Engineering Dendritic Cell Grafts for Clinical Trials in Cellular Immunotherapy of Cancer: Example of Chronic Myelogenous Leukemia

Allan B. Dietz1, Mark R. Litzow2, Dennis A. Gastineau2, Stanimir Vuk-Pavlović1,2

1Stem Cell Laboratory, Mayo Clinic Cancer Center, and 2Division of Hematology, Mayo Clinic, Rochester, Minnesota, USA

Dendritic cells are pivotal regulators of immune reactivity and immune tolerance. The observation that dendritic cells can recruit naive T-cells has invigorated cancer immunology and stimulated clinical trials of dendritic cells in immunotherapy. However, variables inherent in preparation and use of dendritic cell grafts remain to be tested. Here we discuss the role of ex vivo dendritic cell processing for in vivo antigen presentation in clinical trials. As an example of the complexity in a clinical trial of dendritic cell vaccines, we present our ongoing trial in immunotherapy of chronic myelogenous leukemia.

Keywords: antigen-presenting cells; cancer vaccines; cells fusion; dendritic cells; immunotherapy; T lymphocytes
ing tumor-specific immunity. Presently, there are no methods of in vivo manipulation that result in dendritic cells presenting defined antigens. Consequently, methods have been developed for ex vivo engineering of dendritic cell grafts; these methods obviate tumor-borne inhibition of dendritic cell maturation and allow controlled delivery of tumor-associated or tissue-specific antigens.

Dendritic cells can be derived from different hematopoietic progenitors (16,17), but those derived from monocytes are understood best. In the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, monocytes develop into immature dendritic cells (16,17). Immature dendritic cells express higher levels of HLA Class II molecules and co-stimulatory molecules than monocytes; however, they do not express cytokines (such as IL-12) or stimulate proliferation of allogeneic T cells (16). Incubation of immature dendritic cells with lipopolysaccharide or cytokines, such as tumor necrosis factor (TNF)-α or CD40 ligand, results in mature dendritic cells (Fig. 2). These cells express the full array of co-stimulatory molecules, such as cytokines and chemokines, that make them the most effective antigen-presenting cells in vitro (16,17). A recent comparison of mature and immature dendritic cells in a murine model of tumor immunotherapy in vivo concluded that, in general, “the capacity of dendritic cells to induce an antitumor immune response... correlated to their degree of maturation” (18).

**Principles and Practice of Immunotherapy Employing Dendritic Cells: Dendritic Cell “Education” and Processing**

To initiate tumor-specific immunity, dendritic cells must be “educated” for presentation of tumor-associated antigens. Specifically, they must internalize and process antigens, select (“edit”) the resulting peptides, and present them appropriately to the immune system. The repertoire of ex vivo education methods available is vast. It includes dendritic cells interacting with defined peptides, tumor lysates, heat shock proteins, simple and fused recombinant proteins, recombinant viruses, DNA, RNA, and others (Table 1) (14,19-22). Consequently, a method developed for a particular clinical trial is selected on the basis of characterized antigenic signature of the tumor; HLA

![Figure 1. Basic in vivo interactions of dendritic cells (DC), T cells, and tumor cells.](image)

In immunologically peripheral tissues, DC precursors mature and capture antigens from tumor cells. While further maturing, they process antigens and move to lymph nodes where they recruit naïve T cells in an antigen-specific manner. T cells expand and differentiate into tumor-specific cytotoxic T lymphocytes (CTL). CTL can recognize and lyse tumor cells. Tumors, in turn, can secrete inhibitors that impede DC maturation, induce T cell anergy and even kill T cells (dashed arrows). Ex vivo methods eliminate tumor-borne inhibition and allow optimization of DC yields, phenotype, and antigen capture. The scheme is greatly simplified for clarity.

![Figure 2. Dendritic cells can be derived from monocytes under controlled conditions ex vivo.](image)

In the presence of GM-CSF and IL-4, CD14-positive monocytes differentiate into immature dendritic cells. Upon stimulation by inflammatory mediator(s) (e.g., lipopolysaccharide (LPS) or TNF-α), antigen-capturing immature dendritic cells differentiate into antigen-presenting mature dendritic cells. The figure indicates some phenotypic and functional characteristics of cells at different stages of maturity.

