1 INTRODUCTION

Malignant melanoma, the deadliest type of skin cancer, is responsible for about 60,000 deaths worldwide each year. Historically, the only possible cure for melanoma was early detection and surgical removal. Since 1975, after the introduction of dacarbazine as a treatment option, the survival remained unchanged with a dismal 5-year survival of <10%. Interleukin-2 was an option in some countries, with some durable complete remissions, but given only to a select subset due to its severe adverse events.

Huge advances have been made in treatment of malignant melanoma the past 10 years. From almost four decades with dacarbazine (DTIC) as the meagre gold standard, we have seen a paradigm shift emerging this last decade. 2011 stands as a monumental year with the introduction, and FDA approval, of both ipilimumab (a cytotoxic T lymphocyte antigen 4 [CTLA-4] antibody) and vemurafenib (a BRAF inhibitor). From then on, treatment options have been rapidly evolving with addition of PD-1 (programmed cell death protein-1) inhibitors (nivolumab and pembrolizumab) as well as MEK inhibitors (cobimetinib, trametinib and mektovi) in combination with BRAF inhibitors (trametinib, braftovi and vemurafenib). Therapy with the combination of anti-CTLA-4 plus anti-PD-1 (ipilimumab and nivolumab) has so far the highest response with an estimated 5-year survival
of 52%. There is a tremendous improvement, but we still face a challenge to improve the survival rates for half of the metastatic melanoma population as they are without effective treatment.

Administration of immune checkpoint inhibitors (ICIs) unleashes T lymphocyte-mediated immune responses by suppressing the interaction of T cell inhibitory receptors with their cognate ligands on tumour or stromal cells. Anti-tumour T cells play a major role in control of tumour growth, and in studies with checkpoint inhibitors against melanoma, a lack of response to therapy was seen in patients without tumour-infiltrating T cells. Adoptive T cell therapy (ACT) is emerging as a possible way forward in modern cancer therapy filling the gap where checkpoint inhibitors fail to succeed.

The basis of ACT consists of harnessing the patient's own T cells, and there are three approaches: transfer of autologous tumour-infiltrating T lymphocytes (TILs), T cells expressing chimeric antigen receptors (CAR-T) and TCR-directed therapy (see Figure 1). There have been several clinical studies with TIL therapy in melanoma, with promising, but varying degrees of success. ACT using genetically modified T cells to encode receptors that recognize cancer-specific antigens is the basis for CAR and TCR therapy. CARs are restricted to surface antigens which may be complicated to define in solid tumours and whose expression is frequently not restricted to tumour. TCRs recognize both surface antigens and intracellular antigens. Although some very promising results have been achieved with CAR-T therapy against children with B cell lymphoblastic leukaemia and other haematological cancers in adults, it has not shown the same promise for solid tumours. Clinical studies with TCR therapy against melanoma have been investigated since 2006 when Rosenberg and colleagues included the first patients into their study (NCT00393029). We will here review TCR-directed ACT against melanoma and describe some of its strengths and challenges.

FIGURE 1 TCR-based adoptive T cell therapy. Figure shows the process of T cell therapy production for patient use. Graphical elements adapted from Servier Medical Art repository (http://www.servier.com)
2 | TCR BIOLOGY

2.1 | Short overview of the pMHC-TCR complex

The potency of TCRs and their ability to distinguish infected or abnormal cells from self depend on their interaction with peptide-major histocompatibility complex (pMHC). Proteins are digested into peptide chains and displayed by either major histocompatibility complex (MHC) class I or class II molecules to form pMHC complexes that can be recognized by T cells. MHC class I are present on all nucleated cells at their surface, but only professional antigen-presenting cells (APCs), such as dendritic cells, macrophages or B cells, express MHC class II molecules. The general (and simplified) model of peptide presentation says that all cellular proteins are degraded and the resulting peptides are presented on MHC I, whereas extracellular proteins are loaded on MHC II. In humans, MHC I and II proteins are expressed from 3 gene human leucocyte antigen (HLA) regions each: HLA-A, HLA-B and HLA-C for MHC class I and HLA-DR, HLA-DP and HLA-DQ for MHC class II. The TCR heterodimer consists of two different transmembrane polypeptide chains called α and β. Each chain has a constant region anchoring the chain inside the cell membrane, and a variable region, which recognizes and binds their cognate pMHC. The TCR itself does not contain any signalling domains, but is coupled with a CD3 complex containing immunoreceptor tyrosine-based activation motifs (ITAMs) which propagate the intracellular signalling to activate the T cell. TCRs can be activated by only one pMHC complex, considered a strength compared to CARs that require a greater antigen site density.

In order to execute full T cell function, additional co-stimulatory signals are often needed. Major co-stimulatory signals are CD80 on the surface of cytotoxic T lymphocytes (CTL) binding to MHC I, and CD4 on the surface of helper T cells (Th) binding to MHC II. High-affinity TCRs have been shown to be independent of co-receptors. In addition to co-stimulatory molecules, there are also important co-inhibitory molecules that regulate immune responses. Examples of these are CTLA-4 and PD-1, molecules that take part in extinguishing T cell signalling, and treatment directed against these molecules are foundations in modern melanoma treatment today.

2.2 | Immune synapses

The immune synapse (IS) is the interface between a T cell and a target cell presenting the cognate pMHC. The IS has two functions: the first is to organize the TCR signalling machinery to induce changes in T cell function and gene expression, and the second is to allow for direct secretion of pro-inflammatory cytokines and cytolytic molecules towards target cells. Three supramolecular activation clusters (SMACs) morphologically structured like a ‘Bull’s eye’ make up the IS: the central, peripheral and distal SMAC. The distal one is the site where the TCR activation initially occurs. After activation, TCR microclusters move centrally, permitting a stable CD3-ITAM phosphorylation and initiating downstream TCR signalling. The peripheral part stabilizes the IS through interactions between the T cell and intercellular adhesion molecule 1 (ICAM-1) expressed on the target cell. The final step in TCR activation is the site of the central part where cytoplasmic granules are delivered towards the target cell and TCR signalling is terminated.

2.3 | Genetic modification of lymphocytes

Retro- and lentiviral vectors have been the most widely used vectors for inserting transgenes encoding TCRs into T cells for use in clinical practice. Viral-based approaches have been effective, but not without therapeutic limitations. Viral vectors provide efficient gene transfer, high level of transgene expression and broad tropism. Clinical trials have shown that both retro- and lentiviral vectors are safe, although the latter has been shown to have a safer integration profile in haematopoietic stem cells (see Table 1). Firstly, viral vectors integrate semi-randomly into the T cell genome. They appear to have some bias towards transcriptionally active genes. To date, there have been no reported transformation events following viral transfer in melanoma trials, but there remains possibility of viral-induced gene disruption. Secondly, virally integrated sequences can be associated with differentially regulated gene expression, resulting in high-level expression of the TCR in non-homogenous expression in a population.

A double plasmid-based expression system, the sleeping beauty (SB) transposon/transposase system, has similarly been used to introduce TCRs to T cells. The transfection efficiency is lower than the viral one, but the main advantages are the costs and the handling time which are both greatly reduced. Such integrations are also random and could potentially cause undesired mutagenesis. The clinical efficacy of TCR-modified T cells using this approach still has to be demonstrated.

In contrast to stable and permanent expression of the transgene obtained with viral vectors or plasmid DNA transfection, mRNA electroporation avoids genetic integration, but only permits temporary TCR expression (see Figure 1). This increases safety for first-in-human studies and permits dose escalation for each patient. The method has been tested clinically for CAR-T and recently for TCR (NCT03431311).
**TABLE 1** Clinical studies with TCR against malignant melanoma

| Target       | Trial number    | Study period (Status) | Patient no (est.) | Vector/mode                          | HLA allele | Cancer targeted                                      |
|--------------|-----------------|-----------------------|-------------------|--------------------------------------|------------|------------------------------------------------------|
| MART-1       | NCT00509288     | 2007-2011 (C)         | 24                | Retroviral vector                    | HLA-A*0201 | Metastatic cutaneous melanoma2                      |
|              | NCT00612222     | 2008-2011 (T)         | 4                 | Retroviral vector + ALVAC vaccine     | HLA-A*0201 | Metastatic cutaneous melanoma                        |
|              | NCT00910650     | 2009-2019 (C)         | 14                | + dendritic cell vaccine             | HLA-A*0201 | Metastatic cutaneous melanoma3                      |
|              | NCT02654821     | 2012-2019 (A, nr)     | 12 (25)           | Retroviral vector                    | HLA-A*0201 | Metastatic cutaneous and uveal melanoma              |
| MART-1 gp 100| NCT00923195     | 2008-2011 (C)         | 4 (85)            | Retroviral vector + peptide vaccine  | HLA-A*0201 | Metastatic cutaneous melanoma4                      |
| gp100        | NCT00610311     | 2008-2011 (T)         | 3                 | + ALVAC vaccine murine TCR           | HLA-A*0201 | Metastatic cutaneous melanoma                        |
|              | NCT00509496     | 2007-2011 (T)         | 21                | Retroviral vector                    | HLA-A*0201 | Metastatic cutaneous melanoma                        |
| NY-ESO-1     | NCT00670748     | 2008-2016 (T)         | 45                | Retroviral vector                    | HLA-A*0201 | Metastatic cancers, melanoma/RCC and others5         |
|              | NCT01457131     | 2011-2013 (T)         | 2                 | Retroviral vector w/inducible IL-12  | HLA-A*02:01| Metastatic cancers, melanoma and others              |
|              | NCT0150401      | 2011-2016 (T)         | 4                 | Lentiviral vector enhanced TCR       | HLA-A*0201 | Metastatic, cutaneous melanoma                       |
|              | NCT01967823     | 2013-2020 (C)         | 11                | Retroviral vector murine TCR         | HLA-A*0201 | Metastatic cancers, melanoma and others              |
|              | NCT02062359     | 2014-2016 (T)         | 2                 | Retroviral vector CD62L+ T cells     | HLA-A*0201 | Metastatic cutaneous melanoma                        |
|              | NCT02366546     | 2015-2018 (A, nr)     | 91                | Unknown                              | HLA-A*02:01| Metastatic cancers, melanoma and others              |
|              | NCT02457650     | 2015-2019 (R)         | 361               | Unknown                              | HLA-A*02:01| NY-ESO-1 + malignancies (children and adult)        |
|              | NCT02869217     | 2016-2020 (A, nr)     | 221               | Unknown                              | HLA-A*02:01| Metastatic cancers, melanoma and others              |
|              | NCT03638206     | 2018-2023 (R)         | 731 (incl. CAR-T trial) | Unknown                              | HLA-A*02:01| Metastatic cancers, melanoma and others incl. multiple myeloma |
| MAGE9        | NCT01273181     | 2010-2012 (T)         | 9 (97)            | Retroviral vector                    | HLA-A*0201 | Metastatic cancers, melanoma and RCC6               |
| A3           | NCT02153905     | 2014-2018 (T)         | 3 (102)           | Retroviral vector                    | HLA-A*01   | Metastatic cancers, melanoma                         |
| A3           | NCT02111850     | 2014-2023 (R)         | 171 (107)         | Retroviral vector CD4 TCR            | HLA-DP4    | Metastatic cancers, melanoma and others7            |
| A4           | NCT01694472     | 2012-2016 (U)         | 151               | Unknown                              | HLA-A*24:02| Metastatic cancers, melanoma and others              |
| A4           | NCT03132922     | 2017-2020 (R)         | 421               | Lentiviral vector                    | HLA-A*02    | Metastatic cancers, melanoma and others              |

