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CD4+ T cell lymphoma harboring a chimeric antigen receptor integration in *TP53*

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Summary:

Malignant T cell transformation after CAR T cell therapy has been described, but the contribution of CAR integration to oncogenesis is not clear. Here we report a case of a T-cell lymphoma harboring a lentiviral integration in a known tumor suppressor, *TP53*, which developed in a patient with multiple myeloma after BCMA CAR T cell therapy.

Introduction

Chimeric antigen receptor (CAR) T-cell therapies have shown remarkable efficacy and are now approved for several hematologic malignancies.[1] These cellular therapies are typically manufactured using viral vectors for integration of the CAR into T cells.[2] While rare, several cases of T-cell malignancies after CAR T-cell therapies have been reported to date.[3–8] The exact etiologies of secondary T-cell malignancies are uncertain. but one potential mechanism is insertional mutagenesis in T cells. In all reported T-cell malignancies post CAR T-cell therapy to date, the vector integrations occurred in genes of unclear oncogenic potential, making it difficult to establish a causal association with tumorigenesis.[4,8] Here, we describe a case of T-cell lymphoma of the GI tract occurring several weeks after ciltacabtagene autoleucel (cilta-cel), a BCMA CAR T-cell therapy for multiple myeloma. This tumor appears to be a malignancy associated with integration of the CAR vector within a canonical tumor suppressor gene (*TP53*).

Case Report:

A 72-year-old woman with relapsed and refractory high-cytogenetic risk triple-class refractory IgA kappa multiple myeloma had received four lines of myeloma-directed therapy (including two autologous hematopoietic cell transplantations) before treatment with fludarabine-cyclophosphamide lymphodepleting chemotherapy followed by ciltacabtagene autoleucel (cilta-cel), an autologous BCMA-specific 4–1BB/CD3z CAR T cell.[9,10] The treatment course was notable for grade 1 cytokine release syndrome and resulted in a minimal residual disease (MRD) negative complete remission by 1 month after cilta-cel therapy.[11]

Approximately 2 months after infusion, she reported new and persistent nausea, vomiting, anorexia, and intractable watery, non-bloody diarrhea ultimately requiring hospitalization (Supplementary Figure 1). Evaluation for an infectious etiology was negative and a CT scan of the abdomen and pelvis did not show any significant abnormalities. She underwent upper endoscopic and flexible sigmoidoscopic evaluations that revealed a normal-appearing esophagus, stomach, proximal duodenum, and entire examined portion of the colon, with biopsies taken during both procedures.

The histopathological findings of the upper endoscopy were felt to be compatible with a potential immune mediated enterocolitis of unclear etiology. She was administered a variety of treatments including glucocorticoids, tacrolimus, and high-dose infliximab without sustained response. Treatment course was complicated by *cytomegalovirus* (CMV) reactivation requiring treatment with ganciclovir. Eventually, ruxolitinib was started at a dose of 5 mg orally twice daily, then increased to 10 mg orally twice daily.[13] Over the course of 2 weeks, her appetite improved and the diarrhea decreased in frequency, leading to potential hospital discharge planning. However, shortly before discharge, she developed clinical signs of sepsis with blood cultures growing *Stenotrophomonas maltophilia* and *Streptococcus mitis*, and despite broad-spectrum antibiotic therapy, her condition worsened with septic shock and acute respiratory failure. In concert with her family, the clinical team pursued comfort care measures and the patient died with her family at her side.

Molecular Studies:

Subsequent to this case, our institution developed an in-house digital droplet polymerase chain reaction (ddPCR) assay for the detection of the cilta-cel CAR constructs in blood and other biologic specimens. CAR was detected in high abundance in DNA extracted from duodenal biopsy samples with a ratio of 25.12% ciltacel compared to a human DNA (ATP2B4) control (Supplementary Figure 2; Supplementary Table 1).

This prompted a pathological re-examination of the original duodenal biopsy samples (Figure 1A-B; Supplementary Figure 3). Immunohistochemistry (IHC) demonstrated a dense lymphocytic infiltration of the lamina propria positive for TCRα, CD3, CD4, and granzyme B but which did not express TRBC1 chain (a marker for expression of a T-cell antigen receptor constant beta chain; uniform positivity or negativity indicates clonality) or CD8. No lamina propria plasma cells were detected by the BCMA staining. The pathological entity was reclassified as an indolent (CD4+) T-cell lymphoma/lymphoproliferative disorder of the GI tract.[14,15]

T-cell repertoire sequencing in the duodenal biopsy revealed a dominant TCR Vβ11–02 clone representing 83.6% of productive TCR sequences, consistent with a clonal CAR+ lymphoproliferative process (Figure 2A). CAR integration site analysis[16] of the duodenal biopsy identified two dominant insertion sites: the first integrated in reverse orientation in the first intron (chr17: 7683124) of *TP53*, and the second in positive orientation in the first intron (chr22: 20025368) of *TANGO2* (Figure 2B; Supplementary Figure 4). The two insertions were found at similar frequencies (29% and 23%, respectively), which when paired with a single dominant clone by TCR sequencing was most consistent with a single clone bearing CAR in both *TP53* and *TANGO2*. Additional insertions in genes representing minor populations included *MADD* and *IZKF4*, as well as an additional insertion in *TP53* which was identified in a single sequencing read.

p53 protein expression in the duodenal biopsy was assessed using IHC and showed physiologic expression in the crypt epithelial cells (Figure 1C). However, the majority (90%) of T cells showed dim or absent p53, consistent with reduced expression in clonal T cells (Figure 1C).

