

# Neurosphere culture of brain tumor stem cells

## Application note

**Our 3D Tumorsphere Medium XF has been designed to meet your requirements for the extended serial neurosphere culture.**

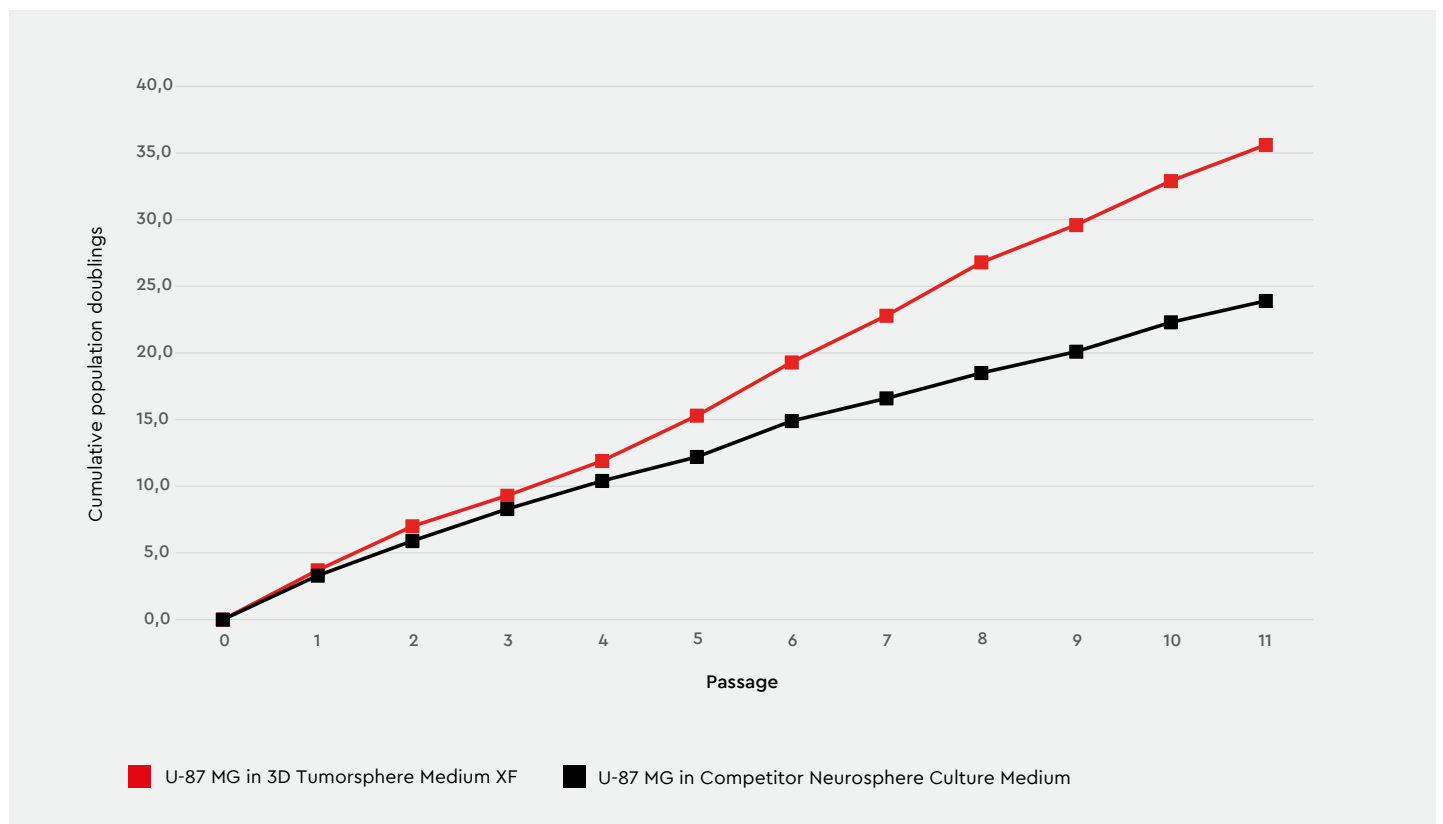
While adherent cultures of brain tumor cells in fetal calf serum-containing standard media undergo changes in the form of partial differentiation, 3D cultures of brain cancer cells as neurospheres in serum-free conditions maintain – and therefore reflect better – the properties of primary tumors [1].

