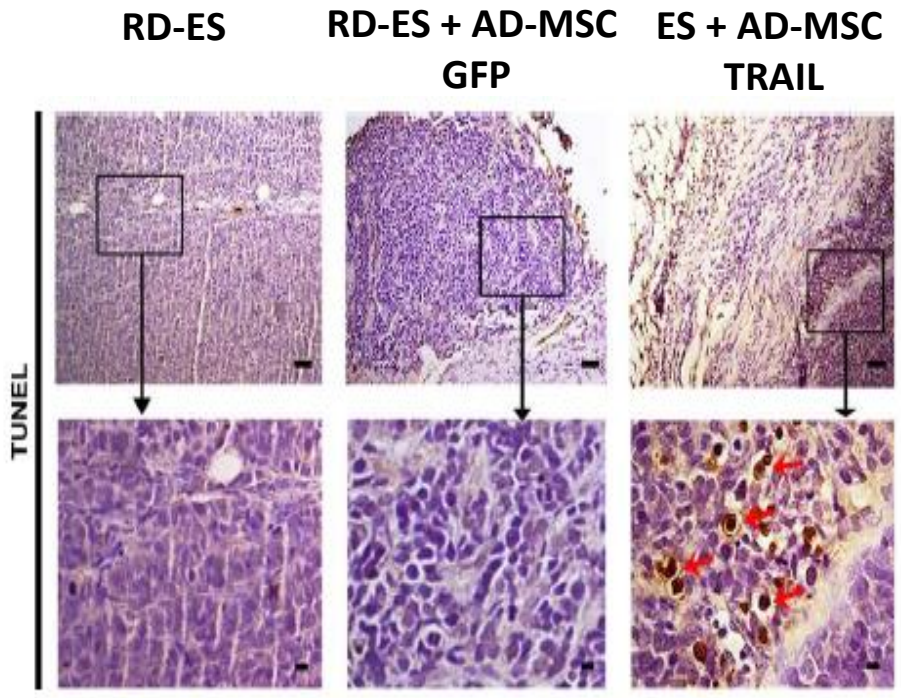
Negative margins[i] → Chemotherapy[f,j] (category 1)

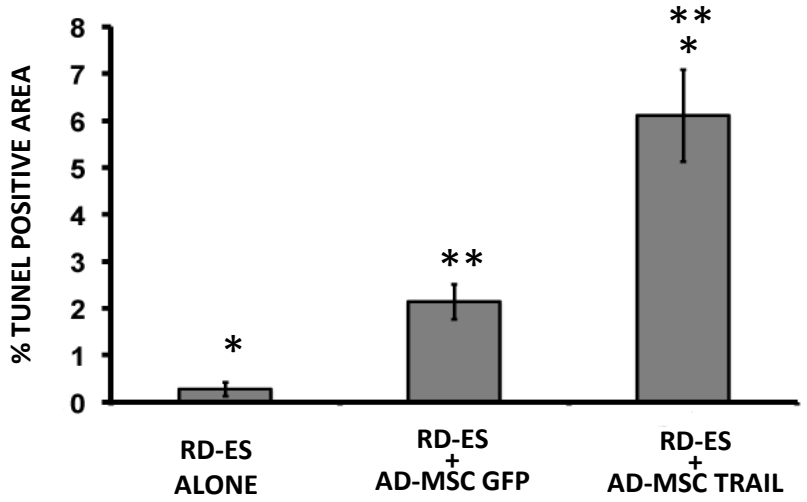
Consider RT[l] and/or surgery to primary site for local control or palliation

MSC in Ewing sarcoma: our experience









Grisendi G et al.
Stem Cells 2015

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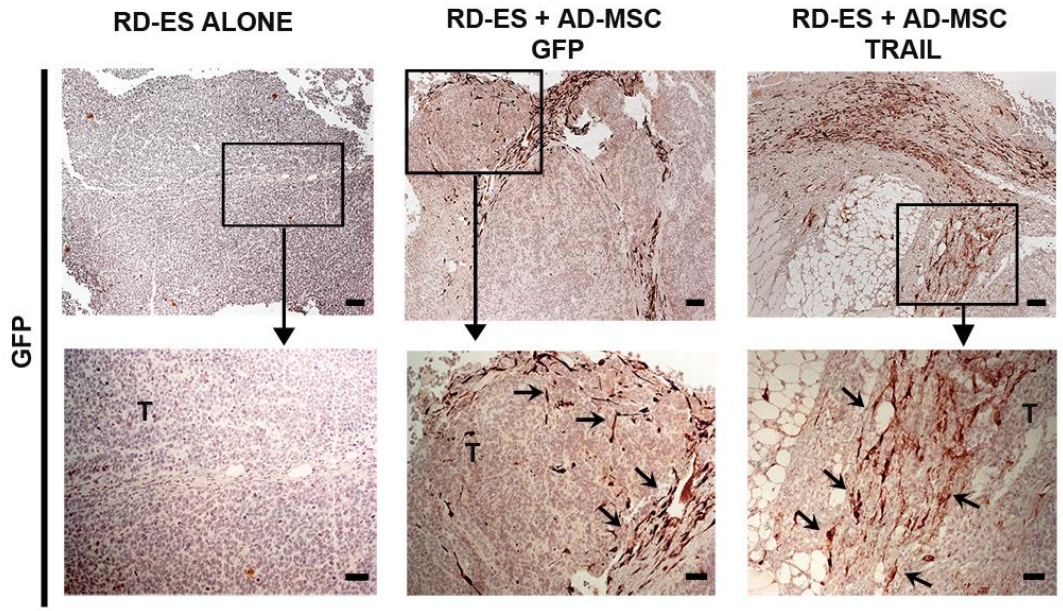
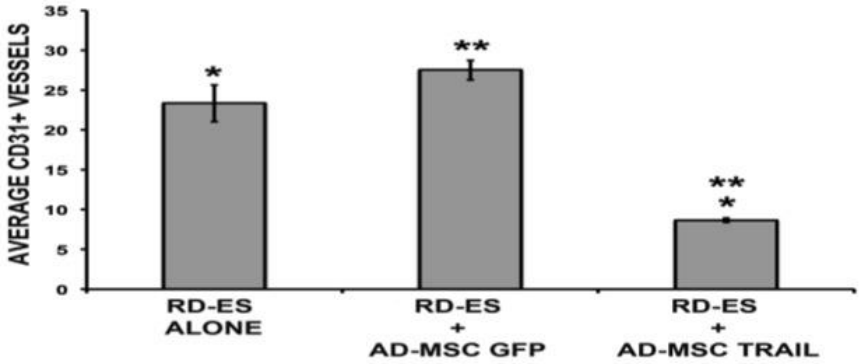
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

AD-MSCs TRAIL engraft into tumor stroma and reduce tumor vascularization





Grisendi G et al. Stem Cells 2015

Take home messages

1. Stem cells are a powerful tool for the future medicine
2. Not only regenerative medicine but also drug development and basic research
3. In cancer stem cells could be used as vehicle to selectivelly deliver anticancer agents into tumor sites, thus enhancing the antitumor efficacy while avoiding systemic side effects

THANK YOU FOR YOUR ATTENTION