

Single- and double-hit events in genes encoding immune targets before and after T cell-engaging antibody therapy in MM

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Key Points

- First report on homozygous *BCMA* gene deletion and subsequent antigen loss in a patient treated with CD3xBCMA bispecific antibody.
- Heterozygous deletions of *BCMA*, *GPRC5D*, and other immune targets in up to 15% of immunotherapy-naïve patients.

T cell-engaging immunotherapies exert unprecedented single-agent activity in multiple myeloma (MM), thereby putting a yet unexplored selective pressure on the clonal architecture. In this study, we report on homozygous *BCMA* (*TNFRSF17*) gene deletion after BCMA-targeting T cell-redirecting bispecific antibody therapy in a heavily pretreated MM patient. Loss of BCMA protein expression persisted over subsequent relapses, with no response to treatment with anti-BCMA antibody drug conjugate. In light of the multiple alternative targets that are emerging in addition to BCMA, we extended our analyses to delineate a more complete picture of genetic alterations that may have an impact on immunotherapy targets in MM. We performed whole-genome sequencing and RNA sequencing in 100 MM patients (50 were newly diagnosed; 50 were relapsed/refractory) and identified a significant proportion of patients with aberrations in genes encoding immunotherapy targets; *GPRC5D* ranked first with 15% heterozygous deletions, followed by *CD38* (10%), *SDC1* (5%), and *TNFRSF17* (4%). Notably, these heterozygous deletions did not lower the expression levels of respective genes, but they may represent a first hit that drives the acquisition of homozygous deletions and subsequent antigen-loss relapse upon targeted immunotherapy. In summary, we show preexisting vulnerability in genes encoding immunotargets before and homozygous deletions after T cell-engaging immunotherapy.

Introduction

Novel T cell-engaging therapies such as chimeric antigen receptor (CAR) T cells or bispecific antibodies (BsAb's) have entered the treatment for multiple myeloma (MM) and show exceptionally high response rates in relapsed/refractory (RR) patients. Beyond BCMA, a plethora of other plasma cell targets is currently under investigation, including SLAMF7,¹ GPRC5D,² CD44v,³ and FCRL5⁴ (also known as FcRH5), with promising preliminary results in clinical phase 1 trials. Yet patients continue to relapse, and in contrast to immunotherapy in aggressive lymphoma, there is no survival plateau after treatment with CAR T cells or BsAb's in MM.

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All requests for raw and analyzed data and materials will be promptly reviewed by the University Hospital Würzburg and the MLL Munich Leukemia Laboratory to determine whether the request is subject to any confidentiality or data protection obligations. Any data and materials that can be shared will be released via a Material Transfer

Agreement. Adherence to the Datenschutz-Grundverordnung (<https://dsgrvo-gesetz.de/> and <https://data.consilium.europa.eu/doc/document/ST-5419-2016-REV-1/en/pdf>) is mandatory for sharing WGS data. Thus, a complete whole genome raw data set for a single patient cannot be shared according to European law. Processed data from which the identification of a patient is not possible can be made available. For requests, contact Leo Rasche via e-mail at rasche_l@ukw.de.

