LB-060

Detection of HER2+ tumor cells using MagSense™ Nanoparticles: Safety and Sensitivity

Farideh Z. Bischoff[1], Rebeca Romero Aburto[2], Erika C. Vreeland[1], John D. Hazle[2], Konstantin Sokolov[2], Kayla E. Minser[1], Todor Karaulanov[1], Giulio Paciotti[1]. *[1]Imagion Biosystems, Inc., San Diego, CA; [2]MD Anderson Cancer Center, Houston, TX*

ABSTRACT

MagSense™ is an *in-vivo* diagnostic for the detection of primary and metastatic disease. The platform consists of tumor targeting superparamagnetic iron oxide nanoparticle (NPs) and a device capable of distinguishing the magnetic signatures of NPs that are free (e.g. flowing through the blood) from those that reach and bind their intended target (e.g., the cancer cell). Our first intended use of this technology is to test the ability of MagSense NPs labeled with an anti-HER2 antibody to detect HER2+ breast cancer in the sentinel and axillary lymph nodes of patients previously confirmed with HER2+ disease.

Our preclinical data on the MagSenseTM-anti-HER2 platform reveal: 1) specific binding and detection of HER2 positive tumor cells in-vitro (5000 cells); 2) specific detection of HER2 positive tumors in-vivo; 3) binding and amplitude of the magnetic signal is proportional to the level of HER2 expression in-vitro and in-vivo; and 4) the nanoconstruct remains stable in circulation. Based on these supportive preclinical data, the MagSense Anti-HER2 NPs are being produced under cGMP along with an R&D version of the MagSense device, for an early stage research clinical trial.

Objective: To support our clinical efforts, initial assessment of NPs safety were conducted. In-vitro efforts focused on the ability of the PEGylated NPs to induce an inflammatory response, activate complement, cause coagulation and platelet aggregation. In-vivo, the safety of the NPs was confirmed by following the degradation of the NPs over time in NGS mice.

Experimental Methods:

- MagSense Anti-HER2 nanoparticles were generated using non-directional EDC chemistry. Once produced the particles were interrogated for particle size, charge, HER2 content and activity and stealth.
- In Vitro binding studies:
  - HER2 positive (BT-474 or MCF7-HER218) and negative (MCF-7) human breast cancer cell lines were incubated with the MagSense Anti-HER2 particles in the presence and absence of a 200 fold excess of free antibody.
  - After 2-12 hours the culture media was removed, the cells washed 3 times with PBS, harvested and isolated by centrifugation. The binding of the particles was evidence by digital photography and the SPMR signal determined using the MagSense device.
- Ex Vivo Lymph Node Co-cultures
  - 10^6 HER2 positive (BT-474) or negative (MCF-7) were co-cultured with with 10^7 splenocytes
  - The cultures were pulsed with MagSense Ani-HER2 particles ± 200 fold excess of free antibody
  - Cells were harvested and magnetic signatures of the co-cultures were determined as above
- In Vivo binding studies:
  - HER2 positive (BT-474) and negative (MCF-7) human breast tumors were generated in either SCID or nude mice
  - Upon the formation of palpable tumors mice received an IV injection of the particles. In a second study a BT474 tumor bearing nude mice received an injection of 500 µg of free anti-HER2 or PBS
  - 24 hours post injection the mice were received a second injection of the MagSense Anti-HER2 particles and the SPMR signal was determined in the MagSense device
- Safety studies:
  - In Vitro:
    - MagSense anti-HER-2 particles were cultured with peripheral blood lymphocytes for 48 hours.
    - The culture supernatants were analyzed for the presence of inflammatory cytokines and mediators using standard techniques such as ELISA
  - In Vivo Clearance of MagSense Particles:
    - Naïve BalbC mice were injected with MagSense Anti-HER 2 particles that over 24 hours were present in the liver.
    - SPMR signal was measured in the mice over time.

In Vitro Pharmacology

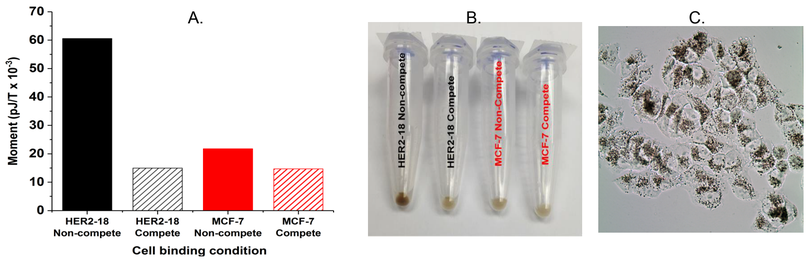

Figure 1. Differential/specific binding and internalization of the MagSense Anti-HER 2 particles into HER2+ versus negative breast cancer cells. SPMR signals from cell pellets reveal that HER2 positive BC cells MCF7-HER218 generate a significant SPMR signal compared to the low expressing cell line. Specificity was further shown by competition. Shown in Figure 1 C is the internalization of the particles into the HER2 overexpressing cells

