

**CURRENT LANDSCAPE OF IMMUNE CHECKPOINT
INHIBITORS IN ADVANCED NON-SMALL CELL
LUNG CANCER- A SYSTEMATIC LITERATURE
REVIEW AND BAYESIAN NETWORK META-
ANALYSIS**

ANAMIKA YADAV

SUPERVISOR- DR. ANDREJS BRAUN

MSc CANCER AND CLINICAL ONCOLOGY
BARTS AND THE LONODN SCHOOL OF MEDICINE AND DENTISTRY
QUEEN MARY UNIVERSITY OF LONDON

SEPTEMBER 2022

WORD COUNT ~ 9000

DECLARATION

As the author of this manuscript, I declare that all the work presented here is original and mine unless otherwise indicated. Microsoft Excel, R programming language and BioRender (<https://biorender.com/>) were used to create the figures. There is an acknowledgement of information obtained from other sources.

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my deepest gratitude to my supervisor, Dr. Andrejs Braun. This endeavour would not have been possible without my supervisor's constant support, feedback, and motivation.

My classmates and friends have also been instrumental in providing me with feedback late at night and moral support throughout the process.

In closing, I would be remiss if I did not acknowledge the support and encouragement from my parents while pursuing my master's degree and, even more so, during my dissertation.

TABLE OF CONTENTS

ABSTRACT	6
ABBREVIATIONS	8
1. INTRODUCTION	
1.1 Lung cancer epidemiology and incidence	9
1.2 Classification of lung cancers	10
1.3 Molecular pathogenesis of NSCLC	12
1.4 Current treatment strategies for NSCLC	13
2. CURRENT LANDSCAPE OF IMMUNOTHERAPY IN NSCLC	18
2.1 Immunity in health and cancer	18
2.2 Efficacy of pivotal trials	22
2.3 Strategies for patient selection	24
3. AIMS AND OBJECTIVES OF THE DISSERTATION	26
4. METHODS	27
4.1 Search strategy	27
4.2 Selection criteria	27
4.3 Data extraction	27
4.4 Quality assessment	28
4.5 Heterogeneity test	28
4.6 Statistical data analysis and primary endpoints	28
5. RESULTS	31
5.1 Systematic literature search	31
5.2 Baseline study characteristic	33
5.3 Risk of bias and methodology assessment	36
5.4 Network graph	37
5.5 Comparison of overall survival	38
5.6 Comparison of progression free survival	45
5.7 Comparison of overall response rate	51
5.8 Bayesian rankings	54
5.9 Assessment of heterogeneity and transitivity	56

6. DISCUSSION	57
6.1 Principal findings	57
6.2 Applicability of included trials	65
6.3 The Bayesian NMA heterogeneity	65
6.4 Strengths and limitations of this analysis	66
6.5 Future research implications	67
7. CONCLUSION	69
8. REFERENCES	70
9. APPENDICES	79

ABSTRACT

BACKGROUND

Immunotherapy has revolutionised cancer treatment with its unprecedented survival advantages over chemotherapy. This Bayesian network meta-analysis (NMA) was conducted to suggest the best treatment regimen for advanced non-small cell lung cancer (NSCLC) in patients with high programmed death ligand-1 (PDL-1) levels via gathering and analysing the available data from randomised control trials (RCT) comparing immune checkpoint inhibitors (ICI) with chemotherapy.

METHODS

Through a systematic search protocol, 18 RCTs with a total of 7259 patients with dichotomous variables overall survival (OS), progression-free survival (PFS) and overall response rate (ORR) as the primary endpoints were incorporated in this Bayesian NMA. Dichotomous variables OS, PFS and ORR, hazard ratios (HRs), and their 95% confidence intervals (CI) were also extracted.

RESULTS

A total of 7259 patients from 18 phase II and III RCTs, including six different treatment regimens, were incorporated in this Bayesian NMA. ICI dual therapy had the highest surface under the cumulative ranking (SUCRA) plot in terms of OS, and PFS (92%, 99%, respectively), followed by ICI monotherapy (OS-62% and PFS-74.8%). In terms of ORR, ICI plus chemotherapy had the highest SUCRA score (77.5%). The addition of chemotherapy to immunotherapy significantly improves the overall response rate, pooled odd's ratio of 0.06 (95% CI 0.02-0.16). However, ICI monotherapy failed to demonstrate superiority over ICI plus chemotherapy in ORR.

CONCLUSION

Based on this Bayesian NMA, ICI dual therapy is most likely the best treatment option for advanced NSCLC. Moreover, the addition of conventional chemotherapy may be an appropriate alternative treatment modality for advanced NSCLC with high PD-L1 levels.

KEYWORDS

Non-small cell lung cancer; immunotherapy; immune surveillance; Bayesian network meta-analysis; literature review

ABBREVIATIONS

ABBREVIATIONS	DEFINITIONS
ALK	Anaplastic lymphoma kinase
BPR	Bayesian probability ranking
CTLA4	Cytotoxic T-lymphocyte associated protein-4
DC	Dendritic cell
EGFR	Epidermal growth factor receptor
HR	Hazard's ratio
ICI	Immune checkpoint inhibitor
IFNy	Interferon-γ
MCMC	Markov-chain monte-Carlo
MEK	Mitogen activated protein kinase-kinase
mTOR	Mammalian target of rapamycin
NMA	Network meta-analysis
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
OR	Odd's ratio
OS	Overall survival
PARP	Poly-adenosine diphosphate-ribose polymerase
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand 1
PFS	Progression-free survival
PI3K	Phosphoinositide 3-kinase
SCLC	Small cell lung cancer
SUCRA	Surface under the cumulative ranking
TAA	Tumour-associated antigens
TAM	Tumour-associated macrophages
TIL	Tumour-infiltrating lymphocytes
TKI	Tyrosine kinase inhibitor
TMB	Tumour mutational burden
WHO	World health organisation

1. INTRODUCTION

1.1 LUNG CANCER EPIDEMIOLOGY AND INCIDENCE

Lung cancer is the most common cause of cancer-related morbidity, with approximately 2.2 million cases accounting for 11.4% of all cancer types diagnosed worldwide and 1.8 million deaths yearly [1]. It is the most frequently occurring cancer in men and a leading cause of cancer-related deaths after prostate (11.7%) and colorectal cancer (10%) [1]. The incidence of lung cancer is highest in people aged 85-89, with about 48,500 cases in the UK alone [2]. The 5-year survival rates are dismally low, with a slight improvement in the past 40 years. Lung cancers diagnosed at earlier stages have better survival outcomes (88%) as opposed to those diagnosed at advanced stages (19%) (Figure 1) [2].

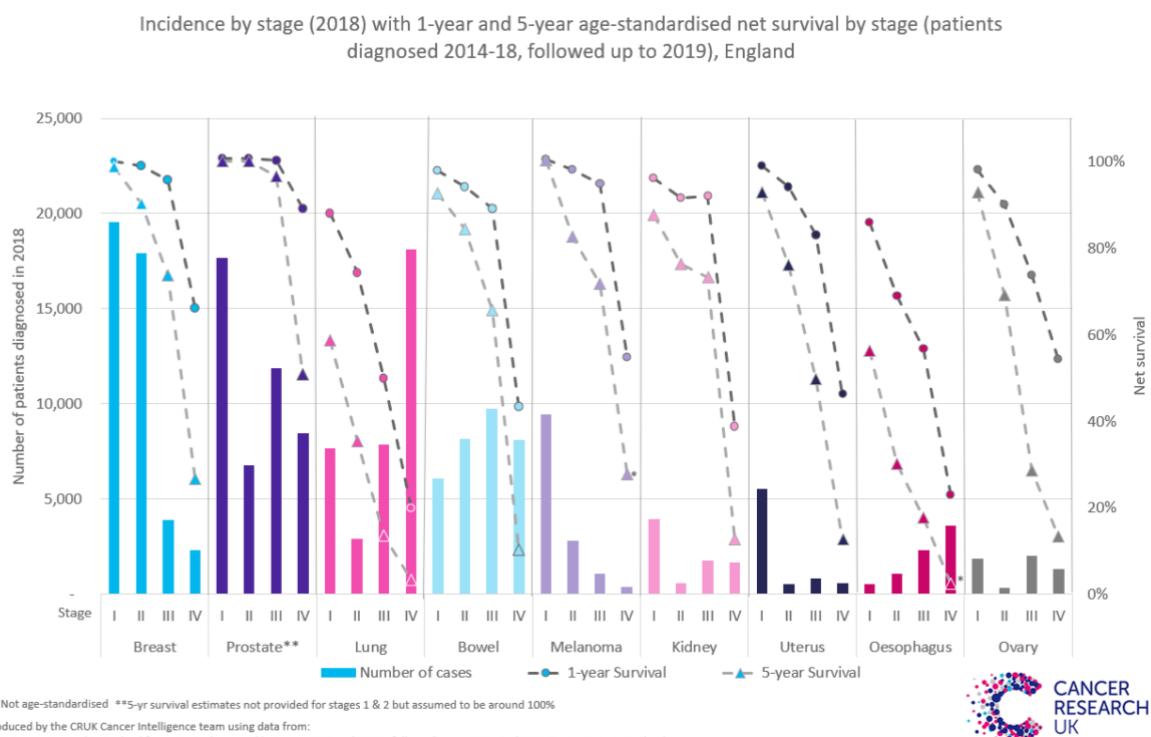


FIGURE 1. INCIDENCE BY STAGE WITH 1-YEAR AND 5-YEAR OVERALL SURVIVAL RATES OF DIFFERENT CANCERS IN 2018 [2].

