



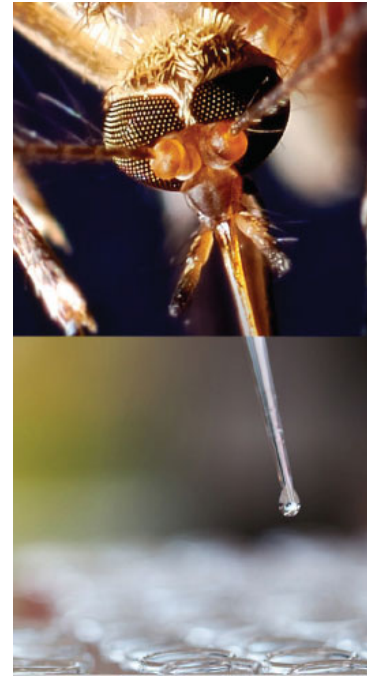
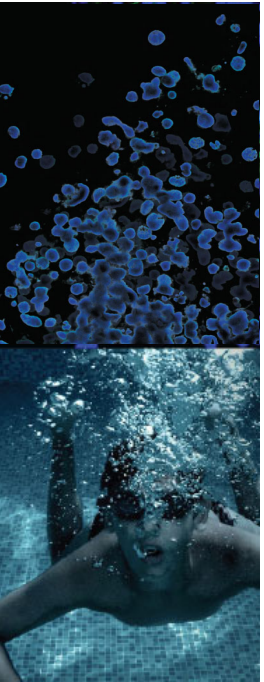
Immune Checkpoint Reporter Cell Lines for Cancer Immunotherapy Drug Screening

Utsav Sharma, PhD
Product Manager, ATCC

Hyeyoun Chang, PhD
Scientist, ATCC

Kevin Tyo, PhD
Scientist, ATCC

Credible Leads to Incredible™



About ATCC

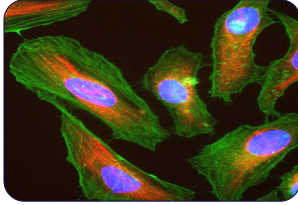
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and a center of scientific excellence in Gaithersburg, MD
- We have the world's largest, most diverse biological materials and information resource for cell culture – the “*gold standard*”
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees

Oncology Portfolio



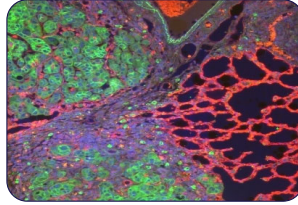
Classical Cell Culture

- ✓ Human Cell Lines
- ✓ Animal Cell Lines
 - ✓ Certified Reference Material
- ✓ Cell Culture Media
 - ✓ Cell Culture Reagents



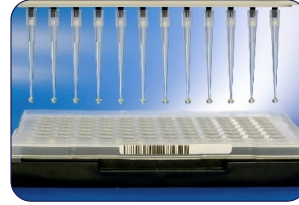
Biomarker Discovery

- ✓ Matched Tumor - Normal Cells
- ✓ Cancer Cell Panels
- ✓ Quantitative Cell Line DNA



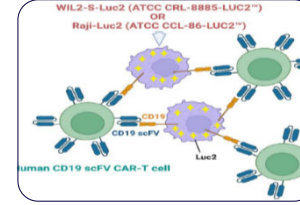
Tumor Biology

- ✓ Cell Lines by Gene Mutation
 - ✓ EMT/MET Reporter Cells
 - ✓ Fluorescent Reporter Labeled Cells
- ✓ Luciferase Labeled Cells
- ✓ Exosomes



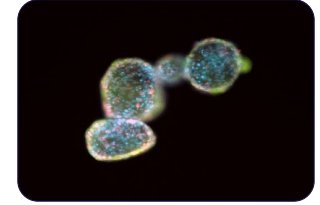
Drug Screening

- ✓ Isogenic Cell Lines
 - ✓ EMT/MET Reporter Cells
- ✓ Primary Cells
 - ✓ hTERT-immortalized Primary Cells
- ✓ iPSC-derived Cells



Immuno-Oncology

- ✓ Primary Immune Cells and Cell Lines
- ✓ THP-1 Reporter Cells
- ✓ Hybridoma Cells
 - ✓ iPSC-Derived Immune Cells
 - ✓ CAR-T Target Reporters
 - ✓ Checkpoint Reporter Cells
- ✓ Assay Ready Immune Cells

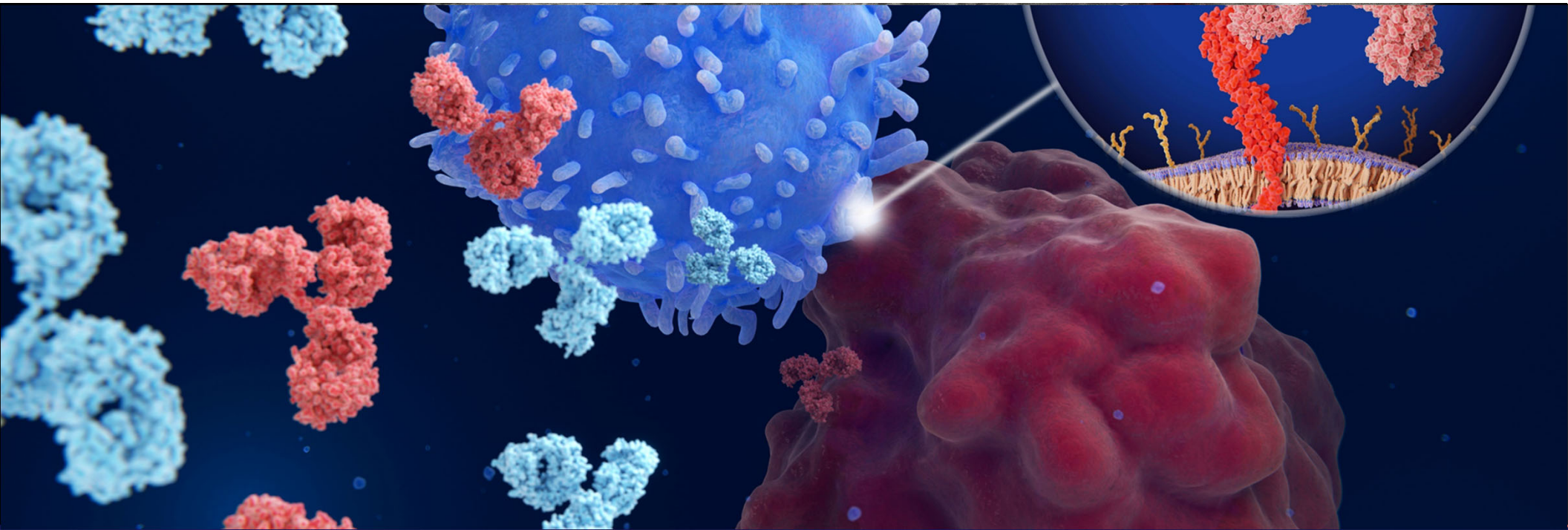


Patient-Derived Models

- ✓ HCMI Organoids
- ✓ HCMI Adherent and Suspension Cell Models
 - ✓ Conditionally Reprogrammed (CRC) Cells
- ✓ Organoid Growth Kits

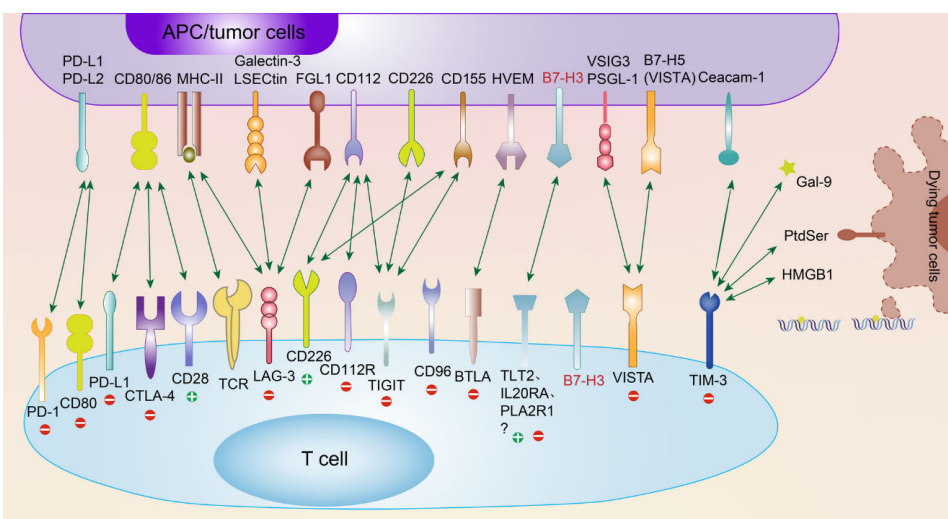
Agenda

- Immune checkpoint inhibitors
- Development of immune checkpoint reporter cell lines
- Comparison of modified and parental cell lines
- Showcasing applications of GAS-Luc2 reporter cell lines
- Summary

