

Persistence of cryopreserved tumor-infiltrating lymphocyte product lifileucel (LN-144) in C-144-01 study of advanced metastatic melanoma

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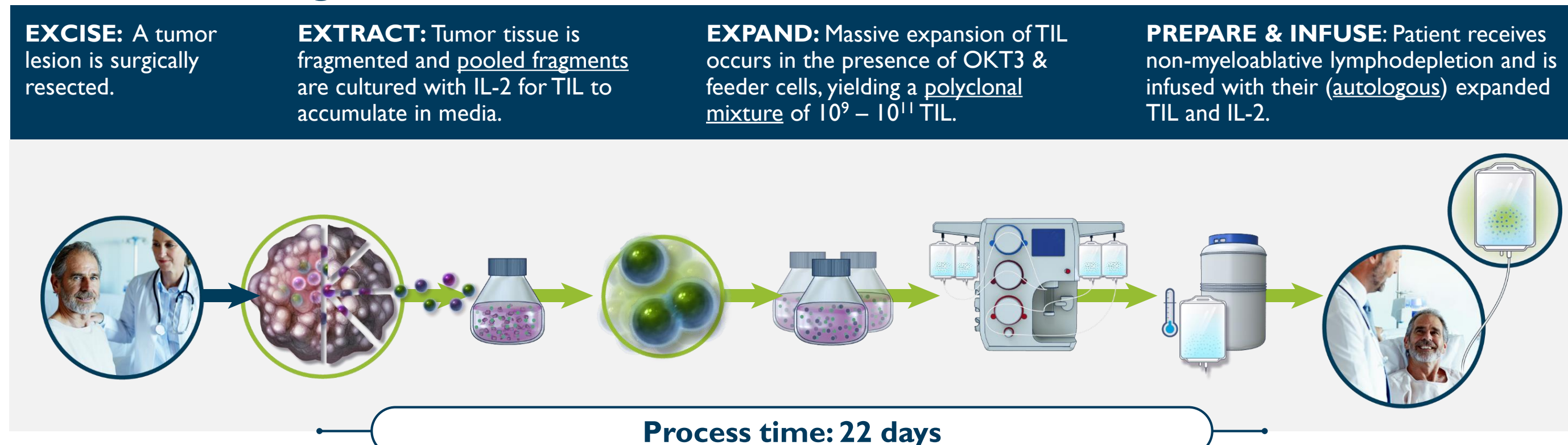
BACKGROUND & INTRODUCTION

- Adoptive cell transfer utilizing tumor-infiltrating lymphocytes (TIL) is recognized as an effective treatment in metastatic melanoma and other solid tumors eliciting durable and complete responses in heavily pretreated patients, presumably by targeting somatic mutations specific to each tumor.¹
- C-144-01 (NCT02360579) is an ongoing Phase 2 multicenter study:
 - Investigational agent: autologous TIL (lifileucel; LN-144)
 - Patient population: unresectable metastatic melanoma who have progressed on checkpoint inhibitors and BRAF/MEK inhibitors (if BRAF mutated)
 - Central manufacturing of cryopreserved TIL, 22-day duration process
- Patients with anti-PD-1-relapse or refractory advanced melanoma have been treated with lifileucel; an objective response rate of 38% (n=47; 1 CR, 17 PR+uPR) has been reported.²
- Here, we analyzed the composition of the initial TIL products and the T cells circulating 42 days post-infusion (D42) to uncover a potential link between clonal diversity, TIL *in vivo* persistence, and anti-tumor activity.
- Since TIL products are preparations of polyclonal autologous T cells, each T cell clone expresses a unique T cell receptor (TCR) that can be identified by its complementary determining region 3 (CDR3).

