計畫編號：DOH96-TD-I-111-TM003

行政院衛生署九十六年度科技研究計畫

樹突細胞腫瘤疫苗治療：抗癌藥與免疫系統互補之研究

研 究 報 告

執行機構：中國醫藥大學

計畫主持人：楊文光

研究人員：許登美、廖珮如、洪政豪、李東城、
湯曉君

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目　　錄

中文摘要

我們已執行完畢之第一相臨床試驗--「自體樹突細胞腫瘤疫苗之惡性膠質癌輔助療法」正在衛生署審核結案中。參與此臨床試驗並接受此療法的十六位病人已追蹤四年以上，其中四位至今尚存活，亦即說，此療法之四年存活率為 25%，和一般療法(經手術、放射線治療)的病人近乎 0%之四年存活率相比較，已經是非常良好之成果。但是，同樣接受此療法的另外四分之三的病人，則沒有得到預期療效。我們一年前所執行之「臨床試驗後轉譯醫學研究」，所獲結果顯示有兩種細胞會影響到此免疫療法之療效。其一是免疫調控細胞(T regulatory lymphocyte)，另一是惡性膠質細胞瘤中之「癌基幹細胞」(Cancer stem-like cells of glioblastoma)。由衛生署經藥物科技國家型計畫資助一年之此研究，乃在探討有何種藥物會針對此兩種細胞產生抑制作用。我們的成果如下：

(1) 研發大量培養 Treg 細胞之方法。

(2) 建立技術平台以發現抑制 Treg 之藥物，像我們用此方式可以發現的 NS38 和 CPT(托樸-抑制藥)(Topo I inhibitor drugs)以及 IL-12 細胞刺激素，都會產生 Treg 之抑制。

(3) 大量培養免疫特殊性之 CTL 的技術。

(4) GBM「癌幹細胞」之大量培養與性質分析。

(5) 發現 celecoxib 可以降低「癌幹細胞」之放射線抵抗性質，此藥物之作用機制乃在增加放射線照射所引致之 mitotic catastrophe，而不會影響(或促進)到 apoptosis 之機制。

中文關鍵詞: 惡性膠質腦瘤，樹突細胞腫瘤疫苗，免疫調控 T 細胞，「癌基幹細胞」，免疫調理藥物，放射線治療增強劑

SUMMARY

The main purpose of this project for the past one year is to established in vitro technical platform of tumor cells and immune lymphocytes for possible new therapeutics discovery to improve the adjuvant immunotherapy of currently incurable human WHO grade IV malignant gliomas. Our results are summarized in the following:

[1] "Cancer stem-like cell" lines of glioblastoma multiforme (GBM) from Chinese patients: We have established permanent cell lines from selected short-term culture tumor cells from two GBM and one malignant oligodendrioglioma patients. Upon exposure to ionizing radiation, while most of the CD133(-) cells died, neurosphere-forming clonogenic CD133(+) cancer stem-like cells apparently survived and emerged as colonies in vitro.

[2]. Cloning of GBM-specific cytotoxic T lymphocytes (CTL). We have utilized post-DC tumor vaccine therapy tumor-infiltration lymphocytes for the isolation of CTL clones from #13GBM patient and from #12MO patient. The purpose is to study the interactions between DC vaccine-induced anti-tumor CTLs and autologous tumor cells, especially the GBM "cancer stem-like cells".

[3]. T regulatory cells from tumor infiltrating lymphocytes of several GBM patients before vaccine therapy. We previously found that tumor infiltrating lymphocytes of GBM patients before autologous dendritic cell-based tumor vaccine therapy comprised CD4(+)CD25(++)CD45RO(+)GITR(+)FOXp3RNA(+) Treg cells in large percentages. A method has been developed for growing large numbers of these Treg from GBM patients.

[4]. Targeting of Treg cells by topoI inhibitors. We have found that topoisomerase I inhibitor drugs, CPT and NS38, at nanomolar concentration can inhibit Treg cells by apparently different mechanisms. While inhibition by NS38 was through cytotoxicity to Treg cells, CPT at relative non-cytotoxic levels appeared to decrease the immuno-suppressive activity of Treg cells.

[5]. Celecoxib sensitization of GBM "cancer stem cells" to therapeutic ionizing radiation. GBM "cancer stem-like cells" are relatively efficient in repairing radiation-induced DNA damages and hence presumed to be the culprit of GBM recurrence after radiotherapy. We have found that celecoxib treatment can sensitize the isolated GBM stem-like cells to therapeutic ionizing radiation by enhancement of mitotic catastrophe. This result is highly significant for possible clinical application.

Keywords: Glioblastoma multiforme, dendritic cell-based tumor vaccine therapy, T regulatory cells, immune modifiers, cancer stem-like cells, radiotherapy enhancement.

INTRODUCTION

Malignant gliomas account for 80% of adult primary brain tumors. The high grade gliomas, including WHO grade IV glioblasetoma multiforme (GBM), are among to the most fatal of human cancers, perhaps reflecting the eccentric biology and cellular origin of these neoplastic cells. Current therapeutic options for GBM patients consist of surgical resection followed by radiation therapy and chemotherapy. Despite the aggressive multimodality approach, prognosis remains poor with only slight increase in post-diagnosis median survival of 8-10 months. For more than a decade, the major goal of this laboratory has been to develop safe and effective, if not life-saving, combination therapy measures for currently incurable and fatal human cancer diseases. After more than six years of translational research, the P.I. has been able to come up with a protocol of dendritic cell-based tumor vaccine therapy, particularly glioblastoma multiforme. On the basis of our pre-clinical translational research, we have modified the procedures of dendritic cell preparation And developed . Since the end of 2002, we have conducted a clinical trial of autologous DC-based tumor vaccine adjuvant therapy aiming at post-surgical residual tumor cells of glioblastoma multiforme and anaplastic astrocytoma. The clinical data of 16 participant patients, followed up to 5 years, showed no apparent adverse effects and likely survival benefits of our vaccine therapy, with statistical analysis for possible factors associated with unsatisfactory responses to the vaccine therapy in some GBM patients. Also, we have isolated the tumor infiltrating lymphocytes from tumor specimen of re-operated GBM patients and found them to comprise mostly immunosuppressive T regulatory cells prior to the DC-tumor vaccine therapy whereas tumor-specific CD8(+) T lymphocytes during and after the vaccination course.