(Continues)
Different modifications of the TCR have been proposed to improve its expression.\textsuperscript{43}

### 3 | ANTIGENS USED IN TCR AGAINST MELANOMA

Melanoma-associated antigens can be characterized by their composition (proteins or carbohydrates), their cellular location (whether intracellular or membranous) and the tumour stage they are expressed in (primary tumour or metastatic).\textsuperscript{44} Major factors determining whether an antigen is a good target or not for ACT are based on its tumour specificity, how well it can generate a T cell response and the prevalence and level of expression on tumour cells.\textsuperscript{45} One of the main differences between CAR and TCR therapies is that CAR-T can recognize antigens in an MHC-independent manner, but are restricted to surface antigens.\textsuperscript{46} Unlike CARs, TCRs are able to recognize all cellular proteins vastly increasing the repertoire of a TCR in comparison.\textsuperscript{28} Chandran and Klebanoff illustrated this difference elegantly with an iceberg representing the human proteome: the part above water representing the (27%) membrane-associated proteins (potential CAR and TCR targets) and the larger portion below the surface representing the (73%) intracellular proteins (exclusively TCR targets).\textsuperscript{28} Finally, TCRs are extremely sensitive and can distinguish single amino acid changes; thus, they can detect point mutations such as neoantigens. We will here give an overview over the most prevalent antigens in melanoma\textsuperscript{47} identified as possible targets for TCR therapy.

#### 3.1 | Melanocyte differentiated and overexpressed antigens

Tissue differentiation antigens are normal non-mutated proteins expressed as a consequence of a specific function of the target tissue. In melanoma tissue, differentiation antigens are called melanocyte differentiation antigens (MDAs) and they are a series of proteins associated with pigment production in melanomas and melanocytes.\textsuperscript{48} These antigens are expressed in epidermal melanocytes and pigmented epithelia of the retina, iris and ciliary body of the eye. Barrow and colleagues revealed that a number of differentiation antigens were strongly expressed in up to 95% of melanoma tumours, regardless of stage.\textsuperscript{49} Well-known examples are tyrosinase, tyrosinase-related proteins (TRP-1 and TRP-2), melanocyte antigen (melan-A/MART-1) and glycoprotein 100 (gp100).\textsuperscript{50} An advantage of this group of antigens is that they are shared antigens and can be found in many different melanoma patients potentiating a ‘off the shelf’ therapy development. One of their major disadvantages is that they are expressed on normal tissues causing a risk for on-target, off-tumour
therapy. Both TRP-1 and TRP-2 are associated with break-

Tyrosinase is a copper-containing glycosylated enzyme

Tyrosinase-related protein (TRP): TRP-1 may have a role

Melan-A/MART-1: MART-1 (melanoma antigen rec-

gp 100, also called Pmel17 (premelanosomal protein 17), is

hTERT: Overexpressed antigens are due to, for example,

MAGE family: Melanoma-associated antigen (MAGE) can be divided into two categories. Category I

Cancer germline antigens (CGAs)

Cancer germline antigens are antigens characterized by their expression in the testis and placenta as well as various tu-

The BAGE family consists of highly homologous polypep-

diagnostic

may function as driver events contributing to oncogenesis through TERT dysregulation and therefore undergo positive selection.

Survivin: he anti-apoptotic protein survivin plays a critical

3.2 Cancer germline antigens (CGAs)

Cancer germline antigens are antigens characterized by their expression in the testis and placenta as well as various tu-

The MAGE family: Melanoma-associated antigen (MAGE):

MAGE-A1-12, MAGE-B and MAGE-C. Category II consist of MAGE-D, MAGE-E, MAGE-F, MAGE-G, MAGE-H and MAGE-L. These antigens are not limited to the X chromosome, but expressed in various tissues such as brain and embryonic tissues. Expression of MAGE-I proteins in tumour cells is associated with poor clinical prognosis. The exact functions of MAGE proteins are not yet clear, but they may function as drivers of tumorigenesis through the regulation of cell cycle progression and restric-

According to findings by Peled et al, MAGE-A3 can be found in about 78% of melanomas. Several clinical trials, some of which are still ongoing, target MAGEs (Table 1). General clinical outcome is so far unpublished, but in one trial cross-reactivity against other MAGEs occurred, as discussed below.

The BAGE family consists of highly homologous polypep-

diagnostic
marker and target for TCR-directed ACT, but does not seem to be a desired clinical target for TCR studies as none are published.

**PRAME** (PRerferentially expressed Antigen in MElanoma) is a cancer germline antigen not expressed in healthy tissues except for testis, ovary, placenta, adrenals and endometrium. In their study, Lescano and colleagues found an ~87% PRAME expression in metastatic melanomas. They found a high frequency of PRAME expression (around 90%) in acral, superficial spreading, nodular and lentigo maligna melanomas, but low in desmoplastic melanomas (about 35%). PRAME is also high in uveal melanomas and is currently used in the clinic as a biomarker for metastatic risk in class I uveal melanoma. Two of the open clinical trials of TCR therapy are targeting PRAME (see Table 1), but no results have been published so far for melanoma.

**NY-ESO-1** is regarded as one of the most immunogenic CGAs, because humoral immune responses against NY-ESO-1 are more frequently observed than any other CGAs. This is also reflected by the number of TCR therapy trials targeting this antigen in melanoma, making up about one third of the studies (Table 1). NY-ESO-1 expression in melanoma is estimated to be 45% and, unlike some of the MAGEs, its expression is high also in primary tumours. Due to its high immunogenicity, NY-ESO-1-positive tumours can induce immune responses causing both spontaneous autoimmune responses and tumour regression.

SSX-2 belongs to the SSX family. It consists of 10 members that are heterogeneously expressed in many cancers, not only melanoma. Expression of SSX in tumours is associated with advanced stages and unfavourable prognosis. An SSX-2 epitope can elicit a cytotoxic T cell response in melanoma patients with HLA-A2 haplotypes. Antibodies against SSX2 can be found in about 18% of melanomas, but so far this antigen has not been targeted in TCR therapy.

In summary, CGAs are frequently expressed antigens that are relatively easy to target for TCR therapy. There is limited peripheral tolerance against them; however, they could potentially still cause on-target/off-tumour toxicity.

### 3.3 Cell membrane proteins

Expression of cell adhesion receptors is upregulated in melanoma. Cell adhesion receptors facilitate cell-matrix or cell-cell contacts with concomitant transduction of signals that contribute to the growth, migration and invasion of tumour cells. The most studied receptors in melanoma are the integrins, melanoma chondroitin sulphate proteoglycan (MCSP), immunoglobulin superfamily molecules, melanotransferrin (MTf) and S100. To our knowledge, no TCRs have to date been proposed to target these proteins. CARs have been developed to treat other cancers and could be applicable for melanoma treatment, for example used in a CAR design to enhance tumour specificity.

**Integrins** are heterodimer receptors composed of paired α- and β-subunits. Changes in heterodimer pairing are seen in various malignant cells. Through up- and downregulation during different stages of cancer, they are able to regulate the activity of invasion and migration while preventing the apoptosis that usually occurs in normal detached matrix-dependent cells. Some integrins can regulate the function of non-cancerous cells in the TME supporting development of the tumour. The basis of many possible variations in malignant cells, including melanoma, makes them a difficult target for TCR.

**Melanoma chondroitin sulphate proteoglycan** (MCSP) is a transmembrane cell surface molecule highly expressed in many melanomas, but also expressed in low levels in normal melanocytes. In melanoma, MCSP plays a role in stabilizing cell-extracellular matrix interactions during early stages of melanoma cells spreading on endothelial basement membranes, and it is involved in the migration, invasion and survival of melanoma cells. So far, only preclinical reports have been published.

**Immunoglobulin superfamily molecules** are present on the cell surface and are involved in cell-cell adhesion and intercellular recognition. In melanoma, the most overexpressed proteins of this group are MUC18 (melanoma cell adhesion molecule/Mel-CAM or CD146) and intercellular adhesion molecule 1 (ICAM-1). MUC18 can be expressed in some normal tissues, subsets of lymphocytes and also non-melanoma tumours; hence, it is relatively unspecific. ICAM-1 is expressed on normal cells and is the ligand for the lymphocyte function-associated antigen 1 (LFA-1). Because they are non-specific targets, they are less likely to be used in TCR.

