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Adoptive cell transfer using autologous tumor infiltrating lymphocytes in gynecologic malignancies

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Abstract

During the last decade, the field of cancer immunotherapy has been entirely transformed by the development of new and more effective treatment modalities with impressive response rates and the prospect of long survival. One of the major breakthroughs is adoptive cell transfer (ACT) based on autologous T cells derived from tumor-infiltrating lymphocytes (TILs). TIL-based ACT is a highly personalized cancer treatment. T cells are harvested from autologous fresh tumor tissues, and after ex vivo activation and extensive expansion, are reinfused to patients. TIL-based therapies have only been offered in small phase I/II studies in a few centers given the highly specialized care required, the complexity of TIL production and the very intensive nature of the three-step treatment protocol. The treatment includes high-dose lymphodepleting chemotherapy, the infusion of the expanded and activated T cells and interleukin-2 (IL-2) injections to increase survival of the T cells. Despite the limited data on ACT, the small published studies consistently confirm an impressive clinical response rate of up to 50 % in metastatic melanoma patients, including a significant proportion of patients with durable complete response. These remarkable results justify the need for larger clinical trials in other solid tumors, including gynecologic malignancies. In this review we provide an overview of the current clinical results, future applications of TIL-based ACT in gynecologic malignancies, and on risks and challenges associated with modern T cell therapy.

Introduction

Traditional cancer treatments, surgery, chemotherapy and radiation, have demonstrated limited efficacy for patients with late-stage and recurrent gynecological malignancies and often cause substantial and long-lasting adverse effects. In the past decade cancer immunotherapy has shown remarkable promise, especially for disease refractory to standard of care approaches. Cancer immunotherapy encompasses a wide range of methods, spanning

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The authors declare no conflicts of interest.

from active immunization to immune stimulatory methods to enhance tumor immunogenicity and improve immune cell trafficking, and cell based approaches using adoptive cell transfer (ACT). Among these strategies, ACT has been demonstrated to be the most effective immunotherapy method that can result in long-term remission (>5 years) and low recurrence rate.

Successful immunotherapy requires the activation, expansion and efficient trafficking of cancer-specific T cells to the tumor microenvironment. T cell-mediated anticancer responses *in vivo* include three basic steps. First antigen-presenting cells capture and process cancer antigens into antigenic peptides and present these in combination with human leukocyte antigen (HLA) molecules for T cell receptor (TCR) recognition on T cells. The second step is T cell activation, which requires the binding of the costimulatory surface molecules B7 and CD28 on antigen-presenting cells and T cells. T cell activation is then followed by trafficking to tumor microenvironment and tumor cell recognition and elimination. In cancer patients this complex system fails to function properly as T cells are often unable to recognize cancer antigens, are unable to traffic into the tumor, and/or become disabled locally by the suppressive tumor microenvironment.

Not surprisingly several studies have demonstrated a clear association between the number of tumor infiltrating lymphocytes (TILs) and patient outcomes. The quantity of TILs and TIL phenotype, especially CD8+ cytotoxic T lymphocytes, predict increased progression-free survival (PFS) and overall survival (OS) in many tumor types including melanoma [1], head and neck squamous cancer [2, 3], colon [4, 5], gastric [6], pancreatic [7, 8], lung [9, 10], breast [11, 12] and ovarian cancer [13, 14]. Although TILs are capable of accessing tumor tissue and recognizing tumor-specific antigens, they are often at low numbers and fail to control tumor growth due to the highly immunosuppressive environment.

Therefore, alongside novel immune therapeutics being developed by the pharmaceutical industry, a huge effort from non-commercial research groups around the world has been made to develop highly individualized cellular cancer therapies, particularly those based on transfer of autologous T cells. These treatments are now becoming more accessible to patients with advanced gynecologic malignancies. In this review we will focus on the role of tumor infiltrating lymphocytes (TILs) in antitumor immune responses and their therapeutic implications in the three main tumor types in gynecologic cancer: ovarian, cervical and endometrial cancers. We will discuss the basic concepts in ACT utilizing autologous TILs and review established protocols and the unique toxicities associated with ACT and their management. Other techniques for adoptive cell transfer exist, including genetically modified T cell receptor and chimeric antigen receptor (CAR) T cell therapy; however this review will focus specifically on adoptive T cell therapy using autologous TILs.

Historical perspective on Adoptive T Cell Therapy

Adoptive cell therapy for the treatment of cancer is a concept that began over 30 years ago. The possibility of utilizing of lymphocytes in cancer immunotherapy was first investigated in animal tumor models, where donor lymphocytes from infiltrating tumors were capable of inducing tumor regression in recipient animals [15, 16]. The commercial production of IL-2