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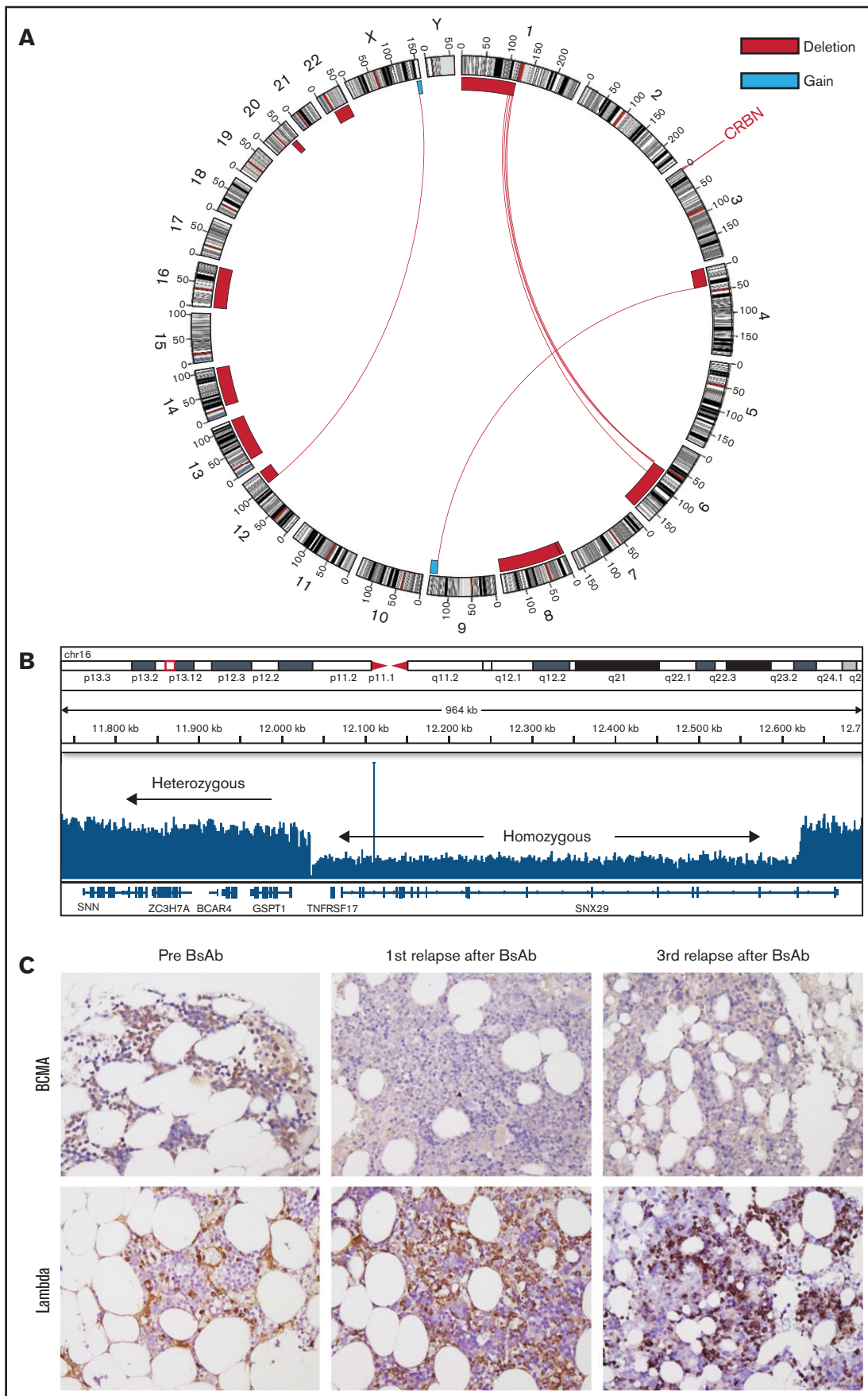


Figure 1.

Recently, we described a first tumor-intrinsic resistance mechanism to TNFRSF17 (known as BCMA)-directed CAR T cell therapy. It helped explain the mystery of homozygous deletions on chromosome 16p encompassing the *TNFRSF17* (*BCMA*) gene as the cause of antigen escape in a patient treated with the BCMA CAR T cell product ide-cel in the KarMMa trial.⁵ At the American Society of Hematology (ASH) 2020 meeting, other groups confirmed biallelic events (del/del and del/mut) as mechanisms of antigen escape after CAR T cell therapy.^{6,7} Notably, biallelic inactivation usually requires 2 independent hits, and heterozygous *TNFRSF17* deletions have been described in BCMA-naïve patients^{8,9} at risk for a second hit. Thus, the pressure of an anti-BCMA-directed immunotherapy could lead to irreversible antigen loss and subsequent treatment failure.

Here, we report the first case of a biallelic *BCMA* gene deletion after BsAb therapy, highlighting that this genomic mechanism of resistance is relevant in both types of T cell treatment: CAR T cell therapy and BsAb therapy. Furthermore, by using whole-genome sequencing (WGS) data, we provide evidence that genomic alterations in genes encoding immunotherapy targets preexist in a relevant proportion of patients before immunotherapy, including heterozygous deletions, which may potentially serve as a predictive biomarker in future trials.

