

SEMINAR

“Newer Treatment modalities for Glioma”

Presenter: Dr Gaurav Bansal

- Dramatic improvement of imaging capabilities over last two decades:
 - Improved anatomic & functional localization
 - Improved surgical orientation & outcome
 - Accurate radiation delivery
- Better understanding of molecular oncogenesis : development of targeted therapies

- Improvement in the delivery of drugs:
 - Local placement of biodegradable polymers in the resection cavity : ‘ **Convection enhanced delivery** ’
- More specifically targeted therapies :
 - Signaling inhibitors as EGF or VEGF receptor inhibitors
 - Gene therapy : attempting to introduce specific genes as p53

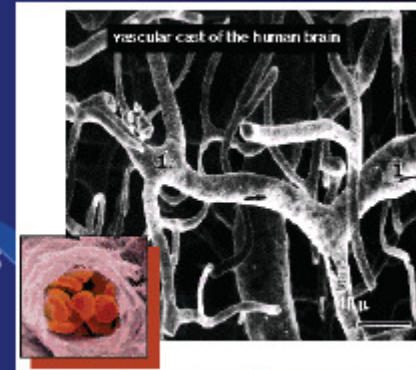
- Immune mechanisms of tumor cell recognition and lysis or toxin directed therapies
- Inhibitors of tumor angiogenesis
- Thermal Ablation Of Brain Tumors
- Brain Cancer Vaccines
- More recently use of Stem cells to target brain tumor cells

Important for effective treatment of primary brain tumors

- Treatment must reach entire volume of CNS.
- Glioma is not a local disease. It is a diffuse disease.
- Should not be toxic to normal brain cells.
- Should limit the development of resistance to the therapy, and should reactivate tumor killing if or when there is a recurrence

Blood-Brain Barrier

- **BBB protects brain from toxic substances**
- **Primarily small, lipophilic molecules readily cross the BBB**
- **98% of potential CNS therapeutics, including monoclonal antibodies, most chemotherapeutics don't cross BBB in sufficient quantities to be effective**
- **Misconception that blood-brain tumor barrier does not impair drug delivery to brain tumors**
- **None of the major pharmaceutical companies have a brain drug delivery division**



Immunology of Gliomas

- Our current understanding of tumor immunity is compelling that immunity important in progression of gliomas.
- Immune Cells are not able to recognize gliomas. Gliomas “Cloak” themselves to killer T immune cells. Down regulate MHC antigens, B7 and CD 11 co-stimulatory molecules on glioma cell surfaces that are important for immune recognition

Immunology of Gliomas

- Gliomas cells actively suppress killer T cell responses by release of immunosuppressive cytokines such as TGFb.
- In fact, it has been shown that the greater the immune response to the tumor the greater the release of immunosuppressive cytokines by the tumor

Advantages to use of Cellular Immune Therapy for Brain Tumors

- Activated immune cells can survey entire CNS One capillary for every two neurons
- Activated Immune Cells Cross the BBB.
- T Cell Immune killing is Selective. Is not toxic to normal brain
- Should retain memory for tumor killing. Should Reactivate when/if recurrence occurs

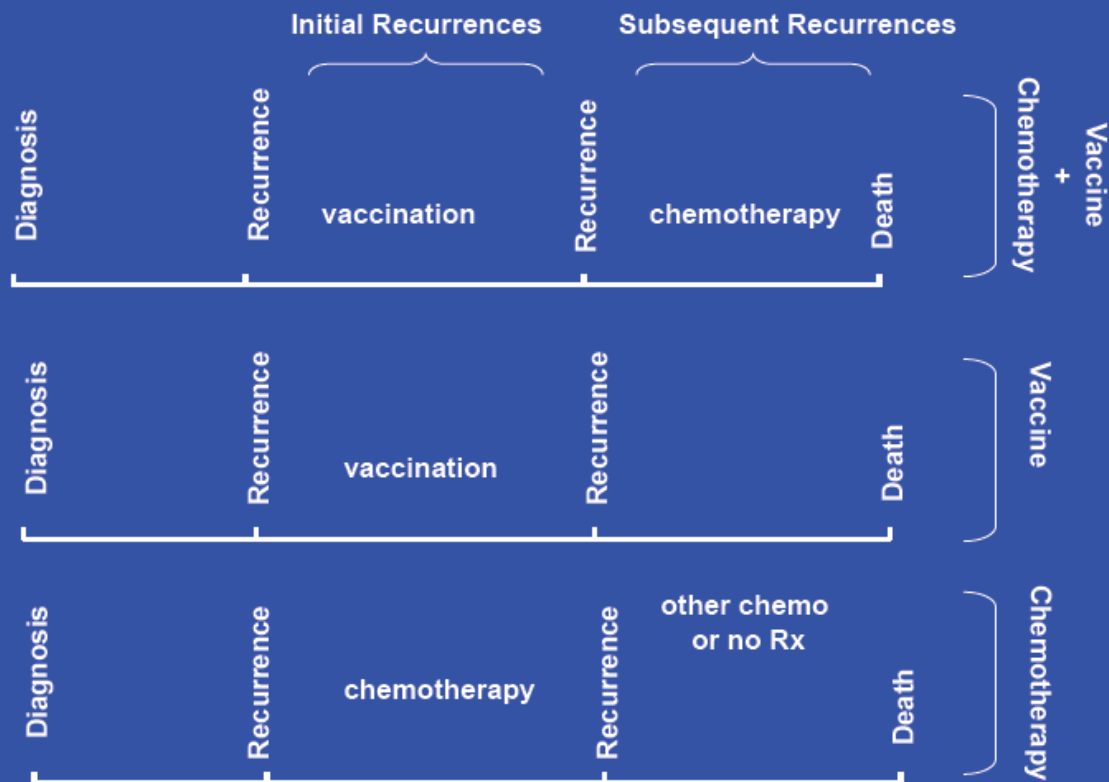
Brain Cancer Vaccines

- Brain Cancer Vaccines Can enhance immune response
- Dendritic Cell Vaccines: Using Antigens from both tumor cell culture and tumor lysate.
- Advantages with dendritic cells: most potent way to present tumor antigens to immune system.
- Do not need to know specific glioma tumor antigens. Dendritic cells select the important tumor antigens. Also Decrease risk of immune resistance by activation of multiple tumor antigens

