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Adoptive T cell therapy targeting somatic p53 mutations

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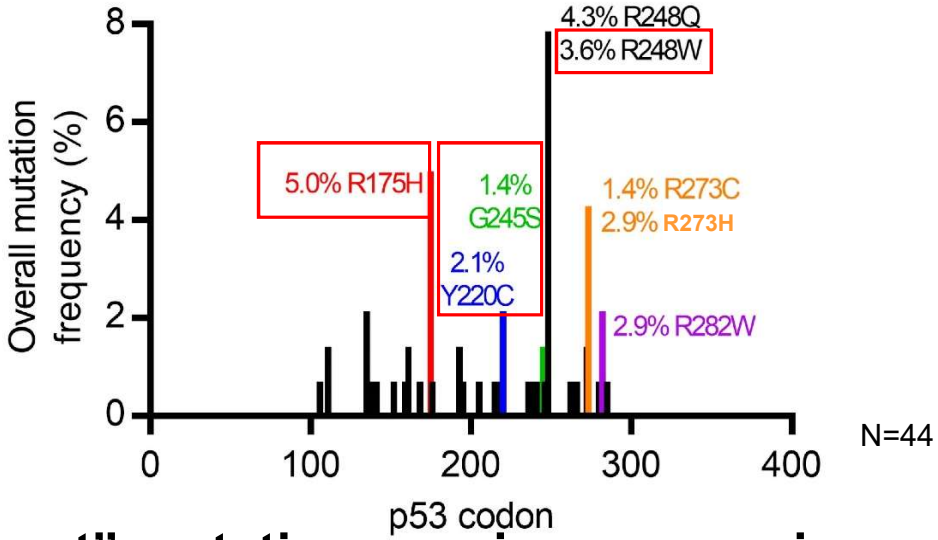
Disclosure

I have nothing to disclose.

The vast majority of *TP53* mutations are potential neoantigen candidates

- ***TP53*** **is the most mutated gene in solid cancers.**
- **Missense mutations (70%), frameshift (12%)**
  → potential neoantigen candidates
- **Missense *TP53* mutations are driver mutations in 27/33 tumor types (TCGA).**
- **Targeting driver mutations, such as *TP53* mutations, can minimize tumor escape due to antigen loss.**

TP53 "hotspot" mutations can be immunogenic



- **Some *TP53* "hotspot" mutations are immunogenic. (Malekzadeh et al, 2019)**

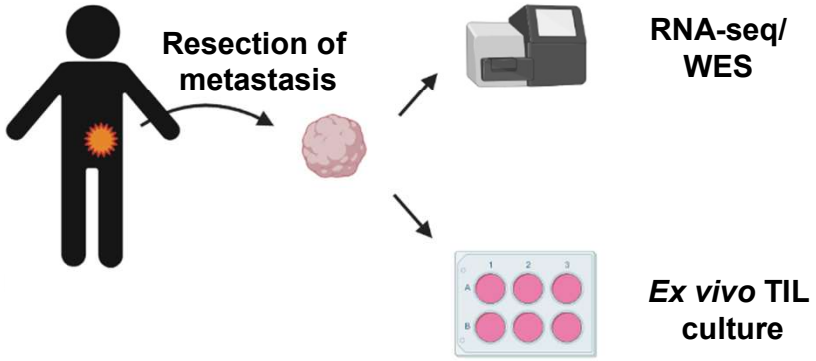
- **Some less frequent "non-hotspot" *TP53* mutations are still recurrent.**
  - **Are they immunogenic?**

Goals:

1. **To develop a library of tumor infiltrating lymphocytes (TILs) /TCRs against ALL *TP53* mutations**
   - **Autologous TIL treatment**
   - **Autologous PBL engineered to express "Off-the-shelf" TCRs**

2. **To evaluate the effectiveness of adoptive T cell therapies directed against shared *TP53* mutations**

Screening ALL *TP53* mutations for recognition by autologous TILs



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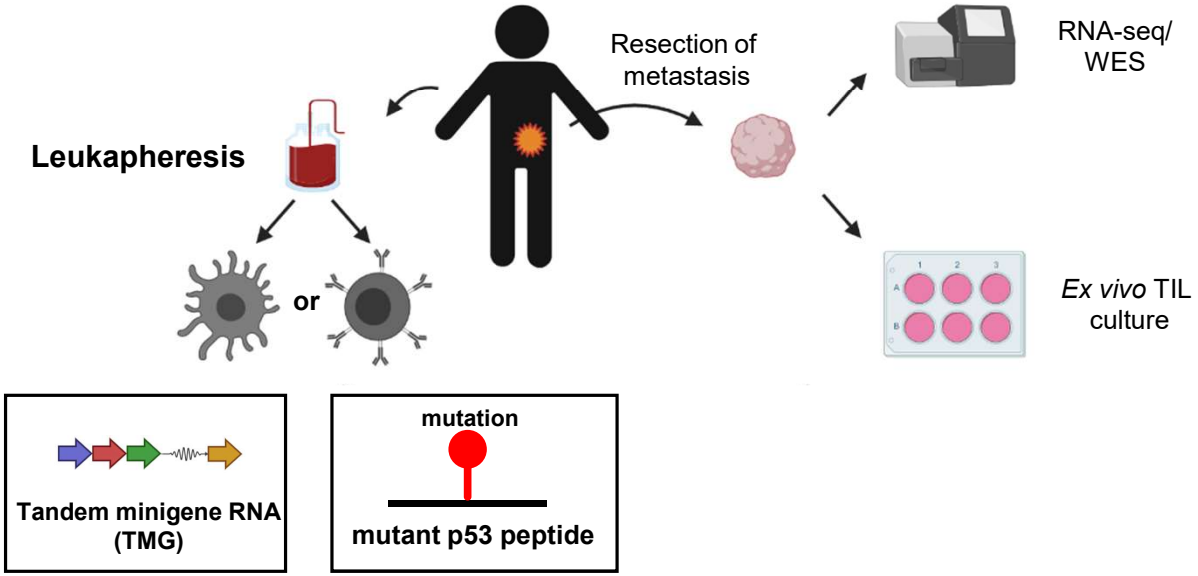

