Tumour Escape from CAR-T Cells

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Over the past decade, CAR-T cells have emerged as one of the most powerful cellular immune therapy approaches in the battle against haematological malignancies. Nonetheless, similar to other immunotherapeutic approaches, tumour cells develop strategies to evade CAR-T cell therapy, often with the support of a highly immunosuppressive and protective tumour microenvironment. To date, antigen loss, immune dysfunction, exhaustion and (microenvironment-mediated) upregulation of anti-apoptotic pathways have been identified as major modes of tumour escape from CAR-T cell therapy. This chapter will focus on our current understanding of these modes of immune escape from CAR-T cells.

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Immune Escape and CAR-T Cell Resistance Related to Antigen Loss

Antigen loss represents the ultimate adaptation of a cancer cell to the selective pressure of targeted immunotherapy. While antigen downregulation or dim expression is a well-known event in lymphoma and myeloma treated with therapeutic IgG antibodies (Plesner et al. 2020; Jilani et al. 2003), complete target loss is a phenomenon typically occurring after T-cell-based therapy, such as CAR-T cell or T cell engaging bispecific antibodies (TCE) therapy, and rarely after treatment with antibody-drug conjugates (ADCs).

In B cell malignancies, CD19 loss has been noted in up to 40% of patients with B cell acute lymphoblastic leukaemia treated with different CAR 19 products (Orlando et al. 2018). Point mutations in CD19 have been described to lead to nonfunctional anchoring of the CD19 protein to the cell membrane and consequently to a loss of surface antigen (Orlando et al. 2018). Deleterious mutations and alternatively spliced CD19 mRNA variants were identified in two other studies (Asnani et al. 2020; Sotillo et al. 2015). In B-ALL with rearrangement of the mixed lineage leukaemia (MLL) gene, some patients relapsed with clonally related acute myeloid leukaemia after treatment with CD19 CAR-T cells, adding a switch to a CD19-negative myeloid phenotype as another mechanism of resistance (Gardner et al. 2016). In DLBCL, the frequency of CD19 loss after CAR19 axicabtagene ciloleucel (axi-cel) treatment was 33% (Neelapu et al. 2017; Neelapu et al. 2019), and alternatively spliced CD19 mRNA species could be identified. In follicular lymphoma and DLBCL treated with CD20 X CD3 bispecific TCE, CD20 loss relapses were seen, but the frequency is yet to be reported (Bannerji et al. 2018). Furthermore, a single case of CD22 loss was described after ADC inotuzumab-ozogamicin treatment in a paediatric patient with B-ALL (Paul et al. 2019). Taken together, antigen loss is a key mechanism of resistance to novel immunotherapies targeting CD19, CD20, and CD22. In myeloma, downregulation of BCMA was recorded in a significant proportion of patients following BCMA CAR-T therapy, but intensity increased back towards baseline in almost all patients (Cohen et al. 2019). However, three case reports described irreversible BCMA loss after anti-BCMA CAR-T cell treatment (Da Via et al. 2021; Samur et al. 2020; Leblay et al. 2020). In two of these cases, homozygous *BCMA* gene deletions were identified as the biological underpinning of antigen loss. In the third case, the authors found a heterozygous *BCMA* deletion together with a *BCMA* mutation, leading to antigen loss. In summary, biallelic events impacting the *BCMA* locus represent one molecular mechanism of antigen loss after BCMA CAR-T therapy. However, these events seem to be rare. In the KarMMa trial, only 4% of patients relapsed without an increase in soluble BCMA, which is thought to be a biomarker of this type of resistance (Munshi et al. 2021). Heterozygous BCMA deletions, present in approximately 7% of anti-BCMA naïve patients, represent a risk factor for BCMA loss-relapse after T-cell-based therapy (Da Via et al. 2021). While a plethora of alternative antigens, such as FCRH5 or GPRC5D, are currently being investigated in early clinical trials (Rasche et al. 2020),

antigen loss for these targets has not been reported thus far. However, MM is a disease associated with high frequencies of copy number aberrations, including deletions impacting genes encoding immunotherapy targets, and we expect biallelic events leading to antigen loss to also be relevant for MM targets other than BCMA. Multispecific CAR-T cells or combinations of monospecific targeted immunotherapies may overcome antigen loss in future trials (Fernández de Larrea et al. 2020).

