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TITLE: Convection-Enhanced Delivery (CED) in an Animal Model of Malignant Peripheral Nerve Sheath Tumors and Plexiform Neurofibromas

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14. ABSTRACT Our experiments for the current study are ongoing. Unfortunately, work will not be completed within the time frame of the DOD grant. We have encountered several challenges in completing this. Work on this project was initially delayed by several months secondary to difficulty in obtaining regulatory approval from both our own IACUC and the DOD. Additionally, though we were able to establish and maintain an MPNST cell line suitable for our needs, we have been unable to maintain a cell line for benign plexiform neurofibroma. We have not been able to successfully establish the xenograft within the sciatic nerve. Using the MPNST cell line we were able to perform in vitro studies, comparing efficacy of erlotinib, rapamycin and imatinib in inhibition of cell proliferation and established that erlotinib is the most potent inhibitor for this particular cell line. The cell line we are using is aggressive and grows rapidly as a flank xenograft though we have not been able to successfully establish the xenograft within the sciatic nerve.					
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Convection enhanced delivery (CED) in an animal model of malignant peripheral nerve sheath tumors and plexiform neurofibromas.

PI: Kaleb Yohay

Introduction

Neurofibromatosis Type 1 (NF1) is a common autosomal dominant neurocutaneous disorder that results in a predisposition to the development of benign and malignant tumors. Plexiform neurofibromas (PN) are complex, often large tumors that usually involve multiple nerves and may cause significant morbidity. Complete surgical resection is rarely feasible. PNs may undergo malignant transformation to become Malignant Peripheral Nerve Sheath Tumors (MPNST). MPNSTs are aggressive, often fatal sarcomas and are the leading cause of death in adults with NF. Currently there are no available effective medical therapies for PNs or MPNST. EGFR is not normally expressed by Schwann cells, however, EGFR expression has been demonstrated in association with the development of benign and malignant peripheral nerve sheath tumors, making this receptor a potential therapeutic target. Effective *in vivo* delivery of traditional chemotherapy and newer molecularly targeted therapeutics to tumors of the nervous system remains a significant obstacle. Systemic delivery is restricted by systemic toxicity, non-targeted distribution and subtherapeutic tumor levels of potentially curative agents. Interstitial infusion, also referred to as convection-enhanced delivery (CED), is a mode of local drug delivery which relies on a pressure-dependent gradient to accomplish uniform infusate dispersion and high drug concentrations directly in tumor or tissue while avoiding systemic exposure and hence dose-limiting toxicity.

In this study we had proposed that CED may be used as a safe, reliable and effective means of delivery of therapeutic agents in animal models of plexiform neurofibromas and malignant peripheral nerve sheath tumors.

Specific Aim 1: To use an animal model to determine the distribution of macromolecules delivered to intraneural PNs and MPNST via CED.

Design: Orthotopic xenograft models of sciatic intraneural NF1 MPNST and PNs in scid mice as described by Perrin et al. (2007a, 2007b) were to be used. Six to eight weeks after implantation, the location and extent of tumor development within the sciatic nerve will be assessed by MRI. The sciatic nerve tumor will be surgically exposed and a silica infusion catheter will be placed within the tumor. HRP-labeled albumin or gadolinium-albumin will be infused. Several infusion rates and volumes will be assessed. Volume of distribution within the tumor and sciatic nerve will be assessed with immunohistochemistry and *in vivo* MRI.

Specific Aim 2: To determine the efficacy CED of the epidermal growth factor receptor (EGFR) inhibitor erlotinib in animal models of intraneural PNs and MPNST.

Design: Orthotopic xenograft models of sciatic intraneural NF1 MPNST and PNs in scid mice were to be used. EGFR positive cell lines were to be used for implantation. Six to eight weeks after implantation, the location and extent of tumor development within the sciatic nerve will be assessed by MRI. Erlotinib or vehicle will be infused into the tumor via CED using the infusion parameters optimized from the results of Specific Aim 1. Tumor growth and angiogenesis will be monitored by *in vivo* contrast enhanced MRI at several time points after infusion and tumor growth will be compared in erlotinib versus vehicle treated animals. Phosphorylated Akt and EGFR will be measured in tumor samples to assess for biologic activity of the erlotinib.

Body

Our experiments for the current study are ongoing. Unfortunately, work will not be completed within the time frame of the DOD grant. However, we are in the process of acquiring additional funding through a private source that we hope will allow us to complete the studies as planned. At the time of completion of the study we will provide the DOD with detailed data and results.