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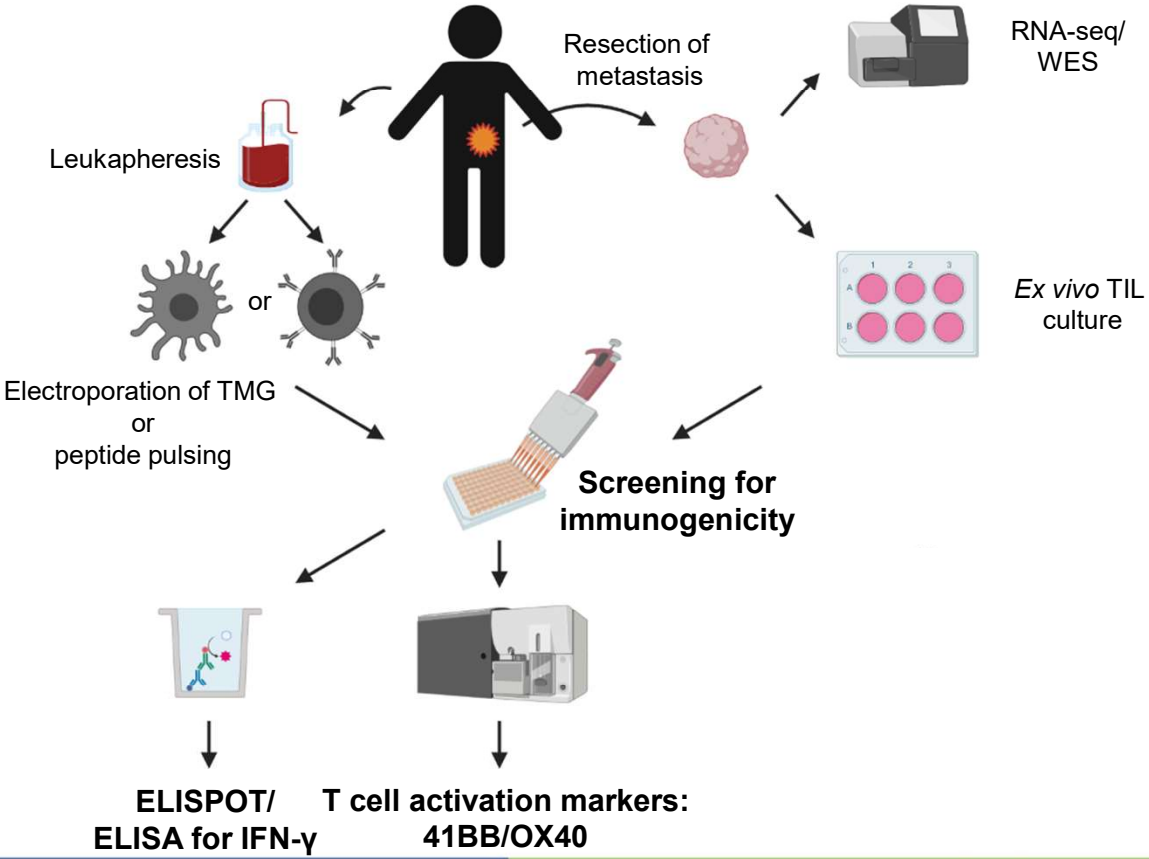

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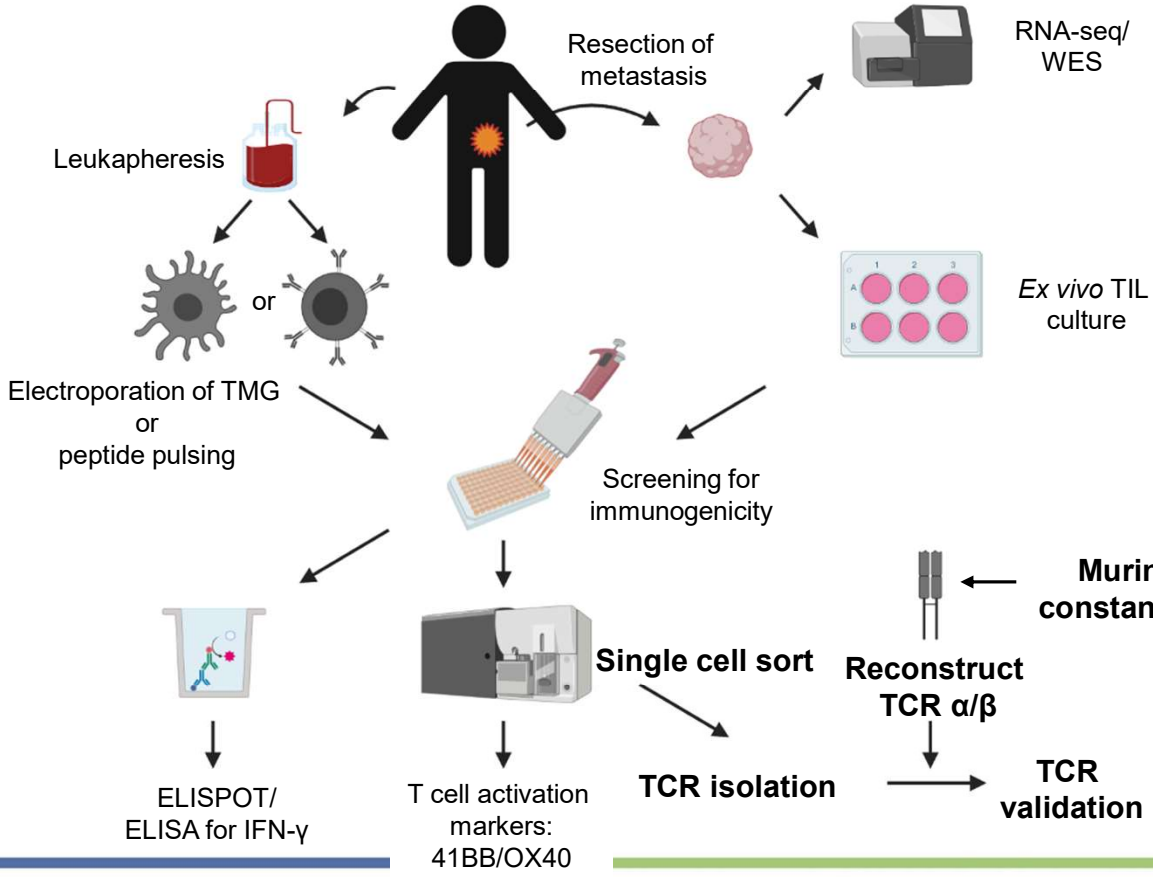

