

## BACKGROUND

Adoptive cell therapy (ACT) is an advanced form of therapy that uses the patient's own immune system to combat cancer. It is currently unknown whether TILs from panNETs can be expanded, and whether they can reach the numbers needed for adoptive infusion in patients.

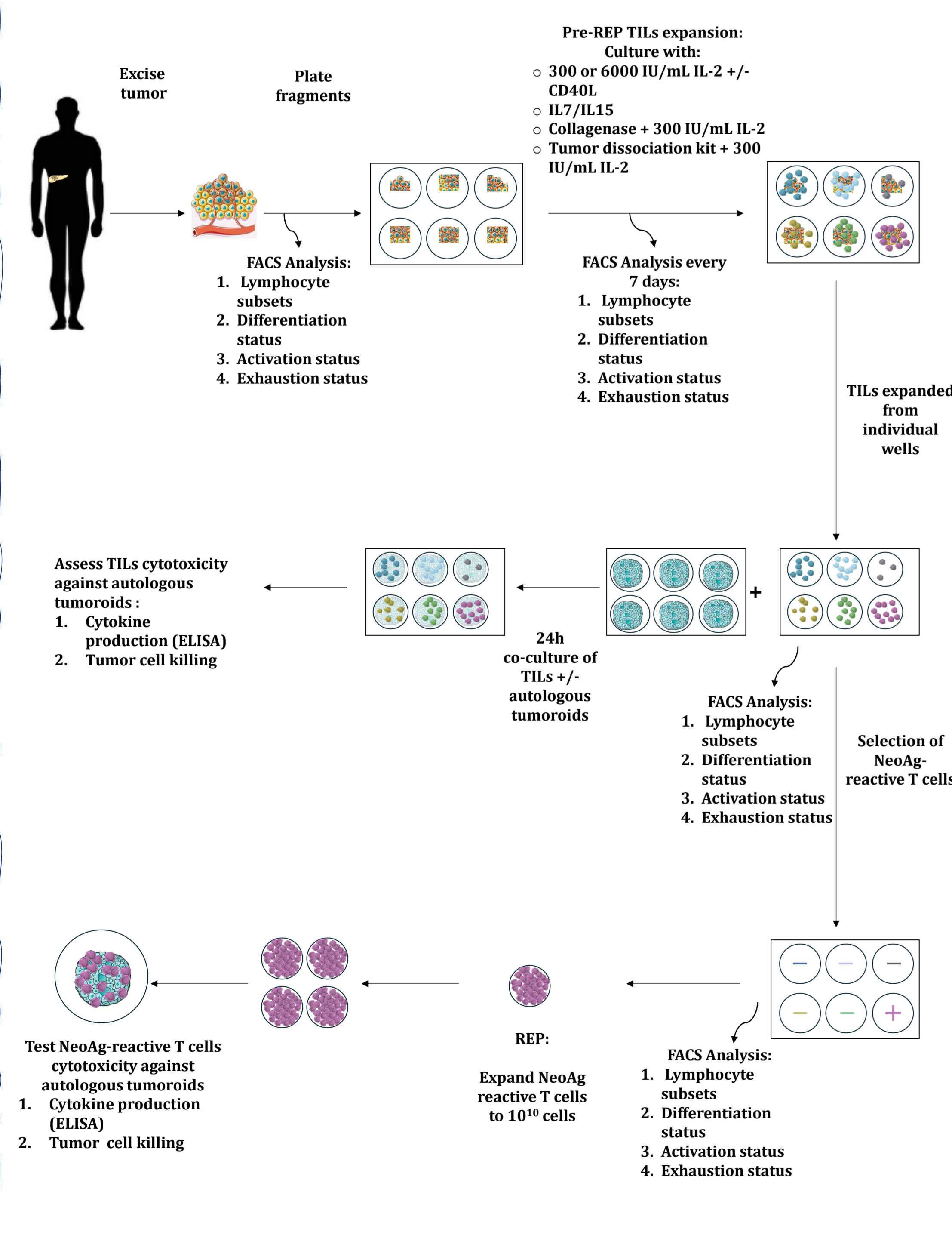
## INCLUSION CRITERIA AND PATIENT DEMOGRAPHICS