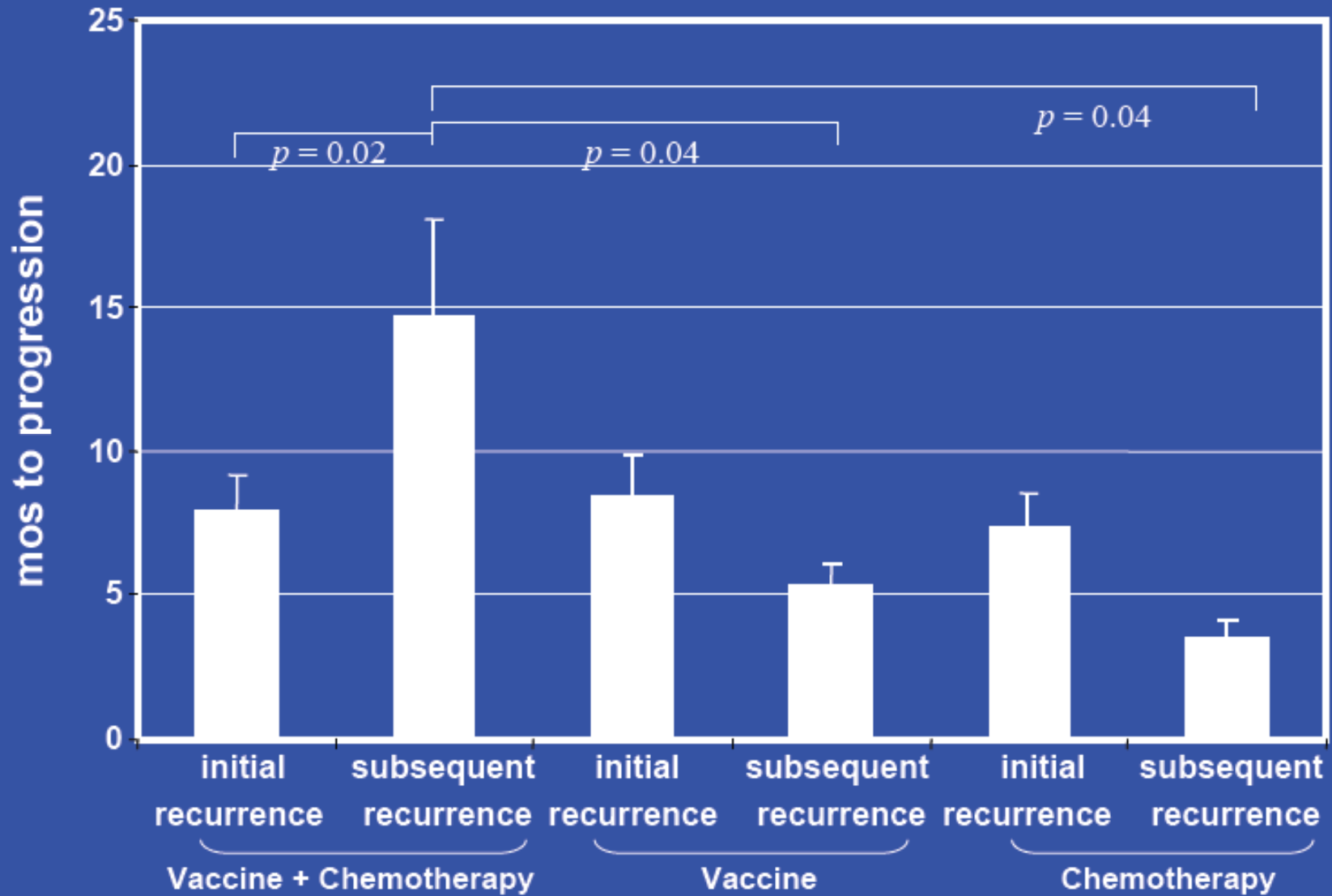
- Phase I study in glioma patients using dendritic cell vaccine (*Cancer Research* 61:842-847, 2001) :
 - treated patients with:
 - TGFb antisense glioma vaccines
- Tumor lysate or cultured tumor cells obtained from surgical specimen
- Mononuclear cells isolated by leukapheresis and differentiated into DCs
- 3 to 4 vaccinations with DCs pulsed with antigens from cultured tumor cells or lysate from whole tumor

53 patients treated with DC Vaccine

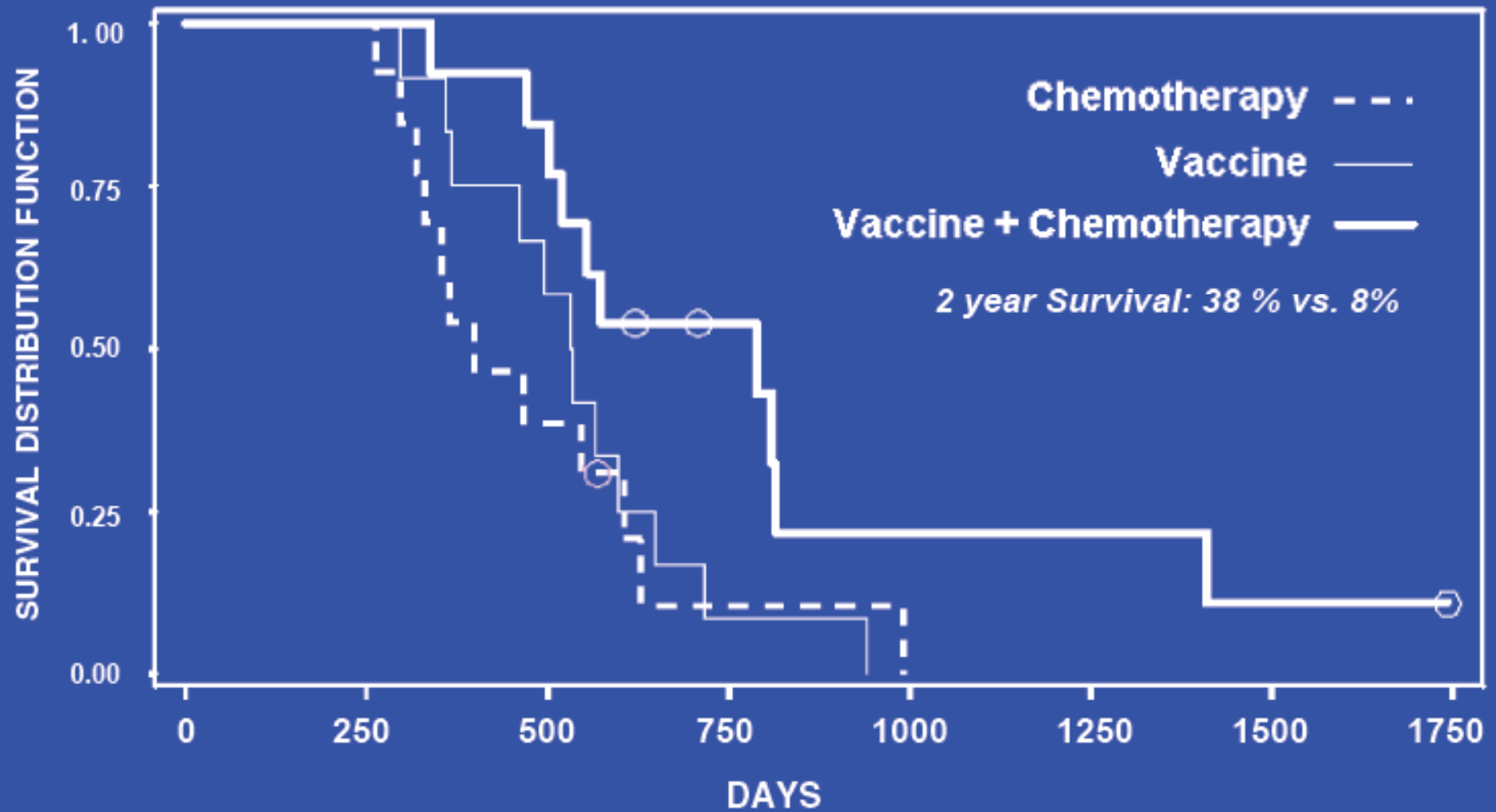
Glioblastoma Multiforme (GBM): Vaccine + Chemotherapy



