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# Product References

**X-VIVO 10, 15, & 20**

## X-VIVO 10 - References

Wu, M. H., S. L. Smith, and M. E. Dolan. November 2001. **"High Efficiency Electroporation of Human Umbilical Cord Blood CD34(+) Hematopoietic Precursor Cells."** Stem Cells 19, no. 6: 492-9.

**X-VIVO 10** Abstract: Human umbilical cord blood provides an alternative source of hematopoietic cells for purposes of transplantation or ex vivo genetic modification. The objective of this study was to evaluate electroporation as a means to introduce foreign genes into human cord blood CD34(+) cells and evaluate gene expression in CD34(+)/CD38(dim) and committed myeloid progenitors (CD33(+), CD11b(+)). CD34(+) cells were cultured in **X-VIVO 10** supplemented with thrombopoietin, stem cell factor, and Flt-3 ligand. Electroporation efficiency and cell viability measured by flow cytometry using enhanced green fluorescent protein (EGFP) as a reporter indicated 31% +/- 2% EGFP(+)/CD34(+) efficiency and 77% +/- 3% viability as determined 48 hours post-electroporation. The addition of allogeneic cord blood plasma increased the efficiency to 44% +/- 5% with no effect on viability. Of the total CD34(+) cells 48 hours post-electroporation, 20% were CD38(dim)/EGFP(+). CD34(+) cells exposed to interleukin-3, GM-CSF and G-CSF for an additional 11 days differentiated into CD33(+) and CD11b(+) cells, and 9% +/- 3% and 8% +/- 7% were expressing the reporter gene, respectively. We show that electroporation can be used to introduce foreign genes into early hematopoietic stem cells (CD34(+)/CD38(dim)), and that the introduced gene is functionally expressed following expansion into committed myeloid progenitors (CD33(+), CD11b(+)) in response to corresponding cytokines. Further investigation is needed to determine the transgene expression in functional terminal cells derived from the genetically modified CD34(+) cells, such as T cells and dendritic cells.

Cavazzana-Calvo, M. and others. 28 April 2000. **"Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease."** Science 288, no. 5466: 669-72.

**X-VIVO 10** Abstract: Severe combined immunodeficiency-X1 (SCID-X1) is an X-linked inherited disorder characterized by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the gammac cytokine receptor subunit of interleukin-2, - 4, -7, -9, and -15 receptors, which participates in the delivery of growth, survival, and differentiation signals to early lymphoid progenitors. After preclinical studies, a gene therapy trial for SCID- X1 was initiated, based on the use of complementary DNA containing a defective gammac Moloney retrovirus-derived vector and ex vivo infection of CD34+ cells. After a 10-month follow-up period, gammac transgene-expressing T and NK cells were detected in two patients. T, B, and NK cell counts and function, including antigen-specific responses, were comparable to those of age-matched controls. Thus, gene therapy was able to provide full correction of disease phenotype and, hence, clinical benefit.

Pawelec, G. and others. June 1999. **"T cell immunosenescence in vitro and in vivo."** Exp Gerontol 34, no. 3: 419-29.

**X-VIVO 10** Abstract: The term "immunosenescence" refers to an age-associated dysregulation of immune function which contributes to the increased susceptibility of the elderly to infectious disease. Although there are age-associated changes measurable in the innate immune system (Pawelec et al., 1998c), it is the adaptive, particularly T cell, system which is most susceptible to the deleterious effects of aging. In this minireview, characteristics of aging in long-term human T cell cultures will be summarized, and the parallels between the in vitro model and in vivo immunosenescence will be documented. The use of culture models to screen for

ways of manipulating immunosenescence in vitro may provide a basis for intervention to ameliorate immunosenescence in vivo.

Pawelec, G. and others. December 1998. **"Extrathymic T cell differentiation in vitro from human CD34+ stem cells."** J Leukoc Biol 64, no. 6: 733-9.

**X-VIVO 10** Abstract: Although it is well established that T cells are derived from CD34+ stem cells in vivo, and that T cells can develop in the absence of a functioning thymus, it has not proven possible thus far to generate human T cells in vitro from CD34+ cells in the absence of any thymic influence. We now present a limiting dilution cloning culture system that supports the differentiation of highly purified human CD34+ cells to CD3+ T cells in vitro in the complete absence of any thymic components. The culture system features the use of a serum-free medium supplemented with a cocktail of cytokines including flt-3 ligand, interleukin-3 (IL-3), stem cell factor (SCF), and IL-2. CD4+ T cell clones capable of mitogen-stimulated proliferation and response to IL-2, and expressing a varied TCR-Vbeta repertoire were obtained under these conditions. This culture system therefore supports human T lymphopoiesis in the absence of any thymic influence and may prove useful for the evaluation of extrathymic T cell differentiation in vitro.

Weich, N. S. and others. 15 November 1997. "Recombinant human interleukin-11 directly promotes megakaryocytopoiesis in vitro." Blood 90, no. 10: 3893-902.

**X-VIVO 10** Abstract: We have investigated the mechanism of action of the thrombopoietic cytokine, recombinant human interleukin-11 (rhIL-11), on megakaryocytopoiesis in vitro. We have shown that rhIL-11-induced murine and human megakaryocytopoiesis are not mediated by thrombopoietin (Tpo). Murine megakaryocytes (MKs) were produced from bone marrow (BM) mononuclear cells cultured with rhIL-11, IL-3, and a combination of the two cytokines. Conditioned media (CM) were collected and assayed for the presence of biologically active Tpo. Tpo activity was not detected in any of the CMs tested. Next, human BM CD34+ cells were cultured in serum-free fibrin clot medium with rhIL-11, IL-3, or rhIL-11 plus IL-3 and an antibody that neutralizes human Tpo activity. No inhibition of either burst-forming unit-MK- or colony-forming unit- MK-derived colony formation was observed. The antibody did partially inhibit steel factor-induced MK-colony formation, suggesting that the actions of this cytokine are mediated, in part, by Tpo. We determined that MKs can be direct targets of rhIL-11 by showing the expression of functional IL-11 receptor on these cells. Total RNA was prepared from cultured human BM CD41+CD14- cells (MKs) and IL-11 receptor alpha chain mRNA was detected in the MKs by reverse transcription-polymerase chain reaction. Analysis of single-sorted CD41+CD14- cells confirmed that the observed IL-11 receptor expression was not due to contaminating CD41- cells in the pool. The presence of rhIL-11 receptor alpha chain protein in the cells was established by Western blot analysis. After a short exposure of purified BM MKs to rhIL-11, enhanced phosphorylation of both its signal transduction subunit, gp130, and the transcription factor, STAT3 was detected, showing a direct activation of receptor signaling by the cytokine. Consistent with the lack of effect of rhIL- 11 on platelets in vivo, IL-11 receptor alpha chain mRNA and protein were not detected in isolated human platelets. These data indicate that rhIL-11 acts directly on MKs and MK progenitors but not on platelets.

