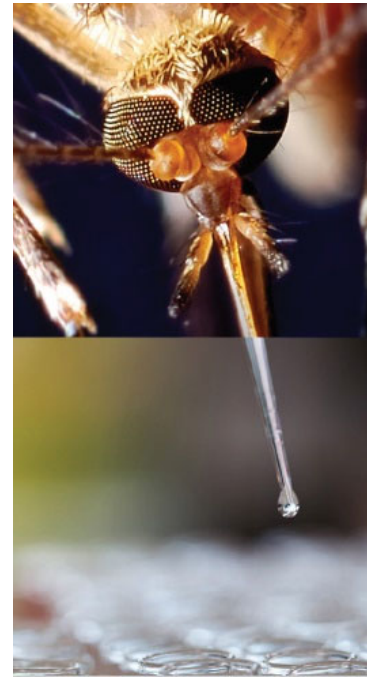
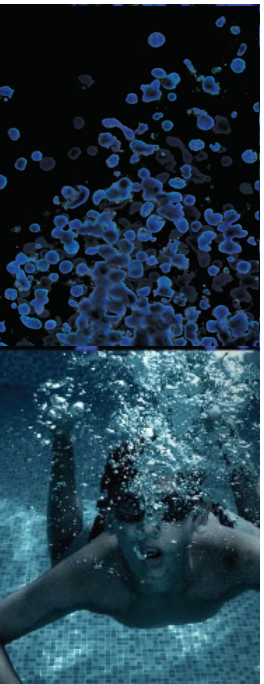




Luciferase Reporter Cancer Cell Lines: Facilitate Your CAR-T Development

John Foulke, MS
Lead Biologist
Immuno-Oncology R&D, ATCC

Credible Leads to Incredible™

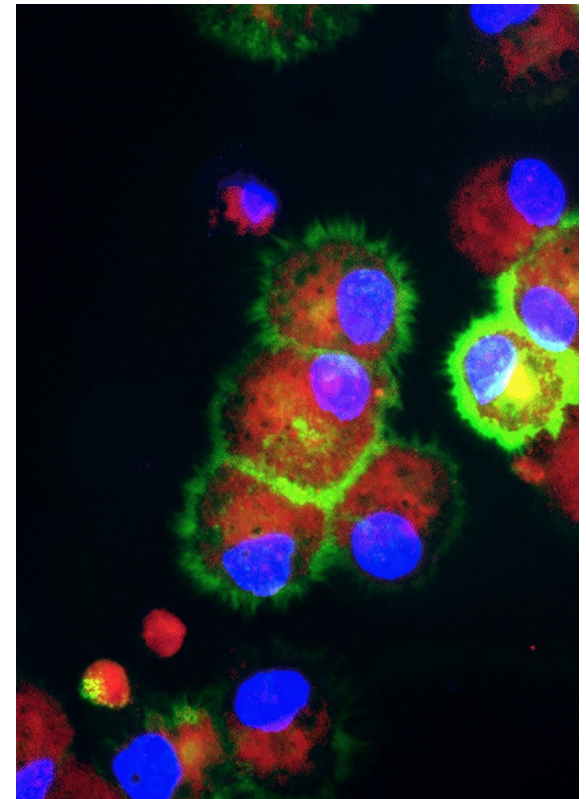


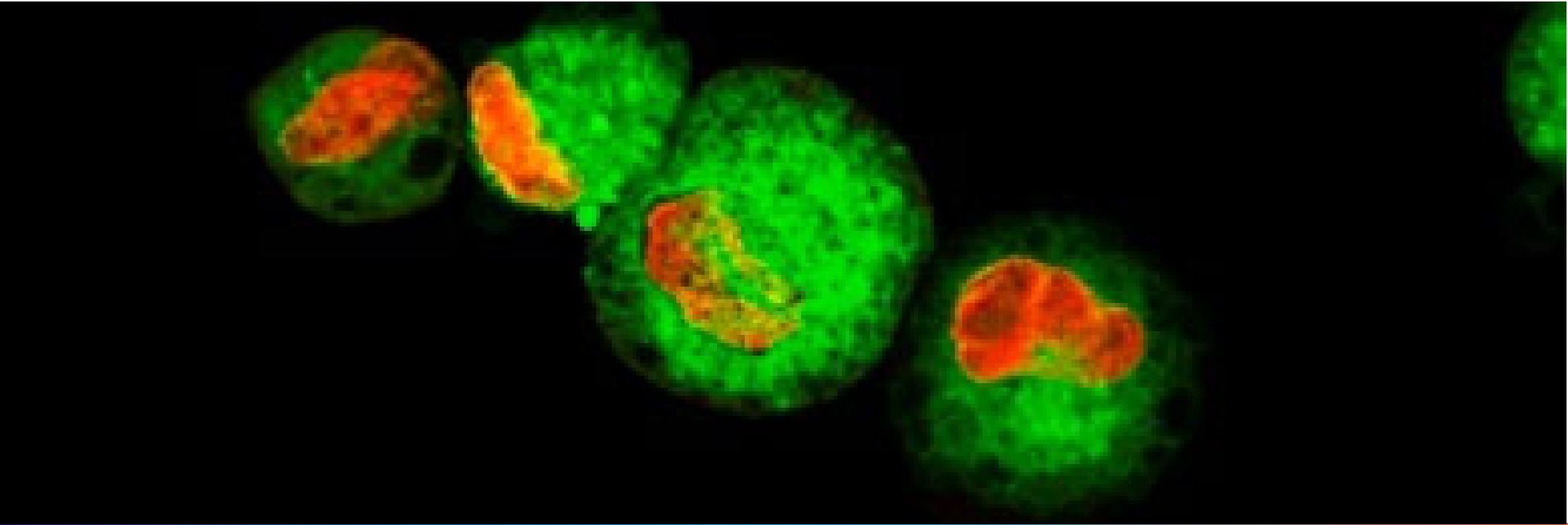
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell biology – the “gold standard”
- Innovative R&D company featuring advanced cell models and immuno-oncology tools
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viruses, and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees

Agenda

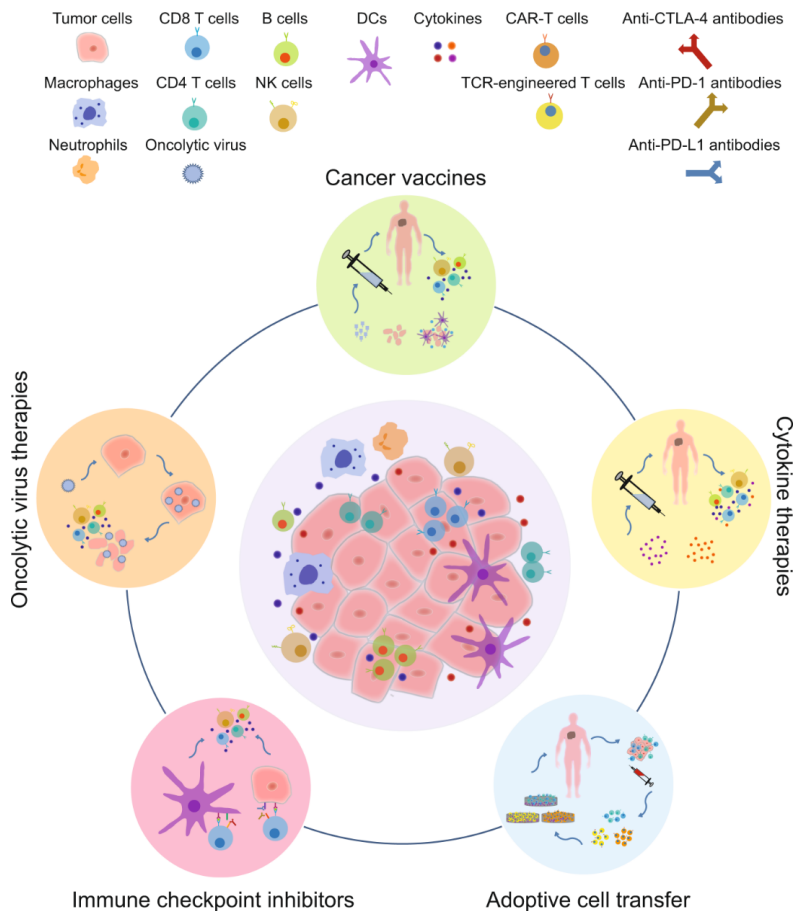
- Introduction
- ATCC CAR-T luciferase reporter cell line generation
- ATCC application data
- Summary





Introduction

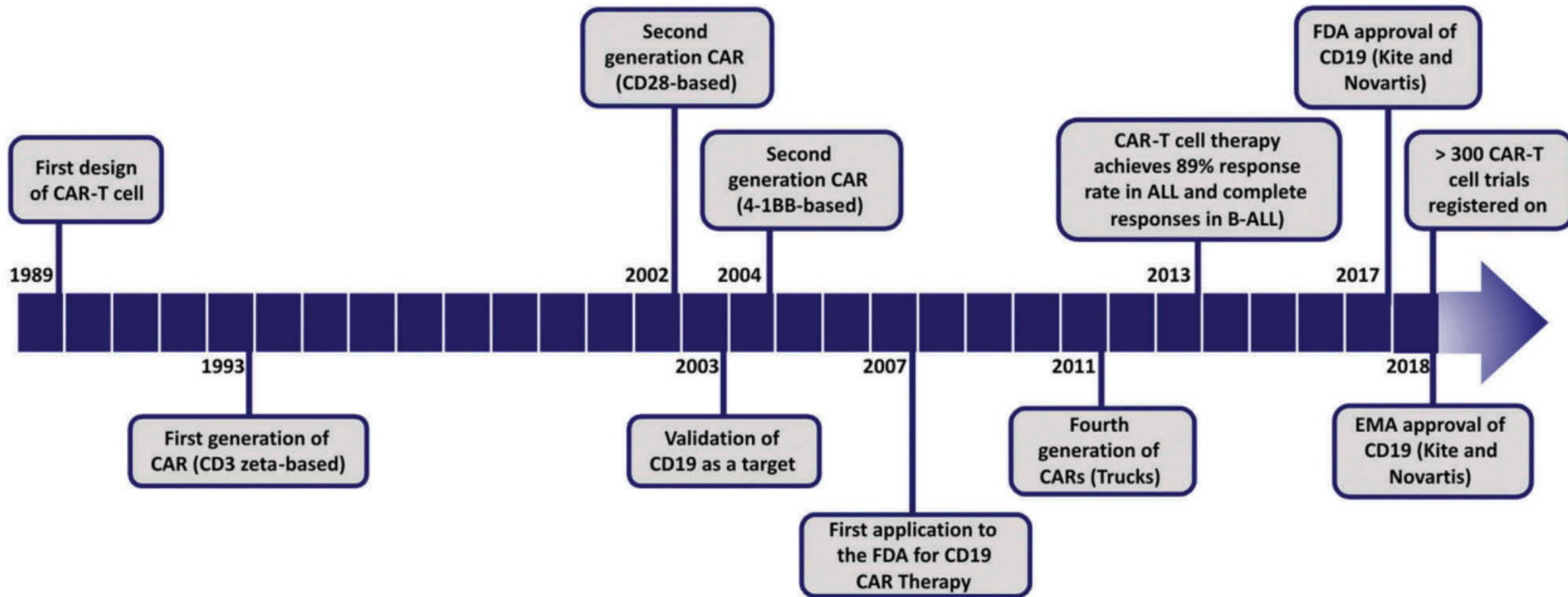
The Major Categories of Immunotherapy



1. Oncolytic virus therapies (eg, talimogene laherparepvec for melanoma)
2. Cancer vaccines (eg, BCG live for bladder cancer and sipuleucel-T for prostate cancer)
3. Cytokine therapies (eg, IFN- α for stage III skin cancer, IL-2 for metastatic melanoma and renal cancer, and GM-CSF for neuroblastoma)
4. Adoptive cell transfer (eg, CD19-targeting CAR T for leukemia and lymphoma and BCMA-targeting CAR T for advanced multiple myeloma)
5. Immune checkpoint inhibitors (eg, CTLA-4 inhibitor for melanoma and PD-1 inhibitors for melanoma & non-small-cell lung cancer)

Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol.* 2020 Aug;17(8):807-821. doi: 10.1038/s41423-020-0488-6. Epub 2020 Jul 1. PMID: 32612154

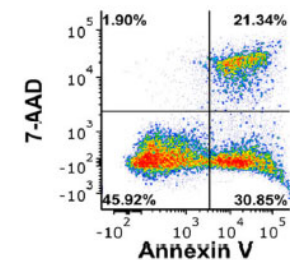
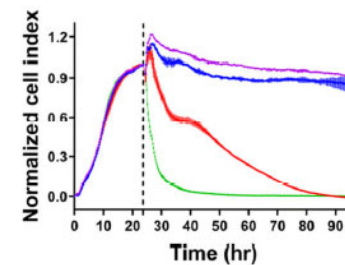
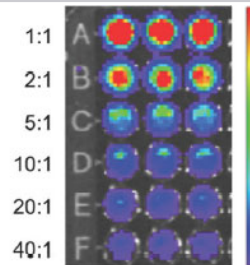
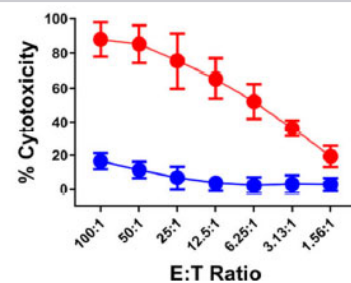
Timeline of CAR-T cell therapy development and progress



Picanco-Castro V et al. e Emerging CAR T cell therapies: clinical landscape and patent technological routes. Human Vaccines & Immunotherapeutics. 2020; 16:6, 1424-1433 DOI: 10.1080/21645515.2019.1689744. PMID: 31702480

Properties of cell-mediated cytotoxicity assays

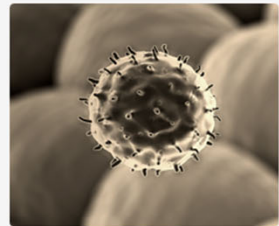
Assay format	Chromium release	Bioluminescence	Impedance	Flow cytometry
Principal measure of cytotoxicity	^{51}Cr release	Luciferase activity	Cell detachment	Live/dead staining phenotype
Radioactive materials needed	Yes	No	No	No
Target cell labeling required	Yes	No	No	Yes
Genetic modification of target cells	No	Yes (reporter gene)	No	Yes
Endpoint/kinetic	Endpoint	Endpoint	Temporal	Endpoint
Real-time measurement	No	No	Yes	No
Maximum time point measured	18 – 24 hours	Days	Days	Days
Ability to measure different cytotoxicity heterogenous targets	No	No	No	Yes
Throughput and automatability	Low	High	High	High



ATCC internal efforts

Cancer immunology research tools

Boost your cancer immunology research with well authenticated and characterized cell models focused on advancing areas such as cancer cell-directed therapies.

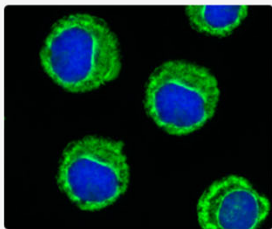


Primary Human Immune Cells

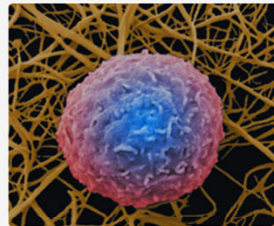
ATCC primary immune cells support complex, physiologically relevant research projects, including toxicity screening, transplantation and graft rejection, inflammation and allergy, vaccine, drug development, and cancer immunology studies.

Progenitor Cells

Hematopoietic progenitor cells are capable of differentiating into all blood cell types. Understanding progenitor cells and the cells that derive from them is critical to many fields, including hematology, immunology, and oncology.



The immune system is central to many forms of cancer, being critical in both its development and its treatment, including immune cell-directed therapies.

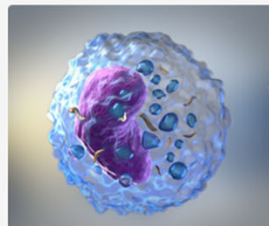


Lymphoid Cells

Lymphoid cells provide long-lasting immunity against microbes and pathogens and are essential in fields such as autoimmunity and cancer immunotherapies. Human primary lymphoid cells provide physiological relevance to your research.

Natural Killer Cells

Natural killer (NK) cells were first identified for their ability to kill tumor cells without activation. NK cells are a focus of many cancer immunotherapies, as they display rapid and potent immunity to metastasis or hematological cancers.



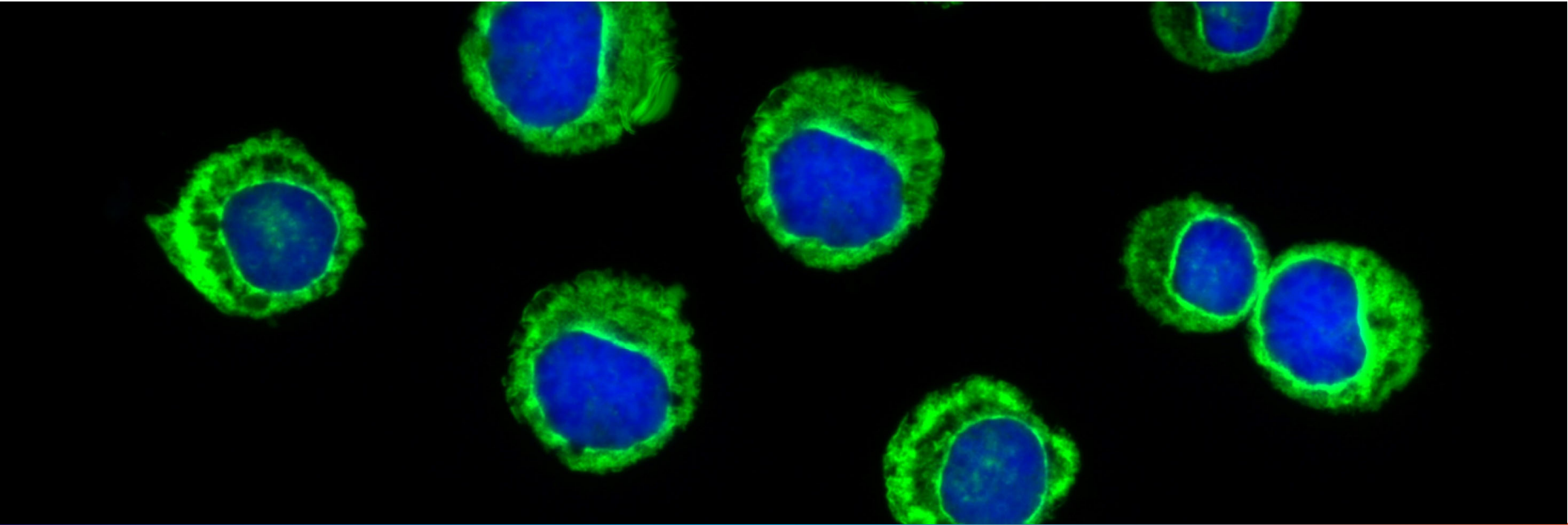
- Generated and released 30 Luciferase reporter cell lines
- Generated 5 new CAR-T Luciferase reporter cell lines
- Endogenous natural expression of CAR-T targets on the cell surface provides a cost-saving measure over screening of patient samples

ATCC human Luc2 cells with common CAR targets (CCLE database)

Cell Line Info			Haematological Tumor CAR Targets											
ATCC LUC2 ID	Cell Line Name	Lineage Subtype	TNFRSF17 (BCMA)	IL3RA (CD123)	SDC1 (CD138)	CD19	MS4A1 (CD20)	CD22	CD38	CD5	FUT3 (LeY)	KLRK1 (NKG2D)	ROR1	WT1
CRL-8885-LUC2	WIL2-S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CCL-86-LUC2	RAJI	Non Hodgkin Lymphoma	3.8807	0.0566	0.3785	7.1104	8.0865	7.5211	5.8276	0.0144	0.0144	0.0286	0.7312	0.0144
HTB-20-LU2	BT474	Breast Ductal Carcinoma	0.1635	0.2141	6.2052	0.0000	0.0000	0.2141	0.0286	0.1506	0.1506	0.0704	0.0286	0.0000
CRL-2630-LU2	FARAGE	Non Hodgkin Lymphoma	1.3785	0.0000	0.0144	6.5132	9.0930	8.6641	3.8032	0.0286	0.0566	0.7049	0.0000	0.1243
CCL-213-LUC2	DAUDI	Non Hodgkin Lymphoma	4.1094	0.3103	0.3103	6.9925	8.8855	9.8023	7.4682	0.0976	0.0841	1.9745	0.3334	0.1763
CCL-121-LUC2	HT1080	Fibrosarcoma	0.0000	0.1890	7.1797	0.0841	0.0704	3.4436	0.0841	0.0286	0.0000	0.0000	1.7570	2.3190
CCL-185-LUC2	A549	NSCLC	0.0000	0.0841	6.5703	0.0704	0.0426	0.2388	2.5008	0.0000	0.0286	0.0000	3.8620	2.1699
CCL-225-LUC2	HCT15	Colorectal Adenocarcinoma	0.0426	0.0566	5.7142	0.0000	0.1763	0.2510	0.0000	0.0144	1.7442	0.0566	0.5460	0.0286
CCL-228-LUC2	SW480	Colorectal Adenocarcinoma	0.1243	0.1890	4.7719	0.0144	0.0000	0.9336	0.0144	0.0144	0.0426	0.1506	1.6041	1.4594
CCL-240-LUC2	HL60	AML	0.2869	0.4436	0.6229	0.1890	0.0000	0.0286	0.6041	0.0000	0.0426	1.1440	0.0704	3.4370
CCL-247-LUC2	HCT116	Colorectal Adenocarcinoma	0.0704	0.0000	4.9303	0.0286	0.1375	2.1210	0.0000	0.0144	0.1506	1.1440	1.2327	0.1243
CRL-1435-LUC2	PC3	Prostate Adenocarcinoma	0.1110	0.0000	7.6454	0.0426	0.0000	0.2388	0.0426	0.1506	2.4222	0.0841	2.0496	1.9964
CRL-1469-LUC2	PANC1	Exocrine	0.2987	0.1635	6.3425	0.0704	0.0144	1.0000	0.0976	0.0286	0.0566	0.0286	4.0036	3.9495
CRL-1555-LUC2	A431	Skin Squamous	0.0000	0.0000	7.7041	0.0000	0.0000	2.0000	0.6323	0.0426	2.8875	0.0704	0.2630	0.0000
CRL-1619-LUC2	A375	Melanoma	0.1375	0.1635	4.5027	0.0144	0.1110	2.9030	0.0000	0.0144	0.0841	0.0841	1.4114	2.9165
CRL-1739-LUC2	AGS	Gastric Adenocarcinoma	0.0000	0.1110	5.0866	0.0000	0.0841	0.5059	0.1110	0.0144	0.1763	0.2510	5.5097	0.0841
CRL-2003-LUC2	TF1	AML	0.0000	1.9928	1.0000	1.6182	0.1763	3.4141	3.1570	0.0144	0.0286	0.2750	0.0144	4.6118
HTB-14-LUC2	U87MG	Glioma	0.2388	0.0566	5.7230	0.0000	0.0000	2.5558	0.1890	0.0426	0.1110	0.2869	0.1506	0.1635
HTB-22-LUC2	MCF7	Breast Carcinoma	0.0000	0.0566	3.9653	0.0000	0.0976	1.5607	0.2016	0.1763	0.1763	0.0000	0.0144	0.0704
HTB-43-LUC2	FADU	Upper Aerodigestive Squamous	0.0000	0.0841	6.3100	0.0976	0.0566	0.2141	0.2987	0.0144	3.7560	0.1890	0.1506	1.7137
HTB-96-LUC2	U2OS	Osteosarcoma	0.2016	0.3785	6.3583	0.0426	0.1243	1.6781	0.0704	0.0976	0.1375	1.2327	3.6508	1.5705