Immune Dysfunction and Exhaustion of CAR-T Cells

In addition to antigen loss, a number of other mechanisms also limit or abrogate the effective recognition of cancer cells by CAR-T cells, either directly conveyed by tumour cells or through rewiring of the microenvironment. In preclinical models, especially in solid tumours, it was shown that tumour-infiltrating CAR-T cells undergo rapid loss of functionality, limiting their therapeutic efficacy. This hyporesponsiveness appears to be reversible when the T cells are isolated away from the tumour and is associated with upregulation of intrinsic T cell inhibitory enzymes (diacylglycerol kinase and SHP-1) and with the expression of surface inhibitory receptors (PD1, LAG3, TIM3, and 2B4) (Moon et al. 2014).

Additionally, in patients with diffuse large B cell lymphoma (DLBCL) treated with axicabtagene ciloleucel (axi-cel), it has been shown that tumour-infiltrating CAR-T cells express the inhibitory receptor PD1 and that they represent only a minor fraction of the immune cells detectable in the tumour (Chen et al. 2020). Of note, immunogenic chemotherapy can enhance the recruitment of CAR-T cells to the tumour bed by inducing the release of chemokines from monocytes, and this can potently synergize with immune checkpoint blockade (Srivastava et al. 2021). In another recent study in DLCBL, interferon (IFN) signalling expression, along with high blood levels of monocytic myeloid-derived suppressor cells (M-MDSCs), IL-6 and ferritin, was associated with a lack of a durable response to axi-cel. The authors showed that high IFN signalling is associated with the expression of multiple checkpoint ligands, including PD-L1, on lymphoma cells and that these levels were higher in patients who lacked a durable response to CAR-T therapy (Jain et al. 2021). However, impairment of IFN signalling, such as through mutations or downmodulation of JAK2 and other pathway components, can confer tumour cell resistance to killing by CAR-redirected T cells (Arenas et al. 2021).

These findings have direct implications for the design of next-generation CAR-T cell protocols: a number of strategies are now being explored to combine immune checkpoint blockade with CAR-T cell therapy, either by coinfusion of genetically modified lymphocytes with monoclonal antibodies or by engineering the cell to produce the relevant scFv (Carneiro and El-Deiry 2020), be resistant to inhibitory signals (Cullen et al. 2010), or even transform signals under activating stimuli (Sutton et al. 2000). Moreover, novel promising compounds have been shown to counteract the activity of T cell inhibitory enzymes (Moon et al. 2014).