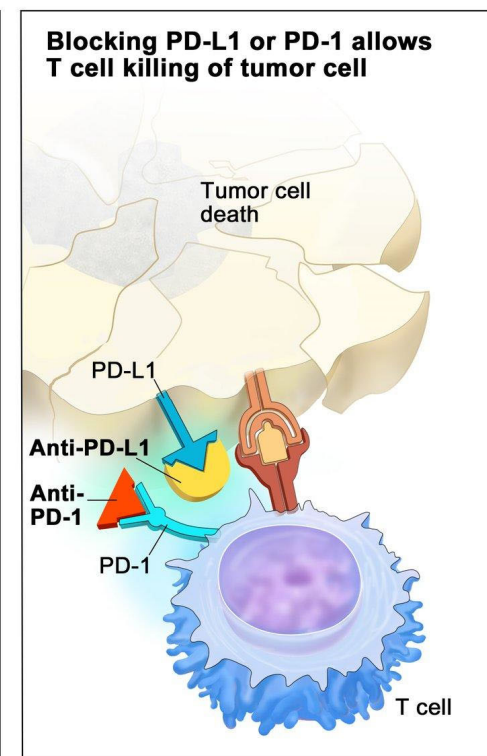
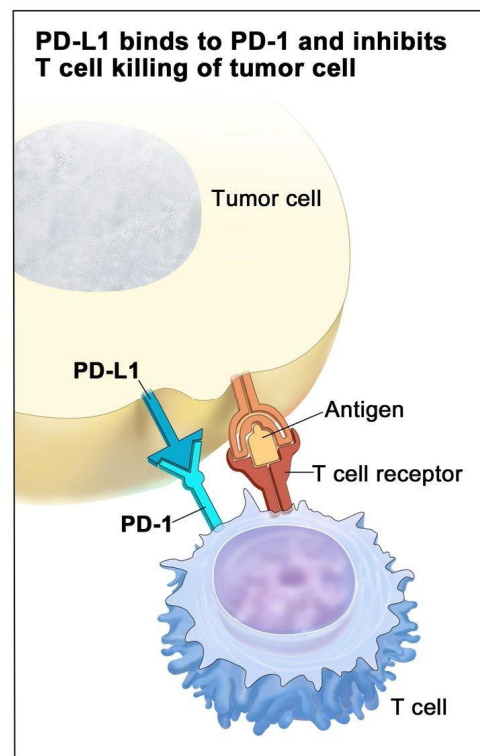


Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICIs)



- Immune checkpoint molecules
 - Markers on the surface of cells communicate “self” to immune cell
 - Cancer cells have increased expression of these markers to evade detection
- Therapeutic antibodies (ICIs) have been developed to bind to the markers to allow immune activation
- A significant breakthrough in the field of oncology and a major step forward as a novel type of cancer immunotherapy

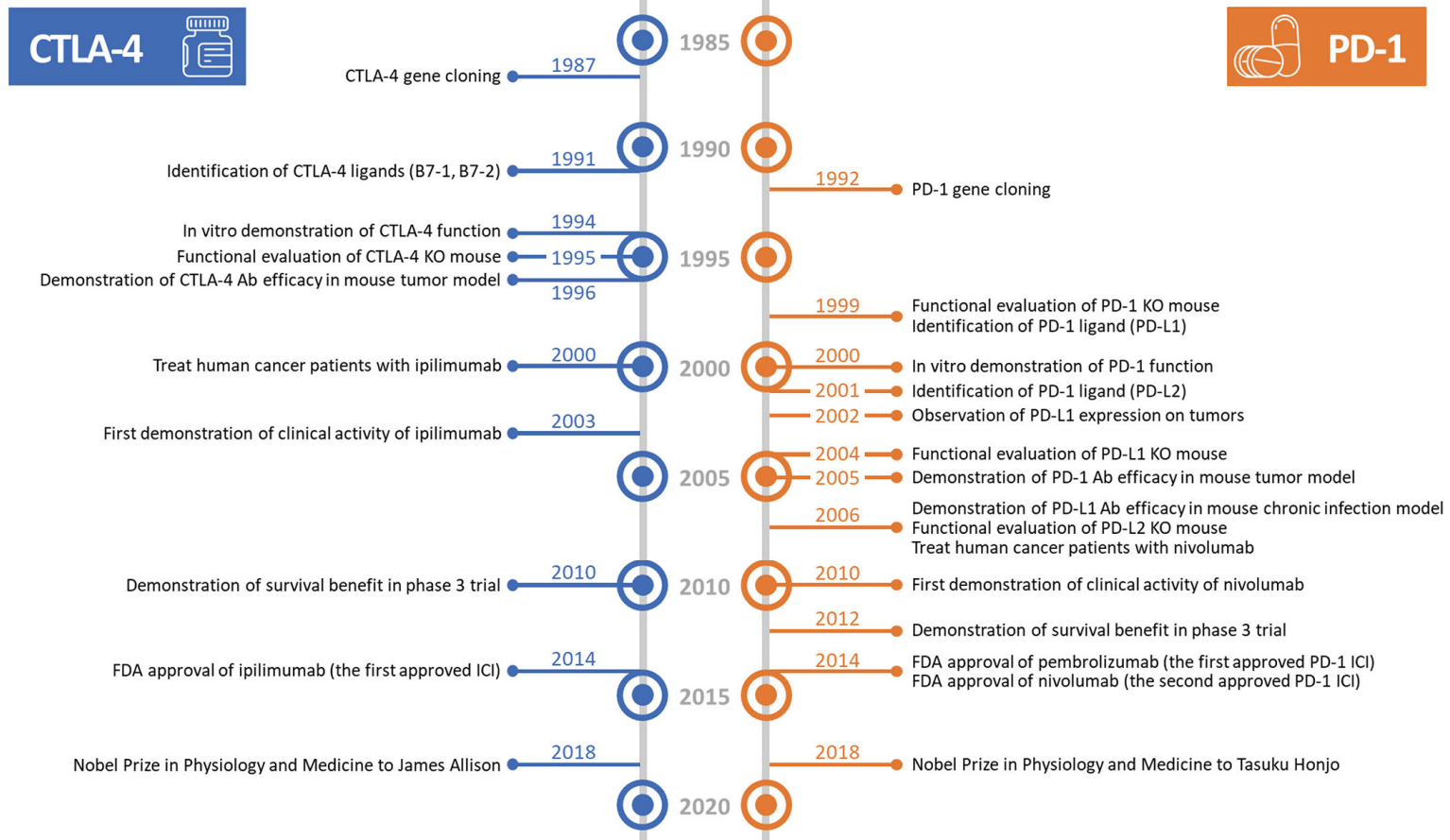


© 2015 Terese Winslow LLC
U.S. Govt. has certain rights

Sources: <https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors>

Zhao, B., Li, H., Xia, Y. *et al.* Immune checkpoint of B7-H3 in cancer: from immunology to clinical immunotherapy. *J Hematol Oncol* **15**, 153 (2022). <https://doi.org/10.1186/s13045-022-01364-7>

ICI timeline and milestones



Source: Immune Netw. 2022 Feb;22(1):e2. <https://doi.org/10.4110/in.2022.22.e2>

Challenges in developing ICIs



In vitro artificial systems

- Cancer cells are engineered to overexpress immune checkpoint molecules
- Non-cytotoxic T cells are engineered to express T cell activation signaling reporter gene
- Solves reproducibility issue, but loses physiological relevance

Syngeneic or humanized mouse models

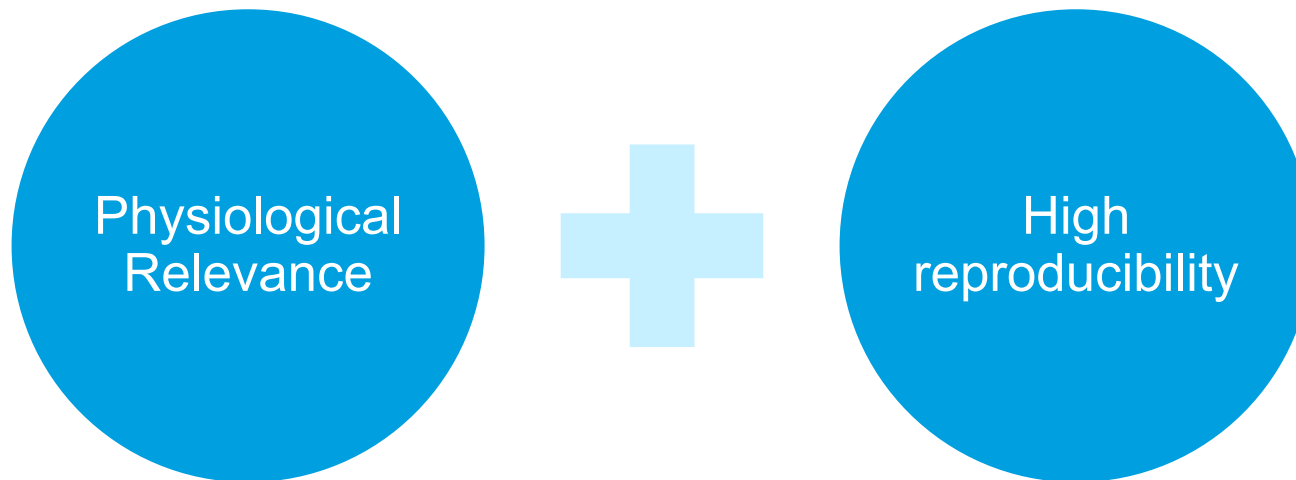
- To understand immunological mechanisms and test potential therapeutics
- Inter-species disparity (mouse vs. human immune system)

