Gene of the month: cancer testis antigen gene 1b (NY-ESO-1)

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ABSTRACT

Cancer testis antigen gene 1B (CTAG1B) and its associated gene product; New York oesophageal squamous carcinoma 1 (NY-ESO-1), represent a unique and promising target for cancer immunotherapy. As a member of the cancer testis antigen family (CTA), the protein's restricted expression pattern and ability to elicit spontaneous humoural and cellular immune responses has resulted in a plethora of novel modalities and approaches attempting to harness its immunotherapeutic anti-cancer potential. Here, we discuss the structure and function of CTAG1B/NY-ESO-1 in both health and disease, immunohistochemical detection, as well as the most promising advances in the development of associated anti-cancer therapies. From cancer vaccines to engineered cellular therapy approaches, a multitude of immunotherapies targeting CTA's are coming to the forefront of oncology. Although the efficacy of such approaches have yet to provide convincing evidence of durable response, early phase clinical trial data has resulted in some exciting findings which will have significant potential to act as a platform for future practice changing technologies.

INTRODUCTION

The cancer testis antigen gene 1B (CTAG1B) was first described in 1997, with subsequent gene product, New York oesophageal squamous cell carcinoma 1 (NY-ESO-1), representing a protein with highly restricted expression to germ cells in normal tissue.[1 2] CTAG1B is part of the cancer testis antigen (CTA) family, a large family of genes/proteins associated with a diverse range of malignancies, including the X chromosome CTA subfamilies MAGE, SSX, NY-ESO-1, as well as a number of non-X chromosome members.[3 4] CTAs are defined by (1) predominant mRNA expression restricted to germ cells in normal tissue, (2) gene activation in certain malignancies and (3) expression in malignancy in a lineage non-specific fashion.[5] Members of the antigen family, including NY-ESO-1, are currently of high interest as potential immunotherapeutic anti-cancer targets, particularly for novel cellular therapy and vaccine approaches, owing to their unique expression profile and high tumour specificity.[1] Additionally, the finding that 40%–50% of patients with cancer with NY-ESO-1 expressing tumours develop spontaneous humoural and CD8+ T cell responses to the antigen, has fuelled significant therapeutic development in this area.[6 7]

STRUCTURE

CTAG1B was originally identified through serological analysis of recombinant copy DNA (cDNA) expression libraries (SEREX) in a patient with oesophageal squamous cell carcinoma.[5] Full length NY-ESO-1 cDNA and putative peptide sequences were obtained, identifying three clones initially. The longest of these being 747 base pairs in length, containing a 543-bp coding region.[5] Further studies have characterised the genomic organisation of CTAG1B. Three exons were identified, spanning over 8 kilobases.[8] The chromosomal location of CTAG1B was identified as the most distal portion of the X chromosome, Xq28. It is now known that around 10% of genes on the X chromosome relate to CTA's.[9]

The NY-ESO-1 protein consists of 180 amino acids, with a molecular mass of 18 kDa. Its glycine-rich, hydrophilic, N-terminal region is known to contain epitopes of humoural and cellular response, with an extremely hydrophobic C-terminal region containing a functionally relevant PCC1 transcription factor domain.[5 10] The secondary structure has been predicted to display a flexible loop or turn conformation within the N-terminal domain, while the C-terminal domain confers a restrained helix conformation.[11] Additionally, the N-terminal hydrophilic domain is predicted to be exposed on the surface of the protein, with subsequent direct contact with immunoglobulin molecules.[11]

FUNCTION IN HEALTHY TISSUE

Expression of NY-ESO-1 in healthy tissue is heavily restricted to the testis and ovary. It has been detected during gonadal development as early as at 13–18 weeks of foetal development, peaking at 22 and 24 weeks before falling (figure 1).[12] In adult males, expression is only consistently maintained in spermatogonia and primary spermatocytes, the early (stem cell) stages of sperm development.[13] In adult female ovarian and endometrial tissue, NY-ESO-1 mRNA, but not protein, has been detected, with unclear biological relevance.[14] It is therefore likely this protein has a function in gametogenesis; however, its functional role and significance are still to be fully elucidated.