Glioblastoma Multiforme (GBM): Time to Progression



Glioblastoma Multiforme (GBM): Survival Graph



Convection Enhanced Delivery (CED)

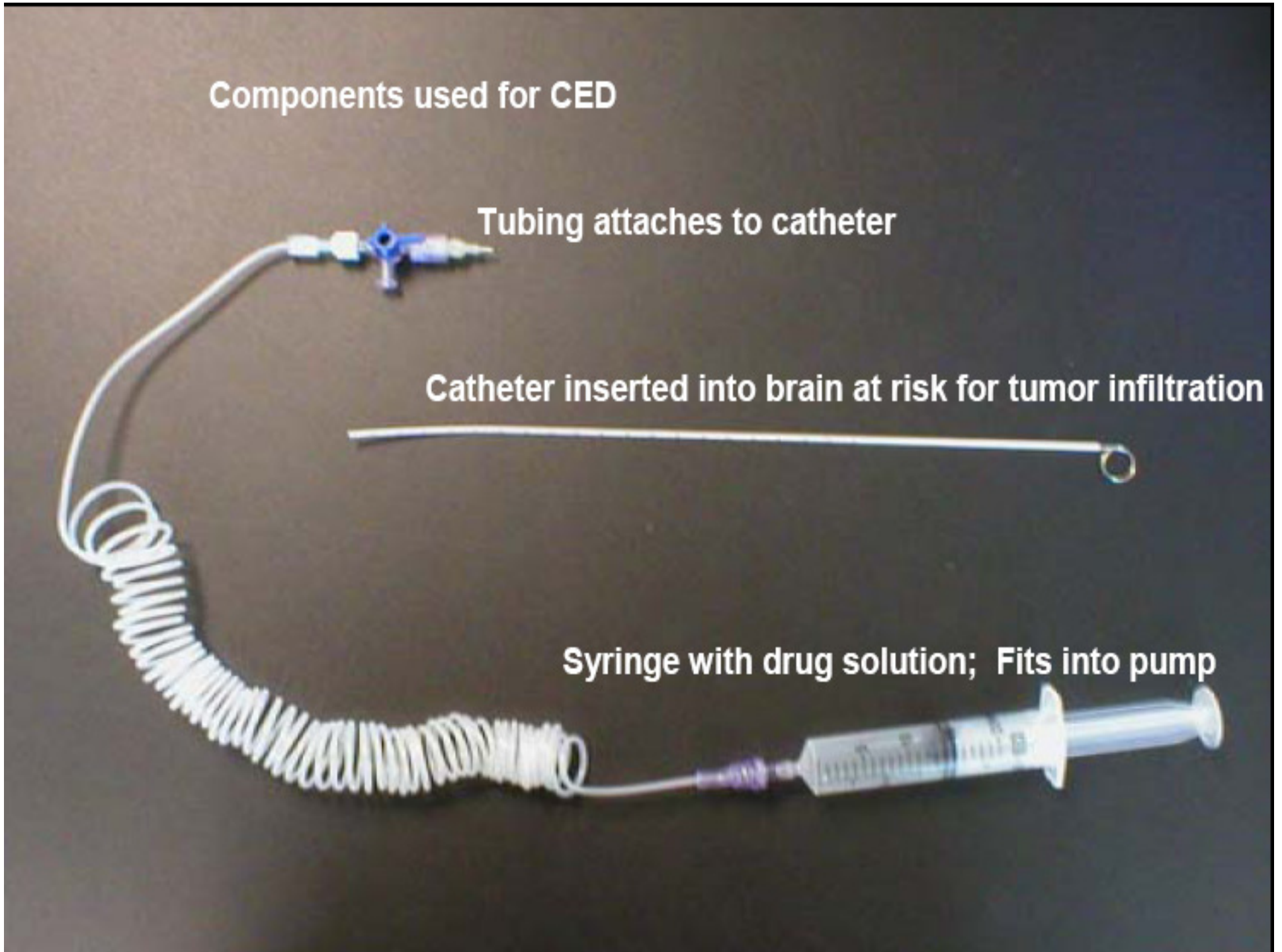
- Convection Enhanced Delivery (CED) is an experimental method to introduce the drug into the area at risk for tumor growth after resection
- By- passes the blood-brain barrier
- Method utilizes placement of catheters, 2-4 small tubes, after the resection
- A microinfusion pump is used to push the drug solution slowly to the brain area at risk of remaining tumor cells using positive pressure
- Infusion lasts for four days while in the hospital

