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Trial Watch
Adoptive cell transfer immunotherapy

Lorenzo Galluzzi,[1,2,3,†] Erika Vacchelli,[1,2,3,†] Alexander Eggermont,[2] Wolf Hervé Fridman,[4,7] Jerome Galon,[4,8] Catherine Sautès-Fridman,[4,6,8] Eric Tartour,[5,7,9] Laurence Zitvogel[2,10] and Guido Kroemer[1,2,5,7,11,*]

[1]INSERM; U848; Villejuif, France; [2]Institut Gustave Roussy; Villejuif, France; [3]Université Paris-Sud/Paris XI; Le Kremlin-Bicêtre, France; [4]INSERM; U872; Paris, France; [5]Université Paris Descartes; Sorbonne Paris Cité; Paris, France; [6]Centre de Recherche des Cordeliers; Paris, France; [7]Pôle de Biologie; Hôpital Européen Georges Pompidou; AP-HP; Paris, France; [8]Université Pierre et Marie Curie/Paris VI; Paris, France; [9]INSERM; U970; Paris, France; [10]INSERM; U1015; Villejuif, France; [11]Metabolomics Platform; Institut Gustave Roussy; Villejuif, France

[†]These authors contributed equally to this article.

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Abbreviations: ACT, adoptive cell transfer; AML, acute myeloid leukemia; APC, antigen-presenting cell; CAR, chimeric antigen receptor; CDR, complementarity-determining region; CIK, cytokine-induced killer; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; IFNγ, interferon γ; IL, interleukin; NK, natural killer; NKT, Vα24 NK; MDSC, myeloid-derived suppressor cell; PBMC, peripheral blood mononuclear cell; PSA, prostate-specific antigen; RCC, renal cell carcinoma; TCR, T-cell receptor; TGFβ, transforming growth factor β; TILs, tumor-infiltrating lymphocytes; TLR, Toll-like receptor; Tregs, FOXP3$^+$ regulatory T cells; VEGFR2, vascular endothelial growth factor receptor 2

During the last two decades, several approaches for the activation of the immune system against cancer have been developed. These include rather unselective maneuvers such as the systemic administration of immunostimulatory agents (e.g., interleukin-2) as well as targeted interventions, encompassing highly specific monoclonal antibodies, vaccines and cell-based therapies. Among the latter, adoptive cell transfer (ACT) involves the selection of autologous lymphocytes with antitumor activity, their expansion/activation ex vivo, and their reinfusion into the patient, often in the context of lymphodepleting regimens (to minimize endogenous immunosuppression). Such autologous cells can be isolated from tumor-infiltrating lymphocytes or generated by manipulating circulating lymphocytes for the expression of tumor-specific T-cell receptors. In addition, autologous lymphocytes can be genetically engineered to prolong their in vivo persistence, to boost antitumor responses and/or to minimize side effects. ACT has recently been shown to be associated with a consistent rate of durable regressions in melanoma and renal cell carcinoma patients and holds great promises in several other oncological settings. In this Trial Watch, we will briefly review the scientific rationale behind ACT and discuss the progress of recent clinical trials evaluating the safety and effectiveness of adoptive cell transfer as an anticancer therapy.

Introduction

For a long time, the immune system has been believed to participate in oncogenesis, tumor progression and response to therapy as a mere bystander, a notion that has now been invalidated. On one hand, components of the immune system, such a B lymphocytes and macrophages, have been shown to facilitate inflammation-driven carcinogenesis,[1-3] while others, such as CD8$^+$ T and natural killer (NK) cells, ensure a constant barrier against oncogenesis (immunosurveillance) that malignant precursors must break to develop tumors.[4] On the other hand, the therapeutic efficacy of several anticancer regimens, including conventional chemotherapeutics as well as targeted agents, appear to rely (at least in part) on the activation of innate or cognate immune effector mechanisms.[5] Thus, the abundance of intratumoral CD8$^+$ and memory T cells has recently been shown to dramatically affect the clinical outcome in multiple oncological settings.[6-9] Along with this conceptual shift, which occurred during the last three decades, therapeutic interventions aimed at activating the immune system against tumors begun to attract an ever increasing interest, from both researchers and clinicians. The promising field of anticancer immunotherapy had been established.[10]

