Enhanced Leukemia Cell Detection Using a Novel Magnetic Needle and Nanoparticles

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Abstract
Acute leukemia is a hematopoietic malignancy for which the accurate measurement of minimal residual disease is critical to determining prognosis and treatment. Although bone marrow aspiration and light microscopy remain the current standard of care for detecting residual disease, these approaches cannot reliably discriminate less than 5% lymphoblasts. To improve the detection of leukemia cells in the marrow, we developed a novel apparatus that utilizes antibodies conjugated to superparamagnetic iron oxide nanoparticles (SPIONs) and directed against the acute leukemia antigen CD34, coupled with a “magnetic needle” biopsy. Leukemia cell lines expressing high or minimal CD34 were incubated with anti-CD34–conjugated SPIONs. Three separate approaches including microscopy, superconducting quantum interference device magnetometry, and in vitro magnetic needle extraction were then used to access cell sampling. We found that CD34–conjugated nanoparticles preferentially bind high CD34-expressing cell lines. Furthermore, the magnetic needle enabled identification of both cell line and patient leukemia cells diluted into normal blood at concentrations below those normally found in remission marrow samples. Finally, the magnetic needle enhanced the percentage of lymphoblasts detectable by light microscopy by 10-fold in samples of fresh bone marrow aspirate approximating minimal residual disease. These data suggest that bone marrow biopsy using antigen-targeted magnetic nanoparticles and a magnetic needle for the evaluation of minimal residual disease in CD34-positive acute leukemias can significantly enhance sensitivity compared with the current standard of care.

Note: J.E. Jaetao and K.S. Butler contributed equally to this manuscript. Requests for reprints: Richard S. Larson, University of New Mexico Cancer Research Facility, Room 223, 2325 Camino de Salud, Albuquerque, NM 87111. Phone: 505-272-6950; Fax: 505-272-5186; E-mail: rlarsen@salud.unm.edu. ©2009 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-09-1083

Introduction
Acute leukemias are bone marrow–derived malignancies for which the development of sensitive detection methods is crucial to improving clinical detection and outcomes. Currently, technologies used to examine bone marrow samples may fail to detect the presence of leukemia cells below 1% to 5% of total leukocytes, i.e., minimal residual disease (1, 2). A major difficulty in detecting minimal residual disease using bone marrow aspiration is that random sampling can neglect areas of focal disease. As a result, opportunities to intensify therapy may be overlooked, leading to relapsed disease. In these cases, the ability to reliably detect residual leukemia cells, when present below 5%, to monitor the efficacy of therapy is critical to improving care (3).

Magnetic nanoparticles have become an increasingly important tool in both targeting and detecting cancer cells. Cellular targeting may be achieved through attachment of receptor-specific ligands, including antibodies. As a result, cell surface receptors expressed on tumor cells, such as CD34 on acute leukemia cells (4), could allow for targeting nanoparticles labeled with antibodies (5–7). In addition, receptor-specific binding of nanoparticles leads to internalization in vitro (8, 9) and in vivo (10, 11), thus increasing the potential number of nanoparticles associated with each cell target. By using superparamagnetic nanoparticles composed of iron oxide (SPIONs), conjugated to anti-CD34 antibodies, we hypothesized that we could create magnetically charged leukemia cells that could be preferentially collected using a magnetic source during standard bone marrow sampling procedures.

Once magnetically charged leukemia cells are collected, nanoparticle binding and lymphoblast collection efficiency of the magnetic needle needed to be assessed. In addition to using standard techniques, such as light microscopy, we used a highly sensitive magnetometer called a superconducting quantum interference device (SQUID; ref. 12) to allow assessment of very small numbers of nanoparticle-coated cells. SQUID magnetometry has been used for clinically detecting magnetic fields under a variety of conditions because of its acute sensitivity. One such method uses a SQUID biosusceptometer, which can detect small aberrations in iron seen in iron-based pathologies such as hemochromatosis and thalassemia-induced iron storage disease (13, 14). Our method uses magnetorelaxometry, whereby nanoparticles are briefly magnetized by a pulsed field, and the SQUIDs detect the nanoparticle magnetization as it relaxes back to equilibrium (15). Pertinent to our study, SQUIDs have three specific properties that make them highly compatible for SQUID relaxometry detection: (a) they are superparamagnetic, (b) the individual magnetic moments of these particles align with a magnetic field, so that cells labeled with sufficient numbers of bound single particles with magnetic moments of ~4 × 10−18 J/T (16) are detectable by SQUIDs, and (c) unbound single particles, even when present in large numbers, do not generate detectable SQUID signals (17). Magnetic moments measured by SQUID relaxometry provide additional information regarding cellular binding and a secondary confirmation of microscopy results from magnetic needle collections.