Within NY-ESO-1, the Pcc1p transcription factor has been identified (between amino acids 89 and 164) which may be integral to its regulation of the cell cycle. Mutation of the parent PCC1 gene in yeast cells can affect expression of several key genes involved in both cell cycle progression and polarised cell growth.[15 16] Furthermore, evidence

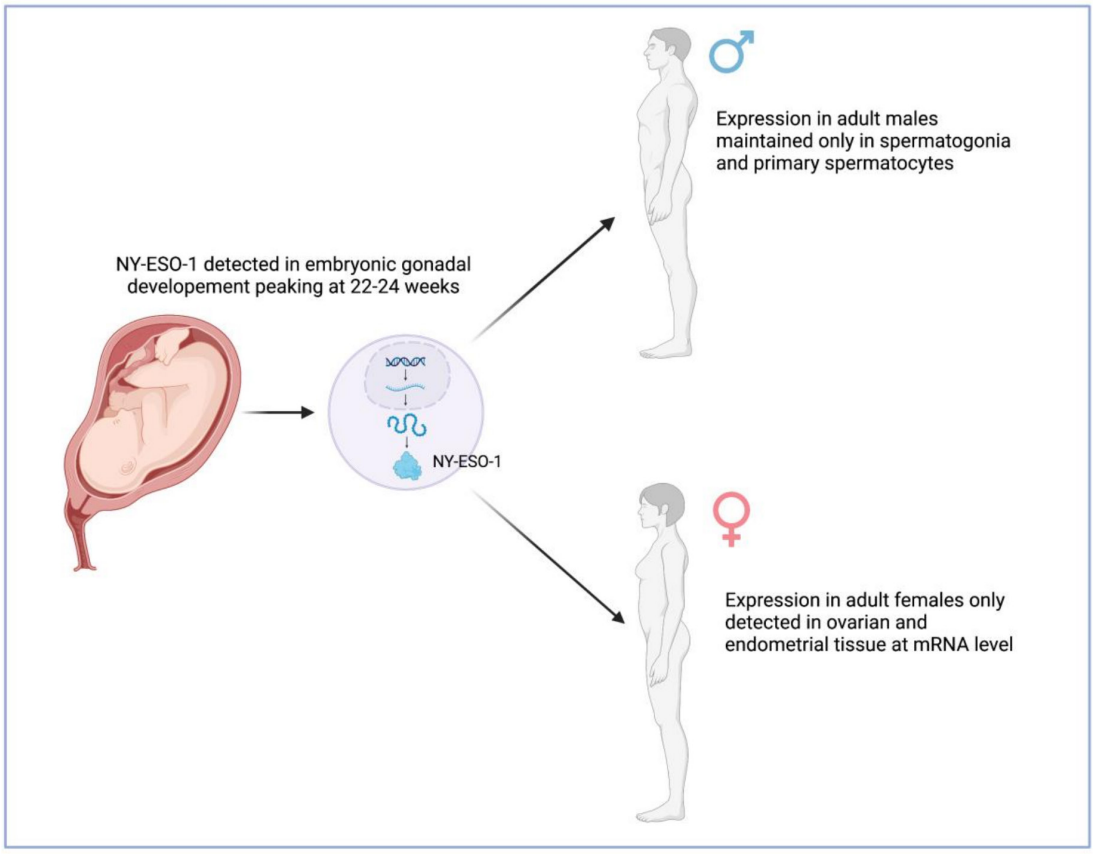

Figure 1 Expression dynamics of New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) in healthy tissue.[12 13] Image created using Biorender.com.

has shown expression of NY-ESO-1 in mesenchymal stem cells, with consistent downregulation observed through the stages of cellular maturation.[17] While data from these studies is suggestive of a physiological role for NY-ESO-1, its true biological function remains unclear, and further work is required in this respect.

