RRx-001 Oxidation of Redox Sensitive Protein Thiols in Tumors Measured by Gd-LC7-SH Enhanced MRI In Preclinical Tumor Models

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Introduction. RRx-001 (**fig. 1a**) is a member of the novel dinitroazetidine-containing class of anticancer agents that profoundly perturbs the thiol redox potential of the cancer cell while subjecting it to damaging ROS/RNS and acting as an epigenetic modifier. Pre-clinical studies indicate that RRx-001 selectively alkylates glutathione and a specific thiol on hemoglobin, resulting in a pro-oxidant effect in tumors [1,2]. Thiol-containing DOTA-based chelates of gadolinium have been reported to provide redox-sensitive MRI contrast enhancement [3,4]. We have investigated the utility of one such molecule, Gd-LC7-SH (**fig. 1b**), in a preclinical MRI investigation of the pharmacodynamics and mechanism of action of RRx-001. We hypothesize that alkylation of thiols in the tumor by RRx-001 will manifest as measurable differences in the post-GdLC7SH change in tumor T1 between untreated and treated animals.

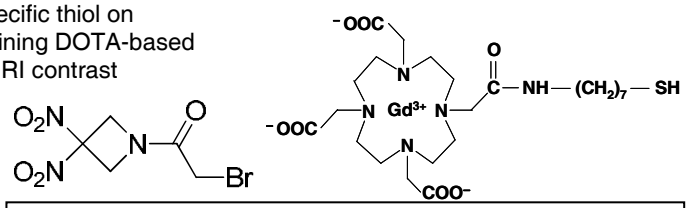

Figure 1. Structures of (a, left) RRx-001 and (b, right) Gd-LC7-SH.

Materials & Methods. Severe Combined Immunodeficient (SCID) mice were inoculated in the flank with either CHP-100 Ewing's Sarcoma, HT-29 colorectal carcinoma, or PANC-1 pancreatic carcinoma cells. Mice were imaged on a 7 Tesla Bruker Biospec® small animal MRI scanner when tumors had grown to 250-400 mm^3 in size. Mice were anesthetized using isoflurane and cannulated at the tail vein for injections using a low dead volume i.v. line (~25 μL dead volume) for administration of Gd-LC7-SH or RRx-001. T1 maps of the tumor were acquired pre-contrast and at various times post-contrast to 60 min post-injection of 0.05 mmol/Kg Gd-LC7-SH. Mice were imaged before treatment and at 1h, 24 h and 72 h post-treatment with 10 mg/Kg RRx-001.

Results. Gd-LC7-SH spontaneously binds to thiol targets following i.v. administration. The fraction of gadolinium that is bound to macromolecular targets such as plasma albumin and exofacial protein thiols (EPTs) is protected from renal clearance, and produces a prolonged decrease in tumor T1 measured by MRI. This is qualitatively illustrated in **figure 2**. Before treatment with RRx-001 (figure 2, top panel) the T1 of both tumor and muscle are significantly lower 60 min post-Gd-LC7-SH relative to pre-contrast T1. After RRx-001 exposure (figure 2, bottom panel) the T1 of both tumor and muscle are higher than expected 60 min post-Gd-LC7-SH, consistent with a rapid clearance of Gd-LC7-SH due to oxidation of exofacial protein thiols & albumin in the tumor. We hypothesize that the larger the decrease in tumor T1 following administration of Gd-LC7-SH, the greater must be the retention of Gd-LC7-SH in the tumor. The change in tumor T1 before *vs.* after treatment with RRx-001 is summarized in **figure 3**. In all 3 tumors before treatment, Gd-LC7-SH produced a prolonged decrease in tumor T1 even at the delayed time point of 60 min post-injection. This decrease was abolished or greatly decreased 1 h post-treatment with RRx-001 in all 3 tumors. Furthermore, in HT-29 and PANC-1 tumors this effect of RRx-001 on tumor T1 post injection of Gd-LC7-SH was apparent even at 72 h post-drug. In all 3 tumor types, the tumor ΔT1 at 1 h post-drug was significantly smaller than pre-drug tumor ΔT1 (p<0.02). In the HT-29 and PANC-1 tumors the ΔT1 at 72 h post-drug remained smaller than baseline ΔT1 (p<0.05). These observations indicate decreased tumor retention of Gd-LC7-SH following treatment with RRx-001, which is consistent with a decrease in availability of reduced albumin and EPTs in the tumor.

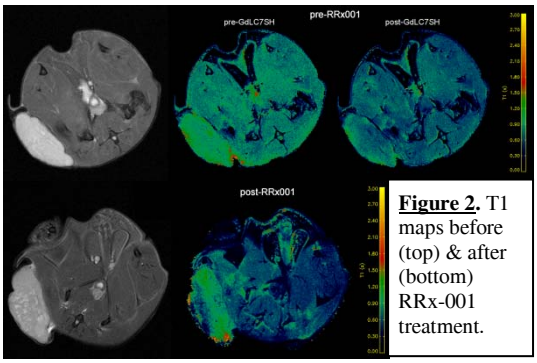

Figure 2. T1 maps before (top) & after (bottom) RRx-001 treatment.

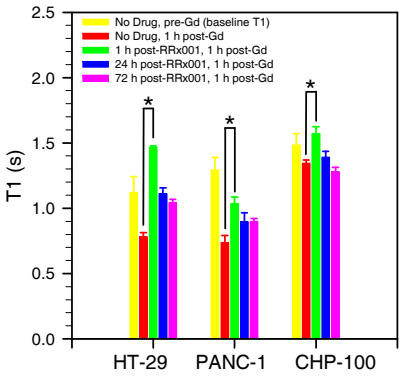

Figure 3. The variation of tumor T1 (mean ± S.E.M., n=4) before *vs.* following treatment with RRx-001 (10 mg/Kg, *i.v.*). In all 3 tumors, Gd-LC7-SH produced a prolonged decrease in tumor T1 evident even at 60 min post-injection (red *vs.* yellow). This decrease was abolished or greatly decreased 1 h post-treatment with RRx-001 (green *vs.* red) in all 3 tumors. In HT-29 and PANC-1 tumors this effect of RRx-001 on post-GdLC7SH tumor T1 was apparent even at 72 h post-drug (blue & pink *vs.* red).

Discussion. The previously reported redox activity of RRx-001 together with its very short half-life in vivo suggests an indirect effect on albumin and exofacial thiols that manifests as smaller ΔT1 values on Gd-LC7-SH MRI imaging. The anti-proliferative activity of RRx-001 may not only be due to glutathione depletion and NO release under hypoxia, but also to an increase in intratumoral ROS burden leading to a direct redox modulation of exofacial thiols integral to tumor protein function. Additional studies are planned to confirm this postulate.

References.

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