

# Exploring anti-PD-1 resistance mechanisms for therapeutic targeting in NSCLC

Haoyi Wu<sup>1</sup>, Jessica M. Konen<sup>1,2</sup>, B. Leticia Rodriguez<sup>1</sup>, Yanhua Tian<sup>1</sup>, Susan Tsang<sup>1</sup>, Jared J. Fradette<sup>1</sup>, Laura Gibson<sup>1</sup>, Don L. Gibbons<sup>1</sup>

1. Department of Thoracic/Head & Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030  
2. Department of Hematology & Medical Oncology, Emory University, Atlanta, GA 30322

THE UNIVERSITY OF TEXAS  
MD Anderson  
Cancer Center

UTHealth  
Houston

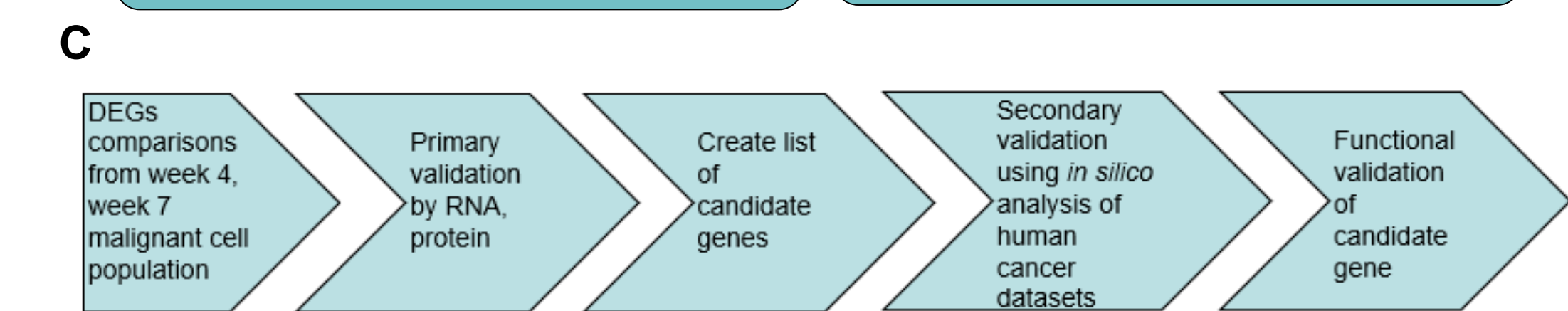
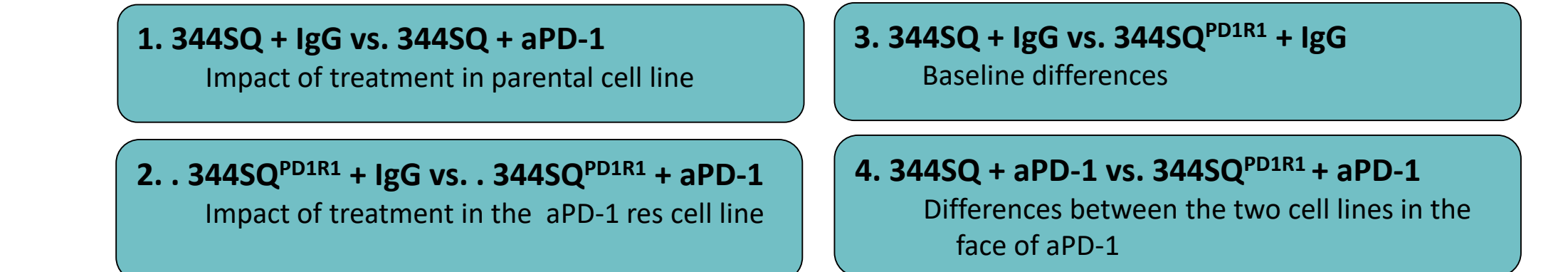
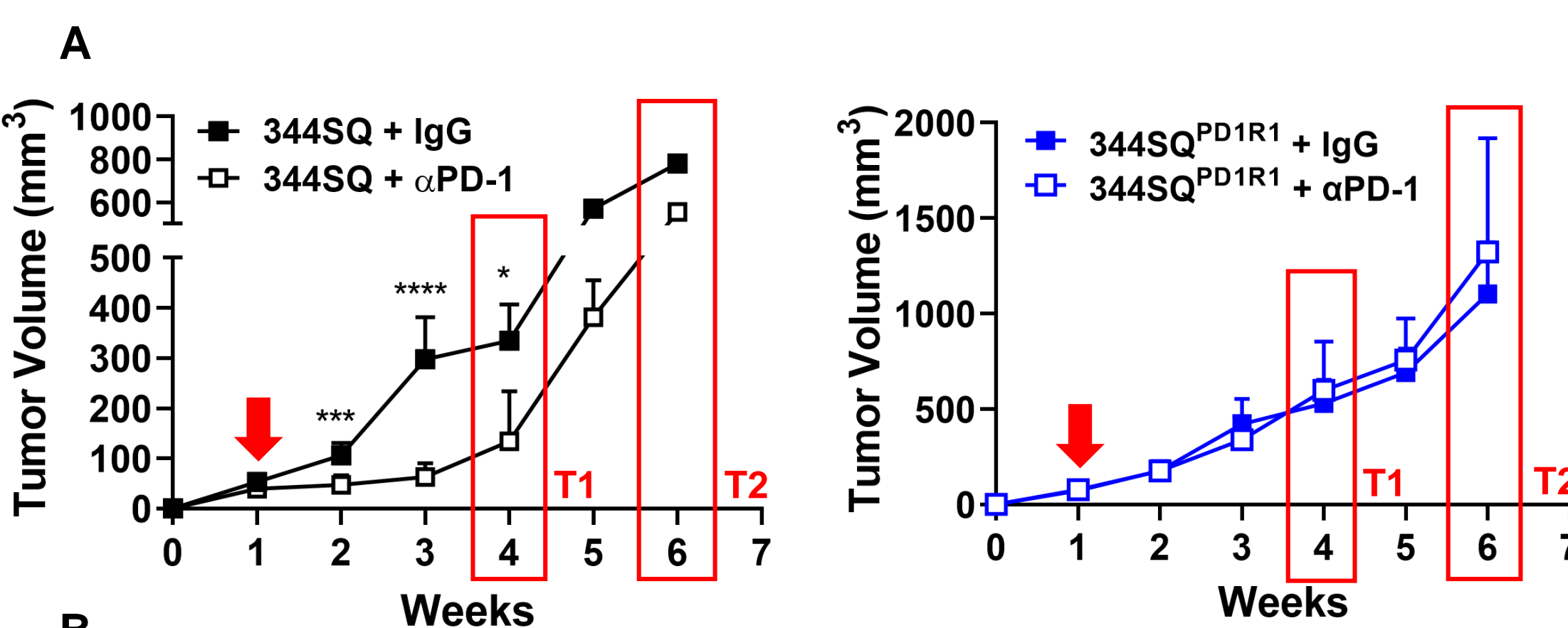
Graduate School of Biomedical Sciences

