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Adoptive T Cell Therapies: A Comparison of T Cell Receptors and Chimeric Antigen Receptors

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Abstract

The tumor-killing properties of T cells provide tremendous opportunities to treat cancer. Adoptive T cell therapies have begun to harness this potential by endowing a functionally diverse repertoire of T cells with genetically modified, tumor-specific recognition receptors. Normally, this antigen recognition function is mediated by an $\alpha\beta$ T cell receptor (TCR), but the dominant therapeutic forms currently in development are synthetic constructs called chimeric antigen receptors (CARs). While CAR-based adoptive cell therapies are already showing great promise, their basic mechanistic properties have been studied in less detail compared with those of $\alpha\beta$ TCRs. In this review, we compare and contrast various features of TCRs versus CARs, with a goal of highlighting issues that need to be addressed to fully exploit the therapeutic potential of both.

The Rise of Adoptive T Cell Cancer Therapies

The success of adoptive T cell therapies, using genetically engineered receptors against cancer antigens, has recently gained the interest of biotechnology and pharmaceutical companies [1]. Much of the attention has focused on synthetic receptors now commonly referred to as CARs. CARs typically link an extracellular, antigen-recognition molecule comprising antibody domains (a **single-chain Fv**, scFv, containing the variable domains of the light and heavy chains; see Glossary), a stalk-like region, a transmembrane region, and intracellular signaling domains derived from proximal T cell signaling machinery.

While many different variations of the CAR format have been studied *in vitro*, and several have now been used in clinical trials, significant questions remain about their mechanistic properties. The answers to these questions should help guide improvements in the use of CARs in adoptive T cell therapies. The field of adoptive cell therapies has recently been reviewed (e.g., [1–5]). As Jensen and Riddell pointed out, conventional TCRs evolved to have a different set of properties than are currently configured in CARs [5]. TCRs accomplish their primary recognition task, ultrasensitive recognition of aberrant intracellular antigens (as peptides bound to proteins encoded by the major histocompatibility complex, MHC), by using a complex assembly of proximal signaling molecules [6]. T cells thereby initiate potent and specific immune responses against foreign agents, such as viruses, or transformed cancer cells.

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There remains considerable interest in engineering conventional TCRs as the recognition element in adoptive T cell therapies, primarily because they are able to recognize a larger array of potential antigens compared with CARs. This feature is possible only because TCRs have evolved the sensitivity to detect low levels of intracellular antigens. The ability to recognize almost any intracellular protein via the MHC system allows TCRs to target more antigens than can antibodies (or scFv-CARs), which recognize only cell surface cancer antigens. In this review, we compare features of conventional TCRs with the array of different synthetic CARs that have been studied.

TCR: Structure and Signaling

The TCR is an $\alpha\beta$ heterodimer that binds to a short peptide bound to a product of the MHC. Each $\alpha\beta$ subunit contains variable (V) and constant (C) region domains, and the latter is followed by a transmembrane region. Each V domain contains three loops, called complementarity-determining regions (CDR1, CDR2, and CDR3), which interact with the peptide (pep)MHC antigen. The conserved docking angle of the six CDRs over the pepMHC antigen is thought to confer maximal signaling capabilities and it places the two most hypervariable loops (CDR3s) over the most diverse portion of the antigen (the peptide) [7].

T cell activation upon TCR binding to the pepMHC antigen involves multiple other cell surface molecules (Figure 1) that collectively initiate and amplify the signal [8]. In fact, the $\alpha\beta$ heterodimer lacks its own intracellular signaling domains and, thus, must associate with a six-subunit complex called **CD3**, comprising three dimers: CD3 $\epsilon\gamma$, CD3 $\epsilon\delta$, and CD3 $\zeta\zeta$ [9]. The cytoplasmic domains of CD3 γ , δ , and ϵ each contain one **immunoreceptor tyrosine-rich activation motif** (ITAM) and each CD3 ζ contains three ITAMs [10]. Accordingly, the TCR/CD3 complex contains a total of ten ITAMs, and 20 possible tyrosine-phosphorylation sites (Box 1), which serve as substrates for the Src-family kinase **lymphocyte-specific protein tyrosine kinase** (Lck).

