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Brief report

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Introduction

Contaminant cancer cells in bone marrow (BM) or mobilized peripheral blood (PB) transfusions have been shown to correlate with disease relapse in the setting of some hematologic malignancies. In recent years, several technologies have been employed to purge tumor cells from autologous transfusions, including antibody-mediated selection for progenitor cells, depletion of tumor cells, genetic modification of tumor cells, selective chemical purging from stem cell preparations in non-Hodgkin lymphoma (NHL) with recent evidence of clinical benefit. Whereas no studies on a similar scale or with similar results exist for multiple myeloma (MM), we selected this hematologic disease as a test case for our purging technology since BM samples from patients with myeloma are consistently infiltrated with tumor, the tumor cells are readily quantified, and autologous transplantation is used to treat patients with this disorder. It should be emphasized however, that the data presented here simply use myeloma as an example and do not argue for or against the clinical utility of autologous transplantation with purging for MM.

The NHL studies that did use purging employed CD34 selection that is time-, cost-, and labor-intensive, and may not efficiently preserve hematopoietic stem cells. We sought to test a different method based on prior studies showing that defined electric field pulses applied to static, small volume samples selectively depleted breast cancer and megakaryocyte cell lines by 2 to 2.5 logs from mixtures with blood cells, and preserved small cells including lymphocytes and CD34+ cells. The principle behind selective pulsed electric field (PEF) purging is that a cell’s cytosol is largely conductive, but the lipid cell membrane does not conduct electricity. The voltage developed across each cell is proportional to the cell’s diameter. Under defined electric field conditions, larger cells are killed without altering the viability of smaller cells, including hematopoietic stem cells (HSCs). Whereas HSCs and resting lymphocytes are generally 6 μm to 8 μm in diameter, myeloma cells and other tumor cells are generally more than 10 μm in diameter.

We have now applied this technology in a modified format that permits continuous and rapid pulsing of clinically relevant numbers of cells (> 10⁹ cells in 30 minutes) at controlled flow rates that negate the effects of cell concealment or cell settling (manuscript in preparation). Applying this technology to myeloma, we define tumor cell depletion by 3 to 6 orders of magnitude without sacrificing functional stem cells.

From Science Research Laboratory Inc, Somerville, MA; the Center for Regenerative Medicine and Technology, Massachusetts General Hospital; and the Jerome Lipper Myeloma Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.


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A.C. and Y.S. contributed equally to this work.

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Reprints: David T. Scadden, Center for Regenerative Medicine and Technology, Cancer Center, Massachusetts General Hospital, Harvard Medical School, 149 13th St, Room 5212D, Charlestown, MA 02129-2000; e-mail: scadden.david@mgh.harvard.edu.

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Study design

Preparation of primary cells and cell lines

PB was obtained from donors at Massachusetts General Hospital (MGH). BM was obtained from NDR (Philadelphia, PA), and 2 mL to 5 mL of discard BM aspirates were obtained from patients with MM at the Dana-Farber/Harvard Cancer Center (institutional review board approval no. 1999-P008 401/4). Bone marrow or peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque centrifugation. Cell lines were obtained from American Type Culture Collection (ATCC; Manassas, VA).

Flowing PEF apparatus

A prototype flowing PEF apparatus was designed and constructed by Science Research Laboratory (SRL). Components include the flowing treatment chamber, Cytopulse (Rockville, MD) electric pulse driver system, Tektronix oscilloscope (Beaverton, OR), PC-based control system, syringe pumps (Harvard Apparatus, Framingham, MA), and 4-way valve and outlet for purging air (manuscript in preparation).23

In vitro assays and staining

For tumor regrowth assays, cells were serially diluted in triplicate in conditioned medium/RPMI/fetal calf serum (FCS) in 96-well plates. After 2 weeks, plates were scored for colony formation (>1 colony of >4 viable tumor cells).

