Active Immunotherapy and Adoptive Cell Transfer as an Effective Cancer Treatment

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Abstract
There are many ways to fight cancer using the body's own immune system. Some methods include the administration of vaccines while others involve stimulatory factors injected near tumors. One promising method is enlisting the help of T cells. To fight cancer effectively, T cells must be able to recognize cancerous antigens and the environment in which these T cells reside must be conducive to their function, survival, and proliferation. This paper discusses a method of providing such an environment called adoptive cell transfer, as well as the elements that effect this protocol and the ways in which the environment can be manipulated to increase the effectiveness of adoptive cell transfer.

Many factors contribute to the observation that the effectiveness of adoptive cell transfer increases as immunodepletion increases, namely, the depletion of regulatory T cells. Additionally, the existence of natural killer cells during adoptive cell transfer has been shown to decrease its effectiveness. Also, increased levels of cytokines IL-7 and IL-15 enhance the function, survival and proliferation of transferred T cells which would increase their effectiveness. Moreover, the results of adoptive cell transfer are more positive when patients' own T cells are used.

These findings show that T cells can be used through adoptive cell transfer as an effective treatment for metastatic melanoma patients, and that there is potential for adoptive cell transfer to be adopted as a widespread effective treatment for cancer.

Introduction

Cancer is a disease characterized by the presence of mutated cells that continue to divide uncontrollably. Cancerous, or malignant, tumors, can spread to other parts of the body especially via the lymph system which can act like a cancer highway, and new tumors can then form in places far from the original tumor source (What is Cancer 2015). These tumors can have negative health effects and in many cases can be life threatening.

According to the World Health Organization more than 14 million new cancer cases were diagnosed in 2012 and there were more than 8 million deaths attributed to cancer. Additionally, the American Cancer Society projects the number of new cancer cases in the United States in 2015 to exceed 1.5 million and the number of deaths to exceed a half million.

Radiotherapy, a common cancer treatment, works by directing high energy beams, including x-rays and gamma rays, at cancer cells to disrupt their DNA and ultimately result in cell death ("Radiation Therapy"). Although radiation therapy can be successful at eradicating cancerous cells, relapse is a common problem because a few cells, or even just one cell, left behind can continue to divide and pose a serious health risk (Wayteck et. al., 2014).

To combat the problem of relapse, chemotherapy is commonly prescribed to ensure that all cancerous cells are eradicated. Chemotherapy works by killing all rapidly dividing cells ("Chemotherapy"). However, chemotherapy has many unwanted side effects as it does not differentiate between healthy rapidly dividing cells and cancerous rapidly dividing cells. This effects many different systems within the body and can cause anemia, hair loss, infection and other unhealthy and unwanted symptoms.

Another form of treatment, immunotherapy, works by using immune elements to target cancerous cells through recognition of cancer antigens. There are two different ways of recognizing cancerous cells through antigens. One way is through tumor specific antigens which are expressed solely in cancerous tumor cells, while the other involves overexpressed antigens which more abundant in cancerous tumor cells than in healthy cells. This method of treatment aims to avoid the side effects of chemotherapy by targeting only cancerous cells and to also thwart the problem of relapse seen in radiation therapy (Wayteck et. al., 2014).

Passive immunotherapy uses stimulatory factors such IL-2 which is injected into the tumor area to stimulate anti-tumor T cells to proliferate, activate, and increase effector functions (Wayteck et. al., 2014). Active immunotherapy, on the other hand, involves CD 8 and CD4 T cells that are primed to recognize specific cancerous antigens and thereby direct immune cells to target and kill these cancerous cells. This report discusses the effectiveness of active immunnotherapy in general and, specifically, a branch of active immunotherapy called adoptive cell transfer.

Methods

To research the effectiveness of active immunotherapy and specifically adoptive cell transfer, relevant information was gathered from many databases and journals. Those databases included: the Touro College Library database, Proquest Science Journals, Pub MEDLINE (EBSCO), and Oxford Journals. The information gathered was narrowed further and analyzed to glean an understanding of the effectiveness of these treatment protocols.

Discussion

A recent study indicates that active immunotherapy can be an effective treatment for cancerous tumors (Raez, et. al. 2003). This study assessed the impact and response of CD8 T cells to tumor-cell-based allogeneic vaccines in patients with advanced non-small-cell carcinoma, commonly known as lung cancer. However, the selection of patients with advanced stages of non-small-cell carcinoma may have resulted in reduced effectiveness of treatment, as cancerous cells may have been too numerous for the body's immune system to handle. Similarly, as only patients previously unresponsive to conventional treatments like chemotherapy and radiation therapy were studied, the results may have been lower than they might be had the body's ability to fight off harmful cells not already been undermined.