The ability of a T cell to be triggered by TCR binding to as few as one pepMHC molecule [11–14] is accomplished in part by the action of coreceptors **CD4** and **CD8**. CD4 is associated with T helper (T_H) cells (and regulatory T cells, T_{regs}) and recognition of class II MHC. CD8 is associated with cytotoxic T lymphocytes (CTLs) and recognition of class I MHC. The synergy of these coreceptors with the TCR is thought to be driven both by their ability to bind to invariant regions of MHC molecules (CD8 especially) and the fact that Lck associates with the cytoplasmic domains of CD4 and CD8, and, thus, can bring this kinase to the TCR/CD3 complex [15,16]. Lck can be either free in the cytosol [17], anchored to the plasma membrane via myristoylation or palmitoylation, or associated with CD4 or CD8 coreceptors [18]. Recently, Gascoigne and colleagues presented evidence that phosphorylation of CD3 ITAMs occurs in a two-step process, first with coreceptor-independent Lck kinase, followed by coreceptor-associated Lck [19].

The exact biophysical mechanism through which TCR binding triggers intracellular signaling and T cell activation is not fully understood. Current theories are centered on several mechanisms: aggregation (clustering), conformational change, and segregation and/or redistribution [20]. Aggregation and/or clustering of the TCR, driven by binding to

pepMHC, leads to a local increase in TCR complexes, which causes T cell signaling via the recruitment of Lck. While T cell triggering has been shown to occur with a single pepMHC, [11–13], it is possible that TCRs interacting with non-agonist pepMHC could facilitate sufficient signaling to initiate activation [21–23]. A conformational model suggests that ITAMs are normally in an inactivated state via association with membrane lipids [24] and, upon TCR binding of pepMHC, a conformational change in CD3 subunits makes ITAMs more accessible for phosphorylation [25–27]. TCR–pepMHC interactions appear to induce a torque-like force on CD3 molecules, which could also expose intracellular ITAMs [28,29]. The segregation and/or redistribution model is based on the premise that cell surface **CD45** molecules are constitutively involved in dephosphorylating ITAMs, but upon TCR engagement of pepMHC, CD45 molecules are redistributed and excluded from the immunological synapse, allowing stable ITAM phosphorylation and signaling [30]. Indeed, studies using a reconstituted non-immune cell system showed that exclusion of CD45 from the immunological synapse following TCR pepMHC engagement was sufficient to permit sustained ITAM phosphorylation [31]. None of these models for T cell triggering are mutually exclusive and all three may be involved [20].

Signaling mediated by the TCR/CD3/coreceptor complex is considered necessary but not sufficient for the full array of T cell functions (e.g., cytokine release, proliferation, and persistence). Additional co-stimulatory signals enhance the sensitivity of T cells, and drive them along a path that yields optimal proliferative and functional capacity [32]. The most well studied co-stimulatory receptor, **CD28**, binds to the ligand B7 on antigen-presenting cells (APCs) [33]. Upon CD28:B7 engagement, the intracellular cytoplasmic domain of CD28 recruits phosphoinositide 3-kinase (PI3K), protein kinase C θ , and RAS guanyl-nucleotide-releasing protein (RASGRP) [34,35]. Another co-stimulatory molecule, **4-1BB**, promotes T cell survival upon binding ligand on the APC surface, in part through the upregulation of antiapoptotic factors, such as B cell lymphoma 2 (BCL-2), BCL extra large (BCL-XL) and BFL-1 [36]. Thus, these intermediates promote T cell activation and survival through various signaling pathways. Following T cell activation, co-stimulatory molecules, such as CD28 and 4-1BB, appear to be dominated by co-inhibitory molecules, including **CTLA-4** and **Programmed cell death protein 1** (PD-1) [37]. Upon binding to B7 or PD ligand 1 (PD-L1), respectively, CTLA-4 and PD-1 recruit phosphatases that dephosphorylate CD3 ζ , Zeta-chain-associated protein kinase 70 (ZAP70), and Linker for activation of T cells (LAT), and thereby extinguish T cell signaling.