for WHO grade IV glioblastoma multiforme (GBM) and grade III anaplastic astrocytoma. Despite current standard therapeutic measures of surgery and radiotherapy, GBM remains an essentially 100% fatal disease sowing survival period of 8-14 months after diagnosis. It is therefore possible to tell whether this dendritic cell-based tumor vaccine therapy is useful or not in 3 years with a relatively small number of tested patients. From this clinical trial we have obtained results that are highly significant and much better than those reported from US, Europe and Japan. For the past 7 months, significant progress has been made by finding a possible important role played by T regulatory lymphocytes (Treg) in immuno-suppressive effects of GBM and also of emergence of tumor-specific cytotoxic T lymphocytes (CTL) as a key benefit of dendritic cell-based tumor vaccine therapy. These results are to be firmly established by using tumor-infiltrating lymphocytes (TILs) of more numbers of vaccinated GBM patients, while the possible effects of radiotherapy and chemotherapy on anti-tumor immune cells are being investigated. A research platform

of the GBM TILs and autologous GBM tumor cells will be developed for the discovery of therapeutic agents (biologicals, chemicals and purified essential components of Chinese traditional medicines and herbs) that may enhance the immune potency and negate the immunosuppression in cancer therapy._We are in an excellent, if not unique, position to address these translational research problems because we have collected the GBM glioma cells, all in short-term cultures and seven as permanent or established cell lines, as well as all autologous peripheral blood mononuclear cells (lymphocytes and monocytes) and six pairs or series of tumor infiltrating T lymphocytes of the same patients prior and after DC-tumor vaccine therapy, of more than 20 GBM patients. Therefore, we have pursue the following specific aims: [1]. To develop a technology platform of human cancer cells and autologous immune T lymphocytes, particularly tumor-infiltrating T lymphocytes of CD4(+)CD25(+)CD45RO(+)foxp3RNA(+) T regulatory phenotypes, tumor-specific cytotoxic CD8(+) phenotype and CD8(+)CD56(+) NKT phenotypes, for characterization of various cancer therapeutic agents with the CFSE-labeling techniques we have developed for tracing cell growth or inhibition kinetics *in vitro*, [2] To search for and characterize possible modifier biologicals and/or chemicals that can curtail or abrogate the negative effects of tumor-associated immuno-suppression of Treg cells while promoting the positive effects of anti-cancer innate and acquired immunity, including modifier drugs and purified compounds of Chinese traditional or herbal medicines that are likely to affect the immune system (but not yet well characterized).

MATERIALS AND METHODS

Tumor specimen. Tumor cells of WHO grade IV glioblastoma multiforme were cultured from surgical specimen of patients who participated in our clinical trial entitled "Dendritic cell-based Adjuvant Immunotherapy of Malignant Brain Gliomas", endorsed by Chang-gun Memorial Hospital institutional review board and approved by Department of Health of Taiwan (Official approval letter DOH-MD0910072534 Notice). For the present proposed translational research, we used the glioma cells, CD8(+) immune CTLs and CD4(+)CD25(+)CD45RO(+)FOXp3 RNA(+) Treg lymphocytes of the same GBM patients of the Phase-I clinical trial, while continuing to collect these valuable GBM tumor/immune cell materials as by-products of the clinical trial. Most importantly, these GBM tumor/immune cell materials will have individual patient's history and data of the disease state, history-pathological features, clinical course and immunotherapeutic outcome for reference. Patients' privacy rights will be strictly protected by anonymous coding and absolutely no public revelation of the patient's private information.

Large-scale cultures of GBM glioma cells. Although we have cultured the tumor

cells of all GBM and AA patients participated in our Phase-I clinical trial, this owes mainly to the special expertise of one investigator (D.M. Yang). Expansion of in vitro glioma cell growth toward established cell line has been carried put for a few GBM cases (#1, #4, #7, #10, #12, #13), each with lots of tumor cells at various serial passage levels stored permanently in liquid N2 freezers. Viability of the tumor cells after thawing is usually 80-90%. The aliquoted GBM tumor cells are thawed and expanded for experimental use. GBM tumor cells of selected patient (e.g.#13) were characterized by karyotype and MHC analysis. This was made respectively by collaboration with Professor Lin, chief cytogeneticist of CMUH, of our adjacent laboratory and the Clinical Laboratory of CMUH. Although the short-term tumor cell culture is initially carried out in autologous plasma or human AB serum-containing medium, the corresponding established cell lines have been derived by using fetal calf serum-containing medium.

Expansion of immune lymphocytes. TILs of some GBM patients (e.g. #7, #12, and #13) comprised more than 50% of CD4(+)CD25(+)FOXp3RNA(+) Treg, which can be separated by FACSorting or by magnetic beads of anti-CD4 and anti-CD25 monoclonal antibodies. Treg cells can be expanded by growing in AIM-V medium containing 200 U/ml interleukin-2 and 10 ng/ml TGF-beta or low levels of PGE2. On the other hand, tumor-specific CD8(+) CTLs are present in large percentages of post-vaccination TILs, which can be separated by immuno-magnetic beads or FACSorting and expanded by growing in AIM-V medium containing low levels of interleukin-2 and anti-CD3 monoclonal antibody OKT3. We have also found that pure CD8(+) CTLs can be isolated by co-cultivation of post-vaccination TILs with small numbers (E:T ratio of 10:1) autologous tumor cells transduced with a engineered membrane-expressing OKT3 gene.

CFSE vital cell-staining method for tracing in vitro cell growth kinetics. CFSE-labeling immunocytofluorometric procedure, has been developed for quantitative measurement of cell growth or cell killing of GBM tumor cells and lymphocytes in vitro, as a general technical platform. This method has been used quite often for tracing immune cells in vivo in experimental mouse models. With the use of immunocytofluorometry, it is simple yet powerful, as illustrated in our semi-annual progress report, presented above. Glioma cells can be similarly labeled and used as targets for assaying the CD8(+) CTL activity not only of cell killing (annexin-V binding and PI staining) but also of cell-growth inhibition.