FUNCTION IN MALIGNANT TISSUE

NY-ESO-1 is widely expressed throughout a diverse array of solid tumours. This includes, but not limited to: melanoma,[18] breast,[19] lung,[20] thyroid,[21] colorectal,[22] ovarian[23] and renal[24] adenocarcinomas; squamous cell carcinomas of various origins[25] and cholangiocarcinoma.[2 26] Although detected across a plethora of tumour types, there is significant variability in expression frequency, ranging from 20% in breast cancer, to 90% in soft-tissue sarcomas.[2 14] Homogenous expression has been noted in both myxoid/round cell liposarcoma (MRCLS) and synovial sarcoma, which has likely contributed to therapeutic investigation in these cancers.[2] Regulation of expression in malignancy has not been fully characterised; however, studies have suggested a tightly controlled, sequential, epigenetic mechanism involving histone deacetylation, methylation and DNA methylation processes.[27]

In addition to the heterogenous expression between tumour groups and individuals, expression levels also vary depending on specific tumour characteristics. In melanoma, NY-ESO-1 has been shown to be associated with reduced tumour infiltrating lymphocytes and tumour progression.[28] Conversely, one large study of 586 malignant melanoma samples revealed that while the MAGE family of CTA's displayed increasing expression in later stage and higher-grade tumours, NY-ESO-1 did not.[29] This potentially correlates with the observation that NY-ESO-1 can induce spontaneous immune responses, and therefore may be susceptible to 'immunoediting', where clonal selection of tumour variants that can evade the immune system drives disease progression.[30] Additionally, expression is likely to be dynamic throughout the clinical course, impacted by both systemic anti-cancer therapy and radiotherapy.[10] As previously mentioned, the presence of the PCC1 transcription factor domain with the NY-ESO-1 protein is suggestive of cell cycle involvement and a possible oncogenic function. Initial work investigating the PCC1 gene was conducted in yeast, where PCC1 mutant cells were shown to be defective for normal cell cycle progression. Additionally, the Pcc1p protein sequence has been shown to be highly conserved from archaebacteria to man, suggestive of a critical cellular function.[15]

Further work completed on melanoma cell lines has shown colocalisation of NY-ESO-1 with MAGE-C1, another CTA family member. While the function of MAGE is also poorly characterised, it has been associated with chromosome segregation and microtubule formation. The MAGE-A subfamily also inhibits the function of TP53, a key tumour suppressor gene, by binding to its DNA binding portion and promoting subsequent oncogenesis.[31]

In synovial sarcoma, the high frequency and uniquely homogeneous expression of NY-ESO-1 is thought to be linked to the pathognomonic t(X:18) translocation.[32] Expression of the abnormal fusion protein, SS18-SSX, has been postulated as the driver for abnormal epigenetic regulation and aberrant NY-ESO-1 expression, leading to a possible oncogenic mechanism in this specific subset of aggressive malignant tumours.[32 33]

IMMUNOHISTOCHEMISTRY

NY-ESO-1 expression within tissue samples has been demonstrated by immunostaining with an antibody against NY-ESO-1 (1:100, clone E978, monoclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA) using a Ventana BenchMark XT autostainer (Ventana Medical Systems, Tucson, Arizona, USA), according to the manufacturer's instructions. Expression of NY-ESO-1 is interpreted as either positive or negative. Positivity is designated as diffuse homogeneous cytoplasmic/nuclear immunoreactivity in more than 50% of the tumour cell. The positive control is typically from testicular tissue.[1]