The orchestrated assembly of these receptor systems, mediated by both extracellular binding to appropriate ligands and intracellular interactions among cytoplasmic domains, provides a complex but exquisitely sensitive mechanism for optimal T cell activity. The ultrasensitivity of the TCR system underlies the potential for targeting intracellular cancer antigens that might be at low levels, whether they are derived from upregulated self-proteins or mutated proteins [38].

Chimeric Antigen Receptor: Structure and Signaling

Chimeric antigen receptors (CARs) represent a class of synthetic constructs typically comprising a single-chain antibody fragment (scFv, VH-linker-VL, or VL-linker-VH), an

extracellular stalk (hinge) domain, a transmembrane domain, and one or more intracellular signaling domains [5,39] (Figure 1). The scFv endows T cells with the ability to respond to cell surface antigens independent of MHC. The CAR format provides an opportunity to potentially exploit (or repurpose) many of the scFv that have been developed against cancer antigens over the past 30 years. Notably, some of these scFv may not have been amenable to expression as full-length antibodies, or the targets for these scFv may have been at too low a level to mediate activity in conventional antibody formats.

The exact mechanism by which CAR binding to antigen leads to signaling is not clear. It has been shown that reorganization of the CAR and CD45 into a synapse, as with the TCR/CD3 system, occurred with a CD19-specific CAR [31]. Presumably, full signaling must be driven by engagement of multiple CAR molecules by multiple antigens on the opposing cell, but how these signals integrate with other endogenous pathways (e.g., TCR/CD3 complexes, co-stimulatory or inhibitory molecules) remains to be determined. Recent studies have shown that some scFv fragments used in CARs stimulate T cells constitutively in an antigen-independent mechanism [40,41]. This constitutive effect appears to lead to more rapid loss of the transferred T cells, and reduced effectiveness.

The possible mechanism associated with the constitutive signaling appears to be linked to the propensity of some scFv to aggregate in the membrane of T cells, although the extent of the constitutive signaling was mitigated by the use of 4-1BB rather than CD28 signaling domains [41]. It remains to be seen whether this is in part due to an overall reduced 'sensitivity' associated with 4-1BB compared with CD28. Nevertheless, these findings indicate that not all scFv will be suitable for use as CARs, and that scFv screening for appropriate V_L/V_H pairs will be required to avoid undesirable antigen-independent signaling.

The hinge region of a CAR typically comprises either immunoglobulin-like CH2-CH3 (Fc) domains from the constant region of immunoglobulin G (IgG) or the spacer domain from either CD4 or CD8 [42,43]. Reports have shown that lengthening or shortening the extracellular domain can optimize activity of an individual CAR [43–45]. The optimal length of the hinge for each CAR may differ, depending on the dimensions of the cell surface antigen that is targeted by the scFv.

Various transmembrane regions have also been incorporated into CARs, including domains from CD3 ζ , CD4, CD8, OX40, and H2-K^b [46]. Incorporation of the CD28 transmembrane region was correlated with higher CAR expression levels compared with transmembrane regions from CD3 ζ , OX40, or CD8 [47,48]. However, CARs with the CD3 ζ transmembrane domain produced more robust signaling, perhaps due to potential trans-signaling with endogenous CD3 ζ [49].

The intracellular signaling domains of CARs (Figure 2) have received the most attention in terms of their impact on T cell activity, T cell persistence, and efficacy. First-generation CARs, containing only the intracellular domain of CD3 ζ , exhibited *in vitro* activity, but mediated minimal *in vivo* efficacy and T cell persistence [50]. Second-generation CARs added co-stimulatory signaling components (primarily CD28 or 4-1BB) in tandem with

CD3 ζ [51–54]. These CARs have shown improved clinical efficacy and persistence [55–57]. Although 4-1BB⁺ CARs mediate lower levels of cytokine release compared with CD28⁺ CARs, 4-1BB⁺ CARs appear to show greater *in vivo* persistence [57,58].

