

TNFRSF17 (B Cell Maturation Antigen, BCMA) alterations and downstream compensatory pathway activation following BCMA-directed CAR T-cell and bispecific T-cell engagers in multiple myeloma

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Abstract

BCMA-directed CAR T-cell and T-cell engager (TCE) therapies produce high response rates in relapsed multiple myeloma, yet most patients progress. Prior work identified *TNFRSF17* alterations mediating antigen escape in up to 43% of TCE progressions. To evaluate the generalizability of these findings and examine antigen-loss-independent resistance, we performed whole-exome sequencing on 32 paired pre- and post-treatment progression bone marrow samples from 15 patients (5 CAR T, 10 TCE); two patients received both agents. *TNFRSF17* alterations were infrequent and restricted to TCE recipients (one concurrent frameshift and missense mutation; two heterozygous copy-number losses); no biallelic loss or CAR T-associated events were observed. In contrast, deleterious/loss-of-function variants in NF- κ B, PI3K-AKT-mTOR, and MAPK/ERK pathways were universal in pre-treatment samples and acquired or clonally enriched in 80% of patients at progression. Acquired events included loss-of-function alterations in *CYLD*, activating mutations in *KRAS*, *NRAS* and *BRAF*, and acquired *TP53* mutations. In this series, antigen escape via *TNFRSF17*/BCMA alteration was uncommon. Instead, pre-existing, acquired and clonally enriched pathogenic variants in NF- κ B, PI3K-AKT-mTOR and MAPK cascades, together with *TP53* mutation, emerged as a frequent finding. These alterations suggest that deregulation of BCMA-downstream survival signaling, enhanced proliferative fitness, and elevation of apoptotic threshold constitute antigen-loss-independent mechanisms of resistance.

INTRODUCTION

The B-cell maturation antigen (BCMA) is a transmembrane receptor and member of the tumor necrosis factor superfamily, essential for plasma cell survival, and minimally expressed on non-hematopoietic tissues, it has emerged as a key therapeutic target in multiple myeloma (MM) (1). Currently, BCMA-targeted bispecific T-cell engagers (TCEs), chimeric antigen receptor (CAR) T-cells, and antibody drug-conjugates (ADC) are all Food and Drug Administration (FDA) approved for the treatment of relapsed MM (2, 3). As the number of BCMA-targeted therapeutics continues to grow, defining the biological basis of treatment resistance is critical for guiding optimal therapy.

Excluding those with primary refractory disease, potential mechanisms of acquired treatment resistance to T-cell redirecting therapy include immune system exhaustion and intrinsic plasma cell resistance via genomic and antigenic changes (4). While BCMA antigen loss is rare (< 5%) following CAR T-cell therapy (5, 6), biallelic or monoallelic deletions coupled with BCMA extracellular domain mutations have been observed in ~ 43% of relapse cases following BCMA TCE therapy in a single series (7). Additionally, resistance to T-cell mediated cytotoxicity through activated NF- κ B signaling has been proposed as an alternative antigen-independent mechanism of resistance (4, 8).

We examined paired pre- and post-treatment samples from patients progressing after BCMA-directed CAR T-cell or T-cell engager therapy with three aims: (1) to determine the frequency of *TNFRSF17*/BCMA antigen escape; (2) to identify activating or loss-of-function variants in downstream survival pathways (NF- κ B, PI3K/AKT/mTOR and MAPK); and (3) to explore alterations that impair apoptotic competence, including *TP53*, the death receptor pathway, and apoptotic regulators, as potential mechanisms of resistance to T-cell-mediated cytotoxicity.

PATIENTS AND METHODS

We identified patients undergoing CAR T-cell or TCE therapy from a prospectively maintained database and those with the requisite samples available were included. All patients provided consent for storage of their samples for research. The study was approved by the Mayo Clinic Institutional Review Board. We analyzed available paired pre- and post-treatment bone marrow samples using whole exome sequencing (WES). Patients were selected based on confirmed disease progression, with available pre- and post-treatment bone marrow samples. Post-treatment bone marrow samples were collected at the time of multiple myeloma disease progression. After standard DNA extraction and library construction protocols, WES was performed on CD138 + sorted cryopreserved bone marrow cells (13 samples), unsorted cryopreserved bone marrow cells (5 samples) and bone marrow trephines (14 samples), see **supplementary methods**. The median bone marrow plasma cell burden was 55% overall (IQR = 30, 80), 30% for unsorted cryopreserved samples (n = 5, range 5–95%), and 65% for trephine-extracted DNA samples (n = 14, range 25–95%). The mean coverage depth was 208x (185x for CD138 + cells, 181x for unsorted cells, 239x for extracted DNA from bone marrow trephines).

Somatic variants were filtered using the following criteria: (1) total read depth ≥ 5 , (2) variant read depth ≥ 2 , (3) variant allele frequency (VAF) ≥ 0.05 , (4) population allele frequency < 0.001, (5) removal of 'low' impact variants, (6) selection of predicted pathogenic variants based on deleterious predictions by SIFT (score ≤ 0.05) and/or deleterious classifications by PolyPhen (probably damaging: ≥ 0.909 ; possibly damaging: 0.447–0.908), or loss-of-function (LoF) classifications (e.g., frameshifts, nonsense mutations, in-frame insertions/deletions), or variants without SIFT or PolyPhen predictions, and (7) removal of noise genes (e.g., MUCs, TTN, ORs). Synonymous variants were excluded. *TNFRSF17* and *TP53* variants of potential clinical relevance were retained regardless of SIFT/PolyPhen class. To assess copy number abnormalities (CNA), the whole exome BAM files were processed using CNVkit and the log ratios of the segments were compared between pre- and post-treatment time points of each paired patient sample to identify relevant gene gains or losses.

