immudex®
PRECISION IMMUNE MONITORING

TCR-T Cell Therapy

Maximize the potential of TCR-T Cell Therapy with antigen-specific solutions

Thomas Holberg Blicher, Bjarke Endel Hansen, Matilda Felicia Weywadt, Hazel Pinheiro and Liselotte Brix
Immudex A/S, Copenhagen, Denmark

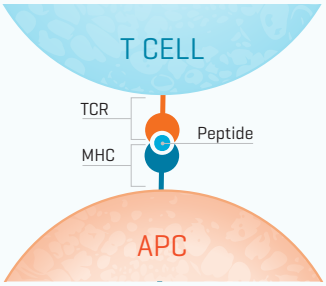
Highlights

- The wide range of cellular immunotherapies
- Overview of approved cellular immunotherapies
- TCR immunotherapies - the diseases to which they are applied and future perspectives
- How antigen-specific solutions and Dextramer® technology can support the development and manufacturing of TCR-T cell therapies:
  - target discovery
  - development and validation of the infusion product
  - GMP manufacturing
  - precision immune monitoring during clinical trials

T-Cell Receptor (TCR) in the Landscape of Cellular Immunotherapies

Cellular immunotherapies coax immune cells into countering disease. Immune cells are isolated, manipulated, and infused into a patient (adoptive cell transfer) to carry out therapeutic action or to activate an endogenous response. Those immune cells may originate from the patient (autologous), from a donor, or from a cell line (allogeneic).

Some therapies simply expand the immune cells *ex vivo* before infusion, while other more recently developed therapies additionally engineer them to express target-specific receptors that direct their activity[1]. Table 1 exemplifies the diversity of immune cell types that have been explored for therapeutic purposes.



Antigen-specific interaction is at the heart of TCR-T cell therapy

- In order to develop an effective T cell-based therapeutic, it is necessary to discover not only the ideal TCR, but also the most suitable target epitope.
- To understand the mechanism of action of the therapeutic, the antigen-specific interaction needs to be well characterized.
- A thorough analysis of antigen-specific responses in T cells is critical to demonstrate efficacy and investigate possible side effects.

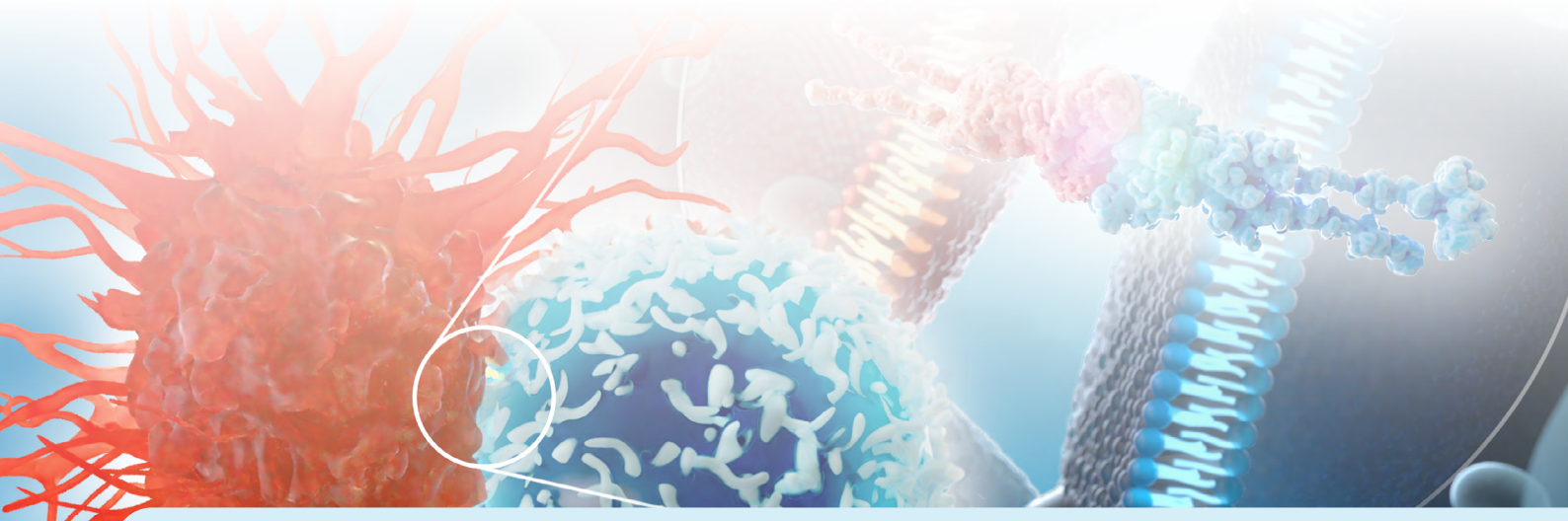


Table 1: **: Overview of immune cells as therapeutic agents**

Cell type	Origin	Manipulation	Comments
T-cell receptor (TCR)-T cells	Peripheral blood (autologous and allogeneic)	Expanded *ex vivo*. Modified to express TCR	▪ Actively pursued to address limitations of CAR-T cells in targeting solid tumors ▪ Evidence of safe and effective use in treating multiple myeloma and acute myelogenous leukemia1 (Table 4) ▪ Other targets and cancers are currently also in clinical trials2 (Table 4).
Chimeric antigen receptor (CAR)-T cells	Peripheral blood (autologous). Allogeneic sources and induced pluripotent stem cells are being explored	Expanded *ex vivo*. Modified to express CAR	▪ Currently, the most successful form of engineered cellular immunotherapy (see Table 2) ▪ Have proven less effective against solid tumors1 ▪ Therapies against viral infections like HIV, hepatitis, HCMV, EBV, and SARS-CoV-23 are in development
Tumor-infiltrating lymphocytes (TILs)	Tumor resection (autologous)	Expanded *ex vivo*.	▪ Penetrate tumor tissue and thus, already recognize tumor-specific antigens ▪ Include cytotoxic (CD8+) and helper (CD4+) T cells, and a small proportion of B and NK cells ▪ Have elicited durable anti-tumor response in patients with certain solid tumors[4]
Natural killer (NK) cells	Peripheral blood (autologous or allogeneic), cell line (NK-92)	Expanded *ex vivo*. Though capable of tumor-recognition, they are now modified to express CAR	▪ Naturally attack tumor cells; release chemokines and cytokines to activate adaptive immunity ▪ Low specificity and suppression by the tumor environment[5]. Is being addressed by CAR and TCR-engineering[6] ▪ Safety profile of allogeneic NK cells is optimal for off-the-shelf strategies[7] ▪ Currently being explored to treat cancer[6] and infectious diseases3
Invariant natural killer T (iNKT) cells	Peripheral blood (autologous and allogeneic)	Expanded *ex vivo*.. More recently, modified to express CAR	▪ Share characteristics with both T and NK cells and express an invariant TCR ▪ Not MHC-restricted[8] due to recognition of glycolipid antigens in CD1d ▪ May be activated by ex vivo-loaded APCs or expanded ex vivo and administered with antigen-loaded APCs
Adoptive B cell transfer	Peripheral blood (autologous)	Antigen loaded *ex vivo*. or modified to express a B cell receptor specific to a tumor antigen	▪ Can generate long-term protection against cancer cells via immunological memory ▪ Co-transplantation of B cells with hematopoietic stem cells promotes immune reconstitution[9,10]
Dendritic cells (DC)	Peripheral blood (autologous)	Expanded *ex vivo*; antigen loaded in certain therapies	▪ Regulate adaptive immunity via cytokine secretion and direct antigen presentation to T cells ▪ Can trigger a strong anti-cancer immune response ▪ Pursued as vaccines[11,12] that prime and expand endogenous antitumor-specific CD8+ T cells[13,14]
Cytotoxic T lymphocytes (CTL)	Peripheral blood (autologous and allogeneic); off-the-shelf cell banks	Selected virus-specific subpopulations or modified to express virus-reactive TCR	▪ Used in transplantation settings to induce immunity against Epstein-Barr virus (EBV), cytomegalovirus (HCMV), adenovirus, BK virus, and human herpesvirus-6 ▪ CTL banks covering numerous MHC alleles offer the option of treating various viral infections in most patients ▪ Autologous, engineered, virus-specific CTLs are used to target virus-associated cancers[1]
Regulatory T cells (Treg)	Peripheral blood (autologous)	Expanded *ex vivo*. Can also be modified to express CAR	▪ T cells that modulate the immune system, maintaining homeostasis and preventing autoimmunity ▪ Used in clinical settings to support organ transplantation, manage graft-versus-host disease, and type I diabetes ▪ Reduce symptoms of colitis, multiple sclerosis, and transplant rejection in mouse models[1]

CONTACT IMMUDEX customer@immudex.com

Approved cellular immunotherapies

The majority of therapeutic immune cells listed in Table 1 are either T cells themselves or are used to stimulate the anti-cancer activity of antigen-specific T cells. A survey of clinicaltrials.gov shows a similar distribution in clinical trials examining the safety and efficacy of these products.

While all cell types listed in Table 1 are currently being explored in at least one active clinical trial, chimeric antigen receptor or CAR-T cell therapies far outnumber other therapy types and are the only form of adoptive cell transfer that has achieved regulatory approval (Table 2).

Tumor-infiltrating lymphocytes (TILs) and natural killer (NK) cells follow CAR-T cells in the number of clinical trials. While TILs may soon receive approval to treat cervical cancer and melanoma, clinical examinations of genetically modified NK cells are in initial stages (Phase I/II)[15].

Besides six CAR-T cell therapies targeting CD19 and B-cell maturation antigen (BCMA), three other cellular immunotherapies have received regulatory approval. The first, Provenge®, is a dendritic cell (DC) vaccine. The other two, Kimmtrak® and Blincyto®, are bispecific T-cell engagers (BiTE).

