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Genetically Modified T-Cell Therapy for Osteosarcoma

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Abstract

T-cell immunotherapy may offer an approach to improve outcomes for patients with osteosarcoma, who fail current therapies. In addition, it has the potential to reduce treatment-related complications for all patients. Generating tumor-specific T cells with conventional antigen presenting cells *ex vivo* is time consuming and often results in T-cell products with a low frequency of tumor-specific T cells. In addition, the generated T cells remain sensitive to the immunosuppressive tumor microenvironment. Genetic modification of T cells is one strategy to overcome these limitations. For example, T cells can be genetically modified to render them antigen specific, resistant to inhibitory factors, or increase their ability to home to tumor sites. Most genetic modification strategies have only been evaluated in preclinical models, however early phase clinical trials are in progress. In this chapter we review the current status of gene-modified T-cell therapy with special focus on osteosarcoma, highlighting potential antigenic targets, preclinical and clinical studies, and strategies to improve current T-cell therapy approaches.

Keywords

Pediatric cancer; Osteosarcoma; Cancer immunotherapy; T-cell therapy; Gene therapy; Chimeric antigen receptors; Tumor antigens

Introduction

Adoptive T-cell therapy refers to the isolation of allogeneic or autologous T cells, followed by *ex vivo* manipulation, and subsequent infusion into patients for therapeutic gain [101]. Channeling the cytotoxic killing and specific targeting ability of T cells through adoptive transfer has the potential to improve outcomes for patients with osteosarcoma. An early example of adoptive T-cell therapy for osteosarcoma was reported by Sutherland et al. [113]. A 14-year-old girl, who had the same human leukocyte antigen (HLA) type as her mother received unmanipulated, maternal lymphocytes. Lymphocytes isolated from the patient post infusion killed osteosarcoma cells *in vitro*, but the patient had only a minimal clinical response prior disease progression and death. Since Sutherland's report, significant advances in immunotherapeutic techniques have taken place.

Cell therapy with conventional T cells has shown promise in several clinical settings [11, 52, 101]. Examples include donor lymphocyte infusions (DLI) after stem cell transplantation to treat CML relapse [61], infusion of Epstein-Barr virus (EBV)-specific T lymphocytes to treat EBV-related lymphomas and nasopharyngeal carcinoma [5, 7, 24, 72, 110], infusion of tumor infiltrating lymphocytes (TILs) to treat melanoma [31, 101], and the infusion of virus-specific T cells to prevent and treat viral-associated disease in immunocompromised patients [42, 64, 65].

Since the *ex vivo* generation of T cells specific for tumor associated antigens (TAA) is often cumbersome, investigators have developed genetic modification strategies to render T cells TAA specific [52, 101, 104]. For example, infusion of T cells genetically modified with chimeric antigen receptors (CAR) specific for GD2 or CD19 has shown promise in early clinical studies for neuroblastoma and CD19-positive hematological malignancies including acute lymphoblastic leukemia and lymphoma [12, 39, 54, 60, 71, 92, 93, 105]. Besides rendering T cells tumor-specific, genetic modifications enable the generation of T cells with enhanced effector functions (Table 1). While these approaches have been mainly evaluated in preclinical models, some are already being actively explored in the clinic. In this chapter we review the current status of gene-modified T-cell therapy for osteosarcoma, highlighting potential antigenic targets, preclinical and clinical studies, and strategies to improve T-cell therapeutic approaches.

T-Cell Therapy Targets for Osteosarcoma

Developing successful antigen-specific T-cell therapy depends on the availability of specific TAA. Once a TAA is identified, TAA-specific T cells can be either generated using conventional antigen presenting cells or by gene transfer to recognize and induce killing of TAA-positive osteosarcoma.

