HHS Public Access

Author manuscript

N Engl J Med. Author manuscript; available in PMC 2024 December 20.

Published in final edited form as:

N Engl J Med. 2023 October 12; 389(15): 1435–1437. doi:10.1056/NEJMc2308544.

Genetic Basis of Relapse after GPRC5D-Targeted CAR T Cells

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TO THE EDITOR:

G protein–coupled receptor, class C, group 5, member D (GPRC5D)–targeted chimeric antigen receptor (CAR) T-cell therapy was recently shown to be safe and active in patients with relapsed or refractory multiple myeloma with limited other therapeutic options.[1] Of the 17 patients treated in our phase 1 study of the GPRC5D-targeted CAR T-cell therapy MCARH109, 12 patients had an objective response and 6 patients had a relapse after an initial response of 3 to 9 months. Although no validated clinical assays to assess GPRC5D expression exist, we reported decreased or loss of protein expression using immunohistochemical analysis in all 6 patients who had a relapse.[1] This finding suggests that genetic alterations may be an important mechanism of antigen escape after GPRC5D-directed CAR T-cell therapy. With the recently approved B-cell maturation antigen–directed CAR T-cell therapies, antigen loss due to genetic alterations appears to be a rare mechanism of resistance.[2,3]

We first investigated the genetic status of *GPRC5D* in a patient (Patient 15) who underwent biopsies of a plasmacytoma in the left posterior chest wall at baseline and a recurrent plasmacytoma at the same site at relapse with nearly 100% tumor. This patient received the maximum tolerated dose of 150×10^6 GPRC5D-directed CAR T cells and had a best response of stringent complete response without minimal residual disease in the bone marrow. Unfortunately, 6 months after infusion, he had a relapse in the chest and femur (Fig. 1A and 1B), extensive bone marrow involvement (90 to 95%), rapidly rising lambda free light-chain levels in the serum, and substantially decreased but still detectable circulating CAR T cells (CAR vector copies per milliliter, 666) (Fig. 1C). We performed a droplet digital polymerase-chain-reaction (PCR) assay on extracted DNA from the plasmacytoma biopsy samples and estimated the GPRC5D copy number to be 2 at baseline and 0.1 at relapse, findings consistent with biallelic loss in a substantial majority of plasma cells (Fig. 1D). We then performed whole-exome sequencing, which revealed two balanced copies of *GPRC5D* at baseline and biallelic loss at relapse consisting of a broader deletion (8.76 Mb) in chromosome 12p in one allele and a more focal deletion (145 kb) encompassing

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

the *GPRC5D* locus in the second allele (Fig. 1E and Fig. S1 and Tables S1 and S2 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Subclonal genetic alterations in *GPRC5D* were not seen at baseline. The bone marrow biopsy sample was not sequenced at relapse; however, immunohistochemical analysis confirmed the loss of GPRC5D in the postprogression bone marrow.

Beyond this patient, we identified that relapses after GPRC5D-directed CAR T-cell therapy were associated with decreased or no expression of GPRC5D messenger RNA (mRNA) (Fig. 1G and Fig. S2 and Table S1). All the patients who had a relapse showed reduced or absent GPRC5D mRNA and protein expression at the time of disease progression (Fig. 1G). Given that two patients who had a response and a relapse had low or no GPRC5D expression at baseline (Patients 10 and 13), further studies are warranted to characterize the heterogeneity of GPRC5D expression and how well immunohistochemical analysis and droplet digital PCR assay capture its expression.

Here, we report that antigen escape after GPRC5D-directed CAR T-cell therapy can be mediated by structural chromosome alterations resulting in biallelic loss of *GPRC5D* in a patient with an initial excellent response. We also noted transcriptional down-regulation of GPRC5D in other patients at relapse, but whether this is transient or durable remains to be determined. Our patients with heavily pretreated multiple myeloma may harbor greater tumor heterogeneity and genomic instability than previously described,[4,5] which can facilitate clonal outgrowth of antigen-negative tumor cells under continuous selective pressure of GPRC5D-targeted CAR T cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by Juno Therapeutics/Bristol Myers Squibb. All the authors employed by Memorial Sloan Kettering Cancer Center (MSKCC) were supported by a National Cancer Institute Core Grant (P30 CA008748). Dr. Mi is supported by the American Society of Clinical Oncology Conquer Cancer Young Investigator Award, the American Society of Hematology Research Training Award for Fellows, Burroughs Welcome Weill Cornell Physician Scientist Program, and the T32 Investigational Cancer Therapeutics Training Program at MSKCC. Dr. Abdel-Wahab is supported by the Paula and Rodger Riney Fund as well as the Sawiris Family Fund. In addition, MSKCC has institutional financial interests relative to Juno Therapeutics/Bristol Myers Squibb.

