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# Melphalan Conjugated Anti-EGFR Antibody ior egf/r$^3$ for Tumor Imaging and Therapy

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Target selective drug delivery by the aid of carrier molecules is one of the most innovative ways to overcome the deprived therapeutic index problem. Epidermal growth factor, EGFR specific conjugates are of prime interest for imaging and therapy. With an ultimate aim to enhance selectivity for cancer treatment, we have prepared antibody-drug conjugate comprising DNA minor groove binding anticancer drug, Melphalan and monoclonal antibody (anti-EGFR ior egf/r$^3$). In vitro assay revealed dose-dependent cytotoxicity on U-87MG and HeLa cell line with IC50 values 1.56µM and 12.5µM post 24h treatment and specific binding of Kd= 4.2nM and 5.6nM respectively. Confocal imaging, DNA electrophoresis, and FACs establish apoptosis and G2/M phase arrest in U-87MG cells post 24h treatment at IC50. 99mTc-Mel-ior/r3 revealed specific activity of 8-12mCi/mg and maximum tumor uptake at 24h p.i in biodistribution and SPECT imaging of U-87MG xenograft. Immunoconjugate showed remarkable tumor regression in established EAT tumors in Balb/c mice.
**Key words:** Antibody, Melphan, Immunoconjugate, Drug delivery, Minor grove binder.

## Introduction

Present cancer chemotherapy is based on the premise that the cytotoxic drugs mostly destroy rapidly proliferating tumor cells. However, in reality, the activity difference of current anticancer drugs against tumor and healthy tissues is relatively small. It is well known that cytotoxic chemotherapeutic agents like paclitaxel, cisplatin, or doxorubicin, cannot distinguish cancer cells from normal cells. This unfortunate feature constitutes the primary basis for various undesirable side effects associated with these drugs in cancer chemotherapy. The amount of the drug required to accomplish clinically effective cell death often causes severe damage towards actively propagating surrounding non-malignant cells. In recent years, the impact that monoclonal antibodies (mAbs) have had in the clinical treatment of cancer has been pronounced [1]. Agents such as Avastin (anti-VEGF) and Erbitux (anti-EGFR, HER1, and c-Erb-1) for colon cancer Rituximab (anti-CD-20) for non-Hodgkin's lymphoma, and Herceptin (anti-Her-2) for breast cancer provide convincing proof that mAb therapies impact disease progression and patient survival [2-4]. Antibody-drug conjugates (ADCs)/ immunoconjugates comprising a monoclonal antibody tethered to a cytotoxic drug have attracted substantial attention for cancer therapy [5-8]. It efficiently unites the targeting advantages of mAbs with the cytotoxic potential of drugs to enhance the specific drug delivery in tumor cells through the antibody-antigen interaction, thereby reducing non-specific toxicities [9]. Some ideal considerations in making effective and well-tolerated antibody-drug conjugates (ADC) include using highly potent drug payloads, addressing aggregation due to drug and linker hydrophobicity, circumventing issues such as multidrug resistance mechanism and off-target toxicity [10]. Promising data were reported for ADCs with drugs such as doxorubicin, maytansinoids, and minor groove binders [11-21]. Upon binding to the antigen on the surface of cancer cells, the ADC-antigen complex internalizes into endosomal/lysosomal compartments through receptor mediated endocytosis.

Epidermal growth factor receptor (EGFR) is the most characterized and widely studied first erbB family member [22-23]. These receptors are single-chain transmembrane polypeptide proteins possessing three different domains: (a) the extracellular domain, which binds ligands that activate the receptor, (b) the transmembrane domain that is involved in dimerization interaction between receptors, and (c) the intracellular tyrosine kinase domain that phosphorylates tyrosine residues on substrate proteins. EGFR receptor is overexpressed in most solid tumors, including breast and ovarian cancer, colon cancer, head-and-neck cancer, and non-small-cell lung cancer (NSCLC), with some breast cancers expressing up to 2 x 106 receptors per cell. The nitrogen mustards are among the earliest effective antitumor drugs used in human cancer chemotherapy. Currently, mechlorethamine and melphalan are two of the most used anticancer agents that are assumed to exert pharmacologic activity by inducing interstrand cross-links in the DNA major groove [24-25]. Melphalan is believed to alkylate the nucleophilic sites in DNA. The mechanism does not involve the formation of intermediary 'aziridinium ion' [26-27]. DNA alkylation results in DNA breaks and cross-linking of the twin strands, thereby hindering DNA replication and transcription. Like other alkylators, Melphalan is cell cycle phase non-specific. Melphalan is extensively used to treat neoplastic diseases, such as myeloma, chronic lymphocytic leukemia, ovarian and breast carcinomas. Still, the emergence of Acute Drug Resistance (ADR) often limits their effectiveness [28-29]. The present work includes the preparation of immunoconjugate (Mel-Ior egf/$r^3$) by conjugating anticancer drug Melphalan (Mel) to monoclonal antibodies (Ior egf/$r^3$) and assessed for its targeted in vitro anticancer efficacy and in vivo tumor imaging through SPECT. The key findings from work are that the cytotoxic effect of Melphalan was maintained; however, as it acts only on cells that are bound to antibodies, effective and specific delivery of the drug is also ensured.