Williams, Stephanie F. and others. 1996. **"Selection and expansion of peripheral blood CD34+ cells in autologous stem cell transplantation for breast cancer."** Blood 87: 1687-91.

**X-VIVO 10** Abstract: Cytopenia after high-dose chemotherapy and autologous stem cell reinfusion is a major cause of morbidity. Ex vivo cultured expansion and differentiation of CD34+ peripheral blood progenitor cells (PBPC) to neutrophil precursors may shorten the neutropenic period further. we explored the use of these ex vivo cultured PBPCs in nine patients with metastatic breast cancer. All underwent PBPC mobilization with cyclophosphamide, VP-16, and G-CSF. Subsequently, they underwent four to five apheresis procedures. One apheresis product from each patient was prepared using the Isolex 300 Magnetic Cell Separation System (Baxter Immunotherapy, Irvine, CA) to obtain CD34+ cells. These cells were then cultured in gas permeable bags containing serum-free **X-VIVO 10** (BioWhittaker, Walkersville, MD) medium supplemented with 1% human serum albumin and 100 ng/ml PIXY321. At day 12 of culture the mean fold expansion was 26x with a range of 6 to 64x. One patient's cells did not expand because of a

technical difficulty. The final cell product contained an average of 29.3% CD15+ neutrophil precursors with a range of 18.5% to 48.1%. The patients underwent high-dose chemotherapy with cyclophosphamide, carboplatin, and thiotepa. On day 0, the cryopreserved PBPCs werre reinfused and on day +1 the 12-day cultured cells were washed, resuspended, and reinfused into eight or nine patients. One patient was not infused with cultured cells. The mean number of cultured cells reinfused was 44.6 x 10(6) cells/kg with a range of 0.8 to 156.6 x 10(6) cells/kg. No toxicity was observed after reinfusion. The eight patients have recovered absolute neutrophil counts >500/microL on a median of 8 days (range 8 to 10 days); the median platelet transfusion independence occurred on day 10 (range 8 to 12 days) and platelet counts >50,000/microL were achieved by day 12 (range 9 to 14) for the seven patients whose platelet counts could be determined. Expanded CD34+ selected PBPC can be obtained and safely reinfused into patients.

Sandstrom, Craig E. and others. 1996. **"Comparison of Whole Serum-Deprived Media for Ex Vivo Expansion of Hematopoietic Progenitor Cells from Cord Blood and Mobilized Peripheral Blood Mononuclear Cells."** Journal of Hematotherapy 5: 461-73.

**X-VIVO 10** Abstract: A whole serum-deprived (WSD) medium was developed and optimized for expansion of colony-forming cells (CFC) in cord blood (CB) mononuclear cell (MNC) cultures. This medium was compared with four commercially available WSD media (commercial media), three WSD media whose compositions have been publicly disclosed (public media), two serum-containing media, and two basal media, for cell and CFC expansion in 10-day CB and mobilized peripheral blood (PB) MNC cultures supplemented with interleukin-3 (IL-3), IL-6, and stem cell factor (SCF). Selected WSD media and both serum-containing media gave significant CFC expansion in CBMNC and PBMNC cultures. The serum-containing human long-term medium gave the greatest cell (3.0-fold) and CFC (25-fold) expansions in CBMNC cultures, whereas our medium maintained the most cells (93% of input) and gave the greatest CFC expansion (6.1-fold) for PBMNC cultures. Of the commercial media, Progenitor-34 gave the greatest cell expansion (1.2-fold) and **X-VIVO 10** gave the greatest CFC expansion (11-fold) for CBMNC cultures, and Progenitor-34 maintained the most cells (83% of input) and gave the greatest CFC expansion (3.1-fold) for PBMNC cultures. Of the public media (including ours), our medium gave the greatest cell (1.4-fold) and CFC (6.1-fold) expansion for CBMNC cultures. Although there were slight correlations between cell and CFC expansion in 10-day CBMNC and PBMNC cultures (r2 of 0.848 and 0.594, respectively), the correlations did not give reliable predictions for medium selection.[1] In addition, the different media favored expansion of different CFC types and performed differently for cultures using different cell sources (CB versus PB). Taken together, these results suggest that media must be carefully screened for the cell source to be cultured and the cell type(s) to be produced (e.g. total cells, CFC).

Zimmerman, Todd M. and others. 1995. **"Clinical Use of Selected and Expanded Peripheral Blood CD34+ Cells: A Preliminary Report of Feasibility and Safety."** Journal of Hematotherapy 4: 527-9.

Abstract: In this report, we describe the preliminary results from a feasibility and safety study on the clinical use of CD34-positive cells cultured from mobilized peripheral blood. Separation and cell expansion were successfully performed, and the patients toleated the infusions without problems and achieved engraftment.

Purdy, Malcolm H. and others. 1995. **"Large Volume Ex Vivo Expansion of CD34-Positive Hematopoietic Progenitor Cells for Transplantation."** Journal of Hematotherapy 4: 515-25.