Whole exome sequencing of the duodenal biopsy identified 23 additional mutations relative to a preinfusion blood sample (Supplementary Table 2; Supplementary Figure 5). Notably, we detected a missense mutation in the tumor suppressor *SOCS1*, with a serine to phenylalanine substitution pS206F in the "SOCS box" domain that regulates protein degradation.[17] SOCS1 is a negative regulator of JAK/STAT signaling[17] and prior studies have established phosphorylated STAT 3 (pSTAT3) as a readout for active JAK/STAT signaling [18]. IHC for pSTAT3 demonstrated strong staining in 80–90% of T cells, consistent with reduced SOCS1 activity leading to increased JAK/STAT signaling (Figure 1D).

Clonal Kinetics:

To understand the kinetics of clonal emergence, we performed TCR sequencing on serial peripheral blood samples from pre- and post-CAR infusion. The TCR Vβ11–02 clone was detected at a frequency of 16.9% of TCR sequences at day 74 post-infusion, coincident with the onset of clinical symptoms (Figure 2A). The same clone was detectable at 0.01% of sequences on day 28 but was not detectable at any prior timepoint either pre- or post-infusion (days 3 and 14 after CAR T cell infusion). In addition, a high frequency TCR Vβ27–01 clone was detected on day 74 post-infusion in the peripheral blood (29.3%), but not at any timepoints prior. This clone was detected at low abundance (<2%) in the duodenal biopsy. To better characterize clonal progression in the blood, we performed whole genome sequencing on preinfusion (day −58), day 14, and day 74 blood samples. We detected a *DNTM3A* Y284* nonsense mutation at a variable allele frequency (VAF) of 7.6% in the preinfusion sample, representing pretreatment clonal hematopoiesis in this patient (Figure 2C). The DNMT3A alteration was stably present at approximately 10% VAF at both day 14 and 74 but was below the limit of detection in the post-infusion GI lymphoma sample. No additional mutations or structural variants in *TP53* were detected. Notably, 22/23 including the SOCS1 pS206F mutation were identified by at least 2 reads in the day 74 peripheral blood whole genome sequencing (WGS), coincident with the emergence of the Vβ11–02 clone (Figure 2C; Supplementary Figure 5; Supplementary table 3). The SBS1 and SBS5 "clock-like" mutational signatures were predominant in both the duodenal biopsy and the day 74 peripheral blood (Supplementary Figure 6).

To further characterize the consequence of genetic alterations, we performed single cell RNA with VDJ sequencing on approximately 18,000 cells from peripheral blood pooled from 3 timepoints (day 14, 28, 74). The TCR Vβ11–02 clone clustered independently (Figure 2D; Supplementary Figure 7) and represented approximately 3% of all cells. Although our analysis was limited by sparse RNA transcripts and low clone frequency, the TCR Vβ11–02 dominant cluster had the lowest mean *TP53* expression (p-value: 0.006 and adjusted p-value: 0.0689). In comparison, abundance of *TANGO2* and the *TP53*-adjacent gene *WRAP53* were not decreased compared to other clusters (Figure 2D).

Discussion

This is the second report of a CAR-T cell derived lymphoproliferative process occurring in the gut after treatment with ciltacabtagene autoleucel[8], and the first description of lymphoma harboring a CAR transgene integration into a common cancer-associated gene.

We identified integration of a lentiviral vector in an antisense orientation in the first intron of *TP53*. Lentiviral vectors show preference for insertion in transcription units, [19] which may disrupt regulatory sequences necessary for gene expression [20] or generate alternate isoforms through splice donor/acceptor sites in the retroviral vector sequence.[21] Immunohistochemistry and single-cell RNA sequencing were consistent with decreased *TP53* transcription and reduced P53 expression in clonal T cells bearing the *TP53*-integrated CAR vector.

It is not clear whether a monoallelic *TP53* CAR vector integration predisposes to transformation[22]. An additional integration in *TP53* was detected as a single event in our sample without clonal expansion. Furthermore, in a study of 58 patients treated with CD19 CAR T cells, 19 unique integration sites were found in the *TP53* transcription unit, and none were associated with clonal expansion, suggesting that monoallelic *TP53* integration alone is not sufficient to transform CAR T cells. [23] Thus, we sought to identify alternative factors that may have contributed to oncogenesis.