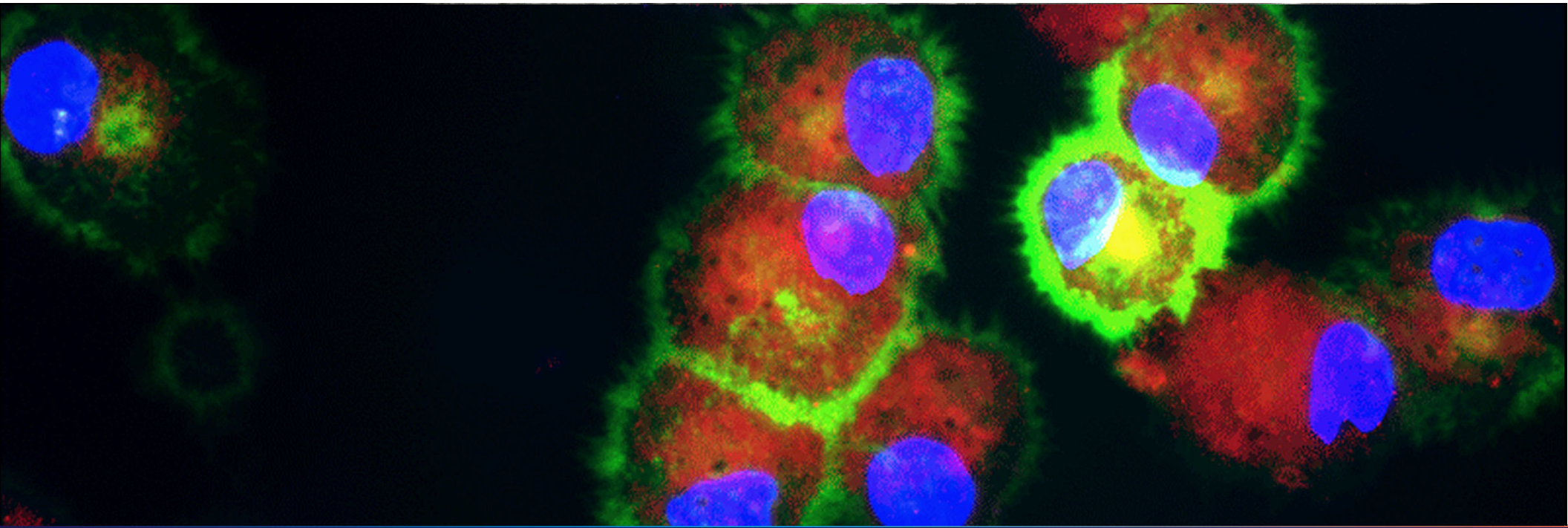
Fully human ex vivo assay models

- Co-culture assays of human T cells and human cancer cells
- Primary immune cells and patient samples adds physiological relevance
- Donor variability issue
- Difficult to reproduce results

What's the solution?

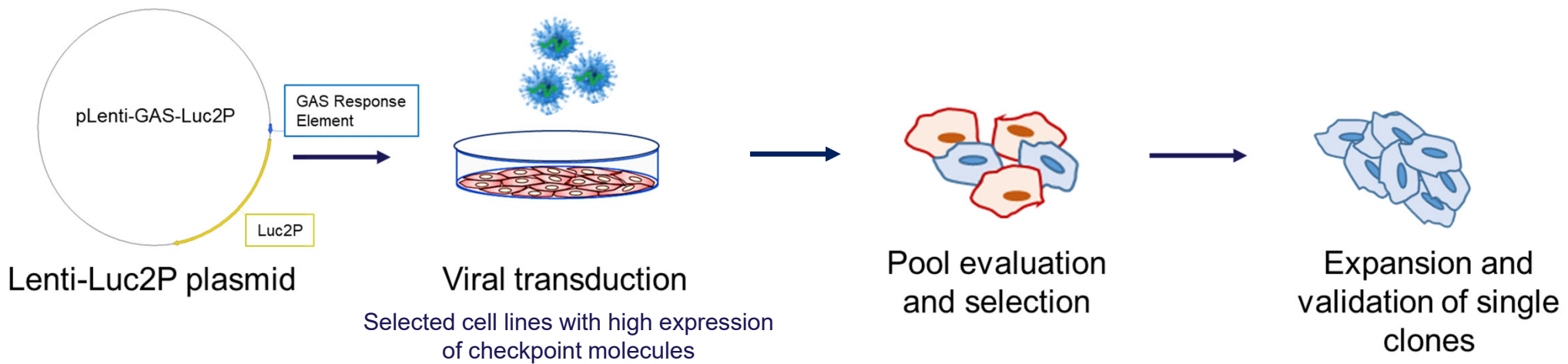
- Physiologically relevant ex vivo reporter assay systems
 - Endogenous expression of checkpoint molecules
 - More predictive model of the in vivo situation
 - Convenient data collection by reporter gene expression
 - Enables reliable measurement of the potency and stability of ICIs



