Glioblastoma (GBM) is the most common primary brain malignancy with limited treatment options. The standard of care for GBM, including maximal safe resection, radiotherapy, and concomitant and adjuvant temozolomide is currently recommended for all patients (1) but only a small proportion of patients survive 2 years or longer and, eventually, virtually every tumor recurs (2). GBM is characterized by a high degree of intra-tumoral cellular heterogeneity. Recently, genome-wide expression profiling in the context of The Cancer Genome Atlas (TCGA) project has led to the identification of initially three, and then four main subtypes of GBM (3,4), namely proneural (P), mesenchymal (M), neural (N) and proliferative (or classical, C), thus revealing the existence of a profound degree of inter-tumoral cellular heterogeneity. An alternative scheme has also been reported in which GBMs clustered in six subtypes that correlated better with survival than histology (5). The exact number of subtypes, their validity and inter-observer reproducibility, and their clinical and prognostic correlate, all are still a matter of debate. However, the opposing picture of the P and M GBM subgroups is widely accepted. The P subtype is strongly associated with secondary GBM, hypermethylator phenotype, younger age, lack of enhancement on MRI and, in selected reports, better prognosis. Conversely, M subtype GBMs tend to be more invasive, displaying angiogenesis, necrosis and contrast enhancement (3,6-8).

Cancer cells with stem cell-like features (CSCs) seem to influence tumor growth and recurrence in many malignancies. Although there is no generally accepted definition of these so-called CSCs in GBM (GSCs), they have been characterized as slow-cycling tumor cells with enhanced self-renewal potential and high tumor-forming potential (9). Analogous to normal stem cells, GSCs seem to be endowed with increased resistance to chemotherapy and radiotherapy, due to the over-expression of DNA damage repair enzymes (10) as well as to metabolic traits which allow GSCs to grow under hypoxic conditions (11,12) thus, shielding them from the oxygen-dependent effects of radiotherapy. Increased expression of membrane proteins associated with drug-resistance has been reported in GSCs, too (13,14). The role of GSCs in determining the molecular subtype of GBM according to TCGA classification is not well understood. However, expression profiling of GSC cultured in vitro from primary GBM appears to divide them into two groups: one is characterized by proneural-like gene expression signature, in which GSCs resemble normal neural stem cells, are CD133-positive, grow as floating spheres and display high invasiveness in vivo (“full” GSCs), whereas the other one shows mesenchymal-like expression signature, with cells more similar to adult normal neural stem cell lines, displaying no CD133 expression, in vitro adherent growth, and low invasive behavior in vivo (“restricted” GSCs) (15).

However, irrespective of the subtype, the discovery of GSCs as the cell population responsible for tumor development and propagation has led to the search of novel targeted therapies aimed at eradicating this cellular core, rather than the differentiated, non-stem tumor bulk. This has led to a push to identify the specific signaling pathways which stem cells depend on. Carcinogenesis is a complex process finely regulated at multiple levels by post-translational modifications (PTMs). Ubiquitination is one of the most important
PTMs responsible for regulating the stability and activity of modified proteins. Deubiquitination has a pivotal role in the ubiquitin system through specific deconjugation of ubiquitin from target proteins. The human genome encodes at least 98 deubiquitinases (DUBs), grouped into six families based on sequence and structural similarity, reflecting the need for specificity in their function (16). The wide functional diversity of DUBs has a profound impact on the regulation of multiple biological processes as cell-cycle control, DNA repair, chromatin remodeling and several signaling pathways that are frequently altered in cancer (17). Consistent with this, altered DUB function has been related to several diseases, including cancer and thus, DUBs are increasingly regarded as a potential target in cancer therapy. The link between genetic damage repair and cancer development is widely documented by the increased tumor rates reported for those disorders associated with deficient DNA repair mechanisms such as Fanconi’s anemia. A critical role in the regulation of several important steps in the Fanconi’s anemia pathway is played by USP1, a member of DUBs, through the deubiquitination of Fanconi’s anemia protein FANCD2 and the subsequent stabilization of CHEK1 (18). USP1, upon forming a molecular complex with UAF1 (WDR48), deubiquinates substrates including PCNA, FANC1, FANCD2, and the short-lived Inhibitor of DNA Binding (ID) proteins ID1, ID2, and ID3 (19). Members of the Id gene family, including Id2, have been proposed as mediators of GBM and are known to be highly expressed in a number of different tumor types (20).