## Abstract

Immune checkpoint blockade is a promising treatment option for patients with non-small cell lung cancer (NSCLC), the most common type of lung cancer. Lung tumors harboring Kras/p53 (KP) mutations express higher levels of PD-L1 and respond better to anti-PD-1/PD-L1 therapy than other Kras subsets<sup>1</sup>. However, despite the success of immunotherapy in treating late-stage lung cancer, tumors can gain acquired resistance through mechanisms that are not well understood.

Therefore, our goal was to elucidate the mechanisms of intrinsic and acquired anti-PD-(L)1 resistance in NSCLC. For this purpose, we developed several anti-PD-(L)1 sensitive and resistant mouse cell lines as working models. Preliminary studies suggest that our resistant cell lines do not exhibit known mechanisms of tumor cell-intrinsic immunotherapy resistance. To further identify genes that drive novel resistance mechanisms on a single cell resolution, we studied the differentially expressed genes (DEGs) between 344SQ (sensitive) and 344SQ<sup>PD1R1</sup> (resistant) tumors treated *in vivo* with either IgG or anti-PD-1. After validation of numerous DEGs at both the mRNA and protein level, we obtained a list of candidate genes. Interestingly, we found methylthioadenosine phosphorylase (MTAP), a housekeeping gene known to play tumor-suppressor roles, to be consistently and significantly upregulated in anti-PD-1 resistant cell lines and tumors. We hypothesized that gene expression changes in immunotherapy resistant tumor cells reprogram the tumor microenvironment to create an immunosuppressive milieu. Our pilot *in vivo* study suggests that MTAP knockdown partially re-sensitizes 344SQ<sup>PD1R1</sup> tumors to anti-PD-1 treatment and modulates intratumoral T cell activation status. The outcome of this project aims to provide novel therapeutic targets in combination with immunotherapy to overcome anti-PD-(L)1 resistance in NSCLC patients.