### Inclusion criteria:

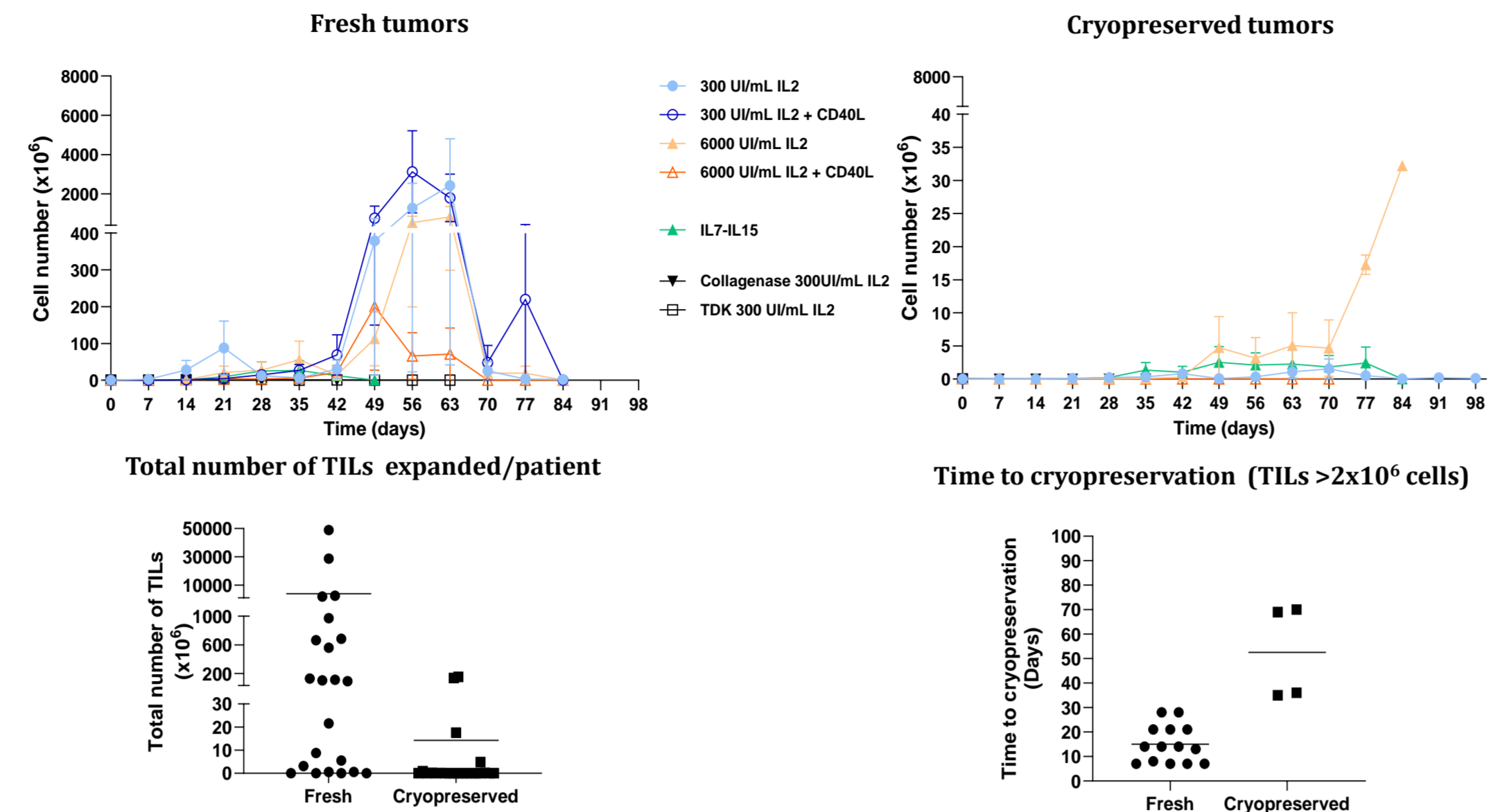
- Patients with G1/G2/G3 well differentiated panNETs metastatic to the liver
- Any line of therapy
- Fresh or cryopreserved samples of panNET liver metastasis available (at least 10 fragments 3 mm<sup>3</sup> each)

Characteristics	Number of patients (n=30)
<b>Age (years)</b>	
Median at diagnosis	69
Median at time of TIL resection	62
<b>Gender</b>	
Male	13 (43%)
Female	17 (57%)
<b>Hereditary syndrome</b>	
Yes	1 (3%)
No	29 (97%)
<b>Grading</b>	
G1	8 (27%)
G2	19 (63%)
G3	3 (10%)
<b>Vascular invasion</b>	
Yes	7 (23%)
No	9 (30%)
Endometrial	14 (47%)
<b>Perineural invasion</b>	
Yes	3 (10%)
No	9 (30%)
Endometrial	18 (60%)
<b>Functional status</b>	
Functioning	6 (20%)
Non-functioning	24 (80%)
Median interval from diagnosis to TIL resection (months)	7 (1-175)
Median times of treatment before metastasis resection	1 (0-2)