Lung cancer survival rates drops drastically despite maximum number of cases (~ 18,000) diagnosed at stage IV. Patients diagnosed at stage II have the best survival outcome followed by stage I and stage III. Taken from [<https://crukcancerintelligence.shinyapps.io/EarlyDiagnosis/>]

1.2 CLASSIFICATION OF LUNG CANCERS

Lung cancer is known to arise from the tracheobronchial tree and alveolar sac lining, eventually leading to pluripotent stem cells that can differentiate into any form of cancer. It is a group of heterogeneous diseases with a wide range of clinicopathological features. WHO has classified lung cancer into two main histological types, non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), accounting for about 85% and 15% of all lung cancer [3] subtypes, respectively (Figure 2). With advances in molecular profiling, the classification systems have been regularly updated with a critical role in selecting the most active regimen for a particular patient. Some molecular signatures may also have prognostic value in lung cancer. For example, testing for EGFR mutations and ALK fusions has enabled oncologists to identify patients most likely to respond to the targeted drugs like TKIs (Erlotinib) and Crizotinib. Moreover, lung cancers have played a crucial role and revolutionised the development of targeted therapies in cancer

NSCLC is further classified into adenocarcinoma, which makes up the most commonly diagnosed NSCLC subtype, followed by squamous cell carcinoma. Incidence of squamous cell neoplasms have dropped drastically in the last century mainly due to decrease in the smoking incidence and changes in the composition of cigarettes. Somatic mutations (mutations occurring only in the tumour cells) and high number of chromosomal aberrations are the hallmark of lung neoplasms (discussed in section 1.3). These molecular aberrations serve as potential biomarkers to screen lung cancers. Figure 2 further shows key oncogenes that drive human pulmonary carcinogenesis.

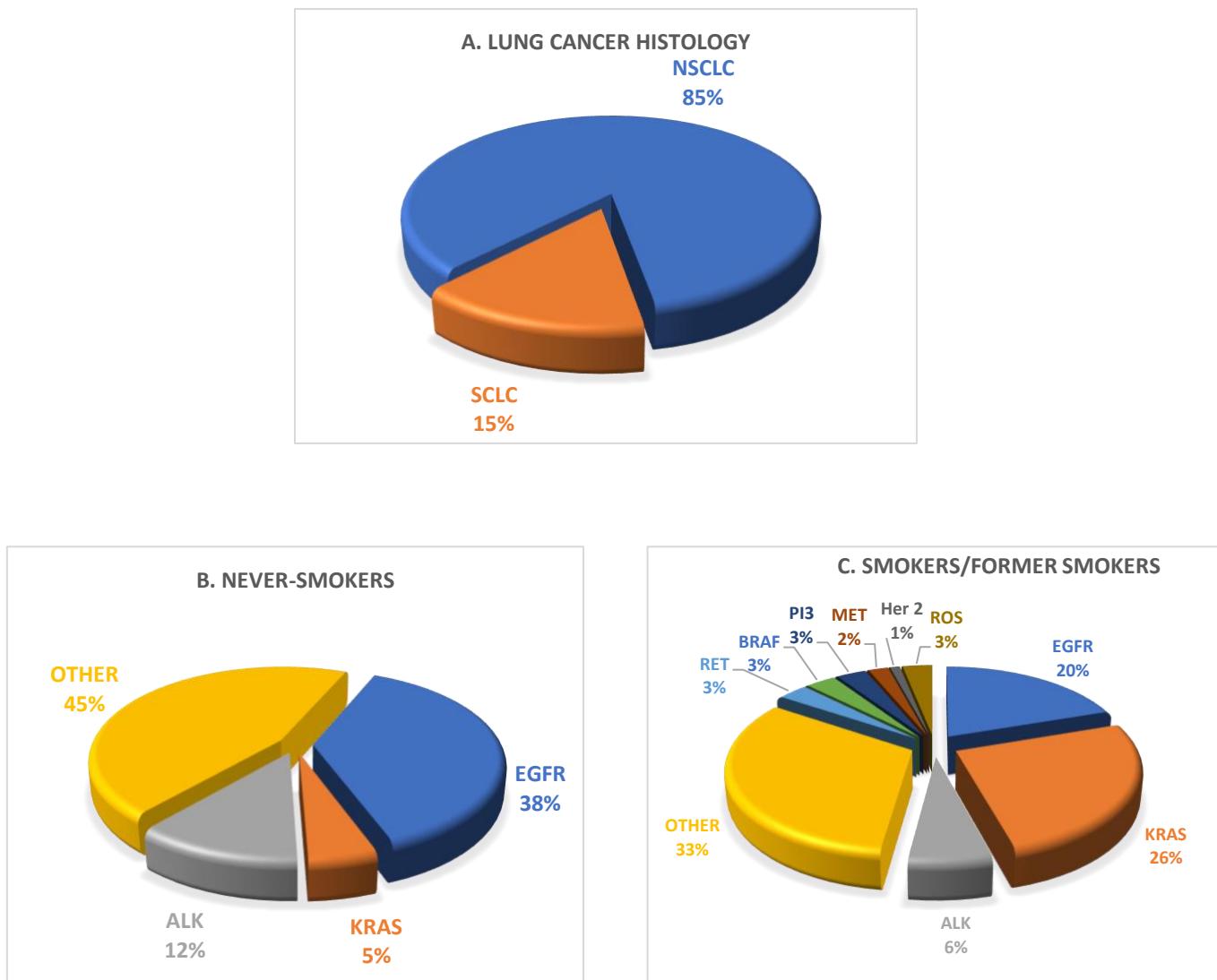


FIGURE 2. LUNG CANCER HISTOLOGY AND MOLECULAR PROFILES.

A) Lung cancer is divided into NSCLC and SCLC.

B) Compared with current and former smokers, never-smokers have different mutation spectra of lung adenocarcinomas. Never-smokers have a higher prevalence of ALK fusions and other targetable mutations (such as EGFR mutations) than current or former smokers. However, 45% of driver mutations have not yet been identified.

C) On the other hand, smokers or former smokers have growing number of pro-oncogenic driver mutations including MET, ROS1, BRAF, HER2 and PI3.

NSCLC- Non small cell lung cancer, EGFR- Epidermal growth factor receptor, ALK- Anaplastic lymphoma kinase, KRAS- Kristen rat sarcoma viral oncogene homolog, MEK- Mitogen activated kinase, kinase, HER-Human epidermal growth factor receptor, PI3K- Phosphoinositide 3-kinase, BRAF- v-raf murine sarcoma viral oncogene homolog B1.

1.3 MOLECULAR PATHOGENESIS OF NSCLC

Tobacco smoking accounts for about 90% of all diagnosed NSCLC cases worldwide [4]. Carcinogens can induce field cancerization effect through induction of enzymes or formation of DNA adducts which eventually results in DNA mis-replication and mutations. NSCLC is more common in former smokers, as suggested by the accumulation of various molecular aberrations due to smoking. It initiates a cascade of tumorigenesis and leads to neoplasms. It is a multi-step process and is remarkably diverse and complex (Figure 3). For example, the development of squamous cell carcinoma is preceded by hyperplasia and squamous dysplasia, eventually resulting in carcinoma-in situ (Figure 3) before it progresses to invasive carcinoma [5,6]. Adenocarcinoma harbours about 26 regions of recurrent arm-level amplifications and deletions, 98 detectable breakpoints and 31 regions of recurrent focal copy number gain and loss [7].

On the other hand, in never-smokers, adenocarcinoma is preceded by atypical adenomatous hyperplasia (AAH). AAH is a lung parenchymal lesion arising in the terminal alveoli. However, it can be sequentially reviewed using Hanahan and Weinberg's hallmarks of cancer elsewhere [8].