## Background



**Figure 1. Single cell RNA-sequencing experimental design and validation.** **A.** 344SQ (sensitive) or 344SQ<sup>PD1R1</sup> (resistant) tumors were subcutaneously implanted into wildtype mice. One week post implantation, IgG or anti-PD-1 were i.p. injected weekly until end point (week 6). Tumors were collected at Time 1 (T1) (344SQ remained sensitive) and Time 2 (T2) (344SQ gained resistance) to be processed for single cell RNA-sequencing. **B.** Four comparisons between tumor models and treatments were performed on both T1 and T2 samples to obtain 8 sets of differentially expressed genes (DEGs) comparisons. **C.** Workflow of DEGs validation, identified from comparisons in Figure 1B.

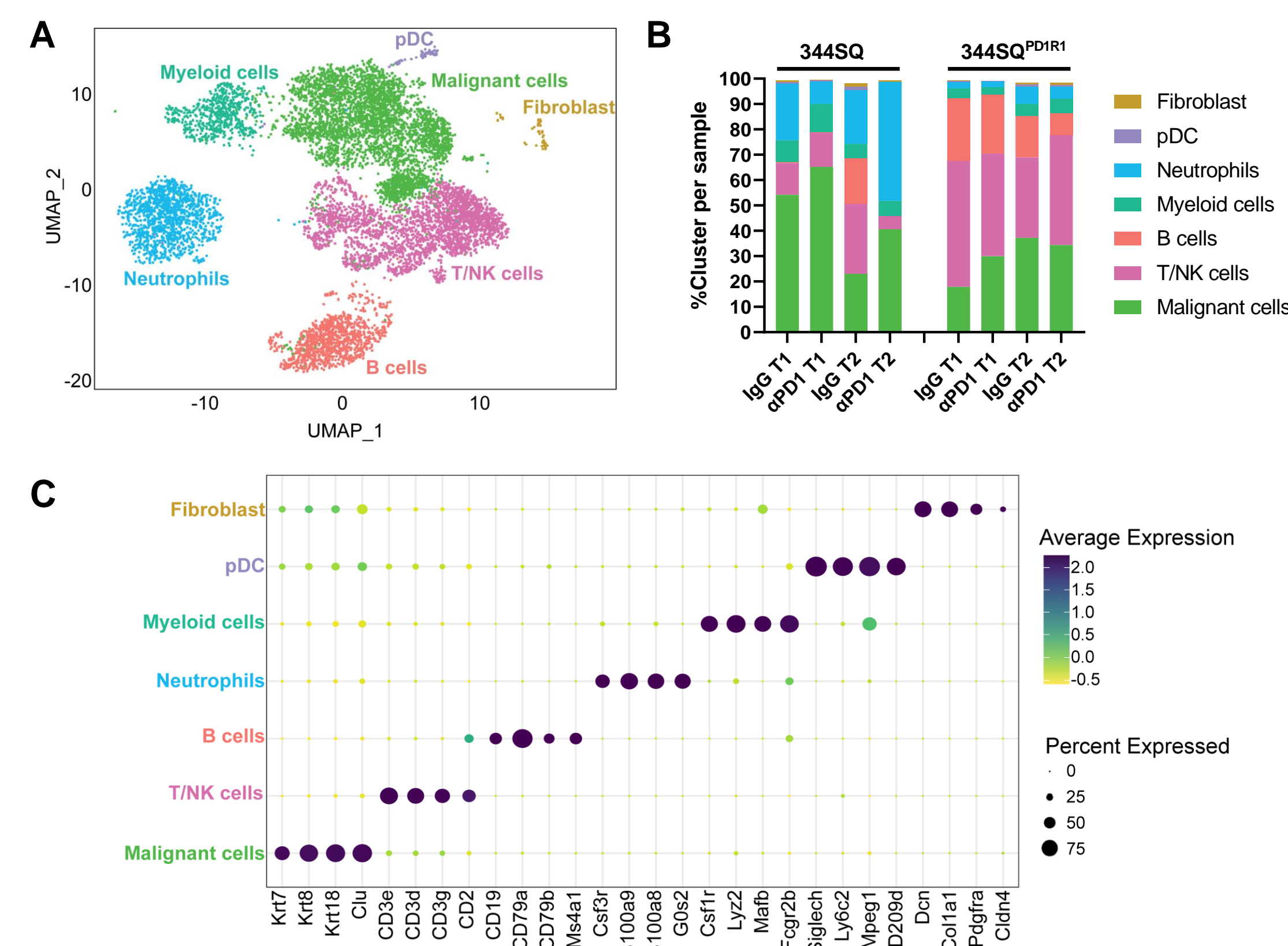
## Funding

CPRIT RP200235  
CPRIT-MIRA RP160562-P3  
NIH R37 CA214609  
NIH F32 CA239292  
2P50CA070907-21A

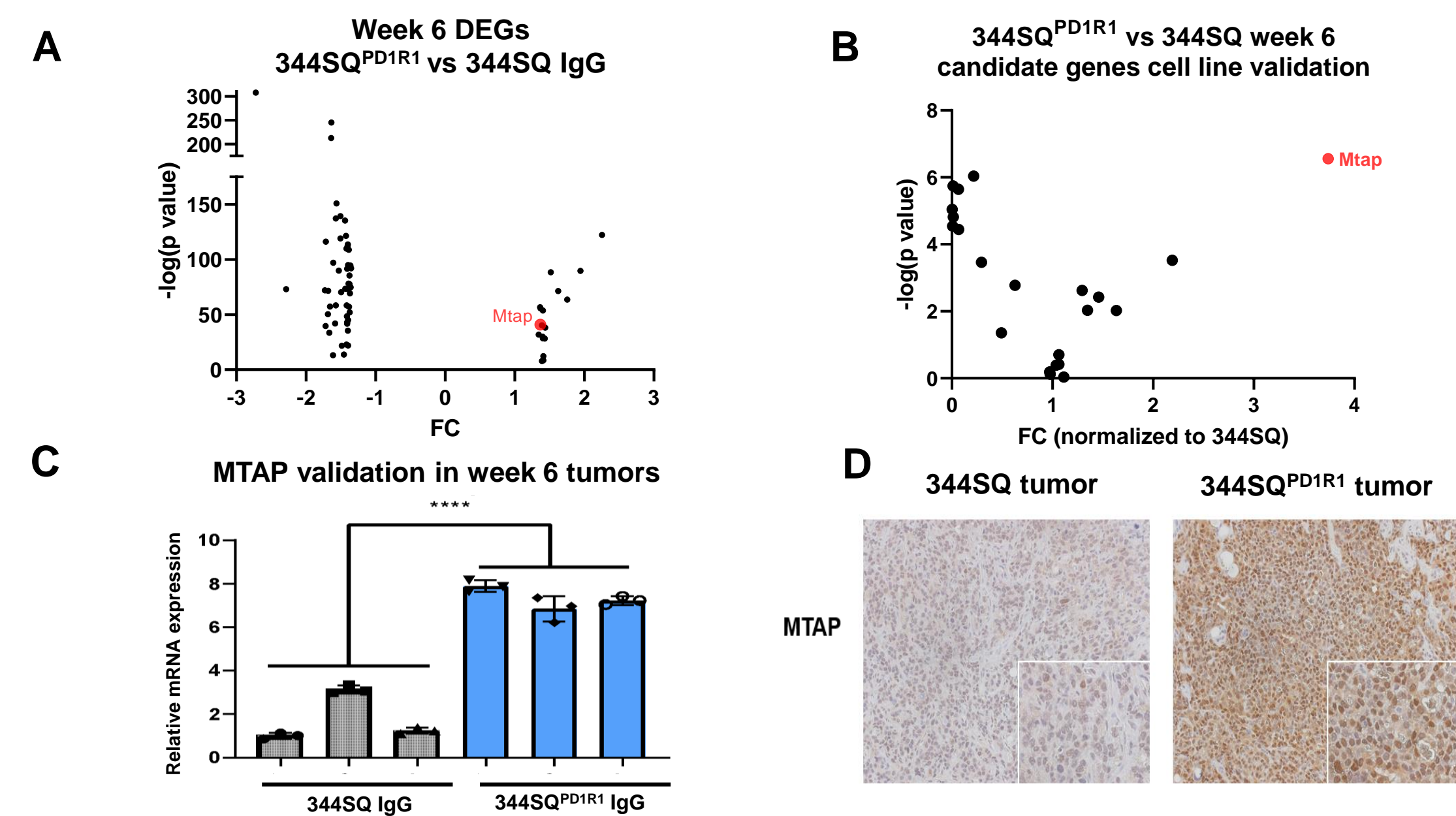