ATCC human Luc2 cells with common CAR targets (CCLE database)

Cell Line Info			Solid Tumour CAR Targets																										
ATCC LUC2 ID	Cell Line Name	Lineage Subtype	MET (C-Met)	CA9 (CAIX)	PROM1 (CD133)	L1CAM (CD171)	CD70	CEACAM5 (CEA)	EGFR	EPCAM (Ep-CAM)	EPHA2 (EphA2)	FAP	GPC3	ERBB2 (HER2)	IL13RA2 (IL13Ra2)	FUT3 (LeY)	MAGEA3	MAGEA4	MLANA (MART1)	MSLN (Mesothlin)	MUC1	MUC16	CTAG1B (NY-ESO-1)	CD274 (PD-L1)	PSCA	FOLH1 (PSMA)	ROR1	KDR (VEGFR2)	
CRL-8885-LUC2	WIL2-S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CCL-86-LUC2	RAJI	Non Hodgkin Lymphoma	2.7506	0.0144	0.0976	0.3896	6.9706	0.1243	0.1635	0.5361	0.4854	0.0566	0.0000	1.2510	0.0566	0.0144	0.0704	0.1243	0.3219	0.1763	0.1763	0.0144	0.0426	0.7485	0.3103	0.0000	0.7312	0.0000	
HTB-20-LUC2	BT474	Breast Ductal Carcinoma	3.4222	0.2141	0.8074	0.8156	0.1635	2.0670	1.5410	7.4039	2.2265	0.0704	0.8400	10.5222	0.0976	0.1506	0.0976	0.0426	0.0286	0.3103	4.6616	0.0286	0.1110	0.1506	5.0700	1.0976	0.0286	0.0000	
CRL-2630-LUC2	FARAGE	Non Hodgkin Lymphoma	1.5607	0.0000	0.0000	0.6229	7.4529	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.4647	0.0000	0.0566	0.0000	0.0000	0.1635	0.0286	0.0704	0.0144	0.0000	3.6005	0.1243	0.0000	0.0000	0.0000	
CCL-213-LUC2	DAUDI	Non Hodgkin Lymphoma	1.4803	0.0144	0.9411	0.1243	6.2919	0.2265	3.0790	0.6599	0.3674	0.0144	0.0000	3.3089	0.0000	0.0841	0.1110	0.1243	0.8797	0.1506	0.8953	3.4854	0.1110	0.5160	0.1763	0.0976	0.3334	0.0286	
CCL-121-LUC2	HT1080	Fibrosarcoma	6.2300	5.4877	0.0841	0.5753	6.8347	0.0566	4.2950	0.4222	7.0611	0.1506	0.1635	4.2510	0.2265	0.0000	7.1033	0.0000	0.3219	0.1506	1.2690	0.0144	6.4000	5.0605	1.5410	0.4957	1.7570	0.0976	
CCL-185-LUC2	A549	NSCLC	6.4411	0.8319	0.0841	2.3477	3.5361	0.1890	5.3459	2.9523	5.7926	0.0566	0.1375	4.2063	0.0000	0.0286	0.8797	0.1375	0.1506	2.0807	1.0000	0.0704	0.0426	1.7866	0.6323	0.4114	3.8620	0.0000	
CCL-225-LUC2	HCT15	Colorectal Adenocarcinoma	4.7613	4.5336	0.0566	0.2141	0.0426	0.2987	3.9241	9.6185	5.6488	0.0286	0.4751	5.9669	0.0000	1.7442	0.2750	0.2265	0.3561	0.4436	1.3448	0.0426	0.0976	1.2869	0.0426	0.0000	0.5460	0.0000	
CCL-228-LUC2	SW480	Colorectal Adenocarcinoma	1.4699	0.5460	0.0841	5.6363	3.1619	0.1635	3.7159	8.5089	5.5647	0.0144	0.0841	3.6159	0.0566	0.0426	0.0976	0.2510	0.0000	2.7929	1.6554	0.1110	0.0841	1.2810	0.2987	0.0000	1.6041	0.0144	
CCL-240-LUC2	HL60	AML	0.5558	0.0000	0.0426	0.3334	0.8797	0.1110	0.2265	0.6323	0.4114	0.2016	0.0426	1.2449	0.5361	0.0426	0.1110	0.1375	0.0704	0.2016	0.5753	0.0144	0.4222	0.2388	0.0976	0.0000	0.0704	0.0976	
CCL-247-LUC2	HCT116	Colorectal Adenocarcinoma	6.7623	0.0704	3.9079	2.7334	0.4114	0.4222	4.7634	8.3334	6.3918	0.1506	0.6135	4.7570	0.0566	0.1506	7.0889	0.2265	0.2016	4.1043	1.7698	0.0566	0.0000	1.5607	0.3674	0.2750	1.2327	0.0841	
CRL-1435-LUC2	PC3	Prostate Adenocarcinoma	6.2038	0.6229	0.0144	3.6769	1.4647	1.4436	4.8283	7.5399	7.8590	0.0704	0.1110	4.1102	1.8400	2.4222	0.8953	0.3561	0.1375	1.7527	3.2750	0.3334	0.0426	2.5261	1.9523	0.2265	2.0496	0.8875	
CRL-1469-LUC2	PANC1	Exocrine	5.9873	0.0426	0.1506	8.2491	5.8202	0.2388	5.8497	4.2342	7.9525	0.0144	0.1110	4.2758	0.0144	0.0566	0.1635	0.2141	0.1243	1.7991	4.4436	0.5656	0.0000	0.0976	1.0704	0.0286	4.0036	0.0426	
CRL-1555-LUC2	A431	Skin Squamous	4.4456	1.9782	0.0144	0.1375	0.0286	1.4489	7.6442	7.1480	5.1094	0.4330	0.0000	4.8435	0.7312	2.8875	0.0286	8.5461	0.1763	0.0286	1.7181	2.6668	0.0426	2.5435	6.4091	0.0144	0.2630	0.0000	
CRL-1619-LUC2	A375	Melanoma	4.5844	2.1985	0.4542	4.1643	6.2223	0.1110	1.4906	0.7740	5.9300	0.3561	0.4222	4.5033	9.0446	0.0841	7.3091	9.6843	0.3896	0.7049	1.9928	0.0144	5.9816	1.7698	1.1243	2.0670	1.4114	2.1603	
CRL-1739-LUC2	AGS	Gastric Adenocarcinoma	4.8324	0.0426	0.1506	6.7570	0.1635	2.1440	3.8012	9.1172	7.1738	0.0976	0.6960	6.2246	0.0000	0.1763	0.1243	0.1506	0.2750	5.7968	7.0095	0.0426	0.0000	1.3505	1.4276	0.0286	5.5097	0.0000	
CRL-2003-LUC2	TF1	AML	0.7740	0.0000	0.0704	0.5261	0.2750	0.1110	0.2141	0.8718	0.4222	0.0704	0.0000	1.5945	0.1635	0.0286	4.3590	0.2141	0.6135	0.0841	3.7991	0.4647	0.0000	0.5460	0.9855	0.0000	0.0144	3.0514	
HTB-14-LUC2	U87MG	Glioma	6.1445	7.5568	0.1375	0.5361	5.2384	0.0841	5.1085	0.9336	4.7698	5.2407	0.0426	2.1890	6.1183	0.1110	1.7949	0.2750	0.4330	0.2016	1.8875	0.0704	0.0000	2.8094	2.8053	0.0000	0.1506	1.7782	
HTB-22-LUC2	MCF7	Breast Carcinoma	3.0036	0.1243	0.0286	4.3491	0.2016	2.0704	0.2630	8.4341	3.3854	0.0566	1.2388	5.0665	0.0704	0.1763	0.2016	0.1763	0.3103	0.0976	5.1922	1.4699	0.0426	0.2630	0.4854	0.0566	0.0144	0.0286	
HTB-43-LUC2	FADU	Upper Aerodigestive Squamous	5.0794	0.4222	0.0704	1.7004	0.5059	0.4114	6.5534	7.0353	6.0027	0.0286	0.6323	5.7578	0.0566	3.7560	0.1110	7.7173	0.5945	1.6781	3.7602	0.6599	0.0976	2.9982	1.3448	1.1043	0.1506	0.0000	
HTB-96-LUC2	UZOS	Osteosarcoma	3.6450	2.0215	0.1110	6.8354	4.9621	0.0704	2.1635	3.6859	6.0721	1.5059	2.3646	5.4343	0.0000	0.1375	4.4060	6.0350	0.3896	4.7296	3.3882	0.0566	0.3103	1.4005	0.9411	1.6323	3.6508	0.0566	