Given the variability in scFv fragments, hinge, and transmembrane domains, it can be difficult to compare results from different studies (e.g., to determine whether CD28 or 4-1BB is optimal for CAR-based therapies). Third-generation CARs, containing two co-stimulatory domains along with the CD3 ζ signaling sequence, have demonstrated promising early results and are likely to be further developed for clinical use [48,59]. The mechanism by which scFv binding to antigen leads to effects on the intracellular domains of CD3 ζ , CD28, and 4-1BB, in terms of recruitment of adaptors and kinases, remains to be seen.

Another emerging area is the use of multiple scFv fragments, each fused to different signaling domains. This combinatorial approach could provide enhanced safety and/or therapeutic efficacy by targeting two or more different cancer antigens. It also raises the possibility for enhanced sensitivities due to synergistic signaling [60,61]. Further safety might also be achieved through the use of inhibitory CARs (iCARs), in which a separate CAR contains a scFv specific for an antigen on normal tissue, fused to an inhibitory cytoplasmic domain, such as PD-1 [61,62]. This system could have the potential to reduce on-target, off-tumor toxicities in either TCR- or CAR-mediated adoptive T cell therapies.

Sensitivity of TCRs and CARs: Impact of Affinity, Receptor Density, and Antigen Density

As emphasized above, based simply on their design, the mechanism by which CAR binding to its cognate antigen leads to T cell activation differs in substantial ways from the mechanism by which TCR binding leads to T cell activation. Even without consideration of co-stimulatory molecules (CD28 and 4-1BB), TCRs mediate activity through a complex of ten subunits that are poised to be triggered by low numbers of pepMHC antigens, and through the action of the coreceptors CD4 and CD8 [11–13] (Box 1). This single ‘fixed’ mechanism associated with conventional TCR/CD3 complexes contrasts with the distinct and varied signaling properties that are likely associated with the array of diverse CAR structures.

Paradoxically, the ability of T cells to be stimulated by as few as one antigen molecule per target cell is accomplished not by high-affinity but by low-affinity TCR:pepMHC interactions. Thus, the physiological affinity range of TCRs is 10^4 – 10^6 M⁻¹ (an equilibrium binding affinity of 1 μ M is considered a ‘high’-affinity TCR in this context). It has been suggested that the ability of a single pepMHC molecule to activate a T cell is in part due to the ability of that pepMHC molecule to bind serially to many TCRs (serial triggering) [14,63], which is consistent with a fast off-rate (i.e., lower affinity). In contrast to TCRs, the affinity of most monoclonal antibodies that have been engineered as scFv fragments for CARs are in the range of 10^6 – 10^9 M⁻¹ (an equilibrium binding affinity of 1 nM is considered a ‘high’-affinity antibody in this context). T cells have evolved an exquisite system of multiple receptor subunits to achieve this high level of sensitivity and yet retain a high degree of specificity. Engineering the affinity of a TCR (micromolar) to the antibody

affinity range (nanomolar) can in fact yield functional self-peptide cross-reactivities because of the low-affinity threshold of the TCR system [64] (i.e., even 300- μ M affinity for such a reaction can result in CD8-dependent activity [65]). CD8 acts to lower the TCR affinity required by approximately 100 times [66], and it reduces the amount of pepMHC required from over 30 molecules per target cell to just one molecule [11–13].