Components used for CED

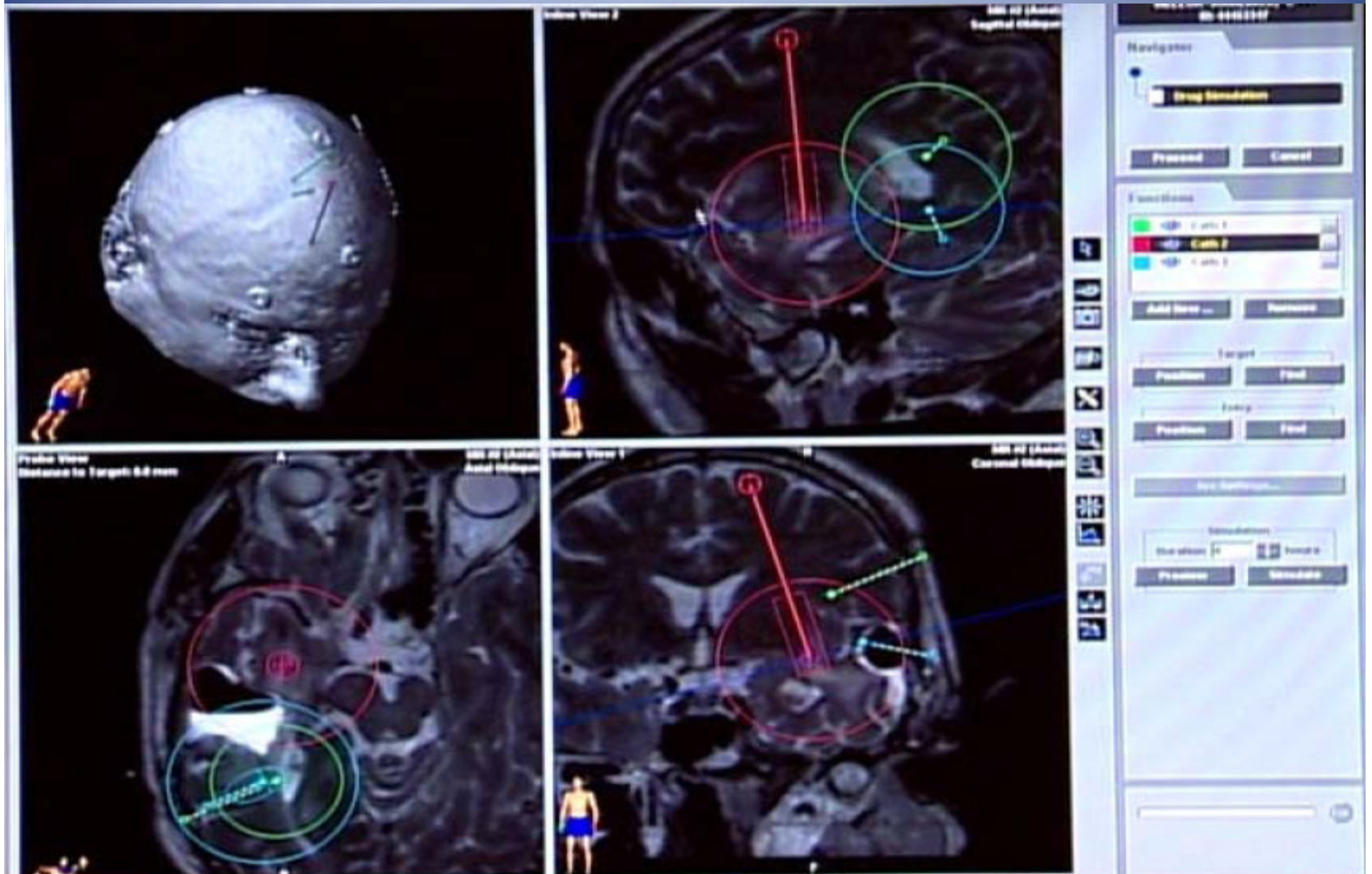
Tubing attaches to catheter

Catheter inserted into brain at risk for tumor infiltration

Syringe with drug solution; Fits into pump



Target Selection



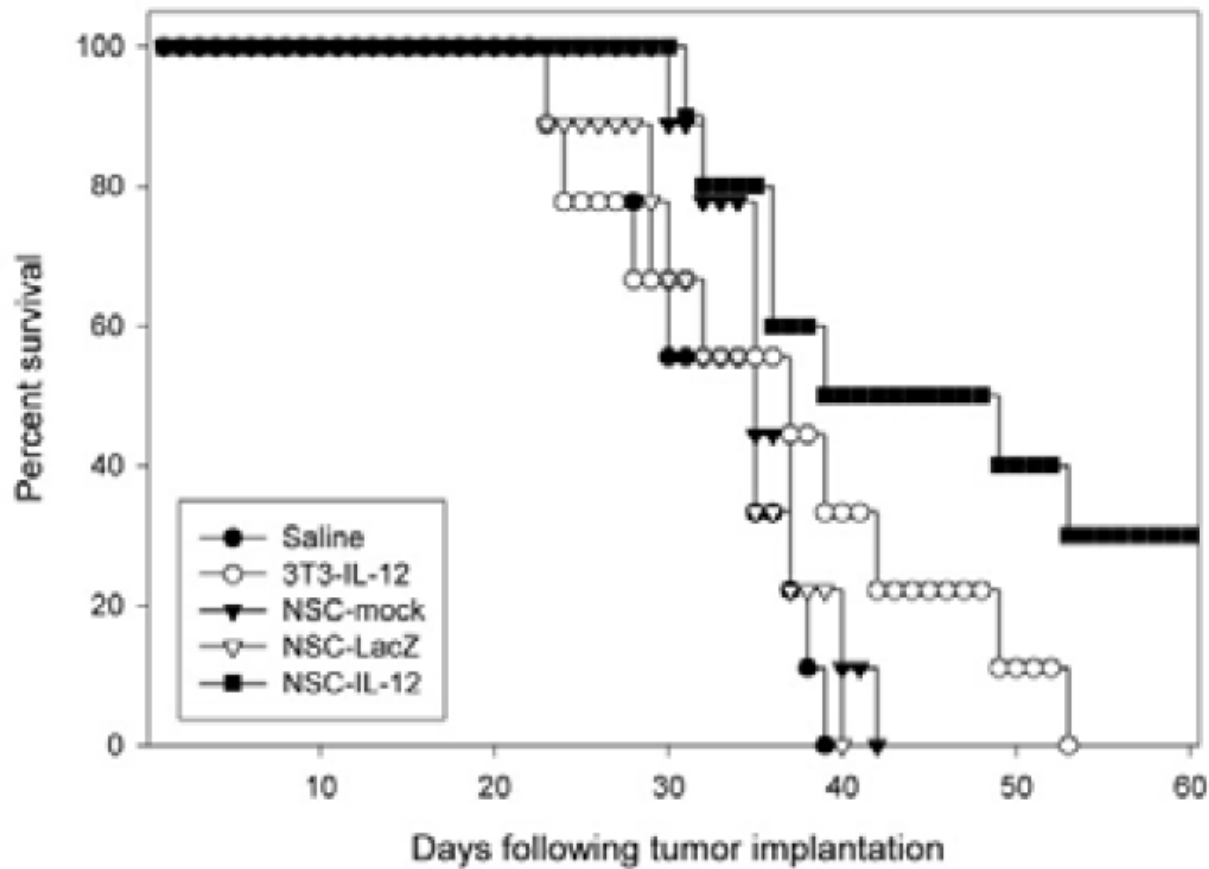
Stem Cells

- Demonstrate ability to track tumor cells through brain and deliver toxic payload
- Now able to harvest neural stem cells from bone marrow. A potential therapeutic source for neural stem cells
- Neural stem cell genetically engineered to secrete IL-12
- Enhanced antitumor immune response
- Increased survival

Stem Cells

- High grade gliomas are highly infiltrative neoplasms
- Tumor outgrowths and microsatellites can serve as microscopic reservoirs for tumor recurrence - despite resection of substantial “normal” peritumoral tissue.
- Current therapeutic strategies fail to adequately target tumor pockets .
- An immortalized neural stem cell (NSC) line demonstrated extensive tropism for *in vivo intracranial pathology including glioma. (K.S.Aboody et al, Cancer Research 62:5627-5694,2002)*
- The migratory characteristics of NSC may provide them with the ability to therapeutically target inaccessible tumor pockets