A second dominant insertion was detected in Transport and Golgi Organization protein 2 Homolog (TANGO2), a gene of poorly described function, associated with developmental disorders[24] but with no known role in oncogenesis and whose transcriptional expression was maintained in our clone. We further detected a mutation in *SOCS1*, a known tumor suppressor that regulates cytokine signaling *via* JAK/STAT pathways.[17] The substitution occurred in the degradation-regulating “SOCS box” domain at a site that has previously been implicated in large B-cell lymphoma.[26] IHC confirmed JAK-STAT pathway activation and the patient experienced a notable clinical improvement after initiating treatment with ruxolitinib, a JAK2 inhibitor. Both *TP53* and *SOCS1* alterations are commonly identified and can co-occur in patients with peripheral T-cell lymphoma[26]; due to limitations of available biospecimen, we are not able to conclusively differentiate the relative contribution of TP53 and SOCS1 alterations to the malignant transformation in this patient.

Notably, a pre-existing *DNMT3A* mutation found in peripheral blood was not enriched in the duodenal biopsy. Mutations in genes associated with clonal hematopoiesis (CHiP) such as *TET2* and *DNTM3A* have been identified in persisting CAR T-cell clones [27], as well as in post-CAR T-cell lymphomas associated with [28] or without[4] CAR integration. This case demonstrates that pre-existing clonal mutations may not necessarily contribute to and are not required for all CAR T-cell transformation scenarios.

In addition to gene disruption *via* insertional mutagenesis, a CAR could potentially contribute to clonal expansion and transformation by providing sustained activating and pro-survival signals. We previously showed that some CARs may cooperate with loss of *TET2* to promote highly proliferative clones in a *MYC*-dependent manner[29]. In these studies, the propensity to sustain lymphoproliferation depended on the CAR design and its costimulatory domain, raising the possibility that different CAR signaling domains may differ in their potential to cooperate with preexisting mutations or insertional disruptions to promote malignant transformation. For instance, in the context of TET2 disruption, CARs with 4–1BB costimulatory domain had a greater likelihood of association with clonal expansion[29].

CAR T-cell-derived lymphomas are rare.[4,6] However, this patient did not demonstrate any obvious masses, or radiologic or cytologic abnormalities to initially suggest leukemia or lymphoma, despite a chronic and severe clinical syndrome with persistent diarrhea and weight loss. Thus, clinicians and pathologists should maintain a high degree of suspicion with otherwise unexplained clinical symptoms consistent with T-cell infiltration, including diarrhea.