However, conventional serum-free media for 3D culturing of neural cancer stem cells as neurospheres struggle to efficiently maintain self-renewing driver subpopulations of cancer stem cells (CSCs). Consequently, the stem cell population in the culture is gradually lost along with its proliferation potential (see Fig. 1, grey chart).

Our 3D Tumorsphere Medium XF is designed to meet your requirements for undifferentiated and extended serial 3D neurosphere culture of most commonly used brain tumor cell lines. Its advanced and defined formulation permits 3D expansion as self-renewing neurospheres while maintaining rigorous cellular selection for stem cell traits, i.e. self-renewal and anoikis resistance. This results in an optimal culture environment for brain CSCs, as is reflected in the sustained serial passage ability of 3D neurospheres with the 3D Tumorsphere Medium XF. Thus, efficient neurosphere expansion allows to easily access cells enriched for sharing these aforementioned stem cell properties (see Fig 1, red chart).

Our 3D Tumorsphere Medium XF is defined and ready to use, providing a standardized

culture devoid of stimuli of uncharacterized origin. This is a significant benefit in terms of neural stem cells, which are highly responsive stem cells that require a reliable and reproducible control of the self-renewal/differentiation axis. The 3D Tumorsphere Medium XF is suitable for a cost-effective, standardized routine culturing of neural cancer cell lines as neurospheres. In contrast to the limited numbers of passages achieved with conventional serum-free formulations, the 3D Tumorsphere Medium XF supports long-term passageable neurosphere cultures of a broad variety of cell lines.



**Fig. 1: Plot of cumulative population doublings of U-87 MG glioblastoma cells during cultivation as 3D neurospheres in the 3D Tumorsphere Medium XF compared to an established commercial neurosphere culture medium.** The 3D Tumorsphere Medium XF maintained neurosphere formation and steady proliferation during the entire culture, which was discontinued after passage 11 with no signs of growth rate inhibition. By comparison, when cultured in the competitor neurosphere culture medium, a steadily declining expansion rate of U-87 MG was observed; this was indicative of the progressive loss of the primitive self-renewing driver cell population.

# Background

The term "brain cancers" encompasses a variety of malignancies of the central nervous system (CNS), e.g. gliomas, meningiomas, pituitary adenomas and nerve sheath tumors. The most prevalent and lethal tumor of the brain is the astrocyte-derived glioblastoma multi-forme [2]. This rapidly growing recurrent cancer is resistant to all conventional treatments. Moreover, malignancies of the brain are difficult to manage because most agents cannot cross the blood-brain barrier and the healthy part of the brain is very susceptible to damage as a result of therapeutic interventions [3]. In research, 3D neurosphere culture of brain cancer cells is considered a biologically relevant in vitro model of the

zones of the tumor that exhibit the highest level of malignancy [5]. The neurosphere culture system was first described by Reynolds and Weiss 1992 [9] and involves the proliferative cultivation of neural stem cells and their progeny as free-floating spherical clusters, so-called neurospheres. In the neurosphere culture system using neural cancer cells, neurospheres presumably develop from individual cancer stem cells (CSC) while closely reflecting and maintaining the genetic and phenotypic traits of the primary tumor [6, 7]. Neurosphere-derived cells express stemness markers that are typical of neural stem cells (NSC), e.g. Nestin and Sox2, but usually lack the differentiation marker GFAP [1, 5].

Brain tumor CSCs, which share multiple properties and hallmarks of NSCs, are a small subset of self-renewing tumorigenic cells that divide fairly slowly [4]. Similarly to the CSC subpopulations contained in the primary tumor, they are multipotent and possess significant differentiation potential for generating cell types of the neural lineage in vitro [5–8].