**X-VIVO 10** Abstract: A large volume culture system was developed for the ex vivo expansion of CD34 positive (+) hematopoietic progenitors, using cells donated by 15 patients receiving high-dose chemotherapy with autologous hematopoietic progenitor cell support (AHPCS). Substantial expansion of myeloid (181-fold) and megakaryocyte (41-fold) progenitors cells was demonstrated, using the conditions that we determined to be optimal: CD34+ progenitors cultured unperturbed for 7 (marrow) or 10 (blood) days in Teflon-coated bags with **X-VIVO 10** medium containing 10% autologous plasma, 100 ng/ml, respectively, of recombinant stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), and granulocyte colony-stimulating factor (G-CSF). The studies demonstrated that (a) CD34 selection was necessary to obtain large, clinically relevant numbers of hematopoietic progenitors, (b) the addition of G-CSF to the baseline regimen of SCF/IL-6 significantly enhanced the expansion of myeloid progenitors, (c) the addition of IL-1 to SCF/IL-

6 did not significantly enhance myeloid progenitor cell expansion, (d) CD34+ G-CSF-mobilized peripheral blood progenitor cells (PBPC) produced higher numbers of myeloid progenitors in culture than CD34+ marrow cells, and (e) long-term tissue culture (LTC) assays demonstrate the preservation of long-term initiating cells in ex vivo culture. The short-term and long-term reconstituting capability of CD34+ PBPC cultured in this system remains to be determined and will be evaluated in a clinical trial where they will be used as the sole source of AHPCS following high-dose therapy.

Abrahamsen, T. G. and others. 1991. **"Stimulatory Effect of Counterflow centrifugal elutriation in large-scale separation of peripheral blood monocytes can be reversed by storing the cells at 37 degrees C."** J. Clin. Apheresis 6: 48-53.

**X-VIVO 10** Abstract: Transfusion of peripheral blood monocytes may be of benefit as adjuvant treatment of leukopenic patients with serious infections. To study the feasibility of this approach, a large-scale monocyte separation procedure employing leukapheresis, density gradient centrifugation, and counterflow centrifugal elutriation was established. By processing 5 to 6 liters of normal donor blood, it was possible to obtain a mean of 1.1 x 10(9) (range: 0.5-1.7 x 10(9) cells) of mononuclear cells, of which 89% (range: 82-94%) were monocytes by Wright's stain morphology. When the elutriation was performed in **X-VIVO 10**, a commercially available, serum-free medium developed for adoptive immunotherapy, spontaneous secretion of superoxide by the monocytes was significantly higher than for monocytes elutriated in Hanks' balanced salt solution without calcium and magnesium or non-elutriated peripheral blood mononuclear cells. This stimulated state of the monocytes was observed both immediately after elutriation and after overnight storage at 4 degrees C, and it was not affected by the type of storage vessel used. Overnight storage of the monocytes at 37 degrees C resulted in a reversal of the stimulated state of the cells. Monocytes elutriated in **X-VIVO 10** and kept overnight at 4 degrees C released high amounts of arachidonic acid. A subsequent decrease in this release was seen after additional storage at 37 degrees C for 18 hours. These observations demonstrate that separation and storage variables have important effects on the state of stimulation of monocytes. Further investigations of such variables may suggest improved procedures for preparation and storage of these cells, as well as possible ways to stimulate monocytes prior to transfusion.

Streck, Richard J. and others. 1990. **"Lysis of Autologous Human Macrophages by Lymphokine-Activated Killer Cells: Interaction of Effector Cell and Target Cell Conjugates Analyzed by Scanning Electron Microscopy."** Journal of Leukocyte Biology 48: 237-46.

**X-VIVO 10** Abstract: Lymphokine (i.e., interleukin 2; IL-2)-activated killer (LAK) cells derived from normal human blood are known to destroy human tumor target cells. Accordingly, immunotherapy modalities using IL-2, either alone or in combination with LAK cells, have been evaluated for eradicating metastatic cancer. In studies conducted to characterize receptors on LAK cell membrane ultrastructures, we observed that LAK cells kill autologous human monocyte-derived macrophages (Mo). In these experiments, peripheral blood mononuclear cells of a healthy adult donor were cultured to generate LAK cells and autologous non-adherent Mo. Thereafter, conjugates were prepared by incubating for 3 h autologous populations of LAK cells and Mo. Examination of the conjugates by scanning electron microscopy (SEM) identified LAK cell-mediated killing of Mo. Moreover, SEM analysis of the LAK cell membrane architecture identified microvilli-like ultrastructures that provided a physical bridge that joind together the LAK cell and Mo. The immunological mechanism(s) underling LAK cell killing of autologous Mo is not known; nevertheless, these conjugates will provide a useful model to study membrane receptor son ultrastructures that mediate the initial stages of cytolysis that include target cell recognition and cell-to-cell adhesion. The results of our observations and the findings of other investigators who have also demonstrated LAK cell killing of autologous normal human leukocytes are discussed in the context of the association of IL-2 and IL-2-activated killer cells with side effects observed in ongoing clinical trials and with autoimmune disorders.

Helinski, Ernest H. and others. 1990. **"Tumor-Cytolytic Human Macrophages Cultured As Nonadherent Cells: Potential for the Adoptive Immunotherapy of Cancer."** Cancer Detection and Prevention 14, no. 4: 471-81.

**X-VIVO 10** Abstract: Tumor-cytolytic lymphokine (e.g., interleukin-2; IL-2)- activated killer cells are currently being evaluated in IL-2 / LAK cell adoptive immunotherapy regimens for the treatment of cancer. Monocyte-derived macrophages (Mo) are also known to be efficient tumor killer cells; accordingly, Mo that have been activated *in vitro* may also be of therapeutic merit. However, attempts to cultivate Mo for morphological and functional studies have often been compromised because Mo adhere rapidly and tenaciously to cultureware. Studies that we have conducted to address this problem have proven successful in developing procedures for the long-term cultivation of non-adherent immunocompetent Mo in serum-free medium using petri dishes containing a thin Teflon liner. The utility of this technology is documented by the results of studies presented herein in which light and scanning electron microscopy was used to analyze tumor-cytolytic human Mo. In these experiments, we demonstrated that nonadherent immunocompetent human Mo can be prepared for detailed examinations of their pleomorphic membrane architecture. Moreover, non-adherent human Mo could readily be collected for preparing conjugates of Mo and tumor cells. It is anticipated that this technology should prove useful for future structure-function studies defining the topographical location and spatial distribution of antigens and receptors on Mo membrane ultrastructures, particularly the microvilli-like projections that bridge together an immunocompetent effector Mo and target cell (e.g. tumor cells and microbial pathogens) and which proved the physical interaction required for the initial phases of a cellular immune response that includes antigen recognition and cell-to-cell adhesion.

## X-VIVO 15 - References

Reya, T. and others. 27 April 2003. **"A role for Wnt signalling in self-renewal of haematopoietic stem cells."** Nature.