In summary, we report a secondary T-cell lymphoma of the GI tract that has integration of the CAR construct into a known tumor suppressor gene with attendant structural disruption of the gene product. Unlike prior reports, pre-existing clonal hematopoiesis did not appear to contribute to oncogenesis. Instead, our data suggest that a mutation in *SOCS1* detectable post-infusion may have cooperated with CAR activity or CAR vector integration in *TP53* to contribute to the development of CAR+ T-cell lymphoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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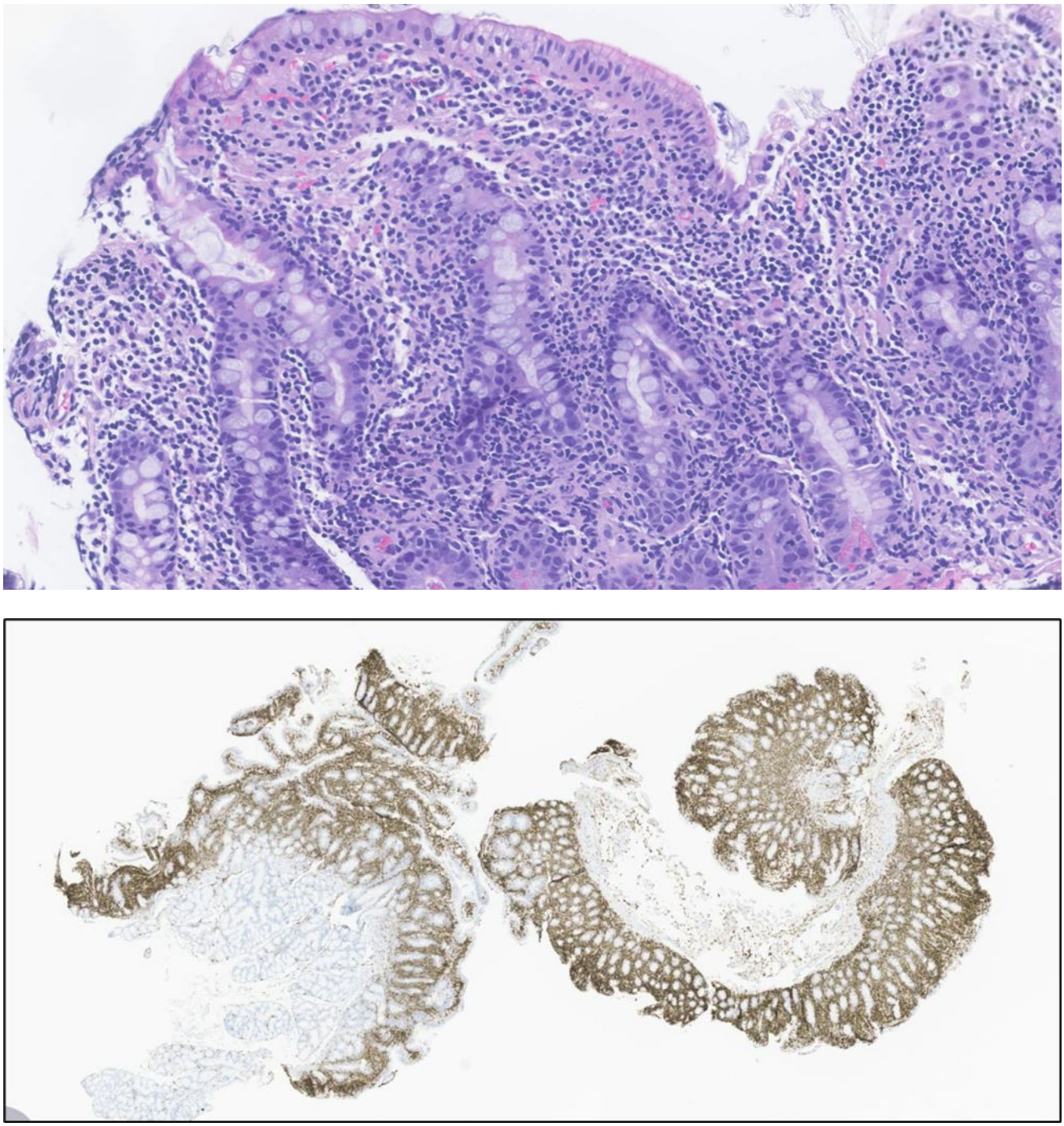
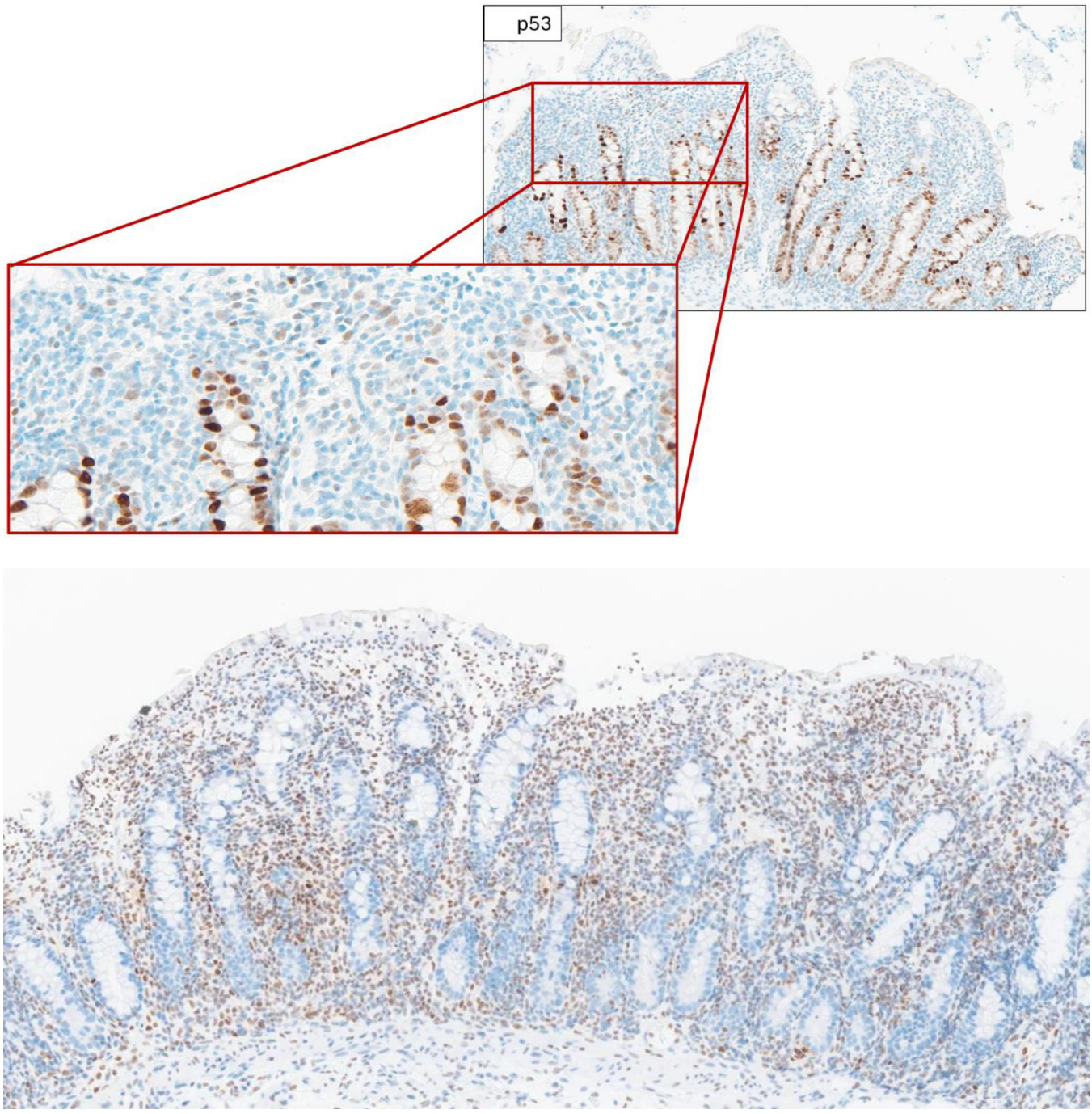