Here, we describe the enhancement of leukemia cell sampling using a novel bone marrow sampling device and nanoparticles. In addition, we examine the sensitivity and ability of the SQUID to quantify cell sampling. This study represents a significant first...
step toward developing enhanced technologies for marrow sampling, which will improve clinical decision making and patient outcomes.

Materials and Methods

Cell culture. U937, Jurkat, and GA-10 cells were purchased commercially from American Type Culture Collection and cultured in RPMI supplemented with 10% fetal bovine serum (FBS; v/v; HyClone), 1% penicillin streptomycin (v/v; Life Technologies-Bethesda Research Laboratories), and 4 μg/mL ciprofloxacin (Bayer). Cells were cultured in an incubator at 37°C with 5% CO₂ and maintained at a cell concentration between 1 x 10⁵ and 1 x 10⁶ viable cells/mL. U937, GA-10, and Jurkat represent myeloid, B-cell, and T-cell lineage leukemia cell lines. Each cell line expresses CD34.

Peripheral blood and bone marrow collection. Peripheral whole blood was obtained from donors through venous puncture and was anti-coagulated in 10 U/mL of heparin (Becton Dickinson). Bone marrow aspirations were performed in patients with acute leukemia who required a bone marrow evaluation as a part of their routine clinical care. Human subjects provided consent in accordance with local and federal guidelines. The patients were placed in the supine position, and the sacral area was draped in a sterile fashion. Local anesthesia was achieved with 1% Xylocaine (Abaxis Pharmaceutical) administered s.c., and periosteally. A Jamshidi needle (Baxter Healthcare Corporation) was inserted into the cortex of the posterior superior iliac spine.

Determination of cell surface antigen expression on live cells. A Quantum Simply Cellular kit (Bangs) was used for quantitation of cellular antigen expression in antibody binding capacity units as per manufacturer’s instructions and described briefly here. The Quantum Simply Cellular bead populations provided a means for constructing a QuickCal calibration curve (antibody binding capacity values versus fluorescence intensity). Cells were compared with antibody-labeled beads, and cell surface antigen expression was quantified in antibody binding capacity units. Approximately 1 x 10⁶ cells and Quantum Simply Cellular beads were incubated with mouse FITC-labeled anti-human anti-CD34 (Caltag). Labeled cells were analyzed for cellular antigen expression using FACSscan (Becton Dickinson) flow cytometry as described previously (18).

Production of ligand-bearing nanoparticles. The nanoparticles were provided with an available surface carboxyl group to which an amino group provided with an available surface carboxyl group to which an amino group was attached using the carbodiimide method modified from a commercial protocol (Ocean NanoTech®) through experimentation and is described briefly here. Ten milligrams of SMAG-TCL 100-nm nanoparticles (Chemical) were aliquoted into a 15-ml conical tube (Greiner Bio-One) and brought to a total volume of 10 mL with double distilled water. N-hydroxysulfosuccinimide (Pierce) and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC; Pierce) were prepared fresh at a concentration of 25 mg/mL each in separate tubes with double distilled water. One hundred microliters each of the EDC and N-hydroxysulfosuccinimide were added to the nanoparticles and incubated at room temperature on a LabQuake shaker (LabIndustries, Inc.) for 20 min. Nanoparticles were brought to pH 8.0 with 50 mmol/L NaHCO₃ (Sigma-Aldrich), 50 μg of anti-CD34 antibody (BD Biosciences) was added, and the mixture was incubated at room temperature on a LabQuake shaker for 2 h. The antibody-nanoparticle mixture was centrifuged at 7,500 relative centrifugal force for 30 min at 4°C. The supernatant was removed and 10 mL of double distilled water was added to the pelletted nanoparticles. The centrifugation parameters were repeated once more and the supernatant was removed. The remaining pellet was resuspended in a total volume of 240 μL PBS (Life Technologies-Bethesda Research Laboratories)/0.5% FBS (HyClone). CD34-conjugated nanoparticles were stored at 4°C before use.