## Experimental Section

### Instant thin-layer chromatography (ITLC).

The quantity of free and bound antibody in radiolabeled immunoconjugate was determined by ITLC as described previously [30]. The ITLC strips were developed using 10 mM EDTA (pH 4.5) and 0.9% saline/10 mMNaOH solution as solvents. The 99mTc labeled complex remained at the bottom, and 99mTcO4- progressed with the solvent front in acetone. Hence the yield of free pertechnetate and complexed ligand was assessed.

**Immunoconjugate (Mel-Ior egf/r$^3$).** For the coupling reaction, 25 µL of 20 mM of Melphalan solution (free amine) was added to 300 µL solution of activated COOH containing mAb antibodies (1 mg in 0.1 M sodium phosphate buffer, pH 7.4). Approximately 40 µL of saturated trisodium phosphate solution was added to maintain the final pH to 8.5–9.0. This was followed by incubation (37°C for 14 h), centrifugation of the reaction mixture, and column gel chromatography to eliminate the unreacted HeLa cells, and changed the buffer to 0.1 M ammonium acetate, pH 5.5. UV absorbance method was employed to determine the protein concentration at 280 nm. Size-exclusion HPLC with 0.1 M ammonium acetate buffer (pH, 7.0) was used for measuring immunoconjugate formation.

**Radiolabeling of Mel-Ior egf/r$^3$.** The immunoconjugate of anti-EGFR was radiolabeled following previous methods [38]. Briefly, to an aqueous solution of Mel- Ior egf/r$^3$ reducing agent stannous chloride was added, followed by freshly eluted 5 mCi 99mTechnetium pertechnetate. The pH of the solution was adjusted to 7–8, followed by incubation at room temperature for 20 min. Subsequently, radio-immunoconjugate was eluted on an exclusion-diffusion column using sodium acetate buffer (pH 7.0).

**Human serum stability evaluation of 99mTc- immunoconjugate.** The human serum stability of radio-immunoconjugate was assessed using the freshly extracted serum from healthy volunteers. The serum was incubated with 99mTc Mel- Ior egf/r$^3$ and kept in a CO2 chamber at 37°C, and analyzed for the presence of complex dissociation employing ITLC. To determine the percentage of free 99mTcO4- at different time intervals, ITLC was run in acetone and pyridine, acetic acid, and water (PAW) (3:5:1.5) as mobile phase.

**Receptor Binding Studies.** The specificity of immunoconjugate towards tumor cell surface receptors was studied by receptor binding assays on U-87 MG and HeLa cell lines maintained in DMEM (10% serum, 10 µM folic acid). Briefly, cells were incubated with varying concentrations (0.001 µM–20 µM) of 99mTc Mel- Ior egf/r$^3$ for 1 h at 37°C in the absence and presence of the 100 folds excess unlabeled immunoconjugate for the calculation of total and non-specific binding, respectively. The protocol used was similar to the previous reported method by our group [30].

**Cytotoxicity of Mel-iorEgf/R3.** Cytotoxicity of Mel-ior Ior egf/r$^3$ on cancerous cell lines was examined using the MTT [3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide] assay [31-32]. Briefly, exponentially growing cells plated in a 96-well micro titre plate were treated with varying concentrations of the immunoconjugate (mM– µM range) for various time intervals viz., 12 h, 24 h, and 48 h for performing the assay.

**Confocal fluorescence microscopy Studies**. Morphology of apoptosis was investigated by labeling the cells with the nuclear stain Hoechst 33258 and visualized under a confocal spectral microscope (fluorescence microscopy) (33-34). U-87 MG and HeLa cells (1 x 106) were pre-plated in a 6-well plate and treated with the IC50 concentration. Cells were grown and harvested as described above. After that, untreated or treated cells were collected by centrifugation and suspended in PBS (phosphate-buffered saline). Finally, the cells were fixed in 4% paraformaldehyde followed by staining the nucleus with Hoechst 33258. The presence of a fragmented or condensed nucleus was used to differentiate apoptotic and control cells.