TAA are potential candidates for immunotherapy, including T-cell therapy, if they are (1) expressed at higher than normal levels on tumor cells compared to nonmalignant host cells, (2) are normally only expressed during fetal development or at immunoprivileged sites, such as the testes, (3) contain novel peptide sequences created by gene mutation, (4) are viral antigens, (5) are antigens produced by epigenetic changes, (6) or are antigens on non-transformed cells in the tumor microenvironment [15, 98, 121]. Unaltered tissue-

differentiation antigens on tumors can also be targets for T-cell immunotherapy, but only if the associated tissues are not essential for life and/or their products can be replaced [121]. For example, CD19-specific T-cell therapy induces regression of CD19-positive malignancies, but also leads to long-term depletion of normal, CD19-positive B cells, which can be remedied by the infusion of intravenous immunoglobulin (IVIG) [12, 39, 54, 60, 92, 105].

For osteosarcoma, numerous TAA have been described that are summarized in Table 2. These include human epidermal growth factor receptor 2 (HER2) [2, 38], interleukin 11 receptor alpha (IL11R α) [46], melanoma associated antigen (MAGE) and g melanoma antigen (GAGE) family members [49], GD2 (a disialoganglioside; not a protein tumor associated antigen) [129], New York esophageal squamous cell carcinoma 1 (NY-ESO-1) [49], clusterin-associated protein 1 (CLUAP1) [48], papillomavirus binding factor [118], fibroblast activation protein (FAP) [130], tumor endothelial marker 1 (TEM1) [103], and B7-H3 [75]. Other TAA for osteosarcoma-targeted T-cell therapy are rapidly being elucidated. Orentas et al. described several potential targets shared amongst multiple pediatric tumors, such as melanoma cell adhesion molecule (MCAM) and glypican-2, which are present on six different pediatric solid tumors, but not expressed in normal tissues [83, 84]. While gene expression data was used to identify these targets, and additional studies are needed to confirm expression on a protein level, the work demonstrates the use of gene expression array data to identify new TAAs.

Genetic Approaches to Render T Cells Specific for Osteosarcoma

Since the *ex vivo* generation of conventional antigen-specific T cells is often cumbersome and unreliable, investigators have developed genetic approaches to rapidly generate antigen-specific T cells. These include the forced expression of α/β T-cell receptors (TCRs) and CARs.

α/β TCR Modified T-Cells

Conventional TCRs are composed of α and β chains that form heterodimers. TCRs recognize peptides, which are derived from proteins and are presented on major histocompatibility complex (MHC) molecules on the cell surface. Isolating TCRs for adoptive T-cell therapy requires the generation of TAA-specific T-cell clones and subsequent isolation and cloning of the TCR α and β chains [120]. In general, a large number of T-cell clones need to be screened, and isolated TCRs often are of low affinity requiring additional affinity maturation. Following isolation, genes encoding the α and β chains are cloned into retroviral or lentiviral vectors, and then used to transduce T cells [98]. Since T cells express endogenous α/β TCRs, mispairing between endogenous α/β and transgenic α/β TCR chain is a common problem. Several approaches have been developed to overcome this limitation, including the introduction of disulfide bonds or use of murine sequences to favor dimerization of transgenic α/β TCR chains [23, 37]. Silencing the expression of endogenous α/β TCR by shRNAs or zinc-finger nucleases is another option [82, 116].

α/β TCRs have been isolated for several TAA including CEA, GP100, MAGEA3, MART1, and NY-ESO-1 [51, 76, 77, 87, 99]. So far the safety and efficacy of α/β TCR T-cell therapy has been evaluated mainly in melanoma patients, but studies have also been conducted for patients with sarcoma, colon cancer and multiple myeloma. One of the first studies in humans with transgenic α/β TCR T cells was conducted by Morgan et al. and demonstrated that the infusion of autologous polyclonal T cells expressing MART1-specific α/β TCRs was safe and induced objective tumor responses in 2 out of 15 lymphodepleted melanoma patients [77]. To increase the response rates, the same group infused T cells expressing high affinity MART1- and gp100-specific α/β TCRs. While response rates increased, several patients developed toxicities including skin rash, uveitis, and/or hearing loss that were not associated with antitumor responses [51]. Recognition of normal tissues expressing low levels of CEA has also been reported for the adoptive transfer of CEA-specific α/β TCR T cells [87]. In contrast, infusion of NY-ESO-1-specific α/β TCR T cells was well tolerated with a response in 4/6 patients with synovial cell sarcoma and in 5/11 patients with melanoma. In addition, an ongoing clinical study indicates that NY-ESO-1-specific α/β TCR T cells induce clinical responses in patients with multiple myeloma without off-targets effects [68]. As mentioned above, affinity maturation is frequently used to increase the activity of α/β TCRs. However, this can lead to recognition of related antigens resulting in severe adverse events [74, 76]. For example, infusion of MAGE A3-specific α/β TCR T cells caused fatal neurotoxicity due to recognition of MAGE A12 as well as fatal cardiac toxicities due to recognition of titin.

