CHAPTER 13

T Cell Receptor Engineered T Cell Therapy in Oncology

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INTRODUCTION

After over a century of debate, it is well accepted today that the immune system is capable of successfully eradicating abnormal cells from the body and that it shapes the evolution of established tumours (Breakthrough, 2013; Dunn et al., 2004; Nauts et al., 1946). In the past two decades, the ability to break immunological tolerance and provide or induce tumour-specific immune responses with significant clinical benefit has been clearly demonstrated. The discovery of immune signalling pathways that can be modulated for therapeutic benefit in oncology resulted in the 2018 Nobel Prize for Medicine (Nobel Prize, 2018). Consequently, the field of immuno-oncology (IO) has exploded in the past decade, and there are now 14 products approved in the United States and Europe including oncolytic virus therapy (talimogene laherparepvec), dendritic cell vaccine therapy (sipuleucel-T), monoclonal antibodies (numerous), cytokine therapy (aldesleukin, interferon) and engineered T cell therapy (tisagenlecleucel and axicabtagene ciloleucel). A deep pipeline of new IO modalities is coming rapidly behind with over 240 new IO agents in development (Pharmaceutical Research and Manufacturers of America TACSCAN, 2017).

T cell therapy has roots in oncology due to the practice of transplantation. In the second half of the last century, bone marrow and stem cell transplants were employed to rescue patients following high dose chemotherapy and to leverage the balance between graft-versus-tumour and graft-versus-host disease to improve outcomes (Horowitz and Bortin, 1990; Margolin et al., 1997; Robertson, 1993; Champlin et al., 2001; Dickinson et al., 2017). This established experience around elements of adoptive T cell therapy, including preinfusion conditioning, cell product freezing and administration and regulatory guidance overlaying these aspects. Expansion of this specialised infrastructure beyond haematology–oncology into other oncology specialties is maturing into what has been named the 'fourth pillar of the global healthcare industry', following pharmaceuticals, biologics and medical devices (Mason et al., 2011).

Adoptive T cell therapy, of which engineered T cell therapy is a subset, is unique in IO in that it has the ability to overcome intrinsic defects in the immune system and

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provide precision targeting to tumours in a way that most other modalities cannot. This therapy became clinically feasible with the emergence of effective viral vector systems able to deliver therapeutic genes with high efficiency. Response rates reported in up to 80% in treatment-resistant tumours have illustrated the potency of this approach, as have a subset of cases of acute off-tumour toxicities, as reviewed later in this chapter (Brudno et al., 2018; D'Angelo et al., 2018b; Linette et al., 2013; Maude et al., 2018; Morgan et al., 2013; Neelapu et al., 2017).

The engineered T cell therapy field has seen significant industry investment since 2011 when Novartis (Basel, Switzerland) made its initial investment in the CD19 chimeric antigen receptor (CAR) programme at the University of Pennsylvania (Philadelphia, PA, USA), following publication of responses in just three patients (Kalos et al., 2011). This spawned a series of significant investments in the space leading to the formation of Juno Therapeutics (Seattle, WA, USA) and Kite Pharma (Los Angeles, CA, USA) in 2012, the partnership of Adaptimmune Therapeutics (Philadelphia, PA, USA and Oxford, UK) with GlaxoSmithKline (GSK) (Brentford, UK), formation of an alliance between Celgene Corporation (Summit, NJ, USA) and bluebird bio (Cambridge, MA), among many others. Novartis's tisagenlecleucel licencing approval for childhood leukaemia came in late 2017, with subsequent approval in diffuse large B cell lymphoma. The second approval in early 2018 was Kite's axicabtagene ciloleucel in B cell lymphoma. The regulatory validation led to the acquisition of Kite by Gilead Sciences (Foster City, CA) for $11.9B in 2017 (Gilead Sciences, 2017), followed closely behind by Celgene's acquisition of Juno for $9B (Celgene, 2018) and the exercise of the GSK option on the Adaptimmune NY-ESO-1 programme. As of mid-2018, there are an estimated 375–497 cell therapy clinical trials in oncology (Tang et al., 2018a; Preti, 2018). Today, there is significant investor interest in this space (Smith et al., 2018). In 2016, $1.7B was raised to support gene and gene-modified cell therapy, which increased to $4.5B in 2017 (Preti, 2018).

T cells generally are engineered for tumour specificity in two ways, through T cell receptors (TCRs) or through CARs. TCRs are the natural receptors that enable the T cell to identify targets, and using them preserves the biological signalling machinery and associated checks and balances of the T cell. CARs are synthetic receptors comprising an extracellular antibody domain for target binding, linked to a transmembrane domain and intracellular signalling chains, which are designed to replicate both TCR signalling and the required costimulatory signalling. These CARs have been reviewed in a separate chapter in this book and elsewhere (June et al., 2018). Since the catalysing events of 2011, the primary industry interest has been with CARs, due to challenges inherent to TCR generation and testing, the independence of CARs from human leukocyte antigen (HLA) restriction and the ability to engineer costimulatory signalling into the CAR. This focus was fuelled by transformative data emerging from various studies in B cell malignancies (Brudno et al., 2018; Lee et al., 2015; Locke et al., 2017; Turtle et al., 2016).

In fact, many erroneously use the term 'CAR' as the moniker for all engineered T cell therapy. However, significant antitumour effects outside of haematologic malignancy, or 'liquid tumours', have not been reported with CARs. In addition, tumour-specific targets, expressed on the cell surface and thus accessible to CARs, are more limited than for TCRs.

These challenges have fuelled a recent surge of interest in TCR-engineered T cells (Lee et al., 2015). Although there is much overlap with CAR technology in terms of manufacturing platform and clinical delivery and management, there are important differences in the discovery and translation of TCRs. We review in this chapter the current state for discovery, clinical data, development considerations and manufacturing for this modality and provide future-looking perspectives for this space.

T CELL RECEPTOR STRUCTURE AND FUNCTION

The TCR is a transmembrane heterodimer comprising an α and a β chain, which assemble inside the cell, are expressed together and are connected by a membrane proximal disulfide bond at the cell surface. To be fully functional, the α and β chains must complex with the invariant CD3-ε, CD3-δ and CD3-γ chains on the cell surface as well as the ξ chain (Fig 13.1A). When identifying a TCR for use in engineering T cells, the α and β chains are provided by gene transfer, and the cell provides the remainder of the complex. The target for a TCR is the HLA, which binds in its groove a short amino acid sequence derived from any protein (foreign or self) which may be expressed within the cell ('class I-restricted', and which is naturally recognised by $CD8^+$ T cells) or taken up by the cell from the external environment ('class II-restricted', and which is naturally recognised by $CD4^+$ T cells) (Fig 13.1B,C).

The short amino acid sequences, or peptides, are generated by the proteasome within each cell and may vary by the type of cell processing the antigen. The transporter associated with antigen processing, or TAP, then brings peptides into the endoplasmic reticulum of the cell, which is a specialised organelle for preparing proteins for cell surface expression or secretion. Peptides are folded into HLA molecules with the help of a series of chaperone proteins, and then the peptide–HLA complex (pHLA) is expressed on the cell surface. Several reviews that describe this process in detail are available (Monaco, 1995; Natarajan et al., 2018). The peptide profile and diversity, as well as efficiency of pHLA formation and surface presentation, is significantly altered in the presence of inflammation and formation of the immunoproteasome, which contains different subunits to the proteasome that routinely functions within cells to enable protein homoeostasis and general immune surveillance (Aki et al., 1994; Ferrington and Gregerson, 2012).

