A novel class of S-Gal™ analogs as ^{1}H MRI *LacZ* gene reporter molecules

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Introduction: Extensive implementation of gene therapy as a therapeutic strategy for cancers has been hampered by difficulties in quantitatively assessing the success of gene transfection and longevity of gene expression. Therefore development of non-invasive reporter techniques based on appropriate molecules and imaging modalities may help to assay gene expression [1,2]. We have evaluated a range of S-Gal™ analogs (Fig 1) as novel ^{1}H MR *lacZ* gene-reporter molecules *in vitro* and have identified C3-GD as an optimal agent for *in vivo* studies.

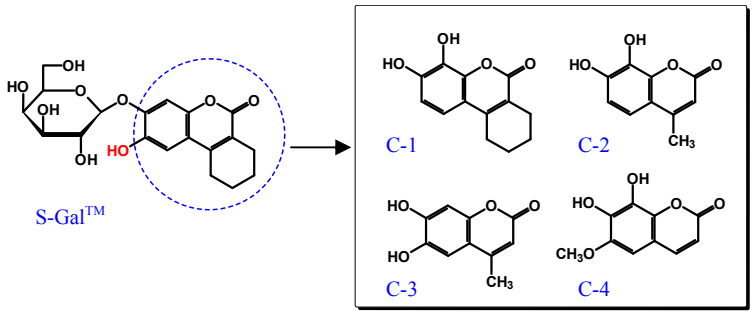

Figure 1: Structure of commercial colorimetric *lacZ* gene-reporter, S-gal™. C1-4 are analogs of the product of cleavage of S-gal™ by β-galactosidase.

Materials and Methods: *In vitro* measurement of T_1 and T_2 of the developed agents was performed at 37°C using a 4.7 T horizontal bore MR system. Each sample contained 15mM Agent + 5mM ferric ammonium citrate (FAC) in agarose with or without 5 units of β-gal enzyme. Nude mice (n=4) implanted with subcutaneous MCF7 (wild type) and *lacZ* transfected MCF7 tumor cells were used to perform the *in vivo* study. A spin echo based pulse sequence was used to quantify the T_1 & T_2 values of the tumor slices. Following baseline imaging, 25µl of a solution with 15 mM C3-GD and 5 mM ferric ammonium citrate (FAC) in water was injected intra-tumorally using a fine needle into each tumor. T_1 & T_2 weighted images were acquired at 1 and 2 hours post injection.

Results and Discussion: In the presence of ferric ions (Fe^{3+}), the agent is cleaved by beta-galactosidase encoded in the *lacZ* gene, and forms a paramagnetic iron chelate.C3-MGD (C3 mono galactoside) and C3-GD (C3 di-galactoside) showed pronounced T_1 and T_2 effects in the presence of β-gal in agarose samples. Due to the better solubility of C3-GD in water, it was used for *in vivo* studies. Following intra tumoral injection of C3-GD + FAC, the MCF7/ *lacZ* tumors showed statistically significant changes in T_1 values (from 1.810±0.16s to 1.515±0.20s) & T_2 values (from 0.049±0.007s to 0.040±0.008s) (Fig 2A) after 2hours whereas the MCF7 (wild type) tumor showed minimal changes in T_1 & T_2 values (Fig 2B) in a representative slice under the same experimental conditions. Our results suggest that C3-Gd is a promising novel Fe-based ^{1}H MR *lacZ* gene reporter molecule.

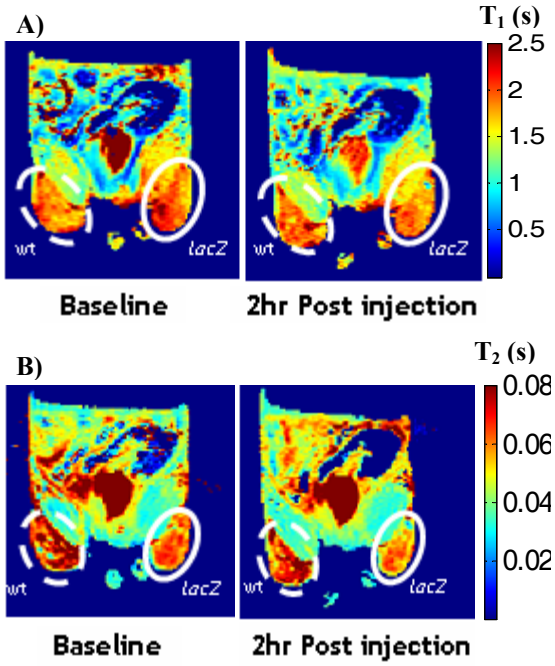

Figure 2: *In vivo lacZ* gene reporter activity of C3-GD. A representative MRI slice of a nude mouse with wild type MCF7 tumor (left - dotted) and *lacZ* transfected MCF7 tumor (right - solid) are shown. A) T_1 maps and B) T_2 maps.

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References:

1. *Nature Biotechnol.* 2000; 18:321.
2. *NMR Biomed.*2008; 21:704.