Inhibitory effects by cell-to-cell contact between effectors and target cells. Some biological effects may be mediated not by secreted factors but by direct cell-to-cell contacts. To examine this aspect experimentally, GBM tumor cells will be co-cultivated with peripheral blood lymphocytes (CD56(+)CD3(+) NK, CD4(+)CD25(-), CD8(+) and

CD4(+)CD25(+)CD45RO cells) or tumor infiltrating CTLs. GBM tumor cells are irradiated, heat-shocked or not treated, while the lymphocytes are vital-labeled with green fluorescent dye like CFSE. Following co-cultivation, changes of T lymphocyte phenotype distribution and proliferative activities can be determined by multi-color cytofluorometric analysis which shows mean fluorescence index, MFI, of FL-1 fluorescence channel and MFI of other channels which respectively indicate the quantity of specific fluorescent antibody binding to the CD markers on individual lymphocytes (i.e. CD4, CD25, CD45RO antigenic sites of different lymphocytes). If co-cultivation with autologous tumor cells (by cell-to-cell contact) down-regulates the surface marker expression of a lymphocyte, the MFI of the particular fluorescent antibody molecules bound to this lymphocyte will decrease accordingly.

RESULTS

[a] T regulatory (Treg) lymphocytes (Fig.1-3). We have found that CD4(+)CD25(++)CD45RO(+)GITR(+)FOXp5RNA(+) Treg cells are the major constituent of tumor infiltrating lymphocytes in most GBM patients prior to or without vaccine therapy: [i] We have isolated these Treg vells from a dozen of GBM patients' surgical specimen. [ii] We have found that the Treg phenotypes such as surface CD25 as well as Foxp3 RNA and their immunosuppressive function can be down-regulated by IL-12, the major cytokine product of mature dendritic cells, as well as by the processed immunogenic autologous glioma cells. [iii] We have developed a scheme for testing or searching for drugs or therapeutics that inhibit the immuno-suppressive activities of Treg by exploiting the isolated Treg cells from GBM TILs. [iv] Using this scheme, we showed that two topoisomerase-I inhibitor drugs, NS38 and CPT, exerted negative effects by different mechanisms on the immunosuppressive function of glioma-infiltrating Treg cells. At 5-20 nM levels in growth medium in vitro, NS38 .is cytotoxic to Treg cells, while CPT is not cytotoxic and yet causes Treg cells not to inactivate the co-cultured T lymphocytes.

[b] Glioblastoma-specific cytotoxic CD8(+) T lymphocytes (CTL) (Fig.4-5). We found that tumor-specific CD8(+) cytotoxic T lymphocytes became the major tumor-infiltrating T lymphocytes of the GBM patients after DC-tumor vaccine therapy in our phase I clinical trial. To characterize the GBM-specific CTLs, we have developed an in vitro cytotoxicity assay using CFSE-labeled autologus GBM tumor cells as the target of effector lymphocytes in tumor infiltrating lymphocyte (TILs) population. Our major findings concerning GBM-specific CTLs: [i] We have isolated these tumor-specific CTLs from TILs of GBM patients after the DC-tumor vaccine therapy. [ii] We have developed in vitro culture procedures to expand the GBM tumor-specific CTLs. [iii] Pre-vac (left panels of Fig. 5) TILs did no kill but instead

promote the growth of autologous glioma cell target. [iv] Post-vaccination TILs (middle and right panels of Fig.5) inhibited the growth and also killed the autologous glioma cell target. [v] We have found that GBM CD133(+) "cancer stem cells", although slightly more immuno-resistant than GBM CD133(-) tumor cells, can still be killed by autologous CTLs but slowly and through the action of interferon-鵜 secreted by CTLs.

[c] Glioblastoma "cancer stem cells" (Figures 6-8). We have found that cancer stem-like cells may emerge after radiotherapy: [i] We established cell lines from surgical specimen of Chinese glioblastoma multiforme (GBM) patients participated in our phase-I clinical trial of autologous dendritic cell-based tumor vaccine adjuvant immunotherapy. [ii] Simulating in vivo radiotherapy, we exposed some GBM patients' tumor cells to high doses of ionizing radiation in vitro and found that many surviving tumor cell colonies contained up to 60% of CD133(+) neuron-sphere forming "cancer stem cells". [iii] Screening for drugs that may affect CD133(+) glioma cells, we found that celecoxib is effective in sensitizing the GBM "cancer stem cells" to ionizing radiation, by enhancing the mitotic catastrophe caused by radiation damages.

DISCUSSION

The purpose of this translational research is to continue our post-clinical trial translational research to improve the adjuvant efficacy of autologous dendritic cell-based tumor vaccine therapy. As we have found that two cells, namely the GBM tumor infiltrating T regulatory lymphocytes and the GBM cancer stem-like cells, are mainly responsible for the malignancy of these fatal human neoplastic diseases, it is important to find some drugs or biological agents that can target these cells to counteract their malignancy effects. We have been able to isolate and expand these two cells in in vitro cell cultures for use in the drug discovery. Our research efforts have led us to some drugs such as topoisomerase-I inhibitors, CPT and NS38, which can curtail the immunosuppressive functions of tumor infiltrating Treg cells, and also celecoxib, which increases the sensitivity of CD133(+) GBM cancer stem-like cells to therapeutic ionizing radiation. These findings can be used for modification of GBM treatment protocols for further clinical trial in our attempts to raise the 4-year survival rates from the 25% achieved by our phase I clinical trial.

Another important implication of our present translational research findings is associated with the question whether or not post-surgical immunotherapy should bee administered before post-surgical adjuvant radiotherapy. We have collected in vitro data to determine the time order of post-surgical adjuvant radiotherapy and adjuvant immunotherapy in newly diagnosed WHO high grades malignant gliomas, in