## Contact info

E-mail: hwu7@mdanderson.org

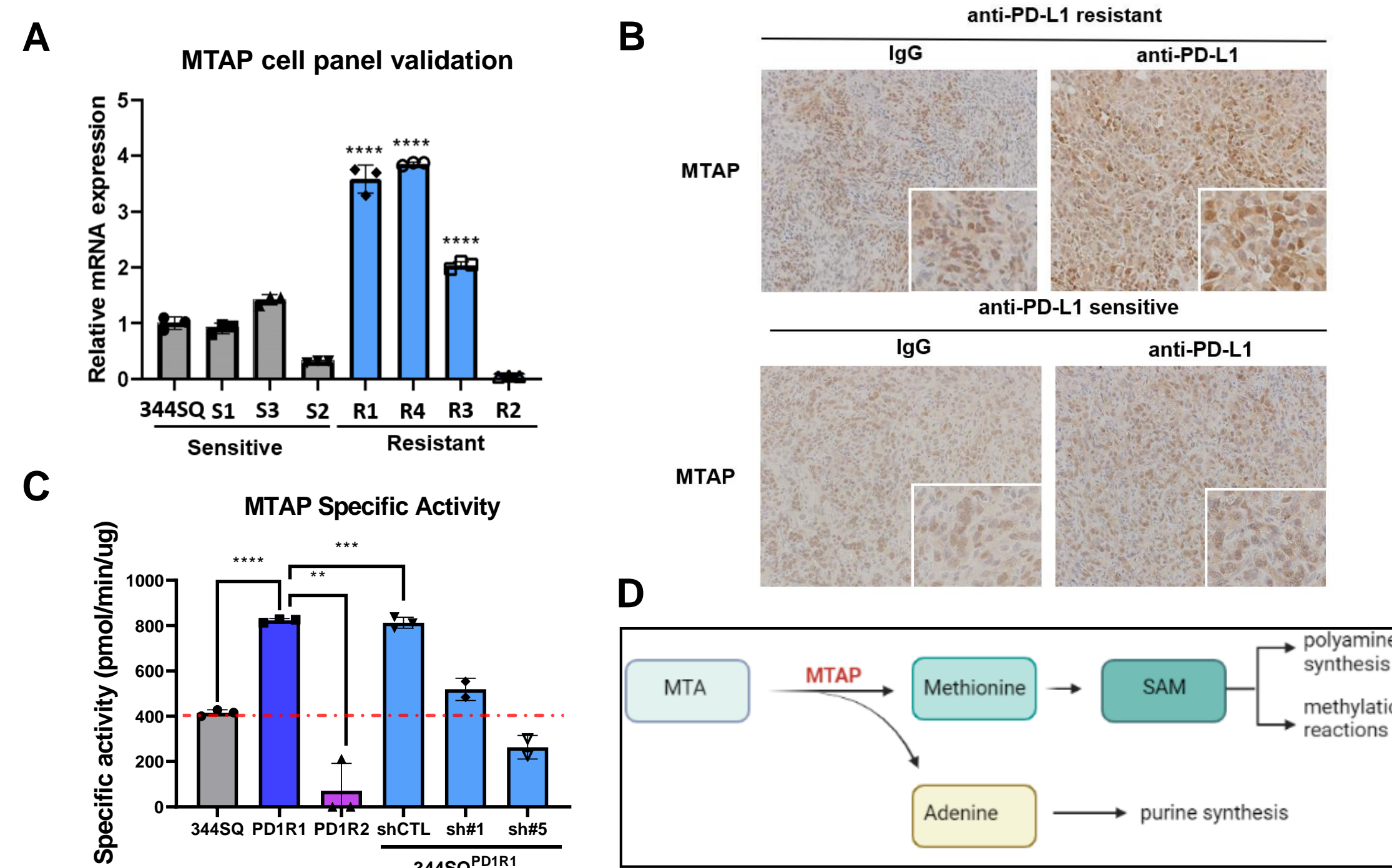
## Results



**Figure 2. Clustering of single cells using markers from literature identified seven distinct populations.** **A.** Unsupervised clustering of pooled samples identified 7 main cell types. **B.** Percentage of the identified cell clusters was graphed within each sample. **C.** Markers from literature used to identify each cluster.

