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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Functional Characterization of *GPRC5D* alteration and Its Impact on Talquetamab Resistance in Relapsed/Refractory Multiple Myeloma

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Background

T-cell engaging (TCE) antibodies show unprecedented efficacy in heavily pretreated penta-refractory multiple myeloma (MM). Responses however are often not durable as resistance mechanisms can arise despite the initial absence of resistance-conferring mutations. Biallelic loss of *GPRC5D* on chr.12p is a rare, yet well-established mechanism of acquired resistance to the first-in-class anti-GPRC5D directed TCE talquetamab (Lee et al., 2022). At the same time, monoallelic *GPRC5D* frameshift and missense mutations are much more common and occur in up to 15% of MM patients (Truger et al., 2021). In this study, we investigated the functional relevance of monoallelic *GPRC5D* alterations and their impact on talquetamab resistance.

Methods

GPRC5D alterations were modeled *in vitro* by using CRISPR-Cas9 editing in the OPM-2 MM cell line. The bioluminescence-based cytotoxic assay was performed using PBMCs as effector cells to measure the resistance to talquetamab. Quantitative PCR (qPCR), immunohistochemistry, bulk whole genome sequencing (WGS), and whole genome bisulfite sequencing (WGBS) were performed for molecular, genomic, and epigenetic characterization in a patient with acquired talquetamab resistance.

Results

Using CRISPR-Cas9, we generated cell line models with mono- vs. biallelic deletions in the *GPRC5D* gene. Clones with biallelic alterations (OPM-2 $^{Del/Del}$) displayed a 96.4%, reduced expression of *GPRC5D* ($p < .0001$), leading to *in vitro* resistance to talquetamab. *GPRC5D* expression levels in monoallelic models (OPM-2 $^{Wt/Del}$) decreased by 57.4% ($p = .0004$) compared to the wild type. Sensitivity to talquetamab in these clones was equally impaired, however, less pronounced than in the biallelic knock-outs. To assess the impact of monoallelic *GPRC5D* deletions in the clinic, we next investigated the case of a heavily pretreated 58-year-old patient with lambda light-chain MM treated at our institution with talquetamab in his 7th line of therapy. qPCR was used to measure *GPRC5D* expression in CD138+ MM cells from the bone marrow of this patient before and at the time of relapse to talquetamab after 15 months of treatment (best response: CR). At the time of relapse, we observed a 94.0% ($p < .0001$) decrease in *GPRC5D* expression upon qPCR assessment as compared to the pre-treatment time point which was confirmed by immunohistochemistry. WGS revealed a novel monoallelic loss of chr.12p encompassing *GPRC5D* in talquetamab-resistant CD138+ MM cells. Since monoallelic alterations seem unlikely as the sole drivers of resistance, we hypothesized that epigenetic modifications may have contributed to talquetamab resistance in this patient. To this end, WGBS was performed and uncovered a significant shift towards hypermethylation of two regulatory regions of *GPRC5D*, most likely resulting in the silencing of the second *GPRC5D* allele.

Time-course experiments are currently ongoing to explore the methylation landscape of our CRISPR-Cas9-generated clones with monoallelic *GPRC5D* deletions. Data for these validation experiments will be presented at the meeting.

Conclusions

Our study provides the first evidence of the functional impact of monoallelic *GPRC5D* alterations resulting in relative resistance to talquetamab *in vitro*. We further report on epigenetic regulation as a potential second-hit mechanism conferring clinical resistance to anti-GPRC5D-directed TCE therapy. This data suggests that patients with monoallelic *GPRC5D* deletions may be at particular risk for developing resistance to talquetamab.

Disclosures Dorado: *Altum Sequencing Co.:* Current Employment. **Haferlach:** *MLL Munich Leukemia Laboratory:* Current Employment, Other: Equity Ownership. **Einsele:** *Sanofi:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *GlaxoSmithKline:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Janssen:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Takeda:* Honoraria, Other: Consulting or advisory role, Travel support; *Amgen:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding; *Novartis:* Honoraria, Other: Consulting or advisory role, Travel support; *Bristol Myers Squibb/Celgene:* Honoraria, Other: Consulting or advisory role, Travel support, Research Funding. **Rasche:** *Sanofi:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria; *BMS:* Consultancy, Honoraria, Research Funding; *Amgen:* Consultancy; *GSK:* Consultancy, Honoraria; *Skyline Dx:* Research Funding; *Roche:* Honoraria. **Waldschmidt:** *Sanofi:* Consultancy; *Takeda:* Consultancy; *Janssen:* Consultancy; *Pfizer:* Consultancy; *Oncopeptides:* Consultancy. **Kortüm:** *GSK:* Honoraria; *BMS:* Honoraria; *Takeda:* Honoraria; *Pfizer:* Honoraria; *Janssen:* Honoraria; *Abbvie:* Honoraria.

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