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REVIEW

Is adoptive T-cell therapy for solid tumors coming of age?

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Among the novel biological therapeutics that will increase our ability to cure human cancer in years to come, adoptive cellular therapy is one of the most promising approaches. Although this is a complex and challenging field, there have been major advances in basic and translational research resulting in clinical trial activity that is now beginning to confirm this promise. The results obtained with tumor-infiltrating lymphocytes therapy for melanoma, and virus-specific CTLs for EBV-associated malignancies are encouraging in terms of both ability to obtain clinical benefit and limited toxicity profile. In both settings, objective responses were obtained in at least 50% of treated patients. However, improvements to the clinical protocols, in terms of better patient selection and timing of administration, as well as cell product quality and availability, are clearly necessary to further ameliorate outcome, and logistical solutions are warranted to extend T-cell therapy beyond academic centers. In particular, there is a need to simplify cell production, in order to decrease costs and ease preparation. Promising implementations are underway, including harnessing the therapeutic potential of T cells transduced with TCRs directed against shared tumor antigens, and delineating strategies aimed at targeting immune evasion mechanisms exerted by tumor cells.

Introduction

Advances in systemic therapy for metastatic cancer, including the development of a variety of new agents targeting important cellular pathways involved in cancer development/progression, have produced, with rare exceptions, relatively short-term benefits for the majority of patients. Moreover, treatment with targeted therapies, that is, for kidney, colorectal and breast cancer, is associated with chronic toxicities, including cardiotoxicity and dermatological toxicity, and considerable costs.[1–3]

Immunotherapy can result in long-term benefit even after short-term treatment. This has been exemplified by the development of IL-2 for the treatment of melanoma and renal cell cancer.[4] It has been hypothesized that the durable responses observed following high-dose IL-2 therapy are because of the induction of a generalized T-cell response initiated during treatment and persisting over a long period. Unfortunately, approaches to immunotherapy other than IL-2 have not achieved major success, and approval for routine use. This is the case of adoptive T-cell therapy (ATCT), considered the most potent immunotherapeutic approach, which, despite a number of ambitious early phase trials, has not yet become part of standard clinical management in medical oncology. However, because of significant advances in our understanding of cancer immunology,[5] in more recent years, cellular immunotherapy is emerging as a novel weapon for the cure of solid tumors.

ATCT involves the expansion, either *ex vivo* (for later reinfusion) or *in vivo*, of immune effector cells capable of tumor killing. This may be nonspecific, as in the case of allogeneic hematopoietic progenitor cell transplantation (HPCT), leukocyte-activated killer cells (LAKs) or cytokine-induced killer (CIK), or may use tumor/antigen-specific *ex vivo* cultures or genetically engineered cells to have tumor-directed specificity.

This article reviews the clinical progress in ATCT, which may provide, in the near future, novel patient and disease-specific approaches to cancer therapy.

Nonspecific ATCT

Allogeneic hematopoietic progenitor cell transplantation

Allogeneic HPCT from a human leukocyte antigen-compatible donor has been utilized as adoptive immunotherapy in metastatic solid tumors since 1996. Several small series have been published, and there has been increasing interest in exploiting graft-versus-tumor effects following allogeneic HPCT for treatment of solid tumors, especially those of the kidney and breast. Non-myeloablative

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(reduced intensity) conditioning regimens with or without the use of donor lymphocyte infusions have been developed to avoid the high treatment-related morbidity and mortality associated with the use of conventional myeloablative conditioning regimens. A graft-versus-tumor effect has been reported in several solid tumors (that is, renal, breast, ovarian, colorectal cancer), and more than 1000 patients with refractory malignancies, who have undergone allogeneic transplantation in European centers, have been reported to the EBMT Registry.[6] In metastatic, cytokine-refractory renal cell cancer (RCC), patients with renal cancer have reported partial or complete disease responses, in the 20–40% range, after allogeneic-transplantation following a reduced-intensity regimen. However, the TRM is still high in the 10–20% rate, due to GVHD and infectious complications, and responses are rarely durable. Experimental evidence suggests that donor-derived T cells and natural killer cells are the main mediators of the graft-versus-RCC effect upon allogeneic HPCT. Isolation of CD8 + CTL clones recognizing several target antigens of graft-versus-RCC effect (for example, mHAs on RCC cells; a peptide epitope derived from human endogenous retrovirus type E; the tumor-associated antigen encoded by the *Wilms tumor 1* gene) has increased our knowledge of the immunology of the disease and has opened the possibility of antigen-specific adoptive cell therapy.[7] The introduction in the clinic of molecularly targeted agents that interfere with neoangiogenesis, both MoAbs and small tyrosine-kinase inhibitor molecules (for example, sunitinib, sorafenib, bevacizumab[8]), has markedly decreased the use of allogeneic transplantation. Though not curative, novel targeted agents may be combined, in perspective, with allogeneic transplantation or other forms of adoptive cell therapy to maximize the chances of cure.

