EA3842
CAPTuR
Contrôle de l'Activation Cellulaire, Progression Tumorale et Résistance thérapeutique

Université de Limoges

Isolation and characterization the Cancer Stem Cells (CSCs): One question many answers …

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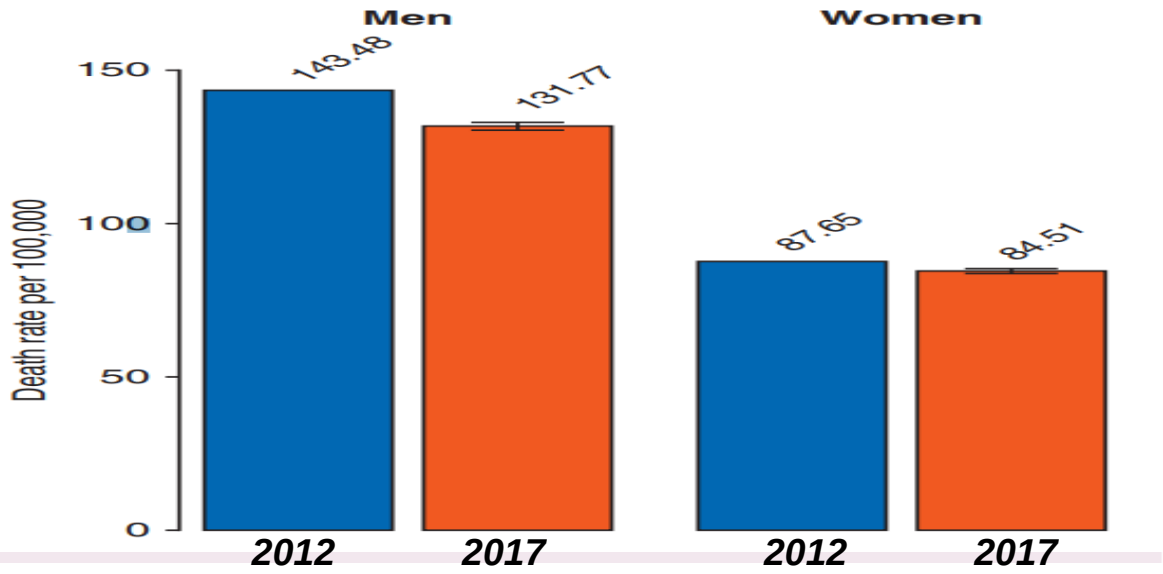
Cancer in European countries

Table 1. Number of predicted deaths and mortality rates for the year 2017 and comparison figures for most recent data (2012), for the EU as a whole, with 95% prediction intervals

European Union		Observed number of deaths 2012	Predicted number of deaths 2017
Men	Stomach	36 304	33 700
	Colorectum	92 508	97 100
	Pancreas	39 812	43 600
	Lung	185 621	183 400
	Prostate	71 810	76 100
	Leukaemias	23 594	24 400
	All cancers (malignant and benign)	745 281	761 900
Women	Stomach	23 501	21 500
	Colorectum	78 027	78 600
	Pancreas	39 692	43 800
	Lung	82 076	92 300
	Breast	91 847	92 600
	Uterus (cervix and corpus)	28 973	29 500
	Leukaemias	18 869	19 500
	All cancers (malignant and benign)	588 140	611 600

Total > 1.30M > 1.35M



Malvezzi M et al, Ann.,Oncol. 2017)

Total Cancer Mortality

Du to the low efficiency of treatments ➔ Cure failure

➔ High rates of recurrences

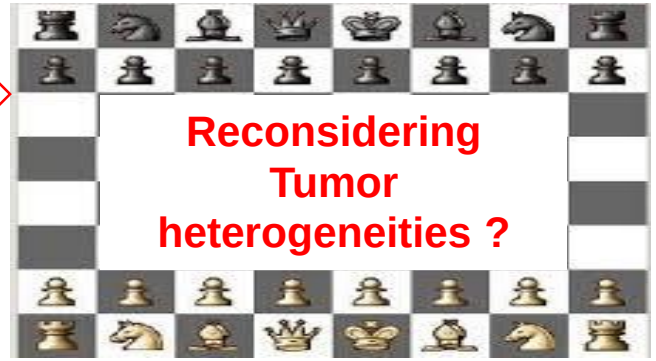
Beliefs and dogmas evolution …

➔ The cancer
One tumor

➔ Different tumors
Different organs

➔ Different genotypes
Different people

➔ Different environments
Different cells



Intratumoral heterogeneity and Cancer Stem Cells (CSCs)