Thus clinical studies so far have not only demonstrated the potency of adoptively transferred α/β TCR-modified T cells but also their clinical limitations. Nevertheless, active exploration of α/β TCR-modified T-cell therapy is warranted for osteosarcoma.

CAR-Modified T Cells

Antigen-specific T cells can also be generated by the transfer of genes encoding CARs [32, 73, 104]. CARs consist of an ectodomain that confers antigen specificity, a hinge, a transmembrane domain, and an endodomain that contains signaling domains derived from the T-cell receptor CD3- ζ chain and co-stimulatory molecules such as CD27, CD28, 41BB, or OX40. Depending on the number of co-stimulatory domains, CARs are referred to as first generation (no), second generation (one), or third generation (two) CARs. CARs targeting multiple pediatric malignancies have been developed [2, 16, 39, 44, 46, 86, 93, 102, 106]. CAR ectodomains are most commonly generated by joining the heavy and light chain variable regions of a monoclonal antibody (MAb), expressed as a single-chain Fv (scFv) molecule. CARs recognize unprocessed antigen on tumor cell surfaces, and do not require peptide presentation on MHC molecules.

CAR T-cell therapy has several advantages compared to α/β T-cell therapy. Because CARs do not require antigen presentation on MHC molecules, generation of CAR T cells for patients does not require HLA matching. This property also renders CAR T cells resistant to tumor escape mechanisms, such as downregulation of HLA molecules and defects in the MHC class I processing pathway. A second advantage is that MAbs already exist for numerous surface antigens, obviating the need of cumbersome α/β TCR isolation.

Additionally, CAR T cells recognize carbohydrate and glycolipid antigens, in addition to protein antigens [73, 104]. Furthermore, CARs confer T-cell specificity in a single molecule unlike artificial α/β TCRs, which require the expression of two molecules that are prone to heterodimerization with the endogenously expressed α/β TCR chains. A potential drawback of CARs is that in general only cell surface molecules are recognized. However, the isolation of scFvs that recognize HLA-molecule/peptide complexes has allowed the generation of CARs that recognize peptides derived from intracellular proteins [107, 127].

Of the TAA expressed in osteosarcoma, GD2, HER2, IL11R α , and FAP are expressed on the cell surface, and have been targeted with CAR T cells in preclinical animal models and/or clinical studies. Pule et al. expressed a first generation GD2-specific CAR on EBV-specific T cells and gave them to 11 children with advanced neuroblastoma [71, 93]. Three of them had complete responses (sustained in two) while an additional two with bulky tumors showed substantial tumor necrosis. Follow up studies are in progress [80], and these encouraging results should justify the exploration of GD2-specific CAR T cells for patients with osteosarcoma.

While HER2 is not gene amplified in osteosarcoma, 60–70 % of osteosarcoma are HER2 positive and HER2-positivity is associated with poor outcomes [38, 79]. T cells expressing a second generation CAR with a CD28- ζ -endodomain showed promising antitumor activity in preclinical animal models [2]. In addition, HER2-CAR T cells had potent antitumor activity against osteosarcoma spheroids, which are enriched in osteosarcoma-initiating cells [97]. However, safety concerns have been raised in regards to targeting HER2 with CAR T cells in humans. One patient, who received high dose chemotherapy followed by the infusion of 1×10^{10} T cells expressing a third generation HER2-specific CAR and IL2 developed respiratory failure within 12 h of infusion and died [78]. Subsequently, up to $1 \times 10^8/\text{m}^2$ T cells expressing a second generation CAR were given to pediatric and adolescent sarcoma patients. While the infusions were safe, infused T cells did not expand significantly post infusion, and antitumor activity of the infused T cells was limited [1].

