Targeted Treatment in Patients with Non-Small Cell Lung Cancer: Use of Bevacizumab and its Biosimilars

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any otherdegree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled "Targeted Treatment in Patients with Non-Small Cell Lung Cancer: Use of Bevacizumab and its Biosimilars"submitted by Umme Tania (17346054) of Spring, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on April, 2021

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Ethics Statement

The study does not involve any kind of animal and human trial.

Abstract

Angiogenesis is one of the pivotal factors contributing to the development and metastasis of non-small cell lung cancer which is one of the most commonly diagnosed types of lung cancer. Bevacizumab, a monoclonal antibody that acts by restricting angiogenesis, is therefore a major choice of medication for advanced and metastatic non-small cell lung cancer treatment. Bevacizumab is essentially a biologic medicine which is a distinct class of biopharmaceuticals. Biologics are considerably expensive and this has resulted in the advent of biosimilars which are highly similar to their reference biologics in terms of quality, efficacy, and safety. As biosimilars are adopted as affordable copies of biologics, they possess great potential to influence the healthcare system. In this literature review, the importance of biosimilars with respect to its cost effectiveness and accessibility to the patients were discussed. The study also focused on the availability of biosimilars of bevacizumab as a potential targeted treatment option for NSCLC. In the meantime, regulatory frameworks established in different countries concerning the introduction of biosimilars in the market were reviewed.

Keywords: Biologics, Biosimilars, Bevacizumab, Non-small cell lung cancer, Regulatory frameworks, Targeted therapy

Dedication

Dedicated to my parents

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List of Acronyms

ADA	Anti-drug antibody
AE	Adverse events
ADR	Adverse drug reaction
AMCP	Academy of Managed Care Pharmacy
ALK	Anaplastic lymphoma kinase
BLA	Biologics License Application
BPCIA	Biologics Price Competition and Innovation Act
CI	Confidence interval
CHO	Chinese hamster ovary
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CMC	Chemistry, Manufacturing and Control
DOR	Duration of response
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
EU	European Union
EEA	European Economic Area
FISH	Fluorescence *in situ* hybridization
FDA	Food and Drug Administration
FOB	Follow-on biologic
GMR	Geometric mean ratio
HER2	Human epidermal growth factor receptor 2
HER4	Human epidermal growth factor receptor 4
HGF	Hepatocyte growth factor
ITT	Intention to treat
IHC	Immunohistochemistry

IND	Investigational New Drug
KRAS	Kirsten rat sarcoma viral oncogene homolog
MAP2K1	Mitogen-activated protein kinase 1
MHLW	Ministry of Health, Labor, and Welfare
NSCLC	Non-small cell lung cancer
NTRK1	Neurotrophic tyrosine receptor kinase 1
NGF	Nerve growth factor
NDA	New drug application
NRAS	Neuroblastoma ras viral (v-ras) oncogene homolog
NAbs	Neutralizing antibodies
ORR	Overall response rate
OS	Overall survival
PFS	Progression free survival
PFS	Progression free survival
PD-1	Programmed cell death protein 1
PD-L1	PD-ligand 1
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PP	Per protocol
PPACA	Patient Protection and Affordable Care Act
rDNA	Recombinant DNA
RT-CPR	Reverse transcription polymerase chain reaction
RBP	Reference biotherapeutic product
RP	Reference product
SBP	Similar biotherapeutic products
SEB	Subsequent-entry biologic
TrkA	Tropomycin receptor kinase A

TRK	Tropomyosin related kinases
TKI	Tyrosine kinase inhibitor
TTP	Time to progress
TEAE	Treatment-emergent adverse events
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WHO	World Health Organization

Chapter 1

Introduction

1.1 Background

Lung cancer irrefutably can be indexed in the major fights to be won within the oncology sector. Also, more than 85% of the reported lung cancer cases consist of non-small cell lung cancer (NSCLC). Global Cancer Observatory regulated by the World Health Organization claimed that lung cancer at present comprises the third place in the list of the most commonly developing malignance globally. Additionally, regardless of the declining fatalities since the 90s, this disease happens to be the utmost vicious type of cancer at the moment (Mello, 2020). Different therapeutic strategies utilized for treating NSCLC are surgery, radiation therapy, chemotherapy, targeted therapy and immunotherapy (Non–Small Cell Lung Cancer: Epidemiology, Risk Factors, Treatment, and Survivorship, n.d.). In spite of the expansion of treatment strategies to the newest chemotherapy regimens, such as chemotherapeutic agents based on platinum, the prognosis concerning NSCLC that is in advanced stage, metastatic and not operable stays to be insignificant. A clinical study performed by Eastern Cooperative Oncology group established an extended comparison between a conventional dosage regimen of cisplatin/paclitaxel and three other platinum based chemotherapeutic agents. The ORR (overall response rate) of the study was found to be 19% with a survival rate of 33% one year and 11% two years. Hence, there is an extreme necessity for novel and better therapies in the treatment of NSCLC, as improvement via existing cytotoxic therapy is proven to be inadequate (Herbst & Sandler, 2004).

A better understanding of the mechanisms of tumor growth at the molecular level has discovered several conceivable targeted therapeutic strategies, including angiogenesis pathways to restrain tumor growth more effectively (Herbst & Sandler, 2004; Lauro et al., 2014; Molina et al., 2008). Angiogenesis has been studied for an extended period of time and is presently reckoned as one of the 10 hallmarks of cancer (Greillier et al., 2016). Vascular endothelial growth factor (VEGF) can be identified as a primary moderator of angiogenesis in tumors. Increased manifestation of vascular endothelial growth factor has been associated with extremely low disease recovery, incorporating an aggravated possibility of recurrence and spread of cancer resulting in fatality

(Reinmuth et al., 2019). The functions of VEGF in governing tumor angiogenesis, retaining existing vascularization, causing the tumor to be resistant towards conventional therapeutic strategies accompanying unfavorable clinical outcome makes the VEGF a suitable target for pharmacologic intervention in the treatment of NSCLC (Herbst & Sandler, 2004). Bevacizumab, a monoclonal antibody synthesized via recombinant DNA technology restricts the biological activity of VEGF by binding to it and hence, blocks the interaction of VEGF with its receptors in the cell surface membrane of endothelium (Reinmuth et al., 2019). In the first line treatment of non-squamous NSCLC, bevacizumab has been the first anti-VEGF biologic to obtain license (Lauro et al., 2014). Since the past 20 years, considerable progress has been brought into existence in cancer treatment through the ongoing development of brand-new biologics, markedly monoclonal antibodies (Busse & Lüftner, 2019).

Biologics can be defined as large molecule therapeutic agents which possess complex nature and are manufactured employing living organisms (Kabir et al., 2019). They are essentially synthesized by applying biotechnological strategies via living microorganisms or cells with genetic modification which make them more costly than other medications. Consequently, access of the patients to biologics is usually restricted by excessive price (Busse & Lüftner, 2019). The emerging expiration of patents and confined biologics accessibility have nevertheless catalyzed a huge opportunity for the evolution of a different division of medicine, that is biosimilar (Kabir et al., 2019). Biosimilars can be introduced into the market where the patents of its reference biologics have come to expire and serve as an attractive strategy to lower the healthcare cost by price competition with the biologics (Busse & Lüftner, 2019). This class of biotherapeutics exerts high potentiality of price reducing. Therefore, increased acceptance of biosimilars can considerably reduce treatment costs and optimize reach to biologic medicines for patients with malignancies such as lung cancer (Cuellar et al., 2019; Rugo et al., 2016). However, the most important issue is that the biosimilars in the cancer treatment should be introduced in a proper process and healthcare providers must possess access to all the relevant data concerning biosimilars, so that they can take conclusive clinical decisions (Mellstedt et al., 2008).

1.2 Objectives of the Study

This study has been conducted to highlight the promising consequences and future implications of biosimilars in the treatment of non-small cell lung cancer.

The objectives of the study are:

1. to summarize the impact of the biosimilars in healthcare community

2. to highlight impact of biosimilars as a cost-effective alternative to increase oncologists' confidence in biosimilars

3. to outline the regulatory framework for approving biosimilars

4. to review the efficacy and safety of both the biologic bevacizumab and its biosimilar(s) in the treatment of NSCLC

1.3 Rationale of the Study

Biological agents or ''biologics" are extensively adapted in the oncology field in order to treat cancer and to provide supportive management of treatment-related side effects (Rugo et al., 2016). However, these medications are markedly more high-priced than their small molecule counterparts (pharmaceutical). Consequently, this has led to the ascent of biosimilars, considered to be the affordable copies of innovator biologics (Rathore & Bhargava, 2020). There is a huge market for biologics used in cancer treatment, and introduction of biosimilars possess fair potential in expansion of entrance towards treatment together with decreased prices. Both the development procedure and licensing process concerning these biotherapeutics require a pre-standardized regulatory pathway which includes a systematic strategy to validate bio-equivalence and conduct comparative clinical studies to verify equivalence in terms of pharmacokinetic (PK) profile, therapeutic efficacy, quality, toxicology profile, and immunogenicity to the reference biologic (Cuellar et al., 2019). In this study, the most relevant dispositions and the regulatory framework aimed at the growth phase and authorization of biosimilar medications are summarized in order to accelerate the rate of approval of biosimilars worldwide (Kang et al., 2020; Santos et al., 2019). The literature review also presents the oncological biosimilar drugs employed in the NSCLC treatment at present with the purpose of providing more precise and

crucial information to oncologists about the quality and safety of the particular medicines, since information is the essential guide to mitigating the basic concerns with regard to the adaptation of biosimilars (Mellstedt et al., 2008; Santos et al., 2019).

Chapter 2

Methodology

For this study, all the information, and data were collected from authentic articles indexed in high impact research databases such as Scopus, Pubmed, ScienceDirect, and Springer, as well as from renowned websites. At first, an outline was prepared to execute the review in a systematic manner. The articles for the study were searched using keywords like biologics, biosimilars, regulatory framework, lung cancer, targeted therapy, and bevacizumab. After going through about 100 articles, 60 relevant papers were chosen to collect information for the study.

Chapter 3

Cancer

3.1 Cancer: What Is It?

Cancer is considered to be among the prime reasons for fatalities and the single most considerable obstruction to accentuating lifespan worldwide in the 21st century (Bray et al., 2018). Cancer being a complicated and dreadful disease or sets of diseases, have caused sufferings to the multicellular living beings for around 200 million years, and evidence of cancers were found amidst the ancestors of modern humans going back more than millions of years. In contrast to infectious diseases and other environmental diseases, cancer is not necessarily initiated by an entity which is foreign to our bodies (Haberkorn, 2007).

In every cancer, cells of an organ or tissue inside the body start to divide in an extremely uncontrollable manner and gradually spread into surroundings. Generally, cells inside the human body multiply to generate newer cells according to the body's requirement and when these cells become older or defective, they face death. However, in case of development of cancer inside the body this orderly process gets hampered. Old and damaged cells do not die and also new cells keep dividing aggressively which form a mass of cells known as tumor.

Tumors which are cancerous are malignant in nature, meaning that they are capable of spreading into and invading surrounding organs. Additionally, a portion can get detached from the tumor and move to another part inside the body via the circulatory or lymphatic system causing another tumor to form there. On the contrary, benign tumors are not capable of spreading and if removed, such tumors generally don't recur, while malignant tumors may relapse (What Is Cancer? - National Cancer Institute, n.d.).

3.2 Types of Cancer

Cancer can fundamentally be classified on the basis of organs/tissues from where the cancer cells generate. To mention a few, the kidney cancer generates in the cells of the kidney, and the brain tumor develops in the neurons of the brain. It can also be categorized according to the group of cells which developed the particular cancer, for example, epithelial cells or myeloma cells.

