SMART G-Rex® for Non-Invasive Monitoring of CAR-T Cell Expansion and Harvest Timing

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BACKGROUND

- Closed G-Rex systems are widely used for CAR-T expansion.
- Conventional monitoring relies on periodic **sample removal** for cell counts and metabolite measurements for assessing culture expansion.
- Invasive sampling may introduce **contamination risk**, additional **operator interventions** and **culture disturbance**.
- SMART G-Rex provides a **non-invasive** monitoring approach that avoids sample removal.



Sensing principle. SMART G-Rex incorporates a resonant sensor that responds to metabolites released during culture. Expanding T cells release small hydrophobic metabolites that the sensor's polymer layer absorbs, shifting its resonant frequency. The SMART Growth Index (SGI) reports this shift as a signal of metabolic activity that can be used to assess expansion.

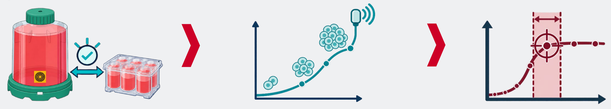
SGI acquisition is simple: place the SMART G-Rex on the SensoRex reader and scan. No sampling, reagents, external probes, tubing, or complex calibration required.

METHODS

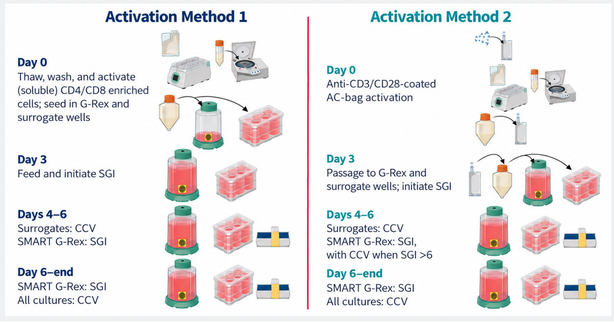
01 Result 1
Surrogate Representativeness

02 Results 2–4
Expansion Monitoring

03 Result 5
Harvest-Window Assessment

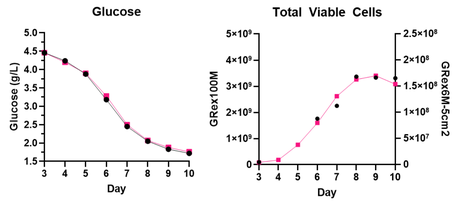


- **Cultures:** T cells from two healthy donors and two tumor patients were evaluated using two CAR-T activation methods (six cultures total).
- **Vessels & measurements:** SMART G-Rex 100M bioreactors with G-Rex 6M surrogate wells; daily glucose measurements and surrogate viable cell counts began on Day 3.
- **Surrogate wells:** Smaller G-Rex 100M vessels served as representative sampling surrogates while maintaining static culture conditions.
- **Representativeness:** Glucose was sampled directly from SMART G-Rex cultures without disturbing the cell layer; cell counts at selected time points confirmed surrogate comparability.



RESULTS

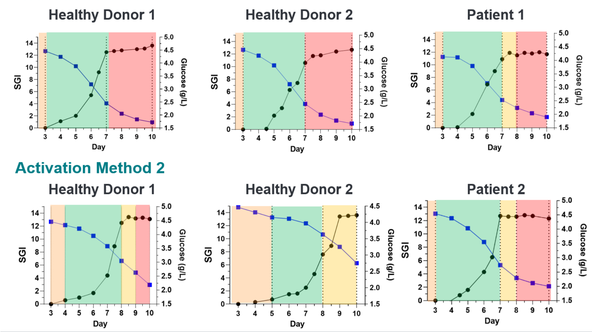
Result 1: Surrogate Cultures Demonstrated Comparable Expansion Kinetics



Representative plots: Healthy donor 2, Method 1 (one of six cultures). Total viable cell counts use vessel-specific axes.

Across all six cultures, G-Rex 6M surrogate wells demonstrated glucose consumption and expansion kinetics comparable to SMART G-Rex cultures, supporting their use as reference cultures for tracking daily cell growth.

Result 2: SGI Reflected Expansion and Growth-Phase Transitions

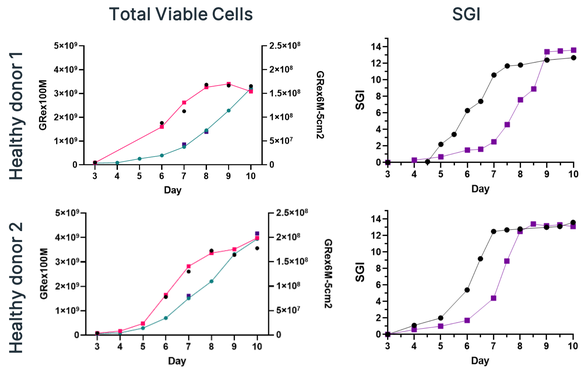


SGI increased during expansion and approached a plateau as growth slowed. Glucose continued to decrease and provided less resolution between growth phases.

