Improving engineered T cell efficacy for treating solid tumors through epigenetic modulation.

T-cell therapy has shown remarkable success in treating certain types of blood cancers, but the same effectiveness has not been observed in solid tumors such as breast, liver, or brain cancer. There are several reasons for this lack of success, such as the high heterogeneity and absence of tumor-specific targets in solid cancers, as well as an immunosuppressive tumor microenvironment that can hinder T cell activity.

Researchers are actively addressing these challenges by engineering T cells to transiently express chimeric antigen receptors (CARs) or specific T cell receptors (TCRs) that recognize and target cancer cells with higher specificity. However, multiple infusions are often required, increasing patient discomfort and treatment costs. To minimize this, strategies to improve the anti-tumor activity of transient-engineered T cells are needed.

Researchers from A*STAR's Institute of Molecular and Cell Biology (IMCB) and Singapore Immunology Network (SIgN), in collaboration with Duke-NUS Medical School, set out to enhance the cytotoxicity of engineered T cells against hepatitis B virus-positive (HBV+) hepatocellular carcinoma (HCC).[1] Their primary objective was to introduce small molecule epigenetic inhibitors during *ex vivo* T cell expansion and assess their impact on T cell cytotoxicity. This strategic approach, when implemented in a clinical setting, enables precise modulation of T cell behavior before they are reintroduced into the patient, offering a promising avenue for tailoring immunotherapeutic interventions to individual patient needs.

Enhancing cytotoxicity of TCR-engineered T cells with G9a/GLP inhibition

The study aimed to enhance the anti-tumor activity of engineered transient-expressing TCR+ T cells that had previously been shown to be effective against HBV+ HCC. The team used this model to screen for small molecule inhibitors targeting epigenetic regulators capable of enhancing the cytotoxicity of these engineered T cells. Over a period of 5 days, T cells derived from healthy donors were treated with 24 carefully selected small molecule inhibitors. After treatment, the drugs were withdrawn and the T cells were transfected with mRNA specific to the HBV envelope S183–191 region, resulting in the generation of engineered TCR+ T cells.

To evaluate the impact of these inhibitors, the researchers utilized 2D and 3D cytotoxicity assays. In the 3D cytotoxicity assay, HepG2-preS1 cells were seeded in a collagen matrix within microfluidic channels, and engineered T cells were introduced into one channel, allowing migration into the matrix toward target cells. Using the Opera Phenix® high-content screening system, the researchers quantified the cytotoxicity and migration of the engineered T cells over 24 hours (Figure 1A-C).

