In vivo persistence of Iovance tumor-infiltrating lymphocytes LN-145 in cervical cancer patients

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IOVANCE BIOTHERAPEUTICS

ADVANCING IMMUNO-ONCOLOGY

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Introduction

Background

- C-145-04 (NCT03108495) is an ongoing phase 2 multicenter study:
  - Investigational agent: Autologous TIL (LN-145/lifileucel)
  - Central manufacturing of cryopreserved TIL, using Iovance's 22-day GMP Generation 2 process.
  - Patient population: Patients with recurrent, metastatic or persistent cervical carcinoma which is not likely to be cured with surgery and/or radiation. Patients have received at least one prior treatment with systemic chemotherapeutic treatment for cervical cancer.
  - Demonstrated efficacy: 44% ORR and 85% DCR.[1]
- Goal of the presented work was to understand the potential impact of the TIL product clonal composition and *in vivo* persistence on antitumor activity.

Overview of TIL Therapy Process

1. A fragment of tumor is surgically isolated from patient.
2. Tumor sample is shipped to the GMP facility where TIL are isolated and multiplied to generate billions of TIL over a 22-day Gen 2 manufacturing process.
3. Patient initiates a week of pre-conditioning therapy to receive TIL.
4. TIL product is administered as a one-time therapy followed by up to 6 doses of IL-2 to support growth and activation of the TIL therapy in the body.

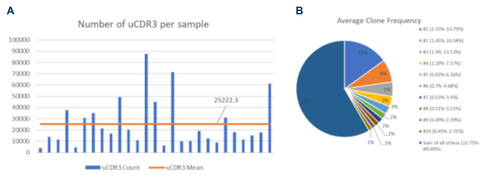
Figure 1: Overview of TIL therapy procedure

Experimental Design and Methods

- The T cell receptor (TCR) repertoire of the TIL lots and corresponding pre- and post-infusion PBMC samples from 27 cervical cancer patients who underwent resection for the purpose of TIL generation was established by RNA-seq:
  - Total RNA was extracted, using Qiagen's RNeasy Mini Kit protocol.
  - TCR beta chain complementarity-determining region 3 (CDR3) was amplified and sequenced by next generation sequencing, using iRepertoire technology (Huntsville, AL).
  - Unique CDR3 sequences (uCDR3) were identified and quantified, using the iRWeb platform.
- The 27 libraries of uCDR3 were analyzed to determine the range of clonality/diversity across TIL lots and whether common clones may be identified:
  - Bioinformatics analyses were performed using custom code, written in Python.
  - Diversity was defined as Shannon Entropy = $-\sum_{i=0}^{10000} p_i \log_2 p_i$, where p_i designates proportion of the clone i.
  - uCDR3 with 100% sequence homology in at least 2 samples were considered Common.
- Potential association of the above TCR repertoire properties with clinical response as assessed by RECIST 1.1 was determined:
  - Student's t-test test was used to calculate nominal p values.
  - Statistical significance was set at p value < 0.05.
- A similar method was used to evaluate the correlation between response and reactivity of the TIL to the E6/E7 epitopes of HPV16/18 as assessed by co-culture with peptide-loaded autologous APC.
- Circulating levels of TIL clones were determined pre- and post-adoptive cell therapy to estimate the *in vivo* persistence of the product.

Results

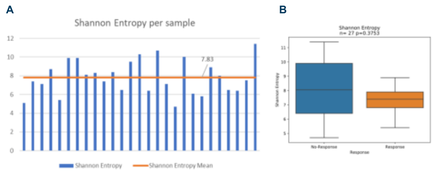
Figure 2. TIL clonal characterization



Unique CDR3 counts were determined. Shown as blue bars are the values obtained for each individual TIL lot. Mean is indicated by the orange line **(A)**. Frequencies of the top 10 most abundant clones in the 27 TIL products were averaged per ranking position. Averages are depicted in the pie chart with each top 10 positions indicated as a colored slice and remaining clones, indicated in dark blue, as the largest slice. Range of frequencies for each of the top 10 clones are listed **(B)**.