**Table 1. Variables in clinical trials employing vaccination with dendritic cells**

<table>
<thead>
<tr>
<th>Source of dendritic cells</th>
<th>Bone marrow, peripheral blood, CD14-positive cells, CD34-positive cells, adherent leukocytes; autologous, allogeneic (matched, unmatched, haploidentical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturation status</td>
<td>Immature, mature</td>
</tr>
<tr>
<td>Ex vivo maturation signals</td>
<td>CD40 ligand, TNF-α, monocyte-conditioned medium, interferon-γ, prostaglandin E1, heparan sulfate, calcium ionophores, etc.</td>
</tr>
<tr>
<td>No. of dendritic cells per dose</td>
<td>1-300 million*</td>
</tr>
<tr>
<td>No. of infusions</td>
<td>Varies among trials</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Intravenous, subcutaneous, intradermal, intralymphatic, intratumoral</td>
</tr>
<tr>
<td>Frequency of vaccination</td>
<td>Weekly, bi-weekly, monthly*</td>
</tr>
<tr>
<td>Source of antigen known</td>
<td>Antigenic peptides, native proteins, modified proteins, DNA, RNA, etc.</td>
</tr>
<tr>
<td>Source of antigen unknown</td>
<td>Tumor cell lysate, tumor cells fused to dendritic cells, apoptotic bodies, heat-shock proteins, RNA, endogenous mutant proteins (i.e., BCR-ABL), etc.</td>
</tr>
</tbody>
</table>

*Data from literature in Table 2.
makeup of the patient; and availability of tumor tissue. For a selected few tumors (melanoma, breast cancer, ovarian cancer, etc.), some immunodominant epitopes from tumor-associated antigens are available. However, for most tumors these epitopes are not known. Hence, methods have been developed to take advantage of the innate capacity of dendritic cells to process antigens, edit the resulting peptides and present them to the effector cells. Such methods include isolation of mRNA from patient's tumor cells and its combination with dendritic cells (23). Another method fuses tumor cells with dendritic cells (24,25). These methods do not rely on antigen isolation and characterization. An additional benefit of such “biological” methods is that they transfer to dendritic cells the entire, though uncharacterized, antigenic signature of tumors. (For a comparison of “biological” methods of dendritic cell education with more “biochemical” methods using defined antigens, see ref. 26).

Our standard clinical-scale protocol for dendritic cells preparation (“manufacturing”) begins with immunomagnetic separation of CD14-positive cells from peripheral blood mononuclear cells derived by leukopheresis (Fig. 3). CD14-positive cells are cultured for seven days with GM-CSF and IL-4, followed by three days with added TNF-α, prostaglandin (PG) E2, IL-18, and IL-6. This protocol yields a defined dendritic cell product containing uniformly mature dendritic cells (27). The CD14-negative fraction of peripheral blood mononuclear cells provides CD8+, CD19+, CD3+, and T cells for assays used to monitor immunity.

Administration of Dendritic Cell Vaccines

Evidence for the selection of a particular route of administration (intravenous, subcutaneous, intradermal, and intralymphatic) or site of injection is only emerging. The effects of a prostate-specific dendritic cell vaccine were independent of the route of administration (intravenous, subcutaneous, intradermal, and intralymphatic) or site of injection is only emerging.

Clinical Trials in Dendritic Cell Immunotherapy

Advances in understanding dendritic cell biology together with developments of methods for dendritic cell graft engineering stimulated clinical trials in cancer immunotherapy. The first successful use of autologous ex vivo-processed dendritic cells for treatment of malignancy was reported in 1996 (31). More recently, trials in several malignant diseases have documented induction of cellular immune response and, in some cases, clinical benefits (Table 2).

Clinical trials listed in Table 2 differ in variables, such as mode of antigen delivery to dendritic cells, method of dendritic cell manufacture, site of infusion, etc, but common is the use of dendritic cells in delivery of tumor-associated antigens. These trials provide the first strong evidence for the capacity of dendritic cells to induce autologous T cell response in vivo and stimulate clinically beneficial immunity. This success has stimulated clinical trials to evaluate dendritic cell therapy for virtually every tumor type worldwide. These trials employ different protocols and a plethora of cell preparation methods (Table 2).