To investigate tumor-intrinsic mechanisms of resistance beyond *TNFRSF17* alterations, we examined pathogenic mutations in the key downstream signaling pathways activated by BCMA: NF- κ B, PI3K/AKT, and MAPK (including the JNK/p38 subset). For the 20 genes overlapping multiple downstream pathways, we assigned each variant to the most biologically relevant pathway according to its primary role in BCMA signaling, **Supplementary Table 1**. In addition, we examined genes associated with tumor resistance to T-cell killing including; apoptosis regulators, the death-receptor pathway, *TP53*, and the unfolded protein response, **Supplementary Table 2**. We categorized variants into three groups: (1) pre-existing variants (detected in pre-treatment samples at VAF ≥ 0.05), (2) acquired variants (detected exclusively in post-treatment samples), and (3) enriched variants [present in pre-treatment samples with a $\geq 20\%$ increase in cancer-cell fraction (CCF) post-treatment].

RESULTS

WES was performed on 32 paired pre- and post-treatment bone marrow samples from 15 patients (5 CAR T, 10 TCE). Two patients received both BCMA-directed CAR T and TCE. Patient C1 received ide-cel, relapsed, underwent intervening therapy, and 5-months later received teclistamab; the ide-cel relapse bone marrow served as the baseline sample for their teclistamab pair. Patient T6 received REGN5459, relapsed, and proceeded directly to cilta-cel as their next-line of therapy. The median age at BCMA treatment initiation was 66 years (range: 46–76), and 9 (60%) were male. Patient and sample characteristics as well as treatment outcomes are summarized in Table 1. The median number of prior lines of therapy was 5 (range: 3–10), and 12 patients (80%) were triple-class refractory. Eight patients (C1, C3, T1, T5, T7-10) were identified to have deletion 17p on fluorescence in-situ hybridization (FISH) testing at diagnosis or relapse and before BCMA-targeted therapy. Patients C3 and T10 had previously received BCMA-targeted CAR T-cell therapy before the BCMA-targeted treatment they received at our institution, Fig. 1. Etentamig (n = 6) was the most common TCE, and cilta-cel (n = 3) the most common CAR T-cell therapy. Best responses to CAR T-cell therapy were stringent complete response or complete response (sCR/CR; n = 2), very good partial response (VGPR; n = 3), and progressive disease (PD; n = 1). Best responses to T-cell engager (TCE) therapy were sCR/CR (n = 5), VGPR (n = 1), partial response (PR; n = 2), stable disease (SD; n = 2), and PD (n = 1).

Table 1

Characteristics of patients with pre- and post-BCMA targeted tumor samples sequenced in the study cohort y, years; BMPC, bone marrow plasma cells; FISH, fluorescence in-situ hybridization; m, months; CAR T, chimeric antigen receptor T-cell; TCE, T-cell engaging bispecific antibody; M, male; F, female; DMSO, dimethyl sulfoxide; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease; Ide-cel, idecabtagene autoleucel; cilta-cel, ciltacabtagene autoleucel; Zevor-cel, Zevorcabtagene autoleucel.

Patient	Sample	Drug	Sex	Age, y	BMPC, %	Sample type	Mean target coverage	FISH diagnosis	Del17p	Best response	PFS, m	OS, m	Status
C1	A: Pre-CAR T		M	74	80	CD138 + DMSO	125	t(11;14)	Yes				
	B: Post-CAR T	Ide-cel			30	CD138 + DMSO	221			CR	10	38	
	C: Post-TCE	Teclistamab		76	80	CD138 + DMSO	180			PD	2	23	Deceased
C2	A: Pre-CAR T		M	69	60	Unsorted DMSO	135	Hyperdiploidy	No				
	B: Post-CAR T	Zevor-cel			55	Extracted DNA	200			VGPR	12	22	Deceased
C3	A: Pre-CAR T		M	70	25	CD138 + DMSO	151	t(11;14), del13q, t(4;14)	Yes				
	B: Post-CAR T	Ide-cel			5	CD138 + DMSO	200			sCR	9	26	Deceased
C4	A: Pre-CAR T		M	69	92.5	CD138 + DMSO	186	t(11;14)	No				
	B: Post-CAR T	Cilta-cel			30	Extracted DNA	170			VGPR	5	6	Deceased
C5	A: Pre-CAR T		M	61	90	Extracted DNA	199	No abnormality	No				
	B: Post-CAR T	Cilta-cel			95	Extracted DNA	181			VGPR	3	11	Deceased
T1	A: Pre-TCE		F	69	50	Extracted DNA	86	No abnormality	Yes				
	B: Post-TCE	Etentamig			20	Unsorted DMSO	233			PR	3	21	Deceased
T2	A: Pre-TCE		M	72	65	Extracted DNA	529	Hyperdiploidy, del13q, t(14;20), gain1q	No				
	B: Post-TCE	Etentamig			95	CD138 + DMSO	221			sCR	6	11	Deceased
T3	A: Pre-TCE		M	62	30	Unsorted DMSO	166	No abnormality	No				
	B: Post-TCE	AMG-701			80	Extracted DNA	123			PR	10	14	Deceased
T4	A: Pre-TCE		F	65	30	Extracted DNA	104	t(14;16), del13q	No				
	B: Post-TCE	Etentamig			5	Unsorted DMSO	169			CR	48	64	Alive
T5	A: Pre-TCE		F	46	20	CD138 + DMSO	154	Hyperdiploidy, del13q, del17p	Yes				
	B: Post-TCE	Etentamig			65	CD138 + DMSO	204			CR	16	37	Alive
T6	A: Pre-TCE		F	64	65	Extracted DNA	691	t(11;14)	No				

