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Mechanisms of resistance to bispecific T cell engagers in multiple myeloma and their clinical implications

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Abstract:
Bispecific T cell engagers (TCE) are revolutionizing patient care in multiple myeloma (MM). These monoclonal antibodies, that redirect T cells against cancer cells, are now approved for the treatment of triple-class exposed relapsed refractory multiple myeloma (RRMM). They are currently tested in earlier lines of the disease, including in first line. Yet, primary resistance occurs in about one third of RRMM patients, and most responders eventually develop acquired resistance. Understanding the mechanisms of resistance to bispecific TCE is thus essential to improve immunotherapies in MM. Here, we review recent studies investigating the clinical and molecular determinants of resistance to bispecific TCE. Resistance can arise from tumor-intrinsic or tumor-extrinsic mechanisms. Tumor-intrinsic resistance involves various alterations leading to the loss of the target antigen such as chromosome deletions, point mutations or epigenetic silencing. Loss of MHC class I, preventing MHC class I:TCR co-stimulatory signaling, was also reported. Tumor-extrinsic resistance involves abundant exhausted T cell clones and several factors generating an immunosuppressive microenvironment. Importantly, some resistance mechanisms impair response to one TCE while preserving the efficacy of others. We next discuss the clinical implications of these findings. Monitoring the status of target antigens in tumor cells and their immune environment will be key to select the most appropriate TCE for each patient, and to design combination and sequencing strategies for immunotherapy in multiple myeloma.-

Conflict of interest: COI declared - see note

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Mechanisms of resistance to bispecific T cell engagers in multiple myeloma and their clinical implications

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Abstract

Bispecific T cell engagers (TCE) are revolutionizing patient care in multiple myeloma (MM). These monoclonal antibodies, that redirect T cells against cancer cells, are now approved for the treatment of triple-class exposed relapsed refractory multiple myeloma (RRMM). They are currently tested in earlier lines of the disease, including in first line. Yet, primary resistance occurs in about one third of RRMM patients, and most responders eventually develop acquired resistance. Understanding the mechanisms of resistance to bispecific TCE is thus essential to improve immunotherapies in MM. Here, we review recent studies investigating the clinical and molecular determinants of resistance to bispecific TCE. Resistance can arise from tumor-intrinsic or tumor-extrinsic mechanisms. Tumor-intrinsic resistance involves various alterations leading to the loss of the target antigen such as chromosome deletions, point mutations or epigenetic silencing. Loss of MHC class I, preventing MHC class I:TCR co-stimulatory signaling, was also reported. Tumor-extrinsic resistance involves abundant exhausted T cell clones and several factors generating an immunosuppressive microenvironment. Importantly, some resistance mechanisms impair response to one TCE while preserving the efficacy of others. We next discuss the clinical implications of these findings. Monitoring the status of target antigens in tumor cells and their immune environment will be key to select the most appropriate TCE for each patient, and to design combination and sequencing strategies for immunotherapy in multiple myeloma.

