Checkpoint Molecule Profiling in Tumor and Immune Cell Lines: Applications for Immuno-oncology Drug Screening

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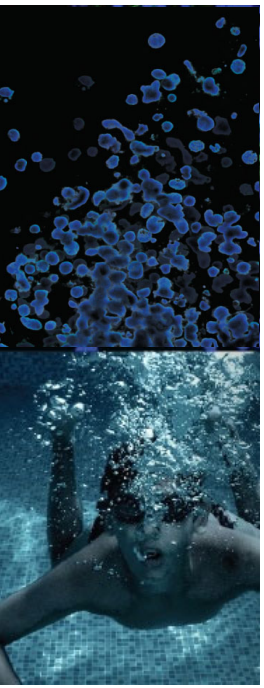
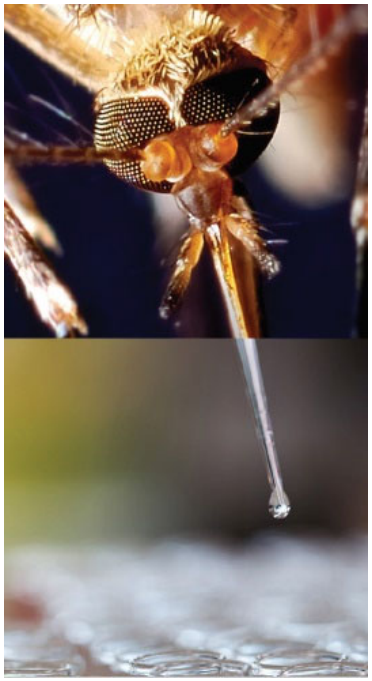
R&D, ATCC

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 450+ employees, over one-third with advanced degrees

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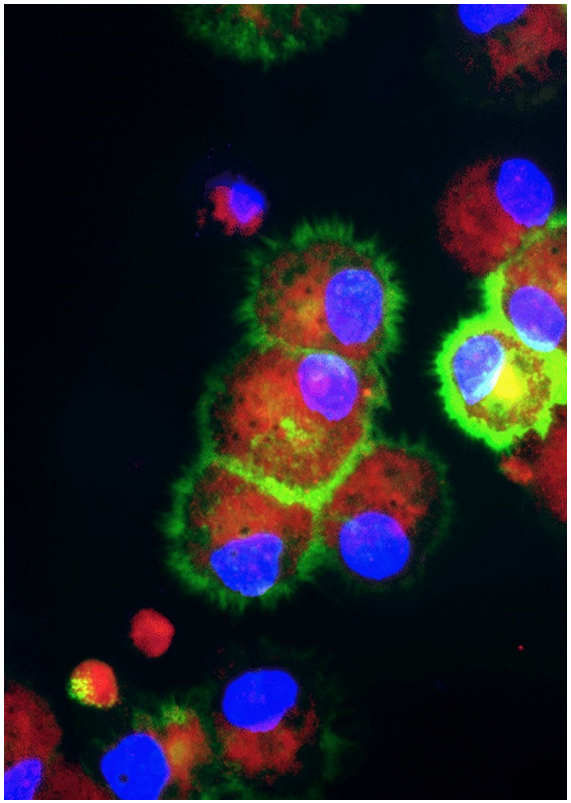
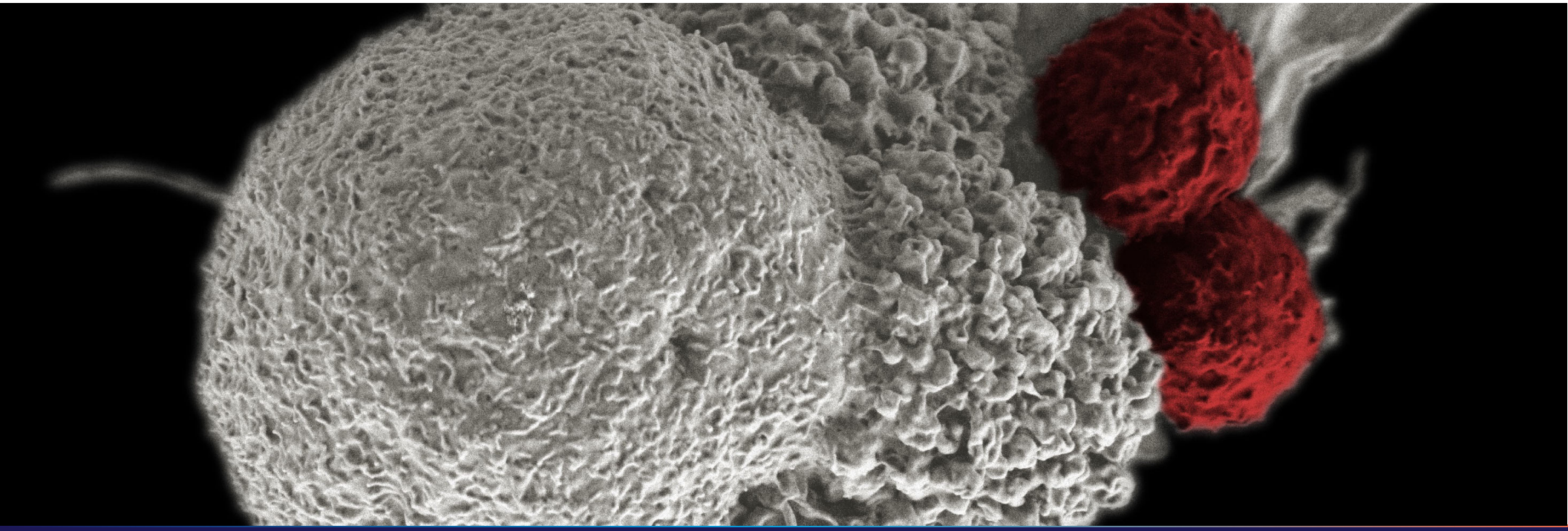
Agenda

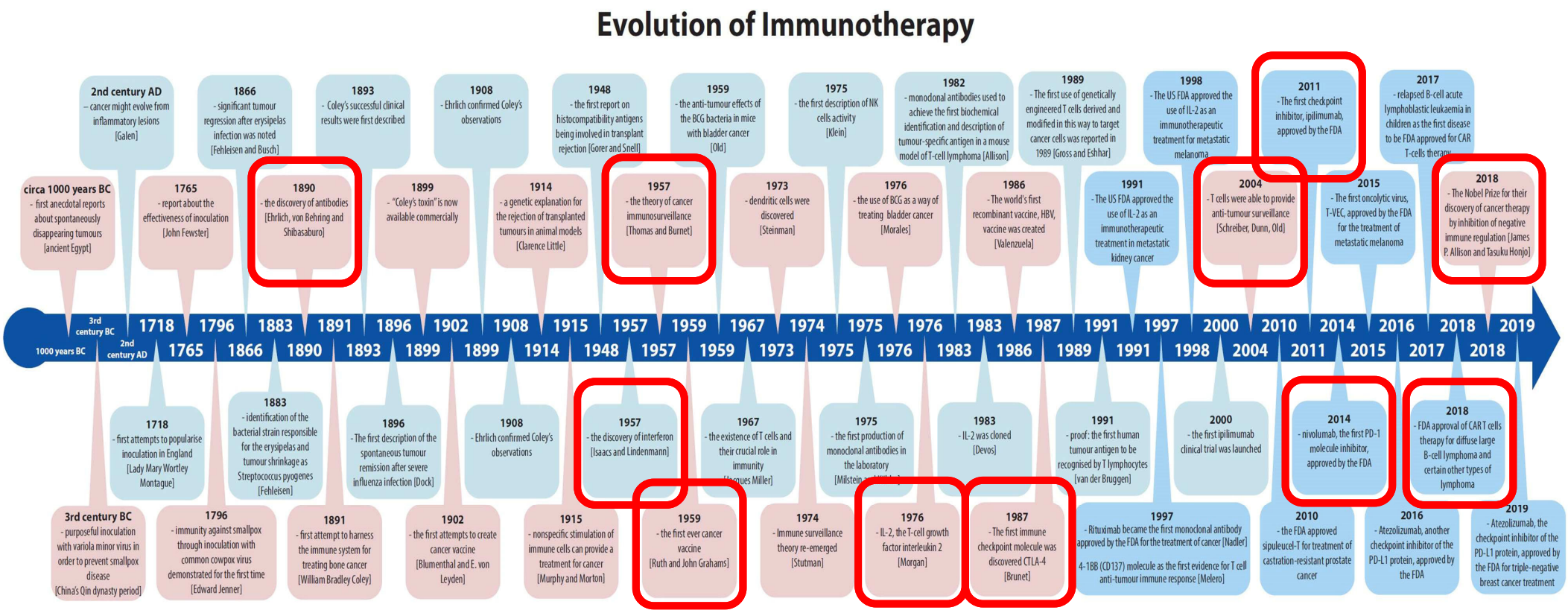
- Introduction
- ATCC internal cell line protein profiling
  - Cancer cell line profiling
  - Immune cell line profiling
- ATCC internal application data
  - IFNγ expression levels of T cell lines
  - TIM-3 checkpoint blocking assay
  - T cell and tumor cell co-culture system for PD-L1 blockade screening
- Summary





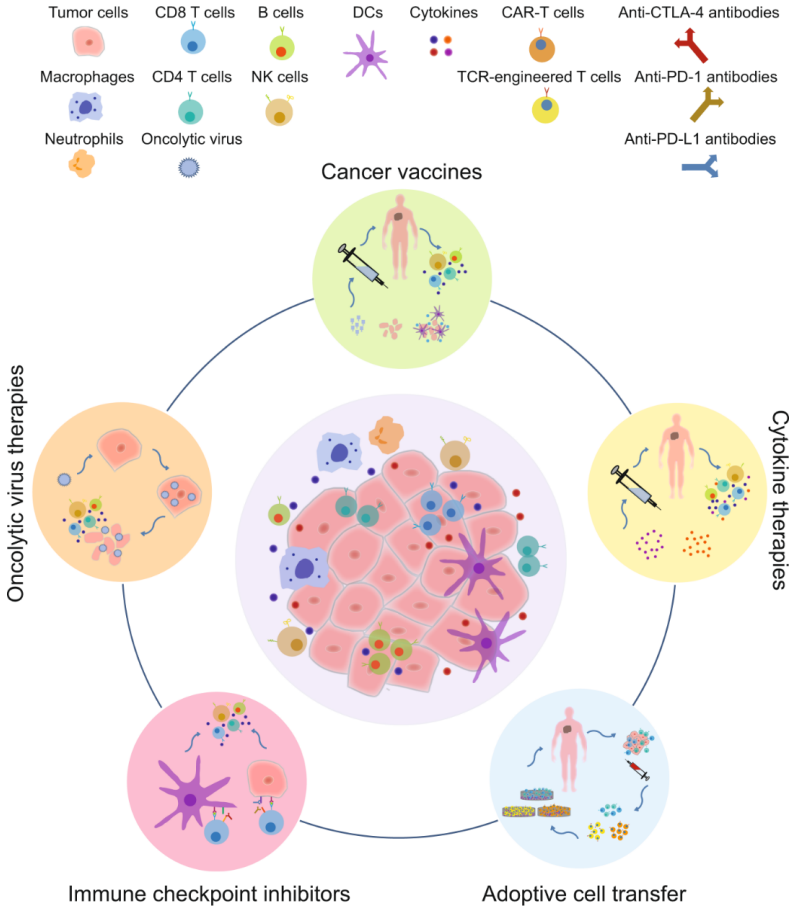
Introduction

History: Immuno-oncology



Dobosz P, Dzieciątkowski T. The Intriguing History of Cancer Immunotherapy. Front Immunol. 2019 Dec 17;10:2965. doi: 10.3389/fimmu.2019.02965. PMID: 31921205

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

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FDA approved immunomodulators

Checkpoint Inhibitors

- **Atezolizumab (Tecentriq®):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with bladder cancer, breast cancer, liver cancer, lung cancer, and melanoma
- **Avelumab (Bavencio®):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with bladder cancer, kidney cancer, and Merkel cell carcinoma, a type of skin cancer
- **Cemiplimab (Libtayo®):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with cutaneous squamous cell carcinoma, basal cell carcinoma, and lung cancer
- **Dostarlimab (Jemperli):** a checkpoint inhibitor that targets the PD-1 pathway; approved for subsets of patients with uterine (endometrial) cancer
- **Durvalumab (Imfinzi™):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with bladder cancer and lung cancer
- **Ipilimumab (Yervoy®):** a checkpoint inhibitor that targets the CTLA-4 pathway; approved for subsets of patients with melanoma, mesothelioma, liver cancer, and lung cancer
- **Nivolumab (Opdivo®):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with bladder cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, lymphoma, melanoma, and mesothelioma
- **Pembrolizumab (Keytruda®):** a checkpoint inhibitor that targets the PD-1/PD-L1 pathway; approved for subsets of patients with bladder cancer, breast cancer, cervical cancer, colorectal cancer, cutaneous squamous cell carcinoma, esophageal cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, lymphoma, melanoma, Merkel cell carcinoma, and stomach cancer. It is also approved to treat subsets of patients with cancers of any type that present with certain genetic mutations (MSI-H, dMMR, or TMB-H).
- **Relatlimab:** a checkpoint inhibitor that targets the LAG-3 pathway; approved in combination with nivolumab (together known as Opdualag™) for subsets of patients with melanoma

Cytokines

- **Aldesleukin (Proleukin®):** a cytokine that targets the IL-2/IL-2R pathway; approved for subsets of patients with kidney cancer and melanoma
- **Granulocyte-macrophage colony-stimulating factor (GM-CSF)**: an immunomodulatory cytokine; approved for subsets of patients with neuroblastoma
- **Interferon alfa-2a:** a cytokine that targets the IFNAR1/2 pathway; approved for subsets of patients with leukemia and sarcoma
- **Interferon alfa-2b (Intron A®):** a cytokine that targets the IFNAR1/2 pathway; approved for subsets of patients with leukemia, lymphoma, melanoma, and sarcoma
- **Peginterferon alfa-2b (Sylatron®/PEG-Intron®):** a cytokine that targets the IFNAR1 pathway; approved for subsets of patients with melanoma

Adjuvants

- **Imiquimod:** an immune adjuvant targeting the Toll-like receptor 7 (TLR7) pathway; approved for subsets of patients with basal cell carcinoma
- **Poly ICLC (Hiltonol®):** an immune adjuvant targeting the Toll-like receptor 3 (TLR3) pathway; approved for subsets of patients with squamous cell carcinoma

Other Immunomodulators

- **Pexidartinib (Turalio™):** a small molecule inhibitor of the KIT, CSF1R, and FLT3 pathways; approved for a subset of patients with tenosynovial giant cell tumor

Due to their effect on overall immune activity and their ability to stimulate immune responses, immunomodulators may cause side effects.