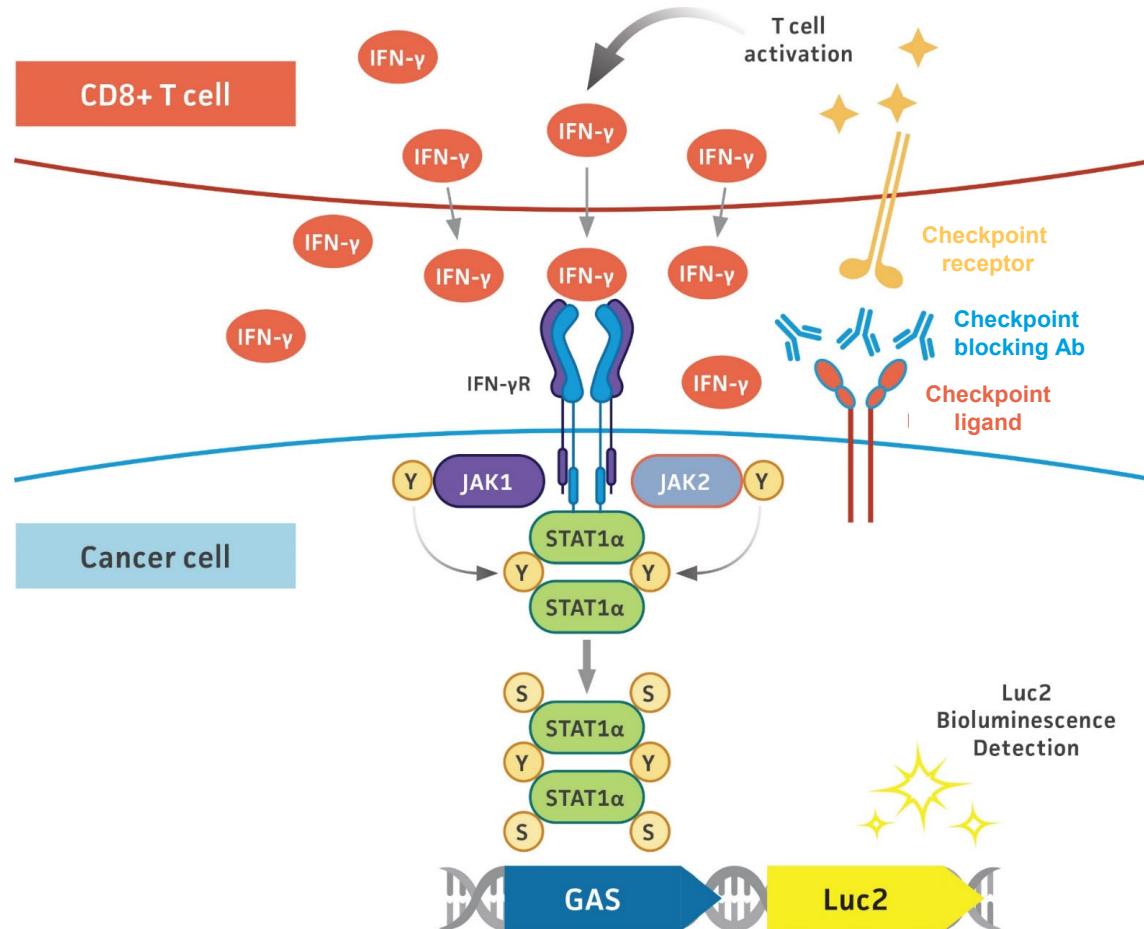


Development of immune checkpoint reporter cell lines

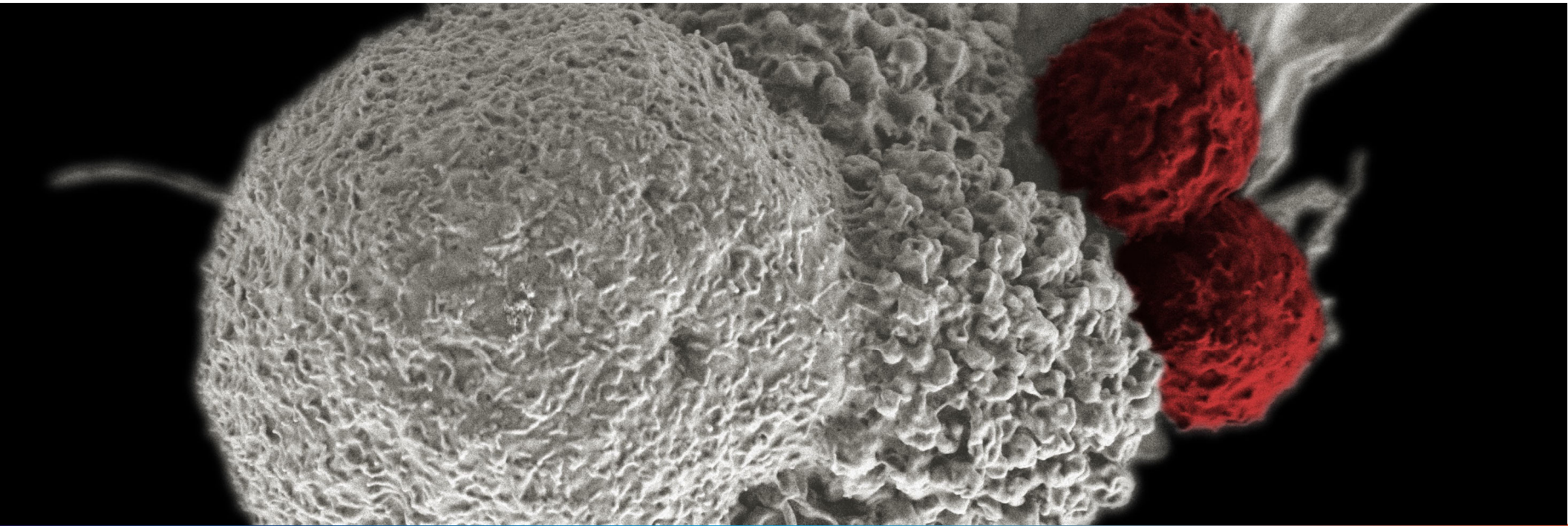
Development of luciferase-expressing cell lines



Mechanism of action



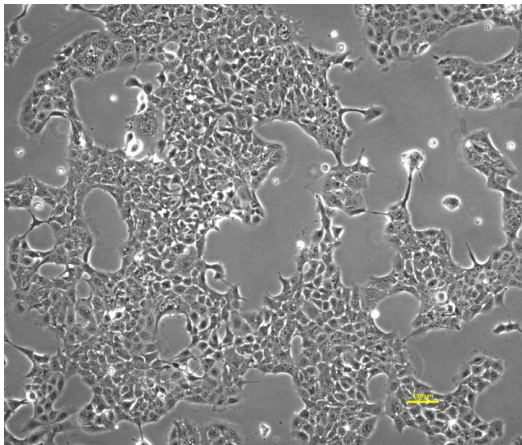
©2023 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.



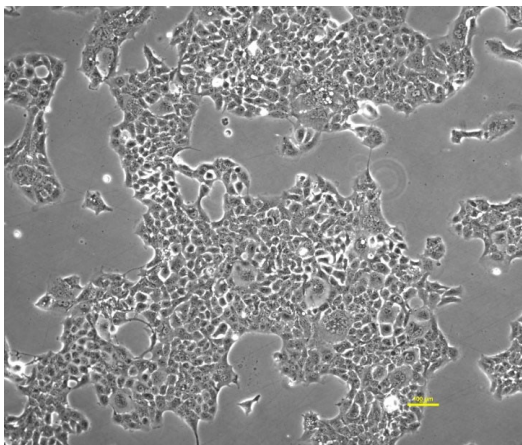
Reporter cell lines maintain physiological relevance.

Morphology (Parental vs. Luciferase)

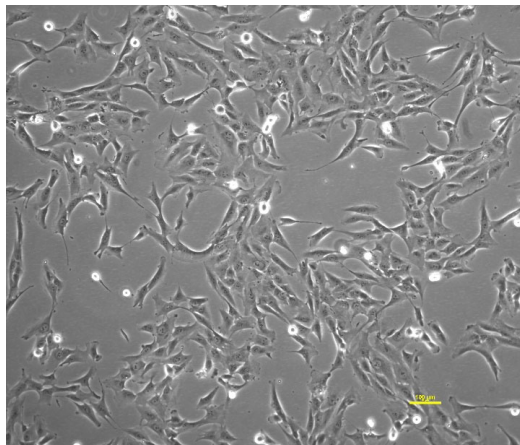
HCC827 Parental



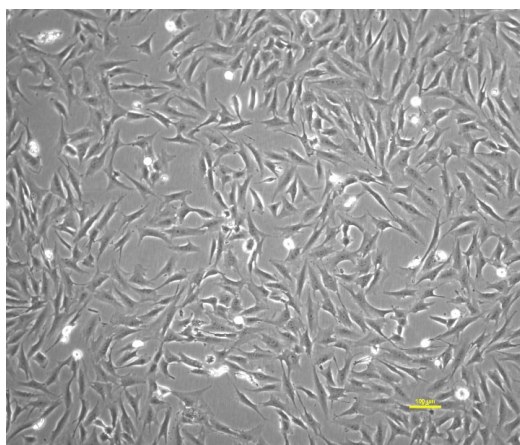
HCC827-GAS-Luc2



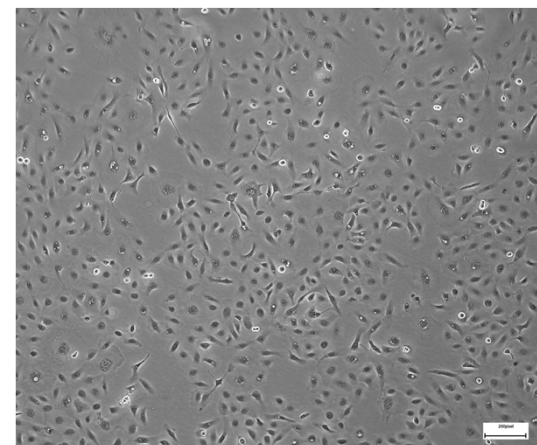
CRL-1427 (MG-63 Parental)



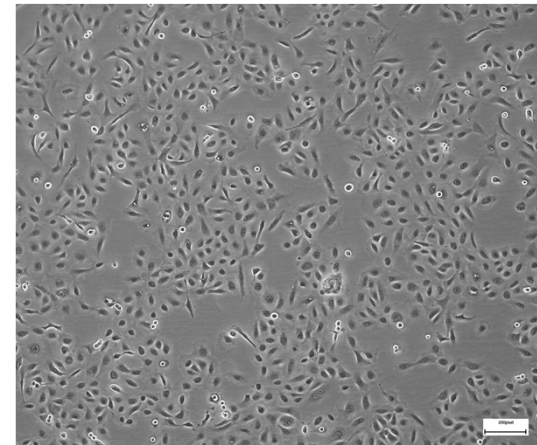
CRL-1427-GAS-LUC2 (MG-63 GAS-Luc2)



CRL-5883 (NCI-H1650 Parental)



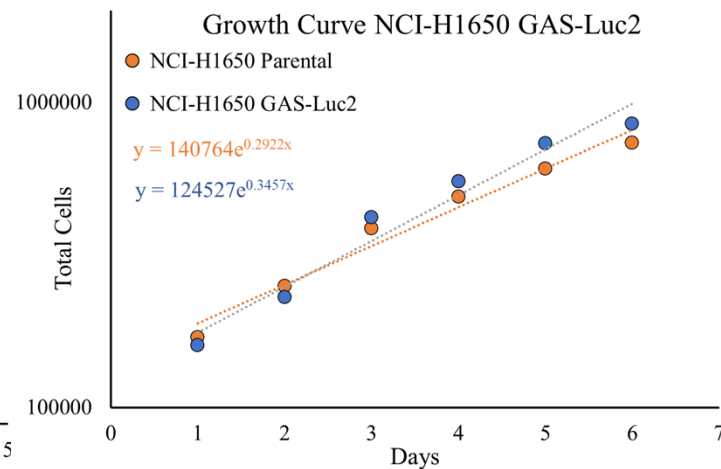
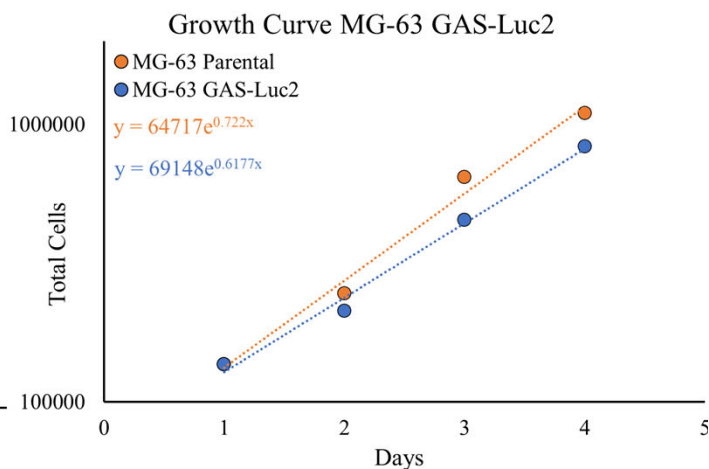
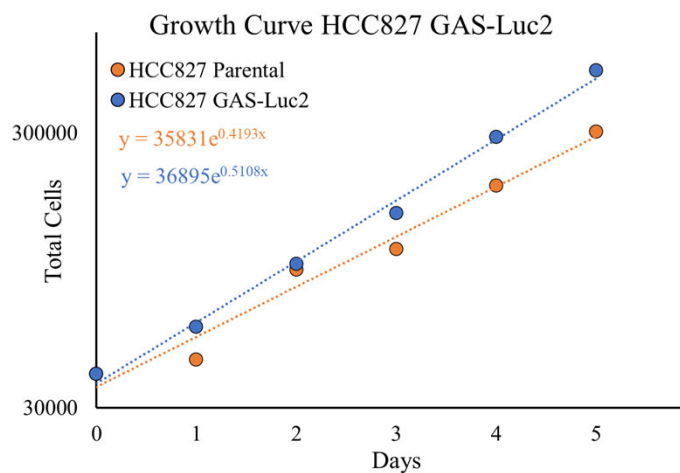
CRL-5883-GAS-LUC2 (NCI-H1650 GAS-Luc2)