Introduction

The prognosis of patients with relapsed refractory multiple myeloma (RRMM) with prior exposure to immunomodulatory drugs (IMiDs), proteasome inhibitors (PI) and anti-CD38 monoclonal antibodies (anti-CD38 mAb) remains poor[1]. In this population, chimeric antigen receptor T cell (CAR-T cell) and bispecific T-cell engagers (TCE) targeting B-cell maturation antigen (BCMA) represent a new standard of care. Idecabtagene vicleucel (ide-cel, anti-BCMA CAR-T) has been approved based on an overall response rate (ORR) of 73% and a median progression-free survival (PFS) of 8.8 months in heavily pretreated triple-class exposed myeloma patients[2]. Ciltacabtagene autoleucel (cilta-cel, another anti-BCMA CAR-T) has also been approved in this population based on an ORR of 97.9% and a median PFS of 34.5 months[3]. Despite this favorable efficacy profile, accessibility and manufacturing process still represent a limitation for broad use of CAR-T cells in multiple myeloma[4]. Bispecific TCE are readily available off-the-shelf monoclonal antibodies able to bind to an antigen on tumor cells and to another antigen on T cells to redirect these lymphocytes toward malignant cells[5]. To date, two bispecific TCE, teclistamab and elranatamab, targeting CD3 on T cells and BCMA on myeloma cells, have been approved for the treatment of triple-class exposed RRMM. In the Majestec-1 study, teclistamab led to an ORR of 63% and a median PFS of 11.3 months in triple-class exposed patients who received a median number of 5 prior lines[6]. In the Magnetismm-3 study (cohort A), elranatamab led to an ORR of 61% and a median PFS of approximately 15 months in triple-class exposed patients who received a median number of 5 prior lines[7]. Bispecific TCE targeting other tumor antigens (i.e. FCRH5, GPRC5D) also demonstrated promising activity in relapsed MM[8]. Recently, talquetamab, another bispecific TCE targeting G-protein coupled receptor family C group 5 member D (GPRC5D), has also been approved in RRMM patients, based on the results of the MONUMENTAL-1 study[9,10]. In a population of advanced, T-cell redirecting agent naive myeloma patients (n=145, 69% triple-class refractory, median of 5 prior lines), talquetamamb (0.8 mg/kg biweekly) single agent demonstrated an ORR of 72% and a median PFS of 14 months. In patients previously exposed to a T-cell redirecting agent (n=51), talquetamab resulted in an ORR of 64% with a median duration of response of 11.9 months. Despite this favorable efficacy profile, nearly one third of patients do not respond to bispecific therapy (primary resistance). Moreover, most responding patients treated with bispecific antibodies will finally develop disease progression (acquired resistance). The present review aims at describing the tumor-intrinsic and tumor-extrinsic mechanisms leading to bispecific TCE resistance.

Clinical determinants of resistance to bispecific TCE

To date, the BCMA-targeting TCE teclistamab and elranatamab are approved for the treatment of RRMM who received at least 3 lines of prior therapy and are triple-class exposed. Data from clinical trials identified several baseline clinical characteristics as predictors of poor response to BCMA-targeted BsAb, including presence of extramedullary disease (EMD), International Staging System (ISS) stage III and refractory status. In Magnetismm-3, patients with EMD had an ORR of 38.5% to elranatamab, in comparison to 71.4% for patients without EMD[7]. Patients with ISS III (versus ISS I-II) and penta refractory disease (versus not penta refractory) also had an inferior response rate to elranatamab. In Majestec-1, ORR to teclistamab was also significantly inferior in patients with EMD or ISS III[6]. Lower response rate to teclistamab in EMD patients could be related to higher level of soluble BCMA in this population[11]. High tumor burden was also associated with lower response rate to elranatamab (bone marrow (BM) plasma cells $\geq$ 50%) and teclistamab (BM plasma cells $\geq$ 60%)[6,7]. In contrast, high cytogenetic risk was not found to significantly impact response rate to these two drugs[6,7]. Talquetamab is to date the only approved BsAb targeting GPRC5D. In Monumental-1, the presence of extramedullary disease was the only baseline clinical characteristic found to significantly influence response rate, with a median ORR of 48.5% and 43.2% in the weekly and biweekly cohorts in patients with EMD, versus 81.8 and 88% in the weekly and biweekly cohorts[9]. ISS, cytogenetic and refractory status did not significantly impact response to talquetamab in this study.