Class I HLA molecules have a broad distribution across tissues in the body with few exceptions, including neurons and some reproductive tissues. Class II HLA molecules have a much more restricted expression pattern. Class II HLA molecules are present on

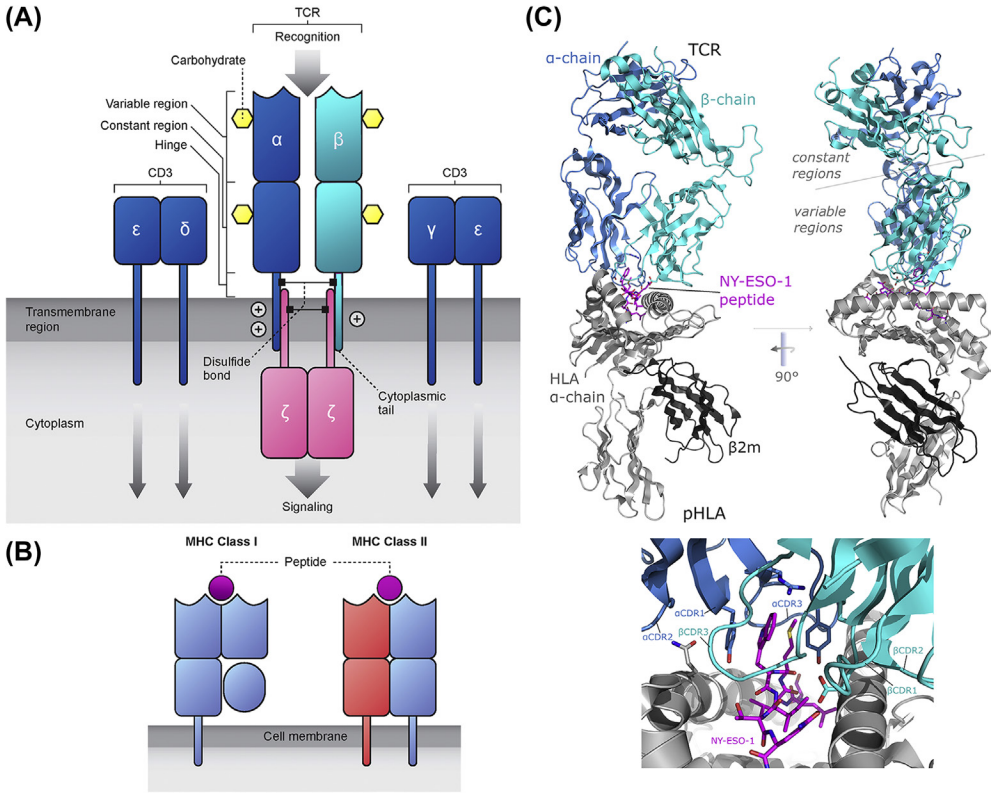

Figure 13.1 T cell receptor (TCR) and peptide–major histocompatibility complex (MHC) interface. (A) Cartoon depicting the α and β chains of the TCR and the association with the receptor complex involving CD3-ε, CD3-δ and CD3-γ chains on the cell surface as well as the ξ chain. (B) Cartoon depicting class I and class II MHC molecules. Class I MHC is composed of an α chain comprising three domains, α1, α2 and α3 (upper right, upper left and lower left, respectively). Expression is stabilised by β2 microglobulin (lower right). Class II MHC is composed of an α and β chain, each with two domains (α1, α2 and β1, β2). (C) Annotated ribbon diagram for the X-ray crystallographic structure of a TCR binding to an HLA-A*02:01 (class I)–restricted pHLA construct presenting the NY-ESO-1 peptide (PDB accession code: 2BNQ) (Chen et al., 2005). The right panel is a 90-degree rotated view of the left. Below is a zoom into the TCR–pHLA interface, showing the orientation of the peptide within the peptide-binding cleft of the HLA molecule, and the peptide and HLA interactions with the TCR complementarity-determining region (CDR) loops, with some key residues depicted (as sticks).

the surface of 'professional antigen-presenting cells', which are specialised cells designed to drive the activation of the adaptive immune response, as well as on some endothelial cells and in the thymus, where they drive T cell development. HLA Class II can be upregulated during inflammation on other tissues (Roche and Furuta, 2015). Many of the neoantigen responses to tumour cells are class II restricted (Linnemann et al., 2015; Marty et al., 2018). Although the TCR repertoire diversity is large (with the potential to

create between 10^{15} and 10^{20} clonotypes), the size of the post-thymic population of T cells in peripheral blood is approximately 10^{12}, containing approximately 10^{7} unique TCRs (Miles et al., 2011; Nikolich-Zugich et al., 2004). The TCR is evolutionarily optimised to recognise the combined peptide HLA complex, contacting both the HLA and peptide at the same time and activating the T cell only when both are properly engaged. Antibody recognition of pHLA has been attempted by some groups, the specificity and hence safety of this approach has not consistently been reported, although the first clinical trial testing this approach has yielded favourable preliminary results (Dao et al., 2013; Liu et al., 2017; Eureka Therapeutics, 2018). Tumours generally express class I HLA, although HLA loss is a known resistance mechanism (Algarra et al., 2004; Aptsiauri et al., 2018; Boegel et al., 2018; McGranahan et al., 2017). Class II HLA is expressed on approximately 50% of tumours (Campoli and Ferrone, 2008). Screening for tumour HLA expression may become an important requirement for eligibility.

Both class I– and class II–restricted TCRs have been clinically tested, although the majority in trials to date are class I restricted (reviewed in more detail in the next section). Class I HLA complexes are encoded by the HLA-A, HLA-B and HLA-C loci. Class II HLA complexes are encoded by the HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ and HLA-DR loci. Across these genes, there are thousands of alleles. However, a few alleles dominate the population for both class I and class II HLA. Generating TCRs to cover three class I HLA alleles is anticipated to cover about 70% of the US and European population (Fig. 13.2). Numbers are similar for class II HLA

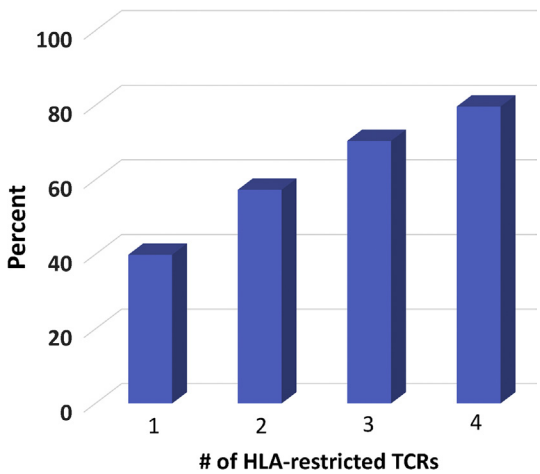

Figure 13.2 Population coverage with multiple T cell receptors (TCRs). This graph represents the percentage of the population that can potentially benefit from a TCR therapeutic. Shown as an example are numbers derived from the class I human leukocyte antigen (HLA) in the US and EU population. The first *bar* represents the frequency of HLA-A*02:01, the most frequent HLA allele in this population. Subsequent *bars* illustrate the nonredundant additive effect of additional HLA-restricted TCRs.

alleles. The allelic overlap for a similar coverage in China, Japan or Korea is similar but would involve alternate HLA, and so to cover a majority of the world population, up to five HLA-restricted TCRs may be required. Developing TCRs restricted to allele superfamilies, which are phylogenetically related HLA particularly in the regions of TCR and peptide binding, could potentially increase population coverage (Harjanto et al., 2014).

The majority of the TCR chains (~95%) are derived from the TCR α and β loci ('α/β T cells'), and the remaining 5% are derived from the γ and δ loci ('γ/δ T cells'). γ/δ T cells are part of the innate immune system and recognise molecular patterns associated with bacteria, fungi and viruses and are not thought to be subject to HLA restriction. γ/δ T cell therapies are in the early stages of clinical development. The majority of work in the TCR field today has utilised α/β T cells, which are capable of the specific recognition of self and foreign protein targets and are the subject of this review.