## METHODS

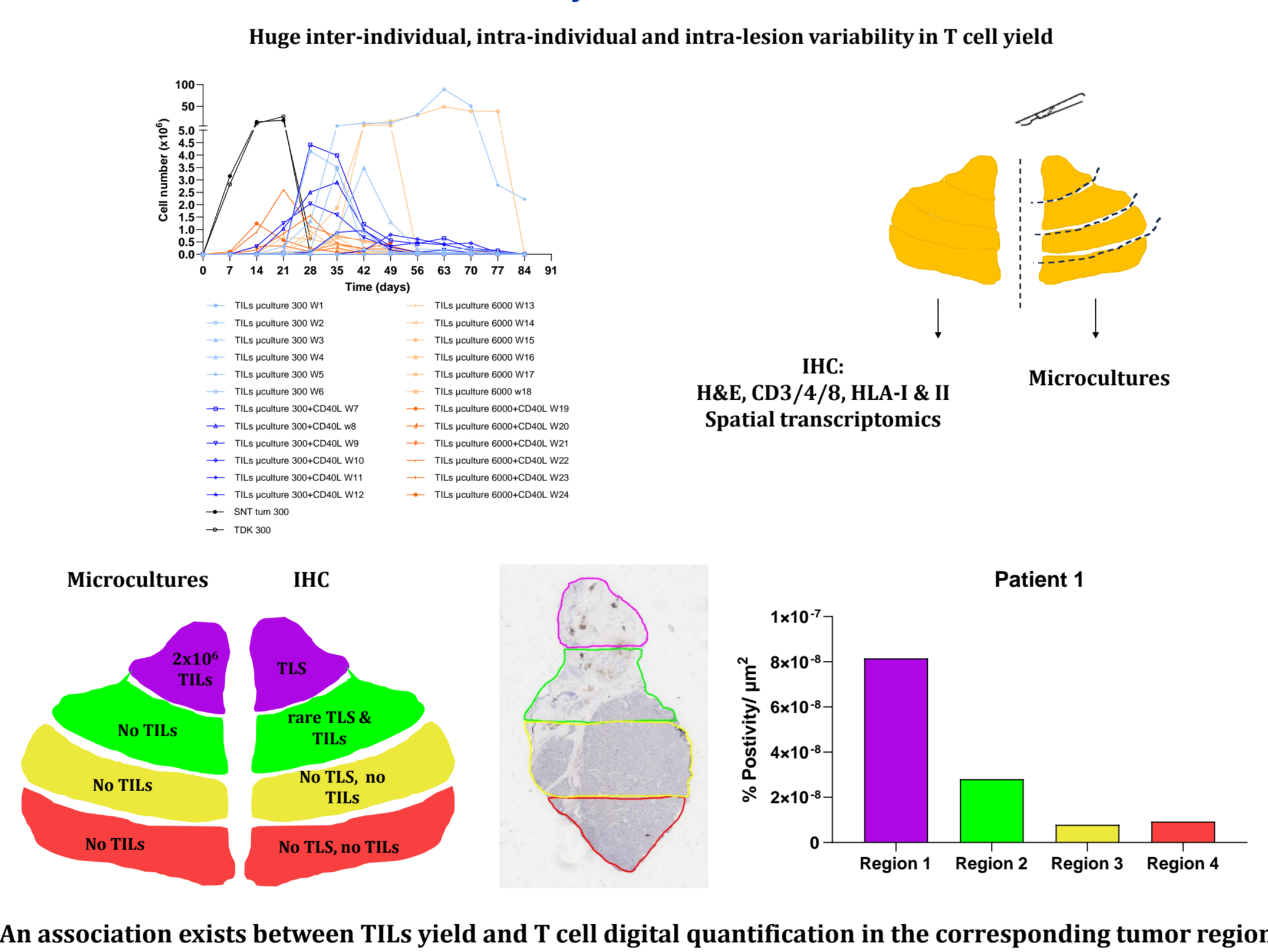


### Can we expand TILs from panNET liver metastases?



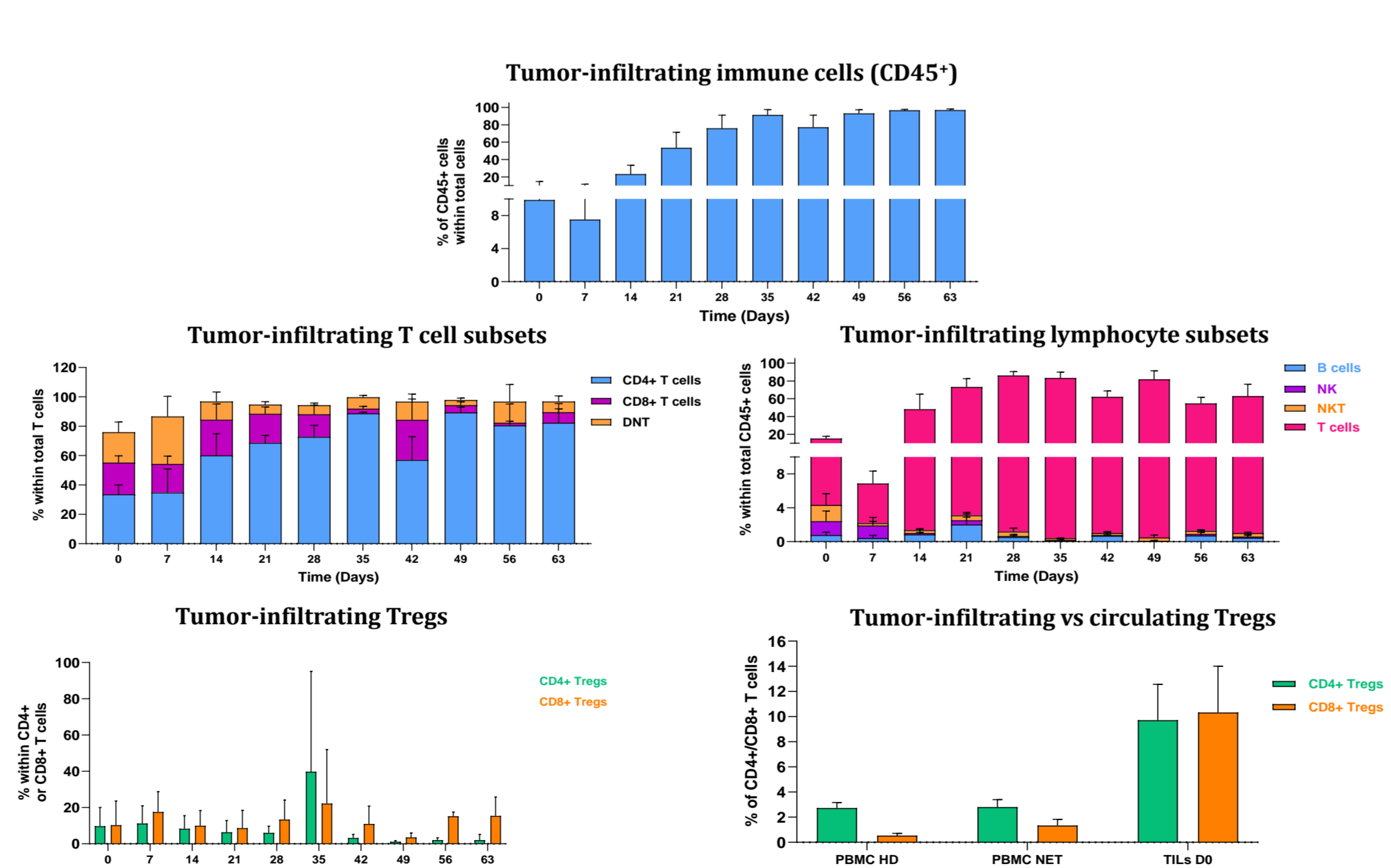
- TILs can be successfully expanded from panNET liver metastasis: Fresh tumors >>> cryopreserved tumors; Microcultures >>> tumor digests; IL2 >>> IL7/15
- No difference in the growth rate of TILs on the basis of: tumour grading; Ki-67, vascular and perineural invasion or prior therapies
- Pre-REP sufficient numbers of TILs (>2x10<sup>6</sup> cells) in 18/30 tumours (60%):
  - 14/20 fresh samples (70%)
  - 4/10 cryopreserved samples (40%)