Chronic Myeloid Leukemia - Example of Dendritic Cell Immunotherapy

Recently we commenced a Phase I clinical trial, testing the safety of autologous dendritic cells injected for immunotherapy of chronic myeloid leukemia (CML). Our approach has taken advantage of the pathognomonic BCR-ABL fusion protein (32). CML is a malignant disorder of hematopoietic stem cells, manifested predominantly as abnormal proliferation of myeloid precursors (33,34). It is diagnosed at a median age between 50 and 60 (35). The disease is characterized by a reciprocal translocation that fuses a portion of the abelson (abl) oncogene on chromosome 9 to the breakpoint cluster region (bcr) on chromosome 22 (36,37). The resulting chromosome 22 contains a hybrid gene producing an active aberrant BCR-ABL tyrosine kinase (38). This kinase underlies malignant transformation and provides a molecular marker for transformed cells.

For many years, therapy of CML consisted of chemotherapy (busulfan, hydroxyurea) that did not significantly alter the natural history of the disease (39). Recombinant human interferon-α (40) improves survival in comparison to chemotherapy (41), but is coupled with considerable side effects and high cost (42). Autologous transplant of hematopoietic stem cells extends survival, but most post-transplant patients still harbor residual leukemia (43). STI571, a specific abl kinase inhibitor, elicits significant cytogenetic responses in only one in three patients (44,45). Allogeneic bone marrow transplant (BMT) from HLA
identical siblings results in durable leukemia-free survival with eradication of bcr-abl-positive cells in more than 60% patients transplanted in the chronic phase. In patients with more advanced disease, the survival rate is lower (46). Allogeneic transplants are best tolerated by younger individuals; they are estimated to be an option for only 15-25% of all CML patients. However, the effectiveness of donor lymphocyte infusions in re-inducing remission after relapse following allogeneic transplant underscores the potency of immune mechanisms in the treatment of CML (47). Altogether, a significant number of patients lack effective treatment options. For this reason, we chose to develop an immunotherapy protocol employing dendritic cell vaccines.

CML is special with regard to antigen presentation by dendritic cells because dendritic cells can be derived from leukemic myeloid precursors. Such dendritic cells express tumor-associated genes. Other hematopoietic malignancies that originate from the same dendritic cell precursor (e.g., acute myeloid leukemia) (48) might be amenable to a similar therapeutic approach. Leukemic dendritic cells can elicit autologous and/or allogeneic T cell responses in vitro (49-52). Consequently, dendritic cells used in our current Phase I clinical trial are derived without exogenous antigen. This simplifies manufacturing in comparison to procedures that employ exogenous antigen(s), but limits the applicability of this paradigm.

For this trial, we manufacture dendritic cells by our standard method of in vitro maturing CD14-positive cells (see above) (27). To determine whether dendritic cells are fully leukemic, we used fluorescence in situ hybridization to test peripheral blood mononuclear cells and the final CML-dendritic cells for the presence of the bcr-abl fusion. Data showed that peripheral blood mononuclear cells from six CML patients contained bcr-abl-positive cells in a range from 65% to 97% (Fig. 4). After selection and maturation, dendritic cell populations contained more than 98% bcr-abl-positive cells. Thus, the method yielded grafts highly enriched in leukemic cells.

The regimen for administration CML-dendritic cells includes four subcutaneous injections given four weeks apart. In the absence of pertinent evidence, the number of doses and their timing was chosen to mini-
in vitro cytokine proliferation stimulated by dendritic cells
the number of circulating
bcr
relate a quantitative measure of tumor burden (e.g.,
assays include delayed-type hypersensitivity, leuko-
and molecular techniques. For all patients, immune
progression of disease or no longer than two years. To
monitoring. After the last infusion, patients are as-
cells, we draw 99 mL of peripheral blood for immune
ex vivo
matured dendritic cells is assessed by
ex vivo
mature dendritic cells were derived from six chronic myeloid leukemia (CML) patients.
Reproduced from ref. 27 by permission of the publisher.

Figure 4. Immunomagnetic separation and ex vivo matura-
tion yield dendritic cells (DC) highly enriched in bcr-abl

mize patient travel. Before each infusion of dendritic
cells, we draw 99 mL of peripheral blood for immune
monitoring. After the last infusion, patients are assessed one month later and every six months until progression of disease or no longer than two years. To guard against toxicity, the initial Phase I study is accruing cohorts at monthly doses of $3 \times 10^6$, $15 \times 10^6$, or $50 \times 10^6$ CD83-positive cells. The lowest number of $3 \times 10^6$ cells was selected because it was found safe in a different immunotherapy protocol (30). The highest dose of $50 \times 10^6$ cells is set by the maximum manufacturable number of CML-dendritic cells from a single unit of apheresis product.