Nowadays, cancer immunotherapy can be subdivided into three major branches: (1) approaches for the relatively "unselective" stimulation of the immune system against tumors, (2) anticancer vaccines (including protein-, peptide- and cell-based vaccines), and (3) adoptive cell transfer (ACT) protocols.[11] Immunostimulatory interventions are exemplified by the systemic administration of lymphocyte-targeting growth factors such as interleukin-2 (IL-2), other pro-immunogenic cytokines such as interferon α (IFNα), or compounds that block immunosuppressive mechanisms, including monoclonal antibodies that are specific for the cytotoxic T lymphocyte antigen 4 (CTLA4) or chemotherapeutics that selectively depletes immunoregulatory cell populations. Immunostimulatory agents given as monotherapy have been associated with consistent rates of tumor regression

*Correspondence to: Guido Kroemer; Email: kroemer@orange.fr
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in melanoma and renal carcinoma patients,[12-15] perhaps because these cancers are able to elicit per se elevated levels of antitumor lymphocytes. Of note, several anticancer agents that are currently used in the clinic also mediate immunostimulatory effects, either by actively triggering immune effector mechanisms or by selectively inhibiting/killing immunosuppressive cells such as FOXP3$^+$ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs).[5,16] These chemotherapeutics might de facto function as combination therapies, mediating both a cytotoxic/cytostatic effect on tumor cells and a stimulatory effect on the immune system.

Vaccines constitute a very appealing approach to cancer immunotherapy, presumably because they would be relatively easy to administer, cheap (especially in the case of peptidic vaccines) and virtually devoid of side effects.[17] Nonetheless, cancer vaccines, encompassing both peptides and dendritic cell (DC)-based approaches, so far have failed to meet the high expectations that they had raised, being associated with modest and often non-reproducible clinical benefits.[11] Perhaps, this can be attributed to the fact that end-stage cancer patients often exhibit immune defects that can compromise their ability to mount a vaccine-driven antitumor response. One notable exception is provided by sipuleucel-T (Provenge®), a DC-based vaccine that has been granted FDA approval for the treatment of asymptomatic or minimally symptomatic, metastatic castration-resistant (hormone refractory) prostate cancer.[18-20] In addition, promising results have been observed in prostate cancer patients receiving prostate-specific antigen (PSA)-targeted poxviral vaccines (PROSTVAC-FS),[21] as well as in melanoma patients treated with a peptidic vaccine combined with high-dose IL-2.[22]

ACT has emerged as an effective form of immunotherapy, with rates of complete durable responses (in specific clinical settings) as high as 40%.[23,24] As a note, ACT must be conceptually differentiated from other cell-based immunotherapies, including the re-infusion of autologous DCs pulsed ex vivo with tumor antigens or tumor cell lysates (aimed at eliciting an anticancer T cell response in vivo) and the infusion of allogeneic T and NK cells (aimed at obtaining a curative graft-versus-disease effect).[25,26]

No ACT-based approach is currently approved by FDA for use in humans. In this Trial Watch, we will briefly review the scientific rationale behind ACT and discuss the progress of recent clinical studies evaluating the safety and efficacy of cell immunotherapy in oncological settings.

Scientific Background

ACT entails the (re)introduction into a conditioned patient of large amounts (often up to 10^{11}) of lymphocytes exhibiting antitumor activity. When possible, notably in the case of melanoma and renal cell carcinoma (RCC) patients (which spontaneously manifest high number of antitumor lymphocytes), the starting material for ACT is constituted by a surgery specimen or biopsy, from which tumor-infiltrating lymphocytes (TILs) are isolated and (in some instances) selected for T-cell receptor (TCR) specificity.[27] Before reinfusion, such lymphocytes are expanded ex vivo in the presence of IL-2 and other growth factors, and optionally activated with immunostimulatory compounds such as anti-CD3 antibodies, alone or combined with tumor-specific antigens.[28] This said, melanoma and RCC constitute relatively privileged settings for immunotherapy, as suggested by the high rate of spontaneous TILs and by fact that immunostimulatory interventions alone are efficient against these tumors but not others.[12-15]