**Melanotransferrin** (MTf) has low expression in normal adult tissues, but higher in foetal tissue and benign pigmented nevi as well as in 90% of melanomas. MTf regulates cell surface localization and transmembrane signalling. Because of its expression in adult tissues, albeit low, it does not seem an attractive target for TCR-directed ACT due to the risk of on-target, off-tumour toxicity.

**S100 proteins** are one of the earliest used markers of melanoma. They are expressed in other tumours as well and used as diagnostic tools only in association with other more specific melanoma markers, such as gp100 or Melan-A. The S100 proteins are also expressed in other cancers and additionally in a wide range of other human disorders, such as inflammatory diseases, cardiomyopathies and neurodegenerative syndromes. Due to its high toxicity potential, S100 is considered an unattractive target for TCR.
3.4 | Neoantigens

Melanoma tends to be incredibly immunogenic, generating neoantigens through chromosomal rearrangements or genetic polymorphisms that can mimic ‘foreign’ infection and thus potentially elicit strong cytotoxic responses. The clinical success of checkpoint blockade and other immunotherapies have been associated with the presence of anti-tumour T cell responses against neoantigens.92,93 There are several advantages of targeting such antigens as they are tumour-specific, thus reducing the risk of toxicity, and immunogenic as they are not expressed in normal tissue. Melanoma is known to have a high mutational burden,94 and with recent technological advances, the identification of neoantigens by next-generation sequencing has become feasible. One of the drawbacks with targeting such antigens is that most immunogenic mutations tend to be patient-specific. This requires extended time to develop personalized treatment.95 One option would be to target frequently mutated driver genes or hotspots for mutation.

Examples of shared melanoma-associated mutations are BRAF, NRAS and others predominantly related to UV exposure. Several reports have shown that neoantigens stimulate predominantly CD4 T cells versus CD8 T cells.95,96 This indicates that CD4 T cell-derived TCRs may be more important when targeting this group of antigens with ACT. Given the time of development of TCR therapy, targeting shared neoantigens is the likely way forward for most clinical trials. Mutant BRAF is an ongogenic driver and occurs in around 40% of melanoma. The most frequent mutation, BRAFV600, occurs in 90% of cases and can be regarded as a public neoantigen. Pharmacological inhibition of the mitogen-activated protein kinase (MAPK) pathway with BRAF and MEK inhibitors has led to major advances in melanoma treatment. But despite initial impressive effect of BRAF and MEK inhibitors, resistance is acquired through recruitment of alternative signalling pathways.97 Approximately 50% of patients treated with the BRAF and MEK inhibitor combination progressed after 9-10 months.98 A recent case report illustrated how TCR therapy can be successfully used to target neoantigens with complete response in a melanoma patient treated with CD4 T cell-derived BRAFV600E-specific TCR-engineered T cells.99 Wide use of such therapy would require targeting a shared neoantigen presented by a frequently expressed HLA allele.

Other frequently mutated genes that could be targeted for TCR therapy are MUM-1, B-catenin, CDK4 and ERBB2Ip.100

While neoantigens expressed by melanoma cells would be the most immunogenic, that is most likely to expand cytotoxic T cells, self-antigens expressed at high levels could potentially break tolerance and activate low-affinity CD8+ T cells. To induce effective anti-tumour responses from such tolerant or low-affinity T cells, the use of checkpoint inhibitors or a combination with self-antigen cancer vaccines may be necessary.100

Identification of neoantigens broadly expressed by melanomas is an area of intense research. Neoantigens vary from patient to patient or even between tumours in the same individual, thus requiring development of highly personalized treatment which can be both cost- and labour-intensive with no guarantee of success.101

4 | CLINICAL TRIALS OF TCR AGAINST MELANOMA

4.1 | Efficacy in clinical trials

Thirty clinical trials of TCR therapy in melanoma were identified in our search (see Table 1). There are published results from only seven of these 30 clinical trials. Overall out of these seven, there have been objective clinical responses in some patients with either partial or complete results published, which have validated genetically modified T cell immunotherapy as a promising melanoma treatment strategy. Limited information on clinical outcome is available, but for certain studies, analysis of the T cells is published.102 Gene expression analysis of T cells pre- and post-infusion showed that the post-infusion T cells upregulated numerous immune checkpoint molecules, indicating that they were activated in vivo, but they did not seem to persist or induce strong clinical responses.

For the first trial described targeting MART-1 (NCT00509288), partial response (PR) was observed in 6/24 patients and the remaining patients had progressive disease (PD).102 In another TCR trial targeting MART-1 in combination with a dendritic cell (DC) vaccine (NCT00910650), 9 of 13 treated patients (69%) showed evidence of initial transient tumour regression measured at day 30.103 Amendments were made during the protocol adjusting cell dose and infusing fresh rather than cryopreserved T cells, and this was associated with increased side effects.

One of the promising TCR therapy trials targeting NY-ESO-1 (NCT00670748) demonstrated objective clinical responses in 11 of 20 (55%) melanoma patients.104 The estimated overall three- and five-year survival rates for the melanoma patients in this trial were both 33% which is comparable to treatment with some ICI. This is so far the only study with NY-ESO-1 TCR in melanoma that has published results with a reasonable number of patients and is therefore very encouraging. Similar to the MART-1 + gp100 study,102 few results on clinical outcome are available for TCR therapy trials targeting MAGEs. A study by Rosenberg’s group, targeting MAGE-A3, is the only study so far demonstrating evidence of cancer regression, seen in four out of seven patients. However, as discussed below, the treatment was associated
with severe toxicity in some patients and the trial was terminated (NCT01273181). Another study from the same group targeted MAGE-A3 using an HLA-DP4-restricted TCR (NCT02111850). This was the first clinical trial reporting an MHC II-restricted TCR. Six of the seventeen patients treated had melanoma, but none of these patients were amongst the four patients demonstrating partial or complete responses. Various MAGEs are targeted by different TCRs in the studies described in Table 1, and their publications are much awaited. There is only one TCR study targeting tyrosinase with published results (NCT01588403, active). In this study, one of three patients demonstrated a PR, but became a complete responder after subsequent high-dose IL-2 therapy, whereas another patient presented with vitiligo post-TCR therapy, showing that the infused T cells were biologically active. These initial clinical results are encouraging, but will need to be validated in a larger patient cohort. There is one study initiated in 2019 (NCT03970382) which incorporates both CD4 and CD8 TCRs specific for neoantigens with or without nivolumab. This is the second study only that includes CD4 T cell-derived TCRs and will be closely followed for clinical results.

Hoping the field will make a leap forward and offering new treatment options for melanoma patients unresponsive to standard treatments today, the importance of publishing unfavourable, or lack of, results is vital knowledge. The publications of results from these clinical trials are therefore awaited with much anticipation.

4.2 | Toxicities related to different antigen use

The most severe toxicities related to TCR therapy have been seen when T cells attack normal tissue, either so-called on-target/off-tumour or off-target autoimmunity. Shared antigens present at low levels in normal tissue can still be targeted effectively on cancer cells in some situations. The frequently tested MART-1 or gp100-specific TCRs were generally associated with vitiligo and severe rash, but patients also experienced uveitis and hearing loss sometimes requiring local steroid administration. The same toxicities were not seen when these antigens were targeted with vaccines, demonstrating the potency of TCR therapy versus vaccination. Off-target toxicity occurs when the TCR unpredictably recognizes another antigen than the intended one. Unexpected deaths were seen after treatment with a MAGE-A3-targeted TCR due to cross-reactivity with related MAGE protein found at low levels in normal brain causing neurotoxicity. Another example of lethal toxicities observed when targeting MAGE-A3 with a different TCR was observed due to cross-reactivity with titin, a protein expressed in cardiomyocytes. The MAGE-A3-specific TCRs used in these studies were either structurally modified or generated in HLA transgenic mice to enhance affinity which can increase the risk of unpredicted cross-reactivity. No evidence of autoimmunity has been seen with targeting of the NY-ESO-1 antigen despite high objective response rates in patients with metastatic melanoma and synovial sarcoma. Careful consideration of both the target antigen is therefore required. Furthermore, despite extensive preclinical testing and predictions using available algorithms and databases, not all cross-reactivity can be foreseen, and therefore, precautions should be taken with first-in-man clinical testing.

5 | CHALLENGES OF THE TUMOUR MICROENVIRONMENT (TME)

The existing relationship between cancer cells and immune cells can be described following the rule of 3 E’s: elimination, equilibrium and escape.

During early phases, transformed cells are actively eliminated by immune cells, thus impeding tumour initiation. Due to the high plasticity of tumour cells, and the eventual development of favourable mutations, a subset of transformed cells can acquire properties that lead to evasion. During “equilibrium”, tumour initiation is achieved by selection and expansion of more immune-resistant clones, but the host’s immune system is able to control tumour growth through continuous elimination of immune-sensitive clones.

The TME is a cellular niche consisting of tumour cells, fibroblasts, immune cells, signalling molecules and the extracellular matrix (ECM). The plasticity of cancer cells, including melanoma, leads to a phenomenon called ‘immune escape’, whereby cancer cells acquire a less immunogenic phenotype and the ability to suppress anti-tumour immune cells within the TME.

5.1 | Cells of the TME

For tumour-reactive T cells to efficiently attack tumour cells, they must home to the tumour site. They rely not only on their tumour-specific TCR, but also on unique selectin-chemokine receptor-integrin combinations, to enable organ-specific targeting. Tumours have evolved multiple ways to block T cell penetrance into lesions, which include down-regulation of the necessary adhesion molecules, chemokines and other pro-migratory molecules not only on tumour cells, but also leveraging the TME for this immunosuppression. Many of the cell types present in the TME known to exert such functions in melanoma are described below.