Cytokine-induced immune effector cells
One of the first prototypes of cytokine-induced immune effector cells are the LAK cells. First described in the early 1980s, LAK cells are cytotoxic effector lymphocytes whose cytolytic activities are not restricted by the MHC and have the ability to kill fresh tumor cells and natural killer-resistant tumor cell lines.[9] LAK cells are generated from PBLs following expansion in the presence of IL-2 during a 5-day culture period. LAK cells demonstrated potent *in vitro* cytotoxicity against susceptible tumor cells and led to the regression of established tumors in animal models.[10,11] In clinical studies, LAK cells had demonstrated modest efficacy against metastatic cancer such as RCC and melanoma.[12] In a randomized controlled trial in the 1990s, adoptive immunotherapy using *ex vivo*-activated T cells showed clinical efficacy in terms of prolongation of relapse-free survival for patients with hepatocellular carcinoma following resection of the primary tumor.[13] In particular, time to first recurrence in the immunotherapy group was significantly longer than that in the control group (38% vs 22% at 5 years), and the immunotherapy group had significantly longer recurrence-free survival ($P = 0.01$) and disease-specific survival ($P = 0.04$) than the control group.
   Closely related to LAK cells, CIK cells are polyclonal T-effector cells generated *in vitro* by incubating PBLs with

anti-CD3 MoAb, IL-2, IL-1α and IFN-γ.[14] This unique subset of non-MHC-restricted CD3 + CD56 + T cells was initially referred to as natural killer-like T cells as, similar to natural killer cells, they do not require previous specific sensitization to induce the recognition of target cells. CIK cells have a high rate of proliferation and demonstrate a potent cytolytic activity. Compared with standard LAK cells, CIK cells possess enhanced cytotoxic activity.[15]
   Over the years, CIK cells have been tested against a variety of tumor targets *in vitro,* including solid tumors.[16,17] However, data on the efficacy of CIK cells *in vivo* are limited. Recently Hontscha *et al.*[18] published the first report of the international registry on CIK cells, which included 426 patients treated within 11 clinical trials. This study confirms that a large-scale expansion of CIK cells *ex vivo* is possible and that their infusion is a safe procedure. Regarding efficacy, the total response rate was 91/384 reported patients, with 24 patients showing a complete response, 27 patients a partial response and 40 patients a minor response. In addition, 161 patients had a stable disease. Because of the heterogeneity of the study populations and the limited data on response rates, no conclusive data on the efficacy of this therapy in patients with solid tumors can be drawn.
   CIK cells show only limited graft-versus-host effects in various mouse models,[19] which suggest their potential use as adoptive immunotherapy following allogeneic transplantation.[20] Allogeneic ATCT with CIK cells might represent an effective alternative to classic donor lymphocyte infusion, helping allogeneic HPCT to successfully meet current challenges like the extension across major human leukocyte antigen barriers and application to solid tumors.[21,22]

Targeted ATCT

A strategy that has proven effective in increasing the efficacy of anti-cancer cell therapy protocols is the *ex vivo* identification of autologous or allogeneic lymphocytes with anti-tumor activity, which are then administered to cancer patients. A number of different approaches have been used to date to obtain tumor-specific T cells, such as *ex vivo* selection of tumor-infiltrating lymphocytes (TILs), based on their capacity to recognize autologous tumor cells, repeated *in vitro* stimulation with tumor-associated antigens (TAAs)/whole tumor cells, or, more recently, genetic modification of T cells using TCRs encoding retroviruses that can convert normal lymphocytes into cells with specific anti-cancer activity.

Tumor-infiltrating lymphocytes
TIL therapy can be considered a targeted T-cell therapy, as these cells are *ex vivo* selected for their capacity to recognize autologous tumor cells. Transfusion of TIL has emerged as the most effective treatment for patients with metastatic melanoma. This approach was first described in 1988,[23] but the decisive improvement in efficacy came in 2002 with the introduction of an immunodepleting preparative regimen given before the adoptive transfer, which resulted in the

clonal repopulation of patients with anti-tumor T cells.[24] Objective tumor regression, including complete responses, was observed in 49–72% of patients with metastatic melanoma refractory to all other treatments; the greater the degree of host lymphodepletion, the more effective was the treatment.[25] Responses can be durable (greater than 5 years), and are seen in all organ sites, including the brain.[26] The observation that cells used for ATCT may cross blood–brain barrier suggests that brain tumors may also be targeted by a similar approach. Despite recent progress in the *ex vivo* production of TIL,[27] which may facilitate the widespread clinical application of this approach, TIL with high avidity for tumor antigens can only be generated from about 50% of patients with melanoma. This hurdle can only be overcome by a different ATCT approach, such as the use of chimeric antigen receptors (CARs) technology.