Cell labeling and sampling. Cultured U937, Jurkat, or GA-10 cells were harvested and washed using sterile PBS. Harvested cells were counted using a hemocytometer (Hausser Scientific). Each sample, unless otherwise specified, contained 1 x 10⁷ cells suspended in 200 μL of cold PBS/0.5% FBS solution and 20 μL of CD34-coupled SMAG-TCL nanoparticles, at a concentration of 41.67 μg [solids]/μL, corresponding to 22.8 μg [Fe]/μL. Cells and CD34 nanoparticles were incubated on ice for 1 h.

The magnetic needle used for cell collection is similar in size to a standard bone marrow biopsy needle. However, the magnetic needle is composed of a 17-cm-long, 1-mm-diameter stainless steel rod, at the end of which are two cylindrical NdFeB magnets 2 mm in length and separated by a stainless steel spacer 2 mm in length. The components of the magnetic needle are contained within a tight-fitting polyimide tube and the needle assembly is then inserted into a removable polyimide sheath. Magnetically labeled cells are attracted to both poles on both magnets and stick to the outside of the sheath. After extracting magnetic material from the marrow, the sheath is removed from the needle and inserted into media where the cells are liberated from the sheath. Additional physical characterizations of the magnetic needle used in this study have been described previously by Bryant and colleagues (19) and more recently by Adolphi and colleagues (16).

For leukemia cell dilution experiments, U937 cells were each diluted in donor whole blood. Cells were incubated with CD34-conjugated nanoparticles for 60 min at 4°C on a nutator (Becton Dickinson). SQUID measurements were taken after incubation and pre–needle draw, post–needle draw, and of the needle draw sample. The needle draws were performed following the nanoparticle incubation by placing the magnetic portion of the needle in the center of the samples in 1.5-mL microcentrifuge tubes for 1 min.

Glass slide preparations were made using a Cyto-centrifuge (Shandon). Slides were stained with either Diff-Quik stain (Dade Behring), which is similar to a Wright-Giemsa stain, or Prussian blue stain, which reveals the presence of iron. Prussian blue staining was performed by TriCore Reference Laboratories. Stained samples were qualitatively assessed for nanoparticle attachment using light microscopy. Light microscopy was performed on an Axiosvert 200 MAT (Zeiss) and captured images were taken with Moticam 2300 using provided Motic Images Plus software (Motic). Percent lymphoblasts in bone marrow diluted in blood were assessed microscopically by the number of lymphoblast cells per 200 leukocytes. The collection enhancement factor was determined by comparison of the lymphoblast percent in the needle and pre–needle draw samples.

Complete leukocyte counts of bone marrow, donor blood, post–needle draw, and needle draw samples were performed by lysis of the erythrocytes using ammonium chloride followed by collection of the leukocytes by centrifugation. Leukocytes were counted using a Beckman Coulter Counter. Absolute lymphoblast counts were then calculated from the total leukocyte counts using the percent lymphoblast counts determined microscopically.

Cell detection using SQUID relaxometry. Cell nanoparticle attachment was quantified using SQUID relaxometry, which involves briefly magnetizing the nanoparticles using a pulsed magnetic field and then detecting the decaying magnetization of bound particles over time using the SQUID sensors. In the case of cell-bound nanoparticles (which are not free to rotate), the decay is due to the Néel mechanism (20), involving internal reorientations of the nanoparticle magnetic moment and gives a detectable signal in the time window required by SQUID relaxometry for nanoparticles with the appropriate core diameter (~24 ± 4 nm iron oxide). The magnetization of unbound nanoparticles in fluid decays by Brownian rotation of the particle, and this relaxation is generally too fast to be detected by our technique. Samples in 1.5 mL microcentrifuge tubes were centered under the seven channel SQUID sensor array (BTI 2004, 4D-Neuroimaging) and placed at a distance of 3.4 cm below the center sensor. A uniform magnetic field of 38 Gauss was produced at the location of the sample for 0.3 s using a square Helmholtz array. After a 50-ms delay immediately following the magnetic field pulse, the decaying magnetization at each sensor was then sampled at a rate of 1 kHz and digitized using a NI-PXI8336 16-channel digitizer and LabVIEW 8.5.1 acquisition software (National Instruments). The pulsing sequence was repeated 10 times and the results averaged to increase the signal to noise ratio.