Myeloid-derived suppressor cells (MDSCs) have the ability to inhibit the T cell response by modulating cytokine...
production. The activation of MDSCs in cancer results in a high expression of both arginase 1 (ARG1) and inducible nitric oxide synthase (iNOS/NOS2). MDSCs require direct cell-to-cell contact with T cells, and at the same time, high NOS2 levels and ARG1 to promote immunosuppression. The expression of ARG1 by MDSCs also depletes the microenvironment of L-arginine and locally impairs the proliferation of T cells. It has also been demonstrated that MDSCs promote a functional skew of CD4+ T cells into CD4+/CD25+ Tregs.

Tumour-associated neutrophils (TANs): Neutrophils make up 50%-70% of circulating leucocytes in humans and are major players in innate immune responses, but have also been observed in close association with tumour cells. Their exact role within the TME is still a subject of controversy with publications both demonstrating pro-tumorigenic and anti-tumour functions of neutrophils. TANs support tumour growth by secreting vascular epidermal growth factor (VEGF) promoting angiogenesis. TANs also secrete ARG-1, TGF-β and iNOS which promote immunosuppressive macrophage phenotypes and inhibit T cell responses.

One of the chemokines shown to recruit neutrophils to melanoma is CXCL-6. Neutralizing antibodies against CXCL-6 were shown to reduce neutrophil recruitment and lead to reduced tumour growth in a melanoma model. Neutrophilia has been associated with poorer prognosis in many cancers, including melanoma.

Cancer-associated fibroblasts (CAFs): There is an increased number of fibroblasts and collagen fibres in the TME. CAFs stimulate expansion of extracellular matrix (ECM) and matrix tension. Melanoma cells are able to affect the behaviour of stromal cells through CAFs in order to promote the recruitment of pro-tumour immune cells. CAFs are known to promote cancer cell proliferation and invasion.

Tumour-associated macrophages (TAMs): Monocytes differentiate to macrophages that can change their phenotypes responding to signals in the TME. Macrophages and cancer cells are known to upregulate the production of various cytokines, including VEGF, platelet-derived growth factor (PDGF) and TGF-β. These cytokines can stimulate angiogenesis to produce new blood vessels to accommodate the increasing metabolic needs. In line with this, Georgouli and colleagues recently demonstrated how myosin activity in melanoma cells reprogrammes the TME by enabling the recruitment of monocytes that differentiate into pro-tumorigenic or M2 macrophages which also support angiogenesis.

Regulatory T cells (Tregs): Tregs have an essential role in sustaining self-tolerance and immune homeostasis by suppressing many physiological and pathological immune responses. Naturally occurring Tregs are produced in the thymus and form a functionally distinct T cell population in the periphery making up 5%-10% of the CD4-positive T cells in peripheral blood. They express transcription factor forkhead box P3 (FOXP3), a factor controlling the expression of proteins capable of mediating Treg suppressive function. In melanoma, high-frequency Tregs correlate with tumour progression and poor survival and have been associated with poor clinical outcome in melanoma patients treated with immunotherapy. Treg functions depend on activation of T cell receptors, but the cells exert their effect in an antigen-non-specific manner. In murine models of melanoma, transient Treg depletion has induced anti-tumour immunity and improved tumour clearance and survival.

Dendritic cells (DCs): DCs are professional antigen-presenting cells. They originate from CD34+ precursor cells in the bone marrow. Mature cells circulate in the blood and can migrate towards peripheral tissues, including tumours. They interact with lymphoid and myeloid cells. Mature DCs express multiple co-stimulatory markers, including CD80 and CD86, which are essential for activation of T cells. In melanoma, DCs are initially activated in the tumour bed in the presence of tumour DNA through the cGAS-STING pathway. Soluble tumour antigens from necrotic melanoma cells are engulfed by DCs and macrophages and proteolytically processed for direct presentation or cross-presentation to naïve T cells in tumour-draining lymph via MHC molecules. Ultimately, functional effector T cells must be recruited to melanomas through a chemokine gradient generated by DCs or tumour-associated stroma. DCs are essential to orchestrate the T cell response to melanoma and thus the generation of melanoma-specific TCRs that could be further used in therapy. Importantly, DCs modified to present melanoma antigens have been exploited as cancer vaccines.

5.2 Mechanisms for escape of T cell recognition

Melanoma cells can escape T cell recognition through the following mechanisms: (a) downregulation of TAAs, (b) changes in antigen-processing machinery that may include proteasome subunits or transporters associated with antigen processing (TAP) and (c) downregulation of MHC molecules. The latter occurs through epigenetic modifications (so-called soft or reversible lesions) or through genetic mutations leading to loss of HLA alleles or β2-microglobulin (hard, non-reversible lesions).

In the case of reversible lesions, these can be recovered by various interventions inducing IFN-γ stimulation. A study characterizing structural and epigenetic HLA class I antigen-processing machinery defects in a panel of 57 metastatic melanomas identified novel, irreversible tapasin mutations associated with HLA haplotype loss as well as selective epigenetic IFN-γ unresponsiveness. Murine models have shown CD8+ T cells specific for tumour antigen/MHC I capable of inducing regression of MHC I-deficient melanoma, provided that the T cells could migrate into the tumour and efficiently produce IFN-γ. These CD8+ T cells were resistant to...
inhibition both through the PD-1/PDL-1 pathway and through TGF-β.\textsuperscript{132} This still depends on the tumour being sensitive to IFN-γ, but IFN-γ resistance through inactivating mutations in the JAK1/2-signalling pathway has been identified in melanoma patients, giving rise to T cell-resistant tumours.\textsuperscript{133}

By reducing antigen presentation alone, melanoma cells are capable of becoming virtually ‘invisible’ to the immune system.\textsuperscript{134–136} Melanoma cells are not only restricted to a single avenue of immune escape, the high antigen load within the tumour microenvironment can contribute itself to T cell exhaustion and failed tumour control, thus adding to the complexity, and difficulty, of curative treatments.\textsuperscript{108}

5.3 Intra-tumoural vasculature and pressure

There are multiple contributing factors to the formation of increased pressure in solid tumours. Ariffin et al highlight four major factors: hyperpermeable and tortuous tumour vasculatures, lack of functional inter-tumoural lymphatic vessels, abnormal TME and solid stress exerted by proliferating tumour cells.\textsuperscript{137} Lowering of tumour interstitial fluid pressure (IFP), for example, by certain cytokine antagonists can improve drug uptake and thereby improve treatment efficiency.

Tumour cells proliferate faster than the formation of its vasculature resulting in abnormal and leaky capillaries compared to our normal vessels. A higher pressure within tumour cells makes it more difficult for T cells to infiltrate the tumour. The abnormalities in vascular structure cause blood stasis, leading to a reduction of oxygen and blood flow in the tumour. The resulting hypoxia leads to an accumulation of extracellular adenosine, which inhibits anti-tumour T cells. Another factor inhibiting T cells can be the transcription factor hypoxia-inducible factor-1α (HIF-1α), often upregulated in response to hypoxia to promote angiogenesis.\textsuperscript{138}

6 INCREASING EFFECTIVENESS OF TCR IN MELANOMA

6.1 Immune checkpoint inhibitors

The use of immune checkpoint inhibitors (ICI) in clinical management of melanoma is firmly sedimented today and is used also for the less responsive mucosal melanomas.\textsuperscript{139} The phase III trials CheckMate-066 (using nivolumab) and Keynote-006 (using pembrolizumab) showed that PD-1 inhibitor monotherapy has an objective response rate about 40%-45% and relatively well-tolerated side effects, about 17% grade 3-4 adverse events (AEs).\textsuperscript{7,140} PD-1 inhibitor monotherapy is therefore widely used today. The Checkmate-067 trial with the combination of CTLA-4 and PD-1 inhibition (ipilimumab and nivolumab, respectively) further increased the overall survival to 52% and also has an effect in patients with asymptomatic brain metastasis, but it comes at a cost with 59% grade 3 or 4 AEs.\textsuperscript{9,141} T cell activation is a complex process that requires more than one stimulatory signal. TCR binding to MHC provides specificity to T cell activation, but further co-stimulatory signals are required. Binding of B7-1 (CD80) or B7-2 (CD86) molecules on the APC with CD28 molecules on the T cell leads to signalling within the T cell.\textsuperscript{142}

CTLA-4 is a negative regulator of T cell function. In resting naive T cells, CTLA-4 is located primarily in the intracellular compartment and stimulatory signals from both TCR and CD28:B7 binding induce upregulation of CTLA-4 on the cell surface. CTLA-4 is a CD28 homologue with much higher binding affinity for B7, but unlike CD28, binding of CTLA-4 to B7 does not produce a stimulatory signal.\textsuperscript{143} The relative amount of CD28:B7 binding versus CTLA-4:B7 binding determines whether a T cell will undergo activation or anergy.\textsuperscript{144} Tregs highly express CTLA-4 and are able to exert a suppressive function of T cells through CTLA-4. The exact mechanism by which anti-CTLA-4 antibodies induce an anti-tumour response is unclear, although it is believed that CTLA-4 blockade affects the immune priming phase by supporting the activation and proliferation of a higher number of effector T cells, regardless of TCR specificity, and by reducing Treg-mediated suppression of T cell responses.\textsuperscript{145} Anti-CTLA-4 treatment with ipilimumab was the first ICI to be introduced in the clinic. After the arrival of anti-PD-1 treatment, anti-CTLA-4 treatment was initially believed to be less used in the future. Anti-CTLA-4 and anti-PD-1 have different mechanisms of function and combination therapy of the two further increased response rates, and it is today considered the most efficient treatment in melanoma.\textsuperscript{9}

PD-1 is a transmembrane receptor that is induced by, and a negative regulator of, T cell activation. PD-1 plays an essential role of maintaining tolerance and preventing autoimmunity. PD-1 also limits productive T cell immunity against pathogens and tumour cells. When exposed to persistent antigenic challenge, they enter a state of exhaustion, and PD-1 is highly expressed on such non-functional, exhausted T cells.\textsuperscript{146} PD-1 ligation is required for inhibition, and it is believed that PD-1 must co-localize with the TCR-CD3 complex or CD28 to exert its function.\textsuperscript{147} Programmed cell death ligand-1 and programmed cell death ligand-2 (PD-L1/PD-L2) (B7-family members) are ligands for PD-1. PD-L1 is broadly expressed on hematopoietic and non-hematopoietic cells, including on a wide variety of tumours, and high levels of PD-L1 expression correlates with unfavourable prognosis.\textsuperscript{148} PD-L2 expression is restricted to APCs like DCs and macrophages. Anti-PD-1 therapy is one of the foundations of modern melanoma treatment.