Cancers which have been classified based on particular cell types are as follows:

i. Carcinoma

Among the different classes, carcinoma happens to be quite frequently occurring cancer. This form of tumor initiates in epithelial cells comprising both interior and exterior surfaces of the body. There are distinctive names for carcinomas starting in different types of epithelial cells. The cancer which begins in fluids or mucus producing epithelial cells is defined as adenocarcinoma. Cancer developing in the breast, lung, and prostate is mostly adenocarcinoma. Basal cell carcinoma originates in the initial layer of epidermis that comprises the utmost lining of the skin. Another kind of carcinoma is squamous cell carcinoma, also known as epidermoid carcinoma that develops in squamous cells present right underneath the outer layer of skin. Transitional cell carcinoma that mostly occurs in the bladder, ureters, and kidneys originates from a class of epithelial cells known as transitional epithelium.

ii. Sarcoma

Sarcoma, another kind of malignancy, begins in the bone tissues and soft tissues such as muscle tissue, fat tissue, blood vessels and lymph vessels as well as fibrous tissues. The most prevalent bone cancer is osteosarcoma. Among soft tissue sarcomas, leiomyosarcoma, kaposi sarcoma, liposarcoma, malignant fibrous histiocytoma, and dermatofibrosarcoma protuberans are the most common ones.

iii. Leukemia

Cancer beginning within the tissue present in bone marrow which forms blood cells is called leukemia. Solid tumors are not developed by these cancers, rather unusual white blood cells (WBC) such as leukemia cells and leukemic blast cells accumulate extensively in the blood and bone marrow. Leukemia can be classified on the basis of how rapidly the cancer spreads e.g., acute or chronic and based on the blood cell types in which the cancer begins e.g., lymphoblastic or myeloid.

iv. Lymphoma

A cancer recognized as lymphoma develops in T cells and B cells (lymphocytes). In this type of cancer, unusual lymphocytes pile up in the lymphatic system along with different organs of the body (What Is Cancer? - National Cancer Institute, n.d.).

The major categories of this disease are:

Hodgkin lymphoma – it usually develops in the B cells and starts in the lymph node located in the upper portion of the body (About Hodgkin Lymphoma | Cancer Research UK, n.d.).

Non-Hodgkin lymphoma – Non-Hodgkin lymphoma essentially starts in both B and T lymphocytes. Non-Hodgkin lymphoma is applied for many types of lymphoma that share few of the similar characteristics however different signs and symptoms (Lymphoma - Non-Hodgkin: Introduction | Cancer.Net, n.d.).

v. Multiple myeloma

Multiple myeloma generates in plasma cells. In this cancer, plasma cells start to grow uncontrollably and express an abnormal protein called myeloma protein (M-protein) that works as a biomarker to monitor the disease (What Is Myeloma? | Cancer Research UK, n.d.).

vi. Melanoma

Melanoma, a kind of cancer, forms in cells called melanocytes producing melanin on the skin. Melanomas can begin in any area of the skin; however, they mostly begin on the chest and back in men and on the arms as well as legs in women. The remaining common sites are the neck and face. If melanoma appears in the eyes, this is generally recognised as intraocular or ocular melanoma (Melanoma Treatment (PDQ®)–Patient Version National Cancer Institute, n.d.).

vii. Brain and spinal cord tumors

Tumors in the brain and spinal cord exist as a bunch of irregular cells growing uncontrollably. They can start anywhere in the brain and show symptoms according to the location. They may

stay as either benign (not cancerous) or malignant (cancerous) (What Are Brain Tumours? | Brain Tumours | Cancer Research UK, n.d.).

viii. Germ cell tumors

Germ cell tumors begin in the cells producing eggs or sperms. Such cancer can develop nearly at any place inside human body where the germ cells may reside and are of two types: benign and malignant (Germ Cell Tumours | Cancer Research UK, n.d.).

ix. Neuroendocrine tumors

Neuroendocrine tumors develop in the cells which secrete hormones into the circulatory system in response to a stimulus generated by the neurons. This condition is accompanied by excess hormone production which leads to several physiological abnormalities. The two categories of neuroendocrine tumors include benign and malignant. Carcinoid tumor is one kind of neuroendocrine tumor which grows slowly. Such tumors are generally found in the gastrointestinal system. The tumors can migrate to the hepatic cells and various parts inside the body, and secrete components such as serotonin and prostaglandins, triggering carcinoid symptoms (What Is Cancer? - National Cancer Institute, n.d.).

3.3 Prevalence of Cancer

Approximately more than eighteen million individuals have encountered cancer in 2018 worldwide. Moreover, around 9.6 million people have confronted death from this disease. About 4% of the recent cases of cancer were reported in low development index countries, about 16% were found in medium development index nations and the remaining were reported within nations indexed as highly developed. Among those cases, approximately five percent of deaths from cancer took place in low development index countries, about 20% took place in medium indexed countries, and the remaining took place in highly indexed developed countries. The lack of cancer records concerning approximately 85% of the global population, especially in low and moderate income nations, influences the reliability of such estimations. Moreover, approximately 50% of cancer cases and 60% of deaths from cancer occurred in Asia; however, only 6.5% of patients from this particular region are registered.

Cancer can be positioned as the primary reason for early death in approximately 100 out of 185 countries. One in five men and one in six women have been estimated to have suffered from cancer in 2018, and one in eight males and one in ten females came experienced death due to cancer. Around the world, the four principal causes of death from cancer are tumors in the lung, colorectum, stomach, and liver. Frequency ratio of cancer on average and by its types differ from country to country and reflect age distribution of population, variation in prevalence and propagation of the primary predisposing factors including socioeconomic condition and availability of medical services to diagnose cancer in a country. In developing countries in terms of economy, displacement of infection-related and poverty-related cancers such as stomach, cervix, and liver cancers by the cancers which appear to be more prevalent in developed countries such as colorectal cancer is on the rise (Cortes et al., 2020).

Lung cancer exists as most commonly diagnosed malignancy regardless of sex and it is the leading cause of death from cancer among males. Among all the cancers, lung cancer is a highly prolific one present in both men and women (11.6 percent of total diagnosed cases) and it also records the most deaths (18.4 percent of total death reports) than any other cancers (Bray et al., 2018).

Chapter 4

Lung Cancer

4.1 Introduction to Lung Cancer

Lung cancer, which is a greatly invasive and fast metastasizing malignancy, comes across as the top killer cancer in every gender all over the world (Lemjabbar-Alaoui et al., 2015). WHO estimates ~~is~~ that the death rates of the distinctive cancer will persist globally in an increasing manner, essentially due to a rise in smoking rate worldwide, specifically in Asian region (Duma et al., 2019). Lung cancer, a diversified disease constitutes different subcategories with distinct physiological and therapeutic implications (Rodriguez-Canales et al., 2016).

A few environmental factors and social habits can be related with the ensuing advancement of lung cancer. Cigarette smoking appears to be the utmost critical one among them which is estimated to account for 85%-90% of lung cancers. The incidence of being diagnosed with cancer is related to the frequency of smoking and exposure to different carcinogenic substances, such as asbestos. Further components related to enhanced risk of lung cancer formation is ionizing radiation, passive smoking, radon and carcinogenic metals e.g. arsenic, chromium, and cadmium, and polycyclic aromatic hydrocarbons. A pre-existing medical condition of pulmonary fibrosis, HIV infection, and intoxication with alcohol are also considered as predisposing factors of developing lung cancer (Duma et al., 2019).

Treatment options for lung cancer include surgery, chemotherapy, radiation therapy, targeted therapy, immunotherapy, photodynamic therapy, laser therapy, microwave and radiofrequency ablation (Treatment | Lung Cancer | Cancer Research UK, n.d.). The type of treatment that needs to be recommended to a cancer patient depends on the type of cancer and its stage.

There are two key forms of lung cancer- the first one is non-small cell lung cancer (NSCLC) inflicting 85% of the diagnosed individuals and another is small cell lung cancer (SCLC) which is found in 15% of the patients suffering from lung cancer (Duma et al., 2019).

4.2 Non-Small Cell Lung Cancer (NSCLC)

Non-small cell lung cancer is a kind of malignancy where cancerous cells generate within the lung tissues (Non-Small Cell Lung Cancer Treatment (PDQ®)–Patient Version - National Cancer Institute, n.d.-b). Approximately 80 to 85 out of 100 cases of reported lung cancer are non-small cell lung cancer (Types of Lung Cancer | Cancer Research UK, n.d.). Use of tobacco is the primary risk factor contributing to non-small cell lung cancer. One of the manifestations of non-small cell lung cancer is persistent cough as well as trouble in breathing (Non-Small Cell Lung Cancer Treatment (PDQ®)–Patient Version - National Cancer Institute, n.d.-a). NSCLC is mostly diagnosed at a metastatic stage (Dafni et al., 2019).

The advancement in comprehensive molecular understanding such as molecular expressions and activities induced attention for the categorization of non-small cell lung cancer into adenocarcinoma, squamous cell carcinoma and large cell lung carcinoma (Rodriguez-Canales et al., 2016). The different types of NSCLC which are described as follows:

i. Adenocarcinoma (ADC)

The most prevalent category of non-small cell lung cancer is adenocarcinoma, comprising nearly 40% of reported lung cancer. This begins in cells known as alveolar cells found within the bronchioles inside the lung and usually expresses specific immunohistochemical tumor markers like napsin A and TTF-1 (Duma et al., 2019). Adenocarcinoma can exhibit distinctive microscopic arrangement integrated in the same tumor which includes acinar, lepidic, papillary, micropapillary, and solid (Rodriguez-Canales et al., 2016).

The WHO also classifies early stages of adenocarcinoma based upon the degree of invasiveness as adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive adenocarcinoma (Duma et al., 2019).

ii. Squamous cell carcinoma (SqCC)

25%-30% of indexed lung cancers happen to be squamous cell carcinomas. This cancer generally forms in the cells positioned at the epithelium of the airway inside the lung. CK5, CK6, p40 along with desmoglein-3 are the immunohistochemical markers principally expressed in this type of cancer cells.

iii. Large-cell lung cancer (LCLC)

Large-cell lung cancers constitute about 5%-10% of the lung cancers reported. The occurrence of this cancer is getting reduced because of recent immuno-phenotyping approaches, enabling improved categorization of inadequately characterized adenocarcinomas and squamous cell carcinomas (Duma et al., 2019).

4.3 Molecular Alterations in NSCLC

A greater number of new molecular alterations has been established in NSCLC in the past years which include oncogenes and tumor suppressor genes. A number of these genes serve as novel prognostic biomarkers or suitable targets for cancer therapies (Rodriguez-Canales et al., 2016).

4.3.1 Epidermal Growth Factor Receptor (EGFR)

EGFR was detected in 2004 and from that time it was evident that EGFR mutations represent a certain subcategory of patients having NSCLC: mostly non-smokers with adenocarcinoma histology, commonly Asian women, along with distinct spread into the central nervous system (Vecchiarelli & Bennati, 2018). In 40%-80% patients diagnosed with non-small cell lung cancer, EGFR is generally excessively expressed. Nearly 10% NSCLC patients found in the US and 35% patients in East Asia possess NSCLC affiliated with mutations in EGFR (Rodriguez-Canales et al., 2016). EGFR mutation is the first molecular alteration identified in NSCLC (Vecchiarelli & Bennati, 2018). The position of the EGFR gene is in the short arm of chromosome number 7 at position 12. This gene encodes for the EGFR transmembrane glycoprotein, a member of the protein kinase superfamily (Rodriguez-Canales et al., 2016).

EGFR mutations generally develop within exon 18-21 which encode a section of the EGFR kinase domain. Heterogeneity is observable in the EGFR mutations, where the allele that is mutant also shows gene amplifications. Around 90% of such mutations occur within exon 19, deleting CTG-CGG which ultimately results into the substitution of leucine by arginine at codon 858 (L858R). Such mutations enhance the EGFR kinase activity which results in the downstream prosurvival signaling mechanism hyperactivation, thus supporting tumor development (Rodriguez-Canales et al., 2016).