The histological heterogeneity observed is associated to **heterogeneous expression of different markers among** cancer cells, termed **intratumoral heterogeneity**. *(Nassar D and Blanpain C Annu. Rev. Pathol. Mech. Dis. 2016)*

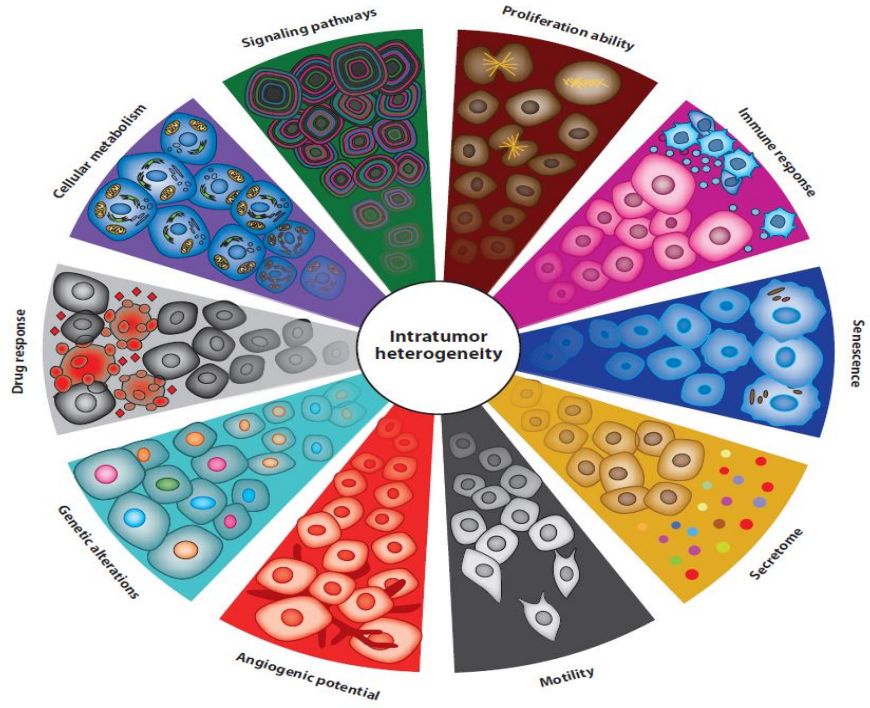
Arising tumors in different patients ➔ Gives a supplementary variability known as **intertumoral heterogeneity** *(Almendro V et al. Annu. Rev. Pathol. Mech. Dis. 2013)*

Heterogeneities in cancer ➔ constitute many biological properties, shared by all tumors. *(Almendro V et al. Annu. Rev. Pathol. Mech. Dis. 2013)*

Tumor heterogeneity (mostly genetic determinants)
➔ Leads to tumor classification

CSCs emerging as a nongenetic determinants of heterogeneity

➔ Related to development pathways and epigenetic modifications
(Kreso A and Dick JE. Cell Stem Cell. 2014)



The Cancer Stem Cells or CSCs

CSC concept ➔ States that tumor growth, analogous to the renewal of healthy tissues, is fueled by small numbers of dedicated stem cells.

Firsts demonstrations related to CSCs

- Acute Myeloid Leukemia (AML) ➔ John Dick et al. Determined a cell subpopulation of primary tumor able to propagate disease in immuno-deficient mice *(Lapidot T, et al. Nature 1994)*

- Exhibit cell-surface marker phenotype similar to the hematopoietic stem cells *(Bonnet D and Dick JE. Nat. Med. 1997)*