particular glioblastoma multiforme. A few of our results would help in this determination. First, we have confirmed the previous finding that serial ionizing radiation in vitro, while killing almost all GBM tumor cells in culture, could not eliminate the CD133(+), neurosphere forming and highly clonogenic cancer stem-like GBM cells. This implies that the adjuvant radiotherapy administered in neuro-oncology practice apparently can not eliminate the residual GBM tumor completely by preventing the DNA-repair efficient cancer stem-like cells from emergence to cause life-threatening GBM recurrence . Second, CD133(+) GBM cells were less sensitive than CD133(-) GBM cells to autologous immune specific CD8(+) cytotoxic T lymphocytes in several-hour acute cytotoxicity assay, but nevertheless became vulnerable to a delayed action of the immune lymphocytes. This implies that a robust adaptive immune response is likely capable of eliminating the residual GBM tumors that include minor stem-like glioma cells but still not sufficient to target the massive stem-like cell population during GBM recurrence after radiotherapy. GBM has been long known to be highly immuno-suppressive, which is possibly due to production of immunosuppressive factors by GBM tumors. Third, pre-incubation with cytotoxic T lymphocytes significantly enhanced the radiation sensitivity of CD133(+) GBM cells as well as CD133(-) GBM cells. As "cancer stem cells" are recently believed to be chief culprit in human malignant neoplasms, therapeutic targeting are being actively investigated, possibly including the known cancer chemotherapeutic agents. We have found that selective COX-2 inhibitor, celecoxib, may enhance the radiation-induced mitotic catastrophe of human CD133(+) GBM cells (Yang et al, to be published). The specific immune targeting of cancer stem-like cells represents an additional approach, especially in combination with radiotherapy. In this regard, recently it has been demonstrated that prior immunotherapy to induce robust specific immune responses can increase the efficacy of subsequent chemotherapy in GBM patients. Fourth, we found that immune CD8(+) cells were highly susceptible to ionizing radiation, even at lower than therapeutic doses, confirming the long known adverse effects of radiaotherapy on the immune system. However, after killing of immune CD8(+) tumor infiltrating T lymphocytes by adjuvant radiotherapy applied to the brain tumor sites, it is expectable that the tumor-specific cytotoxic lymphocytes will be replenished from peripheral lymph node T lymphocytes that are induced by dendritic cell-tumor antigen immunization. All these results point to the advantage of surgery-immunotherapy-radiotherapy (chemotherapy) scheme for newly diagnosed GBM. However, further clinical trials are needed to show the predicted survival benefits of this modified therapeutic approach.

CONCLUSION

Our results suggest that the efficacy of post-surgical autologous dendritic cell-based tumor vaccine adjuvant therapy can be improved by targeting two cell culprits, namely the tumor infiltrating Treg cells and CD133 (+) GBM cancer stem-like cells, which curtail the anti-tumor immune responses and which can lead to fatal GBM tumor recurrence, respectively. These results could be incorporated to revise our current protocol of autologous dendritic cell-based tumor vaccine adjuvant immunotherapy of glioblastoma multiforme. In the previous phase-I clinical trial we have achieved 4-year survival rate of 25% (in comparison to virtually 0% 4-year survival rate of the "historical control glioblastoma patients". Further clinical trials should be instituted to find out if our revised protocol can raise the 4-year survival rate above 25% in the treatment of human glioblastoma multiforme. Given the fact that dendritic cell-based immunotherapy appears to have virtually no adverse effects, thus providing excellent life quality for cancer patients, it is worthwhile to carry out an expanded clinical trial of our dendritic cell-based immunotherapeutic approach with proof-in-principle and technical feasibility.

九十六年度計畫重要研究成果及對本署之具體建議

（本資料須另附乙份於成果報告中）

計畫名稱：樹突細胞腫瘤疫苗治療:抗癌藥與免疫系統互補之研究

主 持 人：楊文光　　計畫編號：DOH94-TD-I-111-TM003

1. 本計畫之新發現或新發明

1) 人類 T 免疫控制細胞(Treg lymphocyte)之新種株

2) 人類抗膠質胚原腫瘤之特殊免疫殺傷淋巴細胞

3) 人類膠質胚原腫瘤細胞之新種株

4) 建立可發現免疫調整藥物之細胞技術平台

5) 樹突細胞腫瘤疫苗製造的新技術和新配方(擬申請專利和技術轉移)

2. 本計畫對民眾具教育宣導之成果

目前僅限於參與臨床試驗受治療之惡性腦瘤病人及其家屬之宣導，以及計畫主持人在學校及學術機構的演講。

3. 本計畫對醫藥衛生政策之具體建議

[1] 希望根據本計畫的研究成果所策劃和改進之樹突細胞腫瘤疫苗癌症免疫治療新方法能夠獲衛生署很快的核准，進行二相臨床試驗，以嘉惠罹患此惡性腦瘤之病人。

[2] 目前第四級惡性腦膠質腫瘤(Recurrent WHO grade IV glioma)病人腫瘤再發時，健保給予 temozolamide，每個月 7 萬元。若以本

研究計劃成果所策劃之樹突細胞腫瘤疫苗臨床試驗，收 40 位病人避免其再發，則起碼可省一千四百萬(7x5x40)之健保 temozolamide 之給付經費。

Figure 1– Immunocytofluorometric analysis of TILs from 3 GBM patients prior to vaccine therapy, showing predominant CD4(+)CD45RO(+)CD25(++) Treg cell population