SQUID analysis. Analysis of the SQUID data was performed using the Multi-Source Analysis program, written in our laboratory using MATLAB (The MathWorks, Inc.). The SQUID system consists of seven sensors that

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7 http://www.oceannanotech.com
were identified with varied expression of CD34. The number of CD34 receptors on the cell surface was quantified using a bead-based technique flow cytometry. This flow cytometry method is extremely sensitive and a leukemia cell line expressing no CD34, and therefore truly CD34 “negative”, was not experimentally observed by us since very low numbers of receptors can be detected (21, 22). U937 which expressed $4.6 \times 10^4$ sites, and two relatively low CD34 expressing cell lines, GA-10 and Jurkat, expressing $6.2 \times 10^5$ and $2.3 \times 10^8$ sites, respectively, were incubated with anti-CD34 antibody–tagged nanoparticles and unconjugated nanoparticles. Following incubation, cytospin slides were prepared and stained for cells and for the presence of iron-containing nanoparticles. The presence of nanoparticles bound to the cell was assessed microscopically. By light microscopy, qualitative differences in the number of anti-CD34 antibody–tagged nanoparticles bound to the three cell lines was observed; U937, the high CD34-expressing cell line (Fig. 1A), shows a high level of CD34 antibody–conjugated nanoparticle association in the cytoplasm preparations. Lower levels of cell association with CD34 antibody–conjugated nanoparticles were found in the low CD34-expressing cell lines GA-10 and Jurkat (Fig. 1B). Very little nanoparticle association was found between any of the cell lines and the unconjugated nanoparticles (Fig. 1A). To confirm the light microscopy results, cells were assessed for nanoparticle binding using SQUID magnetometry, which showed increasingly higher magnetic moment in samples with higher CD34 expression (Fig. 1B). These data suggest increased nanoparticle binding as the number of CD34 receptors increases on the cell surface and indicate that the antibody attached to the nanoparticles has sufficient orientation to provide functionality.

Quantifying binding specificity using SQUID. To understand the capability of SQUID to quantitate nanoparticle binding, we performed two sets of experiments. First, we measured the SQUID signal obtained by adding varying amounts of CD34-labeled nanoparticles to U937 cells. When using $1.0 \times 10^7$ U937 cells, $\sim 9.2 \times 10^{11}$ CD34 receptors were available in the sample. An increasing SQUID signal was observed up to $\sim 7.1 \times 10^{11}$ nanoparticles, which then plateaued, indicating that additional sites for nanoparticle binding were not available once $7 \times 10^{11}$ sites were bound, although up to $3.5 \times 10^{12}$ nanoparticles were added (Fig. 2A and B). Interestingly, unconjugated nanoparticles also produced a detectable signal, indicating that low amounts of cellular nanoparticle uptake can be detected by SQUID, but is not easily visualized by light microscopy and special staining, or that the nanoparticles are agglomerated so they intrinsically produce a small magnetic moment.

In the second set of experiments, we fixed the number of nanoparticles and varied the number of CD34-expressing cells. Using $7.1 \times 10^{11}$ nanoparticles, a peak specific signal was seen with $1.0 \times 10^7$ cells that then plateaued (Fig. 2C and D). Again, background uptake of unconjugated nanoparticles was also detected. Taken together, the findings of these two experiments are consistent and indicate that $\sim 7.1 \times 10^4$ nanoparticles bound per cell, although $9.2 \times 10^4$ CD34 receptors are present, suggesting some level of steric hindrance. Nonetheless, SQUID relaxometry provided a highly sensitive method to quantitatively measure the specific binding of anti-CD34 antibody–bound SPIONs to leukemia cells.

Isolation of CD34-positive cells from peripheral blood using anti-CD34–conjugated nanoparticles and the magnetic needle. Specific binding of anti-CD34 SPIONs to the CD34-positive cells suggests that it may be possible to specifically sample these cells from blood or bone marrow. To achieve this, a magnetic

Results

Analysis of unlabeled and antibody-conjugated nanoparticle binding to human cell lines in vitro. To evaluate ligand-specific nanoparticle binding behavior, three leukemic cell lines were performed thrice; bars, mean; columns, mean; SD.