Tumor-intrinsic mechanisms of resistance

Genetic inactivation of TNFRSF17

Whole genome sequencing (WGS) of myeloma cells before BCMA-targeting TCE therapy and at relapse identified genetic inactivation of *TNFRSF17* gene (encoding BCMA protein) as a common tumor-intrinsic resistance mechanism. Truger *et al.* reported a first case of BCMA antigen loss due to a homozygous deletion of *TNFRSF17* gene[12]. More recently, Lee *et al.* analyzed 14 patients with disease progression on BCMA-targeting TCE therapy, and revealed biallelic *TNFRSF17* inactivation in 6 cases (42.8%), by homozygous deletion (n=1) or mono-allelic loss with mutation (n=5)[13]. Two patients displayed convergent evolution, with the emergence of several resistant clones harboring distinct *TNFRSF17* alterations, highlighting the strong selective pressure imposed by TCE. *TNFRSF17* mutations involved hotspots in the extracellular domain of BCMA, with one missense p.Arg27Pro mutation and two in-frame deletions p.Pro34del (found in 3 patients) and p.Ser30del (in 2 patients). Mutant proteins were still recognized by polyclonal anti-BCMA antibodies and retained the ability to bind APRIL (a proliferation-inducing ligand) and activate the prosurvival NF-κB signaling. However, BCMA extracellular domain mutations abrogated TCE binding and TCE-induced cell death. Importantly, *TNFRSF17* mutations conferred distinct sensitivities to different anti-BCMA TCE. *In vitro*, cells harboring p.Arg27Pro and p.Pro34del mutations were resistant to teclistamab and elranatanab but remained sensitive to alnuctamab, whereas cells with p.Ser30del mutation were resistant to teclistamab but remained sensitive to elranatanab and alnuctamab. These data in cell lines need to be confirmed *in vivo* but suggest that myelomas resistant to one anti-BCMA TCE might still be sensitive to another targeting a different epitope.

Genetic or epigenetic inactivation of GPRC5D

Tumor-intrinsic mechanisms of resistance to the GPRC5D-targeting TCE talquetamab were assessed in two synchronous studies[13,14]. Combining deep WGS and single-cell multi-omics, Derrien *et al.* reported convergent evolution in a patient with a clonal 12p deletion (encompassing *GPRC5D* locus) in the pre-treatment sample. Seven resistant subclones emerged at relapse, each having acquired a distinct second hit in *GPRC5D* (3 frameshift indels, 2 nonsense mutations, 1 in-frame deletion and a large deletion encompassing the transcription start site) leading to the complete loss of GPRC5D protein at the cell surface[14]. Similarly, Lee *et al.* reported 4 talquetamab-resistant cases with biallelic *GPRC5D* inactivation due to homozygous deletion or mono-allelic deletion with mutation (1 frameshift indel, 1 missense and 2 nonsense mutations)[13]. The mutation landscape of *GPRC5D* mostly involves truncating mutations distributed all along the protein sequence, in sharp contrast with the hotspot mutations in *TNFRSF17* that alter TCE recognition while preserving BCMA-mediated pro-survival signaling (**Fig. 1**). Finally, Derrien *et al.* reported two talquetamab-resistant cases with a loss of *GPRC5D* expression due to the long-range epigenetic silencing of its promoter and enhancer regions, in absence of any genetic alteration[14]. This is a proof-of-concept that epigenetic remodeling alone can induce TCE resistance by silencing the transcription of the antigen. Overall, resistance to *GPRC5D*-targeting TCE usually involved a complete inactivation of the target, suggesting that myeloma cells better tolerate the loss of GPRC5D than the loss of BCMA. Consistently, reduced or lost GPRC5D expression was observed in 6/6 cases who relapsed after anti-GPRC5D CAR-T therapy[15], while loss of BCMA expression was rare after anti-BCMA CAR-T therapy (3/71, 4%)[16]. BCMA promotes the growth of MM cells, protects them from apoptosis and promotes immunosuppression in the bone marrow microenvironment[17,18]. These pro-survival effects may prevent the selection of clones with BCMA inactivation, even in the presence of anti-BCMA treatment.

Loss of MHC class I

Using single-cell RNA-seq and TCR tracing, Friedrich *et al.* explored the dynamic response of T cells in myeloma patients treated with anti-BCMA TCE[19]. TCE response was driven by the clonal expansion of effector CD8+ T cells, but also naive T cells. Importantly, MHC class I interaction with tumor cells and

MHC class I:TCR co-stimulatory signaling were required for the functional recruitment and priming of naive T cell clones. Several lines of evidence highlighted the loss of MHC class I as a potential tumor-intrinsic mechanism of TCE resistance beyond loss of the target antigen. First, the expression of MHC class I (*HLA-E*, *HLA-C*) and class II genes (*CD74*) was deregulated in response to TCE treatment. Second, loss of MHC class I surface expression was identified at relapse by flow cytometry in some patients. However, the frequency and causal mechanism of this loss of MHC class I expression remain to be established.