**X-VIVO 15** Abstract: Haematopoietic stem cells (HSCs) have the ability to renew themselves and to give rise to all lineages of the blood; however, the signals that regulate HSC self-renewal remain unclear. Here we show that the Wnt signalling pathway has an important role in this process. Overexpression of activated beta-catenin expands the pool of HSCs in long-term cultures by both phenotype and function. Furthermore, HSCs in their normal microenvironment activate a LEF-1/TCF reporter, which indicates that HCSs respond to Wnt signalling in vivo. To demonstrate the physiological significance of this pathway for HSC proliferation we show that the ectopic expression of axin or a frizzled ligand-binding domain, inhibitors of the Wnt signalling pathway, leads to inhibition of HSC growth in vitro and reduced reconstitution in vivo. Furthermore, activation of Wnt signalling in HSCs induces increased expression of HoxB4 and Notch1, genes previously implicated in self-renewal of HSCs. We conclude that the Wnt signalling pathway is critical for normal HSC homeostasis in vitro and in vivo, and provide insight into a potential molecular hierarchy of regulation of HSC development.

Tuettenberg, A. and others. February 2003. **"Priming of T cells with Ad-transduced DC followed by expansion with peptide-pulsed DC significantly enhances the induction of tumor- specific CD8+ T cells: implications for an efficient vaccination strategy."** Gene Ther 10, no. 3: 243-50.

**X-VIVO 15** Abstract: In recent years, vaccination strategies using antigen-presenting cells (APC) have been under investigation. Antigen delivery using genetic immunization through ex vivo transduction of dendritic cells (DC) is supposed to enhance the induction of antitumor responses in humans by activating a broad range of peptide-specific CD8+ T cells. In this study, we compared the potential of adenoviral (Ad)-transduced versus peptide-pulsed DC to induce melanoma-antigen (Ag)-specific T-cell responses in vitro. Whereas gp100-peptide-pulsed DC induced long- lasting specific CD8+ T-cell responses against single peptides, Ad-transduced DC induced broad and strong, specific immunity against various peptides of the gp100-Ag. Surprisingly, several restimulations led to decreasing gp100-specific and in parallel to increasing anti-adenoviral T-cell responses. Nevertheless, those anti-adenoviral T-cell responses provided an "adjuvant" effect by inducing an early release of high amounts of IL-2/IFN-gamma, therewith enhancing CTL induction in the initiation phase. Based on these data, we suggest a prime/boost vaccination strategy in melanoma

patients--combining the use of Ad-DC and peptide-pulsed DC--to obtain efficient and long-term antitumor T-cell responses.

Geiger, J. D. and others. 1 December 2001. **"Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression."** Cancer Res 61, no. 23: 8513-9.

**X-VIVO 15** Abstract: Dendritic cells (DCs) have been shown to be a promising adjuvant for inducing immunity to cancer. We evaluated tumor lysate-pulsed DC in a Phase I trial of pediatric patients with solid tumors. Children with relapsed solid malignancies who had failed standard therapies were eligible. The vaccine used immature DC (CD14-, CD80+, CD86+, CD83-, and HLA-DR+) generated from peripheral blood monocytes in the presence of granulocyte/monocyte colony-stimulating factor and interleukin-4. These DC were then pulsed separately with tumor cell lysates and the immunogenic protein keyhole limpet hemocyanin (KLH) for 24 h and then combined. A total of 1 x 10(6) to 1 x 10(7) DC are administered intradermally every 2 weeks for a total of three vaccinations. Fifteen patients (ages 3-17 years) were enrolled with 10 patients completing all vaccinations. Leukapheresis yields averaged 2.8 x 10(8) peripheral blood mononuclear cells (PBMC)/kg, and DC yields averaged 10.9% of starting PBMC. Patients with neuroblastoma, sarcoma, and renal malignancies were treated without obvious toxicity. Delayed-type hypersensitivity (DTH) response was detected in 7 of 10 patients for KLH and 3 of 6 patients for tumor lysates. Priming of T cells to KLH was seen in 6 of 10 patients and to tumor in 3 of 7 patients as demonstrated by specific IFN-gamma-secreting T cells in unstimulated PBMCs. Significant regression of multiple metastatic sites was seen in 1 patient. Five patients showed stable disease, including 3 who had minimal disease at time of vaccine therapy and remain free of tumor with 16-30 months follow-up. Our results demonstrate that it is feasible to generate large numbers of functional DC from pediatric patients even in those highly pretreated and with a large tumor burden. The DC can be administered in an outpatient setting without any observable toxicity. Most importantly, we have demonstrated the ability of the tumor lysate/KLH-pulsed DC to generate specific T-cell responses and to elicit regression of metastatic disease.

Banchereau, J. and others. 1 September 2001. **"Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine."** Cancer Res 61, no. 17: 6451-8.

**X-VIVO 15** Abstract: Immunization to multiple defined tumor antigens for specific immune therapy of human cancer has thus far proven difficult. Eighteen HLA A*0201(+) patients with metastatic melanoma received injections s.c. of CD34(+)progenitor-derived autologous dendritic cells (DCs), which included Langerhans cells. DCs were pulsed with peptides derived from four melanoma antigens [(MelAgs) MelanA/MART-1, tyrosinase, MAGE-3, and gp100], as well as influenza matrix peptide (Flu-MP) and keyhole limpet hemocyanin (KLH) as control antigens. Overall immunological effects were assessed by comparing response profiles using marginal likelihood scores. DC injections were well tolerated except for progressive vitiligo in two patients. DCs induced an immune response to control antigens (KLH, Flu-MP) in 16 of 18 patients. An enhanced immune response to one or more MelAgs was seen in these same 16 patients, including 10 patients who responded to >2 MelAgs. The two patients failing to respond to both control and tumor antigens experienced rapid tumor progression. Of 17 patients with evaluable disease, 6 of 7 patients with immunity to two or less MelAgs had progressive disease 10 weeks after study entry, in contrast to tumor progression in only 1 of 10 patients with immunity to >2 MelAgs. Regression of >1 tumor metastases were observed in seven of these patients. The overall immunity to MelAgs after DC vaccination is associated with clinical outcome (P = 0.015).

Gnjatic, S. and others. 26 September 2000. **"Strategy for monitoring T cell responses to NY-ESO-1 in patients with any HLA class I allele."** Proc Natl Acad Sci U S A 97, no. 20: 10917-22.