To extend the benefits of ACT to other types of cancer that are not associated with an intense endogenous immune response, genetic engineering can be employed to convert circulating lymphocytes into cells that exhibit antitumor activity. Thus, normal lymphocytes are isolated from the peripheral blood and genetically manipulated for the expression of TCRs that recognize tumor antigens with high affinity. The implementation of this strategy requires the isolation of very few endogenous cells with antitumor activity, from which rearranged TCR genes can be cloned and incorporated into highly efficient retroviral or lentiviral vectors.[11] Upon transduction newly generated antitumor cells are amplified and treated similar to their tumor-derived counterparts. Importantly, as the TIL phenotype has been shown to affect both their in vivo persistence and their clinical efficacy,[29-32] genetic engineering can be employed to confer additional features to lymphocytes, including (1) increased in vivo persistence, owing to the (over)expression of anti-apoptotic proteins like BCL-2[33] or telomerase, the enzyme that prevents the senescence-associated physiological attrition of telomeres;[32] (2) increased proliferative potential, due to the (over)expression of growth factors, like IL-2, or their receptors;[34] (3) enhanced homing to tumor sites, as a result of the expression of molecules involved in trafficking such as CD62L and CCR7;[35] (4) enhanced antitumor potential, upon the (over)expression of co-receptors such as CD8 or co-stimulatory molecules like CD80 or the blockade of potentially immunosuppressive signals, such as those mediated by transforming growth factor β (TGFβ).[36] Finally, as an alternative to TCRs, the so-called chimeric antigen receptors (CARs) can be employed. These chimeric receptors bind to tumor antigens via antibody-derived complementarity-determining regions (CDRs), yet are coupled to the intracellular machinery for TCR signaling, de facto mediating the functional activation of T cells even when antigens are not displayed in the context of MHC molecules.[37]

In the context of genetically engineered T cells, the choice of the tumor antigen specifically recognized by the TCR (or CAR) is critical, as it dictates both the efficacy and the safety of ACT.[38] So far, encouraging results have been obtained with TCRs recognizing the melanocyte-specific markers MART1,[39,40] MELOE-1[41,42] and gp100,[43,44] the carcinoembryonic antigen (CEA),[45] and cancer-testes antigens (which are expressed by a variety of epithelial cancers)[46] such as NY-ESO-1, MAGE-A1 and MAGE-A3,[47-50] as well as with CARs targeting the B-cell antigen CD19[51-53] and the vascular endothelial growth factor receptor 2 (VEGFR2) (**Table 1**).[54]

In early trials, upon ex vivo expansion and activation, autologous TILs were re-administered to virtually untreated patients, resulting in transitory tumor regression but no durable responses.[55] Perhaps, this was due to the fact that inoculated cells are subjected to a consistent degree of immunosuppression by

Table 1. Examples of tumor antigens exploitable for adoptive cell transfer

Antigen	Tumor type	Notes	Ref.
CEA	Colorectal cancer	Preferentially expressed by gastrointestinal tumors	45
CD19	CLL Lymphoma	B cell-specific antigen	51–53
gp100	Melanoma	Melanocyte-specific antigen	43, 44
MAGE-1	Multiple epithelial malignancies	Cancer-testes antigen	46, 49
MAGE-A3	Multiple epithelial malignancies	Cancer-testes antigen	46, 50
MART-1	Melanoma	Melanocyte-specific antigen	39, 40
MELOE-1	Melanoma	Melanocyte-specific antigen	41, 42
NY-ESO-1	Melanoma Synovial cell carcinoma	Cancer-testes antigen	47, 48
VEGFR2	Multiple types of solid tumors	Overexpressed in tumor vasculature	54

Abbreviations: CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukemia; VEGFR2, vascular endothelial growth factor receptor 2.

endogenous Tregs and MDSCs, and normally fail to persist over long periods in vivo. Moreover, endogenous B, T and NK cells might compete with re-infused TILs for limited amounts of critical cytokines, including IL-7 and IL-15, a phenomenon known as "cytokine sink."[56,57] To circumvent these critical issues, pre-conditioning regimens based on cyclophosphamide (an alkylating agent that at high doses exert consistent immunosuppressive effects), fludarabine (a nucleoside analog commonly used for the therapy of hematological malignancies) and total body irradiation have been developed, resulting in different extents of lymphodepletion.[58] Importantly, the intensity of lymphodepletion has been shown to directly correlate with ACT antitumor efficacy,[58] leading to the introduction of preconditioning lymphodepletion into the clinical practice for ACT.