**DNA Ladder**. The qualitative damage to genomic DNA was estimated by agarose gel electrophoresis. After drug treatment, cells were washed twice with PBS. The cells were lysed by adding lysis buffer (1% NP40 in 20mM EDTA, 50mM Tris HCl pH7.8) for 10 min on ice and centrifuged at 10,000X g for 10 min to obtain supernatants of DNA fragmented fractions. Afterward cells were incubated for 1 h with RNaseA (10µg/ml) at 37˚C, before being digested for 30 min with proteinase K (100µg/ml) at 56oC to obtain cell lysate. DNA was precipitated with 96% ethanol for 24h at -80°C. DNA samples were electrophoresed on 1.4% agarose gel containing ethidium bromide (0.16µg/ml) and visualized and photographed using gel doc.

**Flow Cytometry**. Flow-cytometric analysis of DNA histograms was carried out for the quantitative measurement of cell cycle distribution, and the methodology used was similar to previous work by our group [35]. At 24 h treated cells (at IC50 Conc.) with appropriate controls were detached by trypsinization and fixed in 70% chilled ethanol and stored atleast overnight at -4-8° C. Following fixation cells were washed twice with PBS, and incubated with 200 µg/ml RNase-A for 30 min at 37° C, and stained using 50 µg/ml propidium iodide in PBS at 4° C for 30 min at a density of 0.5 million cells/ml. Cell cycle distribution was studied with the help of FACS Caliber (Becton-Dickinson & Co., USA) flow-cytometer using the Cell Quest (version 3.0.1; Becton Dickinson & Co., USA) and Mod fit LT (version 2.0; verify software House, Inc., USA) softwares for acquisition and analysis. A minimum of 10,000 cells per sample was analyzed.

**Blood Kinetic studies of immunoconjugate, Mel- Ior egf/r$^3$**. Briefly, 10MBq of 99mTc-Mel-ior Ior egf/r$^3$ was injected intravenously (i.v.) via dorsal ear vein of normal rabbit weighing approximately 2–2.5 kg for studying pharmacokinetic. Around 0.1 mL of blood was withdrawn from another ear vein at various time intervals beginning from 5 minutes to 24 h to evaluate the percentage of activity remained using a gamma counter.

**Biodistribution studies in mice.** To examine the distribution of 99mTc-Mel-ior EGF/R3in the right hind leg of U-87 MG (overexpresses the EGF receptor), xenografted athymic nude mice biodistribution studies were done. For the experiment, mice with an approximate weight of 0.17 g and easily palpable tumors were used. 99mTc-Mel-ior EGF/R3 was intravenously inoculated in a volume of 0.1 mL through the tail vein of the animal. Similarly, in another group of animals, a standard drug (1mg 1kg by weight of the animal model) was injected 10 minutes before the injection of the radiolabeled test compounds and biodistribution was studied under receptor saturation conditions. Animals were sacrificed at different time points, and various organs were dissected and analyzed for counts on a gamma counter. The activity left in the animal tail was subtracted from the injected activity to estimate the actual activity administered. Accumulation of 99mTc-Mel- Ior egf/r$^3$ in each organ was stated as a percentage administered dose per gram (%ID=g) of tissue, and the blood volume was assumed to be 7% of total body weight.

**Scintigraphy Studies.** For visualizing the qualitative uptake of 99mTc-Mel- Ior egf/r$^3$ (20 µCi) in athymic nude mice xenografted with U-87 MG cells tumor in the right flank, scintigraphic studies were carried out at different time periods. Images were captured at various time points starting from 15 min to 24 h after post-injection of radioimmunoconjugate.

**Tumor Transplantation.** For the study, 6-8 weeks old male nude mice weighing 15-20 g were obtained from the Institution's central animal facility. The subcutaneous tumor was implanted in mice through intramuscular injection of 15x106 U-87 MG cell suspension (0.1-0.15 mL) into the right hind leg. The formula used for measuring tumor volume is V= π/6 (d1 * d2 * d3), where d1, d2, and d3 signifies three orthogonal diameters measured with a vernier caliper. The experiment was executed after 5 days of tumor implantation when the tumor volume reached 300-400 mm3. On attainment of tumor volume of approximately 5000 mm3, animals were sacrificed to evade discomfort linked to the tumor burden according to the UKCCCR guidelines. Entire experiments were performed as per the guidelines established by CPCEA, Indian National Science Academy (INSA) after taking permission from the institutional animal ethics committee.