While the targets for TCR-based therapies are intracellular peptides bound by MHC, potential targets for CAR-based therapies are cell surface antigens expressed at higher densities on cancer cells, and these densities typically vary from one target to another. A recent study investigated the density of CD20 required to activate T cells expressing a CD20-specific CAR [67]. Using cell lines that expressed CD20 at various densities (very low to high, approximately 200–200 000 molecules/cell), the CAR-CD20 T cells mediated lysis of cells expressing the lowest detectable level of CD20 (approximately 200 molecules/cell), similar to an earlier study with a carbohydrate-specific CAR [68]. Cytokine release required higher densities of CD20, between 200 and 5000 molecules/cell. The results with the CD20-CAR system are also consistent with observations about the need for 30 or more pepMHC ligands on the surface of a target cell in the absence of coreceptor [11,12].

To increase the sensitivity of a CAR towards an antigen expressed at low levels, the scFv binding affinity could be increased [69]. Conversely, recent studies have suggested that the therapeutic index for CAR targeting of antigens with significant expression on normal tissues could be improved by using lower affinity scFv fragments [70–72]. (To our knowledge, affinities cited for a particular CAR, i.e., of the scFv domains, have been measured using the soluble scFv or the full antibody; thus, the actual affinity of a CAR may differ to some extent in the context of the membrane-bound form.) The scFv from nimotuzumab (K_D of approximately 10^{-8} M) mediated tumor cell activity (at or above approximately 340 000 molecules/tumor cell) while ignoring normal fibroblasts with lower epidermal growth factor receptor (EGFR) levels (approximately 15 000 molecules/cell). In the ErbB2 system, an antibody with a K_D value of approximately 1 μ M was found to discriminate between enhanced levels on some ErbB2 tumors and levels found in normal tissues. While the optimal affinity for a scFv in the CAR format varies depending on the antigen density, other parameters, such as the expression levels of the CAR and the propensity of the scFv to aggregate and cause cis-signaling [41], will also impact activity.

Further work on CAR signaling will be needed to better determine whether the intersecting pathways and thresholds initiated in conventional T cells [6], by binding of multiple ligands to TCRs, coreceptors, and co-stimulatory molecules, are quantitatively capable of driving the same polyfunctional responses. The potency and persistence of cytolytic CD8⁺ T cells has been demonstrated in many studies, but the ability to mediate activity, persistence, and localization of CD4⁺ T cells may be important [73,74]. Within T cell subsets, the T cell lineages that might be preferred in the adoptive T cell setting, whether using TCRs or CARs, is also an area of active study [75].

While the many parameters that differ between TCRs and CARs (affinity, ligand structure, and ligand density) have made it difficult to compare directly, a system has been developed that allows comparisons by using the analog of a scFv, a single-chain TCR ($V\beta$ -linker- $V\alpha$)

as a CAR-like receptor [76,77]. This allows the same V regions to be used in a conventional full-length $\alpha\beta$ TCR for comparison with the CAR, where the affinity of the cell-associated forms, the ligand (pepMHC), and ligand density are identical. This system also allowed the direct measurement of CAR affinity using typical monomeric pepMHC antigens, and it enables the rapid analysis of the effects of antigen density by simply 'loading' different concentrations of peptides onto MHC-positive target cells. Using a high-affinity variant of the 2C TCR called m33 (K_d of the TCR and CAR on T cells was identical), it was observed that, in primary CD4⁺ T cells, the full-length TCR had greater sensitivity to pepMHC than the CAR format (with either CD28 or 4-1BB, and CD3 ζ), even though the CAR format was expressed at higher densities on the surface of T cells [77]. Thus, even in the absence of the CD8 coreceptor, the TCR machinery has greater sensitivity.

Adoptive T Cell Therapy: Clinical Outcomes Using TCRs and CARs

Early clinical data from adoptive T cell trials using TCRs and CARs have demonstrated the tremendous potential of redirected T cells in the control of tumors. Some of the most exciting results have come from trials using CAR T cells against hematological cancers. As described in recent reviews [1,3,4], the variability in scFv fragments, co-stimulatory domains, and methods of gene transfer (such as retrovirus, lentivirus, or other newly developed techniques), have made it difficult to compare results. Other parameters, such as pre-existing tumor burden, conditioning chemotherapy, *ex vivo* expansion techniques, and T cell dosage, have further complicated comparisons. To date, the most successful CARs have been those specific for CD19 on B cell malignancies. Treatment with these second-generation CARs has resulted in complete remission in some patients who previously had been unresponsive to more traditional chemotherapy regimens [78–81]. Given that the CD19 antigen is also expressed on nonmalignant B cells, CD19-CAR therapy results in B cell aplasia, which has been treated with exogenous immunoglobulin administration.

