Introduction
Choline kinase (Chk), an enzyme that converts free choline to phosphocholine (PC), is overexpressed in several cancers, including breast cancer [1-2]. Chk contributes to the increased phosphocholine (PC) and total choline (tCho) levels in breast tumors, which can be detected by $^3$P and $^1$H magnetic resonance spectroscopy (MRS) [1-2]. Because Chk is associated with tumor aggressiveness, it can be used as a target for anticancer therapies using gene silencing by RNA interference [1]. We are currently developing systemic RNA interference-based anticancer therapies that target Chk for breast cancer treatment [3]. Because lentiviral vectors have emerged as vectors of choice for long-term, stable in vitro and in vivo gene transfer, we have generated an HIV-based lentivirus to target Chk in vitro and in vivo in MDA-MB-231 breast cancer cells and xenografts [3]. This lentivirus produces double-stranded short hairpin RNA (shRNA) specific to Chk (shRNA-chk), and efficiently transduces MDA-MB-231 breast tumor xenografts as previously shown [3]. Here we have utilized in vivo single-voxel $^3$P MRS to monitor the functional efficacy of lentiviral-mediated Chk silencing following intravenous injection of this lentivirus in mice bearing MDA-MB-231 breast tumor xenografts. We optimized the treatment protocol with this lentiviral vector, which was monitored by quantitation of the ratios of the signal integrals from PC/$^\beta$-nucleoside triphosphate (NTP) and phosphomonoester (PME)/$^\beta$-NTP. Treatment efficacy was monitored by measuring tumor volumes.

Results
The optimal treatment protocol was achieved by two intravenous injections of lentiviral particles encoding for shRNA-chk per week (on days 4 and 7), based on the evaluation of the $^3$P MR spectra and tumor growth curves. Phosphorus MRS was performed twice a week on each day after injecting the lentiviral particles expressing shRNA-chk as compared to controls (Fig. 1). In vivo $^3$P MRS demonstrated that the PME/$^\beta$-NTP (Fig. 2) and PC/$^\beta$-NTP (Fig. 3) ratios significantly decreased in shRNA-chk lentivirus-treated tumors compared to shRNA-luc treated controls. All tumors displayed significant transduction as measured by EGFP expression.

Discussion
Here we have demonstrated that tumoral Chk silencing can be achieved by bi-weekly intravenous administration of lentiviral particles expressing shRNA-chk into SCID mice bearing human MDA-MB-231 breast tumor xenografts. This lentiviral particle-mediated Chk-targeted therapy significantly reduced tumor growth, and decreased proliferation in vivo, which is in good agreement with our previous in vitro studies [1]. Systemic lentiviral shRNA-chk delivery in vivo decreased the tumoral PC and PME levels as monitored by PC/$^\beta$-NTP and PME/$^\beta$-NTP ratios from in vivo $^3$P MR spectra. Decreased PC and PME levels following shRNA-chk delivery are in good agreement with our previous studies [1]. Systemic viral particle-mediated Chk-targeted gene therapy may be feasible for clinical translation, and can be monitored by single-voxel $^3$P MRS in vivo.