Poster #496

GAS-Luc2 Reporter Cell Lines for Immune Checkpoint Drug Screening in Solid Tumors

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Abstract

Cancer immunotherapies that target immune checkpoints, such as immune checkpoint inhibitors (ICIs), antibody-dependent cellular cytotoxicity (ADCC), and antibody-drug conjugates (ADCs), have shown tremendous success in the treatment of solid tumors, including skin, lung, breast, renal, and liver cancers. However, the built-in complexity of immunological models and the variable drug responses among different cancer types have challenged the development and application of these novel immunotherapies. To facilitate large-scale drug discovery for this growing class of immunomodulators, we conducted a comprehensive cell surface protein profiling of ATCC®'s vast portfolio of human tumor and immune cell lines for established and novel immune checkpoint molecules as well as their binding ligands. Based on this protein profiling data, we generated the three immune checkpoint reporter cell lines HCC827-GAS-Luc2 (ATCC® CRL-2868-GAS-LUC2™), MG-63-GAS-Luc2 (ATCC® CRL-1427-GAS-LUC2™), and NCI-H1650-GAS-Luc2 (ATCC® CRL-5883-GAS-LUC2™), which endogenously express high levels of programmed death-ligand 1 (PD-L1), cluster of differentiation 155 (CD155), and B7 homolog 3 protein (B7-H3/CD276), respectively. These reporter cell lines were engineered to contain a gamma interferon activation site (GAS)–response element upstream of a luciferase gene. The luciferase expression is suppressed when the relevant immune checkpoint marker on the cancer cells binds to the corresponding checkpoint protein on T cells. In the presence of a relevant immune checkpoint inhibitor, the GAS-Luc2 reporter cell senses the IFNγ from the activated T cells to produce a luciferase expression–based bioluminescent signal. This signal can be readily detected and quantified to evaluate the efficacy, potency, and dynamics of the checkpoint inhibitor. In addition to drug screening for immune checkpoint inhibitors, these GAS-Luc2 reporter tumor cell lines have also been demonstrated to be effective in detecting paracrine IFNγ signaling for immune checkpoint-targeted ADCC drug development.

Background

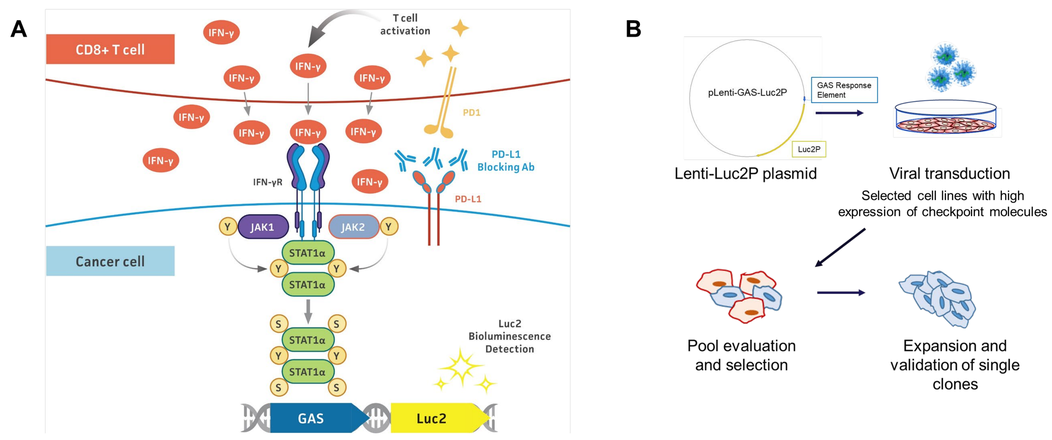

Figure 1: Schematics of the immune checkpoint molecule-expressing GAS-Luc2 reporter system. (A) Disruption of immune checkpoint binding, such as PD-1/PD-L1 recognition, by a blocking antibody activates CD8+ T cells, which then release IFN-γ. IFN-γ activates JAK-STAT signaling in cancer reporter cells, promoting GAS-induced transcription of the luciferase gene, producing an easily detectable bioluminescence signal. Created with BioRender.com. (B) Selected cell lines with high endogenous expression of PD-L1, CD155, or B7-H3 were transduced with lentiviral-GAS-Luc2 plasmids in the presence of 50 µg/mL protamine sulfate (Sigma) for 24 hours. The cells were then enriched by puromycin selection and single cells were isolated by automatic cell sorting (Sony SH800). Expanded single cell clones were evaluated by IFN-γ stimulation. The clones that yielded the highest luciferase signal upon IFN-γ stimulation were selected for future experiments.