©2023 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.



Growth rate (Parental vs. Luciferase)

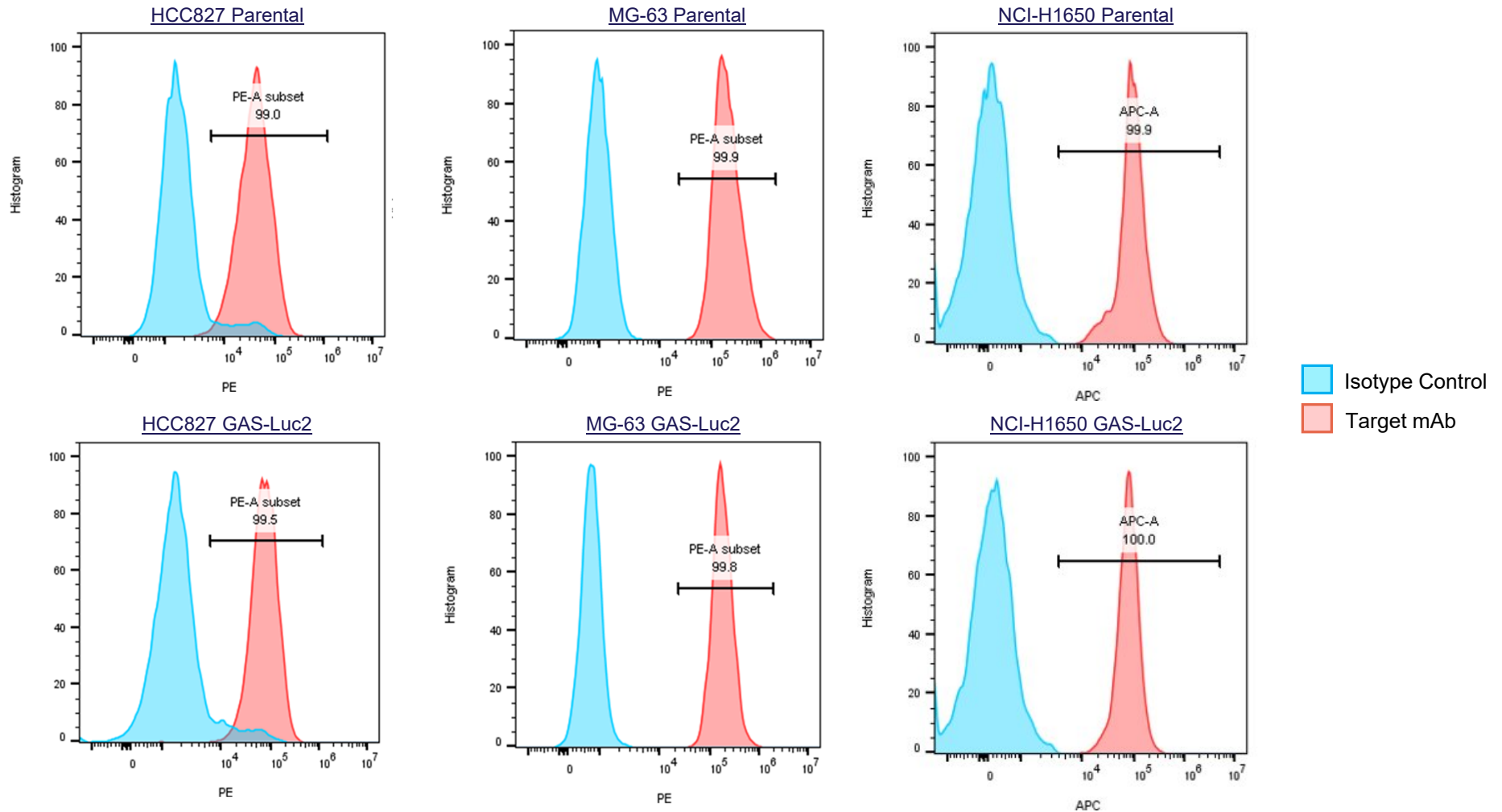


Cell Line	Doubling time (hours)	Growth rate change (%)
HCC827 Parental (CRL2868)	39.7	17.9
HCC827 GAS-Luc2 (CRL-2868-GAS-Luc2)	32.6	

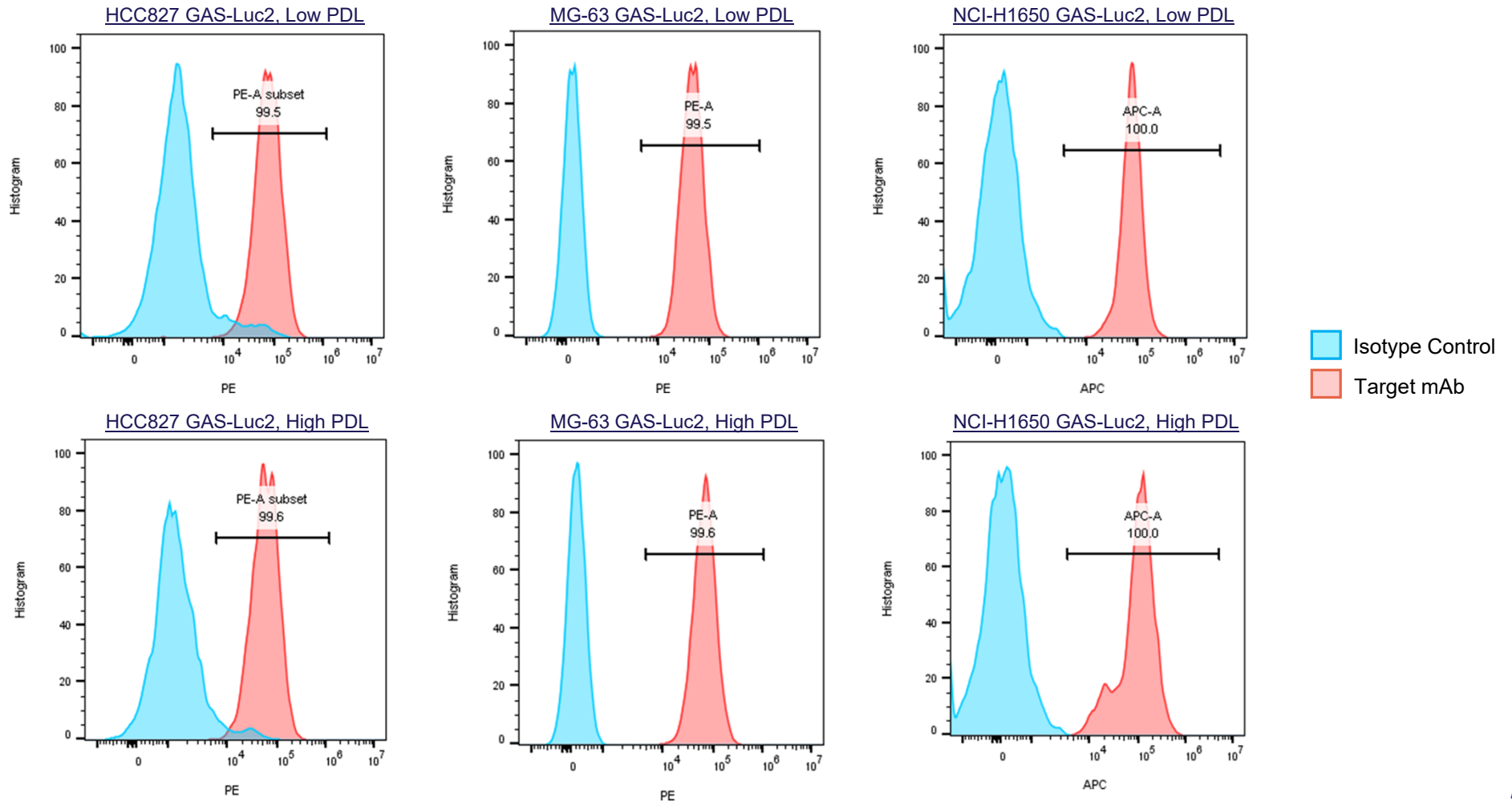
Cell Line	Doubling time (hours)	Growth rate change (%)
MG-63 Parental (CRL-1427)	23	-16.9
MG-63 GAS-Luc2 (CRL-1427 GAS-Luc2)	26.9	