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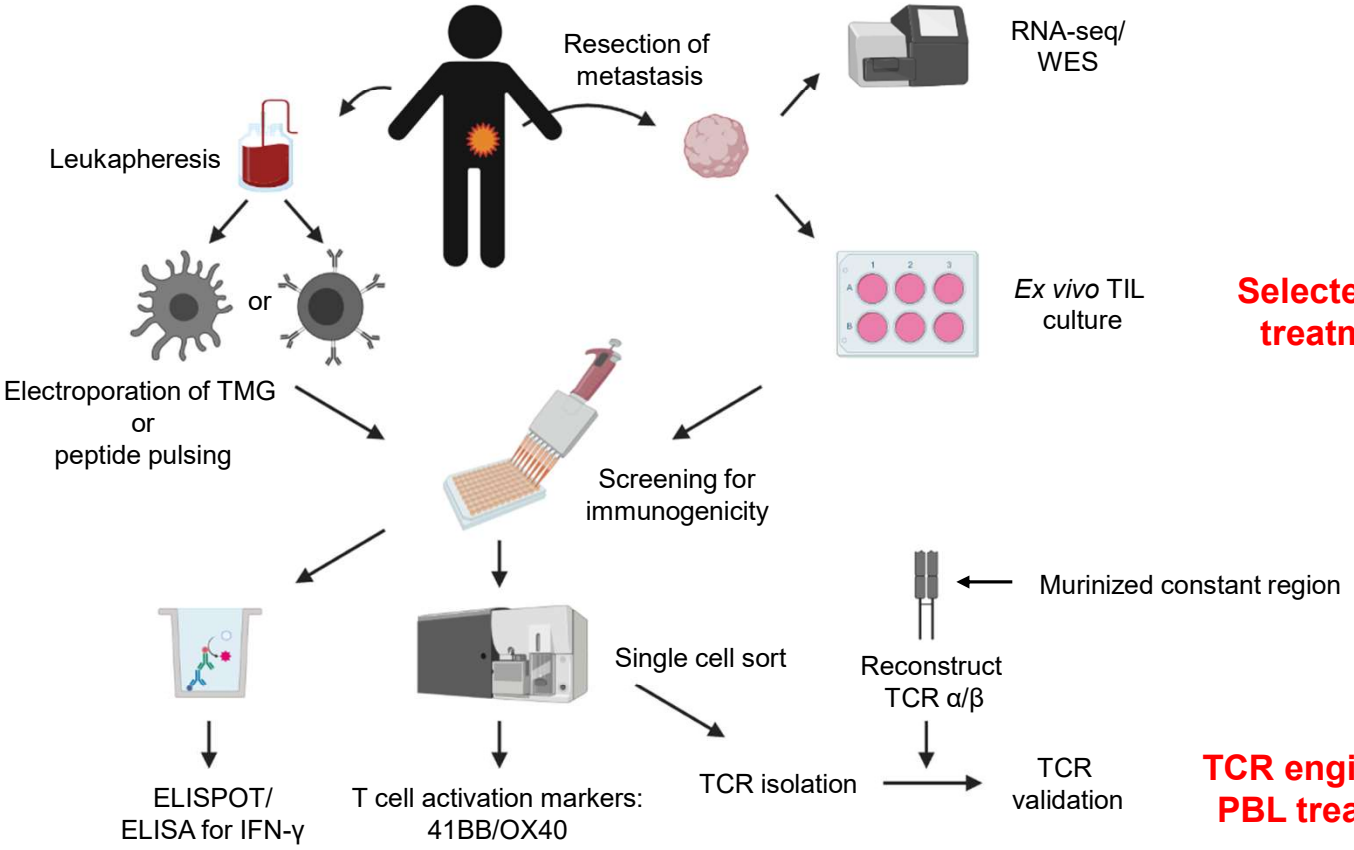

Example: TIL screening for patient 4316 reveals T cell activation against the p53 C135Y mutation

- **Patient 4316: metastatic colorectal cancer with p53 C135Y mutation**
- **24 TIL fragments were grown *ex vivo* and screened.**

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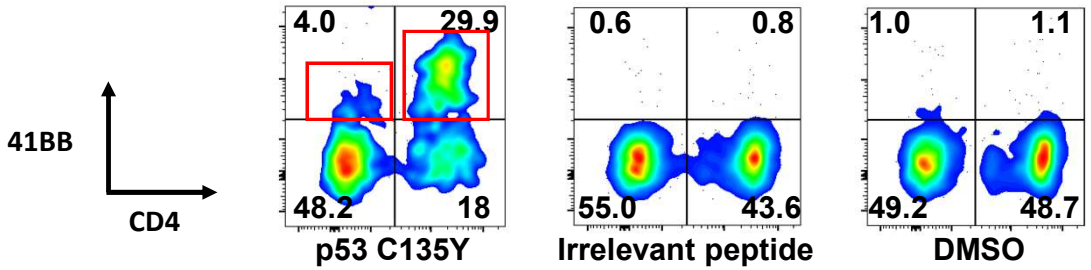
- **24 TIL fragments were grown *ex vivo* and screened.**
- **TIL fragment 22 showed interferon-γ secretion and 41BB upregulation against the p53 C135Y peptide.**



The TCRs isolated from patient 4316 show specificity for p53 C135Y over WT

CDR3A	CAESYSGGYQKVTF
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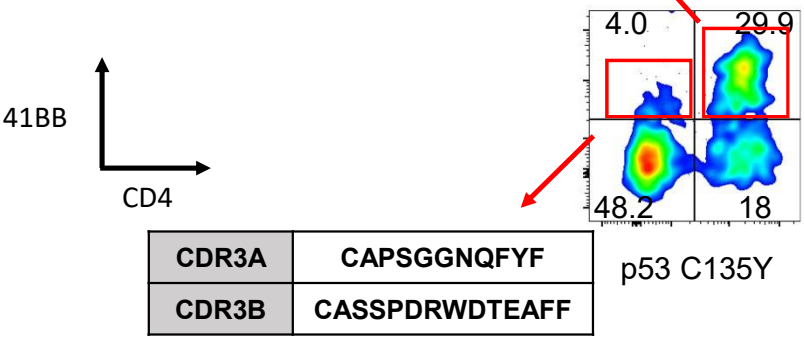