Results

*-": without IFNγ *+": with IFNγ			HLA typing		Inhibitory checkpoint molecule ligands								Co-stimulatory checkpoint molecule ligands								
Cancer type	Cell lines	ATCC® catalog #	HLA class I	HLA class II	PD-L1-	PD-L1+	PD-L2-	PD-L2+	B7-H3-	B7-H3+	B7-H4-	B7-H4+	HVEM-	HVEM+	4-1BBL-	4-1BBL+	ICOS-L-	ICOS-L+	CD155-	CD155+	CD80-	CD80+	CD86-	CD86+
Bladder cancer	S637	HTB-9™	+	-	52096	143325	49	2594	60004	52945	0	0	1593	1783	3085	2831	1322	1464	68780	85293	2092	3069	1909	1993
	HT-1197	CRL-1473™	+	-	40740	45361	1368	68911.5	21853	16451	0	0	1785	2838	0	1852	1682	1837	105114	127213	4220	6126	2120	2878
	HT-1376	CRL-1472™	+	-	27135	51493	1692	8578	74668	66185	0	0	365	1790	0	0	3440	6322	36478	44828	4293	4179	1233	1707
	RT4	HTB-2™	+	-	0	5054	52	518	143148	139442	0	42	717	1602	2395	2961.5	5676	7754	40953	48452	883	1097	1482	1954
	TCCSUP	HTB-5™	-	+	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)	CRL-2271™	+	-	745	6837	0	258	15903	17884	156	123	262	217	626	528	228	240	5236	6395	452	350	923	778
	U-87 MG	HTB-14™	+	-	321	2990	249	246	73474	72722	338	263	4718	3312	2804	3010	339	454	30877	33809	2926	2597	2080	1968
	U-87 MG-Luc2	HTB-14-LUC2™	+	-	15061	40367	0	0	29967	29009	1508	1374	487	706	1717	1370	141	219	36063	43417	1851	1491	984	753
Breast cancer	AU565	CRL-2351™	+	-	2428	11013	0	0	9476	8169	3514	2925	307	831	1289	841	633	856	37017	35953	983	1027	431	454
	BT-20	HTB-19™	+	-	6082	17072	886	4614	44830	44507	711	761	0	0	7297	8831	300	136	203815	235198	8916	9398	1172	1244
	DU4475	HTB-123™	+	-	1912	3232	1082	3774	59238	54996	1941	1317	4014	4293	8298	6525	0	0	36382	32343	8865	6426	2523	1278
	HCC38	CRL-2314™	+	-	13009	126059	3097	16705	220234	208819	2300	1565	6396	7267	1912	3050	1525	1855	132767	134741	5751	4437	2143	1906
	MCF7	HTB-22™	+	-	53	1802	0	0	46613	42793	4324	2944	2197	1972	4821	4165	1583	2402	23280	22977	5720	4584	2867	2424
	MCF7-Luc2	HTB-22-LUC2™	+	-	0	3116	0	2793	56518	53829	575	936	1331	1723	3902	5935	465	1037	20258	22678	1724	5297	1215	2149
	MDA-MB-231	HTB-26™	+	-	11359	20492	986	1880	12979	11668	149	125	456	1031	531	777	14	37	38583	53188	563	428	346	234
	MDA-MB-468	HTB-132™	+	-	221	5046	115	380	16180	16342	806	575	140	438	740	769	401	747	36560	43422	475	464	308	290
	T-47D	HTB-133™	+	-	72	6355	0	0	32581	24851	828	594	597	703	3140	1990	859	683	39364	37651	3038	2166	1620	1325