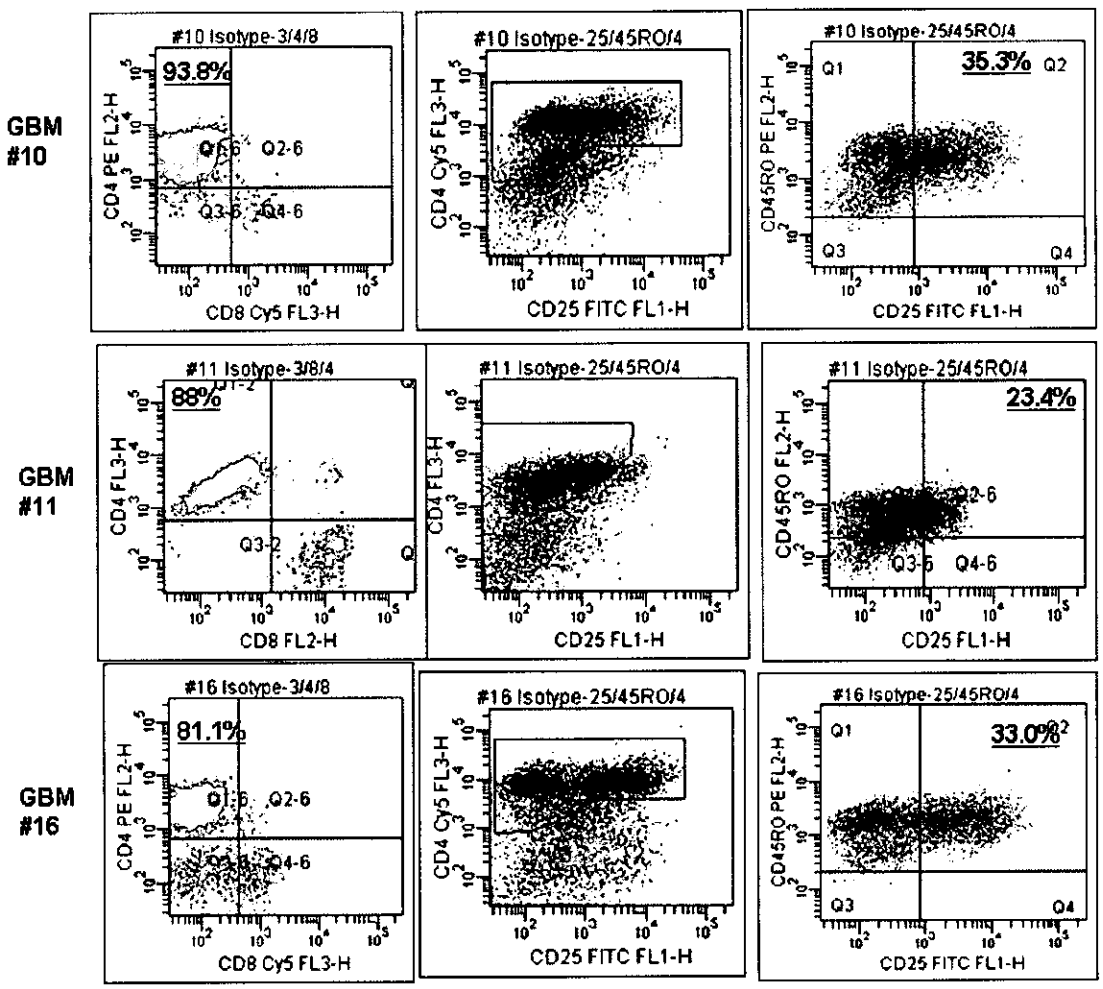


Figure 2 – A general scheme for search of chemotherapeutic agents to inhibit the immuno-suppressive function of Treg cells. Topoisomerase I inhibitors CPT and NS38 are used as an example for demonstrating the feasibility of this scheme

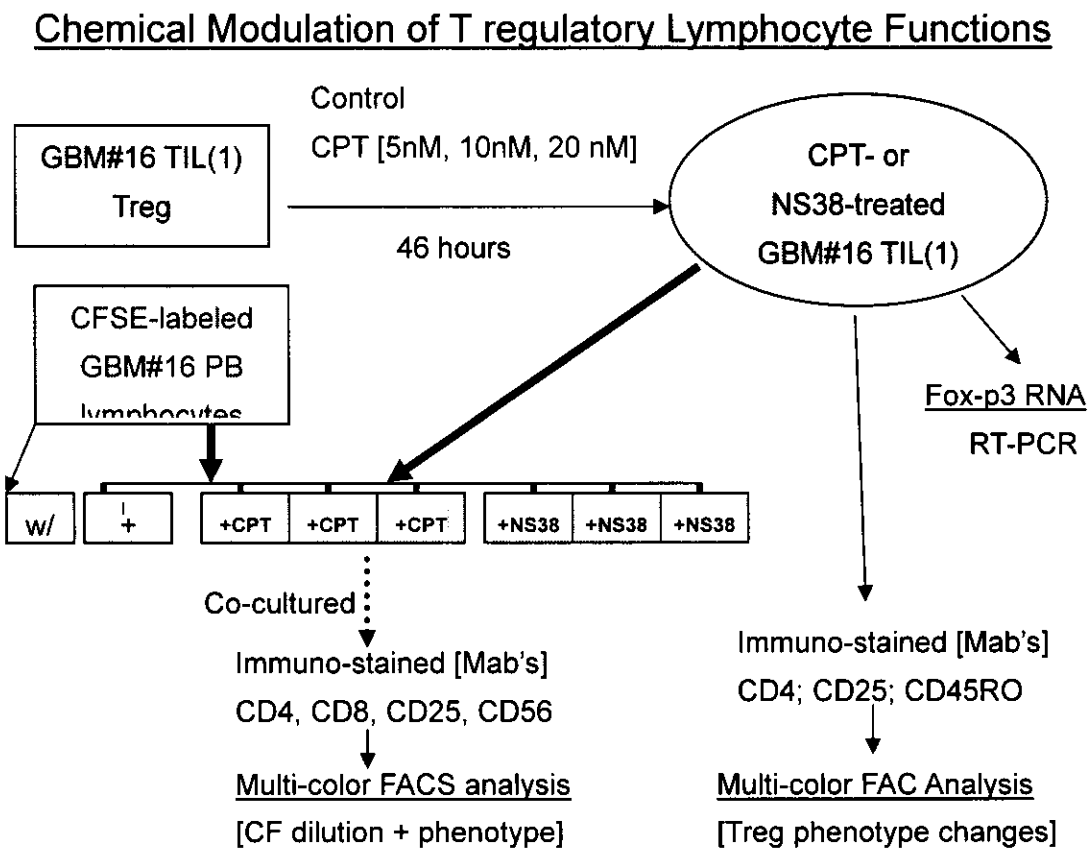


Figure 3 –Topoisomerase-I inhibiting drugs, CPT and NS38, exert inhibitory effects on human Treg immuno-suppressor T lymphocytes (surface CD4 and CD25 (IL-2 receptor-alpha)