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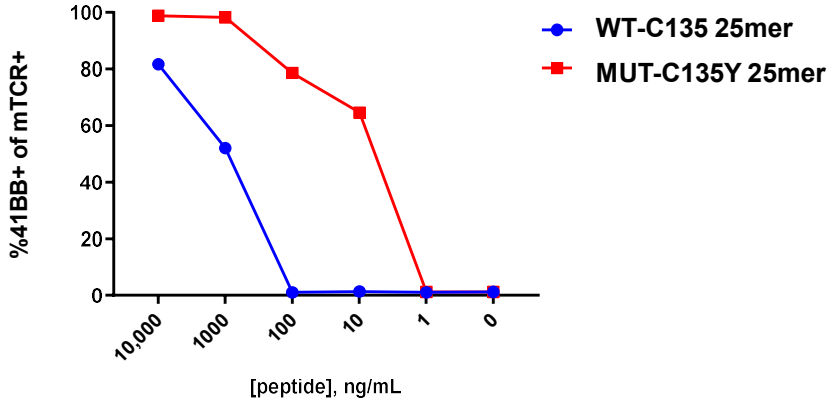
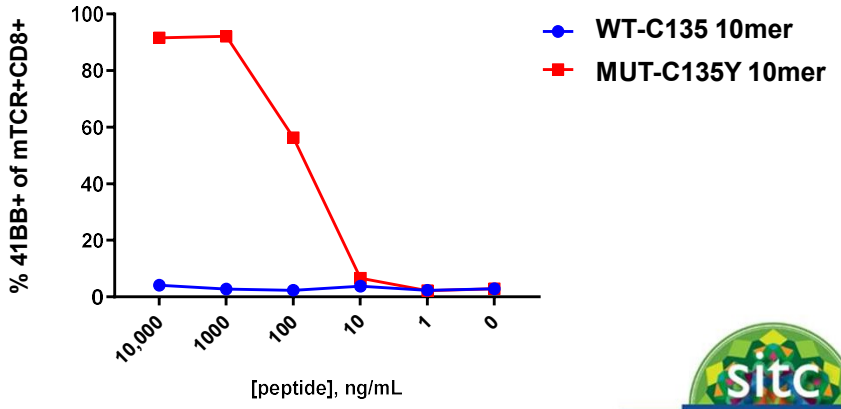
p53 C135Y CD4 TCR-D



p53 C135Y CD8 TCR-B



We have established a library of anti-mutant p53 TILs and TCRs

- T cell responses against mutant p53 in 22% (18/77) of screened patient TILs
  - 46 TCRs (Class I:18, Class II:28)
- *TP53* mutation frequency X HLA frequency = potentially treatable patients
- TCRs we identified can potentially treat 7.3% of all patients with solid cancers.

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→ Autologous TIL treatment against mutant p53

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- Persistence of mutant p53 reactive cells in the peripheral blood at 6 weeks post-treatment was low: median 0.01% (range, 0 to 1.45%)
- High degree of differentiation/exhaustion of the infused TILs
  - 43% PD1 (range, 17 to 64%), 33% TIM3 (range, 2 to 89.5%)

Limitations:

1. Low % of mutant p53 reactive cells
2. High degree of differentiation/exhaustion
3. Mutant p53 reactive cells do not persist

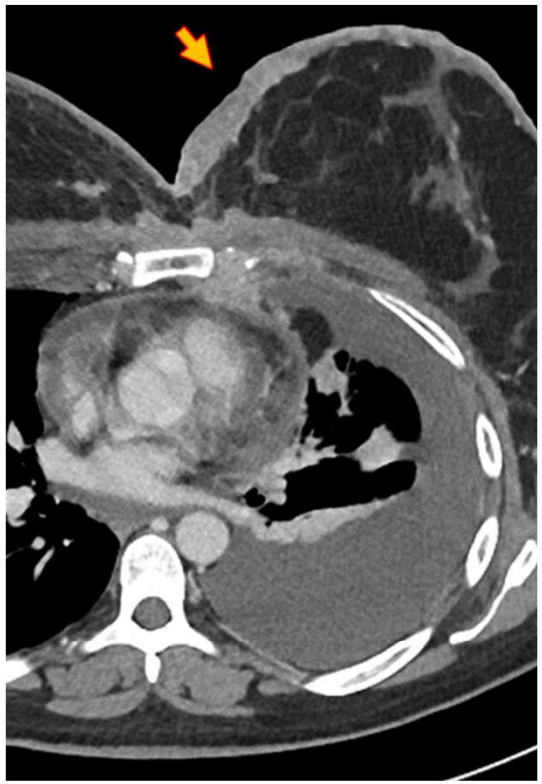
→ Engineer “young” PBLs to express an anti-mutant p53 TCR

Case study of allogeneic “off-the shelf” treatment of patient 4349

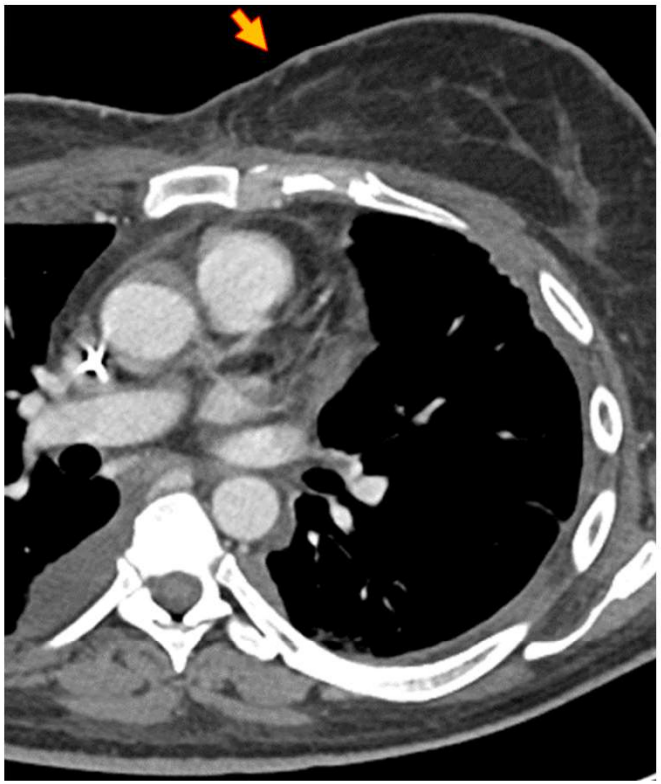
- 48-year-old female with metastatic breast cancer (ER+, PR-, HER2+) with mets to lymph nodes, skin, pericardium and bone
- Progressed through 10 prior chemo-, hormonal, and radiation therapies
- Tumor expressed p53 R175H, HLA A*02
- Adoptive transfer of autologous PBLs retrovirally transduced with an anti-p53 R175H TCR (allogeneic) - restricted by HLA A*02