While CD19-CAR therapies for hematological cancers have shown promising results, CAR therapy of solid tumors has so far not been as effective [82]. Antigens from various types of solid tumor have been targeted with limited antitumor response [83,84]. CAR therapies against solid tumors could be less effective due to the local immunosuppressive environment seen with many solid tumors [85] and might also be due to the use, to date, of suboptimal signaling domain(s) within the CAR construct. Targeting solid tumors is also restricted by the number of cancer-associated antigens that are expressed at sufficient levels for effective responses without on-target/off-tumor toxicities. A trial using a scFv specific for ErbB2 led to unexpected toxicity and ultimately death in one patient as a consequence of lung tissue expressing low levels of ErbB2 [86], but it is possible that affinity-tuning of scFv fragments could minimize these toxicities [70,71]. As described above, recent studies have suggested that some scFv fragments used as CARs (e.g., against mesothelin and GD2) have problems associated with constitutive signaling. It is possible that, as these issues are sorted out, more effective treatments for solid tumors may be achieved [40,41].

Clinical trials using TCRs for adoptive T cell therapy have had some successes in eradicating both solid and hematological tumors [87–91]. Given that most of the targets for TCR-based therapies are self-peptide/MHC antigens, endogenous peripheral T cells against

these targets exhibit low affinity due to negative selection in the thymus [92]. These T cells have reduced effectiveness at promoting antitumor responses. To overcome this problem, affinity-enhanced TCRs have been developed from techniques such as yeast and phage display [93,94]. However, since these TCRs have not undergone negative selection, they carry with them the potential risk of off-target reactivity [64].

Two different affinity-enhanced TCRs against a peptide from the MAGE-A3 protein were recently used in clinical trials, and both resulted in fatalities caused by TCR recognition of structurally similar pepMHC complexes present in noncancerous tissues ('off-target/off-tumor') [95,96]. While these results highlight the risks associated with affinity-enhanced TCRs, the choice of target also has a significant role in off-tumor toxicities. A TCR with nanomolar affinity ($K_d = 730$ nM) has been successfully used to target cancer testis antigen NY-ESO-1 without reports of 'off-target/off-tumor' toxicities [90,91]. To better identify potential target antigens, *in silico* proteome searches have been conducted that analyze target peptides for structural uniqueness [95,97]. This type of analysis could identify potential off-target/off-tumor toxicities associated with self-peptide/MHC antigens that could pose problems with cross-reactivity.

Concluding Remarks

Adoptive T cell therapies using engineered receptors that bind to cancer antigens have received much attention due to early clinical successes. The two major formats of these receptors target different types of antigen. TCRs target the natural ligands for T cells: cancer-associated peptides bound to an MHC product. Optimization of these receptors for adoptive T cell therapies will typically require raising the affinity above the normal levels [92] to mediate the activities of both CD8 and CD4 T cells against the same class I pepMHC antigen. The affinity will need to be 'tuned' for each TCR to avoid cross-reactivities with nontarget peptides.

CARs are MHC-independent receptors and, thus, can function in both CD4 and CD8 T cells. Nevertheless, optimizing a CAR for each cancer antigen will require consideration of the levels of the tumor antigen, the affinity and aggregation propensity of the scFv fragment, and the type of intracellular signaling domain(s) that will drive T cell polyfunctionality and persistence. An alternative approach that has recently gained some interest is to engineer a CAR with a 'universal' recognition motif, such as an Fc receptor (e.g., CD16), allowing these CAR-T cells to be used in conjunction with antibody treatments, such as rituximab [98].