### Can we dissect the intratumor heterogeneity to increase the T cell yield?



An association exists between TILs yield and T cell digital quantification in the corresponding tumor region

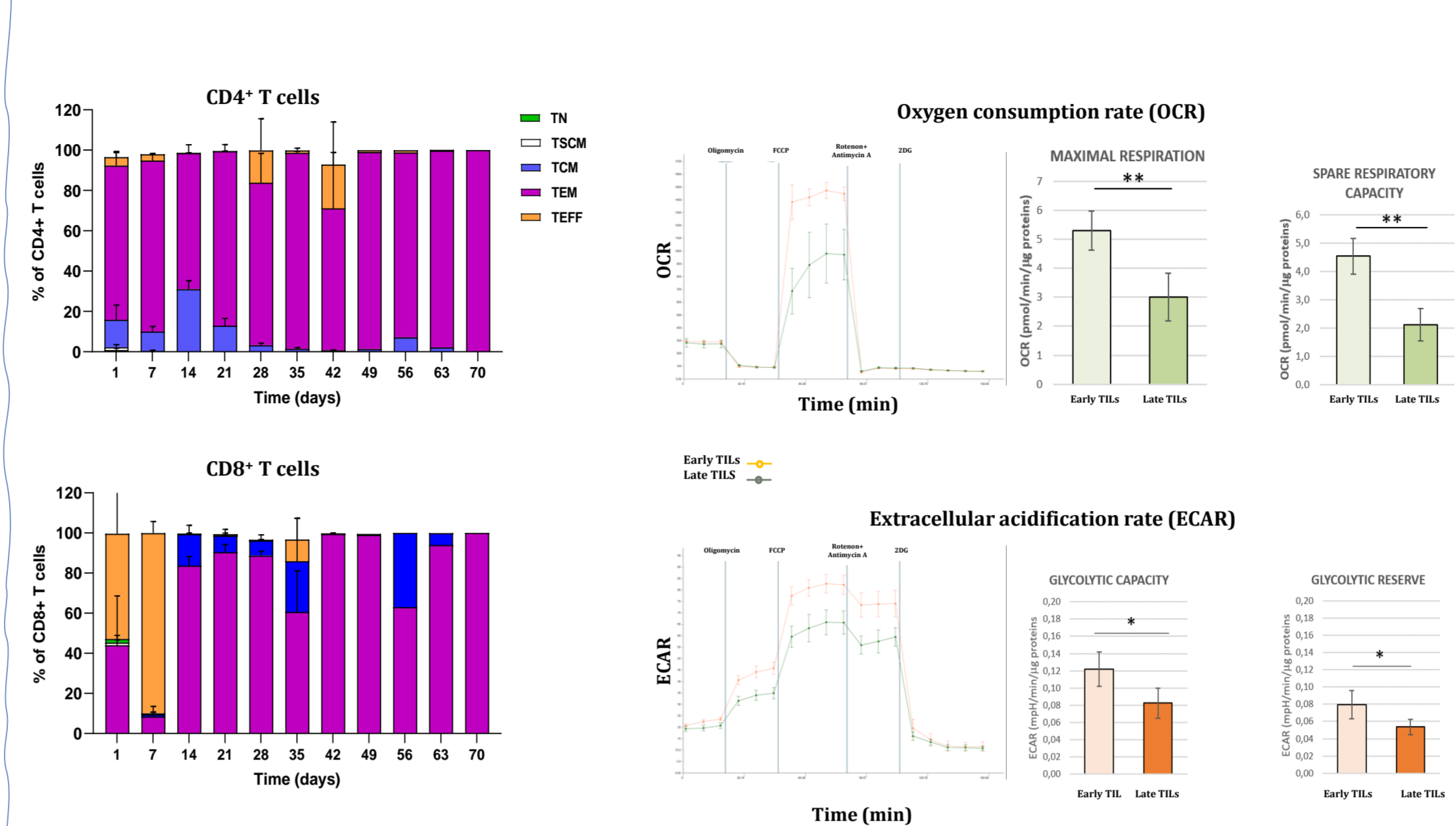
### What are the TIL subsets isolated from panNET liver metastases?