Methods

Patients

We report the case of a 56-year-old patient treated with a CD3xBCMA BsAb who was subject to WGS in compliance with the CARE guidelines. Furthermore, we report on WGS data derived from 100 patients with MM (50 newly diagnosed [NDMM]; 50 relapsed/refractory [RRMM]; supplemental Table 2). All patients gave their written informed consent for scientific evaluations. The study was approved by the Internal Review Board at the University of Würzburg (reference KFO216) and adhered to the tenets of the Declaration of Helsinki.

WGS and RNA-seq

WGS and bulk RNA sequencing (RNA-seq) were performed as previously described.¹⁰ Briefly, WGS libraries were prepared from 1 µg DNA from CD138 purified cells with the TruSeq polymerase chain reaction free library prep kit, and 2 × 150-bp paired-end sequences were generated on a NovaSeq 6000 instrument with a median coverage of 106×. For bulk RNA-seq, 250 ng of total RNA per sample was used to produce stranded RNA libraries (TruSeq Total Stranded RNA, Illumina). Altogether, 2 × 100-bp paired-end reads were sequenced on the NovaSeq 6000 with a median of 64 million reads per sample (bioinformatic approach is provided in the supplemental Data).

Immunohistochemistry

BCMA protein expression was determined by immunohistochemistry using a polyclonal goat anti-BCMA antibody (dilution 1:10; target

retrieval pH 6.1) (AF193, R&D Systems) on paraffin-embedded bone marrow sections according to standard procedures.

Results and discussion

We detected a clonal biallelic deletion at 16p13.13 encompassing the *BCMA* gene in a 56-year-old patient with penta-refractory MM who was receiving daratumumab, pomalidomide, and dexamethasone in his eighth line of therapy. WGS showed a hypodiploid karyotype with monosomy 16 and a 586-kb homozygous deletion involving *BCMA* (Figure 1; supplemental Figure 1; supplemental Table 1). The patient was treated previously on the AMG420 trial, which evaluated a CD3xBCMA bispecific T cell-engager construct at the sixth line of therapy; he achieved a complete response but relapsed after 6 months of treatment. We retrospectively assessed the BCMA status using immunohistochemistry on bio-banked trephine biopsies that showed strong and consistent BCMA expression before the patient participated in the AMG420 trial but loss of BCMA expression at relapse. Notably, BCMA loss was persistent at a subsequent relapse from carfilzomib, lenalidomide, dexamethasone (KRD) salvage therapy 20 months later. Without knowing about BCMA loss at relapse at that time, the anti-BCMA antibody-drug conjugate belantamab mafodotin was administered at the ninth line of therapy, to which the patient was primary refractory (supplemental Figure 2). This is the first case study that shows homozygous *BCMA* gene deletion to be a mechanism of resistance to BCMA-directed BsAb's. In this patient, antigen loss was irreversible, and subsequent BCMA therapy failed, suggesting that targeted immunotherapies, in theory, are an evolutionary bottleneck in the progression of MM. It is still unclear whether the BCMA deletion was a rare preexisting event or whether it developed during anti-BCMA therapy. Highly sensitive single-cell approaches and accounting for spatial heterogeneity will be required to address this question.

To explore the relevance of this resistance mechanism in MM, we screened for genomic alterations in *BCMA* and 20 alternative immunotherapy targets (supplemental Table 2) by using WGS in a group of 100 T cell immunotherapy-naïve patients (supplemental Tables 3 and 4). Indeed, we found heterozygous deletions in *GPRC5D* (15%), *CD38* (10%), *SDC1* (5%), *TNFRSF17* (4%), and *NCAM1* (3%). Overall, heterozygous deletions occurred in 30% of patients, and some patients showed deletions in up to 3 different immunotarget genes. Considering the emerging dual-antigen targeting immunotherapies,^{11,12} it is worth mentioning that 1 patient presented with both heterozygous deletion of *BCMA* and heterozygous deletion of *GPRC5D*. Of note, 15 of 21 targets were encoded on chromosomes involved in hyperdiploid karyotypes or on 1q and were consequently amplified in about half the patients. Single-nucleotide variants (SNVs) in genes encoding immune targets were found at low frequency (Figure 2). With the exception of 3 frameshift mutations in *GPRC5D*, no mutation was nonsense or frameshift, and the functional consequences of gene mutations remain elusive. Nevertheless, we observed 1 biallelic event (del/mut) for *GPRC5D*.

