1108 NOVEL REGULATORS OF IMMTAC-MEDIATED KILLING OF MELANOMA CANCER CELLS REVEALED BY GENOME-WIDE CRISPR-CAS9 SCREENS

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Background ImmTAC molecules are TCR-anti-CD3 bispecific fusion proteins that can redirect polyclonal T cell activation against tumor cells. Tebentafusp, a gp100-directed ImmTAC, is the first TCR therapeutic to demonstrate survival benefit and is approved for the treatment of HLA-A*02:01+ adults with unresectable or metastatic uveal melanoma (mUM).[1] Here, we conducted a pooled whole-genome CRISPR/Cas9 knockout screen to identify proteins/pathways that confer resistance to killing by ImmTAC-redirected T cells *in vitro*.

Methods A cutaneous melanoma cell line (Mel624) was transduced with a whole-genome CRISPR-Cas9 GeCKOv2 library consisting of 123,411 unique gRNA[2] to generate single gene knock-out (KO) tumor cells. These were co-cultured with primary PBMC in the presence or absence of ImmTAC. DNA was collected, subjected to next-generation sequencing, and analysed using MAGeCKFlute pipeline to identify genes and pathways that were enriched following ImmTAC treatment. Baseline (N=70) and matched on-treatment tumor biopsies (at 16 days post treatment [N=35] and at progression [N=14]) from a Phase 2 trial of tebentafusp in HLA-A*02:01+ patients with previously treated mUM (NCT02570308) were used to measure gene expression by bulk RNAseq.

Results Analysis of the genome-wide screen hits revealed 4 major pathways that impact cancer cell resistance to ImmTAC-redirected T cell killing: antigen presentation machinery (APM), IFNγ signalling, cell adhesion and mitochondrial respiration. In addition to classical APM genes (HLA-A, B2M and TAP1/2), endoplasmic reticulum aminopeptidase (ERAP1) appeared critical for ImmTAC-mediated cytolysis, as tumor cells lacking ERAP1 (ERAP1 KO) exhibited resistance to cytolysis. Moreover, KO of IFNGR2 and STAT1 (positive regulators of IFNγ signalling) conferred ImmTAC resistance, whereas KO of PTPN2, a negative regulator of IFNγ, sensitised cells to ImmTAC-mediated cytolysis. Similarly, KO of cell adhesion molecules, ICAM1 and CD58, as well as several mitochondrial ETC Complex I genes, including NDUFB10 demonstrated ImmTAC resistance *in vitro*. To assess the relevance of these findings *in vivo*, we examined tumor biopsies from tebentafusp-treated patients collected at clinically confirmed progression. Patients exhibiting below-median tumor expression of ERAP1, IFNGR2, ICAM1 and NDUFB10 at progression had significantly shorter overall survival (Hazard ratio = 0.13, 0.06, 0.26, and 0.30, respectively).

Conclusions The first genome-wide CRISPR screen of tumor cells using a TCR-CD3 bispecific reveals key genes and pathways involved in tumor resistance and sensitivity to ImmTAC-redirected T cell mediated tumor elimination. These screens provide a valuable resource of druggable targets for combination therapies and development of next generation ImmTAC molecules.

REFERENCES

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Ethics Approval Institutional review board approval was obtained and all participants gave informed prior consent prior to enrolment.

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