**X-VIVO 15** Abstract: NY-ESO-1 elicits frequent antibody responses in cancer patients, accompanied by strong CD8(+) T cell responses against HLA-A2-restricted epitopes. To broaden the range of cancer patients who can be assessed for immunity to NY-ESO-1, a general method was devised to detect T cell reactivity independent of prior characterization of epitopes. A recombinant adenoviral vector encoding the full cDNA

sequence of NY-ESO- 1 was used to transduce CD8-depleted peripheral blood lymphocytes as antigen-presenting cells. These modified antigen-presenting cells were then used to restimulate memory effector cells against NY-ESO-1 from the peripheral blood of cancer patients. Specific CD8(+) T cells thus sensitized were assayed on autologous B cell targets infected with a recombinant vaccinia virus encoding NY-ESO-1. Strong polyclonal responses were observed against NY-ESO-1 in antibody-positive patients, regardless of their HLA profile. Because the vectors do not cross-react immunologically, only responses to NY-ESO-1 were detected. The approach described here allows monitoring of CD8(+) T cell responses to NY-ESO-1 in the context of various HLA alleles and has led to the definition of NY-ESO-1 peptides presented by HLA-Cw3 and HLA-Cw6 molecules.

Bryder, D. and S. E. Jacobsen. 1 September 2000. **"Interleukin-3 supports expansion of long-term multilineage repopulating activity after multiple stem cell divisions in vitro."** Blood 96, no. 5: 1748-55.

**X-VIVO 15** Abstract: Although long-term repopulating hematopoietic stem cells (HSC) can self- renew and expand extensively in vivo, most efforts at expanding HSC in vitro have proved unsuccessful and have frequently resulted in compromised rather than improved HSC grafts. This has triggered the search for the optimal combination of cytokines for HSC expansion. Through such studies, c-kit ligand (KL), flt3 ligand (FL), thrombopoietin, and IL-11 have emerged as likely positive regulators of HSC self-renewal. In contrast, numerous studies have implicated a unique and potent negative regulatory role of IL-3, suggesting perhaps distinct regulation of HSC fate by different cytokines. However, the interpretations of these findings are complicated by the fact that different cytokines might target distinct subpopulations within the HSC compartment and by the lack of evidence for HSC undergoing self- renewal. Here, in the presence of KL+FL+megakaryocyte growth and development factor (MGDF), which recruits virtually all Lin(-)Sca-1(+)kit(+) bone marrow cells into proliferation and promotes their self- renewal under serum-free conditions, IL-3 and IL-11 revealed an indistinguishable ability to further enhance proliferation. Surprisingly, and similar to IL-11, IL-3 supported KL+FL+MGDF-induced expansion of multilineage, long-term reconstituting activity in primary and secondary recipients. Furthermore, high-resolution cell division tracking demonstrated that all HSC underwent a minimum of 5 cell divisions, suggesting that long-term repopulating HSC are not compromised by IL-3 stimulation after multiple cell divisions. In striking contrast, the ex vivo expansion of murine HSC in fetal calf serum-containing medium resulted in extensive loss of reconstituting activity, an effect further facilitated by the presence of IL-3. (Blood. 2000;96:1748-1755)

Kugler, Alexander and others. March 2000. **"Regression of Human Metastatic Renal Cell Carcinoma after Vaccination with Tumor Cell - Dendritic Cell Hybrids."** Nature Medicine 6, no. 3: 332-6.

Abstract: No Abstract

Yang, Shiaolan and others. 1 January 1999. **"Generation of Retroviral Vector for Clinical Studies Using Transient Transfection."** Human Gene Therapy 10: 123-32.

**X-VIVO 15** Abstract: Transient transfection of 293T cells was utilized to produce high-titer murine recombinant retroviral vectors for clinical studies. This system was initially optimized by gene transfer using different retroviral envelope proteins into activated human CD4+ T lymphocytes *in vitro*. Higher titer and infectivity were obtained than with stable murine producer lines; titers of 0.3-1 x $10^7$ infectious units per milliliter for vectors encoding the green fluorescent protein (GFP) were achieved. Virions pseudotyped with envelope proteins from gibbon ape leukemia virus or amphotropic murine leukemia virus resulted in gene transfer of $\geq$50% in CD4+ human T lymphocytes with this marker. Gene transfer of Rev M10 with this vector conferred resistance to HIV infection compared with negative controls in the absence of drug selection. Thus, the efficiency of transduction achieved under these conditions obviated the need to include selection to detect biologic effects in T cells. Finally, a protocol for the production of large-scale supernatants using transient transfection was optimized up to titers of 1.9 x $10^7$ IU/ml. These packaging cells can be used to generate high-titer virus in sufficient quantities for clinical studies and will facilitate the rapid, cost-effective generation of improved retroviral, lentiviral, or other viral vectors for human gene therapy.

Chen, Bing-guan and others. 1998. **"The Role of Tumor Necrosis Factor *alpha* in Modulating the Quantity of Peripheral Blood-Derived, Cytokine-Driven Human Dendritic Cells and Its Role in Enhancing the Quality of Dendritic Cell Function in Presenting Soluble Antigens to CD4+ T Cells *In Vitro*."** Blood 91, no. 12: 4652-61.

> **X-VIVO 15** Abstract: Because dendritic cells (DC) are critically involved in both initiating primary and boosting secondary host immune responses, attention has focused on the use of DC in vaccine strategies to enhance reactivity to tumor-associated antigens. We have reported previously the induction of major histocompatibility complex class II-specific T-cell responses after stimulation with tumor antigen-pulsed DC in vitro. The identification of in vitro conditions that would generate large numbers of DC with more potent antigen-presenting cell (APC) capacity would be an important step in the further development of clinical cancer vaccine approaches in humans. We have focused attention on identifying certain exogenous cytokines added to DC number and function. DC progenitors from peripheral blood mononuclear cells (PBMC) were enriched by adherence to plastic, and the adherent cells were then cultured in serum-free **X-VIVO 15** medium (SFM) for 7 days with added granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). At day 7, cultures contained cells that displayed the typical phenotypic and morphologic characteristics of DC. Importantly, we have found that the further addition of tumor necrosis factor *a* (TNF*a*) at day 7 resulted in a twofold higher yield of DC compared with non-TNF*a*-containing DC cultures at day 14. Moreover, 14-day cultured DC generated in the presence of TNF*a* (when added at day 7) demonstrated marked enhancement in their capacity to stimulate a primary allogeneic mixed leukocyte reaction (8-fold increase in stimulation index [SI]) as well as to present soluble tetanus toxoid and *Candida albicans* (10- to 100-fold increases in SI) to purified CD4+ T cells. These defined conditions allowed for significantly fewer DC and lower concentrations of soluble antigen to be used for the pulsing of DC to efficiently trigger specific T-cell proliferative responses in vitro. When compared with non-TNF*a*-supplemented cultures, these DC also displayed an increased surface expression of CD83 as well as the costimulatory molecules, CD80 and CD86. Removal of TNF*a* from the DC cultures after 2 to 4 days reduced its enhancing effect on DC yield, phenotype, and function. Thus, the continuous presence of TNF*a* over a 7-day period was necessary to achieve the maximum enhancing effect observed. Collectively, our findings point out the importance of exogenous TNF*a* added to cultures of cytokine-driven human DC under serum-free conditions, which resulted in an enhanced number and function of these APC. On the basis of these results, we plan to initiate clinical vaccine trials in patients that use tumor-pulsed DC generated under these defined conditions.

