SMART G-Rex® & SensoRex™ Technology for In-line, Non-Invasive, and Real-Time Monitoring of TIL Expansion

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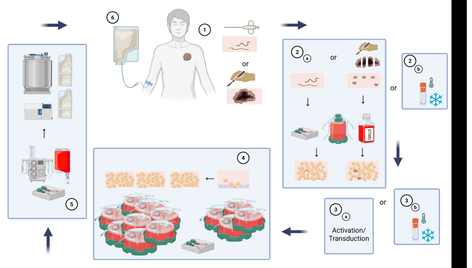
CTMC+ a joint venture between Resilience + UT MD Anderson

BACKGROUND

- TIL manufacturing began as an adaptive academic process in which harvest, especially for the first-phase expansion, was timed to the culture, not to the calendar. Industrial scale-up traded that flexibility for fixed, hardcoded process days.
- Flexibility remains valuable, primary to avoid termination, relieve manufacturing, but could also be used to avoid penalizing a good growers.
- Flexibility only works with reliable readouts. Connventional monitoring requires sampling that disturbs the culture.

METHODS

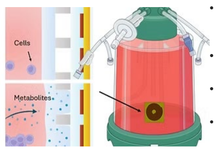
- Both expansion phases of the CTMC TIL platform were evaluated: first-phase expansion (pre-REP) and the second-phase Rapid Expansion Protocol (REP).



01 Tumor resection from the patient **02a** Fragmentation, culture initiation, 1st phase expansion (pre-REP) using proprietary reagents **02b** Fragmentation, optional cryopreservation hold **03a** Activation/Transduction (all soluble) **03b** Optional cryopreservation hold **04** TIL 2nd phase expansion (REP) using proprietary reagents **05** Harvest, concentration/wash, formulation and cryopreservation **06** Infusion back into the patient

- Pre-REP: SMART G-Rex 100M-TF cultures were initiated from a heavily pre-treated, Stage IV melanoma tumor using 5, 10, or 30 tumor fragments to test whether frequency (MHz) readings capture differences in TIL yield (Figure 1).
- The resulting frequency target was then applied prospectively on Day 9 to a metastatic head and neck tumor (TIL4027-HN) and a Stage IV melanoma tumor (TIL5001-MEL) to select early-harvest candidates (Figure 2).
- REP: daily growth of TIL5004-MEL, TIL6015-CRC, and TIL4101-NSCLC was monitored in G-Rex 100M, with extrapolation to G-Rex 500M, to determine whether a pre-plateau harvest threshold could be defined (Figure 3).
- Endpoints were total viable cell number, viability, growth kinetics, and the ability to trigger harvest before a growth plateau.

SMART G-Rex & SensoRex MONITORING TECHNOLOGY



- SMART sensors embedded in the G-Rex platform: no sampling, no culture disturbance
- In-line, real-time frequency (MHz) readout of cell growth
- Growth curves reflect both expansion kinetics and culture quality
- Enables targeted early harvest against pre-defined frequency thresholds
- Applies across pre-REP and REP and scales from G-Rex 100M to 500M

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CONCLUSIONS

- SMART G-Rex technology enabled sampling-free tracking of TIL expansion, generating growth curves that reflect both cell growth kinetics and culture quality throughout manufacturing.
- In the pre-REP, a target of 142 +/- 0.5 MHz identified cultures ready for an early harvest yielding 300 to 500 x10^6 TIL, while glucose readings failed to predict the best growing culture.
- In the REP, a 141 MHz +/- 0.5 MHz next-day-harvest threshold flagged cultures before they reached plateau, suggesting harvest between Day 10 and Day 12 of the REP and preserving post-thaw proliferation capacity.
- Constant non-invasive monitoring also builds knowledge of general culture health and accumulates data for process understanding and characterization.