Immunohistochemical expression of NY-ESO-1 is seen in a variety of sarcomas with highest expression seen in myxoid liposarcoma (88%), synovial sarcoma (49%), myxofibrosarcoma (35%) and conventional chondrosarcoma (28%).[1] A study by Hemminger and Iwenofu[34] demonstrated NY-ESO-1 expression was very specific to MRCLS, and rarely expressed in other forms of liposarcoma.[34] A study by Endo *et al*[1] noted similar results in pretreatment cases only and observed a markedly reduced immunohistochemical expression of NY-ESO-1 in post-treatment myxoid/round cell liposarcomas. This supports the theory that expression of NY-ESO-1 decreases as tumour cells evolve through radiation or chemotherapy exposure.[1] A significant correlation has also been observed between increased immunohistochemical expression of NY-ESO-1 and tumour size, the presence of tumorous necrosis, pleomorphism and an increased round-cell component, as well as advanced stage at diagnosis and poor overall prognosis.[35]

CLINICAL MODULATION

With immunological barriers within the testes restricting development of an adaptive immune response to genetically foreign material, re-expression of CTA's by tumour cells gives rise to the possibility of specific cytotoxic T-cell recognition, and subsequent anti-tumour response.[36] This unique property of CTA's has driven their possible therapeutic use across a variety of novel immunotherapies. Table 1 provides a summary of potential modulation strategies under clinical evaluation.

NY-ESO-1 CANCER VACCINES

Cancer vaccine drug development generally relies on the process of specific tumour antigen selection, followed by exogenous administration (±adjuvant), with subsequent dendritic cell activation.[37] The aim is to stimulate an adaptive immune response to induce durable tumour regression and eradication of residual disease. To date, the only Food and Drug Administration (FDA)-approved anti-cancer vaccine for advanced disease remains sipuleucel-T in the field of prostate cancer.[37 38] This is likely to be due to the complex interplay between tumour intrinsic resistance and extrinsic systemic immunosuppressive mechanisms; however, there is now a re-emerging enthusiasm for anti-cancer vaccine development.[39]

Investigations into vaccination of patients with NY-ESO-1 peptides have been ongoing for over two decades. Initial efforts evaluated the use of intradermal vaccination with 3 human leucocyte antigen (HLA)-A2 binding NY-ESO-1 peptides, derived from NY-ESO-1 sequence homology, for 12 patients with a variety of NY-ESO-1 expressing metastatic tumours.[40] Vaccination was given alone for the first cycle of treatment with the addition of granulocyte-macrophage colony-stimulating factor for subsequent cycles, acting as a non-specific immunopotentiator. Tumour regression or stabilisation of disease was noted in 71% (5/7) of patients in the baseline NY-ESO-1 antibody negative cohort. However, 60% (3/5) of these patients went on to develop disease progression despite strong CD8+ T cell reactivity. The authors concluded that NY-ESO-1 antigen loss or MHC-loss was a possible mechanism to therapy resistance.[22]

In efforts to promote durable anti-cancer responses, vaccine adjuvants have also been investigated. One of the most notable efforts involved the use of the immune stimulating complex (ISCOM) ISCOMATRIX.[41] The complex consists of a saponin adjuvant (QS21) and protein antigen combined within a micellar structure. Saponin adjuvants are a family of water soluble, structurally diverse molecules with proinflammatory properties. Derived from the South American soapbark tree, QS21 is most frequently selected as a vaccine adjuvant, due to its potent immunostimulatory properties.[42] ISCOMATRIX was combined with full-length NY-ESO-1 antigen within the initial double-blind placebo-controlled phase 1 trial, with promising translational data.[41] The vaccine was administered every 4 weeks for 3 doses in 46 patients with NY-ESO-1 positive cancers and minimal residual disease following surgical resection. Overall, this was well tolerated, with circulating CD8+/CD4+ T cells specific for a broad range of NY-ESO-1 epitopes identified. Unexpectedly, despite not being designed to assess clinical impact, patients who received vaccination relapsed less frequently than patients receiving placebo or NY-ESO-1 protein alone. With a median follow-up of 748 days, relapse was detected in 71% (5/7), 56% (9/16) and 11% (2/19) patients in the placebo, protein alone and protein+ISCOMATRIX cohorts, respectively.