Tumor-extrinsic mechanisms of resistance

The response to bispecific TCE treatment is impacted by several tumor-extrinsic factors including the pre-existing T cell landscape, its evolution and the immunosuppressive tumor microenvironment created by myeloma cells and related to previous treatments[19–21]. In a preclinical study, Verkleij *et al.* showed that talquetamab-mediated killing of MM cells is impaired by an increased proportion of several T cell populations, including T cells expressing the exhaustion marker PD-1, activated T cells expressing HLA-DR and regulatory T cells (Treg)[20]. In the transplantable Vk*MYC MM mouse model, T cells upregulated PD-1 expression in response to anti BCMAxCD3 bispecific TCE and diminished in functionality over time, leading to systematic relapse post-treatment[21]. Interestingly, the addition of pomalidomide, an immunomodulatory drug (IMiD), increased the expansion of lytic T cells and short-term efficacy of the TCE, but also induced important toxicity and exacerbated T cell exhaustion, leading to only marginal survival benefit in this preclinical model. In contrast, a combination of the BCMA-targeting TCE with cyclophosphamide was safe and allowed long-term myeloma control by reducing tumor burden, depleting regulatory T cells and preventing TCE-induced T-cell exhaustion. In line with these preclinical studies, Friedrich et al. found that the abundance of exhausted CD8+ T cell clones predicts response failure to BCMAxCD3 bispecific TCE in MM patients[19]. Consistently, van de Donk *et al.* reported baseline immune characteristics predicting unfavorable response to the same TCE, including lower T cell numbers, higher T cells expressing PD-1, TIM-3 or CD38, increased Tregs and CD38+ Tregs, and lower proportion of naive T cells[22]. These studies stressed the importance of the pre-existing T cell repertoire in the response to bispecific TCE therapy. Other factors generate an immunosuppressive environment in multiple myeloma and may contribute to TCE resistance, including the interaction beween MM and bone marrow stromal cells (BMSC), inhibitory cytokines (TGF-ß, IL-6 or IL-10) and myeloid cells[23,24]. The interaction between MM and BMSC has been shown to protect MM cells from T-cell cytotoxicity[25,26]. *In vitro*, the addition of BMSC impaired the talquetamab-mediated lysis of MM cell lines[20]. This protective effect involved cell-cell contact but not BMSC-derived soluble factors nor a reduction in T-cell activation, suggesting the induction of tumor cell-intrinsic resistance mechanisms. Inhibitor myeloid cells such as myeloid-derived suppressor cells (MDSC) and plasmacytoid dendritic cells (pDC) have also been reported to drive an immunosupressive environment favoring MM progression[27–30]. Their potential role in TCE resistance remains to be explored in patients.

Tumor-intrinsic and tumor-extrinsic mechanisms of TCE resistance are summarized in **Fig. 2**.

Clinical implications

The identification of molecular mechanisms underlying TCE resistance provides valuable insights to guide future immunotherapy in multiple myeloma. Before treatment, molecular characterization of the target antigens in tumor cells and of the immune repertoire may help select the most appropriate immunotherapy for each patient. At relapse, understanding the molecular cause of resistance will be instrumental in choosing the next treatment line.