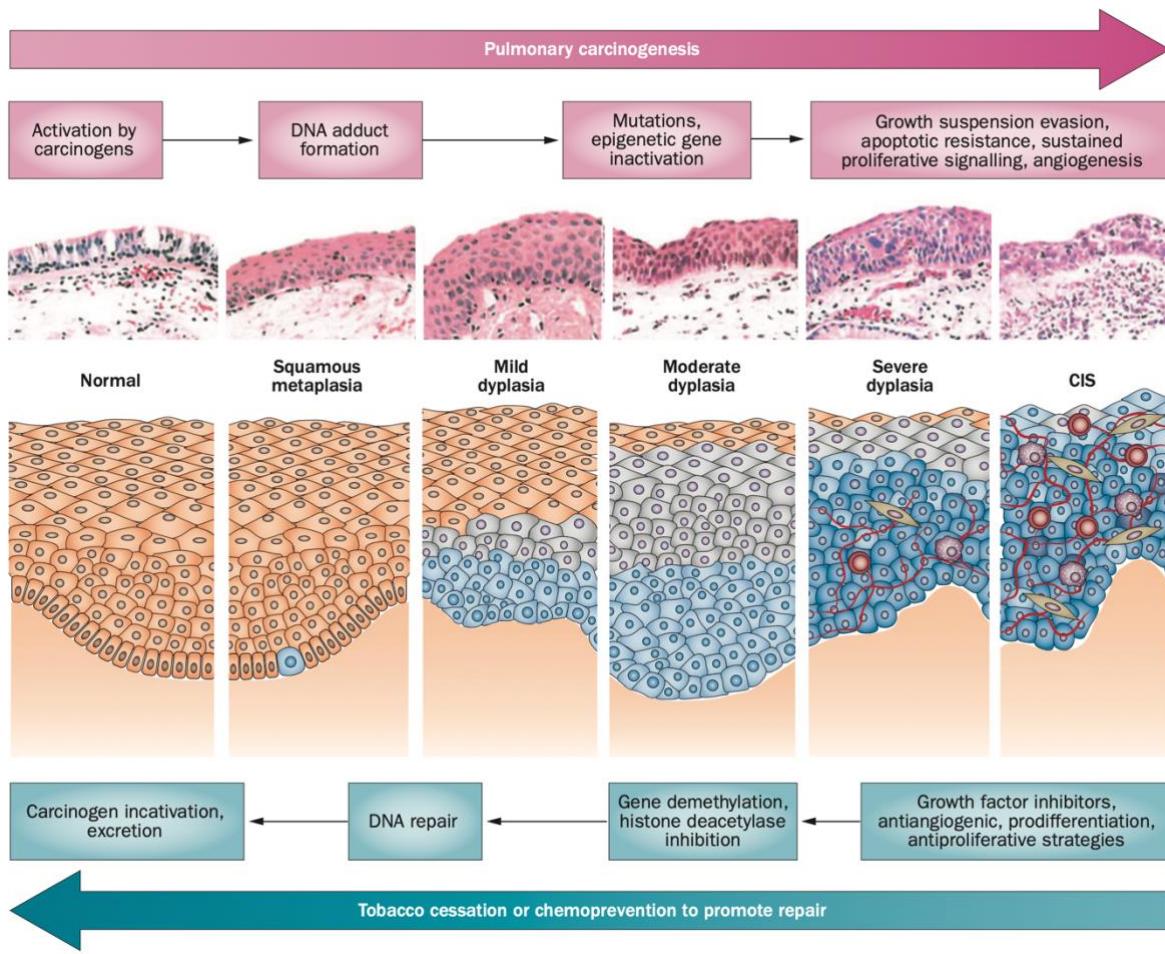


FIGURE 3. MULTISTEP HYPOTHESIS IN THE DEVELOPMENT OF A LUNG CANCER [8].

Constant insults to pulmonary epithelium due to chronic smoking lead to loss of crucial tumour suppressor genes and overexpression of oncogenes. Cumulative molecular aberrations result mainly from the field cancerization effect leading to oncogenic changes in the pulmonary epithelium owing to an accumulation of genetic abnormalities over a long period. Squamous cell carcinoma of the lungs is usually preceded by hyperplastic and dysplastic changes (squamous metaplasia and mild to severe dysplasia) in the epithelium, eventually resulting in CIS and lung neoplasms. CIS- carcinoma in-situ [8].

1.4 CURRENT TREATMENT STRATEGIES FOR NSCLC

As discussed earlier, 40% of the NSCLC cases are diagnosed at advanced stages and have dismally low overall survival rates. Cases diagnosed at stage I have a 5-year survival of 80%, gradually decreasing to 16-30% with stages II and III. Surgical resection is the standard for patients with early-stage NSCLC and a few stage IIIA. Adjuvant chemotherapy has been demonstrated to increase the survival rates by 5-

10% among stage II and IIIA but is associated with significant adverse effects. In managing resectable early-stage NSCLC, video-assisted thoracoscopic surgery (VATS) is an increasingly popular alternative to open thoracotomies [9]. There were no significant differences in long-term oncological outcomes between VATS and the standard procedure after the first year of surgery. Patients with stage I or stage II cancer typically receive anatomical resection (lobectomy) due to the possibility of local recurrence after sub-lobar resections [9].

For medically unfit patients with inoperable tumours, fractionated radiotherapy (4-6 weeks) is an emerging treatment option. As determined by the CHISEL phase 3 trial, stereotactic ablative body radiotherapy (SABR) significantly reduced the number of patients with relapse compared to standard radiotherapy (14% vs 31%; HR 0·32, 95% CI 0·13–0·77) [10]. According to the Lung ART study, postoperative radiotherapy is not beneficial after resectioning mediastinal nodal (N2) NSCLC [11]. After a complete resection of an NSCLC with nodes in the mediastinum, radiation therapy was previously considered an option following surgery. Overall survival rates for postoperative radiotherapy were 66.5%, and for observation, they were 68.5%. The number of cardiopulmonary-related deaths among radiation therapy patients and observation groups was also 16.6% and 2%, respectively. Therefore, postoperative radiotherapy is no longer routinely recommended for mediastinal nodal masses that have been completely removed. Figure 4 shows the current treatment algorithm for the management of NSCLC.

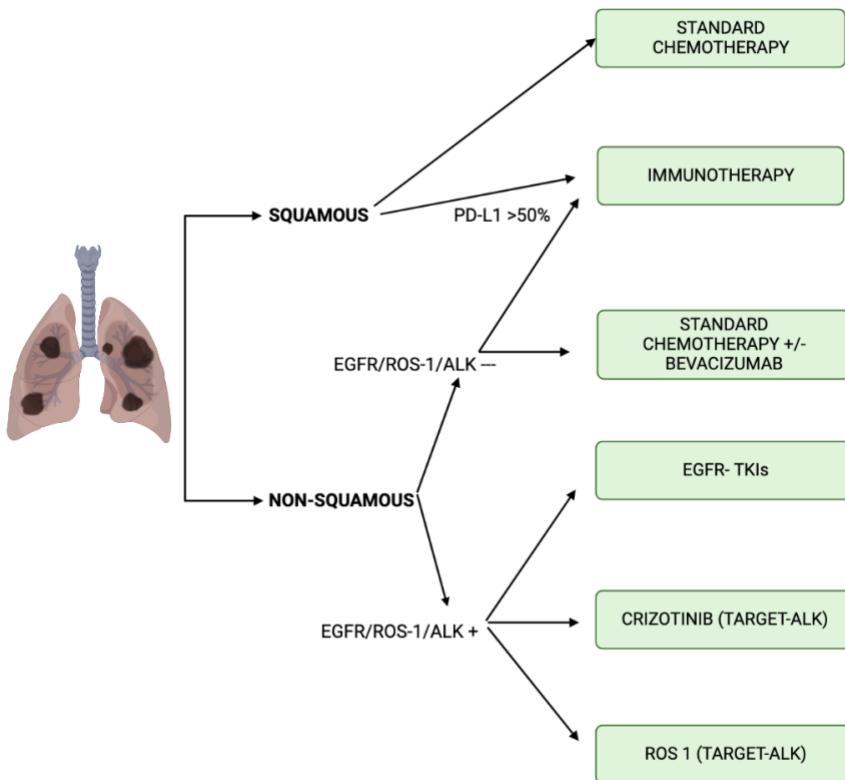


FIGURE 4. TREATMENT ALGORITHM FOR THE MANAGEMENT OF NSCLC

As first-line therapy, the platinum doublet is usually administered for four to six cycles for metastatic NSCLC, with second-line treatment offered if cancer progresses. Recently, improved treatment strategies have resulted from molecular testing of EGFR and ALK. The importance of providing a range of treatment options does not end with ensuring that the best drugs are administered first. Once the best drugs have been administered, a wide range of treatment options becomes increasingly essential. NSCLC- non-small cell lung cancer, EGFR- Epidermal growth factor receptor, ALK- Anaplastic lymphoma kinase, TKI- tyrosine receptor inhibitor, ROS- Reactive oxygen species.