CONTACT

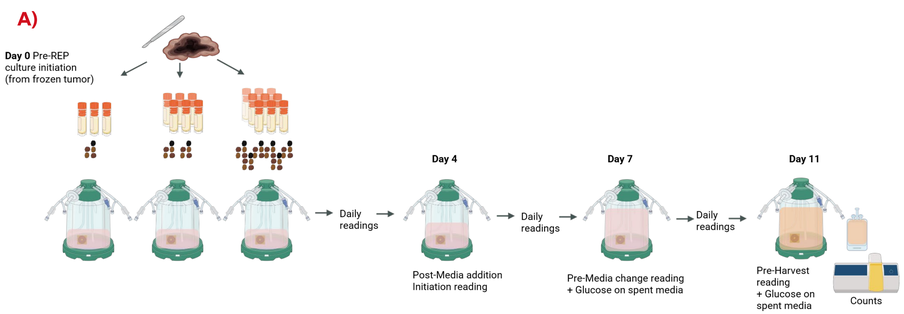
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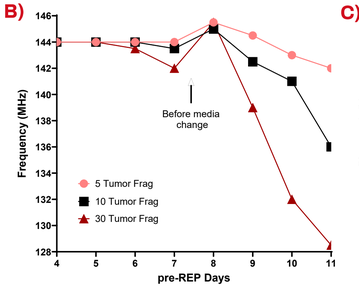
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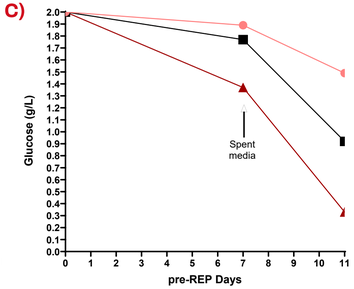
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RESULTS

Figure 1. Testing of SMART G-Rex in Pre-REP: varying tumor tissue input





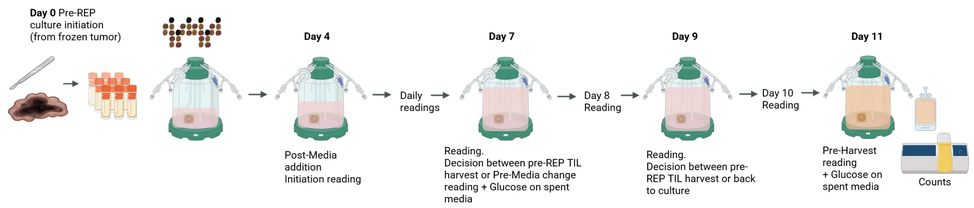


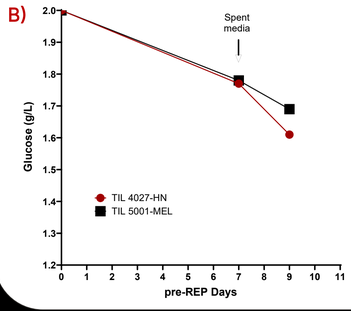
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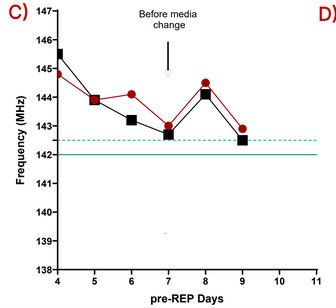
# Tumor Fragment	Day 11 MHz Value	Day 11 Total Viable Cell (x10^6)	Day 11 Viability (%)
5	142	515	97.2
10	136	1,375	97.0
30	128.5	2,281	96.8

Figure 1A) Schematic illustrating the execution of the pre-REP expansion for the initial SMART G-Rex 100M-TF multi tumor fragment study. Briefly, a stage IV Metastatic Melanoma cryopreserved tumor was thawed and used to initiate three single pre-REP cultures using 5, 10, or 30 tumor fragments. Cultures were initiated using CTMC's proprietary reagents. Media addition was performed 4 days post culture initiation and daily frequency reading using the SMART G-Rex and the SensoRex were performed. Seven days post-culture initiation, a media change was performed, and glucose levels were assessed on the spent media using the Flex2 Analyzer. On Day 11 of the pre-REP, all cultures were volume-reduced and harvested before a viable cell count was performed using the NC-200 Cell Counter. **B)** Graph showing frequency results (MHz) for each culture. **C)** Graph showing glucose level results (g/L) for each culture. **D)** Table displaying the frequency, total viable counts and percentage of viable cells for each culture at harvest. Since a frequency of 142 MHz was associated with 500x10^6 pre-REP TIL, setting a frequency target of 142 MHz with an upper limit of 142.5 MHz should lead to a same day harvest product between 300 to 500x10^6 pre-REP TIL.

