

## REFERENCES

- Abbas, A.K., Lichtman, A.H., Pillai, S. (2010). Cellular and Molecular Immunology (6 th ed.). Philadelphia: Saunders.
- Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Frontiers In Pharmacology*, 8, 561.
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*, 68(6), 394-424.
- Chae, Y. K., Arya, A., Iams, W., Cruz, M. R., Chandra, S., Choi, J., & Giles, F. (2018). Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). *Journal for Immunotherapy of cancer*, 6(1), 39.
- Chen, L., & Han, X. (2015a). Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. *The Journal of Clinical Investigation*, 125(9), 3384-3391.
- Chen, N., Fang, W., Zhan, J., Hong, S., Tang, Y., Kang, S., ... & Huang, Y. (2015b). Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *Journal of Thoracic Oncology*, 10(6), 910-923.
- Gao, Y., Yang, J., Cai, Y., Fu, S., Zhang, N., Fu, X., & Li, L. (2018). IFN- $\gamma$ -mediated inhibition of lung cancer correlates with PD-L1 expression and is regulated by PI3K-AKT signaling. *International Journal of Cancer*, 143(4), 931-943.
- Hastings, K., Yu, H. A., Wei, W., Sanchez-Vega, F., DeVeaux, M., Choi, J., ... & Liu, Z. (2019). EGFR mutation subtypes and response to immune checkpoint blockade treatment in non-small-cell lung cancer. *Annals of Oncology*, 30(8), 1311-1320.

Heydt, C., Michels, S., Thress, K. S., Bergner, S., Wolf, J., & Buettner, R. (2018). Novel approaches against epidermal growth factor receptor tyrosine kinase inhibitor resistance. *Oncotarget*, 9(20), 15418.

Hsu, P. C., Jablons, D. M., Yang, C. T., & You, L. (2019). Epidermal growth factor receptor (EGFR) pathway, yes-associated protein (YAP) and the regulation of programmed death-ligand 1 (PD-L1) in non-small cell lung cancer (NSCLC). *International Journal of Molecular Sciences*, 20(15), 3821.

Ikeda, S., Okamoto, T., Okano, S., Umemoto, Y., Tagawa, T., Morodomi, Y., ... & Fujishita, T. (2016). PD-L1 is upregulated by simultaneous amplification of the PD-L1 and JAK2 genes in non–small cell lung cancer. *Journal of Thoracic Oncology*, 11(1), 62-71.

Im, J. S., Herrmann, A. C., Bernatchez, C., Haymaker, C., Molldrem, J. J., Hong, W. K., & Perez-Soler, R. (2016). Immune-modulation by epidermal growth factor receptor inhibitors: implication on anti-tumor immunity in lung cancer. *PLoS One*, 11(7), e0160004.

Inaguma, S., Wang, Z., Lasota, J., Sarlomo-Rikala, M., McCue, P. A., Ikeda, H., & Miettinen, M. (2016). Comprehensive immunohistochemical study of programmed cell death ligand 1 (PD-L1). Analysis in 5536 cases revealed consistent expression in trophoblastic tumors. *The American Journal of Surgical Pathology*, 40(8), 1133.

Lee, C. K., Man, J., Lord, S., Cooper, W., Links, M., Gebski, V., ... & Yang, J. C. H. (2018). Clinical and molecular characteristics associated with survival among patients treated with checkpoint inhibitors for advanced non–small cell lung carcinoma: a systematic review and meta-analysis. *JAMA Oncology*, 4(2), 210-216.

Lin, Y., Wang, X., & Jin, H. (2014). EGFR-TKI resistance in NSCLC patients: mechanisms and strategies. *American Journal of Cancer Research*, 4(5), 411.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. *Methods*, 25(4), 402-408.

- Maier, T., Güell, M., & Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS letters*, 583(24), 3966-3973.
- Mu, C. Y., Huang, J. A., Chen, Y., Chen, C., & Zhang, X. G. (2011). High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Medical Oncology*, 28(3), 682-688.
- Offin, M., Rizvi, H., Tenet, M., Ni, A., Sanchez-Vega, F., Li, B. T., ... & Arcila, M. E. (2019). Tumor mutation burden and efficacy of EGFR-tyrosine kinase inhibitors in patients with EGFR-mutant lung cancers. *Clinical Cancer Research*, 25(3), 1063-1069.
- Ota, K., Azuma, K., Kawahara, A., Hattori, S., Iwama, E., Tanizaki, J., ... & Kage, M. (2015). Induction of PD-L1 expression by the EML4-ALK oncoprotein and downstream signaling pathways in non-small cell lung cancer. *Clinical Cancer Research*, 21(17), 4014-4021.
- Ribas, A., & Hu-Lieskovan, S. (2016). What does PD-L1 positive or negative mean?. *Journal of Experimental Medicine*, 213(13), 2835-2840.
- Roser, M., & Ritchie, H. (2020) Cancer. Our world in data. Published online at OurWorldInData.org. Retrieved from <https://ourworldindata.org/cancer>. [28 February 2020]
- Schabath, M. B., & Cote, M. L. (2019). Cancer progress and priorities: lung cancer. *Cancer Epidemiology and Prevention Biomarkers*, 28(10), 1563-1579.
- Sharma, S. V., Bell, D. W., Settleman, J., & Haber, D. A. (2007). Epidermal growth factor receptor mutations in lung cancer. *Nature Reviews Cancer*, 7(3), 169-181.
- Sul, J., Blumenthal, G. M., Jiang, X., He, K., Keegan, P., & Pazdur, R. (2016). FDA approval summary: pembrolizumab for the treatment of patients with metastatic non-small cell lung cancer whose tumors express programmed death-ligand 1. *The Oncologist*, 21(5), 643.
- Sun, C., Mezzadra, R., & Schumacher, T. N. (2018). Regulation and function of the PD-L1 checkpoint. *Immunity*, 48(3), 434-452.
- Tan, C. S., Gilligan, D., & Pacey, S. (2015). Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer. *The Lancet Oncology*, 16(9), e447-e459.

