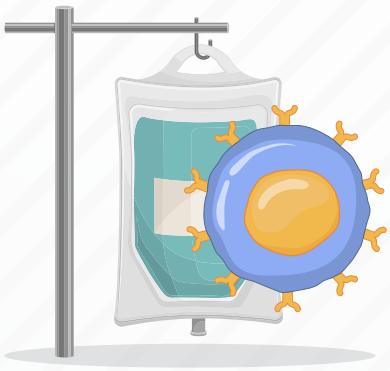
Next-Gen CAR-T: High-Throughput Screening Accelerates Discovery

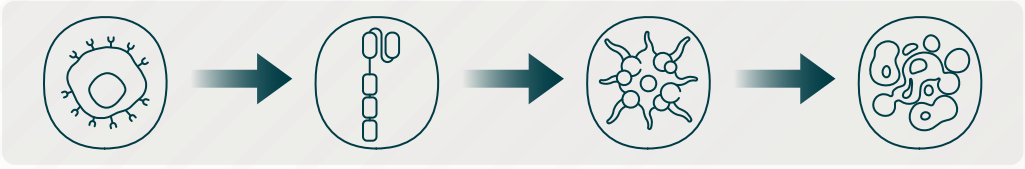
Revolutionizing antibody discovery and CAR construct optimization using automated high-throughput screening cytometry

The Problem and the High-Throughput Screening (HTS) Solution



CAR-T Therapy: Revolutionary but Bottlenecked

Engineered T cells targeting cancer-specific antigens



- FDA-approved for B-cell malignancies
- Durable remissions in patients with refractory malignancies

But...Traditional Development Is Slow

- Antibody screening: Months of work
- Construct testing: Limited throughput
- Manual QC assays: Days per readout
- Limited parameters: 1–3 measurements/ assay
- Long timelines to clinical candidate

HTS: The Game Changer

Automated HTS Cytometry Transforms Every Stage

iQue® HTS Cytometry + Incucyte® Live-Cell Analysis

Traditional workflow
- Limited constructs per week
- Single parameter
- Days for results
- Manual processing

vs.

HTS Cytometry
- 100+ constructs/day
- 20+ parameters simultaneously
- Real-time kinetic data
- Automated analysis

Result

- Vastly higher throughput + dramatically faster timelines
- Significantly shortened development cycles

QC: Quality control; CAR-T: Chimeric antigen receptor T cell

Antibody Discovery and CAR Design Acceleration

Antibody Discovery: From Months to Weeks

The Challenge: Find optimal scFv with:

- High specificity
- Right affinity
- Minimal off-target effects
- Stable expression

Traditional Approach

- Phage display
- Individual clone testing
- Low-throughput validation
- **Timeline: Many months**

HTS Cytometry Approach

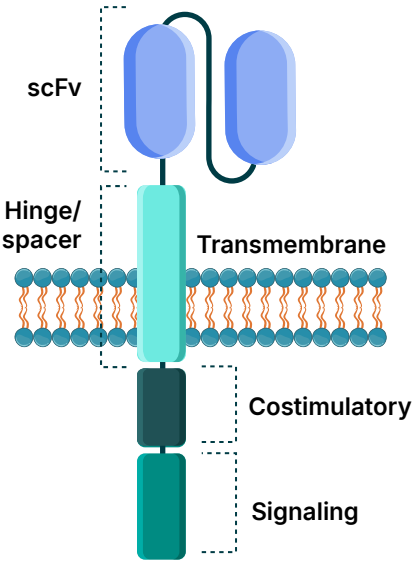
- Parallel screening of antibody libraries in functional CAR-T format
- Test hundreds of scFv candidates simultaneously
- Measure binding + activation + function in one assay
- Screen in relevant T cell context, not just binding
- **Timeline: Weeks**

Key Advantage

Test scFvs directly as CAR constructs—see real functional impact immediately

CAR Design Optimization: Systematic Screening at Scale

CAR Components to Optimize:



Traditional: Test limited configurations (e.g., CD28 vs. 4-1BB)

HTS: Screen 40+ signaling domains in a parallel combinatorial library

Example Success: Novel Domain Discovery

Pooled CAR screening identified BAFF-R costimulatory domain

- Enhanced cytotoxicity vs. standard 4-1BB
- Better outcomes in xenograft models
- Improved persistence under chronic antigen stimulation
- From weeks of sequential testing to days of parallel profiling

scFv: Single-chain variable fragment

QC at Every Stage—Accelerated

Integrated QC Throughout Development

Use Case 1: Functional Validation (CD19-Targeted CAR-T)