Figure 2. Testing of SMART G-Rex in Pre-REP: target frequency for harvest trigger study





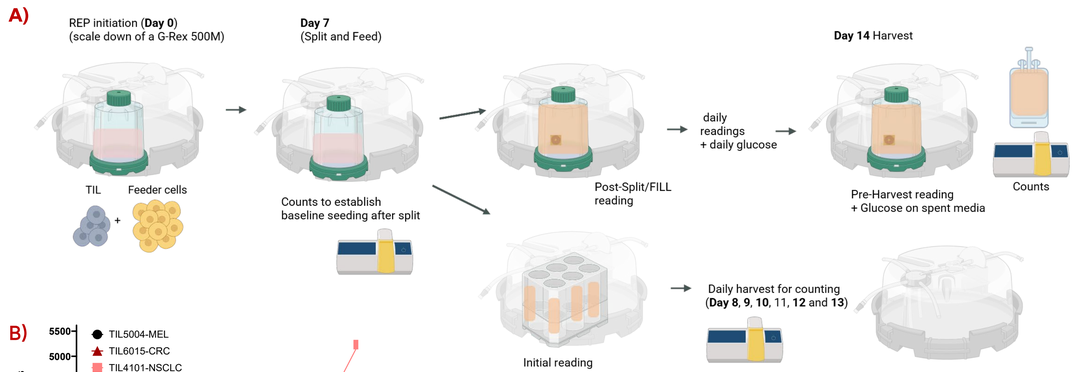


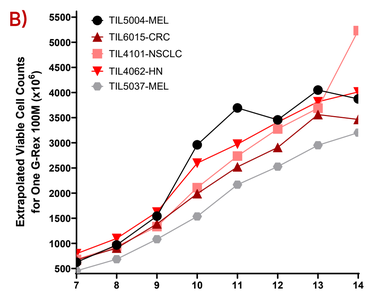
D)

Tumor ID (TIL line ID)	Day 9 MHz Value	Day 9 Total Viable Cell (x10^6)	Day 9 Viability (%)
TIL4027-HN	142.9	269	96.5
TIL5001-MEL	142.5	306	98.9

Figure 2A) Iteration on the Fig. 1A schematic. The differences are on Day 7 and Day 9 pre-REP when decision will be made after the frequency reading. On Day 7, if the frequency is 142 MHz with an upper limit of 142.5 MHz, this triggers a pre-REP harvest. If not, a media change is performed. On Day 9, if the frequency is 142 MHz with an upper limit of 142.5 MHz, this triggers a pre-REP harvest. If not, the culture is returned for expansion until a Day 11 harvest. **B)** Graph showing glucose level results (g/L) for each culture. **C)** Graph showing frequency results (MHz) for each culture. **D)** Table displaying the frequency, total viable counts and percentage of viable cells of each culture at harvest.

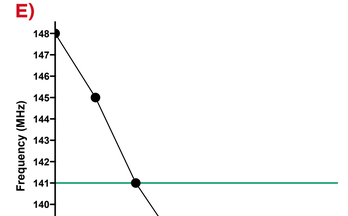
Figure 3. Testing of SMART G-Rex in Pre-REP: target frequency for harvest trigger study