4.3.2 Anaplastic lymphoma kinase (ALK)

Anaplastic lymphoma kinase alterations comprise nearly 3% to 7% of lung adenocarcinomas. Generally, they are more prevalent among young non or light smokers, people having adenocarcinoma histology, and in EGFR/KRAS wild type tumors (Vecchiarelli & Bennati, 2018). Originally, ALK had been detected in translocation of chromosomes resulting in the generation of fusion proteins comprising the COOH- terminal from ALK kinase domain and the NH2-terminal segments from various genes. The most common fusion partner of ALK is nucleophosmin representing 80% of translocation concerning ALK, however a minimum of six other fusion partners have been determined. In most of the cases, ALK rearrangements do not overlap with the rest of the mutations developed in non-small cell lung cancer, like EGFR and KRAS alterations. The commonly used techniques to identify ALK alterations are FISH (fluorescence in situ hybridization), IHC (immunohistochemistry), and RT-CPR (reverse transcription polymerase chain reaction) (Rodriguez-Canales et al., 2016).

4.3.3 Human Epidermal Growth Factor Receptor 2 (HER2)

In around 1% to 2% of NSCLCs, human epidermal growth factor receptor 2 alterations have been detected, mostly in females, non-smokers, and people with histology of adenocarcinoma (Hirsch et al., 2016). ERBB2, EGFR2, and NEU are the other names for HER2 which is a member of the EGFR family and possesses a significant part in growth, differentiation, and survival of cells. A member of the EGF receptor family of receptor tyrosine kinases is encoded by HER2 and it is situated at chromosome 17 at position 12 (Rodriguez-Canales et al., 2016). Most of the HER2 alterations are mainly in frame insertions in exon 20 (Hirsch et al., 2016). Insertion in the exon 20 leads to enhanced kinase activity of HER2 and accelerated downstream signaling, causing increased survival, invasiveness, and tumorigenicity. The HER2 protein does not contain its own ligand-binding domain and hence cannot bind with growth factors. However, HER2 does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signaling pathways, like mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) associated pathways (Rodriguez-Canales et al., 2016).

4.3.4 ROS Proto-Oncogene 1, Receptor Tyrosine Kinase (ROS)

The ROS proto oncogene 1 exists in the chromosome 6 at position 22. This gene is a member of the tyrosine kinase insulin receptor family, and functions as an integral membrane protein possessing tyrosine kinase activities serving as a differentiation receptor (Rodriguez-Canales et al., 2016). ROS1 rearrangements are generally identified in around 1–2% of lung adenocarcinomas reported (Vecchiarelli & Bennati, 2018).

ROS1 gene rearrangements found in NSCLC are affiliated with adenocarcinoma, and mostly develop in non and light smokers and patients aged less than 50 years. Such mutations are usually completely unique compared to EGFR and KRAS mutations, and rearrangements in ALK. In NSCLC, numerous distinct rearrangements in ROS1 had been identified such as SLC34A2-ROS1, EZR-ROS1, TPM3-ROS1, and CD74-ROS1 (Rodriguez-Canales et al., 2016).

4.3.5 Ret Proto-Oncogene (RET)

RET translocations have been identified in 1–2% of NSCLCs, and are more prevalent in non-smokers and juvenile NSCLC patients having histologies of adenocarcinoma or adenosquamous carcinoma (Hirsch et al., 2016). The gene of RET is situated in the long arm of chromosome 10 at position 11.2, and a tyrosine kinase that essentially takes part in the cell growth, migration and differentiation is encoded by the gene. RET rearrangements being recently discovered, are mostly mutually exclusive from other molecular alterations for instance EGFR, KRAS, ALK, and ROS1 which essentially suggests that rearrangements in RET represent a novel and precise molecular subgroup in NSCLC (Rodriguez-Canales et al., 2016).

4.3.6 NTRK1 (TrkA) Fusions

Translocations of NTRK1 are rarely found molecular alteration in the NSCLC and a comprehensive study reported only 1 patient having such mutation out of 1378 screened patients (Hirsch et al., 2016). Neurotrophic tyrosine receptor kinase 1, another name tropomyosin receptor kinase A (TrkA) happens to be a protein that is encoded by the NTRK1 gene. This gene can be found in chromosome 1q21-22. A distinct member of the tropomyosin related kinases (TRK) superfamily of tyrosine kinase receptors is NTRK1. This protein functions as a regulator

of development and differentiation of cells following the MAPK, PLC-c, and PI3K pathways when the nerve growth factor (NGF) ligand stimulates it (Rodriguez-Canales et al., 2016).

4.3.7 MET

MET signalling may become activated through amplification or slice mutation of exon 14 in the MET receptor gene. The MET receptor generally remains inactivated through binding of casitas B- lineage lymphoma (c-CBL). Absence of the binding site in c-CBL because of the splice mutation in exon 14 result in reduced receptor ubiquitylation and faulty degradation of receptors contributing to MET signaling activation. The MET alterations can be found among around 1-3% patients having NSCLC, usually in the absence of other molecular alterations (Hirsch et al., 2016).

MET sends signals to the cytoplasm from the extracellular matrix via interaction with hepatocyte growth factor (HGF) ligand as well as controls various biological activities including proliferation, invasion, survival, and mortality. This gene is situated on the long arm of chromosome 7 at position 3 (Rodriguez-Canales et al., 2016).

4.3.8 Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)

Kirsten rat sarcoma viral oncogene mutation is the most commonly occurring alteration in human cancers. It can be identified in around 30% of lung adenocarcinoma (Passiglia et al., 2020). In lung squamous cell carcinoma, KRAS alterations are rarely observed (Rodriguez-Canales et al., 2016). In contrast to EGFR mutations, ALK rearrangements, and other molecular alterations found in NSCLC, the incidence rate of KRAS mutations is greater in Western people and smokers (Passiglia et al., 2020). KRAS, an oncogene, can be spotted in the short arm of chromosome 12 at position 12.1, and mainly the KRAS protein is encoded by this gene which takes part in regulation of cell division (Rodriguez-Canales et al., 2016). KRAS essentially activates cell growth whenever it receives a signal from another protein identified as EGFR. KRAS remains switched “off” mostly and gets ‘‘on’’ when it receives a signal from EGFR. Upon activation, it makes the cell grow. In this way, EGFR and KRAS function combinedly to regulate cell growth and development (Briefs & Applied, n.d.). Mutations in exon 2, 3 and 4 of

the KRAS oncogene result in an intrinsic initiation of the MAPK pathway which consequently leads to greater cell growth and tumor formation (Passiglia et al., 2020).

4.3.9 B-Raf Proto-Oncogene, Serine/Threonine Kinase (BRAF)

B-Raf is a serine or threonine kinase encoded by BRAF gene that assists in the transmission of signals via chemicals from the exterior to the nucleus of the cell. BRAF plays a part in the RAS/MAPK signaling pathway which is a significant cascade of molecules regulating crucial processes of cells, for example, differentiation, division, migration, and apoptosis. The long arm of chromosome 7 at position 34 is the location of this BRAF gene (Rodriguez-Canales et al., 2016). Such alterations are observed in around 2% patients having NSCLC, primarily in smokers and people with adenocarcinoma histology (Hirsch et al., 2016).

4.3.10 Neuroblastoma RAS Viral (V-Ras) Oncogene Homolog (NRAS)

Neuroblastoma RAS viral oncogene can be found in the short arm of chromosome 1 at position 13.2, and a protein named NRAS is encoded by it which is included initially in directing cell multiplication. In spite of the fact that this may function as one of the processes of developing malignancy in NSCLC, it is an uncommon event as it has been found in less than 1% of patients suffering from NSCLC. Such genomic changes occur more often in patients with adenocarcinoma histology and smoking history (Rodriguez-Canales et al., 2016).

4.3.11 v-AKT Murine Thymoma Viral Oncogene Homolog 1 (AKT1)

The respective gene of AKT1 can be found in the long arm of chromosome 14 at position 14q32.32. The serine/threonine protein kinases are encoded by the AKT1 gene which is located in different kinds of cells. They play a significant part in signalling processes regulating cellular proliferation, differentiation and also help cells to survive. AKT1 works as a mediator of the downstream PI3K pathway, which assists in the control of apoptosis. AKT1 mutations can be quite uncommon sometimes and can be found only in 1% of NSCLC patients (Rodriguez-Canales et al., 2016).

4.3.12 Mitogen-Activated Protein Kinase 1 (MAP2K1)

MEK1 protein kinase is expressed by the MAP2KI gene. MEK1 protein kinase happens to be an important molecule for the signal transduction pathway recognized as RAS/MAPK, it carries out the transmission of chemical signals from the exterior to the nucleus of the cell. The RSA/MAPK pathway aids in controlling the multiplication, separation of cells, along with cell apoptosis. The gene can be found in the long arm of chromosome 15 somewhere in the middle of position 22.1 and 22.33. Substantial alterations in the MAP2K1 gene had been detected in less than 1% of total NSCLC reports and it is observed in adenocarcinoma more than squamous cell carcinoma (Rodriguez-Canales et al., 2016).

4.3.13 Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Alpha (PIK3CA)

The PIK3CA gene exists in the long arm of chromosome 3 at position 26.3. The respective gene generally provides instruction to encode a subunit of PI3K defined as p110 alpha protein which is essentially an enzyme. The PI3K signal transduction is quite essential for numerous cellular activities which include proliferation, migration and survival of cells. Although PIK3CA is one of the most prevalently identified mutated oncogene in various types of cancer, the incident rates determined in NSCLC are comparatively lower (Rodriguez-Canales et al., 2016).

4.3.14 HAGLROS

HARGLOS is a lncRNA with 699 bp length contributing to the development of malignancy. It was demonstrated that this lncRNA is quite over expressed and associated with accelerating growth and metastasis of tumor among patients with NSCLC. Through conducting an analysis of public datasets, it was validated that HAGLROS is notably expressed in NSCLC samples in comparison to regular tissues (Chen et al., 2020).

4.4 Treatments Available for NSCLC

4.4.1 Surgery

The types of surgery available for NSCLC include wedge resection, lobectomy, pneumonectomy and sleeve resection (Non-Small Cell Lung Cancer Treatment (PDQ®)–Patient Version - National Cancer Institute, n.d.-a). Lobectomy, which can be defined as the surgical resection of a

single lobe of the lung, is fundamentally approved as the optimum technique for early-stage NSCLC (Duma et al., 2019). This treatment will prove to be conceivable, if the tumor is entirely resectable and the patient is capable of tolerating the proposed surgical procedure (Molina et al., 2008).

4.4.2 Chemotherapy

Chemotherapy can be defined as utilization of cytotoxic drugs to destroy cancer cells. These drugs essentially function by disrupting the continuous growth of cancer cells. Chemotherapy may be administered on its own in the non-small cell lung cancer treatment. It may also be adopted before or after radiotherapy, or simultaneously with radiotherapy (Chemotherapy Treatment | Lung Cancer | Cancer Research UK, n.d.).

4.4.2.1 Neoadjuvant Chemotherapy

One of the promising benefits of this chemotherapeutic approach is initial treatment of micrometastases which reduces the size of the tumor enabling entire resection; and also has better endurance in comparison with the adjuvant chemotherapeutic approach (Duma et al., 2019). Meta-analyses of randomized trials conducted for neoadjuvant chemotherapy displayed a remarkable survival benefit over only surgery comprising a hazard ratio of 0.8 that equalized to a 5% survival advantage at 5 years (Blumenthal et al., 2018).

4.4.2.2 Adjuvant Chemotherapy

The adjuvant chemotherapeutic approach for early-stage NSCLC is justified by the phenomenon that the most frequently occurring failure following possibly remedying surgery is the distant metastases. Cisplatin-based combination chemotherapeutic regimen is the main course of adjuvant chemotherapy that is prescribed to stage II and IIIA disease patients following surgical intervention (Duma et al., 2019). In a combined investigation of 5 studies conducted with this approach, there was a complete survival advantage of 5.4% at 5 years (Watanabe et al., 2017).

4.4.2.3 Chemotherapy for Advanced Disease

Systemic treatment is required for the patients having tumors that are metastatic in nature. The standard therapeutic approach was a platinum-based doublet chemotherapy (carboplatin/cisplatin

along with gemcitabine, vinorelbine, or paclitaxel) before immunotherapy was introduced to treat NSCLC. Numerous trials have shown that in NSCLC patients, such therapy employing doublets has equivalent efficacy accompanied by variation in their toxicological profile. A multitargeted antifolate, pemetrexed had been evaluated together with cisplatin, and in comparison, with cisplatin and gemcitabine in every type of NSCLC patient. In a predetermined investigation regarding survival in different types of NSCLC (squamous vs. non squamous), a notable survival benefit supporting cisplatin/pemetrexed was found in subjects having non squamous histology. Well tolerance was established in favor of the pemetrexed combination and, hence, was identified as the conventional therapy in patients with non-squamous histology (Duma et al., 2019).