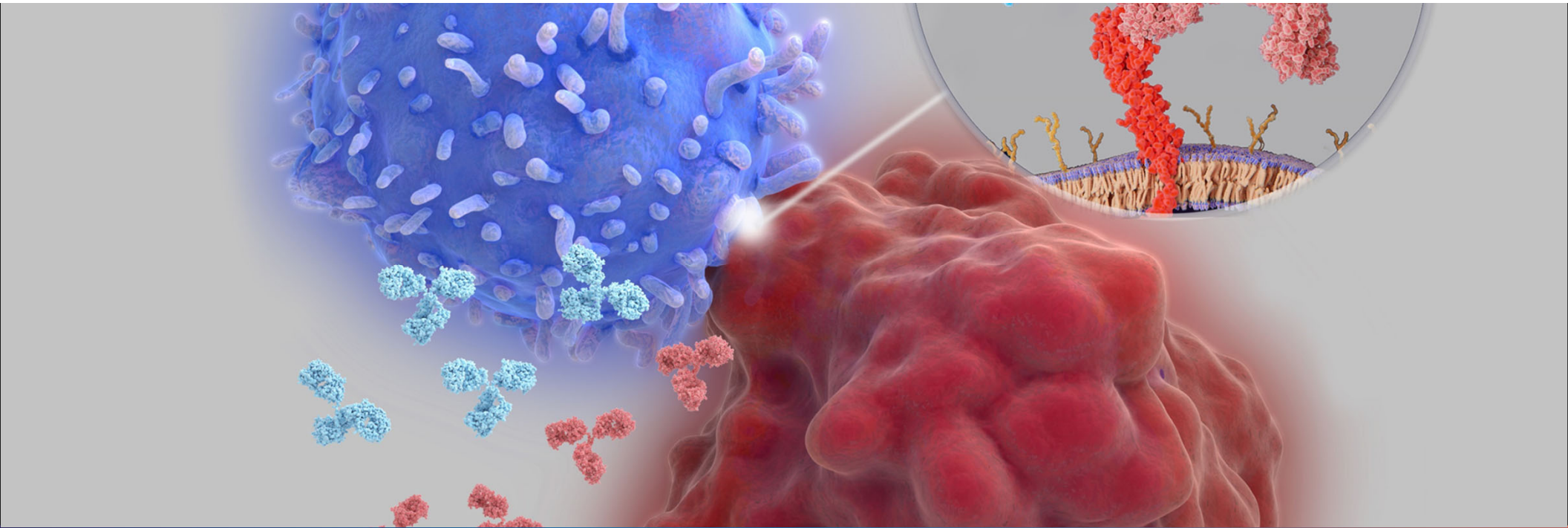
Cell Line	Doubling time (hours)	Growth rate change (%)
NCI-H1650 Parental (CRL-5883)	59.9	-15.4
NCI-H1650 GAS-Luc2 (CRL-5883 GAS-Luc2)	48.1	

Target expression (Parental vs. Luciferase)



Target expression (Low PDL vs. High PDL)

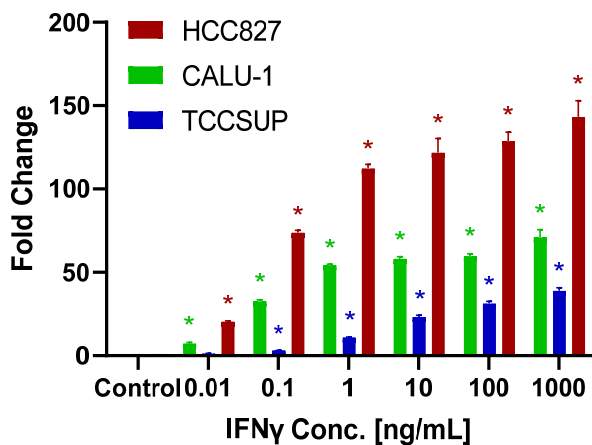




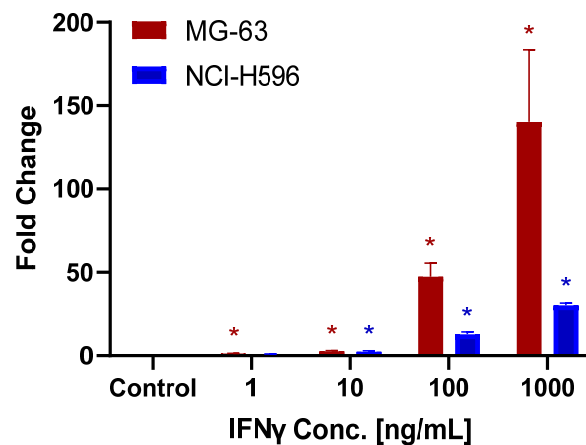
Showcasing applications of GAS-Luc2 reporter cell lines

Evaluation of luciferase-expressing cell lines

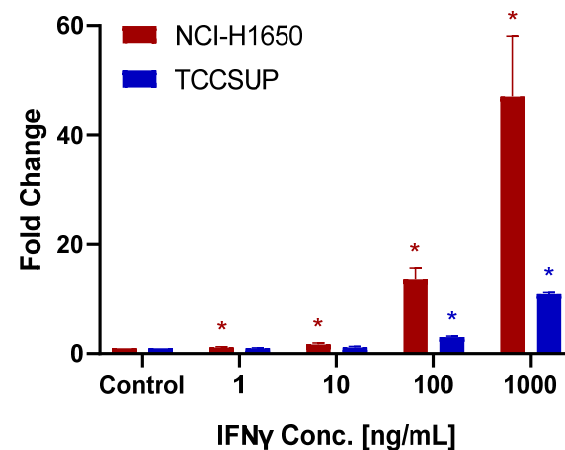
**PD-L1 GAS-Luc2
Candidate Cell Lines**



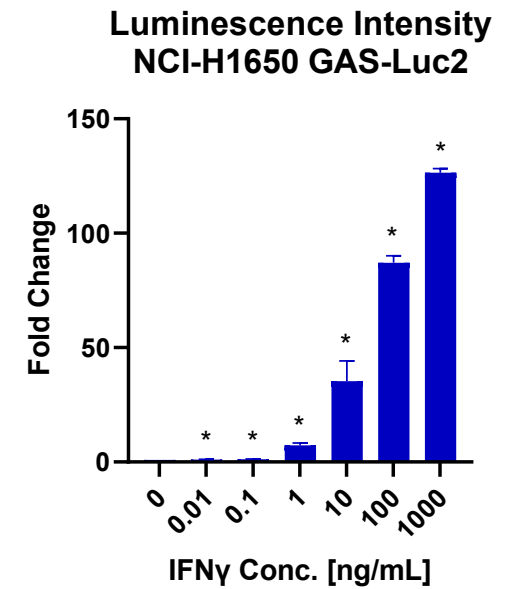
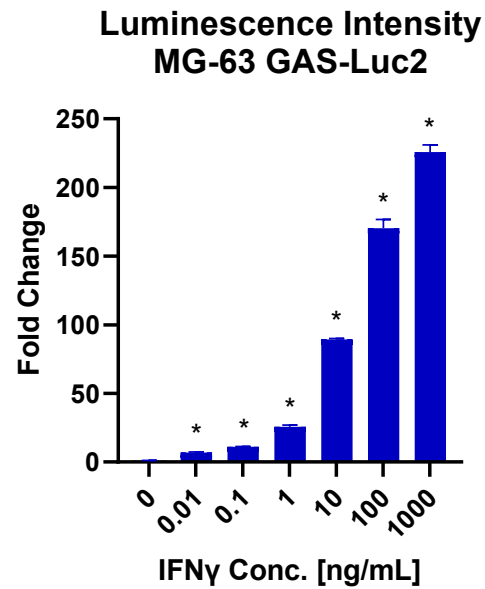
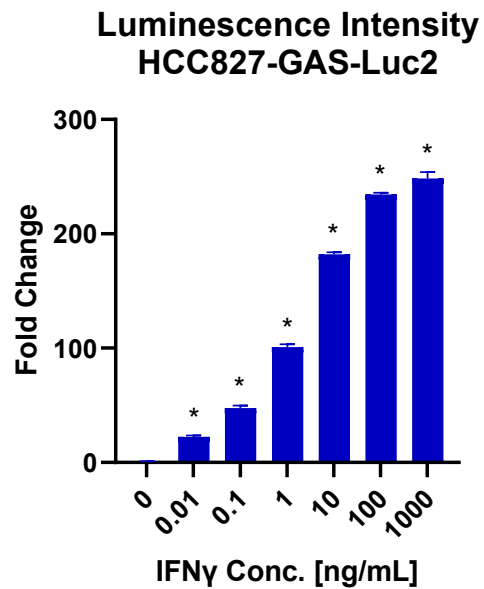
**CD155 GAS-Luc2
Candidate Cell Lines**



