Concurrent targeting of tumors and their microenvironment using CAR-T cells specific for fibroblast activation protein alpha

Larina Tzu-Wei Shen, Afsana Islam, Daiki Fujita, Gunarta I Ketut, Kenji Nakamaru, Dan MacLeod
Optieum Biotechnologies Inc.

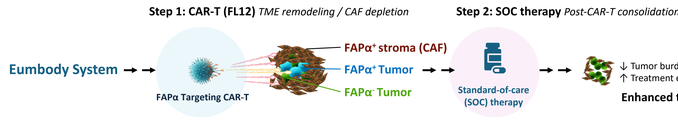
OPTIEUM BIOTECHNOLOGIES
Abstract No. 1521

Eumbody Selection → Avidity Tuning → Functional Optimization → In vivo Efficacy → TME Remodeling

Background

- Cancer-associated fibroblasts (CAFs) contribute to an immunosuppressive tumor microenvironment, limit CAR-T efficacy in solid tumors and response to standard therapies
- High-avidity CAR-T cells can induce antigen depletion and T cell exhaustion through excessive receptor engagement
- Optimal tuning of CAR binding avidity remains a key challenge for improving CAR-T functional fitness and persistence

Therapeutic Strategy: Sequential Targeting of Tumor and Microenvironment



Eumbody System: An iterative functional selection platform for optimization of scFv-CAR T Cells

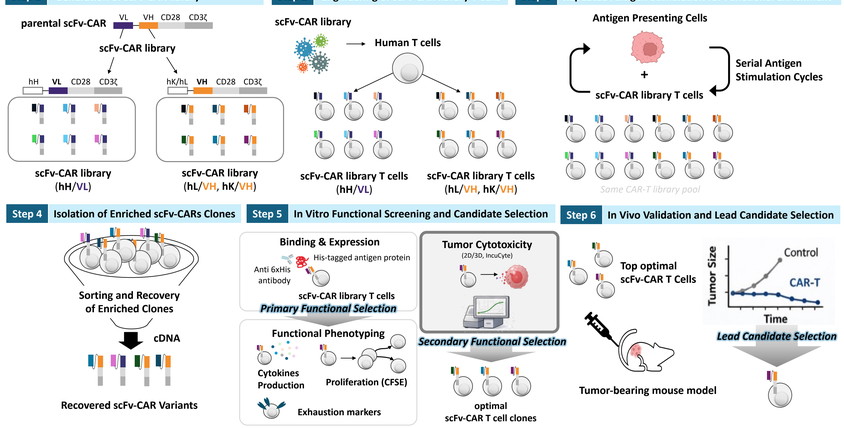


Figure 1. Eumbody System for iterative functional optimization of scFv-CAR T cells. A diverse scFv-CAR library is generated through variable region recombination and expressed in primary human T cells. Library CAR-T cells undergo repeated antigen stimulation to enrich functionally superior clones under sustained activation pressure. Enriched populations are sorted, and recovered scFv sequences are re-engineered into CAR constructs. Candidate CAR-T cells are evaluated through in vitro functional screening, including antigen binding, cytokine production, proliferation, exhaustion profiling, and tumor cytotoxicity (2D/3D assays). Top-performing candidates are advanced to in vivo tumor models for efficacy validation and lead selection.

Eumbody System: *CAR Discovery Engine*

5 Key Characteristics of Eumbody Derived CAR
1. Potent and persistent anti-tumor efficacy
2. Less risk of antigen escape (trogocytosis)
3. Better growth / expansion
4. Optimized cytokine profile
5. Persistence of CAR-T cells *- Exhaustion* *- Naïve / Central Memory subset*

Model validation for target expression

Cell line	Status	% FAPα^{+} cells (of live cells)	FAPα surface expression MFI
A375	FAPα^{negative}	0.1	93
U87MG	FAPα^{high}	86.7	13248
K562/FAPα	FAPα^{high}	99.1	138740
IMR32	FAPα^{negative}	0.19	38
U2OS	FAPα^{low}	12	701
U251MG	FAPα^{low}	9	5109
GBM-CAF	FAPα^{low}	35	3976
TIG-3 (fibroblast)	FAPα^{high}	98.4	12902

Table 1. Characterization of FAPα expression across cell line models used in this study. FAPα expression levels were quantified by flow cytometry as percentage of FAPα-positive cells within the live cell population and corresponding surface expression (MFI).