Patients diagnosed with advanced or metastatic NSCLC are usually treated with the intent of improving survival. Cytotoxic chemotherapy agents are the mainstay for such patients, which may alter based on patient-related factors like performance status (PS), histological subtype and other comorbidities [12]. Different chemotherapeutic regimens, including platinum (carboplatin or cisplatin) combined with paclitaxel, and nab-paclitaxel, are administered to patients with PS 0-1 [13]. However, no single combinatorial regimen has proved to be advantageous over the other. Some studies have demonstrated clear evidence that pemetrexed has a higher survival benefit than

platinum but is associated with severe toxicities [14,15]. For patients with PS-2, only one drug regimen is advised, mainly not platinum [16]. Due to increasing toxicities in patients with PS-3 associated with cytotoxic drugs, palliative care is the primary intent of the treatment for such patients.

NSCLC is a heterogeneous disease with specific ontologies. Multiomics and preclinical research have made it possible to pave the way for personalised medicine. Novel agents targeting (Figure 5) the various signalling pathways MEK inhibitors, PI3K/mTOR pathway inhibitors, notch, hedgehog inhibitors, and targeted gene therapies have yielded optimistic results in multiple clinical trials [17-20].

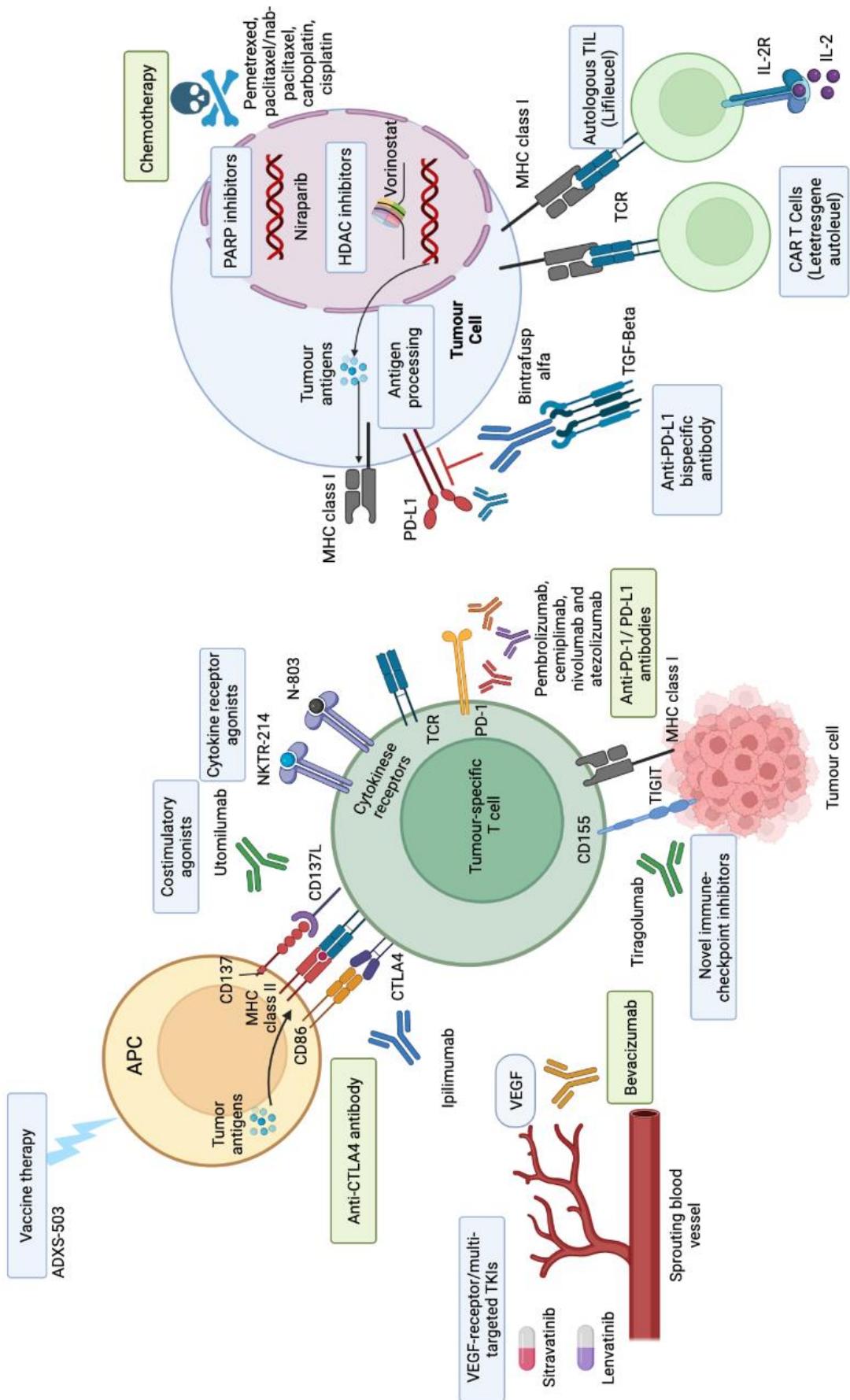


FIGURE 5. EXISTING AND NOVEL THERAPEUTIC TARGETS IN NSCLC.

Immune profile of NSCLC is depicted above and shows FDA approved treatment modality as well as novel therapeutic targets. Examples of few individual drugs are given within each modality.

APC-Antigen presenting cell, TAA-Tumor associated antigen, IL- interleukins, TCR- T cell receptor, HDAC- Histone deacetylase, TIL- tumor-infiltrating lymphocyte, TKI- Tyrosine kinase inhibitor, TIGIT- T cell immunoreceptor.

2. IMMUNOTHERAPY FOR NON-SMALL CELL LUNG CANCER: CURRENT STATE-OF-THE-ART

Over the past two decades, we have gained a better understanding of the molecular underpinnings of NSCLC. Thus, the first-line treatment paradigm has been completely changed for patients with advanced-stage disease. Molecular biomarkers have been developed because of oncogenic driver alterations, which have contributed to the development of these therapies. Many modern therapeutic efforts involve immune-checkpoint inhibitors (ICIs), specifically therapeutic antibodies targeting PD-1/PD-L1. Incidence-based mortality for NSCLC has decreased almost double since 2013 (3.2% from 2006-2013 & 6.3% from 2013-2016) [21].

Unlike chemotherapy, ICIs can exert a durable response even after the cessation of immunotherapy, indicating a possibility of generating a long-lasting, tumour-specific memory [22]. Due to varied heterogeneity in NSCLC, patients tend to develop clinical resistance against single-agent regimens. Studies have demonstrated that the most effective antitumour strategy would be to target the non-redundant immunological pathways [23]. Consequently, complete activation of endogenous tumour immunity can occur in the host. A four-pronged approach would call for the induction of immunogenic death of cancer cells: abrogation of immune suppression, enhancement of antigen presentation or adjuvanticity, and stimulation of immunological effector cells' activation and survival.

2.1 IMMUNITY IN HEALTH AND CANCER

The immune system has paradoxically been demonstrated to constrain and promote tumour progression through conceptual developments over the past two decades. In the nineteenth century, the immune system was first deployed for treating cancer.

Back in the late 1880s, Busch and Fehleisen investigated the relationship between immune status and cancer. Infections caused by *Streptococcus pyogenes*, a common skin infection, caused tumours to regress spontaneously [24]. It is well known that immune systems are complex and can distinguish between self and non-self-antigens, thus preventing foreign pathogens from infecting their hosts. There are two components of an immune system: the innate immune system and the adaptive immune system, each responsible for simultaneously eliminating pathogens [25]. Cancer immunoediting involves elimination, equilibrium, and escape in its most complex form (Figure 6) [26].

During the elimination phase, innate and adaptive immune systems can recognize and destroy cells that have escaped cell-intrinsic mechanisms of tumour suppression. Rarely, tumour subclones that survive elimination may progress to the equilibrium phase, where they are slowed and, inevitably, stymied over time [27]. It is possible, however, for tumour cells to develop immunogenic subclones with reduced immunogenicity due to constant pressure from the adaptive immune system and increased tumour mutational burden. The immune system can detect and destroy these subclones in some cases. It is possible that this selection process does not involve the death of tumour clones but instead involves other features like lost antigen presentation, diminished PD-L1 expression due to epigenetic mutations or lowered T-cell production [28]. During the last phase, there is clear evidence of clinical disease progression. There is compelling evidence from various clinical trials and patients receiving immunotherapy suggestive of reoccurrence of cancer immunoediting in part or entirely as a response to immunotherapy [29].

Despite optimistic results, like every other treatment modality, immunotherapy poses critical barriers owing to innate (primary) and acquired immunological resistance (secondary) (Figure 6). Resistance to ICIs is mainly driven by the genomic and proteomic profile of NSCLC [23]. Mutations regulate immune responses and self-tolerance in inhibitory immune-signalling pathways. The function of these pathways is to prevent uncontrolled reactions and collateral damage during physiological immune responses. Cells that express inhibitory signals such as PD-L1 enable cancerous cells to evade the immune response. It is also possible to attribute immunotherapy resistance to oncogene addiction. Other main mechanisms include the upregulation of co-existing signalling pathways like PTEN and the Wnt-catenin pathway that modulate the TME [23]. Production of cytokines and growth factors by TME results in an immunosuppressive atmosphere which further adds to the risk of developing resistance to ICIs.