Re-infusion protocols have also been progressively refined to improve the persistence of TILs in vivo and to exacerbate their antitumor efficacy. Thus, although the co-infusion of cells with IL-2 is now a routine approach, several other possibilities are being explored. IL-2 has indeed been shown to correct the intrinsic anergy or TILs,[59] and to promote the expansion of antitumor T cells in vivo.[60] However, recent results indicate that this cytokine also stimulate immunosuppressive cell populations including Tregs,[61-63] a phenomenon that may compromise its clinical benefits. New approach include, but are not limited to: (1) the co-infusion of cytokines other than IL-2, encompassing IL-7, IL-12, IL-15 and IFNγ, which stimulate immune effector and memory functions;[64-67] (2) the co-infusion of angiogenesis inhibitors, which facilitate the extravasation of re-infused cells into the tumor;[68] (3) the co-infusion of Toll-like receptor (TLR) agonists, to limit endogenous immunosuppression;[69] and (4) the co-infusion of immunostimulatory chemotherapeutics, such as metronomic cyclophosphamide.[5,70]

Recently, ACT attempts based on B cells and NK cells have been investigated. On one hand, adoptively transferred B cells that exhibit antitumor activity in vitro reportedly mediate the generation of T-cell responses against xenografted breast cancer in mice.[71] However, B cell-based ACT protocols have not yet been evaluated in clinical settings, perhaps due to the fact that B cells also mediate immunosuppressive and pro-tumorigenic effects, at least in some models of carcinogenesis.[1,3] On the other hand, in spite of encouraging preclinical results and of the established efficacy of allogeneic NK cells for the therapy of acute myeloid leukemia (AML),[72-74] NK cells failed to mediate antitumor effects in metastatic melanoma and RCC patients,[75] perhaps owing to their limited persistence in vivo.[76] Conversely, promising results have been obtained with the infusion of so-called "young TILs," i.e., unselected, minimally cultured, bulk TILs whose production is relatively rapid and does not involve individualized tumor-reactivity screening steps.[77-79] By reducing the costs and technical constraints that are associated with the ex vivo amplification and activation of TILs, this new approach may de facto increase the number of centers that will be able to offer ACT immunotherapy to eligible cancer patients. Of note, while the clinical efficacy of ACT is largely believed to depend on $CD8^+$ T cells, the infusion of $CD4^+$ T cells alone also mediates durable responses in melanoma patients.[48] The cellular and molecular circuitries that underlie these observations remain to be precisely elucidated. As a possibility, infused cells (be they $CD8^+$ of $CD4^+$ cells) may initiate antitumor responses that result in the expansion of the tumor-specific TCR repertoire, and hence in the elicitation of robust immune reaction against cancer cells.[48,80] Recent preclinical data demonstrate that oncogene-targeting T cells are superior to oncogene-specific drugs in the eradication of oncogene-addicted tumors, as the latter (but not the former) leave the tumor vasculature intact, allowing for the generation of resistant tumor clones.[81] Perhaps, this superiority relies on the paracrine effects of IFNγ secreted by T cells, provoking the destruction of tumor vessels and impeding the growth or resistant cells.

Al least theoretically, the most prominent side effects of ACT relate to: (1) the specificity of re-infused cells, which might destroy normal tissues expressing the same antigen recognized by genetically engineered TCRs (or CARs);[82] (2) the secretion by re-infused TILs of large amounts of cytokines/chemokines;[83,84] (3) the possibility that transduced TCR chains might recombine with endogenous ones, resulting in the acquisition of unwarranted antigen specificity and graft-versus-host disease.[85] So far, however, only a few cases of severe/lethal adverse reactions have been reported,[45,83,84] suggesting that, in the vast majority of settings, ACT constitutes a safe clinical procedure.

Published Clinical Trials

Since the advent of ACT, the efficacy and safety of this intervention has been evaluated in multiple oncological settings, and the results of these early (Phase I/II) clinical studies have been published in some 30 high impact papers (**Table 2**).