**Tumor Regression.** Approximately 0.1-0.15 mL (in saline) volume of the immunoconjugate was intravenously injected via the tail vein of tumor-bearing mice for the treatment studies. Afterward, animals were housed under a controlled environment, and tumor volume was routinely observed every alternative day with a vernier caliper. The formula used for calculating the tumor volume was V= π/6 (d1 * d2 * d3), where d1, d2, and d3 are the three orthogonal diameters measured using caliper.

## Results and Discussion

There is a new wave of molecularly targeted anticancer therapies under development and in the early stages of clinical use. These provide many advantages over conventional chemotherapy for cancer treatment. The benefits include improvised limiting features of narrow therapeutic windows, drug resistance, and non-specific toxicities. With the increased understanding of carcinogenesis and improved tools in cancer biology, targeted cancer therapy has made significant advances. Various therapeutic agents directed against EGFR and HER-2 has provided promising alternatives to traditional chemotherapy in search of better cancer treatments [30]. Effective delivery to the disease site is a prerequisite for high efficacy and low toxicity of any drug substance. Antibodies can participate in this context by facilitating the transport of a drug cargo within the body, thereby invoking the often-cited "magic bullet" concept, as put forward by Ehrlich over a century ago [36]. Conjugation of a drug to an antibody makes it possible to achieve excellent localization of the drug at the desired site within the body. This increases the effective drug concentration within this target area, thereby optimizing the therapeutic effect of the agent. Radiolabeled monoclonal antibodies provide alternative routes for radionuclide delivery to target cells for radioimaging and antineoplastics for chemotherapy [33-34]. Herein we have prepared an immunoconjugate with mAb and Melphalan, anticancer drug, targeting EGFR receptors

**Synthesis of Antibody-drug Conjugate.** Various methods and approaches are reported for the conjugation of mAb and the drug molecule. We have conjugated antineoplastic agent, Melphalan with free NH2 at pH 9 to the activated –COOH group of the mAb by using coupling agent EDC and sulpho NHS. As assessed by cobalt binding assay, on an average of 1:25::mAb(ior/r3): Mel molecules could be randomly conjugated with insignificant loss of immunoreactivity.

**Quality control of radiolabeled immunoconjugate (Mel- Ior egf/r$^3$).** The radiolabeling efficacy of radioimmunoconjugate was examined through ascending paper chromatography on ITLC-SG (Instant Thin Layer Chromatography-Silica Gel) strips. Under physiological conditions, the complexation of immunoconjugate with 99mTc was suitably stable. The specific activity of radiolabeled Mel- Ior egf/r$^3$ was 20-30 mCi/mg of protein. ITLC-SG results for percentage radiolabeling were>97 % in 0.9 % saline and acetone and with less than 2 % free pertechnetate (Rf = 0.7-1.0). This signifies the presence of less than 2.0% radio colloids and quantitative reduction of pertechnetate. The 99mTc loading of the mAb conjugate (Mel-ior Ior egf/r$^3$ ) was achieved very fast (<1 h) under mild conditions (pH 7, at 25°C) thereby avoiding degradation of the protein [35].

**Human Serum Stability Studies.** Radioimmunoconjugate was examined for in vitro human serum binding, which is an imperative factor that delays blood washout during imaging, leading to a poor target-to-background ratio. Adequately stable up to 24 h, the radioimmuno conjugate has only 3% dissociation in serum post 24 h of incubation. There by demonstrating non-significant transcomplexation of the radioimmunoconjugate in the biological system [37]. This will provide a lower target to-background ratio during in-vivo imaging and the suitability of Mel- Ior egf/r$^3$ for tumor imaging application.

**Receptor binding studies.** To examine the affinity of prepared immunoconjugate towards cancerous cells receptor binding experiment was performed [37].

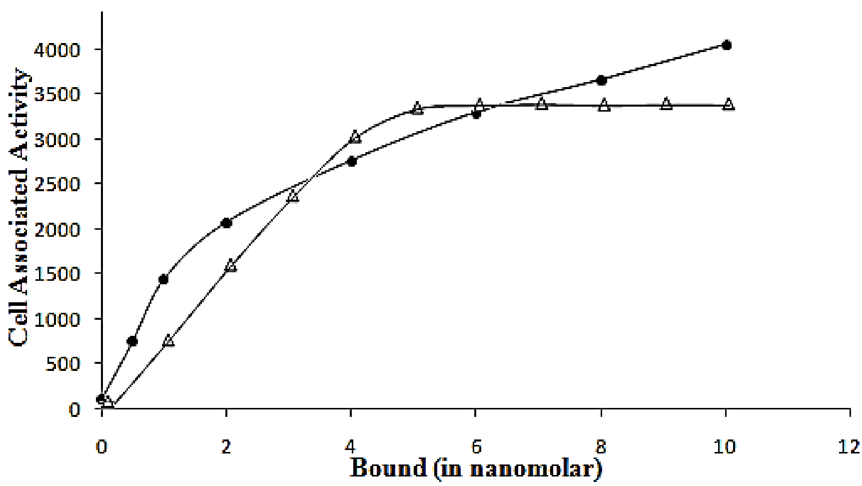


Figure 1: a) Saturation binding of 99mTc-Mel-ior EGF/R3to U-87 MG (•) and HeLa (Δ) human tumor cell lines with increasing concentrations.

