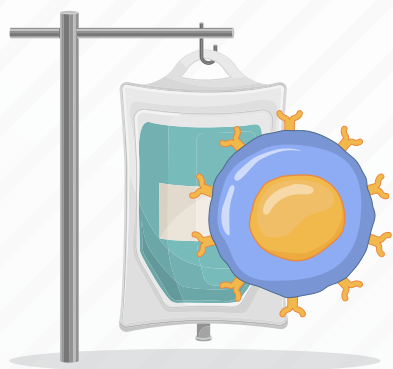


Next-Gen CAR-T: High-Throughput Screening Accelerates Discovery

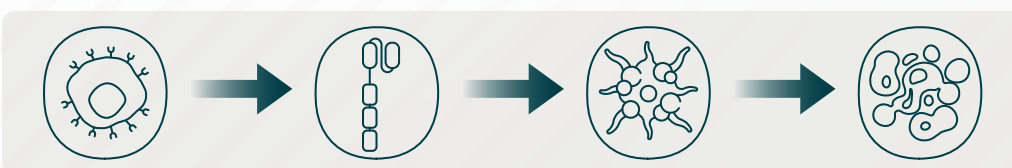
Understanding high-throughput screening approaches in antibody discovery and CAR construct optimization

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

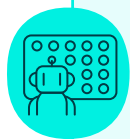
Modern platforms combine flow cytometry and live-cell analysis capabilities



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

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



Vastly higher throughput + dramatically faster timelines



Significantly shortened development cycles

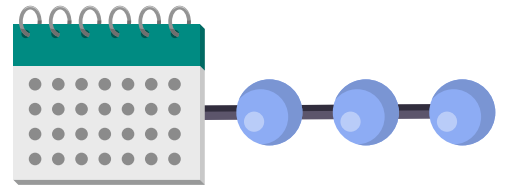
Antibody Discovery and CAR Design Acceleration

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

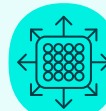


Low-throughput validation

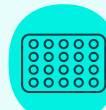


Timeline: Many months

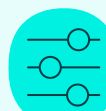
HTS Cytometry Approach



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

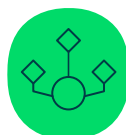
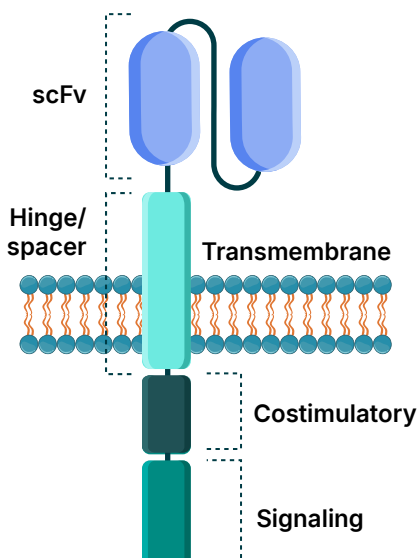
Key Advantage



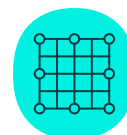
Test scFvs directly as CAR constructs—see real functional impact immediately