Assessment of Response to Therapy

Generally, the relationship between cellular immu-

nity in vivo and the clinical status has not been est-
blished well. CML provides the opportunity to cor-
relate a quantitative measure of tumor burden (e.g.,
the number of circulating bcr-abl-positive cells) with
parameters of cellular immunity. Response to infu-
sions of ex vivo matured dendritic cells is assessed by
changes in clinical signs and immune parameters.
Clinical response (remission, stable disease, and pro-
gression) is evaluated by hematological, cyto
genetic, and molecular techniques. For all patients, immune
assays include delayed-type hypersensitivity, leuko-
cyte proliferation stimulated by dendritic cells in vi-
tro, and induction of cytokines specific for T helper cells.
In HLA-A2-positive patients, additionally we employ
HLA-tetramer-binding assay specific for PR1, a pep-
tide derived from proteinase 3, an antigen associated
with CML cells (53) and PR1-specific or bcr-abl-spe-
cific CTLp assay. The primary endpoint of this trial is
the safety of leukemic dendritic cell vaccines; the sec-
ondary end-point is clinical and/or immunologic
response. The immune assays currently available for
monitoring do not predict the clinical response well.
However, it is generally believed that monitoring T
cell activity is an acceptable laboratory parameter for
assessment of immune response.

Future Trends in Dendritic Cell-based
Immunotherapy

Some concepts in experimental immunotherapy
differ from other modes of cancer therapy. For exam-
ple, chemotherapy is replete with Phase I and Phase II
trials that were terminated for excessive toxicity and/or lack of efficacy. However, most trials of den-
dritic cell-based immunotherapy report some success
in control of disease (Table 2). These trials employed
different sources of dendritic cells, levels of dendritic
cell maturity, “education” methods, dosage, fre-
quency, and route of administration. This makes it dif-
cult to define variables most relevant for induction
and maintenance of antitumor immunity and necessi-
tates large-scale comparative studies to define opti-
mal protocols.

An interesting possibility has been raised re-
cently by successful application of allogeneic den-
dritic cells fused to autologous renal cell carcinoma
cells (54). If this paradigm is generally applicable, it
will be possible to create banks of preprocessed and
cryopreserved normal dendritic cells. Such dendritic
cells will be readily available for “education” and ap-
lication saving on processing time for the manufac-
turer and on the inconvenience of leukopheresis for
the patient. Combining allogeneic dendritic cells
with tumor-borne mRNA (23) might provide further
flexibility, as this education method requires small
numbers of tumor cells, e.g., those obtained by fine
needle biopsy. However, the use of tumor-borne
mRNA in allogeneic dendritic cells still lacks the
proof of principle, as it is not known whether auto-
logous (ie, patient’s) HLA molecules would be ex-
pressed by allogeneic dendritic cells for proper anti-
gen presentation.

Immunotherapy of chronic cancer can engender
biological challenges, such as development of tumor
evasion. Evasion is a response to selective pressure
imposed by immunotherapy. This phenomenon can
employ mechanisms that include anergy, immuno-
suppression, and loss of HLA molecules. In addition
to stimulation of these mechanisms, immunotherapy
can result in expansion of tumor cells lacking the
molecular target, particularly if initiated by recognition
of a single tumor-associated antigen. Consequently, it
is possible that some tumors will require lifelong re-
peated immunizations with the changing antigens of
the tumor as it evades therapeutic immunity (15).

Combining cellular immunotherapy with other
therapeutic modalities could mitigate some of these
problems. So far, clinical trials have been conducted
in the patients with limited life expectancy. Immu-
notherapy could be more effective at earlier stages of
disease before cytotoxic and radiation therapy damage hematopoiesis and immunity. In the patients rescued by hematopoietic stem cell transplantation after tumor burden reduction by high-dose chemotherapy, dendritic cell therapy could boost restoration of immunity and elimination of residual disease. The effects of tumor-borne immunosuppression could be countered by neutralizing immunosuppressive molecules (53), whereas detectable antigen presentation (56) might be offset by differentiating agents.

Clinical dendritic cell therapy is some five years old, yet it has demonstrated its potential in treating a variety of malignancies. Numerous variables inherent in the method require evaluation, optimization, and standardization. At the same time, they provide ample opportunities for basic, translational, and clinical research in the future.

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References

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Correspondence to:
Allan B. Dietz
Stem Cell Laboratory
Mayo Clinic
200 First Street SW
Rochester, MN 55905, USA
dietz.allan@mayo.edu