Jonuleit, H. and others. December 1997. "**Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cell under fetal calf serum-free conditions."** Eur J Immunol 27, no. 12: 3135-42.

> **X-VIVO 15** Abstract: Culture conditions for human dendritic cells (DC) have been developed by several laboratories. Most of these culture methods, however, have used conditions involving fetal calf serum (FCS) to generate DC in the presence of granulocyte-macrophage colony-stimulating factor and interlenkin (IL)-4. Recently, alternative culture conditions have been described using an additional stimulation with monocyte-conditioned medium (MCM) and FCS-free media to generate DC. As MCM is a rather undefined cocktail, the yield and quality of DC generated by these cultures varies substantially. We report that a defined cocktail of tumor necrosis factor (TNF)-alpha, IL-1beta and IL-6 equals MCM in its potency to generate DC. Addition of prostaglandin (PG) E2 to the cytokine cocktail further enhanced the yield, maturation, migratory and immunostimulatory capacity of the DC generated. More importantly, culture conditions also influenced the outcome of the T cell response induced. DC cultured with TNF-alpha/IL-1/IL-6 or MCM alone induced CD4+ T cells that release intermediate levels of interferon (IFN)-gamma (low), IL-4 (neg) cells TNF-alpha/IL-1/IL-6 promoted growth of IFN-gamma (intermediate), IL-4 (neg) CD8+ T cells. Addition of PGE2 again only further polarized this pattern enhancing IFN-gamma production by alloreactive CD8+ T cells in both cultures without inducing type 2 cytokines. Taken together, the data indicate that the defined cocktail TNF-alpha/IL-1/IL-6 can substitute for MCM and that addition of PGE2 further enhances the yield and quality of DC generated. TNF-alpha/IL-1, IL-6 + PGE2-cultured DC seem to be optimal for generation of IFN-gamma-producing DC4/CD8+ T cells.

Steinbrink, K. and others. 15 November 1997. **"Induction of tolerance by IL-10 treated dendritic cells."** J Immunol 159, no. 10: 4772-80.

> **X-VIVO 15** Abstract: Dendritic cells (DC) form a specialized system for presenting Ag to naive or quiescent T cells and consequently play a central role in the induction of T and B cell immunity. In this study we used DC generated from peripheral progenitors to analyze the effect of IL-10 on the accessory function of human DC. We demonstrate that immature DC, harvested on days 9 to 11 and exposed to IL-10 for the last 2 days of culture, show a strongly reduced capacity to stimulate a CD4+ T cell response in an allogeneic MLR in a dose-dependent manner. In contrast, fully mature DC are completely resistant to the effects of IL-10. These results were obtained in both an alloantigen-induced MLR and an anti-CD3 mAb-induced response of primed and naive (CD45RA+) CD4+ T cells. FACS analysis revealed inhibition of the up-regulation of the costimulatory molecules CD58 and CD86 and the specific DC marker CD83 in DC pretreated with IL-10. These data suggest that IL-10 inhibited the development of fully mature DC. Furthermore, DC precultured with IL-10, but not controls, induced a state of alloantigen-specific anergy in CD4+ T cells and of peptide-specific anergy in the influenza hemagglutinin-specific T cell clone HA1.7. Analysis of the supernatants of these anergic T cells revealed a reduced production of IL-2 and IFN-gamma compared with that in control cells. Collectively, these data suggest that IL-10 converts immature DC into tolerogenic APC, which might be a useful tool in the therapy of patients with autoimmune or allergic diseases.

Jonuleit, H. and others. 15 March 1997. **"Induction of IL-15 messenger RNA and protein in human blood-derived dendritic cells: a role for IL-15 in attraction of T cells."** J Immunol 158, no. 6: 2610-5.

> **X-VIVO 15** Abstract: IL-15 is a pleiotropic cytokine with IL-2-like functions. As IL-15 was shown to be mitogenic for T cells, we wondered whether human blood-derived dendritic cells (DC), as the primary stimulators of T cell responses, are able to produce IL-15. To test our hypothesis, DC were grown under serum-free conditions from human peripheral blood using granulocyte-macrophage CSF and IL-4. Cultures were assayed for IL-15 mRNA production at various time by semi-quantitative reverse transcription-PCR. Low baseline signals were detected from days 0 to 5 of culture. A significant increase was detected from days 5 to 9 of the culture. A significant increase was detected from days 5 to 9 of the culture. When DC were further enriched by immunomagnetic beads to >98% purity as determined by CD83 staining, IL-15 mRNA signals were exclusively found in the CD83+ fraction. This increase in mRNA signals was paralleled by IL-15 protein release from days 9 to 12 as detected by CTLL-2 assay and ELISA. In addition, protein levels were increased >10-fold by adding paramagnetic beads to the cultures, thereby inducing phagocytic activity. Furthermore, DC supernatants were tested for chemokinetic and chemotactic activities for T cells in a checkerboard filter assay. It was shown that supernatants express chemokinetic and chemotactic activity for T cells. This activity was blocked almost completely by addition of an anti-IL-15 mAb. Our data show that human blood DC contain IL-15 mRNA and produce functional protein that is induced in culture. Protein release is triggered by phagocytic activity. Furthermore, DC-derived IL-15 has chemotactic and chemokinetic activities for T cells, suggesting a role for IL-15 as an attractant of T cells during the initial DC/T cell interaction.