Molecular characterization of the targets to select the first immunotherapy line

TCE resistance by loss of the target antigen requires the inactivation of the two copies of the gene. Pre-existing deletions or mutations of TCE targets may thus favor the emergence of resistance. A representative example is the talquetamab-resistant case published by Derrien *et al.* in which a pre-existing 12p deletion (encompassing *GPRC5D*) allowed the emergence of 7 resistant subclones, each harboring a distinct second hit[14]. Similarly, Lee *et al.* described 3 patients harboring pre-treatment 16p (encompassing *TNFRSF17*) or 12p deletions who developed subclones resistant to BCMA (respectively GPRC5D)-targeting TCE following acquisition of second hits[13]. Screening of target alterations in large cohorts of TCE treatment-naive MM revealed recurrent heterozygous deletions of *TNFRSF17* (3-8%), *GPRC5D* (13-15%) or *CD38* (10%)[12,13,31,32]. Of note, patients with 16p deletion (encompassing *TNFRSF17*) have increased deletion frequencies of other chromosomes and may be more vulnerable to the biallelic loss of other genes[32]. Altogether, heterozygous deletion of one target occurs in ~30% of MM. Other targets like *FCRL5* and *SLAMF7*, located on chromosome arm 1q, are recurrently gained in RRMM. In addition to deletions, rare somatic mutations of *GPRC5D* were identified in TCE-naive MM[12], as well as somatic (1.1%) and germline (0.7%) *TNFRSF17* mutations, including a recurrent p.Pro33Ser germline variant notably encountered in a patient with primary refractory disease to anti-BCMA TCE[13]. Screening these events may improve TCE response by prioritizing target genes with two intact copies in MM cells, although the predictive value of monoallelic target alterations at baseline remains to be demonstrated in clinical series. In addition to their genomic status, the baseline expression of target antigens may influence TCE response. The two talquetamab-resistant cases with epigenetic *GPRC5D* silencing belonged to the t(11;14) molecular group[14] that displays the lowest *GPRC5D* mRNA expression[20]. *In vitro*, the efficacy of talquetamab was superior in patient-derived MM cells with high *GPRC5D* expression. Whether a low baseline expression may facilitate acquired TCE resistance by epigenetic silencing of the target, e.g. by extension of inactive chromatin marks, will need to be examined in large clinical cohorts.

Molecular profiling of the microenvironment

The abundance of exhausted-like T cell clones was associated with TCE response failure, providing a rationale for immune monitoring before treatment[6]. This could be done by cytometry or single-cell RNA/VDJ-seq. The feasibility of integrating single-cell RNA-seq analyses in clinical trials was already demonstrated in MM[8] and could allow monitoring the evolution of T cell clones as well as their phenotypic trajectories. Similarly investigation of the other bone marrow microenvironmental components, both soluble (cytokines) and cellular (Tregs, BMSC, MDSC, pDC) and their status may provide information about creating a permissive environment for optimal clinical activity of TCE. However, additional studies are required to establish straightforward measures and cut-offs on specific cell populations that could be used in clinical practice.

Adjusting the sequence of immunotherapies in MM

Resistance mechanisms also inform the strategy of immunotherapy sequencing in MM. To date, limited clinical data regarding TCE sequencing are available. In patients receiving anti-BCMA TCE as first subsequent therapy after talquetamab (n=19), the ORR was 57.9%, which is close to ORR in Majestec-1 or Magnetismm-3 studies[33]. In Monumental-1, patients receiving talquetamab as subsequent therapy after BCMA TCE (n=18), the ORR was 44.4%, in comparison with 71.7% in prior TCE naïve patients (0.8 mg/kg cohort)[10]. Complete inactivation of a target, e.g. by homozygous deletion, likely precludes response to other immunotherapies targeting the same antigen. For example, in the case reported by Truger *et al.*, bi-allelic loss of *TNFRSF17* following BCMA-targeting TCE led to an absence of response to subsequent treatment with an anti-BCMA antibody-drug conjugate[1]. By contrast, mutations in the extracellular domain of BCMA can impair the binding of one TCE but not another[13]. Patients may thus benefit from sequential or combined TCE targeting different BCMA epitopes. *TNFRSF17* mutations were less frequent in resistant MM after BCMA-targeting CAR-T than BCMA-targeting TCE. This could be an argument for CAR-T usage in first immunotherapy line, especially with the advent of more efficient CAR platforms like T-Charge[34].