Following these promising initial investigations, a randomised, double-blind phase 2 study was subsequently completed.[43] One hundred and ten patients with resected NY-ESO-1 positive melanoma were randomised to receive either NY-ESO-1 vaccine or NY-ESO-1 vaccine+ ISCOMATRIX. Vaccination was given via three doses intramuscularly at 4-week intervals, followed by a further dose at 6 months. Despite vaccine recipients developing strong antibody responses to NY-ESO-1, there was no statistically significant difference in relapse between the two arms.[34]

The use of mRNA vaccines in clinical medicine has recently seen huge advances due to the COVID-19 global pandemic.[44] A result of these developments has been the promise of adopting this technology for use in cancer vaccine development. One such approach in development by BioNTech uses a nanoparticle liposomal RNA vaccine (BNT111), composed of mRNA encoding four tumour associated antigens (NY-ESO-1, MAGE-A3, tyrosinase and transmembrane phosphatase with tensin homology), for the treatment of patients with advanced, checkpoint inhibitor (CPI) experienced melanoma expressing at least one of these tumour associated antigens.[45] BNT111 is optimised for mRNA translation in immature dendritic cells and augmenting antigen presentation on HLA class I+II molecules, resulting in clonal expansion of antigen specific T cells. Within the exploratory phase one trial, BNT111 (alone or in combination with CPI) was administered at escalating doses in seven cohorts, with patients undergoing eight vaccinations followed by optional continued monthly treatment. It was found that vaccination mediated durable objective responses in CPI experienced patients. These clinical responses were accompanied by strong CD4+ and CD8+ immunity against NY-ESO-1 and MAGE-A3 vaccine antigens and appear comparable to results from similar adoptive T cell approaches.[45] BNT111 is currently under further clinical investigation (NCT04526899) as a single agent or in combination with the anti-PD1 inhibitor, cemiplimab.

Table 1 Summary of clinical strategies under evaluation for anti-cancer efficacy using cancer testis antigen gene 1B/New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) pathway

Therapy	Description	Tumour types **(National Clinical Trial number)**
T-cell receptor-transduced T-cells (TCR-T) targeting NY-ESO-1	Autologous T cells engineered to express an affinity-enhanced T cell receptor recognising NY-ESO-1	► Soft-tissue sarcoma(s) (NCT05620693) ► Synovial sarcoma (NCT01343043) ► Sarcomas (combined with radiotherapy) (NCT02319824) ► Non-small cell lung cancer (NCT03029273) ► Non-small cell lung cancer (with pembrolizumab) (NCT03709706) ► Oesophageal or gastric cancer (combined with interleukin 2) (NCT01795976) ► Ovarian cancer (NCT03691376) ► Any tumour (NCT01343043)
Dendritic cell vaccine	Peptide-pulsed autologous dendritic cell vaccine containing NY-ESO-1 protein	► Sarcoma (combined with atezolizumab) (NCT02609984) ► Melanoma (NCT00798629) ► Any tumour (combined with nivolumab) (NCT02775292) ► Any tumour (combined with pembrolizumab) (NCT02122861)
NY-ESO-1 protein vaccine	NY-ESO-1 protein administered with and without immune adjuvants	► Any tumour (NCT00299728) ► Any tumour (combined with sirolimus) (NCT01522820) ► Prostate cancer (NCT00292045) ► Melanoma (NCT01810016) ► Myelodysplastic syndrome and acute myeloid leukaemia (combined with decitabine and nivolumab) (NCT03358719)
Combined NY-ESO-1 protein vaccine(s)	Mixed protein-based vaccines administered with or without immune adjuvants	► Dual HER2 and NY-ESO-1 expressing cancer (NCT00291473) ► Dual MAGE-A3 and NY-ESO-1 expressing cancer in multiple myeloma (NCT00090493) ► Dual LAGE-1A and NY-ESO-1 expressing cancer (NCT03515551)
NY-ESO-1 overlapping peptides vaccine	NY-ESO-1 overlapping peptides (eg, OLP-4) administered with and without immune adjuvants	► Ovarian cancer (NCT00616941)
NY-ESO-1 DNA vaccine	NY-ESO-1 plasmid DNA cancer vaccine	► Prostate, bladder, oesophageal, non-small cell lung cancer, and sarcoma (NCT00199849) ► Ovarian cancer (NCT00112957)
Ipilimumab	Trial of ipilimumab in patients with spontaneous pre-existing immune response to NY-ESO-1	► Melanoma (NCT01216696)