TCR chains contain constant regions that are shared across members, as well as variable chains. Each variable chain contains three complementarity-determining regions (CDRs) named CDR1, CDR2 and CDR3, which constitute the target-binding domain of the molecule and govern the TCR-binding variability. CDR3 and CDR1 primarily contact the peptide, and CDR2 primarily contacts the HLA molecule. These regions may be modified to enhance pHLA binding affinities and specificities. The use of higher affinity TCRs for effective tumour immunotherapy is thought by many to improve anti-tumour responses (Aleksic et al., 2012; Schmitt et al., 2017; Tan et al., 2015) (further discussed in the subsequent section, Clinical Experience With T Cell Receptor Engineered T Cells).

In the presence of pHLA, microclusters of engaged TCR begin to form on the T cell surface, and these ultimately come together in the presence of coreceptors to form an immunologic synapse, containing not only the TCR but also costimulatory molecules necessary to activate and prevent anergy of the T cell. This dynamic of microcluster and synaptic formation drives what is known as functional avidity of the T cell, which is the ability of the T cell to recognise and respond to antigen. Functional avidity can be quite sensitive despite a fairly low TCR–pHLA affinity (Slifka and Whitton, 2001). TCRs have been shown to be exquisitely sensitive sensors of target antigen, with fewer than 10 target antigens capable of triggering cytokine production and cytotoxicity (Purbhoo et al., 2004). This is far more sensitive than current generation CARs, several of which have been shown to have a threshold activation limit of thousands of targets (Walker et al., 2017), although the sensitivity is likely to differ based on the antibody affinity and signalling mechanism. The greater sensitivity of TCRs may result from the longer dwell times, formation of an organised immunological synapse (although this is not required for activity), and longer and more robust signalling in TCR-activated T cells (Davenport et al., 2018).

IDENTIFYING TARGETS AND DEVELOPING T CELL RECEPTORS FOR CLINICAL USE

Analyses of the human proteome predict that approximately 30% of all proteins contain transmembrane regions, and only a subset of those would contain extracellular domains capable of targeting by antibodies (Almen et al., 2009). Cell surface markers tend to have shared functions on normal tissues or be lineage-specific antigens expressed on a tissue subset. Examples of the latter include CD19, CD20, CA125, PSCA, PSMA as well as others. When expressed on nonessential tissues, such targets are attractive for cancer immunotherapy. Notwithstanding this, the majority of tumour-specific antigens are expressed intracellularly (Table 13.1). Targeting major epithelial cancer indications such as lung and colon cancer may require the targeting of intracellular targets, as cell surface lineage antigens have not been well tolerated in these indications (Morgan et al., 2010; Parkhurst et al., 2011).

There are several different classes of antigens that are targetable by TCRs (Table 13.1). Characteristics of an ideal tumour antigen are thought to be (1) specificity for the tumour, (2) a high level of presentation on the cell surface, (3) requirement for tumour cell survival (driver gene product) and (4) presence in all tumour cells ('clonal expression pattern', although tumour cells may not be clonal). Therefore, the ideal tumour antigen is thought to be 'clonal' driver genes, which to date have primarily been characterised as

Table 13.1 Classes of Antigens Targetable by T Cell Receptors.

Antigen Type	Intracellular (I) Extracellular (E)	Examples[a]	References
Cancer testis	I	NY-ESO-1[a], MAGE-A3[a], MAGE-A4[a], MAGE-A10[a]	(Walker et al., 2000; Medicine, 2018)
Oncofetal	I	AFP	(Melchers, 2012)
Lineage	I, E	CD19[a], CD20[a], CEAa, HER2neua, gp100[a]	(Tufts Center for the Study of Drug Development, 2016; Tang et al., 2018a,b)
Alternate splice	I		(Chen et al., 2005; Fendly et al., 1990)
Neoantigen	I	BRAF, m-KRAS[a], SSX-1	See Table 13.3
Viral	I	EBV[a], HPV[a]	(Tashiro and Brenner, 2017; Arsic et al., 2015)
Edited RNA	I	Cyclin I	(Sah and Seniya, 2015)
Oncogene, mutated tumour suppressor	I	WT-1[a], mp53	(Kanodia et al., 2008)

[a]Tested in the clinic.

Table 13.2 Mutation Frequencies.

Gene Mutation Locus	Cancer Indication	Cancer Incidence (USA)	Cancer Mortality (USA)	Mutation Frequency (%)
EGFR V30_R297del/ viii/de2-7EGFR	GBM	18,000	13,000	25–64
	BRCA	25,2710	40,610	68
BRAF V600E	COAD + READ	128,660	47,750	10–20
	SKCM	87,110	9730	44
AKT1 E17K	BRCA	252,710	40,610	3–10
	COAD + READ	128,660	47,750	6
PIK3CA H1047R	BRCA	252,710	40,610	13.5
	COAD + READ	128,660	47,750	1.8–3.6
PIK3CA E542K	BRCA	252,710	40,610	5.3
	COAD + READ	128,660	47,750	3.9 (2.8–8.4)
EGFR L858R	LUAD	75,650	52,996	4.9
IDH1 R132H	GBM	18,000	13,000	6.1
	LGG	2000–3000	–	70.4
	AML	21,380	10,590	8–13
DNMT3A R882H	AML	21,380	10,590	10.7
H3F3A and HIST1H3B K27M/ K28M	DIPG paediatric glioma	160–240	160–240	95
GNAS R201C	Pituitary cancer	14,230	–	20
	Intraductal pancreatic	4025	–	41–66
TP53 R175H	COAD + READ	128,660	47,750	6.6
	PAAD	53,670	43,090	2.7
	BRCA	252,710	40,610	1.7
	LUAD	75,650	52,996	1

Data on mutation frequencies: combination of IntOGen, COSMIC and literature.
GBM frequency: https://www.roche.com/dam/jcr:f2283374-01e4-4050-9461-fc8f03c49738/en/backgrounder_glioblastoma__concise_guide.pdf.
Pituitary tumour frequency: https://www.cancer.net/cancer-types/pituitary-gland-tumor/statistics; Freq = 5%–7.5%
PAAD.
DIPG frequency: https://smhs.gwu.edu/isb/systems-disorders/brain-spinal.
All other stats: Estimated from total cancer freq. x subtype freq. (US stats: SEER Cancer Statistics, National Cancer Institute. Bethesda, MD, US stats 2017). *AML*, Acute myeloid leukemia; *BRCA,* Breast cancer; *COAD*, Colorectal adenocarcinoma; *GBM,* Glioblastoma; *LGG*, Low grade glioma; *LUAD*, Lung cancer adenocarcinoma; *PAAD*, Pancreatic adenocarcinoma; *READ*, Renal adenocarcinoma; *SKCM*, Skin cutaneous melanoma

viral antigens or neoantigens (McGranahan et al., 2016; Tashiro and Brenner, 2017). Shared neoantigens are rare, and with the combination of low-expression frequency combined with HLA restriction (approximately 10%–50% of the total population expressing the required HLA type for neoepitope presentation), the frequencies of shared neoantigens are challenging for clinical development of engineered T cell therapy (Table 13.2). Instead, some groups are working to identify patient-specific T cells to address

these targets (Gros et al., 2014; Tran et al., 2014). Targeting clonal driver genes may not be necessary to deliver meaningful therapies to patients. Effective antitumour responses, including complete responses, have been shown when targeting other antigens, as reviewed in the following section. While TCRs that bind to many peptide fragments from an individual target can be identified, it is paramount to antitumour activity in patients to select peptides known to be actually processed and presented on tumour tissue.