References

- Rosenberg et al. CCR 2011
- SITC Nov2018

Figure 1. A 22-day process results in TIL products of non-selected polyclonal autologous T cells

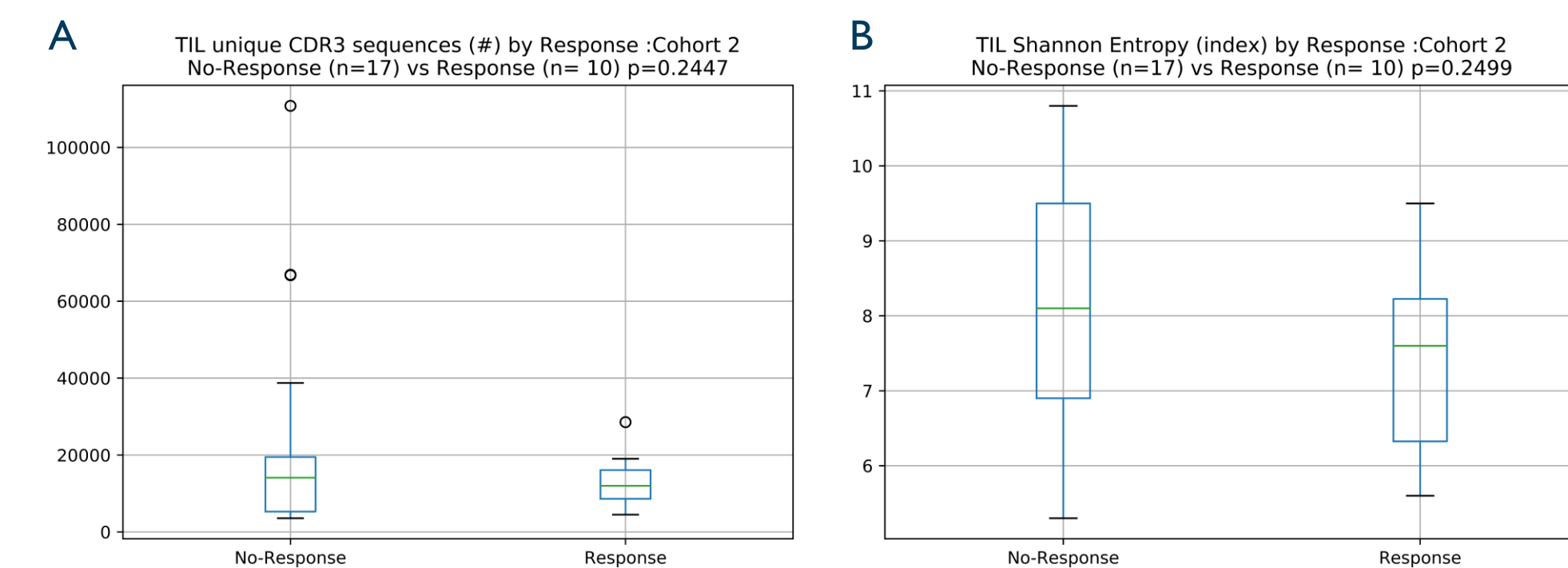


MATERIALS & METHODS

- TIL products corresponding to 27 patients who underwent resection for the purpose of TIL generation and their matching D42 PBMC samples were analyzed.
- Total RNA was extracted, using Qiagen's RNeasy[®] Mini Kit protocol (Germantown, MD).
- CDR3 were amplified and sequenced by Next Generation Sequencing, using iRepertoire technology (Huntsville, AL).
- Custom python scripts were used to identify CD3 clones of interest and perform statistical analyses and generate figures.