A median 17,793 different TCR were identified across TIL lots, with the 10 most frequent clones, on average, making up ~42% of the total repertoire. No correlation between clonality and clinical response was observed.

Figure 3. TIL clonal diversity characterization

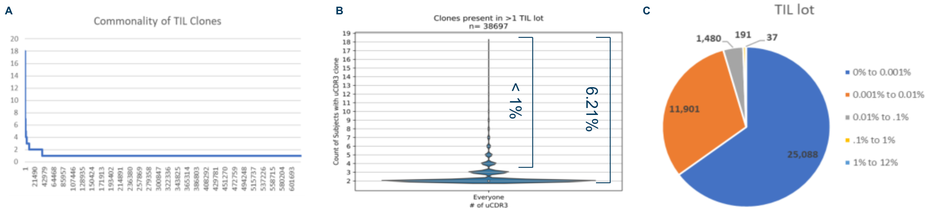


Shannon Entropy, a measure of clonal diversity which accounts for both abundance and evenness of the uCDR3 present in each of the 27 TIL lots, was calculated. Index values are shown as blue bars and mean is indicated by the orange line **(A)**. Diversity indices were sorted between the 2 Response and No-response groups to assess a potential association. Box plots show the median, lower and upper quartiles, and variability; sample size (n) and p value are indicated above the graph **(B)**.

Clonal diversity varied across samples and was not associated with clinical response.

The TIL product in cervical cancer is highly polyclonal, similar to what was observed for melanoma[2], despite the fact that cervical cancer has been categorized as a medium mutational load disease.

Figure 4. TIL clone commonality



D

# of Subjects with common clones	# Common clones	# of Clones associated with response	# of Clones associated with no-response
2 to 3	34486	0	0
4 to 10	4104	0	1463
11 to 18	107	0	14

The 623,167 uCDR3 identified were assessed for the number of times each was encountered among the 27 TIL lots. Each unique clone is indicated with an index on the x axis and the number of encounters per clone plotted on the y axis **(A)**. Number of TIL lots across which the 38,697 uCDR3 found more than once were encountered is shown on the y axis. Surface area of the violin plot is proportional to the corresponding number of uCDR3 **(B)**. The number of Common uCDR3 falling into frequency ranges at which the common clones were represented in their respective TIL lots, as indicated in the legend of the pie chart, are shown **(C)**. Association of each Common uCDR3 with clinical response was assessed by Student's t-test. Listed are the ranges of the number of subjects in whom uCDR3s were detected, followed by the number of clones falling in the indicated subject range. The last two columns indicate the number of clones for which a significant association was noted with either Response or No response **(D)**. Potential epitopes corresponding to the 107 uCDR3 found in more than 10 lots were predicted using a database of TCR sequences with known antigen specificities. Results are listed along with the number of times each antigen was encountered **(E)**.

Only about 6% of all uCDR3 were found in more than 1 TIL lot and less than 1% in more than 3 lots. Most Common uCDR3 represented low frequency clones in the TIL product and a substantial portion corresponded to non-cancer-related antigens. The presence of Common uCDR3 did not correlate with clinical response.

E

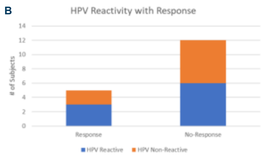
Epitope	# of Clones
CMV	21
EBV	10
CMV, EBV	9
CMV, Influenza-A	3
Influenza-A	2
CMV, DENV	2
CMV, Influenza-A	1
CMV, Tuberculosis	1
CMV, EBV, Influenza-A	1
CMV, HIV-1	1
CMV, TriticumAestivum	1
DENV	1
EBV, Influenza-A	1
HIV-1	1

The TIL product is comprised of a mostly unique repertoire of T cells, reflecting a patient-specific repertoire of tumor antigens.