CLINICAL EXPERIENCE WITH T CELL RECEPTOR–ENGINEERED T CELLS

A handful of small TCR-engineered T cell therapy studies have been carried out to date (Table 13.3), and initial studies were spearheaded by Steven Rosenberg and colleagues at the National Cancer Institute (NCI). The first two studies were carried out in the setting of metastatic melanoma, following promising results in a subset of patients given ex vivo expanded autologous tumour-infiltrating lymphocytes (TILs), which demonstrated, for the first time, that adoptive T cell therapy was capable of fully eradicating bulky tumours (Dudley et al., 2002; Rosenberg and Dudley, 2004). Given TILs cannot be manufactured for the majority of patients, the idea arose to engineer tumour specificity into a patient's own circulating T cells, with the aim to bring tumour-specific adoptive T cell therapy to a broader patient population.[1]

Initial Three Studies of Engineered T Cell Therapy

The first melanoma antigen targeted was MART-1, a melanocyte differentiation antigen upregulated in melanoma. MART-1 is expressed in 80%–90% melanoma cases, frequently recognised by melanoma TIL and expressed at lower levels in normal melanocytes that are present in the skin, hair follicles, eye and inner ear. A natural affinity HLA-A2–restricted TCR, DMF4, was used in the study (Morgan et al., 2006). Engineered T cells were infused following deep systemic lymphodepletion. High-dose IL-2 was given post infusion to support T cell survival and function. Responses were modest with 4 of the 31 patients treated (13%) having objective tumour regression. One patient was reported to have had a complete regression that lasted for 23 months. A second patient exhibited a complete regression of an axillary mass and a 90% reduction in the size of a

[1] More recently, this group has demonstrated impressive clinical responses in individual patients infused with isolated tumour-infiltrating lymphocytes (TILs) reactive against mutant proteins expressed in tumour cells. Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, et al. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014;344(6184):641–5; Zacharakis N, Chinnasamy H, Black M, Xu H, Lu YC, Zheng Z, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nat Med 2018;24(6):724–30. This technology does not use genetic engineering and requires the isolation of these reactive T cells that require the enrichment of reactive T cells against a small repertoire of dominant truncal mutations arising in tumours. Emerging technologies to isolate T cells able to mediate effective immune responses against neoantigens are fuelling this highly personalised form of adoptive immunotherapy.

Table 13.3 T Cell Receptor (TCR)–Engineered T Cell Therapy Studies.

Antigen	TCR Name	TCR Source	Patient Conditioning	Total Infused Cell Dose ($\times 10^{9}$)	# pts	ORR (%)	TCR Related Toxicity	References
HLA-A*02:01–restricted MART-1	DMF4	Human TIL	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	7.4–34.4	31	4/31 (13)	No significant toxicity	(Morgan et al., 2006; (Johnson et al., 2009))
HLA-A*02:01–restricted MART-1	DMF5	Human TIL	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	1.5–107.0	20	6/20 (30)	On-target toxicity in skin, eyes and ear	(Johnson et al., 2009)
HLA-A2–restricted gp100	gp100	Human HLA-A*02:01 transgenic mouse	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	1.8–110.0	16	3/16 (19)	On-target toxicity in skin, eyes and ear	(Johnson et al., 2009)

HLA-A2–restricted CEA	CEA	Human HLA-A*02:01 transgenic mouse; contains engineered sequence	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	0.2–0.4	3	1/3 (33); (74%–99% drop in serum CEA across patients)	Severe transient colitis	(Parkhurst et al., 2011)
HLA-A2–restricted NY-ESO	NY-ESO-1^{c259}	Human PBMC; contains engineered sequence	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	9–130	38	22/38 (58)	No significant toxicity	(Robbins et al., 2015)
HLA-A2–restricted MAGE-A3	MAGE-A3	Human HLA-A*02:01 transgenic mouse	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance.	28–79	9	5/9 (56)	Mental status changes, death, resulting from recognition of related target in the CNS	(Morgan et al., 2013)
HLA-A1–restricted MAGE-A3	MAGE-A3^{ca3a}	Human PBMC; contains engineered sequence	2 dy cy (60 mg/kg) or 200 mg/m^2 melphalan	2.4–5.3	2	NA	Death	(Linette et al., 2013)

Continued

Table 13.3 T Cell Receptor (TCR)–Engineered T Cell Therapy Studies.—cont'd

Antigen	TCR Name	TCR Source	Patient Conditioning	Total Infused Cell Dose ($\times 10^{9}$)	# pts	ORR (%)	TCR Related Toxicity	References
HLA-A2–restricted NY-ESO	NY-ESO-1^{c259}	Human PBMC; contains engineered sequence	200 mg/m^2 melphalan	1.0–10.0	24	16/20 (80)	Autologous GVHD	(Rapoport et al., 2015)
HLA-A24–restricted MAGE-A4	MAGE-$A4_{143-151}$	Human PBMC	None; post infusion MAGE-A4 vaccine administered	0.2–5	10	0/10 (0)	None	(Kageyama et al., 2015)
HLA-DPB1*04:01–restricted MAGE-A3	MAGE-A3	Human PBMC; constant region replaced with murine chains	7 days and post infusion IL-2 support: 2 dy cy (60 mg/kg) followed by 5 dy flu (25 mg/m^2); high-dose IL-2 post infusion (720,000 IU/kg/8 h) to tolerance	0.1–123	17	4/17 (24)	Elevated ALT/AST and creatinine.	(Lu et al., 2017)
HLA-A24–restricted WT-1	TAK-1	Human PBMC	None; post infusion WT1 vaccine administered	0.2–1.0	8	0/8 (0)	No significant toxicity	(Tawara et al., 2017)
HLA-A2–restricted NY-ESO	NY-ESO-1^{c259}	Human PBMC; contains engineered sequence	4 days: 4 dy flu (30 mg/m^2) and 2 dy cy (1800 mg/m^2)	0.5–14.4	12	6/12 (50)	No significant toxicity	(D'Angelo et al., 2018)

dy, day; ORR, overall response rate.

liver lesion which was resected 10 months later, with the patient remaining disease-free 9 years after treatment (Johnson et al., 2006). No significant toxicity was reported.

To improve response rates (RRs), a higher affinity, naturally occurring HLA-A2–restricted TCR for MART-1, DMF5, was then selected for clinical study. No other changes were made to the clinical design, and with the higher affinity TCR, RRs increased to 6/20 (30%) (Johnson et al., 2006). A second higher affinity HLA-A2–restricted TCR specific for another melanocyte differentiation antigen, gp100, was generated in an HLA-A2 transgenic mouse and tested in parallel. Three of 16 patients (19%) experienced objective responses (Johnson et al., 2006). All but two patients eventually relapsed, with one DMF5-treated patient (a partial responder) and one gp100-treated patient (a complete responder) having ongoing responses at 8 years. Severe 'on-target, off-tumour' toxicities were observed, which were significant but clinically manageable. The higher frequency of objective responses and on-target/off-tumour toxicity in these studies suggest that TCRs with increased affinity may confer greater antitumour potency.

The same group led a similarly designed clinical trial in colorectal cancer, using an HLA-A2–restricted TCR specific for carcinoembryonic antigen (CEA). CEA is a glycoprotein that is overexpressed in many epithelial cancers and is present in normal epithelial cells in the gut. This TCR also contained a single amino acid change for augmented recognition of the CEA peptide on colon cancer cell lines (Parkhurst et al., 2011). Three patients with colon cancer were treated, with one patient experiencing a 6-month partial response. However, patients developed severe inflammatory colitis, with grade 1 diarrhoea in one patient and grade 3 diarrhoea in two patients requiring administration of oral corticosteroids. While these autoimmune toxicities resolved in 4–6 weeks, the trial was terminated early.

All together, these three studies suggest that differentiation antigens may not be ideal targets for TCRs due to predictable on-target, off-tumour toxicity. Consequently, subsequent studies have focused on tumour-specific targets such as cancer testis antigens, as described next (e.g., NY-ESO-1, MAGE family members), or mutated antigens (e.g., mutated KRAS) and viral antigens.