In Clinical Trials

Immunomodulator targets under evaluation in clinical trials include:

- **CD40:** Activating this co-stimulatory pathway can kickstart adaptive immune responses
- **CD47:** This surface protein acts as a "don't eat me!" signal that protects cancer from being consumed by certain immune cells; blocking CD47 can improve anti-tumor function of these immune cells
- **CD73 or A2AR:** Blocking these pathways can help prevent the production of immunosuppressive adenosine
- **CD137 (also known as 4-1BB):** activating this co-stimulatory pathway can help promote the growth, survival, and activity of cancer-fighting T cells
- **CSF1/CSF1R:** blocking this pathway can help reprogram cancer-supporting macrophages
- **CTLA-4:** blocking this pathway can help promote expansion and diversification of cancer-fighting T cells
- **CXCR4:** blocking this pathway can promote the migration and recruitment of immune cells
- **GITR:** activating this pathway can help prevent immunosuppression and increase the survival of cancer-fighting T cells
- **ICOS:** activating this co-stimulatory pathway on T cells can help enhance immune responses against cancer
- **IDO:** blocking this enzyme's activity can help prevent cancer-fighting T cells from being suppressed
- **IL-2/IL-2R:** activating this cytokine pathway can help promote the growth and expansion of cancer-fighting T cells
- **LAG3:** blocking this pathway may be able to help prevent suppression of cancer-fighting T cells
- **OX40:** activating this co-stimulatory pathway can help promote T cell survival after activation
- **PD-1/PD-L1:** blocking this pathway can help prevent cancer-fighting T cells from becoming "exhausted," and can restore the activity of already-exhausted T cells
- **STAT3:** activating this intracellular signaling protein can help stimulate adaptive immune responses
- **STING:** activating this protein in the DNA-sensing pathway can help stimulate immune responses against threats such as viruses and cancer
- **Toll-like receptors (TLRs):** activation of these innate immune receptors can help stimulate vaccine-like responses against tumors
- **TIGIT:** blocking this pathway may be able to help prevent suppression of cancer-fighting T cells
- **TIM3:** blocking this pathway may be able to help prevent suppression of cancer-fighting T cells

Clinical trials for checkpoint therapies reached 829 in 2021

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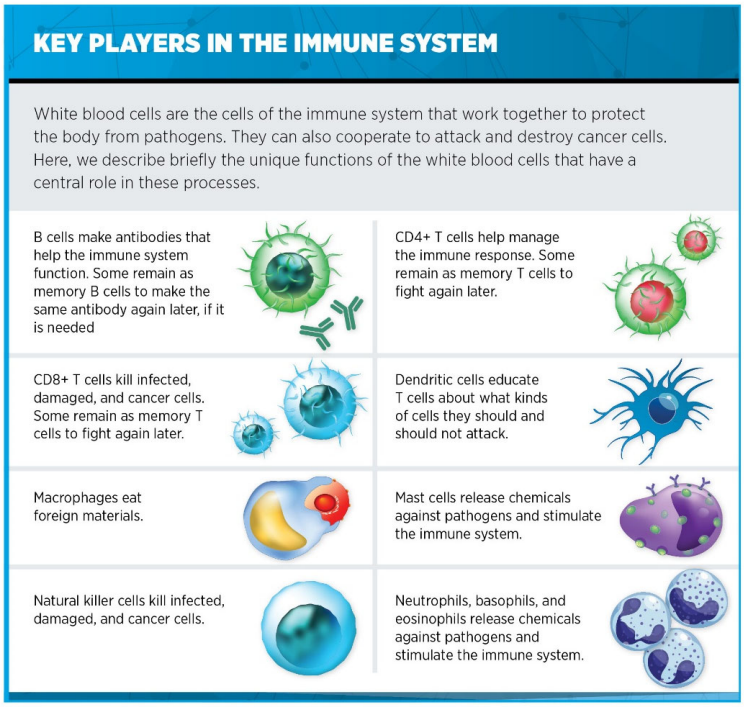
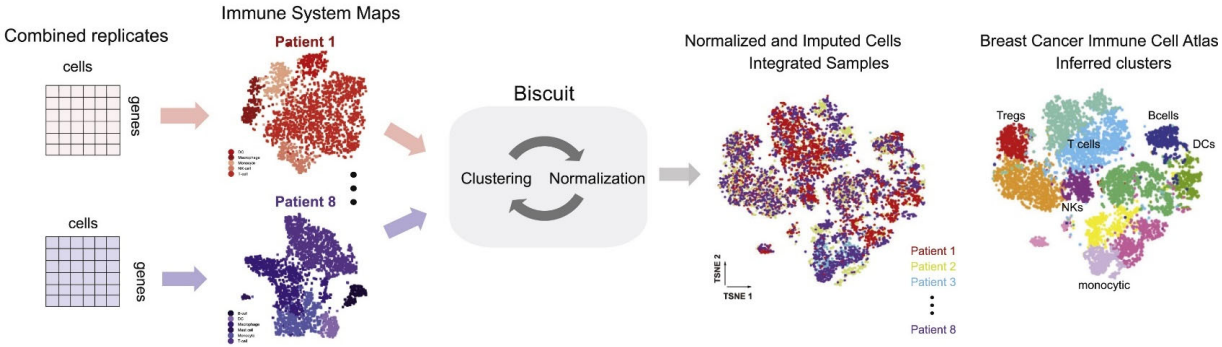
Single-cell Cancer Immunology

Classical Immunology

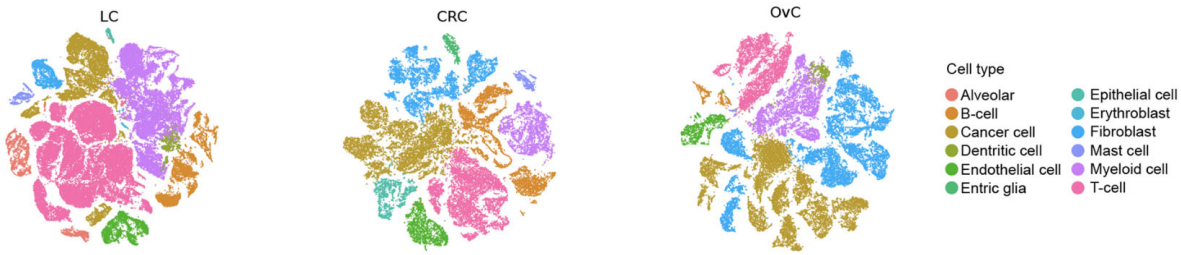


American Association for Cancer Research (AACR) Cancer Progress Report 2020

Single-cell Immunology

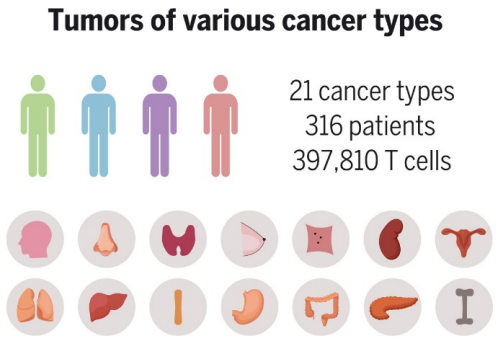


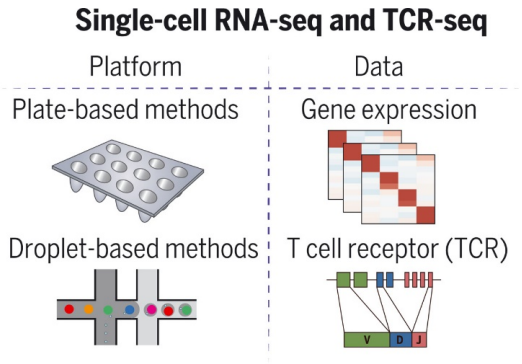
Azizi et al. Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment. Cell. 2018 Aug 23;174(5):1293-1308.e36. doi: 10.1016/j.cell.2018.05.060. Epub 2018 Jun 28. PMID: 29961579

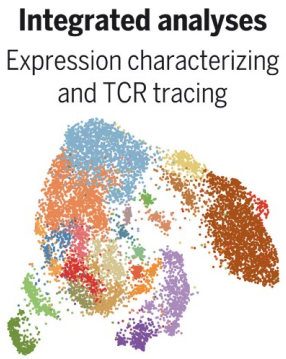


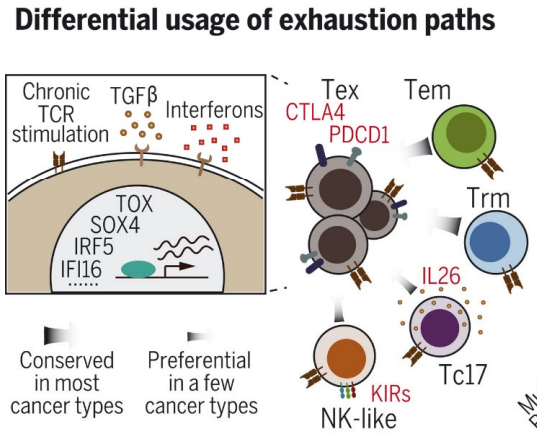
Qian et al. A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. Cell Res. 2020 Sep;30(9):745-762. doi: 10.1038/s41422-020-0355-0. Epub 2020 Jun 19. PMID: 32561858

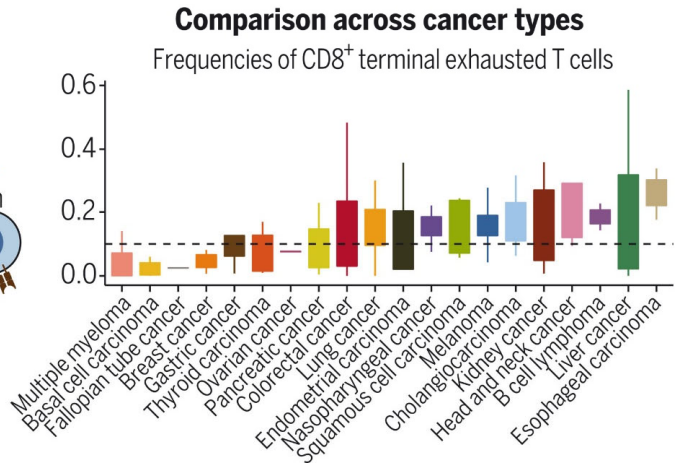
Pan-cancer Single-cell Landscape of Tumor-infiltrating T Cells

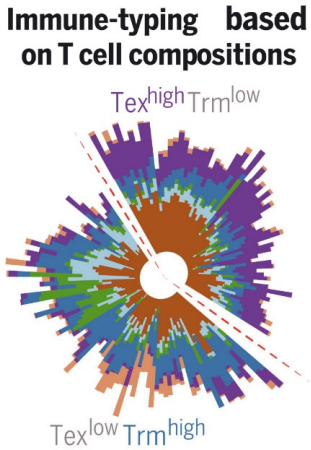












Systematic analysis of a human pan-cancer T cell atlas.

"We analyzed approximately 390,000 T cells from 316 patients of 21 cancer types by means of scRNA-seq. Combining gene expression profiles and T cell receptor sequences, we investigated the heterogeneity and dynamics of tumor-infiltrating T cells and performed a systematic comparison of T cells among cancer types. Additionally, we provided a T cell composition–based immune-typing scheme. KIR, killer cell immunoglobulin-like receptor; IL26, interleukin-26."

Zheng et al. Pan-cancer single-cell landscape of tumor-infiltrating T cells. Science. 2021 Dec 17;374(6574):abe6474. doi: 10.1126/science.abe6474. Epub 2021 Dec 17. PMID: 34914499.