The impressive clinical results provided by the Rosenberg group over the last 10 years require confirmation (and demonstration of reproducibility) in a prospective multi-center setting, also in view of the considerable toxicity reported in these trials. Weber *et al.*[28] in their recent white paper on TIL, suggest an approach using centralized cell expansion facilities (in a few key centers) that will receive specimens and ship expanded TIL infusion products to participating centers to ensure maximal yield and product consistency. If successful, this approach will definitively answer the question of whether TIL therapy can enter mainstream treatment for advanced melanoma. Though some early studies did seem to demonstrate that TIL can be grown in culture from patients with other solid tumors, with variable yields, no clinical data are so far available outside the setting of melanoma.

T-cell lines specific for TAA
In recent years, progress in the field of biotechnology has allowed for the characterization of tumor cells, with identification of tumor-specific or tumor-associated antigens, leading the way to the definition of protocols to obtain good manufacturing practice-grade cellular products for cell therapy trials.

In patients with metastatic melanoma, clinical evidence obtained in separate independent trials, showed that a proportion of patients treated with melanocyte antigen related to T-cells-1-specific CD8+ CTLs, had clinically meaningful responses.[29,30] The efficacy of infusing *ex vivo* expanded T cells directed against defined TAA was strengthened by Hunder *et al.*[31] who demonstrated a durable clinical remission in a single patient affected by refractory metastatic melanoma treated with autologous cancer testis antigen NY-ESO-1-specific CD4+CTLs. However, the number of TAA identified till now is relatively limited, compared with the plethora of molecules present on tumor cells that may contribute to the stimulation of a protective immune response. To overcome this problem, during the past few years, the use of DCs pulsed with whole tumor cell preparations, namely tumor extracts or apoptotic tumor cells, to cross-prime CTLs has been investigated.[32–36] Despite the large body of preclinical studies, clinical trials in humans have not yet been reported.

ATCT with T cells specific for viral antigens. Among the bottlenecks that till now have limited a wider use of T-cell therapy for human tumors, one could be the very low frequency of tumor-specific lymphocytes circulating in patients with cancer,[37] or the limited ability to induce T cell lines with protective anti-tumor activity with current knowledge and available technologies. With the exception of TIL therapy in melanoma, and in a few other cancer types,[38] the only other human solid cancer setting in which tumor-specific T cells have been used with success is virus-related cancer, in particular EBV-associated nasopharyngeal carcinoma.[39,40]

In the last 15 years, a number of reports demonstrated the effectiveness of ATCT directed against EBV antigens for the treatment of EBV-related hematological malignancies in the immunocompromised host.[41–46] EBV-related post transplant lymphoproliferative disease constitutes a highly immunogenic lymphoproliferation whose onset is greatly favored by the host immunodeficiency status. Thus, ATCT in this setting is expected to have a great chance of success. Adoptive transfer of polyclonal CTLs specific for viral latency antigens, in the context of EBV-associated malignancies arising in the immunocompetent host, such as Hodgkin's lymphoma and nasopharyngeal carcinoma, is hampered by a number of factors. EBV-specific CTLs are dominated by reactivity against viral proteins not expressed by these tumors.[47,48] Moreover, the transferred CTLs have to compete with endogenous lymphocytes for cytokines and biological niches, and, once CTLs reach the tumor site, they have to overcome the inhibitory barriers exerted by the tumor environment.[49] Notwithstanding these limitations, when the frequency of circulating T cells against the target antigen on a tumor is high, as is the case for viral antigens, ATCT can be very effective in destroying large tumors in humans. Indeed, the results of the clinical cell therapy trials conducted till now in nasopharyngeal carcinoma and Hodgkin's lymphoma patients demonstrate that administration of an avid anti-tumor T cell targeting a highly expressed antigen can result in cancer regression.[39,40,50] In particular, independent phase I–II studies demonstrated that clinical and immunological responses can be obtained in patients with radiotherapy- and chemotherapy-resistant, stage IV EBV-related nasopharyngeal carcinoma by administration of EBV-specific autologous polyclonal CTL therapy; among the patients treated for refractory/resistant disease, 50% showed disease control.[39,40,51,52]