ATCC CAR-T Reporter Cell Line Generation

Graphical abstract

Chimeric Antigen Receptor T (CAR-T) Cells

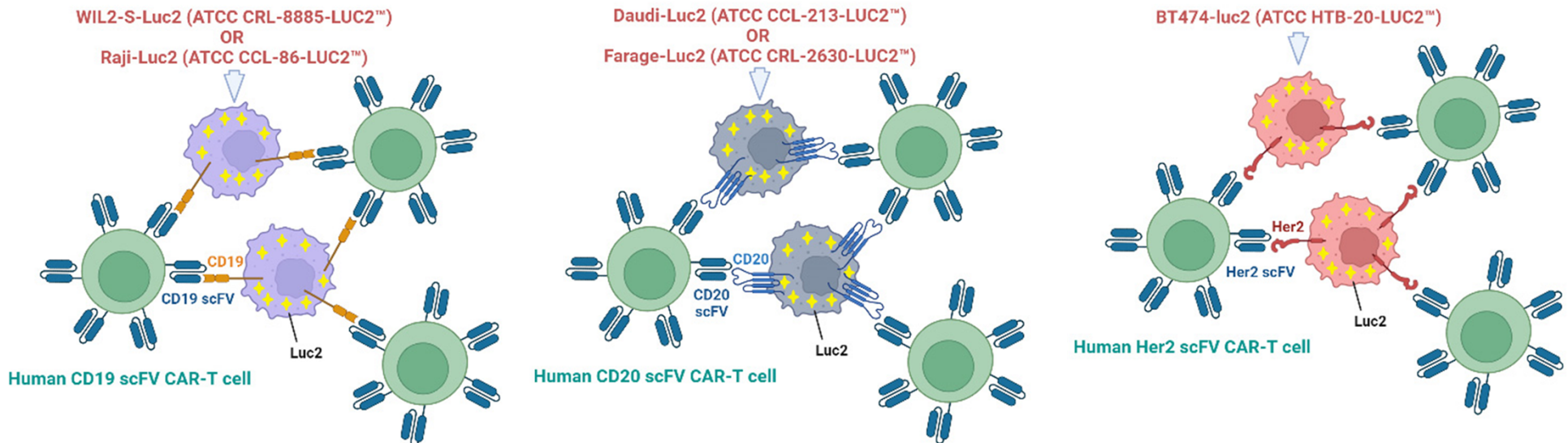
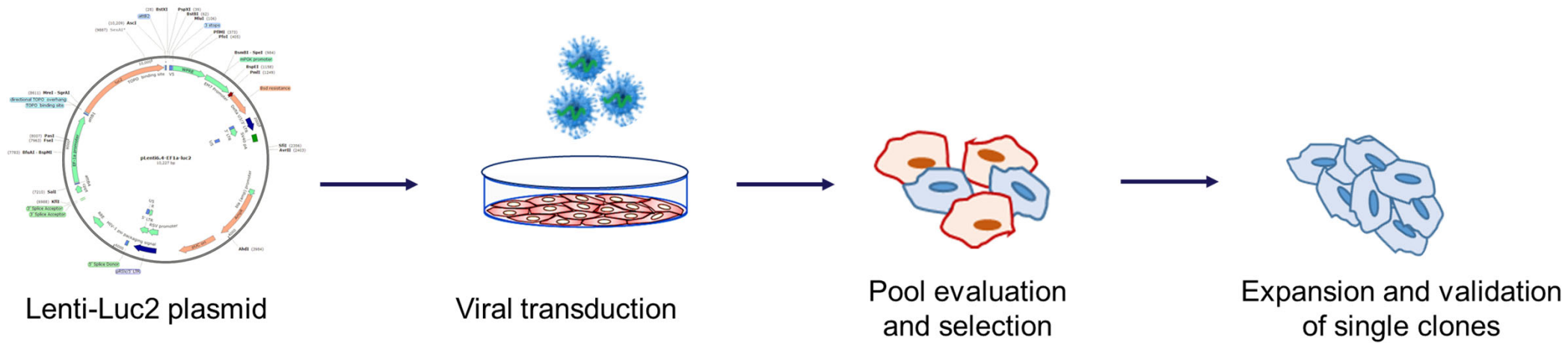


Table 1. ATCC luciferase-expressing cell lines

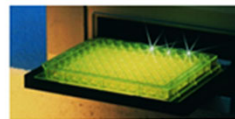
Created with BioRender.com

Luciferase Cell Line	ATCC® No.	Tissue/Disease	Target
WIL2-S-Luc2	CRL-8885-LUC2™	B Lymphoblastoid Cell	CD19
Raji-Luc2	CCL-86-LUC2™	Burkitt's Lymphoma	CD19
Daudi-Luc2	CCL-213-LUC2™	Burkitt's Lymphoma	CD20
Farage-Luc2	CRL-2630-LUC2™	Lymphoma	CD20
BT-474-Luc2	HTB-20-LUC2™	Breast ductal carcinoma	HER2

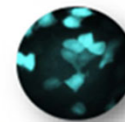
Generation of CAR-T Luciferase reporter cell Lines



Light signal is read by a **luminometer** or a **CCD camera**



Lytic -OR- Live Assay



Morphology of CAR-T Luciferase Reporter Cell Lines is similar to parental cell line

CD19

CD20

HER2

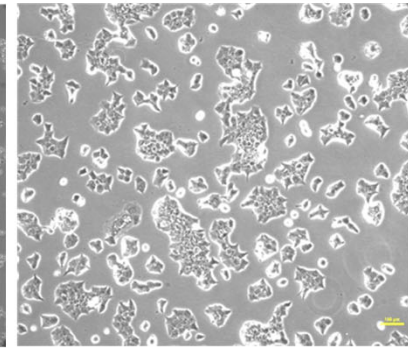
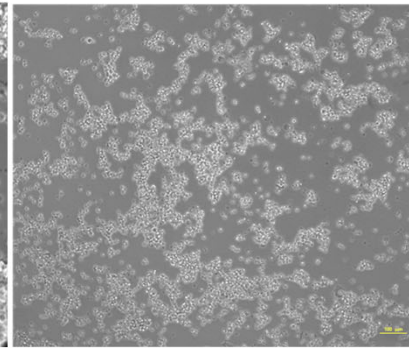
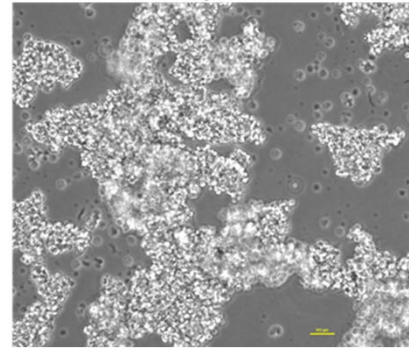
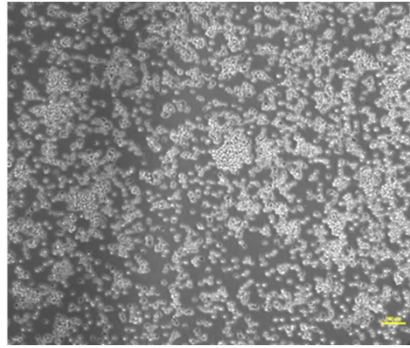
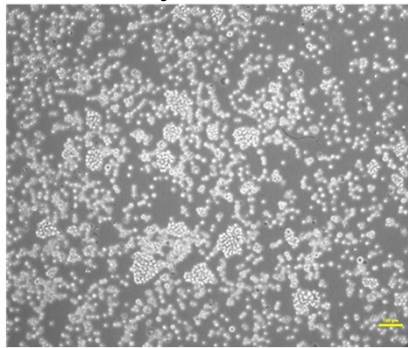
Raji-Luc2

WIL2-S-Luc2

Farage-Luc2

Daudi-Luc2

BT-474-Luc2



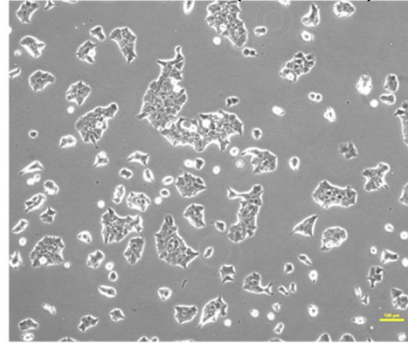
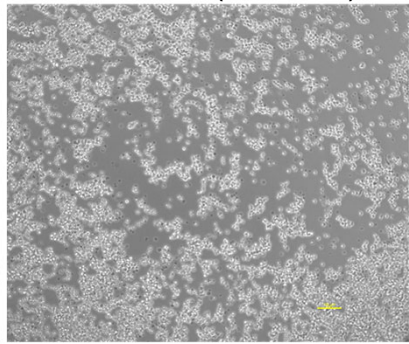
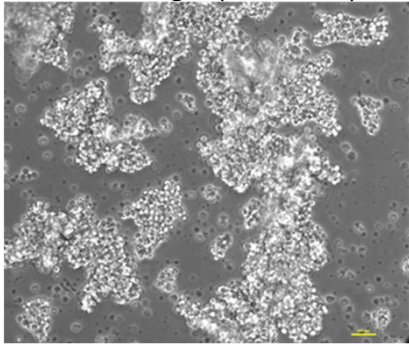
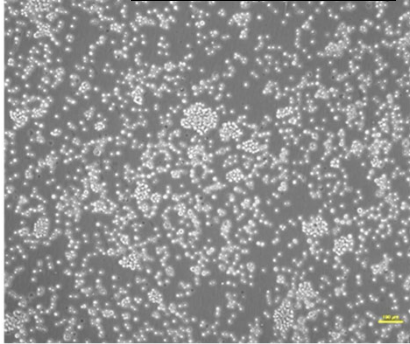
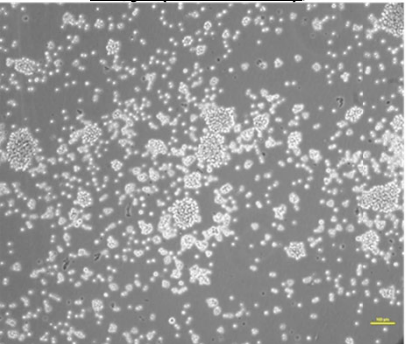
Raji (Parental)

WIL2-S (Parental)

Farage (Parental)

Daudi (Parental)

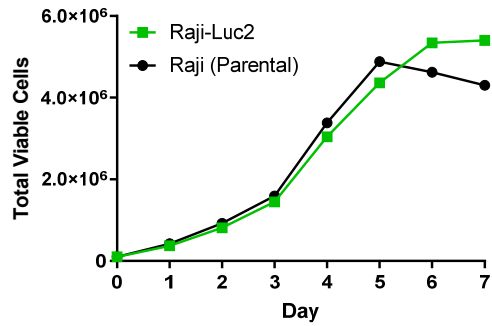
BT-474 (Parental)



Growth Curves of CAR-T Luciferase Reporter Cell Lines are similar to the parental cell line

