Innovative cell-based therapies in onco-hematology: what are the clinical facts?

Background and Objectives. One of the few measurable clinical results obtained by the use of somatic cells in onco-hematology is the clear-cut effect donor leukocyte infusions (DLI) in patients with chronic myeloid leukemia (CML) who relapse after an allogeneic bone marrow transplantation (BMT). From then on much research has focused on the use of cells to treat different aspects of oncologic diseases from leukemia relapse to developments in BMT recipients.

Methods and Information Sources. In this review we critically and schematically summarize the cell-based therapies which have led to a clinical application and recapitulate the results.

Results and State of the Art. Although the overall numbers of successfully treated patients is small, therapy has been shown to be safe and effective in a variety of clinical contexts in onco-hematology.

Perspectives. Preliminary data will have to be validated in well designed clinical trials with cells generated by reproducible methods and in accredited structures working according to Good Manufacturing Practices (GMP).

Key words: cell therapy, immunotherapy, T cell mediated therapy, gene therapy, leukemia and lymphoma, bone marrow transplantation.
anergic T cells from B–CLL patients by exposing them in vitro to anti-CD3 and anti-CD28 antibodies immobilized on clinical grade magnetic beads and then infusing them into the patient. A phase I study has been reported demonstrating the feasibility of this procedure and showing some clinical response at the very first follow-up. More importantly, the activated T cells expanded at a dramatic rate and showed activation markers such as cytokine production and surface molecule expression.

Finally, the adoptive transfer of autologous T lymphocytes, similarly activated in vitro, has already been validated in a phase I study in patients with B-cell non-Hodgkin’s lymphoma (B–NHL) following stem cell transplantation (see below).8

**Vaccination against B-cell tumors**

The immunoglobulin idiotype determinants of clonal B cells are attractive tumor-specific cell surface antigens for cell therapy. Indeed idiotypic proteins coupled to KLH and emulsified in an adjuvant were used in a clinical trial to vaccinate patients with follicular lymphoma through a series of 5 subcutaneous immunizations.9 In 49% of patients a specific immune response was generated against the idiotype and the median duration of freedom from disease progression and overall survival of responsive patients were significantly longer than those of patients who did not mount an immune response. A similar trial, with the addition of a local injection of granulocyte-macrophage colony-stimulating factor (GM–CSF) to boost vaccination, was later reported. Twenty patients in chemotherapy-induced first clinical remission were treated. Eight of 11 evaluable patients achieved molecular remission and circulating tumor-specific cytotoxic CD8+ and CD4+ T cells were observed in 19/20 patients.6

Following this pivotal information, many similar trials with few or no modifications (e.g. the use of idiotype pulsed dendritic cells) have been reported and a recent review10 and references therein clearly summarize all these trials. The first conclusion is that the preparation of individual idiotypic proteins is technically and financially very demanding, although the clear induction of an efficacious immune response can be demonstrated in approximately 50% of patients. However, given the intrinsic characteristics of this disease, the real impact on clinical parameters such as survival or freedom from relapse does remain to be fully established. Phase III studies are ongoing and their results greatly needed.11 Other complicating issues include the advent of alternative therapeutic options (e.g. rituximab), as well as the need to perform careful studies in homogeneous cohorts, for example patients showing a good immune status at the time of vaccination.

Using the same vaccination strategies in MM patients, in whom induction of an immune response is the first goal, has led to less positive results12 (and references therein), even though the idiotypic immunoglobulin is easier to purify from these patients. An impressive number of clinical studies have been performed with several different formulations which also include the use of dendritic cells (DC); these are extensively discussed in the previously cited review.13 Only a small proportion of patients have shown an immune response and positive clinical results. This low response rate is likely to reflect the more profound immunosuppressive status of the patients, and the presence of high levels of circulating antigen which may have rendered the patients anergic or may neutralize the possible antibody/T-cell response. Indeed encouraging results were published very recently in 6 patients with stage I IgG disease who were immunized with the autologous purified M component together with interleukin-2 (IL–2) and GM–CSF: a reduction of blood tumor mass was observed in 4/6 patients, even though there was no significant change in the serum M protein levels.14

An alternative to direct vaccination with the idiotypic protein has been envisaged through the use of DNA vaccination. Direct cloning of the DR regions is being explored in the context of both lymphomas and myeloma and information on the best procedures for DNA preparation and modalities of vaccination are available.9 Preliminary results in 25 patients with low grade B–NHL who had achieved a clinical remission after single agent or combination chemotherapy suggest that immune responses develop relatively slowly and begin to be detectable between 8 and 12 weeks post-vaccination. About 60% of the responders to the DNA structure have also shown evidence of a specific anti-idiotype immune response.