Bone cancer	HOS	CRL-1543™	-	+	13031	41473	2927	9075	60530	61277	289	305	211	552	1127	1210	0	0	99713	124829	841	815	443	400
	MG-63	CRL-1427™	+	-	0	7362	0	0	84745	79181	443	819	368	730	4326	4901	0	0	303805	268365	2894	6552	1339	2968
	Saos-2	HTB-85™	+	-	6082	32705	0	0	7455	7136	332	329	897	1244	2525	1975	0	0	58992	70813	1726	1733	1644	1525
	U-2 OS	HTB-96™	+	-	5929	36019	290	5915	63080	64082	548	333	830	1152	2321	2660	784	778	112962	124648	2554	1124	3008	3045
Colon cancer	Caco-2 [Caco2]	HTB-37™	+	-	0	471	0	0	32201	30175	1315	1209	1900	1817	4255	5817	1060	661	44423	39942	6756	4849	4146	3170
	HCT-15	CCL-225™	-	+	474	3790	35	0	12896	12520	137	94	513	947	369	251	0	21	33045	34475	411	140	441	335
	LoVo	CCL-229™	-	+	468	17697	0	0	20338	19572	347	346	975	2481	1581	1647	775	1080	24870	36144	903	1271	1044	1010
Head & Neck cancer	A-253	HTB-41™	+	-	2070	16019	123	3176	43926	41341	18	0	46	477	1431	2558	3380	3887	67935	83057	3303	3051	721	985
	FaDu	HTB-43™	+	-	2733	37007	205	13372	39475	31090	0	0	138	855	1640	0	3643	4161	60462	62858	2728	2720	1904	1951
	FaDu-Luc2	HTB-43-LUC2™	+	-	6965	29601	0	0	24921	20048	269	333	421	448	1159	1591	484	557	35527	40460	1019	1334	2147	2183
Liver cancer	C3A [HepG2/C3A]	CRL-10741™	+	-	0	2114	0	2698	18098	16938	441	453	1362	2682	1243	2171	394	511	54751	59271	1729	1914	1136	1100
	SK-HEP-1	HTB-52™	-	+	2428	11013	0	0	9476	8169	3514	2925	307	831	1289	841	633	856	37017	35953	983	1027	431	454
Lung cancer	A549	CCL-185™	+	-	1512	9611	0	2476	34719	33139	0	0	764	752	943	1345	2547	3209	87047	88786	719	1227	810	1078
	Calu-1	HTB-54™	+	-	53834	114947	3528	10080	18438	19072	588	604	921	2119	2993	3444	0	0	94510	114947	3240	3268	1210	1254
	NCI-H1650 [H-1650, H1650]	CRL-5883™	+	-	3491	15369	1050	5615	127539	134041	1738	1422	263	476	8605	9501	0	0	153954	191949	9642	7584	1455	916
	NCI-H226 [H226]	CRL-5826™	-	+	49391	145367	10764	24379	73926	101793	640	767	0	672	2378	2758	3006	2629	136158	229665	2143	2477	1220	897
	NCI-H441 [H441]	HTB-174™	+	-	13424	34487	359	1782	34363	32832	887	1044	383	829	2762	2540	246	260	59151	73580	2841	3133	3440	3250
	NCI-H460 [H460]	HTB-177™	+	-	7193	19574	921	2778	55359	49738	885	1089	0	742	2375	3040	189	615	78046	86814	2342	3040	3792	3223