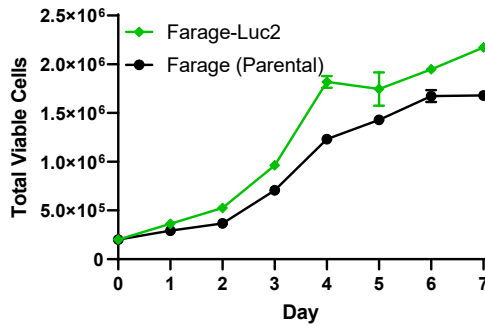
CD19

Raji-Luc2



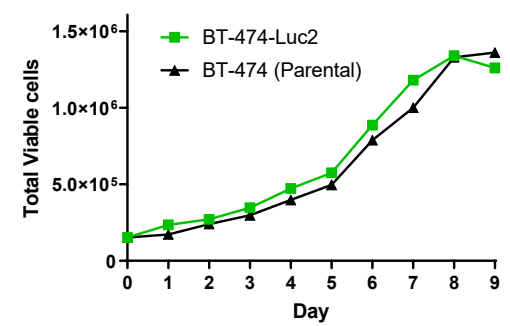
CD20

Farage-Luc2

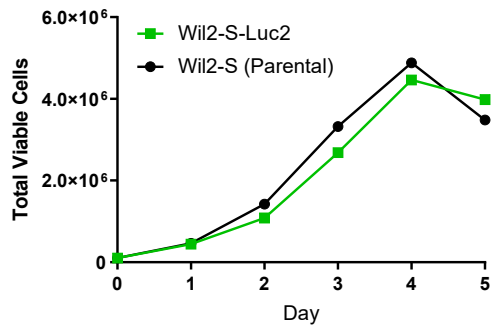


HER2

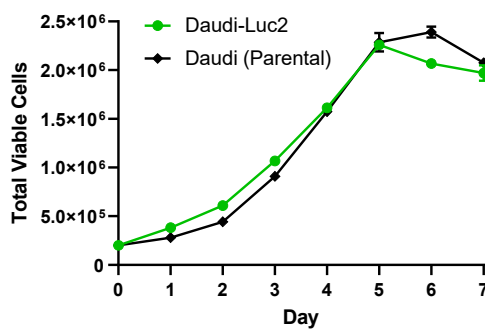
BT-474-Luc2



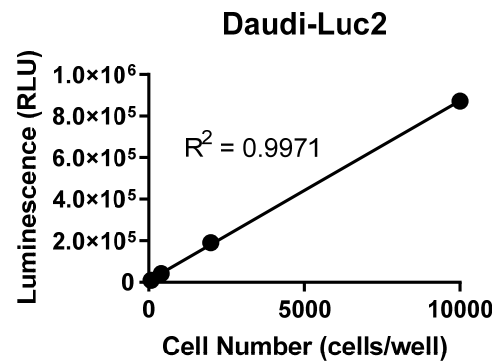
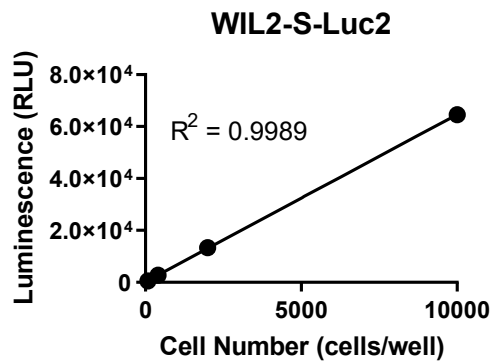
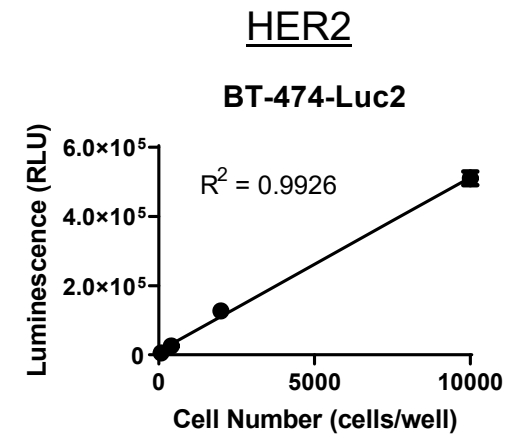
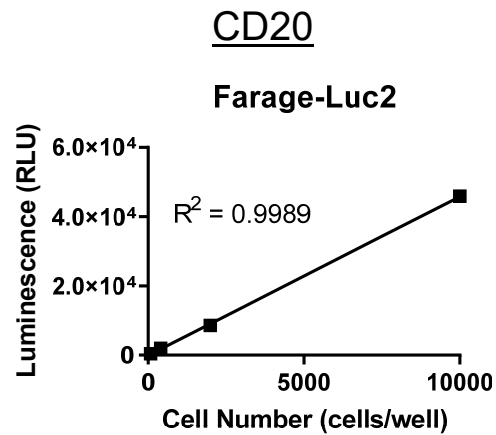
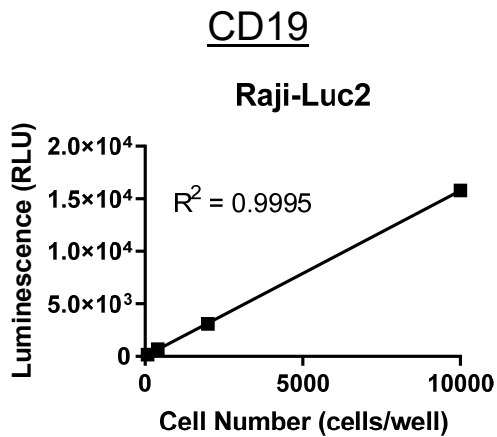
WIL2-S-Luc2



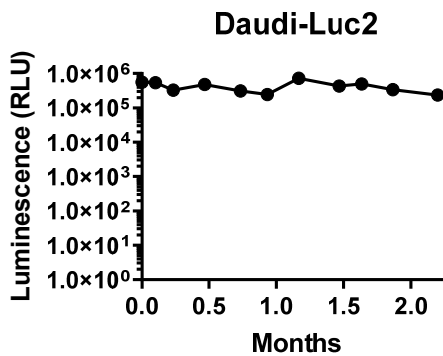
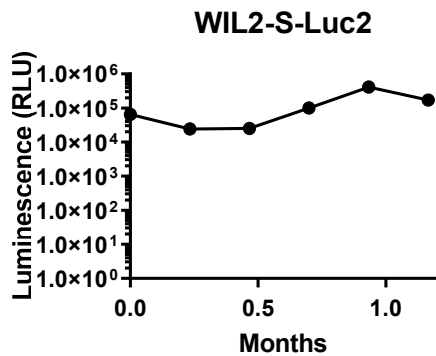
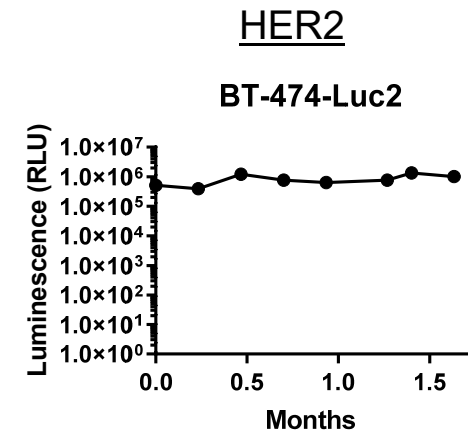
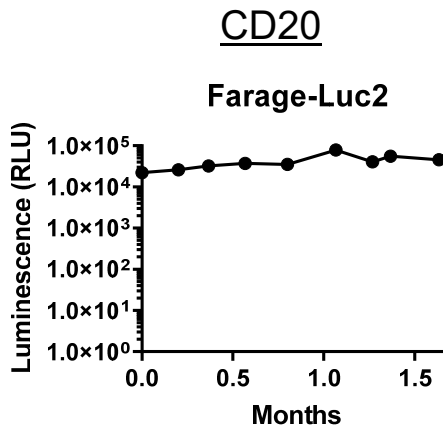
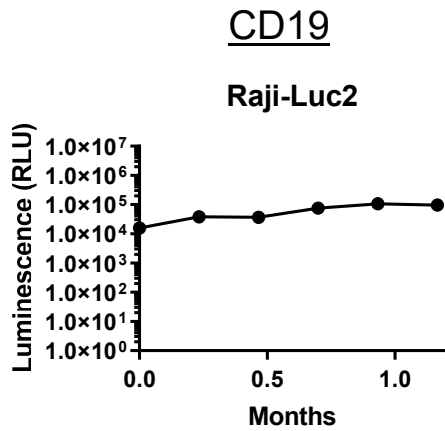
Daudi-Luc2



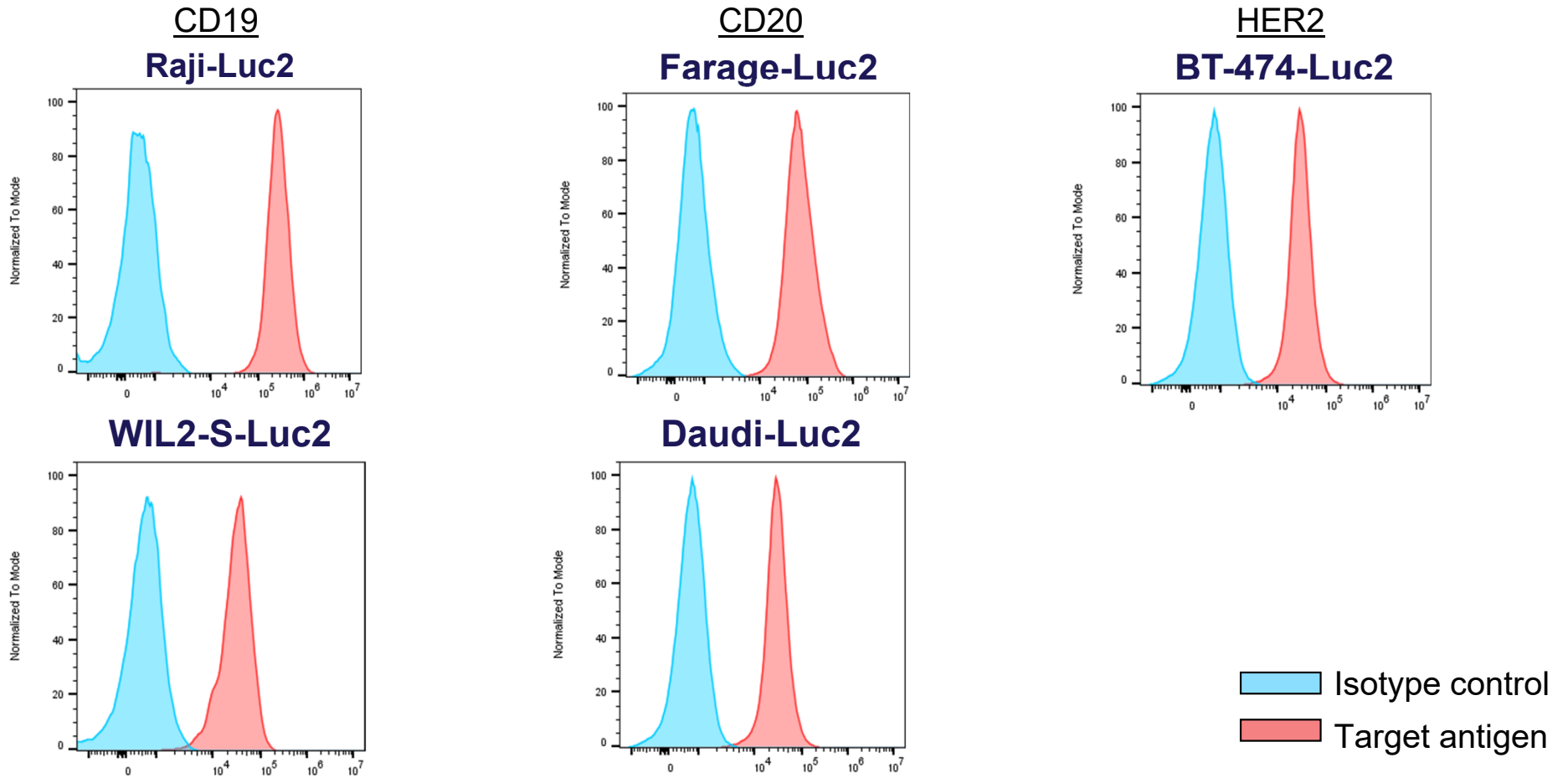
Luciferase activity has a linear correlation between bioluminescence intensity and cell number