**Figure 1.** Analysis of nanoparticle binding using light microscopy and SQUID. Human leukemic cell lines U937, GA-10, and Jurkat. Photomicrographs show 20× imaging of Prussian blue staining for the presence of iron-oxide nanoparticles on cells incubated with either CD34 antibody–conjugated or unconjugated nanoparticles. Scale bar, 20 μm (A). Nanoparticle (NP) binding was confirmed by SQUID magnetometry (B). Nanoparticle conjugated with CD34 (+) or unconjugated nanoparticles (−) are indicated. Experiments were performed thrice; columns, mean; bars, SD.
needle based on a standard bone marrow sampling needle was developed (Fig. 3). The magnetic needle consists of very strong rare earth magnets attached at the bottom of a stainless steel rod (Fig. 3A). To allow collection of cells from the needle after sampling, the needle is covered by a removable polyimide sheath (Fig. 3C). Once covered by the sheath, the needle is lowered into a bone marrow or blood sample for 1 minute to collect nanoparticle-bound cells (Fig. 3B). After sampling, the needle sheath is removed and placed in media to allow removal of collected cells from the sheath and assessment of these cells by preparation of cytospin slides. To confirm binding, samples were placed under the SQUID sensor system and assessed by SQUID magnetometry (Fig. 3D).

Figure 3. Representation of magnetic needle and sampling of spiked sample. Shown is the schematic of the magnetic needle covered with a polyimide sheath prepared for insertion into a bone marrow sample (A), as well as the sheath-covered needle itself (C) and the magnetic needle inserted into an experimental sample (B). Sample tubes are placed into a sample holder on a platform under the SQUID sensors (D).
Cancer Research

To assess the capacity to isolate CD34-positive cells using anti-CD34-labeled nanoparticles and the magnetic needle, we "spiked" human blood with a leukemia cell line, U937 (Table 1). U937 cells were serially diluted in whole blood from a healthy volunteer, incubated with ligand-bearing nanoparticles, and collected using the magnetic needle. The presence of nanoparticle-bound cells in the magnetic needle samples was verified using Diff-Quik and Prussian blue stained cytology. Notably, the collection enhancement of CD34-expressing cells increased at lower absolute numbers and percentages. When these slides were examined by light microscopy, only U937 cells and neutrophils were seen. Other types of leukocytes were not present. Prussian blue staining indicated that the neutrophils in the peripheral blood had phagocytosed nanoparticle; otherwise, the enhanced collection of U937 cells would have been markedly increased.

**Sampling of bone marrow in patients with CD34-positive leukemia.** Next, we assessed our ability to harvest CD34-expressing leukemia cells from patient samples. Bone marrow aspirate from three patients with known acute leukemia were serially diluted in donor whole blood and the lymphoblasts were extracted using the magnetic needle. There was a significant increase in the number of lymphoblasts harvested from each patient bone marrow sample and each dilution (Table 2; Fig. 4). Examination of the percent lymphoblasts present in the pre–needle draw samples to the post–needle draw samples shows a consistent increase in the percent lymphoblasts sample harvested with the magnetic needle. The increase in percent lymphoblasts was greatest in the more dilute samples, similar to the previous experiments with a leukemia cell line and showing the utility of the magnetic needle for isolating the rare lymphoblasts.

Once again, only the neutrophils from the blood used for dilution were seen in addition to the lymphoblasts. Neutrophils were only present in these samples due to the use of donor blood to dilute the bone marrow and should not present a significant factor in collection of lymphoblasts from pure bone marrow specimens. To address the collection of blasts in the absence of blood dilution, a second lymphoblast percentage without the neutrophil component was calculated. As neutrophils have a very different morphology than lymphoblasts, neutrophils were easily indentified visually and excluded when examining needle collection slides microscopically (Fig. 4). Excluding the neutrophils, virtually 100% of the cells identified were lymphoblasts. Since neutrophils are primarily present in these samples because peripheral blood was used to dilute the number of blasts to determine the detection limits of this technique, we would not anticipate the same number of neutrophils in undiluted specimens.