To target IL11R α , Huang and colleagues developed a second generation CAR that contains the natural ligand (IL11) as a CAR ectodomain [46]. IL11R α -specific CAR T cells recognized and killed IL11R α -positive osteosarcoma cells, and caused regression of lung metastases in the KRIB metastatic osteosarcoma model in vivo. Lastly, T cells expressing a second generation FAP-specific CAR, which not only target FAP-positive osteosarcoma cells but also FAP-positive stromal cells [53], have shown promising antitumor activity in preclinical models, which are discussed in the section “Genetic Modification to Overcome Tumor-Mediated Immunosuppression.”

In summary CAR T cells have shown promising antitumor activity in preclinical animal models, and the initial clinical experience is encouraging. However, several challenges remain including in vivo T-cell expansion and persistence, the inhibitory tumor microenvironment, T-cell trafficking to tumor sites, and safety. As reviewed in the next section (Table 1), we and others believe that additional genetic modifications of T cells have the potential to overcome these obstacles.

Genetic Approaches to Enhance the Effector Function of Osteosarcoma-Specific T Cells

Enhancing T-Cell Expansion and Persistence In Vivo

Dramatic T-cell expansion and long-term persistence post infusion of adoptively transferred T cells has been observed in lymphodepleted patients post hematopoietic stem cell transplantation or in patients that have been lymphodepleted with chemotherapy and/or radiation prior to T-cell transfer [31, 42]. Since T-cell expansion post antigen recognition requires the provision of costimulation, investigators have included signaling domains in CAR endodomains derived from costimulatory molecules including CD27, CD28, 4-1BB, and OX40. Numerous preclinical studies have documented the benefit of added costimulation [13, 14, 94, 109], however only one study so far has done a “head to head comparison” in humans. Savoldo et al. compared CD19-specific CARs with a ζ - or CD28. ζ -domain [105]. While CD28 costimulation resulted in expansion of adoptively transferred CAR.CD28. ζ T cells in contrast to CAR. ζ T cells, the effect was limited. Limited expansion was also observed in our clinical trial with HER2.CAR.CD28. ζ T cells for patients with sarcoma [1]. Both of these studies were conducted without lymphodepleting chemotherapy and/or radiation. Thus, aggressive lymphodepletion prior to T-cell transfer might enhance CAR.CD28. ζ T-cell expansion in vivo. Other options include vaccination post infusion to boost T-cell expansion. Lastly, most studies have been conducted with unselected T cells. Recent studies indicate that it might be advantageous to express CARs in T cells that are specific for viruses, so that infused cells could be boosted by vaccination (e.g., influenza) [25] or by viruses, which are present latently in humans (e.g., EBV) [93]. In addition, expressing CARs in T-cell subsets like effector memory T cells has the potential to enhance T-cell persistence [3, 114].

Genetic Modifications to Overcome Tumor-Mediated Immunosuppression

Malignant cells including osteosarcoma and their supporting stroma have developed an intricate system to suppress the immune system [4, 34, 36, 96, 119]. They (1) secrete immunosuppressive cytokines such as transforming growth factor (TGF β) or IL10, (2) attract immunosuppressive cells such regulatory T cells (Tregs) or myeloid derived suppressor cells (MSDCs), (3) inhibit dendritic cell maturation, (4) express molecules on the cell surface that suppress immune cells including FAS ligand (FAS-L) and PD-L1, and (5) create a metabolic environment (e.g., high lactate, low tryptophan) that is immunosuppressive.

Three broad approaches have been developed to overcome tumor immunosuppression [66]: (1) increasing CAR T-cell activation, for example by enhanced co-stimulation (discussed above) or by local production of transgenic cytokines, (2) engineering CAR T cells to be resistant to the immune evasion strategies used by the tumor, or (3) targeting the cellular components of the tumor stroma. Any one may affect more than one mechanism of tumor immunosuppression.