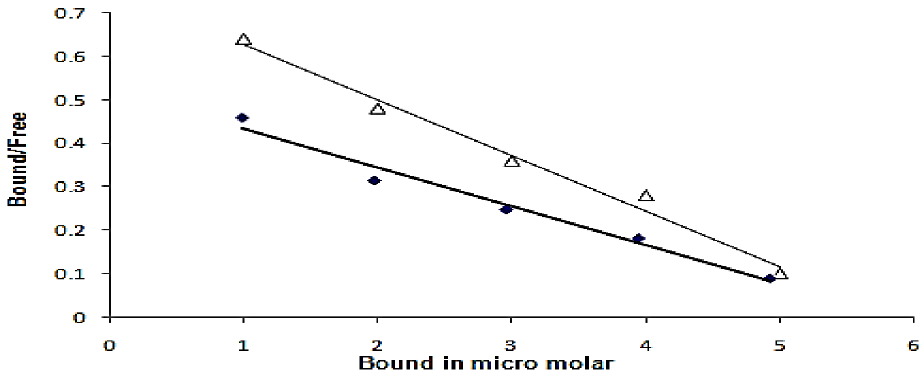


Figure 1: b) Scatchard Plot of the specific binding data to the ratio of bound to free (B/F) of Mel- Ior egf/$r^3$ on U-87 MG ( ♦) and HeLa (Δ) and cell lines.

tumor cell lines. The binding constant, Kd was found to be 4.2 nM and 5.6 nM for U-87 MG and HeLa cell lines, respectively (Figure 1. a, b). These two observations concluded that the expression of EGFR receptors on U-87 MG cells were more than that were present on the HeLa cells.

**Cytotoxicity of Mel-ior EGF/R3 by MTT Assay.** To evaluate the cytotoxic activity of Mel- iorEGF/R3on cancerous cells, we treated U-87 MG and HeLa cells with Mel-ior EGF/R3conjugate. The surviving fractions of Mel-ior EGF/R3 were evaluated to determine its dose- responsive properties by mitochondrial apoptotic assay individually on both cell lines. The result determined a concentration-dependent enhanced activity with an IC50of 1.56 µmol/L when U-87 MG cells were incubated for 24 h for Mel- Ior egf/$r^3$ (Figure 2). The IC50 value for the HeLa cell line was found to be 12.5 µmol/L, which was a higher concentration than that of the IC50 on U-87 MG cells, which reveals the expression of the EGFR receptor on the U-87 MG cells is more as compared to HeLa cells. The lower inhibitory concentration of Mel- Ior egf/$r^3$ for malignant glioma cells (Melphalan alone IC50 = 37 µM) will thus limit the off- target exposure and will be able to differentiate normal and target cells.

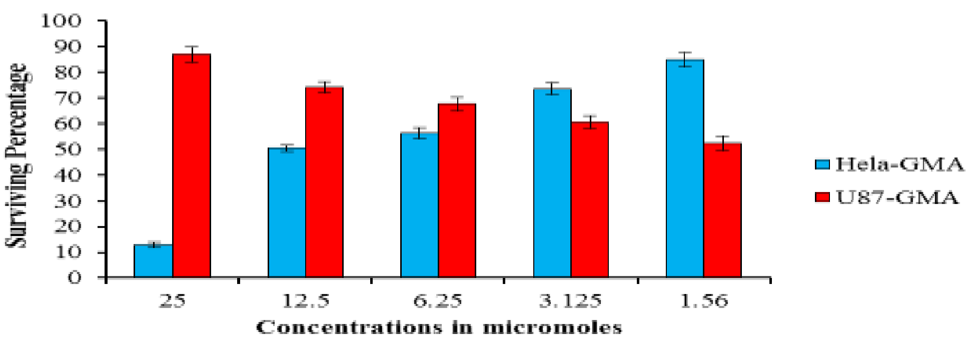

Figure 2: Colorimetric estimation of the mitochondrial activity for Cytotoxicity of Mel- Ior egf/r$^3$ (MTT assay) at different concentrations on U-87 MG and HeLa human cancer cell lines.