Vaccinations were delivered in three courses. After completion of the first course, only patients with stable disease progression or positive response against non-small-cell carcinoma as determined by CT scan, coupled with low toxicity, continued to a second course of vaccination. Again, patients with no tumor progression and non-life threatening levels of toxicity continued on to a third course of vaccination (Table 1). The patients were evaluated at the beginning and end of each course to study clinical effects and to determine toxicity levels (Raez, et. al. 2003).

The results of the above study, as shown in Table 2, indicate an increase in CD8 T cell response in all but one patient. Clinically, however, the results were less profound. Only 27% of the patients

Table 1

		Course 1				Course 2				Course 3			
Study entry	1	2	4	6	7	8	10	12	13	14	16	18	19
Weeks on Study													
Pre-entry-evaluation	x												
Immunization		1	2	3		4	5	6		7	8	9	

Table 2, Clinical and Immunological response

Patient # HLA	Response	Fold titer increase	Previous	Survival (months)	Time to progression (months)	Preimmune	First course	Second course	Third course
1005 A1	PD	190	C+R	10	-	0	190	ND	ND
1012 A1	NE	NE	C	15	-	0.2	ND	ND	ND
1001 A2	PD	25	C+S	18	-	0	25	ND	ND
1002 A2	PD	1.6	C+S	22	-	41	65	ND	ND
1009 A2	PD	6.5	C	3	-	2	13	ND	ND
1010 A2	PR	41	S	27+	3	3.8	46	88	157
1011 A2	PD	19	C	11	-	3	30	57	ND
1013 A2	PD	34	C+R+S	2	-	5.2	164	178	ND
1014 A2	SD	19	C+S	13+	3	1.6	30	30	25
1015 A2	PD	0	C+R	7	-	0	0	ND	ND
1003 non	SD	134	S	31+	26+	1	134	113	84
1004 non	SD	424	C+R	23	11	0	424	232	>450
1006 non	PD	9.3	C+S	30+	-	16	150	ND	ND
1007 non	SD	14	C+R+S	29+	23+	1.2	2.8	0.8	0/17
1008 non	PD	32	C	6	-	5.6	178	ND	ND

PD=progressive disease; PR=partial response; SD=stable disease; ND=not determined; C=chemotherapy; R=radiation therapy; S=supportive care; NE=not evaluable

showed stable disease progression while another 7% showed a partial response. This total clinical response of 33% suggests that immunotherapy may be a viable option but requires further study to assess clinical effectiveness. (Raez, et. al. 2003)

The vaccine used in this study was developed by Dr. N. Savaraj through modification of a cell line harnessed from a patient in 1994. The cells were rendered incapable of colonizing ensuring they would not cause any harm to study participants. However, perhaps a more effective albeit more expensive method, demonstrated at the Johns Hopkins School of Medicine (Perica, et.al. 2015), may be to extract a patient's own CD8 T cells from the vicinity of the tumor and subsequently culture them in vitro. This would ensure that the T cells would be reactive to the patient's own cancerous cells. Using pharmaceuticals to regulate the patient's regulatory T cells, the CD8 T cells harnessed in vitro can be reinserted with free rein to target cancerous cells (Perica, et.al. 2015).

Adoptive cells transfer is a treatment in which immune cells harnessed in vitro are transferred to a patient to give him specific immune functions. To treat cancer patients using adoptive cell transfer, a piece of a tumor is removed from the body, so that T cells can be removed from the tumor, and stimulated to grow rapidly in vitro. These T cells are then infused back into the patient to target and kill cancer cells (Figure 1).

When attempted on patients with metastatic melanoma, adoptive cell transfer showed potential as an effective treatment although admittedly it had limited clinical results. A trial of 20 metastatic melanoma patients were treated with adoptive cell transfer and given IL-2 to stimulate the transferred T cells. The results revealed tumor regression in multiple sites in 11 of the 20 patients studied (Phan, Rosenberg, 2013). However, it should be noted that although IL-2 promotes the growth and function of transferred T cells, its addition may also help regulatory T cell