CSC in Solid Tumors

Breast Cancer	**- Identification of CSC as tumorigenic breast cancer cells** **- ALDH1 as a CSC marker in human mammary cancer**	*Al-Hajj Met al. PNAS. 2003* *Ginestier C, et al. Cell Stem Cell. 2007*
Brain cancer	**Identification of stem cell in human brain tumors, based on CD133 marker**	*Singh SK, et al. Canc. Res. 2003*
Ovarian Cancer	**CSCs in ovarian cancer aggressiveness**	*Bapat Saet al. Can. Res. 2005*
Prostate Cancer	**Identification of tumorigenic prostate CSCs**	*Collins AT, et al. Canc Res. 2005*
Colon cancer	**-Phenotypic characterization of human colorectal CSC** **- Identification and expansion of human colon CSCs**	*Dalerba P, et al. PNAS. 2007* *Ricci-Vitiani L, et al. Nature. 2007*
Lung Cancer	**Identification and expansion of the tumorigenic lung CSC populations**	*Eramo A, et al. Cell Death Differ. 2008*
Pancreatic Cancer	**CSCs function in tumor growth and metastatic activity pancreatic cancer**	*Hermann PC, et al. Cell Stem Cell. 2007*
Liver Cancer	**Characterization of CSCs in hepatocellular carcinoma**	*Yang ZF, et al. Canc Cell. 2008*

CSCs characterization: An older story !!!

➔ **Sajiro Makito postulated the evidence of Cancer Stem Cells, in ascites tumor…. in 1956**

FURTHER EVIDENCE FAVORING THE CONCEPT OF THE STEM CELL IN ASCITES TUMORS OF RATS*

By Sajiro Makino
Zoological Institute, Hokkaido University, Sapporo, Japan

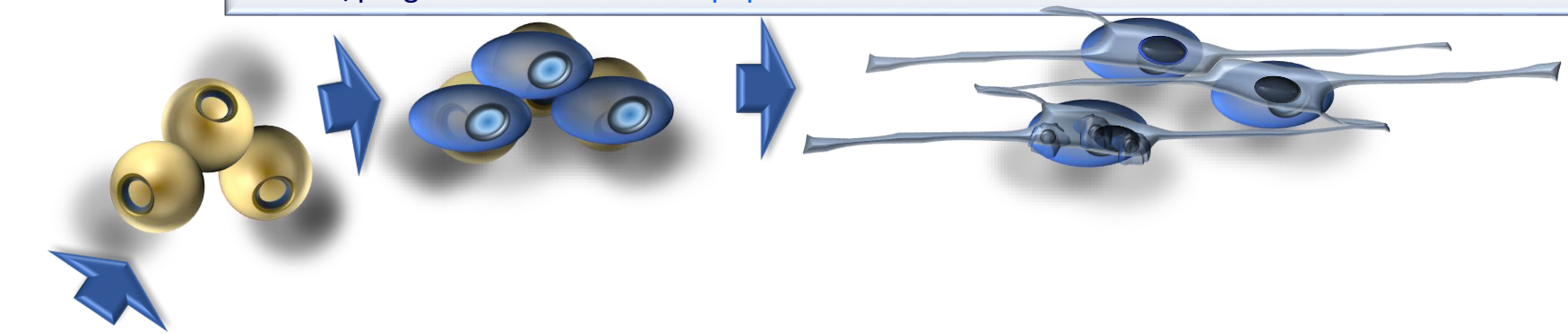
Cytological investigations based on morphological and statistical analysis of the chromosomes in several ascites tumors of rats conducted by the present author in collaboration with his co-workers have revealed the existence of the stem line (or stem lines) of tumor cells as primary contributors to the growth of the tumor. It has been shown that in each of the tumors studied, populations of tumor cells persist characterized by a high frequency of definite chromosome patterns specific to the kind of tumor differing from those of ordinary tissue cells (Makino and Kanô, 1951; Makino, 1952a, b; Makino and Kanô, 1953). The stem cells are persistent through serial transfers by dividing in a regular mitotic manner (Makino, 1952b; Makino and Nakahara, 1953a, b). They also remain unaltered in chromosome individuality through heteroplastic transplantations and through treatment with chemicals (Makino, 1952a; Makino and Tanaka, 1953a, b; Tanaka *et al.*, 1955). Since differences in the genetic constitution of these stem cells are correlated with differences in the genetic behavior of the tumor, it is the stem cells which determine the genetic behavior of a tumor. Further data to be presented in this paper supplement the evidence of the stem cells and strengthen the stem-cell hypothesis.