**Confocal Microscopy.** Morphology of tumor cells was investigated to authenticate the presence of apoptosis by confocal microscopy staining with blue fluorescent DNA dye Hoechst 33258 [33].

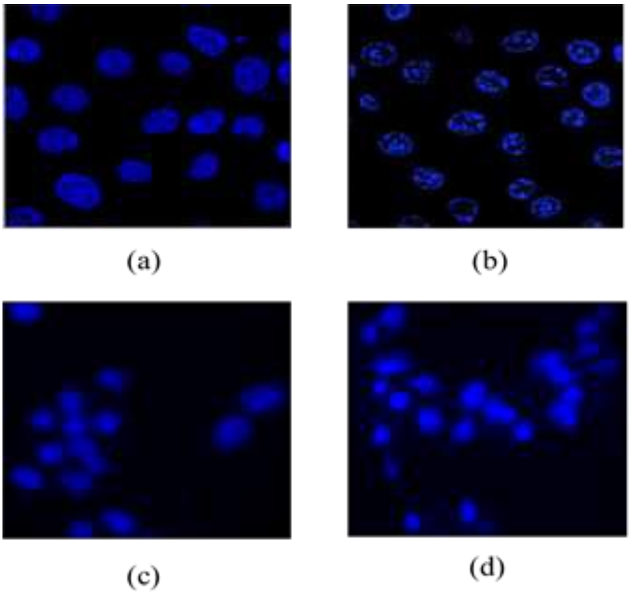


Figure 3: Confocal microphotographs of HeLa (a, b) and U-87 MG (c, d) cells after treatment, where a, c represents control and (b, d) represents treatment with the Mel- Ior egf/r$^3$ followed by Hoechst dye 33258 staining.

It was found that the nucleus treated with immunoconjugate Mel- Ior egf/r$^3$ at IC50 concentration of 1.56 and 12.5μmol/L for U-87 MG and HeLa cells respectively became convoluted and budded off into several fragments as seen in Figure 3. (b, d) for U-87 MG and HeLa respectively. In addition, no apparent nuclear condensation and fragmentation were observed under control experiments condition. Typical characteristics of apoptosis-like nuclear condensation and fragmentation demonstrated that the leading cause of cell death induced by Mel- Ior egf/r$^3$ was apoptosis for U-87 MG and HeLa cells. Apoptosis caused by the mAb conjugated to Melphalan drug was more pronounced than Melphalan being alone, which could be due to the targeted delivery by the mAb.

**DNA Ladder Assay.**To further associate the results of confocal microscopy, we performed the DNA ladder assay on DNA isolated from U-87 MG cells. Figure 4 indicates the presence of apoptosis in the treated cells at IC50 concentration for 24 h. Further, by electrophoresing the treated DNA, we confirmed that cancerous cellular death is due to apoptosis and not necrosis which is also established by the presence of fragmentation in the nuclei of the cells.

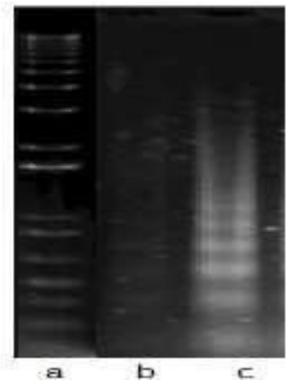


Figure 4: DNA Electrophoresis of U-87 MG cells. Cells were subjected to Mel-ior EGF/R3for 24 h at IC50. (Lane a: marker; Lane b: control; Lane c: treated)

**Cell Cycle Analysis.** The biological activity of immunoconjugate on U-87 MG cancer cell lines was determined by cell cycle analysis using Flow Cytometry Analysis. Figure 5 depicts the effect of IC50 concentration of Mel- Ior egf/$r^3$ on the cell cycle progression of U-87 MG cells. When cells were treated with an IC50 dose of 1.56 µmol/L for 12 h, some cells were found in the apoptotic stage (Figure 5. b). At this time point, the percentage of G2 cells was significantly higher than untreated controls. In the other group, in which cells were treated for 24 h at IC50 concentration, a significant enhancement in the percentage of apoptotic cells was observed compared to untreated controls. A notable boost in the percentage of G2 cells was also observed. The reason for observed apoptosis and G2/M phase arrest in glioma cells could be the presence of melphalan which is known for anticancer potential through DNA minor groove binding.