**Vaccination against other hematologic malignancies**

In the last few years an increasing number of relevant leukemia-associated antigens [e.g. bcr-abl fusion oncogene, WT–1 (Wilms’ tumor antigen), HTERT telomerase, minor histocompatibility antigens (mHAs), proteinase 3 (PR3) and myeloperoxidase (MPO)] have been identified. In particular, PR3 is the target of autoimmune attack in Wegener’s granulomatosis and the PR1-derived peptide can be used to elicit cytotoxic T lymphocytes (CTL) from HLA A2.15 normal donors in vitro. T-cell immunity to PR1 is present in healthy donors and in many patients with CML who are in remission. Using PR–1/HLA A2 tetramers, a significant correlation was found between cytogenetic remission after interferon (IFN)–α treatment and the presence of PR1 CTL. Moreover, PR1 CTL were also identified in some allogeneic transplant recipients who achieved molecular remission, thus demonstrating that the PR1 self-antigen is also recognized by allogeneic CTL.16 In this case, a peptide vaccination strategy is cur-
clones were generated for 5 to 12 weeks. They were then monitored that EBV DNA could return to normal levels.14,15

in the same institutions had done so. It was also documented that EBV-induced lymphomas post-HSCT in patients showing high loads of EBV genome. None of the patients (over 60) developed post-transplant proliferative disorder (PTLD) whereas 11% of historical controls patients maintained cytotoxicity, depleted of CD4+ cells and propagated in vitro for 5 to 12 weeks. They were then transfected into three patients 28 to 35 days after BMT. All the patients had reconstituted CMV-specific cytotoxic T cells against cytomegalovirus (CMV)

T cells against Epstein-Barr virus (EBV)

In the last few years many clinical trials have documented the efficacy of the adoptive transfer of EBV-specific CTL in different clinical contexts: in a large study, donor derived EBV-specific T-cell lines were used as prophylaxis for EBV-induced lymphomas post-HSCT in patients infected with CMV and subsequent restimulation. Clones propagated in vitro by co-culture of peripheral blood lymphocytes from seropositive donors with autologous skin fibroblasts infected with CMV and subsequent restimulation. Clones were selected for cytotoxicity, depleted of CD4+ cells and propagated in vitro for 5 to 12 weeks. They were then transferred into three patients 28 to 35 days after BMT at escalating doses ranging from 3.3×10^6 cells/m^2 to 1×10^7 cells/m^2. CD8+ CMV-specific cytotoxic clones could be demonstrated to persist in vivo for up to one month without toxicity. Subsequently the same group presented the results of the clinical efficacy of this phase I study. All 14 patients had reconstituted CMV-specific cytotoxic T lymphocytes by days 42 to 49 after BMT. All the patients maintained cytotoxic T lymphocyte responses specific for CMV for at least eight weeks after the completion of T-cell therapy; none had CMV viremia or CMV disease. Interestingly, the cytotoxic T-cell activity declined in patients deficient in CD4+ T helper cells, suggesting that T helper cells are required to maintain the CD8+ cells.

More recently, a similar study was published on 8 stem cell transplant recipients who had been unsuccessfully treated with antiviral chemotherapy, with CMV DNA in the blood after more than 4 weeks, and who had no detectable CMV-specific proliferative responses in vitro. Donor T cells were prepared by repetitive stimulation
with CMV antigen. After 4 stimulations in vitro, the T cells had lost their initial alloreactivity toward the patient and showed anti-CMV activity. The patients received a single dose of $10^7$ cells/m² at a variable time after BMT (median 120 days; range 79–479); no toxicity was observed and within 5 to 31 days viral DNA could no longer be detected in 5 out of 7 evaluable patients. Six out of 8 patients started to show a CMV-specific immune response in vitro and the viral load in all 7 evaluable patients declined with a maximal reduction after 20 days.28