Table 2: **FDA and EMA-approved cellular immunotherapies**

Approved Therapy	Type	Approved for (diseases)
Kymriah® (tisagenlecleucel)	Autologous CD19-directed CAR-T cell product	Expanded *ex vivo*. Modified to express TCR.
Tecartus® (brexucabtagene autoleucel, formerly KTE-X19)	Autologous CD19-directed CAR-T cells	Adult large B cell lymphoma and adult follicular lymphoma. Now recommended for routine use on the National Health Service in the UK[16]
Tecartus® (brexucabtagene autoleucel, formerly KTE-X19)	Autologous CD19-directed CAR-T cells	Adult mantle cell lymphoma
Breyanzi® (lisocabtagene maraleucel)	Autologous CD19-directed CAR-T cell product	Large B cell lymphoma in adults, including diffuse large B cell lymphoma, high-grade B cell lymphoma, primary mediastinal large B cell lymphoma, and follicular lymphoma grade 3B
Abecma® (idecabtagene vicleucel; ide-cel)	B-cell maturation antigen (BCMA)-directed CAR-T cells	Multiple myeloma
Carvykti® (ciltacabtagene autoleucel)	Autologous BCMA-directed CAR-T cells	Adults with refractory or relapsed multiple myeloma[17]
Ebvallo® (tabelecleucel)	Allogeneic therapy using donor T cells trained to recognize donor B cells infected with Epstein-Barr virus (EBV)	Adults and children with relapsed or refractory EBV+ post-transplant lymphoproliferative disease. Approved by EMA[18]
Provenge® (Sipuleucel-T)	Activated antigen-presenting cells (APC) within autologous peripheral blood mononuclear cells	Metastatic castration-resistant prostate cancer. EMA approval withdrawn by holder
Kimmtrak® (tebentafusp-tebn)	Bispecific gp100 peptide-MHC-directed CD3 T-cell engager (BiTE)	Major histocompatibility complex (MHC)-matched adult patients with unresectable or metastatic uveal melanoma[19,20]
Blincyto Blinotumomab	BiTE that binds CD19 of B cells and CD3 on T cells	Acute lymphoblastic leukemia[21]

TCR – a powerful alternative to CAR

Engineered T-cell therapies constitute a rapidly evolving frontier in what is commonly called the fourth pillar of cancer treatment after surgery, chemotherapy, and radiotherapy[22]. Among T-cell therapies, CAR-T cell therapies have accrued the most visibility as they solely have received regulatory approval (Table 2). However, the use of CAR has shown certain limitations, two of which stand out. First, CAR molecules can only recognize surface membrane proteins, which limits target options. Only 20–30% of the human proteome are membrane-bound proteins[23] and only a small fraction of those is expressed in a cell of interest and with sufficient tissue specificity to serve as a therapeutic target. Second, the distribution of surface targets for CAR is not limited to cancerous cells in a tissue. On-target, off-tumor activity is a real challenge. Current CAR-T cell therapies eliminate both cancerous and healthy cells in the target tissue. While in some cases the depletion of healthy cells can be managed clinically, as was the case for Emily Whitehead[24] who had no detectable B cells after a CAR-T therapy to treat her B cell lymphoma, that off-tumor activity renders CAR-T cell therapies unsuited to treat cancers in survival-critical tissues.

Use of the TCR has recently gained traction as an alternative to CAR. Like CARs, TCRs also bind cognate targets on the surface of cells to trigger the cytotoxic functions of antigen-specific T effector cells. Unlike CARs, the targets of TCRs are antigens from practically any cellular compartment that are bound to major histocompatibility complexes (MHC). This means that the antigen space is vastly greater for TCR than CAR and allows targeting proteins expressed exclusively in cancerous cells, potentially boosting specificity beyond that achieved with CAR-T cell therapy. This feature makes TCR therapies a promising alternative where the CAR approach has failed[25]. The nature of MHC-peptide recognition via TCR imposes important considerations, however. First, patients selected for TCR-T cell therapy must express the target antigen within the context of a particular MHC allele[2]. Second, while on-target, off-tumor reactivity may be avoided via careful antigen choice, cross-reactivity of the TCR can lead to off-target, off-tumor effects. The TCR must be selected carefully to ensure that any cross-recognition does not overlap with highly expressed self-antigens[26].

Table 3: **Chimeric antigen receptors versus T-cell receptors**

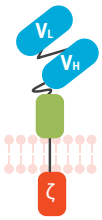
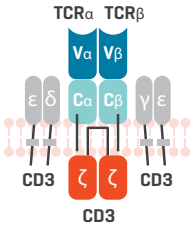
	CAR	TCR
Molecular format	 An antibody-derived recognition domain conjugated to an intracellular CD3 domain that phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs), and to costimulatory receptors	 A heterodimer comprised of an α and β chain, which forms complexes with an intracellular CD3 domain that phosphorylates ITAMs. CD8 and CD4 co-receptors enhance TCR antigen sensitivity by binding MHC I or II.
Mode of action	Recognizes targets independent of MHC	Recognizes only targets bound to MHC. Thus, therapies must be MHC-matched
Target types	Recognizes only cell surface proteins, glycoproteins, and glycolipids	Targets peptides from any cellular compartment, including intracellular proteins which are more commonly specific to the tumor
Sensitivity	Requires multiple target surface molecules to elicit a response	Can elicit an immune response to as little one MHC molecule[27,28]
Side effects	More likely to cause cytokine release syndrome. Currently approved therapies targeting CD19 result in complete loss of B cells that must be managed clinically	Off-target, off-tumor toxicity (cross-reactivity) can result in side effects[26]. The use of tumor-associated antigens as targets of a TCR therapy can also result in on-target off-tumor toxicity
Limiting factors	Factors that can reduce efficacy include antigen escape and limited tumor infiltration	Factors that can reduce efficacy include mispairing between engineered and endogenous heterodimer chains and downregulated MHC expression in tumor cells

Table 3 highlights key distinctions between CAR and TCR-based therapies. The differences emphasize the utility of both strategies in coaxing the immune system into therapeutic action and the importance of using sensitive but highly specific receptors to restrict that activity to diseased and not healthy cells (Table 3).

CONTACT IMMUDEX customer@immudex.com

Can CARs mimic TCRs?

Researchers have designed CARs that behave like a TCR, inducing T cell cytotoxic activity in response to a pMHC. However, their engineering is more complicated than that of their TCR counterpart and they generally exhibit lower antigen sensitivity[27].

Disease areas relevant for TCR immunotherapies

Especially in the case of cancer treatments, exclusivity of antigen expression is a key factor in the success of cellular immunotherapy. Given that specificity of expression in cancer cells is found among intracellular antigens, engineering cells with TCRs vastly expands the space of targetable proteins.

There are currently no approved therapies involving adoptive transfer of TCR-T cells. However, the number of clinical trials testing this approach has more than doubled since 2016. A review by Schafer[2] and colleagues (2022) found 120 trials in clinicaltrials.gov as of October 2021. At that time, 52 (43%) of those trials were active. Table 4 summarizes the 120 trials, organized by disease. The vast majority address solid tumors, though some test candidates to treat hematological malignancies and viral infections. Among a diverse repertoire of over 30 TCR targets that have entered clinical trials, by far the most tested is NY-ESO-1, and most restricting MHC alleles belong to the A2 superfamily. A quarter of the trials surveyed are monotherapies. Other trials include concomitant administration of interleukin 2 – a cytokine that stimulates the expansion of T cells – and/or DC vaccines or targeted therapies like Pembrolizumab and Ipilimumab[2].

The future promise of TCR immunotherapy

To date, no clinical trial for a TCR-T cell therapy has moved beyond phase II. Nevertheless, several trials have achieved improved therapeutic outcomes in solid tumors compared to CAR-T cell therapies. A couple of features of TCRs may account for that difference. TCRs can access intracellular antigens which show higher tumor specificity than surface proteins. TCRs may also be more sensitive and thus, able to trigger a response to low-abundance targets. Finally, TCRs show no tonic signaling, an underlying cause of toxicity, T cell anergy and T cell exhaustion observed in CAR-T cell therapies[29]. Thus, while there are still many hurdles to overcome – not the least of which is the cost and complexity of manufacturing – the mechanism and diverse target availability of TCRs open numerous therapeutic strategies to explore.

For example, the search for effective targets has expanded to products of non-coding regions in the human genome. Tumor cells overexpress[30] these so-called "dark antigens," making them a promising new angle in neoantigen development[31]. TCR engineering is also not restricted to T cells. TCRs have been expressed in NK cells[7,32.] which presents an exceptional opportunity for off-the-shelf therapies capable of responding to malignant cells lacking antigen presentation via MHC I[32]. Such therapies would be a welcome alternative in situations where TIL and TCR-T cell therapies lose efficacy as tumors downregulate MHC-peptide expression to escape attack[33,34]. Finally, infused TCR-T cells are being engineered to prime and activate antigen-specific endogenous T cells against other tumor antigens, so-called epitope spreading[35].

TCR-T cell therapies combine features of already proven CAR-T cell therapies with benefits of TCR bispecific treatment modalities. Based on learnings from existing clinical trials and the diversity of ideas pursued to enhance the therapeutic potential of TCR approaches, it is reasonable to expect increased investment in research and development, and a soon-to-come first approval of a TCR-T cell therapy.

Table 4: **Indications, target antigens, and restricting MHC alleles pursued in clinical trials of TCR-T cell therapies** (as of October 2021)

Disease	Number of clinical trials	Target antigens	Restricting MHC
Various solid tumors	31	p53, CEA, MAGE-A4, NY-ESO-1, MAGE-A3, MAGE-A10, MAGE-A6, KRAS G12V, MAGE-A1, PRAME, KRAS G12D	A*0201, DP*04, A*1101, A*2402, A*01, A*0206, DPB1*0401, A*02, DP*0401, DP*0402
Melanoma	19	gp100, MART-1, tyrosinase, NY-ESO-1, p53, MAGE-A3, MAGE-A12, MAGE-C2	A*0201, A*02
Neoplasms	13	NY-ESO-1	A*0201, A*0205, A*0206
HPV-associated solid cancers (cervical, oropharyngeal, uterine, vulvar)	11	HPV-antigen (e.g., E6, E7)	A*0201
Soft tissue sarcoma	4	NY-ESO-1, MAGE-A4	A*0201, A*0206, A*02
Liver	9	HVB-antigen, alpha-fetoprotein	A*0201, B*5801, Cw*0801, A*0203
Renal	6	TRAIL/DR4, HERV-E, NY-ESO-1, p53, MAGE-A3/MAGE-A12	A*1101, A*0201 (TRAIL not restricted)
Lung	5	WT1, MAGE-A10, NY-ESO-1, KK-LC-1	A*0201, A*0206, A*0101
Myeloid leukemia and myelodysplastic syndromes	5	WT1, PRAME	A*0201
Various hematological malignancies	4	HA-1, HA-1H, NY-ESO-1	A*0201
Multiple myeloma	3	NY-ESO-1	A*0201
Nasopharyngeal	3	LMP1, LMP2, EBNA1	A*0201, A*2402, A*1101
Head and Neck	3	EBV-antigen, HPV-antigen, MAGE-C2	A*0201 and others unspecified
Pancreatic	2	KRAS G12V, mesothelin	A*1101, A*0201
Ovarian	1	NY-ESO-1	A*0201, A*0205, A*0206
Skin (non-melanoma)	1	Merkel cell polyomavirus-antigen	A*0201
Colorectal cancer	1	TGF β II	A*02
Thyroid	1	Thyroglobulin	A*0201
CMV infection	2	CMV-antigens	A*1101, A*0201, A*2402
EBV infection	1	EBV-antigen	A*1101, A*0201, A*2402
HIV infection	2	gag	A*02

Source: Supplementary data from Schafer *et al.* 2022[2]

CONTACT IMMUDEX customer@immudex.com

Developing and Manufacturing a TCR-T Cell Therapy

While the details of how a TCR-T cell therapy is developed and subsequently manufactured depend on the nature of the cellular product itself, the process can be generally described in four steps.