More than half of the 14 relapses after BCMA-targeting TCE studied by Lee *et al.* did not involve *TNFRSF17* genetic alterations[13]. Part of these cases are likely explained by tumor-extrinsic factors like T cell exhaustion[19]. Such mechanisms may also prevent response to subsequent TCE targeting other antigens. By contrast, all sequenced relapses after GPRC5D-targeting TCE were driven by genetic or epigenetic GPRC5D inactivation[13,14], which should not impair the efficacy of immunotherapies targeting other antigens. Consistent with these predictions, immune and genome profiling of a few patients with sequential immunotherapies suggests that T cell exhaustion precludes response to subsequent immunotherapy lines whereas genetic inactivation of an antigen does not impair response to another immunotherapy targeting another antigen or epitope[35]. Importantly, the frequency of heterozygous deletions encompassing TCE target gene loci increases significantly between NDMM and RRMM[12]. Myeloma cells also contribute to create an immunosuppressive bone marrow by several means[29,36–38]. As a result, the immune microenvironment gets compromised during MM progression[39], and RRMM display features of T cell exhaustion[40]. Both effects are likely to limit the efficacy of TCE in advanced disease and argue for their use in early treatment lines.

Conclusion and future perspectives

Other potential resistance mechanisms

Both tumor-intrinsic and tumor-extrinsic resistance mechanisms of TCE resistance have been elucidated, but they were so far analyzed separately. Joint analyses of tumor and immune cell responses to TCE treatment will be useful to understand how tumor cells may influence T cell response, and to estimate the proportion of cases in which resistance remains unexplained. Interestingly, some post-TCE relapses displayed subclonal target inactivations affecting most but not all tumor cells[13,14]. Treatment escape in the remaining subclones may involve undetected target alterations (e.g. mutations in very small clones), or other resistance mechanisms yet to be characterized. Epigenetic inactivation was investigated for *GPRC5D*[14] but not *TNFRSF17*. In addition, γ-secretase can shed BCMA protein from the cell surface and release soluble BCMA (sBCMA) into the blood[41]. An activation of this process may allow TCE escape through the removal of the target antigen from MM cells, and interference of the drug with sBCMA. High sBCMA levels were associated with increased tumor burden, extramedullary disease and lower response to anti-BCMA TCE[11,42,43]. *In vitro*, high sBCMA levels decreased the binding of anti-BCMA antibodies to MM cells[44] and the efficacy of anti-BCMA CAR-T and TCE[45]. Interestingly, structural genomic rearrangements leading to the overexpression of BCMA and higher sBCMA levels were identified in MM after anti-BCMA CAR-T / TCE treatment[45]. Finally, two studies reported down-modulation of IFN-γ signaling as an acquired mechanism of resistance to HER2-targeting TCE in gastric and breast cell lines, conferring resistance to killing by active T lymphocytes[46,47]. Inhibition of IFN-γ signaling has not been reported so far in TCE- treated MM.

Development of trispecific antibodies and drug combinations

To limit target-related mechanisms of resistance, strategies using multitarget T-cell engagers are currently being investigated. One option is to combine bispecific antibodies. The phase 1b study RedirecTT-1 evaluated the combination of biweekly talquetamab and teclistamab in RRMM patients (median of 4 prior lines, 79.6% triple class refractory). The combination demonstrated high efficacy with an ORR of 86.6% including 40.2% complete response, and a median PFS of 20.9 months[48]. Another option for multitarget approach is the use of trispecific antibodies, targeting CD3 and 2 distinct targets on plasma cells. A phase 1 study is currently evaluating a trispecific antibody targeting CD3xBCMAxGPRC5D in relapsed myeloma patients (NCT05652335). To limit tumor extrinsic mechanisms of resistance (i.e. T-cell exhaustion) several ongoing studies evaluate bispecific antibodies with IMiDs (i.e. lenalidomide, pomalidomide), anti CD38 antibodies (i.e daratumumab) or immune checkpoint inhibitors (i.e. cetrelimab) that have been shown to promote T-cell activity[49,50]. Initial efficacy and safety results of a talquetamab + pomalidomide combination were promising in