NSC-IL-12 therapy prolonged survival compared to controls



Conclusions

- Primary NSC can be engineered with close to 100% efficiency using adenoviral mediated gene transfer
- NSC demonstrate extensive and potent tropism for disseminating intracranial glioma cells
- Migratory, tumor “tracking” NSC serve as superior therapeutic protein delivery vehicles compared to non-migratory fibroblasts
- NSC-IL-12 therapy can induce strong cytotoxic T-cell responses against tumor outgrowths and microsattelites, whereas 3T3-IL-12 cannot

Molecular Alterations in Gliomas

Self-sufficiency in growth signals

- EGF/EGFR
- PDGF/PDGFR
- Ras

Sustained Angiogenesis

- VEGF/VEGFR
- bFGF
- HIF-1a
- EGF/EGFR

Evading apoptosis

- p53 mutation
- bcl-2
- PI3 Kinase-AKT
- PTEN
- c-Myc

Limitless Replicative Potential

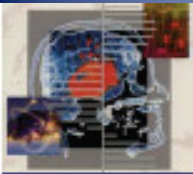
- Telomerase reactivation

Insensitivity to Growth inhibitory signals

- pRb
- p16
- TGF β

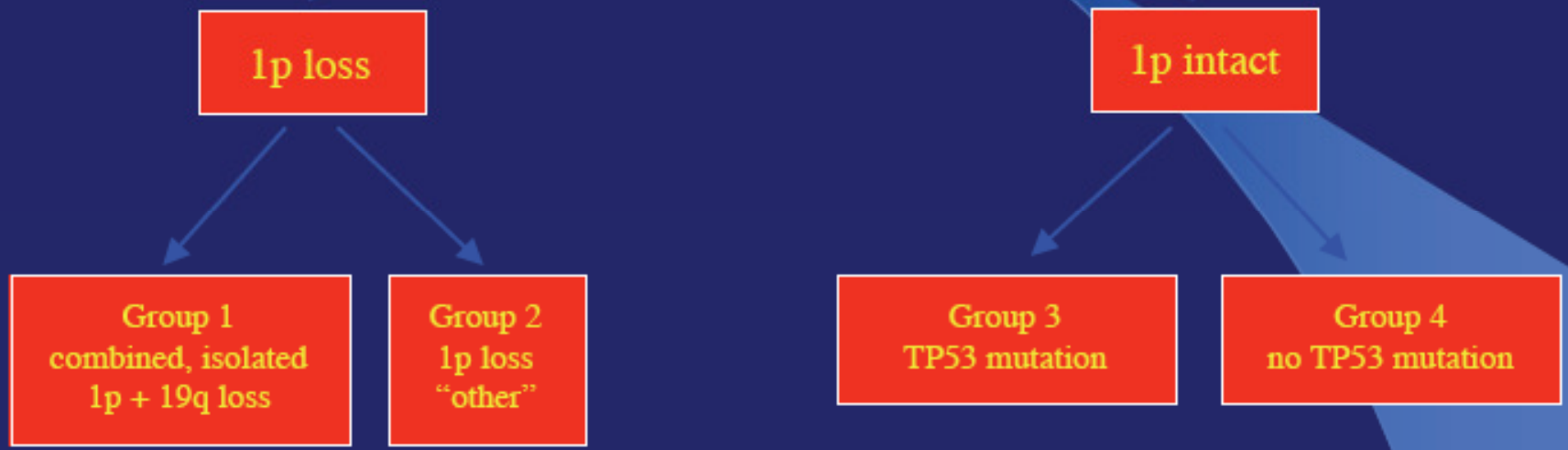
Tissue Invasion

- Matrix Metalloproteases



Glioma chemosensitivity is associated with genetic abnormalities:
1p32-36, 19q LOH

anaplastic oligodendrogliomas, astrocytomas



Response rate:	100%	100%	33%	18%
Response duration:	>31 mos	11 mos	7 mos	5 mos
Survival:	>123 mos	71 mos	71 mos	16 mos

Adapted from Louis, Holland & Cairncross, 2001. *Am J Pathol* 159:779-786.

Targeted Therapy

- What are these targets?
 - VEGF (vascular endothelial growth factor)
 - EGF (epidermal growth factor)
 - Genetic mutations (RAS, RAF)
 - mTOR pathway
 - Etc.

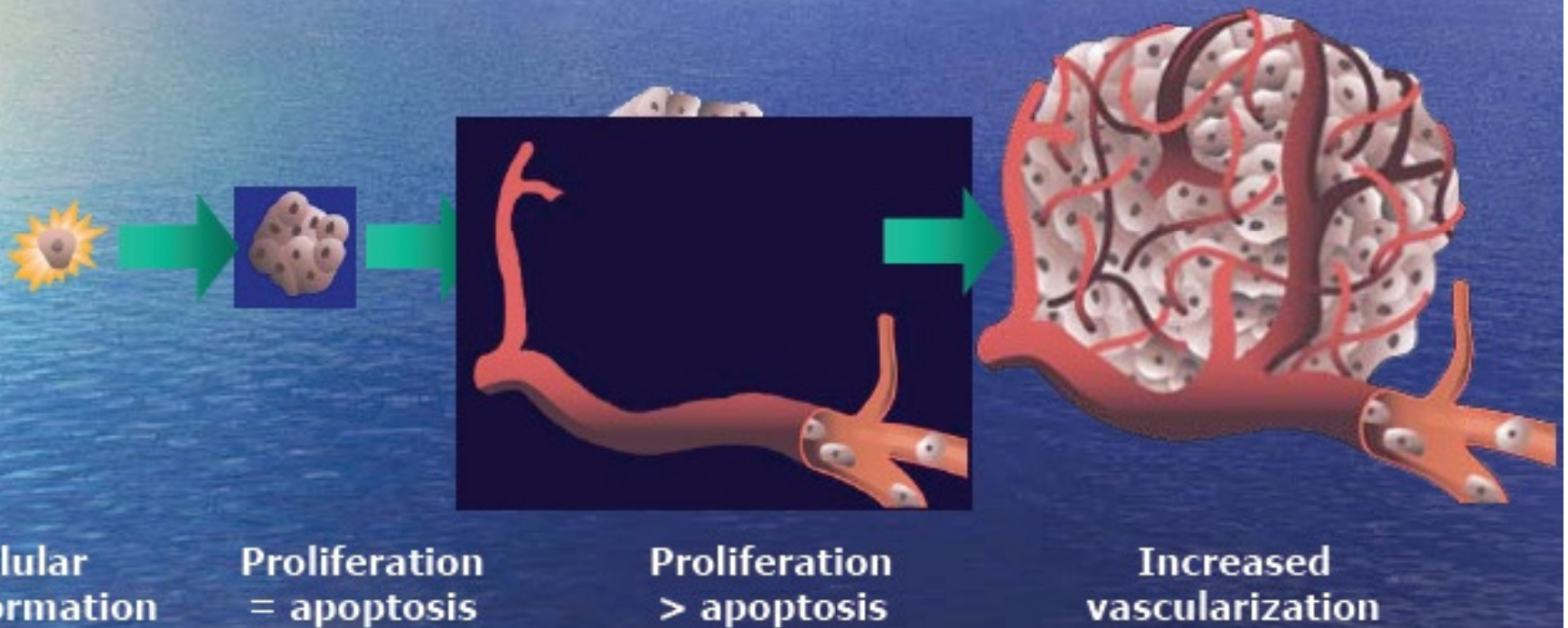
How do we target?