Patient 4349 showed a tumor regression by 55%

Pre-treatment



Day+ 60



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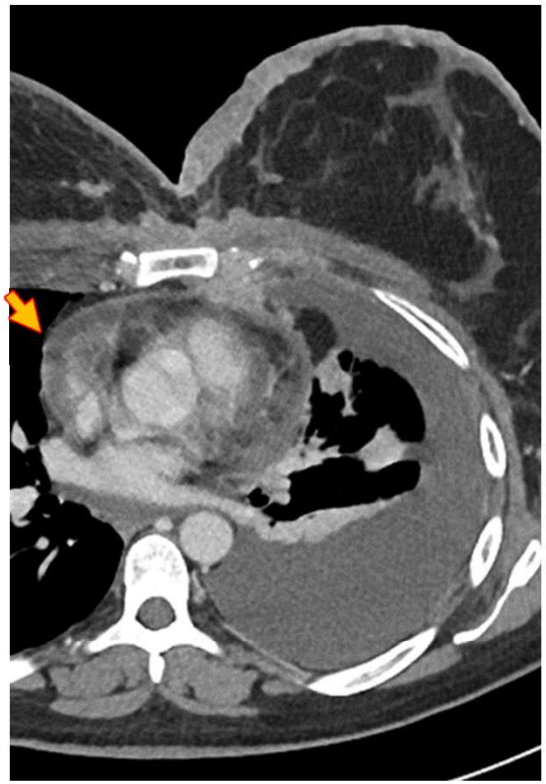

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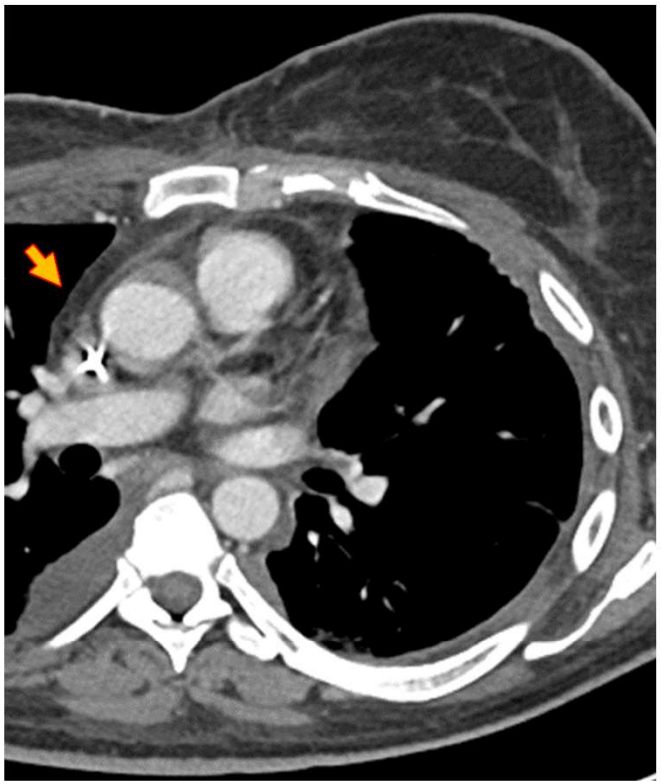

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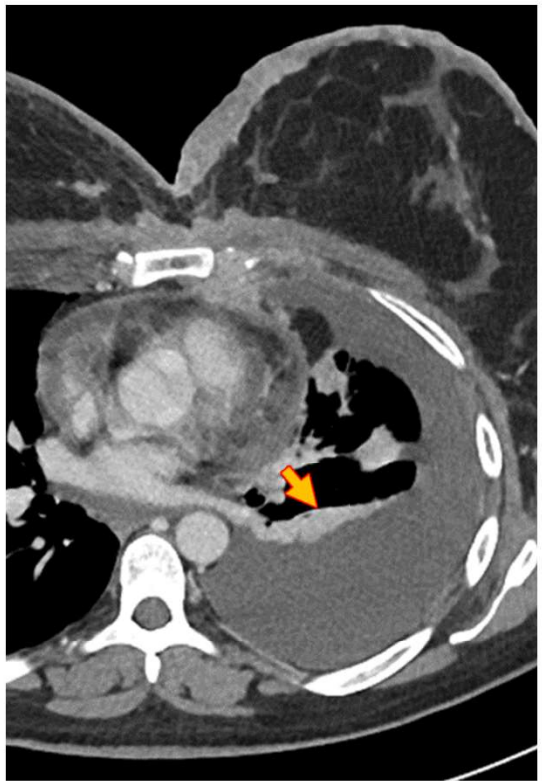