- First, promising candidate TCRs and appropriate target peptide-MHC complexes (pMHCs) are identified that meet the defined criteria of a therapeutic strategy. After the discovery phase, the identified TCR candidates are optimized to deliver the desired therapeutic effect with minimal off-target reactivity.
- Second, one or more TCR-pMHC pairs are characterized *ex vivo* to assess binding strength and determine any potential cross-reactivity. A selection of those candidates is then examined in a biological system to validate the TCR interaction with the target pMHC, assess cross-reactivity in physiological context, and ultimately generate a validated infusion product that can be produced under good manufacturing practice (GMP). Antigen-specific T cells are also isolated from biological samples and expanded, and the presence of antigen-presenting cells (APC) is assessed in relevant tissues to ensure appropriate TCR recognition.
- In the third step, the T cell number, identity, and purity of the infusion product is scrutinized to ensure that it meets lot release criteria for use in clinical trials.
- Finally, the infusion product is tested in clinical trials and the infused TCR-T cells are systematically monitored in patients.

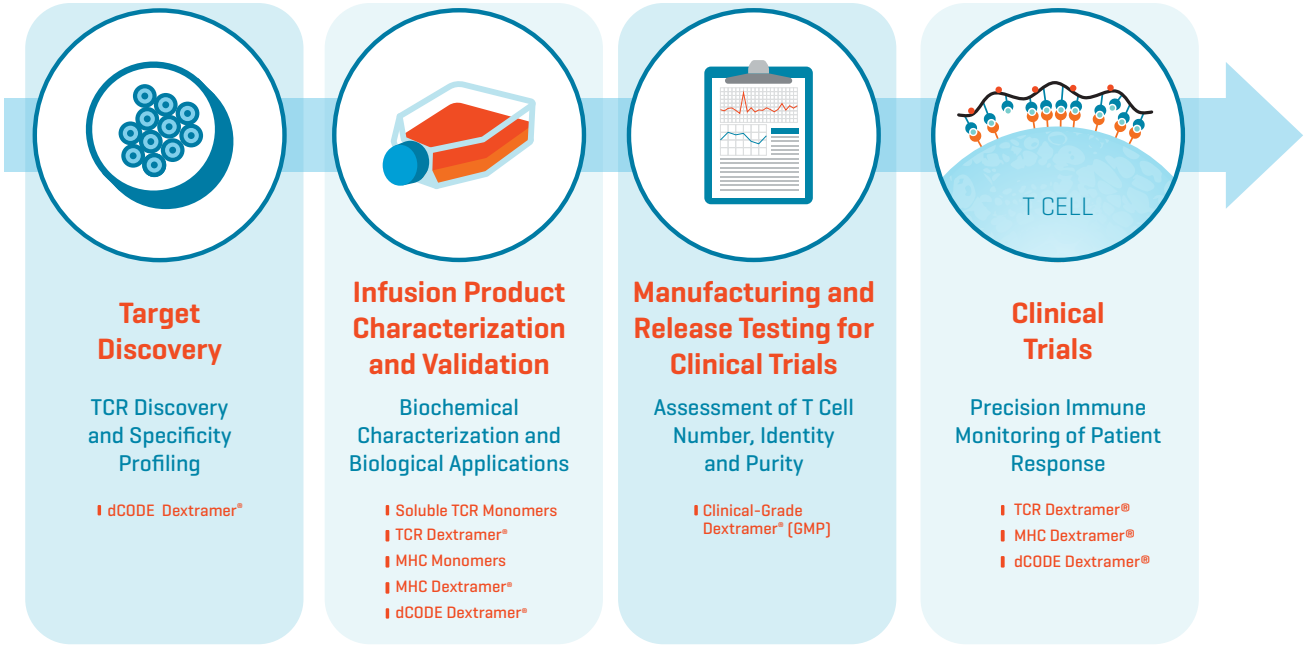


Figure 1: Steps in the development and manufacturing of a TCR T cell therapy that benefit from highly sensitive and flexible Dextramer® reagents

These four steps to develop and manufacture a TCR-T cell therapy call for in-depth examination of TCR function, T cell phenotype, and product performance. The construct flexibility of Dextramer® reagents is a good match for the diversity of potential targets and creative approaches in TCR engineering. Plus, the sensitivity of Dextramer® reagents ensures reliable and reproducible results. Finally, with both research- and GMP-grade products, you can ensure continuity of reagents from development to manufacturing.

In the following section, we explore the goals of the four steps and discuss the advantages of Immudex products in progressing candidates from target discovery, product validation and release testing, to precision immune monitoring after infusion.

Step 1: Target Discovery

The goal of this first step is to identify target pMHCs and candidate TCRs that have the potential to elicit a desired therapeutic response. The processes in identifying each are intimately linked as, ultimately, the nature of their interaction is at the core of your TCR-T cell therapy. Target pMHCs are selected based on a strong binding affinity between peptide and MHC, but that choice is informed by the availability of epitope-reactive TCR clonotypes that show clinically relevant phenotypic characteristics. TCR discovery also builds on finding T cell clones that expand upon binding target pMHCs with balanced affinity and specificity. The resulting candidate TCRs may then also be optimized through protein or gene engineering strategies to create modified TCRs with enhanced affinity for specific pMHCs that trigger the desired effect with minimal off-target activity.

Discovery of Candidate TCRs and Target pMHCs

TCR Discovery

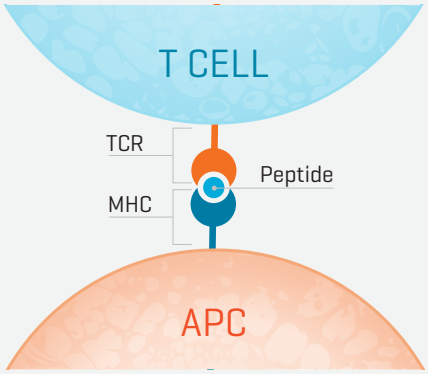
Single-cell V(D)J sequencing and TCR discovery at scale using dCODE Dextramer® libraries

↓

Candidate TCR(s)

↓

TCR Optimization



pMHC Binding Affinity

Assess pMHC binding affinity using loadable* MHC Monomers

↓

Specificity Profiling

Large scale epitope recognition profiling using dCODE Dextramer® libraries

↓

Target pMHC(s)

Figure 2: Overview of how Immudex products can support the discovery of candidate TCRs and target pMHCs.

Approach and methods

1. **Shortlist candidate pMHCs**

   One approach to generating this list is to start with whole-exome sequencing or mass spectrometry[36–38] of, for example, tumor samples to identify tumor-specific antigens or neoantigens. The resulting sequences are filtered via bioinformatic analysis and neoepitopes are predicted *in silico*. Another important assessment is to compare the candidate epitopes to self, with the aim of reducing the likelihood of off-target activity.

2. **Assess pMHC binding affinity**

   Use MHC monomers (e.g., on coated plates) loaded with candidate epitopes to assess the strength of binding affinity between the MHC and each peptide. Mass spectrometry has also been used to measure pMHC stability[39].

Create plates coated with Peptide-Receptive MHC Monomers

3. **Explore specificity and identify high-affinity TCRs for candidate pMHCs**

   Once candidate epitopes with good binding affinity to the MHC have been identified, large-scale epitope recognition profiling can be done with DNA-barcoded libraries of candidate pMHCs to assess immunogenicity and identify epitope-reactive TCR clonotypes. This method allows quantifying the different clonotypes. Combined with co-staining for T-cell differentiation and activation markers, the method also links analyses to clinically relevant functional responses. The aim is to see which epitopes are being seen by T cells as these will lead to relevant TCRs for your therapeutic strategy.

*Peptide-receptive monomers are available as easYmers® MHC I powered by immunAware and U-Load® MHC II monomers.