Adequate culture systems that induce efficient expansion of brain tumor cells without diminishing their self-renewal capabilities or differentiation potential are an essential prerequisite for research aimed at identifying ways of eradicating brain tumor CSCs with targeted, holistically curative therapies.

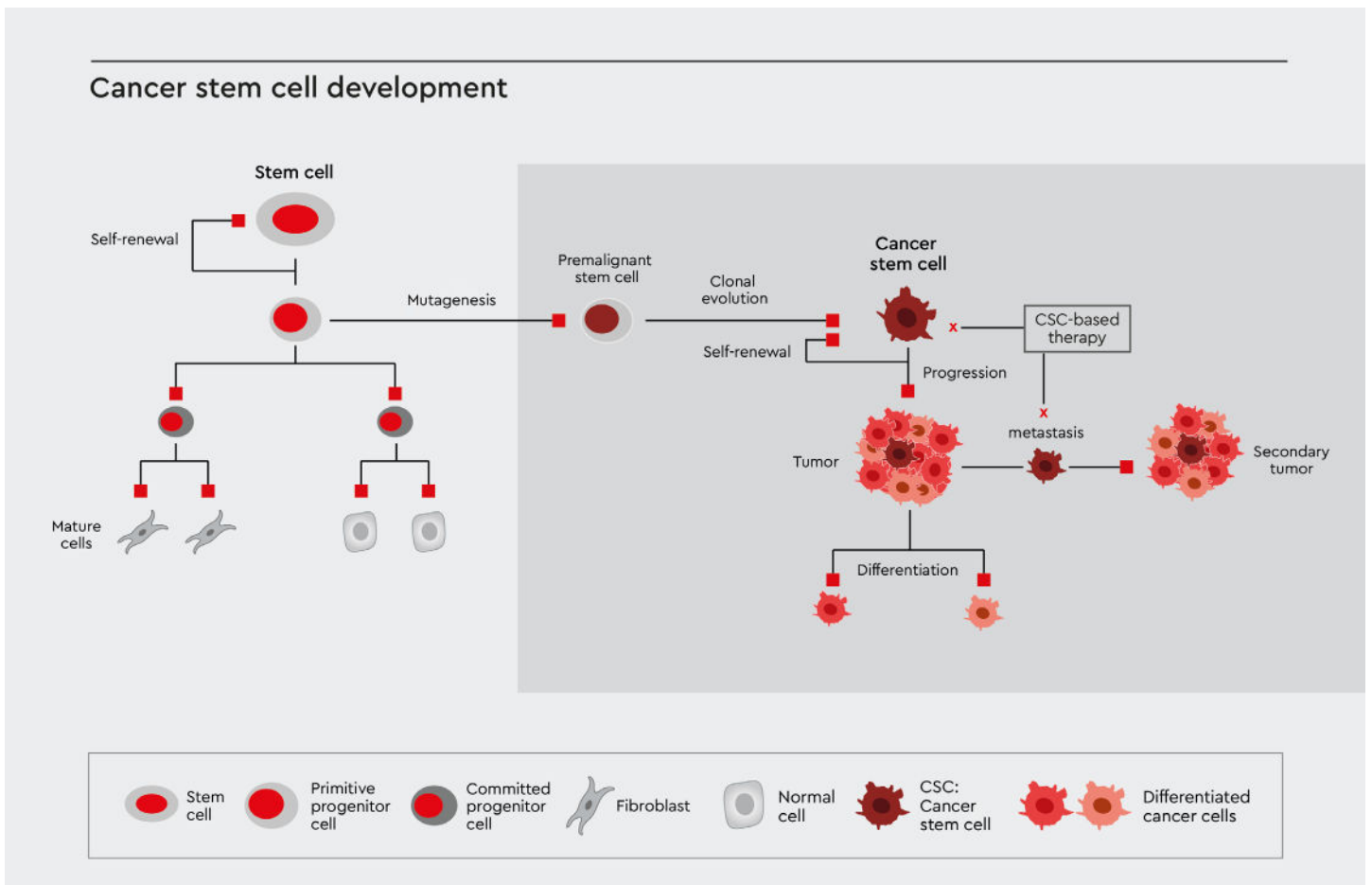


Fig. 3: Schematic overview on the origin, evolution and fate of cancer stem cells (CSCs).

