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A Cryopreserved Tumor Infiltrating Lymphocyte (TIL) Product for LN-144, Generated with an Abbreviated Method Suitable for High Throughput Commercial Manufacturing Exhibits Favorable Quality Attributes For Adoptive Cell Transfer

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BACKGROUND

Classical methods of generating tumor infiltrating lymphocytes (TIL) for adoptive cell transfer (ACT) involve multiple ex-vivo incubation steps to yield a fresh (non-cryopreserved) infusion product.

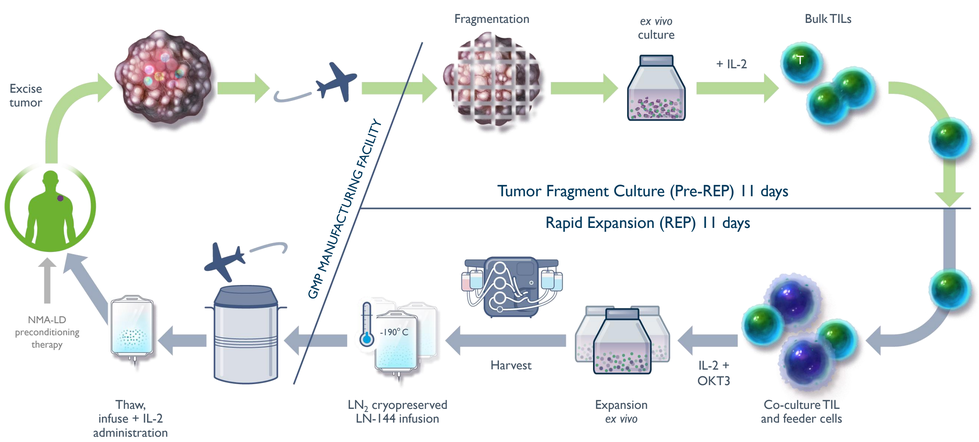
Iovance's first generation (Gen 1) process produced a dose of fresh TIL in approximately 6 weeks. Iovance has developed a second generation (Gen 2) TIL manufacturing process which abbreviates the ex-vivo culture duration to 22 days.

Iovance's Gen 2 process is suitable for centralized manufacturing and yields a cryopreserved TIL infusion product that brings convenience in scheduling, logistics, and delivery to the clinical sites.

The cryopreserved TIL infusion product for LN-144 produced by our Gen 2 process has comparable quality attributes to the non-cryopreserved TIL infusion product for LN-144 generated by our Gen 1 method.

The Gen 2 TIL manufacturing method represents a flexible, robust, closed, and semi-automated cell production process that is amenable to high throughput TIL manufacturing on a commercial scale.

Iovance Cryopreserved LN-144 Manufacturing Process (22 days)



STUDY OBJECTIVES

- TIL infusion products (LN-144) generated by both Iovance's proprietary Gen 1 and Gen 2 manufacturing processes were assessed to determine comparability in terms of the following quality attributes:
  1. Cell count (dose), viability, growth rate of REP phase
  2. T-cell purity and phenotypic expression of co-stimulatory molecules on T-cell subsets
  3. Average relative length of telomere repeats
  4. Ability to secrete IFNγ in response to CD3,CD28,CD137 engagement
  5. Diversity of T-cell receptors present in the final infusion product.

Table 1. Process Improvements Gen 1 → Gen 2

PROCESS STEP	GEN 1	GEN 2	IMPACT
Fragment Culture	≤21 days, multiple bioreactors, multiple operator interventions	≤11 days, single closed bioreactor, no intervention	Shortens culture, reduces interventions
TIL selection	IL-2 expanded TIL cryopreserved, tested, selection based on phenotype, thaw, rest, co-culture	Bulk TIL direct to co-culture	Reduces steps, eliminates testing, increases clonal diversity
Harvest/ Wash	Manual volume reduction and harvest. Manual wash and concentration	Closed semi-automated volume reduction and harvest. Automated wash and concentration	Reduces operator interventions, reduces processing time, maintains functionally closed system
Formulation	Fresh hypothermic product (2- 8^{0}C)	Cryopreserved product (≤ -150^{0}C)	Allows for global trials through increased flexibility in shipping and patient scheduling
Manufacturing Time	38 day process time	22 day process time	Turnaround to patient, clean room throughput, lower cost of goods