	HCC827	CRL-2868™	+	-	9795	60468	3725	8477	41249	47178	1817	1721	879	0	3726	3399	162	0	58497	105562	5176	7123	2222	1917
	NCI-H1299	CRL-5803™	+	-	278	3436.5	0	92	37817	36030	0	0	0	0	2768	3391	2961	4373	196936	184904	3765	3790	909	662
	NCI-H1975 [H-1975, H1975]	CRL-5908™	+	-	2483	23447	490	4677	70851	62007	0	0	368	1729	227	208	535	1455	168919	175547	3665	4409	1160	1412
	NCI-H596 [H596]	HTB-178™	+	-	18669	40780	1275	3245	84320	77592	0	0	6	275	0	0	3410.6	3890	255616	311989	5243	2880	1349	1078
Melanoma	A-375 [A375]	CRL-1619™	+	-	1255	27782	0	433	52580	40341	0	0	566	1127	0	0	755	544	30126	37903	3133	2863	1237	1077
	A375-KRAS	CRL-1619G-1™	+	-	40740	45361	1368	68911.5	21853	16451	0	0	1785	2838	0	1852	1682	1837	105114	127213	4220	6126	2120	2878
	A375-KRAS-Luc2	RL-1619G-1-LUC2	+	-	109294	117180	0	966	12826	13191	735	816	0	60	3526	3450	0	0	128469	160467	4777	5130	1723	1784
	RPMI-7951	HTB-66™	+	-	10229	26724	2662	8763	65180	80081	0	0	523	1646	0	0	1930	1297	66083	91229	883	1097	1482	1954
	SH-4	CRL-7724™	+	-	1291	12124	0	0	54016	44759	0	68	2556	3350	108	2006	1142	760	66235	65168	3429	4483	1078	1507
	SK-MEL-24	HTB-71™	+	-	400	17538	1000.5	750	26932	17137	27	60	236	1187	2903	3177	6613	5316	45197	75332	888	826	2945	2605
Ovarian cancer	ES-2	CRL-1978™	+	-	57764	89033	718	5906	11970	11255	405	390	1161	1368	2730	1971	188	0	92087	122142	1453	1620	3210	3510
Pancreas cancer	AsPC-1	CRL-1682™	-	+	0	6325	155	2800	28044	26743	297	397	1147	2666	1415	1444	310	546	32180	49052	825	1290	3033	3095
	PANC-1	CRL-1469™	+	-	1049	0	0	0	20419	21694	421	473	1276	976	2031	2093	331	196	33618	34518	2265	2625	2005	1878
	PANC 10.05	CRL-2547™	+	-	27818	43052	1359	4174	15027	17384	0	0	996	1402	1802	3716	847	857	40464	48360	2628	4485	1485	2323
Prostate cancer	PC-3	CRL-1435™	-	+	18303	47222	346	2725	31886	29497	641	230	203	1704	5474	2108	0	0	91370	122713	2503	0	555	0
	PC-3-Luc2	CRL-1435-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	CRL-1555™	+	-	13020	37809	1660	6635	64875	61082	996	1792	2656	5120	2623	4203	1369	1757	130495	152286	2272	2824	1078	893
	A-431-Luc2	CRL-1555-LUC2™	+	-	2868	41277	688	3235	14291	12967	458	463	446	1021	845	942	0	10	39458	41452	618	709	528	573
Uterine cancer	HEC-1-A	HTB-112™	+	-	0	0	0	0	23302	21501	337	373	418	449	1401	1471	199	136	46400	41305	2300	1860	628	722