Strobl, Herbert and others. 1997. flt3 **"Ligand in Cooperation with Transforming Growth Factor - beta1 Potentiates in vitro Development of Langerhans-Type Dendritic Cells and Allows Single-Cell Dendritic Cell Cluster Formation Under Serum-Free Conditions."** Blood 90, no. 4: 1425-34.

Woffendin, C. and others. 2 April 1996. **"Expression of a protective gene-prolongs survival of T cells in human immunodeficiency virus-infected patients."** Proc Natl Acad Sci U S A 93, no. 7: 2889-94.

> **X-VIVO 15** Abstract: The resistance of acquired immunodeficiency syndrome (AIDS) to traditional drug therapy has prompted a search for alternative treatments for this disease. One potential approach is to provide genetic resistance to viral replication to prolong latency. This strategy requires the definition of effective antiviral genes that extend the survival of T cells in human immunodeficiency virus (HIV)- infected individuals. We report the results of a human study designed to determine whether a genetic intervention can

prolong the survival of T cells in HIV-infected individuals. Gene transfer was performed in enriched CD4+ cells with plasmid expression vectors encoding an inhibitory Rev protein, Rev M10, or a deletion mutant control, deltaRev M10, delivered by gold microparticles. Autologous cells separately transfected with each of the vectors were returned to each patient, and toxicity, gene expression, and survival of genetically modified cells were assessed. Cells that expressed Rev M10 were more resistant to HIV infection than those with deltaRev M10 in vitro. In HIV-infected subjects, Rev M10-transduced cells showed preferential survival compared to deltaRev M10 controls. Rev M10 can therefore act as a specific intracellular inhibitor that can prolong T-cell survival in HIV-1-infected individuals and potentially serve as a molecular genetic intervention which can contribute to the treatment of AIDS.

Strobl, Herbert and others. 1996. **"TGF-beta1 Promotes In Vitro Development of Dendritic Cells from CD34+ Hemopoietic Progenitors."** The Journal of Immunology 157: 1499-507.

**X-VIVO 15** Abstract: Several studies have demonstrated that dendritic cells (DC) can be generated in vitro from CD34+ hemopoietic progenitor cells. The growth requirements for these cells are poorly characterized, however. In particular, undefined serum/plasma components seem to significantly contribute to in vitro DC development. We report here that the cytokine combination granulocyte-macrophage CSF (GM-CSF) plus TNF-*a* and stem cell factor (SCF) commonly used for the in vitro generation of DC in serum/plasma-supplemented medium is, in the absence of serum supplementation, very inefficient in inducing DC development. We further demonstrate that supplementation with TGF-*B*1 is required for susbtantial DC development to occur in the absence of serum. Culture of CD34+ cells under serum-free conditions with TGF-B1 plus GM-CSF, TNF-*a*, and SCF strongly induces DC differentiation. This culture condition is even more efficient than culturing CD34+ cells with GM-CSF plus TNF-*a* and SCF in the presence of cord blood plasma. The proportions and total yields of cells with typical DC morphology and CD1a molecule expression are higher. The allostimulatory capacity of DC from TGF-B1-supplemented, but not plasma-supplemented, cultures express the Birbeck granule marker molecule Lag and display numerous Birbeck granules. Cells with distinct monocytic features are less frequently observed in TGF-B1-supplemented serum-free cultures. The addition of neutralizing anti-TGF-B1 Ab abrogates the observed TGF-B1 effects.

## X-VIVO 20 - References

Tuettenberg, A. and others. February 2003. **"Priming of T cells with Ad-transduced DC followed by expansion with peptide-pulsed DC significantly enhances the induction of tumor- specific CD8+ T cells: implications for an efficient vaccination strategy."** Gene Ther 10, no. 3: 243-50.

**X-VIVO 20** Abstract: In recent years, vaccination strategies using antigen-presenting cells (APC) have been under investigation. Antigen delivery using genetic immunization through ex vivo transduction of dendritic cells (DC) is supposed to enhance the induction of antitumor responses in humans by activating a broad range of peptide-specific CD8+ T cells. In this study, we compared the potential of adenoviral (Ad)-transduced versus peptide-pulsed DC to induce melanoma-antigen (Ag)-specific T-cell responses in vitro. Whereas gp100-peptide-pulsed DC induced long- lasting specific CD8+ T-cell responses against single peptides, Ad-transduced DC induced broad and strong, specific immunity against various peptides of the gp100-Ag. Surprisingly, several restimulations led to decreasing gp100-specific and in parallel to increasing anti-adenoviral T-cell responses. Nevertheless, those anti-adenoviral T-cell responses provided an "adjuvant" effect by inducing an early release of high amounts of IL-2/IFN-gamma, therewith enhancing CTL induction in the initiation phase. Based on these data, we suggest a prime/boost vaccination strategy in melanoma patients--combining the use of Ad-DC and peptide-pulsed DC--to obtain efficient and long-term antitumor T-cell responses.

Luft, T. and others. 15 August 1998. **"Type I IFNs enhance the terminal differentiation of dendritic cells."** J Immunol 161, no. 4: 1947-53.

**X-VIVO 20** Abstract: This study identifies type I IFNs as activating cytokines in a serum-free system in which human dendritic cells (DC) were generated from CD34+ progenitor cells. After 14 days of culture in GM-CSF, TNF-alpha, and IL-4, CD34+ progenitors gave rise to a population of large, immature DC expressing CD1a and CD1 1b but lacking CD14, CD80, CD83, CD86, and CMRF44. During the next 2 wk, this population spontaneously matured into nonadherent, CD1a(low/-), CD1 1b(low/-), CD14-, CD80+, CD83+, CD86+, CMRF44+ DC with high allostimulatory activity in the MLR. To examine which factors influenced this maturation, 25 different cytokines or factors were added to the immature DC culture. Only type I IFNs (alpha or beta) accelerated this maturation in a dose-dependent manner, so that after only 3 days the majority of large cells acquired the morphology, phenotype, and function characteristics of mature DC. Furthermore, supernatants from cultures containing spontaneously maturing DC revealed low levels of endogenous IFN production. Because of the similarity of the activation of DC in our culture system with the phenotypic and functional changes observed during Langerhans cells activation and migration in vivo, we investigated the effect of IFN-alpha on human Langerhans cell migration. IFN-alpha also activated the migration of human split skin-derived DC, demonstrating that this effect was not limited to DC derived in vitro from hemopoietic progenitor cells. DC activation by type I IFNs represents a novel mechanism of immunomodulation by these cytokines, which could be important during antiviral responses and autoimmune reactions.