In the advanced stages of NSCLC, ICIs have changed the treatment landscape dramatically. As checkpoint blockade is increasingly resistant to secondary and primary resistance, alternative immune modulation approaches are required. One of the most researched approaches is the addition of chemotherapy and antiangiogenic agents to the standard immunotherapy regimens. Several RCTs have demonstrated a favourable survival outcome for such patients owing to the ability of cytotoxic agents to modulate different immune responses and induction of immunogenic cell death (discussed in subsequent sections).

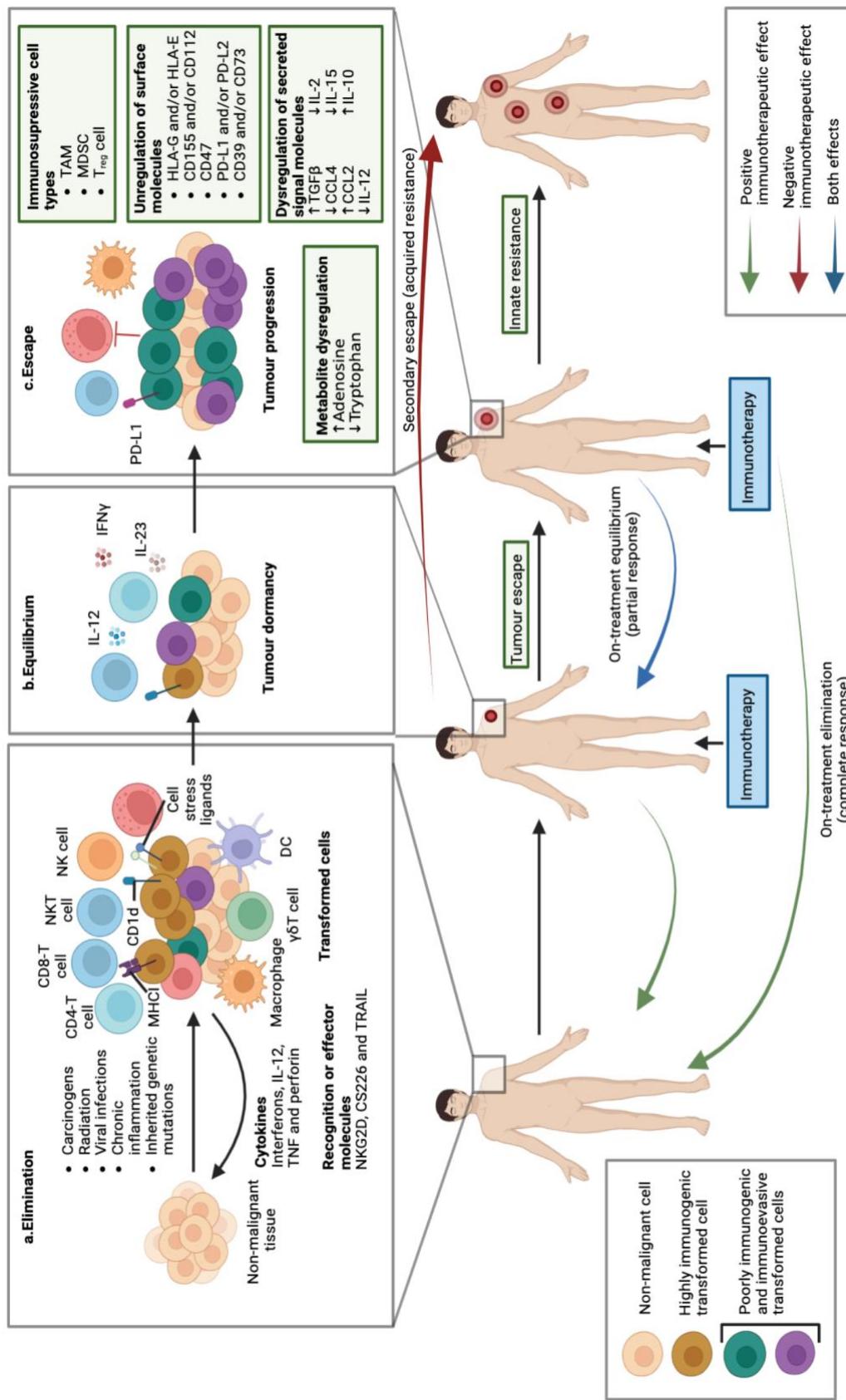


FIGURE 6. CANCER IMMUNOEEDITING DEMONSTRATING 3 ES.

A) Three phases are involved in cancer immunoediting: elimination, equilibrium, and escape. As a result of intrinsic tumour suppression, both the innate and adaptive immune mechanisms work together during the elimination phase to eradicate transformed cells before they become clinically detectable. Once tumour cells have been destroyed, immunoediting has been completed. **B)** During survival, the tumour develops a state of equilibrium over which its growth is restricted. Adaptive immune systems modify the production of these proteins in a tumour to alter its immunogenicity. **C)** This phase is characterized by the activation of immuno-suppressive and immuno-evasive pathways, which allows the tumours to grow unhindered. An escapee tumour is visible to the naked eye and has escaped from the body. Immunotherapy without promoting antitumour immunity after cancer-induced immune suppression will not produce an objective response. It is, therefore, necessary to consider innate resistance.

Usually, secondary escape occurs when cancer cells circumvent or suppress the immune response to the tumour, resulting in acquired resistance to chemotherapy. On the other hand, tumours can be driven back into the elimination stage by the immune system, resulting in complete response. Even though immunotherapy cannot permanently remove all tumour-induced immune suppression, the tumour may enter a partial response during treatment if it cannot remove all tumour-induced immune suppression. DC-Dendritic cell, NK- natural killer cell, IL- interleukins, TNF- Tumor necrosis factor, MHC- Major histocompatibility complex, PD-L1- Programmed cell death ligand 1, TAM- tumor associated macrophage

2.2 EFFICACY OF PIVOTAL TRIALS

Pembrolizumab, Atezolizumab and Nivolumab are the three main single-agent ICI that have been vigorously tested for safety and efficacy in phase III clinical trials. OAK, a double-blind phase III study [30], compared ICI-single agent, Nivolumab, with Docetaxel in both the histologies. PDL-1 levels were not mandated for inclusion in the study. Interim analyses revealed a significant improvement in the atezolizumab arm with the control arm, 13.8 vs 9.6m, respectively, HR 0.73 (0.62-0.87. Patients in the experimental arm had much better safety profiles (15% vs 43%). Some studies have also demonstrated that patients with brain metastases benefit greatly from Atezolizumab. Similar clinical benefits were also observed in another phase III study, CHECKMATE 017, which compared Nivolumab with standard doublet chemotherapy as a second-line treatment option for patients with advanced non-small cell lung cancer and squamous histology [31]. As part of this study, patients were given nivolumab 3 mg/kg every two weeks or Docetaxel 75 mg/m² every three weeks until the disease progressed, or unacceptable toxicities occurred. The OS with Docetaxel was significantly shorter, 6.2m vs 9.2m; HR, 0.59; 95% CI(0.44-0.79), while the ORR with nivolumab was 20%, whereas the ORR with Docetaxel was 9%. A 1%, 5%, or 10% stratification of PD-L1 expression failed to demonstrate any predictive value [31].

According to the primary efficacy analysis, nivolumab did not improve overall survival or progression-free survival for patients with PD-L1 TPS of 1%. On the other hand, CHECKMATE 026 study, which compared Nivolumab with Docetaxel in untreated NSCLC patients, failed to show any survival benefits [32]. Further exploratory subgroup analysis did not show a significant difference in PFS and OS among patients with PD-L1 and TPS over 50%. In the exploratory analysis based on the missing 243 missense mutations in the TMB, patients with a high rate of TMB had longer PFS

(9.7m vs 5.8 m; HR, 0.62; 95% CI, 0.38-1.01). OS remained unchanged regardless of TMB.

All in all, several studies (Table 1) have demonstrated a definite survival advantage over standard doublet chemotherapy and a better toxicity profile. Despite the heterogeneous cut-offs and diagnostic methods employed in those trials, whether PD-L1 expression should be considered when selecting patients for second-line immunotherapy remains unclear. Immunohistochemistry can be used to detect PD-L1 expression in tissue. To categorize the expression of PD-L1 on tumour cells, the 22C3 pharmDx assay (Agilent) was used in the pivotal trials of pembrolizumab [33,34].