Figure 1

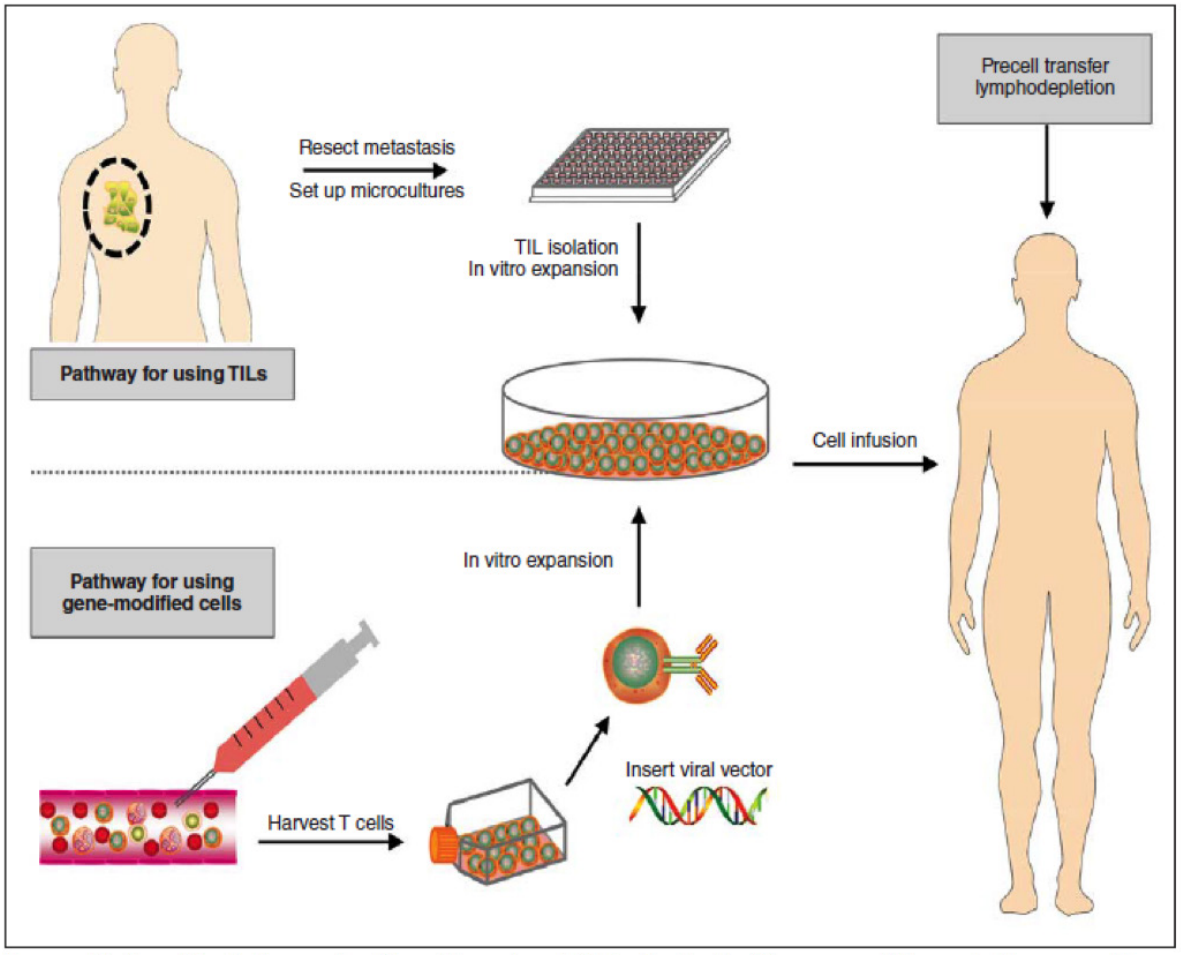

Figure. — Adoptive cell transfer therapy using either autologous tumor-infiltrating lymphocytes (TILs; upper panel) from excised tumors or autologous peripheral blood lymphocytes gene-modified to express engineered T-cell receptors or chimeric antigen receptors (lower panel). Patients undergo a preparative lymphodepleting regimen prior to cell infusion.
Phan, Rosenberg, 2013

suppress these transferred T cells thus countering the intended effect and perhaps limiting the overall effectiveness of the treatment. This indicates that adoptive cell transfer may have a place in the pursuit of a cure for cancer but it does require some further fine tuning.

Depletion of immune cells and other immune elements before adoptive cell transfer of CD 8 T cells in mice has been shown to increase the effectiveness of transferred T cells (Gattinoni et. al., 2005). It has been proposed that depleting the immune system of its natural elements keeps regulatory T cells from turning off anti-cancer CD 8 T cells transferred during adoptive cell transfer. Additionally, adoptive cell transfer helps lower the immune system's tolerance of the cancerous "self-antigens" by selecting and activating highly specific T cells, mostly CD 8 T cells, and by changing the body's internal environment to one that is more receptive to these cells.