CTLA-4 and PD-1 have been the main targets for ICI therapy; however, new targets are emerging as promising
additional candidates and are under clinical trial: lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin domain 3 (TIM-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT) and AXL.

LAG-3 is expressed on activated CD4+ and CD8+ T cells, Tregs, B- and NK cells as well as DCs. Its interaction can lead to inhibition of CD4+ and CD8+ T cell proliferation and decreased cytokine secretion, but the precise molecular mechanisms by which LAG-3 mediates the TCR signalling pathway and function are still largely unknown. Studies with Lag-3 targeting antibody (relatlimab and others) in melanoma are on their way and early results in patients who had progressed on a PD-1 inhibitor showed a disease control rate of relatlimab plus nivolumab of 45%. Given that it also appears to be relatively well tolerated, 9% grade 3-4 AEs, it could be a good potential partner for TCR therapy.

TIM-3 is a negative regulator that is expressed on CD4+ and CD8+ T cells, Tregs, B cells, NK cells, DCs, mast cells and macrophages. It serves as a negative regulator of Th1 response and Th1-related production of TNF-α and IFN-γ. It can induce immunological tolerance leading to asthma, allergies and autoimmune disease. In melanoma, high expression of TIM-3 has been associated with CD8 T cell exhaustion. There is too little clinical evidence so far to suggest TIM-3 targeting therapy can be a good companion for TCR therapy in the near future.

TIGIT is expressed on both Tregs and activated T and NK cells. CD4+ Tregs impede T cell responses to tumours. They express multiple inhibitory receptors that support their suppressive functions, including TIGIT. In melanoma patients, Tregs exhibit increased TIGIT expression and decreased expression of its competing co-stimulatory receptor CD226 as compared with CD4+ effector T cells. Such an increased TIGIT/CD226 ratio correlates with increased Treg frequencies in tumours and poor clinical outcome upon immune checkpoint blockade. Use of anti-TIGIT and anti-CD226 therapy together with TCR against melanoma in the future is uncertain.

AXL is a tyrosine kinase. Ligand binding leads to activation of downstream signalling pathways by PI3K and ERK/MAPK. Induced expression of AXL in tumours correlates with disease progression and decreased survival. AXL expression is higher in tumour cells resistant to immunotherapy, targeted therapies and chemotherapy, compared to treatment-sensitive cells. Inhibition of AXL in combination with the PD-1 inhibitor pembrolizumab is currently in a phase IIb/II clinical trial for treatment of metastatic melanoma (NCT02872259).

### 6.2 Radiation therapy (RT)

Melanoma has traditionally been regarded as a radiation-resistant disease. However, in the aftermath of ICI therapy we have seen clinical trials combining radiation therapy (RT) with ICIs in combination with ipilimumab monotherapy, in combination with anti-PD-1 (nivolumab or pembrolizumab) (and two unpublished studies: NCT02407171 and NCT02562625) or the ongoing ABC-X study where combination therapy of ipilimumab + nivolumab is given to patients with brain metastasis alone or in combination with stereotactic radiotherapy (NCT03340129). These studies have showed good tolerance of the combination of RT and ICI and a possible synergistic effect. RT has immediate effects on the TME by causing local cytokine release. It causes a window of opportunity for reconstitution of the TME. In the target field, RT eliminates many of the immune cells and especially those of lymphocytic origin. After RT, there is an increased immune infiltration in the radiation field. Whether conventional RT or stereotactic radiation, fractionated or single dose of RT is the best when it comes to harnessing the benefits together with ICI, or possibly TCR, is still uncertain. Schaue et al observed that fractionated radiation maintained a low number of regulatory T cells (Tregs), while a single high-dose RT resulted in an increase in Treg representation, supporting a ‘shoot to harm, not to kill’ mentality even when it comes to a RT-immunotherapy synergy. Achieving abscopal effect with radiation therapy in melanoma patients is sought after, but, alas, seen very rarely.

### 6.3 β-blockers

In 1966, Edlich et al published work showing that the administration of low doses of adrenaline and noradrenaline in rodents with melanoma was associated with a relevant reduction in tumour blood flow and an unchanged blood flow in the normal skin. The influence of β-adrenergic receptor (β-AR)-driven stress on the immune response and the role of stress in suppressing anti-tumour immune response is well documented. Increased β-AR signalling reduces pro-inflammatory cytokine secretion from monocytes and macrophages. In melanoma biopsies, β-AR expression has been detected indicating that human melanoma may be directly sensitive to β-blocker treatment; however, there is no similar evidence about α-AR expression. Kokolus et al found in their retrospective study in melanoma patients receiving anti-PD-1 therapy that the 62 (out of 195) patients taking pan-β-blockers lived longer than those who did not. Their results were reproduced by a β-2-selective blocker, but not a
β-1-selective blocker, suggesting the main effect on immunotherapy being mediated by β-2-selective blocker. De Giorgi et al report some conflicting evidence about β-blockers in melanoma and its beneficial effect. A Case-control studies from the UK, the Netherlands and the United States could also not show beneficial effect in melanoma patients receiving ICI therapy. A recent study in a murine melanoma model showed enhanced effect of vaccine in combination with β-blockers due to a direct effect of β-blockers on naïve CD8+ T cells which have high levels of β-AR. In contrast to mice, human memory CD8+ T cells express higher levels of β-AR than naïve CD8+ T cells. Depending on the infused subset of T cells or the sequence of infusion and treatment with β-AR, β-blockers could thus potentially influence the efficacy of TCR therapy, either directly or through enhancing secondary endogenous immunity (see Figure 2). Until large double-blinded, randomized, clinical trials will be available, no final conclusions can be drawn on the possible benefits of β-blocker use together with ICI therapy or, potentially, TCR.

6.4 | Microbiome

The role of the gut microbiome and its effects on immunotherapy are under tremendous scrutiny. Preclinical models gave evidence that differential composition of the gut microbiome can influence therapeutic responses to anti-PD-1 therapy at the level of the TME. How the gut microbiome might modulate the response to ICIs in melanoma patients has so far been published in two clinical studies. Results from both indicated the gut microbiome may modulate responses to anti-PD-1 immunotherapy in melanoma patients (see Figure 2).

Wargo and her team prospectively collected microbiome samples (both oral/buccal and gut/faecal) from 112 patients with metastatic melanoma starting treatment with anti-PD-1 therapy and studied together with response to ICI therapy. In responders, they found a gut microbiome with high diversity and abundance of Ruminococcaceae and Faecalibacterium and hence favourable, enhancing systemic and anti-tumour immune responses. This response was mediated by increased antigen presentation and improved effector T cell function in the periphery and TME. In non-responder patients, they identified an unfavourable gut microbiome, with low diversity and high relative abundance of Bacteroidales. They had a limited intra-tumoural lymphoid and myeloid infiltration, and weakened antigen presentation capacity mediated an impaired systemic and anti-tumour immune response. Gajewski and colleagues collected stool samples from 42 patients with metastatic melanoma before receiving ICI therapy (anti-PD-1 or CTLA-4). A significant association was observed between commensal microbial composition and clinical response. Bacterial species more abundant in

**FIGURE 2** Factors impacting on the clinical success of a TCR in melanoma. Figure summarizes factors with possible influence on T cell therapy (TCR). Green arrows indicate a positive influence on efficacy, and red arrows indicate negative influences. Graphical elements adapted from Servier Medical Art repository (http://www.servier.com)
responders included operational taxonomic units (OTUs) of *Bifidobacterium longum*, *Collinsella aerofaciens* and *Enterococcus faecium*. Their cohort also suggested that a ratio of favourable OTUs to unfavourable OTUs was the strongest predictor of clinical response.

The Zitvogel laboratory did not study melanoma patients, but 249 advanced lung, renal and urothelial cancer patients treated with anti-PD1. Importantly, they found that patients receiving antibiotics before or soon after anti-PD-1 relapsed sooner and had an overall survival that was less than half compared to patients that did not receive antibiotics. Based on samples for 100 patients with lung and renal cancer, they found a significantly increased representation of the OTU *Akkermansia muciniphila* in anti-PD-1 responders compared to non-responders. In non-responding lung cancer patients, they found a higher frequency of *Staphylococcus haemolyticus* and *Corynebacterium aurimucosum*. In mice, they demonstrated that faecal transfer from a responder patient could rescue unresponsiveness to anti-PD-1 in mice recolonized with faecal microbiome from non-responder patients.

These three studies identified completely different bacterial OTUs in the gut, and it is tempting to speculate that this could be due to the geographic differences, but it does show that we are still lacking many answers when it comes to the microbiome. However, it is clear that the gut microbiome can modulate the response to ICI therapy, especially from the PD-1/PD-L1 axis. And, importantly, the use of antibiotics before or soon after initiating ICI treatment should be avoided, as it can alter the microbiome-balance in the gut towards a low diversity and unfavourable disposition.