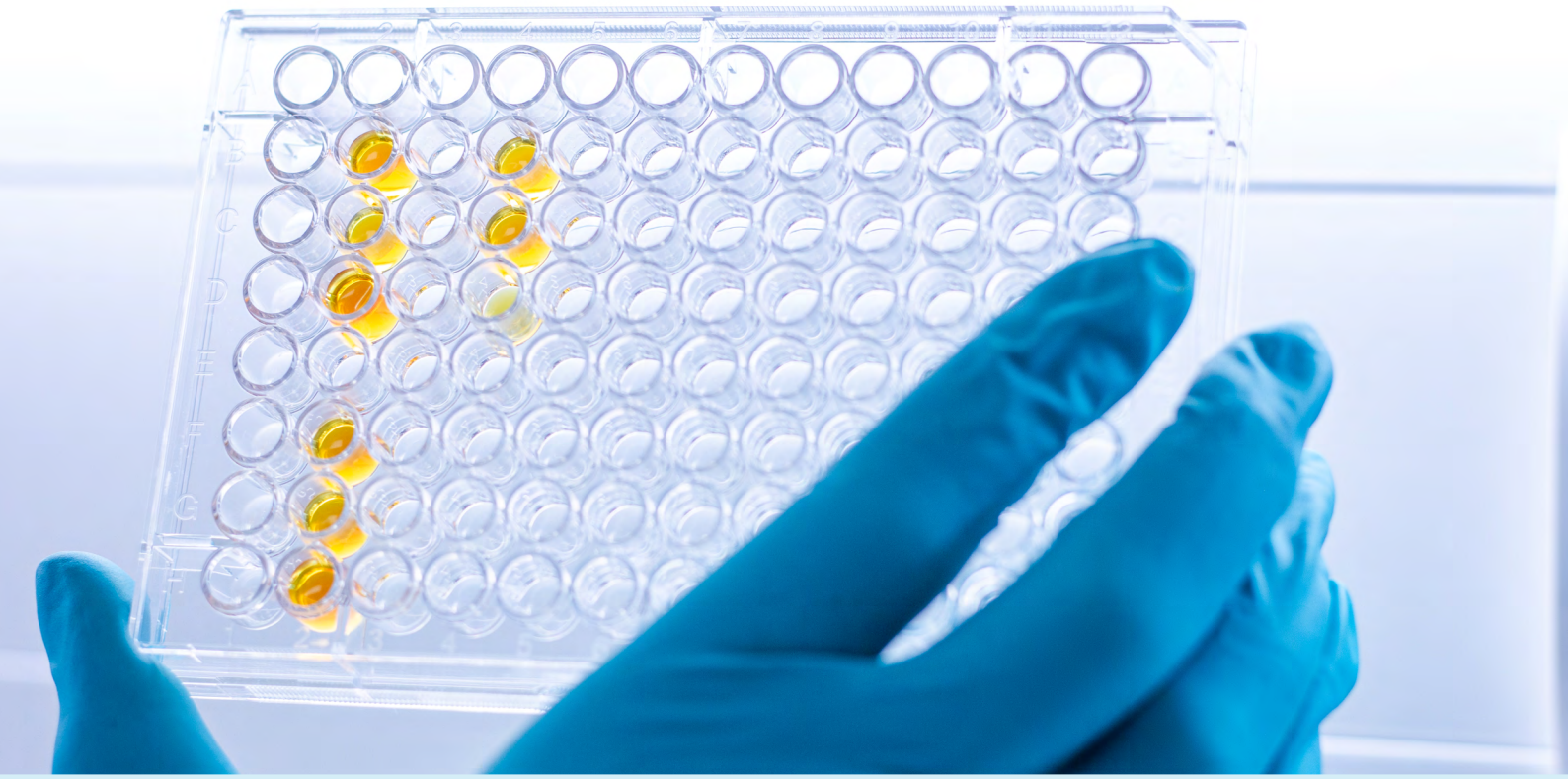
CONTACT IMMUDEX customer@immudex.com

Using Immudex products for Target Discovery

MHC Dextramer® and dCODE Dextramer® reagents support the aims of this first step in developing TCR-T cell therapy (see Figure 2 and table below). A unique set of features sets them apart from alternative research tools in facilitating experiment design and delivering highly precise results:

- High avidity: Dextramer® technology ensures that even low-affinity TCRs are reliably detected, so you don't miss potential TCR candidates.
- Broad allele selection: Dextramer® technology – dCODE Dextramer® reagents and loadable MHC monomers and multimers – offers the broadest selection of MHC I and MHC II alleles. Custom alleles can be ordered through Immudex custom solutions and services.
- Setup flexibility: You can create libraries either with ready-to-use dCODE Dextramer® reagents or load your own peptides on U-Load dCODE Dextramer® reagents.

Aim	Immudex product	How to use
Specificity profiling of pMHC candidates	MHC Dextramer® dCODE Dextramer®	MHC Dextramer® multimers are used to assess potential immunogenicity of candidate epitopes. Profiling can be scaled up to simultaneously screen over a thousand epitopes with DNA-barcoded dCODE Dextramer® Reagents (Figure 3)
Finding TCRs of therapeutic value	dCODE Dextramer®	dCODE Dextramer® technology can be used to identify TCRs that bind candidate pMHCs40. Create a library of customized, barcoded multimers to isolate, screen, and sequence TCRs. With up to 1000 different barcodes available, the libraries can cover several specificities for concurrent analysis (Figure 3)
Phenotyping T cells responsive to candidate pMHC	dCODE Dextramer®	Combine sequencing of DNA-barcoded reagents with single-cell multi-omic analysis to phenotypically characterize T cells that potentially recognize the pMHC. The information provides insights into the function of identified TCRs in a therapeutic setting.



Specificity Profiling and TCR Discovery with dCODE Dextramer® Technology

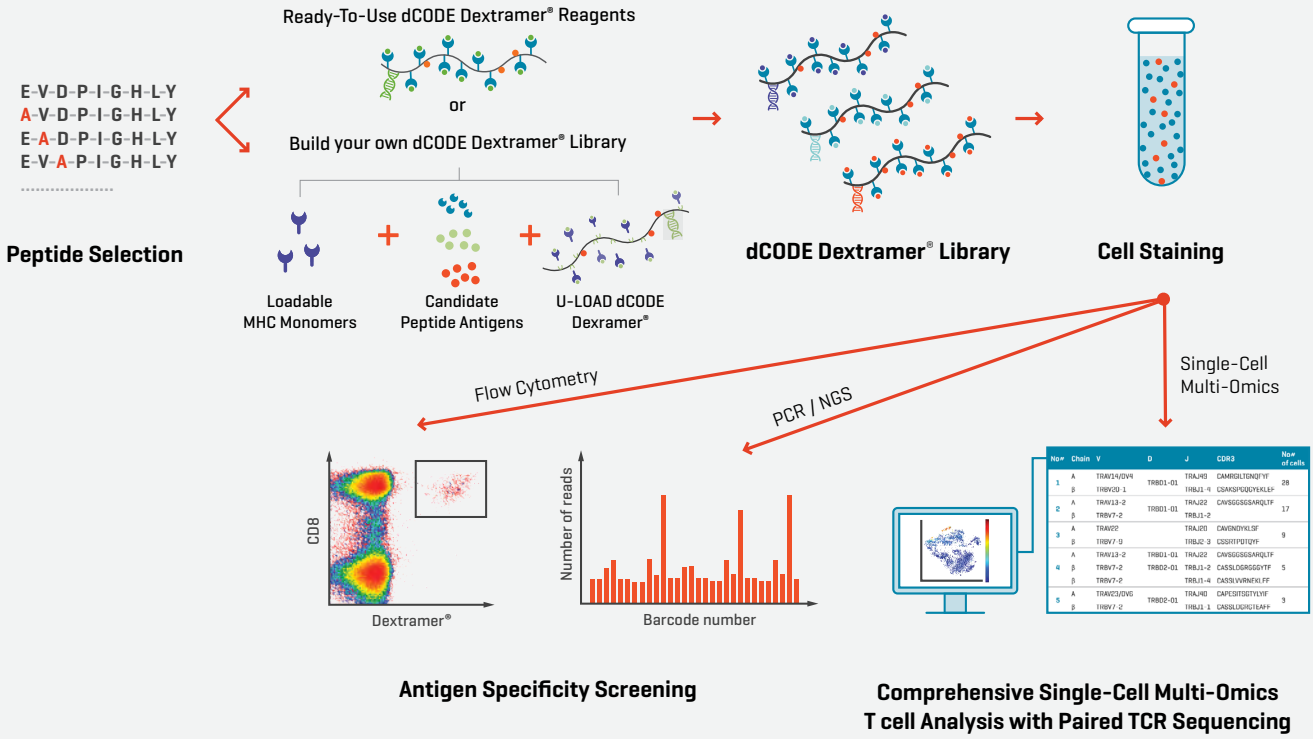


Figure 3: Multiplex screening of large epitope panels using dCODE Dextramer®. dCODE Dextramer® library is created using ready-to-use dCODE Dextramer® reagents or alternatively by building your own library using U-Load dCODE Dextramer® and loadable* MHC Monomers incubated with the candidate peptides. After staining the cell sample with the dCODE Dextramer® library, FACS-sorting is used to isolate the MHC-multimer-binding cells based on PE fluorescence intensity. The antigen-specificities are then identified by sequencing the DNA barcodes. Positive populations are validated by flow cytometry. TCR discovery can be performed at scale using libraries of dCODE Dextramer® reagents[40], enabling up to 1000 different specificities to be screened in the same sample. The advantage of this single-cell dCODE Dextramer® approach is that it enables you to simultaneously identify the TCR sequences as well as the epitopes with which the TCRs interact.

Identifying shared tumor epitopes from endogenous retroviruses for high-avidity cytotoxic TILs using MHC Dextramer®

Six candidate HLA-A2 epitopes derived from cancer- associated endogenous retroviruses (HERVs) were evaluated as targets for CD8+ TILs in breast cancer patients. MHC Dextramer® reagents were used to efficiently identify all T cell populations specific to the epitopes predicted to be shared among the cancer patients[41]. A high prevalence of CD8+ T cells specific to 3 of the 6 tested epitopes were observed in the tested individuals.

Find out more: immudex.com/bonaventura

*Peptide-receptive monomers are available as easYmers® MHC I powered by immunAware and U-Load® MHC II monomers.

CONTACT IMMUDEX customer@immudex.com

Important considerations for target discovery

MHC Dextramer® and dCODE Dextramer® reagents support the aims of this first step in developing TCR-T cell therapy (see Figure 2 and table below). A unique set of features sets them apart from alternative research tools in facilitating experiment design and delivering highly precise results:

- High avidity: Dextramer® technology ensures that even low-affinity TCRs are reliably detected, so you don't miss potential TCR candidates.
- Broad allele selection: Dextramer® technology – dCODE Dextramer® reagents and loadable* MHC monomers and multimers – offers the broadest selection of MHC I and MHC II alleles. Custom alleles can be ordered through Immudex custom solutions and services.
- Setup flexibility: You can create libraries either with ready-to-use dCODE Dextramer® reagents or load your own peptides on U-Load dCODE Dextramer® reagents.

