

# Gd-encapsulating Anti-HER2 Immunoliposomes for MR Monitoring of Targeted Drug Delivery

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## Purpose

The goal of this study was to characterize the distribution of HER2 receptor targeted immunoliposomes in a mouse model of human breast cancer and compare this to non-targeted sterically stabilized liposomes.

## Introduction

Liposomes are currently being investigated as a means of improving delivery of chemotherapeutic agents to tumors. Sterically stabilized liposomes have been studied because they remain in the bloodstream longer than conventional liposomes, which are quickly cleared by the reticuloendothelial system. Improved targeting may be achieved by conjugating liposomes with antibodies specific for certain tumor cell receptors. It has been demonstrated in multiple HER2-overexpressing human breast tumor xenograft models, that treatment with doxorubicin-loaded anti-HER2 immunoliposomes (ILs) produces significantly increased antitumor cytotoxicity and significantly less systemic toxicity than free doxorubicin<sup>1</sup>. We have previously utilized MR to characterize the uptake of different compositions and doses of sterically stabilized liposomes. The purpose of this study is to characterize and compare (targeted) anti-HER2 liposomes with non-targeted liposomes. We are ultimately interested in developing magnetic resonance (MR) methods utilizing gadolinium (Gd) encapsulating anti-HER2 liposomes to monitor chemotherapy treatment.

## Methods

Sterically stabilized POPC/Chol/PEG-PE liposomes (3:2:0.3 molar ratio) encapsulating GdDTPA-BMA were prepared by the freeze thaw method then extruded. The mean diameter determined by dynamic light scattering was  $90 \pm 30$  nm. A portion of these liposomes were conjugated with sF<sub>1</sub> anti-HER2 antibody as previously described<sup>1</sup>. Nude mice implanted with 17 $\beta$  estradiol pellets were implanted in the shoulder region with  $2 \times 10^7$  cells from the human breast tumor line BT474M1+6, which overexpresses HER2. For imaging studies, mice were anesthetized with an intra-peritoneal injection of 0.05 ml/kg ketamine/xylazine. The mice were imaged at 21-28 days post-implantation when tumors had reached a diameter of 1-2 cm. Mice were injected retro-orbitally with either non-targeted (n=2) or anti-HER2 targeted (n=2) liposomes (0.1 or 0.05 mmol/kg GdDTPA-BMA, respectively). Images were acquired immediately after administration of liposomes to verify blood pool enhancement and at 8, 16, 24, and 44 hrs post-injection.

Imaging experiments were performed on a 1.5 T GE Signa clinical scanner using the conventional Medical Advances wrist coil. Animals were imaged in pairs in a customized holder. An axial image was obtained for tumor localization, followed by a coronal T1-weighted high resolution 3DFGRE image over the whole tumor (TR= 22 ms, TE=4.2 ms, FOV 8 x 8 cm, imaging matrix 512 x 256, slice thickness 0.7 mm, 2 NEX, image resolution 157 X 157 X 700 microns).

Signal intensity (SI) was calculated for regions of interest (ROIs) in various tissues (tumor, liver, vessel and muscle) for both types of liposomes. Signal intensities were normalized to a vitamin E phantom.

## Results and Discussion

Both types of liposomes accumulated in tumors over time, maintaining or increasing concentration for up to 24

hrs (Figure1). Signal intensity rose initially in the liver, beginning to decrease at 24 hrs (Table 1). The increase in blood vessel SI seen immediately after injection was twice as high for the POPC liposomes as for the Anti-HER2 liposomes, consistent with GdDTPA-BMA dose. It is in agreement with previous results which showed a linear relationship between liposome encapsulated GdDTPA-BMA dose and SI in this range<sup>2</sup>.

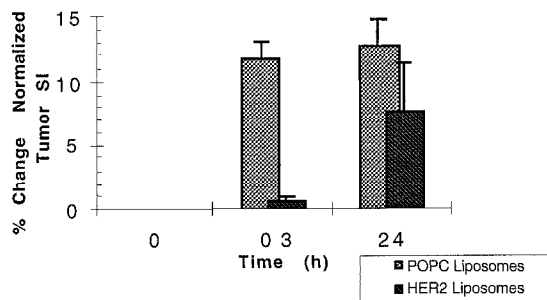


Figure 1. Percent change in signal intensity of tumor with time after administration of POPC or HER2 liposomes containing GdDTPA-BMA.

LIPOSOME	TISSUE	$\Delta$ SI (%)	
		0.33 HRS	24 HRS
Anti-HER2 Gd-POPC (targetted)	TUMOR	7.40	7.61
	LIVER	27.83	16.33
	VESSEL	127.11	63.34
Gd-POPC (non-targetted)	TUMOR	11.74	12.85
	LIVER	32.93	9.62
	VESSEL	253.05	80.28

Table 1. Percent change in normalized signal intensity in selected tissues at 0.33 and 24 hrs post-injection of each type of liposome.

An MR detectable change in tumor SI was observed for anti-HER2 liposomes. Similar tissue uptake patterns were seen for the anti-HER2 and POPC liposomes, with the lower Gd dose administered in the anti-HER2 ILs resulting in a lower percent change in SI. Increasing tumor SI was seen up to 24 hrs post-injection despite a drop in blood vessel SI for both types of liposomes. This was consistent with mouse tumor xenograft studies of doxorubicin-containing anti-HER2 ILs which showed high tumor/blood concentration ratios at 67 hrs post-liposome injection<sup>1</sup>. Our results suggest that MRI may be an effective method for monitoring IL drug delivery in patients.

## References

1. Park JW, Hong K, Kirpotin D et al, Cancer Letters 1997; 118: 153-160.
2. Wilmes LJ, Gaber MH, Saeed M et al, Proc. ISMRM 7th Meeting, 1999; 1180.

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