Patient	Sample	Drug	Sex	Age, y	BMPC, %	Sample type	Mean target coverage	FISH diagnosis	Del17p	Best response	PFS, m	OS, m	Status
	B: Post-TCE	REGN5459			50	CD138 + DMSO	160			CR	26	60	
	C: Post-CAR T	Cilta-cel		66	70	Unsorted DMSO	201			PD	1	32	Alive
T7	A: Pre-TCE		F	72	30	CD138 + DMSO	156	Hyperdiploidy	Yes				
	B: Post-TCE	Etentamig			7.5	CD138 + DMSO	180			sCR	31	65	Alive
T8	A: Pre-TCE		M	63	25	Extracted DNA	123	t(4;14), Del17p, gain1q	Yes				
	B: Post-TCE	REGN5459			80	CD138 + DMSO	265			VGPR	7	17	Deceased
T9	A: Pre-TCE		F	58	50	Extracted DNA	495	Hyperdiploidy, del13q	Yes				
	B: Post-TCE	Etentamig			92.5	Extracted DNA	90			SD	1	8	Deceased
T10	A: Pre-TCE		M	50	95	Extracted DNA	182	Gain1q	Yes				
	B: Post-TCE	Teclistamab			90	Extracted DNA	168			SD	2	14	Deceased

TNFRSF17:

At the BCMA gene locus, *TNFRSF17*, only two mutational events were detected across all pre- and post-treatment samples, Table 2. Both events occurred in the post-treatment sample of patient T7 who had a prolonged remission with etentamig therapy (PFS 31 months). The events included a frameshift deletion (p.Thr92ArgfsTer9) at a VAF of 15% and a missense mutation (p.Pro23Ser) at a VAF of 17% (extracellular domain). Neither mutation was identified at any frequency in T7's pre-treatment sample. We did not identify *TNFRSF17* alterations in any CAR T-cell post treatment samples. Additionally, patients C1 and T6 who had previously received BCMA-targeted CAR T-cell therapy, did not have any baseline alterations in *TNFRSF17*. Only two patients (T2 & T5), had a single copy-number loss at the *TNFRSF17* gene locus on post-treatment samples. Both had received TCE therapy and obtained deep responses (CR & sCR) with PFS exceeding 6-months. Neither T2 nor T5 had any other mutational abnormality at the *TNFRSF17* gene locus. There were no cases of biallelic *TNFRSF17* gene loss.

Table 2

TNFRSF17 variants, TNFRSF17 copy number abnormalities, and variants in downstream pathways (NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, and JNK/p38) among patients with pre- and post-BCMA targeted tumor samples sequenced in the study cohort. *TNFRSF17*, TNF Receptor Superfamily 17; PFS, progression free survival; CAR T, chimeric antigen receptor T-cell; TCE, T-cell engaging bispecific antibody; M, male; F, female; sCR, stringent complete response; CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease.

Patient	Sample	Age, Sex	<i>TNFRSF17</i>	CNA <i>TNFRSF17</i>	Pre-existing variants	Acquired post- treatment variants	Enriched post- treatment variants	Best response	PFS, m
C1	A: Pre-CAR T	74 M	-	Gain	NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-CAR T		-	Diploid		-	-	CR	10
	C: Post-TCE		-	Gain		PI3K	-	PD	2
C2	A: Pre-CAR T	69 M	-	Diploid	NF- κ B, MAPK/ERK				
	B: Post-CAR T		-	Diploid		PI3K, JNK/p38, MAPK	-	VGPR	12
C3	A: Pre-CAR T	70 M	-	Diploid	NF- κ B, PI3K-AKT-mTOR, JNK/p38				
	B: Post-CAR T		-	Diploid		-	-	sCR	9
C4	A: Pre-CAR T	69 M	-	Diploid	NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-CAR T		-	Diploid		PI3K, JNK/p38	JNK/p38	VGPR	5
C5	A: Pre-CAR T	61 M	-	Diploid	NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-CAR T		-	Diploid		NF- κ B, PI3K-AKT-mTOR	-	VGPR	3
T1	A: Pre-TCE	69 F	-	Diploid	NF- κ B, PI3K-AKT-mTOR, JNK/p38				
	B: Post-TCE		-	Diploid		-	-	PR	3
T2	A: Pre-TCE	72 M	-	Diploid	NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-TCE		-	Hetero loss		PI3K	PI3K-AKT-mTOR, MAPK/ERK, JNK/p38	sCR	6
T3	A: Pre-TCE	62 M	-	Diploid	JNK/p38				
	B: Post-TCE		-	Diploid		NF- κ B, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38	-	PR	10
T4	A: Pre-TCE	65 F	-	Diploid	NF- κ B, PI3K-AKT-mTOR, JNK/p38				
	B: Post-TCE		-	Diploid		-	-	CR	48
T5	A: Pre-TCE	46 F	-	Diploid	JNK/p38				
	B: Post-TCE		-	Hetero loss		NF- κ B	-	CR	16
T6	A: Pre-TCE	64 F	-	Diploid	MAPK/ERK, JNK/p38				
	B: Post-TCE		-	Diploid		-	MAPK/ERK	CR	26
	C: Post-CAR T		-	Diploid		-	-	PD	1