**Table 1. Enhancement of U937 cell collection with the magnetic needle**

<table>
<thead>
<tr>
<th>U937 cells</th>
<th>Percentage of U937 cells</th>
<th>After harvest</th>
<th>Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4 × 10^4</td>
<td>66% (4%)</td>
<td>66% (9%)</td>
<td>1.00 (0.19)</td>
</tr>
<tr>
<td>3.2 × 10^5</td>
<td>33% (3%)</td>
<td>49% (3%)</td>
<td>1.49 (0.23)</td>
</tr>
<tr>
<td>1.6 × 10^6</td>
<td>21% (2%)</td>
<td>34% (8%)</td>
<td>1.62 (0.21)</td>
</tr>
<tr>
<td>8.0 × 10^6</td>
<td>13% (2%)</td>
<td>20% (6%)</td>
<td>1.55 (0.28)</td>
</tr>
<tr>
<td>4.0 × 10^7</td>
<td>7% (0%)</td>
<td>12% (6%)</td>
<td>1.85 (0.87)</td>
</tr>
<tr>
<td>2.0 × 10^8</td>
<td>3% (0%)</td>
<td>8% (4%)</td>
<td>2.65 (1.2 )</td>
</tr>
<tr>
<td>1.0 × 10^9</td>
<td>2% (0%)</td>
<td>4% (1%)</td>
<td>2.75 (0.35)</td>
</tr>
<tr>
<td>5.0 × 10^9</td>
<td>1% (N/A)</td>
<td>4% (N/A)</td>
<td>3.5 (N/A)</td>
</tr>
<tr>
<td>2.5 × 10^10</td>
<td>0.5% (N/A)</td>
<td>4% (N/A)</td>
<td>8.0 (N/A)</td>
</tr>
<tr>
<td>1.25 × 10^11</td>
<td>0.5% (N/A)</td>
<td>5% (N/A)</td>
<td>10.0 (N/A)</td>
</tr>
</tbody>
</table>

NOTE: The amount and percentage of U937 cells is indicated. Experiment was performed in triplicate. SD is shown in parentheses. Abbreviation: N/A, no SD was available.

**Table 2. Enhancement of collection of lymphoblasts from bone marrow samples**

<table>
<thead>
<tr>
<th>Bone marrow dilution in blood</th>
<th>Pre-needle percentage of lymphoblasts</th>
<th>Percentage of lymphoblasts after harvest</th>
<th>Percentage of lymphoblasts without neutrophils after harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1:64</td>
<td>32.0%</td>
<td>6.0%</td>
<td>3.5%</td>
</tr>
<tr>
<td>1:128</td>
<td>27.0%</td>
<td>3.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>1:256</td>
<td>19.5%</td>
<td>2.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>1:512</td>
<td>13.0%</td>
<td>1.0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>1:1024</td>
<td>5.5%</td>
<td>1.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>1:2058</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

NOTE: The amounts and percentages of lymphoblasts are indicated. Needle draws were performed on three individual patients.

Discussion

Cellular targeting using superparamagnetic particles is not a new clinical strategy (23); however, the evolution of this technology has provided new opportunities for its use as a clinical tool. Within the area of nanoparticles, several techniques, including antibody-bound (24), small-molecule modification (25), and aptamer-based (7) technologies have been studied. Since the accurate detection of residual disease has previously been shown to lead to response-based improvements in leukemia-free survival, we investigated the binding of ligand-bearing nanoparticles to CD34+ cells to enhance collection of lymphoblast cells using a magnetic needle as an initial step toward the development of a tool to increase the sensitivity of detection of residual disease in acute leukemia patients. We found the binding of nanoparticles to CD34+ cell lines was dependant on cell line receptor expression and the presence CD34 antibody ligand on the nanoparticle surface. We also showed the capacity for ligand-bearing particles to bind to CD34+ cell lines and patient leukemic cells and for these cells to be collected using a magnetic needle at levels that improve upon standard bone marrow sampling.

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marrow aspirate technique. Finally, these results suggest that ligand-bearing nanoparticles in combination with magnetic needle extraction present a novel option for oncologists to use targeted leukemic sampling to improve detection of low levels of disease.