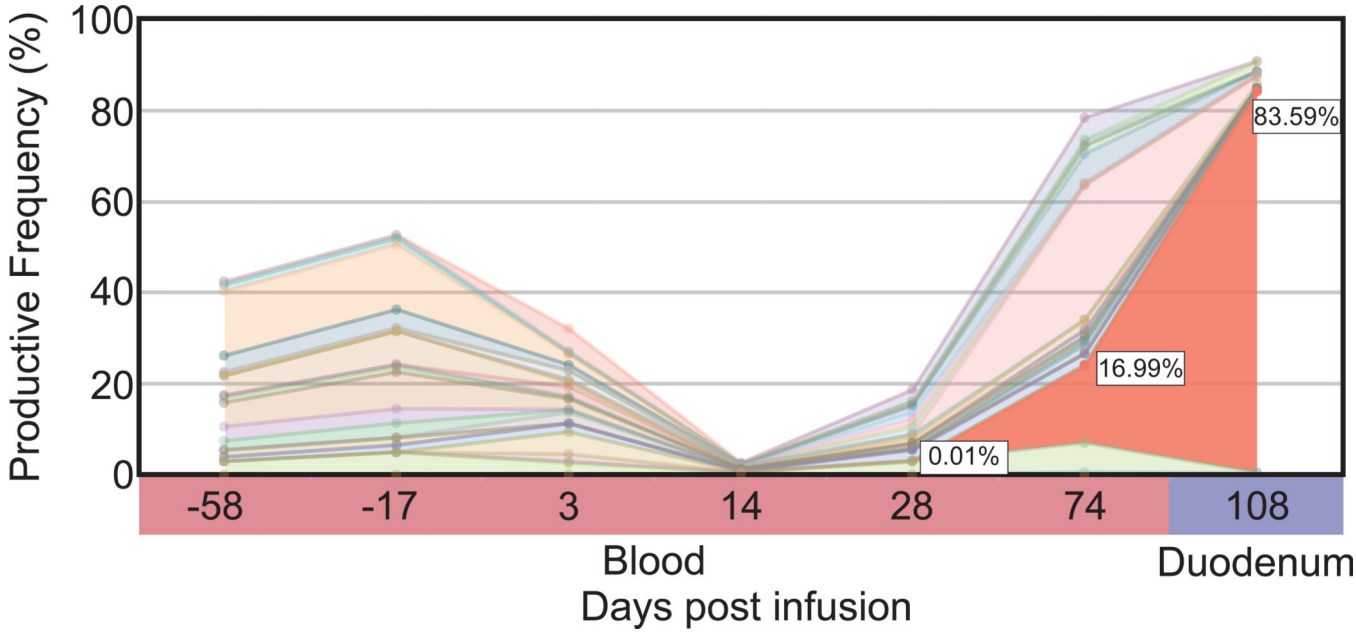