A recent study looked at 13 metastatic melanoma patients who received immunodepleting chemotherapy specifically targeted to regulatory T cells. The patients were then injected with in vitro cultured T cells as well as IL-2. Because the patients first received immunodepleting chemotherapy, in this study the addition of IL-2 avoided the adverse effect of activating regulatory T cells. This allowed for highly favorable results (Table 3) that were significantly more efficient than the results of the study involving non-small-cell carcinoma patients. Of the 13 patients, 6 showed positive clinical responses, and 4 showed mixed responses consisting of considerable shrinkage of at least one tumor. Although the study was relatively small and was limited to melanoma patients, it nevertheless demonstrates the potential of adoptive cell transfer in treating cancer patients (Dudley et. al., 2002).

In a similar trial, metastatic melanoma patients were treated with different levels of immunodepletive therapy prior to adoptive cell

Table 3

Patient Demographics, Treatments Received, and Clinical Outcomes.

		Treatment						
Patient	Age/Sex	Cells infused (10^-10)	CD8/CD4 phenotype (%)	Antigen specificity	IL-2 (doses)	Sites of evaluable Metastases	Response duration (months)	Autoimmunity
1	18/M	2.3	11/39	Other	9	Lymph(axillary nodes mesenteric pelvic)	PR (24+)	None
2	30/F	3.5	83/15	MART-1 gp100	8	Cutaneous, subcutaneous	PR (8)	Vitiligo
3	43/F	4.0	44/58	gp100	5	Brain, cutaneous, liver,lung	NR	None
4	57/F	3.4	56/52	gp100	9	Cutaneous, subcutaneous	PR (2)	None
5	53/M	3.0	16/85	Other	7	Brain, lung, lymph nodes	NR-mixed	None
6	37/F	9.2	65/35	Other	6	Lung, intraperitoneal, subcutaneous	PR (15+)	None
7	44/M	12.3	61/41	MART-1	7	Lymph nodes, subcutaneous	NR-mixed	Vitiligo
8	48/M	9.5	48/52	gp100	12	Subcutaneous	NR	None
9	57/M	9.6	84/43	MART-1	10	Cutaneous, subcutaneous	PR (10+)	Vitiligo
10	55/M	10.7	96/2	MART-1	12	Lymph nodes, cutaneous, subcutaneous	PR (9+)	Uveitis
11	29/M	13.0	96/3	MART-1	12	Liver, pericardial, subcutaneous	NR-mixed	Vitiligo
12	37/F	13.7	72/74	MART-1	11	Liver, lung, gallbladder, lymph nodes	NR-mixed	None
13	41/F	7.7	92/8	MART-1	11	Subcutaneous	NR	None

PR = Partial Response, NR = No Response

Dudley et. al., 2002

transfer in order to establish a level of immunodepletion that would best enhance this treatment protocol (Rosenberg et. al., 2011). One group of 43 patients received a nonmyeloablative preparative regimen, a less toxic method of immunodepletion. A second and third group of 25 patients each, received a more toxic total body irradiation of 2 Gy and 12 Gy (Dept. of Homeland Security 15), respectively, in addition to a non-myeloablative preparative regimen. All three groups were subsequently treated with adoptive cell transfer (Rosenberg et. al., 2011).

The results of this study (Table 4) showed that at higher levels of immunodepletion positive clinical outcomes following adoptive cell transfer increased. In the group that received only a non-myeloablative preparative regimen, 49% of subjects showed an overall response while 5% showed complete response. The group that received 2 Gy of total body irradiation exhibited slightly higher incidence of overall response at 52%, but showed a large increase in complete response at 20%. The group that received 12 Gy of total body irradiation showed a marked overall response of 72% with complete response at 40%, indicating that the greater the immunodepletion the greater the clinical outcome of adoptive cell transfer (Rosenberg et. al., 2011).