- Immediately after tumor resection: mainly T cells and NK cells, rare B cells; with time, only T cells
- T cells: mainly CD8<sup>+</sup> T cells immediately after resection, then mostly CD4<sup>+</sup> T cells
- % Tregs similar to that observed in other cancers: Breast (20%) ovarian (23%) RCC (10%) HCC (15%)
- Tregs accumulate within panNETs liver metastases

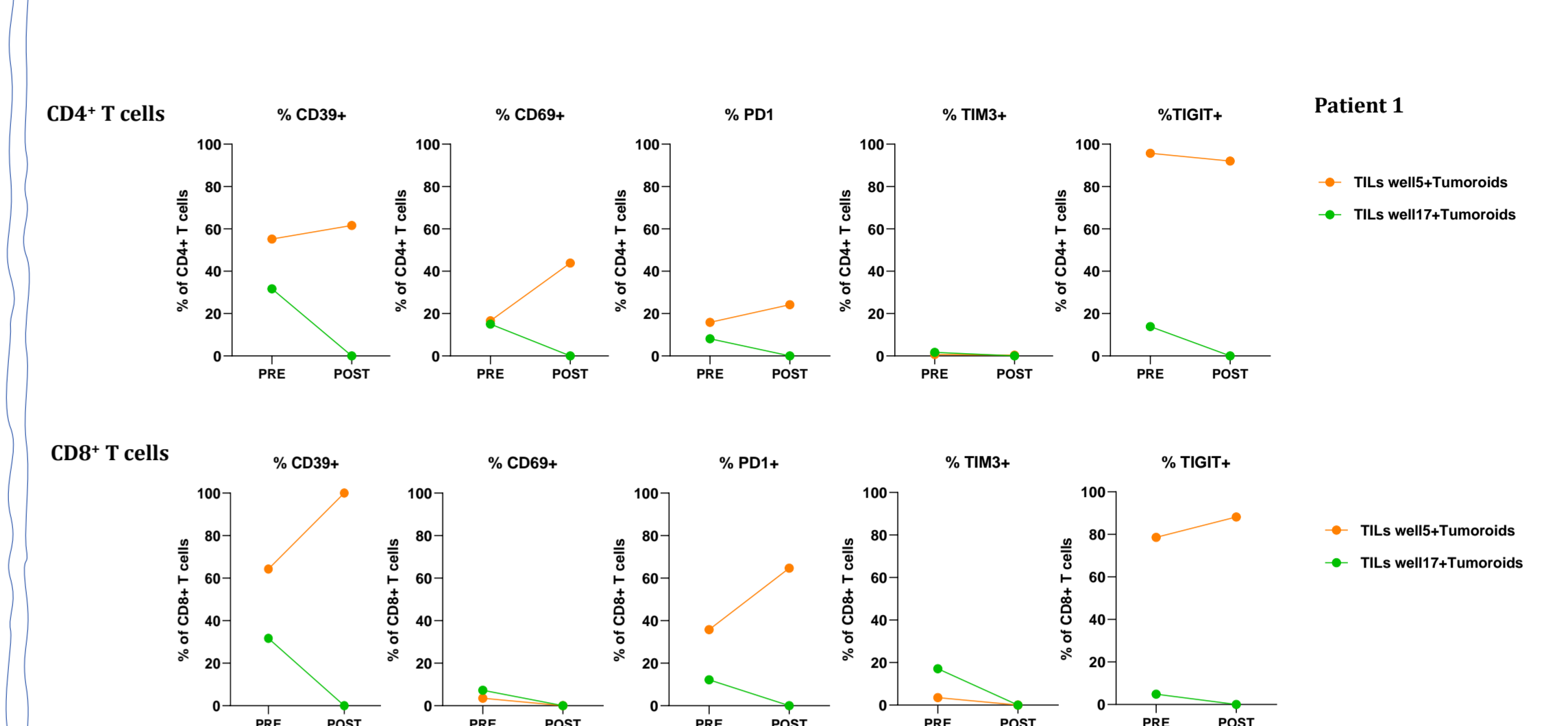
## RESULTS

### What is the differentiation and metabolic status of TILs over time?



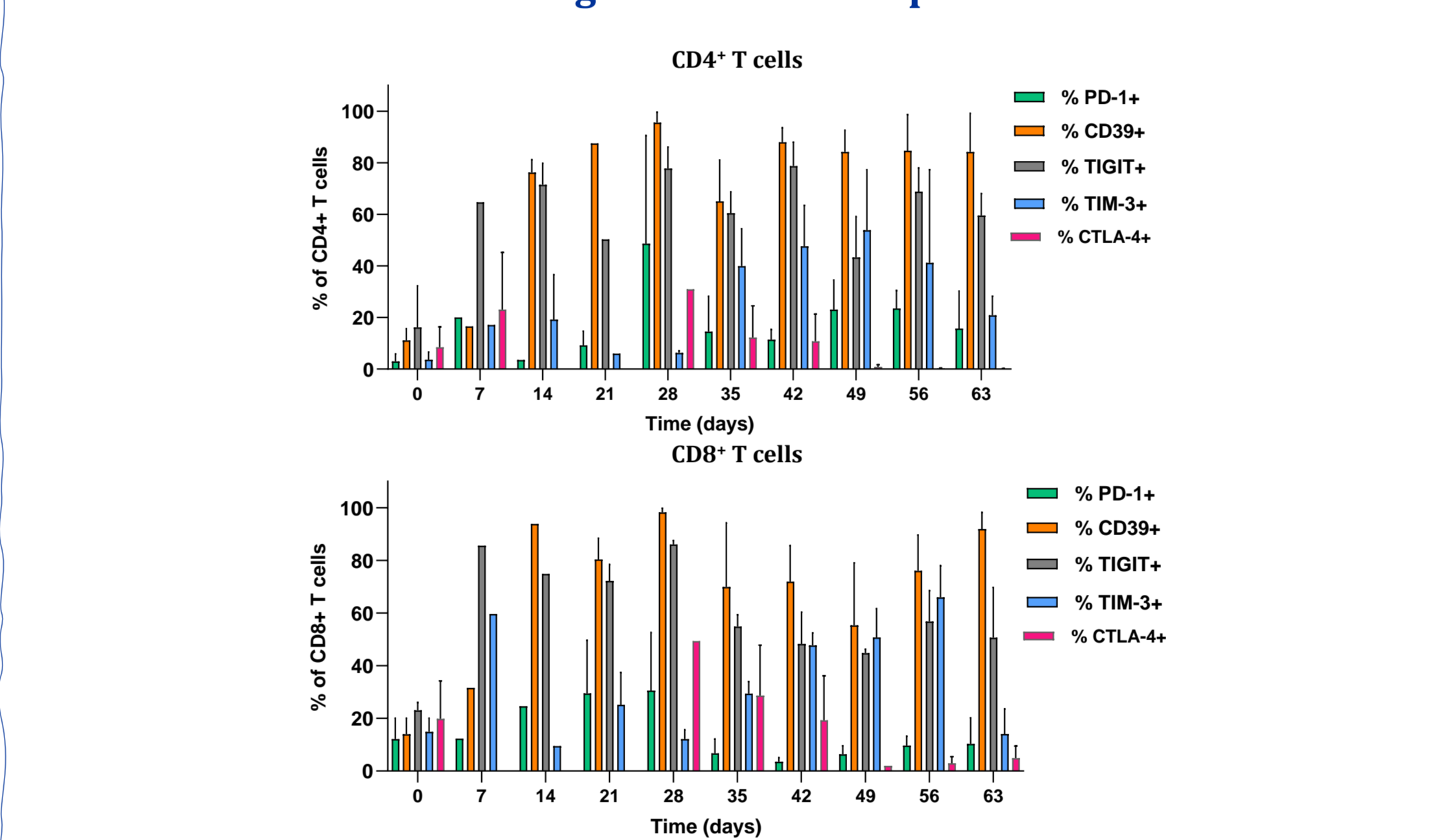
- CD4<sup>+</sup> T cells: differentiation status is consistent overtime