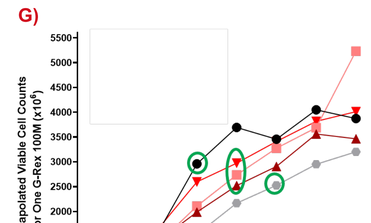


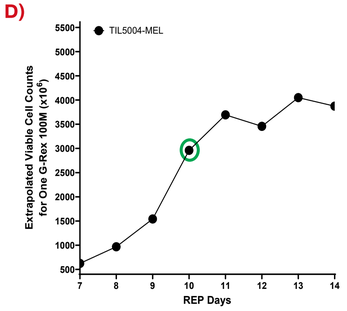


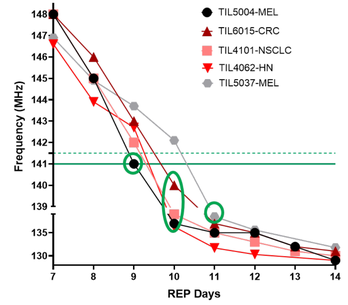
C)

TIL Line	D14 REP Viability at Harvest (%)
5004-MEL	98.9
6015-CRC	97.9
4101-NSCLC	97.7
4062-HN	97.6
5037-MEL	97.9









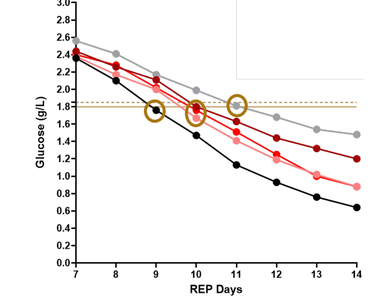


Figure 3A) Schematic illustrating execution of REP expansion for the SMART G-Rex 100M-TF. Two Stage IV Metastatic Melanoma tumors (MEL), one Metastatic Colorectal Cancer tumor (CRC), one Lung Cancer tumor (NSCLC) and one Metastatic Head and Neck tumor (HN) cryopreserved pre-REP TIL lines were thawed, rested, and activated before initiating a REP for each activated TIL line in a G-Rex 100M. CTMC's proprietary feeders, iTR-56, were added to the culture before expanding for 7 days. On REP Day 7, each line was resuspended, counted (NC-200) and split into a Smart G-Rex 100M (top of the schematic) or five wells of a G-Rex 10M 6-well plate. Every subsequent day, a well of the 6 well plate was harvested and counted (NC-200) and its total viable cell count was extrapolated to the cell number of the SMART G-Rex 100M, while the SMART G-Rex 100M had daily frequency readings and media sampling for glucose level tracking (Flex2) **B)** Graph showing daily extrapolated total viable cell counts as well as the real Day 14 G-Rex 100M cell count. Data presented for each TIL line expanded (n=5) as one color per line. **C)** Table displaying the percentage of viable cells of each culture at harvest (Day 14 REP). **D)** Graph focusing on the first TIL line to "plateau" during the expansion (TIL5004-MEL). The optimal harvest day (prior to reaching a growth "plateau") for this line is highlighted with a green circle (REP Day 10). A REP harvest on Day 10 for TIL5004-MEL would have equated to 3 x10^9 TIL per G-Rex 100M which if extrapolated to a G-Rex 500M would be 15x10^9 TIL/flask **E)** Graph showing frequency results (MHz) for TIL5004-MEL through the REP expansion. A green line was drawn at the frequency on REP Day 9 , 141 MHz, to highlight the frequency value that should have been targeted for a "next day harvest", before reaching the growth "plateau". **F)** 141 MHz was applied as a frequency target (green line, with an upper limit of 141.5 MHz, green dotted line) to all the daily frequency readings for all TIL line REP (n=5). For each line, the REP day reaching this frequency target (or below) was highlighted with a green circle. The CRC, NSCLC and HN all reached the target frequency on Day 10. Their daily extrapolated total viable cell counts on Day 11 REP (green circles in **G**) were all over 2 x10^9 TIL per G-Rex 100M which if extrapolated to a G-Rex 500M would all be over 10 x10^9 TIL/flask. The same observation was made for the 2nd MEL line but a day later (**F** and **G**). **H)** Graph showing daily glucose level results (g/L) for each culture and the target for the "next day harvest" based on the glucose levels of TIL5004-MEL on REP Day 9. Glucose levels predicted the same "next day harvest" as the frequency for all TIL lines but required daily sampling of each flasks.