4.4.3 Radiation

Radiation therapy is a treatment option where x-rays possessing high energy or different kinds of radiation are utilized to cause destruction of cells with malignancy or inhibit the cells from developing. There are two types of radiation therapy: external radiation therapy and internal radiation therapy. The types of the radiation therapy provided is dependent on the type and stage of the NSCLC being treated (Non-Small Cell Lung Cancer Treatment (PDQ®)–Patient Version - National Cancer Institute, n.d.-a). The most commonly executed radiation treatment can be identified as external radiation therapy, a radiation therapy provided via a device from the exterior of the body. Few stage I NSCLC patients or those unable to go through surgery can be treated with such therapy as an optimum alternative therapeutic option (Lung Cancer - Non-Small Cell: Types of Treatment | Cancer.Net, n.d.). Recently, new technologies and techniques used in radiation oncology and imaging provide opportunities to optimize the advantages rendered by loco-regional treatments, extend treatment to new classes of patients for instance those suffering from oligometastatic disease and reduce usual tissue toxicity. Additionally, novel agents (e.g. targeted therapies) are now available which can be incorporated with radiation therapy to improve treatment (Thomas et al., 2020).

4.4.4 Targeted Therapy

Targeted therapeutic approach is a type of therapy where the tumor's distinctive genes, proteins, or the environment of tissues which essentially promote tumor growth and survival are targeted in order to inhibit tumor growth and survival (Molina et al., 2008).

4.4.4.1 Epidermal Growth Factor Receptor (EGFR) Inhibitors

Numerous Phase III clinical studies have demonstrated the efficacy, reduced toxicity, and improved quality of life with EGFR tyrosine kinase inhibitors (TKI) over conventional cytotoxic chemotherapy as first-line treatment in EGFR mutation-positive NSCLC patients. Due to such remarkable outcomes, nearly all recommendations supported the administration of first-line EGFR tyrosine kinase inhibitors such as gefitinib or erlotinib in patients with EGFR activating mutations (Hirsch et al., 2016).

Second generation inhibitors such as afatinib and dacomitinib are irreversible inhibitors that target HER2 (human epidermal growth factor receptor 2) and HER4 (human epidermal growth factor receptor 4) as well as EGFR, as opposed to first generation inhibitors, which are reversible competitive ATP inhibitors that only target EGFR. Both afatinib and dacomitinib rendered higher PFS (progression free survival) in comparison to gefitinib (Herbst et al., 2018). In spite of the greater RR (response rate) acquired by TKIs of EGFR, after 6 to 12 months of treatment, the disease continues to progress in the majority of patients, and resistance to the agent develops. However, the precise mechanisms that contribute to the patients being resistant are still ambiguous. A secondary missense mutation in exon 20 T790M is found among 40-60% of patients, which can be treated by osimertinib, a third-generation EGFR inhibitor (Duma et al., 2019; Vecchiarelli & Bennati, 2018)

4.4.4.2 Anti-EGFR Monoclonal Antibodies

The functions and outcomes of anti-EGFR monoclonal antibodies are not found to be related to the existence of activating EGFR mutation. When combined with systemic cytotoxic agents, two Phase III clinical trials evaluating anti-EGFR monoclonal antibodies detected a a notable improvement in OS (overall survival) for cetuximab (hazard ratio= 0.87; P<.04) or necitumumab (hazard ratio= 0.84; P<.01) (Duma et al., 2019).

4.4.4.3 ALK Inhibitors and ROS-1 Inhibitors

Crizotinib has been approved for ALK mutant patients of NSCLC as the first-generation inhibitors of ALK. It can be characterized as an oral TKI of ALK, ROS1, and MET kinases. Two non-specified Phase III clinical trials have displayed a greater PFS (progression free survival) and ORR (overall response rate) improvement obtained by crizotinib than chemotherapy, for ALK mutation-positive NSCLC in any treatment setting (Vecchiarelli & Bennati, 2018).

Most of the patients who earlier received treatment with crizotinib have been found to benefit from second generation inhibitors of ALK (alectinib, ceritinib, and brigatinib) (Herbst et al., 2018). Ceritinib is capable of efficiently inhibiting most of the crizotinib-resistant ALK secondary mutations causing no anti hepatocyte growth factor receptor (MET) activity (Vecchiarelli & Bennati, 2018). Ceritinib has demonstrated better effectiveness over traditional chemotherapeutic agents in the front line treatment arrangement in a trial named ASCEND-4, providing an increased PFS (16.6 months in the ceritinib group vs 8.1 months in the chemotherapeutic approach group) and a higher response duration (23.9 months vs 11.1 months) (Duma et al., 2019).

Alectinib enables the inhibition of most of the attained ALK resistance mutations and the rearrangement during the transfection of oncogene RET, but not MET and ROS1. A phase II evaluation assessed the effectiveness relating to alectinib in patients having crizotinib resistance (NP28673) and found a 50% ORR along with a median PFS of 8.9 months (Vecchiarelli & Bennati, 2018).

Lorlatinib is the latest ALK inhibitor, and it is the preferred treatment for alectinib resistance. It is effective against all known ALK inhibitor resistance mutations (Duma et al., 2019).

4.4.4.4 KRAS Mutation Suppressors

Development of targeted therapy against KRAS mutation has been quite difficult, even though numerous therapeutic candidates were discovered in *in-vitro* studies. The combination of selumetinib which is an oral MEK inhibitor with docetaxel increased PFS in patients who previously received treatment for NSCLC with KRAS mutation in advanced stage (5.3 months

using combination of selumetinib with docetaxel vs 2.1 with placebo plus docetaxel) (Hirsch et al., 2016).

4.4.4.5 RET Inhibitors

Tyrosine kinase inhibitors that target multiple mutations have displayed pharmacological activity against RET kinase and such TKIs include sunitinib, vandetanib, cabozantinib, alectinib, sorafenib, apatinib, lenvatinib and ponatinib (Hirsch et al., 2016).

4.4.4.6 Vascular Endothelial Growth Factor Receptor Inhibitors

Bevacizumab, an anti-vascular endothelial growth factor monoclonal antibody, was approved by the US FDA in 2006. By binding to the vascular endothelial growth factor (VEGF) and thus, restricting the interaction between VEGF and its receptor, it aids in the prevention of angiogenesis and endothelial cell proliferation (Ruiz-Ceja & Chirino, 2017).

According to the findings of the Eastern Cooperative Oncology group 4599 Phase III trial, the Bevacizumab arm had a median survival of 12.3 months compared to 10.3 months in the chemotherapy alone arm. Due to bleeding side effects reported in the Phase II trial, this trial was limited to patients with nonsquamous cell lung cancer (Duma et al., 2019).

4.4.4.7 Other Targetable Alterations

Patients with NSCLC who have a BRAF mutation are sensitive to BRAF inhibitors. Both BRAF inhibitors, vemurafenib and dabrafenib, show sensitivity when used alone or in combination with the MEK inhibitor trametinib (Herbst et al., 2018). Responses to Vemurafenib, an oral small-molecule TKI, were observed in 42% of patients in a limited Phase II trial, with a median PFS of 7.3 months (Duma et al., 2019).

Patients having NSCLC who have exon 14 skipping may benefit from MET inhibitors like crizotinib or cabozantinib (Herbst et al., 2018).

The FDA has approved larotrectinib, an oral tropomyosin receptor kinase inhibitor, for the treatment of advanced stage tumors with a neurotrophic receptor tyrosine kinase (NTRK) gene fusion, no known acquired resistance mutation, and no other ideal alternative treatment choices.

Especially for NTRK-positive NSCLC, larotrectinib is suggested after progression on earlier treatment with chemotherapy or immunotherapy (Duma et al., 2019).

4.5 Immunotherapy

Immunotherapy has drastically altered the dimension of NSCLC treatment (Duma et al., 2019). The immune system's main function is to recognize and kill neoplastic cells before they turn out to be clinically meaningful. The immune system's safe-keeping of normal healthy cells is regulated by an equilibrium of activating and inhibitory pathways. Malignancies can alter this equilibrium and escape immune surveillance in order to survive. The programmed cell death protein 1 (PD-1)/PDL-1 is one such pathway (Duma et al., 2019).

Anti-PD-1 therapy has added a new dimension in the cancer treatment of NSCLC. Pembrolizumab is approved for the first line treatment of advanced tumors expressing PD-ligand 1 (PD-L1) >50%, while nivolumab and atezolizumab received approval in second or further lines (Vecchiarelli & Bennati, 2018).

Chapter 5

Biologics and Biosimilars

5.1 Introduction to Biologics and Biosimilars

The term biologics has been linked with a novel group of therapeutics based on proteins being synthesized through living organisms which include microorganisms and even animals for instance bacteria and mammalian cells. Unlike the conventional drugs, for example aspirin, this novel genre of drug happens to be intrinsically more complicated and is not synthesizable inside a lab by solely chemical procedures. Biological therapeutics include therapeutic proteins such as hormones, monoclonal antibodies, conjugated proteins, and few polypeptides. The therapeutic products are structurally complex and are essentially macromolecules, which are more rigorous to develop and formulate as a drug than small molecule drugs, however, provide the benefit of being more target-specific and greatly efficacious in terms of their clinical functionality. Fundamentally, the active product has been synthesized following a biological process such as fermentation and a particular cell culture expression system or a biotechnological technique like recombinant DNA (rDNA) technology, or collecting from a living organism (Huynh-Ba, 2009). Earlier, biological products were primarily synthesized mainly using the purified extracts retrieved from animal blood and tissue. However, with scientific progress, particularly in the field of recombinant DNA technology, nowadays biologics are being successively synthesized from genetically reprogrammed cell lines of microorganisms to achieve a mass production of a specific biological therapeutic (Brougher, 2014).

The treatment of numerous acute and chronic diseases has been revolutionized by the development of biologics with improved specificity. The explosion in the expansion of the therapeutics has been significant in the management of therapeutic approaches in terms of cancer (monoclonal antibodies), autoimmune disease, diabetes (insulin) and anemia (erythropoietin replacements).

Due to the blooming achievement of proteins and monoclonal antibodies manufactured by recombinant DNA technology in the clinical and commercial arena, many pharmaceutical companies have aspired to play a crucial role in the biological therapeutics field. Moreover,

nanobodies, soluble receptors, fusion proteins, immunotherapies, synthetic vaccines, immunoconjugates, modified proteins (glycosylated and pegylated), and other biotherapeutic therapies have all been developed since then for the expansion of biotherapeutic range. These treatments have come to exist as a result of new technologies and a better understanding of cell line production and identification, expression, and engineering of proteins (Kabir et al., 2019).

However, therapies employing biologics are quite costly and this puts a major economic stress on the public health care system (Kabir et al., 2019). Unfortunately, access to prospective life savior biologics is quite restricted in several regions of the world. As the patent expiry of these biological agents is approaching, there has been enormous enthusiasm in developing and introducing biosimilars as a cost saving approach for healthcare systems and to extend global access to leading biological therapies (Rugo et al., 2016).

A biosimilar is a biotherapeutic that contains a similar active substance and is reckoned to be equivalent with respect to quality, potency, efficacy, safety, and immunogenicity to a previously licensed reference biotherapeutic product (originator), but the name, appearance, and packaging of the product may differ (Bradford and Gary, 2014). To be precise, in terms of safety and potency, there must not be any clinically meaningful distinction between biosimilar products and the reference products (Rehman et al., 2018). Any differences present in the biosimilar drugs must be limited solely in the therapeutically inactive components in the medicines. Biosimilar medicines are designed to be administered in the same route, in an equivalent dose, and to treat identical diseases as the reference products (Santos et al., 2019).