ANALYTICAL METHODS & INSTRUMENTATION

- **Cell Count and Viability:** Final formulated infusion products were sampled and assayed for total nucleated cells, total viable cells, and viability determined by acridine orange / DAPI counterstain using the NC-200 automated cell counter. Process Development lots were assayed on the Nexcellom Cellometer K2 Cell Viability Counter using acridine orange / propidium iodine dual florescent staining.
- **Phenotypic markers :** Formulated infusion products were sampled and assayed for identity by immunofluorescent staining. Percent T-cells was determined as the CD45$^+$,CD3$^+$ (double positive) population of viable cells. Frozen satellite or sentinel vials for each process were thawed and assayed for extended phenotypic markers including CD3, CD4, CD8, CD27, and CD28. Fresh infusion products were acquired on the BD FACS Canto II, and extended phenotypic markers on thawed infusion products were acquired on the Bio-Rad ZE5 Cell Analyzer
- **Average relative length of telomere repeats:** Flow-FISH technology was used to measure average length of telomere repeat. This assay was completed as described in the DAKO® Telomere PNA Kit/FITC for Flow Cytometry protocol. Briefly, 2.0 x 10^6 TIL cells were combined with 2.0 x 10^6 human cell line (1301) leukemia T-cells. The DNA was denatured at 82°C for 10 minutes and the PNA-FITC probe was hybridized in the dark overnight at room temperature. Propidium Iodide was used to identify the cells in G0/1 phase.
- **Immune function:** The ability of the infusion product to secrete IFNγ upon reactivation was measured following co-culture with antibody coated beads (LifeTechnologies, anti-CD3, anti-CD28 & anti-CD137). After 24 hours culture supernatants were harvested, frozen, thawed, and assayed by ELISA using the Quantikine IFNγ ELISA kit (R&D systems) according to manufacturers instructions.
- **T-cell receptor diversity:** RNA from infusion products was isolated and subjected to a multiplex PCR with VDJ specific primers. CDR3 sequences expressed within the TIL product were semi-quantitatively amplified and deep sequenced to determine the frequency and prevalence of unique TIL clones. Sequencing was performed on the Illumina MiSeq benchtop sequencer. Values were indexed to yield a score representative of the relative diversity of T-cell receptors in the product.

RESULTS

Figure 1. Total viable cells, growth rate, and viability

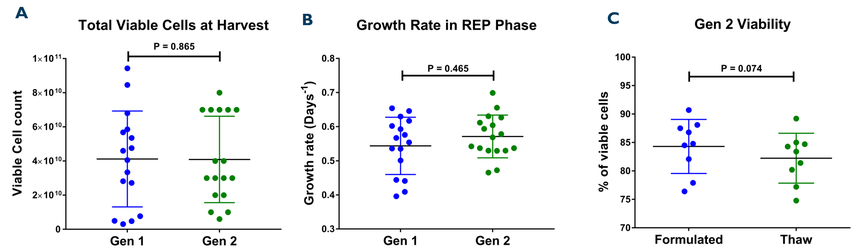


Figure 1. On Day 22 the volume reduced cell product is pooled and sampled to determine culture performance prior to wash and formulation. (**A**) Samples are analyzed on the NC-200 automated cell counter as previously described. Total viable cell density is determined by the grand mean of duplicate counts from 4 independent samples. The Gen 2 process yields a TIL product of similar dose to Gen 1 (Gen 1 mean = 4.10x10^{10} ± 2.8x10^{10}, Gen 2 mean = 4.12x10^{10} ± 2.5x10^{10}). (**B**) The growth rate is calculated for the REP phase as $gr = ln(N(t)/N(0))/t$. (**C**) Cell viability was assessed from 9 process development lots using the Cellometer K2 as previously described. No significant decrease in cell viability was observed following a single freeze-thaw cycle of the formulated product. Average reduction in viability upon thaw and sampling is 2.19%.

Figure 2. Gen 2 products are highly pure T-cell cultures which express costimulatory molecules at levels comparable to Gen 1