➔ Existence of a **stem lines of tumor cells** as a primary contributors of tumor growth
➔ These cells are **persistent by regular** mitotic **divisions**
➔ They are unaltered by chemical treatments ……. chemo-resistance!
➔ Their genetic constitution determines the genetic behavior of the tumor
…. These cells **provide genetic background of the tumor**

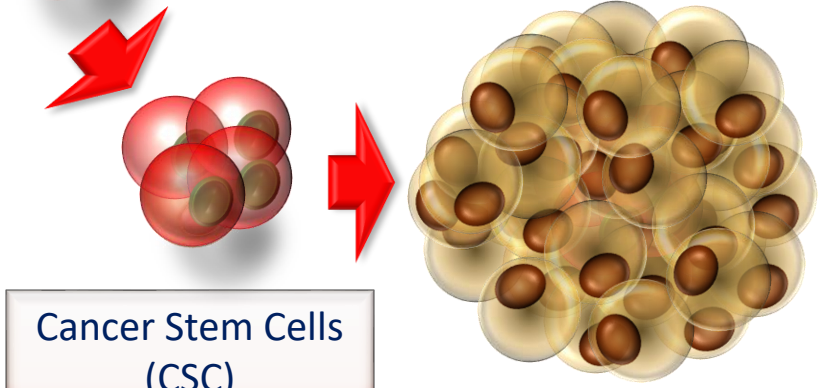
Makino Sajiro. Ann N Y Acad Sci.1956

Normal Cells/Cancer Cell … Not the same cells!!

Normal Cells have normal Differentiation and functions.
Cell growth, proliferation, maturation and differentiation are physiological and controlled process ➔ Followed by Natural/programmed Cell death: apoptosis.

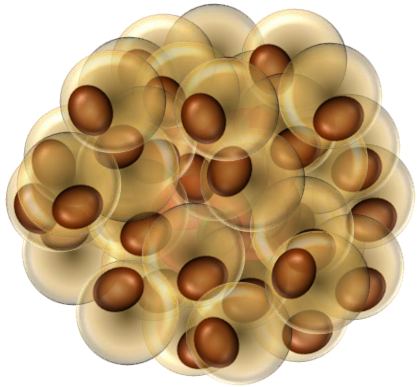


Stem Cells/ progenitor



Cancer Stem Cells (CSC)

Tumor regeneration (Recurrence)



Treatment resistance

Cancer Cells have No differentiation et no functionality.
Cell growth, proliferation and maturation are not controlled process. Apoptosis deficiency ➔ Raising up of proliferative cell clones

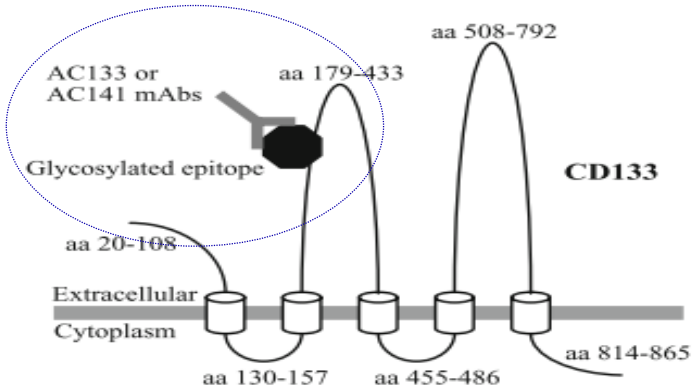
How to identify CSCs ?

- **Small subpopulation fractions**

- **Definition based on cell surface markers expression**
  CD133, CD44….CD34 (Hematopoietic Stem Cell marker)

- **Determination complexes !!!**

Eg: CD133 marker



(Bidlingmaier et al., 2008)