Patient	Sample	Age, Sex	TNFRSF17	CNA TNFRSF17	Pre-existing variants	Acquired post-treatment variants	Enriched post-treatment variants	Best response	PFS, m
T7	A: Pre-TCE	72 F	-	Diploid	NF-κB, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-TCE		Frameshift + missense	Diploid		JNK/p38	-	sCR	31
T8	A: Pre-TCE	63 M	-	Diploid	NF-κB, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38				
	B: Post-TCE		-	Diploid		PI3K-AKT-mTOR	-	VGPR	7
T9	A: Pre-TCE	58 F	-	Diploid	NF-κB, MAPK/ERK, JNK/p38				
	B: Post-TCE		-	Diploid		NF-κB, PI3K-AKT-mTOR, MAPK/ERK, JNK/p38	MAPK/ERK, NF-κB	SD	1
T10	A: Pre-TCE	50 M	-	Diploid	PI3K-AKT-mTOR, JNK/p38				
	B: Post-TCE		-	Diploid		PI3K-AKT-mTOR, MAPK/ERK, JNK/p38	-	SD	2

Pre-existing variants in BCMA Downstream pathways:

In pre-treatment samples (n = 15), we identified 108 unique deleterious or LOF variants across the NF-κB (n = 23), PI3K-AKT-mTOR (n = 32), MAPK/ERK (n = 18), and JNK/p38 (n = 35) pathways, Fig. 2. All patients had at least one deleterious or LOF variant in their pre-treatment samples, with a median of 6 variants per patient (range: 1–21). A complete list of these pre-existing mutations is provided, **Supplementary Table 3**. There were recurrent baseline activating or likely-activating mutations in the MAPK (including JNK/p38) pathway, specifically: *BRAF* (C5: p.Gly504Arg, T2: p.Glu626Lys, T6: p.Val640Glu), *KRAS* (C4: p.Gly12Cys, T9: p.Gln61His), and *MAP3K9* (T8: p.Ala772AsnfsTer18, T1: p.Glu771Ala, T1, T10: p.Lys774Thr). Similarly, recurrent LOF variants in key negative regulators of the NF-κB pathway were identified including *NFKBIA* (C3: nonstop p.Ter318SerextTer5, T1: frameshift p.Gln271HisfsTer2), *CYLD* (T1: p.Gln77Ter), and *TRAF3* (C4: p.Glu283Ter). Among patients with PFS ≤ 3 months the median number of pre-existing mutations was 10.5 variants, compared with 6 variants in those with PFS > 3 months (p = 0.21). Patients C3 and T10 whom had both received prior BCMA-targeted CAR T-cell therapy external to our centre, had 4 pre-existing variants each.

Acquisition of New Downstream BCMA Variants Post-Treatment:

Analysis of post-treatment samples revealed 53 unique acquired deleterious or LOF variants within NF-κB (n = 9), PI3K-AKT-mTOR (n = 27), MAPK/ERK (n = 8), and JNK/p38 (n = 9), Fig. 3. Eleven out of 15 patients had acquired a deleterious or LOF variant post therapy, with a median of one variant per patient (range: 0–12). The acquisition rate of unique post-treatment variants was comparable between CAR T-cell and TCE recipients (median 1.5 vs. 1 new variant per patient, p = 0.68), and for patients with PFS ≤ 3 months or > 3 months (median 2 vs. 1 new variant per patient, p = 0.63). Recurrent or convergent variants included the missense *DUSP5* p.Ala220Met in three patients (C2, C4, T7), identical frame-shift insertion-deletion events in *TAB3* in two patients (C4 & T10: p.Thr430AsnfsTer18 & p.Ile429MetfsTer28), and identical missense *VCAM1* mutations in two patients (C5 & T9: p.Ile523_Leu524delinsArgMet), **Supplementary Table 4**. In the NF-κB pathway, several variants predicted to enhance NF-κB signaling were identified including *CYLD* (T9: p.Leu423Phe & T5: p.Thr521IlefsTer6), and *NFKBIA* (T3: p.Asp28dup). Activating or likely activating mutations in the RAS/RAF pathway were acquired in three patients, including *BRAF* (T3: p.Val640Glu), *NRAS* (C2: p.Gln61His), and *KRAS* (T10: p.Gly60Val). Acquired variants in the PI3K-AKT-mTOR pathway occurred in nine patients, including the FGFR family (C4, T3, T9: *FGFR1/2/3/4*), *ERBB2* (T3: p.Thr306Met, T9: p.Asp171Del & p.Ile172delinsMetSer), *AKT2* (T3: p.Arg392Lys and p.Asp324Glu), *IRS2* (C1: p.Ser308Leu), *RICTOR* (T9: p.Pro378Ser), *PIK3CA* (T9: p.Gly118Asp) and a frameshift deletion in *MTOR* (T9: p.Pro568ArgfsTer29).