One half of these studies were performed in small cohorts of (often metastatic) melanoma patients,[23,43,47,52,77,82,86-94] while the other half tested ACT in clinical settings as diverse as

hematological malignancies,[23,43,47,52,77,82,86-90,92-94] RCC,[47,95,96] hepatocellular carcinoma,[97] ovarian cancer,[98] neuroblastoma,[99] metastatic colorectal carcinoma,[45] and head and neck squamous cell carcinoma.[100]

In about one third of these studies, patients were re-infused with autologous cells that had been non-specifically activated in vitro, for instance by the administration of IL-2 or anti-CD3 antibodies, alone or in combination with anti-CD28 antibodies.[23,86,89,92,96,101-105] Alternatively, patients received lymphocytes that had been specifically activated against the tumor by the ex vivo administration of dead cancer cells in the presence of the Calmette-Guerin bacillus,[95] or by the co-culture with living tumor cells,[93] with DCs pulsed with tumor antigens,[87,91] with DCs pulsed with cancer cell lysates,[97] or with an artificial antigen-presenting cell (APC) system that can educate antitumor lymphocytes to acquire both a central memory and an effector memory phenotype.[94] In two studies, patients received young TILs,[77,79] whereas in one trial ex vivo expanded Vα24 NK (NKT) cells were intravenously administered in tumor-feeding arteries, in conjunction with the nasal administration of αGalCer-pulsed APCs.[100] In the remaining studies, the re-infused material consisted in genetically modified cells, including peripheral blood mononuclear cell (PBMC)-derived T cells engineered to express tumor antigen-specific TCRs,[43,45,47,82,92,98] CARs[51,52,99,106] or IL-2.[88]

In most cases, patients received classical lymphodepleting regimens based on cyclophosphamide + fludarabine, alone or in combination with total body irradiation,[23,47,52,79,82,86,89,92,101,104,106] and were given cells together with high-dose IL-2.[23,25,43,45,47,51,52,77,79,82,86,88,89,91,92,94-98,100-102,104-106] As an alternative, cells were co-infused with a course of low-dose IL-2 over 6 d,[87] or low-dose IFNγ.[93]

In two of these trials no antitumor effects were observed,[97,98] and in two other studies ACT was associated with limited (though assessable) therapeutic responses.[96,105] Apart from these notable exceptions, the results of the clinical trials conducted so far on ACT are very encouraging, reporting response rates as high as 70% and a very low incidence of severe side effects (**Table 2**). Although they were often performed in small patient cohorts and the therapeutic protocols often differed from trial to trial, these phase I/II studies demonstrate that ACT is efficient and safe for the treatment of some types of cancer, in particular melanoma. This paved the way for ongoing studies that evaluate alternative ACT protocols or the applicability of ACT to other oncological settings.

Ongoing Clinical Trials

At present, there are around 35 ongoing, early (Phase I/II) clinical trials that test the safety and efficacy of ACT in oncological indications (source www.clinicaltrials.gov). Thirteen of these studies are performed in melanoma and RCC patients. In addition, ACT, alone or combined with established procedures, is being evaluated as a therapy for tumors as different as hematological malignancies (including multiple myeloma and several types of leukemia and lymphoma), sarcomas, cancers of the reproductive trait (including cervical, Fallopian tube, ovarian and prostate cancer), neoplasms of the central nervous system

Table 2. Published clinical trials evaluating adoptive cell transfer in cancer patients

Site	Tumor type(s)	Phase	Notes	Ref.
Hematological neoplasms	BCL	I	IL-2-activated T cells + IL-2 2 PRs + 5 SDs out of 7 patients	103
	CLL	I	Anti-CD19 CAR-engineered T cells + IL-2 1 PD, 2 SDs and 3 PRs out of 9 patients	51
	CLL Follicular lymphoma LCL MCL SLL	I/II	Anti-CD19 CAR-engineered T cells + IL-2 1 CR, 1 SD, 6 ORs out of 8 patients	52, 106
	Multiple myeloma Plasma cell neoplasms	I/II	CD3/CD28-costimulated T cells ± vaccine upon HSCT No ORs but some immunological responses	105
	NHL	I	CD3/CD28-costimulated T cells following HSCT 5 CRs, 7 PRs, 4 SDs out of 16 patients Transient dose-dependent infusion toxicities	102
Kidney	Renal cell carcinoma	I	CD3/CD28-costimulated T cells + IL-2 Some metastatic regression Mild toxicity	96
	Renal cell carcinoma	II	CD3-activated, dead tumor cells + Calmette-Guerin bacillus-primed $CD4^+$ cells + IL-2 9 CRs + 5 PRs out of 39 patients	95
Multiple tumors	Metastatic melanoma Metastatic SCC	II	Anti-NY-ESO-1 TCR-engineered T cells + IL-2 ORs in 4/6 SCC patients and 5/11 melanoma patients	47
	Advanced solid tumors NHL	I	CD3-stimualted $CD4^+$ cells + IL-2 1 CR, 2 PRs and 8 MRs out of 31 patients	101