RESULTS

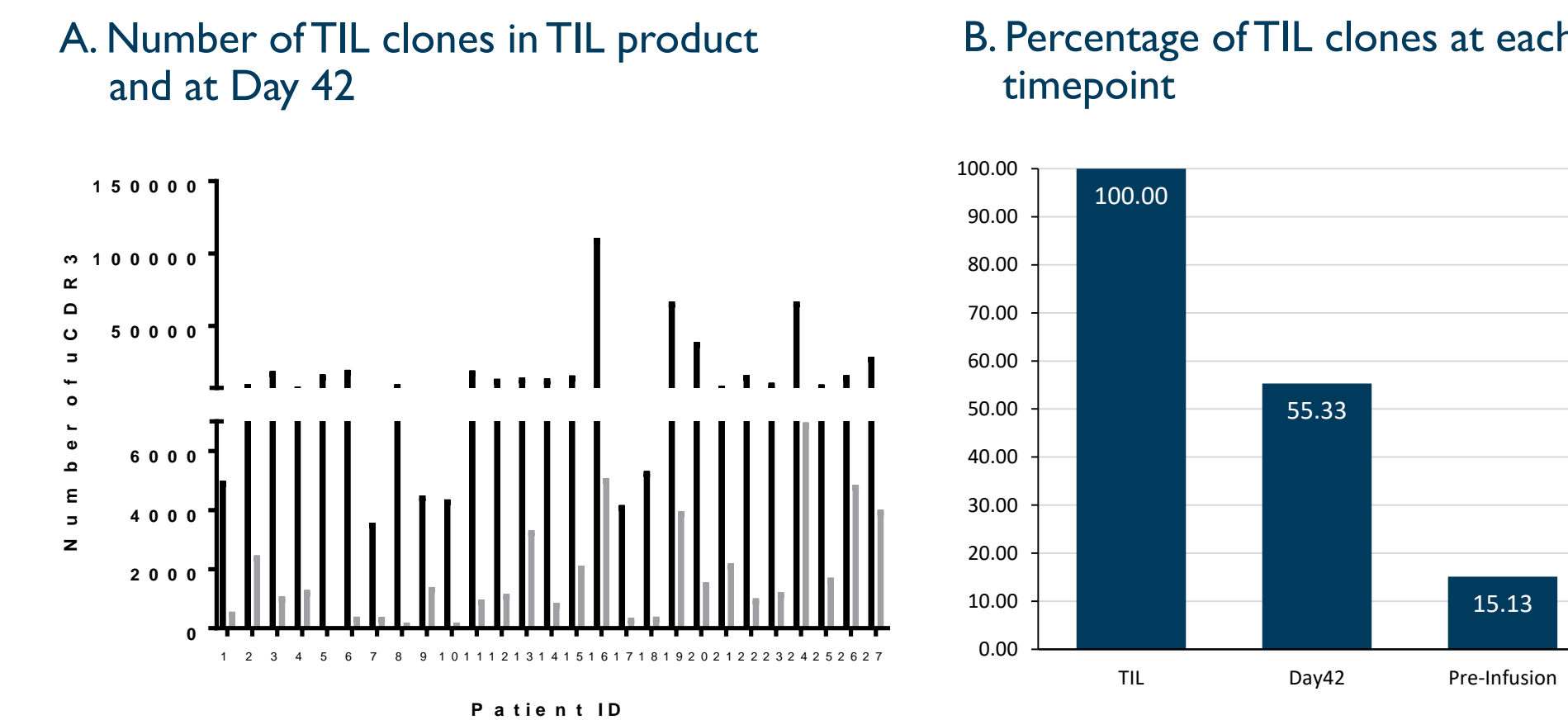
Figure 2. No association between the number or diversity score of TCR clonotypes and clinical response



Assessment of diversity of TIL products. Post sequencing, the unique CDR3 sequence (uCDR3) counts were determined and plotted (A). For the same samples, the Shannon Entropy was also calculated and plotted (B). The median for both variables are indicated with the green line inside the boxplots. The groups are based on the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) assessments, with the 'Response' group including subjects with a partial or complete response and the 'No-Response' groups including subjects with stable or progressive disease. The average number of uCDR3 was 17544 [3574-110797] across TIL products, with Shannon diversity indexes varying from 2.7 to 10.8.

Anti-tumor T cells are present in the TIL infusion products of low and high diversity, confirming that bulk preparations can recover relevant TIL without prior knowledge of tumor antigens.