In our studies, attachment of nanoparticles to cells was ligand and receptor expression dependant whether assessed qualitatively, by light microscopy, or quantitatively, by SQUID magnetometry. Increased nanoparticle attachment was observed on cells that expressed higher levels of CD34 when compared with cell lines that had lower expression. Interestingly, our titration studies indicated that there was a limit to the number of nanoparticles that bound to a cell. Nanoparticles (7.1 x 10^4) bound to each cell, although 9.2 x 10^4 receptors were present. The limit of nanoparticle binding to a cell may be a result of steric considerations, either based on cell size or access to the receptor, or incomplete coupling of the nanoparticles with antibodies directed against CD34. Since U937 has a reported diameter of between 10 and 20 μm, and the nanoparticles used in this study have a diameter of 140 nm, and a rough calculation indicates that ∼1.6 x 10^4 to 6.5 x 10^4 nanoparticles can bind to the surface of a cell. Our observed limit seems to be in close agreement with this theoretical calculation.

Magnetic needle extraction enhanced the number of CD34-expressing cells visible by microscopy in both spiked U937 cells as well as freshly collected patient bone marrow samples diluted into blood. The enhancement increased as the percentage of CD34-expressing cells was lowered in the sample. Notably, because we were required to dilute samples, we were required to use human peripheral blood, which, unlike bone marrow, had a high percentage of neutrophils (50–70%). Our enhancement was observed in spite of the nonspecific uptake of nanoparticles by neutrophils and their resultant harvesting. As we move to a formal clinical trial, we anticipate testing bone marrow samples with a low percentage of leukemic blasts and few neutrophils, as the rule rather than the exception, because neutrophils are not as prevalent in marrow as blood. Accordingly, the future clinical use of this device may show even higher collection enhancement than we observed. Nonetheless, the number of lymphoblasts collected on the needle remained fairly constant regardless of dilution factor, and at the lowest dilutions, the number of lymphoblasts in the pre–needle sample approaches the number of lymphoblasts recovered on the needle (data not shown).

Nanoparticles, in the absence or presence of cells but without a targeting ligand, also showed a measurable background SQUID signal. This background is derived from two sources: nanoparticle agglomeration visible by light microscopy in samples without cells as well as nonspecific uptake and binding of naked nanoparticles to cells. Agglomeration of the nanoparticles causes a signal similar to cell-nanoparticle binding and was visible by SQUID relaxation magnetometry. Agglomeration has not been described in previous studies using nanoparticles as agents in magnetic resonance imaging studies. However, our experiments indicate that a low level of agglomeration is present in virtually all nanoparticle preparations and is reflective of the high sensitivity of the SQUID magnetometry relative to other methods. Additionally, nanoparticle aggregation was easily distinguishable from cell-nanoparticle binding by microscopy. Nonspecific cellular attachment and uptake of nanoparticles may also occur but was not easily visualized by light microscopy. The nonspecific uptake of nanoparticles has been reported by previous investigators (8, 9), which showed that cell lines preferentially take up nanoparticles regardless of ligand. Nonetheless, our background SQUID signals were lower than the specific nanoparticle-cell signal and could easily be subtracted.

The magnetic needle, when combined with microscopy, proved to enhance the collection and identification of CD34-expressing cells. The ultimate goal of this study is to develop this technique for future use in clinical trials and we are now in a position to test the ex vivo extraction of leukemia cells from patient bone marrows in a formalized clinical trial. In addition, this sampling modality seems to have the capacity to identify minimal residual disease earlier, which may improve survival and reduce therapy-related patient toxicity. A key advantage of the nanoparticles used in this study is their potential for in vivo use for the detection and possible treatment of leukemias. In the future, we intend to directly inject the antibody-labeled nanoparticles into the bone marrow and then specifically harvest or kill CD34-expressing cells.

Disclosure of Potential Conflicts of Interest

N.L. Adolphi has an equity interest in ABQMR and nanoMR, small companies developing magnetic resonance–based biosensing technologies. ABQMR and nanoMR did not support this work. The other authors declared no potential conflicts of interest.

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Figure 4. Percentage of lymphoblasts in pre-needle and needle harvest samples of three leukemia patients. The patient samples were diluted 1:1024 in donor blood, and the lymphoblast percentage was calculated both before and after needle harvest. Since neutrophils were present primarily due to dilution of sample with blood so that the percentage of blasts was low, the percentage of lymphoblasts was calculated with neutrophils included in the WBC count (A) and excluded from the WBC count (B).
References
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