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The 12th World Congress on CONTROVERSIES IN MULTIPLE MYELOMA (COMy)

Development of *in vitro* model system for functional asessment of immunotherapy efficacy in Multiple Myeloma

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INTRODUCTION

Multiple myeloma (MM) is a chronic hematologic malignancy in which patients undergo sequential regimens involving multiple lines of therapy[1]. Teclistamab, a recently approved bispecific antibody targeting B-cell maturation antigen (BCMA) on MM cells and CD3 on T cells, redirects T-cell-mediated cytotoxicity and has demonstrated clinical efficiency[2]. Nevertheless, therapeutic resistance frequently emerges, posing a significant challenge. Thus, elucidating the mechanisms underlying resistance is essential for optimizing immunotherapy efficacy and improving patient outcomes.

THE AIM

This study aims to establish an *in vitro* platform combining co-culture assays and CRISPR-based perturbations to functionally investigate resistance to immunotherapy in MM. It is intended to support systematic studies of resistance pathways and provide a versatile tool to explore immunotherapy in MM.

METHODS

Co-cultures of target and effector cells were established using MM cell lines (OPM-2 and AMO-1) as target cells, and Jurkat cells engineered to express CD8, or primary T cells isolated from peripheral blood of healthy donors as effector cells. Multi-parametric flow cytometry was performed to characterize cell phenotypes and quantify basal BCMA expression. Teclistamab dose-response studies (0.1-10 nM) were conducted to optimize culture conditions and identify the parameters required for a robust T-cell-mediated cytotoxicity. In parallel, nucleofection was optimized for the efficient delivery of CRISPR-Cas9 constructs to enable CRISPR-mediated gene perturbations.

RESULTS

Selective sensitivity of OPM-2 to teclistamab treatment

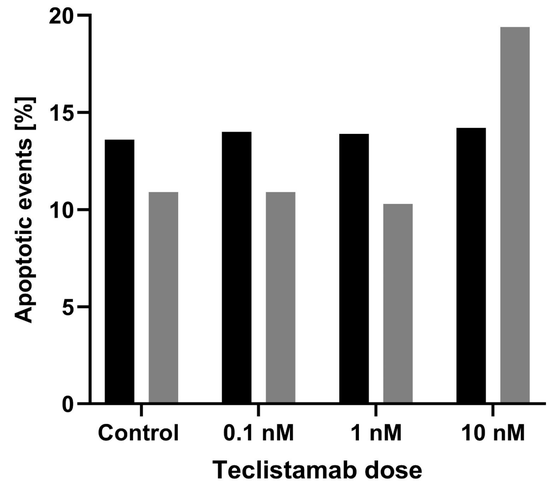

Figure 1. Preliminary dose-response analysis of teclistamab-induced apoptosis after 24h. AMO-1 and OPM-2 multiple myeloma cell lines were co-cultured with Jurkat CD8⁺ T cells and treated with increasing concentrations of teclistamab (0.1-10 nM). Both cell lines exhibit a comparable phenotype with consistent BCMA expression. Apoptotic events were quantified by flow cytometry.