Figure 2: Heat map based on protein profiling data of selected cancer cell lines for immune checkpoint molecule expression by flow cytometry. Immune checkpoint molecule ligand expression levels in cancer cell lines under basal (-) and 100 ng/mL IFN-γ stimulated (+) conditions were profiled. HLA class was defined by either low expression (-) or high expression (+). Table values represent median fluorescence intensity sample values subtracted by isotype control MFI. Each column was color-coded separately to avoid cross comparison.

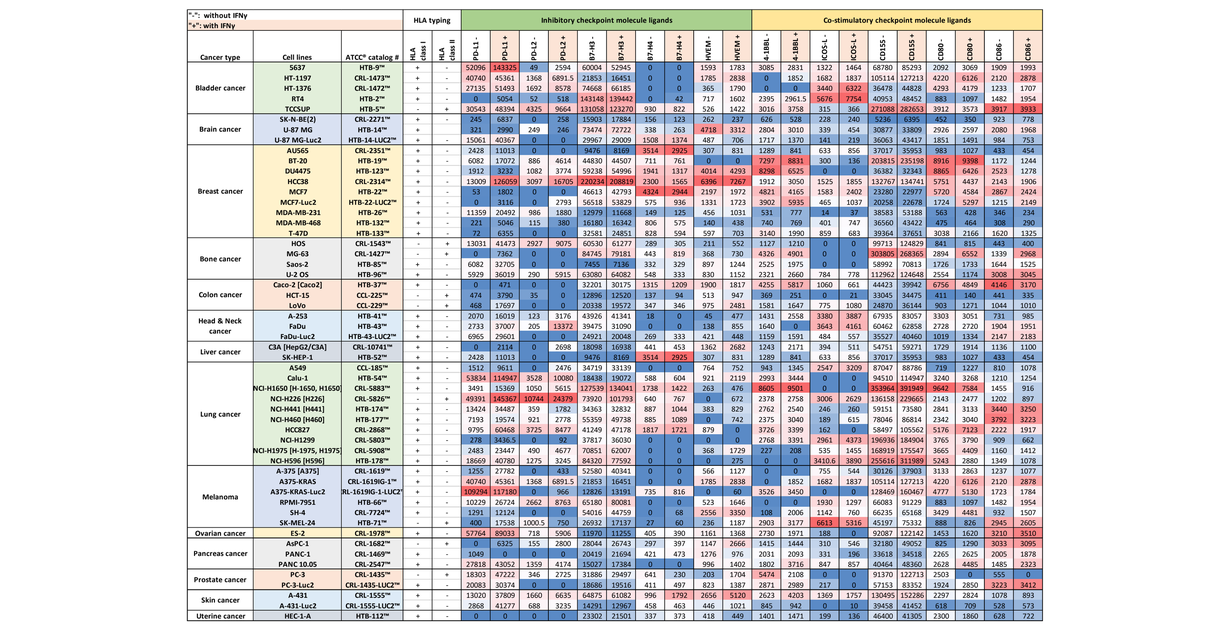

Figure 3: Cytokine stimulation of monoclonal GAS-Luc2 cell lines with IFNγ or CD8+ cytotoxic T cell-conditioned media. (A-C) The luminescence intensity of the (A) HCC827-GAS-Luc2, (B) MG-63-GAS-Luc2, or (C) NCI-H1650-GAS-Luc2 cell line upon IFNγ stimulation (0.01-1,000 ng/mL). (D-F) The luminescence intensity of the (D) HCC827-GAS-Luc2, (E) MG-63-GAS-Luc2, or (F) NCI-H1650-GAS-Luc2 cell line upon stimulation with conditioned media collected from non-activated or activated CD8+ cytotoxic T cells. N=3 in all experiments. *P < 0.05.