- CD8<sup>+</sup> T cells: switch between the first and second week of culture: TEFF → TEM
- Young TILs show better respiratory capacity and glycolytic capacity than old TILs

### Does the exposure to autologous tumouroids affect TILs phenotype?



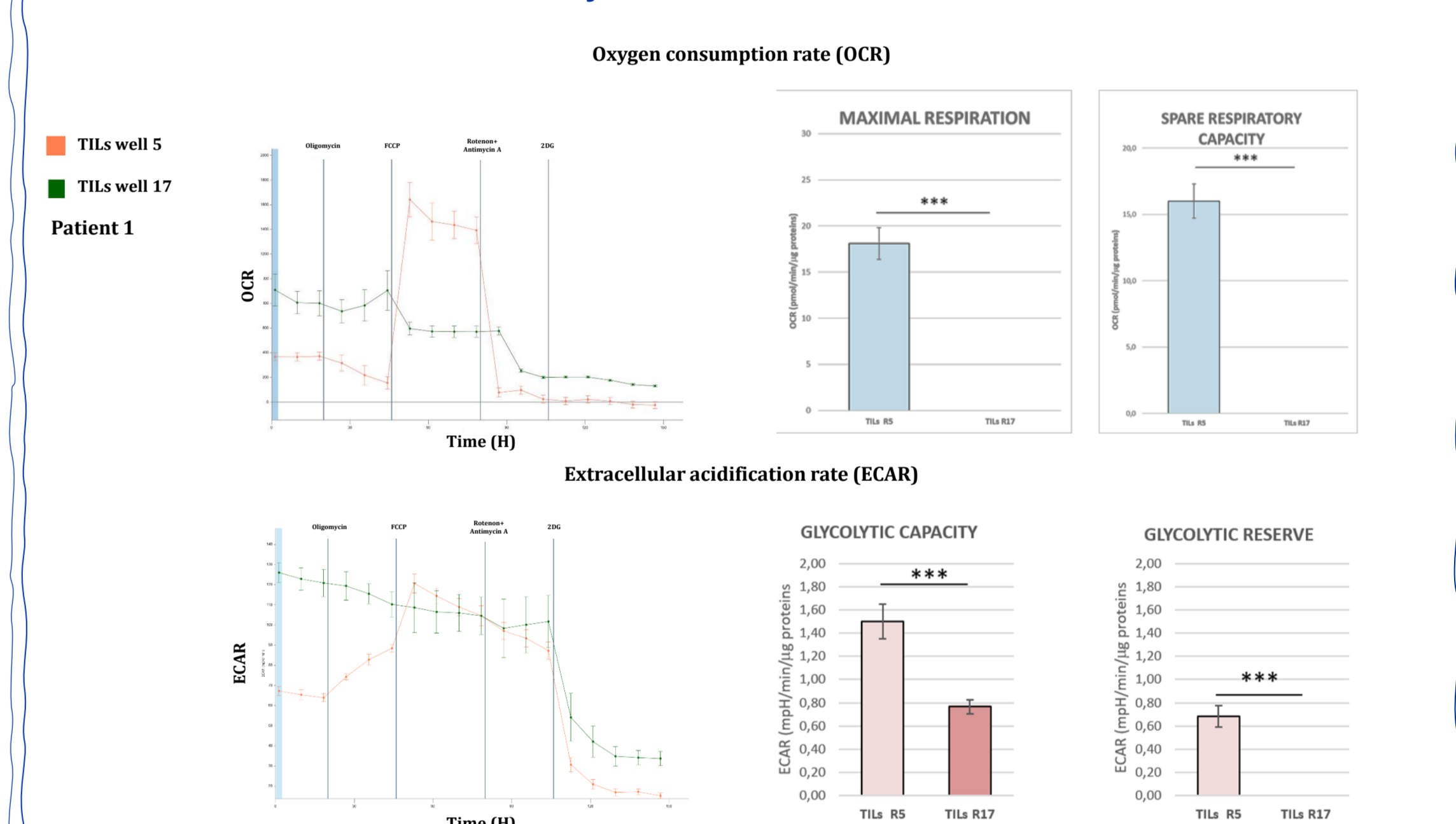
- The activation and exhaustion phenotype of TILs with high anti-tumor activity and TILs with no anti-tumor activity is different before coculture
- Exposure to autologous tumouroids induces phenotypic changes consistent with lymphocyte activation in TILs showing anti-tumor activity