CLINICAL TRIALS	TREATMENT	MEDIAN SURVIVAL	p-VALUE
First line: ICI vs chemotherapy			
RECK <i>et al.</i>	Pembrolizumab vs platinum doublet	HR 0.60 (95% CI 0.41–0.89)##	0.005
MOK <i>et al.</i>	Pembrolizumab vs platinum doublet	16.7 months vs 12.1 months	0.0018
CARBONE <i>et al.</i>	Nivolumab vs platinum doublet	14.4 months vs 13.2 months	NS
First line: ICI + chemotherapy vs chemotherapy			
PAZ-ARES <i>et al.</i>	CBDCA-(nab)PTX ± pembrolizumab	15.9 months vs 11.3 months	<0.001
GANDHI <i>et al.</i>	CDDP/CBDCA-PEM ± pembrolizumab	NR vs 11.3 months	<0.001
SOCINSKI <i>et al.</i>	CBDCA-PTX-beva ± atezolizumab	19.2 months vs 14.7 months	0.02
Second-line			
BRAHMER <i>et al.</i>	Nivolumab vs docetaxel	9.2 months vs 6 months	<0.001
BORGHAEI <i>et al.</i>	Nivolumab vs docetaxel	12.2 months vs 9.4 months	0.002
HERBST <i>et al.</i>	Pembrolizumab vs docetaxel	10.4/12.7 months vs 8.5 months	<0.001
RITTMAYER <i>et al.</i>	Atezolizumab vs docetaxel	13.8 months vs 9.6 months	0.0003
Adjuvant in stage III			
Antonia <i>et al.</i>	Durvalumab vs placebo	NR vs 28.7 months	0.0025

HR: hazard ratio; NS: not significant; NR: not reached; CBDCA: carboplatin; PTX: paclitaxel; CDDP: cisplatin; PEM: pemetrexed; beva:bevacizumab. #: no median survival available in the manuscript.

TABLE 1. LANDMARK TRIALS AND THEIR SELECTED RESULTS COMPARING ICI AND CHEMOTHERAPY

Several studies have demonstrated significant improvement in the survival outcomes with immunotherapy in first- and second-line settings [30-41].

2.3 STRATEGIES FOR PATIENT SELECTION

An illustration of the first-line treatment algorithm can be found in Figure 7. No other biomarkers have been approved for use in selecting patients for immunotherapy other than the immunohistochemical expression of PD-L1. Different companies use different PD-L1 assays. For instance, Dako 28-8 and Ventana SP263 are used for Nivolumab and Durvalumab, respectively, resulting in different cut-off values and interpretations. However, some patients with high levels of PD-L1 do not respond to an anti-PD-1/PD-L1 inhibitor, while others with low levels of PD-L1 do. The presence of PD-L1 is still a partial indicator in this regard.

On the other hand, researchers are constantly working on new biomarkers, such as TMB, to predict the response of patients with NSCLC to ICIs [42]. Neoantigens are more likely to be produced in tumours with a high TMB. MHC may induce a T cell response depends on the immunogenicity of neoantigens. ICIs may facilitate tumour regression by reactivating an immune response that has been suppressed or stifled. Several studies have demonstrated that ICIs have greater efficacy against NSCLC with high TMB [43]. Despite some initial optimistic results, there has limited clinical application of TMB due to its lack of accuracy in predicting OS [44]. For example, studies like CHECKMATE 227 [45], OAK [30] and MYSTIC [43] considered TMB as an independent marker. In these studies, TMB was achieved by most patients, and the rate of high TMB (>10 muts/MB) was generally between 23%-30%. Furthermore, a significant correlation was found between haematological and tissue TMB, implying that it is easier to test the blood TMB. Additionally, TMB results may vary depending on the sequencing platform, and this testing method can be expensive for routine clinical usage, just as PD-L1. Albeit the clinical limitations, TMB can serve as a

complementary biomarker to PD-L1. However, future prospective trials are warranted to elucidate and establish the clinical utility of TMB as a potential investigational biomarker. Moreover, refinement measures that focus on harmonizing assays and the uniform identification of predictive cut-off values could help us understand the nuances of this biomarker.

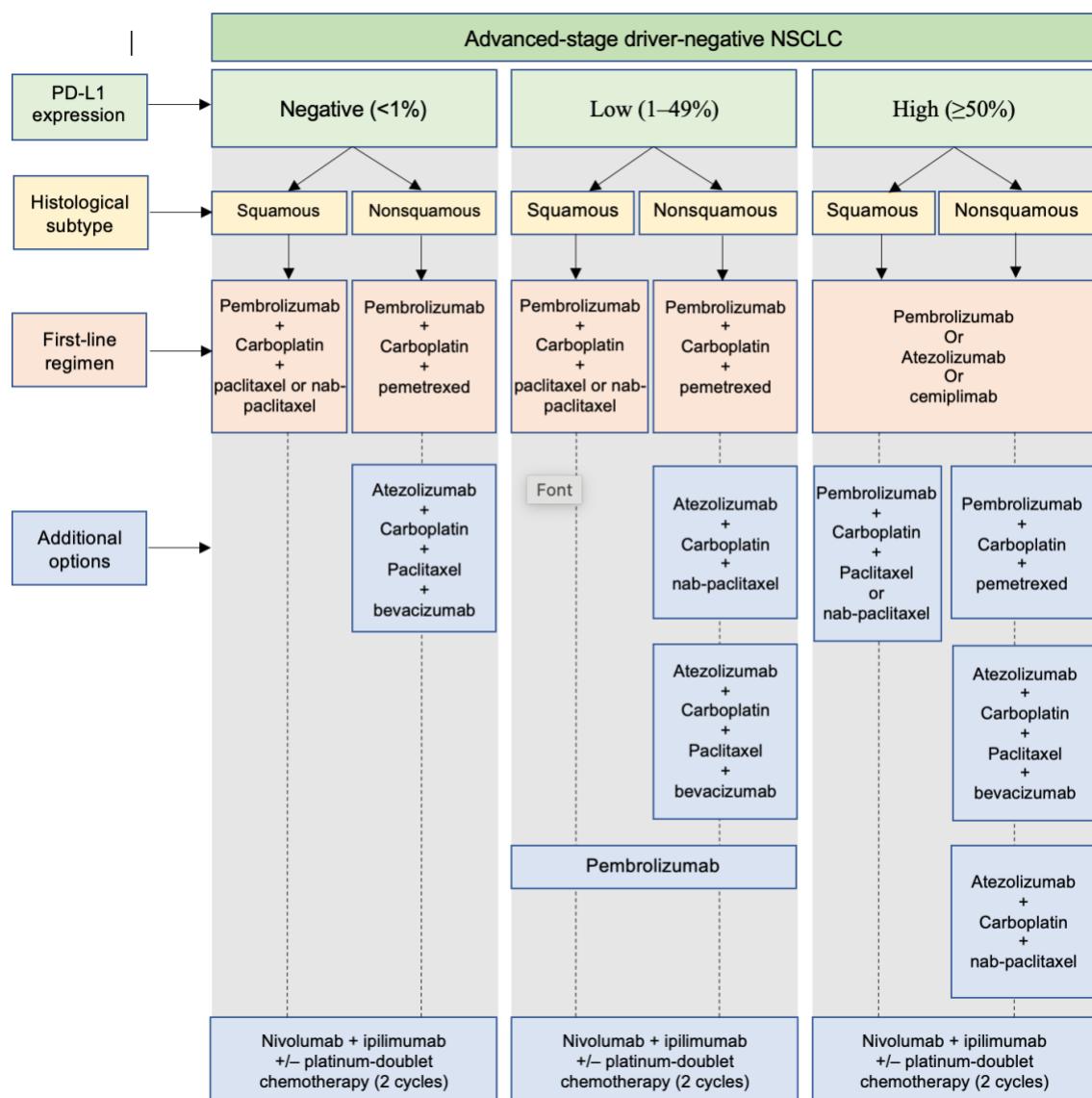


FIGURE 7. PROPOSED ICI-BASED MANAGEMENT OF PATIENTS WITH ADVANCED NSCLC (DRIVER NEGATIVE).

3. AIMS AND OBJECTIVES OF THE DISSERTATION

Until a more profound discovery can be made to identify a potentially curative intervention in this field, the initiative is intended to provide incremental patient benefits. It has been demonstrated that chemotherapy and anti-PD-1 inhibitors can have synergistic antitumor effects when combined, as cytotoxic agents release high levels of tumour antigens and restore immune surveillance. Several randomised controlled trials have been conducted to test the effectiveness of ICIs in combination with chemotherapy, antiangiogenic agents, and ICIs in combination with other ICIs. In light of the increasing number of studies on ICI combinations, we must determine the most effective strategy for future clinical trials comparing ICI combinations with standard chemotherapy.