Phases were defined by cell doubling times: log growth <36 h, lag/transition 36–60 h, plateau >60 h. Lag precedes log growth, and transition follows it.



Result 3: SGI Reflected Activation Method-Dependent Expansion Differences



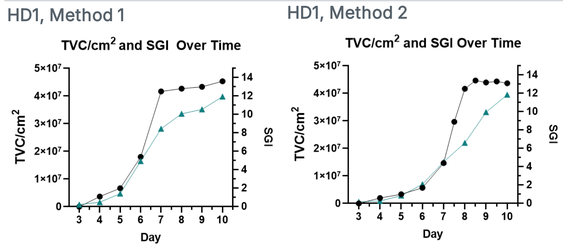
Activation Method 2 exhibited delayed expansion relative to Method 1 in both healthy donors, and SGI reflected the corresponding shift in culture kinetics.

Cell expansion (left panels)
- Method 1: SMART G-Rex (left axis)
- Method 1: G-Rex6M (right axis)
- Method 2: SMART G-Rex (left axis)
- Method 2: G-Rex6M (right axis)

SGI (right panels)
- Method 1
- Method 2

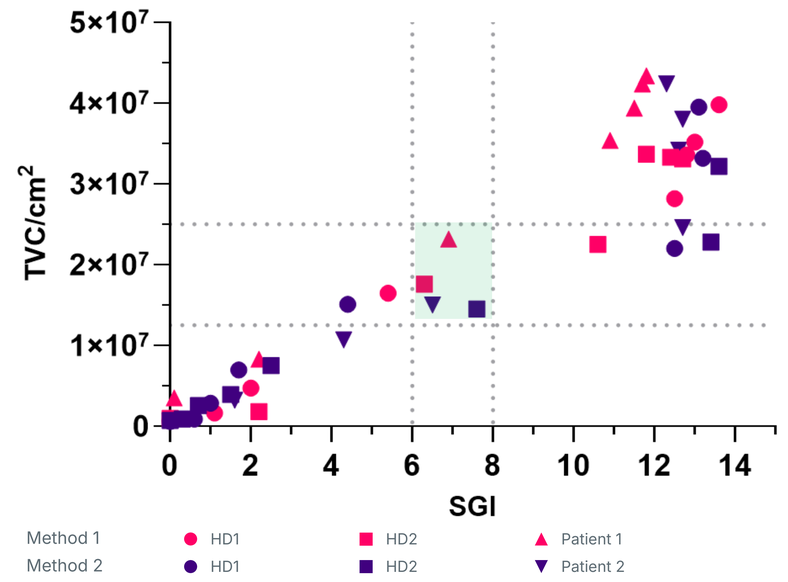
RESULTS (CONTINUED)

Result 4: SGI Tracked Viable Cell Density



Representative plots: Healthy donor 1, Methods 1 and 2. SGI closely tracked viable cell expansion across both activation methods, capturing the increase in cell density and subsequent changes in expansion kinetics. Although the timing of expansion differed between methods, SGI exhibited a consistent relationship with culture growth.

Result 5: SGI 6–8 Aligned With the Target Harvest-Density Window



Across tested cultures, an SGI range of approximately 6–8 aligned with the target harvest-density window (1.25–2.5 × 10^7 TVC/cm², show in green overlay), supporting further evaluation of SGI as a mechanism to inform harvest timing.

Conclusions

- SGI tracked CAR-T expansion kinetics across healthy donor and patient-derived cultures.
- SGI reflected activation method-dependent differences in expansion.
- SGI provided additional insight into growth-phase transitions compared with glucose monitoring.
- SGI range of ~6–8 aligned with the target harvest-density window across all conditions.

Manufacturing Implications

- Non-invasive SGI acquisition without removal of culture sample.
- Improved visibility into cell expansion within closed culture systems.
- Straightforward measurement workflow.
- Data-driven harvest timing decisions, potentially without cleanroom entry if using continuous remote monitoring.
- Reduced operator interventions and manufacturing complexity.

Future Directions

- Evaluate additional healthy donor and patient-derived cultures.
- Investigate relationships between SGI, cell expansion, and other quality attributes.
- Evaluate SGI performance across different G-Rex vessel sizes.
- Evaluate continuous, incubator-integrated SGI monitoring.
- Determine if harvest timing can be predicted with early SGI readings.