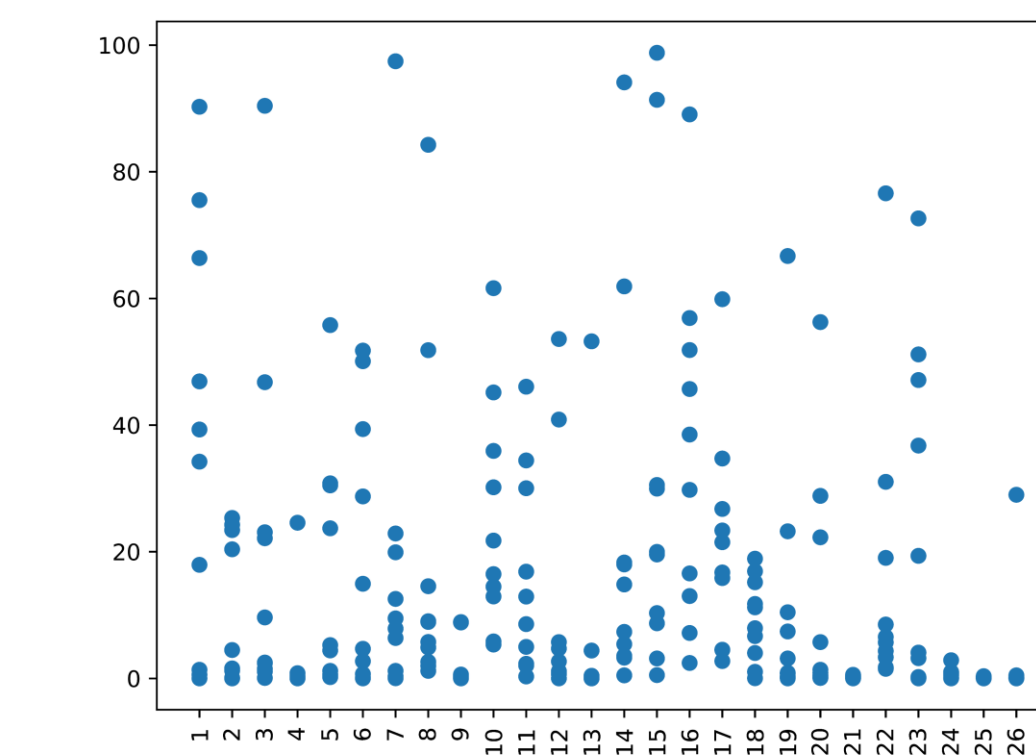
Figure 3. Persisting TIL in 100% of patients at D42



Evaluation of shared clones between TIL products and D42 PBMC. Numbers of shared CDR3s were determined by measuring the number of CDR3 clones detected in circulation at D42 that were also present in the corresponding TIL product. Shared CDR3s were detected in all PBMC samples analyzed, at levels varying from 28 to >6,900 clones. They are plotted as grey bars next to the initial CDR3 numbers plotted as black bars (A). Clone frequencies were calculated for TIL, D42, and pre-infusion samples to assess whether TIL clones pre-existed in patient blood (n=15). Results are shown as means in a bar chart of shared clone frequency sums. Of the 29,745 shared clones identified, 69% (20,480) were not detectable pre-infusion (B).

In vivo persisting TIL clones are not present or present at much lower frequency in the blood pre-TIL infusion, suggesting tumor antigen-specific expansion.