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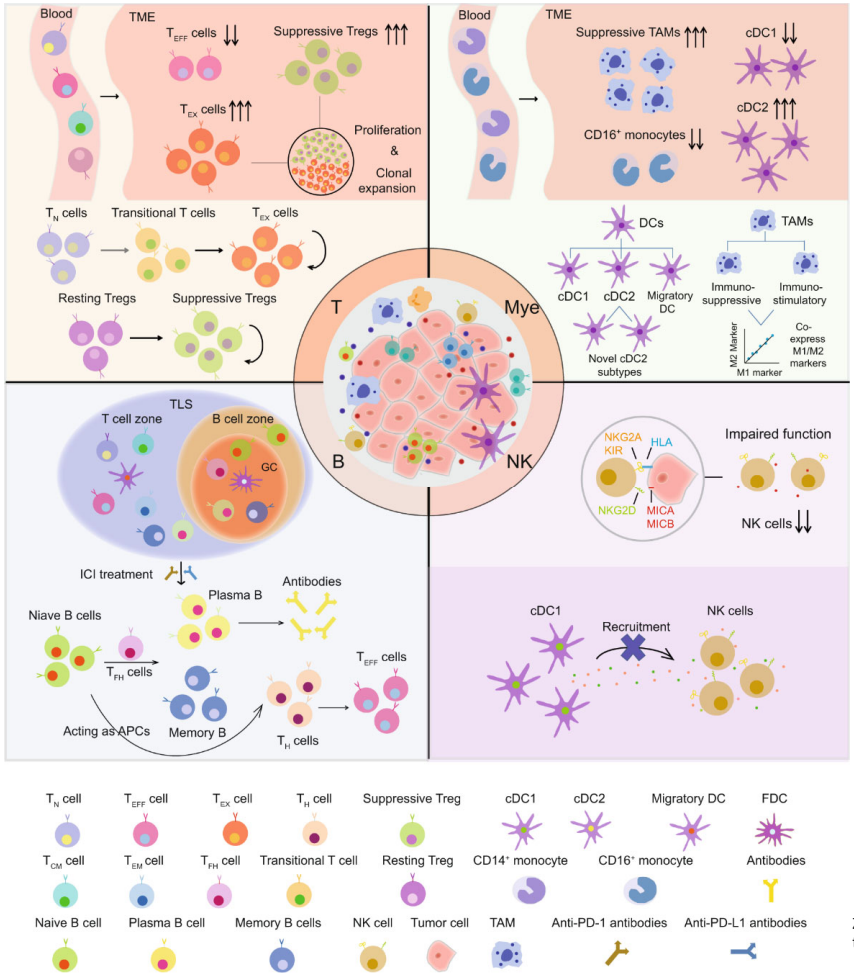
Functional Properties and Dynamic Changes of Immune Cells in the Tumor Microenvironment



- T cells in peripheral blood infiltrate into tumors and undergo functional state transitions, possibly driven by the immunosuppressive microenvironment.
- Myeloid cells in blood are mainly monocytes, including $CD14^+$ and $CD16^+$ subsets, while these cells tend to differentiate into macrophages and DCs in tumors. The TME sculpts them to harbor immunosuppressive phenotypes.
- NK cells exert cytotoxic functions, yet they show reduced cell numbers, impaired cytotoxic function and an impeded orchestrating effect for immune responses exemplified by the hampered cDC1 recruitment in the TME.
- B cells play important roles in antitumor immunity and ICI treatment. The activated B cells can differentiate not only into plasma B cells to produce antibodies to clear cancer cells but also into active T-cell-mediated immune responses.

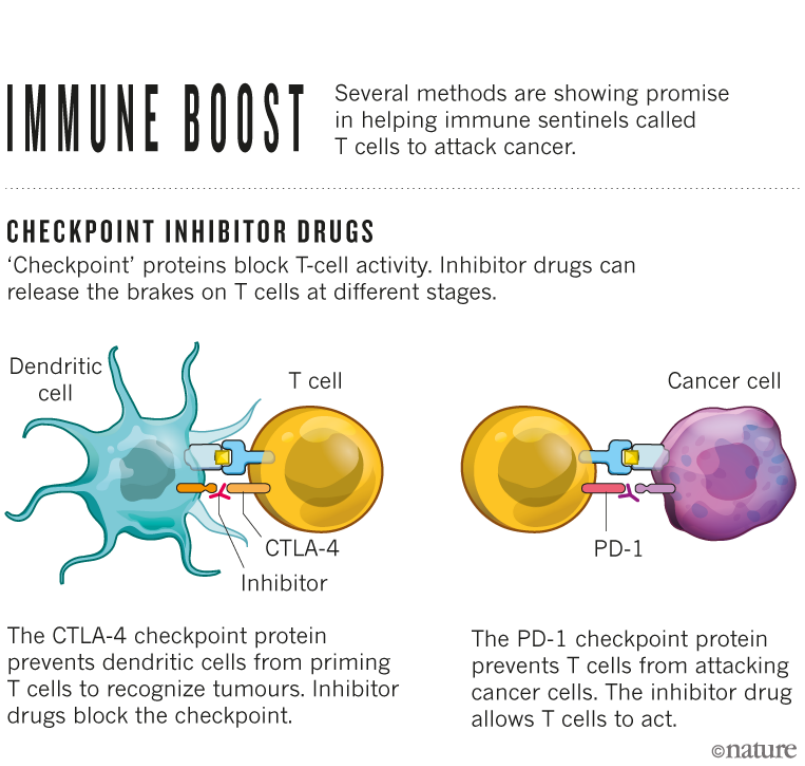
Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. Cell Mol Immunol. 2020 Aug;17(8):807-821. doi: 10.1038/s41423-020-0488-6. Epub 2020 Jul 1. PMID: 32612154

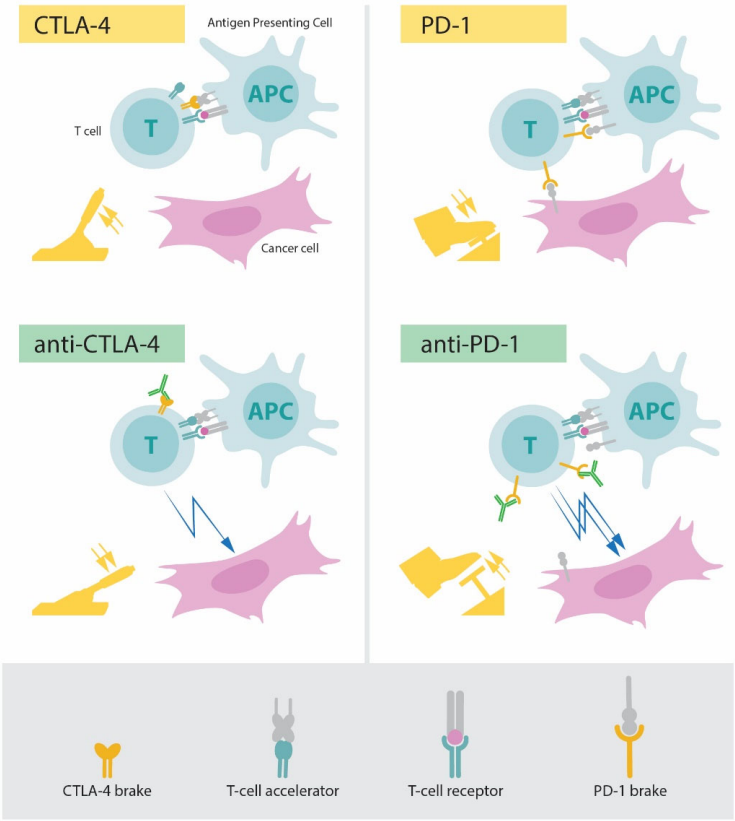
Immune Checkpoints – Negative Immune Regulation

The Nobel Prize in Physiology or Medicine 2018

James P. Allison

Tasuku Honjo





Press release: The Nobel Prize in Physiology or Medicine 2018. NobelPrize.org. Nobel Prize Outreach AB 2022. Tue. 5 Apr 2022. https://www.nobelprize.org/prizes/medicine/2018/press-release/
Image of JP Allison: https://en.wikipedia.org/wiki/James_P._Allison; Image of Tasuku Honjo: https://en.wikipedia.org/wiki/Tasuku_Honjo

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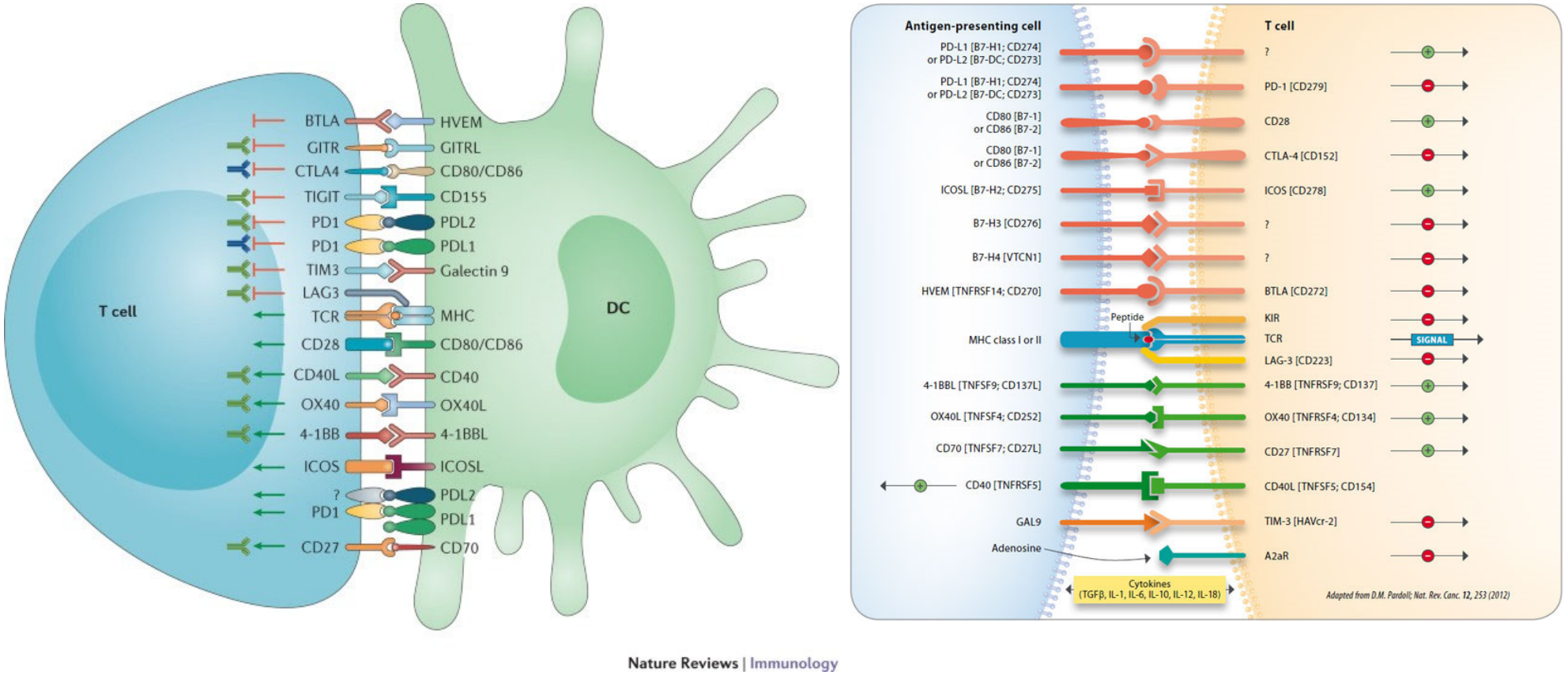
T Cell Checkpoints



Nature Reviews | Immunology

Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. Nat Rev Immunol. 2018 Feb;18(2):91-104. doi: 10.1038/nri.2017.112. Epub 2017 Oct 9. PMID: 28990586

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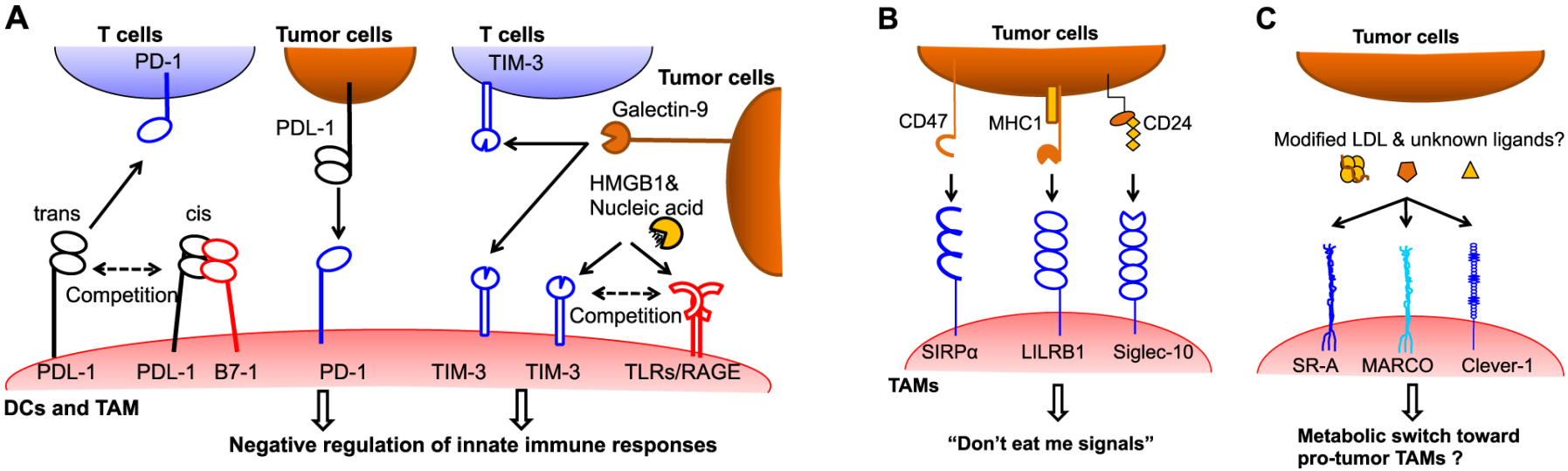
Myeloid Cell Checkpoints



Potential immune checkpoint molecules on myeloid cells. **a** Tumor-associated macrophages and dendritic cells (DCs) express several T cell immune checkpoints and their ligands. While the trans interaction between PD-L1 on myeloid cells and PD-1 on T cells critically regulates T cell activation, the cis interaction between PD-L1 and B7-1 on antigen-presenting cells competitively inhibits the trans interaction. TAMs and DCs also express PD-1, which negatively regulates innate immune responses. TIM-3 on DCs also negatively regulates cytokine production, either directly by interacting with galectin-9 on tumor cells or indirectly by the sequestration of the HMGB1-nucleic acid complex (ligands for RAGE and TLRs). **b** ITIM-containing receptors SIRPα, LILRB1, and Siglec-10 have emerged as key receptors that negatively regulate cellular phagocytosis through the recognition of CD47, MHC class 1, and CD24, respectively. Note that PD-1 on macrophages is also known to regulate phagocytosis. **c** Through the recognition of certain ligands in the TME, several scavenger receptors might contribute to metabolic switching toward protumor TAMs, given that genetic ablation or pharmacological inhibition of these receptors can direct TAMs into antitumor phenotypes

Nakamura K, Smyth MJ. Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. Cell Mol Immunol. 2020 Jan;17(1):1-12. doi: 10.1038/s41423-019-0306-1. Epub 2019 Oct 14. PMID: 31611651