in the 1970s allowed researchers to expand TILs *in vitro* while maintaining their effector function. Rosenberg and colleagues at the National Cancer Institute (NCI) pioneered the use of TILs as a source of tumor-specific T-cells that could be expanded *ex vivo* in the 1980s [17]. Subsequently, human trials were initiated using TILs and IL-2. Rosenberg et al. published the first trial using adoptive T cell transfer in humans in 1988. They treated 20 patients with metastatic melanoma with tumor infiltrating lymphocytes that were expanded *ex vivo* and re-infused in combination with IL-2 after a single dose of cyclophosphamide. There was 55% objective response by RECIST criteria [18]. This study laid the groundwork for ACT in cancer treatment, although, the duration of response was usually short and a follow-up study demonstrated a response rate of 34% [19]. A significant breakthrough in ACT was the addition of a lymphocyte-depleting chemotherapy regimen prior to TIL transfusion [20, 21], which improved the rates and duration of response and the persistence of tumor-specific T-cells in the circulation [22]. Several similar trials of TIL infusion in metastatic melanoma have been reported, with variation of the lymphocyte-depletion regimen, and objective responses ranging from 38% to 56% [20, 23, 24]. Parameters that correlated with response include number of TILs infused, number and percentage of CD8+ TILs, CD8+ phenotype [24], and telomere length[25]. Most of the pioneering work in TIL ACT was done in melanoma patients. Although less accessible than checkpoint blockade, autologous TIL therapy in recurrent/metastatic melanoma is arguably the most effective therapy with response rates >50% and durable complete response rates of >20% [20] and thus holds a promise for other intractable solid tumors.

Adoptive T Cell Transfer: Concepts and strategies for this treatment modality

ACT is defined as the infusion of T cells extracted from autologous tumor tissue, after *ex vivo* activation and multiple rounds of expansion. ACT takes advantage of the presence of enriched tumor-specific T cells found in tumors and aids these cells in completing their antitumor immune function. ACT allows T cell activation and expansion to bypasses the normal host immune regulatory responses, which would prevent rapid and massive expansion of the effector T cells with tumor antigen specificity. Final TIL infusion products consist of CD8+ and CD4+ T cells at highly variable ratios, as well as a very small fraction of γδ T cells[26]. The efficacy of TIL therapy was traditionally primarily attributed to CD8+ T cells; however, newer studies suggest that tumor-reactive CD4+ T cells can also be identified in TILs preparations[26], and many of the patients achieving long-lasting complete remission, were actually treated with TIL products largely dominated by CD4+ T cells[24].

TIL manufacturing is a complex and labor-intensive process, thus it needs to be carried out in a Good Medical Practices (GMP) compliant specialized laboratory. In earlier protocols selected TIL manufacturing took 5-8 weeks, which often led to high patient drop out due to clinical deterioration. New modified protocols thus abandoned culture selection and use single bulk TILs extracted from the resected specimen. Unselected TILs first undergo expansion using IL-2 to yield at least 40–50 million total cells, which takes 2–4 weeks[27]. At this point cryopreservation of the cells is possible for future use, which allows more

flexibility and also to save cell product for non-progressing patients for later use. During the final 2 weeks using rapid expansion protocols (REP) T cells are cultured with anti-CD3 antibody and IL-2 resulting in a 1,000-fold to 3,000-fold expansion of the T cells to generate clinical grade TIL products from about 90 % of tumor samples[27] . This whole process of "young TIL" generation takes 4–6 weeks, and typically a total of 2-20×10^{10} TILs can be produced and infused [27]. Besides the shortened preparation time the final infusion product contains younger cells with high expression of co-stimulatory receptor molecules (such as CD27), longer telomere length and great diversity in anti-tumor recognition, thus future clinical trials on ACT with TIL are unlikely to require T cell selection based on antitumor reactivity.

In order to enhance the efficacy of ACT, infusion of the final product is preceded by lymphodepleting cytoxan and fludarabine treatment. Preconditioning lymphodepletion results in multiple benefits: 1.) removal of endogenous lymphocytes in the patient prior to the TIL infusion, which could compete for cytokines and the infused IL-2, 2.) removal of endogenous T regulatory cells which can inhibit effector T cell functions and decrease TIL expansion within the patient after infusion, 3.) improve the persistence of the TILs after infusion into the patient [28–32]. T cell infusion is then followed by IL-2 treatment, which promotes T cell activation, proliferation and augments antitumor cytolytic activity [33]. Figure 1 outlines the basic steps of ACT.

Given the improved outcomes with utilizing chemotherapy for lymphodepletion, a more aggressive second method of lymphodepletion has been tested utilizing total body irradiation (TBI) in combination with cyclophosphamide and fludarabine. At the NIH this has been explored through two clinical trials in melanoma. When 12 Gy TBI was utilized in addition to chemotherapy for lymphodepletion response rate increased to 72%, but unfortunately this approach added significant additional toxicity to treatment [29].

Established Protocols and Controversies for ACT with Tumor Infiltrating Lymphocytes

The most established, widely studied and utilized protocol for ACT infusion is based on data generated by Steven Rosenberg at the NCI and still serves as a basis for contemporary clinical trials. Classic ACT protocols for TILs begin with a pre-treatment myeloablative chemotherapy course beginning 7 days prior to the administration of the T Cells. On Day -7 and -6 patients are given IV infusions of cyclophosphamide 60mg/kg/day followed by an infusion of mesna at 3mg/kg/hour for 23 hours followings each cyclophosphamide infusion. On days -7 to -3 (or in some protocols days -5 to -1) patients receive IV infusions of fludarabine 25mg/m2. For patients receiving TBI, in addition to the above outlined chemotherapy regimen, the patients also receive on day -3 to day -1, 2Gy of TBI twice a day for a total of 12Gy. On Day 0 the adoptive cell transfer occurs. On day 1-4 an IV infusion of high dose IL-2 is administered at 600,000 to 720,000 IU/kg every 8 hours for up to 5 days. Table 1 outlines a general protocol with inclusion of prophylactic medications generally recommended for these patients [20, 22, 23, 28, 29].