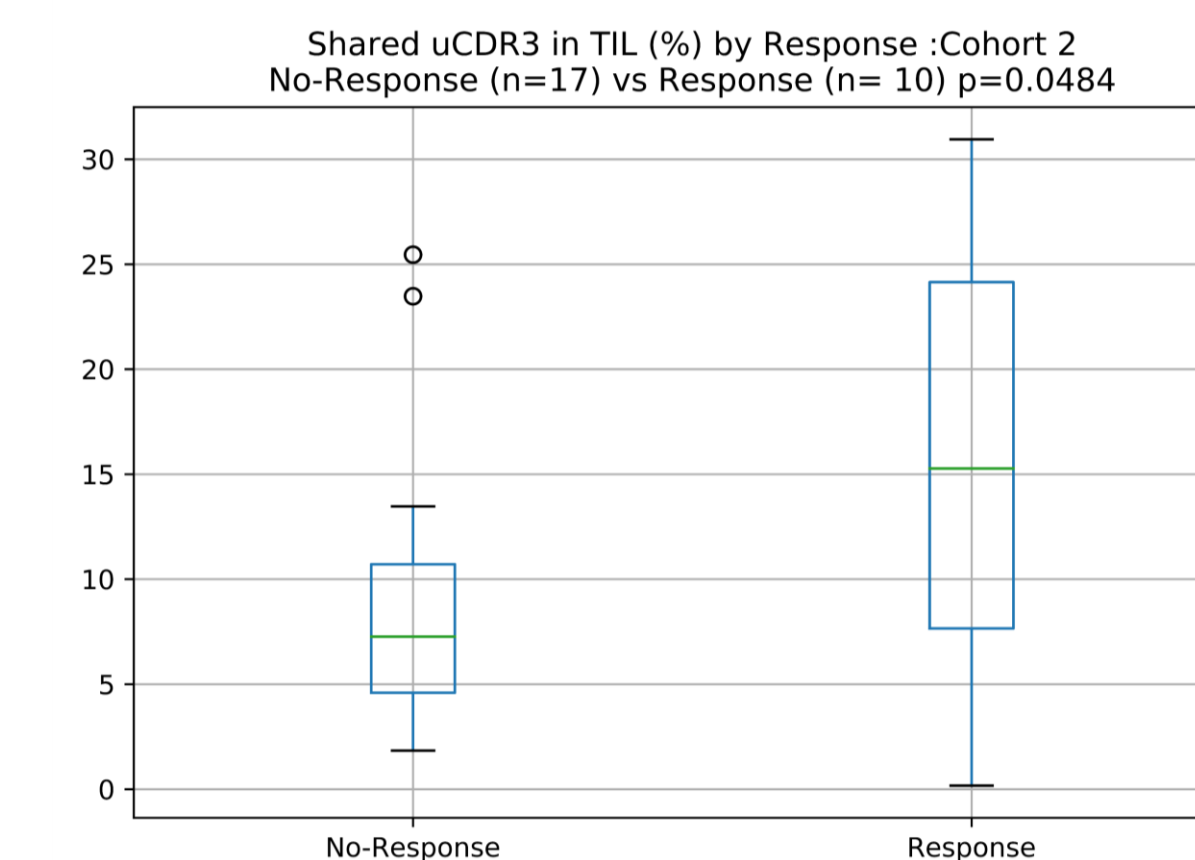
Figure 4. Random pattern of TIL expansion



Assessment of TIL rank of the top 10 persisting clones. The rank of the top 10 most frequent persisting clones at D42 were identified in the TIL product. Each blue dot represents the rank as a percentage within the TIL product for each of the top ranking clones. The most frequent clones in the TIL product correspond to the lowest values; similarly, the lowest frequency clones in the TIL product, correspond to the values closer to 100. Persisting clones were found at both high and low levels in the TIL product. uCDR3 clones represented at either high or low frequencies in the TIL product could persist for at least 6 weeks post-infusion.

The abundance of a clone in the TIL product does not correlate with its abundance at D42. This is indicative of the cyclical expansions TIL experience in vivo as they encounter their cognate antigen.