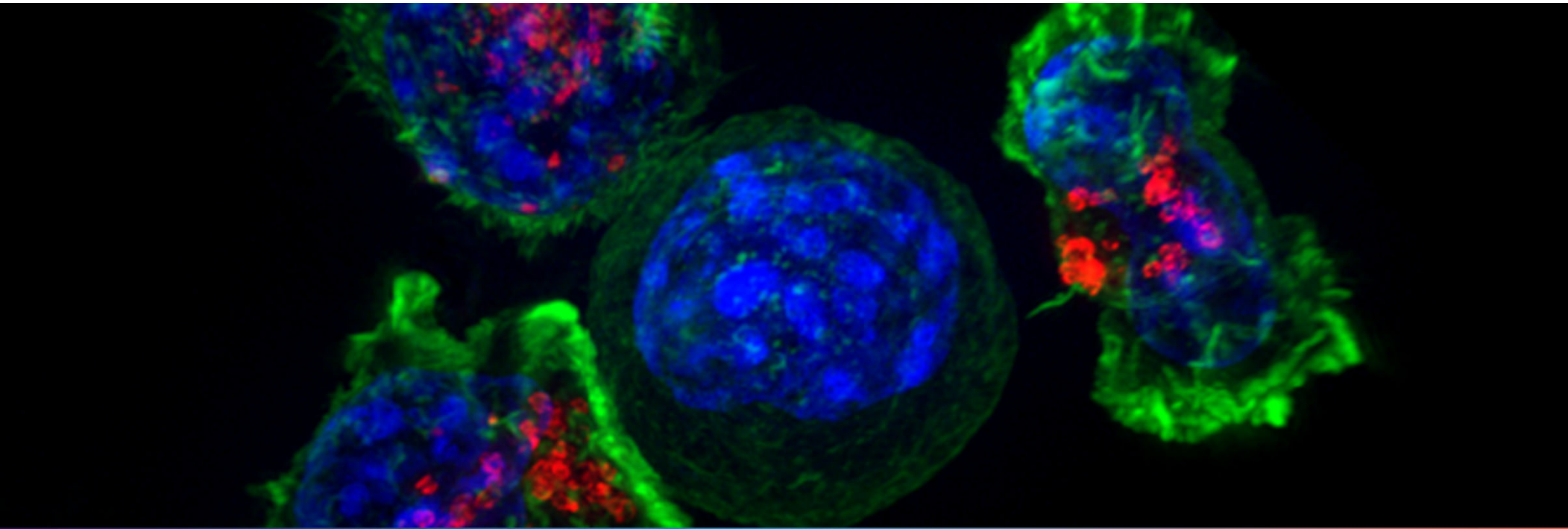


Luciferase expression is stable for over 30 population doublings



Antigen expression is stable for over 30 population doublings

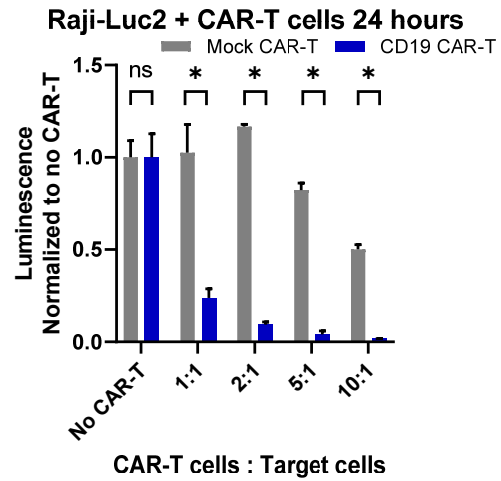




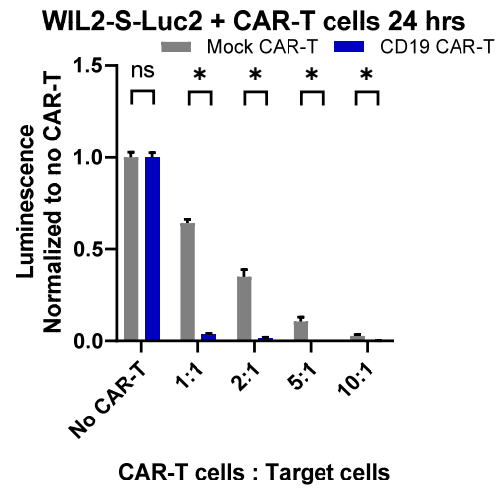
ATCC Application Data

Luminescence Assay

A

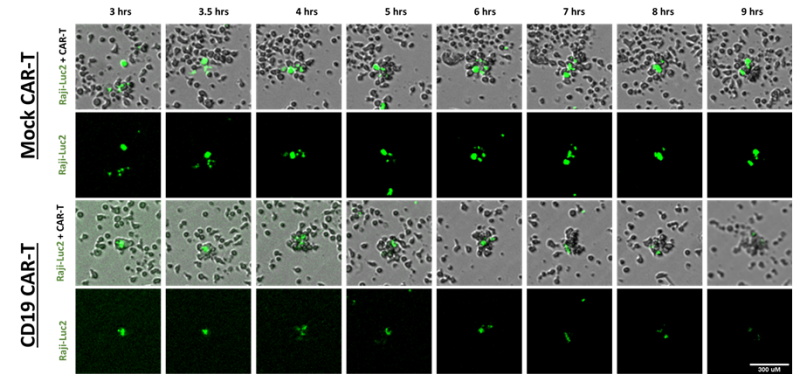


B

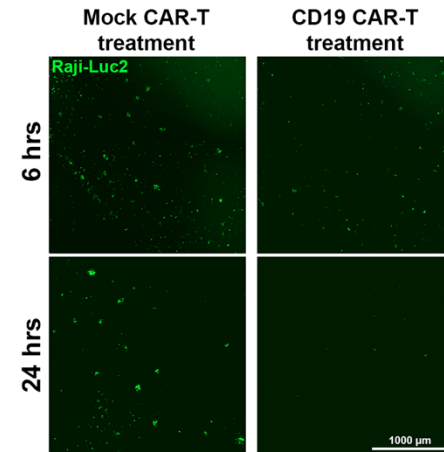


Live Cell Imaging Assay

C

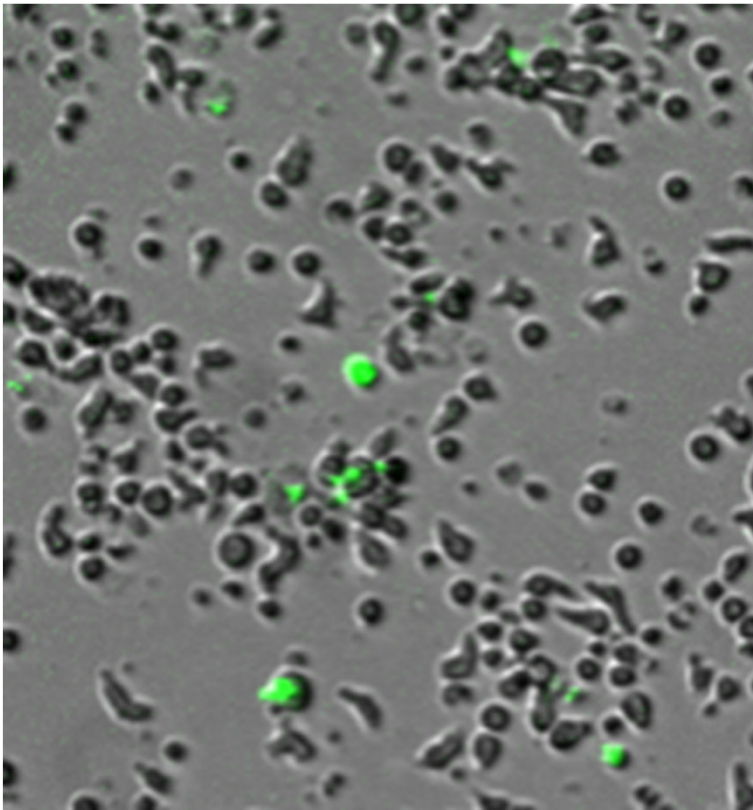


D

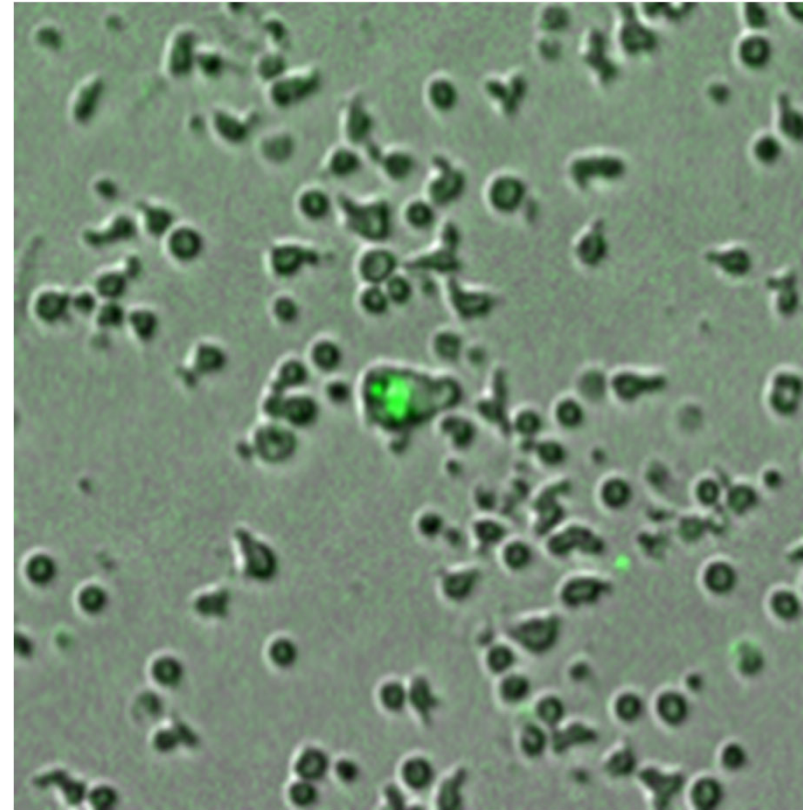


Live cell imaging assay

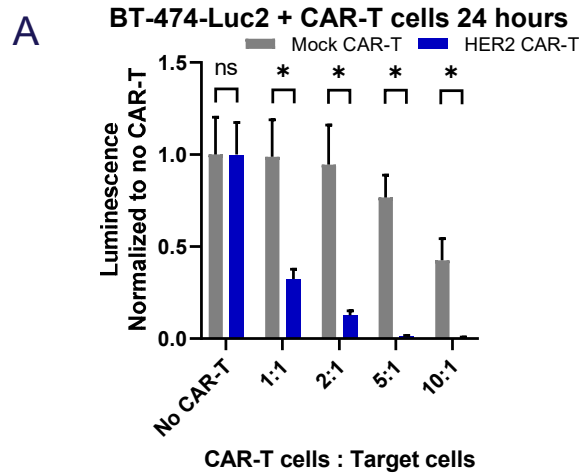
Raji-Luc2 + Mock CAR-T



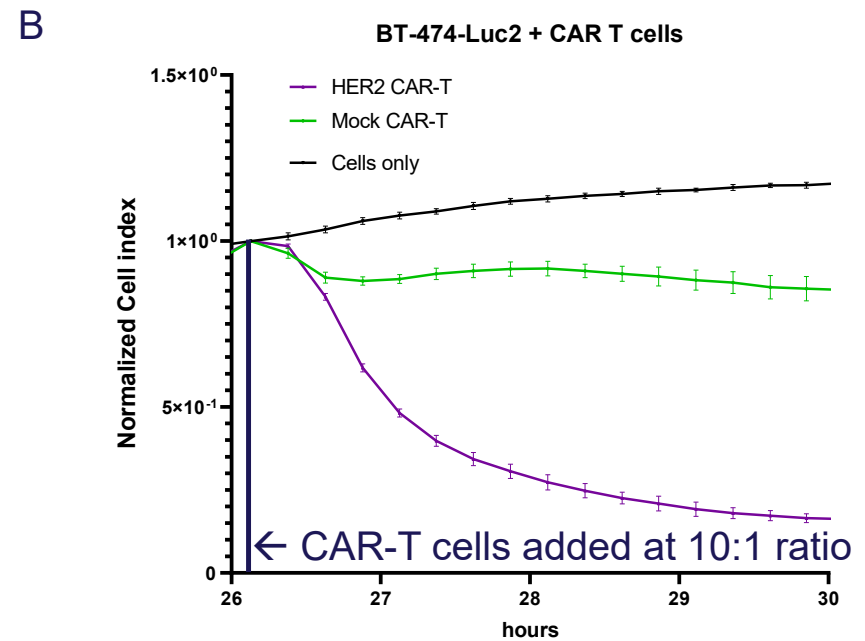
Raji-Luc2 + CD19 CAR-T



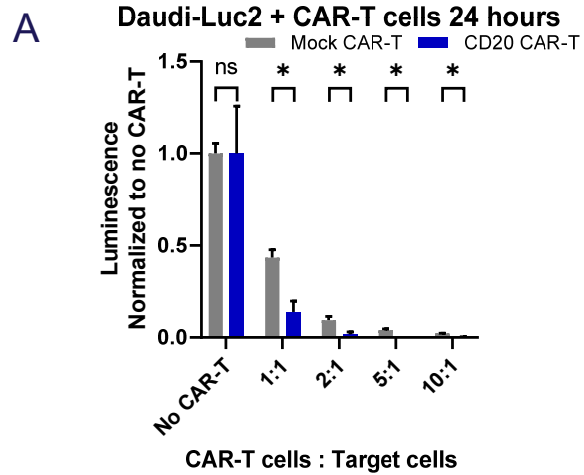
Luminescence Assay



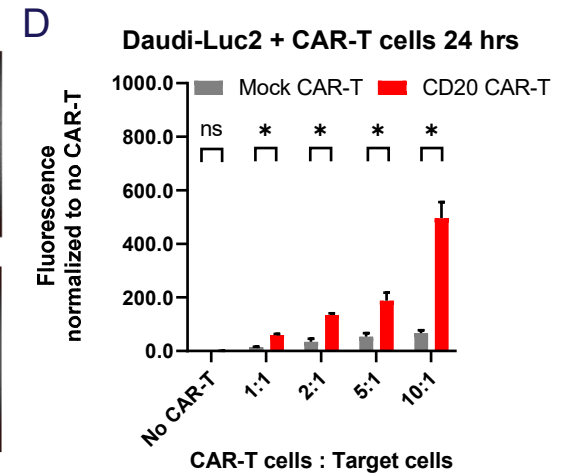
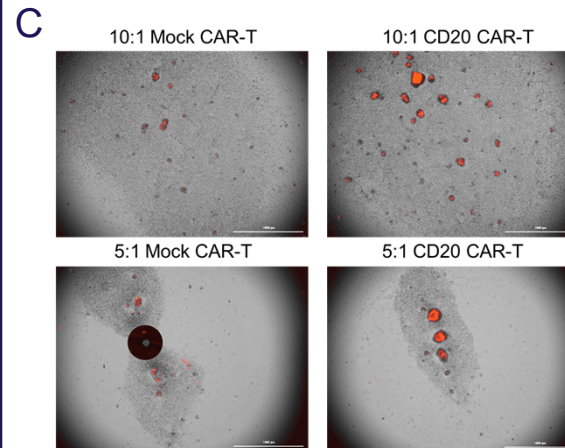
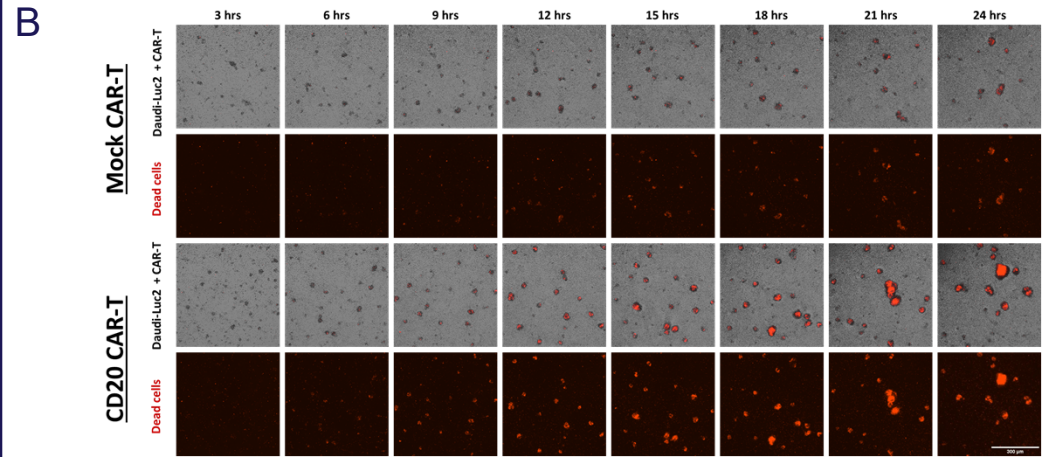
xCelligence Live Cell Assay