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



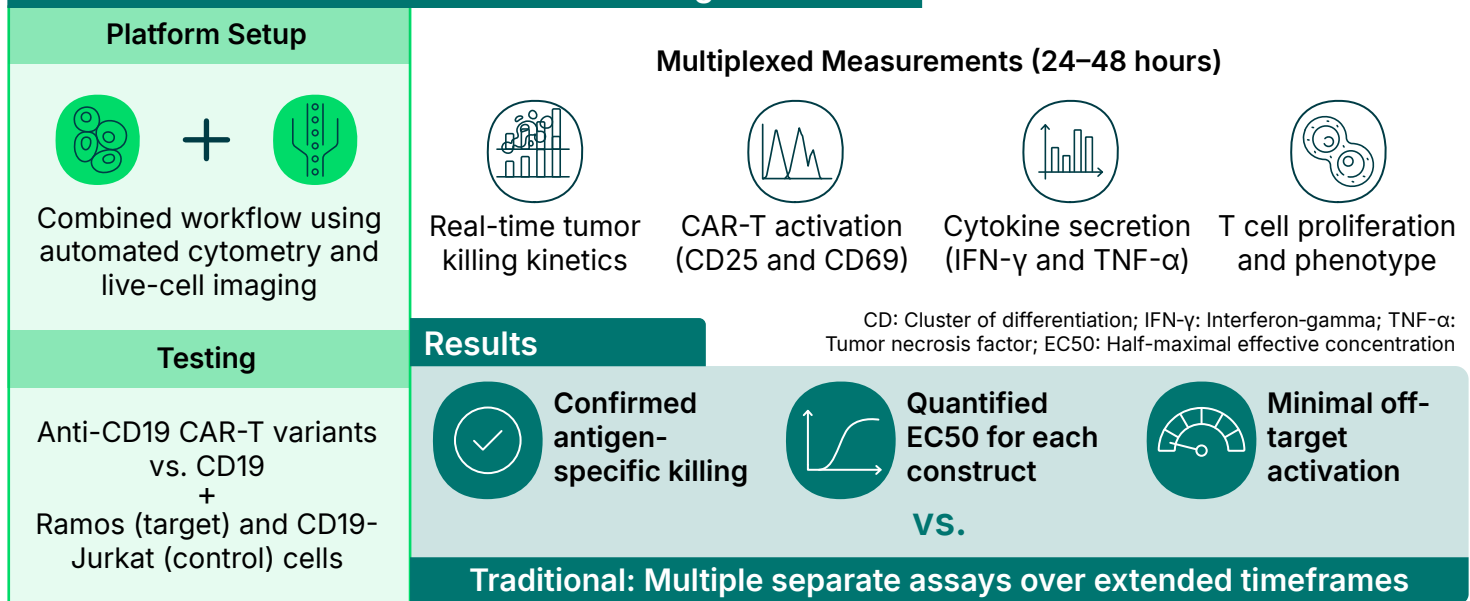
From weeks of sequential testing to days of parallel profiling