### Can we unleash the antitumor activity of TILs from panNET liver metastases through immune checkpoint inhibition?



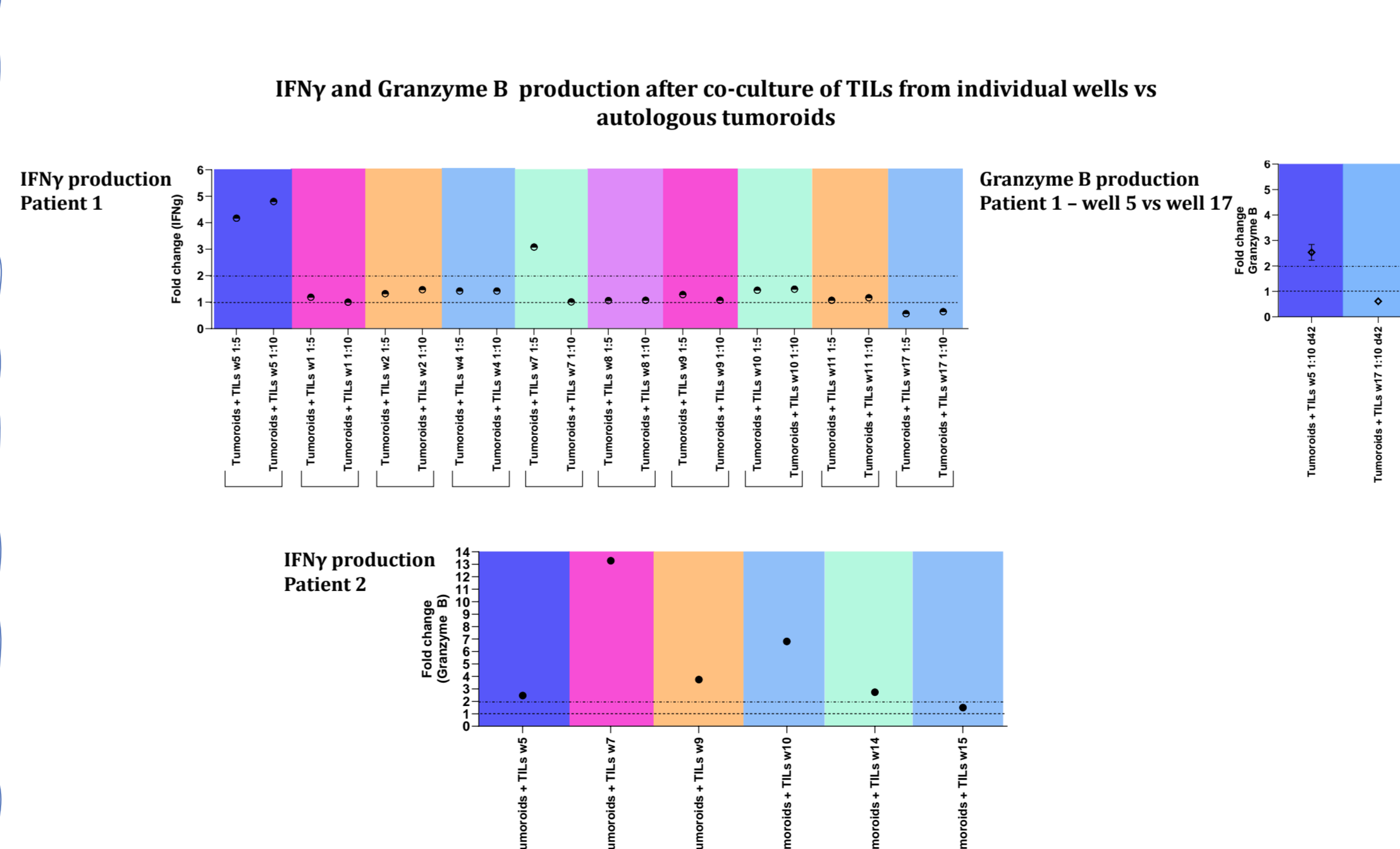
- Immediately after tumor resection, low expression of immune checkpoint
- Low expression of PD-1 and CTLA-4 over time
- The expression of CD39, TIGIT and TIM3 tends to increase over time in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells