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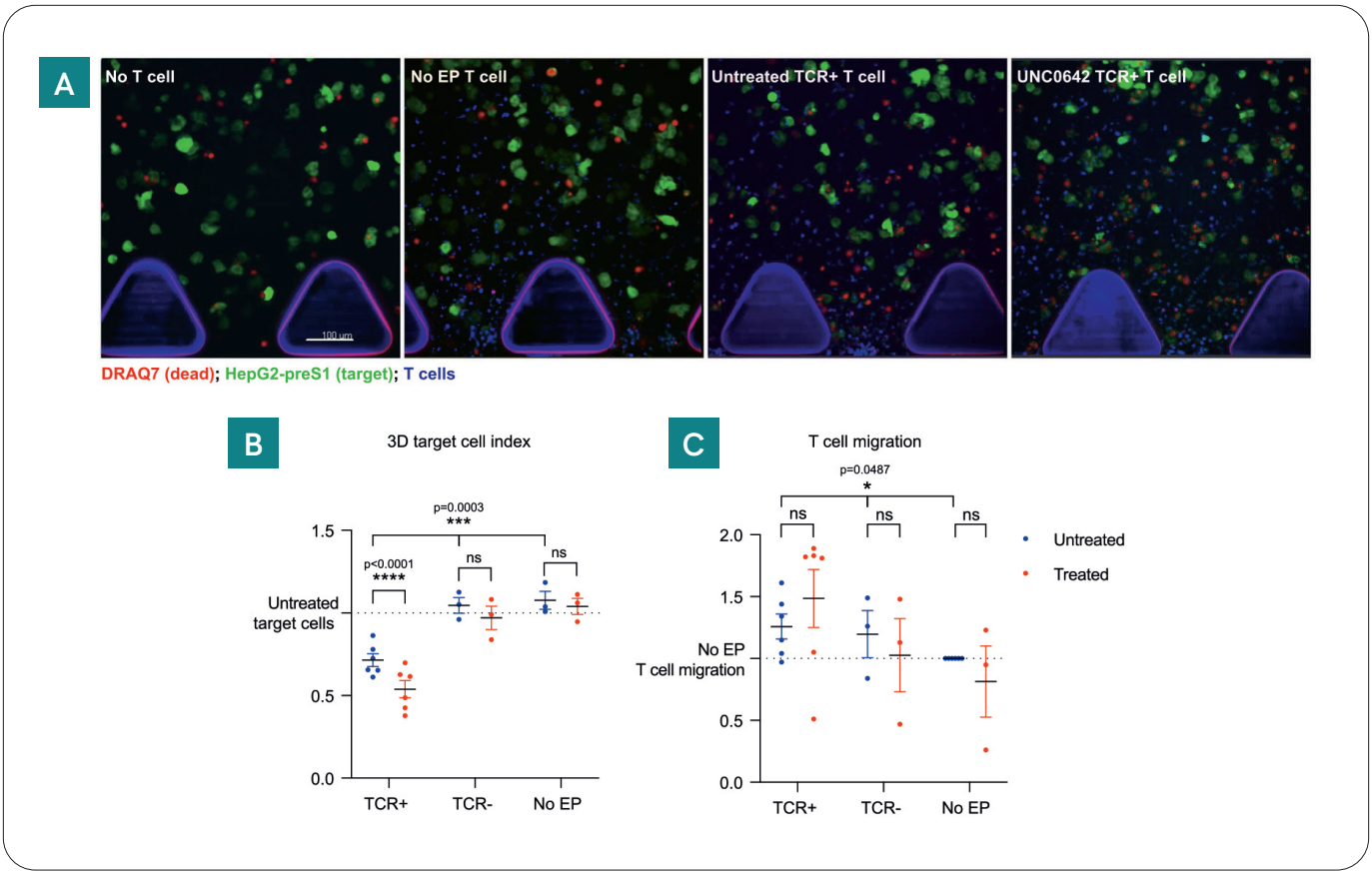

Figure 1: G9a/GLP inhibitors improve the cytotoxicity of TCR-engineered T cells. A) Representative images showing live (green) and dead (red) cells in the 3D cytotoxicity assay, with quantification of B) normalized live target cell count after addition of untreated or UNC0642-treated TCR+ T cells, TCR- mock-electroporated T cells (TCR-) and naïve T cells (no EP electroporation), normalized to no T cell controls and C) number of invading T cells within the 3D matrix, normalized to no EP controls. TCR- and no EP: N = 3 biologically independent donors; TCR+: N = 6 biologically independent donors. Data are analyzed with two-way ANOVA with matching within donors. All data are shown as mean ± SEM. *P<0.05, **P < 0.01, ***P < 0.001, ****P<0.0001, ns not significant. Source data are provided as a Source Data file.

Image source: Lam, M.S.Y., Reales-Calderon, J.A., Ow, J.R. et al. G9a/GLP inhibition during *ex vivo* lymphocyte expansion increases *in vivo* cytotoxicity of engineered T cells against hepatocellular carcinoma. Nat Commun 14, 563 (2023). Image licensed under Creative Commons License 4.0.

The team discovered that several selective inhibitors of G9a/GLP, enzymes regulating histone methylation, showed the most substantial increase in target cell killing. In particular, UNC0642-treated TCR+ T cells showed the most significant rise in cytotoxicity, leading to its selection for subsequent experiments.

As shown in Figure 1B, treatment with UNC0642 increased the cytotoxicity of engineered TCR+ T cells, an effect that was not observed in mock electroporated TCR- T cells or naïve non-electroporated T cells. This finding indicates that the observed increase in target cell killing was linked to the presence of the engineered TCR. Importantly, UNC0642 treatment did not seem to affect the migratory ability of TCR+ T cells, as shown in Figure 1C. This suggests that UNC0642 selectively enhances the intrinsic cytotoxicity of T cells without affecting their migratory capabilities.

Overall, the findings of the cytotoxicity assays suggest that G9a/GLP inhibition during T cell expansion, particularly with UNC0642, boosts the cytotoxicity of engineered TCR+ T cells against target HCC cells without compromising their migratory abilities.