CAR T cells can be engineered to produce immunostimulatory cytokines by transgenic expression of cytokines such as IL-15 [45, 95], which improves CAR T-cell expansion and

persistence in vivo. In addition, it renders T cells resistant to the inhibitory effects of Tregs by activation of the phosphoinositide 3-kinase (PI3K) pathway [90]. Alternatively, transgenic expression of IL12 in CAR T cells acts directly to enhance T-cell activity [18, 56, 89, 131]. In addition it reverses the immunosuppressive tumor environment by triggering the apoptosis of inhibitory tumorinfiltrating macrophages, dendritic cells, and MDSCs through a FAS-dependent pathway [56], resulting in enhanced antitumor activity of adoptively transferred T cells in several preclinical animal models. While there are safety concerns in regards to constitutive IL12 expression, there are several mechanisms available to restrict IL12 production to activated T cells at the tumor site by using inducible expression systems [131].

Conversely, instead of themselves being engineered to produce cytokines, CAR T cells can be engineered to be resistant to cytokines such as IL4 and TGF β that inhibit their cytolytic function. TGF β is widely used by tumors as an immune evasion strategy [128], since it promotes tumor growth, limits effector T-cell function, and activates Tregs. These detrimental effects of TGF β can be negated by modifying T cells to express a dominant-negative TGF β receptor type II (DNR), which lacks most of the cytoplasmic kinase domain [8, 33]. DNR expression interferes with TGF β -signaling and restores T-cell effector function in the presence of TGF β , and a clinical study evaluating this strategy is in progress for patients with EBV-positive lymphomas [6].

It is also possible to engineer T cells to actively benefit from the inhibitory signals generated by the tumor environment, by converting inhibitory into stimulatory signals [67, 123, 125]. For example, linking the extracellular domain of the TGF β RII to the endodomain of toll-like receptor (TLR) 4 results in a chimeric receptor that not only renders T cells resistant to TGF β , but also induces T-cell activation and expansion [123]. Chimeric IL4 receptors are another example of these “signal converters.” Many tumors secrete IL4 to create a TH2-polarized environment, and two groups of investigators have shown that expression of chimeric IL4 receptors consisting of the ectodomain of the IL4 receptor and the endodomain of the IL-7R α or the IL-2R β chain enable T cells to proliferate in the presence of IL4 and retain their effector function including TH1-polarization [67, 125].

Silencing genes that render T cells susceptible to inhibitory signals in the tumor microenvironment may also improve T-cell function. For example, many tumor cells express FAS ligand, and silencing FAS in T cells prevents FAS-induced apoptosis [30]. Besides silencing genes, expression of a constitutively active form of serine/threonine AKT (caAKT), which is a major component of the PI3K pathway, in T cells has also been shown to improve their function [112]. caAKT-expressing T cells sustained higher levels of NF- κ B and had elevated levels of anti-apoptotic genes such as Bcl2, resulting in resistance to Tregs and TGF β .

Lastly, most solid tumors have a stromal compartment that supports tumor growth directly through paracrine secretion of cytokines, growth factors, and provision of nutrients, and contributes to tumor-induced immunosuppression [22, 40, 91]. For example, we have shown in preclinical studies that T cells expressing CARs specific for FAP expressed on cancer associated fibroblasts (CAFs) have potent antitumor effects [53]. In addition, combining

tumor-specific CAR T cells with FAP-specific CAR T cells enhanced antitumor effects. While recently some concerns have been raised in regards to targeting FAP [100, 117], our findings indicate that targeting CAFs has the potential to improve the antitumor effects of adoptively transferred CAR T cells. Targeting the tumor vasculature with CARs to enhance T-cell therapy for solid tumors has been explored by others [19, 81]. Targeting the tumor vasculature with vasculature endothelial growth factor receptor (VEGFR)2-specific CAR T cells in addition to tumor cells synergized in inducing tumor regression in several syngeneic, preclinical tumor models [17]. In addition, transgenic expression of VEGFR2-specific CARs and IL12 in T cells was sufficient to eradicate tumors, indicating that combining countermeasures might potentiate effects [18].

While many of the discussed genetic modification strategies have not been explored in osteosarcoma models, these strategies could be readily integrated in current T-cell therapy approaches for osteosarcoma.