FL1, FL8 and FL12: In Vitro Functional Screening and Candidate Selection

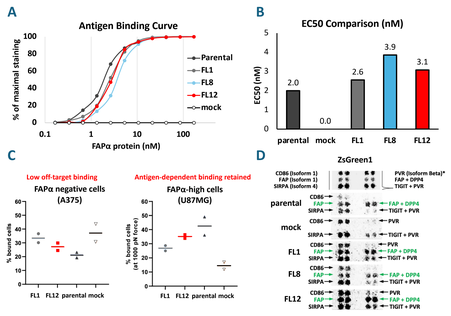


Figure 2. Eumbody System enables tuning of CAR binding avidity through scFv selection. A) Antigen binding curves demonstrate right-shifted binding profiles of selected scFv-CAR T cells relative to the parental. B) EC50 analysis indicates reduced apparent affinity of selected clones. C) Functional avidity assessed by Z-Movi under differential antigen density shows low binding to FAPα^{negative} cells (A375) while maintaining antigen-dependent binding to FAPα^{high} cells (U87MG). D) Retrogenix screening confirms target specificity with no significant off-target interactions beyond FAPα-associated targets.

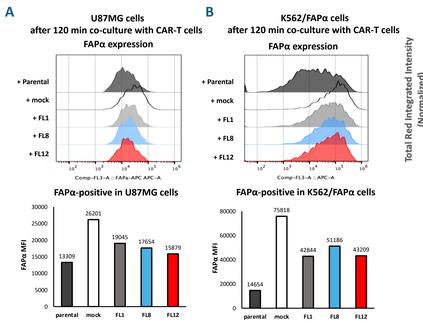


Figure 3. Reduced CAR binding avidity preserves target antigen expression following CAR-T engagement. A) FAPα expression on U87MG cells following 120 min co-culture with CAR-T cells. B) FAPα expression on K562/FAPα cells under the same conditions. Higher-avidity CAR-T cells (parental) induce greater loss of surface antigen, whereas selected lower-avidity clones (FL1, FL8, FL12) preserve antigen expression, consistent with reduced trogocytosis.

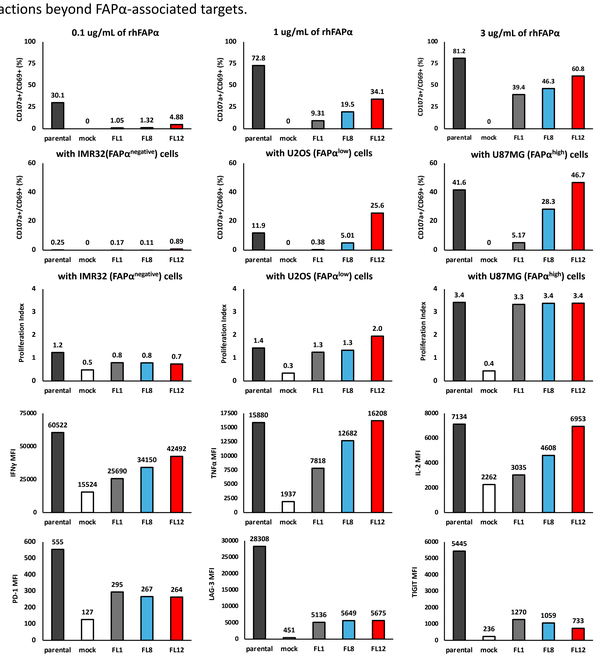
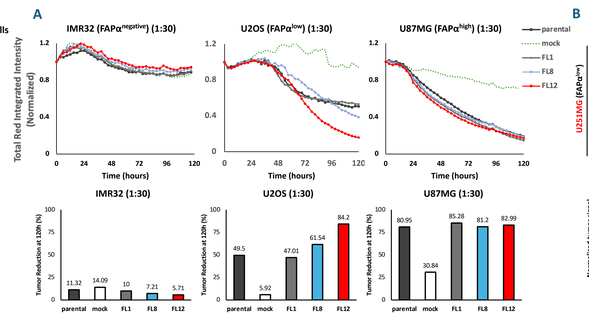


Figure 4. CAR optimization by Eumbody enables controlled activation, preserves functional responses, and limits T cell exhaustion. A) Activation (CD107a/CD69) across increasing antigen concentrations shows reduced excessive activation in selected clones while maintaining antigen responsiveness. B) Proliferation assays demonstrate preserved antigen-dependent expansion without non-specific proliferation. C) Cytokine profiling indicates FL12 maintains levels comparable to parental CAR-T cells without exaggerated responses after stimulation with immobilized rhFAPα protein (3 μg/mL, 20 h). D) Exhaustion markers (PD-1, LAG-3, TIGIT) are reduced in selected clones vs parental CAR-T cells after stimulation with immobilized rhFAPα protein (3 μg/mL, 72 h).