NY-ESO-1 Studies

The next series of TCR-engineered T cell studies that were conducted drew from the learnings of these early studies and continue to serve as the flagship programme for the field today. A publicly available HLA-A2–restricted TCR specific for the NY-ESO-1 cancer testis antigen (Robbins et al., 2008) was engineered for higher affinity (NY-ESO-1^{c259}) by a company specialising in TCR protein science (Avidex Ltd., Abingdon, UK). This TCR was advanced into the clinic by the Rosenberg team at the NCI (Bethesda, MD, USA) using a similar design as described earlier. NY-ESO-1 has broad expression across many oncology indications, with particularly high prevalence and intensity of expression in the rare tumour type synovial sarcoma (Lai et al., 2012;

Velazquez et al., 2007). Therefore, both melanoma and synovial sarcoma patients were included in this study, which allowed comparison to earlier melanoma studies, while evaluating the therapy in an indication where expression levels are extremely high. Eleven of 18 synovial sarcoma patients (61%) and 11 of 20 (55%) melanoma patients experienced objective clinical responses, in the absence of any significant toxicity. Finally, a sweet spot between improved efficacy and safety seemed to have been achieved (Robbins et al., 2011, 2015).

The NY-ESO-1^{c259} TCR was taken further forward into clinical development by Adaptimmune, which carried out additional studies. A first study evaluated the safety and activity of NY-ESO-1^{c259}, which are autologous T cells (CD4$^+$ and CD8$^+$) expressing NY-ESO-1^{c259}, in patients with relapsed or refractory multiple myeloma (MM). Following a myeloablative preparative regimen and autologous stem cell transplant (ASCT), patients received the engineered T cell infusions, which were well tolerated, with no significant toxicity. Autologous graft-versus-host disease was observed in a subset of patients, which had previously been reported with the transfer of activated T cells post ASCT (Hammami et al., 2018). The RR (per IMWG, 2011 criteria) was 76%, although the specific contributions of the engineered T cells versus the ASCT was difficult to determine (Rapoport et al., 2015, 2017).

A follow-on study to the Rosenberg study in synovial sarcoma patients was also conducted. In the first cohort of this study, the conditioning regimen was simplified to 4 days from 7 (fludarabine 30 mg/m^2/day for 4 days and cyclophosphamide 1800 mg/m^2/day for 2 days), and no supportive IL-2 was administered. Further, a different manufacturing process was employed. A key initial aim of the study was to demonstrate comparable responses in patients, given these changes. The overall RR for the 12 patients treated with NY-ESO-1^{c259}T cells was 50% (one complete response and five partial responses), comparable to the prior study. Time to response was 6 weeks (range 4–9). Median duration of response was 31 weeks (range 13–72) (D'Angelo et al., 2018b).

This study was further amended to explore the safety and efficacy of this TCR in patients receiving further reduced conditioning therapies (cyclophosphamide 1800 mg/m^2/day for 2 days with no fludarabine or fludarabine at 30 mg/m^2/day for 3 days and cyclophosphamide 600 mg/m^2/day for 3 days) and to evaluate the effect of low NY-ESO-1 expression on clinical response. These studies are ongoing and only interim analyses have been presented as conference proceedings (Mackall et al., 2017; D'Angelo et al., 2018a). Efficacy in the absence of fludarabine conditioning was suboptimal (RR = 20%). Patients receiving fludarabine-containing regimen of lower intensity than that of cohort 1 had lower peak persistence, lower RR (27%) and shorter durability of response (16 weeks vs 31 weeks for cohort 1). Responses were observed across a spectrum of NY-ESO-1 expression, and there is evidence of clinical benefit in a patient population refractory to available therapies.

The MAGE-A3 Experiences

Another cancer testis antigen with excellent expression across multiple tumours is MAGE-A3. MAGE is a large family of proteins, many of which have shared homology. Many MAGE family members have tumour-specific expression, but a subset, such as MAGE-A12, has limited expression in normal tissue as well. Therefore, when selecting a MAGE peptide target, it is important to be aware of MAGE homology and understand the expression profile of all MAGE members encompassed by the target. An example of toxicity resulting from homologous MAGE peptides occurred in a study with an HLA-A2–restricted MAGE-A3 TCR taken forward by the Rosenberg group. This TCR recognised MAGE-A3 and also peptides produced by MAGE-A9 and MAGE-12. MAGE-A12 has expression in the central nervous system (CNS), but MAGE-A12 expression patterns were not fully understood before the study start. Nine patients were treated, and five had objective clinical responses. However, three patients had serious CNS toxicity and two died due to TCR-mediated on-target toxicity in the brain (Morgan et al., 2013).

Another MAGE-A3 study was carried out by Adaptimmune, using an HLA-A1–restricted TCR specific for the MAGE-A3 member of the MAGE family of proteins. In this case, lethal toxicity was also observed, but for different reasons that were entirely unrelated to MAGE-A3 as a target. Similar to the NY-ESO-1^{c259} TCR, this MAGE-A3 TCR (MAGE-A3^{ca3a}) was an affinity-enhanced human TCR. The first patient treated was a melanoma patient, who experienced acute cardiac toxicity and died 5 days following infusion. The study was paused. After extensive study, it was determined that the patient had extensive underlying cardiovascular disease, and there was no evidence across primary cell and molecular assays that the TCR recognised cardiac myocytes or related peptides. After review by the FDA and Recombinant Advisory Committee of the National Institutes of Health, the study was restarted. A patient with treatment-refractory MM was then enrolled, after being fully informed of the prior death and the ensuing investigation and conclusions. This patient also suffered acute cardiac toxicity and died 4 days after infusion (Linette et al., 2013). Subsequent investigations revealed that the MAGE-A3^{ca3a} was recognising a peptide that was unrelated to the MAGE-A3 peptide, which had a sequence homology that was too different from the MAGE-A3 peptide to be picked up in preclinical screens used at the time. Furthermore, the cross-reactive peptide was derived from the protein titin that is not expressed in standard 2D culture systems, as it is the largest protein in the human genome and is too energetically costly for cells to routinely produce, therefore the cross reactivity was not detected in routine screens of cardiomyocyte cells. As a result of this, researchers in the field now know that mapping the target peptide using single amino acid replacement ('x-scanning') to determine the consensus binding sequence for a TCR, as well as 3D culture systems where possible, is important for assessing the safety of TCRs preclinically (Cameron et al., 2013; Harper et al., 2018).

A third study targeting MAGE-A3, this time expressed in the context of a class II HLA molecule HLA-DPB1*0401, was conducted by the Rosenberg group at the NCI. The study utilised only $CD4^+$ T cells, and 4 of 17 patients (24%) experienced objective clinical responses (Lu et al., 2017). No significant toxicity was observed. This was a dose escalation study and RRs may be higher as more patients are treated at full dose, although notably, the one complete responder was in an early dose cohort. This study underscores the continued merit of MAGE-A3 as a target, demonstrates the feasibility of targeting a class II–restricted epitope and shows the therapeutic potential of $CD4^+$ T cells alone.

Studies Without Pre-infusion Conditioning

Two studies have been carried out in Japan targeting HLA-A24–restricted peptide targets WT1 and MAGE-A4. Neither of these studies used preconditioning regimens, and no responses or adverse events were observed. The studies attempted to use vaccination approaches as a way to stimulate the infused cells, as an alternative to preconditioning. Vaccination did not yield enhanced persistence of engineered T cells in vivo, to overcome the lack of preconditioning. Although the reason for the lack of responses is not truly understood (i.e., it may be related to TCR affinity or the choice of peptide target as well), this study may highlight the importance of preconditioning for the potentiation of the current generation of TCR-engineered T cells (Kageyama et al., 2015; Tawara et al., 2017).