- Monoclonal Antibodies (IV)
 - Literally bind to the cell surface and block
 - Avastin
- Small Molecule (oral)
 - Bind to cell surface and block
 - I.e. Tarceva
- Tyrosine kinase inhibitors (oral)
 - Block a pathway in the cell
 - I.e. Gleevec
- Multi-kinase inhibitors (oral)
 - Block multiple pathways in the cell
 - i.e. Nexavar/Sorafenib, Sutent/Sunitinib
- Immunomodulators (oral)
 - Unknown mechanism
 - I.e. Thalomid, revlimid

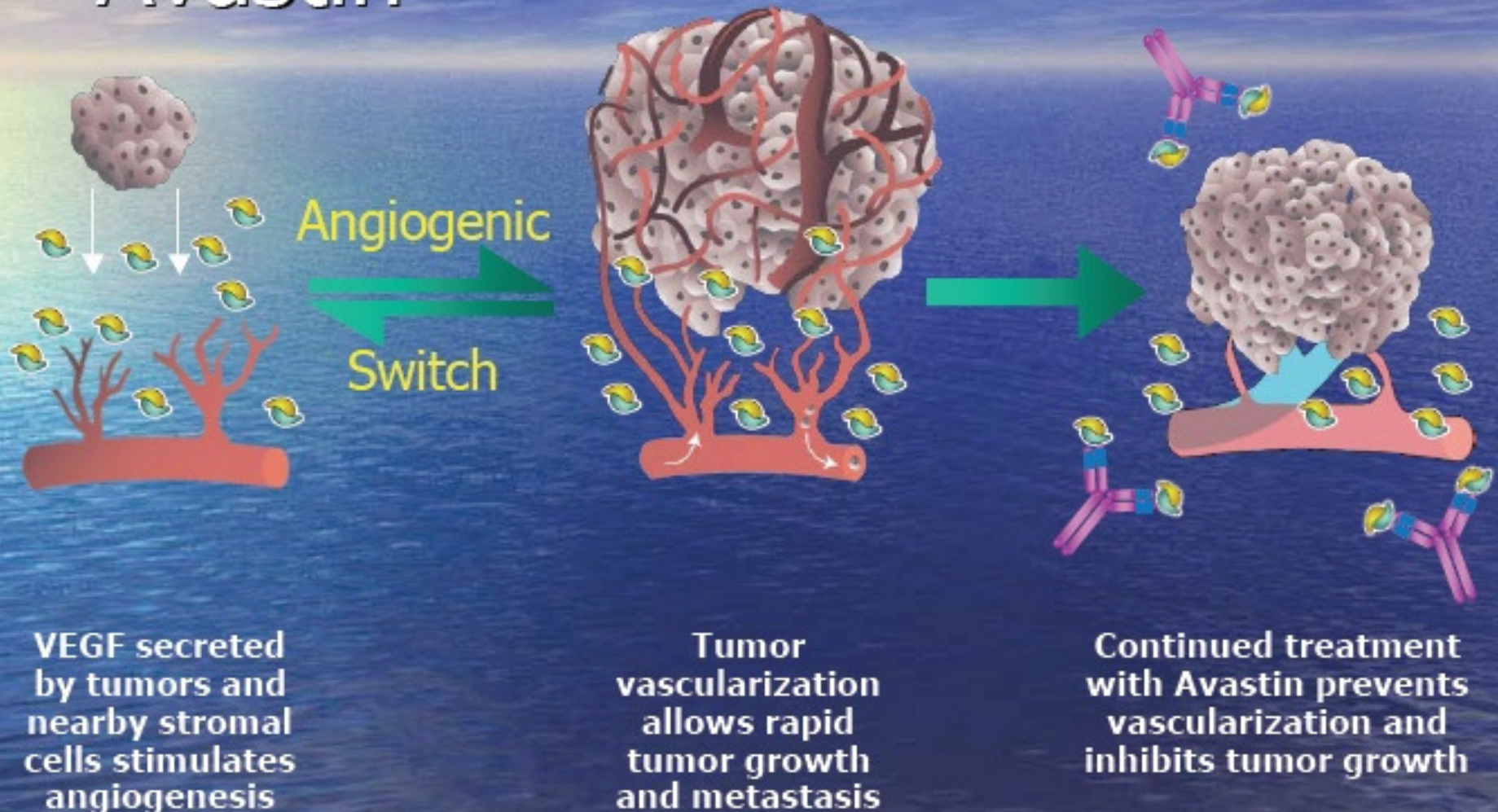
Is there a target in Gliomas?

- Glioblastoma Multiforme (GBM)
 - Pathologically show increased blood vessel density
 - Laboratory models and animal models predict enhanced angiogenesis (increased blood vessels) as a mode of growth.

Characteristics of Cancer



Antiangiogenic Therapy With Avastin



Anti-angiogenic Drugs

- Anti-angiogenic drugs inhibit new blood vessel formation
 - Thalidomide
 - Avastin (rhuMAb-VEGF)
 - CC 5013 (Revimid™)
 - PTK 787/ZK 222584
 - EMD-121974
 - ZD6474
 - LY317615 (Enzastaurin)
 - Cannabinoids(inhibit genes needed for production of VEGF)

Cell Signal Transduction

- Enzymes within the cell control
 - Growth
 - Function
 - Resistance to standard treatment
- Drugs that block or alter these enzymes inhibit tumor cell growth and resistance to treatment

Cell Signal Transduction Modulators

Enzyme	Drug
O6-alkylguanine-DNA alkyltransferase	O6 benzylguanine
Farnesyl transferase inhibitor	R115777 (Zarnestra)
Inhibition of protein kinase C	Tamoxifen Bryostatin
Protein kinase C-alpha	LY900003 (ISIS 3521)
Inhibition of enzyme mTOR	Rapamycin (tablets) CCI-779 (iv) RAD001

Growth Factor Inhibitors

- Normal cell growth is controlled by growth factors
 - Vascular endothelial growth factor
 - Platelet derived growth factor
 - Tyrosine kinase
 - Epidermal growth factor
- Blocking the production or action of these growth factors halts tumor growth

Growth Factor Inhibitors

- ZD 1839 (Iressa)
- OSI 774 (Tarceva)
- AEE788 (EGFR)
- STI 571 (Gleevec)
- SU 5416
- Transtuxumab (Herceptin)
- SB431542 (TGF-beta)