Figure 1. WGS, read coverage track, and immunohistochemistry. (A) Circos plot with copy number variations, structural variations, and single-nucleotide variants based on WGS data at progression after therapy with CD3xBCMA BsAb. Outer track runs clockwise from chromosome 1 to Y. Inner track shows gains >1 Mb in blue and losses >1 Mb in red. Red lines inside the circle represent interchromosomal translocations with variant allele frequency (VAF) >0.1. Genes with mutations (*CRBN*) are depicted in red. (B) Read coverage visualized with Integrative Genomics Viewer illustrates copy number variations in the short arm of chromosome 16 comprising heterozygous deleted regions caused by monosomy 16 and a homozygous deletion of 586 kb from 12.036.001 to 12.622.000 (hg19 reference genome) including *TNFRSF17* (*BCMA*). (C) BCMA protein expression was determined by immunohistochemistry using a polyclonal goat anti-BCMA antibody on paraffin-embedded bone marrow sections. Lambda light chain staining (immunohistochemistry) served as positive control (magnification, x200).

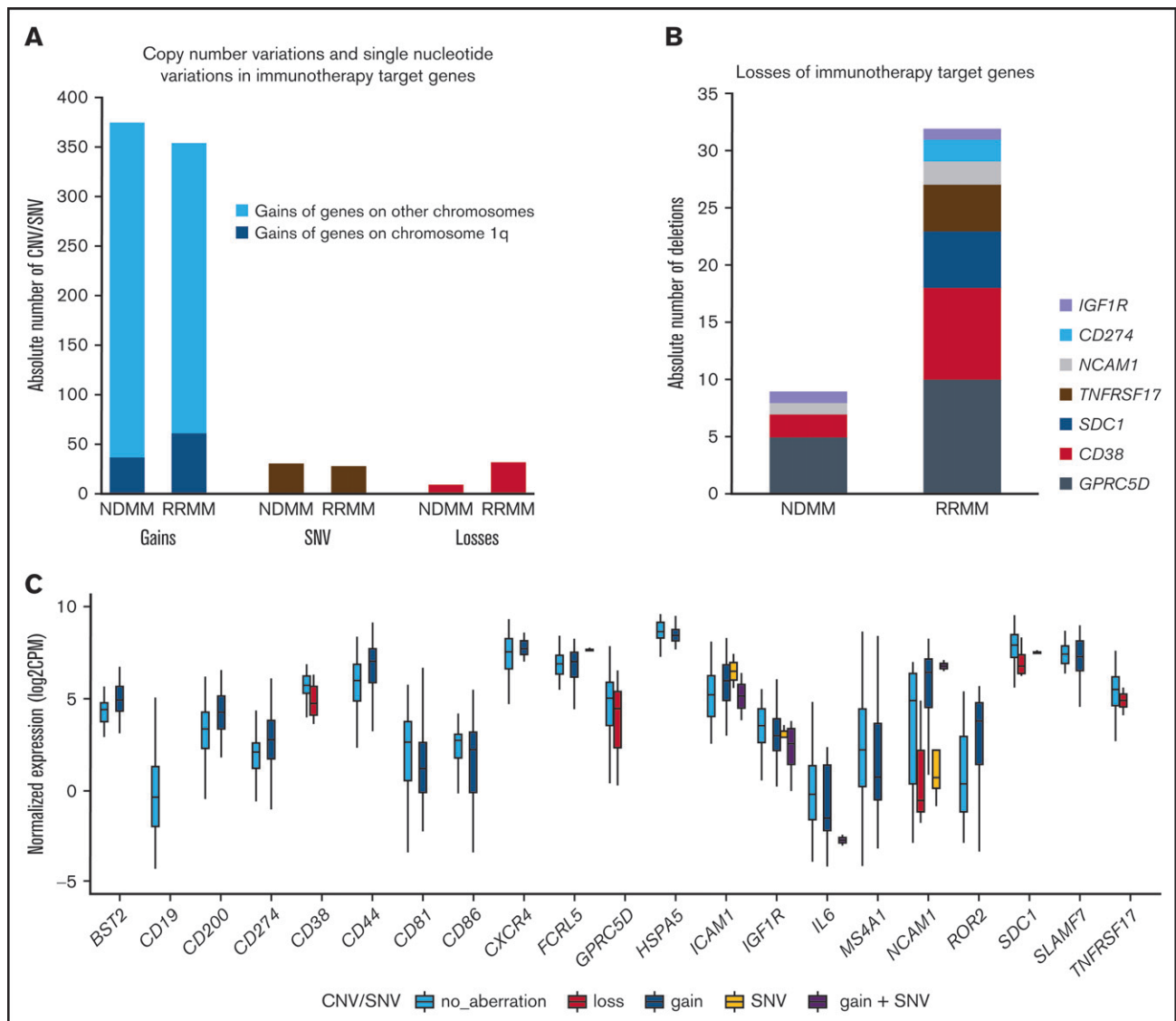


Figure 2. Overview on WGS and expression data for immunotherapy targets. (A) Gains, single-nucleotide variants (SNV), and deletions of 21 immunotherapy targets in 50 NDMM and 50 RRMM patients. (B) Deletions of target genes. (C) Expression of immunotherapy target genes in 44 NDMM and 28 RRMM patients. Box-and-whisker plot shows the minimum, the maximum, the sample median, and the interquartile range between the lower and upper quartiles. Depicted are gains of at least 3 patients and losses, SNVs, as well as copy number variation (CNV) (gains) accompanied by SNVs of at least 2 patients. CPM, counts per million.

The genomic makeup of MM changes from baseline to relapse,⁹ and 1q gain or amplification is more common at relapse (supplemental Figure 3). Consequently, in our cohort, gain of *FCRL5* (19 vs 30 patients; $P = .045$) and *SLAMF7* (19 vs 31 patients; $P = .027$, Fisher's exact test) were significantly enriched in RRMM. Furthermore, as expected, we observed a clear trend for more deletions in pretreated patients (Figure 2) because karyotypes become more complex at later stages of the disease.¹³ This trend was confirmed when comparing our data to the data set from the CoMMpass trial, which included NDMM patients (supplemental Table 5).