Enrichment of Pre-Existing Downstream BCMA Variants Following Treatment:

Enrichment analysis identified 11 pre-existing variants with ≥ 20% CCF increase post-treatment, potentially indicating clonal selection under therapeutic pressure, Fig. 4. Enrichment in downstream pathways was more pronounced post-TCE (3 patients, 10 variants) than post-CAR T (1 patient, 1 variant). Most enriched variants (n = 6) occurred in sample T2B, a TCE-recipient who had 6-month PFS with etentamig. T2 demonstrated simultaneous enrichment of variants across MAPK, PI3K, and JNKp38 pathways, including expansion of several PI3K variants predicted to enhance PI3K signaling; *PIK3CB* (p.Ser781Phe) and *DEPTOR* (p.Lys58Asn). MAPK was the most frequently involved pathway (5 variants, 3 patients) with expansion of several known activating mutations including *BRAF* (T6: p.Val640Glu), *KRAS* (T9: p.Gln61His), as well as the less well characterized *BRAF* (T2: p.Glu626Lys), **Supplementary Table 5**.

Pre-existing, acquired, and enrichment of variants associated with resistance to T-cell cytotoxicity:

In pre-treatment samples, we identified 7 unique deleterious or LOF mutations in apoptotic, death-receptor, and unfolded protein response pathways in 5 individual patients, Fig. 5. Most notably, we observed pre-existing subclonal LOF mutations in core apoptotic machinery: *PMAIP1* (C1), *APAF1* (T2), *BAX* (T1), as well as the death-receptor pathway *CFLAR* (T1), **Supplementary Table 6**. Acquired deleterious missense mutations in *TP53* were observed in three patients (C3, T2, T8), as well as acquired mutations in *ERN1* (T10B: p.Ser846Arg & p.Asp844_Val845insCysArgSerArgPro). No apoptotic, death-receptor or unfolded protein response mutations were enriched between pre- and post-treatment samples.

DISCUSSION

In this study of paired pre- and post-treatment bone marrow samples from 15 patients progressing after BCMA-directed CAR T-cell or TCE therapy, genomic alterations at the *TNFRSF17* (BCMA) locus were uncommon. Only one patient (T7, post-TCE) acquired coding mutations in *TNFRSF17*, and heterozygous copy-number loss was observed in just two additional TCE-treated patients. No biallelic loss or CAR T-associated *TNFRSF17* alterations were identified. In contrast, we observed frequent pre-existing, acquired, and clonally enriched deleterious variants in the key downstream BCMA signaling pathways, and in pathways linked to resistance to T-cell mediated cytotoxicity (including TP53 and core apoptotic machinery). These findings suggest that tumor-intrinsic rewiring of survival, proliferative, and apoptotic signaling represents an important potential antigen-loss-independent mechanism of resistance.

Our findings on antigen escape differ in frequency from the series reported by Lee et al., in which approximately 43% of TCE progressions and 20% of CAR T-cell progressions harbored biallelic *TNFRSF17* loss or monoallelic loss combined with extracellular domain mutations (7). In that study, a clustering of mutations mapping to the BCMA extracellular domain between amino acids in positions 27 and 34 coupled with monoallelic loss of *TNFRSF17* was observed in a number of teclistamab and elranatamab recipients, with such mutations abrogating the binding of TCEs to BCMA. Despite a similar number of progression events, we observed a *TNFRSF17* mutation frequency of only 9% (1/11 TCE progressions) following BCMA-targeted TCE and no occurrences following BCMA-targeted CAR T-cell therapy. Conceivably the TCE agents used may have contributed to the lower frequency of *TNFRSF17* alterations observed in our series. Herein, the majority of patients received etentamig rather than teclistamab or elranatamab, and differences in epitope binding, affinity, and dosing schedules could alter the selection pressure for antigen escape (9). Analogous to the original work and recent findings from Maura et al., we observed that antigen loss or alteration remains rare following CAR T-cell therapy (6, 7, 10–12). These results support the contrasting selective pressure exerted by continuous TCE exposure compared to CAR T-cell therapy. Of note, patient T7 who demonstrated two distinct acquired *TNFRSF17* variants only possessed a single acquired downstream pathway signaling variant (*DUSP5*).

Beyond antigen modulation, we systematically examined likely pathogenic variants in pathways that mediate the pro-survival effects of BCMA signaling. Pre-existing deleterious or LOF variants were universal in pre-treatment samples. Recurrent activating or likely activating alterations were identified in the MAPK cascade, including *BRAF*, *KRAS*, and *RAF1*. Sustained MAPK signaling could provide a tumor-intrinsic bypass mechanism under therapeutic BCMA engagement (13). Activation of NF- κ B through LOF variants in key negative regulators was also frequent. These included nonsense mutations in *TRAF3*, and *CYLD*, and LOF alterations in *NFKBIA* (including frameshift & nonstop variants) (14–16). Recent functional studies have shown that *TRAF3* and *CYLD* inactivation constitutively activates NF- κ B, raises the apoptotic threshold and drives antigen-independent resistance to both BCMA- and GPRC5d-directed therapies (8). Consistent with this, the two patients in our series who harbored pre-existing *TRAF3* or *CYLD* nonsense mutations (C4 & T1) experienced relatively short PFS.