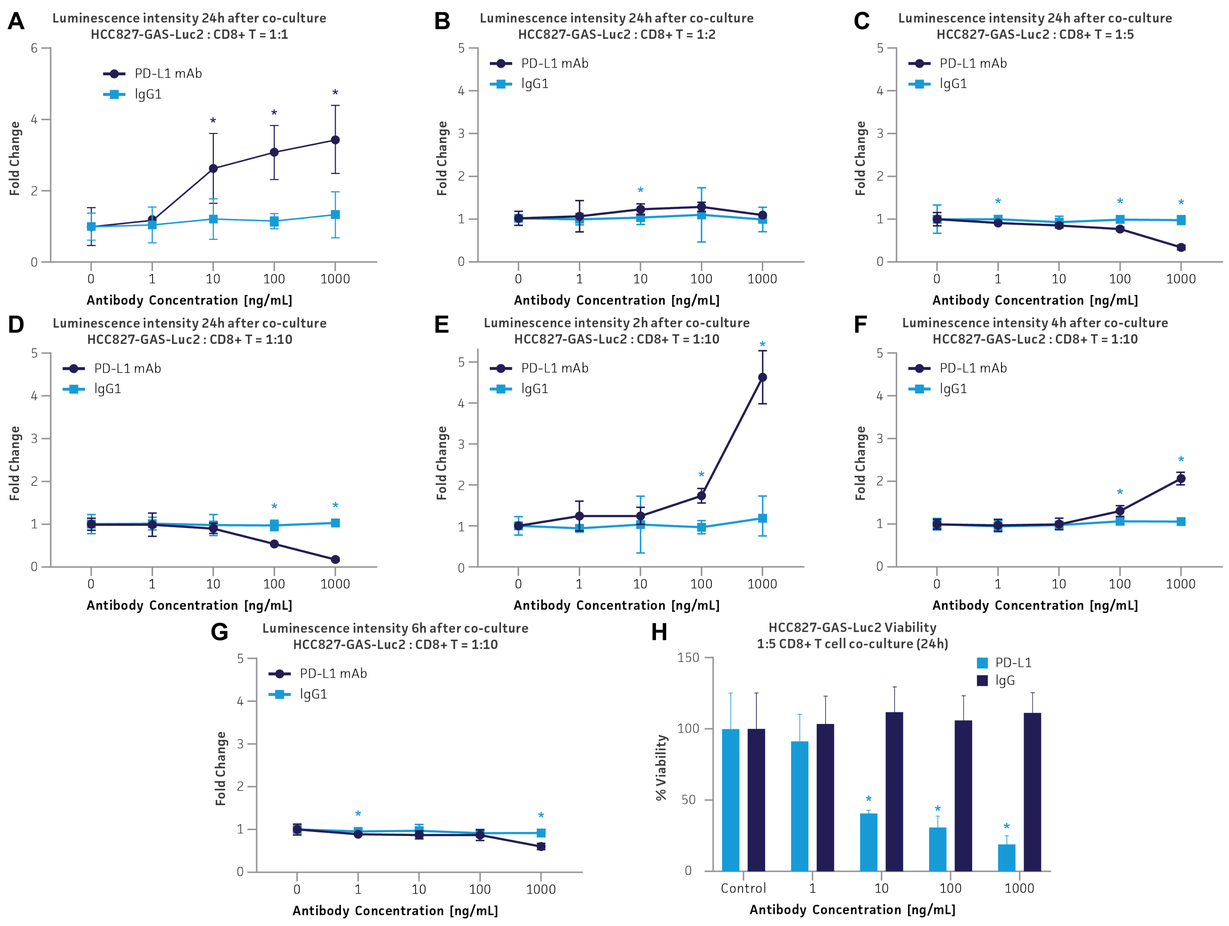

Figure 4: Co-culture of monoclonal HCC827-GAS-Luc2 with primary human CD8+ cytotoxic T cells at varying cell ratios and co-culture durations in the presence of a PD-L1 blocking antibody. (A-D) The luminescence intensity from HCC827-GAS-Luc2 cells after 24-hour co-culture with CD8+ cytotoxic T cells at a (A) 1:1, (B) 1:2, (C) 1:5, and (D) 1:10 ratio of target cells to effector cells. (E-G) The luminescence intensity from HCC827-GAS-Luc2 cells after co-culture at a 1:10 ratio with CD8+ cytotoxic T cells for periods of (E) 2 hours, (F) 4 hours, and (G) 6 hours. (H) The viability of HCC827-GAS-Luc2 after 24 hours of co-culture with a 1:5 ratio with CD8+ cytotoxic T cells for 24 hours. During the co-culture, the cells were administered with either PD-L1 mAb or isotype control IgG (1-1,000 ng/mL). N=3 in all experiments. *P < 0.05.

