

## **Biodistribution and Tumor Specificity of a Multimutated Adenovirus**

Scott J. King and Timothy P. Cripe

Division of Pediatric Hematology/Oncology, Department of Pediatrics

**Introduction:** Conditionally replicative adenoviruses hold great promise as a selective oncolytic therapy, but are dependent on expression of viral receptors on the cancer cell. The major limitation to the use of these viruses as therapy for many cancers is low expression of the adenovirus receptor, CAR, resulting in a decreased efficacy of adenoviral replication. In addition, the vast majority of adenovirus is found in the liver following systemic administration. Adenoviruses have been genetically modified to bypass the requirement for CAR in an effort to achieve improved infection of CAR(-) cells. One common modification is the insertion of a positively charged, lysine-rich polypeptide, designed to improve binding to negatively charged cell surface molecules such as heparan sulfates. We have previously demonstrated this modification improves viral uptake into rhabdomyosarcoma cells that lack CAR expression by 10-fold. The biodistribution and tumor specificity of such modified viruses, however, is largely unknown. **Rationale/Hypothesis:** Due to increased uptake by rhabdomyosarcoma cells, we hypothesized that the polylysine modification on the adenovirus fiber knob would increase delivery of virus to a xenograft rhabdomyosarcoma tumor following intravenous administration. In addition, because the virus we used selectively replicates in tumor cells, we hypothesized that amplification of the conditionally replicative virus would occur over time selectively in the tumor compared with other tissues. **Methods:** Viruses were injected via tail vein into nude mice harboring xenograft tumors. Mouse tissues and the xenograft were harvested at 24 and 96 hours. DNA was extracted from tissue. Using quantitative PCR (qPCR), the biodistribution of a wild type virus and the polylysine-modified virus were compared. **Results:** Tumors were grown, mice were successfully injected with virus, and DNA was prepared. Initial qPCR data with the modified virus at 24 hours showed highest levels in liver, cardiac and skeletal muscle, and lung. No virus was detectable in the tumor. This pattern of distribution differs significantly from other studies that demonstrated unmodified virus typically is found mostly in the liver (>95%), with small amounts in the lung. Confirmation of these results and of those with the unmodified virus, however, were inconclusive, due to repeated contamination of the viral qPCR reaction (shown by positive signal in negative controls). Initial efforts to eradicate the contamination using new PCR reagents, a new location for pipetting, and new procedures were not successful in the allotted time. **Conclusions:** Our data preliminarily suggest that the insertion of a polylysine peptide in the adenovirus fiber knobs dramatically alters its systemic biodistribution. The sensitivity of qPCR is sufficiently high that contamination easily becomes a significant technical burden.