The Rosenberg protocol is the most widely used in clinical trials, however the administration of high dose IL-2 provides a treatment challenge with significant toxicity, thus it is only administered in highly specialized centers. There is a well-known variation in the side effect profile of high dose IL-2 among patients, ranging from mild to life-threatening toxicities. The toxicities of high dose IL-2 are mostly related to capillary leak syndrome (CLS). CLS is a condition in which fluid and protein leaks from the capillaries leading to hypotension, hypoalbuminemia, and an overall decrease in plasma volume resembling septic shock. The typical clinical course of CLS has a common timeline. First, within 2-3 hours after the first or second dose of IL-2, patients may experience fevers and chills. Patients also develop hypotension and tachycardia shortly after infusion, which is typically maintained throughout the remainder of the IL-2 treatment course. Oliguria can also develop and is typically seen in the first 24 hours after infusion. Nausea, vomiting, and diarrhea have also been seen closer to the end of the treatment course. Capillary leakage symptoms usually progressively worsen through the treatment course and manifest as edema, weight gain, pulmonary congestion, in addition to the above outlined symptoms. Lab abnormalities which can be prominent and therapy limiting are a rise in serum creatinine and thrombocytopenia. All of these side effects quickly dissipate once the IL-2 therapy is completed [34]. The Rosenberg group has also published guidelines for the management of side effects associated with high dose IL-2 as outlined in the referenced book *Principles and practice of the biologic therapy of cancer*[35].

The rationale for high dose IL-2 is that this treatment helps support the newly transferred T cells *in vivo*. However, new emerging evidence suggests that this therapy may promote the re-constitution of T regulatory cells, which is associated with a worse clinical response to ACT [36]. Several groups are now exploring the use of lower doses of IL-2 to determine if an equivalent treatment response rate can be achieved. Two different lower dose IL-2 regimens have been evaluated: one regimen is administering 2MIU/day of IL-2 for 14 days and the other is utilizing an intermediate decrescendo infusion cycle (continuous infusions of IL-2 at 18MIU/m2 over 6 hours, then 12 hours, then 24 hours, followed by 4.5 MIU/m2 over 24hours for 3 days). The first lower dose regimen was tested in a pilot study of 6 patients with metastatic melanoma and 2/6 had an ongoing complete response at 30 and 10 months, 2/6 had stable disease at 4 and 5 months and 2 patients had progression of disease shortly after infusion[37]. In the decrescendo regimen 22 patient with progressive metastatic melanoma were analyzed and 2 had complete responses at 33 and 16 months, 7 had partial responses, 10 had stable disease, and 3 had progressive disease [38]. The response utilizing the decrescendo method is equivalent to response rates in patients receiving high dose IL-2. While both of these regimens represent a possible alternative to high dose regimens, these alternative regimens are still in their infancy and deserve further exploration. There are currently at least eight ongoing trials exploring the use of lower dose regimens of IL-2 regimens in conjunction with ACT that are actively recruiting patients.

Adverse events and clinical management of patients receiving ACT

While adverse events and toxicities are frequently observed with high dose IL-2 administration, in general the infusion of autologous T cells into a patient is well tolerated and side effects within the first 24 hours of infusion are mild. A study examining adverse

events in T cell transfer patients was conducted from 1998 to 2008 and involved 381 T cell infusions to 180 patients. In this study there were no grade 3-4 infusion reactions within 24 hours, and only 45 mild (grade 1-2) adverse events within 24 hours. The most common grade 1-2 reaction was nausea and vomiting, which was attributed to the DMSO cryoprotectant (media in which the T cells are stored) and hypotension from the diphenhydramine premedication. Other side effects included culture negative fevers and chills [39].

While the initial infusion is well tolerated, several long-term adverse effects have been seen, which fall into two broad categories: auto-immune toxicity and cytokine associated toxicity. Auto-immune toxicity can be broken down into both “on target” and “off target” side effects. On target effects occur when the infused T cells recognize the tumor associated antigens (TAAs) they were intended to recognize, however some of the TAAs are not specific to the tumor and are found in normal tissue. As the T cells recognize the TAAs in normal tissue, a host augmented immune response is elicited and a form of Graft-Versus-Host (GVH) disease occurs. In melanoma, for example, T cells that recognize TAAs such as MART-1 and gp100 not only target melanoma cells with this antigen, but also normal skin and uvea cells leading to vitiligo and uveitis [22]. Typically the stronger avidity the T cell receptor has to the TAAs, the more effective the T cell transfer is at treating the tumor cells, however higher rates of on target auto-immunity are seen [40]. While some of these on target auto-immunity side effects are mild, others can be life threatening. There are several case reports of patient deaths related to on target auto-immunity after T cell infusions [41, 42]. Off target effects occur when infused T cells with a specific T cell receptor to a TAA, cross-react with another antigen on normal tissue. There has only been one study with reported off target effects but the 2 patients who developed this complication both died. The patients had metastatic melanoma and received T cell infusion with and engineered HLA-A1-restricted MAGE-A3 TCR. After the second death occurred, autopsy revealed that the TCR was cross-reacting with a peptide on striated cardiac muscle cells (Titin). Unfortunately this cross-reactivity would have been very difficult to predict and was only discovered after autopsy revealed the cause of death in the second patient [43]. This represents the only off-target toxicity reported in the literature, however given the silent nature and the severity of this toxicity, it represents a significant concern. In order to mitigate these potentially severe, life-threatening and possibly treatment limiting toxicities, a “safety switch” has been proposed and tested in human patients. To create a “safety switch” T cells are genetically modified to express iCasp9 which is a modified distal effector of the intrinsic apoptotic pathway. When iCasp9 is activated by AP1903, it activates the terminal effector caspase 3 which leads to rapid apoptosis of the transferred T cells. This safety switch essentially allows for conditional elimination of adoptively transferred T cells by administering AP1903 if patients develop auto-immune toxicity associated with the T cell transfer [44]. This safety switch will likely be most useful in patients who develop life threatening auto-immunity associated with T cell transfer and will have limited clinical utility for mitigating milder toxicities associated with T cell transfer.