Table 1. *A summary of putative cancer stem cells from cancer*

		Cancer stem cells						
Cancer	Species*	Definition	Fraction	Frequency† (cells)	Tumorigenic *in vivo*‡	Replating‡	Origins	I
AML	H	$CD34^+CD38^-$	0.2–1%		Y		Myeloid progenitors	
APML	H	$CD34^-CD38^+$			Y		Myeloid progenitors	
B-ALL (ETV6-RUNX1)	H	$CD34^+CD38^-CD19^+$	1.1%		Y		B progenitors	
B-ALL (p190 BCR-ABL1)	H	$CD34^+CD38^-CD19^+$	1.1%		Y		B progenitors	
B-ALL (p210 BCR-ABL1)	H	$CD34^+CD38^-CD19^+$			Y		B progenitors	
MPD in $JunB^{-/-}$ mice	M	Sca-1^+c-Kit^+Thy$1.1^{int}Lin^-$		60	Y		Long term-HSC	
CML blast crisis	H	$CD34^+CD38^+IL3R\alpha^+CD45RA^+$				Y	Granulocyte/monocyte progenitors	
Medulloblastomas	H	$CD133^+$	6–21%	100	Y	Y	Stem cells/progenitors	
Glioblastomas	H	$CD133^+$	19–29%	100	Y	Y	Stem cells/progenitors	
Ependymomas	H	$CD133^+Nestin^+RC2^+BLBP^+$	0.001–1.5%	10,000	Y	Y	Radial glia cells	
Breast cancer	H	$ESA^+CD44^+CD24^{-/low}Lin^-$	0.5–5%	200	Y		Stem cells/progenitors	
Melanomas (metastatic)	H	$CD20^+MCAM^+$	20.0%			Y	?	
Lung adenocarcinoma	M	$SP\text{-}C^+CCA^+$					Bronchioalveolar stem cells	

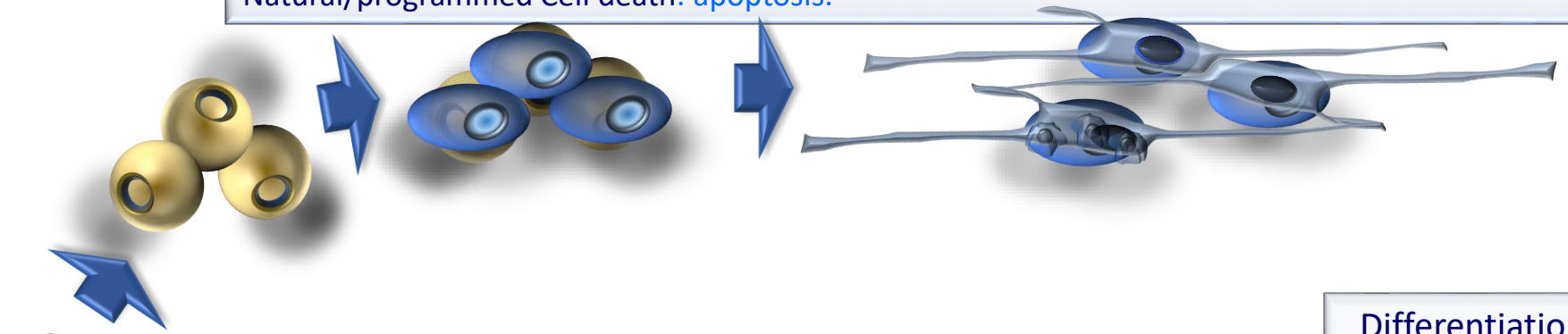
(Guo W et al. Pediatr. Res. 2006)

- CD133+ cells with **glycosylated motif** ➔ Marker of CSC (GBM model)
  (Kemper K, et al. P. Can. Res. 2010)

- CD133- cells ➔ Also able to regenerate tumors, less proliferation properties
  (Beier D, et al. Can. Res. 2007

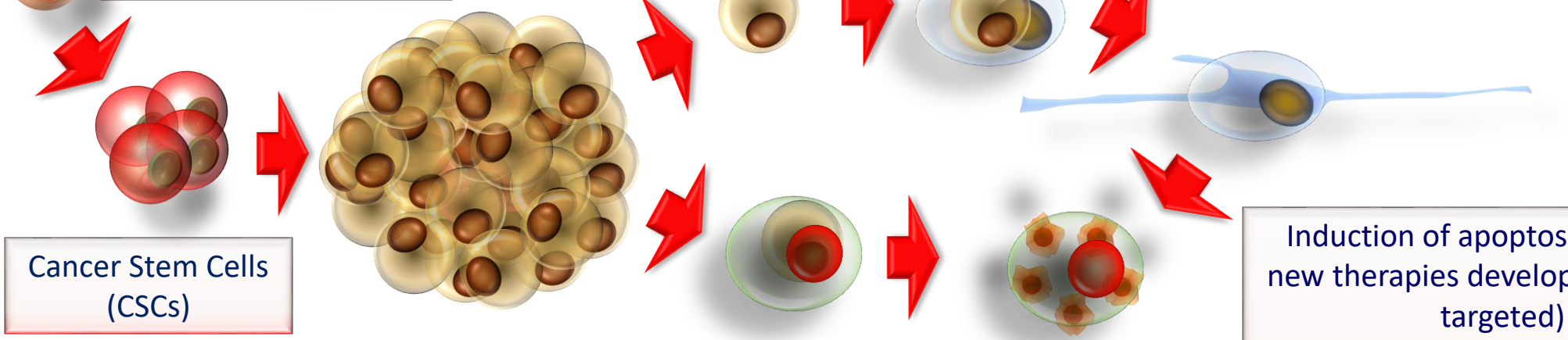
Which Strategy to tackle the CSCs ??