After BCMA-directed therapy, most patients (75%) acquired new deleterious or LOF variants, distributed across the NF- κ B, PI3K, and MAPK pathways. The highest frequency of acquired variants was observed in the PI3K/AKT/mTOR axis, including deleterious missense mutations in positive regulators of the axis such as *AKT2*, *ERBB2*, *RICTOR*, and *IRS2*. Although the functional consequences of these specific variants have not been experimentally validated, activating mutations of these genes could promote cell survival and raise the apoptotic threshold, potentially reducing sensitivity to T-cell mediated cytotoxicity (17). LOF alterations in negative regulators of NF- κ B were also acquired, these included a frameshift deletion and missense mutation in *CYLD*, as well as an in-frame insertion in *NFKBIA*. In parallel, activating or predicted activating mutations were acquired in the core MAPK cascade (*KRAS*, *BRAF*, and *NRAS*). Taken altogether, this pattern of acquired mutations supports enhanced downstream BCMA survival signaling under therapeutic pressure.

Clonal enrichment of pre-existing variants was more pronounced after TCE than after CAR T-cell therapy, and was particularly striking in patient T2, who demonstrated simultaneous expansion of clones harboring alterations across the MAPK, PI3K, and JNK/p38 pathways. Enrichment patterns supported the positive selective pressure placed on these survival pathways, with expansion of established or putative activating drivers such as *KRAS* p.Gln61His, *BRAF* p.Val640Glu and *BRAF* p.Glu626Lys (18, 19). Consistent with these observations, a recent case report described a patient who relapsed following BCMA CAR T-cell therapy with a dominant *BRAF* V600E subclone, subsequent treatment with MAPK inhibition produced a meaningful clinical response – supporting the concept that activating MAPK-pathway mutation can drive progression after BCMA therapy (19).

Collectively, the acquisition and clonal expansion of variants in the NF- κ B, PI3K/AKT/mTOR, and MAPK cascades suggest that tumour cells with constitutive or enhanced activity in these BCMA-downstream survival pathways may possess a selective advantage under the pressure of BCMA-directed therapy (8, 16, 18). As physiological BCMA ligation activates the NF- κ B, PI3K/AKT, and MAPK cascades that are critical for plasma-cell survival, therapeutic interruption of the upstream signal may favor clones that have deregulated or can rapidly deregulate these pathways. Critically however, CAR T-cells and TCEs primarily kill myeloma cells through T-cell-mediated cytotoxicity rather than simply interrupting BCMA survival signaling. Thus, even if a clone becomes independent of BCMA, it must still resist the apoptotic programs delivered by the engaged T cell. In this context, mutations that produce constitutive NF- κ B, MAPK or PI3K/AKT signaling may raise the intrinsic apoptotic threshold, enhance proliferative fitness, and reduce sensitivity to T-cell cytotoxicity (8, 17, 18, 20). Additionally, constitutive NF- κ B activation has been shown to promote an immunosuppressive microenvironment that can suppress T-cell activity (8).

Other variants associated with impaired apoptotic competence included *TP53* deletion or mutation which is known to raise the apoptotic threshold and reduce the sensitivity to both granzyme- and death-receptor mediated killing (21, 22). Loss of TP53 function emerged as a recurrent feature in our series and

aligns with recent large-scale genomic analyses (6). We identified acquired, near-clonal TP53 missense mutations in three patients at progression. Maura et al., similarly, found *TP53* mutation to be one of the strongest genomic predictors of primary refractoriness after both CAR T-cell and T-cell engager therapy, and their murine models further demonstrated that heterozygous *Trp53* loss reduces *Tnfrsf17* expression and impairs the depth and durability of response to an anti-BCMA TCE (6). In addition, several patients carried LOF variants in core components of the mitochondrial apoptotic pathway (*PMAIP1*, *APAF1*) (23, 24).

This study is subject to several important limitations. The cohort size is modest, with variation in prior therapy, sample types, and plasma-cell purity. Specifically, Enrichment estimates should be interpreted cautiously where paired samples differed in processing. The genomic impact of BCMA targeted therapy was highly heterogeneous among patients. Functional validation of the identified variants was not performed, and concurrent RNA or protein expression data are lacking. Copy-number analysis was limited to whole exome-derived estimates, which may under-detect focal microdeletions.

The relative rarity of *TNFRSF17* genomic loss after CAR T-cell and TCE therapy indicates that BCMA expression is frequently retained at progression and may remain a viable therapeutic target. At the same time, the frequent acquisition or enrichment of MAPK, PI3K and NF- κ B pathway alterations, together with recurrent *TP53* mutations and loss-of-function variants in core apoptotic regulators, demonstrates that antigen-independent resistance mechanisms are common. These findings expand the conceptual framework of resistance beyond antigen loss and support strategies that simultaneously target BCMA and the key survival, proliferative, and apoptotic pathways that enable tumor cells to withstand T-cell-mediated cytotoxicity.