Cytokine associated toxicity is non-antigen related toxicity that occurs when widespread inflammation is induced by T cells recognizing their antigens thereby generating a host immune response. The widespread inflammation induced by T cell infusions is termed

cytokine release syndrome (CRS) and is characterized by skin eruption, fevers, rigors, hypotension and hypoxia [45]. Cytokine release syndrome occurs when a large number of lymphocytes are activated and release inflammatory cytokines. Symptoms can arise days to weeks after the T cell infusion and usually coincide with the time point of maximal in vivo T cell expansion [45]. Elevated levels of INF-gamma, IL-6, TNF-alpha, IL-2, GMCSF, IL-10, IL-8, IL5 and fractalkine have been reported [46–48], however recent literature suggests that IL-6 is the central mediator of toxicity associated with CRS [49]. This is due to broad expression of the IL-6 receptor on many immune cells and when IL-6 level is high, this cytokine can initiate trans-signaling, which initiates a pro-inflammatory IL-6 mediated signaling cascade. Currently CRS is a clinical diagnosis with limited biomarkers to evaluate a patient with suspected CRS. The most obvious biomarker for CRS would be elevation in cytokine levels; however, there are several reasons why measuring cytokine levels in a patient presents a challenge. Many hospital labs do not have Clinical Laboratory Improvement Amendments (CLIA) validated assays for measuring cytokines and to implement this would be a costly undertaking. Baseline cytokine level variations are seen in patients and are highly dependent on their medical comorbidities or cancer diagnoses / disease burdens. “Normal ranges” for cytokines are not well established further complicating the establishment of clinical biomarkers. Therefore, currently the routine measurement of cytokine levels in patient’s receiving ACT is not recommended. The only widely available biomarker that might be of some value is C-Reactive Protein (CRP) [48]. CRP has been shown to serve as a reliable surrogate for IL-6 activity [50]. However, CRP is not specific to CRS and any other inflammatory stimulus modifies its levels, most notably infection, limiting CRP’s ability to serve as a diagnostic tool. Nevertheless, in CRS after infection has been ruled out, CRP may serve as a reliable biomarker to follow the severity of CRS and document improvement in CRS. The Common Terminology Criteria for Adverse Events Version 5(CTCAEv5.0) offers a grading scale for measuring the severity of CRS based on symptomatology [51]. Table 2 outlines the grading system. It is important to keep in mind that mild CRS is expected. When T cells react to TAAs they will release cytokines to induce tumor lysis. The goal of managing a patient on T cell therapy with CRS is not to completely eradicate CRS, but to manage the symptoms and prevent end organ damage. When referencing the CTCAE scale as outlined in Table 2, it becomes evident that one of the most important factors when assessing severity of CRS is hypotension. A patient should have a documented and accurate baseline blood pressure prior to infusion of any T cell therapy in order to assess the severity of CRS should it develop [45]. In patients with Grade 1-2 CRS supportive care with IV or oral hydration and anti-pyretics are typically recommended depending on their co-morbid conditions. Patients with grade 3 or higher CRS should be monitored closely in an ICU setting and should be given tocilizumab +/− corticosteroids in addition to supportive care as needed. Tocilizumab is a humanized, immunoglobulin G1 kappa (IgG1kappa) anti-human IL-6R mAB [52].Tocilizumab prevents IL-6 from binding to both cellular and soluble IL-6 receptors thereby abrogating pro-inflammatory IL-6 mediated signaling cascade. Tocilizumab has been shown to be an effective treatment for CRS [46, 53]. A recent publication in *Blood* from an expert panel recommends that tocilizumab be administered over 1 hour at a dose of 4mg/kg in an adult with the option to repeat the dose if clinically indicated within 24-48 hours [45]. The same expert panel recommends that corticosteroids be used as second line therapy based on their experience of quicker

resolution of symptoms with Tocilizumab and that evolving evidence suggests the use of corticosteroids may decrease the anti-tumor efficacy of the infused T cells[48].