CLINICAL DEVELOPMENT CONSIDERATIONS

As described above, TCR-engineered T cell therapy has the potential to deliver significant, and in some cases durable, responses in patients with bulky and treatment-resistant disease. Approaches that demonstrate significant improvement over available therapies in areas of significant unmet medical need can move from early proof-of-concept studies to pivotal studies rapidly, using surrogate end points (in the timescale of 5 years). The regulatory landscape has been evolving to support such rapid development, with access to accelerated development programmes and increased regulatory involvement during the development phase of the product, such as Breakthrough and PRIME designation in the United States and Europe, respectively. While this is not unique to adoptive cell therapy, many of the products currently in advanced stage of development have attained this status. As part of the 21st Century Cures Act, the FDA has developed new designations, such as Regenerative Medicine Advanced Therapy designation, to further expedite the development of promising life-saving drugs in the field of cell- and tissue-engineered therapies. The same Act also includes provisions for in silico control arms for rare patient populations, thus supporting feasibility for pivotal study designs for indications where there are poor comparators or more limited access to patients. High RRs (e.g., in the range of 20%–30% or greater) also present an opportunity for testing and exclusion/

progression of new technologies or technology enhancements in pilot studies (e.g., around 10 patients), which can be prosecuted quickly. To do this well demands a close functional alignment between translational research, manufacturing sciences and clinical development.

Patient Diagnostics

Understanding which patients are likely to benefit from therapy is attractive for patients and for payers, given the cost and complexity of engineered T cell therapy today. Patients can be evaluated to ensure that they express the matching peptide–HLA antigens. Sequencing technology is required for the HLA typing test, as suballeles (e.g., HLA-A*02:01, HLA-A*02:02 and so forth) have been shown to bind to the peptide and the TCR with varying efficiencies, which therefore may affect safety and efficacy profiles. Sequencing-based tests already exist on the market, and validation of the precision and sensitivity of these tests to regulatory standards for the targeted HLA suballeles is required in tandem with marketing approval and commercialisation. Solid tumour biopsies are currently required for tumour screening. 'Liquid biopsy'–based analyses, where patient blood is assessed for solid tumour cell markers, have generally not yet demonstrated sufficient sensitivity, although this technology is rapidly developing (Arneth, 2018; Siravegna et al., 2017; Wan et al., 2017). The requirement for solid tumour biopsy poses risks to patients, which can be serious depending on the site of tumour and the procedure required to obtain a biopsy. Therefore, because of this risk, an investigational device exemption is often required as part of initial regulatory submission for first-time-in-human clinical trials for regulators to determine risk:benefit for patients enrolling on the study (in the United States, this is a separate submission to the Center for Devices and Radiological Health-FDA). A companion diagnostic is also required for tumour antigen identification and requires validation alongside clinical development.

Toxicity Considerations

Potential adverse effects of engineered T cell therapy broadly fall into three categories: (1) expected toxicities associated with on-target activity of the transferred cells, (2) unanticipated toxicities associated with off-target or bystander effects and (3) toxicities associated with the therapeutic regimen. Cytokine release syndrome (CRS) and CAR-T cell–related encephalopathy syndrome (CRES) are common toxicities associated with activity of engineered T cell therapy that have been described extensively by others in the field (Lee et al., 2014; Neelapu et al., 2018). The frequency and intensity of both CRS and CRES in TCR-engineered T cell studies has been lower than observed in CAR studies, yet it is unknown whether this is a feature of the receptor, the target itself, target density or tumour bulk and tumour accessibility. Assessment and management of CRS and CRES toxicities are applied to both CAR and TCR therapies. On-target toxicities also include the eradication of nontumour cells that express the tumour target

('on-target, off-tumour'), such as CD19 or CD20, and can also apply to TCRs depending on which target is selected.

Off-target activity is a far more challenging issue with TCRs than with CARs. The reason for this stems from the extreme cross-reactive nature of TCRs, as required by nature for them to be able to recognise the estimated 10^{17} naturally occurring potential peptides (Sewell, 2012). Recognition of an 'off-target' peptide has been reported twice now in clinical studies, as described earlier.

Although patients are 'matched' for HLA so that they will be able to respond to TCR therapy, an exogenous TCR may manifest alloreactivity against the patient's other peptide/class I HLA complexes. TCRs can be screened for this reactivity, although in vitro assays may predict alloreactivity that might not manifest in vivo (Melenhorst et al., 2010; Senra et al., 2018) or conversely may fail to predict alloreactivity that is specific for rare HLA types or individual tissue types expressing specific peptides.

Pre-infusion Conditioning

Adoptive T cell therapy is potentiated by 'preconditioning' with cytoreductive chemotherapy. Standard regimens today include cyclophosphamide and fludarabine, although other regimens have been used (Table 13.1). The mechanism of preconditioning in enhancing the efficacy of adoptive T cell transfer is three-fold: (1) reduction of immunosuppressive cell populations (e.g., regulatory T cells and myeloid-derived suppressor cells), (2) an increasing antigen presentation by inducing cell death and (3) increasing the prevalence of T cell proliferative cytokines IL-7 and IL-15 that results in an increase in T cell reactivity and expansion (Gattinoni et al., 2005; Muranski et al., 2006; Wrzesinski and Restifo, 2005). The preconditioning regimen can result in common toxicities such as lymphopenia, pancytopenia, thrombocytopenia, febrile neutropenia and opportunistic infections that are unrelated to the T cell therapy itself and may require intervention.

BIOMARKERS AND IMPROVING RESPONSES

The cancer immunity cycle is a useful framework for thinking about biomarkers of response and mechanisms of resistance for TCR-engineered T cell therapy (Dunn et al., 2004; Chen and Mellman, 2013). Successful immune responses against tumours require (an) antigen(s) expressed on the tumour to which the immune system is not tolerized, processing and presentation of (the) antigen(s), priming and activation of the immune system to the antigen(s), trafficking of T cells into sites of antigen positive tumours and, finally, recognition and killing of the tumour. The body undergoes a continuous cycle of cancer immunity, and when tumours become established, it is due to a breakdown at one or more of these steps. TCR-engineered T cells are designed to overcome limitations in T cell priming and activation, overcoming immune exhaustion and tumour cell recognition, but first-generation products remain susceptible to many of the preexisting

mechanisms of resistance resulting from a breakdown of the steps in this cycle. The universe of drug, biologic and genes capable of enhancing T cell therapy is too large to empirically test. Active biomarker and correlative research designed to elucidate the mechanism of action and resistance is therefore an essential component of any programme in TCR therapy to drive next-generation improvements. This research is dependent on collection of serum, blood and tumour pre- and postadoptive transfer of T cells. Additional genes can be included alongside the TCR to improve potency, and well-designed combinations with engineered T cells may also be used to improve responses. This section provides a high-level view of a few notable areas of correlative investigation that are important for the successful development of TCR-engineered T cell therapies.

Target Antigen Expression

Adequate presentation of pHLA antigen is essential. Reduced or complete loss of HLA class I expression is a known mechanism of resistance to immunotherapy. Immunohistochemistry for HLA is an insensitive technique, which can detect the continued presence of HLA on tumour but can fail to detect low levels still capable of recognition by higher affinity TCRs. Therefore, molecular techniques such as sequencing, in situ RNA hybridisation (RNAish), and NanoString panels are beneficial. Defects in the antigen-processing machinery also have been described. Loss of heterozygosity (LOH) has recently been described as a primary mechanism of immunologic resistance to neoantigens, which typically are only presented in the context of a single HLA allele (McGranahan et al., 2017; Chowell et al., 2018). Immunologic responses against nonmutated tumour-associated antigens may be less subject to this resistance mechanism, as multiple peptides can be presented from each tumour-associated antigen, forcing the tumour to select for loss of multiple HLA genes or pan HLA loss, thus rendering the tumour then susceptible to natural killer (NK) cell attack (Alari-Pahissa et al., 2014; Moretta et al., 2014). Efficient processing and presentation of the target peptide must occur within the tumour environment and can change depending on the tumour microenvironment. Confirmation of peptide presentation on tumour, through mass spectrometry or cell-based cytotoxicity assays, should be conducted before initiating clinical programmes.

