Background Thyroid cancer incidence is rising,1 and most thyroid cancer deaths are attributed to a subset of de-differentiated, treatment-refractory, metastatic tumors. Thyroid stimulating hormone receptor (TSHR),2,3 making TSHR a compelling target for advanced thyroid cancer diagnostics and therapeutics. We therefore developed a novel TSHR-targeted chimeric antigen receptor (CAR) T cell therapy to treat these aggressive thyroid cancers.

Methods TSHR-CAR constructs were synthesized using a single chain variable fragment derived from thyroid auto-antibody clone KI-70 and cloned into a lentiviral CAR construct containing 4-1BB and CD3ζ. We then generated TSHR-CART cells by transducing T cells derived from normal donors. TSHR-CART demonstrated potent antigen-specific in vitro and in vivo antitumor activity. NOD-SCID-γc-/- (NSG) mice were inoculated subcutaneously with a TSHR-overexpressing thyroid cancer cell line, THJ529, and were randomized by tumor volume to treatment with TSHR-CART cells or control untransduced T cells (UTD). Treatment with TSHR-CART cells resulted in dose-dependent antitumor activity and prolonged survival (figure 1).

Results Anaplastic thyroid cancers (ATC) are reported to downregulate TSHR. Our TSHR immunohistochemistry results corroborated these findings and displayed attenuated or no TSHR protein expression, precluding successful TSHR-CART treatment (figure 2). We therefore sought to sensitize these tumors with mitogen-activated protein kinase (MEK) inhibitors, which have been shown to upregulate TSHR expression in patients with metastatic thyroid cancer.4,5 We verified that TSHR expression was upregulated in patient-derived xenograft (PDX) ATC models after one week of daily administration of the MEK inhibitors trametinib and R05126766 (figure 3). After confirming that MEK inhibition does not dampen TSHR-CART effector functions (not shown), we tested combination therapy of TSHR-CART with MEK and BRAF inhibition in vivo. NSG mice were engrafted with ATC BRAF-mutant PDX tumors and were randomized by tumor volume to daily oral treatment with placebo or trametinib (MEK inhibitor) plus dabrafenib (BRAF inhibitor). One week later, mice received either UTD or TSHR-CART. Because MEK/BRAF inhibitors alone inhibit tumor growth, treatment groups receiving placebo were implanted one week later than groups receiving MEK/BRAF inhibitors to achieve similar tumor volumes at the time of TSHR-CART treatment. Mice conditioned with trametinib plus dabrafenib and subsequently treated with TSHR-CART showed superior antitumor activity (figure 4).

Conclusions Collectively, our findings indicate that MEK/BRAF inhibition of de-differentiated thyroid cancers upregulated TSHR expression and enhanced TSHR-CART antitumor activity. This work represents a viable strategy to improve outcomes of patients with aggressive, metastatic thyroid cancers.

REFERENCES

Abstract 324 Figure 1 TSHR-CART cells demonstrate potent antitumor activity in thyroid cancer TSHR+ xenograft models. TSHR-CART cells exhibit dose-dependent antitumor activity (left panel) and prolonged survival (right panel) compared to untransduced (UTD) T cells in mice bearing subcutaneous TSHR+ THJ529 cells (n = 5 mice/group).

Abstract 324 Figure 2 TSHR expression is lost in advanced thyroid cancers. H-score quantitation of TSHR expression is shown. H-scores provided are based on 0 – 3 IHC scoring and percent of total areas each score (0x%0 + 1x%1 + 2x%2 + 3x%3 = H Score; 0 – 300 range with statistical analysis. * p =0.0261, ** p =0.0083, *** p =0.0004, **** p <0.0001, Kruskal-Wallis test.
Abstract 324 Figure 3  MEK inhibitors upregulate TSHR expression in ATC. H-score quantitation of TSHR expression (n=5 mice/group; * p<0.05, ** p <0.01).

Abstract 324 Figure 4  Sequential treatment of MEK/BRAF inhibition followed by TSHR-CART cell therapy demonstrates enhanced antitumor activity. Schema of treatment strategy (left panel). On day 0 or 7, NSG mice were engrafted with 5 mm3 ATC PDX. The day 0-innoculated mice, upon achieving tumor volume of ~100 mm3, were treated daily for 7 days with trametinib (1.5 mg/kg) plus dabrafenib (12.5 mg/kg) orally through ad libidum treated diet gel access to upregulate TSHR. On day 11, mice received 10 x 106 cells of either UTD or CART intravenously. Tumor volume of mice treated with UTD or TSHR-CART +/- trametinib + dabrafenib (right panel).