Finally, with an alternative in vitro methodology utilizing CMV antigen loading of in vitro–generated DC and co-cultures for 14 to 21 days, 16 patients received an infusion of $1\times10^6$ cell/kg at a median of 36 days following BMT after the first episode of CMV viremia. No infectious toxicity was noted. Three patients developed grade 1 acute graft-versus-host disease (GVHD) and 8 patients cleared viral DNA without antiviral drugs. Only 2 patients had a second episode of CMV reactivation compared to 45 of 72 contemporary controls. In four of five evaluable patients there was a massive in vivo expansion of tetramer binding cells, of which up to 35% were positive for CD8 and CD3.29

**Anti-leukemic T cells**

Several approaches to induction of an anti-tumor T-cell response have been investigated, including stimulation/selection of tumor-specific T cells and depletion of alloreactive cells.

**Unmanipulated (total or selected) allogeneic donor T cells**

Adoptive immunotherapy with DLI after allogeneic HSCT has provided an effective means of augmenting the graft-versus-leukemia (GVL) response, particularly for patients with CML or MM, although at the cost of inducing GVHD. In a recent review of the EBMT-95 survey and of the North American survey (USA-97) complete remission rates varied from 80% for CML patients in cytogenetic relapse to 36% for patients with CML in transformed phase. Moreover complete remissions were observed in 26% patients with AML/MDM, in 15% with ALL and in 29% with MM. Most responses were durable and were associated with improved survival.30 No clear-cut explanation exists for the different GVL efficacies of allogeneic T cells in different tumor types. It is, however, likely that different direct antigenic properties or indirect co-stimulatory conditions (such as antigen presentation by leukemia-derived dendritic cells or down-regulation of the in vivo T-cell recognition by some property of the neoplastic cell type) are responsible for the variable GVL effects. The response to DLI is presumably mediated by donor T cells directed against either tumor-associated antigens or minor histocompatibility antigens. Many experimental approaches have suggested the possibility of separating GVHD and GVL.

In a large study centered around the careful evaluation of the role of the amount of cells administered, patients were treated with a so-called bulk dose regimen, the median dose of lymphocytes infused being $1.5\times10^7$/kg (range 0.6–5.3). For those treated with the escalating dose regimen the median lymphocyte dose was $1.9\times10^7$/kg (range 0.01–3.3). While the probability of achieving remission did not differ, the incidence of GVHD was much lower using the escalating dose regimen not because of the total amount of cells, but rather due to the different schedule of administration.31

Attempts to separate the GVHD and the GVL effect have been made by CD4 positive selection or CD8 depletion.32,33 Indeed very recent results suggest that DLI may be mediated by CD8+ T clones of donor origin detectable in the patient’s peripheral blood before DLI, which expand in vivo in the months after DLI and which are directed against antigens expressed by recipient tumor cells.34 In one particular myeloma patient who had received CD4+ cells for DLI, it was possible to demonstrate that the addition of CD4+ cells had facilitated the expansion and the functional activation of pre-existing circulating CD8+ cells.

An alternative approach considers that, since most of the GVHD reaction appears to be mediated by type I immune effector mechanisms, it may be possible to use TH2 cell graft augmentation in the reduced intensity SCT setting. TH2 cells were generated in vitro using an artificial APC system (anti-CD3/anti-CD28 coated beads and contemporary presence of IL–4). The phase I trial demonstrated the feasibility of this approach since recipients had rapid and complete engraftment at day 14 at a TH2 dose of $25\times10^6$/cells/kg. Four to six recipients had grade 0 acute GVHD through day 190 and rapid and durable anti-tumor effects were observed.35

**In vitro expanded cytotoxic cells**

Cells activated and expanded in the presence of interferon-α followed by anti-CD3 monoclonal antibody and IL-2 have been termed cytokine-induced killer cells (CIK) and recently shown to be CD8+ T cells which display MHC unrestricted T-cell receptor (TCR)-independent cytotoxicity against malignant targets. A central role for NKG2D mediated recognition has been suggested for these cells36 which co-express CD56, produce TH1 type cytokines, and undergo rapid, up to 1000-fold in vitro expansion. Infusion of these cells has been shown to reduce the risk of tumor recurrence in patients with hepatocellular carcinoma following surgical resection.37
In vitro depletion/inactivation of alloreactive cells