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# CSC Neurosphere culture protocol

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## I. Initiation of CSC neurosphere culture

### Materials

- 3D Tumorsphere Medium XF (C-28070 or C-28075)
- Phosphate Buffered Saline w/o Ca<sup>++</sup>/Mg<sup>++</sup> (PBS, C-40232)
- Detach-Kit (C-41210)
- 6-well Suspension Culture Plates (e.g. Greiner Bio One, No. 657 185)
- Cultured neuronal cancer cell line (for initial neurosphere culture set-up)

*Use aseptic techniques and a laminar flow bench.*

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### Harvest the adherent cells

The neural cell culture to be used for the setup of neurospheres should be in good condition. Harvest the neural cells as a single cell suspension according to your standard procedure. Resuspend the cells in a small volume, e.g. 3 – 5 ml, of the 3D Tumorsphere Medium XF.

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### Count the cells

Count the cells using your routine method and adjust the volume with 3D Tumorsphere Medium XF to obtain a concentration of 1 million cells/ml.

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### Set up the viable culture

Seed the cells in appropriate suspension culture vessels at 10,000 cells/ml, e.g. 40,000 cells in 4 ml of 3D Tumorsphere Medium XF in each well of a 6-well suspension culture plate.

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### Allow the neurospheres to grow

Incubate the culture for 4 – 10 days, depending on the cell type used. Add one half of the culture volume of fresh 3D Tumorsphere Medium XF every 3 – 4 days. Do not change the medium.

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### Passage of the neurosphere culture

The neurospheres should be passaged (section II. below) before they start to develop a dark center. Depending on the cell type used optimal passage should occur after 4 – 10 days.

**Note:** If a defined size range of tumorspheres is required, e.g. 100 – 200 µm, filters may be used for fast and easy sorting of tumorspheres according to size. Use a 200 µm cell strainer to filter the tumorspheres and collect the flow-through. Then, filter the flow-through again using a 70–100 µm cell strainer and flush back and collect the tumorspheres retained in the filter. The remaining fraction of tumorspheres will have a defined range of diameters from approx. 100 to 200 µm.

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## II. Serial passage of CSC neurosphere cultures

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### Collect the neurospheres

Transfer the 3D Tumorsphere Medium XF containing the neurospheres into 15 ml conical tubes using a serological pipet.

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### Wash the neurospheres

Repeat the sedimentation (step 7) with an equal volume of PBS. Gently aspirate the PBS leaving approximately 200 µl in the conical tube.

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### Break down remaining cell aggregates

Pipet the spheres up and down 10 – 20 times using a 1000 µl pipet tip to generate a single cell suspension. Aspirate the cell suspension as normal but tilt the pipet tip slightly at the bottom of the tube when expelling the cells. The shear forces generated facilitate the break-up of any residual cell aggregates. Perform a visual check to confirm that no large cell aggregates remain. Immediately after trituration, add twice the volume of Trypsin Neutralization Solution (TNS).

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### Determine the cell number and viability

Make up to 5 ml with fresh 3D Tumorsphere Medium XF and determine the cell number and viability. Centrifuge the cells for 5 minutes at 300 x g. Discard the supernatant and resuspend the cells in fresh 3D Tumorsphere Medium XF at 1 million cells/ml.

**Note:** Alternatively, the cells may be resuspended in buffer, e.g. PBS w/o Ca<sup>++</sup>/ Mg<sup>++</sup> plus 0.5% albumin plus 2 mM EDTA, and used for further experiments and/or analytical procedures.

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### Gravity sedimentation of the neurospheres

Allow the spheres to settle by gravity sedimentation for 10 – 12 minutes at room temperature. Aspirate the supernatant, but leave approximately 200 µl in the conical tube. Do not aspirate the neurospheres.

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### Enzymatic digestion of the neurospheres

Add 1 ml of Trypsin-EDTA to the neurospheres and incubate for 2 – 4 minutes at room temperature. Keep the spheres resuspended in the trypsin solution by pipetting up and down once every 30 seconds. Avoid sedimentation of the spheres.