Luminescence Assay

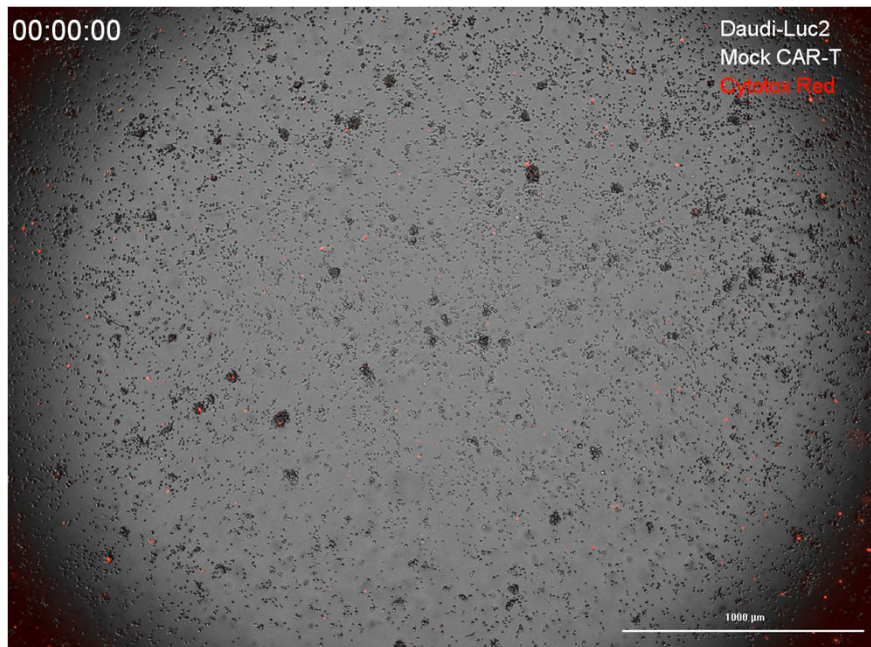


Live Cell Imaging Assay

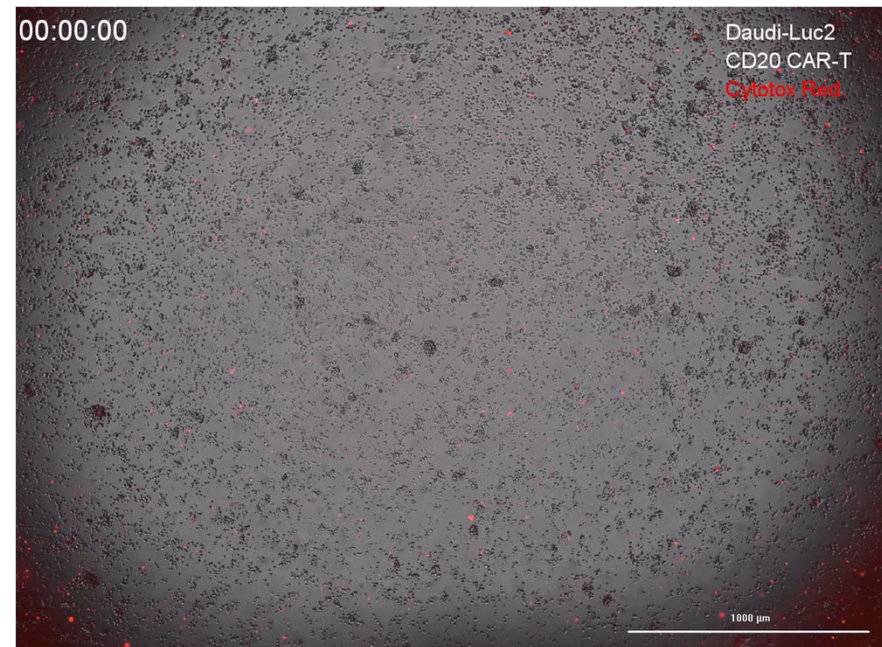


Live cell imaging assay

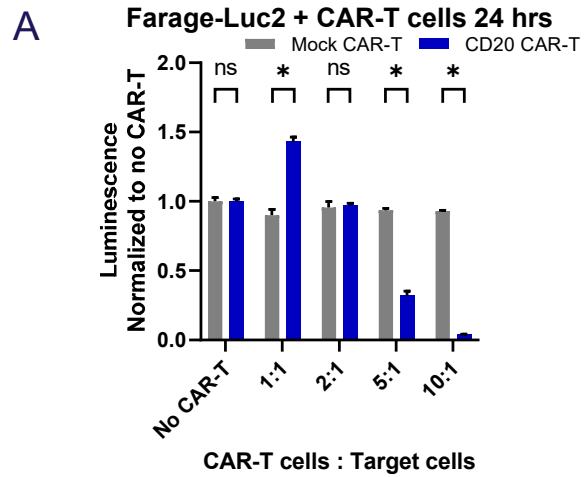
Daudi-Luc2 + Mock CAR-T



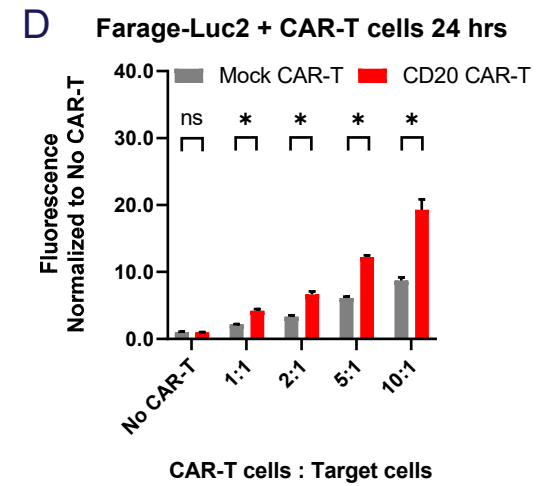
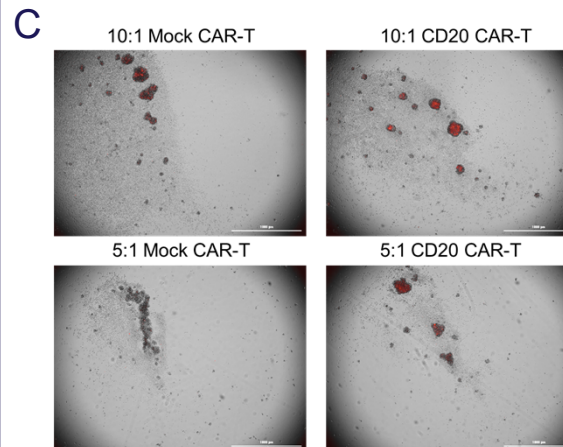
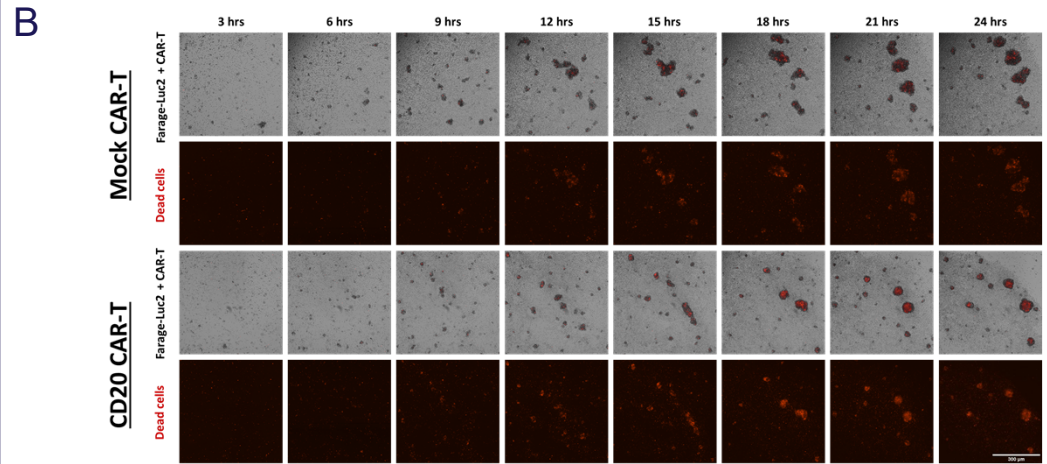
Daudi-Luc2 + CD20 CAR-T



Luminescence Assay

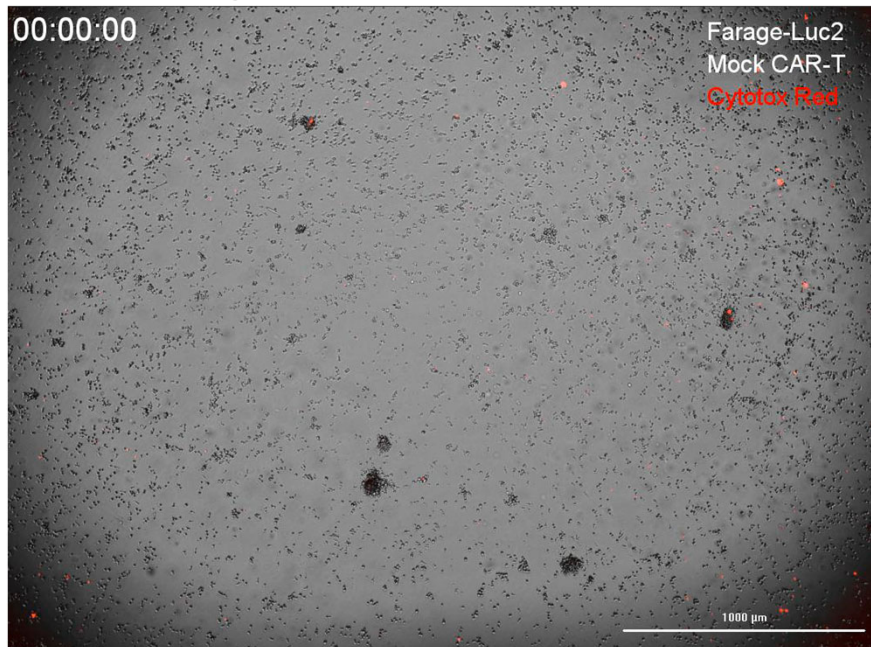


Live Cell Imaging Assay

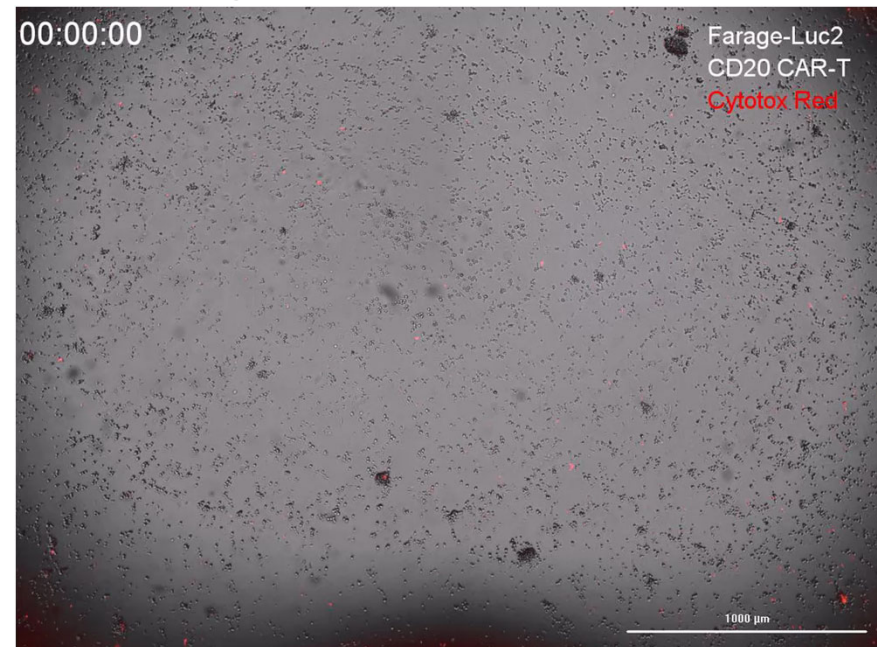


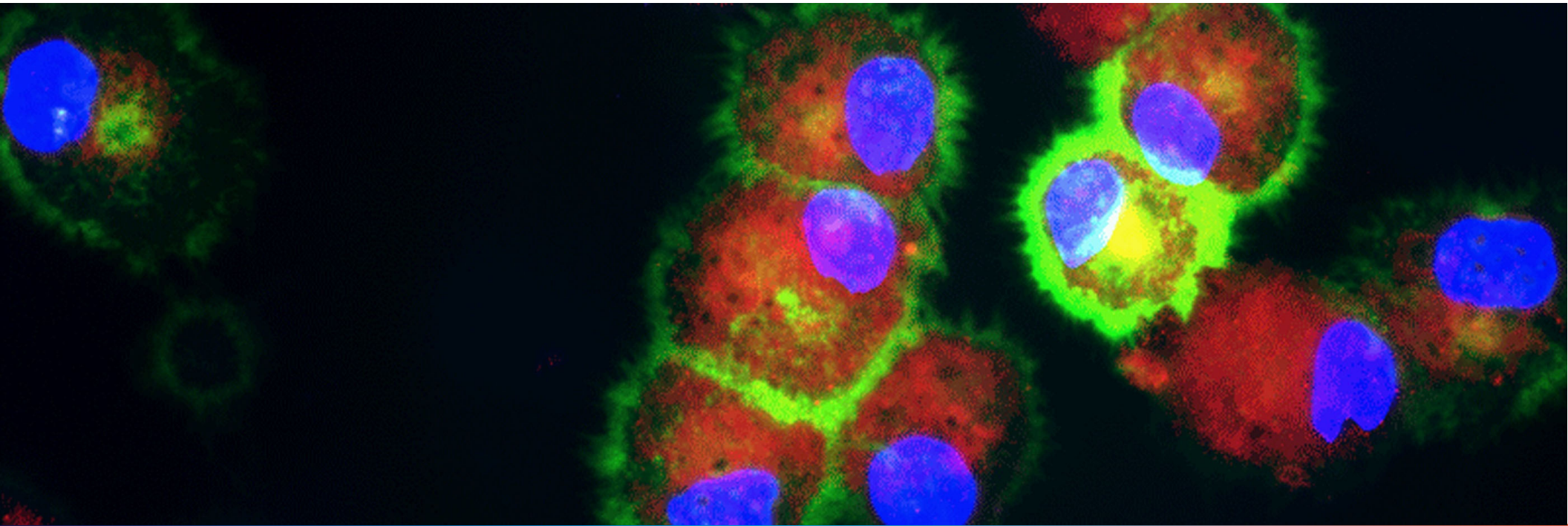
Live cell imaging assay

Farage-Luc2 + Mock CAR-T



Farage-Luc2 + CD20 CAR-T

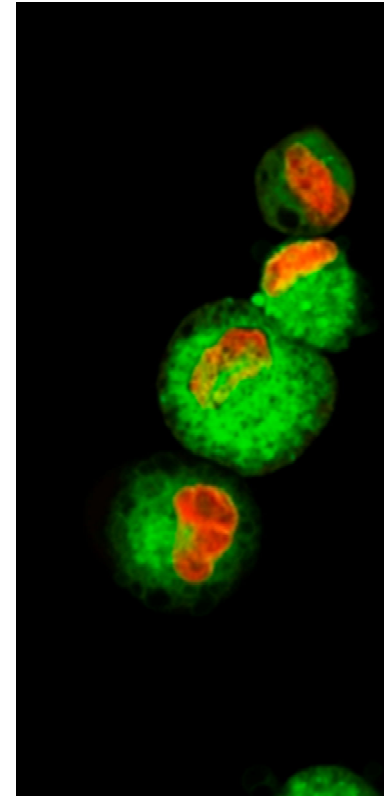




Summary

Summary

- Our results demonstrate luciferase reporter system is a simple, robust, and highly sensitive means to measure biological processes.