We have encountered several challenges in completing this study as proposed. Several factors have contributed to our inability complete the work as planned. Work on this project was initially delayed by several months secondary to difficulty in obtaining regulatory approval from both our own IACUC and the DOD. Additionally, though we have been able to establish and maintain an MPNST cell line suitable for our needs, we have been unable to maintain a cell line for benign plexiform neurofibroma. As such, we modified our proposal to utilize only an MPNST xenograft model and not a PN xenograft model. Additionally, in order to maximize our chance of success in the treatment efficacy experiments, we spent additional time testing agents to compare efficacy to erlotinib in vitro, including rapamycin and imatinib.

At this point we have characterized our MPNST cell line, established that erlotinib is the most promising of the three agents tested for this particular cell line, and we established the surgical procedures necessary for the sciatic xenograft model. The cell line we are using is aggressive and grows rapidly as a flank xenograft. However, to date we have not been able to successfully establish the xenograft within the sciatic nerve.

Key Research Accomplishments

Characterization of the status of signaling molecules of MPNST cells: Phosphorylated EGFR positive status was confirmed for our cell line.

Determination of the surgical procedures: Utilizing mouse cadavers, we have refined our techniques for tumor cell implantation and drug delivery using convection-enhanced delivery (CED).

Relative Growth of MPNST cells in vivo treated with rapamycin, imatinib or erlotinib: Elotinib demonstrated the most potent growth inhibition in vitro of the agents tested (See Supporting DataFig 1).

Establishment of a MPNST flank xenograft tumor model: Utilizing our MPNST cell line we were unable to establish a sciatic xenograft model but were able to establish a successful flank xenograft model (see Supporting Data Fig 2).

Reportable Outcomes

None.

Conclusions

We were unable to complete the experiments as proposed for the reasons detailed above. We cannot make any conclusions about the relative feasibility or efficacy of convection enhanced delivery in malignant peripheral nerve sheath tumors or plexiform neurofibromas.

References:

Perrin GQ, Fishbein L, Thomson SA, et al., Plexiform-like neurofibromas develop in the mouse by intraneural xenograft of an NF1 tumor-derived Schwann cell line. J Neurosci Res, 2007. 85(6): p. 1347-1357.

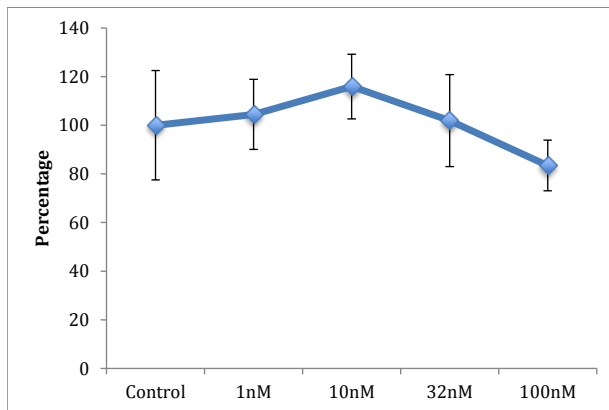
Perrin GQ, Li H, Fishbein L, et al., An orthotopic xenograft model of intraneural NF1 MPNST suggests a potential association between steroid hormones and tumor cell proliferation. Lab Invest, 2007. 87(11): p. 1092-1102.

Appendices: None.

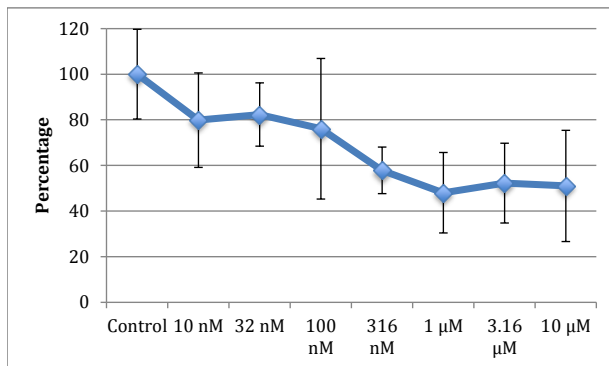
Supporting Data:

Figure 1:

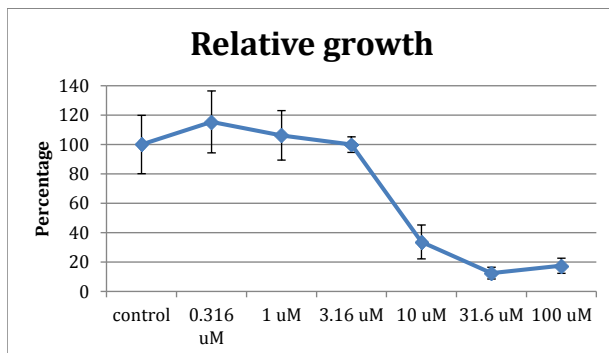
Relative Growth of MPNST cells in vivo treated with rapamycin, imatinib or erlotinib.



Rapamycin



Imatinib



Erlotinib (least soluble; max = 50um)

Figure 2:

MPNST flank xenograft tumor volume.