Adoptive T cell transfer in ovarian cancer

Ovarian cancer represents an attractive disease for ACT for several reasons. Ovarian cancer is an immunogenic tumor and over 50% of women with ovarian cancer have CD3+ TILs within tumor islets, which lead to markedly improved survival [54]. Patients with tumors harboring increased TILs had a median progression free survival (PFS) of 22.4 months, vs. 5.8 months in patients without CD3+ TIL infiltration. Overall survival (OS) was similarly improved in patients with TILs at 50.3 months vs. 18 months in patients without TILs. Other studies confirmed these findings and showed that the presence of CD8+ TILs in high-grade serous ovarian cancer was significantly associated with improved survival [14], with a dose response relationship and a median survival of 2.8 years in patients with low CD8+ TIL infiltration vs. 5.1 years in patients with high CD8+ TIL infiltration [55]. It has also been observed that ovarian cancer patients with a higher mutational burden have an improved treatment response, PFS and OS [56]. When analyzing a tumor from the perspective of harvesting and expanding TILs for ACT, the more immunogenic the tumor is, the more likely there will be a high number of TILs present in the resected tumor for ex vivo expansion and reinfusion.

In 1991, the first ACT trial in ovarian cancer was completed with 17 total patients receiving TIL infusions, either alone, after one dose of cyclophosphamide, or in combination with standard chemotherapy [57]. The response rates were mixed, not statistically significant, and clouded by the variable therapies the patients were receiving at the time of the infusion. While this data did not provide significant insight into the role of TIL infusion in ovarian cancer patients, it did demonstrate for the first time that this treatment strategy was safe. In 1994, a second TIL trial was completed in eleven patients with platinum refractory disease. In this trial eight patients received intra-peritoneal injections of TILs in combination with recombinant IL-2, while 3 patients received recombinant IL-2 alone. This study again failed to show a significant benefit to patients, and resulted in grade 3 peritonitis and anemia with the treatments [58]. However, this pilot study showed that ACT holds promise even in a very aggressive tumor type and in patients with limited effective treatment options. Four out of eight patients showed evidence of clinical activity via ascites reduction (2/4 patients), tumor and CA-125 reduction (1/4 patients), and surgically confirmed stable disease and decreased CA125 values (1/4 patients). In 1995, the first study demonstrating clear clinical benefit of ACT in ovarian cancer patients was published. In this case control study, TILs were employed as maintenance therapy in 13 patients after primary cytoreduction and platinum based chemotherapy. This showed significantly improved 3 year overall survival (100 vs 67.5%) and 3 year disease free interval (82.1 vs. 54.5%)[59]. Table 3 summarizes these completed trials. Since these early publications, significant advancements have been made in the methodology of ACT, and these early trials often failed to utilize pre-conditioning with lymphodepletion and T cell maturation and maintenance with IL-2 infusions. Several new ovarian cancer clinical trials utilizing the principles that established the efficacy of ACT in other solid tumors are currently enrolling (Table 4).

Adoptive T cell transfer in Cervical Cancer

Standard of care chemotherapy choices for patients with metastatic or recurrent cervical cancer are largely for palliation only. Cervical cancer is almost always an HPV driven disease, which harbors viral antigens that are foreign to the immune system, allowing for specific tumor targeting by T-cells. TIL infiltration in cervical cancer is also associated with favorable patient outcomes. The intraepithelial CD8+ TIL : Treg ratio conferred improved survival, [60], while decreased 5-year survival was reported in patients with lower numbers lower CD4+:CD8+ ratio, and higher numbers of Tregs in cervical cancer [61].

These findings led to the first clinical trial using TIL-based ACT in cervical cancer patients, and results were published by Stevanovic et al in 2015[62]. In this NCI-based phase I/II clinical trial 9 patients with metastatic or locally advanced refractory or recurrent cervical cancer who had previously received platinum-based chemotherapy or chemoradiation, underwent surgical resection of tumor specimen. TILs were extracted from surgically resected tumor fragments and ex vivo expanded. Samples with lymphocyte outgrowth were tested for reactivity to HPV-16 and -18 E6 and E7 proteins. Patients received non-myeloablative lymphocyte-depleting chemotherapy with cyclophosphamide and fludarabine, followed by 33×10^9 to 152×10^9 HPV-reactive TIL infusion and several doses of high dose IL-2 [62]. In the 2015 publication of results, 3 of 9 women had objective responses (two complete responses and one partial response). The two complete responses were still ongoing at 54 and 46 months at the time of a second publication [63]. A higher percentage of HPV-reactive TIL in the infusion and the frequency of HPV-reactive T-cells in peripheral blood 1 month after the infusion was associated with an improved response to therapy. Interestingly, for the 2 patients with a CR, the infused TILs were predominantly CD8+ cells for 1 patient and predominantly CD4+ cells for the other patient. TIL infusion was safe and as expected most toxicity resulted from the preconditioning lymphodepleting chemotherapy before the infusion.

Recently updated data on the clinical trials registry shows 18 patients enrolled with 2 complete responses (11.1%), 3 partial responses (16.7%), 1 stable disease (5.6%), and the remaining 12 with progressive disease (66.7%). Although the numbers are small, these results are impressive and suggest that this highly personalized therapeutic modality also warrants further investigation in cervical cancer.