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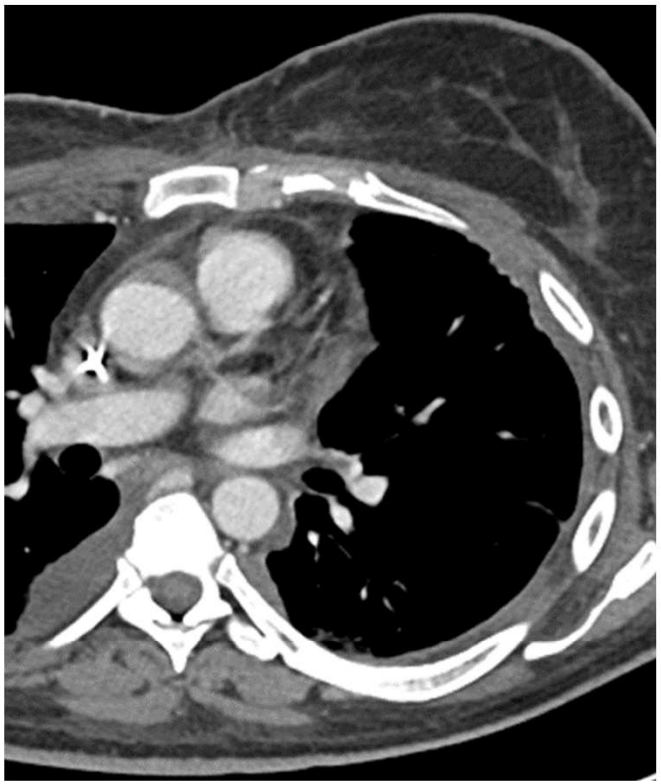
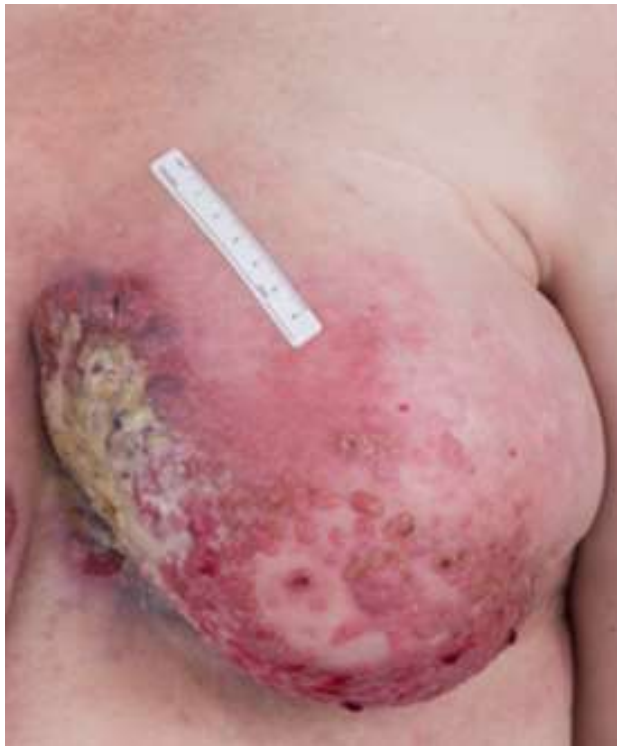


Patient 4349 had a near resolution of the skin lesions

Pre-treatment



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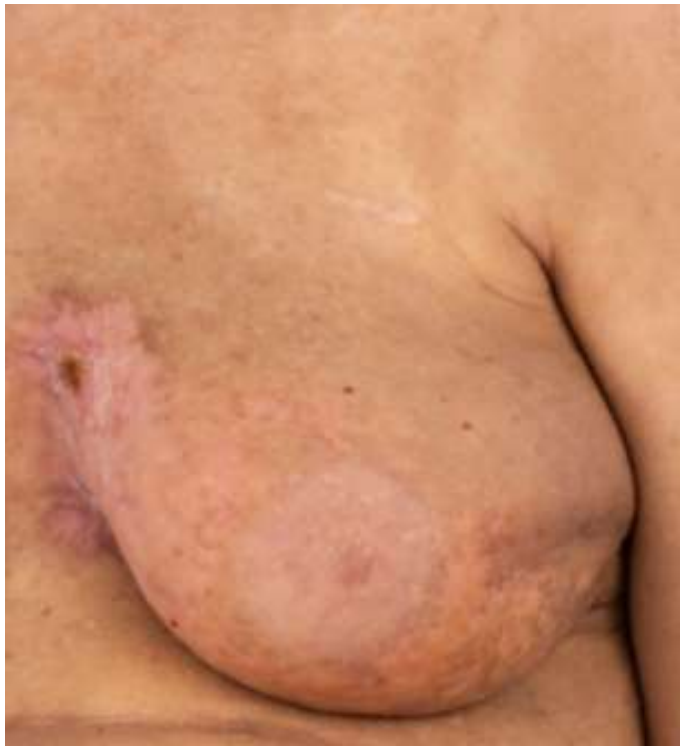


PR for 6 months

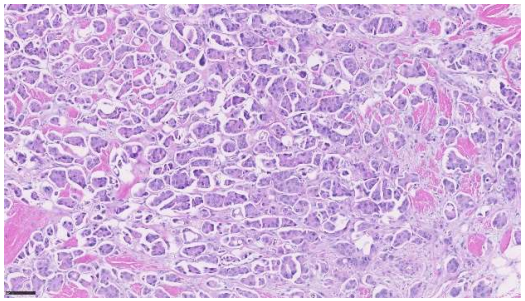
Patient 4349 received higher numbers of mutant p53 reactive cells that persisted longer

	Autologous selected TIL treatments	Anti-p53 R175H TCR engineered PBLs
Number of infused cells (cells/patient)	8.1e10 (2.1e10-1.2e11)	5.3e10
% mutant p53 reactive cells in the infusion product	8.9% (1-51%)	64%
Persistence at 6 weeks post-treatment	0.01% (0-1.45%)	14%
Exhaustion/differentiation	43% PD1 (17-64%) 33% TIM3 (2-89.5%)	13% PD1 22% TIM3

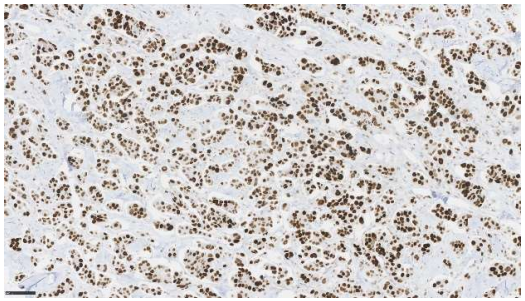
The biopsies of skin lesions show necrosis of tumor cells and infiltration of CD8 T cells following adoptive T cell therapy

Day 0 (Cell therapy)

