# Appendix E1

# MR imaging of Antigen-loaded Dendritic Cell Migration in a Pancreatic Carcinoma Model

## Generation and Antigen Loading of Bone Marrow–derived Dendritic Cells

Bone marrow–derived dendritic cells were prepared as previously described (19). Briefly, bone marrow cells from the tibia and femur were flushed by using RPMI 1640 medium (Sigma-Aldrich). These cells were suspended in 2 mL of ammonium chloride potassium lysis buffer (Sigma-Aldrich) and were incubated for 5 minutes at room temperature to eliminate red blood cells. Cells were cultured at approximately $4 \times 10^5$ cells/mL in dendritic cell medium: That is, RPMI 1640 medium supplemented with 10% fetal calf serum (Sigma-Aldrich), L-glutamine (2 mmol/L), penicillin and streptomycin (100 IU/mL), 20 ng/mL granulocyte macrophage colony-stimulating factor (Sigma-Aldrich), and 20 ng/mL IL-4 (Sigma-Aldrich). After 7 days, loosely adherent cells were harvested. For flow cytometry experiments, dendritic cells were seeded in six-well plates at a density of approximately $1 \times 10^6$ cells per well. Next, dendritic cells were stained with the following antibodies: APC anti-mouse CD86 (BD Pharmingen) and PE anti-mouse CD40 (BD Pharmingen). Finally, fluorescence-activated cell sorter analysis was performed with a flow cytometer, and the results were analyzed by using FlowJo Software (TreeStar). The expression of dendritic cell markers was quantified as positive and negative percentages ($n = 6$).

For antigen loading of dendritic cells, apoptotic Panc02 cells were generated by means of four rapid freeze-thaw cycles, as previously described (20,21). In brief, Panc02 cells were subsequently treated with two rapid freeze-thaw cycles (−140°C/21°C; one cycle consisted of 10 seconds in liquid nitrogen followed by 10 minutes at room temperature). Dendritic cells generated on day 7 were washed twice in phosphate-buffered saline, counted, and resuspended in dendritic cell medium. Then, Panc02 cell lysates were added (100 μg lysates per $5 \times 10^5$ dendritic cells in 1 mL medium) and incubated with dendritic cells for 3 hours. Tumor antigen labeled cells were harvested, washed, and resuspended in RPMI 1640.

## SPIO Nanoparticle Labeling of Antigen-loaded Dendritic Cells

A sterile suspension of FeOlabel Texas Red nanoparticles (GENOVIS), fluorescently labeled SPIO nanoparticles, was diluted in serum-free OptiMEM media (Life Technologies) to an iron concentration of 0.8 mg/mL. An equal volume of 4.5 μg/mL of protamine sulfate (Sigma-Aldrich) transfection agent diluted 1:50 in OptiMEM was mixed with the diluted SPIO suspension and incubated for 15 minutes at room temperature to coat the SPIO particles with cationic lipid. Antigen-loaded dendritic cells were placed in a flask with fresh dendritic cell medium, and protamine sulfate–coated SPIO was added to the mixture to give final concentrations of 20 μg iron per milliliter and 0.5 μg protamine sulfate per milliliter. The dendritic cells were then incubated for 3 hours at 37 °C in a 5% $CO_2$ atmosphere to permit cellular uptake of the SPIO particles. Aliquots of labeled cells and unlabeled (control) cells,

which were exposed to neither iron particles nor protamine sulfate, were stained with trypan blue, transferred to a hemocytometer, and examined with a microscope. Dead (trypan blue–positive) and live cells were counted to calculate overall viability (expressed as a percentage of overall cells counted). Labeling efficiency was determined by using fluorescence microscopy. Approximately $3 \times 10^5$ SPIO-labeled dendritic cells were fixed in formalin for 30 minutes at room temperature. Cell suspensions (10 μL) were placed on microscope slides and evaporated. These samples ($n = 6$) were overlaid with hard-set mounting medium with DAPI nucleic acid counterstain (Vector Laboratories, Burlingame, Calif), and cover slips were placed. Cells were considered labeled if intracytoplasmic red fluorescence could be detected. Slides were observed with an LSM 510 META confocal microscope with a 405-nm laser (Carl Zeiss Microscopy, Thornwood, NY). Percentages of labeled dendritic cells were determined in 10 fields (×20) on each slide per group.