ADOPTIVE T CELL THERAPY

While cancer vaccines have shown the ability to develop both humoural and cellular immune responses, these responses are frequently short lived and rarely achieve durable clinical significance. In efforts to improve depth and durability of response, autologous cellular therapy strategies have been employed through the development of T cell receptor (TCR) based therapies.

TCR therapies are autologous T cell products, engineered with modified TCRs specific for peptides from intracellular cancer antigens, presented through the HLA framework.[46 47] T cells are isolated from patient's peripheral blood following apheresis, prior to ex vivo expansion and modification with the use of retroviral and lentiviral vectors (figure 2).[32] Prior to reinfusion, patients are conditioned with lymphodepleting chemotherapy, usually in the form of cyclophosphamide and fludarabine-based regimens.[47]

Due to the high expression frequency of NY-ESO-1 in synovial sarcoma and MRCLS, these tumour types have seen the most attempts to explore TCR therapy as a therapeutic strategy.[47] Seminal work by Robbins *et al*[48] resulted in the identification of two NY-ESO-1 specific TCR variants of interest, engineered through CDR3α and CDR2β amino acid substitutions and directed against the SLLMWITQC NY-ESO-1 epitope.[48] These

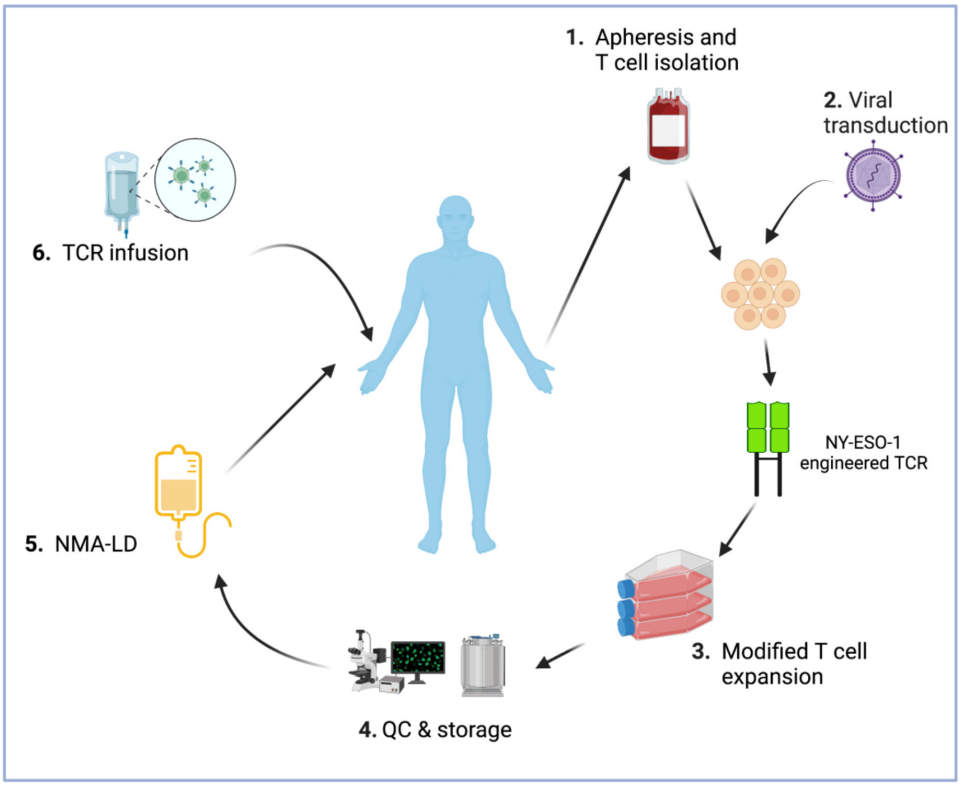

Figure 2 Manufacture and administration steps in New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) targeting T cell receptor (TCR) therapy. (1) Apheresis and T cell isolation. (2) Viral transduction. (3) Modified T cell expansion. (4) Quality control (QC) and storage. (5) Non-myeloablative lymphodepletion. (6) TCR infusion. Image created using Biorender.com.

displayed dramatically enhanced specificity for NY-ESO-1 + tumour cells by TCR gene modified CD4+ cells. This technology was translated into the clinic and reported in 2015, where 18 patients with synovial sarcoma and 20 patients with metastatic melanoma were treated with autologous TCR transduced cells plus adjuvant interleukin 2.[49 50] Objective clinical responses, defined as complete response (CR) or partial response (PR), were seen in 61% (11/18) synovial sarcoma patients (two CRs) and 55% (11/20) patients with melanoma (four CRs).

A further attempt to target NY-ESO-1 with TCR therapy was published in 2018.[51] Twelve patients with metastatic synovial sarcoma were treated with a similar affinity enhanced NY-ESO-1 targeting TCR therapy. Antitumour response, with 1 CR and 5 PRs, was seen in 50% (6/12) of patients. Additionally, circulating modified T cells were detectable in all treated patients, which persisted in responders for over 6 months. Translational data confirmed regenerative pools of modified T cells producing a continuous and sustained immune response. An ongoing phase 2 trial is evaluating NY-ESO-1 targeting TCR therapy in synovial sarcoma or MRCLS, with outcomes eagerly awaited (NCT03967223).