Enhancing engineered T cell efficacy *in vivo*

Given the encouraging *in vitro* data, the team decided to examine the ability of UNC0642 treatment to enhance the cytotoxicity of engineered TCR+ T cells *in vivo*. To simulate HCC, an orthotopic mouse model was established by intrahepatically injecting luciferase-expressing HepG2-2.2.15 target cells into NSG mice. The mice were then intravenously administered either untreated or UNC0642-treated TCR+ or mock electroporated TCR- T cells intravenously on specific days following tumor cell injection (Figure 2A). Tumor burden was then monitored using the bioluminescence *in vivo* imaging system (IVIS®) at 7 and 21 days post-tumor injection (Figure 2B).

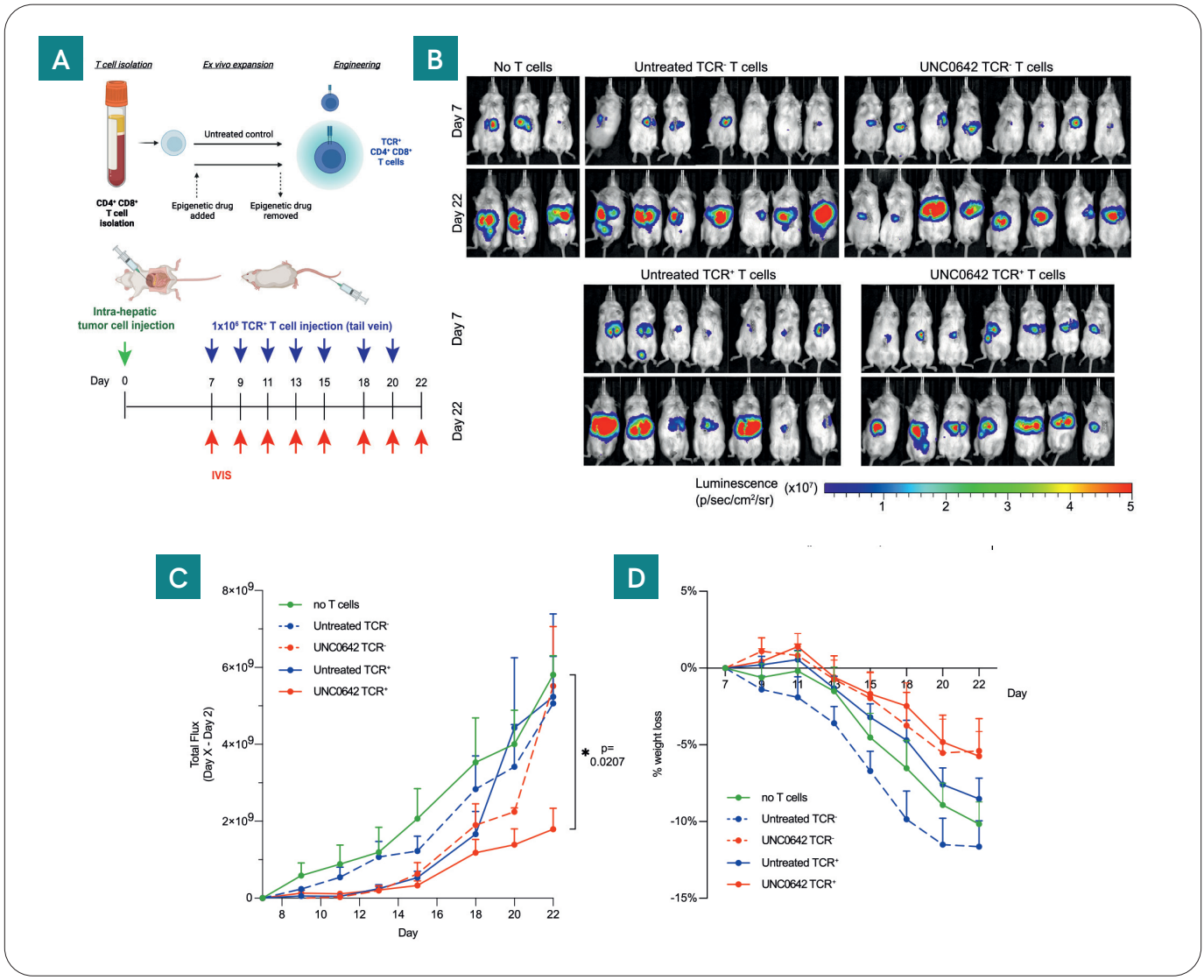

Figure 2: UNC0642 treatment improves the anti-tumor activity *in vivo*. A) Overall design of *in vivo* experiment. 2 M HepG2-2.2.15-luc cells were injected intrahepatically at the start of the experiment. At day 7, mice were allocated into 5 groups, and 1 M of untreated or UNC0642-treated TCR+ or TCR- T cells were injected intravenously. Tumor volume was monitored by IVIS imaging and T cell injections were repeated every 2 days until day 21 post-tumor injection. Created with BioRender.com. B) Images taken using the IVIS on day 7 and on day 21 post-tumor injection. All mice analyzed are shown. C) Tumor volume was tracked using bioluminescence and shown as total photon flux (p/s) relative to day 7 post-tumor injection over time. Data at day 21 are analyzed with one-way ANOVA with multiple comparisons. N = 3 for no T cell control mice; 8 for UNC0642-treated TCR- T cell mice; and 7 for all other conditions. D) Percentage loss of body weight. Data are analyzed with one-way ANOVA with multiple comparisons, N > 3 mice per condition, as shown in B.

Image source: Lam, M.S.Y., Reales-Calderon, J.A., Ow, J.R. et al. G9a/GLP inhibition during ex vivo lymphocyte expansion increases *in vivo* cytotoxicity of engineered T cells against hepatocellular carcinoma. Nat Commun 14, 563 (2023). Image licensed under Creative Commons License 4.0.

As shown in Figure 2C, the UN0642-treated engineered T cells successfully restricted tumor growth throughout the 22-day observation period. Untreated TCR+ T cells managed to impede tumor growth until day 18, with regression observed after day 20, indicating that UNC0642 treatment enhances the ability of the engineered T cells to inhibit tumor progression. An interesting observation was that UNC0642-treated TCR- T cells were also able to restrict tumor growth for the initial 20 days of the study, followed by regression on day 22. This finding is noteworthy as TCR- T cells typically lack specific cytotoxicity against tumors due to the absence of a designed TCR.