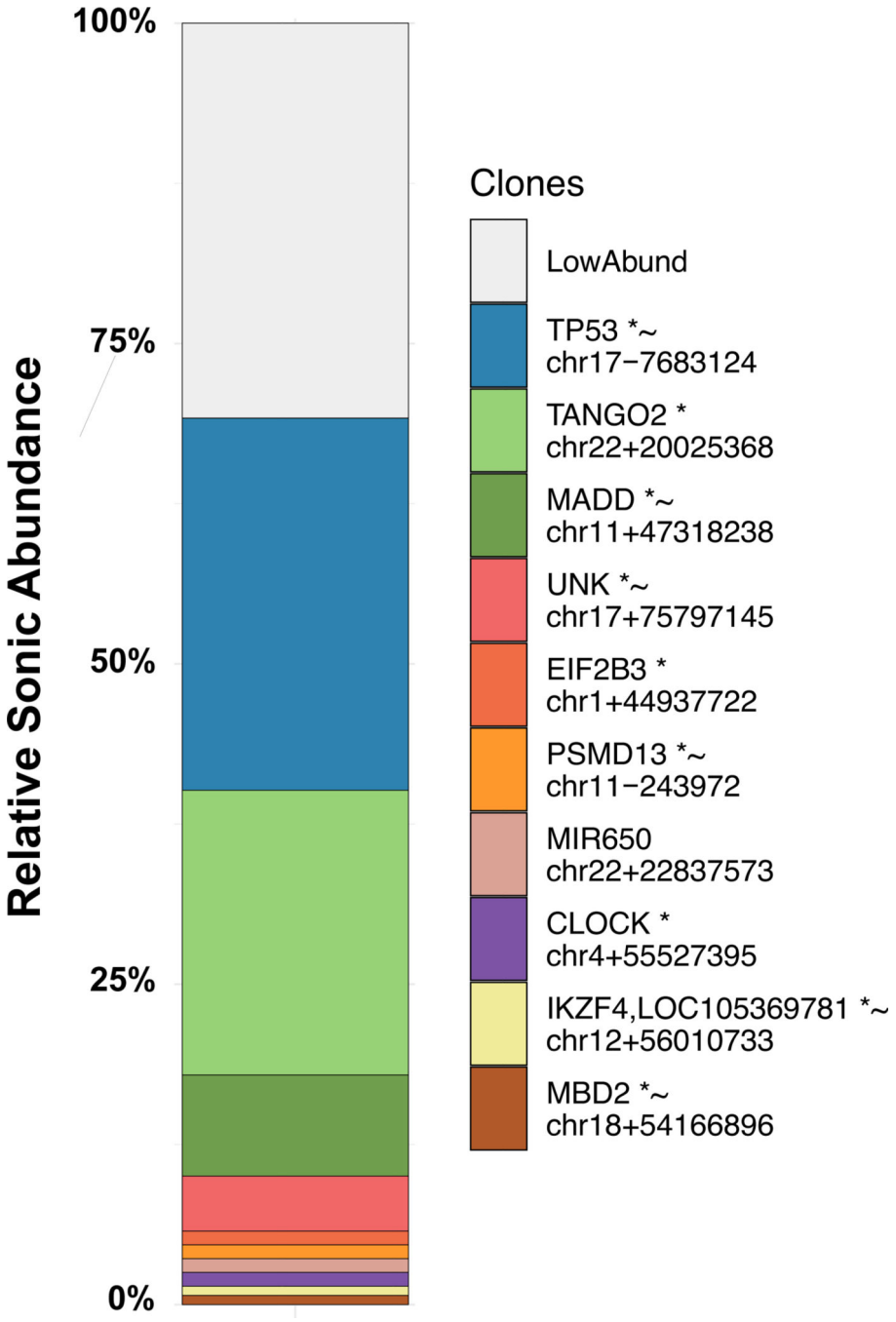
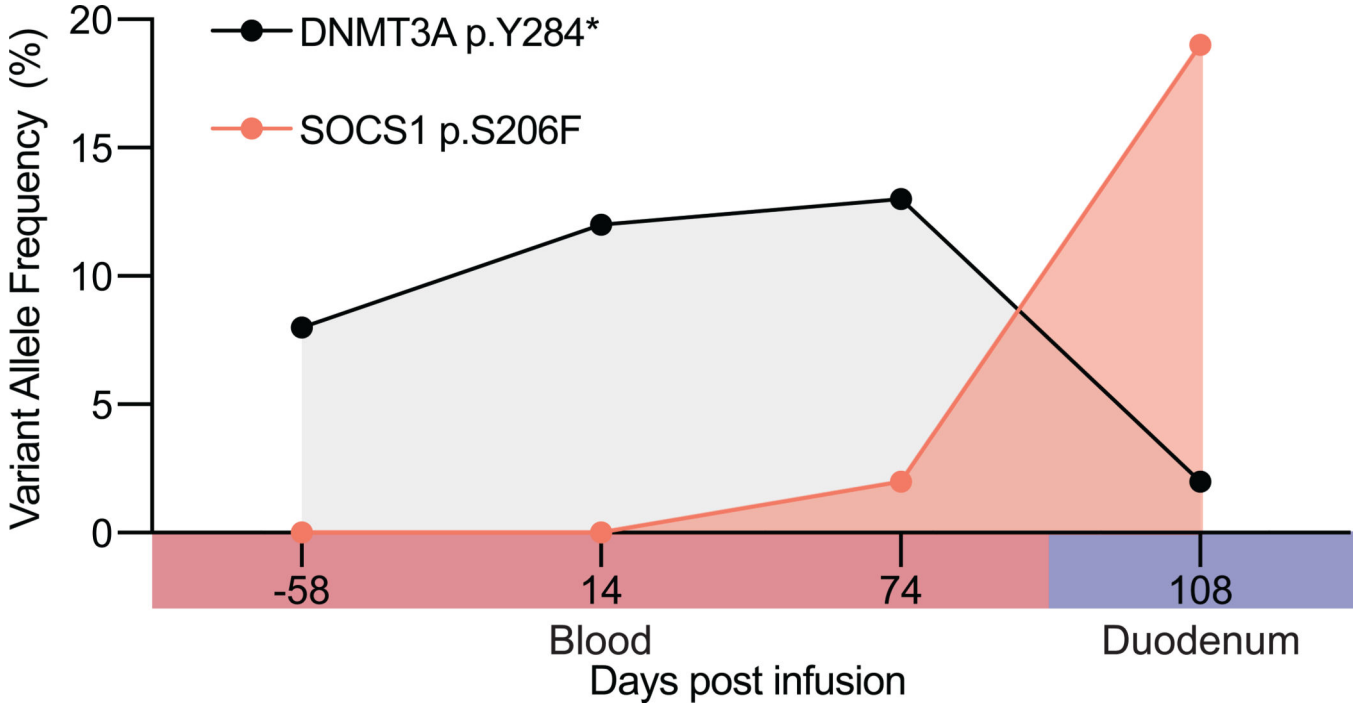