A Bayesian NMA allows comparisons from direct and indirect evidence and different combinatorial regimens to a common comparator. Due to the absence of head-to-head trials, all first-line ICI combinations currently available for advanced NSCLC patients were compared for efficacy in this study. The Bayesian inference approach was used for systematic reviews and network meta-analyses.

A benefit-to-risk ratio for ICIs will be calculated by comparing the data extracted from various RCTs to determine if anti-PD-1/PD-L1 immunotherapy is the "new paragon" in treating advanced NSCLC and, if so, which regime provides the most significant benefit. In addition to providing answers to unmet clinical needs, statistical analyses will provide insights into current challenges in managing advanced NSCLC.

4. METHODS

4.1 SEARCH STRATEGY

A systematic literature review (SLR) was conducted based on Prisma guidelines (*Appendix 1*) [46]. A pre-defined systematic protocol was generated from PRISMA-P 2015 statement, which defined the population, intervention, comparator, and outcome (PICO) framework. A comprehensive search of databases from PubMed, clinicaltrials.gov, EMBASE, CENTRAL, HTA, Scopus, and Google scholar was conducted. The search was limited to phase II and III randomized control trials related to advanced or metastatic NSCLC. A manual search was also conducted for the reference list across the original studies. The keywords used were ‘advanced NSCLC’, ‘metastatic NSCLC’, ‘immunotherapy’, ‘immune checkpoint inhibitors’, ‘Pembrolizumab’, ‘Atezolizumab’, ‘Cemiplimab’, ‘Durvalumab’, ‘PD-L1 inhibitors’ (*Appendix 2*). The language was set to English.

4.2 SELECTION CRITERIA

According to the PICO framework, all the eligible studies (phase 3 clinical trials) were subjected to inclusion criteria (*Appendix 3*) to minimize bias and heterogeneity. Exclusion criteria are also enumerated in *Appendix 3*.

4.3 DATA EXTRACTION

Full article texts of the clinical trials were examined for the main title, summary, and supplementary materials, along with evaluating the eligibility criteria. The data extraction manual was accompanied during the data collection (*Appendix 4*). Baseline characteristics and primary endpoints were extracted from the trials and tabulated in a spreadsheet (*Supplementary sheet 1*) by AY. The data extracted for clinical

appraisal were: - trial name, trial identifier, author and year, sample size, the number of participants belonging to the high PDL-1 (>50%) level subgroup, the histological subtype of NSCLC, intervention arm, comparator arm, time of analysis along with the primary and secondary endpoints. In addition, dichotomous variables OS, PFS and ORR along with HRs and their 95% CI were also extracted.

4.4 QUALITY ASSESMENT

The Cochran's Collaboration's Risk of Bias tool (ROB2) was used to evaluate factors such as randomness, double blindness, and integrity of the outcome data (*Appendix 5*) [47]. The risk of bias was assessed according to the following criteria: - low risk, high risk and some concerns. 'Low risk' is applicable in the current analysis. The result of the assessment can be found in supplementary sheet 2.

4.5 HETEROGENEITY TEST

The Higgins I^2 and X^2 statistics were used to evaluate in-between trial heterogeneity of included studies [48]. A fixed effect model will be employed if the *p*-value for $X^2 > 0.1$ and I^2 's <50%. Otherwise, a random effect model will be chosen if the I^2 statistic is >50% or the *P* value for $X^2 < 0.1$, signifying the presence of statistical heterogeneity among the clinical trials.

4.6 STATISTICAL DATA ANALYSIS AND PRIMARY ENDPOINTS

The primary endpoints in this network meta-analysis were the HRs for PFS and OS and the odd's ratio for ORR (dichotomous variables). PFS and OS survival analyses were presented as 95% confidence intervals (CI). A summary of the effect sizes was calculated by calculating the Odds Ratio (OR) based on the ORR. A Bayesian

inference approach was taken to conduct this network meta-analysis. Direct and indirect comparisons were generated to produce a hierarchical model of the best treatment regimens in the treatment of advanced NSCLC. A random-effects model was selected due to high heterogeneity between the studies based on Higgin's ρ statistic with a priori log-normal (-2.76, 1.672).

The control arm (comparator-doublet chemotherapy) was used as a standard therapeutic arm in this Bayesian NMA. The Bayesian model estimated the summary effects by their HRs and 95% CI. The posterior distribution of the Bayesian model was obtained using Markov Chain Monte Carlo Simulation (MCMC). For every five upshots, five Markov chains were run in parallel simulation with 500/5000 burn-ins and 50,000 iterations. At the conclusion of every ten simulations, the autocorrelation was thinned. The convergence of the model was assessed using Gelman-Rubin diagnostics (via trace and density plots, cut-off value 1.05). Furthermore, the surface under the cumulative ranking curves (SUCRA plots) was calculated using the Bayesian approach to calculate overall ranking probabilities and is presented in a *rankogram*. A higher SUCRA value indicated an increased probability that the treatment regimen is the best. Further analysis by the frequentist approach was also conducted to calculate SUCRA scores of pairwise meta-analyses for all three survival outcomes (OS, PFS and ORR) to validate the findings of Bayesian analysis.

The node-splitting analysis was conducted to rule out any inconsistency in the Bayesian Hierarchical Model among the closed loops in each network. Furthermore, the consistency of results and robustness were confirmed using sensitivity analysis. Egger's test with a funnel plot was performed to determine publication bias across the

studies. A *p*-value <0.05 would indicate asymmetry and the absence of a publication bias. Fundamental assumptions of a Bayesian NMA are transitivity, consistency, and similarity. The statistical analysis was conducted in software R (version 4.0.5) and R GeMTC package (version 1.0.1). The complete code for this Bayesian NMA and required data sets are available on GitHub at the following link:

[https://github.com/anamika0509/BayesianNMA_NSCLC.](https://github.com/anamika0509/BayesianNMA_NSCLC)

5. RESULTS

5.1 SYSTEMATIC LITERATURE SEARCH

A total of 4310 publications were identified from various databases and conferences during preliminary research. Following this, 2098 duplicates were removed, and 2212 publications were screened. Finally, 180 full-text articles were assessed for eligibility. Non-pertinent articles were removed via thorough consideration and a total of 18 trials and patients were incorporated in this Bayesian NMA. The selection protocol for the literature search is shown in Figure 8. All updated data were included in the pooled network. Patients received one of the following treatment regimens: Pembrolizumab, Atezolizumab, Cemiplimab, Durvalumab + Tremelimumab, Nivolumab, Avelumab, Sintilimab + Doublet chemotherapy, Pembrolizumab + Doublet Chemotherapy, Pembrolizumab + Ipilimumab or doublet chemotherapy. All the treatments mentioned above were further categorized into six different treatment nodes: ICI-monotherapy, ICI-dual therapy, ICI-doublet chemotherapy, ICI dual therapy-doublet chemotherapy, ICI- and doublet chemotherapy.