CARs can mediate activity against even relatively low-density antigens (e.g., 100–1000 molecules per tumor cell) by achieving adequate surface levels on the T cells and a sufficient affinity of the linked scFv fragment. Accordingly, the approach provides opportunities to target novel cancer-associated antigens where a less sensitive therapeutic format used in the past (e.g., soluble antibody forms) might have led to escape due to 'antigen loss variants' (i.e., the antigen was at a level below the therapeutic efficacy of that format). Choosing the optimal format and scFv affinity for adoptive T cells also should be able to take advantage of a therapeutic window that is influenced directly by the number of

target molecules on cancer cells versus normal cells (see Outstanding Questions). In the case of combinatorial CAR systems (including iCARs), the parameters of CAR density, scFv-affinity, and cytoplasmic domains will all need to be optimized to match the tumor and tissue antigen densities.

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Glossary

4-1BB	a co-stimulatory molecule (also known as CD137) expressed on the surface of T cells. 4-1BB promotes the expression of the antiapoptotic factors, such as BCL-2, BCL-XL, and BFL1
CD3	a cell-surface molecule expressed on T cells. CD3 subunits associate with the T cell receptor $\alpha\beta$ heterodimer and contain intracellular signaling motifs for T cell activation. Three CD3 heterodimers (CD3 $\epsilon\gamma$, CD3 $\epsilon\delta$, and CD3 $\zeta\zeta$) occur within each TCR/CD3 complex
CD4	a coreceptor molecule expressed on the surface of the T _H and T _{reg} subsets of T cells. CD4 binds to class II MHC molecules, and the intracellular domain associates with the kinase Lck
CD8	a coreceptor molecule expressed on the surface of cytotoxic T cell subset (also known as cytotoxic T lymphocytes, CTLs). CD8 binds to class I MHC molecules, and the intracellular domain associates with the kinase Lck
CD28	a co-stimulatory molecule expressed on the surface of T cells. Upon engagement with its ligand (B7), CD28 signals via various intracellular pathways (such as PI3K and RAS) to promote expression of antiapoptotic and prosurvival genes
CD45	a regulatory molecule expressed on the surface of T cells. CD45 has intracellular phosphatase activity that inhibits the initiation of T cell signaling. During T cell activation, CD45 dephosphorylates the inhibitory tyrosine of Lck, and it is redistributed on the perimeter of the immunological synapse to permit sustained phosphorylation of the TCR/CD3 complex, and additional downstream signaling
Cytotoxic T lymphocyte-associated protein 4 (CTLA-4)	a co-inhibitory protein expressed on the surface of T cells. After T cell activation, CTLA-4 competes with CD28 for binding to B7. Upon engagement with B7, CTLA-4 recruits phosphatases that extinguish T cell signaling

Immuno-tyrosine activation motifs (ITAMs)	occur within the intracellular domains of CD3 molecules and contain the tyrosine residues that become phosphorylated upon T cell activation
Lymphocyte-specific protein tyrosine kinase (Lck)	a kinase expressed in T cells and responsible for early T cell signaling. Lck can be associated with the intracellular domains of coreceptors CD4 and CD8, associated with membrane lipids, or free within the cytosol. Lck initiates signaling via phosphorylation of tyrosine residues within the intracellular domains of CD3 molecules
Major histocompatibility complex (MHC)	a cell surface molecule. Two main categories of MHC, class I and class II, are recognized by CD8 and CD4 T cells, respectively. Class I MHC comprises α_1 , α_2 , and α_3 domains along with β_2 microglobulin. The α_1 and α_2 domains bind to a short intracellular peptide (8–10 amino acids), and the complex (pepMHC) is transported to the cell surface
Programmed cell death protein 1 (PD-1)	a co-inhibitory protein expressed on the surface of T cells. Upon engagement with its ligands PD-L1 and PD-L2, PD-1 leads to signaling and induction of apoptosis
Single-chain variable fragments (scFv)	comprise the antibody heavy-and light-chain variable fragments linked by an in-frame amino acid linker (typically 15–25 amino acids long). In the context of CARs and adoptive T cell therapies, scFv fragments bind to cell surface antigens on a cancer cell and, thus, are the recognition element of the CAR construct

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Trends

T cells engineered to express receptors (TCRs) for cancer antigens show great promise.