### Do tumor-reactive and bystander TILs have similar metabolism?



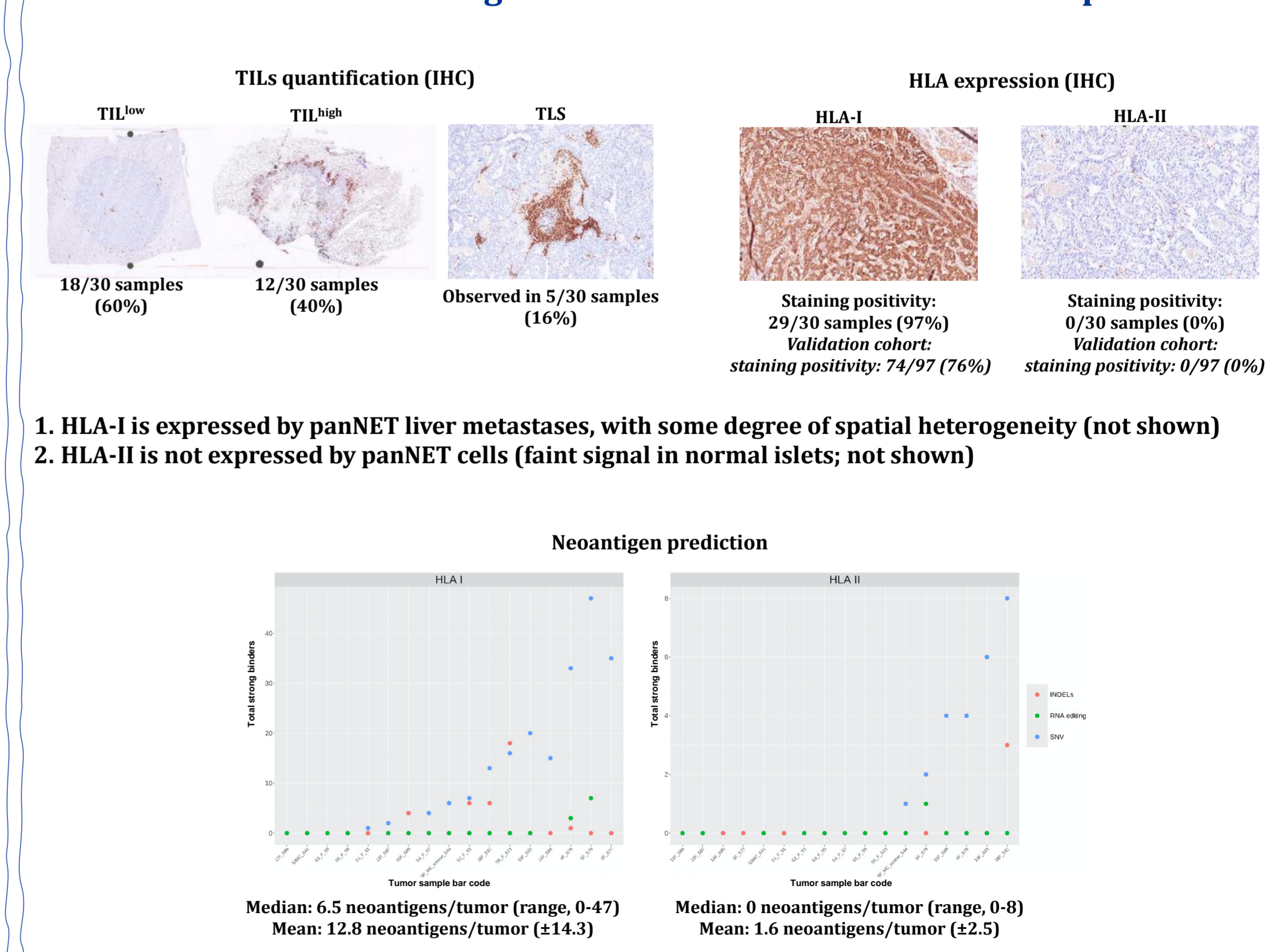
TILs showing anti-tumor reactivity have better respiratory and glycolytic metabolism

### Do TILs expanded from different regions of the tumor exert similar anti-tumor activity?



TILs exert heterogeneous anti-tumor activity:  
 → TIL cultures comprise both anti-tumor reactive T cell clones and bystander lymphocytes  
 → Isolation and expansion of tumor-reactive TILs may enhance the efficacy of TILs adoptive transfer

### What are the immunological characteristics of included samples?



- HLA-I is expressed by panNET liver metastases, with some degree of spatial heterogeneity (not shown)
- HLA-II is not expressed by panNET cells (faint signal in normal islets; not shown)

## CONCLUSIONS

- TILs can be successfully expanded from panNET liver metastasis to reach clinically meaningful numbers (success rate ~60%)
  - Differentiation and metabolic status of CD8<sup>+</sup> T cells changes overtime in *in vitro* cultures
    - Huge inter-individual, intra-individual and intra-lesion variability in T cell yield
- Huge intra-lesion variability of anti-tumor activity of TILs: presence of both tumor-reactive and bystander TILs within the same tumor
  - Isolation and expansion of tumor-reactive TILs may enhance the efficacy of TILs adoptive transfer.