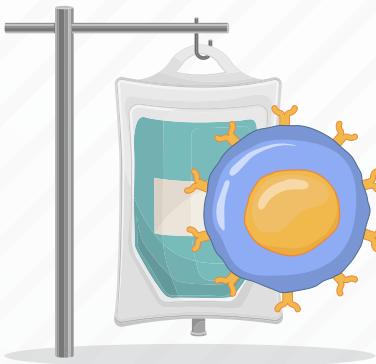


Next-Gen CAR-T: High-Throughput Screening Accelerates Discovery

Understanding high-throughput screening approaches in antibody discovery and CAR construct optimization

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

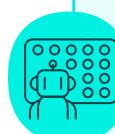
Modern platforms combine flow cytometry and live-cell analysis capabilities



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

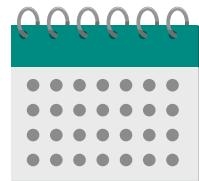
Antibody Discovery and CAR Design Acceleration

Antibody Discovery: From Months to Weeks



The Challenge: Find optimal scFv with:

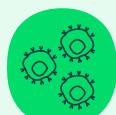
- High specificity
- Right affinity
- Minimal off-target effects
- Stable expression



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

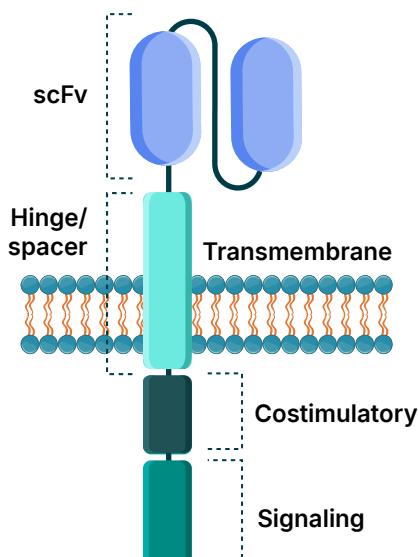
Key Advantage



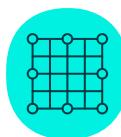
Test scFvs directly as CAR constructs—see real functional impact immediately

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

QC at Every Stage—Accelerated

Integrated QC Throughout Development

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

Platform Setup	Multiplexed Measurements (24–48 hours)			
 +  Combined workflow using automated cytometry and live-cell imaging	 Real-time tumor killing kinetics	 CAR-T activation (CD25 and CD69)	 Cytokine secretion (IFN-γ and TNF-α)	 T cell proliferation and phenotype
Testing	Results  Confirmed antigen-specific killing  Quantified EC50 for each construct  Minimal off-target activation			
Anti-CD19 CAR-T variants vs. CD19 + Ramos (target) and CD19-Jurkat (control) cells	VS. Traditional: Multiple separate assays over extended timeframes			

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

Use Case 2: Exhaustion Profiling Under Chronic Stimulation

Challenge	Specialized Kits Track:		
 Will CAR-T cells maintain function long-term?	 Exhaustion markers (PD-1, TIM-3, and LAG-3) expression	 Proliferative potential	 Cytokine production capacity (IFN-γ and TNF-α)
HTS Solution	Results  Identify CAR designs maintaining function under pressure  Traditional: Single endpoint only  HTS: Continuous monitoring = predictive data		
 Continuous antigen challenge assay (10 days)	PD-1: Programmed cell death protein 1; TIM-3: T cell immunoglobulin and mucin-domain containing-3; LAG-3: Lymphocyte activation gene		

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

Critical Challenge	Test Panel			
 HER2 CAR-T toxicity in solid tumors	AU565 (high HER2)	MDA-MB-231 (low HER2)	MDA-MB-468 (negative)	 HER2 CAR-T variants
Problem	Real-Time Monitoring:			
 Attacks healthy tissue with low HER2 expression	 Strong killing of high HER2  Minimal activity vs. low HER2  No activity vs. negative			
HTS Solution	Speed: Results in days vs. extended traditional timelines Scale: Screen 10+ CAR variants × 5+ cell lines in one 384-well experiment			
 3D spheroid specificity screen	HER2: Human epidermal growth factor receptor 2			
Outcome	Identify tumor-selective, safer CAR designs before clinical testing			

The Integrated Platform and Impact

Automated HTS Cytometry Platform Capabilities

Flow Cytometry Systems:



20+ parameter flow cytometry



384-well throughput



Cell phenotype + secreted proteins (same well)



Automated software analysis

+

Live-Cell Analysis Systems:



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 throughput (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



Example platforms for integrated HTS workflows include the [iQue® HTS Cytometry](#) and [Incucyte® Live-Cell Analysis Systems](#) from Sartorius



White paper: [Phenotypic and Functional Characterization of CAR-T Cells with 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-throughput Image Cytometry for CAR-T. Cytometry Part A](#) • [Sarikonda et al. \(2021\) - Best Practices for CAR-T Flow Cytometry. Cytometry Part B](#)