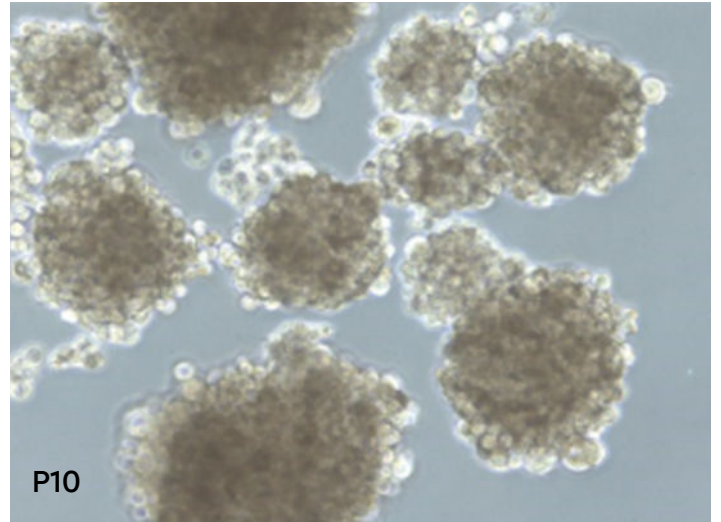
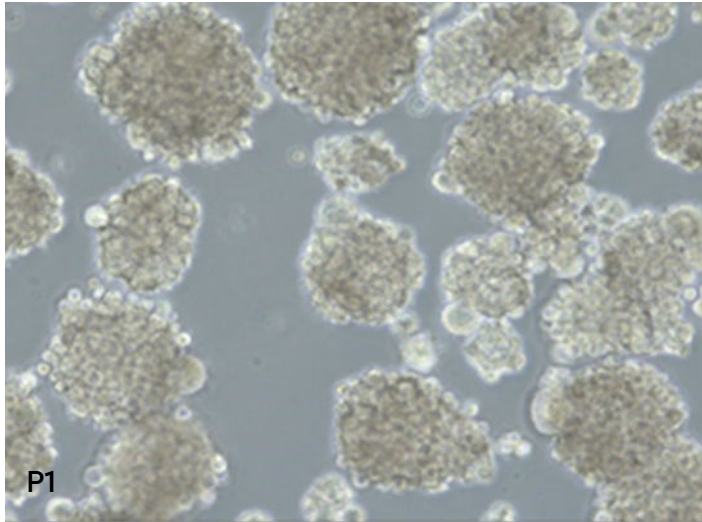
**Note:** The optimal incubation time required to achieve complete dissociation in the following step II. 10 (below) must be determined empirically by the user for each cell type. While 2 – 3 minutes will be optimal in most cases, neurospheres of some cell lines may need longer incubation. If a completely defined dissociation process is preferred, a recombinant trypsin solution may be used as an alternative dissociation reagent according to the supplier's instructions.