The comparable biologic drug products are recognized as similar biotherapeutic products (SBPs) by the World Health Organization (WHO), biosimilars by the European Medicines Agency (EMA) of the European Union (EU), follow-on biologics (FOBs) by the US Food and Drug Administration (FDA), and subsequent-entry biologics (SEBs) by Health Canada. In few events, the term “biosimilar” has been utilized, and hence it is quite essential to analyze variations in biosimilar definitions in various regions (Table 1).

Table 1: Definitions of biosimilar according to different country's regulatory bodies (Biosimilar Medicines: Overview | European Medicines Agency, n.d.; Kabir et al., 2019).

Terminology	Given By	Definition
SBP	WHO	A biotherapeutic product that is, with respect to quality, safety, and effectiveness, analogous to a previously approved reference biotherapeutic product.
Biosimilar	EMA	A biosimilar is a biological medication that is very similar to another biological medicine that has already been licensed (reference product).
FOB	US FDA	A product that is greatly similar to the reference biologic in terms of safety, purity, and potency, with no clinically significant variations.
SEB	Canada	A biologic drug that receives entrance to the market after a previous version was approved in Canada and has been displayed to be similar to a reference biologic product.
Biosimilar	Korea	Biological drugs that have been shown to be bioequivalent in terms of quality, safety, and effectiveness to a formerly licensed reference biologic.

According to the various definitions mentioned above, it is evident that in the definition of biosimilars, there are three universal characteristics:

1. The biosimilar drug needs to be a biological drug;
2. The reference biologic requires ~~it~~ to be a previously approved biological product;
3. It is absolutely necessary to exhibit high bioequivalence in quality, effectiveness and toxicology (Wang & Chow, 2012).

In order to confirm biosimilarity to a reference biologic, the US Food and Drug Administration (FDA) applies a totality-of-the-evidence approach that involves analytical research, animal studies, and clinical trials to make a comparison of human pharmacokinetics (PK), pharmacodynamics (PD), clinical effectiveness, and safety, including immunogenicity. (Cuellar et al., 2019).

Although it is quite necessary to acknowledge the fact that biosimilars are not identical to their generic versions, and thus therapeutic equivalence is not exhibited by default. The term "generic" medication is generally adopted in order to refer to drugs composed of small molecules which are structurally and therapeutically equivalent to their respective reference drug. In contrast, biologics are more difficult to be characterized structurally. In terms of size, biologics are exceedingly bigger than chemically synthesized drugs. They contain hundreds of amino acids which are combined in a biochemical process following a definite sequence. Consequently, biologics usually own several secondary and tertiary structural and also post-translational modification alterations. Glycation, oxidation, glycosylation, sulfide crosslinking, etc. which fundamentally prompt structural alterations, can be found within the identical lot of biological products. Since small variations are quite hard to eliminate, extremely similar biosimilars and their reference products do not have the similar therapeutic effectiveness. Due to the complex large molecular structure, there are various challenges in the manufacture of these biotherapeutic products in comparison with conventional small molecule generic drugs.

Biosimilar market is well established in the United States and European countries (Kabir et al., 2019). Aside from Europe and the United States, biosimilars have been launched as alternatives to biological pharmaceuticals in Japan, Australia, and a few other nations. The market for biosimilars is expected to expand significantly, with advantages in terms of new advanced technologies, market competition, patent expiration, the development of reliable, quality drugs at reasonable costs, and global demand all contributing to the expansion of the biosimilar market.

The success resulting from biosimilars can greatly prompt the sustainability of pharmaceutical business in the upcoming decade (Rehman et al., 2018).

5.1.1 Advantages of Biologics and Biosimilars

- Biologics is essentially capable of binding with target sites which are most likely tricky or unfeasible for small molecule drugs.
- Biologics are capable of providing improved economical return in comparison to that of small molecule drugs (Kabir et al., 2019).
- As the clinical evaluation and biosimilar approval processes are simplified along with an efficient manufacturing process, the overall costs of biosimilars development tend to be lower than reference products. Because of the price competition, biosimilar's introduction can greatly lessen the expenses of originator biological products.
- By reduction of the costs, biosimilars possess the potential of allowing the budget reallocation to novel treatments or reinvestment (Cuellar et al., 2019).
- The availability of biosimilars is considered to be a way to expand access to biotherapeutic products as they are fundamentally providing more treatment options (Kang et al., 2020).
- Biosimilars have the promise to enhance the use of biologics (Health Care System Benefits | Pfizer Biosimilars, n.d.).
- The capability of biosimilars in increasing the efficiency of the healthcare system is incredible. Hence, it is assumed that biosimilars will grant a positive impact on the public health and healthcare system (Santos et al., 2019).

5.2 Manufacturing Process of Biologics and Biosimilars

Manufacturing process of both biologics and biosimilars involves a step wise process which includes cell line development, cell culture recovery and purification of the product. The whole process can be elucidated by these subsequent 4 steps:

i. Cell line development: A specific cell line requires to be engineered that contains the required gene which will function for the transcription of the preferred biologic.

After a disease target has been detected and a protein that is fundamentally the preferred biologic has been designed to interact with the target, biologic manufacturing will begin. Cells go through transfection, screening, and cloning in this first step, and a cell line that is capable of producing the desired protein product with the optimum yields is chosen.

Transfection is one of the processes in recombinant DNA technology in which the gene encoding the desired protein is incorporated within an expression vector and introduced in the host cell (Nathan et al., 2018). After that, culture, screening and evaluation of the transfected cells are carried out to identify the cells which can produce the preferred product in the controlled environment (Lai et al., 2013). Finally, a stock culture of cells possessing identical genetic arrangement is obtained after repeating the selection technique for numerous times. Cell banks (master and working cell banks) are created from reservoirs of the final clone, and by utilizing those, the subsequent batches of the biologic may be produced. The cell line production process can take many years and is crucial to confirming that a quality product can be manufactured and delivered to customers (Nathan et al., 2018).

Several factors are needed to be taken into consideration while performing the cell line selection. Few of the biological products can be synthesized utilizing organisms most commonly used, for example, *E. coli*, or yeast, or plant cells. Many biologics, however, have highly complex features structurally and can only be synthesized in mammalian cells. For instance, few such agents contain saccharide units adjoining them by a process known as glycosylation; and also, glycosylation pattern works as a determinant of the biofunctional properties of the biologic (Nathan et al., 2018). Furthermore, the risk of an immunological reaction to the drug may get enhanced if non mammalian derived cell lines are utilized (Dumont et al., 2015). Selection of a cell line becomes more complex, since each type of cell line, such as chinese hamster ovary (CHO), has innumerous variants. CHO cells are highly utilized cells for a cell line; however, few earlier biological agents are synthesized employing murine hybridoma cells (Lai et al., 2013).

ii. Expansion and cell culture: The selected cells are mass produced to synthesize the biologic.

Cell culture, or developing cells from a cell bank stored at a laboratory, is the successive step in the biologics manufacturing process. Cells are defrosted out of a reservoir and put in petri dishes or flasks with a liquid medium containing the nutrients the cell requires to grow. The cells are

consecutively transferred into larger sized vessels known as bioreactors as more cells are achieved through cell division. The culture media and development specifications in manufacturing of biologics are proprietary to the manufacturer of biologic and may have an effect on the cells' growth as well as the structural and functional properties of the biologic they express (Nathan et al., 2018).

It is critical to preserve the unique condition that the cells need to replicate at each stage of the cell culture phase (Litten & Grampp, 2012). Conditions are generally maximized to advance growth of cells in the prior stages of the expansion process. Amid sequential steps within bioreactors, the conditions are optimized as well in order to advance the synthesis of the preferred substance. Minor alterations in the environment can influence the cells and modify their generated proteins. Because of this, strict maintenance amid the bioreactor stage is required to guarantee the final product's quality, consistency, and safety. Such maintenance incorporates inspections performed amid generation to screen and, if suitable to identify corrective measures to specific process parameters. To achieve this necessary regulation, scientists carefully screen factors like temperature, pH, nutrient concentration, cell density, and oxygen levels (Nathan et al., 2018).

iii. Recovery and purification: Harvesting the product from the cells or culture media and separation of the biological product from the unwanted substances.

The desired biologic must be isolated from the cells and growth media after the cell culture process is completed, which is recognized as the downstream processing. Based on the size, molecular weight, and/or electrical charge of the biological molecule, several removal techniques are employed to retrieve the biological molecules from the cell culture.

After the isolation and purification steps, the initial product that is attained possesses a bulk of molecules having similar size, molecular weight and charge. The initially retrieved product is identified as the active pharmaceutical ingredient (API) (Nathan et al., 2018). Minor changes while performing the process of separation and purification, can alter structural attributes and the final product's constitution, hence the therapeutic payoff (Litten & Grampp, 2012).

iv. Formulation: Preparation of the biologic for the utilization by and for the patients.

The final step in the manufacturing of biologics is formulation. In this step, the drug substance is formulated into a suitable dosage form that is injectable since it cannot be administered orally. Injectable formulation usually involves mixing the drug substance with sterile solutions where buffer solutions and other excipients may be present, not necessarily every time. This assures that biologics stability increases and drug delivery properties enhance (Nathan et al., 2018). It is suggested that protein (biologic) stability is improved by the combination of some compatible excipients. Hence, excipients are considered to be an integral part of the biologic drug formulations. The excipient determination is dependent upon the characteristics of the API, route of administration, dosage form, and target patient (Prasad et al., 2020). After executing all these processes, the mixture is then transferred to a vial or syringe.

This formulation could affect the structure and function of the drug substance. Quality is ensured by performing analytical testing just like drug substance testing. Also, compatibility with the packaging is studied so that there is no interaction or reaction between the API and components of the administration device (e.g., vials or syringes). After all the product specifications are met, the medicine is labeled, and sent for packaging and distribution (Nathan et al., 2018).

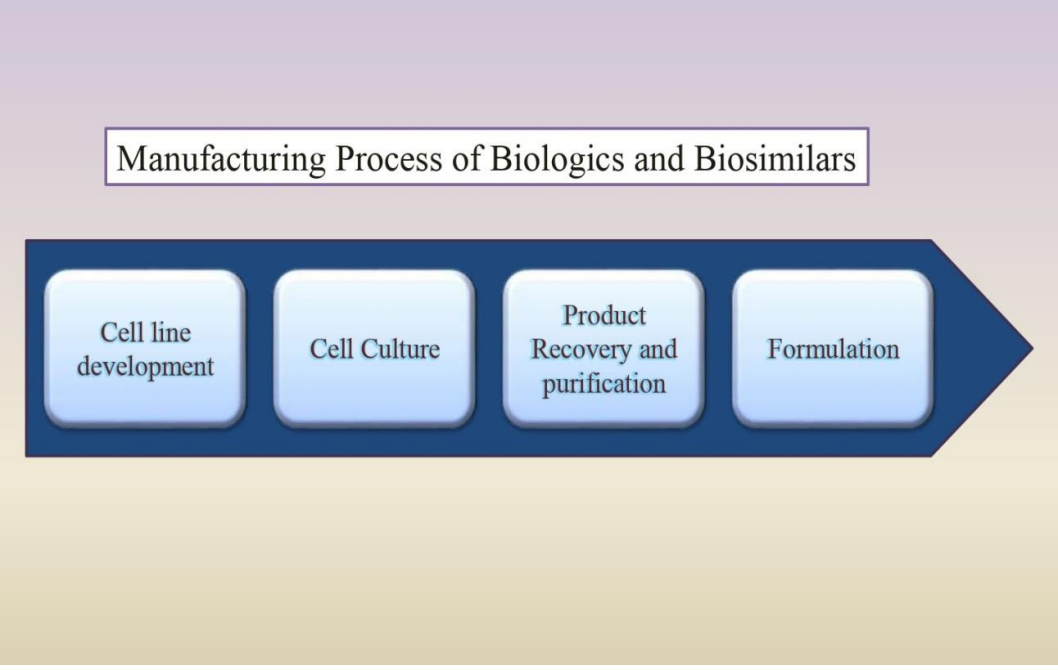

Figure 1: The manufacturing process of biologics and biosimilars (Adapted from Litten & Grampp, 2012)).

