1. INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN.

This Investigational New Drug application describes a proposed Phase I clinical trial that will evaluate the toxicity of intra-tumoral administration of an E1A/B-deleted, replication incompetent adenovirus (Ad), containing dominant negative EGFR-CD533, a truncated EGFR (ERBB1) molecule lacking the 533 COOH-terminal amino acids. The ultimate objective will be clinical application of Ad-EGFR-CD533 as a potential radiosensitizing agent in combination with current standard therapy for high-grade malignant gliomas, glioblastoma multiforme (GBM), to include tumor resection followed by post-operative irradiation to doses near 60 Gy and chemotherapy.

The Ad-EGFR-CD533 contains, under control of a CMV promoter, the EGFR-CD533 that has been shown to completely lack any transforming and/or mitogenic activity of EGFR (ERBB1) in autocrine growth regulated human carcinoma and malignant glioma cells. The activity of EGFR-CD533 as a potent radiosensitizing agent will be described below and in Sections xxx of this application.

The Phase I trial will be conducted in the context of standard treatment. The toxicity of Ad-EGFR-CD533 will be assessed at four dose levels. The study will follow a standard Phase I design in which patients will be given increasing doses of Ad-EGFR-CD533 starting at $1 \times 10^{10}$ pfu in log and half log increments, 3 patients per group, will be given to a maximum total dose of $1 \times 10^{13}$ pfu. Dose limiting toxicity (DLT) will be assessed for the entire treatment course following NCI Common Toxicity Criteria (see Protocol for details) in order to establish the maximum tolerable dose (MTD).

Patients with imaging results compatible with recurrent high grade glioma will be subjected to an initial biopsy using a single drill hole through the skull bone. If frozen section analysis is suggestive of GBM, the biopsy access will be used to place a catheter, under stereotactic guidance, ending in the center of the tumor with appropriate distance (specify in cm) from vital structures of the brain if this applies. Upon establishment of the recurrent high grade glioma diagnosis, patients will be infused with Ad-EGFR-CD533 using an established positive pressure continuous infusion method that has been shown to achieve satisfactory and relatively uniform distribution of the infusate within the infused brain/tumor tissue. Three days (72 h) after beginning the infusion the patient will be subjected to craniotomy for standard resection of the tumorous tissues; while multiple samples will be submitted for standard histopathology, substantial tissue samples will be processed for molecular correlative studies examining the following: transduction rate of tumor cells, expression levels of the transgene EGFR-CD533 and its suppression of ERBB1(EGFR) and ERBB2-4 expression/activity. Patients will be followed, as specified in the protocol, for the remaining lifespan or until recurrence.
GBM is one of the most deadly neoplasms currently occurring at an incidence of 2/100,000 equaling 6000 new cases/year, mostly treated at academic medical centers. At the MCV/VCU multidisciplinary Neuro-Oncology Center a minimum of 80 new cases are seen annually. Although the standard surgery/radiotherapy has prolonged the median survival of patients from 6 to 13 months many different attempts of further improving outcome have failed. Currently, an accelerated/concomitant-boost radiation trial, initiated at MCV/VCU Radiation Oncology, has completed accrual in a national Radiation Therapy Oncology Group (RTOG) trial. Besides the dismal prognosis, malignant gliomas are uniquely suited for a trial of local administration of DN EGFR-CD533 since most of these tumors not only over-express EGFR (and other ERBB species) but also express constitutively active mutant receptors, e.g., EGFRvIII, that may not be attacked by other methods of functional EGFR disruption.

Much of the mechanistic evidence for EGFR inhibition as a powerful mode of tumor radiosensitization has been generated by our laboratory. ERBB1 in concert with other ERBB receptor Tyr kinases (RTKs) mediates an immediate, major cytoprotective/pro-survival response involving MAPK/PI3K/AKT and additional downstream cytoplasmic protein kinases. Importantly, the radiation-induced activation of cellular signaling responses can be linked to transcriptional regulation, apoptosis responses and DNA repair through recently established functional relationships between DNA strand break (DSB) recognition/repair and RTK function. A major challenge in attacking RTKs for radiosensitization is posed by the finding from our group that all RTKs expressed by a given tumor cells are simultaneously activated and lead to a broad activation of the cytoplasmic protein kinase network. Most recently we have also demonstrated that compensatory receptor activation responses are occurring upon selective inhibition of EGFR, including activation of ERBB3 and IGF-1R [1]. We have also demonstrated that EGFRvIII is prominently activated by radiation than the EGFR wild-type receptor and that this leads to a 5-fold enhancement of the activation of downstream effectors, such as MAPK [2]. Thus, the selective activation of RTKs, mediating radiorsistance, is complex and is likely to fail if simplistically applied to clinical testing. Relative to mAb C225 and tyrphostin kinase inhibition for EGFR inactivation we have demonstrated that DN EGFR-CD533 acts as an effective pan-ERBB inhibitor, including IGF-1R, in tumor cells. Thus, in future studies it may be important to establish the RTK expression profile of tumor cells prior to treatment. We carried our studies on the mechanisms of EGFR-CD533 radiosensitization through preclinical in vivo studies using various human carcinoma and malignant glioma cell xenograft tumors and have established at the molecular levels that the mechanisms established in vitro apply in vivo. While the requirement for local administration of Ad-EGFR-CD533 may represent a disadvantage, its prolonged expression for at least 28 days after a single in vivo administration has to be viewed as a major advantage considering the apparent systemic toxicity of C225 and the tyrphostin ZD1839 at concentration effective in tumors.