Using the same NCI Rosenberg protocol, NCT03108495 is currently open at multiple locations across the US and in several large European centers and recruiting patients with metastatic, recurrent or persistent cervical cancer. TILs are extracted from resected tumor, expanded exponentially *ex vivo* to yield 10^9-10^{11} TILs in 4-6 weeks. Patients receive non-myeloablative lymphodepletion with cyclophosphamide and fludarabine prior to T cell infusion, which followed by high dose IL-2. Patients who experience relapse after the first infusion on this trial are currently allowed to undergo a second tumor harvest and another cycle of lymphodepletion with second TIL infusion followed by high dose IL-2 injections.

Adoptive T cell transfer in uterine cancer

The role of TILs in endometrial cancer has been less well studied. Similarly to other tumor types, several studies have demonstrated that the presence of TILs in endometrial cancer specimens, specifically high CD8+ T cells and a higher ratio of CD8+/FoxP3+ T cells are correlated with improved DFS and OS [64]. Despite the evidence that the immune system plays and important role in endometrial cancer, as of now, no trial results have been reported on the use of autologous TILs in treating endometrial cancer.

Over the last few years, endometrial cancer classification has shifted from a histologic classification to a molecular-based classification. For example, a recent analysis of high-risk endometrial cancers from the PORTEC consortium demonstrated enhanced immune infiltration in tumors characterized as POLE-mutated or MSI-high [65]. The TCGA was then queried for expression of immune markers to validate this finding, and the POLE-mutant and MSI-high subsets demonstrated significantly higher levels of CD3, CD8 and other immune cell markers, confirming the relationship between tumor subset and immune infiltration. These findings contribute to the growing body of evidence suggesting that higher neoantigen load and higher somatic mutational load correlates with tumor sensitivity to immune-based therapies [66–69]. As we continue to learn more about the molecular profiles of endometrial cancer, we may be better able to characterize tumors amenable to immune-based approaches, including TIL therapy, for future studies.

Currently there is one TIL ACT trial at the NIH accepting patients with metastatic endometrial cancer (NCT01174121) in addition to other metastatic solid tumors. In this trial patients receive TILs and IL-2 after lymphocyte-depleting chemotherapy with cyclophosphamide and fludarabine, with or without the checkpoint inhibitor pembrolizumab; no data has been published yet on the results of this study.

Conclusions and future directions

TIL based ACT has been widely studied in tumor types outside of gynecologic cancers and has shown significant improvement in clinical outcomes. Adoptive T-cell transfer for gynecologic tumors faces unique challenges because of the inherent heterogeneity of tumor parenchyma, the complexity of the tumor microenvironment, and tumor occurrence in areas with limited therapeutic accessibility. Despite these challenges, this treatment modality represents a promising future for the treatment of gynecologic cancers. Currently there are many ongoing clinical trials in the three main gynecologic tumor types that are set to mature in the next 5 years. Until this data is reported, it is unclear if the impact on survival in these patients will be as profound as what has been reported in melanoma. However initial studies suggest that this treatment will provide a significant improvement in patient outcomes and the data is currently evolving.

The widespread use of ACT for patients has several hurdles to overcome. The extraction and expansion of TILS requires an individual approach to each patient and this represents a significant cost in this treatment modality. Also TILs must be expanded and evaluated after transport in a highly specialized, GMP facility and for that reason ACT is currently limited

to highly specialized centers. Also the administration of high dose IL-2 is currently only done in highly specialized centers due to the nature of the side effects that can occur related to this therapy. Lastly, management of patients with both auto-immune toxicities and cytokine release syndrome requires special clinical expertise. The manufacturing and expansion of lymphocytes will need to be streamlined in order to offer this therapy to patients on a wider scale and at smaller institutions. Providers will also need to be trained in managing the medications and toxicities associated with this treatment modality in order to offer this to patients on a widespread scale.

As we move into the future of ACT, the use of engineered T cells has gained popularity in place of TIL extraction and expansion for multiple reasons. T cells can be isolated from the patient's peripheral blood and engineered with a specific receptor to target a known TAA on the patient's tumor. This allows the generation of a highly specific T cell that can be obtained from the peripheral blood and overcomes the need for tumor extraction and expansion of TILS. This also allows for expansion of a high volume of T cells and is not dependent on the amount of lymphocyte infiltration into the tumor. The engineered T cells will have a highly avid T cell receptor to the TAA and can also be engineered to have other co-stimulatory mechanism to improve patient outcomes. The use of Chimeric Antigen Receptor (CAR) T cells provides a patient with a lymphocyte that has both the T cell receptor (TCR) specific to the TAA, but also a costimulatory "activation" signal that allows the lymphocyte to bypass the possible downregulation by the tumor micro-environment or the host immune system. There are multiple ongoing clinical trials in gynecologic tumor sites utilizing engineered T cells for the treatment of these diseases. While each immunomodulating treatment strategy aims to overcome one of the mechanisms of immune tolerance that tumors create to persist in the body, the most promising outcomes are likely to come from the combination of multiple immune based therapies in conjunction to overcome tolerance. The use of ACT will be one component in the "cocktail" of immune treatments patient's will receive in order to achieve improved clinical outcomes. Many clinical trials are ongoing that combine checkpoint inhibition, ACT, immune based vaccines, epigenetic modifying agents, traditional cytotoxic chemotherapies and others to identify the most optimal combination for treatment outcomes.