GVHD may be inhibited by deletion of alloreactive cells or induction of tolerance. In a first study, induction of tolerance to the host cells was attempted. Bone marrow from a donor mismatched with the recipient for one HLA haplotype was co-cultured with irradiated cells from the recipient for 36 hours in the presence of CTLA4-Ig, an agent that inhibits B7-CD28-mediated co-stimulation and induces antigen-specific tolerance. In the 11 studied patients the haploidentical marrow engrafted, 3 had acute GVHD confined to the gastrointestinal tract but 5 died of infectious diseases. Five patients were alive in CR after 134 to 863 days.40

An alternative approach is the selective depletion of alloreactive T cells in vitro, prior to infusion. This has been done by co-culturing donor PBMC with irradiated host PBMC for 3 days and then adding RFT5-dgA (an anti-CD25-immunotoxin) to deplete activated alloreactive cells. Subsequently, 1-8×106 treated cells /kg were-infused into 15 pediatric patients from HLA mismatched family donors. Two patients developed grade I acute GVHD. In one additional patient grade II GVHD developed. The immunotoxin treatment was efficacious in removing the CD25-positive T cells. These data suggest that the treatment should be considered effective for allorepletion. Moreover the T cells infused seemed able to cure or prevent major infections. Five patients with leukemia were included and in three cases they relapsed.41 Thus whether allorepletion also decreases leukemia-specific T cells has yet to be ascertained.

In a very similar approach EBV-LCL were obtained from a host and used in vitro to stimulate donor PBMC for 3 days and then depleted with the same molecule as above. The first results of the phase I study have recently been presented: the cells were infused in escalating doses after haplo-identical HSCT for relapsed/refractory hematologic malignancies. Nine patients were treated. One patient had severe GVHD after the first infusion (lower dose) and another after the second infusion (higher dose). After 9 months of follow-up, 8/9 patients are alive and 5/9 are disease-free. Immune reconstitution at the low dose (which is similar to that infused with the graft) was slow, as expected, whereas all 3 patients infused with the higher dose achieved normal cell numbers by 4 months post-HSCT, suggesting that allorepleted T-cell infusion may improve immune reconstitution.42

Minor histocompatibility antigen-specific T-cell clones

In HLA-identical donor-recipient pairs, alloreactive donor T cells may recognize minor histocompatibility antigens (mHag) expressed on recipient cells. mHag exclusively expressed on recipient cells of hematopoietic origin such as HA-1 and HA-2 or on lineage-specific hematopoietic cells such as HB-1 (or as recently suggested also by Y chromosome specifically in the female to male transplant direction) may result in GVL reactivity in the absence of severe GVHD. When three mHag HA-1 and/or HA-2 positive patients with disease relapse after allogeneic-stem cell transplantation with DLI from their mHag HA-1 and/or HA-2 negative donors were studied, the emergence of HA-1 and HA-2 specific CD8+ T cells was observed in the blood of the recipients 5 to 7 weeks after DLI. Cloned tetramer-positive T cells isolated during remission specifically recognized malignant progenitor cells.43 Moreover, in these patients, when peripheral blood T cells were stimulated with pre-transplant bone marrow and then IFNγ-secreting cells directly cloned and sorted, CTL clones specific for mHag were indeed demonstrated to be present and to be monoclonal or oligoclonal in nature.44

However, it is worth noting that, although several antigens have already been characterized as leukemia-specific or highly expressed on leukemic cells, and the in vitro expansion of specific CTL has been shown to be possible,45-47 this approach has not yet been transferred to the clinic in a large number of patients. One patient with accelerated phase CML after allogeneic SCT was treated with leukemia-reactive CTL obtained from her HLA identical donor. Cells were co-cultured in the presence of IL-2 for over a month. The cells were infused at 5-week intervals to a cumulative dose of 3.2×109 cells. Complete eradication of the leukemic cells was observed.48 The same group is now trying to expand these results to a larger number of patients. A recent preliminary report describes the results obtained after administration of in vivo expanded CTL administered to 3 AML and 5 CML patients; 3 patients achieved CR (2 in CML and 1 in AML) and 1 patient had PR (AML). The protocol does still need to be optimized in order to produce sufficient numbers of cytotoxic T cells.49-51 Alternatively, dendritic cells transduced with HA-1 cDNA may significantly reduce the time required to obtain CTL in vitro.52