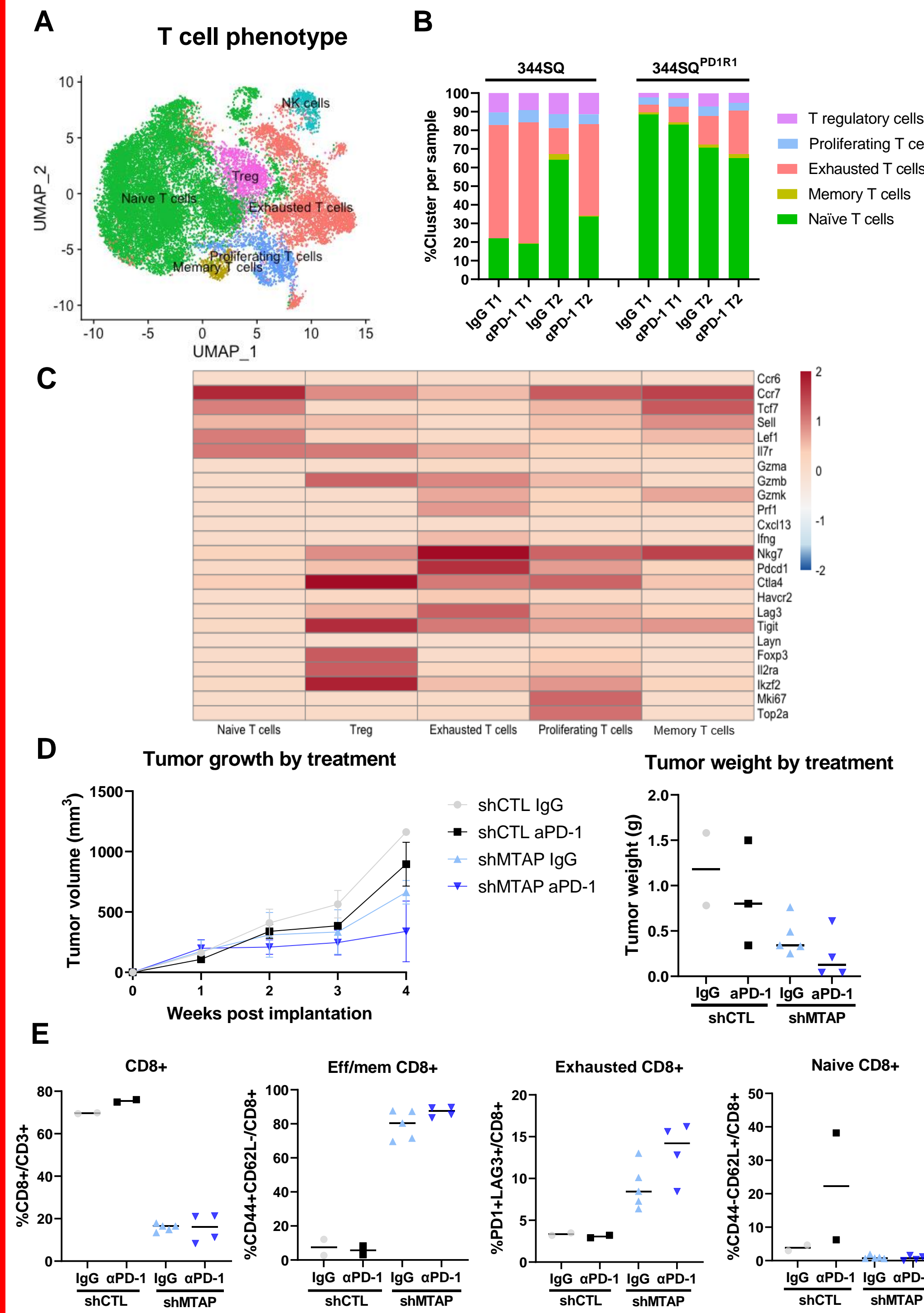


**Figure 3. MTAP is significantly upregulated in resistant tumor in multiple scRNA-seq DEGs comparisons and its expression is validated.** **A.** Volcano plot of week 6 344SQ<sup>PD1R1</sup> vs 344SQ IgG DEGs comparison from which MTAP (red) was identified. MTAP was similarly upregulated in week 4 344SQ<sup>PD1R1</sup> vs 344SQ IgG and αPD-1 treated DEGs comparisons (not shown). **B.** A list of candidate genes from T2 comparisons were validated by qPCR in cell lines, with MTAP being the highest upregulated in 344SQ<sup>PD1R1</sup>. **C.** RNA validation of MTAP levels by qPCR in IgG treated week 6 344SQ<sup>PD1R1</sup> and 344SQ tumors. **D.** Protein validation of MTAP levels by IHC in baseline SQ tumors.



**Figure 4. MTAP expression and enzymatic activity are further validated in different resistance models.** **A.** Validation of MTAP RNA levels by qPCR from panel of 344SQ sensitive and resistant cell lines. **B.** IHC of MTAP in IgG/αPD-L1 treated tumors derived from sensitive/resistant KP GEMM cell lines. **C.** Assay based on MTAP (from cell lysate) conversion of MTA to adenine, which was converted to 8-dihydroxyadenine by xanthine oxidase. Absorbance read at 305nm for 30 min at kinetic mode<sup>2</sup>. **D.** Schematic depicting MTAP housekeeping function and downstream metabolites produced (made with BioRender).

## Results



**Figure 5. Pilot *in vivo* experiment shows MTAP KD in malignant cells partially re-sensitizes resistant tumors to anti-PD-1 treatment and alters T cell activation state.** **A.** Clustering of T cell population from scRNA sequencing using markers from literature revealed 6 sub-populations with differential stages of T cell activation. **B.