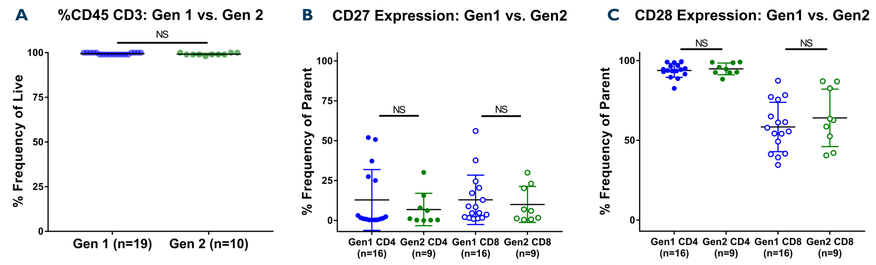


Figure 2. (**A**) Fresh formulated drug products were assayed for identity by flow cytometry for release. Gen 1 and Gen 2 processes produce high purity T-cell cultures as defined by CD45+,CD3+ (double positive) phenotype. (**B & C**) Cryopreserved satellite vials of formulated drug product were thawed and assayed for extended phenotype by flow cytometry as previously described. Gen 1 and Gen 2 products express similar levels of costimulatory molecules CD27 and CD28 on T-cell subsets. Costimulatory molecules such as CD27 and CD28 are required to supply secondary and tertiary signaling necessary for effector cell proliferation upon T-cell receptor engagement. P-value was calculated using Mann-Whitney 't' test.

Figure 3. Gen 2 products trend toward longer relative telomere lengths

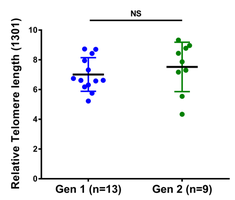


Figure 3. Flow-FISH technology was used to measure the average length of the telomere repeat as previously described. The RTL value indicates that the average telomere fluorescence per chromosome/genome in Gen 1 is 7.5 % ± 2.1%, and Gen 2 is 8.4% ± 1.8% of the telomere fluorescence per chromosome/genome in the control cells line (1301 Leukemia cell line). Data indicate Gen 2 products on average have comparable telomere lengths to Gen 1 products. Telomere length is a surrogate measure of the length of ex-vivo cell culture.

Figure 4. Gen 2 drug products secret IFNγ in response to CD3, CD28, and CD137 engagement

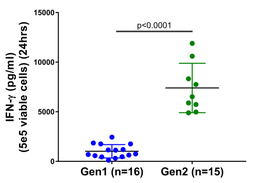


Figure 4. Cryopreserved drug products were thawed and incubated with ab-coated beaded as previously described. Data is expressed as the amount of IFNγ produced by 5x10^5 viable cells in 24hrs. Gen 2 drug products exhibit an increased ability to produce IFNγ upon reactivation relative to Gen 1 drug products. The ability of the drug product to be reactivated and secrete cytokine is a surrogate measure of in-vivo function upon TCR binding to cognate antigen in the context of HLA.

Figure 5. Gen 2 drug products have an increased diversity of unique T-cell receptors

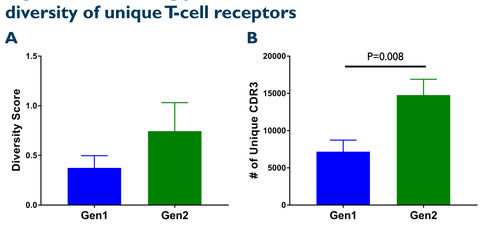


Figure 5. T-cell receptor diversity: RNA from 10x10^6 TIL from Gen 1 and Gen 2 infusion products were assayed to determine the total number and frequency of unique CDR3 sequences present in each product. (**A**) Unique CDR3 sequences were indexed relative to frequency in each product to yield a score representative of the overall diversity of T-cell receptors in the product. (**B**) The average total number of unique CDR3 sequences present in each infusion product. TIL products from both processes are composed of polyclonal populations of T-cells with different antigen specificities and avidities. The breadth of the total T-cell repertoire may be indicative of the number of actionable epitopes presented on tumor cells.

CONCLUSIONS

The Iovance Gen 2 manufacturing process produces a TIL infusion product (LN-144) with comparable quality attributes to Gen 1.

- Gen 2 produces similar doses of highly pure TIL. T-cell subsets are in similar proportions and express costimulatory molecules at comparable levels relative to Gen 1.
- Gen 2 TIL trend toward longer relative telomere length commensurate with reduced ex-vivo culture period.
- Gen 2 TIL display an increased diversity of TCR receptors which, when engaged, initiate robust secretion of IFNγ, a measure of cytolytic effector function.
- The Gen 2 abbreviated 22-day closed expansion process with cryopreserved infusion product presents a scalable and logistically feasible TIL manufacturing platform that allows for the rapid generation of clinical scale doses for cancer patients in immediate need of a novel therapy option.

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- All authors are employees of Iovance Biotherapeutics, Inc. and may have stock options.