Table 4

Regimen	No. of Patients	Partial Response n (%)	Complete Response n (%)	Overall Response n (%)
No TBI	43	16 (37)	5 (12)	21 (49)
2 GY TBI	25	8 (32)	5 (20)	13 (52)
12 GY TBI	25	8 (32)	10 (40)	18 (72)
Total	93	32 (34)	20 (22)	52 (56)
TBI=total body irradiation, TIL=tumor-infiltrating lymphocyte				

Phan, Rosenberg, 2013

One possible method to increase the overall effectiveness of adoptive cell transfer and such positive results is to harness a patient's own CD 8 T cells to ensure that the T cells are as specific to the cancer cell antigens as possible to. However, this is likely a complicated task as it would require development of a "new drug" for every patient. Another method that may enhance the results of adoptive cell transfer is to take a sample of the patients own cancer cells and test CD 8 T cell reactivity to the cancer antigens thereby ensuring a highly reactive T cell response. In another experiment, 20 metastatic melanoma patients received a less intense form of immunodepletion called nonmyeloablative preparative regimen. However, no patient received total body irradiation. In this trial, however, T cells were taken directly from the actual patients' tumor and grown quickly in vitro, as opposed to using previously developed anti-tumor T cells. This may have helped ensure T cells would be highly specific and highly reactive to the patients' own cancerous cells. Of the 20 patients treated, 10 had an overall response, of which 2 had a complete remission and 8 had a partial remission. Additionally, 4 patients had stable disease, and 6 had progressive disease for an overall response of 70% (Besser et. al., 2010). The results show that nonmyeloablative preparative regimen, coupled with adoptive cell transfer that uses cells harnessed from patients own tumors can be an effective treatment. Furthermore, analysis of the results may show that the results are more positive then what is seen on the surface. Of the 6 patients with progressive disease after treatment, all 6 started with stage M1c melanoma, which represents the stage in which the tumor has traveled to vital organs (excluding the lungs) or when the tumor has traveled to other areas and the patient shows elevated levels of low-density lipoprotein ("How is Melanoma Staged"). At this stage the cancer cells may have been too numerous for the transferred T cells. However, all patients with stages below M1c melanoma and even some patients with stage M1c melanoma showed at least some response to adoptive cell transfer, either complete response, partial response, or stable disease. This indicates that adoptive cell transfer using patient's own T cells, combined with nonmyeloablative preparative regimen for immunodepletion is an effective treatment for early stages of metastatic melanoma patients.

This trial, in which patients received only nonmyeloablative preparative regimen for immunodepletion prior to adoptive cell transfer, significantly outperformed the previously mentioned trial in which patients received only nonmyeloablative preparative regimen for immunodepletion prior to adoptive cell transfer. One possible explanation may be that this trial used T cells taken from removed portions of the patients own tumor, ensuring that the T cells used were highly reactive to the patients cancerous cells.

As previously stated, immunodepletion prior to adoptive cell transfer increases the effectiveness of the treatment. However, immunodepletion effects the entire immune system, which can have life threatening side effects. Therefore further study to determine key components that effect the effectiveness of adoptive cell transfer and how to precisely block them may be helpful.

The most obvious immune element that would reduce the effectiveness of adoptive cell transfer is regulatory T cells. When developing T cells and B cells, the body has mechanisms through which it ensures than no immune cell is released that reacts to self-antigens; however, the system is not foolproof. Regulatory T cells restrain the few immune cells that get through those mechanisms and suppress them ensuring that no self-cells are targeted by the immune system.

It has been shown that regulatory T cells suppress anti-cancer CD 8 T cells in vitro (Antony et. al., 2005). It would be logical to hypothesize that regulatory T cells would also effect the T cells transferred during adoptive cell transfer. To test for a correlation between regulatory T cells and adoptive cell transfer, tumor bearing mice were treated with adoptive cell transfer. Some mice were then injected with anti-Thy-1.2 antibody and complement to suppress function of regulatory T cells, while others were given active regulatory T cells. The results showed that when regulatory T cell function was turned off the transferred T cells destroyed the cancerous cells. However, when active regulatory T cells were added the size of the tumors continued growing exponentially (North, 1982). This shows that regulatory T cells have a large impact on the effectiveness of adoptive cell transfer.

Although regulatory T cells decrease the effectiveness of adoptive cell transfer, simply turning them off while leaving the rest of the immune system intact would allow surviving immune cells that recognize self-antigens to attack patients' healthy cells causing autoimmune disease.

One method to reduce the effect of regulatory T cells on adoptive cell transfer without effecting the entire immune system may be to induce apoptosis only in regulatory T cells in close proximity to the tumor. This would allow the transferred T cells to operate in an environment conducive to their function, while allowing regulatory T cells in other areas of the body to operate freely avoiding autoimmune disease.