scFv: Single-chain variable fragment

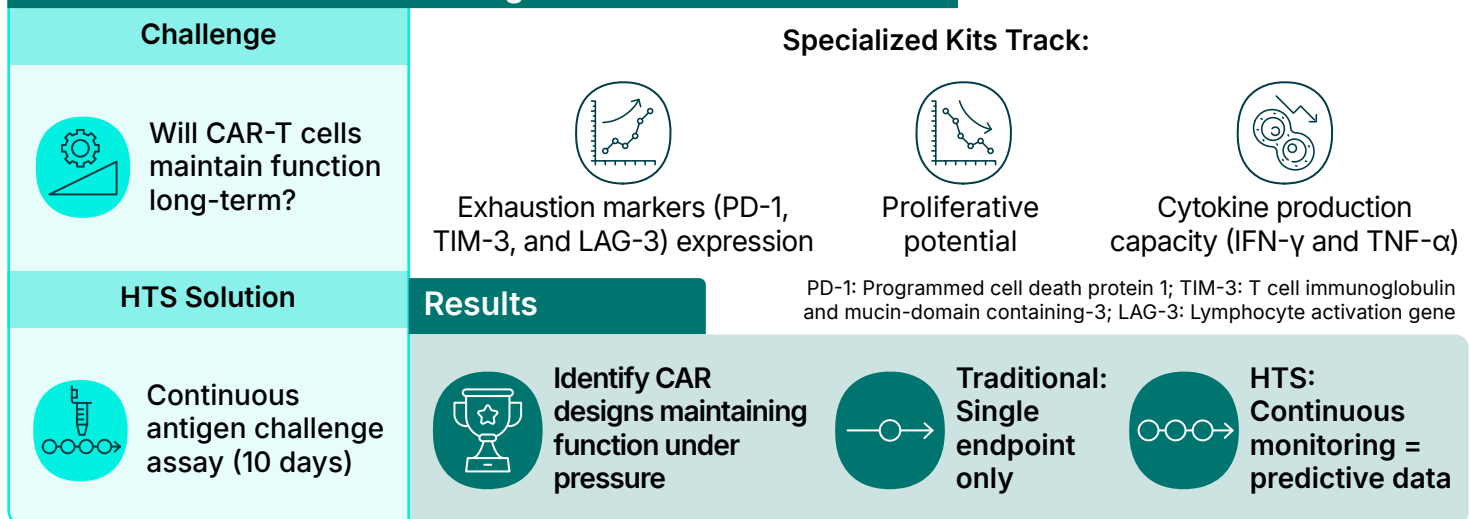
QC at Every Stage—Accelerated

Integrated QC Throughout Development

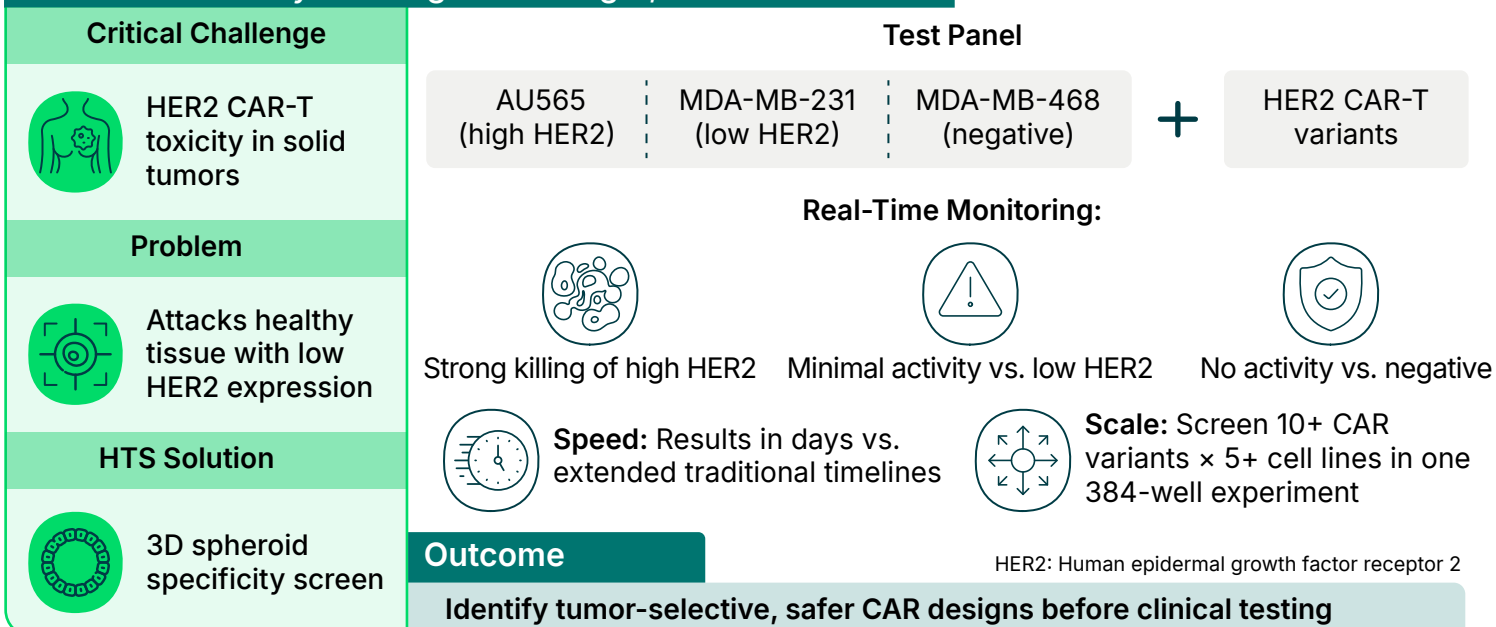
Use Case 1: Functional Validation (CD19-Targeted CAR-T)



Use Case 2: Exhaustion Profiling Under Chronic Stimulation



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



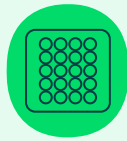
The Integrated Platform and Impact

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:



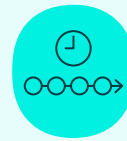
Real-time kinetic
imaging (days-weeks)



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

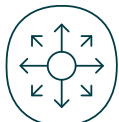
- Product potency
- Identity/purity
- Release testing

The Bottom Line: HTS Delivers



Speed

Dramatically faster
development timelines
(shortening months)



Scale

Vastly higher 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



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](#)