CHAPTER 1: INTRODUCTION

1.1. Background

Cancer is the second most common cause of death worldwide (Roser & Ritchie, 2020). It was accounted in 2018 that lung cancer occupies the largest part of the cancer incidence and cancer-related death of the world (Bray et al., 2018). Eighty five percent cases of lung cancer are histologically categorized as Non-Small Cell Lung Carcinoma (NSCLC), and 10-35% of NSCLC was caused by the Epithelial Growth Factor receptor (*EGFR*) mutation (Schabath & Corte, 2019). The current first-line drugs for NSCLC with *EGFR* mutation are tyrosine kinase inhibitor (TKI) such as gefitinib, erlotinib, and afatinib (Hsu et al., 2019). They act specifically on blocking the mutated EGFR protein to stop the signal transduction, and the therapy has better results compared to chemotherapy (Heydt et al., 2018). However, all patients are eventually developing acquired resistance to the first line of TKI therapies mostly after 6-12 months of treatment initiation and afterwards the resistance also occurs on the second line and later throughout the remedy (Lin et al., 2014; Heydt et al., 2018). One way to develop the therapy is to look for another treatment besides TKI.

The Programmed Death-1/Programmed Death Ligand-1 (PD-1/PD-L1) pathway inhibitor might be useful to treat TKI-resistant *EGFR*-mutated NSCLC because of its known benefit on NSCLC patients (Sul et al., 2016). Often used by cancer to hide from immunosurveillance (Chen & Han, 2015a), PD-L1 protein is actually intended to create immune tolerance such as to inhibit the immune rejection of a fetus conceived in the womb (Sun et al., 2018). It is considered as a good specific drug target as it is not expressed in normal tissue in general (Inaguma et al., 2016). Immunohistochemistry to detect the presence of PD-L1 molecule is the standard procedure to determine whether a patient will derive benefit from PD-1/PD-L1 inhibitor (Sul et al., 2016). PD-1/PD-L1 inhibitor blocks the PD-L1 expressed by cancer cells from interacting with the PD-1 receptor of active immune cells (mostly killer T-cells), thus enabling the immune cells to initiate the tumor-killing mechanism. The drug was found to prolong the overall survival of advanced NSCLC patients compared to chemotherapy. However, the result of

PD-1/PD-L1 inhibitor on NSCLC which have mutated *EGFR* as its driver gene is not predictable (Lee et al., 2018). On NSCLC, the expression of PD-L1 on the cell surface indicates that the patient would derive benefit from PD-1/PD-L1 inhibitor (Sul et al., 2016). The problem is that the result of PD-1/PD-L1 inhibitor on *EGFR*-mutated NSCLC is not predictable with the Immunohistochemistry test, as EGFR overstimulation was known to cause upregulation on PD-L1 expression without the stimulus from the active immunocytes (T-cells) (Lee et al., 2018). The false assumption will lead to unsuccessful PD-1/PD-L1 inhibitor, as the absence of active immune cells means no tumor-killing activities. Interferon-gamma (IFN-γ) secreted by T-cells was known as the primary stimulus of immune-induced PD-L1 expression on PD-L1 expressing cancer cells (Chen & Han, 2015a).

This study is observing the difference of PD-L1 expression profile between *EGFR*-mutated NSCLC which derive benefit from PD-1/PD-L1 inhibitor with those which are not. *EGFR* mutation can be categorized into several subtypes according to the alteration of the protein sequence. The two most common *EGFR* mutations on NSCLC are deletion on exon 19 (45% of *EGFR*-mutated NSCLC) and L858R mutation on exon 21 (40-45%), which means the substitution of codon Leucine on 858 with Arginine (Sharma et al., 2007). Hastings and his colleagues (2019) try to analyze clinical records and outcomes of institutions that use PD-1/PD-L1 inhibitor after EGFR-TKI resistance and found that NSCLC with L858R mutation has a promising overall response rate, compared to the other common mutation (Hastings et al., 2019).

To understand the mechanism which lies behind the better outcomes of the subtype L858R, this study measured the RNA expression and DNA copy number of PD-L1 of *EGFR*-mutated NSCLC. The subjects of the experiment were NSCLC cell lines; H1650 cell line with *EGFR* mutation exon 19 deletion (del 19), H1975 cell line with *EGFR* L858R & T790M mutation, and an A549 cell line with wild-type *EGFR*. These cells were cultured and treated with IFN-γ (30 minutes, 6 hours, and 24 hours of induction) as the representative of T cell infiltrates. The quantification of the *PD-L1* transcription level (RNA) and the DNA copy number will be done with quantitative Polymerase Chain Reaction (qPCR). The experiment also compares the expression with and without IFN-γ exposure.

1.2. Research Questions

The questions which this research tries to answer is: Does *PD-L1* expression mechanism of NSCLC with *EGFR* mutation subtype L858R different from the NSCLC with *EGFR* mutation subtype exon 19 deletion?

1.3. Objectives

The objective of this experiment are:

1. To determine the *PD-L1* DNA copy number of the NSCLC cell lines A549, H1975, and H1650.

2. To determine the baseline *PD-L1* mRNA expression level of the NSCLC cell lines A549, H1975, and H1650.

3. To determine the effect of IFN-γ exposure towards *PD-L1* mRNA transcription level on the NSCLC cell lines A549, H1975, and H1650.

1.4. Scope of Work and Limitation

This research explores the difference of *PD-L1* DNA copy number and mRNA expression of NSCLC cell lines with different *EGFR* mutation subtype. The *PD-L1* mRNA expression consists of the baseline expression and the IFN-γ induced expression. The direction of research is to confirm the finding of Hasting et al. (2019) that NSCLC patients with *EGFR* mutation subtype L858R have a better response to PD-1/PD-L1 blocker therapy compared to the NSCLC patients with *EGFR* mutation subtype exon 19 deletion. Although there are many parameters should be known in order to definitively understand how the PD-1/PD-L1 blocker have better outcomes on the subtype L858R, this research only focused on how *EGFR* mutation, IFN-γ exposure, and DNA copy number affect the *PD-L1* mRNA expression.