**B7-H3 GAS-Luc2
Candidate Cell Lines**

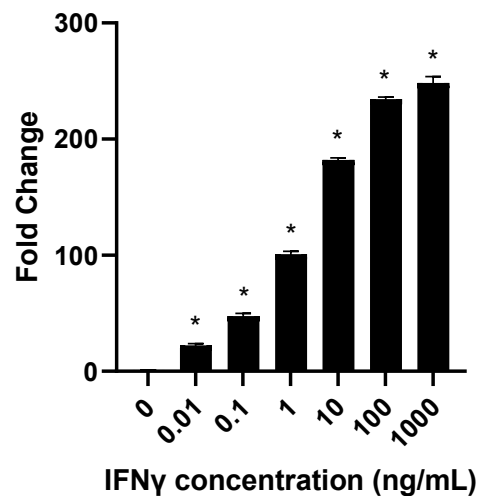


Luciferase expression upon cytokine stimulation

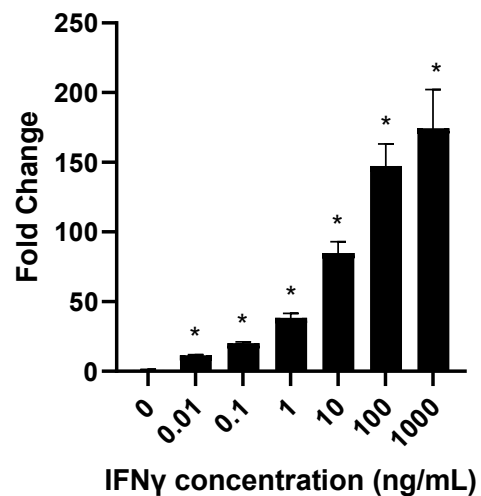


Luciferase expression at PDL>30

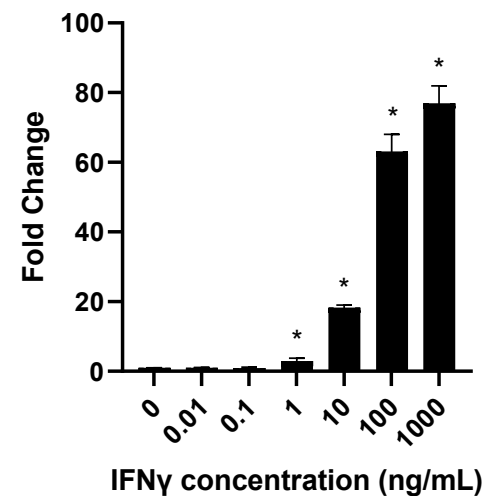
**Luminescence Intensity
HCC827 GAS-Luc2, high PDL**



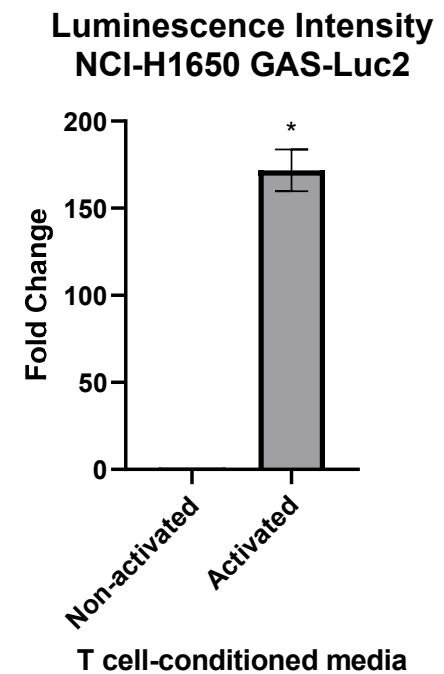
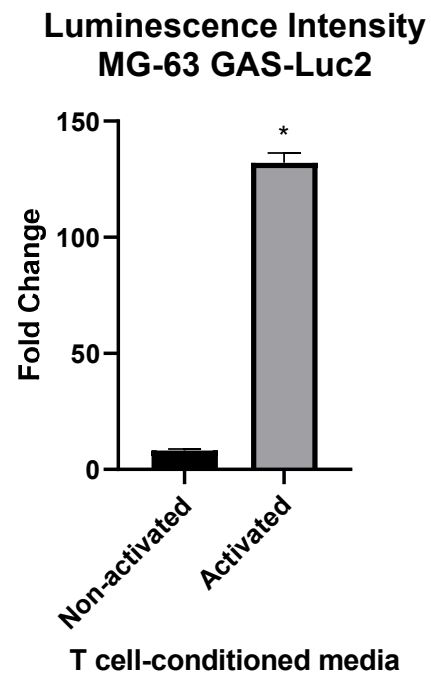
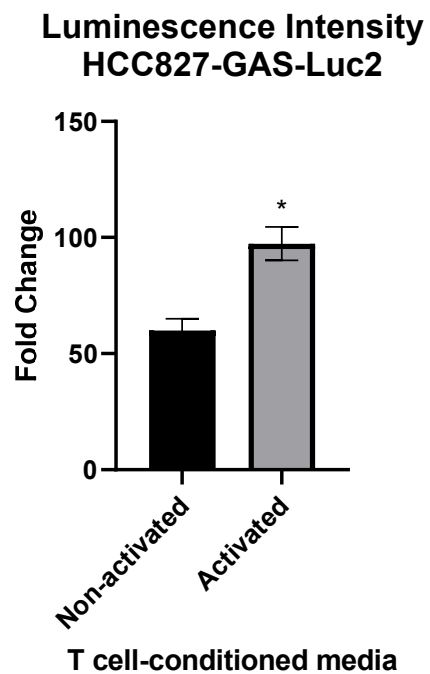
**Luminescence intensity
MG-63 GAS-Luc2, high PDL**