Figure 5. Correlation of HPV reactivity with clinical response

A

		Response	
		Response (HPV+ Status)	No-Response (HPV+ Status)
HPV Reactivity	Reactive	3 (3)	6 (3)
	Non-Reactive	2 (1)	6 (4)

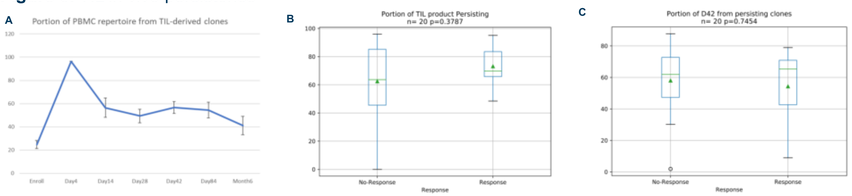


TIL were tested for reactivity against HPV by co-culture with peptide-loaded autologous APC. Reactivity and response information was available for 17 subjects **(A)**. Violin plots were created for the response and no-response groups. Subjects are shown as belonging to the Response and No-Response groups on the x-axis with HPV reactivity shown in blue and non-reactivity shown in orange **(B)**.

No correlation was found between HPV reactivity and response

HPV reactivity is not associated with response to TIL therapy, aligned with the expected wide recognition of peptide antigens by TIL.

Figure 6. TIL *in vivo* persistence



Twenty subjects with available TIL and pre- and post-infusion timepoints were analyzed for the frequencies of TIL-derived uCDR3 present in the circulation (Shared clones). The average of the sums of those frequencies are plotted **(A)**. The sum of frequencies of Shared clones in the TIL product **(B)** and in the Day 42 samples **(C)** are plotted for the Response and No-response groups. The median is indicated with a green line and the mean with a green triangle for each group.

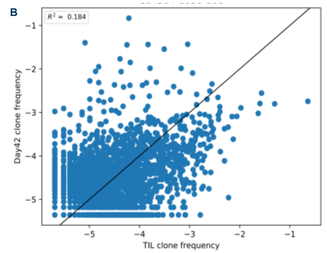
TIL-derived clones could be detected at variable frequencies in all patients and at all time points, with no correlation with response.

***In vivo* persistence suggests that the memory T cells that comprise the TIL product can be long-lived.**

Figure 7. TIL/Day42 clone distribution are not correlated

A

Subject	R^2
1	0.385167
2	0.345023
3	0.342798
4	0.242643
5	0.061519
6	0.389739
7	0.036278
8	0.183967
9	0.306159
10	0.178368
11	0.133733
12	0.294695
13	0.034656
14	0.046466
15	0.202798
16	0.014487
17	0.245905
18	0.174322
19	0.313835
20	0.05963



Pearson correlation was determined between the frequency of shared uCDR3 in the TIL product and at Day 42. R-squared values are listed for 20 subjects **(A)**. Example of a correlation plot representing the average R2 is shown **(B)**.

The low R^2 values demonstrate the absence of correlation between clone distributions in the TIL products and 6 weeks post-infusion.

Clone abundance in the TIL infusion product does not predict persistence.

Conclusions

1. The TIL product generated from cervical tumors was highly polyclonal, with a median of ~17K clonotypes per lot.
2. Composition of the TIL product was unique to each patient, with less than 1% of common clones in more than 3 lots, many of which targeted non cancer-related antigens.
3. HPV reactivity of TIL did not predict response.
4. Long-term TIL persistence was observed in all patients, that was not associated with clinical response.
5. Overall, our findings imply that identifying a common TCR to target cervical cancer across patients, may be difficult due to a lack of common neoantigen.

References

[1] Jazaeri, *et al.* JCO (ASCO) 2019.
[2] Gontcharova, *et al.* *J Immuno Cancer* (SITC) 2019.

Conflicts of Interest

- Research support: Iovance, BMS, Aravive, AZ, Pfizer, Eli Lilly, Merck
- SAB: NuProbe; AvengeBio
- Consulting: GLG; Guidepoint