## 7 | CONCLUSIONS AND FUTURE PERSPECTIVES

From this review, we conclude that that TCR therapy has enormous clinical potential in melanoma treatment. Compared to CAR-T, TCR therapy has been far less clinically tested. ACT in solid tumours generally comes with numerous challenges, many of which we have discussed. Great advantages of TCR therapy are that they can target all cellular proteins, are highly sensitive to low antigen densities and can detect as little as one amino acid change caused by mutation. Additionally, TCRs can be highly tumour-specific, although clinical trials have shown that care must be taken to avoid toxicity. This is especially true for enhanced, high-affinity TCRs that can be cross-reactive to epitopes present in normal tissue, related or unrelated to the target antigen. A disadvantage of TCR remains in their HLA restriction. Less frequent HLA alleles are not likely to be exploited unless highly personalized therapy is applied. This is not a consideration when patient-specific neoantigens are targeted for autologous treatment. Targeting neoantigens is a safer approach, although more time consuming and expensive. With further technology development, this may become more widely applicable in the future. There are many antigens identified in melanoma, as we have discussed above, but in order to reach a larger number of patients, efforts should be made to identify TCRs with different HLA restrictions.

One major challenge in solid cancers in general is that the redirected T cells have to overcome the often highly immunosuppressive TME. Even with a very potent TCR, the T cells might be trapped at the tumour border or get eliminated before they can reach their target. One future possibility might lie in introducing the expression of homing receptors or pro-inflammatory cytokines in tumour-directed TCR-modified T cells.

Combination of ICIs and TCR therapy is starting to enter clinical trials and may make the tumours more permissive to T cell attack. TCR therapy will most probably continue to be tested in melanoma patients that have progressed on ICIs. Combined application with ICIs, radiotherapy, vaccines or other modalities will likely be needed to improve efficacy (see Figure 2). Interestingly, very few CD4 compared to CD8 T cell-derived TCRs have so far been tested, but are now emerging. CD4 T cells can also be cytotoxic; in addition, they exert an important helper function as well as activating other players of the immune system. CD4 T cell responses can alter the TME and contribute to establish long-lasting T cell memory.

Despite tremendous improvements in melanoma survival rates, largely attributed to immunotherapy, half of patients with stage IV melanoma experience disease progression today. We believe these patients will be a major focus for TCR therapy. Future research and clinical trials will provide answers concerning the most efficient TCR approach for melanoma; will it be by using CD4 or CD8 TCRs, in combination with drugs or RT, or even by harnessing the microbiome?

### ACKNOWLEDGMENT

The authors wish to thank Prof. Gunnar Kvalheim (Oslo University Hospital, Oslo, Norway) for his support. We are also grateful to the Research Council of Norway and the South-Eastern Norway Regional Health Authority for funding the TCR research.

### CONFLICT OF INTEREST

All authors declare no potential conflicts of interest.

### AUTHOR CONTRIBUTIONS

AKW-M. wrote, reviewed and revised the manuscript, and prepared and revised the table and the figures. SW reviewed
and revised the manuscript, and revised the table and the figures. EMI wrote, reviewed and revised the manuscript, prepared and revised the table, and revised the figures. All authors have agreed to the published version of this manuscript.

**ORCID**

Sébastien Wälchli [https://orcid.org/0000-0001-5869-1746](https://orcid.org/0000-0001-5869-1746)

Else Marit Inderberg [https://orcid.org/0000-0002-6147-3536](https://orcid.org/0000-0002-6147-3536)

**REFERENCES**

1. Khazaee Z, Ghorat F, Jarrahi AM, Adineh HA, Sohrabivafa M, Goodarzi E. Global cancer and mortality of skin cancer by histological subtype and its relationship with human development index (HDI); an ecological study in Kazaei, Z.; 2018. World Cancer Res J. 2019;6:e1265.

2. Tsao H, Atkins MB, Sober AJ. Management of cutaneous melanoma. *N Engl J Med*. 2004;351(10):998-1012.

3. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol*. 1999;17(7):2105.

4. Hodi FS, O’Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Eng J Med*. 2010;363(8):711-723.

5. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010;363(9):809-819.

6. Weber JS, Hodi FS, Wolchok JD, et al. Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma. *N Eng J Med*. 2013;369:2105-2117.

7. Robert C, Ribas A, Schachter J, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma. *J Clin Oncol*. 2017;35(7):785-792.

8. Spathis A, Katoulis A, Damaskou V, et al. BRAF mutation status in primary, recurrent, and metastatic malignant melanoma and its relation to histopathological parameters. *Dermatol pract*. 2019;9(9):54-62.

9. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab in untreated melanoma. *N Engl J Med*. 2019;381(16):1535-1546.

10. Pardoll D. Cancer and the immune system: basic concepts and targets for intervention. *Semin Oncol*. 2015;42(4):523-538.

11. Besser MJ, Shapira-Frommer R, Izhaki O, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clin Cancer Res*. 2013;19(17):4792-4800.

12. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res*. 2011;17(13):4550-4557.

13. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378(5):439-448.
34. Tendeiro Rego R, Morris EC, Lowdell MW. T-cell receptor gene-modified cells: past promises, present methodologies and future challenges. Cytotherapy. 2019;21(3):341-357.

35. Wang X, Riviere I. Manufacture of tumor- and virus-specific T lymphocytes for adoptive cell therapies. Cancer Gene Ther. 2015;22(2):85-94.

36. Sadelain M, Papapetrou EP, Bushman FD. Safe harbours for the integration of new DNA in the human genome. Nat Rev Cancer. 2012;12(1):51-58.

37. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature. 2018;558(7709):307-312.

38. Ellis JS, Yao S. Retrovirus silencing and vector design: relevance to normal and cancer stem cells? Curr Gene Ther. 2005;5:367-373.

39. Peng PD, Cohen CJ, Yang S, et al. Efficient nonviral sleeping beauty transposon-based TCR gene transfer to peripheral blood lymphocytes confers antigen-specific antitumor reactivity. Gene Ther. 2009;16(8):1042-1049.

40. Field A-C, Vink C, Gabriel R, et al. Comparison of lentiviral and sleeping beauty mediated αβ T cell receptor gene transfer. PLoS One. 2013;8(6):e68201.

41. Zhao Y, Zheng Z, Robbins PF, Khong HT, Rosenberg SA, Morgan RA. Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines. J Immunol. 2005;174(7):4415-4423.

42. Beatty GL, Haas AR, Maus MV, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res. 2014;2(2):112-120.

43. Eisenberg V, Hoogi S, Shamul A, Barliya T, Cohen CJ. T-cells "à la CAR-T(e)" - Genetically engineering T-cell response against NY-ESO-1. Cancer Immunol Res. 2019;7(9):1062-1074.

44. Herlyn M, Koprowski H. Melanoma antigens: immunological and biological characterization and clinical significance. Annu Rev Immunol. 1988;6:283-308.

45. Ilyas S, Yang JC. Landscape of tumor antigens in T cell immunotherapy. J Immunol. 2015;195(11):5117-5122.

46. Fagerberg L, Jonasson K, von Heijne G, Uhlén M, Berglund L. Prediction of the human membrane proteome. Proteomics. 2010;10(6):1141-1149.

47. Pitcovski J, Shahar E, Aizenshtein E, Gorodetsky R. Melanoma antigens and related immunological markers. Crit Rev Oncol Hematol. 2017;115:36-49.

48. Hod H. Well-defined melanoma antigens as progression markers for melanoma: insights into differential expression and host response based on stage. Clin Cancer Res. 2006;12(3):673-678.

49. Barrow C, Browning J, MacGregor D, et al. Tumor antigen expression in melanoma varies according to antigen and stage. Clin Cancer Res. 2006;12(3):764-771.

50. Kawakami Y, Elyahu S, Delgado CH, et al. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci U S A. 1994;91(14):6458-6462.

51. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006;314(5796):126-129.

52. Topalian SL, Gonzales MI, Parkhurst M, et al. Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med. 1996;183(5):1965-1971.

53. Moore T, Wagner CR, Scurti GM, et al. Clinical and immunologic evaluation of three metastatic melanoma patients treated with autologous melanoma-reactive TCR-transduced T cells. Cancer Immunol Immunother. 2018;67(2):311-325.

54. Pak BJ, Chu W, Lu SJ, Kerbel RS, Ben-David Y. Lineage-specific mechanism of drug and radiation resistance in melanoma mediated by tyrosinase-related protein 2. Cancer Metastasis Rev. 2001;20(1-2):27-32.

55. Khalil DN, Postow MA, Ibrahim N, et al. An open-label, dose-escalation phase I study of anti-TYRP1 monoclonal antibody IMC-20D7S for patients with relapsed or refractory melanoma. Clin Cancer Res. 2016;22(21):5204-5210.

56. Hotblack A, Holler A, Piapi A, Ward S, Stauss HJ, Bennett CL. Tumor-resident dendritic cells and macrophages modulate the accumulation of TCR-engineered T cells in melanoma. Mol Ther. 2018;26(6):1471-1481.

57. Kerkar SP, Sanchez-Perez L, Yang S, et al. Genetic engineering of murine CD8+ and CD4+ T cells for preclinical adoptive immunotherapy studies. J Immunother. 2011;34(4):343-352.

58. Hoashi T, Watabe H, Muller J, Yamaguchi Y, Vieira WD, Hearing VJ. MART-1 is required for the function of the melanosomal matrix protein PMEL17/GP100 and the maturation of melanosomes. J Biol Chem. 2005;280(14):14006-14016.

59. Kawakami Y, Battles JK, Kobayashi T, et al. Production of recombinant MART-1 proteins and specific anti-MART-1 polyclonal and monoclonal antibodies: use in the characterization of the human melanoma antigen MART-1. J Immunol Methods. 1997;202(1):13-25.

60. Kurnick JT, Ramirez-Montagut T, Boyle LA, et al. A novel autocrine pathway of melanoma escape from immune recognition: melanoma cell lines produce a soluble protein that diminishes expression of the gene encoding the melanocyte lineage melan-A/MART-1 antigen through down-modulation of its promoter. J Immunol. 2001;167(3):1204-1211.

61. Gordan JD, Vonderheide RH. Universal tumor antigens as targets for immunotherapy. Cytotherapy. 2002;4(4):317-327.

62. Zanetti M. A second chance for telomerase reverse transcriptase in anticancer immunotherapy. Nat Rev Clin Oncol. 2017;14(2):115-128.

63. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957-959.

64. Grossman D, McNiff JM, Li F, Alitieri DC. Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. J Invest Dermatol. 1999;113(6):1076-1081.

65. McKenzie JA, Liu T, Jung JY, et al. Survival promotion of melanoma metastasis requires upregulation of α5 integrin. Carcinogenesis. 2013;34(9):2137-2144.

66. Nitschke NJ, Bjoern J, Met O, Svane IM, Andersen MH. Therapeutic vaccination against a modified minimal survivin epitope induces functional CD4 T cells that recognize survivin-expressing cells. Scand J Immunol. 2016;84(3):191-193.

67. Inderberg EM, Wålchli S. Long-term surviving cancer patients as a source of therapeutic TCR. Cancer Immunol Immunother. 2020;69(5):859-865.

68. Wang D, Wang J, Ding N, et al. MAGE-A1 promotes melanoma proliferation and migration through C-JUN activation. Biochem Biophys Res Commun. 2016;473(4):959-965.
indicate specific roles in lineage differentiation. *Hum Reprod.* 2008;23(10):2194-2201.

70. van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science.* 1991;254(5038):1643-1647.

71. Simpson AJG, Caballero OL, Jungbluth A, Chen Y-T, Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer.* 2005;5(8):615-625.

72. Barker PA, Salehi A. The MAGE proteins: Emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. *J Neurosci Res.* 2002;67(6):705-712.

73. Sang M, Wang L, Ding C, et al. Melanoma-associated antigen genes—an update. *Cancer Lett.* 2011;302(2):85-90.

74. Yang B, O’Herrin SM, Wu J, et al. MAGE-A, mMage-b, and MAGE-C proteins form complexes with KAP1 and suppress p53-dependent apoptosis in MAGE-positive cell lines. *Cancer Res.* 2007;67(20):9954-9962.

75. Brasseur F, Rimoldi D, Liénard D, et al. Expression of MAGE genes in primary and metastatic cutaneous melanoma. *Int J Cancer.* 1995;63(3):375-380.

76. Peled N, Oton AB, Hirsch FR, Bunn P. MAGE A3 antigen-specific cancer immunotherapeutic. *Immunotherapy.* 2009;1(1):19-25.

77. Morgan RA, Chinnasamy N, Abate-Daga D, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother.* 2013;36(2):133-151.

78. Boël P, Wildmann C, Sensi ML, et al. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity.* 1995;2(2):167-175.

79. Goodison S, Urquidi F, Münz C, Gannagé M. The tumor antigen NY-ESO-1 mediates direct recognition of melanoma cells. *Cancer Immunol Immunother.* 2005;54(12):2177-2181.

80. Lezcano C, Jungbluth AA, Nehal KS, Hollmann TJ, Busam KJ. PRAME expression in melanocytic tumors. *Am J Surg Pathol.* 2012;36(5):629-632.

81. Leczcano C, Junghbluth AA, Nehal KS, Hollmann TJ, Busam KJ. PRAME expression in melanocytic tumors. *Am J Surg Pathol.* 2018;42(11):1456-1465.

82. Velazquez EF, Junghbluth AA, Yancovitz M, et al. Expression of the cancer/testis antigen NY-ESO-1 in primary and metastatic malignant melanoma (MM)—correlation with prognostic factors. *Cancer Immunol Immunother.* 2007;56(10):1121-1129.

83. Fonteneau JF, Brilot F, Münz C, Gannagé M. The tumor antigen NY-ESO-1 mediates direct recognition of melanoma cells by CD4+ T cells after intercellular antigen transfer. *J Immunol.* 2016;196(1):64-71.

84. Smith HA, McNeel DG. The SSX family of cancer-testis antigens as target proteins for tumor therapy. *Clin Dev Immunol.* 2010;2010:150591.

85. Conrad K, Küpper J-H. Chapter 33—Tumor-associated autoantibodies. In: Shoenfeld Y, Meroni PL, Gershwin ME, eds. *Autoantibodies,* 3rd edn. San Diego, CA: Elsevier; 2014:275-287.

86. Simon B, Uslu U. CAR-T cell therapy in melanoma: a future success story? *Exp Dermatol.* 2018;27(12):1315-1321.

87. Rathinam R, Alahari SK. Important role of integrins in the cancer biology. *Cancer Metastasis Rev.* 2010;29(1):223-237.

88. Price MA, Colvin Wanshura LE, Yang J, et al. CSPG4, a potential therapeutic target, facilitates malignant progression of melanoma. *Pigment Cell Melanoma Res.* 2011;24(6):1148-1157.

89. Wiesinger M, März J, Kummer M, et al. Clinical-scale production of CAR-T cells for the treatment of melanoma patients by mRNA transfection of a CSPG4-specific CAR under full GMP compliance. *Cancers (Basel).* 2019;11(8):1198.

90. Suryo Rahmanto Y, Dunn LL, Richardson DR. The melanoma tumor antigen, melanotransferrin (p97): a 25-year hallmark—from iron metabolism to tumorigenesis. *Oncoogene.* 2007;26(42):6113-6124.

91. Ordóñez NG. Value of melanocytic-associated immunochemical markers in the diagnosis of malignant melanoma: a review and update. *Hum Pathol.* 2014;45(2):191-205.

92. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Eng J Med.* 2014;371(23):2189-2199.

93. Gubin MM, Artymov MN, Mardis ER, Schreiber RD. Tumor neoantigens: building a framework for personalized cancer immunotherapy. *J Clin Invest.* 2015;125(9):3413-3421.

94. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature.* 2013;500(7463):415-421.

95. Ott PA, Hu Z, Keskin DB, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature.* 2017;547(7622):217-221.

96. Kreiter S, Vormehr M, van de Roemer N, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature.* 2015;520(7549):692-696.

97. Long GV, Fung C, Menzies AM, et al. Increased MAPK reactivation in early resistance to dabrafenib/trametinib combination therapy of BRAF-mutant metastatic melanoma. *Nat Commun.* 2014;5(1):5694.

98. Menzies AM, Haydu LE, Carlino MS, et al. Inter- and intra-patient heterogeneity of response and progression to targeted therapy in metastatic melanoma. *PLoS One.* 2014;9(1):e85004.

99. Yeatch JR, Lee SM, Fitzgibbon M, et al. Tumor-infiltrating BRAFV600E-specific CD4+ T cells correlated with complete clinical response in melanoma. *J Clin Invest.* 2018;128(4):1563-1568.

100. Palermo B, Franzese O, Donna CD, et al. Antigen-specificity and DTIC before peptide-vaccination differently shape immune-checkpoint expression pattern, anti-tumor functionality and TCR repertoire in melanoma patients. *Oncoimmunology.* 2018;7(12):e1465163.

101. Grzywa TM, Paskal W, Wlodarski PK. Intratumor and intertumor heterogeneity in melanoma. *Transl Oncol.* 2017;10(6):956-975.

102. Abate-Daga D, Hanada K, Davis JL, Yang JC, Rosenberg SA, et al. Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and DTIC before peptide-vaccination differently shape immune-checkpoint expression pattern, anti-tumor functionality and TCR repertoire in melanoma patients. *Oncoimmunology.* 2014;3(12):e1458876.

103. Chodon T, Comin-Anduix B, Chmielowski B, et al. Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Cancer Immunol Immunother.* 2014;63(9):967-975.

104. Robbins PF, Kassim SH, Tran TLN, et al. A pilot trial using CheckMate 067, a phase I/II epitope drive therapeutic immune responses to cancer. *Nature.* 2015;520(7549):692-696.

105. Rathinam R, Alahari SK. Important role of integrins in the cancer biology. *Cancer Metastasis Rev.* 2010;29(1):223-237.

106. Price MA, Colvin Wanshura LE, Yang J, et al. CSPG4, a potential therapeutic target, facilitates malignant progression of melanoma. *Pigment Cell Melanoma Res.* 2011;24(6):1148-1157.
106. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009;114(3):535-546.

107. Linette GP, Stadtmauer EA, Maus MV, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in melanoma and melanoma. Blood. 2013;122(6):863-871.

108. Marzagalli M, Ebelt ND, Manuel ER. Unraveling the crosstalk between melanoma and immune cells in the microenvironment. Semin Cancer Biol. 2019;59:236-250.

109. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. Br J Cancer. 2018;118(1):9-16.

110. Sackstein R, Schatton T, Barthel SR. T-lymphocyte homing: an underappreciated yet critical hurdle for successful cancer immunotherapy. Lab Invest. 2017;97(6):669-697.

111. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009;9(3):162-174.

112. Fujimura T, Kambayashi Y, Aiba S. Crosstalk between regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) during melanoma growth. Oncoimmunology. 2012;1(8):1433-1434.

113. Wu L, Saxena S, Awaji M, Singh RK. Tumor-associated neutrophils in cancer: going pro. Cancers (Basel). 2019;11(4):564.

114. Verbeke H, Struyf S, Berghmans N, et al. Isotypic neutralizing antibodies against mouse GCP-2/CXCL6 inhibit melanoma growth and metastasis. Cancer Lett. 2011;302(1):54-62.

115. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer. 2019;122(6):863-871.

116. Verbeke H, Struyf S, Berghmans N, et al. Isotypic neutralizing antibodies against mouse GCP-2/CXCL6 inhibit melanoma growth and metastasis. Cancer Lett. 2011;302(1):54-62.

117. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer. 2019;122(6):863-871.

118. Verbeke H, Struyf S, Berghmans N, et al. Isotypic neutralizing antibodies against mouse GCP-2/CXCL6 inhibit melanoma growth and metastasis. Cancer Lett. 2011;302(1):54-62.

119. Schmidt H, Bastholt L, Geertsen P, et al. Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. Br J Cancer. 2019;122(6):863-871.