FL12: In Vivo Validation and Lead Candidate Selection



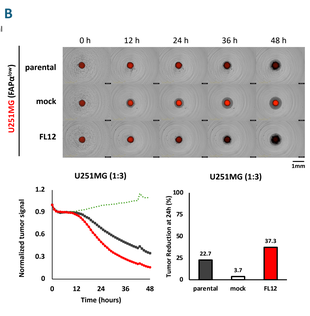


Figure 5. FL12 CAR-T cells exhibit antigen-dependent cytotoxicity and enhanced efficacy in 3D tumor spheroid models. A) IncuCyte 3D assays across cell lines with varying FAPα expression show minimal activity in FAPα^{negative} IMR32 cells and increased cytotoxicity in FAPα^{low} (U2OS) and FAPα^{high} (U87MG) models. FL12 demonstrates enhanced tumor reduction compared to FL1 and FL8, correlating with antigen expression. B) In 3D spheroid models (U251MG, FAPα^{low}), FL12 induces faster tumor clearance at E:T=1:3 compared to parental and mock controls, with greater efficacy in the FAPα-high model. Tumor burden was measured as normalized red integrated intensity; tumor reduction (%) was calculated at 24 hr.

Figure 6. FL12 CAR-T cells mediate durable tumor control in a U87MG CDX model with no observable systemic toxicity. A) At E:T=1 (2x10^6 U87MG cells and 2x10^6 CAR-T cells), FL12 demonstrates sustained suppression of tumor growth compared to FL1 and FL8. Body weight remains stable across all groups, indicating tolerability. Quantification at Day 30 shows ~81% tumor growth inhibition for FL12. B) Dose-response analysis across E:T ratios (0.3, 1, and 3; 2×10^6 U87MG cells with 6×10^5, 2×10^6, and 6×10^6 CAR-T cells) demonstrates robust, dose-dependent efficacy of FL12, with maintained activity even at low effector levels.

Extension to Tumor Microenvironment Models

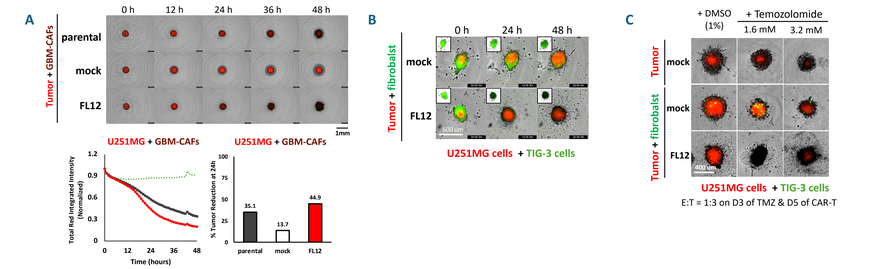


Figure 7. FL12 CAR-T cells overcome stromal-mediated resistance and restore temozolomide sensitivity in 3D tumor models. A) In CAF-containing GBM spheroids (~30% FAPα^{+}), CAF-specific killing cannot be resolved due to mixed signal; tumor cytotoxicity is used as the functional readout, demonstrating that FL12 remains effective in a stromal-rich microenvironment. B) In fibroblast-containing spheroids (~95% FAPα^{+}), FL12 induces loss of green signal, indicating direct elimination of FAPα-high stromal cells.
C) Stromal (FAPα^{high}) cells reduce temozolomide efficacy, which is restored following FL12-mediated stromal depletion, supporting improved combination therapeutic potential.

Conclusion

CAR optimization by Eumbody system generates a functionally optimized CAR-T cell – FL12 with improved fitness, sustained antitumor activity, and the potential to remodel the tumor microenvironment.