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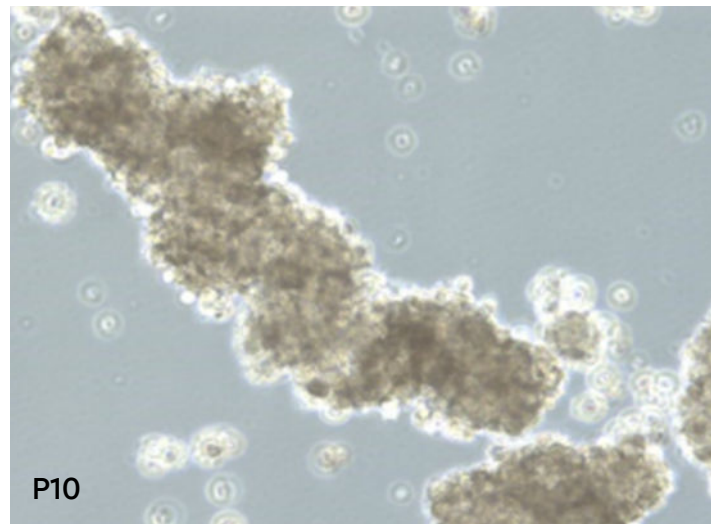
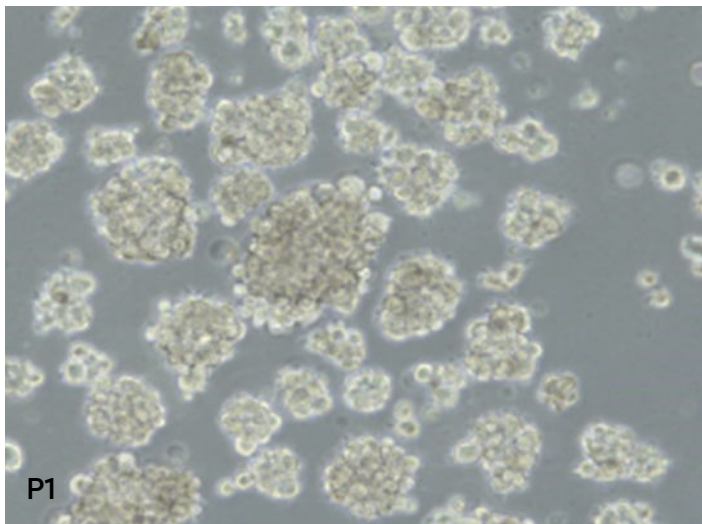
### Plate the Cells

Reseed the cells at 10,000 cells/ml in new suspension culture vessels. Typically, 6-well plates with 40,000 cells in 4 ml of medium per well are used.

### 3D Tumorsphere Medium XF



### Competing medium



**Fig. 3:** U-87 MG cells cultured as 3D neurospheres (passage 1 and 10) in 3D Tumorsphere Medium XF (C-28070). In passage 1, the competing medium (lower left) exhibits neurosphere formation comparable to that of the 3D Tumorsphere Medium XF (upper left). However, the culture in 3D Tumorsphere Medium XF results in significantly more cell mass already in this early passage (upper left). In serial passage 10, the competing medium produces slowly proliferating shapeless cell clusters (lower right), while the 3D Tumorsphere Medium XF consistently results in uniform, quickly growing neurospheres (all images are at 100x magnification).

## Products

Media	Size	Catalog number
3D Tumorsphere Medium XF	250 ml	C-28070
3D Tumorsphere Medium XF, phenol red-free	250 ml	C-28075

## Related Products

Media	Size	Catalog number
Dulbecco's PBS, without Ca <sup>++</sup> /Mg <sup>++</sup>	500 ml	C-40232
DetachKit	3 × 125 ml	C-41210
Primary Cancer Culture System	250 ml	C-28081
Cancer Cell Line Medium XF	250 ml	C-28077

## References

1. Lee, J., et al., Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell*, 2006. 9(5): 391-403.
2. McLendon, R.E. and J.N. Rich, Glioblastoma Stem Cells: A Neuropathologist's View. *J Oncol*, 2011. 2011: 397195.
3. Lawson, H.C., et al., Interstitial chemotherapy for malignant gliomas: the Johns Hopkins experience. *J Neurooncol*, 2007. 83(1): 61-70.
4. Vescovi, A.L., R. Galli, and B.A. Reynolds, Brain tumour stem cells. *Nat Rev Cancer*, 2006. 6(6): 425-36.
5. Schiffer, D., et al., Glioblastoma cancer stem cells: Basis for a functional hypothesis. *Stem Cell Discovery*, 2012. 02(03): 122-131.
6. Gursel, D.B., et al., Glioblastoma stem-like cells-biology and therapeutic implications. *Cancers (Basle)*, 2011. 3(2): 2655-66.
7. Yuan, X., et al., Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene*, 2004. 23(58): 9392-400.
8. Gunther, H.S., et al., Glioblastoma-derived stem cell-enriched cultures form distinct subgroups according to molecular and phenotypic criteria. *Oncogene*, 2008. 27(20): 2897-909.
9. Reynolds, B.A. & Weiss, S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, 1992. 255: 1707-1710.

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