Table 2. Published clinical trials evaluating adoptive cell transfer in cancer patients (cont.)

Site	Tumor type(s)	Phase	Notes	Ref.
Skin	Melanoma	n.a.	MART-1-specific CTLs generated in vitro using aAPCs 1 CR, 3 PRs, 3 SDs out of 10 patients Some IL-2- and lymphopenia-associated toxicity	94
		I	$CD8^+$-enriched young TILs ± myeloablative lymphodepletion 9/33 ORs (3 CRs) without TBI; 11/23 ORs (2 CRs) with TBI	79
	Metastatic melanoma	I	TILs + IL-2 upon non-myeloblative lymphodepletion 18 ORs (3 CRs + 15 PRs) out of 35 patients Some IL-2- and lymphopenia-associated toxicity	86
		I	Anti-MART-1 TCR-engineered PBMCs 2 ORs out of 15 patients and durable engraftment	43
		I	MART-1-specific cells generated upon co-culture with MART-1-pulsed DCs + low dose IL-2 3 ORs out of 11 patients Mild (grade 1–2) adverse effects	87
		I	Autologous CTLs upon previous fludarabine or not 3 MRs or SDs out of 9 patients Fludarabine improved in vivo persistence	104
		I	TILs + IL-2 upon non-myeloblative lymphodepletion 20 CRs out of 93 patients, which exhibited 100% 3 y survival	23
		I/II	IL-2-engineered TILs + IL-2 1 PR out of 12 patients and persistent IL-2 expression	88
		I/II	MART-1-specific T cells + IL-2 and IFNα 6 ORs (2 LTCRs) out of 14 patients	91
		I/II	PBMC-derived T cells generated upon co-culture with melanoma cells + low dose IFNα 1 CR, 1 PR, 3 SDs out of 10 patients	93
		II	Autologous TILs + IL-2 ± myeloablative lymphodepletion Up to 72% ORs (with TBI) out of 93 patients 1 death	89
		II	Anti-MART-1 and anti-gp100 human or murine TCR-engineered PBMCs 30% (human TCR) and 19% (murine TCR) ORs Toxicity to normal melanocytes in the ear and skin	82
		II	Young TILs + IL-2 10 ORs and 4 SDs out of 20 patients Transient and manageable toxicity	77
		II	Autologous TILs or MART1-specific PBMC-derived T cells + IL-2 9 CRs and PRs in the brain out of 26 patients 1 subarachnoid hemorrhage without consequences	92
Various	Colorectal cancer	I/II	Anti-CEA murine TCR-engineered T cells 1 ORs out of 3 patients Transient colitis in all patients	45
	HCC	I	iDCs, CIK cells and CTLs + tumor lysate-pulsed DCs Early increase in the $CD8^+/FOXP3^+$ ratio	97
	HNSCC	II	NKT cells in combination with alphaGalCer-pulsed DCs 5 ORs out of 10 patients	100
	Neuroblastoma	I	Anti-GD2 CAR-engineered EBV-specific CTLs ORs in 50% of patients	99
	Ovarian cancer	I	Anti-FR TCR-engineered T cells + IL-2 No anticancer responses IL-2 related mild (grade 3–4) toxicity in 5/8 patients	98