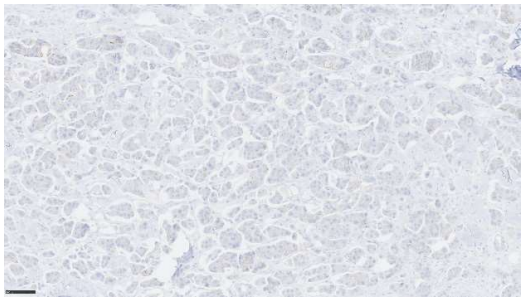
H&E



p53



CD8



Scale bar = 100 µm

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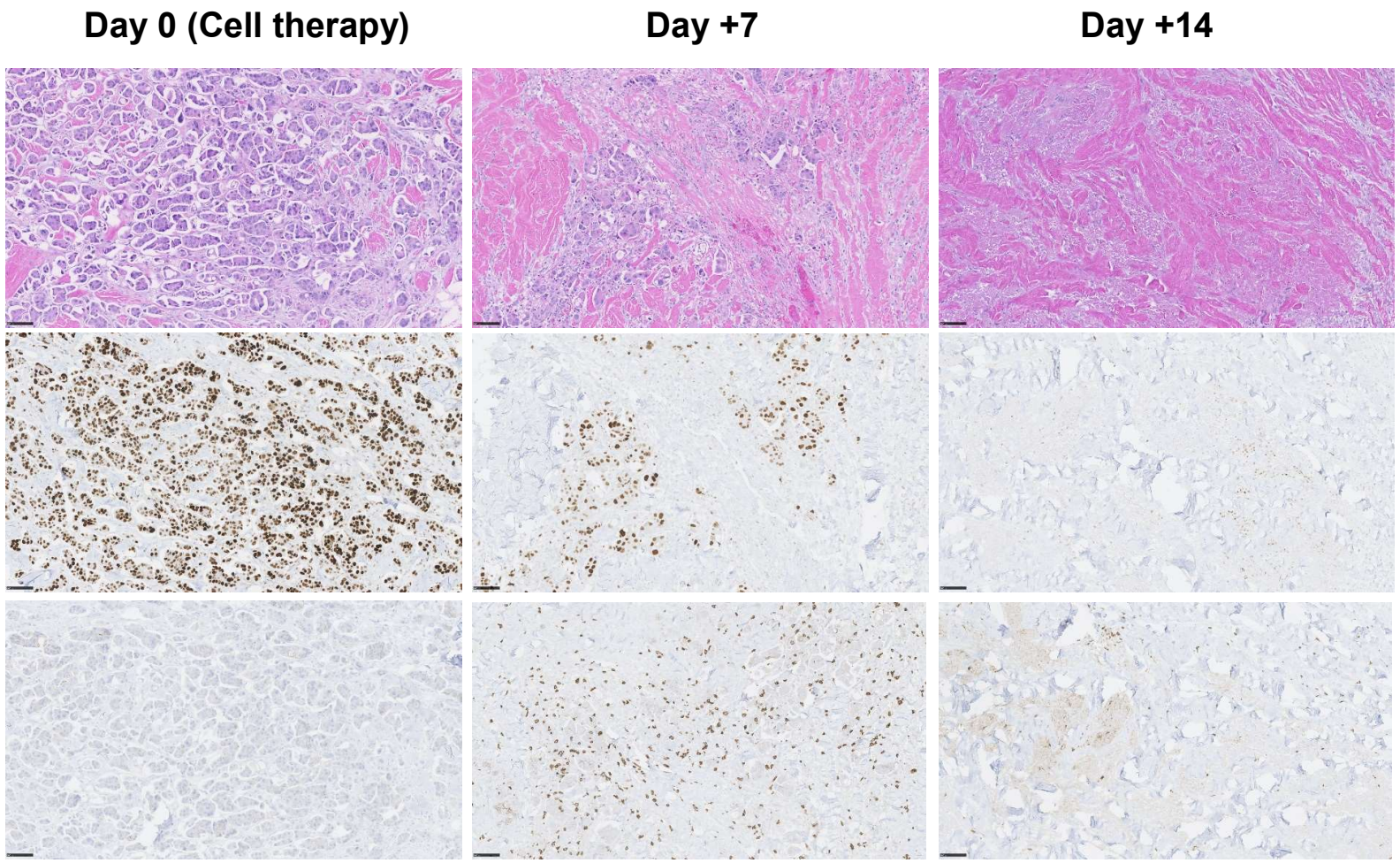
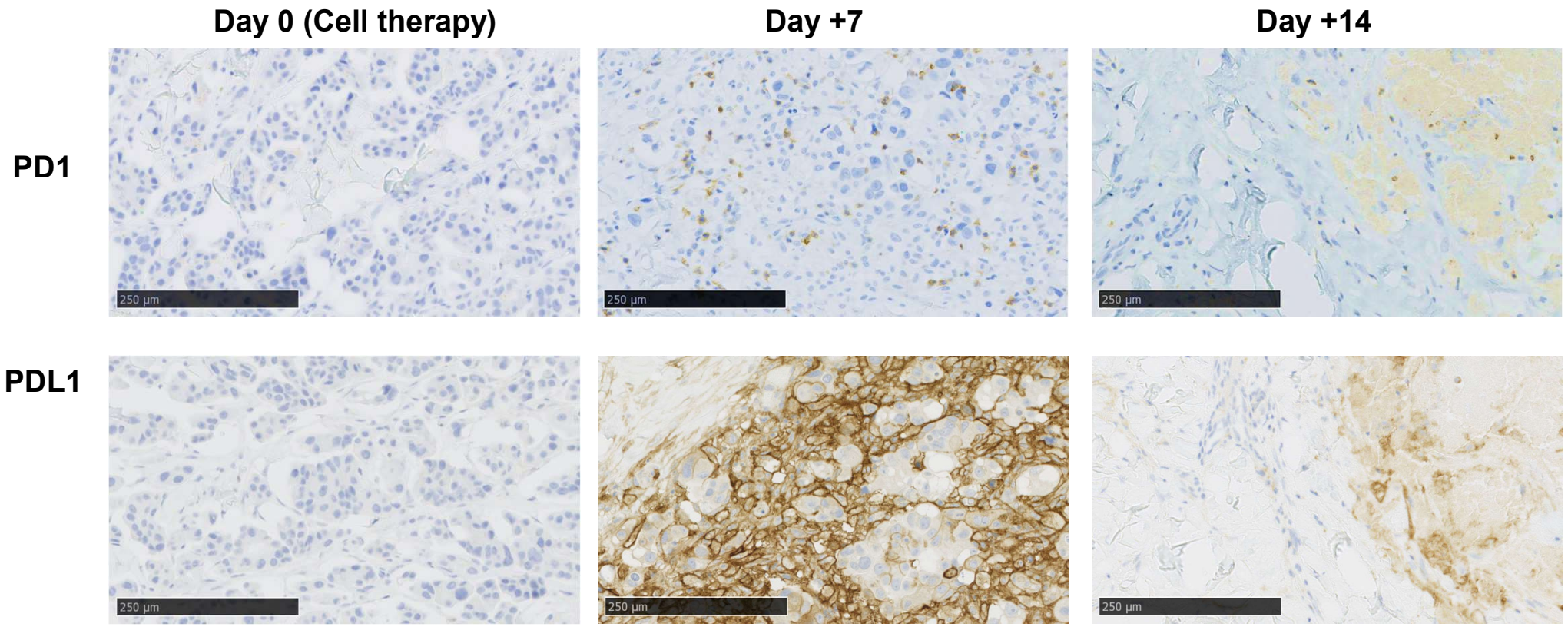