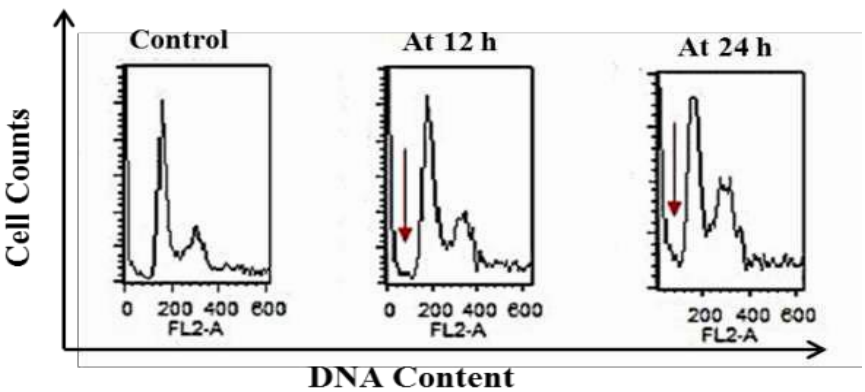

Figure 5: Histograms of Flow Cytometry at IC50 concentration of immunoconjugate on U-87 MG cells where representations are (a) untreated cells (control) (b), (c) post 12 and 24 h of treatment.

**Blood Kinetics.** Blood clearance was studied in normal rabbits after administration of 99mTc-immunoconjugate via the dorsal ear vein. At different time intervals, 0.5 mL blood sample was withdrawn from the dorsal vein of the other ear. The radio-immunoconjugate blood clearance in rabbits showed a bi-exponential pattern (Figure 6) with 94% of injected activity clearance within 3.2 h p.i. The rapid blood-pool washout indicates the stability of immunoconjugate and insignificant retention in organs, which will be of added advantage in scintigraphic imaging.

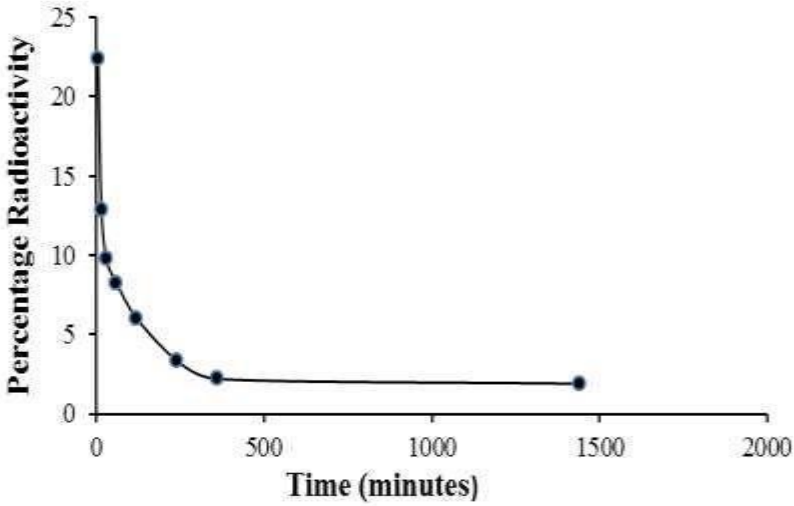


Figure 6: Blood kinetics study of Mel- Ior egf/r$^3$ post injecting 10 MBq radioactivity intravenously through the dorsal ear vein in healthy rabbits.

**Biodistribution Studies.** To examine the in vivo action along with the tumor-targeting efficacy of radio-immunoconjugate, biodistribution studies were performed in athymic nude mice xenografted with EAT tumor. The percentage activity distribution of Mel- Ior egf/r$^3$ in various organs of mice at different time intervals is shown in figure 7. Localization of the radioactivity in the liver, kidneys, and spleen was low compared to other organs at early hours but increased 24 h post-injection. The in vivo distribution shows major accumulation in kidneys (6.72 ± 0.23 %ID/g) at 2 h and reaches up to 17.24 ± 0.55 %ID/g at 24 h. In vivo stability of 99mTc-Mel- Ior egf/r$^3$ was established based on a non-significant accumulation of activity in the small intestine up to 8 h post-injection. The primary route of metabolic clearance from the animals was primarily through the urinary bladder. A good clearance of the radiolabeled immunoconjugate can be seen in the small intestine. The tumor accumulation was 2.46 ± 0.2 %ID/g at 2 h, which progressively increased to 7.42 ± 1.01 %ID/g at 24 h post-injection. An escalation in the target-to-non target ratio has been observed as the background activity gradually cleared from the system. The high specificity of Mel-ior EGF/R3results in selective uptake and distribution of the radiolabeled receptor-ligand at the tissues, which are known to contain a substantial concentration of the target receptor. In vivo biodistribution analysis displays that 99mTc-Mel- Ior egf/$r^3$ can be further utilized for tumor imaging purpose keeping in view the reduced non-specific retention of immunoconjugate in liver at the initial hours.