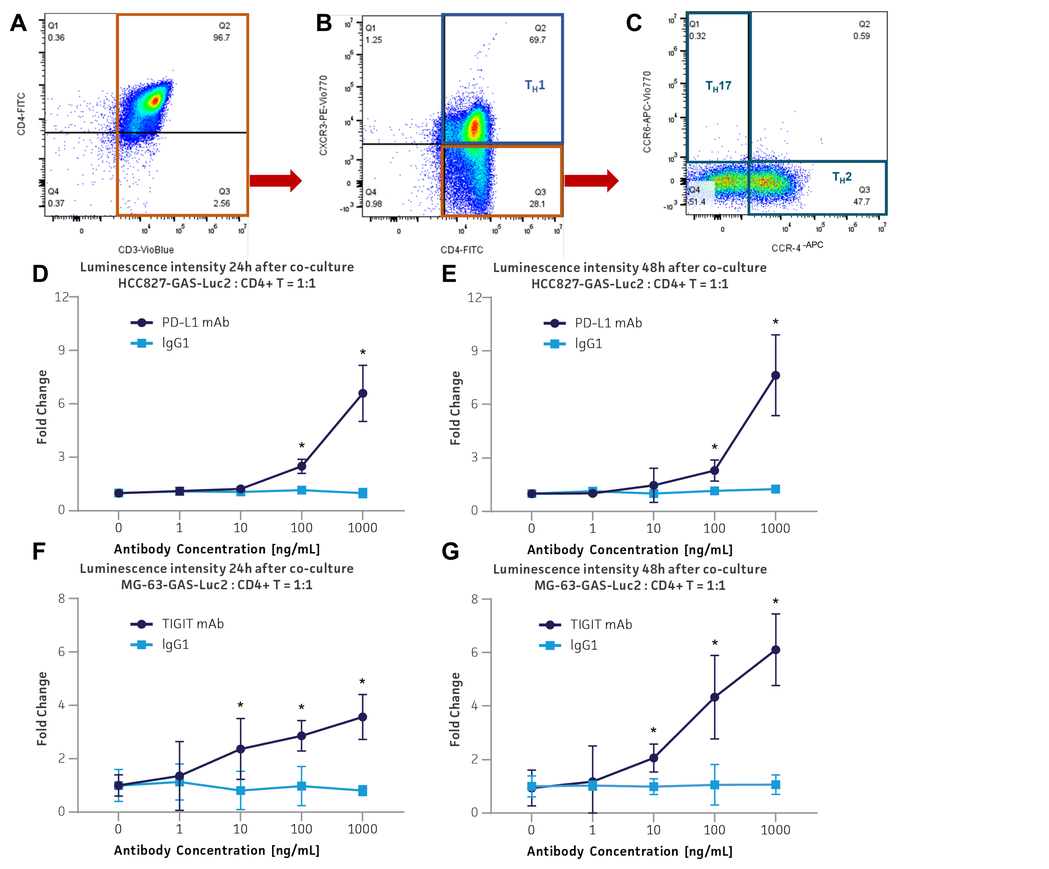

Figure 5: Co-culture of monoclonal HCC827-GAS-Luc2 or MG-63-GAS-Luc2 cells with primary human CD4+ helper T cells in the presence of a respective blocking antibody after CD4+ helper T cell subset phenotyping. (A-C) The flow cytometry analysis of helper T cell subsets T_H1, T_H17, and T_H2. (D-E) The luminescence intensity from HCC827-GAS-Luc2 cells after co-culture at a 1:1 ratio with CD4+ helper T cells for (D) 24 hours or (E) 48 hours in the presence of a PD-L1 mAb or isotype control IgG1 (1-1,000 ng/mL). (F-G) The luminescence intensity from MG-63-GAS-Luc2 cells after co-culture at a 1:1 ratio with CD4+ helper T cells for (F) 24 hours or (G) 48 hours in the presence of a TIGIT mAb or isotype control IgG (1-1,000 ng/mL). N=3 in all experiments. *P < 0.05.

Figure 6: Co-culture of monoclonal NCI-H1650-GAS-Luc2 with primary human CD56+ NK cells in the presence of a B7-H3 ADCC antibody. (A-B) The luminescence intensity from NCI-H1650-GAS-Luc2 after co-culture with CD56+ NK cells at a 2:1 ratio of target to effector cells for (A) 24 hours or (B) 48 hours. During the co-culture, the cells were administered with either B7-H3 mAb or isotype control IgG (1-1,000 ng/mL). N=3 in all experiments. *P < 0.05.

Conclusion

- The expansive protein profiling of cancer cell lines for numerous immune checkpoint molecules and their ligands provides crucial information that facilitates immune checkpoint molecule interaction studies, checkpoint assay development, and cancer immunotherapy screening.
- Based on the protein profiling data, we developed three cancer reporter cell lines with a high endogenous expression of PD-L1, CD155, or B7-H3. The naturally high expression of the immune checkpoint molecules on the surface of the reporter cells facilitates the immune checkpoint inhibitor drug candidate binding, promoting the immune cell activation and subsequent release of IFN-γ by the immune cells, resulting in the intracellular IFN-γ-IFN-γR JAK-STAT GAS signaling activation and luciferase expression by the reporter cells.
- The reporter cell lines present robust, responsive, and reproducible luciferase expression upon signaling activation that enables reliable measurement of the potency and stability of the relevant immune checkpoint inhibitors that trigger immune/tumor microenvironmental cell-mediated immune responses.
- By maintaining physiological relevance and stable expression of the checkpoint ligand owing to the endogenous expression, these reporter cell lines effectively eliminate the donor variability issue commonly experienced by using primary cell models.

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