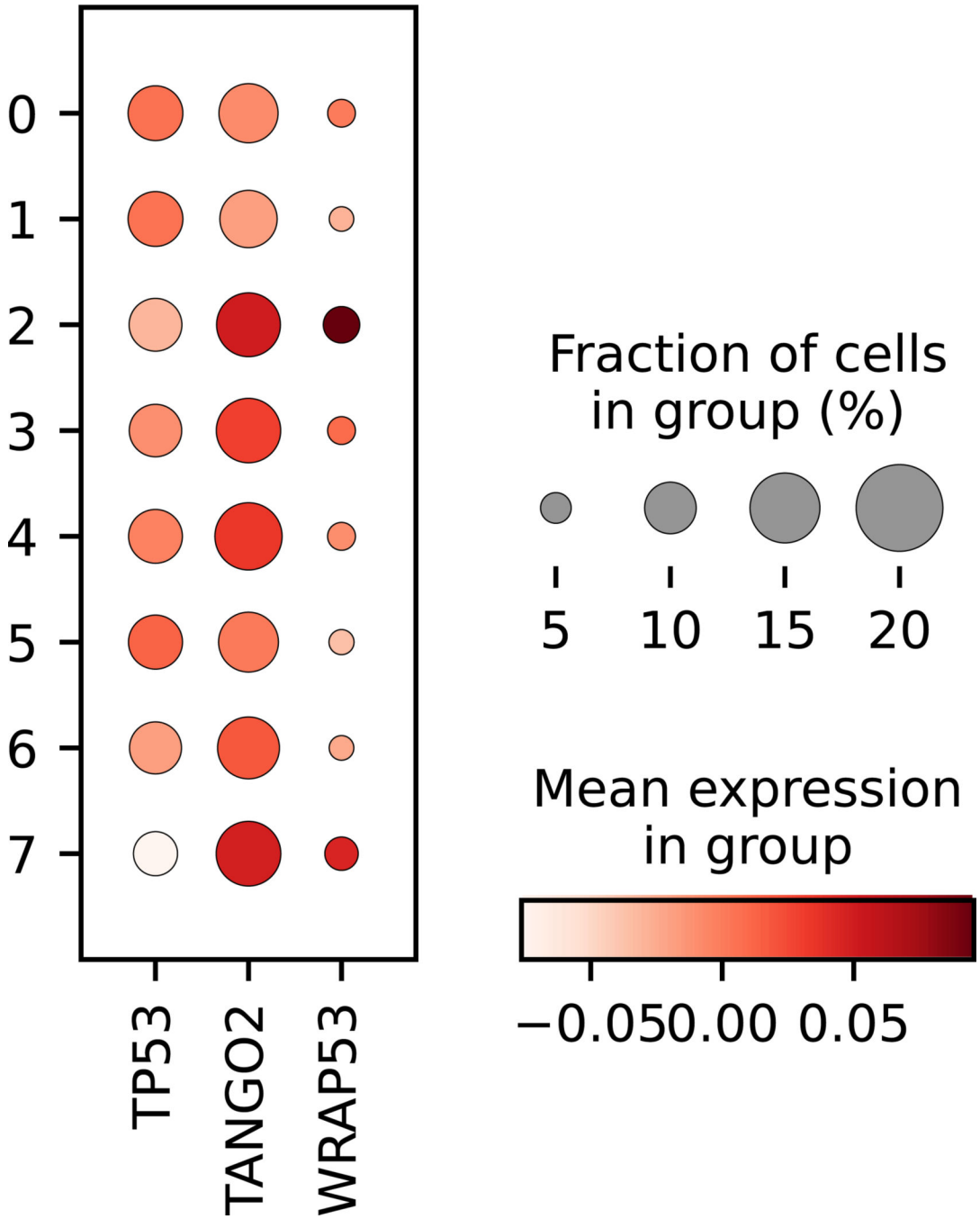