Aim	Immudex product
Focus on the TCR-pMHC interaction	The TCR-pMHC interaction is the "make or break" feature of your TCR-T cell therapy. So, characterize it well and leverage that knowledge to achieve the desired therapeutic effect. That information is also essential in the final formulation of your cellular product. Ideally, you want to infuse only the antigen-specific T cells, which you can select and enrich based on their interaction with the right pMHC. The affinity of the TCR-pMHC interaction is important for your TCR-T cell therapy but does not always have to be high. Anti-cancer TCRs tend to have a mid-range affinity and even low-affinity interactions between a TCR and pMHC can be functional. Therefore, detecting and analyzing even lower-affinity TCRs is important to avoid missing candidates. The ideal window of TCR affinity for a TCR-T cell therapy is still an open question and may depend on the engineering strategy. A TCR with very high affinity can remain bound to target cells, preventing it from engaging with another target cell. Conversely, weak affinity limits binding to cells with high antigen presentation levels, allowing other tumor cells to escape cytotoxic attack.
Know your target pMHC	Figure out the target epitope and MHC for your therapeutic strategy preferably before working on a candidate TCR. The opposite strategy – figuring out what pMHC a given TCR recognizes – is doable but challenging. It is also possible to simultaneously find TCRs and their corresponding epitope via single-cell V(D)J sequencing of TCRs, but that does not narrow down which disease-relevant specificities to screen. The optimal affinity of the peptide-MHC interaction for your TCR-T cell therapy depends on the overall interaction. The affinity must ensure stable epitope presentation on the cell surface, but the amount of epitope needed depends on TCR affinity. A TCR with high affinity requires less epitope. Furthermore, epitopes from a protein that is highly expressed may be sufficiently represented on the cell surface for activation even with low pMHC affinity. The key is to demonstrate a stable epitope presentation, especially when a pool of peptides may compete for MHC binding. Thus, high pMHC affinity and abundance are good goals.
Retain biological context	Use biological samples for TCR discovery. Natural TCRs are more likely to lead to productive interactions because they have been through thymic selection.
Think ahead to the desired final product	As you identify a target pMHC, bear in mind that personalized therapies using patient-specific T cells and epitopes are limited in scale and turnaround time. Finding T cells that recognize universal epitopes, like shared cancer mutations, can simplify cell processing and accelerate production.

Step 2. Characterization and validation of the infusion product

Having narrowed down candidate TCRs and pMHCs to selected pairs that exhibit optimal interaction and affinity in the context of your therapeutic strategy, the pairs progress to the next step. In step 2, receptors and epitopes are further characterized and validated before selecting those that will be advanced to GMP manufacturing and clinical trials.

Assays in both cell-free and biological contexts are performed to scrutinize the interaction between TCRs and pMHCs. *Ex vivo* assays focus on determining binding strength and possible cross-reactivity. Subsequent testing within biological systems serves to first confirm that the TCR recognizes and responds to the target epitope as expected and without unforeseen cross-reactivity. Then, corresponding antigen-specific T cells are isolated and expanded for *in vitro* characterization. Finally, APCs are examined in several tissues to confirm that the target antigen is present predominantly and at sufficient levels in target tissues.

Approach and methods

1. **Detect potential cross-reactivity in cell-free assays**

   Produce the candidate TCR as a soluble monomer and immobilize it for a fitting detection assay (e.g., on a plate for ELISA, Figure 4). Apply libraries of pMHC monomers with potential off-target antigens to evaluate cross-reactivity. Various approaches have been used to generate the library of potential off-target antigens. For example, you can replace each amino acid in the epitope sequence with an alanine to create a pool of similar but distinct antigens. A more extensive library can be generated by systematically substituting each position of the peptide sequence with each of the natural amino acids.

2. **Assess TCR-pMHC binding strength in cell-free assays**

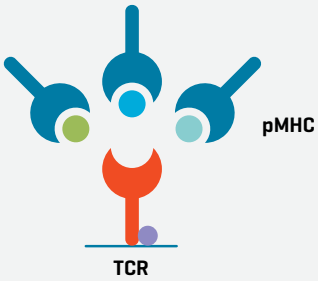
   Plate- or bead-based assays can be used to measure the strength and duration of binding between soluble monomers of the TCRs and pMHCs (Figure 4). Alternatively, surface plasmon resonance can be used by immobilizing TCR monomers onto a chip and allowing soluble pMHC monomers to flow over them.

Biochemical Characterization of the Infusion Product

Cross-Reactivity

Screen TCR specificity and cross-reactivity with immobilized Soluble TCR Monomers and libraries of pMHC Monomers including potential off-target antigens

- Plate-based assays/ELISA



Binding Strength

Assess TCR:pMHC binding strength using immobilized Soluble TCR Monomers and pMHC Monomers

- Surface Plasmon Resonance
- Lateral flow assays
- Plate-based assays

Figure 4: Biochemical characterization of the infusion product using Immudex's products in cell-free assays.

3. **TCR validation and cross-reactivity assessment in a biological system**

   Use flow cytometry to demonstrate the binding of the TCR to the target pMHC, to assess surface expression levels of the TCR[42], and to determine TCR avidity by serially diluting the target pMHC and using staining intensity as a proxy of TCR-pMHC affinity[43]. Likewise, cross-reactivity within the context of a biological system can be explored with flow cytometry[44,45]. Detect cross-reactive clones binding to a library of potential off-target pMHC to assess the specificity of the candidate TCR interaction[46,47]. Finally, you can combine characterization of T cell clonotypes and T cell recognition with functional assays by using DNA-barcoded pMHC reagents.

CONTACT IMMUDEX customer@immudex.com

TCR-accepting T-cell platform enables T-cell engineering with enhanced specificity and prediction of cross-reactivity®

A TCR-accepting T-cell (TnT) platform was developed and validated for use in profiling the functional display, engineering, and cross-reactivity of tumor-specific TCRs. Binding detection by flow cytometry was done using cognate peptide-MHC I Dextramer® reagents[43].

Read the case study: immudex.com/vazquez-lombardi

4. **Isolation and enrichment of antigen-specific T cells**

   Identify and isolate antigen-specific T cells using reagents with the target pMHC. Use exposure to the target pMHC also to stimulate expansion. Additionally, ascertain that the antigen-specific T cells recognize target cells (e.g., tumor cells) to confirm the biological relevance of the selected epitope.

5. **APC detection in target and other tissues**

   Detect antigen-specific APCs in biological samples spanning both target and other tissues using flow cytometry and reagents displaying the candidate TCRs[48]. Examining several tissues is important to determine if APCs present in other tissues can lead to on-target off-tissue reactivity or toxicity. If properly designed, reagents with the candidate TCRs could be used to detect APCs in tissues via in situ hybridization techniques. By the same token, it may be possible to measure loss of target antigen by assessing levels of pMHC expression on APCs either via in situ staining or flow cytometry. Expression levels could be inferred from the median fluorescence intensity and would help determine the levels of pMHC expression that are sufficient for TCR recognition. Information about the expression of the target antigen in patient samples could be helpful to stratify and select patients for subsequent clinical trials.

Biological Validation and Characterization

TCR Validation

MHC Dextramer® or dCODE Dextramer® for:

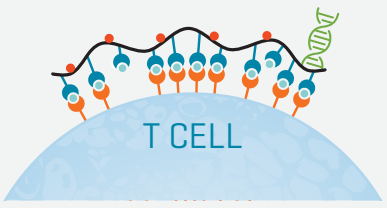
Demonstration of TCR interaction with the target pMHC

Assessment of TCR surface expression level

Investigation of TCR:pMHC complex affinity

Characterization of pMHC-reactive T cell clonotypes

- Flow cytometry
- NGS/single-cell multi-omics



Cross-Reactivity

Investigation of potential off-target antigens using MHC Dextramer® or dCODE Dextramer®

- Flow cytometry
- NGS/single-cell multi-omics

T Cell Isolation

Isolation, enrichment and expansion of antigen-specific T cells using MHC Dextramer® for *in vitro* characterization or confirmation of recognition of patient tumor cells.

APC Detection

Detection of APCs and assessment of target pMHC expression level in biological samples using TCR Dextramer®

- Flow cytometry
- In-situ staining

Figure 5: Overview of applications for Immudex products in the biological validation and characterization of the infusion product.

Using Immudex products to characterize and validate the infusion product

Immudex produces high-quality, comprehensively tested TCR and pMHC monomers to support the biochemical characterization and validation of the TCR-pMHC interaction (see Figure 4 and table below). TCR monomers are optimized to bypass limitations in stability and purity resulting from the inclusion of transmembrane domains. Their functionality and specificity for the target pMHC versus a control pMHC is verified by flow cytometry.

pMHC monomers are a long-standing staple of the Immudex product portfolio and offer extensive configurational flexibility. You can select from an unparalleled menu of MHC I or MHC II alleles, choose GMP-grade products, and determine if you wish to work with loadable* or ready-to-use monomers.

Several high-quality TCR or MHC monomers are bound to a dextran backbone to produce corresponding Dextramer® reagents. Coupled to fluorescent probes, TCR Dextramer® and MHC Dextramer® reagents support highly sensitive flow cytometric analyses of cell identity, quantity, phenotype, and cross-reactivity (see Figure 5 and table below). These reagents retain the quality features of their component monomers (see above), but the dextran backbone confers exceptional avidity to Dextramer® reagents. That avidity ensures that even low-affinity TCR-pMHC interactions are detected reliably and sensitively.