The receptors include conventional TCRs and CARs.

TCRs and CARs have distinct signaling properties and antigen sensitivities.

Adoptive T cell therapies provide an ultrasensitive, polyfunctional modality.

Box 1**Comparison of TCR and CAR Signaling Machinery**

Table I provides a comparison of the signaling machinery in TCRs and CARs. TCRs associate with endogenous signaling molecules and coreceptor, which provides TCRs with more ITAMs and tyrosine phosphorylation sites compared with CARs. TCRs typically have affinities in the micromolar range for pepMHC, while CARs typically have higher affinities for their ligands normally in the nanomolar range. Despite having lower affinity and expression levels, TCRs are more sensitive (i.e., mediate activity at a lower density of antigens) than CARs.

Table I
Comparison of TCR and CAR signaling machinery

Receptor Property	TCR	CAR
Number of subunits in receptor complex	10	1
Number of ITAMs	10	3
Number of tyrosines as substrates	20	6
Coreceptor, co-stimulator involvement	Yes (CD4, CD8, CD28, etc.)	None known
Typical range of affinities for antigen	10^4 – 10^6 M ⁻¹	10^6 – 10^9 M ⁻¹
Number of surface receptors per T cell	~50 000	>50 000 but varies
Minimum number of antigens required on target cells	1	>100

Outstanding Questions

What are the extracellular structural domains and intracellular signaling domains of CARs that promote optimal T cell activation?

Does T cell activation via the different forms of CAR quantitatively replicate activation via conventional TCRs with regard to polyfunctional capabilities?

How does TCR binding to pepMHC and CAR binding to cell surface ligand biophysically transmit early signaling and T cell activation?

What are the relations between scFv affinity, CAR expression levels, and intracellular signaling domains with constitutive signaling?

Which cancer targets (pepMHC or nonMHC antigens) will be expressed at sufficient levels to be safely targeted without on-target/off-tumor toxicities?

Under what circumstances will TCR-mediated therapies be preferable to CAR-mediated therapies (or vice versa)?

Figure 1. Structural Components of T Cell Receptor (TCR) and Chimeric Antigen Receptor (CAR) Signaling

(A) TCRs comprise an $\alpha\beta$ heterodimer that binds to peptide major histocompatibility complex (pMHC). (B) CARs are single-chain molecules that contain a single-chain variable fragment (scFv) recognition domain capable of binding to cell surface antigens. In complex with each TCR are CD3 subunits and a coreceptor (CD4 or CD8) associated with Lymphocyte-specific protein tyrosine kinase (Lck). CARs contain intracellular signaling domains from CD3 ζ and a co-stimulatory molecule (typically CD28 or 4-1BB). Signaling is initiated by Lck-mediated phosphorylation of immuno-tyrosine activation motifs (ITAMs) within the cytoplasmic domains of CD3.

(A)	CD3ζ (residues 52–164)
	ITAM 1 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
	ITAM 2 RGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGM
	ITAM 3 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(B)	CD28 (residues 180–220) RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
(C)	4-1BB (residues 214–255) KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

Figure 2. Common Signaling Domain Sequences Used in Chimeric Antigen Receptors (CARs)
 (A) The CD3 ζ cytoplasmic domain comprises three immuno-tyrosine activation motifs (ITAMs; in red) that become phosphorylated upon ligand engagement and serve as docking domains for downstream signaling molecules. (B) The CD28 cytoplasmic domain contains an ITAM-like sequence (red) and two proline-rich motifs (blue) that provide docking domains for recruitment of co-stimulatory signaling molecules. (C) The 4-1BB cytoplasmic domain contains two acidic motifs (blue) that provide sites for TRAF molecules to associate.

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