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B Cell Checkpoints

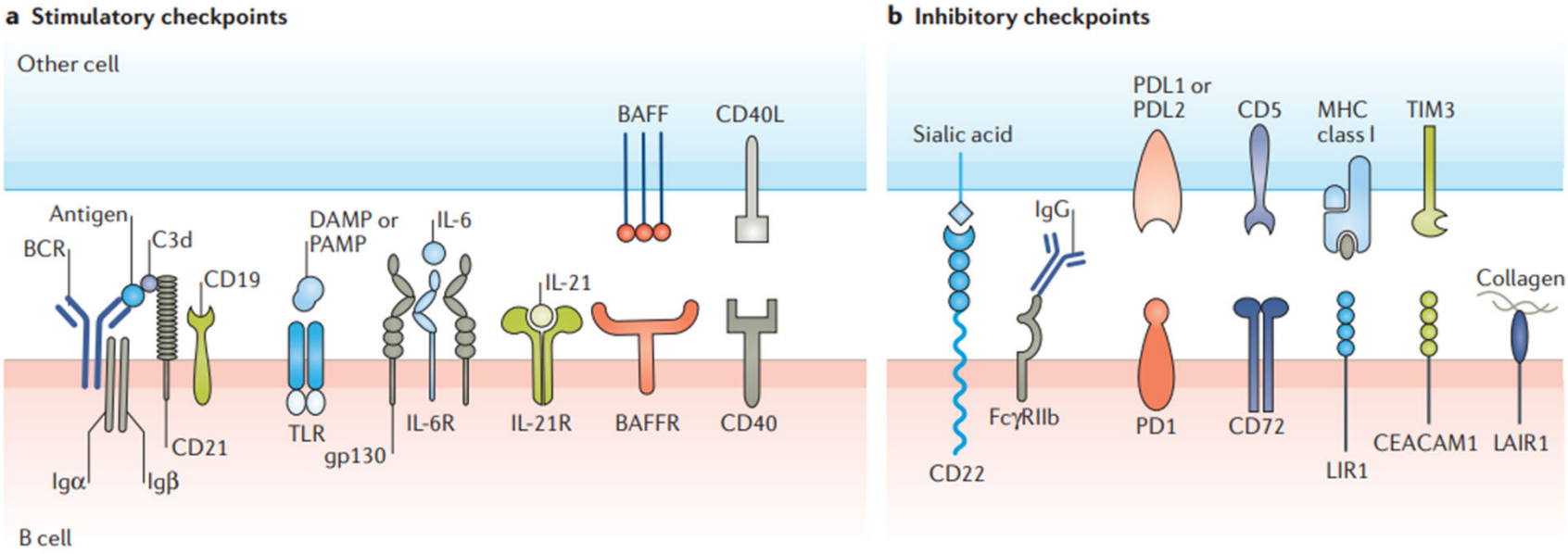

Fig. 2 | **Examples of B cell stimulatory and inhibitory checkpoints.** B cells express a repertoire of stimulatory and inhibitory receptors, the relative levels of which change through the course of B cell development. B cell functions are modulated by the balance of expression and colocalization of stimulatory checkpoint receptors (part **a**) and inhibitory checkpoint receptors (part **b**). Some Toll-like receptors (TLRs) are also expressed in the endosome. BAFF, B cell activating factor; BAFFR, B cell activating factor receptor; BCR, B cell receptor; CD40L, CD40 ligand; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; DAMP, damage-associated molecular pattern; FcγRIIb, low-affinity immunoglobulin-γ Fc region receptor IIb; IL-21R, IL-21 receptor; IL-6R, IL-6 receptor; LAIR1, leukocyte-associated immunoglobulin-like receptor 1; LIR1, leukocyte immunoglobulin-like receptor 1; PAMP, pathogen-associated molecular pattern; PD1, programmed cell death 1.

Rubin SJS, Bloom MS, Robinson WH. B cell checkpoints in autoimmune rheumatic diseases. Nat Rev Rheumatol. 2019 May;15(5):303-315. doi: 10.1038/s41584-019-0211-0. PMID: 30967621.

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FDA approved LAG3 + PD1 combo 3/18/2022

U.S. Food and Drug Administration Approves First LAG-3-Blocking Antibody Combination, nivolumab and relatlimab-rmbw, as Treatment for Patients with Unresectable or Metastatic Melanoma

03/18/2022

CATEGORY: Corporate/Financial News

Opdualag *is a first-in-class, fixed-dose dual immunotherapy combination treatment of the PD-1 inhibitor nivolumab and novel LAG-3-blocking antibody relatlimab*[1]

In RELATIVITY-047, Opdualag *more than doubled median progression-free survival compared to nivolumab monotherapy, an established standard of care*[1,2]

Relatlimab is the third immune checkpoint inhibitor from Bristol Myers Squibb, adding to the Company's growing and differentiated oncology portfolio

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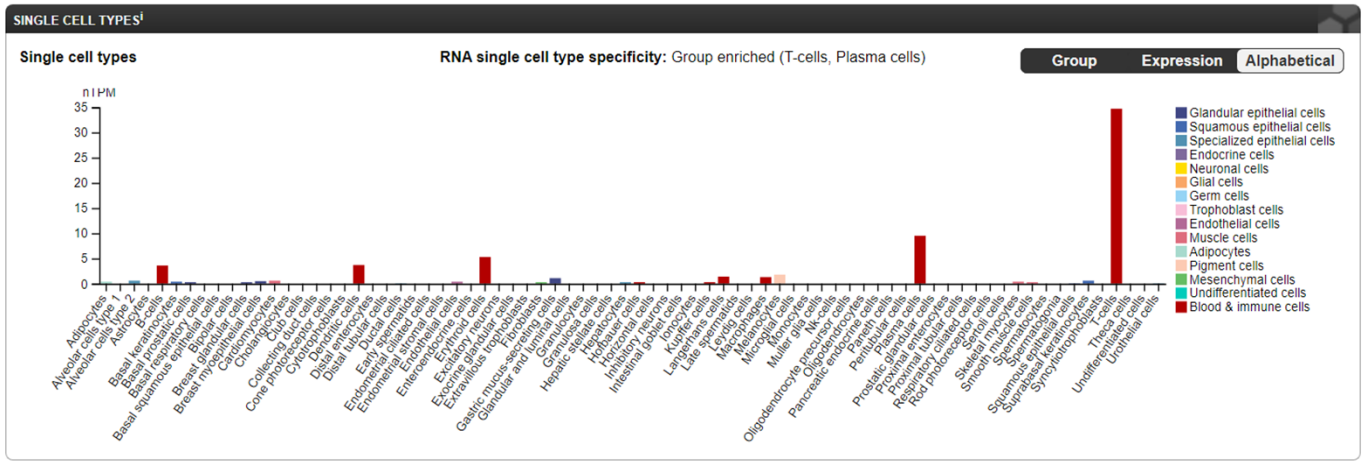
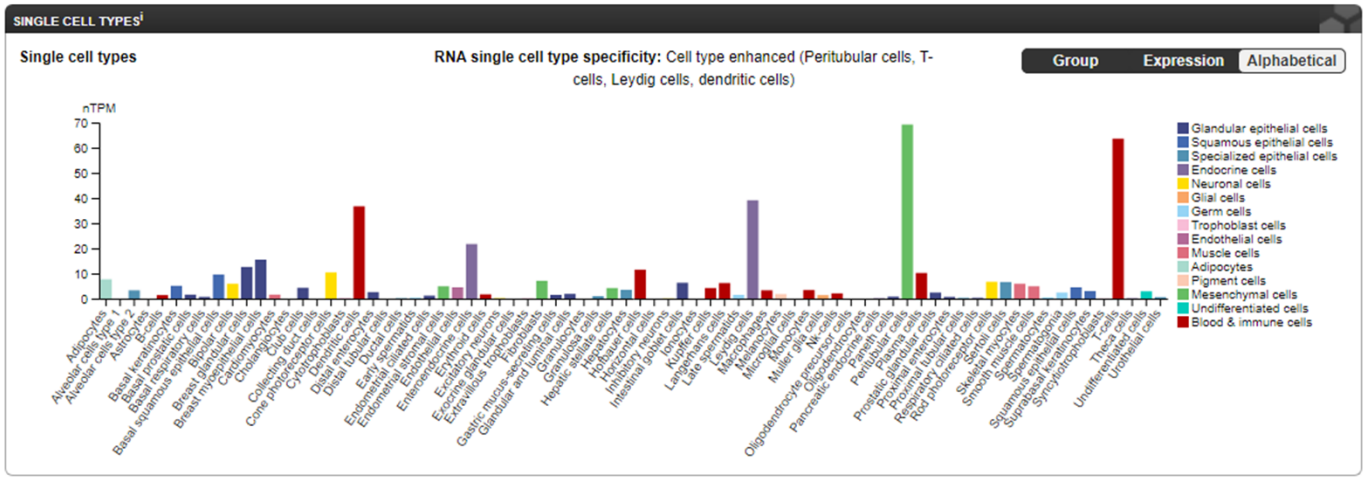
PD1 vs. LAG3 at Single-cell Level https://www.proteinatlas.org/

PDCD1 (PD1)

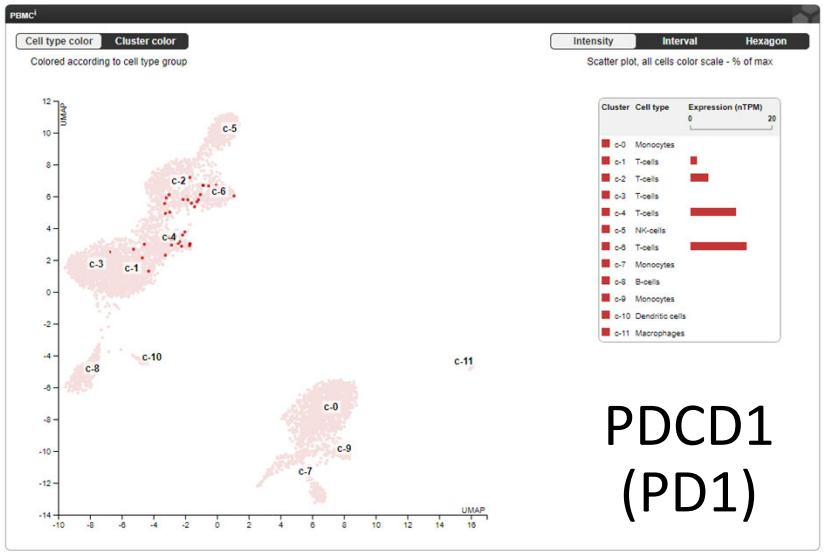


LAG3

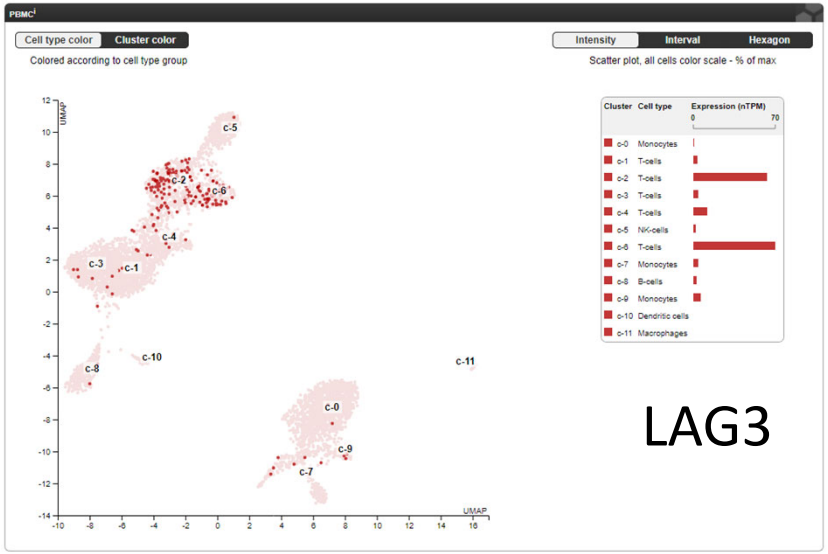


PD1 vs. LAG3 at Single-cell Level

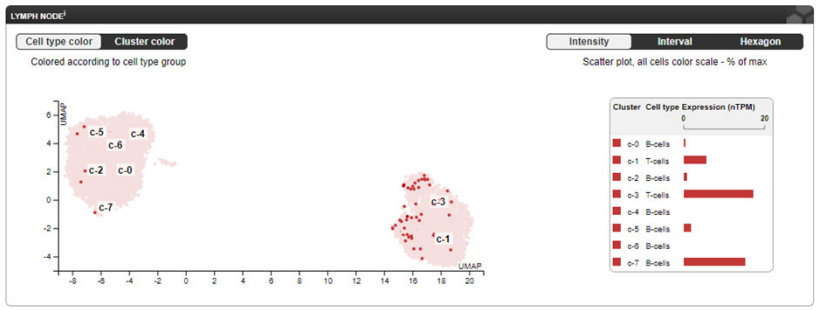
https://www.proteinatlas.org/

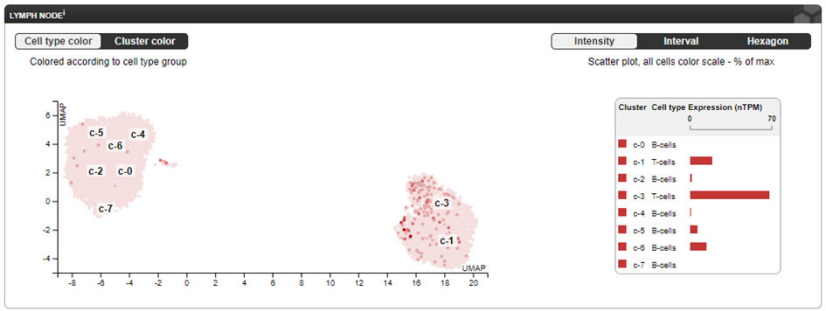


PDCD1 (PD1)