In further analysis, mice receiving UNC0642-treated TCR+ or TCR- T cells lost less weight than those receiving untreated TCR+ or TCR- T cells or no T cells (Figure 2D). Histological analysis confirmed the targeted action of injected TCR+ T cells on the liver tumor in both untreated and UNC0642-treated groups. Automated segmentation for CD3+ regions in multiple liver histological sections revealed an increased presence of T cells in the livers of mice receiving UNC0642-treated engineered T cells.

In summary, the *in vivo* data strongly suggest that UNC0642 treatment enhances the anti-tumor activity of engineered TCR+ T cells in a mouse model of HCC. This enhancement is potentially attributed to improved T-cell persistence and heightened cytotoxicity in the liver.

Conclusion

The high heterogeneity, lack of tumor-specific targets, and immunosuppressive tumor microenvironment of solid tumors are all significant hurdles for the development of T cell therapies. Traditional approaches have often fallen short in achieving the same success witnessed in blood cancers, meaning that innovative approaches are needed to translate this success to solid tumors. This study focuses on overcoming these challenges by introducing small molecule inhibitors targeting the G9a/GLP enzymes during *ex vivo* T cell expansion.

The results demonstrate that G9a/GLP inhibition, specifically with UNC0642, significantly improves the cytotoxicity of engineered TCR+ T cells against HBV+ HCC cells. Importantly, this enhancement is observed without compromising the migratory abilities of the T cells, highlighting the precision of this epigenetic modulation. The promising *in vitro* data are further validated in vivo, where UNC0642-treated TCR+ T cells demonstrate superior anti-tumor activity, effectively restricting tumor growth in a mouse model of HCC.

The study not only addresses the challenge of enhancing T cell efficacy against solid tumors but also reveals unexpected outcomes. UNC0642-treated TCR- T cells, which lack specific cytotoxicity, exhibit a notable ability to delay tumor progression. This finding indicates there may be broader implications of G9a/GLP inhibition beyond engineered TCR+ cells.

Overall, the study demonstrates how epigenetic modulation could be a promising avenue for future developments in cancer immunotherapy, offering promise for more effective and targeted T-cell therapies in the complex landscape of solid tumors.

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Reference

1. Lam, M.S.Y., Reales-Calderon, J.A., Ow, J.R. et al. G9a/GLP inhibition during *ex vivo* lymphocyte expansion increases *in vivo* cytotoxicity of engineered T cells against hepatocellular carcinoma. ***Nat Commun*** 14, 563 (2023). https://doi.org/10.1038/s41467-023-36160-5

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