Abstract 87 Figure 3  RUNX1-mediated HNSCC cell metastasis in MAPK pathway via stimulating OPN. (A) The migration ability of FaDu cells transfected as above were assessed by wound-healing assay. Representative images were obtained at 0h and 24h (magnification 40x). (B) The migration and invasion ability detected by transwell assays. Representative images of FaDu cells from migration and invasion assays experiment were obtained at 24h (magnification 12x). (C) Immunoblotting analysis for protein markers expression levels of the MAPK pathway in FaDu cells transfected as above. (D) The graph of tumor growth/volumes curve at the indicated time intervals (left). Tumor weights were quantified at the end of the experiment (right). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Results  In our study, RUNX1 expression was increased with disease progression in patients with HNSCC (figure 1). The silencing of RUNX1 significantly decelerated the malignant progression of HNSCC cells, reduced Osteopontin (OPN) expression in vitro, and weakened the tumorigenicity of HNSCC cells in vivo (figure 2). Moreover, we demonstrated that RUNX1 activated the MAPK signaling by directly binding to the promoter of OPN in tumor progression and metastasis of HNSCC (figure 3).

Conclusions  Our results may provide new insight into the mechanisms underlying the role of RUNX1 in tumor progression and metastasis and reveal the potential therapeutic target in HNSCC.

Ethics Approval  The study was approved by the Ethics Board of BenQ Medical Center, the Affiliated BenQ Hospital of Nanjing Medical University.

Consent  Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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88 DEVELOPMENT OF A 3D ORGANOID AUTOLOGOUS TIL CO-CULTURE PLATFORM FOR HIGH THROUGHPUT IMMUNO-ONCLOGY STUDIES

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Background  The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitulate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogeneous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations.

3D organoid cultures typically contain neoplastic epithelium; however, they fall short in representing tumor to tumor-infiltrating lymphocytes (TILs) interactions, limiting their ability to generate a clinically relevant response to immunotherapeutics. Addition of immune cells from unrelated donors to organoids can simulate that microenvironment but is complicated by T cell alloreactivity. Here we describe 3D patient-derived xenograft organoid (PDXO) co-cultures with matching autologous human TILs to recapitulate the tumor-specific immune response, leveraging confocal high content analysis and luminescent multiplex assays. This platform allows the evaluation and high throughput screening of novel immune targeting agents to determine impacts on patient-derived T cell function, T cell infiltration, and tumor cytotoxicity.

Methods  Surgical resections from patients were used to generate patient-derived xenografts and tumor-infiltrating lymphocytes in parallel. PDX were resected and digested to establish PDXO. TILs and organoids from the same patient were fluorescently labeled and cultured together for four days to evaluate tumor infiltration and drug cytotoxicity in 3D cultures. CellIn-sight CX7 high content imaging platform was used to track TILs and cancer cells and evaluate T cell infiltration and tumor cell killing in the presence and absence of immunomodulatory therapies.

Results  PDXO were established to mimic in vivo tumor biology. Tumor-specific TILs were successfully expanded and characterized by flow cytometry. Co-culture resulted in TIL infiltration in organoids from day one in culture and increased over four days. Cytotoxicity and TIL infiltration were quantified using fluorescent dyes via high throughput imaging platform. Significantly enhanced TIL infiltration was observed in autologous co-cultures compared to non-autologous co-cultures. The established unique autologous PDXO immune organoid co-cultures could be used as an improved simulation of the modular activity of therapeutic agents in patient-specific T cells against their own tumors.

Conclusions  Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating IO therapeutics with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

Ethics Approval  The study was approved by Champions Oncology’s Institutional Animal Care and Use Committee (IACUC).

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Cellular therapies

89 FIRST-IN-HUMAN CAR T TARGETS CLEAVED MUC1, THE GROWTH FACTOR RECEPTOR FORM

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Background  Minerva Biotechnologies has opened a Phase I 1st-in-human CAR T clinical trial, NCT-04020575, for metastatic breast cancers at the Fred Hutchinson Cancer Research Center. HuMNC2-CAR44 targets the truncated extra cellular domain of MUC1* (muk 1 star), which is the transmembrane cleavage product that remains after MUC1 is cleaved and the

Background T-cell based immunotherapies such as CAR-T, bispecific mAb, transgenic T cells and checkpoint blockade have profound efficacy in multiple tumor types but share a common limitation – target antigen (Ag) escape. One approach to address this limitation has been therapy directed at a ‘parallel’ target (e.g. CD22 after CD19 loss), however, these lineage markers are frequently lost together. Here, we describe an alternate, broadly applicable, approach: potentiating fasL/fas-signaling to increase localized bystander killing of Ag tumor cells and thereby prevent Ag escape.

Methods We used a CRISPR/Cas9 library to screen for tumor expressed molecules that inhibit or facilitate T-cell killing. We then evaluated one candidate – fas– using murine transgenic T cells, murine and human CAR-T cells, bispecific mAb redirected PBMC, and tumoral RNAseq data from a large CAR-T clinical trial.

Results GFP-specific (JEDI) CD8 T cells were co-cultured with on-target (GFP+) and bystander (mCherry+) lymphoma cells that had been transfected with a CRISPR/Cas9 library; this screen revealed several tumor-expressed candidate molecules inhibiting or facilitating T-cell killing. Notably, we observed a marked dependence on fas for on-target tumor killing and then, surprisingly, an exquisite dependence on fas for localized bystander tumor killing. (figure 1). Because bystander tumor killing appeared critically fas-dependent, we hypothesized that potentiating fas-signaling might increase bystander killing. An in vitro screen of small molecules that modulate fas-pathway revealed several candidates, including inhibitors of histone deacetylases (HDAC), inhibitors of apoptosis proteins (IAP) and Bcl-2 family members in murine and human systems (figure 2). To validate these candidates, we demonstrated that HDAC increased GFP-specific T cell killing of both on-target and bystander lymphoma cells, in a completely fas-dependent manner (figure 3). Similarly, using a bispecific antibody-based system, we demonstrated increased, fas-dependent, T cell killing of both on-target and bystander human lymphoma cells with inhibitors of IAP and bcl-2 family members (e.g. MCL1).