LAG3

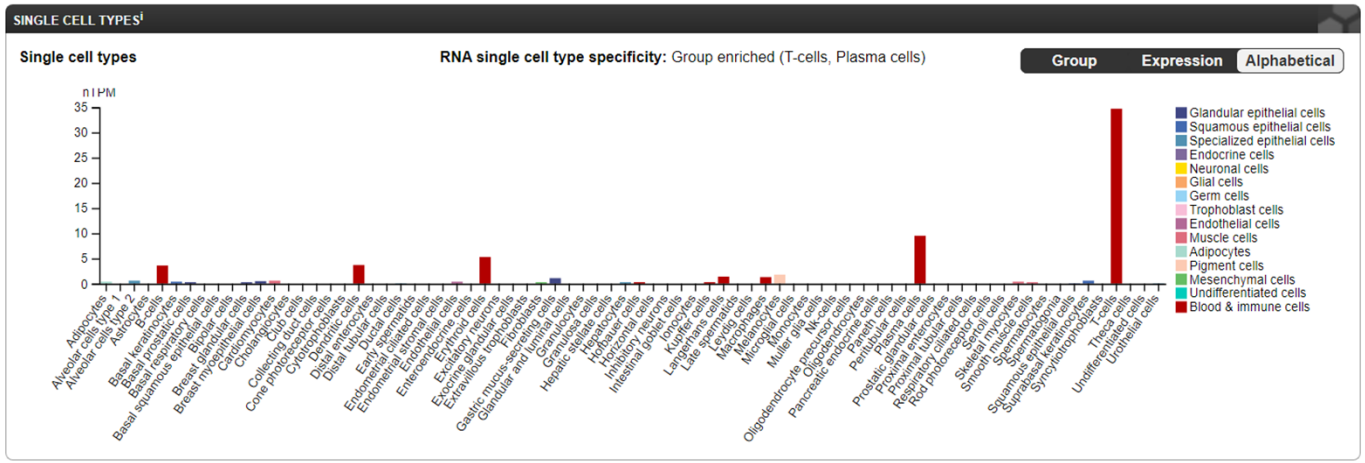




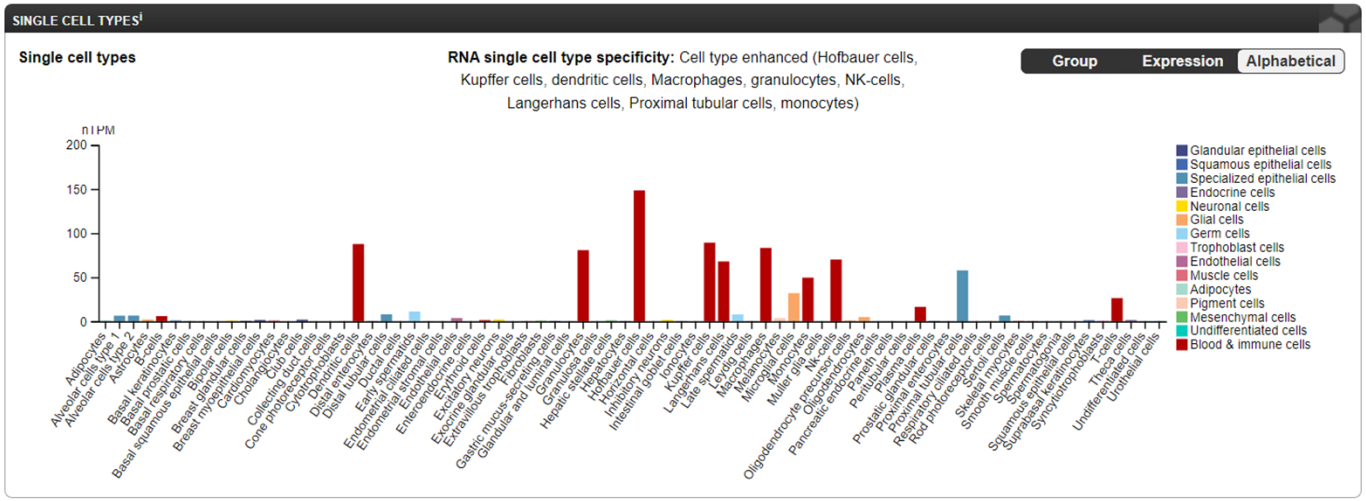
PD1 vs. TIM3 at Single-cell Level

https://www.proteinatlas.org/

PDCD1
(PD1)

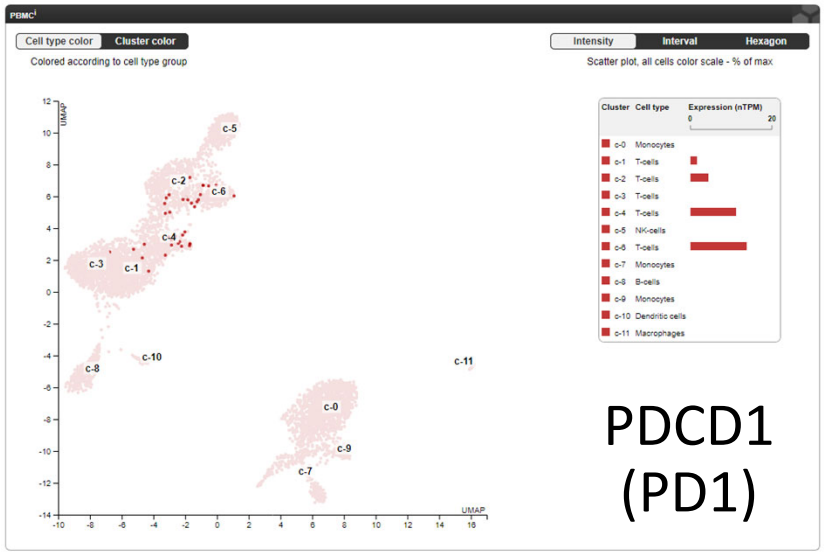


HAVCR2
(TIM3)

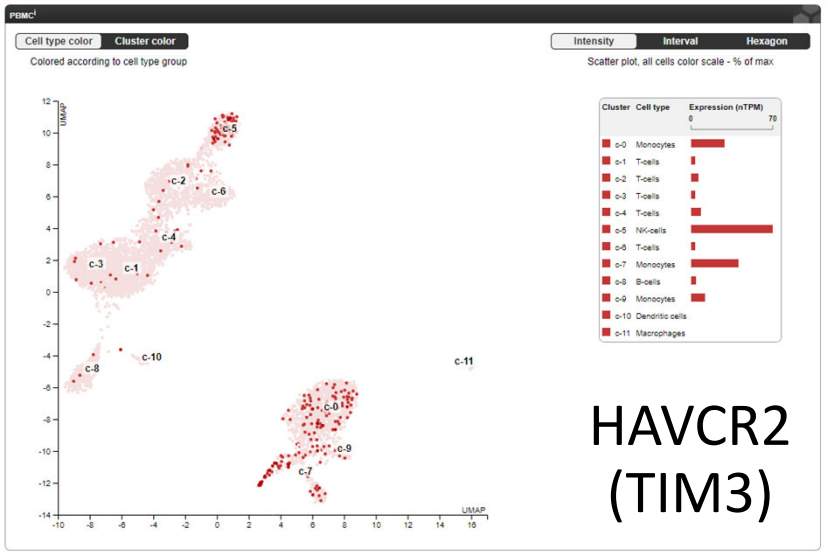


PD1 vs. TIM3 at Single-cell Level

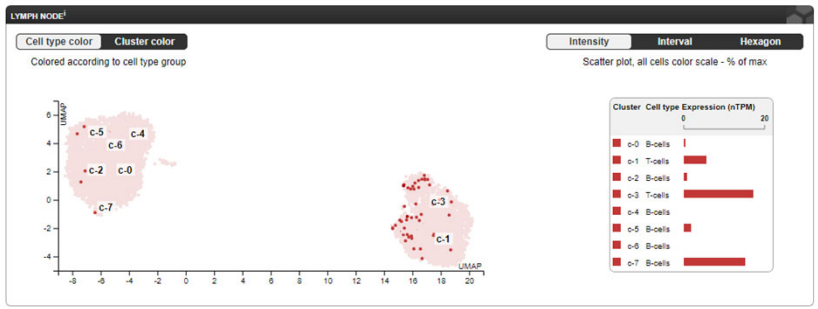
https://www.proteinatlas.org/

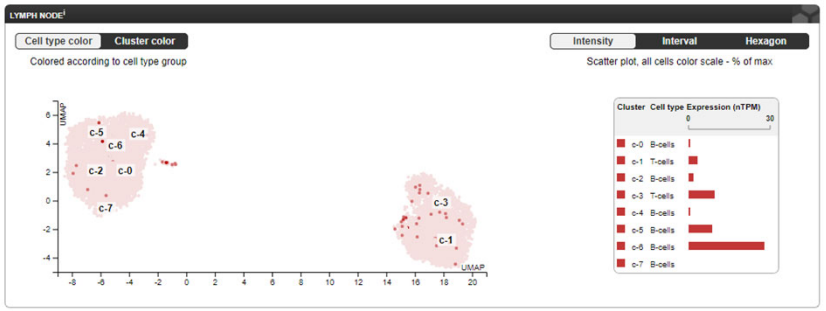


PDCD1 (PD1)



HAVCR2 (TIM3)

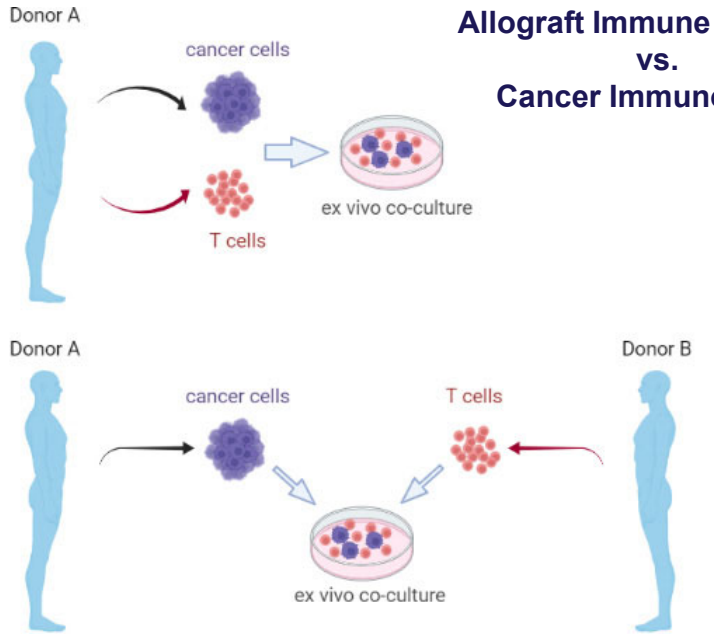




Challenges in Cancer Immunotherapy

Top 10 Challenges in Cancer Immunotherapy
1. Development of pre-clinical models that translate to human immunity
2. Determining the dominant drivers of cancer immunity
3. Understanding organ-specific tumor immune contexture
4. Understanding the molecular/cellular drivers of primary vs. secondary immune escape
5. Elucidating the benefit of endogenous versus synthetic immunity
6. Effective and efficient assessment of cancer immunotherapy combinations in early-phase clinical studies
7. The impact of steroids and immune suppression on cancer immunotherapy and autoimmune toxicities
8. Maximizing personalized approaches through composite biomarkers
9. Developing improved regulatory endpoints for cancer immunotherapy
10. Optimizing long-term survival with multi-agent immunotherapy combination regimens

Adapted from: Immunity 2020 Jan 14;52(1):17-35. doi:10.1016/j.immuni.2019.12.011



Allograft Immune Rejection vs. Cancer Immunology ?

- Immunotherapy drug development has been a difficult endeavor
- Current methods for studying immunotherapies are time consuming, expensive, or not physiologically relevant
- Clear need for a human cell-based model that has endogenous natural expression of checkpoint molecules

ATCC Internal Efforts

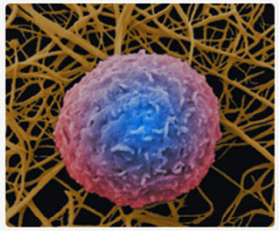
Cancer immunology research tools

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

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

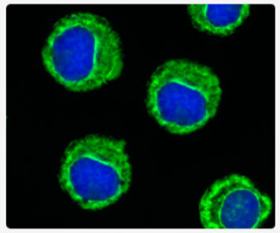


Lymphoid Cells

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.

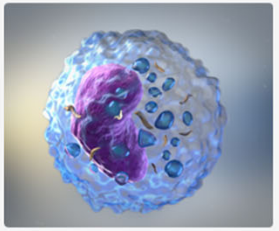
Progenitor Cells

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.

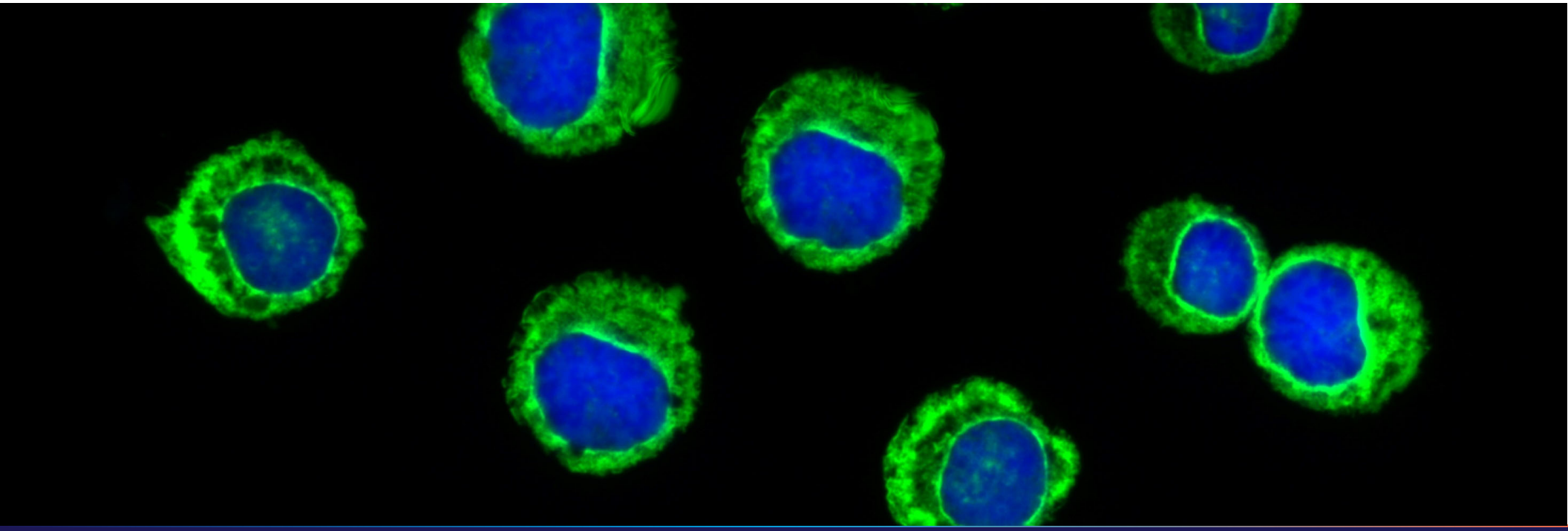


Natural Killer Cells

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.