Despite the promise of high efficacy from cellular therapies, this novel treatment modality can be associated with substantial and life-threatening toxicity. This is usually owing to both lymphodepletion with cytotoxic chemotherapy, and the immune mediate cytokine release syndrome (CRS). The overall severity and grade of immune-mediated toxicity (including immune effector cell-associated neurotoxicity syndrome), however, appear to be less significant that that seen in anti-CD19-tagetting CAR-T cell therapy.[47] Furthermore, clinical experience with these therapies is allowing additional insight to be gleaned, enabling more effective preventative toxicity management strategies.

One major challenge with TCR therapies is the restriction of use in patients with the HLA A*02:01 haplotype.[32] While this has the highest incidence within Caucasian populations, lower expression levels are seen within Asian and African populations.[52] This leaves a large unmet need for patients with alternative HLA haplotypes. Additionally, manufacture time and logistics mean that application of this technology may be limited to specialist centres.[47]

FUTURE DIRECTION(S)

With significant progress in NY-ESO-1 clinical modulation, additional efforts are required to enhance efficacy and durability of clinical responses. Preclinical efforts which may proceed into clinical trials include the use of CAR-T cell therapy in NY-ESO-1 positive multiple myeloma,[53] and the development of bispecific antibodies targeting NY-ESO-1 and other antigens.[54] Additionally, combination therapy with well-established immune checkpoint inhibitors are yet to be reported (table 1).

CONCLUSION

Modulation of CTAG1B/NY-ESO-1 remains an exciting area for cancer therapy development. The ability to induce spontaneous cellular and humoural immune responses, coupled with heavily restricted expression, often limited to malignant tissue, will likely lead to ongoing investment and investigation into associated technologies. From vaccines to adoptive cellular therapy, NY-ESO-1 targeting therapies have been at the forefront of novel immunotherapeutics and have seen significant progress in the field. There are however ongoing concerns that will need to be addressed, including HLA restriction and costly manufacture processes, but these are likely to be adjusted for with ongoing

technological advances. Overall, CTAG1B/NY-ESO-1 represents a unique gene/protein which has the potential to pave the way for practice changing cancer therapies.

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