T cells modified to express chimeric receptors
A strategy to broaden the reactivity against shared cancer-associated antigens present on multiple tumor types consists of grafting specificities for antigens expressed on tumor cells through genetic manipulation.[53] Investigators have developed artificial TCRs, also referred to as CARs, isolated from high-avidity T cells that recognize cancer antigens. CAR molecules usually combine the antigen-binding domain of the variable regions of a specific MoAb with the CD3ζ endodomain of the TCR/CD3 complex (so-called first-generation CARs). CAR transduction by retroviral or lentiviral vectors redirects lymphocyte specificity to these cancer antigens, allowing recognition of specific

antigens expressed on the surface of different types of tumor cells. The first pre-clinical and clinical studies using T cells expressing CARs concerned the targeting of B-cell hematological malignancies, such as CLL, CD19-positive ALL, B-cell lymphomas and Hodgkin's lymphoma.[54,55]

Subsequently, the approach has been extended to solid tumors.[56–59] These studies demonstrated that normal human lymphocytes genetically engineered to express a TAA, such as the disaloganglioside GD2 or the cancer/testis antigen NY-ESO-1, can mediate cancer regression *in vivo,* and have opened opportunities for enhancing and extending the ATCT approach to patients with a wide variety of cancer types, including synovial cell sarcoma and melanoma.

However, in these initial human trials, T lymphocytes expressing first-generation CARs showed limited expansion and relatively short persistence. This result likely reflects the failure of artificial CAR molecules to fully activate T cells after antigen engagement on tumor cells, especially when the tumor cells lack expression of co-stimulatory molecules (such as CD80 and CD86) that are required for sustained T-cell activation, growth and survival.

Table 1 summarizes the clinical results of ATCT in solid tumors obtained till now.

Future directions

Despite its great potential, ATCT for cancer control still has a marginal role in the management of patients with solid tumors, although its use in the setting of melanoma seems ready for development as a routine therapy. This is because of limitations inherent to the technologies and products used, and to the financial and structural burden that are associated with cell therapy (Table 2). Indeed, the extensive infrastructure needed for exploiting such approaches still restricts their use to academic centers with specific programs in the field. The major obstacle for a wider application of ATCT to treat human cancer is the personalized nature of the approach. Most of the currently

Table 1 Clinical results of various adoptive T-cell therapy approaches in solid tumors

Type of ATCT	Clinical setting	Pros	Contra	Comments	Reference(s)
Allogeneic progenitor cell transplantation	Kidney/breast/other solid tumors Advanced disease	Evidence of GVT effect, partial or complete responses	Short-lasting responses in the majority of patients. High TRM and morbidity.	Limited interest/ongoing studies. Potential platform for other adoptive immunotherapy approaches.	3
LAK cells	RCC/melanoma/HCC Advanced disease	Modest efficacy in RCC and melanoma, prolongation of relapse-free survival in HCC	Highly toxic approach. Early data not confirmed.	Approach abandoned.	9, 10
CIK cells	Various solid tumors	Non-MHC-restricted cells. Easy production. Low Toxicity	Limited data on their efficacy *in vivo*.	Promising preclinical data. Alternative to classic DLI in programs of allogeneic Tx.	13, 15, 17
TIL	Melanoma, metastatic disease	High rate of tumor regression/complete response (curability in some patients)	At present limited to metastatic melanoma, Single center data. Requirement of host lymphodepletion (selected patients).	Potential applicability in various solid tumor. Confirmatory multicenter studies required.	20–25
Virus-specific CTLs	Nasopharyngeal carcinoma. Stage IV	Clinical benefit observed in heavily pretreated patients	Response rate associated to levels of LMP1/LMP2 specific T cells in the infused product	Potential applicability in various virus-related solid tumors.	33, 34, 46
Ag-specific T cells	Melanoma. Metastatic disease	Clinical response in a proportion of patients	Requirement of definition of TAA. One study limited to one patient, requires to be confirmed in a large cohort of patients.	Possible applicability only in the presence of known TAA.	26, 27, 28
Whole tumor-specific CTLs	Various solid tumors	Feasibility and safety *in vivo* (one study). Infusion of anti-tumor CTLs with broad specificity. The approach does not require the definition of a specific tumor Ag	No data on efficacy. Labor intensive.	Requirement of clinical studies.	29, 30
Gene-modified T cells	Various solid tumors	Long-lasting responses	Use of lymphodepleting CT.	Possibility to extend ATCT approach to patients with wide variety of cancer.	50–53

Abbreviations: ATCT = adoptive T-cell therapy; CIK = cytokine-induced killer; DLI = donor lymphocyte infusion; GVT = graft-versus-tumor-effect; HCC = hepatocellular carcinoma; LAK = leukocyte-activated killer cell; LMP = latent membrane protein; RCC = renal cell carcinoma; TAA = tumor-associated membrane antigen; TIL = tumor-infiltrating lymphocyte; Tx = transplantation.