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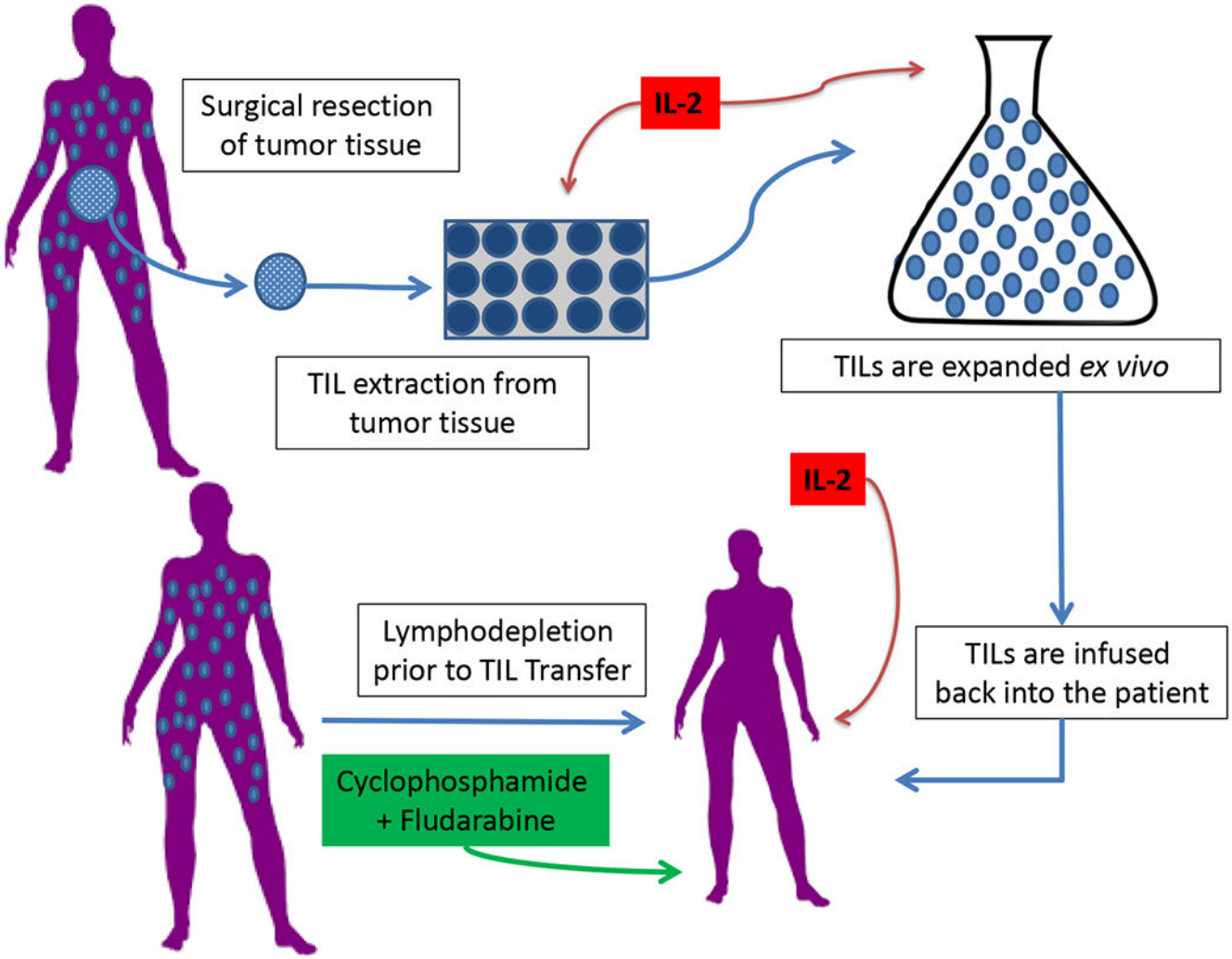

Figure 1:
Outline of adoptive Cell therapy

Table 1:

Outline of the Rosenberg Protocol for ACT

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Therapy												
Cyclophosphamide 60 mg/kg	✓	✓										
Fludarabine 25 mg/m2	✓	✓	✓	✓	✓							
TBI - 2Gy BID (if given)					✓	✓	✓					
TIL Infusion								✓				
IL-2								✓	✓	✓	✓	✓
Filgrastim35 mcg/kg/day									✓	✓	✓	✓
TMP/SMX4 160mg/800mg	✓			✓				✓				
Fluconazole5 400 mg po						✓	✓	✓	✓	✓	✓	✓
Valacyclovir po or Acyclovir IV6						✓	✓	✓	✓	✓	✓	✓

Details : IL-2 should begin within 24 hours of T cell infusion, Filgrastim should be continued until neutrophil count exceeds 1×10^{9}/L for 3 days or greater than 5×10^{9}/L, the TMP/SMX4 should be adjusted to daily 3 times per week (Monday, Wednesday and Fridays) and continued until the CD4 count is above 200×2, Fluconazole should be continued until the absolute neutrophil count is above 1000/mm^{3}, In patients positive for HSV valacyclovir or acyclovir should be continued until the CD4 count is higher than 200×2.