Recently, it was suggested that the protein FasL-Fc can be used to deplete regulatory T cells located only in tumors (Chen et. al., 2007). To confine the protein FasL-FC to the tumor, a protein is incorporated into cell membranes and acts as a trap for the Fc portion of FasL-Fc, not allowing it to escape the confines of the tumor (Chen, Zheng, Tykocinski, 2000). To test this suggestion, tumor bearing mice were injected with FasL-Fc in the tumor region. The results showed a significant increase in apoptosis of regulatory T cells in the tumors treated with FasL-Fc, indicating this may be a viable option to decrease the regulatory T cell effect on adoptive cell transfer without effecting the entire immune system (Chen et. al., 2007).

To test the actual effect of the protein FasL-Fc on adoptive cell transfer, tumor bearing mice were treated with both FasL-Fc and adoptive cell transfer. The results were a significant retardation of tumor growth in a large portion of mice. Additionally, complete tumor regression was seen in 53% of the mice (Chen et. al., 2007). This indicates that using the protein FasL-Fc may be a viable option to eliminate the effect of regulatory T cells on adoptive cell transfer while allowing the immune system to operate regularly and without the risk of autoimmune effects.

To determine the impact of other immune elements on adoptive cell transfer, tumor bearing mice were tested and the results analyzed. In many studies, after irradiation of cancer bearing mice lacking regulatory T cells, effectiveness of adoptive cell transfer increased. This indicates that regulatory T cells are not the only elements responsible for decreased effectiveness of adoptive cell transfer (Gattinoni et. al., 2005).

One hypothesis is that Natural Killer cells act as sinks for cytokines responsible for survival, proliferation, and function of transferred CD 8 T cells (Gattinoni et. al., 2005). Host Natural Killer cells, also in need of cytokines, compete for the same cytokines necessary to support the transferred T cells leading to a limited amount of cytokines available to support the transferred T cells. To test this hypothesis, tumor bearing mice lacking endogenous B cells and T cells, including regulatory T cells, were treated with anti-NK1.1 antibody to decrease the number of Natural Killer cells to the number found after irradiation of the entire immune system. They were then treated with adoptive cell transfer and compared to mice not given the anti-NK1.1 antibody and treated with adoptive cell transfer. The results showed that removal of Natural Killer cells increased the effectiveness of adoptive cell transfer, indicating that Natural Killer cells play an important role in the function of transferred CD 8 T cells (Gattinoni et. al., 2005).

One way to avoid the effects of cytokine sinks may be to precisely determine which cytokines the Natural Killer cells sink, and stimulate an increase of their production giving the transferred T cells access to those cytokines.

To determine the effects of cytokines on adoptive cell transfer, different cytokines were removed and added to tumor bearing mice treated with adoptive cell transfer. Results indicated that the cytokline IL-7 was needed to maintain survival and continued growth of transferred T cells, but was not necessary to maintain function of the T cells. Conversely, IL-15 was necessary to maintain function, but did not play a part in survival and proliferation of transferred T cells. This shows that increasing both IL-7 and IL-15 at the same time increases the transferred T cells effectiveness against tumors, while decreasing both IL-7 and IL-15 at the same time would decreases the transferred T cells effectiveness against tumors (Gattinoni et. al., 2005).

These findings indicate that there may be a path by which regulatory T cells can be eliminated from the area of the tumor (Chen et. al., 2007), and transferred CD 8 T cells can be stimulated to proliferate and enhance their effector functions without effecting the entire immune system (Gattinoni et. al., 2005).

Conclusion

In conclusion, increasing CD 8 T cell count alone is not the most effective way to use T cells to fight cancer. Instead immunodepletion together with an increase in anti-cancer T cells delivered through adoptive cell transfer is an effective approach that has been shown to be effective in humans with metastatic melanoma. Other factors besides immunodepletion also effect the results of adoptive cell transfer such as cytokines, and the origin of the T cells injected. The key to adoptive cell transfer success with limited side effects, is the balance of all elements involved which is a large task and requires further study but does seem possible in the future.

However, for now, adoptive cell transfer is still a relatively new method of treatment and still requires immunodepletion for its effectiveness. This can bring with it its own problems, such as an increased risk of infection and a limited ability to fight infection. Additionally, most trials of adoptive cell transfer on humans have been limited to melanoma patients, making further research necessary for it to be used to fight other cancers. To that effect, further studies are currently underway to broaden this treatment to fight other cancers including, lymphoma, leukemia, and neuroblastoma (Deng et. al., 2014) giving hope to cancer patients all over the world.

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