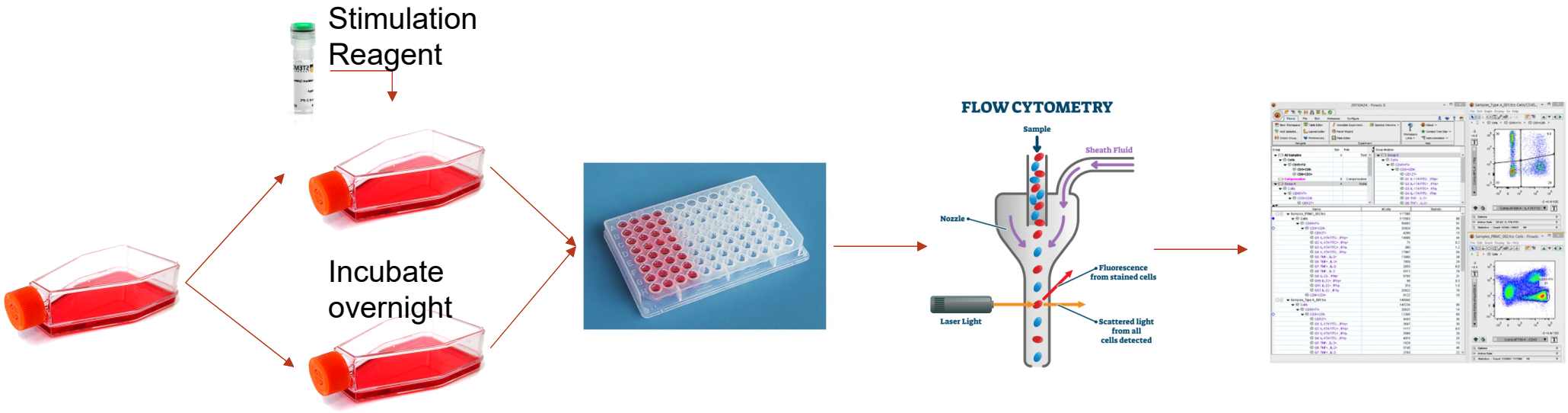
- Protein profiled over 60+ tumor cell lines, over 10+ immune cell lines, and primary human immune cells for established and novel checkpoint molecules
- Provides physiologically relevant cell models for developing cancer immunotherapies
- Endogenous natural expression of checkpoint molecules on the cell surface provides a cost-saving measure over screening of patient samples
- Versatility: We provide you cell tools instead of assay kits.



ATCC Internal Cell Line Protein Profiling

Simple FACS Experiments for Protein Profiling

ATCC internal:
Protein profiled over 60+ tumor cell lines, over 10+ immune cell lines, and primary human immune cells for established and novel checkpoint molecules



Tumor Cell Lines – Protein Profiling by FACS

"-": without IFNγ
"+": with IFNγ

Type	Cell Lines	HLA typing		Checkpoint inhibitory molecules										Checkpoint co-stimulatory molecules									
		HLA I	HLA II +	CD274 (PD-L1) -	CD274 (PD-L1) +	CD273 (PD-L2) -	CD273 (PD-L2)+	CD276 (B7-H3) -	CD276 (B7-H3) +	B7-H4 -	B7-H4+	HVEM-	HVEM+	4-1BB L(CD137 L)-	4-1BB L(CD137 L)+	CD275 (ICOS L) -	CD275 (ICOS L) +	CD155-	CD155+	CD80-	CD80+	CD86-	CD86+
Bladder Cancer	5637	+	-	52096	143325	49	2594	60004	52945	0	0	1593	1783	3085	2831	1322	1464	68780	85293	2092	3069	1909	1993
	HT-1197	+	-	40740	45360.5	1368	6891.5	21853	16451	0	0	1785	2838	0	1852	1682	1837	105114	127213	4220	6126	2120	2878
	HT-1376	+	-	27135	51493	1692	8578	74667.5	66185	0	0	365	1790	0	0	3440	6322	36478	44828	4293	4179	1233	1707
	RT4	+	-	0	5054	52	518	143148	139442	0	42	717	1602	2395	2961.5	5676	7754	40953	48452	883	1097	1482	1954
	TCCSUP	-	+	30543	48394	4325	9664	131058	123270	930	822	526	1422	3016	3758	315	366	271088	282653	3912	3573	3917	3933
Brain Cancer	SK-N-BE(2)	+	-	245	6837	0	258	15903	17884	156	123	262	237	626	528	228	240	5236	6395	452	350	923	778
	U-87 MG	+		321	2990	249	246	73474	72722	338	263	4718	3312	2804	3010	339	454	30877	33809	2926	2597	2080	1968
	U-87 MG-Luc2	+	-	15061	40367	0	0	29967	29009	1508	1374	487	706	1717	1370	141	219	36063	43417	1851	1491	984	753
Breast Cancer	AU565	+	-	2428	11013	0	0	9476	8169	3514	2925	307	831	1289	841	633	856	37017	35953	983	1027	433	454
	BT-20	+	-	6082	17072	886	4614	44830	44507	711	761	0	0	7297	8831	300	136	203815	235198	8916	9398	1172	1244
	DU4475	+	-	1912	3232	1082	3774	59238	54996	1941	1317	4014	4293	8298	6525	0	0	36382	32343	8865	6426	2523	1278
	HCC38	+	-	13009	126059	3097	16705	220234	208819	2300	1565	6396	7267	1912	3050	1525	1855	132767	134741	5751	4437	2143	1906
	MCF-7	+	-	53	1802	0	0	46613	42793	4324	2944	2197	1972	4821	4165	1583	2402	23280	22977	5720	4584	2867	2424
	MCF-7-Luc2	+	-	0	3116	0	2793	56518	53829	575	936	1331	1723	3902	5935	465	1037	20258	22678	1724	5297	1215	2149
	MDA-MB-231	+	-	11359	20492	986	1880	12979	11668	149	125	456	1031	531	777	14	37	38583	53188	563	428	346	234
	MDA-MB-468	+	-	221	5046	115	380	16180	16342	806	575	140	438	740	769	401	747	36560	43422	475	464	308	290
	T-47D	+	-	72	6355	0	0	32581	24851	828	594	597	703	3140	1990	859	683	39364	37651	3038	2166	1620	1325
Bone Cancer	HOS	-	+	13031	41473	2927	9075	60530	61277	289	305	211	552	1127	1210	0	0	99713	124829	841	815	443	400
	MG-63	-	+	0	7362	0	0	84745	79181	443	819	368	730	4326	4901	0	0	303805	268365	2894	6552	1339	2968
	Saos-2	+	-	6082	32705	0	0	7455	7136	332	329	897	1244	2525	1975	0	0	58992	70813	1726	1733	1644	1525
	U-2 OS	+	-	5929	36019	290	5915	63080	64082	548	333	830	1152	2321	2660	784	778	112962	124648	2554	1174	3008	3045
Colon Cancer	Caco-2	+	-	0	471	0	0	32201	30175	1315	1209	1900	1817	4255	5817	1060	661	44423	39942	6756	4849	4146	3170
	HCT-15	-	+	474	3790	35	0	12896	12520	137	94	513	947	369	251	0	21	33045	34475	411	140	441	335
	LoVo	-	+	468	17697	0	0	20338	19572	347	346	975	2481	1581	1647	775	1080	24870	36144	903	1271	1044	1010
Head and Neck Cancer	A-253	+	-	2070	16019	123	3176	43926	41341	18	0	45	477	1431	2558	3380	3887	67935	83057	3303	3051	731	985
	FaDu	+	-	2733	37007	205	13372	39475	31090	0	0	138	855	1640	0	3643	4161	60462	62858	2728	2720	1904	1951
	FaDu-Luc2	+	-	6965	29601	0	0	24921	20048	269	333	421	448	1159	1591	484	557	35527	40460	1019	1334	2147	2183

ATCC

Tumor Cell Lines – Protein Profiling by FACS

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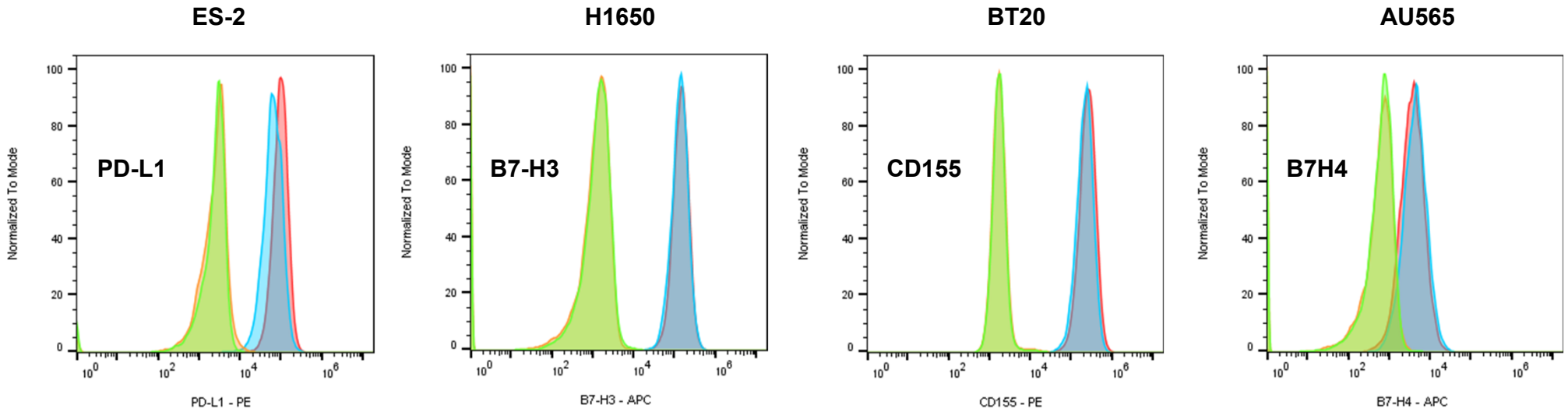
"+": with IFNγ

		HLA typing		Checkpoint inhibitory molecules										Checkpoint co-stimulatory molecules									
Type	Cell Lines	HLA I	HLA II +	CD274 (PD-L1) -	CD274 (PD-L1) +	CD273 (PD-L2) -	CD273 (PD-L2)+	CD276 (B7-H3) -	CD276 (B7-H3) +	B7-H4 -	B7-H4+	HVEM-	HVEM+	4-1BB L(CD137 L)-	4-1BB L(CD137 L)+	CD275 (ICOS L) -	CD275 (ICOS L) +	CD155-	CD155+	CD80-	CD80+	CD86-	CD86+
Liver Cancer	C3A	+	-	0	2114	0	2698	18098	16938	441	453	1362	2682	1243	2171	394	511	54751	59271	1729	1914	1136	1100
	Sk-Hep-1	+	-	2428	11013	0	0	9476	8169	3514	2925	307	831	1289	841	633	856	37017	35953	983	1027	433	454
Lung Cancer	A549	+	-	1512	9611	0	2476	34719	33139	0	0	764	752	943	1345	2547	3209	87047	88786	719	1227	810	1078
	CALU-1	+	-	53834	114947	3528	10080	18438	19072	588	604	921	2119	2993	3444	0	0	94510	114947	3240	3268	1210	1254
	H1650	+	-	3491	15369	1050	5615	127539	134041	1738	1422	263	476	8605	9501	0	0	353964	391949	9642	7584	1455	916
	H226	-	+	49391	145367	10744	24379	73920	101793	640	767	0	672	2378	2758	3006	2629	136158	229665	2143	2477	1202	897
	H441	+	-	13424	34487	359	1782	34363	32832	887	1044	383	829	2762	2540	246	260	59151	73580	2841	3133	3440	3250
	H460	+	-	7193	19574	921	2778	55359	49738	885	1089	0	742	2375	3040	189	615	78046	86814	2342	3040	3792	3223
	HCC827	+	-	9795	60468	3725	8477	41249	47178	1817	1721	879	0	3726	3399	162	0	58497	105562	5176	7123	2222	1917
	NCI-H1299	+	-	278	3436.5	0	92	37817	36029.5	0	0	0	0	2768	3391	2961	4373	196936	184904	3765	3790	909	662
	NCI-H1975	+	-	2483	23446.5	490	4677	70850.5	62007	0	0	368	1729	227	208	535	1455	168919	175547	3665	4409	1160	1412
	NCI-H596	+	-	18669	40780	1275	3245	84320	77592	0	0	0	275	0	0	3410.58	3890	255616	311989	5243	2880	1349	1078
Melanoma	A375	+	-	1255	27782	0	433	52579.5	40340.5	0	0	566	1127	0	0	755	544	30126	37903	3133	2863	1237	1077
	A375-KRAS	+	-	40740	45360.5	1368	6891.5	21853	16451	0	0	1785	2838	0	1852	1682	1837	105114	127213	4220	6126	2120	2878
	A375-KRAS-Luc2	+	-	109294	117180	0	966	12826	13191	735	816	0	60	3526	3450	0	0	128469	160467	4777	5130	1723	1784
	RPMI-7951	+	-	10228.5	26724	2662	8763	65180	80081	0	0	523	1646	0	0	1930	1297	66083	91229	883	1097	1482	1954
	SH-4	+	-	1291	12124	0	0	54015.5	44758.5	0	68	2556	3350	108	2006	1142	760	66235	65168	3429	4481	932	1507
	SK-MEL-24	-	+	400	17538	1000.5	750	26932	17136.5	27	60	236	1187	2903	3177	6613	5316	45197	75332	888	826	2945	2605
Ovarian Cancer	ES-2	+	-	57764	89033	718	5906	11970	11255	405	390	1161	1368	2730	1971	188	0	92087	122142	1453	1620	3210	3510
Pancreas Cancer	AsPC-1	-	+	0	6325	155	2800	28044	26743	297	397	1147	2666	1415	1444	310	546	32180	49052	825	1290	3033	3095
	PANC-1	+	-	1049	0	0	0	20419	21694	421	473	1276	976	2031	2093	331	196	33618	34518	2265	2625	2005	1878
	PANC 10.05	+	-	27818	43052	1359	4174	15027	17384	0	0	996	1402	1802	3716	847	857	40464	48360	2628	4485	1485	2323
Prostate Cancer	PC-3	-	+	18303	47222	346	2725	31886	29497	641	230	203	1704	5474	2108	0	0	91370	122713	2503	0	555	0
	PC-3-Luc2	+	-	20083	30374	0	0	18686	19516	411	497	823	1387	2871	2989	217	0	57153	83352	1924	2850	3223	3412
Skin Cancer	A-431	+	-	13020	37809	1660	6635	64875	61082	996	1792	2656	5120	2623	4203	1369	1757	130495	152286	2297	2824	1078	893
	A-431-Luc2	+	-	2868	41277	688	3235	14291	12967	458	463	446	1021	845	942	0	10	39458	41452	618	709	528	573
Uterian Cancer	HEC-1-A	+	-	0	0	0	0	23302	21501	337	373	418	449	1401	1471	199	136	46400	41305	2300	1860	628	722