5.3 Regulatory Framework of Biologics and Biosimilars

Most of the biologics such as vaccines and most recombinant drugs are licensed by the US FDA under the roof of a subsection of the Public Health Service Act of 1944 (Nagel, 2018). Submission of an Investigational New Drug (IND) application with required data regarding the product development and non-clinical animal study trials needs to be reported in order to conduct clinical trials. The provisions of the IND regulations provide an allowance for the interstate transportation of drugs and biological products to conduct clinical investigations. These human clinical trials are intended to provide necessary data to support a biologics license application (BLA) since the Public Health Service Act asserts that a biologics license should be effective for any biological drug product that is questing to be introduced into the interstate market place. The bulk of the BLA or NDA applications fundamentally consist of non-clinical and clinical inspection reports, the proposed labeling for the biological agent backed by the data, and adequate CMC (chemistry, manufacturing and control) information to confirm that the product meets the required specification of efficacy and potency. A review team evaluates the application if it contains all the required information and all the data provided are reliable for granting the license to commerce. When the review team is convinced by all the data provided and the application passes the review, the FDA approves the product and issues a letter which serves as a license that will offer the allowance for its commercialization (Flannery et al., 2018).

In 2005, a year before the first biosimilar was authorized, the European Medicines Agency was the first governing body to issue regulations regarding biosimilars. The European Union (EU) member states have the authority to implement any regulations relating to the production, growth, and approval of biosimilar drugs. The WHO followed the guidelines given by EMA to set global guidelines in 2009. Biosimilar regulatory framework by the WHO established a set of internationally acknowledged guidelines concerning the launch of quality, safe, efficacious biosimilar products. The primary aim of the WHO regulatory system is to support and also validate that regulatory systems regulated by locals follow the global regulations of biosimilar production. Other countries eventually accepted these regulations without any alterations, while only a few countries established their distinctive standards. Japan and Korea each launched their own biosimilar regulatory frameworks in 2009. Australia implemented the guidelines established by the EMA without any alterations. Malaysia and Singapore brought some alterations to adjust

theirs to match with the standards established by the EMA guidelines. Brazil and Cuba followed the regulations given by the WHO in order to construct their own biosimilar regulations. India also adopted the current trends in the regulatory guidelines and established their own regulatory framework in 2012. The United States was a latecomer to the regulatory pathway concerning biosimilars, with authorization for biologics granted under the Public Health Service Act. In 2010, the Patient Protection and Affordable Care Act (PPACA) included the Biologics Price Competition and Innovation Act (BPCIA), which established a new licensing process for biosimilars. This ensured the availability of biosimilars at reasonable costs to the people by the BPCIA, and it also upheld innovation by companies synthesizing reference biologics (Kabir et al., 2019).

Regulatory standards for follow-on biologics in various regions, such as the European Union (EU), the United States, and the Asia-Pacific region are similar yet a little different (Wang & Chow, 2012).

5.3.1 European Union (EU)/ EMA

A reference product that is not licensed in the European Economic Area (EEA) can be utilized for some clinical and in vivo non clinical trials, according to the regulations provided by the EMA on similar biological medicinal products, but it must meet alike scientific and regulatory requirements as those applied by the EMA and be indicative of the reference product in the EEA.

Biosimilar development is reliant on ‘comparability studies,' which are needed to prove bioequivalence to the originator biologic. A thorough head-to-head analysis to compare between the biosimilar and the reference biologic is generally required. In order for biosimilars to be approved in the EU, a benefit *versus* risk ratio must be displayed based on the evidences obtained for safety and efficacy in human clinical trials, backed up by evidences from non-clinical trials and quality of products, as well as established scientific information on the reference product's safety and efficacy acquired during clinical utilization. The clinical studies done for the comparison are distinctively developed to identify clinically relevant differences with respect to effectiveness and safety between the biosimilar and the originator biological agent, and to ascertain bioequivalence as well as resolve issues which remained unaddressed from prior analysis or operational investigation.

According to the regulations given by EMA, when the biological drug product having various licensed indications encounters significant alterations while performing the production process, for instance, new site of manufacture or new dosage form, these changes can bring about potential effects upon the product's therapeutic outcome which are cautiously analyzed by conducting comparability studies.

EU has a robust regulatory structure in order to protect the safety of the patients through monitoring, reporting, assessing and taking preventive measures for ADRs (adverse drug reactions) of every drug, including the biotherapeutics. To protect public health, regulatory bodies constantly monitor and analyze every biological medication and take appropriate regulatory steps (e.g., attaching warning information to labeling details or limiting use) (Rathore & Bhargava, 2020).

5.3.2 United States (US)/ FDA

The Patient Protection and Affordable Care (PPAC) Act of 2010 created an expedited licencing process for biotherapeutics in order to promote the production of biosimilars in the United States (US) (Rathore & Bhargava, 2020).

A biosimilar that received licence for indications which have not been inspected while executing clinical studies of the biosimilar needs extrapolation of biosimilar indication. An applicant requires demonstrating adequate scientific justification for the extrapolation of clinical data. For instance, in March 2015, the US FDA approved Sandoz's Zarxio for all of Amgen's Neupogen indications (Stanton, 2016). Biosimilar manufacturers may select a condition of use that is sufficiently sensitive to identify clinically relevant variations between the biosimilar and the original biologic, according to the US FDA standards. In few events, healthy volunteers are perhaps the most responsive group to examine when tracing for variations in immune responses since they are not immunocompromised and thus are more likely to manifest uncompromised immunological reactions (Rathore & Bhargava, 2020).

FDA published the draft regarding labeling guidance in 2016. This guidance states that the reference product and its biosimilar have similar labeling. Additionally, the US FDA has the recommendation that labels of biosimilars must possess relevant data of clinical trials from the

reference biologic rather than biosimilar trials' data. To differentiate the biosimilar drug from the reference product and other biosimilars of the same product, the proposed nomenclature includes an individual, non-meaningful, and four-letter suffix (Rathore & Bhargava, 2020).

The FDA's regulatory arrangements for safety of biosimilars are quite similar to those of EMA. Furthermore, manufactures of biosimilars must coordinate with FDA authorities regarding post-marketing supervision strategies, and if necessary, further post-marketing or clinical trials may be needed to comply with the FDA regulations (Kumar & Sigala, 2016). In order to offer patients and providers more trust in biosimilars, professional organizations such as the Academy of Managed Care Pharmacy (AMCP) have been introduced. AMCP recently assembled a multidisciplinary investigation team to develop a framework to evaluate the protection and efficacy of biosimilars (Olech, 2016).

5.3.3 Japan/ MHLW

In 2009, the regulatory guidelines for Japan were established and those are fundamentally based upon the European Medicines Agency's regulations. The Ministry of Health, Labor, and Welfare (MHLW) is the organization in charge of scientifically evaluating and approving pharmaceutical products for use in Japan (Rathore & Bhargava, 2020). Also, MHLW encountered advanced challenges of maintaining the regulations regarding biosimilar products. Recombinant plasma proteins, recombinant vaccines, PEGylated recombinant proteins, and highly processed and characterized non-recombinant proteins are included in the framework of the standards. Polyglycans like heparin which is a low weight molecule were ruled out of the guideline, in contrast with the EU. Synthetic peptides are also eliminated, since the intended synthetic peptides can be simply identified using analysis in terms of structure and can be classified as generic drugs (J. Wang & Chow, 2012).

According to the guidelines provided by Japanese authority, the manufacturers of biosimilars have to validate equivalency in the pharmacokinetic profile between the biosimilar and the reference biologic following every route of administration identified for the originator biologic. Additionally, the equivalence may be displayed by employing a pharmacodynamic indicator that is affiliated with the therapeutic outcomes if possible. A confirmatory clinical trial is required to

show clinical comparability of biosimilar products, however, in specific cases comparable PK-PD investigations may be adequate to establish therapeutic similarity (Arato, 2016).

The evaluation of comparability must be conducted following sequential steps for quality characterization and in non-clinical, pharmacokinetic, and clinical trials. Therefore, the comparability is detected depending on “totality of evidence”; not only on the outcomes of a certain assessment. Thus, demonstration of quality characteristics of the biosimilar and the originator product being highly similar is a comprehensive quality characterization practice and possesses great importance. In case of considering extrapolation of indication, the drug’s mechanism of action along with the specificities regarding the target populations ought to be taken into account and inspected. A biosimilar should possess a nonproprietary name/trade name which will clearly mention that it is a biosimilar product and the name requires to be effortlessly distinctive from the originator product and other biosimilars (Rathore & Bhargava, 2020).

5.3.4 World Health Organization (WHO)

The World Health Organization (WHO) officially established guidance on the comparison of similar biotherapeutic products (SBPs) in 2009 in order to ensure better access to secure and reliable SBPs around the world through global regulatory harmonization concerning licensing. Regulations given by the WHO are adjusted with the EMA’s standards as both recommend stepwise comparability methods for classification of the product's quality characteristics, supported by clinical and non-clinical assessments. The number of non-clinical and clinical evidence needed is based on the product type and reviewed on a case-by-case basis identical to the EMA guidelines. The WHO guidance set the basis for several countries' regulatory authorities as they developed their respective regulatory standards based on its principles (Rathore & Bhargava, 2020).

Manufacturers must apply a comprehensive quality report that involves a complete classification of the commodity, evidence of reliable and rigorous manufacturing, and a comparability assessment between the SBP and the reference biotherapeutic product (RBP) in the quality section, both of which serve as the foundation for a potential reduction in non-clinical data requirements. This theory suggests that reduction in data is only feasible for the non-clinical and clinical aspects of the development program, and that potential distinctions between the

biosimilar and the selected reference biologic discovered during the comparability assessment will necessarily require the collection of more non-clinical and clinical data (Wang & Chow, 2012).

Chapter 6

Biologics in Non-Small Cell Lung Cancer

Biologic agents providing targeted therapy render an innovative therapeutic approach. The utilization of biologics targeting and then modulating certain biologic processes in cells of tumor has continued to emerge together with the advancing knowledge regarding cancer cell biology (Kelly & Huang, 2008). The biologics that are used in combating non-small cell lung cancer have been listed in Table no. 2. Among the biologics listed, biosimilars for only Bevacizumab received approval from the US FDA and the EMA for the treatment of NSCLC.

Table 2: The US FDA approved biologics in the treatment of NSCLC (Ruiz-Ceja & Chirino, 2017)

Biologics	Therapeutic class	Year of approval
Bevacizumab	Anti VEGF-A	2006
Atezolizumab	Anti-PD-1	2016
Pembrolizumab	Anti-PD-1	2016
Necitumumab	Anti-EGFR	2015
Nivolumab	Anti-PD-1	2015
Ramucirumab	Anti VEGF-2	2014

Cetuximab	Anti-EGFR	2008
Durvalumab	Anti-PD-1	2018
Ipilimumab	CTLA-4 inhibitor	2020
Cemiplimab-rwlc	Anti-PD-L-1	2021

6.1 Bevacizumab

Bevacizumab, also recognized as Avastin, is a humanized recombinant monoclonal antibody working against vascular endothelial growth factor, comprising human IgG1 framework segment and segment that is complementary to antigen-binding sourced from a murine antibody (A.4.6.1) which inhibits the interaction between vascular endothelial growth factor and its receptor (Herbst & Sandler, 2004). This is the only biologic in the group of biologics that has biosimilars approved to be used in the treatment of NSCLC.

In 2004, bevacizumab was approved by the FDA manufactured by both Genentech and Roche and in 2006, it was approved as first-line treatment for patients having no squamous NSCLC (Ngo & Chen, 2020; Ruiz-Ceja & Chirino, 2017). It has been licensed for the treatment of the disease in the United States when administered along with paclitaxel and carboplatin and in European Union together with platinum-based chemotherapy (Reck et al., 2020).

Many cancer types, including non-small cell lung cancer (NSCLC), require angiogenesis as a contributing factor to tumor cell formation, proliferation, and metastasis. Vascular endothelial growth factor (VEGF), a prime regulator of vascular growth, is overly expressed in numerous cancer types and is affiliated with tumor cell proliferation, elevated micro-vessel density, and poorer prognosis (Reck et al., 2020). Bevacizumab is the first angiogenesis inhibitor to

accomplish success in the clinical development phase for treatment of NSCLC (Greillier et al., 2016).