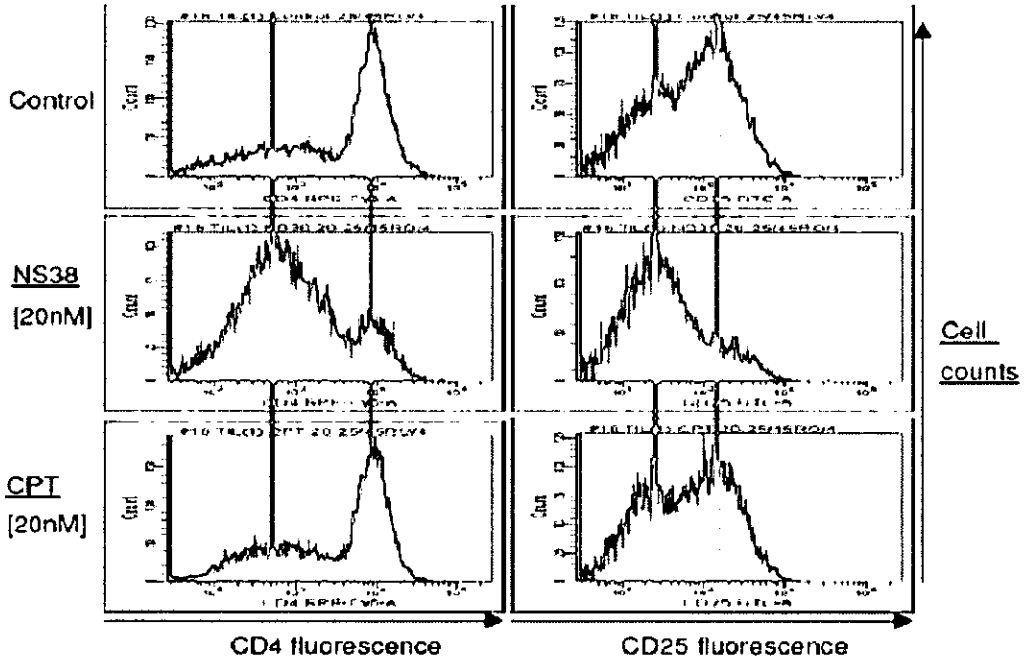
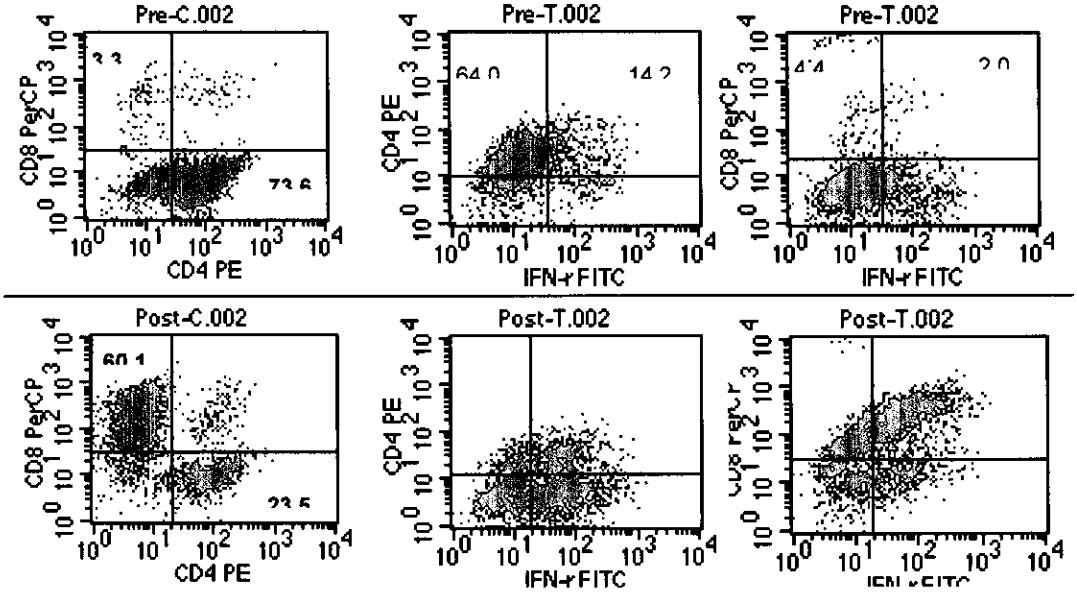


Figure 4 – Isolation and characterization of GBM-specific CD8(+) cytotoxic T lymphocytes from GBM#7 patients before and after autologous DC-tumor vaccine adjuvant immunotherapy



TILs	CD4 total	[INF-γ (+)	INF-γ(-)]	CD8 total	[INF-γ (+)	INF-γ(-)]
Pre-vaccination	73.6%	[14.2% :	64.0%]	3.3%	[2.0% :	4.4%]
Post-vaccination	23.5%	[19.6% :	9.2%]	60.1%	[46.4% :	21.7%]

Figure 5 - Susceptibility of CFSE-labeled GBM tumor cells to cytotoxic activities of TILs isolated before(pre-vac), after 8 vaccines (+8 Vac) and after complete vaccination (post-vac)

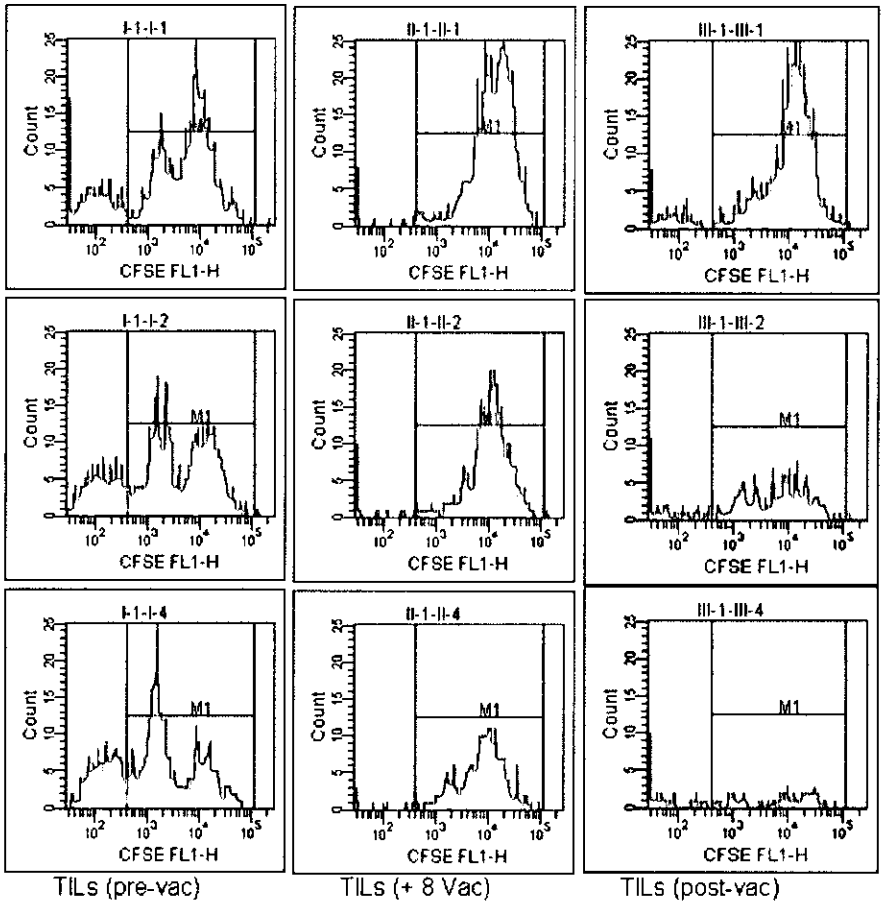


Figure 6– Emergence of high clonogenic CD133(+) "stem cells" in survivors of GBM#13 GBM cells after 1x[10 Gy], 2x[10 Gy] and 3x[10Gy] of ionizing radiation

