# Daidzein-BSA-GdDTPA/CyTE777 a novel contrast material for functional and molecular targeting of ovarian carcinoma tumors.

**H. Migalovich-Sheikhet[1], V. Kalchenko[2], F. Kohen[1], and M. Neeman[1]**

[1]Biological Regulation, Weizmann Institute of Science, Rehovot, Israel, [2]Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel

**Introduction:** The estrogen receptor (ER) is present in approximately two-thirds of human ovarian tumors, part of them express the ERβ subtype. Additionally, ERβ receptors are expressed in different tumors like colon and prostate (1), (2) and thus these receptors can serve as recognition sites for targeting various agents. The isoflavone daidzein has a selectivity for ERβ subtype, and carboxy derivatives of isoflavones have been reported to target tumors expressing ERβ (3). In this study we describe the use of 7-(O)-carboxymethyl daidzein (4), a reactive derivative of daidzein, as a targeting moiety to the MLS human epithelial ovarian carcinoma tumor model for MRI and NIR spectroscopy. We used a macromolecular imaging system based on a combination of functional targeting (due to the enhanced vascular permeability and interstitial convection) with molecular targeting (using cell surface molecules expressed by tumor cells/tumor stroma cells). This system gave us advantage of specific localization and retention of the imaging agent in the target site.

**Materials and methods:**

Contrast material: BSA-GdDTPA (5) and 7-(O)-carboxymethyl daidzein (3) were synthesized as described, BSA-GdDTPA was reacted with the NHS derivative of 7-(O)-carboxymethyl daidzein-(daidzein-NHS) in $NaHCO_3$, 0.1M, pH 8. The product was dialyzed and then Gd was introduced as described (5).

NIR material: BSA- CyTE-777: CyTE-777-NHS (6) (in dry DMF) was added slowly with stirring to BSA (in $NaHCO_3$, 0.1M, pH 8.5). The reaction was stirred overnight and the product was dialyzed against $NaHCO_3$, 0.1M, pH 8.5, PBS, DDW and lyophilized. Daidzein-BSA-CyTE-777: daidzein -NHS (in dry DMF) was added to BSA-CyTE-777 in 1 ml of $NaHCO_3$, 0.1M, pH 8.5, and the reaction was stirred overnight. The product was purified by dialysis against $NaHCO_3$, 0.1M, pH 8.5, and against PBS. This product was used for injection.

MRI measurements: *In-vitro:* Studies were performed on a horizontal 4.7 T Bruker (Germany) Biospec spectrometer using a whole-body birdcage RF coil. $R_1$ measurements spin echo images were acquired at 8 different repetition times ranging between 2000 and 100 ms; 2 averages, field of view 4X4 cm, slice thickness 1 mm, matrix 128 X 128). *In-vivo*: Studies were performed using a whole-body birdcage RF coil and an actively radio-frequency decoupled 1.5 cm surface coil embedded in a Perspex board and a birdcage transmission coil. $T_1$ weighted 3D gradient-echo (GE) images, with pulse flip angles of $5^0$,$15^0$,$30^0$, $50^0$ and $70^0$ were acquired to determined the $R_1$ values. The acquisition parameters: TR 10ms; TE 3.561ms; 2 averages; field of view 4X4X4 cm; 128X128X128 pixels.

MRI in-vivo model: CD-1 nude mice were inoculated with $2.5 \cdot 10^6$ MLS tumor cells/mouse. The tumor bearing mice were injected with BSA-GdDTPA or daidzein-BSA-GdDTPA. $R_1$ was measured 24h after injection of the contrast material.

Fluorescence imaging studies: The tumor bearing mice were injected with daidzein-BSA-CyTE-777 (1mg/mice) + BSA-FAM (1mg/mice) (as a non specific competitor) or BSA-CyTE-777 (1mg/mice) as control. The NIR signal in the whole animal was monitored by at 24, 48 and 72h on an IVIS 100 system (Xenogen inc., USA).

**Results:**

*MRI*: The specific $R_1$ relaxivity of daidzein-BSA-GdDTPA was measured to be 181.82 $mM^{-1}s^{-1}$ per BSA (fig.1A, B). Preliminary experiments suggested specific accumulation of contrast material in the tumor of daidzein-BSA-GdDTPA injected mice relative to mice injected with BSA-GdDTPA (24h post-injection; fig.1C).