the MonumenTAL-2 study[51]. Phase 1b studies combining bispecific antibodies with anti-CD38 monoclonal antibodies demonstrated promising response rates[52,53]. The ongoing phase 1-2 study TRIMM-3 evaluates efficacy and safety of teclistamab or talquetalab in combination with anti PD-1 cetrelimab (NCT05338775). Moreover, ongoing clinical trials evaluated the combination of BCMAxCD3 bispecific antibodies with gamma secretase inhibitors (GSI), to decrease soluble BMCA levels implicated in BCMA bispecific resistance (NCT04722146**)**. Combination of TCE plus the GSI nirogacestat led to a promising response rate, but high-grade immune events were reported in the cohort with early administration of GSI during teclistamab priming doses[54]. Combining bispecific TCE with cyclophosphamide may also improve T-cell persistence and function, as demonstrated in preclinical models[21]. Altogether, combinations with various therapeutic classes holds great promise to improve the efficacy of TCE in myeloma, notably by limiting T cell exhaustion.

Authorship

Contribution: All authors performed literature review, wrote and edited the manuscript.

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Table and Figure Legends

Fig. 1: Spectrum of TNFRSF17 and GPRC5D mutations identified in post-TCE MM relapses. Somatic mutations identified in two studies[13,14] are indicated on the protein structure. Extracellular, transmembrane and cytoplasmic domains are annotated with a color code. TNFRSF17 mutations define hostpots in the extracellular domain and impact a single amino acid. By contrast, GPRC5D mutations are truncating and distributed all along the protein sequence.

Fig. 2: Tumor-intrinsic and -extrinsic mechanisms of TCE resistance in MM. Only resistance mechanisms evidenced in humans or preclinical models are represented.

Table 1: Clinical impact of resistance mechanisms. Table 1 summarizes the molecular alterations associated with TCE resistance and their clinical impact.

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Alteration	Disease stage	Frequency	Detection technique*	Clinical impact	Reference(s)**
16p loss (*TNFRSF17*)	Pre-treatment screening	3-4% of TCE-naive MM	WGS	May facilitate bi-allelic target inactivation by second hit	1,2
TNFRSF17 mutation	Pre-treatment screening	1.1% (somatic) and 0.7% (germline) of TCE-naive MM	WGS	May facilitate bi-allelic target inactivation by second hit	2
12p loss (*GPRC5D*)	Pre-treatment screening	13-15% of TCE-naive MM	WGS	May facilitate bi-allelic target inactivation by second hit	1,2,3
GPRC5D mutation	Pre-treatment screening	4% TCE-naive MM	WGS	May facilitate bi-allelic target inactivation by second hit	1
Low GPRC5D expression	Pre-treatment screening	TBD	RNA-seq	Associated with reduced talquetamab efficacy *in vitro*. May facilitate epigenetic inactivation of the target	3,4
Abundance of exhausted T cell clones	Pre-treatment screening	TBD	scRNA/VDJ-seq	Predicts response failure to BCMA-targeting TCE	5
TNFRSF17 homozygous deletion	At relapse	1/14 relapses post-BCMA-targeting TCE	WGS	Precludes response to other BCMA-targeting therapy	1,2
TNFRSF17 p.Arg27Pro	At relapse	1/14 relapses post-BCMA-targeting TCE	WGS	Confers resistance to teclistamab and elranatanab	2
TNFRSF17 p.Pro34del	At relapse	3/14 relapses post-BCMA-targeting TCE	WGS	Confers resistance to teclistamab and elranatanab	2
TNFRSF17 p.Ser30del	At relapse	2/14 relapses post-BCMA-targeting TCE	WGS	Confers resistance to teclistamab	2
Bi-allelic genetic GPRC5D inactivation	At relapse	5/7 post-talquetamab relapses	WGS	Likely precludes response to other GPRC5D-targeting therapy	2,3
Epigenetic GPRC5D inactivation	At relapse	2/3 post-talquetamab relapses	scMultiome (RNA-seq + ATAC-seq)	Likely precludes response to other GPRC5D-targeting therapy	3

* Indicated techniques are those used in the original references.

** 1 Truger et al., Blood Adv 2021; 2 Lee et al., Nat Med 2023; 3 Derrien et al., Nat Cancer 2023; 4 Verkleij et al. Blood Adv 2021; 5 Friedrich et al., Cancer Cell 2023.