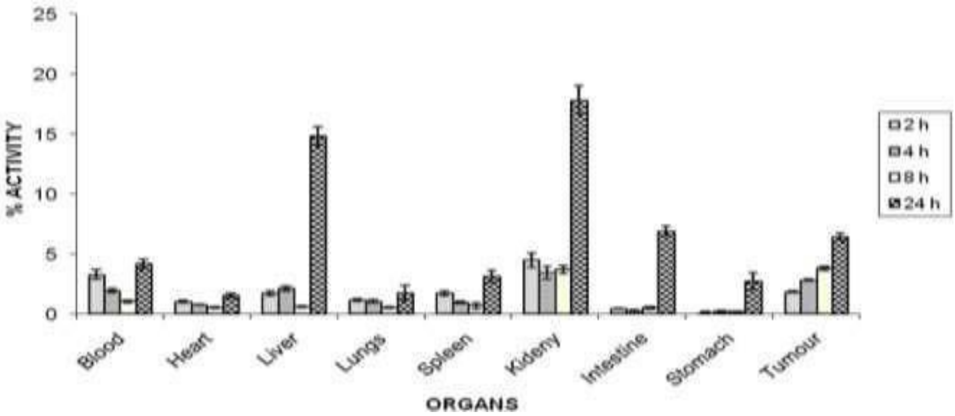


Figure 7: Biodistribution of 99mTc-Mel- Ior egf/$r^3$ in mice with EAT cells tumor implant following intravenous injection. Data from groups of five mice are expressed as mean %ID/g ± S.D.

**Tumor Regression Analysis.** In vivo tumor growth inhibition activity of the mAb antibody conjugated to an anticancer drug, Melphalan (Mel-ior Egf/R3) was evaluated against U-87 MG tumor grafted nude mice. In untreated controls, tumor growth was exponential initially, which declined (reduced tumor volume) after treatment of immunoconjugate at consecutive alternate days. All the treated animals survived for additional ten days compared to the untreated control (Figure 8). These results could be attributed because the over expression of EGFR makes the immunoconjugate internalize into the tumor via the targeted receptor. Thus the reason for improved cytotoxicity is the enhanced receptor-mediated transport. The conjugate is selective active to the cancer cells expressing the EGFR. The target specificity of the prepared immunoconjugate and its aptness as a diagnostic agent and as an antiproliferative agent through DNA fragmentation on binding with DNA minor groove are highlighted in the above studies.

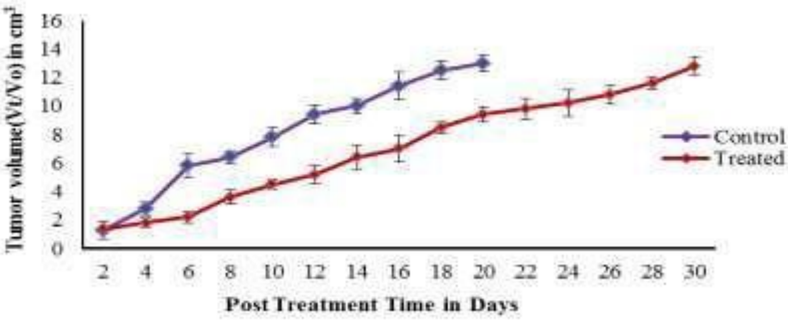


Figure 8: Effect of Mel- Ior egf/r$^3$ on U-87 MG implanted nude mice.

**Conclusion**

In view of the wave of antibody therapeutics making its way, we have successfully prepared an antibody-drug conjugate (ADC), Mel-Ior EGF/R3, to detect and treat cancer. The well-tailored Mel-Ior EGF/R3 demonstrated excellent anticancer activity through apoptosis in malignant glioma due to the minor groove binder drug, Melphalan. Moreover, the radio-immunoconjugate is also prepared in high radiochemical yield, purity, and it retained the in vitro and in vivo stability. 99mTc-Mel-ior EGF/R3established outstanding tumor uptake in biodistribution and scintigraphy imaging studies performed in xenografted mice. These positive outcomes will aid in developing and evaluating ADCs targeted toward cancer and will help guide ongoing research. It will be interesting to see how combinations of mAbs and chemotherapy or radiation can improve clinical responses. The new generation of mAb technologies now under development in the laboratory further enhances the efficacy and toxicity profiles mAb therapies.

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