# Strategy for Brain Cancer Stem Cell sorting with SdFFF.
# Routine BTSC sub-populations preparation for biosensor calibration.

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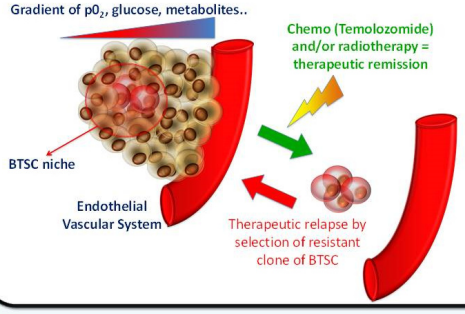
## Aim of the study

**Background :** Glioblastoma and medulloblastoma are one of the most aggressive brain tumors. Such as several types of solids tumors, their high tumorogenicity is based on their high heterogeneity and the presence of Brain Tumor Stem cells (BTSC) which explains tumor growth, spreading and the number of relapses after treatment. Therefore, BTSC characterization and isolation are of high importance in cancer studies. As major difficulties reside in the isolation (low amounts in tumors < 1-2 %) and in the characterization (lack of specific markers) of these potential biological targets, new technology development without immuno-labelling could be of great interest. In that way, our labs works since 10 years in the development of specific non-invasive microwave biosensors able to detect and characterized BTCS (BioCapteur and CCRMES projects). In the frame of the SUMCASTEC project (Semiconductor-based Ultrawideband Micromanipulation of CAncer STEm Cells, Horizon 2020 Framework Programme FET OPEN, N° 737164) we developed a novel non-invasive micro-optofluidic lab-on-chip (LOC) platform able to deliver ultra-wide broadband radiation to compare cell spectral signatures, image subcellular features, and hence modulate BCSCs microenvironment in order to differentiate it, and then reduced their aggressiveness. **Goal:** in order to properly calibrate the biosensor response regarding to the BTCS properties (specificity, sensitivity…), we need to produce purified and calibrated population of BTSC, in that way, we purpose to use Sedimentation Field Flow Fractionation, a non-invasive and label-free method to sort BTSC of various degrees of differentiation.

## Glioblastoma

- **Primary brain tumor : 2% of all cancer and 70% of brain tumors.**
- **Grade IV astrocytoma (WHO).**
- **Dark vital pronostic: relative percentage of survey 30% after 1Y and only 9.8 % after 5Y**
- **Total surgical resection is very difficult (invasive tumor).**
- **Important pool of quiescent Brain Tumor Stem Cells (BTSC) which resist to chemo- and radiotherapy.**
- **Specific micro-environment with important neo-vascularisation, exosomes and miR secretion by BTSC controlling tumorogenesis, neo-vascuarisation and tissue infiltration**

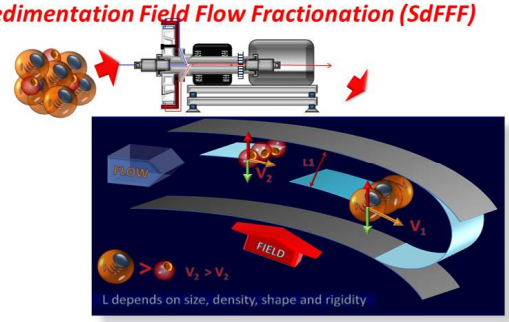



**BTSC :**

- **Purification (SdFFF)**
- **Identification**
- **Detection (BioCapteur project)**
- **Characterization (biological analysis)**

**is our first interest in order to develop specific therapies or to induce selective differentiation into chemo- and radiotherapy sensitive cells = SUMCASTEC project.**

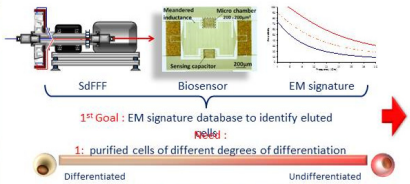
## Sedimentation Field Flow Fractionation (SdFFF)




Cell sorting methods could be classify by their application scale (macro and micro- or nano-methods) or by the principle of sorting such as the use of immunological recognition (FACS, MACS), or intrinsic biophysical properties of cells. Among these last methods, we developed since 15 years, prototypes and applications for sedimentation field flow fractionation (SdFFF), a non-invasive macro-method based on cell size, density, rigidity, respecting cell viability, functionality and differentiation state. SdFFF have been successfully used to sort, without any labeling, normal and cancer stem cells (CSC) from different glioblastoma, neuroblastoma or colorectal cancer cell lines.