In 2004, a randomized Phase II trial was performed in colorectal cancer patients, constructing a comparison of the chemotherapy doublet carboplatin-paclitaxel to the same chemotherapy protocol plus bevacizumab, at doses of 7.5 or 15 mg/kg. Patients who were administered with high dose bevacizumab had an increased response rate than patients who received chemotherapy alone (31.5 percent vs 18.8 percent), a prolonged time-to-progression (TTP) (7.4 months vs 4.2 months; p=0.023), and an improved overall survival (OS) (17.7 months vs 14.9 months; p=0.63). The Eastern Cooperative Oncology Group (ECOG) completed a major randomized Phase III study in 2006, enrolling 878 patients having no squamous NSCLC in stages IIIB-IV. Patients received carboplatin-paclitaxel every three weeks for six cycles, with or without 15 mg/kg of bevacizumab. After that, bevacizumab was used as a maintenance treatment before signs of the disease progression appeared. Overall survival (OS) was substantially increased (12.3 months vs 10.3 months; p=0.003), progression-free survival (PFS) was considerably prolonged (6.2 months vs 4.5 months; p<0.001), and objective response rate (RR) was significantly higher (35 percent vs. 15%; p<0.001) in patients receiving bevacizumab. Well-tolerance among the patients was observed while treating utilizing bevacizumab in the experimental regimen; however more major bleeding incidents were detected than in the control group (Lauro et al., 2014).

Bevacizumab was mostly well tolerated among the patients and it did not appear to raise the frequency or severity of nausea/vomiting, neuropathy, or renal toxicity, which are common side effects of carboplatin/paclitaxel chemotherapy. Hypertension, thrombosis, proteinuria (with intermittent nephrotic syndrome), and epistaxis were recorded as adverse effects in Phase I and II trials. The key safety issue in patients with NSCLC seems to be severe bleeding incidents (hemoptysis/hematemesis), with squamous cell histology as a potential risk factor (Herbst & Sandler, 2004). Squamous histology and hemoptysis of grade 2 or higher were reported to be the only exclusion conditions for bevacizumab therapy in the Annals of Oncology in 2012 (Lauro et al., 2014).

6.1.1 Mechanism of Action

Angiogenesis exhibits a crucial role in the origination of new arrangement of the blood vessels during embryogenesis, however, it mostly remains inactive throughout the adult body and temporarily gets activated during wound healing as well as the female reproductive cycle. Angiogenesis is strongly regulated by a complicated interaction of pro- and anti-angiogenic components, although it can get activated by developing solid tumors. This whole process is recognized as "angiogenic switch" which is also identified as a hallmark of solid tumors (Garcia et al., 2020). Angiogenesis can be defined as the production of new micro-vessels from pre-existent blood vessels. Numerous distinctive cells play their roles in the process, for instance macrophages, pericytes, and endothelial cells which are harmonized by an intricate combination of pro-angiogenic and anti-angiogenic constituents. Tumor development and metastasis was aided by pathological angiogenesis. The formation of irregular, crooked, dilated, poorly-organized blood vessels with increased permeability is the prime characteristic of tumor angiogenesis. Such vascular abnormalities result in the formation of a microenvironment which can be described by interstitial hypertension, hypoxia and acidosis along with a subsequent elevated generation of VEGF (Lauro et al., 2014).

VEGF happens to be a pro-angiogenic component which interacts with membrane receptors and its intracytoplasmic domain possesses tyrosine kinase activity (Lauro et al., 2014). There are six identified members of the VEGF family, however, VEGF-A, also recognized as a vascular permeability factor, is most likely to exhibit the most significant influence in angiogenesis during the cancer development process. VEGF-A exhibits its effects via binding fundamentally to the VEGF receptor-2 (VEGF-2), expressed primarily on endothelial cells (Midgley & Kerr, 2005). In NSCLC, VEGF expression is linked to a worse prognosis or more aggressive pattern (Melosky et al., 2018).

In vitro, bevacizumab binds to a continuous epitope in VEGF and prevents VEGF from binding to VEGFR (vascular endothelial growth factor receptor) located on the membrane of endothelial cells, blocking interaction between the ligand and receptor, and downstream signaling. Bevacizumab inhibits VEGF signaling and may have antitumor effects by inhibiting new vessel development, deterioration of newly developed vessels, regularization of the vasculature to allow

cytotoxic chemotherapy to be administered more effectively, and direct impacts on cancerous cells. Bevacizumab restricts effects prompted by VEGF which include cell development, enhanced permeability, generation of nitric acid and cell migration *in vitro*. Clinical data found indirectly from patients backs up the concept that bevacizumab has mainly cytostatic effects, suggesting it stops new blood vessels from growing (Melosky et al., 2018).

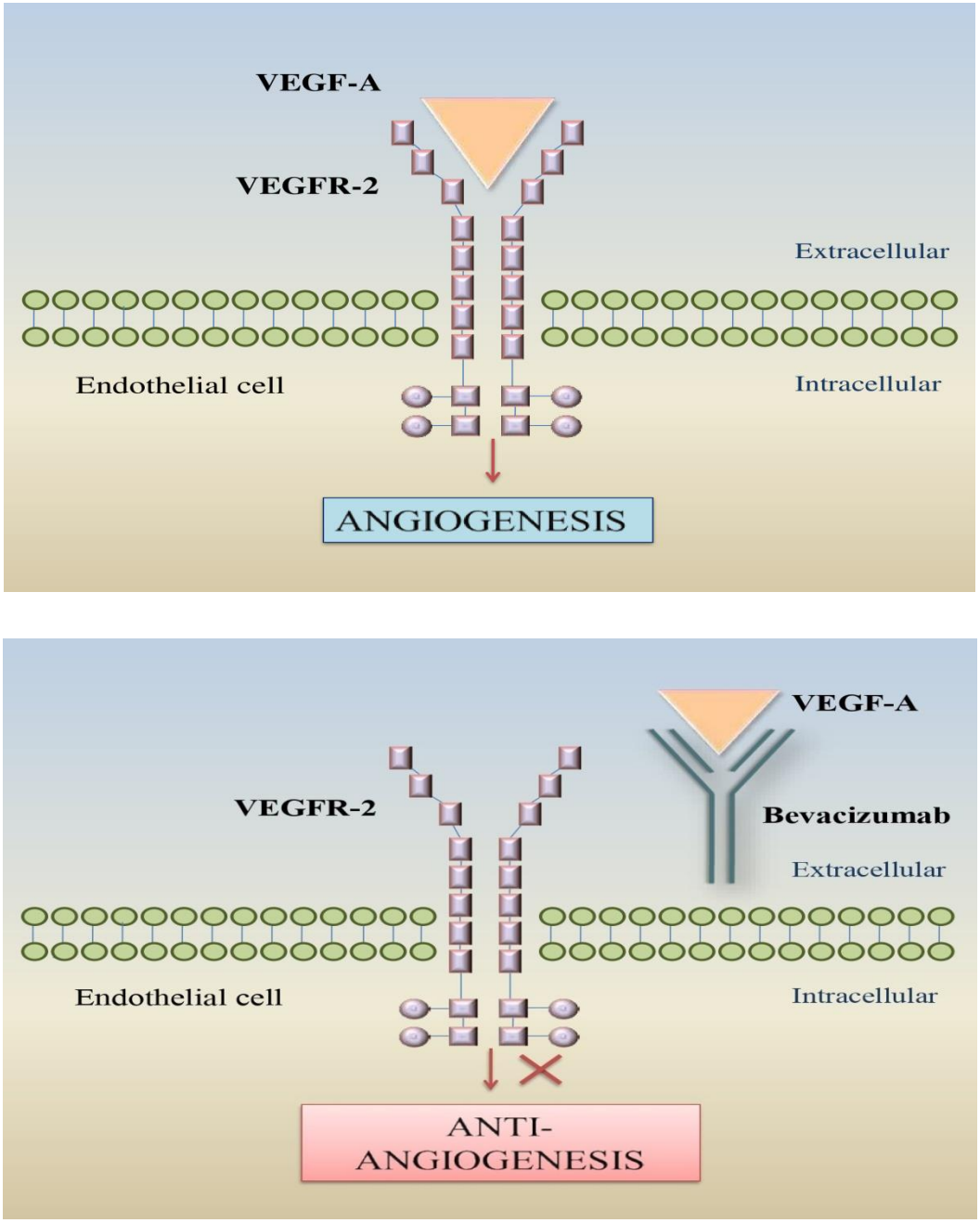

Figure 2: Mechanism of action of bevacizumab (Adapted from Kotowski et al., 2018)

6.2 Biosimilars in Non-Small Cell Lung Cancer

In the United States, the patent on bevacizumab came to expire in July 2019 and in the European Union, it will come to expiry in January 2022 which has prompted several pharmaceutical companies to seek approval for biosimilars (Table 3) (Ngo & Chen, 2020).

Table 3: Approved and candidate biosimilars of bevacizumab (Avastin) (Busse & Lüftner, 2019; Cuellar et al., 2019)

Product name	**Manufacturing company**	**Development status**
ABP 215 (Mvasi, Bevacizumab-awwb)	Amgen	Approved by the FDA in 2017 and in 2018, by the EMA
PF-06439535(Zirabev, Bevacizumab-bvzr)	Pfizer	Approved by the FDA and EMA in 2019
SB8	Samsung Bioepis and Merck	Approved by the European Commission in 2020
BAT1706	Bio-Thera Solutions	Completed Phase III clinical studies
BCD-021	Biocad	Completed Phase III clinical studies

FKB238	Centus Biotherapeutics	Completed Phase III clinical studies
CT-P16	Celltrion	Undergoing Phase III clinical studies
BEVZ92	mAbxience Research	Undergoing Phase III clinical studies
HD204	Prestige Biopharma	Preparing to conduct Phase III clinical studies
JHL1149	JHL Biotech	Conducting Phase I clinical studies
RPH-001	R-Pharm	Completed Phase I trial
ERG12021	EirGenix	Undergoing preclinical trials
BX0510	BioXpress	Undergoing preclinical trials

Bevacizumab-awwb (Mvasi) and bevacizumab-bvzr (Zirabev) are the two biosimilars of bevacizumab in the United States receiving approval in 2017 and 2019 respectively. Both also got licensed in the European Union by the European commission in 2018 and 2019 respectively (Ngo & Chen, 2020). SB8, another biosimilar of bevacizumab, acquired authorization from the

European commission in 2020 (Syed, 2020). The remaining biosimilars mentioned in Table ~~2~~ 3 are in different phases of the clinical trial.

6.2.1 ABP 215 (Mvasi, Bevacizumab-awwb)

ABP 215, a biosimilar to bevacizumab is a recombinant humanized monoclonal antibody. ABP 215 is the first biosimilar of bevacizumab to receive license in both the United States and ~~the~~ European Union. ABP 215 has been approved in the United States and the European Union for the treatment of a variety of cancers, including non-small cell lung cancer (Seo et al., 2018).

6.2.1.1 Structural and Functional Similarity Assessment

To evaluate the similarity of ABP 215 and bevacizumab in terms of structure and function, an extensive analytical approach was developed and implemented. The primary structure, higher order structure, particles and accumulates, product associated materials and impurities, thermal forced degradation, biological activities, general properties, and process associated impurities were thoroughly evaluated in the similarity evaluation for both ABP 215 and the reference bevacizumab (Seo et al., 2018). These findings indicate that ABP 215 is analytically largely similar to the reference bevacizumab, with small variations that are unlikely to affect biological function or efficacy (M. Thomas et al., 2019).