Luft, T. and others. June 1998. **"A serum-free culture model for studying the differentiation of human dendritic cells from adult CD34+ progenitor cells."** Exp Hematol 26, no. 6: 489-500.

**X-VIVO 20** Abstract: The antigen-presenting capacity of dendritic cells (DCs) makes them attractive potential cellular adjuvants for vaccination strategies. Currently, most in vitro culture systems for the production of these DCs include serum. However, this is undesirable because serum contains growth factors that vary between individuals and could affect DC development. Unless the patient's own serum is used, foreign antigens and the risk of infection will detract from the usefulness of these cells in clinical strategies. In this study we investigated the production of DCs from CD34+ progenitor cells of cancer patients or normal donors under serum-free conditions. We have established a model system for the investigation of DC development and maturation. Dendritic cells that developed from myeloid precursors accumulated after 2 weeks in an intermediate CD1a, CD80-, CD83- stage. Intermediate DCs adhered to plastic surfaces, expressed Birbeck granules, and were negative for CD2 and CD14. In the presence of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha, interleukin-4 promoted the development of these stages. Spontaneous maturation of intermediate DCs into fully activated DCs expressing CD83 and costimulatory molecules occurred asynchronously over then ensuing 2 to 3 weeks. This maturation involved increased expression of CD80, CD83, CD86, CMRF-44, HLA-A, -B, -C, and -DR as well as downregulation of CD1a and CD11b. Activated DCs are characterized by the lack of adherence to plastic surfaces and the absence of Birbeck granules. By day 28, these cells were non-phagocytic, potent antigen-presenting cells with an irreversible phenotype. This serum-free system offers advantages in that the process of differentiation and maturation of committed DCs is extended over a period of more than 28 days, allowing investigators to study the effects of individual cytokines or other supplements during distinct phases of DC development in a defined environment.

Jonuleit, H. and others. December 1997. **"Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cell under fetal calf serum-free conditions."** Eur J Immunol 27, no. 12: 3135-42.

**X-VIVO 20** Abstract: Culture conditions for human dendritic cells (DC) have been developed by several laboratories. Most of these culture methods, however, have used conditions involving fetal calf serum (FCS) to generate DC in the presence of granulocyte-macrophage colony-stimulating factor and interlenkin (IL)-4. Recently, alternative culture conditions have been described using an additional stimulation with monocyte-conditioned medium (MCM) and FCS-free media to generate DC. As MCM is a rather undefined cocktail, the yield and quality of DC generated by these cultures varies substantially. We report that a defined cocktail

of tumor necrosis factor (TNF)-alpha, IL-1beta and IL-6 equals MCM in its potency to generate DC. Addition of prostaglandin (PG) E2 to the cytokine cocktail further enhanced the yield, maturation, migratory and immunostimulatory capacity of the DC generated. More importantly, culture conditions also influenced the outcome of the T cell response induced. DC cultured with TNF-alpha/IL-1/IL-6 or MCM alone induced CD4+ T cells that release intermediate levels of interferon (IFN)-gamma (low), IL-4 (neg) cells TNF-alpha/IL-1/IL-6 promoted growth of IFN-gamma (intermediate), IL-4 (neg) CD8+ T cells. Addition of PGE2 again only further polarized this pattern enhancing IFN-gamma production by alloreactive CD8+ T cells in both cultures without inducing type 2 cytokines. Taken together, the data indicate that the defined cocktail TNF-alpha/IL-1/IL-6 can substitute for MCM and that addition of PGE2 further enhances the yield and quality of DC generated. TNF-alpha/IL-1, IL-6 + PGE2-cultured DC seem to be optimal for generation of IFN-gamma-producing DC4/CD8+ T cells.

Romani, N. and others. 27 September 1996. **"Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability."** J Immunol Methods 196, no. 2: 137-51.

**X-VIVO 20** Abstract: Two methods to generate human dendritic cells from hematopoietic precursor cells in peripheral blood have recently been published. One approach utilizes the rare CD34+ precursors and GM-CSF plus TNF-alpha. The other method makes use of the more abundant CD34- precursor population and GM-CSF plus IL-4. Here we report a method that is based on the latter approach. However, the GM-CSF and IL-4 treated cells are not stable mature dendritic cells, e.g., the characteristic morphology and non-adherence of dendritic cells is lost if the cytokines are removed. We describe the need for a monocyte-conditioned medium to generate fully mature and stable dendritic cells. This is achieved by adding a 3 day 'maturation culture' to the initial 6-7 day culture in the presence of GM-CSF and IL-4. Macrophage-conditioned medium contains the critical maturation factors. Mature dendritic cells are defined by their pronounced display of motile cytoplasmic processes ('veils'), their high capacity to induce proliferative responses in resting T cells, particularly in naive umbilical cord T cells, their down- regulated antigen processing ability, and their characteristic phenotype: expression of CD83, high levels of MHC molecules and CD86, lack of CD115 and perinuclear dot-like CD68 staining. These features are stable for at least 3 days upon withdrawal of cytokines and conditioned media. IL-4 can be replaced by IL-13. When CD34+ progenitors are depleted from blood, there is only a minor reduction in the yield of dendritic cells by this method. We have adapted the method to consider several variables that are pertinent to clinical use, including a change from fetal calf serum to human plasma and to media approved for clinical use like X-VIVO or AIM-V. 1% plasma and RPMI 1640 are currently optimal. Additional reagents used for cell culture (Ig. cytokines) and cell separation (immunomagnetic beads) are approved for or already used in clinical applications. For 40 ml blood, the yield is 0.8-3.3 x 10(6) mature dendritic cells as defined by the expression of the new dendritic cell-restricted marker CD83. CD83+ cells constitute between 30 and 80% of all cells recovered at the end of the culture period. Yields can be enhanced up to six-fold if the blood donors are pretreated with G-CSF. Stable, mature dendritic cells generated by this method should be a powerful tool for active immunotherapy.