Platform

Incucyte® + iQue® combined workflow

Setup

Anti-CD19 CAR-T variants vs. CD19
+
Ramos (target) and CD19- Jurkat (control) cells

Multiplexed Measurements (24–48 hours)

- Real-time tumor killing kinetics (Incucyte®)
- CAR-T activation (CD25 and CD69) (iQue®)
- Cytokine secretion (IFN-γ and TNF-α) (iQue®)
- T cell proliferation and phenotype (iQue®)

CD: Cluster of differentiation; IFN-γ: Interferon-gamma; TNF-α: Tumor necrosis factor; EC50: Half-maximal effective concentration

Results

- **Confirmed antigen-specific killing**
- **Quantified EC50 for each construct**
- **Minimal off-target activation**

vs.

Traditional: Multiple separate assays over extended timeframes

Use Case 2: Exhaustion Profiling Under Chronic Stimulation

Challenge

Will CAR-T cells maintain function long-term?

HTS Solution

Continuous antigen challenge assay (10 days)

iQue® Human T Cell Exhaustion Kit tracks:

- Exhaustion markers (PD-1, TIM-3, and LAG-3) expression
- Proliferative potential
- Cytokine production capacity (IFN-γ and TNF-α)

PD-1: Programmed cell death protein 1; TIM-3: T cell immunoglobulin and mucin-domain containing-3; LAG-3: Lymphocyte activation gene

Results

- **Identify CAR designs maintaining function under pressure**
- **Traditional: Single endpoint only**
- **HTS: Continuous monitoring = predictive data**

Use Case 3: Safety Profiling—"On-Target, Off-Tumor" Risk

Critical Challenge

HER2 CAR-T toxicity in solid tumors

Problem

Attacks healthy tissue with low HER2 expression

HTS Solution

3D spheroid specificity screen

Test Panel

AU565 (high HER2) | MDA-MB-231 (low HER2) | MDA-MB-468 (negative) + HER2 CAR-T variants

Real-Time Incucyte® Monitoring

- Strong killing of high HER2
- Minimal activity vs. low HER2
- No activity vs. negative

- **Speed:** Results in days vs. extended traditional timelines
- **Scale:** Screen 10+ CAR variants × 5+ cell lines in one 384-well experiment

HER2: Human epidermal growth factor receptor 2

Outcome

Identify tumor-selective, safer CAR designs before clinical testing

The Integrated Platform and Impact

iQue® HTS Cytometry Platform

- 20+ parameter flow cytometry
- 384-well throughput
- Cell phenotype + secreted proteins (same well)
- Automated ForeCyt® software analysis

+

Incucyte® Live-Cell Analysis

- Real-time kinetic imaging (days–weeks)
- 96/384-well automated acquisition
- Label-free and fluorescent detection
- Continuous monitoring in the incubator

Applications Across the CAR-T Pipeline

Discovery
- Antibody screening
- CAR construct libraries
- Specificity profiling

Optimization
- Killing kinetics
- Activation profiles
- Cytokine production

Expansion and QC
- Proliferation tracking
- Phenotype maintenance
- Exhaustion monitoring

Manufacturing
- Product potency
- Identity/purity
- Release testing

The Bottom Line: HTS Delivers

Speed
Dramatically faster development timelines (shortening months)

Scale
Vastly higher through-put (100s of conditions simultaneously)

Depth
20+ parameters vs. 1–3 traditional (richer biological insight)

Predictive power
Real-time kinetics (better clinical translation)

Safety
Comprehensive specificity profiling (reduces clinical risk)

Result

More effective CAR-T therapies, reaching patients faster

WILEY

SARTORIUS

Further Resources

White paper: Phenotypic and Functional Characterization of CAR-T Cells with Advanced Flow Cytometry and Live-Cell Analysis

Webinars:
- CAR-T: Why Not Me?
- The Importance of Immune Profiling in CAR-T Therapies

Key Publications:
- Wang *et al.* (2021) - High-throughputimage cytometry for CAR-T. *Cytometry Part A*
- Sarikonda *et al.* (2021) - Best practices for CAR-T flow cytometry. *Cytometry Part B*