Normal Cells have normal Differentiation and functions.
Cell growth, proliferation, maturation and differentiation are physiological and controlled process ➔ Followed by Natural/programmed Cell death: apoptosis.



Stem Cells/ progenitor

Differentiation-based Therapy

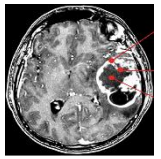


Cancer Stem Cells (CSCs)

Induction of apoptosis based on new therapies development (CSCs-targeted)
(New drugs, new resistance process…)

Cancer Cells have No differentiation et no functionality.
Cell growth, proliferation and maturation are not controlled process. Apoptosis deficiency ➔ Raising up of proliferative cell clones

CSCs biological markers : glioblastoma case



Cell surface markers used for CSC isolation
➔ Different depending on the type of cancer considered

- **Brain➔** CD133+ CD49f+ CD90+
- **Breast ➔** ALDH+ ESA+ CD44+ CD24-/low
- **Colon ➔** CD133+ CD44+ CD166+ EpCAM+ CD24+
- **Lung ➔** CD133+ ABCG2++
- **Melanoma ➔** CD20+
- **Pancreatic ➔** CD133+ CD44+ EpCAM+ CD24+
- **Prostate ➔** CD133+ CD44+ CD24-

In Glioblastoma (GBM)

Bradshaw A et al, Front in Surg. 2016

- **CD133, CD44 ➔** Cell surface markers → **Both are controversial !!!**
- **NANOG, SALL4, SOX2, c-Myc, Olig2, Bmi1, KLF4 ➔** Transcription Factors
- **STAT3 ➔** Cell signaling protein/Transcription Factor

→ **Transcription Factors mean ➔ Nucleus localization ?**

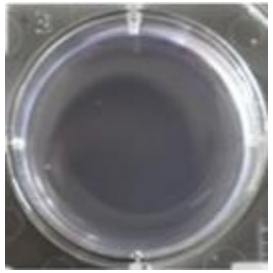
- **Nestin ➔** Intermediate Filament → **Well known as a neural progenitor marker**

CSC characterization ➔ Needs to analyze the whole panel of markers
➔ needs to use a combination of techniques (Flow Cytometry, Immuno-cyto and histochemestry, molecular biology (PCRs) …)

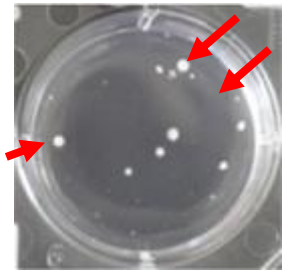
Additional methods/assays to validate CSC characterization

- Evaluation of the **survival and proliferative ability** of a single cell to grow ➔ **Clonogenicity assay**