Colony-forming cell (CFC) and long-term culture-initiating cell (LTC-IC) assays were performed as described.1

All antibodies and staining reagents were used according to company protocols (BDPharmingen, San Diego, CA, and Molecular Probes, Eugene, OR). Flow cytometry was performed as described in Figure 1.
Engraftment of NOD/SCID/β2m<sup>−/−</sup> mice

Six- to 8-week-old nonobese diabetic–severe combined immunodeficient (NOD/SCID)/β2m<sup>−/−</sup> mice were obtained from the Jackson Laboratory (Bar Harbor, ME), handled, maintained, and underwent transplantation as described.<sup>21</sup>

Results and discussion

Selective PEF purging of carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled myeloma cell lines (RPMI8226), mixed with PBMCs or bone marrow cells, was achieved at 1.4 kV/cm (Figure 1A-B). After PEF, viable cells were small and confined largely to the “lymphocyte gate” of a forward- and side-scatter plot, known to contain stem and progenitor cells, while the high forward- and side-scatter myeloma cells largely disappeared (Figure 1B, left). Purging of myeloma cells (CFDA-SE<sup>+</sup>) as well as monocytes (CD14<sup>+</sup>), with preservation of lymphocytes (CD3<sup>+</sup>/CD19<sup>+</sup>), was also evident.

Dose response to PEFs was evaluated. We noted purging of tumor cells (4 logs) with preservation of small lymphocytes (Figure 1C). While a representative experiment is shown, PEF consistently purged cancer cells by 3 to 4 logs, (4- to 5-log limit of detection) with preservation of small blood cells. Monocytes (CD14<sup>+</sup>), larger than lymphocytes and stem cells, were also purged by about 2 logs at 1.35 kV/cm. Experiments involving higher cell densities, up to 10<sup>7</sup> cell/mL, demonstrated similar tumor purging with preservation of small cells (data not shown), validating the utility of this system for large-scale tumor purging.

In order to quantify the purging of truly viable, proliferation-competent myeloma cells, tumor regrowth cultures were established (Figure 1D). Comparison between unpulsed and pulsed tumor cell regrowth showed 5 to 6 logs or greater purging (limit of detection for < 10<sup>6</sup> starting tumor cells). The mean log purging using RPMI8226 cells seeded into PBMCs, assessed by flow cytometry or tumor regrowth assay, is shown in Figure 1E (n = 6). These data suggest that flow cytometry enumeration, unable to distinguish between replication-competent cells and osmotically damaged but morphologically intact cells, underestimates the extent of tumor purging. Further, the lysing of cells by the PEF process releases large amounts of genomic DNA that cannot be effectively removed even with DNase treatment. Therefore, DNA polymerase chain reaction for detection of MM cells after PEF was not effective even with DNase treatment. Therefore, DNA polymerase chain reaction for detection of MM cells after PEF was not effectively applied.

After PEF purging, the capacity of human BM cells to engraft irradiated NOD/SCID/β2m<sup>−/−</sup> BM was preserved, with more than 5% human CD45 cells after 7 weeks (Figure 1F). Preservation of engraftment capacity has also been demonstrated in NOD/SCID mice. Furthermore, CFC and LTC-IC frequency in vitro were similar or slightly enriched after PEF purging at 1.40 kV/cm, the upper limit and current standard field strength for most efficient tumor purging and progenitor cell preservation, and frequency decreased at and above 1.45 kV/cm (Figure 1G). These results demonstrate the functional and differentiation competence of the PEF-treated progenitor cells in vitro and in vivo.

Myeloma cells from the BM of patients with MM were killed at 1.40 kV/cm, demonstrated by trypan blue staining (Figure 2A) and flow cytometry (CD45<sup>dim</sup>CD138<sup>+</sup> CD38<sup>hi</sup> cells; Figure 2B). Percent survival of myeloma or small CD34<sup>+</sup> cells after PEF treatment is summarized in Figure 2C (n = 4). In each case, the CD45<sup>dim</sup>CD38<sup>hi</sup>CD138<sup>+</sup> population was purged at or near the limit of detection of 3 logs (due to small starting cell numbers); CD34<sup>+</sup> cells were also affected at that pulse dose but with lower cell kill, preserving 50% ± 16%.

The data presented here confirm the principle of size selectivity using PEF technology, and demonstrate the utility of the flowing PEF technique for rapid purging of myeloma cells from progenitor and stem cell specimens. This method is appropriate for clinical sample volumes, and may provide a useful strategy for tumor cell purging from autologous BM or mobilized PB stem cell preparations. Overall purging efficacy, volume of specimen, and procedure time are significantly improved compared with our prior report.<sup>17</sup> The patient sample data presented here emphasize the potential clinical utility of this approach.

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References


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