Immune Regulation

Negative immune regulators are a common mechanism of resistance to immunotherapy and include a wide range of mechanisms including metabolic restriction (e.g., limited amino acids, hypoxia, pH and high adenosine signalling), negative immune regulators (e.g., TFG-β, adenosine, IDO/TDO) and immunosuppressive cell subsets (e.g., monocytes and regulatory T cells) (Amobi et al., 2017; Bronte and Zanovello, 2005; Corbet and Feron, 2017; Dahmani and Delisle, 2018; Hammami et al., 2012; Liu et al., 2016; Ohta, 2016; Porta et al., 2018). Often, multiple mechanisms are at play. Unsupervised analyses of gene

signatures associated with these pathways and association with an increase in intensity of a given pathway following engineered T cell therapy in responding versus nonresponding patients can help to elucidate which pathways may be targeted for improved responses.

Immune Contexture

The immune contexture in the tumour is a predictor of response to immunotherapy (Fridman et al., 2017). Immunohistochemistry should be used to characterise the tumour composition, level of immune infiltrate and the nature of the immune infiltrate, if present. Immunohistochemistry can be used for routine analyses on formalin-fixed paraffin-embedded tissue, but availability of fresh tissue opens the possibility for techniques such as single-cell sorting and gene expression signatures, which can unmask deeper insights.

T Cells

Understanding correlations between the phenotype, gene expression profile or function of the infused product with clinical responses and defining the T cell subsets post infusion that engage the tumour and confer immunity is key. This knowledge will support process improvements, establish critical quality attributes (CQAs) of potent products, potentially to establish screening criteria for patient selection and identify mechanisms through which product potency and durability could be enhanced.

Response Assessments

Positive data demonstrating mechanism of action of infused T cells may come from responses that are measured outside of standard response criteria (i.e., response evaluation criteria in solid tumours [RECIST] (Eisenhauer et al., 2009)). Although such responses may not support regulatory approvals, they can provide important insights to response and resistance mechanisms. For example, a patient may progress according to RECIST when the sum of the longest diameter of all target lesions increases 20% from baseline. However, individual lesions may have disappeared or significantly reduced in size. The heterogeneity of tumours across lesions is well established (McGranahan and Swanton, 2017), and responses in a subset of lesions can provide evidence for an active product, while sequencing of the relapsing tumour can provide clues to specific mechanisms of resistance (Grasso et al., 2018; Paulson et al., 2018; Tran et al., 2016). Using alternative response measurements, such as the sum of index lesion diameters, and careful assessment of individual patients for such patterns could assist in identifying such correlates of response.

MANUFACTURING AND SUPPLY CHAIN

To date, adoptive T cell therapy has developed through the use of autologous donors, and the early commercial approvals are for autologous products. The field is advancing technologies to support off-the-shelf cell sources to simplify the supply chain and reduce

costs, which should improve accessibility. This section discusses these two approaches in turn, as well as general development considerations resulting from the emerging manufacturing science and technology particular to cell therapy.

Autologous Products

The manufacturing process for all engineered T cell therapies (CAR or TCR) comprises a shared combination of steps that include white blood cell collection, T cell purification, T cell activation, transfer of the therapeutic gene and (in a majority of cases) expansion and final product formulation and cryopreservation. Comprehensive understanding of the critical process parameters and CQAs of the product that are responsible for effective responses in patients are likely to continue to rapidly evolve for the next 10–20 years, not unlike the early days of antibody manufacture towards the end of the last century.

The majority of engineered T cell products today are manufactured using a patient's own cells, which present unique challenges:

- Firstly, it is not possible to build up product stock, as each patient has his/her own product, unless the patient is identified early on and the product is manufactured at risk before the patient needs it. Manufacturing early would avoid this issue but has significant resource and opportunity cost (e.g., utilising finite manufacturing capacity and potentially crowding out other patients who might benefit). However, manufacturing delays or failures have a direct impact on patients who are progressing rapidly and may ultimately result in the patient not being healthy enough to receive the cells. Recent data with TCRs, as discussed earlier in this chapter in the NY-ESO-1^{c259} experience, suggest that responses may be progressive rather than acute as observed with CARs. Hence, the health status of the patient and the time taken to manufacture and deliver therapy for the patient are critical factors in TCR clinical trials. Excess capacity for manufacturing is required to allow for the necessary flexibility in patient scheduling and treatment, even for products that can be frozen at the beginning and end. Careful management of the 'patient journey' and balancing of the above tensions requires specialised tracking tools as well as dedicated and accountable personnel effort within manufacturing and clinical departments.
- Secondly, each patient represents a different starting material, with variances in cell composition and immunologic health that can lead to different manufacturing outcomes. It is therefore important to evaluate this variation in manufacturing process qualification and validation. Identification of CQA of starting material that predict successful manufacturing outcomes might improve manufacturing success rates, as it would provide manufacturers the opportunity to trigger a planned adjustment in the process to accommodate suboptimal material.
- Thirdly, manufacturing scaling consists of scaling out of key machinery and clean room space, which can be done in a modular fashion as need increases, which is in

contrast to antibody production where the required scale must be predicted and built into the facility design upfront.

- Finally, there is a high supply chain risk inherent in the manufacture of engineered T cell therapy due to specialised reagents, equipment and disposables. For example, critical shortages in viral vector availability has been slowing progress in the field (Kolata, 2017), and a projected limitation of serum supply (Brindley et al., 2012) has been reported. Where possible, the supply chain for critical reagents should be controlled through in-house manufacture or supply agreements with key vendors. Risk assessments can identify areas where back-up supplies might be substituted in when necessary, once it is demonstrated that their use does not alter the final product.

Off-the-Shelf Products

T cells from one person cannot typically be broadly given to others because the infused cells may see the recipient as foreign and attack tissues, causing graft-versus-host disease (Geneugelijk et al., 2014) unless the donor and recipient are closely matched for their tissue type or HLA genes. Similarly, the infused cells will be rejected by the recipient, unless they are somehow made to be invisible to the recipient immune system. 'Off-the-shelf' products are under development and use several approaches, including (1) using healthy donor T cells and genetically editing out the TCR so that the cells cannot recognise and attack the recipient (Poirot et al., 2015), (2) using non α/β T cells such as γ/δ T or NK cells, which will not induce graft-versus-host disease but can be retargeted using CARs or TCRs and (3) genetically editing stem cells to avoid expression of endogenous TCR genes and including additional edits that render the cells invisible to the recipient (e.g., HLA class I and II deletions) and then differentiating the cells into T cells (Gornalusse et al., 2017; Themeli et al., 2013). The first two approaches are undergoing clinical testing, and it is early days for clinical proof of concept (Qasim et al., 2017).

Development Considerations

The rapid development of cell therapy, as described earlier, carries unique challenges for manufacturing development because there is potentially much less time for optimisation of manufacturing protocols, preparation for commercial scale up and launch (Lunger, 2018). The emergent science around autologous T cell manufacture and supply chain makes this even more challenging. Companies are faced with a difficult decision on whether to commit to investment into the manufacturing supply chain at risk to ensure readiness for rapid commercialisation or wait for clear clinical proof of concept first, which may delay clinical development. Furthermore, many novel engineered T cell therapies emerge from academic centres. Utilising data from early phase or academic processes may require the development of more robust manufacturing processes and may require additional comparability work, depending on stage of development. The key to managing these considerations is to be aware of them and for the enterprise to

clearly understand the development pathway, state of the manufacturing science and prospects for follow-on pipeline products that can benefit from an investment in a robust manufacturing platform and thus derisk early investment. Companies that have a strong cell therapy supply chain in place following a successful product have a significant competitive advantage in this space.