TBD: To be determined; WGS: whole genome sequencing.

Figure 1

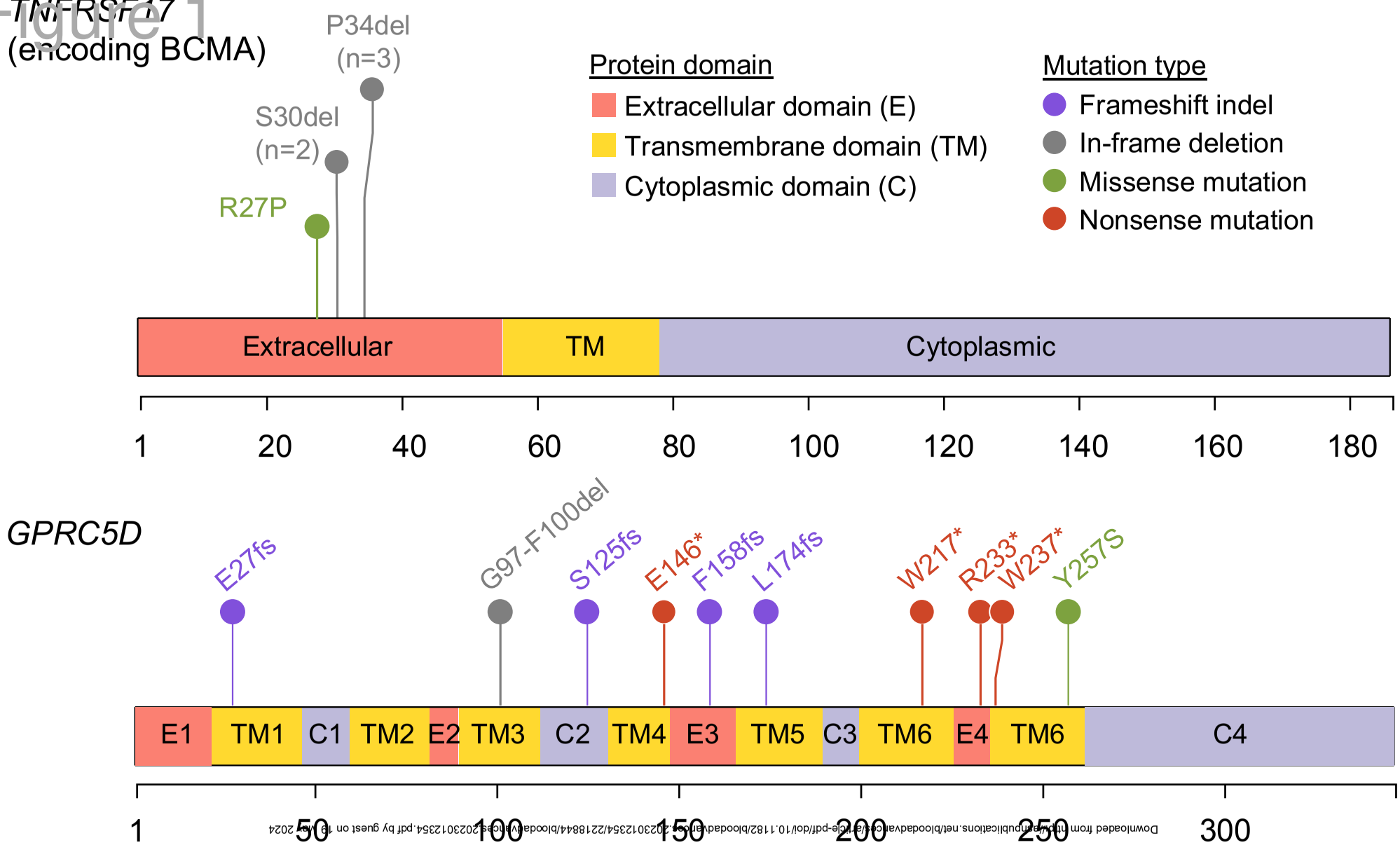

Figure 2