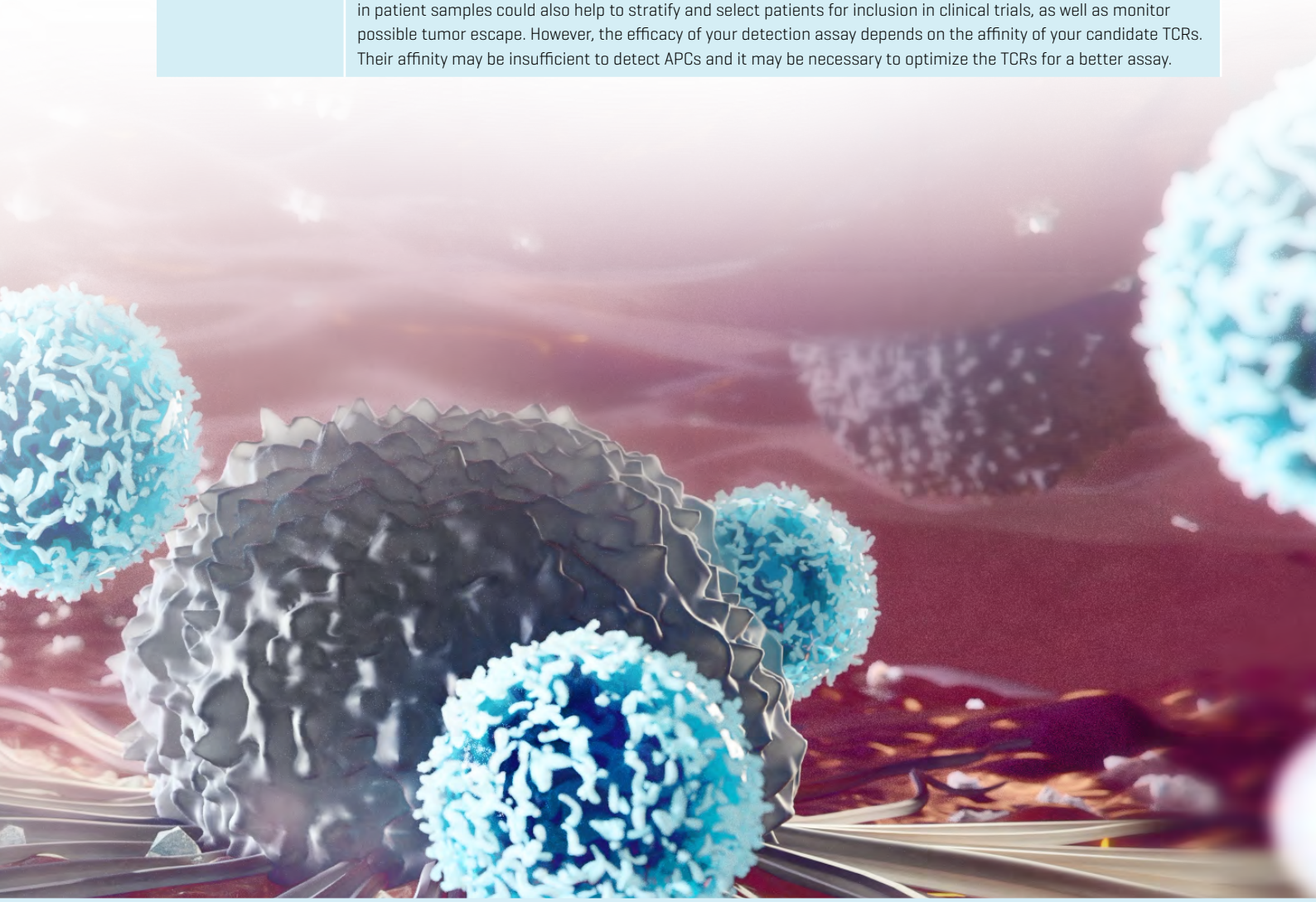
Aim	Immudex product	How to use
Assessing cross-reactivity and binding strength in a cell-free system	TCR monomers pMHC monomers	Use high-quality monomers of your candidate TCR and target pMHCs for suspension or immobilized assays. Immudex monomers are produced and released under stringent quality control requirements to ensure that your results are robust and reliable (Figure 4)
TCR validation and cross-reactivity assessment in a biological system	MHC Dextramer® dCODE Dextramer®	Use MHC Dextramer® reagents for highly sensitive and accurate characterization of TCR performance in binding target and off-target epitopes[44,45], even for low-affinity interactions. The immunogenicity assessment can be scaled up with dCODE Dextramer® reagents, where the unique DNA barcode of each pMHC multimer allows screening up to 1000 epitopes simultaneously[46,47] (Figure 3 and Figure 5)
Isolation of antigen-specific T cells	MHC Dextramer®	The exceptional avidity of MHC Dextramer® reagents boosts the detection, and consequently isolation of antigen-specific T cells even when they exhibit low affinity for the target pMHC (Figure 5)
APC quantification in target and other tissues[48]	MHC Dextramer®	Carefully tested for functionality, TCR Dextramer® reagents allow you to detect and quantify APCs in target and other tissues by flow cytometry, or potentially in situ hybridization (Figure 5). Our scientists can also help you optimize the reagents to create a reliable assay.

*Peptide-receptive monomers are available as easYmers® MHC I powered by immunAware and U-Load® MHC II monomers.

CONTACT IMMUDEX customer@immudex.com

Important considerations for characterization and validation of the infusion product

Aim	Immudex product
Pay due attention to cross-reactivity	TCRs are naturally cross-reactive. In fact, binding to off-target antigens has resulted in dangerous to lethal toxicity[26]. While on-target off-tumor reactivity can be controlled by the choice of target, off-target reactivity is more difficult to determine. Currently, the only way is to test potential antigens in a range of body tissues that look similar enough to trigger killing. Therefore, screening candidate TCRs against a library of potential off-target antigens is vital[46,47] (Figure 3 and Figure 5). This screening library should include a panel of "usual suspects" to avoid self-reactivity, and a panel of highly expressed proteins like titin in addition to sequence permutations of the target epitope. The human proteome atlas can also be used to predict possible off-targets based on expression levels
Understand the challenge of specificity	In general, specificity is challenging in T-cell therapeutics. The success of CAR-T cell therapy against B-cell lineage markers (e.g., CD19, BCMA) may be because the loss of healthy B cells along with cancerous ones is manageable. Similarly, on-target off-tumor cross-reactivity can always be a potential issue with CAR-T cell therapy. The expanded repertoire of potential targets for TCR-based therapeutics allows for more opportunities to find epitopes and overcome hurdles due to lacking specificity
Screen for cross-reactivity, and screen again	Screening for potential off-target cross-reactivity is vital to avoid toxicity and should be done extensively. If you optimize a TCR candidate to increase affinity, continually check that modifications have not introduced undesired cross-reactivity. Given that positive and negative selection of TCRs is imperfect in the body, you must check for autoreactivity as well. Even at low affinity, highly expressed self-peptides can lead to toxicity. Therefore, cross-reactivity screening should always include epitopes of highly expressed proteins and antigens that you may suspect
Plan APC detection carefully	Quantifying APCs in various tissues[48] is important to ensure that the target antigen is present predominantly in the target tissue, and thus, avoid side effects or toxicity. Furthermore, data on APC localization in tumor tissue can inform the development of more targeted attacks on solid tumors. Measuring expression of the target antigen in patient samples could also help to stratify and select patients for inclusion in clinical trials, as well as monitor possible tumor escape. However, the efficacy of your detection assay depends on the affinity of your candidate TCRs. Their affinity may be insufficient to detect APCs and it may be necessary to optimize the TCRs for a better assay.



Step 3. Release testing of manufactured TCR-T cells for clinical trials

After validating candidate TCR-pMHC pairs in cell-free and biological systems, as well as establishing their manufacture as an infusion product under GMP, typically one is advanced to be tested in clinical trials. To that end, the infusion product must meet predefined lot release criteria in T-cell identity, number, and purity.

Approach and methods

All three parameters for lot release can be measured by flow cytometry using sensitive, clinical-grade reagents with the target pMHC. Flow cytometry allows you to count the number of cells in a sample, ascertain that the antigen-positive T cells have the correct specificity, and determine the percentage of antigen-positive T cells relative to all cells in the sample[49–53]. Finally, the fluorescence intensity of antigen-positive T cells is a measure of their TCR expression levels.

Using Immudex products for release testing of the infusion product

To meet the regulatory requirements of release testing, Immudex produces the Clinical-Grade Dextramer® (GMP) reagents for the reliable and unbiased determination of identity, purity, and number of antigen-specific T cells in a production lot. Clinical-Grade Dextramer® (GMP) reagents undergo an extensive number of in-process and final product quality control checks. They have a defined shelf life, stated expiry date, and a certificate of analysis that makes them suitable for the manufacturing and quality control of investigational and commercial pharmaceutical products.

Release Testing using MHC Dextramer®

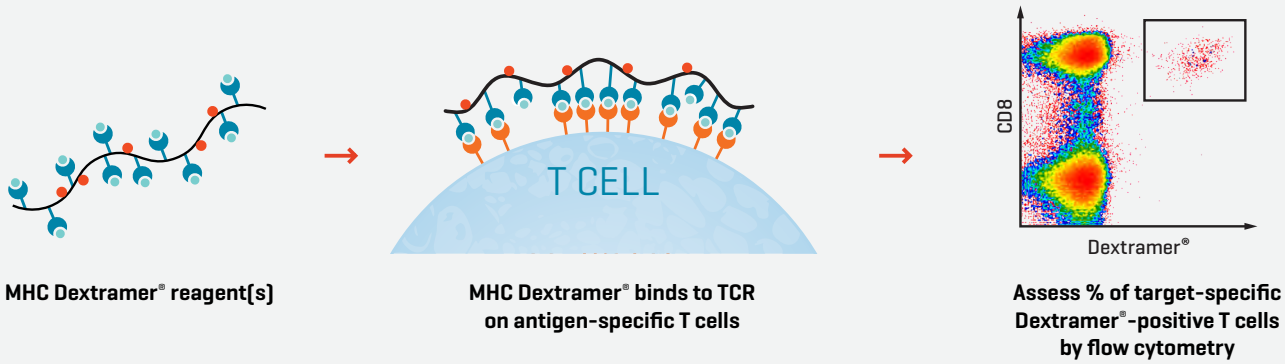


Figure 6: Release testing of the infusion product. Clinical-grade Dextramer® (GMP) reagents enable both the identity and purity of the T cells to be analyzed in a single step.

Important considerations

Aim	Immudex product
Count well, because numbers matter	Accurate enumeration of the number of antigen-specific T cells in a lot is essential for clinical trials to ensure that variation in outcomes is not attributable to differences in cells infused. Furthermore, administering too high a dose of cells can lead to toxicity. Make sure your testing delivers unbiased cell numbers for accurate dosing and minimized risk of side effects. Some therapeutic strategies involve combining T-cell specificities. The performance of the combination will depend on the right balance of specificities, making an accurate cell count all the more important. Also bear in mind that a limited number of cells can be infused, and clinically significant effects may require a minimum number of delivered T cells.
Know what your product delivers	It is imperative to ensure that your infusion product is delivering T cells of the correct specificity. Even in TCR-T cell therapies that reinfuse whole populations rather than isolating antigen-specific T cells, you will need to know the percentage of therapeutically functional T cells in the population.

CONTACT IMMUDEX customer@immudex.com

Step 4. Precision immune monitoring of patient response

For this final step, your infusion product is in clinical trials and your goal is to track how well the TCR-T cell therapy is working in patients. Furthermore, most FDA-approved and investigational immunotherapies for cancer are designed to augment, activate, or replace antigen-specific T cell immunity. Thus, experimentally detecting antigen-specific responses in T cells is an essential readout in demonstrating the efficacy and investigating possible side effects of your product.