** Percentage of each T cell sub-types out of total T cells for each sample. **C.** Markers used to identify each T cell subpopulation phenotype. **D.** 344SQ<sup>PD1R1</sup> shCTL, shMTAP tumor growth curves over 4 weeks (left). Tumor weights at week 4 end point (right). **E.** Flow cytometry of CD8+ T cells isolated from 344SQ<sup>PD1R1</sup> CTL KD and MTAP KD tumors.

## Conclusions

- We have developed and validated a working model to study anti-PD-(L)1 resistance in KP mutant lung cancer
- scRNA-sequencing analysis identifies high T/NK infiltration in the resistant tumor, but these T cells mostly remain at a naïve state
- Anti-PD-1 resistant model has significantly higher MTAP expression and enzymatic activity than the sensitive cell lines
- Pilot *in vivo* study suggests MTAP KD decreases tumor growth at baseline and partially re-sensitizes resistant tumors to anti-PD-1 treatment, potentially by pushing CD8+ T cell activation states closer toward an effector/memory phenotype

## Future Directions

- Optimize the *in vivo* functional validation experiment with MTAP KD and MTAP OE cell lines to better characterize both the lymphoid and myeloid population in the tumor microenvironment
- Identify upstream regulators of MTAP expression in the context of anti-PD-1 resistance
- Further *in silico* analysis using human cancer datasets and *in vitro/in vivo* functional studies are needed to refine the list of candidate genes

## References

- Skoulidis et al. *Cancer Discovery*. 2015 August ; 5(8): 860–877.
- Christopher et al. *Cancer res*. 2002; 62(22), 6639–6644.