## U87-MG cells : BioCapteur project

**Goal :**

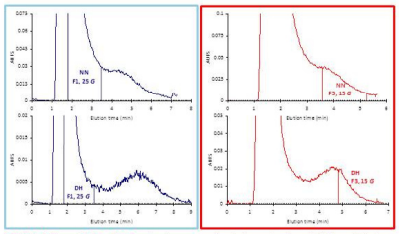



1st Goal : EM signature database to identify eluted cells :

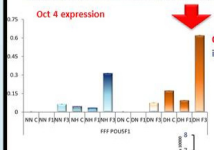
1: purified cells of different degrees of differentiation

Differentiated — Undifferentiated

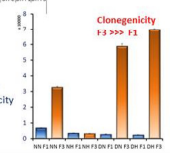
2. Correlated the differentiation state to EM signature



**SdFFF elution at 25 and 15 g,** allowing respectively the collection of differentiated cells (F1 fraction) and BTSC in F3 fraction. We observed a shift in fractogram profiles DH vs NN with an increased retention according to Bertrand et al. 2009 (Int. J. Oncol); where differentiated cells eluted first in contrast to CSC

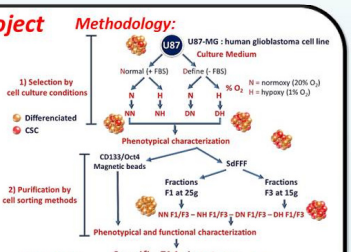



**Oct 4 expression** = High stemness increase for :
- DH vs NN,
- F3/Control and F1



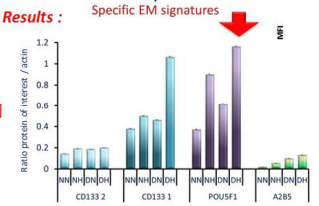

**High stemness increase for :**
- DH vs NN,
- F3/Control and F1 : lower proliferation, higher clonegenicity

**Methodology:**



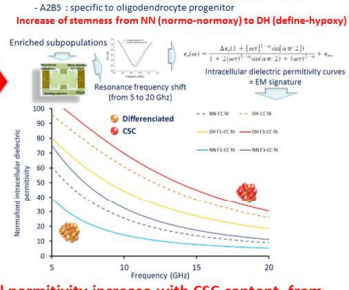

**Results :**

Specific EM signatures




3 markers of cancer stem cells were studied (Western-blot/Proteome array):
- CD133 (2 isoforms), specific but controversial marker of glioma CSC
- Oct4 (self-renewal of undifferentiated embryonic stem cells)
- A2B5 : specific to oligodendrocyte progenitor

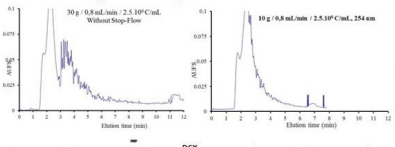
**Increase of stemness from NN (normo-normoxy) to DH (define-hypoxy)**

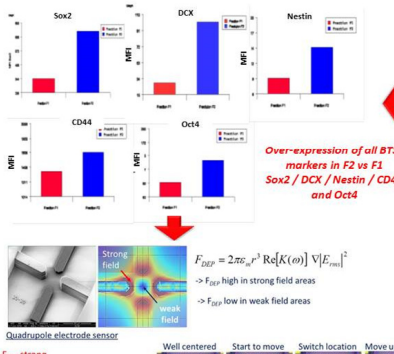



**Cell permitivity increase with CSC content, from NN to DH conditions, and from F1 to F3 fractions**

## LN18 cells : SUMCASTEC

**Methodology: SdFFF Optimization**




**Over-expression of all BTSC markers in F2 vs F1 Sox2 / DCX / Nestin / CD44 and Oct4**

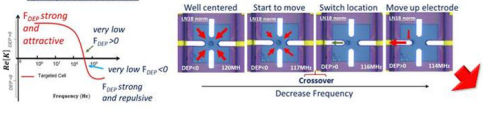



Quadrupole electrode sensor

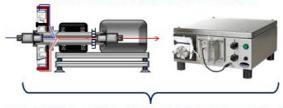
$$F_{DEP} = 2\pi\varepsilon_m r^3 \mathrm{Re}[K(\omega)]\ \nabla|E_{rms}|^2$$

-> $F_{DEP}$ high in strong field areas

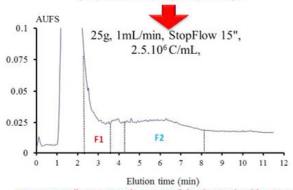
-> $F_{DEP}$ low in weak field areas




**SdFFF on-line coupled with Postnova PN3000 XPT Particle Size Detector**

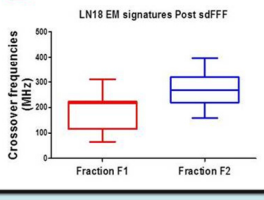


**Bio-physical calibration of elution conditions: Addition of a stop-flow step dure to the cell population polydispersity**




**Fraction collection in the way of biological calibration. At the ligth of previous GBM cell line elution, only two fractions were collected**

**Measurement strategy response of cell crossover frequency : depends on biological properties**




**Cell crossover frequency increase with BTSC content, from F1 to F2 fractions *. Correlation between biological properties and sensor response (*preliminary results)**

## Conclusion

**Conclusion :** Association of cultural conditions and SdFFF cell sorting allowed to prepare enriched population with different BTSC contents. All the sorted fractions present specific stemness properties ranging from differentiated to very undifferentiated sub-populations. In each the cases, whatever the technologies on which the sensor are based, we obtained very specific responses, demonstrated a good correlation between biological state/BTSC content and sensor response. Then it was possible to routinely prepare BTSC populations in order to calibrate sensors, which will be further used, in particular for the SUMCASTEC project, for clinical application to detect and modify BTSC into more therapies sensitive population.

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