ATCC

Tumor Cell Lines – Protein Profiling by FACS

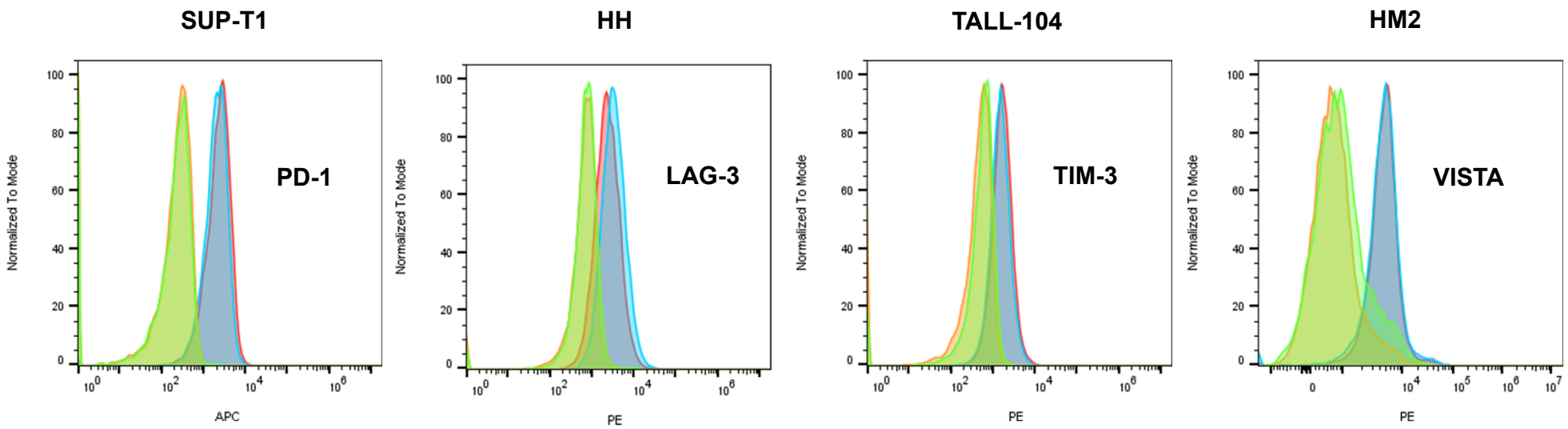


Immune Cell Lines – Protein Profiling by FACS

	HLA typing		Checkpoint inhibitory molecules Receptors (mainly expressed by T cells)							Checkpoint co-stimulatory molecules Receptors (mainly expressed by T cells)							Immune cell marker	
Cell Lines	HLA-A, B, C	HLA-DP, DQ, DR	PD-1	CTLA4	LAG-3	TIM-3	BTLA	VISTA	TIGIT	4-1BB	ICOS	CD30	CD28	OX40	GITR	CD226	CD4	CD8
Jurkat E6-1	+	-	45	0	71	43	202	2406	17	0	77	3463	0	0	156	11054	275	14
TALL-104	+	-	75	2	159	1090	301	1051	0	30	501	36	14507	319	243	99	58	85358
MOLT-3	+	-	230	71	107	42	191	377	32	0	929	672	4353	273	303	149	143	617
HH	+	+	243	24	1046	749	606	3878	1995	42	68	214676	512	1368	610	26814	29347	121
Hut 78	+	+	231	20	416	267	1114	2884	88	240	1014	13216	431	3661	9674	901	7852	397
SUP-T1	+	-	2076	219	81	20	487	1339	18	0	54	1	15430	876	32	656	29250	81122
HM2	+	-	361	46	120	0	464	4075	221	0	518	531	56	854	322	736	229	5412
MJ(G11)	+	+	272	91	348	281	2740	1607	4727	501	9072	51092	0	15528	37952	2987	21023	101
CCRF-CEM	+	-	108	13	81	111	222	119	53	7	347	567	5884	163	479	342	9641	6306
Primary CD8+ T cells	+	-	812	98	274	10745	623	1378	88	57	1567	71	607	119	720	4268	0	223247
Primary CD4+ T cells	+	-	921	106	35	1381	756	1029	32	43	2252	862	6477	380	1040	5041	7916	21

ATCC

Immune Cell Lines – Protein Profiling by FACS



Immune Cell Lines – RNAseq Profiling, CCLE Expression 22Q1 Public

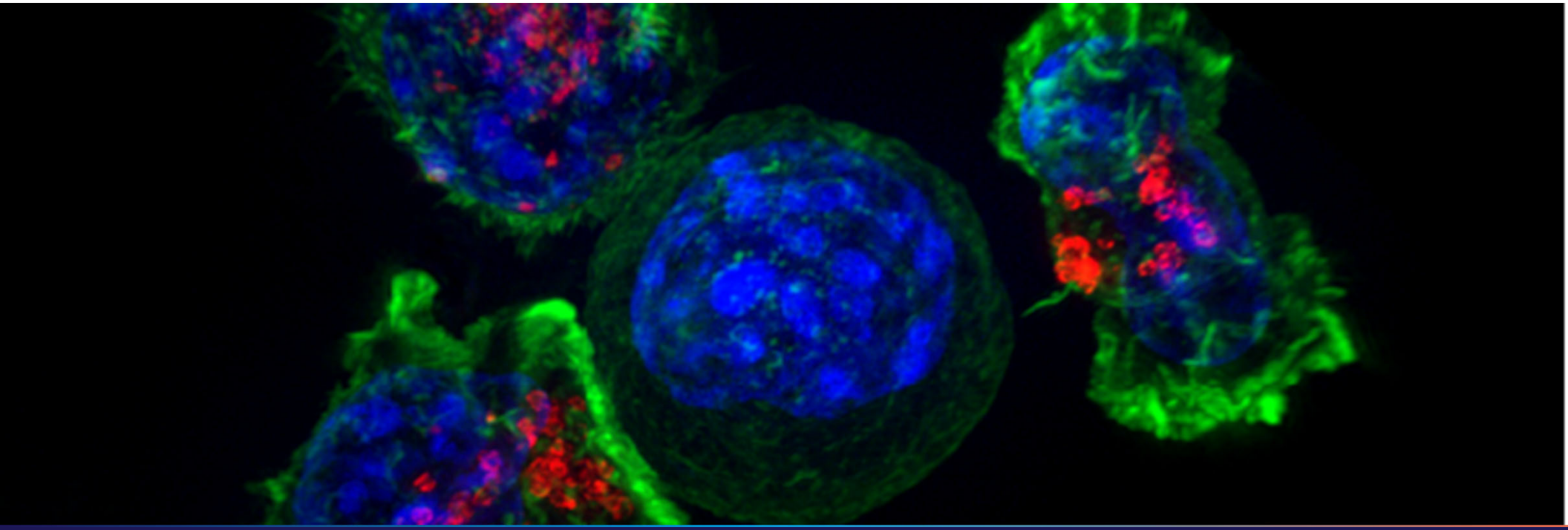
vs.. ATCC JURKAT Clone E6-1

Depmap ID	Cell Line Name	Primary Disease	Lineage	Lineage Subtype	HLA-A	HLA-B	HLA-C	HLA-DPA1	HLA-DPB1	HLA-DQA1	HLA-DQB1	HLA-DRA	HLA-DRB1
ACH-000995	JURKAT	Leukemia	Blood	ALL	8.058912	8.030281	7.862017	0.505891	0.344828	0.495695	2.495695	0.536053	1.655352
Not in CCLE	TALL-104												
ACH-000964	MOLT3	Leukemia	Blood	ALL	8.553744	7.295631	7.458940	0.298658	0.097611	0.124328	0.028569	0.505891	0.214125
ACH-000061	HH	Lymphoma	Lymphocyte	Non Hodgkin Lymphoma	9.954545	11.320575	9.395041	8.428904	8.307292	8.269594	8.348905	9.473036	8.979854
ACH-000509	HUT78	Lymphoma	Lymphocyte	Non Hodgkin Lymphoma	8.630304	9.389007	7.058316	7.738430	6.146492	6.785289	5.242603	8.722159	6.956638
ACH-000953	SUPT1	Leukemia	Blood	ALL	6.669310	4.892877	7.060696	0.925999	0.310340	0.084064	0.137504	1.269033	0.443607
Not in CCLE	HM2												
ACH-000077	MJ	Lymphoma	Lymphocyte	Non Hodgkin Lymphoma	10.667821	12.210458	9.767853	9.383510	8.652558	8.528571	5.261907	9.940901	8.963272
Not in CCLE	CCRF-CEM												

PDCD1	CTLA4	LAG3	HAVCR2(TIM3)	BTLA	VSIR(VISTA)	TIGIT	TNFRSF9(4-1BB)	ICOS	TNFRSF8(CD30)	CD28	TNFRSF4(OX40)	TNFRSF18(GITR)	CD226	CD4	CD8A
1.007196	0.014355	0.298658	0.526069	0.594549	2.533563	0.014355	0.014355	0.516015	1.735522	0.485427	0.485427	0.000000	0.432959	2.121015	1.550901
1.748461	0.367371	1.400538	0.545968	0.111031	3.063503	0.056584	0.014355	1.321928	1.819668	3.480265	5.216455	0.411426	0.286881	1.367371	2.039138
0.056584	0.111031	4.532317	0.084064	0.310340	0.124328	5.159064	0.367371	0.014355	9.074489	0.176323	0.097611	0.042644	4.235727	7.587815	0.042644
0.443607	1.378512	3.008989	0.731183	0.176323	3.478972	0.526069	1.427606	3.738768	5.506208	0.137504	2.972693	4.167519	1.555816	5.837691	1.765535
3.320485	0.111031	0.226509	0.137504	0.028569	0.226509	0.028569	0.056584	0.731183	0.070389	5.176323	0.739848	0.070389	0.422233	6.942045	6.602439
0.056584	3.314697	0.084064	0.097611	0.028569	0.545968	6.095713	3.364572	6.504938	7.551362	0.014355	6.657211	6.397632	4.207112	7.196430	0.056584

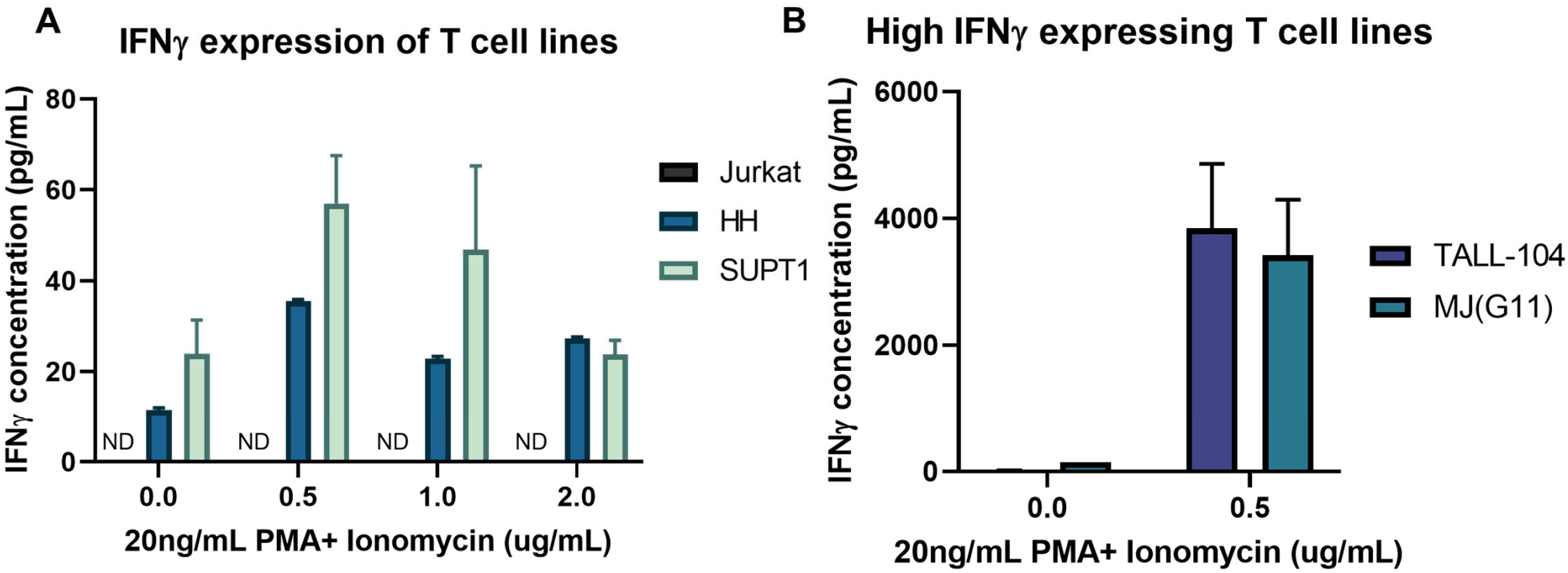
***Green denotes CCLE RNAseq profiling data different from ATCC internal protein profiling data