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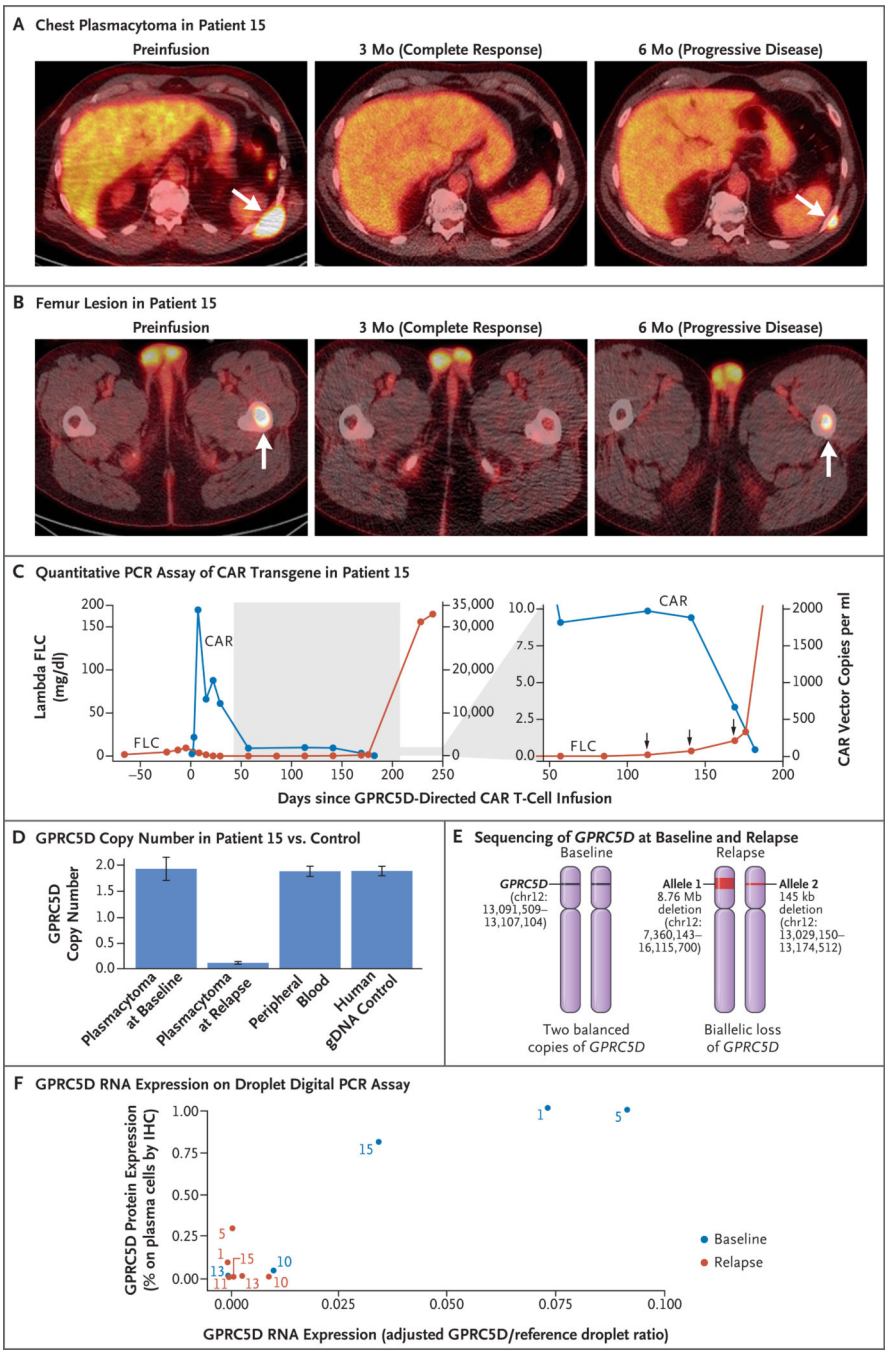

Figure 1. Mediation of Antigen Loss after GPRC5D-Directed CAR T-Cell Therapy by Biallelic Deletion of *GPRC5D* and Transcriptional Down-Regulation.

In Panel A, an ^{18}F-fluorodeoxyglucose (FDG)–avid plas-macytoma in the left posterior chest wall that was detected in Patient 15 at pretreatment baseline was undetectable at month 3 when he had a complete response; the plasmacytoma reappeared at month 6 when he had progressive disease. In Panel B, an FDG-avid osseous lesion in the left proximal femur that was detected in Patient 15 at pretreatment baseline was undetectable at month 3 when he had a complete response; the lesion reappeared at month 6 when he had progressive disease. Panel C shows quantification of serum lambda free light chain (FLC) (red) and G

protein–coupled receptor, class C, group 5, member D (GPRC5D)–directed chimeric antigen receptor (CAR) T cells by quantitative polymerase-chain-reaction (PCR) assay of the CAR transgene (blue) in Patient 15. At right is an expanded view focusing on the time period leading up to relapse. The arrows indicate when FLC levels started to rise while CAR T cells were still detected by quantitative PCR assay. Panel D shows the estimated GPRC5D copy number in the chest-wall plasmacytoma that was biopsied at baseline and relapse in Patient 15. A droplet digital PCR assay was performed on extracted DNA, and the GPRC5D copy number was estimated by comparing the droplet counts of GPRC5D with droplet counts of the reference gene (*EIF2C1*), for which 2 copies per cell are expected. The patient's peripheral blood and a human genomic DNA (gDNA) standard (Promega 6304A) served as controls. In Panel E, whole-exome sequencing reveals two balanced copies of GPRC5D in the chest-wall plasmacytoma at baseline and acquired biallelic loss of *GPRC5D* in the recurrent chest-wall plasmacytoma at relapse, resulting from a broader and a more focal deletion in chromosome 12p. The red bands show the deleted regions proportional to the chromosome. The plasmacytoma biopsy samples at baseline and relapse were sequenced at a depth of 250 times, and the peripheral blood mononuclear cells were sequenced at a depth of 150 times. In Panel F, GPRC5D RNA expression measured by droplet digital PCR assay correlates with GPRC5D protein expression measured by immunohistochemical (IHC) analysis. A droplet digital PCR assay was performed on extracted RNA, and the ratio of droplet counts of *GPRC5D* to droplet counts of the reference gene (*B2M*) was measured and adjusted on the basis of the tumor fraction reported on pathological analysis. All relapse samples have decreased or absent GPRC5D expression.