6.2.1.2 Pharmacokinetics Similarity Assessment

To compare the pharmacokinetic profiles of ABP 215 and the reference bevacizumab, researchers conducted a randomized, single-blind, single-dose, parallel, 3-arm PK analysis in healthy male subjects. A total of 202 people were randomly allocated in 1:1:1 ratio to receive either a single 3 mg/kg IV infusion of ABP 215 (n = 68) or the reference bevacizumab (the FDA approved: n = 67; the EU licensed: n = 67) (M. Thomas et al., 2019). A geometric means ratio (GMR) found from the result for C_{max} and AUC_{inf} was 0.99 (90% CI can range from 0.93-1.03) and 0.99 (90% CI can range from 0.95-1.04), respectively while comparing between ABP 215 and the reference bevacizumab-US. While comparing ABP 215 and the reference bevacizumab-EU, similar results of GMRs were obtained which were 1.03 (90% CI= 0.98-1.08) and 0.96 (90% CI = 0.92-1.01) (Ngo & Chen, 2020). The primary PK parameters of serum concentration versus time, AUCinf, and Cmax, as well as the secondary PK parameters of AUClast, were

comparable across the groups. The 90% CIs of the ratios of the means is completely found within the pre-established bioequivalence parameters of 0.80–1.25, confirming ABP 215 and bevacizumab RP's PK similarity. Over the course of sampling, ABP 215 and bevacizumab RP showed an equivalence in mean serum concentration-time profiles (M. Thomas et al., 2019).

6.2.1.3 Efficacy

642 patients were randomly assigned to receive ABP 215 (n = 328) or bevacizumab RP (reference product) (n = 314) medication. ORRs (overall response rates) were comparable between the ABP 215 and bevacizumab RP groups in the ITT (intention to treat) population with NSCLC (39.0% vs 41.7%) (M. Thomas et al., 2019). PFS (progression-free survival) and OS (overall survival) were also similar in all treatment groupings. For ABP-215, PFS obtained was 60.1% and for bevacizumab RP, it was 60.2%. The risk ratio for ORR while comparing ABP-215 and bevacizumab RP, was 0.93 (90% CI; 0.80 - 1.09). The two-sided 90% CI (confidence interval) for ORR was also contained within 0.67 to 1.5, the pre specified equivalence range (Thatcher et al., 2019). Both secondary and sensitivity tests of the primary and secondary efficacy endpoints, including risk difference (RD) of ORR, DOR (duration of response), and PFS, yielded the same potency outcomes. When auxiliary covariates were used in the primary efficacy model (central radiology analysis of the ITT population), similar results were obtained; the RR of ORR was 0.90 (90% CI: 0.77 - 1.05) (M. Thomas et al., 2019). The RR (response rate) attributed to ORR in the PP (per-protocol) population was 0.94 (90 percent CI, 0.80–1.010), and the RR in the tumor response set was 0.93 (90 percent CI, 0.80–1.09), based on the primary, autonomous, blinded radiologists' analysis.

According to the primary, independent, blinded radiologists' study, the risk ratio of ORR and 90 percent CI between ABP 215 and bevacizumab RP were within the pre established equivalence limit, suggesting that clinical efficacy in ABP 215 and bevacizumab RP is comparable (Thatcher et al., 2019).

6.2.1.4 Safety

Most adverse events were present in grade I or II in terms of severity. Adverse events grade ≥3 was generally affiliated with anti-VEGF toxicities which include hypertension, gastrointestinal

perforations, complications in wound healing, pulmonary hemorrhage and proteinuria, and were mostly similar between treatment groups. Serious AEs had been seen in 85 (26.2%) and 71 (23.0%) patients treated with ABP-215 and bevacizumab RP respectively. These serious adverse effects included febrile neutropenia, pneumonia, pulmonary embolism, anemia, dyspnea, and hemoptysis [(3.4% vs 2.6%), (1.9% vs 1%), (1.9% vs 1.6%), (1.5% vs 1.9%), (0.9% vs 1.9%), (0.9% vs 1.3%), (0.9% vs 1.6%) respectively] (Thatcher et al., 2019). The number of fatalities found for ABP 215 and bevacizumab RP were also comparable; 4.0% and 3.6% respectively (M. Thomas et al., 2019).

6.2.1.5 Immunogenicity

Immunogenicity profiles were comparable in patients with NSCLC receiving treatment with ABP 215 or bevacizumab RP. Overall, 11 patients (4 in ABP-215 and 7 in bevacizumab RP groups) were observed developing the binding of ADAs (anti-drug antibody) at any time while conducting the whole course of the investigation (M. Thomas et al., 2019).

6.2.2 PF-06439535 (Zirabev, Bevacizumab-bvzr)

The second biosimilar of bevacizumab approved by the FDA was PF-06439535 (Ngo & Chen, 2020). It was manufactured by Pfizer and got its approval in 2019 (Cuellar et al., 2019).

6.2.2.1 Structural and Functional Similarity Assessment

An extensive comparative investigational studies have shown that PF-06439535 has the same amino acid sequence as reference bevacizumab from the EU (bevacizumab-EU) and the US (bevacizumab-US), as well as functional similarities (Reinmuth et al., 2019). N-linked oligosaccharide profiling showed that the products had comparable amounts of N-linked oligosaccharides. The prevalent charge isoforms were found to be identical using imaged capillary electrophoresis. Blockade of VEGF-induced cell proliferation in human umbilical vein endothelial cells and attachment to the four main VEGF isoforms showed similar biologic behavior (Melosky et al., 2018).

6.2.2.2 Pharmacokinetics Similarity Assessment

Similarities among PF-06439535, bevacizumab-US and bevacizumab-EU in terms of pharmacokinetics were compared in 102 healthy male subjects by following a single intravenous dose (Melosky et al., 2018). Results demonstrated that in comparison with bevacizumab-EU, PF-06439535 had GMRs for C_{max} and AUC_{inf} of 1.04 (90% CI= 0.98-1.11) and 0.98 (90% CI = 0.92-1.05); subsequently, GMRs for C_{max} and AUC_{inf} of 1.10 (90% CI = 1.04-1.17) and 1.03 (90% CI = 0.96-1.10) when compared with bevacizumab-US (Ngo & Chen, 2020).

6.2.2.3 Efficacy

The efficacy profile of the possible biosimilar bevacizumab PF-06439535 was compared to that of bevacizumab-EU in combination with paclitaxel and carboplatin (Melosky et al., 2018). In the ITT community, 45.3 percent of patients in the PF-06439535 group (95% CI 40.01 - 50.57) and 44.6 percent of patients in the bevacizumab-EU class (95% CI 39.40 - 49.89) obtained an analytical response by week 19 that was validated by week 25. The ORR risk ratio was 1.015, with a 95 percent confidence interval of 0.863–1.193 and a 90 percent confidence interval of 0.886 - 1.163. The risk variation of the unstratified overall response rate was detected to be 0.653%, with a 95% CI of −.06608–0.07908. All the three CIs is placed completely within the pre-specified equivalence limit of 0.73–1.37. Therefore, similarity between the respective biosimilar and bevacizumab- EU was confirmed for overall response rate according to the findings (Reinmuth et al., 2019).

6.2.2.4 Safety

While establishing comparison to the reference product, the safety assessment revealed that PF-06439535 had less treatment-related adverse effects (15.2 percent in PF-06439535, 25.7 percent in bevacizumab-EU, and 18.2 percent in bevacizumab-US) (Ngo & Chen, 2020).The most common severe TEAEs (treatment-emergent adverse events) were pneumonia, febrile neutropenia, and neutropenia, both of which appeared at equal rates across treatment classes. There were no clinically significant variations in the prevalence of any TEAEs of particular concern amongst the classes (Reinmuth et al., 2019).

6.2.2.5 Immunogenicity

In the immunity groups, the prevalence of immunogenicity was modest, with similar numbers of patients with ADAs (anti-drug antibodies) and NAbs (neutralizing antibodies) in both treatment classes. Five (1.5%) of 339 patients in the PF-06439535 group and five (1.4%) of 350 patients in the bevacizumab-EU group were ADA-positive in the overall post-treatment evaluation (Reinmuth et al., 2019).

6.2.3 SB8 (Aybintio)

SB8 is a biosimilar to bevacizumab, a monoclonal anti-VEGF antibody approved in the EU manufactured by Samsung bioepis and merck.

6.2.3.1 Physicochemical and pharmacokinetic similarity assessment

SB8 has physicochemical and pharmacodynamic characteristics that are close to that of the reference drug bevacizumab, and pharmacokinetic equivalence has been demonstrated in healthy subjects and patients with non-small cell lung cancer (NSCLC). For the primary endpoints of area under the concentration-time curve (AUC) and maximum serum concentrations (Cmax), the 90% confidence interval for geometric mean ratios is within the predefined bioequivalence margin of 80.00–125.00 percent. All these findings elucidated the bioequivalence of SB8 to the reference bevacizumab-EU (Syed, 2020).

6.2.3.2 Efficacy

The percentage of patients in the SB8 and the reference bevacizumab groups who achieved the highest ORR in the full analysis set (all randomized patients using the intention-to-treat principle) was 47.6% and 42.8 percent, respectively. The leading ORR's risk ratio was 1.11 (90 percent CI, 0.975 - 1.269), which was below the pre - defined equivalence margin (0.737–1.357). The proportion of patients achieving best ORR in the per-protocol range (patients undergoing at least the first two cycles of combined chemotherapy with tumor evaluation, without significant protocol variations impacting the primary efficacy assessment) was 50.1 % in the SB8 group and 44.8 % in the reference bevacizumab group. The risk variation in the highest ORR was 5.3 percent (95% confidence interval; 2.2–12.9%). Both the lower margin and the upper margin were confined within the pre-specified equivalence margin (-12.5% - 12.5%). The primary research was expressed in sensitivity tests, which validated the primary analysis' robustness (Reck et al.,

2020). SB8 was also equivalent to comparison bevacizumab in the FAS at the end of the study in terms of median progression free survival (PFS) (8.5 months in SB8, 7.9 months in bevacizumab RP groups; 95% CI 0.83 - 1.18), median overall survival (OS) (14.9 months in SB8, 15.8 months in bevacizumab RP groups; 95% CI 0.83 - 1.28), median response duration (7.7 months in SB8, 7 months in bevacizumab RP groups; 95% CI 0.81 - 1.37). All these comparable outcomes in terms of clinical efficacy demonstrates the bioequivalence of SB8 and the reference bevacizumab (Syed, 2020).

6.2.3.3 Safety

There were 758 patients in the safety set (for SB8 n= 378; for bevacizumab RP, n= 380). Adverse events were registered in 348 (92.1%) and 346 (91.1%) of SB8 and BEV patients, respectively; mostly they were grade 1 or 2. The most common grade 3 AEs were neutropenia, hypertension, anemia, and reduced count of neutrophils. Other important adverse effects included nausea, alopecia, complications in wound healing, bleeding which were also similar between the groups (Reck et al., 2020).

6.2.3.4 Immunogenicity

Throughout the analysis, the SB8 and BEV classes in the had similar rates of positive ADA outcomes, including up to cycle 7 (SB8, n= 46/341; BEV, n= 34/337) and EOT (n=55/341; n= 37/337) (Reck et al., 2020).

Chapter 7

Conclusion

Targeted therapy has altered the landscape of medication procedure for non-small cell lung cancer, a complicated and aggressive form of cancer that requires a multidisciplinary approach for its optimum treatment. Biologics are receiving more acceptances since they hold a prime position in the targeted therapy for being more target specific. However, biological medicines are confined to a distinct group of population due to the financial obstacle rendered by them. Biosimilars have acquired entrance to the market as a cost-effective alternative in order to flourish the framework of treatment employing biologics. Biosimilars are not only thriving in the context of pharmaceutical companies but they are also blooming among the patients because of their cost benefits. Regardless of the advantages, there are few concerns about switching to the biosimilars in terms of quality and efficacy. Regulatory bodies can play a crucial role in mitigating such concerns by tailoring robust, more detailed, and harmonized approval pathways for biosimilars so that only quality and effective biosimilar medicines can attain commercialization. Researchers need to review the data retrieved for the biosimilars and make them accessible to the healthcare providers to aid in their decision-making process and to the patients for maintaining the transparency.

Future Directions

More comprehensive studies are required to identify the novel targets for non-small cell lung cancer in order to develop biological medicines and their biosimilars against those targets. Additionally, regulatory authorities from different regions should work together to establish a harmonized regulatory framework for biosimilars. Well-defined policies are required along every phase of biosimilar processing starting from the clinical development of a product to its commercialization. Ultimately, this will result in the development and approval of more quality, effective and safe biosimilars widening the access to biotherapeutics.

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