Table 2:

Common Terminology Criteria for Adverse Events grading scale for cytokine release syndrome

Grade of Severity	Symptomatology
Grade 1	Fever with or without constitutional symptoms
Grade 2	Hypotension responding to fluids; hypoxia responding to <40% O2
Grade 3	Hypotension managed with one pressor; hypoxia requiring ≥ 40% O2
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death
Other considerations	Also consider reporting other organ dysfunctions including neurological toxicities such as: Psychiatric disorders: Hallucinations or Confusion; Nervous system disorders: Seizure, Dysphasia, Tremor, or Headache

Table 3:

Completed clinical trials in TIL ACT trials in ovarian cancer

Author	Phase	Pre-conditioning chemotherapy	Ovarian Cancer Eligibility	TIL	IL-2 dose and regimen	Study Size (patients)	ORR (%)	CR (%)
Aoki et al. 1991	III	Cisplatin or FCAP	Stage III-IV	IV	None	17	82	0
Freedman et al. 1994	I	None		Bolus Intraperitonal	Low-dose IL-2 intraperitoneal	11	0	0
Ikarashi et al. 1994	I/II	FCAP or CAP	Stage II, III or IV	IV	None	12	–	–
Fujita et al. 1995	I/II	FCAP or CAP	Stage II, III or IV	IV	None	13	–	–
Freedman et al. 2000	I	None		Bolus Intraperitoneal	IL-2 or IFN-γ	2	0	0

Abbreviations: TIL, tumor infiltrating lymphocytes; FCAP, 5-fluorouracil, cyclophosphamide, adriamycin, cisplatin; CAP, cyclophosphamide, Adriamycin, cisplatin; IL-2, interleukin-2; IFN-γ, interferon-gamma; ORR, overall response rate; CR, complete response; pt(s), patient(s).

Table 4:

Ongoing clinical trials utilizing TIL therapy alone for the treatment of ovarian cancers

Trial ID	Year Opened	Sponsor	Ovarian Cancer Eligibility	ACT Type	Primary Outcome	Planned Enrollment	Estimated Date of completion	Status
NCT00003887	1998	Fred Hutchinson Cancer Research Center	Ovarian Carcinoma	Peripheral blood lymphocyte therapy	Determine the feasibility of donor lymphocyte infusion as adoptive immunotherapy	Not listed	2003	Completed enrollment, data pending
NCT00228358	2003	University of Washington	HER2-positive Ovarian, Epithelial Cancer, Recurrent Ovarian Germ Cell Tumor, Ovarian Epithelial Cancer, Stage IV Ovarian Germ Cell Tumor	Ex vivo-expanded HER2-specific T cells	Feasibility and Safety of infusing HER2 specific T cells	8	2011	Completed enrollment, data pending
NCT00101257	2004	Fred Hutchinson Cancer Research Center	Advanced ovarian cancer	Autologous CD4+ Antigen Specific T Cell Clones	Safety and toxicity of autologous CD4-positive antigen-specific T cells Determine the duration of in vivo persistence of this drug in these patients	18	2010	Completed enrollment, data pending
NCT00562640	2007	Memorial Sloan Kettering Cancer Center	Fallopian Tube, Ovarian, Primary Peritoneal Cancer	Wilms' tumor gene (WT1) peptide sensitized autologous T cells	Safety and tolerability Mean tolerated dose of autologous WT1 peptide-specific T cells Quantitation of alterations in the concentration of peptide-specific T cells in the blood at defined intervals post infusion Effects of the adoptively transferred T cells on the growth and progression of cancer	21	2018	Active Not Recruiting
NCT01174121	2010	National Cancer Institute (NCI)	Metastatic ovarian cancer	Re-stimulated tumor-infiltrating lymphocytes (TILs)	determine the ability of autologous TIL to mediate tumor regression	332	2024	Recruiting

Trial ID	Year Opened	Sponsor	Ovarian Cancer Eligibility	ACT Type	Primary Outcome	Planned Enrollment	Estimated Date of completion	Status
NCT02482090	2015	Herlev Hospital	Metastatic Ovarian Cancer	Re-stimulated tumor-infiltrating lymphocytes (TILs)	Determine the safety of the administration of TIL therapy including lymphodepleting chemotherapy and Interleukin-2 for patients with metastatic Ovarian Cancer	6	2017	Completed enrollment, data pending
NCT01883297	2015	University Health Network of Toronto	Recurrent, Platinum Resistant High Grade Serous Ovarian, Fallopian Tube, or Primary Peritoneal Cancer	Re-stimulated tumor-infiltrating lymphocytes (TILs)	Number occurrences and severity of side effects	9	2023	Recruiting
NCT02876510	2017	MD Anderson Cancer Center	Relapsed and or refractory solid tumors (including ovarian cancer)	Endogenous CD8+ T cells (ACTolog T cell product)	Incidence of adverse events	31	2019	Recruiting
NCT03412526	2018	Sheba Medical Center	Metastatic ovarian cancer	Ex-vivo expanded autologous Tumor Infiltrating Lymphocytes	Objective Tumor responses and assess adverse events	15	2022	Active Not Recruiting