Abbreviations: aAPC, artificial antigen-presenting cell; BCL, B cell lymphoma; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CIK, cytokine-induced killer; CLL, chronic lymphocytic leukemia; CR, complete response; CTL, cytotoxic lymphocyte; DC, dendritic cell; EBV, Epstein-Barr virus; FR, folate receptor; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; HSCT, hematopoietic stem cell transplantation; iDC, immature DC; LCL, large cell lymphoma; LTCR, long-term complete response; MCL, mantle cell lymphoma; n.a., not available; NHL, non-Hodgkin lymphoma; NK, natural killer: NKT, Vα24 NK; IFN, interferon; IL, interleukin; MR, minor response; n.a., not available; OR, objective response; PBMC, peripheral blood mononuclear cell; PD, progressive disease; PR, partial response; SCC, synovial cell carcinoma; SD, stable disease; SLL, small lymphocytic leukemia; TBI, total body irradiation; TIL, tumor infiltrating lymphocyte.

(including malignant glioma, glioblastoma, medulloblastoma and neuroectodermal tumors), as well as nasopharyngeal, breast, lung and hepatocellular carcinomas (**Table 3**).

In many cases, the infused material consists of ex vivo expanded and activated TILs, though the use of young TILs (NCT01118091; NCT01319565; NCT01369888) and of cytokine-induced killer (CIK, $CD3^+CD56^+$ non-MHC-restricted, NK-like T lymphocytes)[107] cells (NCT00815321; NCT01232062; NCT01395056) is also being investigated. Sometimes, TILs are collected a few weeks after the administration of tumor antigen-specific vaccines (NCT00791037; NCT00834665; NCT01312376). Moreover, distinct approaches of genetic engineering are being undertaken, including the production of lymphocytes expressing tumor antigen-specific TCRs (NCT00720031; NCT00871481; NCT00910650; NCT01212887), CARs (NCT00968760; NCT01218867; NCT01318317; NCT01416974) IL-12 (NCT01236573) or IFNγ (NCT01082887). One particularly interesting study entails the engineering of lymphocytes for the expression of an inducible suicide fusion protein, which might be employed to resolve ACT-related toxicity (NCT00730613). Conditioning regimens largely overlap with those employed in previous successful studies, with a high prevalence of non-myeloablative lymphodepletion (cyclophosphamide + fludarabine), alone or combined with total body irradiation. In one instance, cells are infused in the absence of conditioning and after three cycles of low-dose (sub-efficient) radiotherapy, aimed at stimulating immune responses

Table 3. Ongoing clinical trials evaluating adoptive cell transfer in cancer patients*

Site	Tumor type(s)	Phase	Notes	Ref.
Breast	$HER2^+$ breast cancer	I/II	Autologous ex vivo expanded HER2-specific T cells	NCT00791037
		I	Autologous ex vivo expanded HER2-specific T cells + cyclophosphamide	NCT01219907
	TNBC	n.a.	CIK cells ± DCs	NCT01232062 NCT01395056
CNS	Glioblastoma	I	CMV-activated T cells alone or combined with a DC-based vaccine	NCT00693095
	Malignant glioma	I	Autologous $CD8^+$ T cells expressing inducible suicide fusion protein and an IL-13 chimeric immunoreceptor	NCT00730613
	Medulloblastoma Neuroectodermal tumors	I/II	Tumor-specific T cells alone or combined with a DC-based vaccine	NCT01326104
Hematological neoplasms	AML CML MDS	II	Autologous CIK cells ± imatinib	NCT00815321
	BCL	I	Anti-CD19 CAR-engineered $CD8^+$ cells + IL-2 upon HSCT	NCT00968760
	BCL B-CLL Multiple myeloma	I	Anti-κ light chain CAR-engineered T cells	NCT00881920
	CLL	I	Anti-CD19 CAR-engineered $CD8^+$ cells after chemotherapy	NCT01416974
	NHL	I/II	Anti-CD19 CAR-engineered $CD8^+$ cells upon myeloablative conditioning and PBSCT	NCT01318317
	Multiple myeloma	I	CD3/CD28-costimulated T cells following HSCT	NCT01239368
	Multiple myeloma	I/II	Vaccine primed-autologous T cells following HSCT	NCT00834665
Multiple sites	Breast carcinoma Colorectal carcinoma Gastric cancer Lung cancer Ovarian cancer Pancreatic cancer	I	Anti-CEA TCR-engineered T cells + IL-2	NCT01212887
	Breast carcinoma HCC Nasopharyngeal cancer	I	$CD8^+$-enriched young TILs + IL-2	NCT01462903
	Metastatic melanoma RCC	I/II	Anti-VEGFR2 CAR-engineered $CD8^+$ cells + IL-2	NCT01218867
Reproductive tract	Cervical neoplasms	I/II	Low-dose radiotherapy followed by autologous TILs	NCT01194609
	Fallopian tube cancer Ovarian cancer Primary peritoneal cancer	I	CD3/CD28-costimulated vaccine-primed autologous T cells	NCT01312376
	Prostate cancer	I	Anti-PSMA TCR-engineered T cells	NCT01140373