In addition to using WGS, we performed transcriptome analysis for 72 of the 100 patients for whom genetic material was available. Strikingly, gains, heterozygous deletions, and SNVs did not result in significant changes in the gene expression of the immunotherapy targets (Figure 2), suggesting that expression of these genes is not

solely regulated by gene copy number; with respect to loss of expression, both alleles have to be dysfunctional. In addition, we found tumor suppressor genes with implications in hematologic malignancies within common deleted regions such as *CDKN1B*¹⁴ and *REX1*¹⁵ on chromosome 12 deleted in 100% and 93% of patients with del(12p), respectively, or *LITAF*¹⁶ deleted in all patients with del(16p); thus, one might speculate whether loss of them may provide a growth advantage to the affected clones.

The current treatment landscape of MM is rapidly changing, and we expect therapy to move toward the use of T cell–based immunotherapies upfront. Our study supports the use of immunotherapies earlier in the disease process, because the frequency of deletions and mutations in genes encoding immunotherapy targets was lower in patients with NDMM vs those with RRMM. Our early data suggest that biallelic *BCMA* gene deletion is a key immune evasion mechanism for *BCMA*-

targeting T cell therapies. It also argues for *BCMA* expression testing before BCMA therapy, especially in patients pretreated with anti-BCMA. Similarly, this mechanism of resistance to T cell–based therapies will affect alternative antigens, and we detected genomic vulnerability in their encoding genes in a significant number of treatment-naïve patients. Combination of different immunotargets (eg, multispecific CAR T cells) might be a promising approach for overcoming drug resistance caused by loss of a single antigen.^{11,17}

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Authorship

Contribution: M.S. Truger, L.R., and K.M.K. conceived of and designed the study; J.D., X.Z., L.H., A. Ruckdeschel, S.H., L.H., J.P., M.S. Topp, A. Riedel, L.R., K.M.K., and H.E. provided study material or patients; W.W., M.M., and M.S. Truger analyzed bioinformatic and statistical data; L.R., M.S. Truger, K.M.K., J.D., X.Z., A. Ruckdeschel, M.J., A. Riedel, H.E., C.H., and N.W. interpreted data; M.C.D.V. and N.B. analyzed additional data sets; L.R., M.S. Truger, and K.M.K. wrote the paper; and all authors reviewed and approved the paper.

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