DECLARATIONS

Data sharing

Original data will be shared upon reasonable request, please contact Kumar.Shaji@mayo.edu

Disclosure of Conflicts of Interest:

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Authorship contributions: MJR and SK designed the study and co-wrote the paper. MJR, SZ, DJ, and YL assisted in patient identification and sample procurement. MJR collected the clinical data. MJR & SD analyzed the data. All authors critically reviewed the manuscript, suggested edits, and all authors approved the final version of the manuscript draft for submission.

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Figures

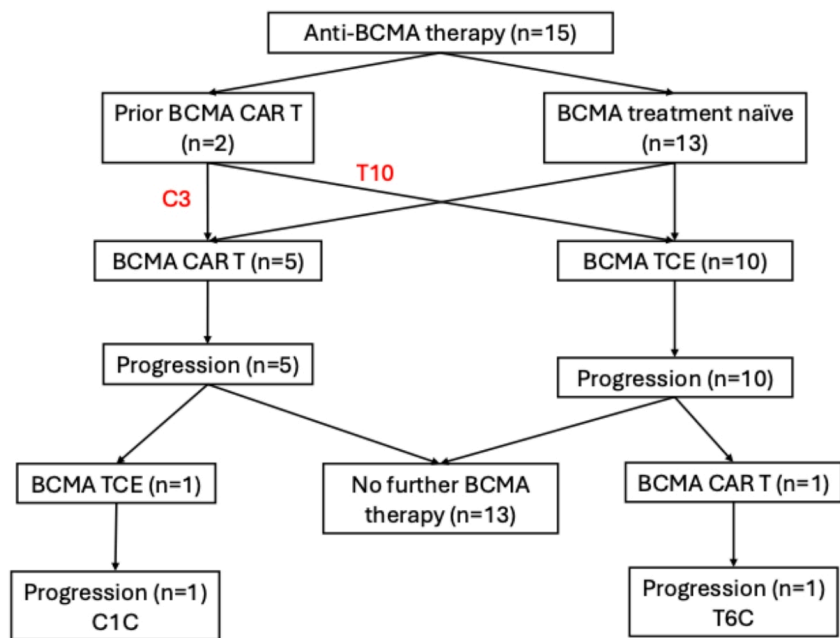


Figure 1

Consort diagram summarizing patient flow and treatment sequence.



Figure 3

Oncoplot of acquired pathway mutations following BCMA-directed therapy. Oncoplot showing deleterious or loss-of-function variants detected exclusively in post-treatment bone marrow samples (i.e., absent from the paired pre-treatment sample) across four key survival pathways (MAPK/ERK, JNK/p38, PI3K-AKT-mTOR and NF- κ B). Each column represents an individual patient (C1–C5: CAR T recipients; T1–T10: T-cell engager recipients). Rows represent mutated genes, ordered by mutation frequency. Tile color indicates variant classification (Missense, Nonsense, Frameshift or In-frame indel). The vertical color bar to the left of the gene names indicates pathway membership. The top annotation indicates treatment agent (CAR T vs TCE). Percentages on the right indicate the proportion of patients harboring an acquired mutation in each gene. Blank columns represent patients in whom no qualifying acquired variants were identified. Only variants meeting predefined filtering criteria (total depth ≥ 5 , variant depth ≥ 2 , VAF ≥ 0.05 , population allele frequency < 0.001 , and non-benign SIFT/PolyPhen predictions) are shown.