Microenvironment-Mediated Tumour Resistance to CAR-T Cells

Immune suppression or exhaustion is not the only mechanism by which tumour cells can become less susceptible to CAR-T cell-mediated cytotoxicity. In many haematological cancers, the bone marrow tumour microenvironment (BMME) is known to upregulate antiapoptotic mechanisms in tumour cells through tight cross-talk of mesenchymal stromal cells (MSCs) and tumour cells. Remarkably, tumour cell lysis by T and NK cells is also largely mediated via activation of extrinsic and intrinsic apoptosis pathways (Hanabuchi et al. 1994; Falschlehner et al. 2009; Carneiro and El-Deiry 2020; Cullen et al. 2010; Sutton et al. 2000). Thus, the idea that BMMSCs might also induce resistance to T and MK cell-mediated cytotoxic activity through upregulation of antiapoptotic mechanisms has recently been tested, and the results showed that MM cell-BMMSC interactions can indeed protect MM cells from conventional cytotoxic T cells and from (daratumumab redirected) NK cells (McMillin et al. 2012; de Haart et al. 2013; de Haart et al. 2016). These studies were recently extended to CAR-T cells by testing a panel of nine different MM-reactive CAR-T cells that were reactive to three different MM-associated antigens (CD138, BCMA, and CD38) with different target affinities and with different costimulatory domains (CD28, 4-1BB, or CD28 plus 4-1BB) (Holthof et al. 2021a). In the absence of BMMSCs, $BCMA^{bb2121}$ CAR-T cells, high affinity CD38 CAR-T cells, and intermediate affinity CD38 CAR-T cells containing CD28 costimulatory domains showed high levels of anti-MM cell lysis, whereas other CAR-T cells showed moderate cytotoxic activity against MM cells. BMMSCs did not modulate the lytic activity of highly lytic CAR-T cells but readily protected MM cells against all other CAR-T cells with intermediate killing capacity. Overall, a strong inverse correlation was demonstrated between the lytic capacity of the CAR-T cells and the extent of BMMSC-mediated protection. Furthermore, the BMMSC-mediated protection of MM cells from these CAR-T cells was readily abrogated by inhibition of survivin, MCL-1, and Xiap using the small molecule FL118. Thus, the results confirmed that BMMSC-mediated immune resistance was mediated by negative regulation of apoptotic pathways. In addition, the importance of the tumour stroma in the efficacy of CAR-T cells has also been suggested in a solid tumour mouse model, where destruction of the tumour stroma contributed to eradication of large tumours by HER2-specific CAR-T cells (Textor et al. 2014). Based on these studies, overcoming BMMSC-mediated immune resistance seems possible by increasing the overall avidity and killing activity of CAR-T cells. This may be achieved by designing CARs containing high affinity antigen recognition domains, tandem CARs, or dual CAR strategies (van der Schans et al. 2020). Alternatively, using the CD28 costimulatory domain (Drent et al. 2019; Drent et al. 2017) or engineering CAR-T cells with cytotoxic effector molecules can upregulate CAR-T cell activity. Indeed, it has recently been demonstrated that BMSMSC-mediated immune resistance towards the NK cell line KHYG-1 can be abrogated by engineering it with a CD38 CAR and/or with a DR5-specific, optimized TRAIL variant (Holthof et al. 2021b). CAR-T cells may also be equipped with caspase-independent apoptotic molecules, such as granzyme-A (Borner and Monney 1999).

In addition, a number of earlier and recent studies indicate the importance of apoptotic pathways for the efficacy of other CAR-T cells. For instance, CD19 CAR-T cells were previously found to benefit from combination with the BCL-2 inhibitor ABT-737 (Karlsson et al. 2013). Recently, similar results were observed when third-generation CD19 CAR-T cells were combined with another BCL-2 inhibitor, ABT199 (Yang et al. 2019). Finally, two independent loss-of-function screens in ALL cell lines identified impaired death receptor pathways as another mechanism of resistance to CD19-targeted CAR therapy. Loss of FADD, BID, and tumour necrosis factor-related apoptosis-inducing ligand 2 (TRAIL2) in leukaemia cells was shown to render them more resistant to cytotoxicity and to drive T cell exhaustion upon prolonged stimulation (Singh et al. 2020; Dufva et al. 2020). The combination of CAR-T cells with the SMAC mimetic compound birinapant significantly improved the lysis of malignant cells (Dufva et al. 2020). Thus, when increasing the lytic capacity of CAR-T cells is not possible or desirable, especially if the target antigen is not entirely tumour-specific, tumour cells can be made more sensitive by combining CAR-T cells with small molecules targeting regulatory proteins in the intrinsic and extrinsic apoptotic pathways, as shown in these studies.

Key Points

1. Loss of expression of the target antigen on tumour cells via the selective immune pressure of CAR-T cells is a major mechanism of CAR-T cell therapy failure.
2. T cell exhaustion of CAR-T cells can decrease their function.
3. Multiple other cells in the tumour microenvironment can contribute to inhibition of CAR-T cell function.

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