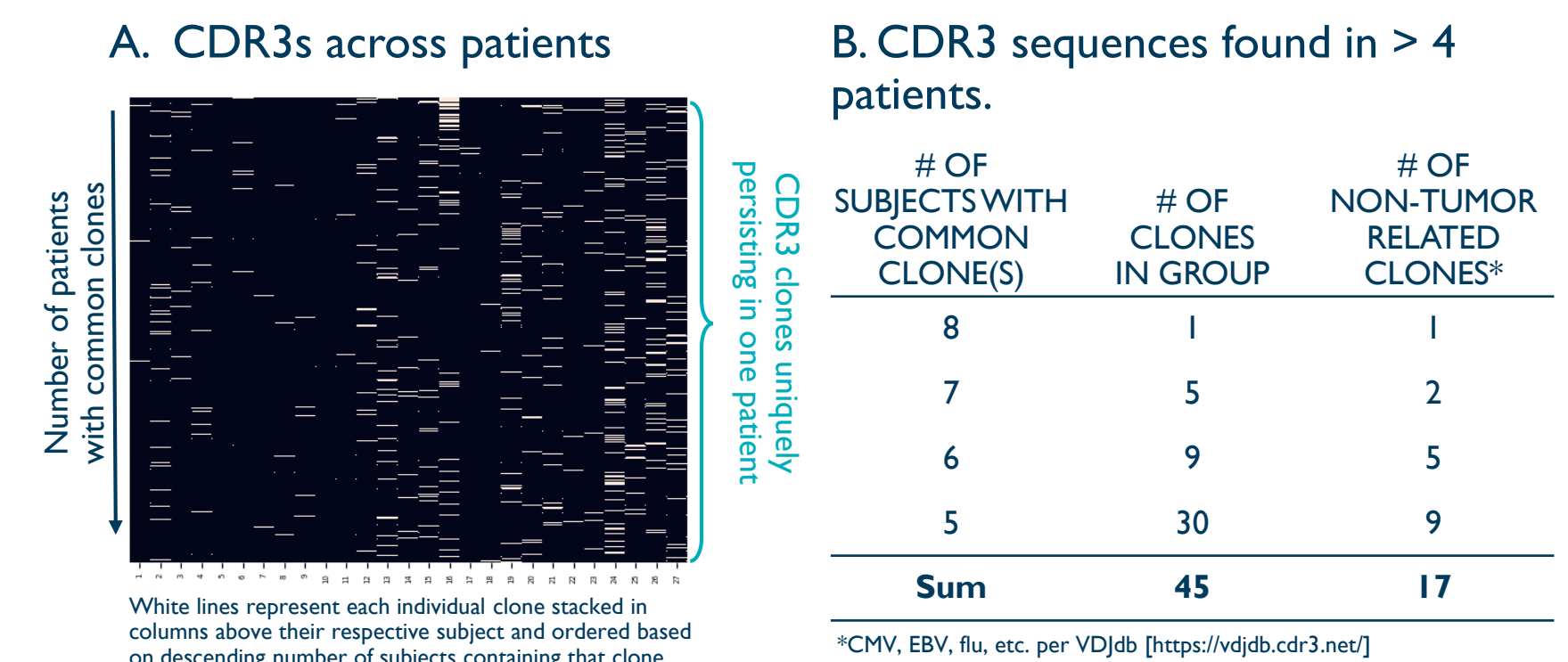
Figure 5. Slight correlation between D42/TIL overlap and clinical response



Correlation analysis of persisting T cell clones and clinical response. Number of clones detected in both the TIL product and D42 PBMC samples were divided by the number of unique CDR3 clones in the TIL products to determine a percentage of persisting clones and provide a measure of overlap between the composition of the infusion product and T cells circulating *in vivo*. Results are shown as box plots.

Clinical response may be associated with in vivo TIL persistence.

Figure 6. High patient-specificity of in vivo persisting TIL



Analysis of clonotypes:

- A total of 47,508 persisting clones were identified among the 27 subjects.
- CDR3 sequence alignments revealed 45,944 (96.7%) sequences found in only 1 subject (A).
- 45 sequences were found in more than 4 subjects. 17 (37.8%) corresponded to CDR3s previously identified to recognize non-tumor-related epitopes (B).
- No association between any common clone and clinical response was observed.

The T cell repertoire, including potentially tumor-specific clones, is unique in each Iovance TIL infusion product preparation.

CONCLUSION

- 100% of Iovance TIL infusion products demonstrate substantial level of *in vivo* persistence 6 weeks post-infusion.
- The TIL product is highly polyclonal and number of unique clones or diversity index are not related to clinical response.
- In vivo* fate of individual TIL clones is irrespective of their frequency in the infusion product, reflecting their specific antigen reactivities.
- The patient TIL products are comprised of unique TCR repertoires, which are highly specific to each patient.

Overall, the data support using a polyclonal product such as bulk TIL to treat solid tumors with their associated unique, patient-specific, mutational and neoantigen spectra.