120. Jacobs JFM, Nierkens S, Figdor CG, de Vries IJM, Adema GJ. T-cell recruitment by chemotactant receptors BLT1 and CXCR3 regulates antitumor immunity by facilitating CD8+ T cell migration into tumors. J Immunol. 2016;197(5):2016-2026.

121. Halilovic A, Bol KP. The use of dendritic cell vaccinations in melanoma: where are we now? Melanoma Manag. 2016;3(4):247-250.

122. Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T. The urgent need to recover MHC class I in cancers for effective immunotherapy. Curr Opin Immunol. 2016;39:44-51.

123. Chang C-C, Pirozzi G, Wen S-H, et al. Multiple structural and epigenetic defects in the human leukocyte antigen class I antigen presentation pathway in a recurrent metastatic melanoma following immunotherapy. J Biol Chem. 2015;290(44):26562-26575.

124. Chheda ZS, Sharma RK, Jala VR, Luster AD, Haribabu B. Chemoattractant receptors BLT1 and CXCR3 regulate antitumor immunity by facilitating CD8+ T cell migration into tumors. J Immunol. 2016;197(5):2016-2026.

125. Mahmoud F, Shields B, Makhouli I, et al. Immune surveillance and metastasis in melanoma: a pooled analysis. J Clin Oncol. 2014;32(19):2145-2155.

126. Woo S-R, Fuertes M, Corrales L, et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014;41(5):830-842.

127. Niu G, Bowman T, Huang M, et al. Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. Oncogene. 2002;21(46):7001-7010.

128. Cesana GC, DeRaffele G, Cohen S, et al. Characterization of CD4+CD25+ regulatory T cells in patients treated with high-dose interleukin-2 for metastatic melanoma or renal cell carcinoma. J Clin Oncol. 2006;24(7):1169-1177.

129. Nizar S, Meyer B, Galustian C, Kumar D, Dalgleish A. T regulatory cells, the urgent need to recover MHC class I in cancers for effective immunotherapy. Immunity. 2016;45(2):e974959.

130. Ariffin AB, Forde PF, Jahangeer S, Soden DM, Hinchion J. Releasing pressure in tumors: what do we know so far and where do we go from here? A review. Cancer Res. 2014;74(10):2655-2662.

131. Aruffo A, Forde PF, Jahangeer S, Soden DM, Hinchion J. Releasing pressure in tumors: what do we know so far and where do we go from here? A review. Cancer Res. 2014;74(10):2655-2662.

132. Niu G, Bowman T, Huang M, et al. Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. Oncogene. 2002;21(46):7001-7010.

133. Cesana GC, DeRaffele G, Cohen S, et al. Characterization of CD4+CD25+ regulatory T cells in patients treated with high-dose interleukin-2 for metastatic melanoma or renal cell carcinoma. J Clin Oncol. 2006;24(7):1169-1177.

134. Long GV, Atkinson VG, Lo S, et al. Long-term outcomes from the randomized Ph 2 study of nivolumab (nivo) or nivo+ipilimumab (ipi) in patients (pts) with melanoma brain
metastasis 8mets): anti-PD-1 brain collaboration (ABC). Ann Oncol. 2019;30(suppl_5):v533–v563.

142. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. Am J Clin Oncol. 2016;39(1):98-106.

143. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. Annu Rev Immunol. 2001;19:565-594.

144. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182(2):459-465.

145. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008;224:166-182.

146. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219-242.

147. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of pProgrammed cell death protein-1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol. 2004;173(2):945-954.

148. Driessens G, Kline J, Gajewski TF. Costimulatory and coinhibitory receptors in anti-tumor immunity. Immunol Rev. 2009;229(1):126-144.

149. Lee WJ, Lee YJ, Choi ME, et al. Expression of lymphocyte-activating gene 3 and T-cell immunoreceptor with immunoglobulin and ITIM domains in cutaneous melanoma and their correlation with programmed cell death 1 expression in tumor-infiltrating lymphocytes. J Am Acad Dermatol. 2019;81(2):2175-2186.

150. Tundo GR, Sbardella D, Lacal PM, Graziani G, Marini S. On the role of TIGIT in antitumor immunity. Eur J Cancer. 2010;46(8):1948-1958.

151. Kin NW, Sanders VM. It takes nerve to tell T and B cells what to do. J Leuk Biol. 2006;79(6):1093-1104.

152. Yang EV, Kim S-J, Donovan EL, et al. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun. 2009;23(2):267-275.

153. Kokulos KM, Zhang Y, Sivik JM, et al. Beta blocker use correlates with better overall survival in metastatic melanoma patients and improves the efficacy of immunotherapies in mice. Oncoimmunology. 2017;7(3):e1405205.

154. De Giorgi V, Geppetti P, Lupi C, Benemei S. The role of β-blockers in melanoma. J Neuroimmune Pharm. 2016;11(1):1-11.

155. Monney L, Sabatos CA, Gagliardino M, et al. H1-specific cell surface protein Tim-3 regulates macrophage activation and severity of autoimmune disease. Nature. 2002;415(6871):536-541.

156. Fourcade J, Sun Z, Benalloua M, et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J Clin Oncol. 2010;207(10):2175-2186.

157. Yu X, Harder K, Gonzalez L, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009;10(1):48-57.

158. Fourcade J, Sun Z, Chauvin J-M, et al. CD226 opposes TIGIT to disrupt Tregs in melanoma. JCI. Insight. 2018;3(14):e121157.

159. Müller J, Krijgsman O, Tsoi J, et al. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. Nat Commun. 2014;5:5712.

160. Hiniker SM, Reddy SA, Maeccker HT, et al. A prospective clinical trial combining radiation therapy with systemic immunotherapy in metastatic melanoma. Int J Radiat Oncol Biol Phys. 2016;96(3):578-588.

161. Liniker E, Menzies AM, Kong BY, et al. Activity and safety of radiotherapy with anti-PD-1 drug therapy in patients with metastatic melanoma. Oncoimmunology. 2016;5(9):e124788.

162. Combining immunotherapy with radiotherapy. [press release]. 2018 Lurie Cancer Center Multidisciplinary Head and Neck Cancer Symposium: The ASCO Post. Presented November 11, 2018.

163. Formenti SC, Rudqvist N-P, Golden E, et al. Radiotherapy induces responses of lung cancer to CTLA-4 blockade. Nat Med. 2018;24(12):1845-1851.

164. Schaue D, Rattikan JA, Iwamoto KS, McBride WH. Maximizing tumor immunity with fractionated radiation. Int J Radiat Oncol Biol Phys. 2012;83(4):1306-1310.

165. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med. 2012;366(10):925-931.

166. Edlich RF, Rogers W, DeShazo CV Jr, Aust JB. Effect of vasoactive drugs on tissue blood flow in the hamster melanoma. Cancer Res. 1966;26(7):1420-1424.

167. Kin NW, Sanders VM. It takes nerve to tell T and B cells what to do. J Leuk Biol. 2006;79(6):1093-1104.

168. Yang EV, Kim S-J, Donovan EL, et al. Norepinephrine upregulates VEGF, IL-8, and IL-6 expression in human melanoma tumor cell lines: implications for stress-related enhancement of tumor progression. Brain Behav Immun. 2009;23(2):267-275.

169. Kokulos KM, Zhang Y, Sivik JM, et al. Beta blocker use correlates with better overall survival in metastatic melanoma patients and improves the efficacy of immunotherapies in mice. Oncoimmunology. 2017;7(3):e1405205.

170. De Giorgi V, Geppetti P, Lupi C, Benemei S. The role of β-blockers in melanoma. J Neuroimmune Pharm. 2020;15(1):17-26.

171. McCourt C, Coleman HG, Murray LJ, et al. Beta-blocker usage after malignant melanoma diagnosis and survival: a population-based nested case-control study. Br J Dermatol. 2014;170(4):930-938.

172. Livingstone E, Hollestein LM, van Herk-Sukel MP, et al. Beta-Blocker use and all-cause mortality of melanoma patients: results from a population-based Dutch cohort study. Eur J Cancer. 2013;49(18):3863-3871.

173. Filling JJ, Finnes HD, Kottschade LA, Allred JB, Markovic SN. Effects of commonly used chronic medications on the outcomes of ipilimumab therapy in patients with metastatic melanoma. Melanoma Res. 2016;26(6):609-615.

174. Daher C, Vimeux L, Stoeva R, et al. Blockade of β-adrenergic receptors improves CD8(+) T-cell priming and cancer vaccine efficacy. Cancer Immunol Res. 2019;7(11):1849-1863.

175. Estrada LD, Agac D, Farrar JD. Sympathetic neural signaling via the beta2-adrenergic receptor suppresses T-cell receptor-mediated human and mouse CD8(+) T-cell effector function. Eur J Immunol. 2016;46(8):1948-1958.

176. Sivan A, Corrales L, Hubert N, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science. 2015;350(6264):1084-1089.
177. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. *Science*. 2018;359(6371):97-103.

178. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. *Science*. 2018;359(6371):104-108.

179. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. 2018;359(6371):91-97.

180. Vetizou M, Trinchieri G. Anti-PD1 in the wonder-gut-land. *Cell Res*. 2018;28(3):263-264.

181. Drakes DJ, Rafiq S, Purdon TJ, et al. Optimization of T-cell receptor-modified T cells for cancer therapy. *Cancer Immunol Res*. 2020;8(6):743-755.

182. Appay V, Zaunders JJ, Papagno L, et al. Characterization of CD4(+) CTLs ex vivo. *J Immunol*. 2002;168(11):5954-5958.

183. Bos R, Sherman LA. CD4+ T-cell help in the tumor milieu is required for recruitment and cytolytic function of CD8+ T lymphocytes. *Cancer Res*. 2010;70(21):8368-8377.

How to cite this article: Winge-Main AK, Wälchli S, Inderberg EM. T cell receptor therapy against melanoma—Immunotherapy for the future?. *Scand J Immunol*. 2020;92:e12927. [https://doi.org/10.1111/sji.12927](https://doi.org/10.1111/sji.12927)