- Characterized and authenticated using cell morphology, growth kinetics, and STR analysis.
- Extensively validated for stable expression of high bioluminescent signals.
- Provide great convenience in measuring target cell killing in CAR-T functional evaluation.
- Well-characterized CAR-T luciferase reporter cell lines from ATCC provide excellent tools for studying CAR-T biofunction and validating new CAR-T agents for cancer immunotherapy.



www.atcc.org/immuno-oncology

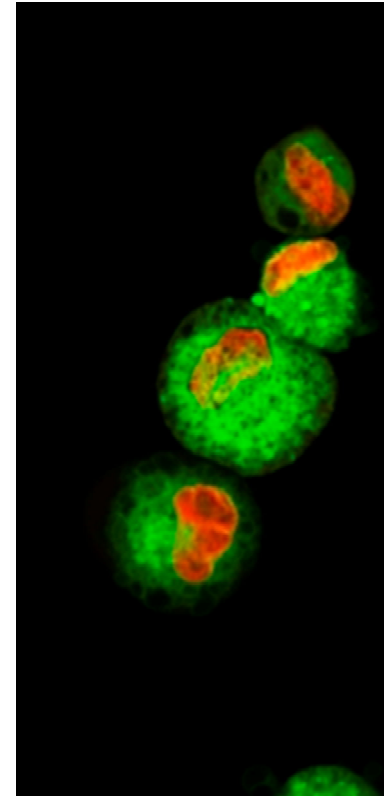
Learn more: www.atcc.org/immuno-oncology

Coming soon!

Does Differentiation Matter? Comparing the Toxicological Response Between Airway Epithelial Models

Presenter: Kevin Tyo, PhD

November 2, 12:00 ET



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Behind the Biology



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