Control population



CSC enriched cells



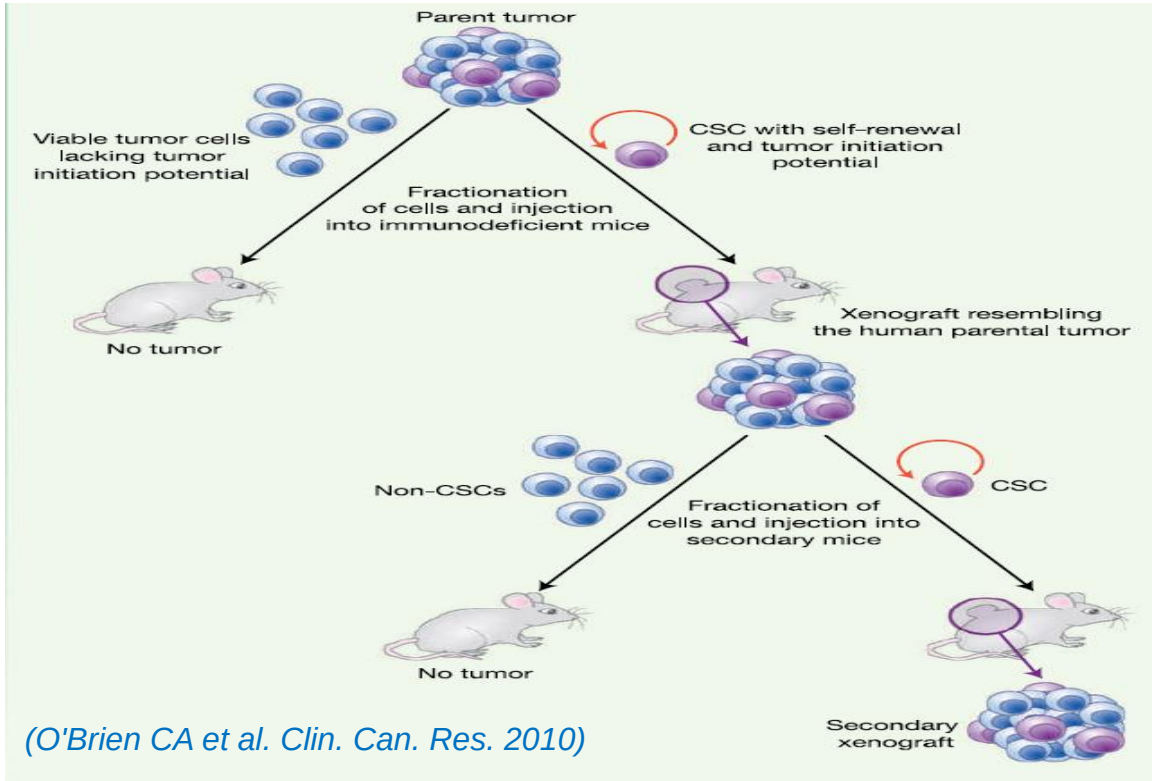
➔ The number of colonies and their sizes were evaluated

- Evaluation of the CSCs self-renewal capacity
  ➔ **Sphere formation assay**

➔ Ability to maintain a panel/percentage of cells with stemness-like properties in the new developed tumor

- **Heavy protocols**
- **Long time consuming**

From Cheray M…and Lalloue F, J Cell Mol Med. 2017



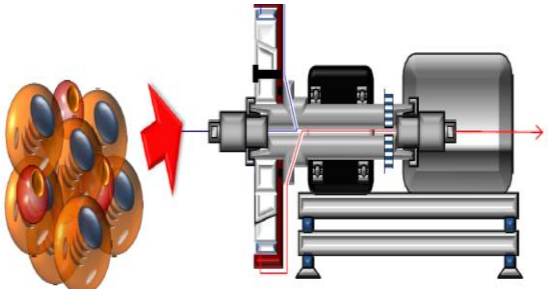
(O'Brien CA et al. Clin. Can. Res. 2010)

CSC studies : new challenges

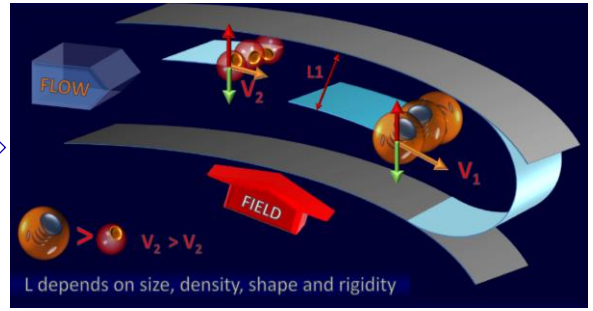
- Explore other CSCs properties : biophysical characteristics