Table 3. Ongoing clinical trials evaluating adoptive cell transfer in cancer patients* (cont.)

Site	Tumor type(s)	Phase	Notes	Ref.
Skin	Metastatic melanoma	II	TILs + IL-2 upon non-myeloblative lymphodepletion	NCT00604136
		I/II	MART-1-specific TILs + IL-2 followed by IL-2 and IFNα	NCT00720031
		I/II	NY-ESO-1-specific TILs + IL-2 ± ipilimumab	NCT00871481
		II	Anti-MART-1 TCR-engineered PBMCs plus MART-126–35 peptide-pulsed DCs + IL-2	NCT00910650
		I	Melanoma reactive (DMF5) TILs + IL-2	NCT00924001
		n.a.	TILs + IL-2 upon non-myeloblative lymphodepletion	NCT01005745
		I/II	IFNγ-engineered TILs + IL-2	NCT01082887
		II	$CD8^+$-enriched young TILs + IL-2	NCT01118091
		I/II	IL-12-engineered TILs + IL-2	NCT01236573
		II	Young TILs + IL-2	NCT01319565
		I/II	Young TILs + IL-15	NCT01369888
		II	TILs + IL-2 upon non-myeloblative lymphodepletion	NCT01468818
Soft tissues	Adult liposarcoma Adult soft tissue sarcoma Adult synovial sarcoma	I	NY-ESO-1-specific $CD8^+$ T cells + IL-2, upon conditioning with low-dose IFNγ and cyclophosphamide	NCT01477021

Abbreviations: AML, acute myeloid leukemia; BCL, B cell lymphoma; B-CLL, B cell chronic lymphocytic leukemia; CAE, carcinoembryonic antigen; CAR, chimeric antigen receptor; CIK, cytokine-induced killer; CML, chronic myeloid leukemia; CMV, cytomegalovirus; CNS, central nervous system; DC, dendritic cell; HCC, hepatocellular carcinoma; HSCT, hematopoietic stem cell transplantation; IFN, interferon; IL, interleukin; MDS, myelodysplastic syndrome; n.a., not available; NHL, non-Hodgkin lymphoma; PBMC, peripheral blood mononuclear cell; PBSCT, peripheral blood stem cell transplantation; PSMA, prostate-specific membrane antigen; TBI, total body irradiation; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes; TNBC, triple negative breast cancer; VEGFR2, vascular endothelial growth factor receptor 2. *started after January, 1st 2008 and not completed or terminated at the day of submission.

(NCT01194609). Most often patients receive cells together with high-dose IL-2, though the co-infusion of IL-15 (NCT01369888) or DC-based vaccines (NCT00693095; NCT00910650; NCT01326104) is also being tested.

Concluding Remarks

Thanks to the work from several laboratories worldwide, our knowledge on the molecular and cellular circuitries that underlie the long-term effectiveness of ACT has greatly advanced. ACT based on autologous TILs has already been associated with consistent rates of durable remissions in exquisitely immunosensitive cancers such as melanoma. Moreover, the use of genetically engineered circulating T cells constitutes a promising approach for the treatment of several other (solid and hematological) malignancies. Results from ongoing trials will clarify whether the clinical benefits of ACT truly extend to poorly immunosensitive tumors. Unfortunately, ACT is far from becoming a routine clinical practice, as its implementation is laborious and associated with elevated costs. In this sense, the development of simplified techniques for the ex vivo expansion, activation and genetic engineering of lymphocytes might allow an increasing number of cancer centers to offer ACT as a therapeutic option.

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