Very precise monitoring of antigen-specific T cells is needed to track the long-term performance of the infusion product and correlate its function to patient response and activation of endogenous immune cells. Numerous questions are explored during monitoring, like what happens to the therapeutic TCR-T cells, do side effects emerge after time, and what response biomarkers correlate with the persistence of TCR-T cells, overall patient survival, and other clinical goals.

Approach and methods

1. **Endogenous T cell response and tracking of TCR-T cells**

   Monitor the kinetics and persistence of the infused T cells in patient blood samples[54]. Use multiparametric flow cytometry to screen endogenous cellular immune responses. Employ a repertoire of antigens to capture and thoroughly characterize important T cell population subsets. To distinguish and identify the therapeutic TCR-T cells, use single-cell multi-omics, which also allows you to determine which clonotypes are expanding. Finally, it may be possible to determine the level of pMHC expression on patient APCs by e.g. flow cytometry to assess possible target antigen loss.

2. **Identification of clinically relevant response biomarkers**

   Examine numerous biomarkers that distinguish responder patients from non-responders. For example, determine the presence of T cells with certain characteristics or mRNA expression profiles. Depending on the biomarkers examined, a battery of methods may be necessary, but results should be connected to clinical goals and the behavior of the tracked therapeutic T cells.

3. **Determination of epitope spreading and post-infusion T cell phenotypes**

   In the case that a biopsy of target tissue (e.g., tumor tissue) is available after infusion, T cell clones can be isolated and phenotyped to assess functional changes. Such a biopsy also allows determining if novel epitopes develop that enhance the activity of the TCR-T cell therapy. Use flow cytometry with the target pMHC and a set of new epitopes (characterized from the biopsy or predicted) to detect binding to the infused TCR T cells.

Precision Immune Monitoring using Dextramer® Technology

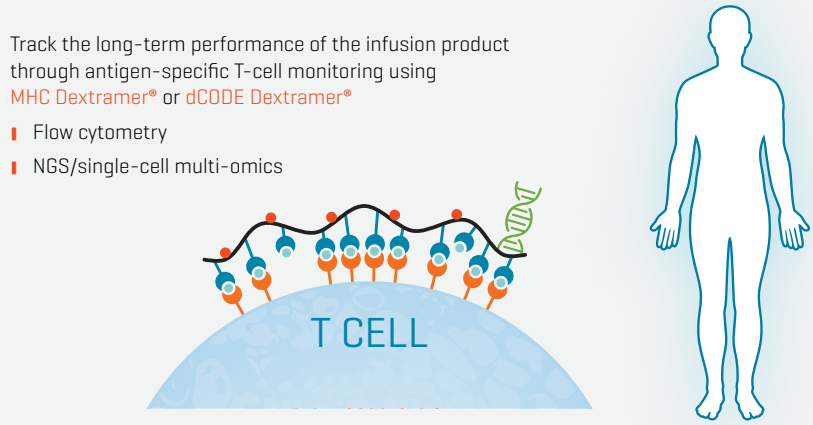


Figure 7: Precision immune monitoring of patient response in clinical trials of TCR-T cell therapies.

Using Immudex products for precision immune monitoring

The breadth of parameters assessed in clinical trials calls for a wide range of assays and reagents. However, all are linked to the behavior of the infused TCR-T cells and endogenous immune responses. Thus, the highly sensitive and accurate detection of various immune cell subpopulations is the core in achieving the goal of this step. This is where Dextramer® reagents excel. With high avidity, strong fluorescent signal, and broadly flexible configuration, Dextramer® reagents enable the detailed investigation of antigen-specific responses in patients.

Important considerations

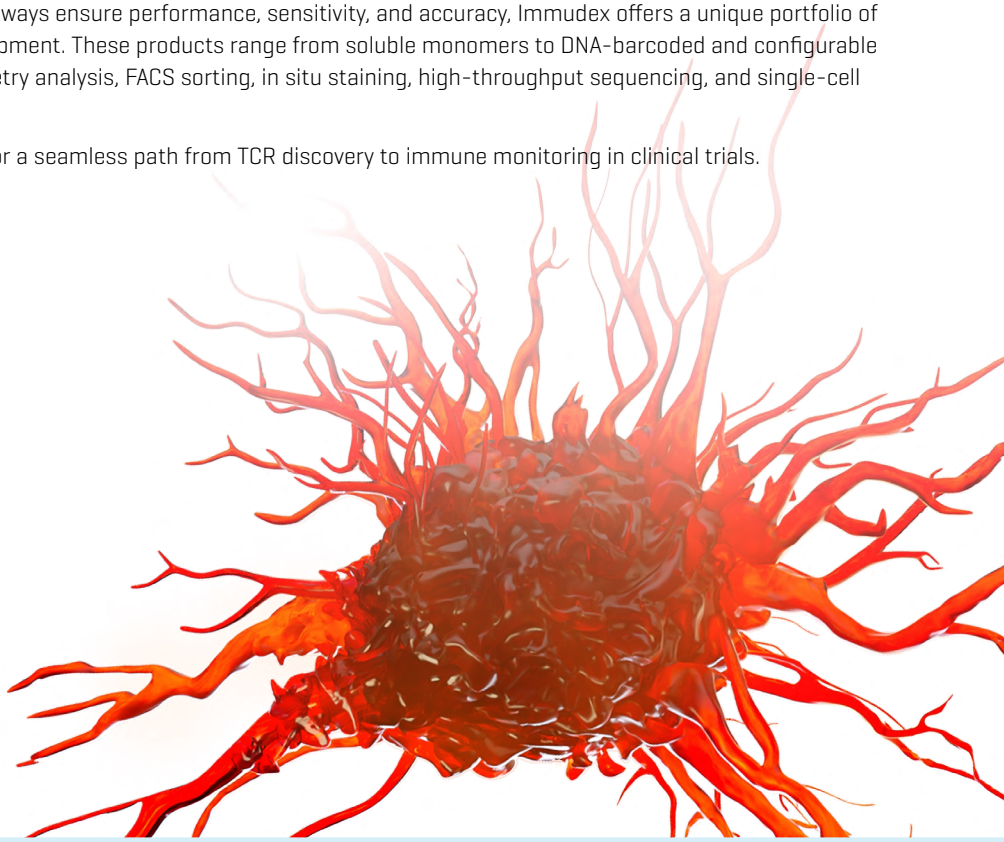
Aim	Immudex product
Perform comprehensive monitoring	Monitoring CD8+ T cells or the CD4+/CD8+ ratio is insufficient to properly characterize patient response. For example, CD4+ T cells are dominant in anti-tumor responses and associated with response patterns of immune checkpoint therapies. Experience with CAR-T cell therapy has also highlighted the importance of comprehensively evaluating a diversity of immune cell responses. Bear in mind that most therapies are target-specific. A tumor under attack by T cells, however, may downregulate that target and come back with new cancerous cells lacking the target. That escape mechanism is worth monitoring
Look for epitope spreading	Epitope spreading is a topic for solid tumors, especially those that exhibit heterogenous anatomy with different layers and a hypoxic center. In cancer, the diversification of epitope specificity from the initial target to other epitopes can boost or extend the efficacy of the therapy. .

Conclusion

The development of TCR-based therapeutics to tackle cancer and other diseases opens a vast landscape of possibilities in coaxing T cells into serving medicinal purposes. Clinical trials presage advances on the horizon, while research reveals new approaches beyond current strategies. The promise of each development hinges on ascertaining the nature, scope, and side effects of the TCR-pMHC interaction. Thus, the tools used to characterize that interaction must accommodate the diverse potential of TCR-based therapies as well as novel means of discovery, optimization, and manufacture.

Building on Dextramer® Technology to always ensure performance, sensitivity, and accuracy, Immudex offers a unique portfolio of flexible reagents to advance TCR development. These products range from soluble monomers to DNA-barcoded and configurable multimers. They are used in flow cytometry analysis, FACS sorting, in situ staining, high-throughput sequencing, and single-cell multi-omics.

Finally, the portfolio is conceptualized for a seamless path from TCR discovery to immune monitoring in clinical trials.