Genetic Modification of T Cells to Improve Homing to Tumor Sites

T-cell homing to solid tumor sites might be limited. For example, Kershaw et al. evaluated the safety and efficacy of first generation folate receptor (FR)- α CAR T cells in patients with ovarian cancer [58]. Infused T cells persisted less than 3 weeks in all but one patient, and did not specifically home to tumor sites as judged by ¹¹¹Indium scintigraphy. No antitumor activity was observed. Since then, several investigators have shown in preclinical models that the expression of chemokine receptors in CAR T cells that recognize chemokines secreted by solid tumors can enhance T-cell homing. For example, transgenic expression of chemokine receptors CCR2b or CXCR2 in T cells enhances trafficking to CCL2- or CXCL1-secreting solid tumors including melanoma and neuroblastoma [26, 57].

Improving Safety of T-Cell Therapy

Toxicities can be divided into four categories: (1) toxicities due to genetic modification (which have not been observed with genetically modified T cells in humans so far [9]), (2) “on target organ” toxicities (e.g., depletion of normal B cells post CD19-CAR T cells [54]), (3) “on target, off organ” toxicities (e.g., liver toxicity of carbonic anhydrase IX CAR T cells to target renal cell carcinoma [62]), and (4) systemic inflammatory syndromes [39, 54, 92].

Genetic safety switches have been developed to selectively destroy genetically modified T cells once adverse events occur. The most widely used suicide gene strategy for T-cell therapy is to introduce the herpes simplex virus thymidine kinase (HSV-tk) gene into the T cells. HSV-tk phosphorylates acyclovir, valacyclovir, and ganciclovir to toxic nucleosides [21]. T cells transduced with HSV-tk are robustly killed in the presence of these medications and clinical studies demonstrate effectiveness of the strategy. A drawback to utilizing HSV-tk as a safety switch for T-cell therapy is the immunogenicity of HSV-tk, and that some patients require acyclovir, valacyclovir, or ganciclovir to treat herpetic diseases. Therefore, genetic safety switches using non-immunogenic human components have been developed, such as inducible caspase 9 that can be activated by a dimerizer [28, 111]. Once exposed to the dimerizer, genetically modified T cells rapidly undergo apoptosis. Another approach

includes the transgenic expression of CD20, rendering T cells sensitive to the clinically approved CD20 MAb rituximab [47]. While suicide gene switches can selectively kill infused cells, systemic inflammatory syndromes might be difficult to control with this approach since resident immune cells, which are activated by the infused T cells, most likely contribute. Recent studies indicate that IL6 plays a critical role in these syndromes, and the infusion of the IL6 receptor MAb (tocilizumab) alone or in combinations with steroids and TNF α MABs (infliximab) proved to be effective [39, 54, 92].

While suicide switches are one strategy to prevent “on target, off organ” toxicities, other strategies include the generation of T cells that are only fully activated if they encounter a unique “antigen address” at the tumor site. Examples include the development of T cells expressing two CARs in which one TAA-specific CAR has an endodomain with a ζ -signaling domain, and a second CAR, specific for another TAA, provides costimulation [59, 63, 126].

Combinatorial T-Cell Therapy

As for other cancer therapies, combinatorial therapies hold the promise to improve T-cell therapy for cancer [122]. These can be divided into approaches that (1) kill tumor cells without affecting T cells, (2) enhance the expression of TAA, (3) improve T-cell expansion and persistence, and (4) reverse the inhibitory tumor microenvironment. For example the BRAF inhibitor vemurafenib has no adverse effects on T-cell function, and combining vemurafenib with adoptive transfer of T cells enhances antitumor effects in preclinical animal models of melanoma [29, 69]. Increasing the expression of TAA in cancer cells can be achieved with epigenetic modifiers such decitabine [20, 27].

Combining T-cell therapy with blocking antibodies specific for negative regulators of T-cell responses such as the cytotoxic T-lymphocyte-associated protein (CTLA-4) and programmed cell death 1 (PD-1) is one strategy to increase their function [55, 88, 115, 124]. The role of CTLA-4 as a negative regulator of T-cell responses has been well demonstrated in CTLA-4-deficient mice and preclinical tumor models. Based on these studies, an antibody to block human CTLA-4, ipilimumab, was developed, and a phase III randomized clinical trial showed that 23 % patients with metastatic melanoma survive more than 4 years following ipilimumab treatment, leading to FDA approval in March 2011 [43].