Figure 1. Pathological staining from the duodenal biopsy.

A) Dense lymphocytic infiltrate that is B) CD4 positive by immunohistochemistry. C) p53 staining shows dim or absent expression in T cell infiltrate; low power with inset. E-F) Phosphorylated STAT3 (pSTAT3) IHC showed positive staining.







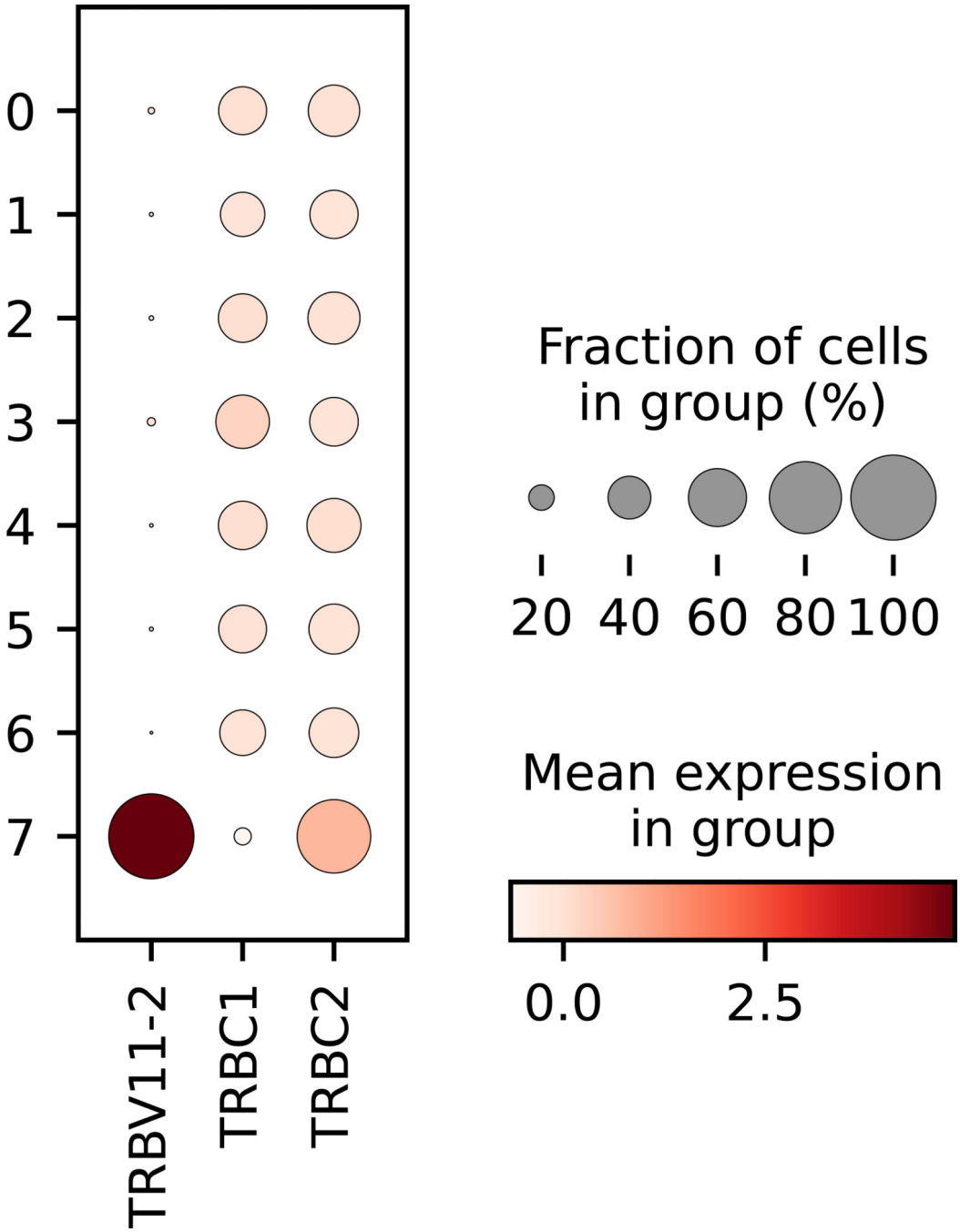


Figure 2. Molecular studies from the duodenal biopsy and peripheral blood.

A. Frequency TCR clones by VDJ immunosequencing in peripheral blood and duodenal biopsy at indicated days post CAR T cell infusion. Selected clones are among the ten most abundant in at least one timepoint. The most abundant clonotype in the duodenal biopsy is highlighted in red. 2B. Frequency of CAR integration sites in the duodenal biopsy sample. Cell clone sizes associated with unique integration sites are quantified using the SonicAbundance method. * Indicates an integration site within a gene; ~ indicates an annotated cancer-associated gene. Numbers below gene names indicate chromosome and

chromosomal positions. LowAbund indicates pooled low abundance integration sites. C. Variant allele frequency (VAF) of indicated DNTM3a and SOCS1 mutations in peripheral blood and duodenal biopsy at indicated days post CAR T cell infusion; peripheral blood sample results are from whole genome sequencing and the duodenal biopsy from whole exome sequencing. D. Single cell RNA expression dot plots of mean expression and frequency of indicated genes (x axis) by leiden cluster (y axis).