CONTACT IMMUDEX customer@immudex.com

References

1. Weber, E. W., Maus, M. v. & Mackall, C. L. The Emerging Landscape of Immune Cell Therapies. *Cell* **181**, 46–62 (2020).
2. Shafer, P., Kelly, L. M. & Hoyos, V. Cancer Therapy With TCR-Engineered T Cells: Current Strategies, Challenges, and Prospects. *Front Immunol* **13**, 781 (2022).
3. Mohammadi, M., Akhoundi, M., Malih, S., Mohammadi, A. & Sheykhhasan, M. Therapeutic roles of CAR T cells in infectious diseases: Clinical lessons learnt from cancer. *Reviews in Medical Virology* Preprint at https://doi.org/10.1002/rmv.2325 (2022).
4. de Sousa, E. *et al.* Targeting Neoepitopes to Treat Solid Malignancies: Immunosurgery. *Front Immunol* **12**, (2021).
5. Cózar, B. *et al.* Tumor-Infiltrating Natural Killer Cells. *Cancer Discov* **11**, 34–44 (2021).
6. Liu, S. *et al.* NK cell-based cancer immunotherapy: from basic biology to clinical development. *Journal of Hematology & Oncology 2021 14:1* **14**, 1–17 (2021).
7. Mensali, N. *et al.* NK cells specifically TCR-dressed to kill cancer cells. *EBioMedicine* **40**, 106–117 (2019).
8. Liu, Y. *et al.* iNKT: A new avenue for CAR-based cancer immunotherapy. *Translational Oncology* vol. **17** Preprint at https://doi.org/10.1016/j.tranon.2022.101342 (2022).
9. Page, A., Hubert, J., Fusil, F. & Cosset, F. L. Exploiting B cell transfer for cancer therapy: Engineered B cells to eradicate tumors. *International Journal of Molecular Sciences* vol. 22 Preprint at https://doi.org/10.3390/ijms22189991 (2021).
10. Page, A. *et al.* Efficient adoptive transfer of autologous modified B cells: a new humanized platform mouse model for testing B cells reprogramming therapies. *Cancer Immunology, Immunotherapy* **71**, 1771–1775 (2022).
11. Cheever, M. A. & Higano, C. S. PROVENGE (sipuleucel-T) in prostate cancer: The first FDA-approved therapeutic cancer vaccine. *Clinical Cancer Research* **17**, 3520–3526 (2011).
12. Liau, L. M. *et al.* First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J Transl Med* **16**, 1 (2018).
13. Plumas, J. Harnessing dendritic cells for innovative therapeutic cancer vaccines. *Curr Opin Oncol* **34**, 161–168 (2022).
14. Filin, I. Y., Kitaeva, K. v., Rutland, C. S., Rizvanov, A. A. & Solovyeva, V. v. Recent Advances in Experimental Dendritic Cell Vaccines for Cancer. *Front Oncol* **11**, (2021).
15. Karvouni, M., Vidal-Manrique, M., Lundqvist, A. & Alici, E. Engineered NK Cells Against Cancer and Their Potential Applications Beyond. *Front Immunol* **13**, 213 (2022).
16. First CAR T-cell therapy recommended on NHS. *European Pharmaceutical Review* https://www.europeanpharmaceuticalreview.com/news/178922/first-car-t-cell-therapy-recommended-on-nhs/ (2023).
17. Jaber, N. Carvykti Approval Marks Second CAR T-Cell Therapy for Multiple Myeloma. *National Cancer Institute* https://www.cancer.gov/news-events/cancer-currents-blog/2022/fda-carvykti-multiple-myeloma (2022).
18. Atara claims first world approval for off-the-shelf T-cell therapy. *Pharmaphorum* https://pharmaphorum.com/news/atara-claims-first-world-approval-for-off-the-shelf-t-cell-therapy/ (2022).
19. FDA approves tebentafusp-tebn for unresectable or metastatic uveal melanoma. *FDA* https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-tebentafusp-tebn-unresectable-or-metastatic-uveal-melanoma (2022).
20. Mullard, A. FDA approval of Immunocore's first-in-class TCR therapeutic broadens depth of the T cell engager platform. *Nat Rev Drug Discov* **21**, 170 (2022).
21. Rafiq, S., Hackett, C. S. & Brentjens, R. J. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nature Reviews Clinical Oncology* vol. 17 147–167 Preprint at https://doi.org/10.1038/s41571-019-0297-y (2020).
22. Abrantes, R., Duarte, H. O., Gomes, C., Wälchli, S. & Reis, C. A. CAR-Ts: new perspectives in cancer therapy. *FEBS Letters* Preprint at https://doi.org/10.1002/1873-3468.14270 (2022).
23. Kingwell, K. T cell receptor therapeutics hit the immuno-oncology stage. *Nat Rev Drug Discov* (2022) doi:10.1038/D41573-022-00073-7.
24. Luk, A. From leukemia to cancer-free: how CAR-T immunotherapy saved Emily Whitehead. *Parker Institute for Cancer Immunotherapy* https://www.parkerici.org/the-latest/from-leukemia-to-cancer-free-how-car-t-immunotherapy-saved-emily-whitehead/ (2022).
25. Sanderson, J. P. *et al.* Preclinical evaluation of an affinity-enhanced MAGE-A4-specific T-cell receptor for adoptive T-cell therapy. *Oncoimmunology* **9**, (2020).
26. Linette, G. P. *et al.* Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* **122**, 863 (2013).
27. Salter, A. I. *et al.* Comparative analysis of TCR and CAR signaling informs CAR designs with superior antigen sensitivity and in vivo function. *Sci Signal* **14**, (2021).
28. Harris, D. T. *et al.* Comparison of T Cell Activities Mediated by Human TCRs and CARs That Use the Same Recognition Domains. *J Immunol* **200**, 1088–1100 (2018).
29. Ajina, A. & Maher, J. Strategies to Address Chimeric Antigen Receptor Tonic Signaling. *Mol Cancer Ther* **17**, 1795–1815 (2018).
30. Xiang, R. *et al.* Increased expression of peptides from non-coding genes in cancer proteomics datasets suggests potential tumor neoantigens. *Communications Biology 2021 4:1* **4**, 1–12 (2021).
31. Boehringer Ingelheim and Enara Bio enter Strategic Collaboration and Licensing Agreement to discover novel shared antigens for cancer immunotherapies. *EnaraBio* (2021). https://enarabio.com/news/boehringer-ingelheim
32. Morton, L. T. *et al.* T cell receptor engineering of primary NK cells to therapeutically target tumors and tumor immune evasion. *J Immunother Cancer* **10**, e003715 (2022).
33. Dhatchinamoorthy, K., Colbert, J. D. & Rock, K. L. Cancer Immune Evasion Through Loss of MHC Class I Antigen Presentation. *Front Immunol* **12**, 469 (2021).
34. Mansilla-Soto, J. *et al.* HLA-independent T cell receptors for targeting tumors with low antigen density. *Nature Medicine 2022 28:2* **28**, 345–352 (2022).
35. Jæhger, D. Adoptive transfer of T cells surface-tethered with IL-12 promotes antigen spreading for enhanced anti-tumor efficacy. *SITC* https://s3.us-east-1.amazonaws.com/repertoire-assets.investeddigital.com/downloads/2020-11-SITC-DTU-Cell-Tethered-IL-12-antigen-spreading.pdf (2020).
36. Zhang, X., Qi, Y., Zhang, Q. & Liu, W. Application of mass spectrometry-based MHC immunopeptidome profiling in neoantigen identification for tumor immunotherapy. Biomed *Pharmacother* **120**, (2019).
37. Newey, A. *et al.* Immunopeptidomics of colorectal cancer organoids reveals a sparse HLA class I neoantigen landscape and no increase in neoantigens with interferon or MEK-inhibitor treatment. *J Immunother Cancer* **7**, (2019).
38. Wilson, E. A. & Anderson, K. S. Lost in the crowd: identifying targetable MHC class I neoepitopes for cancer immunotherapy. *Expert Rev Proteomics* **15**, 1065–1077 (2018).
39. Jappe, E. C. *et al.* Thermostability profiling of MHC-bound peptides: a new dimension in immunopeptidomics and aid for immunotherapy design. *Nat Commun* **11**, (2020).

40. Zhang, W. *et al. A framework for highly multiplexed dextramer mapping and prediction of T cell receptor sequences to antigen specificity. Sci. Adv* vol. 7 (2021).

41. Bonaventura, P. *et al.* Identification of shared tumor epitopes from endogenous retroviruses inducing high-avidity cytotoxic T cells for cancer immunotherapy. *Sci Adv* **8**, (2022).

42. Silva, D. N. *et al.* Process Development for Adoptive Cell Therapy in Academia: A Pipeline for Clinical-Scale Manufacturing of Multiple TCR-T Cell Products. *Front Immunol* **13**, 2842 (2022).

43. Vazquez-Lombardi, R. *et al.* High-throughput T cell receptor engineering by functional screening identifies candidates with enhanced potency and specificity. *Immunity* **55**, 1953-1966.e10 (2022).

44. Bunse, M. *et al.* CXCR5 CAR-T cells simultaneously target B cell non-Hodgkin's lymphoma and tumor-supportive follicular T helper cells. *Nature Communications* 2021 12:1 **12**, 1–19 (2021).

45. Ma, Q. *et al.* A novel TCR-like CAR with specificity for PR1/HLA-A2 effectively targets myeloid leukemia *in vitro* when expressed in human adult peripheral blood and cord blood T cells. *Cytotherapy* **18**, 985–994 (2016).

46. Bentzen, A. K. *et al.* T cell receptor fingerprinting enables in-depth characterization of the interactions governing recognition of peptide–MHC complexes. *Nat Biotechnol* **36**, 1191–1196 (2018).

47. Yarmarkovich, M. *et al.* Cross-HLA targeting of intracellular oncoproteins with peptide-centric CARs. *Nature* **599**, 477–484 (2021).

48. Zhu, X. *et al.* Visualization of p53264–272/HLA-A*0201 Complexes Naturally Presented on Tumor Cell Surface by a Multimeric Soluble Single-Chain T Cell Receptor. *The Journal of Immunology* **176**, 3223–3232 (2006).

49. Rapoport, A. P. *et al.* NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nat Med* **21**, 914–921 (2015).

50. Walseng, E. *et al.* A TCR-based Chimeric Antigen Receptor. *Scientific Reports 2017 7:1* **7**, 1–10 (2017).

51. Proics, E. *et al.* Preclinical assessment of antigen-specific chimeric antigen receptor regulatory T cells for use in solid organ transplantation. *Gene Therapy 2022* 1–14 (2022) doi:*10.1038/s41434-022-00358-x*.

52. Foy, S. P. *et al.* Non-viral precision T cell receptor replacement for personalized cell therapy. *Nature 2022* 1–10 (2022) doi:*10.1038/s41586-022-05531-1*.

53. Stevens, J. *et al.* A closed, autologous bioprocess optimized for TCR-T cell therapies. *Authorea Preprints* (2022) doi:*10.22541/AU.165727810.00978327/V1*.

54. Hong, D. S. *et al.* Autologous T cell therapy for MAGE-A4+ solid cancers in HLA-A*02+ patients: a phase 1 trial. *Nature Medicine 2023 29:1* **29**, 104–114 (2023).

Resources

Cell Therapy

Explore how Dextramer® reagents support the development and manufacturing of effective cell therapies.

Learn more:*immudex.com/cell-therapy*

MHC Dextramer®

- Sensitive and reliable detection of antigen-specific T cells

Learn more: *immudex.com/dextramer*

TCR Discovery

Explore how TCR discovery can advance the development of novel T-cell therapies.

Learn more: *immudex.com/tcr-discovery*

Clinical-Grade (GMP) Dextramer®

- Extended battery of QC checks
- Established shelf-life

Learn more: *immudex.com/dextramer-gmp*

Case Studies and Application Notes

Immerse yourself in educational content exploring the applications of Dextramer® technology.

Learn more: *immudex.com/education*

dCODE Dextramer®

- Analysis of antigen-specific T cells by NGS or single-cell multi-omics

Learn more: *immudex.com/dCODE*

TCR Solutions

- Soluble TCR Monomers
- TCR Dextramer®

Learn more: *immudex.com/tcr-solutions*

MHC Monomers

- Ready-to-Use Monomers
- Peptide-Receptive Solutions

Learn more: *immudex.com/monomers*

Contact us

Immudex ApS
Bredevej 2A
2830 Virum
Denmark

Email: customer@immudex.com
Tel.: +45 29 13 42 24

For research use only. Not for use in diagnostic or therapeutic procedures.

MM0140.01