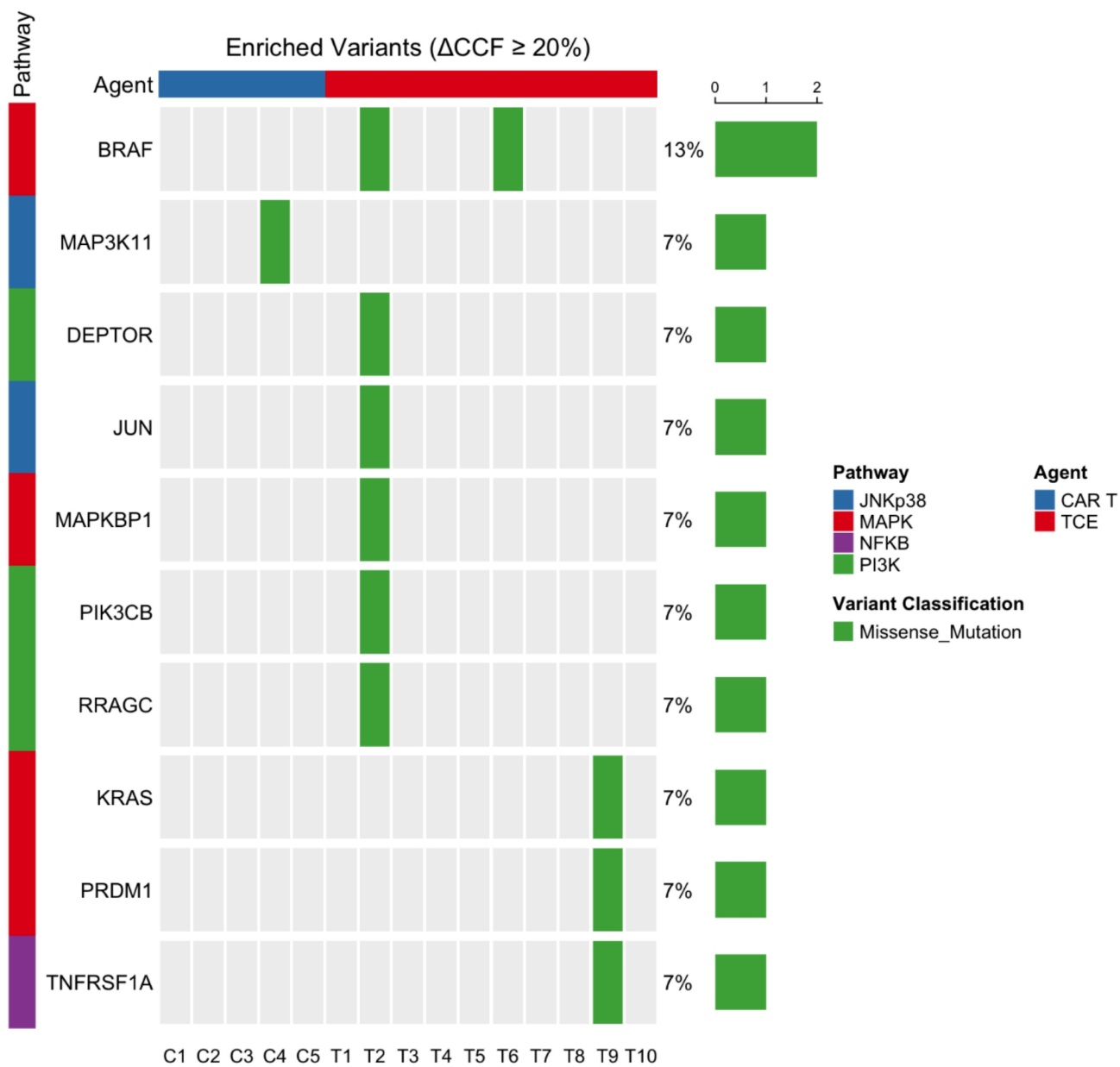


Figure 4

Oncoplot of variants showing clonal enrichment ($\Delta\text{CCF} \geq 20\%$) following BCMA-directed therapy. Oncoplot showing deleterious missense variants present in both pre- and post-treatment samples that underwent significant clonal enrichment (increase in cancer clonal fraction ≥ 20 percentage points) after BCMA-directed therapy. Each column represents an individual patient (C1–C5: CAR T recipients; T1–T10: T-cell engager recipients). Rows represent mutated genes, ordered by mutation frequency. All enriched variants were classified as missense mutations (green tiles). The vertical color bar to the left of the gene names indicates pathway membership (MAPK/ERK, JNK/p38, PI3K-AKT-mTOR or NF- κ B). The top annotation indicates treatment agent (CAR T vs TCE). Percentages on the right indicate the proportion of patients in whom enrichment of the indicated variant was observed. Blank columns represent patients in whom no qualifying enrichment events were identified.

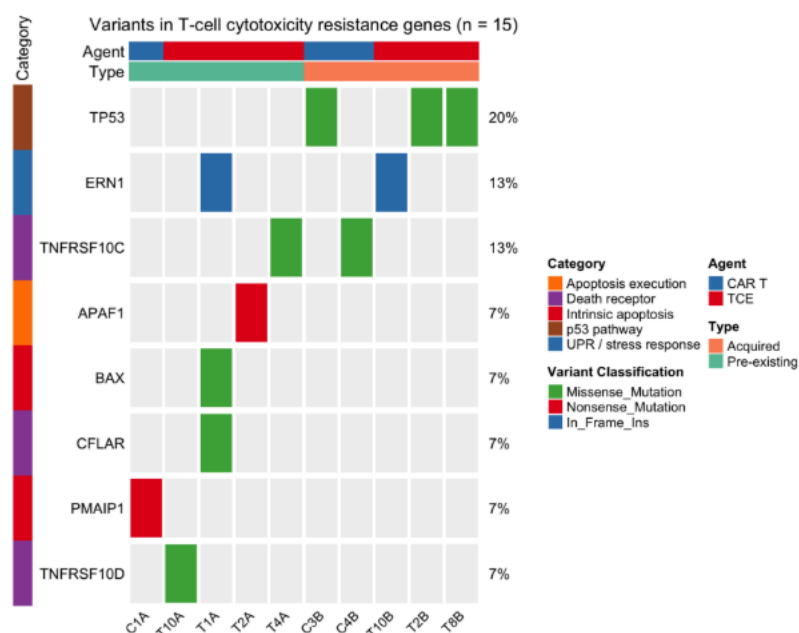


Figure 5

OncoPrint of variants in genes associated with resistance to T-cell-mediated cytotoxicity. Pre-existing and acquired mutations in apoptotic regulators, death-receptor pathway genes, unfolded-protein-response components and *TP53* identified across the 15-patient cohort. Columns are ordered by mutation timing (pre-existing → acquired); percentages are calculated against the full cohort (n = 15).

Supplementary Files

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