There also appears to be an intention to enable a more flexible research-to-GMP transition for emerging manufacturing technologies. A lack of late-stage clinical phase appropriate requirements in Europe and lack of harmonisation of regulations across countries can be a barrier to rapid development of early cell and gene therapies there. Lifecycle management and Quality by Design concepts and their associated learnings from other biologics support a risk-based and staged approach to cell and gene therapy manufacturing development activities (PDA, 2018).

Present day manufacturing methods are rapidly evolving, and there is a lack of harmonisation in the industry about what the best methodology is for vector generation and gene transfer, cell expansion and supply chain. The optimal cell phenotype and dose for sustained responses are not well understood. With the expansion of scientific discoveries leading to greater clinical experimentation, constant improvement in methodology, a trend to automation and harmonisation of regulatory science are expected in the future. The unique aspects of engineered T cell therapy manufacturing and recent regulatory approvals have triggered several recent updates in the FDA guidance, towards a more modern framework for cell and gene therapies. Reflecting the complexity in this space, FDA commissioner, Scott Gottlieb, acknowledged in 2018 that the bulk of regulatory review effort is shifting from clinical to manufacturing aspects of the dossier (Gottlieb, 2018).

PERSPECTIVES

Initial efforts in engineered T cell therapy in fact began in HIV, with the development of the first CARs in the late 1990s, at a time when there were fewer classes of antiretroviral drugs, drug resistance was a major issue and many patients had no available treatment options (Dropulic and June, 2006; Levine et al., 2006; Mitsuyasu et al., 2000; Walker et al., 2000). Significant precompetitive investment was poured into the development of engineered T cell therapy for HIV, and approximately 20 studies were carried out (Dropulic and June, 2006; Medicine, 2018). The learnings from these studies, along with the emergence of a discreet number of biotechnology companies to develop engineered T cell therapy, seeded the field with learnings and talent which today is now almost entirely focused in IO. Continued strategic precompetitive investment into cell therapy infrastructure will help to establish areas of excellence, such as what has occurred in the United States, in Philadelphia (Cellicon valley), Atlanta (Georgia Tech) and Houston (MD Anderson), as well as in the United Kingdom (Catapult) and in Italy (San

Raffaele/TIGET), to name a few of the more established examples. These centres of excellence can help address workforce and space and experienced leadership, which are all current constraints for this rapidly expanding industry. Investment dedicated to deepening our scientific understanding of the mechanisms behind successful immunotherapy, with academic freedom and open sharing of data, may be as instrumental to the success of this emerging field as it was for the monoclonal antibody field that was emergent over a quarter of a century ago (Melchers, 2012).

Clinical trials with TCR-engineered T cells have been going on for well over a decade now. Like CAR-engineered T cells, the targets and indications where favourable efficacy and safety profiles were observed are very narrow, with the majority of the clinical studies being inadequate to support further product development, especially in the setting of solid tumours. The promise with TCRs versus CARs today is that the TCR class of product enables a much broader repertoire of tumour-specific targets, many of which enable specific targeting of solid tumours. However, even once a target and TCR have been identified as safe and specific, there remain significant hurdles to efficacy in solid tumours related to deficiencies in the cancer immunity cycle. These include T cell trafficking and retention, loss of antigenicity (e.g., β2M loss, LOH, immunosuppression) and silencing of reactive T cells by inhibitory mechanisms in the tumour microenvironment. This is made even more complicated by the known intrapatient tumour heterogeneity, which results in selection for tumour cells with the ability to inhibit or evade immune recognition under immune surveillance. To address these challenges, improvements can be engineered into the cellular product or provided in combination. The potential combinations are nearly endless, and so an aggressive investigation into the mechanisms of response and resistance that feeds rapidly back to product development is an essential part of all clinical development in this space. This reality creates a tension between the need to rapidly develop and test improvements in early phase studies and the need to define a product for late clinical development and regulatory approval. Partnerships between industry and academic organisations can be leveraged to cultivate rapid innovation in translational research and early phase exploratory studies.

The 2017 US approvals of tisagenlecleucel and axicabtagene ciloleucel, subsequent expansion of indications and approvals in the European Union prove that engineered T cell therapy can become a commercial therapeutic modality. Patient-specific manufacturing and associated costs remain a significant barrier, and clear value inflection for the industry is a long game. There is a significant focus on off-the-shelf products to address challenges around cost, delivery and chain of custody, such as allogeneic or universal T cell products. However, it is not yet known whether autologous products may be functionally superior because of their composition. First, autologous products contain non–gene-modified T cells with specificity towards the patient's own tumour. In cases where the tumour has a higher nonsynonymous mutational burden (i.e., more potential immunogenic neoantigens), for example, ex vivo expansion of this patient's cells

followed by administration in a lymphopenic environment may induce these cells to contribute to antitumour responses. Moreover, autologous products contain mixed populations of effector and memory cells that may result in longer persistence and prolonged tumour control. Continued investments are merited for both the development of off-the-shelf products as well as improved autologous products (e.g., shorter manufacturing time and increased robustness). It is likely that universal donor T cells, derived from a renewable stem cell source, may eclipse present day allogeneic donor T cells. Complexities of this technology include the need for multiple genetic editing events to allow long-term persistence and avoid graft-versus-host disease, thorough characterisation of the stem cell banks, robust and efficient T cell differentiation process into functional and persisting T cells and the consequent design and implementation of manufacturing processes that can deliver cell product at clinical scale.

Engineered T cell therapy is also competing with other IO modalities. In 2016, 130 biotechnology companies and 20 pharmaceutical companies were developing a variety of IO therapies (Development TCftSoD, 2016). In 2017, over 240 new IO agents were in development (Pharmaceutical Research and Manufacturers of America TACSCAN, 2017). This translates in the past 5 years into a significantly different landscape for patient recruitment on oncology studies (Tang et al., 2018b). Specifically, the broadening approvals of checkpoint blockade are narrowing the oncology space suitable for engineered cell therapy development, particularly in solid tumours. In indications where checkpoint inhibition is an approved therapy, which is ever expanding, engineered cell therapy is studied only after patients have relapsed or been shown to be resistant to checkpoint inhibitors. These patients are later stage, and cross-resistance to immunotherapy will be present in a subset of patients, further complicating development. Moving into earlier lines of therapy will avoid cross-resistance and will likely first require combination with checkpoint inhibitors. Additionally, many patients fail to respond or recur after checkpoint inhibitor therapy. Biomarkers that predict resistance to checkpoint blockade are now being identified (e.g., cold tumours defined by poor T cell infiltration, PDL-1 negativity and low mutational burden), and this may open more space for cell therapy clinical development in these indications.

In this environment, engineered cell therapy has the added hurdle to overcome with investigators and patients because of the specialised training required for administration and safety monitoring and the lead time between enrolment and infusion because of the autologous product. In addition, these studies typically have complex treatment schedules and invasive procedures, which may be less attractive for some patients. Timely and robust manufacture, compelling science and promising preclinical results, experienced centres able to screen large numbers of patients for both HLA and antigen, excellent clinical trial operation teams, sharing of real-time translational research data with investigators and streamlining of study procedures and sample collection and shipment are elements that influence the willingness of oncologists and their staff to enrol their

patients into early studies where benefit:risk is unknown. It is the realisation and interconnection of all these elements that will make game-changing advances possible in the treatment of solid tumours, thereby opening a bright new era in oncology healthcare.

Only a decade ago, engineered T cell therapy was a mostly academic venture focused primarily in infectious disease and moving into oncology. Since that time, it has become the first gene therapy approved product in the United States in history. In a small subset of oncology indications, this therapy is fulfilling the promise of a genetically engineering human immune system to treat disease with transformational outcomes. Successes in oncology are likely to be increasing in difficulty, and it is unknown how long the road will be to achieving stable success in manufacturing and product definitions. However, the ability to engineer the immune system to fight disease is now within grasp.

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