*Fluorescence imaging studies*: Specific tumor targeting of the daidzein-BSA-CyTE777 conjugate was validated by *in vivo* fluorescence imaging (fig.1D). Mice were injected s.c. with $2.5 \cdot 10^6$ MLS tumor cells/mouse. Six days after the tumor inoculation, the doubly labeled BSA was injected i.v. via the tail vein. In mice that were injected with CyTE777-BSA-daidzein and BSA-FAM (as a non-specific competitor), the specific NIR fluorescence was observed in the tumor area even after 72 hours after administration (n=2-4). Mice that were injected with non-targeted material CyTE777-BSA did not show NIR fluorescence from 24 hours after administration (n=2).

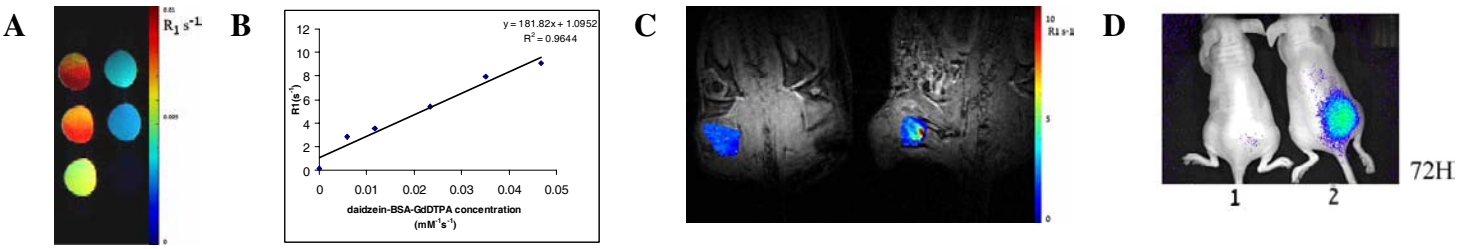


**Figure 1 Daidzein-BSA-GdDTPA as a contrast material for MRI.** R1 map **(A)** and R1 relaxivity measurement **(B)** of Daidzein-BSA-GdDOTA. The $R_1$ relaxivity was 181.82 $mM^{-1}s^{-1}$ per BSA. **R1 maps of the tumor 24h after injection of the contrast material (C)**. MLS tumor bearing mice were injected with BSA-GdDTPA (left) and daidzein-BSA-GdDTPA (right). **In Vivo Imaging of daidzein-BSA-CyTE777 localization in MLS tumor-bearing CD-1 nude mice (D).** MLS tumor bearing mice were injected with 1) Control BSA-CyTE777; 2) daidzein-BSA-CyTE777 + BSA-FAM. The NIR fluorescence after 72 hours is shown.

**Conclusion:** This study describes the use of 7-(O)-carboxymethyl daidzein as a targeting moiety in the MLS human epithelial ovarian carcinoma model. The specific delivery of the doubly labeled BSA to the tumor was shown by NIR imaging and was consistent with preliminary in vivo MRI studies.

**Acknowledgement:** Supported by the Israel Science Foundation ISF 391-02 and by NIH R01 CA075334.

**References:**

1. Deroo, B. J. and Korach, K. S. Estrogen receptors and human disease. J Clin Invest, *116:* 561-570, 2006.
2. Imamov, O., Shim, G. J., Warner, M., and Gustafsson, J. A. Estrogen receptor beta in health and disease. Biol Reprod, *73:* 866-871, 2005.
3. Somjen, D., Stern, N., Knoll, E., Sharon, O., Gayer, B., Kulik, T., and Kohen, F. Carboxy derivatives of isoflavones as affinity carriers for cytotoxic drug targeting in adrenocortical H295R carcinoma cells. J Endocrinol, *179:* 395-403, 2003.
4. Kohen, F., Lichter, S., Gayer, B., DeBoever, J., and Lu, L. J. The measurement of the isoflavone daidzein by time resolved fluorescent immunoassay: a method for assessment of dietary soya exposure. J Steroid Biochem Mol Biol, *64:* 217-222, 1998.
5. Dafni, H., Landsman, L., Schechter, B., Kohen, F., and Neeman, M. MRI and fluorescence microscopy of the acute vascular response to VEGF165: vasodilation, hyper-permeability and lymphatic uptake, followed by rapid inactivation of the growth factor. NMR Biomed, *15:* 120-131, 2002.
6. Hilderbrand, S. A., Kelly, K. A., Weissleder, R., and Tung, C. H. Monofunctional near-infrared fluorochromes for imaging applications. Bioconjug Chem, *16:* 1275-1281, 2005.