Gefitinib (Iressa)

- Growth factors of the epidermal growth factor (EGF) family have been implicated in cancer development and progression
- In GBM, the epidermal growth factor receptor (EGFR) contributes to malignant progression
- Gefitinib inhibits EGFR
 - Inhibits cell division and causes cell death
- Ability to cross the blood-tumor barrier
 - Responses of intracranial metastases secondary to non-small cell lung cancer

Mammalian Target of Rapamycin (mTOR)

- mTOR plays a significant role in promoting tumor growth via multiple pathways
- Rapamycin binds intracellularly to FKBP12
- The resultant complex inhibits mTOR activity

EGFR and mTOR Inhibitors

- The potential synergistic effect of a combination of EGFR and mTOR inhibitors
 - In cervical squamous cell carcinomas a combination of mTOR and EGFR inhibition resulted in significant tumor growth delay
 - Combination treatment with mTOR and EGFR inhibitors have resulted in a synergistic effect that suppressed growth of glioma cell lines

Nuclear Hormone Receptor (NHR) Family PPAR γ

- Expressed in adipose tissue
- Expressed prominently in a variety of cancer cells
- possesses tumor suppressive activity
- Pioglitazone is a highly selective agonist for PPAR γ
 - FDA approved for type 2 diabetes

Isotretinoin (Accutane)

- Another NHR, the retinoic acid receptor (RAR), is expressed in glioblastoma cells.
- The ligand for RAR, all-trans retinoic acid (ATRA), inhibits growth of glioblastoma cell in vitro.

Phase I Trial of Conditionally
Replication-Competent Adenovirus
(Delta-24-RGD-4C) for Recurrent
Malignant Gliomas

Charles Conrad, M.D. – Co-P.I

Frederick Lang, M.D. – Co-P.I.

Juan Fueyo, M.D. – Co-investigator

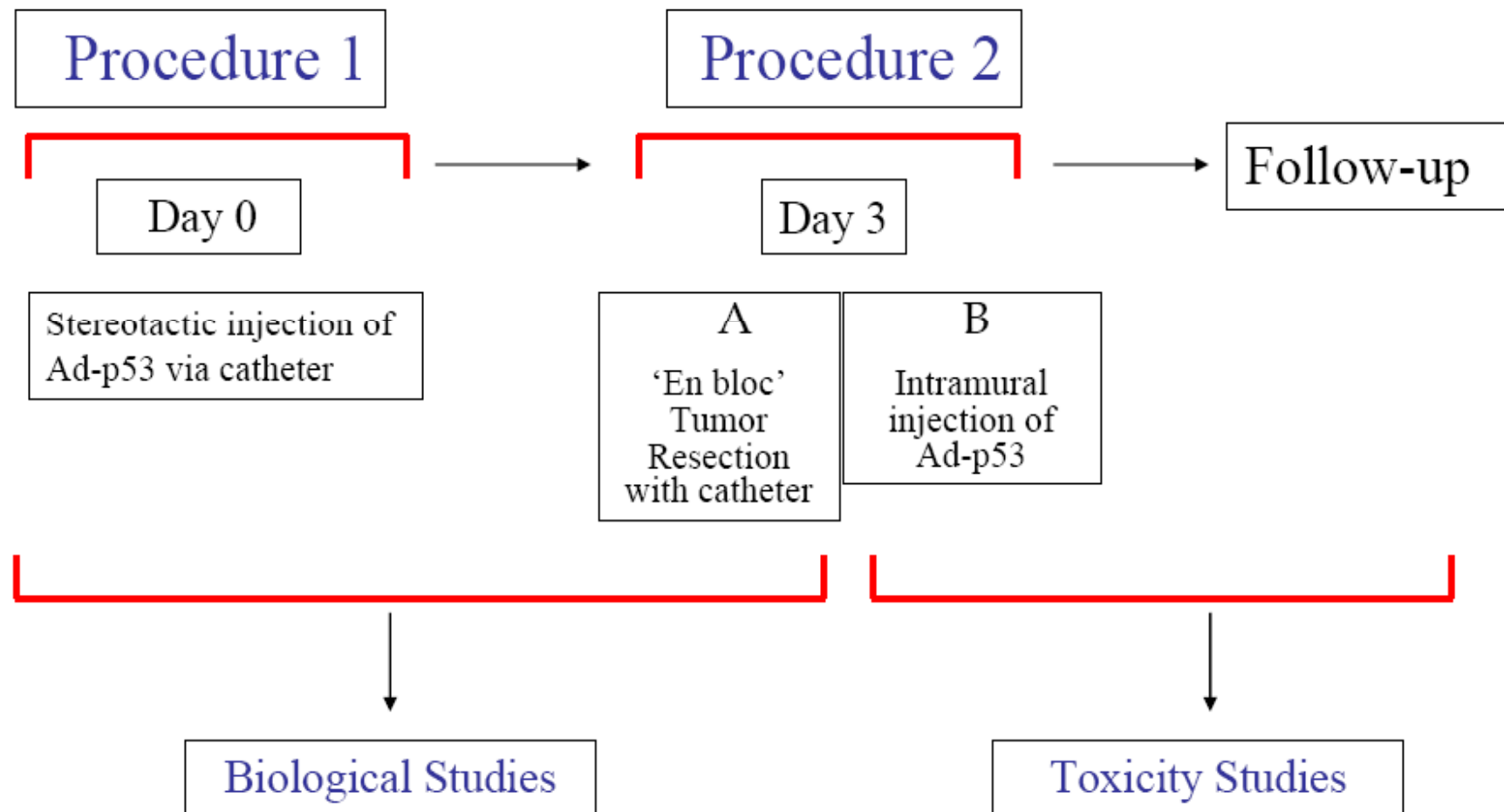
W. K. Alfred Yung, M.D. – Co-investigator

NIH Recombinant DNA Advisory Committee
Review (March 10, 2004)

Ad-p53 Trial: Objectives

- To determine the qualitative and quantitative **toxicity of Adp53** administered by intratumoral injection.
- To determine the **maximum tolerated dose (MTD)** of Ad-p53 administered by intratumoral injection in patients with recurrent malignant gliomas.
- To determine the **biological effects at the molecular level of** intratumoral administration of replication-deficient adenovirus vector containing wild-type p53 gene (Ad-p53) in human malignant gliomas by analyzing the expression and distribution of exogenous p53 protein.

Ad-p53 Clinical Trial for Recurrent Malignant Gliomas: Treatment Schematic



Ad-p53 Clinical Trial:

- No significant toxicities were observed
- Maximum Tolerated Dose (MTD) was reached at 1×10^{12} viral particles
- Two-stage design was well tolerated and without complications
- No Ad-p53 virus was detected systemically
 - (blood, urine, sputum, or feces)
- **Selectivity:** Ad-p53 can infect and transduce exogenous p53 in both normal and tumor cells.
- **Delivery:** With the injection technique used, p53 distribution is limited to 5-6 mm from injection site.