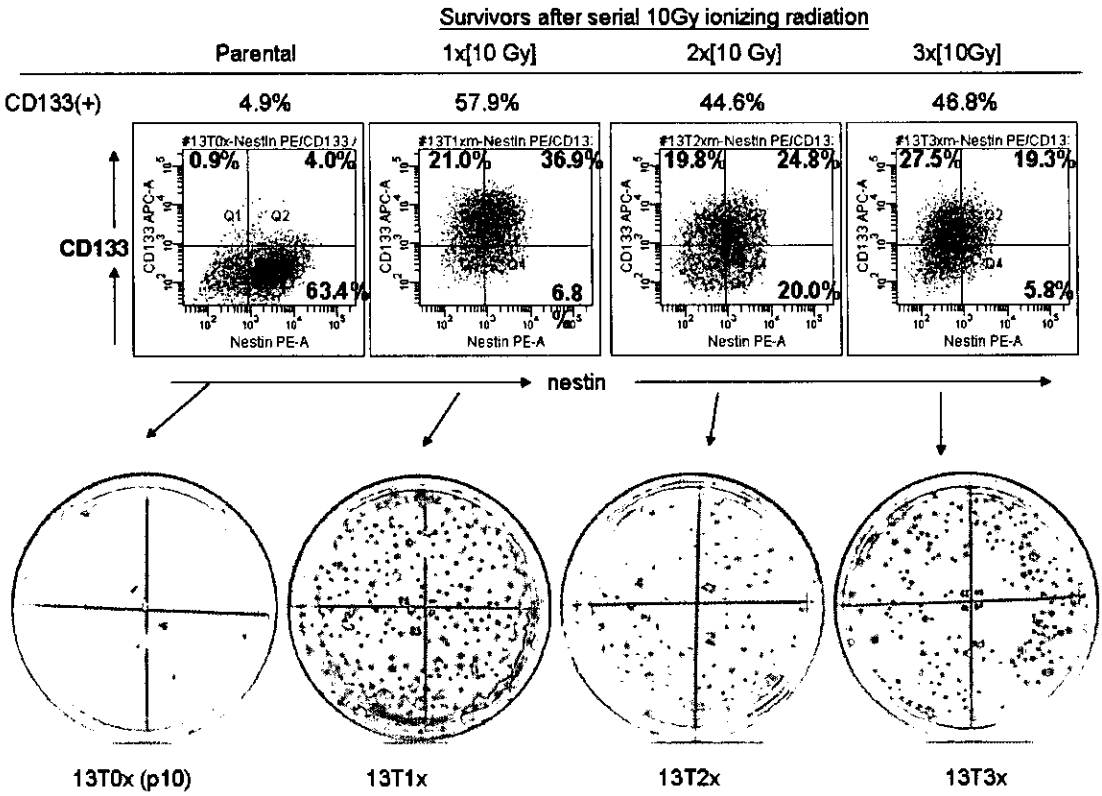


Figure 7 - Celecoxib sensitizes GBM#13T1x tumor cells to ionizing radiation

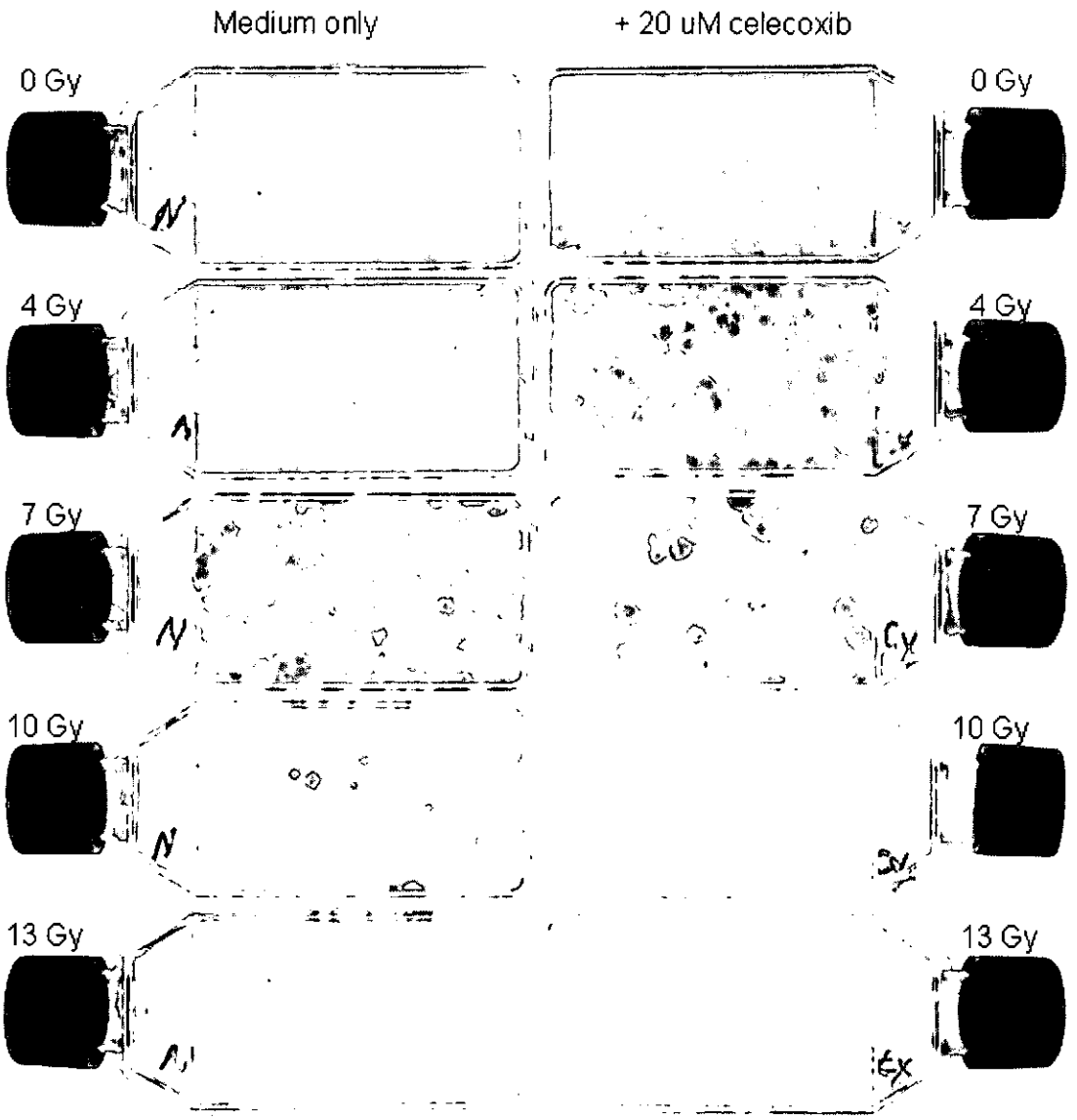
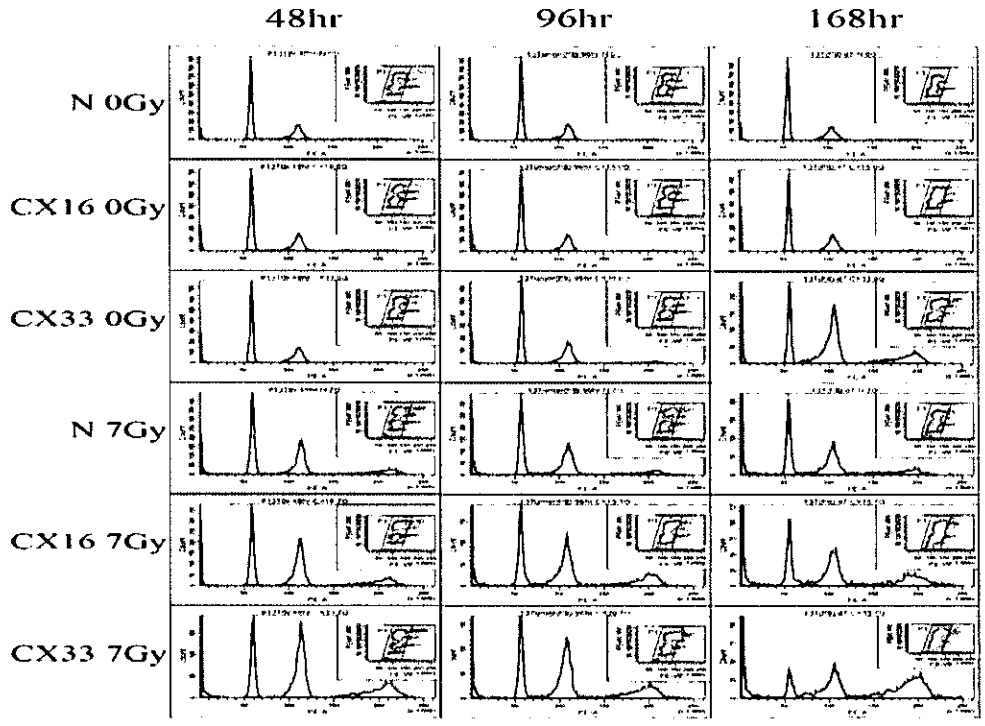