Primary T cells exhibit two-fold higher cytotoxicity than Jurkat CD8⁺ cell line

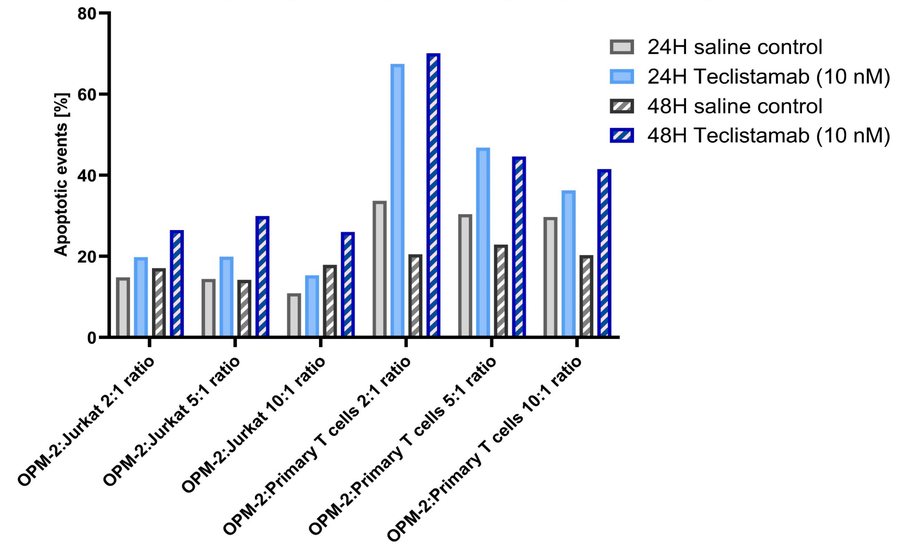

Figure 2. Comparison of the response to treatment with teclistamab of OPM-2 cells. In this experiment, the following were compared: cytotoxicity of different effector cells (Jurkat CD8⁺ and primary T cells), different incubation times, and different effector-to-target ratios.

High efficiency CRISPR-Cas9 delivery in OPM-2

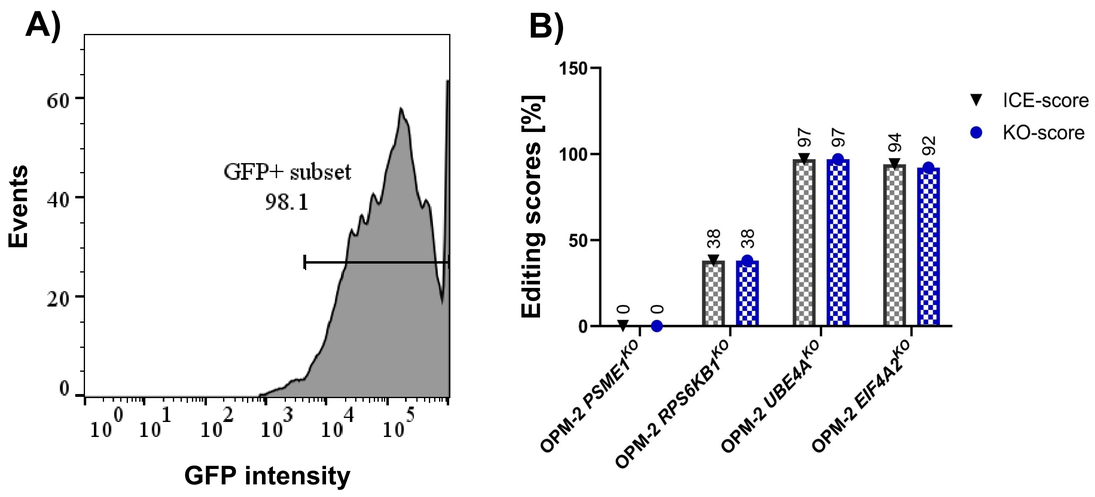

Figure 3. A) Representative flow cytometry histogram depicting CRISPR-Cas9 delivery in OPM-2. The nucleofection efficiency was determined based on eGFP-tagged Cas9 system. **B)** Genomic validation of CRISPR-Cas9 editing efficiency in OPM-2 across multiple target genes. The ICE score indicates the total percentage of the population with successful indels at the target locus, while the KO score represents the proportion of cells with functional gene disruption.

Optimized culture conditions

Table 1. Summary of optimized conditions for teclistamab-mediated cytotoxicity assays. Model was systematically refined by testing teclistamab different doses, incubation times, and effector-to-target cells ratios.

Parameter	Optimized value	Impact on data quality
Teclistamab concentration	10 nM	Max effect ~20% induced apoptosis
Incubation time	48 hours	A more potent cytotoxic effect
Target cells	OPM-2 cell line	More sensitive than AMO-1
Effector cells	Primary T cells isolated from peripheral blood	Superior cytotoxic potential
Effector-to-target cells ratio	2:1 for primary T cells and 5:1 for Jurkat CD8⁺	A more potent cytotoxic effect

CONCLUSION

We have established a versatile *in vitro* platform for studying T-cell-mediated cytotoxicity and dose-response in MM. CRISPR-Cas9 delivery for gene perturbations was optimized in OPM-2 cells, achieving >95% transfection efficiency and gene knockout efficiencies ranging from 30–95% depending on the target gene. This system provides an experimental framework for investigating the molecular mechanisms underlying T-cell-mediated killing and therapeutic response in MM, while providing a foundation for future drug target discovery.

REFERENCES

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2. Pillarisetti, K. et al. Teclistamab is an active T cell–redirecting bispecific antibody against B-cell maturation antigen for multiple myeloma. Blood Adv. 4, 4538–4549 (2020).

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