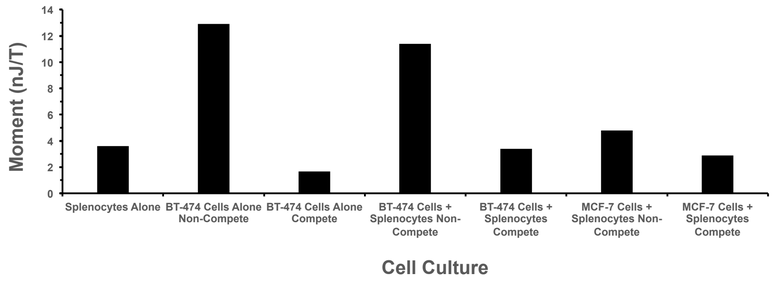

Figure 2. Specific detection of HER2+ Breast Cancer in a splenocyte/cancer cell co-culture

In Vivo Pharmacology/Diagnosis

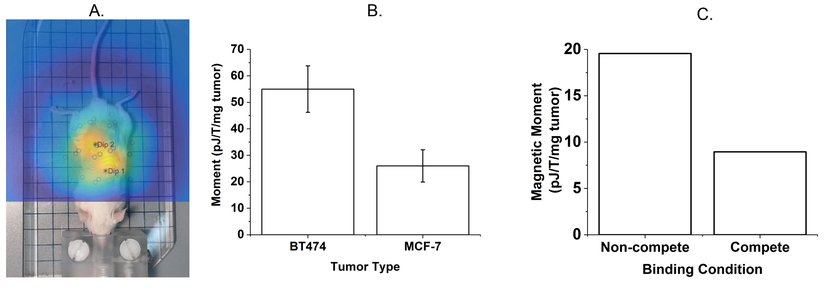

Figure 3. A) Dipole reconstruction for the detection of HER2 positive tumor: Model BT474 cells implanted in SCID mice. B) Differential detection of HER2 overexpressing (BT-474) and under-expressing (MCF-7) breast tumors in vivo. BT-474 generate a significantly higher SPMR signal vs MCF-7 tumors ($p < 0.03$). C). Specificity of SMOR detection of HER2 tumors in vivo demonstrated by competition.

In Vitro and In Vivo Toxicology

Table I. MagSense does not induce an inflammatory response, activate complement or change coagulation profiles in vitro

Factor	Range from 3 Donors	
	MagSense	Endotoxin/Positive Control
IL-1β	BLD	250-400 pg/mL
IL-8	BLD	2000-5000 pg/mL
TNFα	BLD	700-1000 pg/mL
IFNγ	BLD	300-900 pg/mL
iC3B	BLD	350 µg/mL
Leukocyte Recruitment	Baseline	2000FU
Hemolysis	BLD	90%
Coagulation	No Impact	Activation of PT, PTT and Thrombin
Platelet Activation	No impact on Collagen Induced Platelet Aggregation	Collagen Induce Platelet Aggregation
BLD: Below the Limit of Detection		

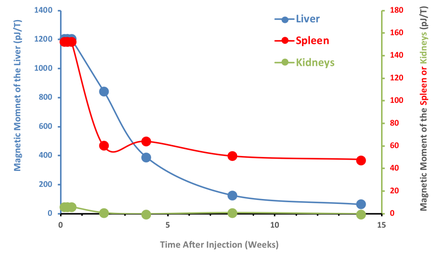

Figure 4. MagSense Nanoparticles are biodegradable. Biodegradation is supported by a loss of SPMR signal from key organs over time

CONCLUSIONS

We report on the extensive pre-clinical development and functionality of PEGylated and antibody-conjugated NPs for *in-vivo* and *ex-vivo* detection of HER2+ tumor cells by Magnetic Relaxometry (MRX). We observed: 1) specific binding and detection of HER2 positive tumor cells in-vitro; 2) specific detection of HER2+ tumors in mice; 3) binding and amplitude of magnetic signal to be proportional to the level of HER2 expression in-vitro and in-vivo; 4) the nanoconstruct remains stable in circulation; 5) the particles do not induce a pro-inflammatory response nor activate complement; 6) the particles are biodegradable; and do not induce acute or delayed signs of morbidity in vivo.

Our results support the first intended clinical use of the MagSense™ platform for the in vivo detection of HER2 amplified tumor cells. Based on these supportive preclinical data, the MagSense Anti-HER2 NPs are being produced under cGMP along with an R&D version of the MagSense device, for an early stage research clinical trial.

We wish to also acknowledge Dale Huber, C.I.N.T., Albuquerque, NM