➔ **SdFFF technique (UNILM) :**

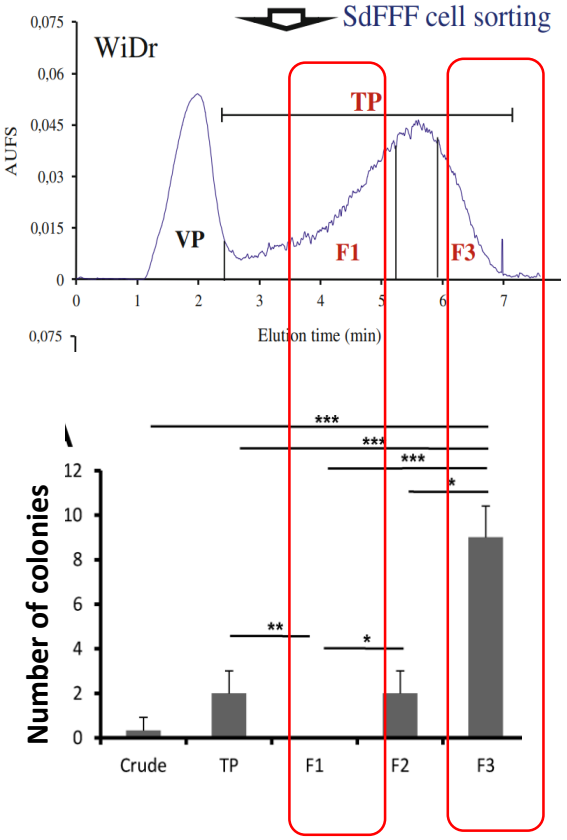
➔ **Sedimentation Field Flow Fractionation : cell sorting based on cell size, density, rigidity**



Flow

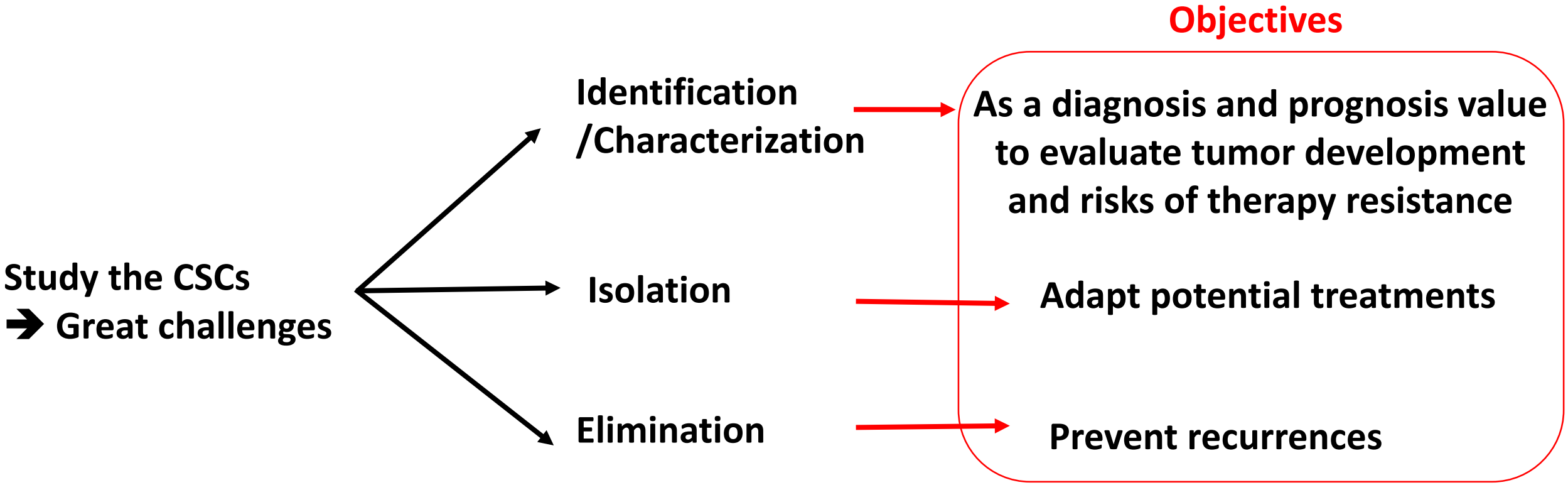


Field



Melin C. et al. Anal Chem.

Summary



Build new tools ➔ eg: Utilize the Dielectrophoresis (DEP) properties

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Thank you

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