Similarly, combining T-cell therapy with MABs that block PD-1 and/or its ligands (PD-L1 and PD-L2) is another promising approach. A recent clinical trial evaluating the safety and efficacy of a PD-L1 antibody reported encouraging objective clinical response rates in patients with advanced melanoma, renal cell carcinoma, and non-small-cell lung cancer [10]. In addition, a recent report demonstrated the benefit of combining PD-1 blockade with the adoptive transfer of HER2-CAR T cells in a preclinical melanoma model [50].

As mentioned in section “Enhancing T-Cell Expansion and Persistence In Vivo,” the administration of vaccines is an attractive strategy to boost adoptively transferred T cells in vivo. Several groups have shown that vaccines augment the effectiveness of adoptive T-cell therapy in preclinical animal models [70, 85, 108]. Besides provision of antigen, providing potent co-stimulation, and/or cytokines was critical for the observed effects. However,

limited experience is available in humans except for an ongoing clinical trial in which patients are vaccinated with an autologous DC vaccine post α/β TCR T-cell transfer.

Lastly, reversing the immunosuppressive tumor microenvironment with small molecule inhibitors is another approach to enhance the antitumor activity of adoptively transferred T cells. For example blocking STAT3 in combination with the adoptive transfer of T cells resulted in enhanced antitumor effects [35, 41].

Conclusions

T-cell therapy has shown promising results in early phase clinical studies especially for hematological malignancies. For solid tumors, including osteosarcoma, T-cell therapy has shown promise in preclinical studies but formidable challenges remain in developing safe and effective T-cell therapies for patients with osteosarcoma. These include target antigen selection, limited in vivo T-cell expansion and persistence, T-cell trafficking to tumor sites, and the hostile tumor microenvironment. Genetic modification of T cells and combining T-cell transfer with other therapies holds the promise to overcome some of these obstacles.

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Table 1

Genetic modifications for T-cell therapy for osteosarcoma

Goal	Introduced gene class	Example
Antigen-specificity	Receptors	$\alpha\beta$ TCR, CAR
T-cell expansion	Costim molecules	CD80, 41BBL
	Domains of costim molecules	CD27, CD28, 41BB, OX40
	Cytokines	IL12, IL15
Resistance to inhibitory tumor environment	Costim molecules	CD80, 41BBL
	Domains of costim molecules	CD27, CD28, 41BB, OX40
	Cytokines	IL12, IL15
	Dominant negative receptors	DN TGF β receptor
	Chimeric cytokine receptors	IL4/IL2, L4/IL7
	shRNAs	FAS
	Constitutive activated kinases	AKT
Improve T-cell homing to tumor sites	Chemokine receptors	CCR2b or CXCR2
Safety	Inducible suicide genes	HSV-tk; caspase 9
	Cell surface markers	CD20

DN dominant negative, *HSV-tk* Herpes simplex virus thymidine kinase, *IL* interleukin, *TGF β* transforming growth factor β

Table 2

Tumor associated antigens expressed in osteosarcoma

Target antigen	Cell surface expression	Preclinical in vivo studies ^a	Clinical studies ^b
B7-H3	+	–	–
CLUAP1	–	–	–
FAP	+	–	–
GAGE 1,2,8	–	–	–
GD2	+	–	–
Glypican-2	–	–	–
HER2	+	+	+
IL-11R α	+	+	–
MAGE A1–6,10, 12; C2	–	–	–
MCAM	–	–	–
NY-ESO-1	–	–	–
Papillomavirus binding factor	–	–	–
TEM1	+	–	–

CLUAP1 clusterin-associated protein 1, *FAP* fibroblast activation protein, *GAGE* G melanoma antigen, *GD2* disialoganglioside, *HER2* human epidermal growth factor receptor 2, *IL11R α* interleukin 11 receptor α , *MAGE* melanoma associated antigen, *MCAM* melanoma cell adhesion molecule, *NY-ESO-1* new York esophageal squamous cell carcinoma 1, *TEM1* tumor endothelial marker 1

^aUsing an osteosarcoma model

^bIncluding osteosarcoma patients