Starting from this background, in a recent work Lee et al. have further defined a role for USP1 in maintaining the stem-cell properties of GSCs and thus in the regulation of GBM aggressiveness and resistance to therapies (21). In the paper, the authors focus on USP1 after an analysis of public genomic and proteomic databases and demonstrated that this protein is correlated with glioma malignancy. Consistently, analysis of GSC cultures showed that USP1 expression paralleled expression of Sox2, which is a stemness marker. Then, the author inhibited USP1 expression in GSCs cultures, using both lentivirus-mediated short-interference RNAs and pharmacological inhibition through pimozide. As expected, USP1 blockade induced apoptosis and strongly reduced clonogenic potential of GSCs. Noticeably, such an effect was not obtained in normal NSCs, confirming that the stemness-maintaining activity of DUBs is unique of neoplastic cells. In a further, elegant set of experiments, the authors confirmed the causal link between USP1 expression and stabilization of ID1 and CHK1 in GSCs. ID proteins are key regulators in gliomagenesis and in the maintenance of the stem cell phenotype of GSCs (22); consistently, USP1 down-regulation decreased the expression of stemness markers while increasing that of differentiation markers, whereas the opposite occurred when USP1 was up-regulated. Moreover, USP1 blockade, through inhibition of DNA checkpoint kinases like CHK1, resulted in a reduced radio-resistance of GSCs.

Interestingly, in a previous work by our group (23), we showed that the multikinase-inhibitor staurosporine-derivative UCN-01 inhibited the growth of GSCs, both in vitro and in vivo, through the blockade of CHK1 and of the receptor tyrosine kinase pathway. Taken together, these evidences support the assumption that, to achieve a therapeutic response against GBM, it is fundamental to target not only the stemness and/or growth factors pathways (like RTKs or ID), but also the DNA-repair machinery. Consistent with this, in an in vivo GSC-derived GBM model in mice, USP1 inhibition leads to reduced tumor growth and prolonged survival of animals. Interestingly, GBM-bearing mice treated with irradiation and the USP1 inhibitor pimozide survive longer than controls and single-treatment arm, with tumor cells showing increased DNA damage and reduced expression of stemness markers.

**Conclusions**

Lee et al. have provided early results describing a role of USP1 in regulating critical regulators of DNA damage repair and stem cell maintenance in GBM. Moreover, they showed that genetic or pharmacologic inhibition of USP1 significantly impairs in vitro clonogenic ability and in vivo tumorigenic potential of GSCs.

While the cellular and molecular complexity of GBM is gradually unraveled, it is crucial to notice that the therapeutic strategies that are more likely to carry a therapeutic success necessarily hit more targets at the same time. DUBs have the advantage to act not only on molecular heterogeneity, targeting several pathways at a time, but also on cellular heterogeneity, reducing the stem cell properties of GSCs and therefore hitting the cellular core of GBM. Moreover, their key role in blocking DNA damage repair enzymes increases the susceptibility of GSCs to chemo-radiation.

The results showed in this work, together with the recent success of clinically targeting the ubiquitin proteasome system in cancer, reinforce the concept of DUBs as...
appealing targets for the development of new specific therapies against human malignancies, including GBM. So far, no DUB-targeted strategies have reached clinical trials and many challenges remain before translating this information into clinical benefits for cancer patients. Thus, more work is required to achieve a better understanding of the basic mechanism of USP1 function and regulation for the development of USP1 inhibitors useful as anticancer drugs for GBM treatment. Hopefully, the knowledge derived from this and similar studies will provide new insights into the multiple questions still open in relation to DUBs and may lead to the introduction of DUB-targeting strategies as an essential component of molecular therapies against cancer.

Acknowledgements
The authors wish to thank Prof. Roberto Pallini for critical reading of the manuscript.

Footnote
Provenance: This is a Guest Editorial commissioned by Section Editor Hongcheng Zhu (Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

References