Limitations of the procedures

As a general comment pertinent to all the proposed in vitro T-cell manipulations, we would like to highlight the problems inherent to in vitro culture of T cells which alters their functionality. As one clear example, in the setting of the suicide-gene manipulated T lymphocytes which have been used to implement allogeneic BMT procedures, analysis of the three published studies46-48 and of the available data from ongoing studies53 suggests that the incidence of acute GVHD observed in vivo after the administration of the gene-manipulated T cells is lower than expected. These preliminary results raise questions about the alloreactivity of retrovirally transduced lymphocytes. Indeed the studies show, as detailed below, that the in vitro culture rather than the gene
transduction itself, is directly altering the immune function of the T cells.

The T-cell receptor (TCR) repertoire was analyzed in on T lymphocytes which had been cultured and transduced under identical conditions as those utilized for one of the clinical studies. In all the samples analyzed, the TCRγδ subfamilies were represented with a significant skewing towards a minority of subfamilies. Importantly, identical abnormalities were found in control cells grown under similar conditions but not transduced and were not present if cells were initially stimulated by anti-CD3/anti-CD28 beads instead of the OKT3 stimulator. Similar data have been reported by other groups. Interestingly, this skewing was not described in other studies with anti-CD3/anti-CD28 in solution. Rather, several groups observed an increased proportion of CD45RO cells when a combination of soluble anti-CD3 and anti-CD28 antibodies was used, and this was also observed when umbilical cord lymphocytes were employed. These results are in agreement with the hypothesis that a transition from the naïve to memory stage may occur during in vitro culture. When directly tested, the alloreactive capacity of transduced and cultured T lymphocytes was found to be reduced and, more importantly, the frequency of CTL precursors was significantly affected by the length of time in culture, 14 days having less dramatic effects than 28 days.

Finally, it is possible that in vitro manipulation may selectively affect T cells. For example, in the context of the suicide gene-manipulated T cells it was observed that the frequency of anti-EBV T cells was lower in gene-modified cells than in similarly cultured but untransduced cells and was much lower than in fresh peripheral blood mononuclear cells, demonstrating an effect of both the culture and of the transduction and/or selection. Replacing the initial anti-CD3 (OKT3) activation by CD3/CD28 restored the anti-EBV response. More recently, an in vitro survival advantage was observed for anti-CMV T lymphocytes with respect to the anti-EBV T cells, perhaps because anti-EBV cells belong to the effector memory compartment while CMV-specific T lymphocytes express mainly a terminal effector phenotype.

Thus, although in vitro culture is likely to have limited the effectiveness of manipulated T cells in vivo, ongoing studies leading to better knowledge of immune cell regulation may allow their functional activity to be improved further for the future perspective of hematopoietic graft manipulation as a tool for efficient immunotherapy.

Conclusions

Cellular therapy has been shown to be safe and effective in a number of clinical contexts in hematopo-inology. The knowledge of the regulation of the immune system has only started to be applied as cell-based therapeutic approaches. Indeed, surprisingly few clinical trials have yet been conducted to explore the therapeutic potential of cell-mediated therapies in spite of growing knowledge providing the scientific rationale for these procedures. One limitation is certainly posed by the technical constraints and costs inherent to ex vivo manipulations, as well as the recently introduced requirements to perform these procedures under good manufacturing practice (GMP) conditions. Nonetheless, continuous progress is likely to lead to improvements in the protocols already employed, as well as to the successful application of new somatic cell therapy procedures in the clinic, with the aim of increasing immunity against tumor cells or infectious agents, or limiting the toxicity of GVHD.

All authors have equally contributed to the collection of data, discussion and selection of results to be presented as well as to the writing of the manuscript.

This work was supported by the Associazione Italiana contro la Leucemia (AIL-Sezione Paolo Belli), the Associazione Italiana contro il Cancro (AIRC), and the Italian Ministry for University and Research (MURST, project FIRB no.RBAU01H8SX-001).


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Innovative cell based therapies in onco-hematology