Scale bar = 100 µm

The biopsies of skin lesions show an increase in PD1+ T cells and upregulation of PDL1 following adoptive T cell therapy



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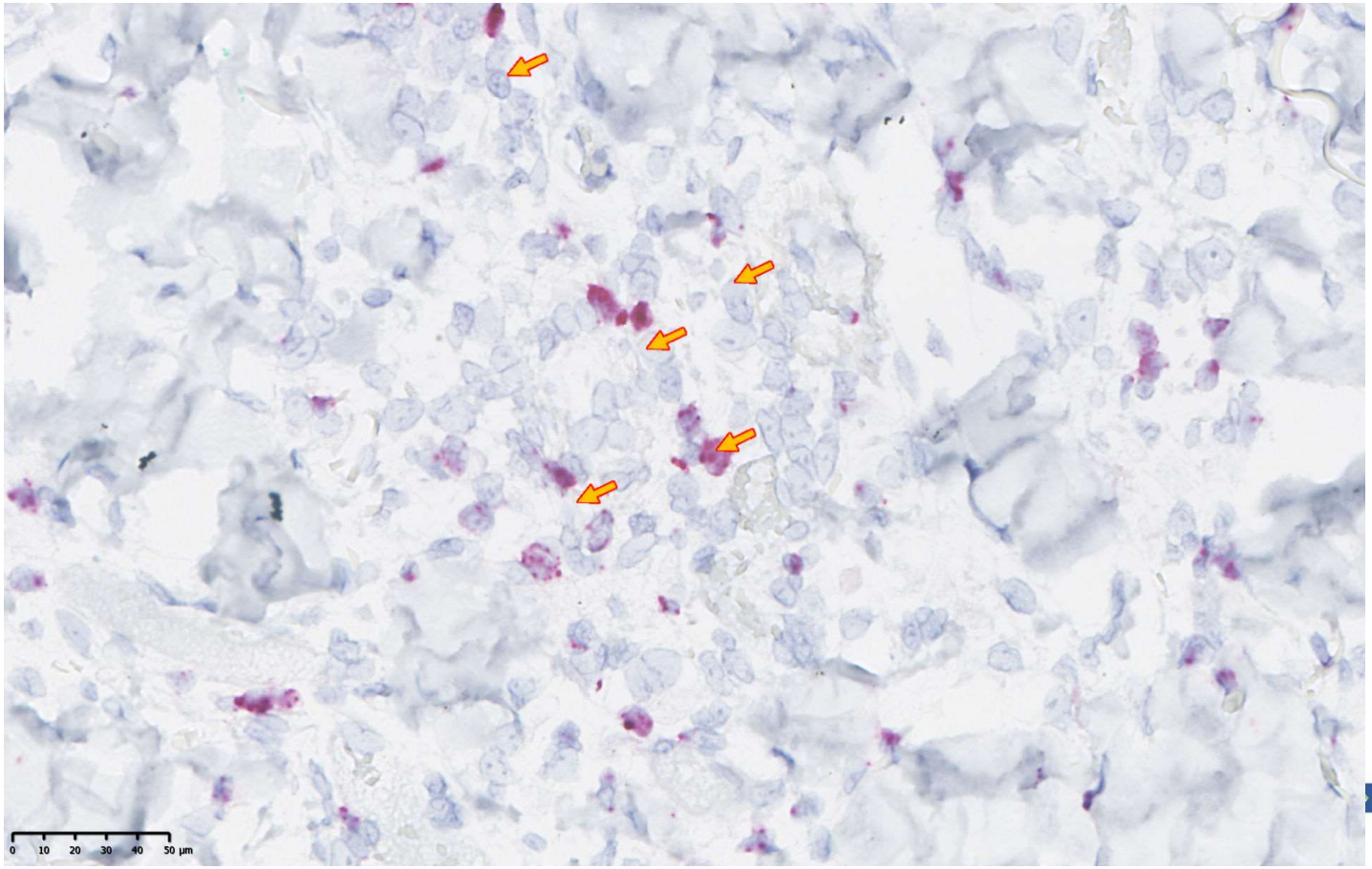
RNA scope targeting MSGV1 3' UTR reveals tumor-infiltrating transduced T cells *in situ*



Scale bar = 50 µm

Summary:

1. **We have built a library of TCRs against shared *TP53* mutations that can be used in an autologous and "off-the-shelf" manner.**

2. **The patients who received selected TIL products generally did not respond likely due to poor persistence and exhausted/differentiated phenotypes of transferred TILs.**

3. **The BrCa patient was treated with a larger number of T cells expressing the allogeneic p53 R175H TCR and had a PR with a better persistence of mTCR+ T cells.**

Future plan:

- **We will develop a clinical protocol to treat additional patients with the anti-mutant p53 TCRs.**

- **We are considering combining an anti-PD1 therapy with the TCR-engineered PBL therapy.**