ATCC

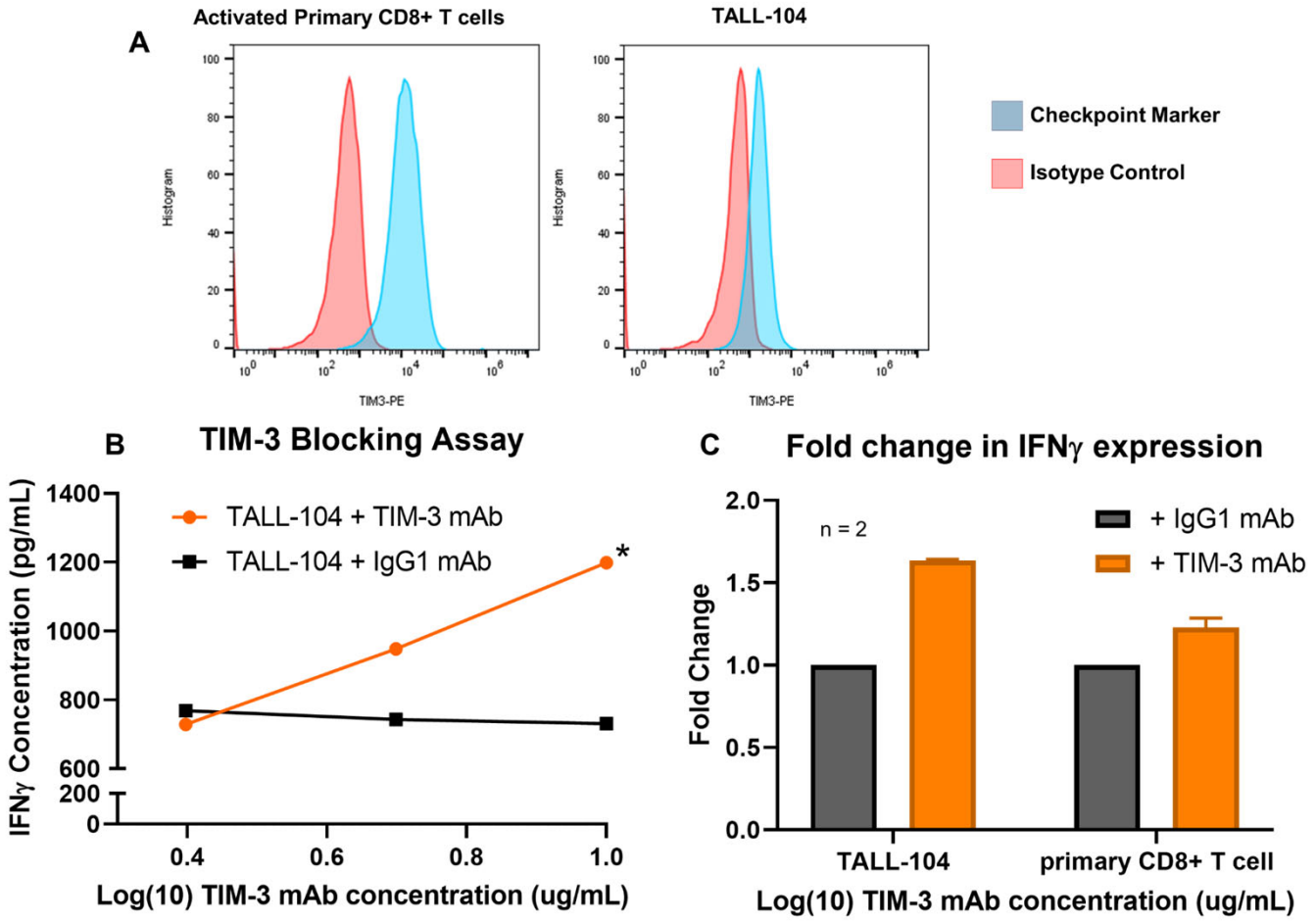


ATCC Internal Application Data

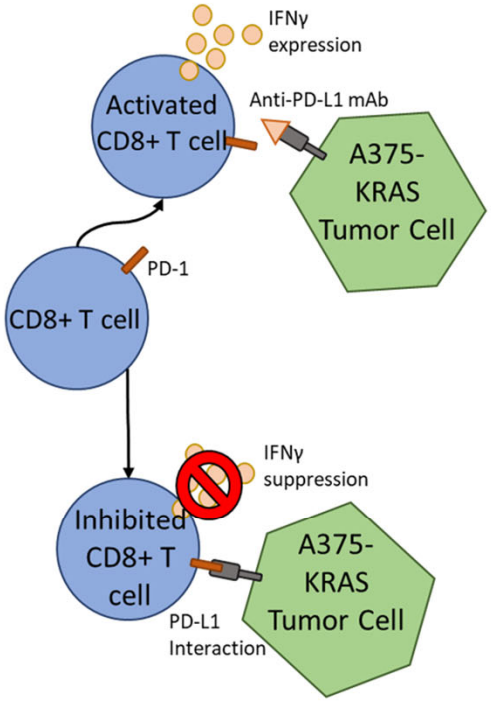
IFNγ Expression Levels of T Cell Lines with High Expression of Checkpoint Receptors

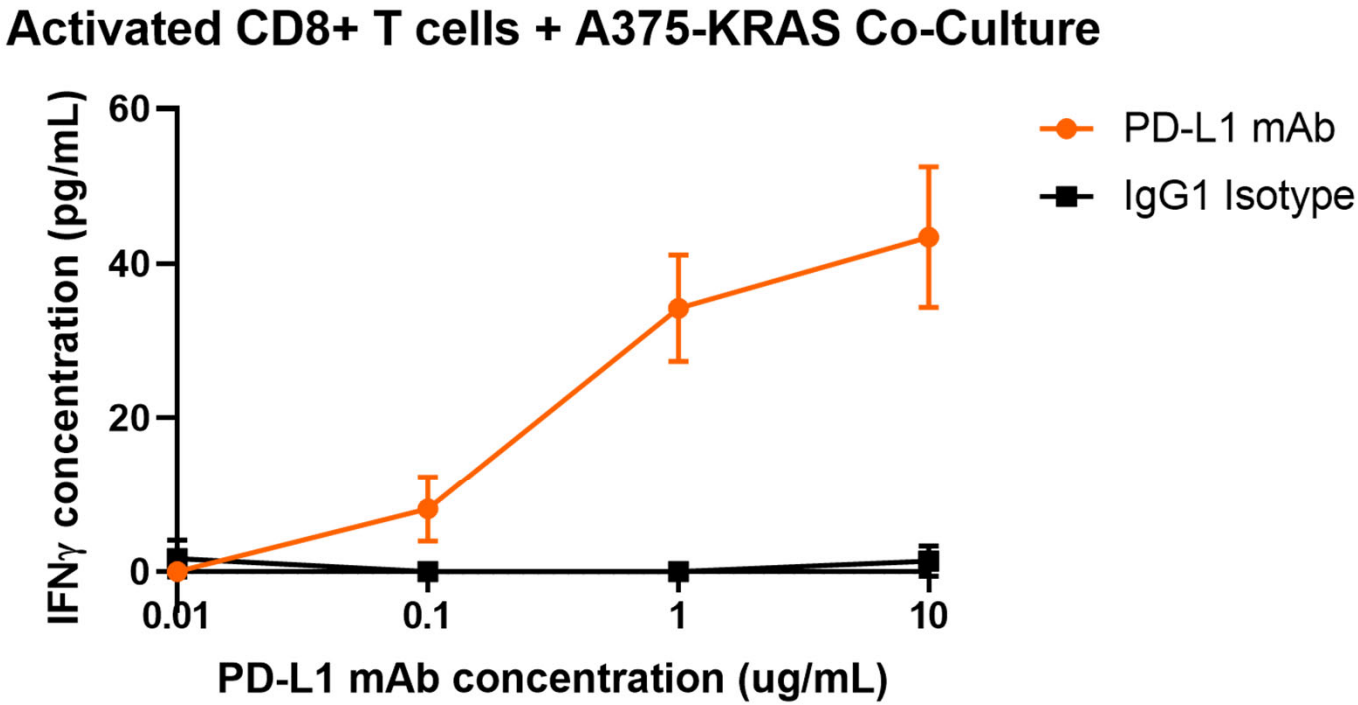


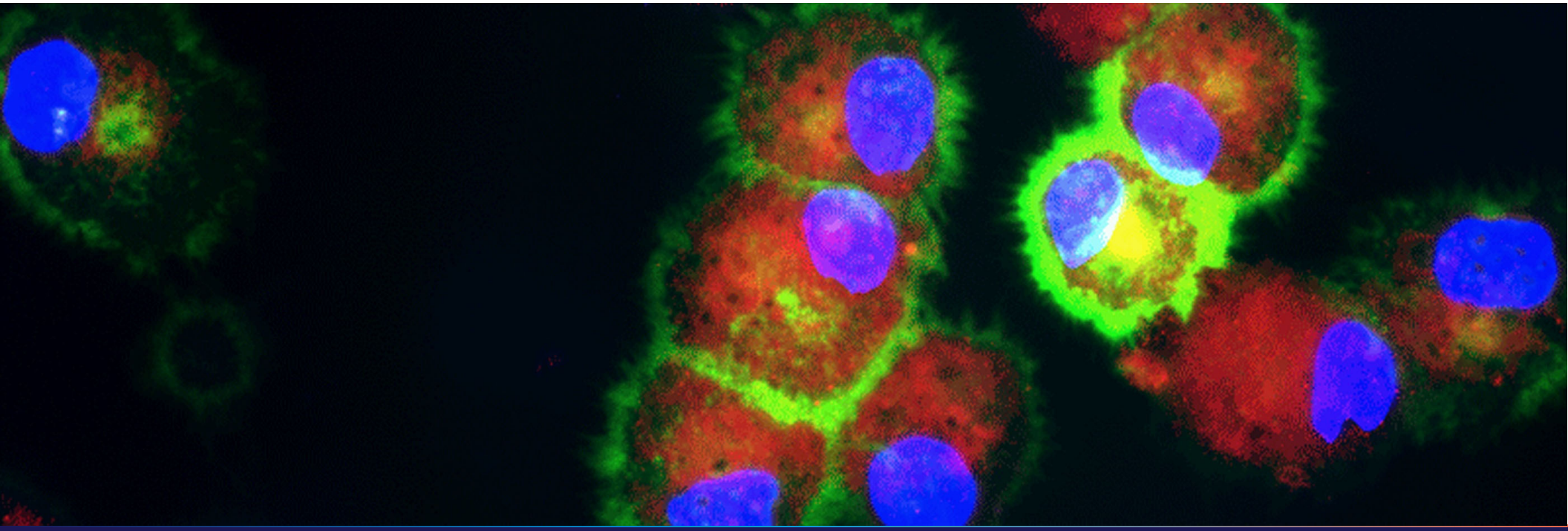
TIM-3 Checkpoint Molecule Blocking Assay with High Expression T Cells



Primary T Cell and Checkpoint High Expressing Tumor Cell Co-culture System for PD-L1 Blockade Screening



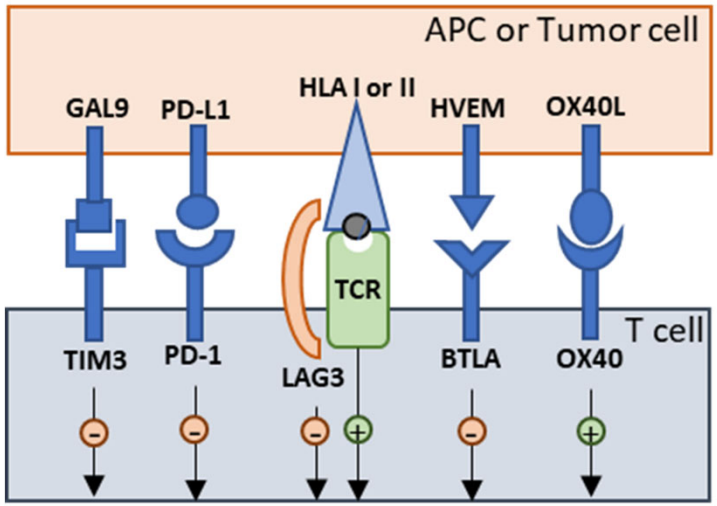




Summary

Summary

- Immune checkpoint blockades are a promising method for treating cancer.
- Current evaluation methods are expensive or not physiologically relevant.
- ATCC has complied a comprehensive data set of checkpoint molecule expression levels of human tumor cell lines and immune cells.
- These cells can be incorporated into simple blocking assays or be integrated into co-culture testing systems.
- ATCC provides a relevant and accessible model system for studying checkpoint molecule interactions and screening biologics as cancer immunotherapy treatments.



Versatility: ATCC provides you the tools; You design the studies.

ATCC

Thank you and questions

Coming attractions:

Tips and Techniques for Propagating your Viral Strains
May 12 at 12:00 PM EST
Adria Allen, MS, Alexander Piccirillo, MS, and Megan Yockey, BS

Genomic Data Quality: Connecting the Dots Between Bioinformatics and Physical Materials
May 19 at 12:00 PM EST
Jonathan Jacobs, PhD

Tips and Techniques for Successfully Culturing Organoids
May 26 at 12:00 PM EST
Steven Budd, MS, MBA, and James Clinton, PhD

For more ATCC immunological resources visit:

www.atcc.org/immuno-oncology