**Luminescence Intensity
NCI-H1650 GAS-Luc2, high PDL**

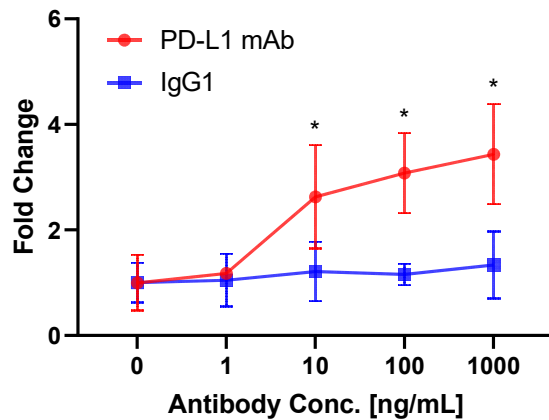


Luciferase signal upon CD8+ T cell-CM stimulation

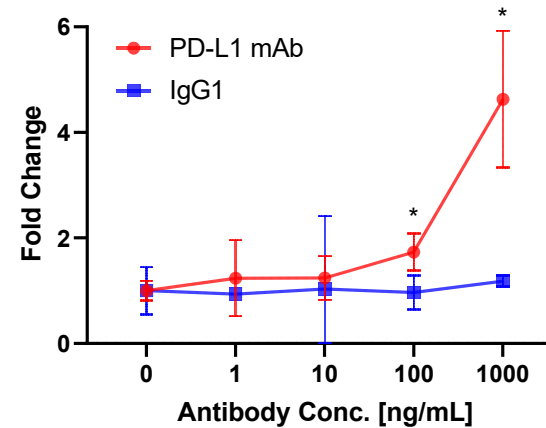


Luciferase signal upon co-culture with CD8+ T cells

Luminescence intensity 24h after co-culture
HCC827 GAS-Luc2 : CD8+ T = 1:1

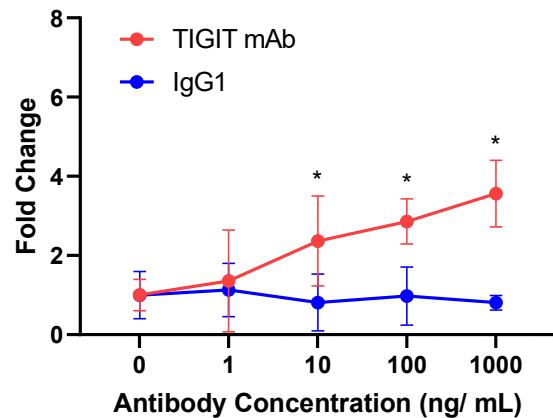


Luminescence intensity 2h after co-culture
HCC827 GAS-Luc2 : CD8+ T = 1:10

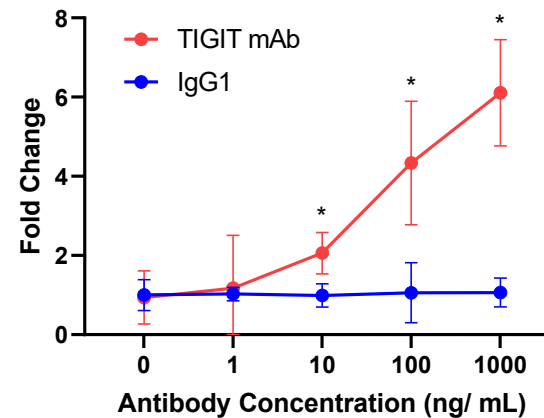


Luciferase signal upon co-culture with CD4+ T cells

Luminescence intensity 24h after co-culture
MG-63 GAS-Luc2 : CD4+ T = 1:1

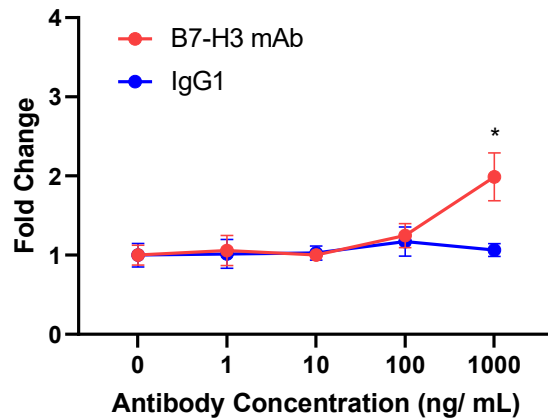


Luminescence intensity 48h after co-culture
MG-63 GAS-Luc2 : CD4+ T = 1:1

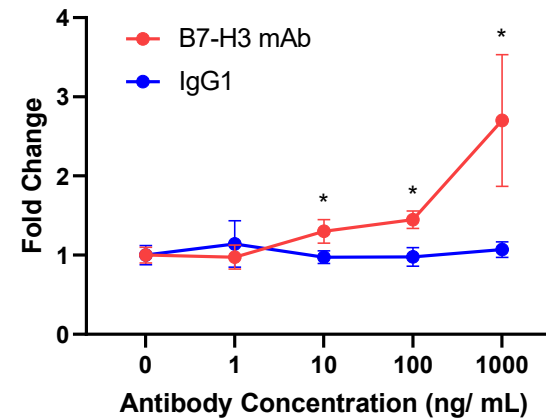


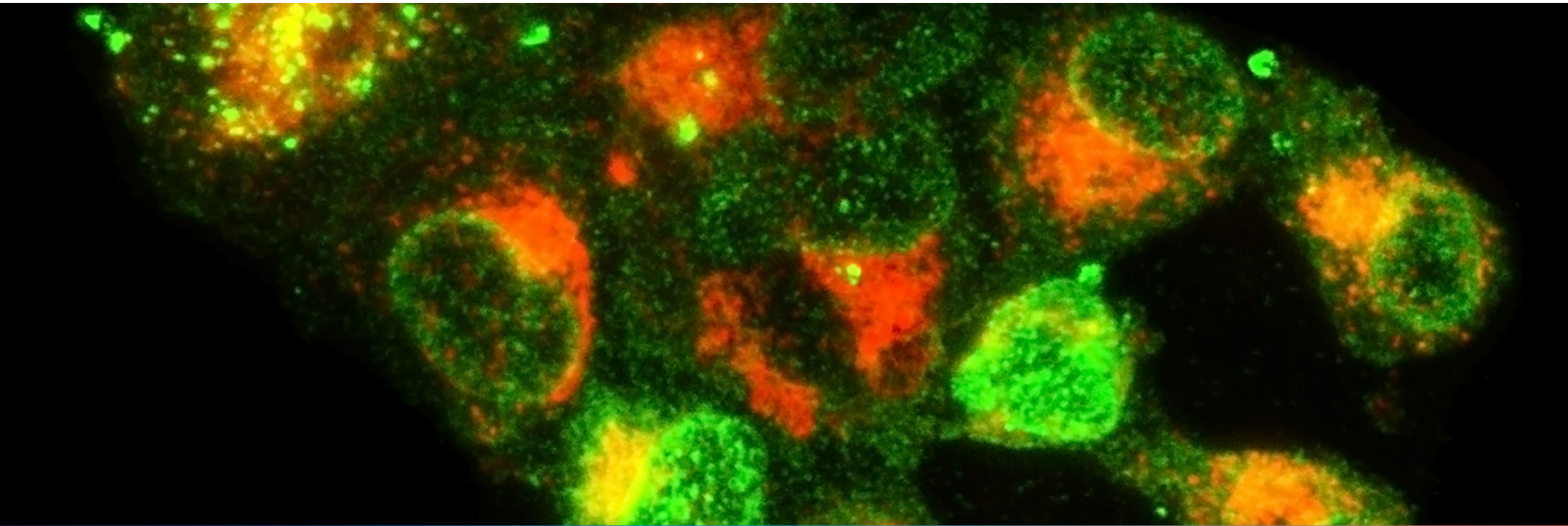
Luciferase signal upon co-culture with NK cells

Luminescence intensity 24h after co-culture
NCI-H1650 GAS-Luc2 : NK = 2:1



Luminescence intensity 48h after co-culture
NCI-H1650 GAS-Luc2 : NK = 2:1



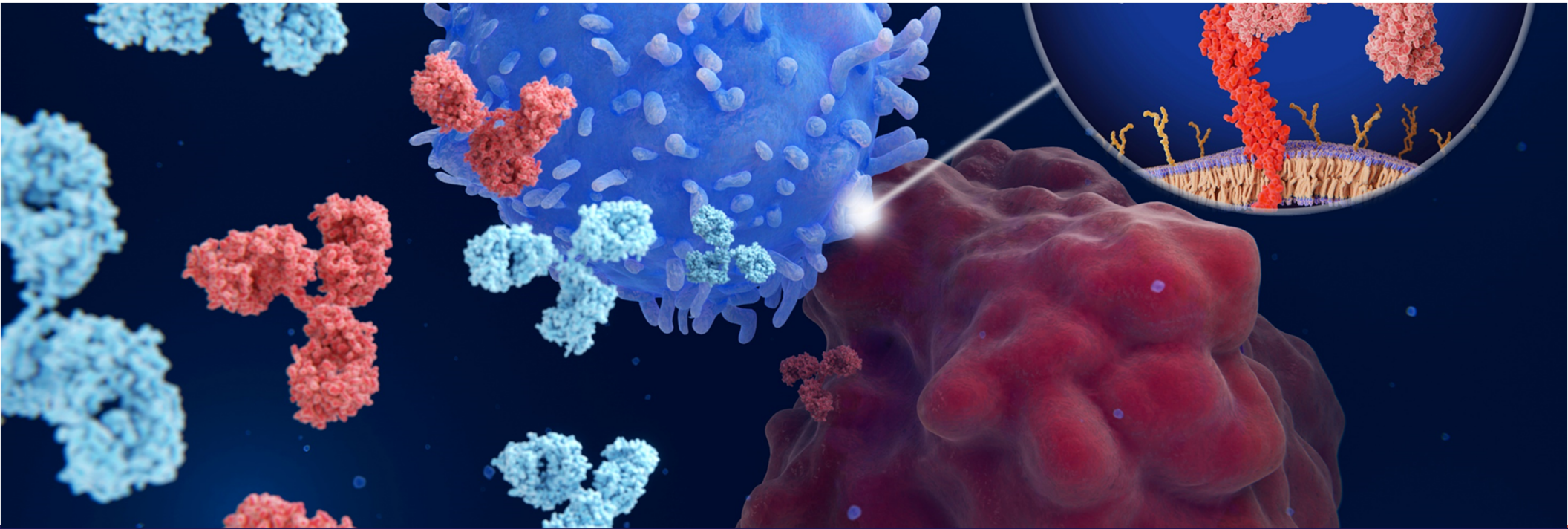


Summary

Summary

- Immune checkpoint inhibitors have immense potential, yet challenges remain.
- Performed protein profiling on our cell line portfolio for use as new models.
- Immune checkpoint reporter cancer cell lines were developed based on the protein profiling data.
- These models provide stable and robust luminescence signaling via transcription of the luciferase reporter gene.
- Can be used as a convenient detection of immune checkpoint blockade-induced signaling resulting from T cell activation.
- The endogenous high expression of PD-L1, CD155, and B7-H3 from the respective HCC827, MG-63, and NCI-H1650 GAS-Luc2 reporter cell lines, delivers physiological relevance to the immune checkpoint assay.

www.ATCC.org/immuno-oncology



Thank you!

New Products:



CAR-T Target Reporter-Labeled Tumor Cells

- Access CAR-T potency and efficacy
- High endogenous expression of CAR-T target antigens
- Available for CD19, CD20, and HER2

Checkpoint Luciferase Reporter Cells

- Enables screening of checkpoint inhibitor molecules
- Wide range of targets such as PD-L1/2, CD-155, B7-H3, and PD-1
- Luciferase will be expressed under the control of GAS or NFAT

Human Cancer Models Initiative (HCMI)

- 2-D and 3-D patient-derived models available
- Diverse genetic backgrounds of the same cancer types
- Culturing protocols and organoid growth kits