Table 2 Current limitations toward a widespread application of adoptive T-cell therapy

Problem	Possible solutions
Limitations inherent to product quality	
Need for a better selection of tumor-directed T-cells	Implementation of novel biotechnologies
	Modification of effector cells or APCs by genetic engineering (CARs)
Poor effector cell-survival capacities	Use of naïve T-cell populations
	Implementation of novel biotechnologies
	Modification of effector cells by genetic engineering (expression of co-stimulatory molecules on T cells)
	In vivo support of ATCT with cytokines or peptide vaccines
Limitations inherent to product activity in vivo	
Immune evasion strategies/ inhibitory effect of regulatory T cells	Modification of effector cells or APCs by genetic engineering (induce resistance to the action of inhibitory cytokines)
	Condition the patient before ATCT by a lymphodepleting chemotherapy or MoAbs
Limitations inherent to production	
Personalized ATCT generation	Centralization by contract cell-producing biotechs
	Use of third party-banked products
Limited GMP standardization	Creation of ATCT networks
High production costs	Centralize production of specific ATCT
	Maintain an active GMP production lab

Abbreviations: ATCT = adoptive T-cell therapy; CAR = chimeric antigen receptor; GMP = good manufacturing practice.

available strategies are patient-specific, labor-intensive and require highly specialized expertise. Availability of contract cell-producing biotechs or cell banks will allow the extended use of ATCT to non-specialized centers, increasing application of this strategy. It is noteworthy, however, that even an academic good manufacturing practice facility, when keeping a number of protocols running, is able to decrease production costs considerably, reducing the costs of a cell product lot to a figure very close to that needed for 'off the shelf' products such as MoAbs or other targeted therapies.[42]

Implementation of the existing protocols for selection of tumor-directed T cells by exploiting novel biotechnologies, such as genetic engineering, with the aim of obtaining cellular products of higher specificity and purity, and easing translation of these technologies into clinical scale protocols for cell production, needs to be further pursued. The clinical efficacy and safety of this approach, however, must be carefully evaluated clinically, possibly within multicenter clinical trials. Furthermore, a better understanding of the mechanisms favoring *in vivo* cell selection, survival and functional activity, will ameliorate the quality of cell therapy products. In particular, the use of naïve T-cell populations, as well as *in vivo* support of ATCT with different cytokines or peptide vaccines could increase cell therapy efficacy. Likewise, means to provide the

co-stimulation signal lacking in tumor cell targets, by incorporating co-stimulatory endodomains, such as CD28, into CAR molecules (so-called second-generation CARs) seems to induce enhanced expansion and persistence of CAR-transduced lymphocytes.[60]

Finally, a barrier to the function of infused tumor-directed T cells in immunocompetent hosts is the display of tumor-mediated immune evasion strategies.[61] An elegant approach to improve the resistance of cell products to tumor-derived inhibitory cytokines is to provide the cell with the machinery needed to overcome inhibition through genetic modification. It has been shown that EBV-specific CTLs made transgenic for a dominant-negative transforming growth factor-β receptor, in which the intracellular signaling domain is truncated, are rendered resistant to the detrimental effects of transforming growth factor-β, secreted by Hodgkin's lymphoma cells.[62] Alternatively, it has been suggested that conditioning the patient before T-cell transfer by a lymphodepleting chemotherapy could have a role in favorably modifying the tumor microenvironment, by reducing levels of both regulatory T cells and regulatory cytokines.[63]

Conclusions

The management of the majority of human cancers with radiochemotherapy and recently developed targeted agents is still suboptimal, due to persistence of refractory/relapsing disease, and the increased toxicity observed with increased efficacy of therapeutic regimens. Novel tumor-specific therapeutic modalities may offer equal or increased efficacy, coupled with a considerable decrease in overall toxicity. Among these novel approaches, cell therapy offers a unique opportunity to restore anti-tumor immune surveillance,[64] and it is therefore conceivable that application of this strategy will increase in the next few years.

Conflict of interest

The authors declare no conflict of interest.

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