Figure 8 - Celecoxib (16, or 33 microM in medium) enhances the cell cycle alterations of G2/M blockage, aneuploid disorder and mitotic catastrophe induced by 7Gy ionizing radiation in GBM13T0x (passage 10) cells



Celecoxib (16, or 33 μM in medium) enhances the cell cycle alterations of G2/M blockage, aneuploid disorder and mitotic catastrophe induced by 7Gy ionizing radiation in GBM13T0x (passage 10) cells

Figure 9 - GBM 13T3X3 cells 72 hours after irradiation with 4Gy ionizing radiation or without and culture in growth medium containing 0 microM (N), 15 microM (CX15) or 30 microM of celecoxib, which enhanced the radiation-induced cell cycle anomalies of CD133(+) glioma cells.

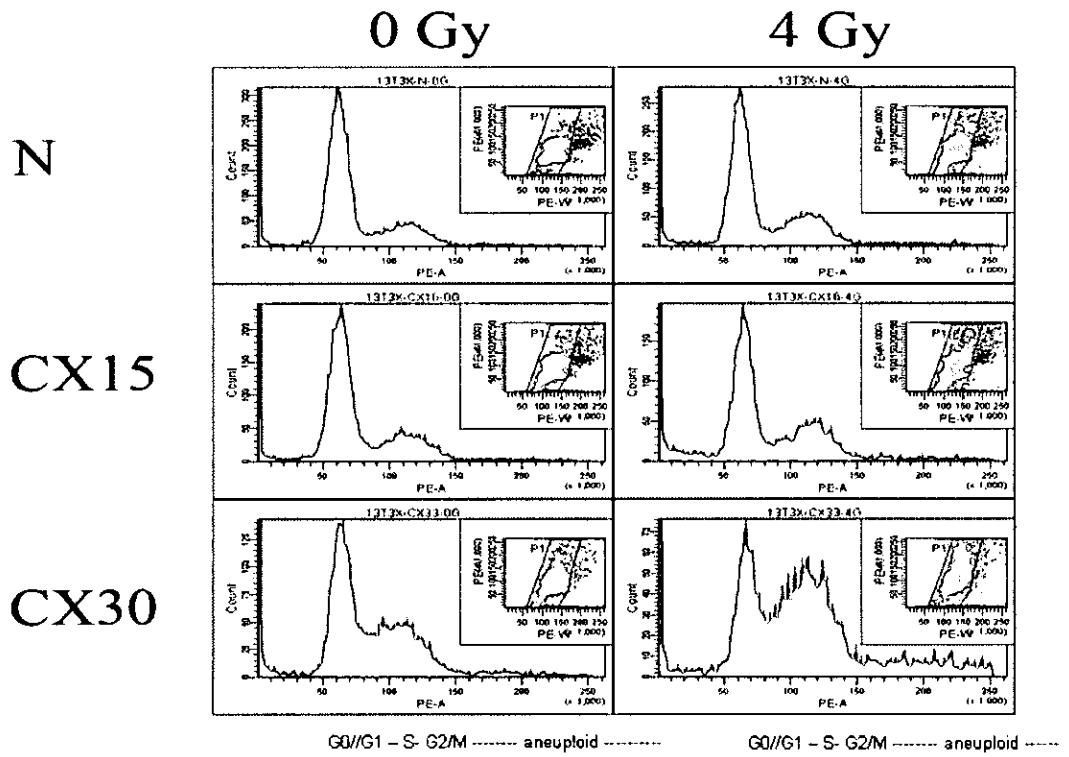


Figure 10 –Celecoxib sensitizes CD133(+) stem-like cells of GBM13T2xm to ionizing radiation