ThermoFisher Scientific Inc. (2018). Introduction to gene expression: getting started guide. Available at [https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4454239\\_IntrotoGeneEx\\_GSG.pdf](https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4454239_IntrotoGeneEx_GSG.pdf).

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., ... & Olsson, I. (2015). Tissue-based map of the human proteome: EGFR. Available at <https://www.proteinatlas.org/ENSG00000146648-EGFR/tissue>.

Wang, S., He, Z., Wang, X., Li, H., & Liu, X. S. (2019). Antigen presentation and tumor immunogenicity in cancer immunotherapy response prediction. *Elife*, 8, e49020.

Yi, M., Jiao, D., Xu, H., Liu, Q., Zhao, W., Han, X., & Wu, K. (2018). Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Molecular Cancer*, 17(1), 1-14.

## APPENDICES

1. Details of Figure 4.1. *PD-L1* DNA copy number of A549, H1975, and H1650 cell lines. Two different experiments were done: A549 cell line determination of *PD-L1* DNA copy number was done separately from the other cell lines.

Samples	Copy Number	
	Min	Max
A549 Cell Lines	1.9	1.93
Whole Blood (Control)	1.91	2.09
H1975 Cell Lines	1.05	1.23
H1650 Cell Lines	2.86	3.73
Whole Blood (Control)	1.82	2.2

2. Details of Figure 4.2. Normalized *PD-L1* qPCR Ct values of A549, H1975, and H1650; and Figure 4.4. Relative *PD-L1* mRNA baseline expression of H1975, and H1650 cell lines, A549 cell line as control. #Ct value of PD-L1 and GAPDH of each cell lines could also be attached in this section

		$\Delta C_t$ (normalized to GAPDH)				$\Delta\Delta C_t$ (A549 as ctrl)		$2^{-\Delta\Delta C_t}$		StDev	$\Delta C_t$ Mean	$\Delta C_t$ StDev			
		Batch 1		Mean 1		Batch 2		Mean 2							
		1	2	1	2	1	2	1	2						
A549	0.5h	17,0658	16,7518	16,6038	16,7795	0,0000	0,0000	1,0000	1,0000	1,00	0	16,77			
	6h	17,5940		17,5206											
	24h	15,5955		16,2143											
H1975	0.5h	9,6807	9,8403	8,7112	8,7761	-6,9114	-8,0034	120,3773	256,6115	188,49	96,33214	9,31			
	6h	10,0822		9,0682											
	24h	9,7581		8,5489											
H1650	0.5h	17,01423	17,0130	13,87273	13,9846	0,2613	-2,7949	0,8344	6,9400	3,89	4,317347	15,50			
	6h	17,04931		14,14669											
	24h	16,97552		13,93439											

3. Details of Figure 4.3. Relative *PD-L1* mRNA expression after induction with IFN- $\gamma$ .

	IFN- $\gamma$	PD-L1 $2^{-\Delta\Delta C_t}$			
		Batch 1	Batch 2	Mean	Stdev
A549	0.5h	2,4252	1,8743	2,1498	0,389524
	6h	24,4105	21,7937	23,1021	1,850388
	24h	7,9787	11,6576	9,8181	2,601407
H1975	0.5h	2,7961	1,4967	2,1464	0,918766
	6h	5,2328	2,8542	4,0435	1,681869
	24h	4,5440	1,8045	3,1743	1,937113
H1650	0.5h	1,067595	3,299624	2,1836	1,578283
	6h	14,34936	7,274049	10,8117	5,003001
	24h	14,59206	13,24548	13,9188	0,952171

4. Details of Figure 4.5. Relative *PD-L1* mRNA expression after IFN- $\gamma$  induction, A549 as control for H1975 and H1650.

Cell line	IFN- $\gamma$	2 $^{\wedge}$ - $\Delta\Delta Ct$			
		Batch1	Batch2	Average	Stdev
A549	0.5h	2,4252	1,8743	2,15	0,389523
	6h	24,4105	21,7937	23,10	1,850388
	24h	7,9787	11,6576	9,82	2,601407
H1975	0.5h	467,3695	355,6777	411,52	78,97799
	6h	955,0542	999,7903	977,42	31,63319
	24h	259,8137	366,3177	313,07	75,30969
H1650	0.5h	1,1064	21,9078	11,51	14,70879
	6h	20,9318	69,8014	45,37	34,55605
	24h	5,6065	70,8581	38,23	46,13983