Gene Therapy

- Process by which genetic material is transferred to somatic cells to bring about a therapeutic effect
- Can be achieved by either replacing defective or missing genes or introducing new functions to the host's cells
- Approaches of gene therapy differ in the selection of the candidate gene or genetic material for a specific biologic effect, mode of delivery (in vivo/ vitro), & vehicle for transfer

- Most gene therapy approaches for brain tumors have used viruses for in vivo transfer
- Retroviral and Adenovirus vectors used mainly
- Gene therapy approaches:
 - Gene transfer mediated drug targeting (suicide gene therapy)
 - Transfer of tumor suppressor genes and cell cycle modulators
 - Genetic immune modulation
 - Antiangiogenic gene therapy
 - Use of cytopathic-oncolytic viruses

Antisense drugs

- Approx. 30,000 genes in our genome can be transcribed into about 85,000 different mRNA,
- Each used in the cell as a template to synthesize a different protein.
- Conventional pharmaceutical drugs (small chemicals), peptides, or proteins (for example, hormones), and antibodies (which are very large proteins) typically bind to the target protein directly to treat a disease.
- Antisense drugs are designed to bind to the mRNA of a target protein, inhibiting the protein production process

Antisense Drug Discovery and Research & Development

THERAPEUTIC GOAL

Identify Target Gene Sequence

Design Antisense Inhibitor

Synthesis of Antisense Inhibitor

Cell Culture Screening

Lead Inhibitor of Target Gene

Sufficient Quantities of Drug

Pilot Animal Efficacy
& Dose Response, Time
Course Studies

Toxicology Animals
Phase I, II, III
market

- Antisense compounds are designed to have the right nucleotide sequence to bind specifically to and interfere with its associated mRNA
- inhibit the production of protein
- offers almost unlimited scope for the development of new and highly specific therapeutics.
- AIIMS – trial for antisense drug carried out
 - 17 patients were enrolled
 - Results yet to be disclosed

Clinical Trials of Cotara

- *What is Cotara?*
- Cotara is an experimental new treatment for brain cancer
- links a radioactive substance designed for medical uses-->a radioactive isotope—to a targeted monoclonal antibody.
- This monoclonal antibody is designed to target—to bind to—a type of DNA that is exposed only on dead and dying cells

- Cancer tumors have a significant number of dead and dying cells at their center,
- Cotara's targeting mechanism enables it to hone in on these dying tumor cells and deliver its radioactive "payload" to the center of the tumor.
- Cotara then literally destroys the tumor "from the inside out" by delivering radiation directly to the cells inside the tumor mass.

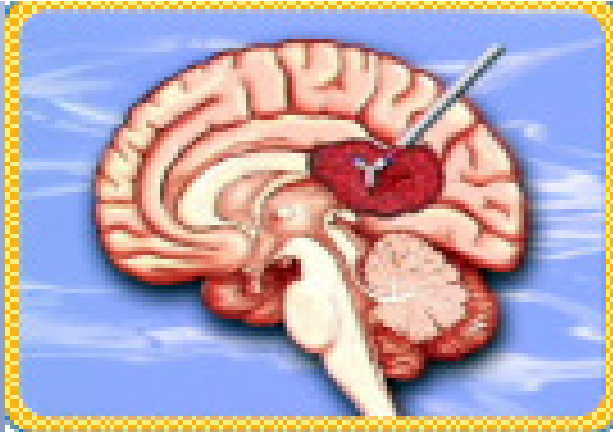
- **Patient Eligibility:**

- Any pt with 1st or 2nd recurrence of GBM

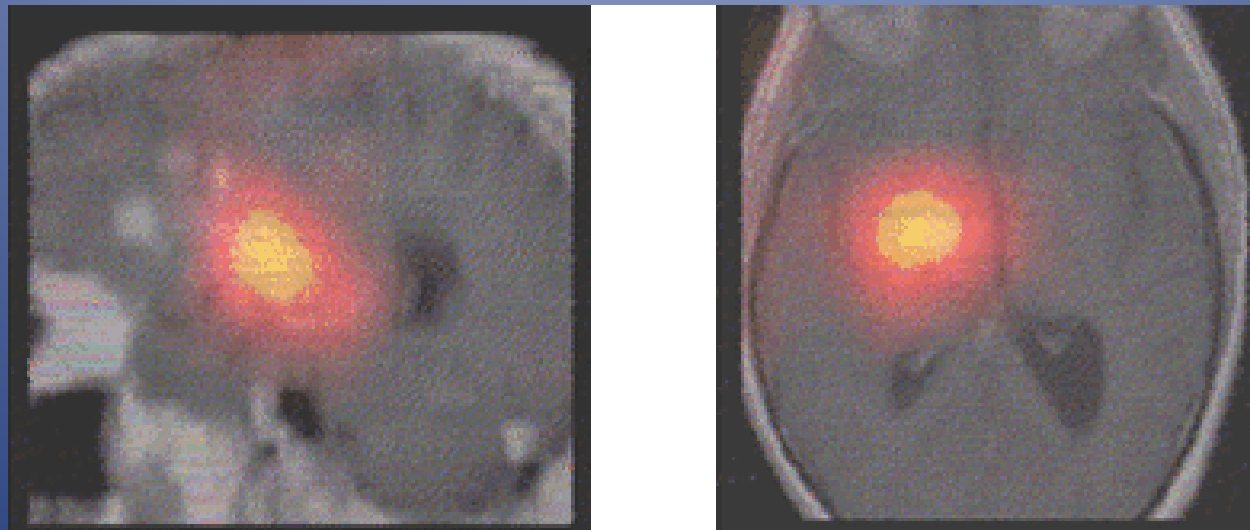
- Patients who have had prior surgery, chemotherapy, or some forms of radiation treatment may be eligible to participate.

- excluded if they have received radiosurgery, brachytherapy or other local therapies

- Cotara is delivered through a special method, called convection enhanced delivery (CED)
- Uses a catheter to bypass the BBB and target the specific tumor site in the brain.
- AIIMS currently one of the centres for Cotara trials



Cotara® is administered directly into the tumor to increase its safety and tumor-destroying potential



SPECT analysis :Cotara can be easily tracked by scanning devices to be sure it is entering the tumor as seen here, and is not dispersing in healthy tissues.

Next Steps in Therapy of Malignant Brain Tumors

- Decrease or Eliminate need for open craniotomy in our 21st Century ORs. Consider microwave ablation of tumors.
- Insert dendritic cells into necrotic tumor
- Insert stem cells to tract microscopic tumor
- Increase numbers of naïve T cells
- Add Therapies against New Molecular targets
- Combine with Selective opening of blood brain tumor barrier for Drug Delivery

Thank You