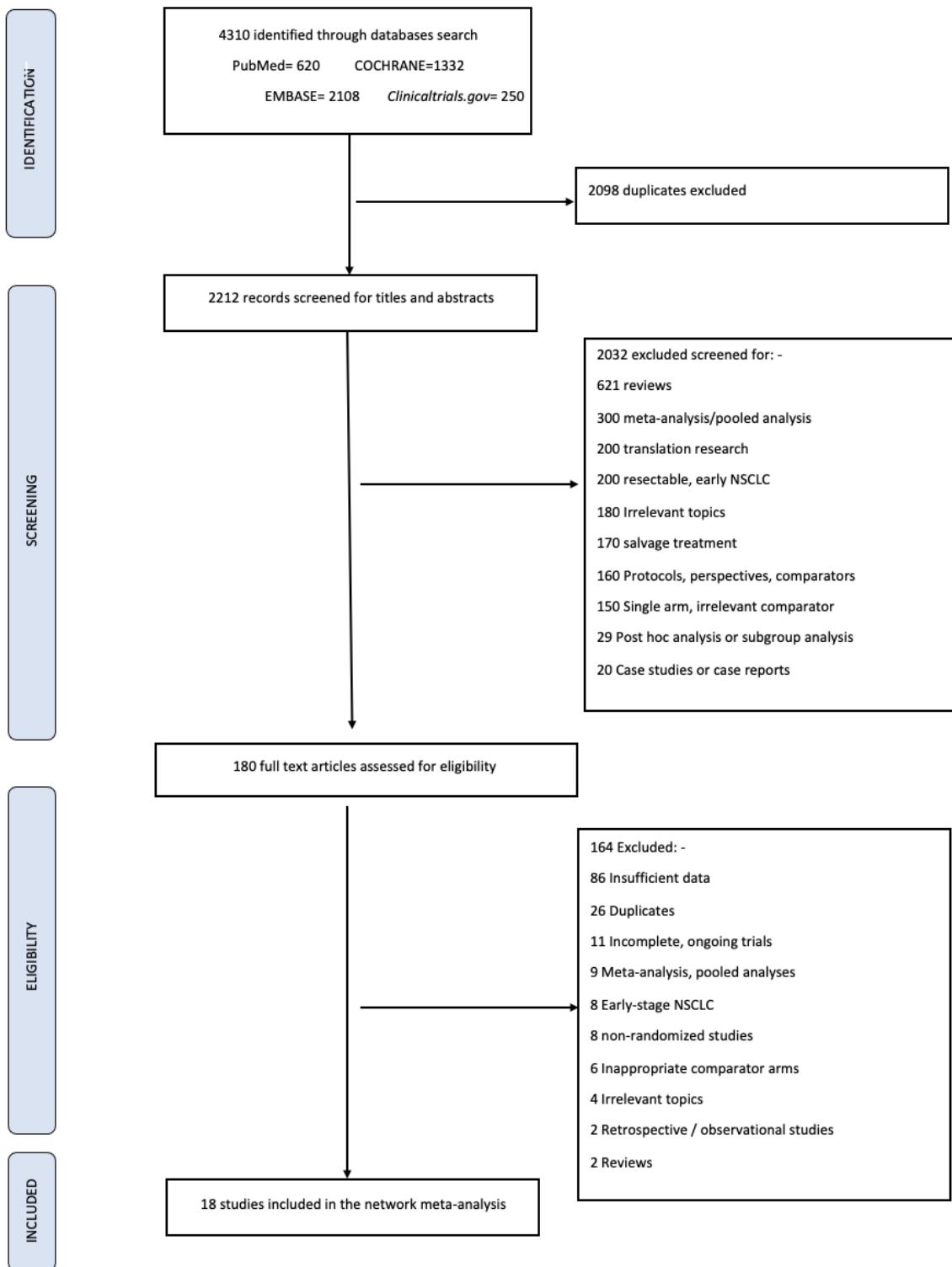


FIGURE 8. PRISMA FLOWCHART ILLUSTRATING TRIAL RETRIEVAL AND SELECTION

Adherence to PRISMA 2015 guidelines and based on inclusion/exclusion criteria resulted in selection of 18 RCTs for this Bayesian NMA.

PRISMA-Preferred reporting items for systematic reviews and meta-analyses, NMA- Network meta-analysis

5.2 BASELINE STUDY CHARACTERISTICS

Details of the study characteristics are summarized in Table 2A and 2B. All the included studies were two-arm trials except for MYSTIC [43], a three-arm trial wherein participants were randomized to receive either anti-PDL1 or PD-1 therapies or chemotherapy. There were considerable differences in the inclusion criteria of a few studies regarding the definition of high PDL-1 levels. For example, in the MYSTIC study, researchers administered anti-PDL1 therapy to patients with PD-L1 levels $\geq 20\%$. In contrast, most studies included patients in the intention-to-treat group if they had PD-L1 levels $\geq 50\%$. 2 studies were conducted with squamous lung cancer, 6 with non-squamous and 10 with mixed histology types. Nine trials directly compared the efficacy of ICI monotherapy with chemotherapy, six trials compared ICI plus chemotherapy with chemotherapy, one trial compared ICI dual therapy plus chemotherapy with chemotherapy, and three trials compared ICI dual therapy with chemotherapy. Moreover, only IMpower-130 wild-type populations were included in the Bayesian NMA in order to minimize heterogeneity [49].

STUDY NAME	REF	AUTHOR/YEAR	HISTOLOGY	PDL-1/PD-1 ? LEVELS : n	INTERVENTION ARM (A)	CONTROL ARM (B)
KEYNOTE 024 [50]	[50]	Martin Reck ; 2016	NSCLC	PD-L1 >50% (100)	Pembrolizumab	Doublet chemotherapy
KEYNOTE 042	[51]	Tony SK Mok ; 2019	NSCLC	PD-L1 >50% (47)	Pembrolizumab	Doublet chemotherapy
KEYNOTE 407	[52]	Luis Paz-Ares ; 2020	Squamous	PD-L1 >50% (26)	Pembrolizumab + Doublet Chemotherapy	Doublet chemotherapy
KEYNOTE 189	[53]	L Gandhi ; 2018	Non-squamous	PD-L1 >50% (202)	Pembrolizumab + Doublet Chemotherapy	Doublet chemotherapy
KEYNOTE 598	[54]	Michael Boyer ; 2020	NSCLC	PD-L1 >50% (100)	Pembrolizumab + Ipilimumab	Pembrolizumab
KEYNOTE 021 (cohort C)	[55]	Corey J Langer ; 2016	Non-squamous	NR	Pembrolizumab + Doublet Chemotherapy	Doublet chemotherapy
KEYNOTE 010 (a)	[40]	Roy S Herbst ; 2015	Non-squamous	PD-L1 >50% (442)	Pembrolizumab (2mg/kg)	Doublet chemotherapy
KEYNOTE 010 (b)	[40]	Roy S Herbst ; 2015	Non-squamous	PD-L1 >50% (442)	Pembrolizumab (10mg/kg)	Doublet chemotherapy
IMPOWER 110	[56]	Roy S Herbst ; 2020	NSCLC	PD-L1 >50% (37)	Atezolizumab	Doublet chemotherapy
IMPOWER 130	[49]	Howard West ; 2019	Non-squamous	PD-L1 >50% (19)	Atezolizumab + Doublet chemotherapy	Doublet chemotherapy
MYSTIC	[43]	Naiyer Arizvi ; 2020	NSCLC	PD-L1 >50% (30)	Durvalumab or Durvalumab + Tremelimumab	Doublet chemotherapy
OAK	[30]	Achim Rittmeyer ; 2016	NSCLC	PD-L1 >50% (34.6)	Atezolizumab	Doublet chemotherapy
EMPOWER-LUNG 1	[57]	Ahmet Sezer ; 2021	NSCLC	PD-L1 >50% (79)	Cemiplimab	Doublet chemotherapy
ORIENT-11	[58]	Yunpeng Yang ; 2020	Non-squamous	PD-L1 >50% (42)	Sentilimab + Doublet chemotherapy	Doublet chemotherapy
ORIENT-12	[59]	Caicun Zhou ; 2021	Squamous	PD-L1 >50% (34)	Sentilimab + Doublet chemotherapy	Doublet chemotherapy
CHECKMATE 026	[35]	DP Carbone ; 2017	NSCLC	PD-L1 >50% (214)	Nivolumab	Doublet chemotherapy
CHECKMATE 9LA	[60]	Luis Paz-Ares ; 2021	NSCLC	PD-L1 >50% (174)	Nivolumab + Ipilimumab + Doublet chemo	Doublet chemotherapy
JAVELIN LUNG 200	[61]	Fabrice Barlesi ; 2018	NSCLC	NR	Avelumab	Doublet chemotherapy

TABLE 2A. BASELINE CHARACTERISTICS OF 18 CLINICAL TRIALS INCLUDED IN THE BAYESIAN NMA.

Trial breakdown according to primary and secondary outcomes. All the clinical trials were critically evaluated for efficacy of various treatment regimens for advanced NSCLC. For more details on clinical trial data, see supplementary notes, Table _ OS- Overall survival, PFS- Progression free survival, ORR- objective response rate, n- number, NR- Not reached, ICI- Immune checkpoint inhibitors

STUDY NAME	REF	SAMPLE SIZE	HAZARD'S RATIO PFS (95% CI)	HAZARD'S RATIO OS (95% CI)	ARM A- ORR	ARM B- ORR
KEYNOTE 024	[50]	305	0.50 (0.37-0.68)	0.60 (0.41-0.89)	44.80%	27.80%
KEYNOTE 042	[51]	1274	0.81 (0.67-0.99)	0.69 (0.56-0.85)	39.00%	32%
KEYNOTE 407	[52]	559	0.56 (0.45-0.70)	0.64 (0.49-0.85)	57.90%	38.40%
KEYNOTE 189	[53]	616	0.52 (0.43-0.64)	0.49 (0.38-0.64)	47.60%	18.9%
KEYNOTE 598	[54]	568	1.06 (0.86-1.30)	1.08 (0.85-1.37)	45.40%	45.4%
KEYNOTE 021 (cohort C)	[55]	123	NR	NR	55.00%	29.00%
KEYNOTE 010 (a)	[40]	1034	0.59 (0.44-0.78)	0.54 (0.38-0.77)	30.00%	8.00%
KEYNOTE 010 (b)	[40]	1034	0.59 (0.45-0.78)	0.50 (0.36-0.70)	29.00%	8.00%
IMPOWER 110	[56]	572	0.63 (0.45-0.88)	0.59 (0.40-0.89)	38.30%	28.60%
IMPOWER 130	[49]	724	0.64 (0.54-0.77)	0.79 (0.64-0.98)	NR	NR
MYSTIC	[43]	1118	1.05 (0.72-1.53) ; 0.76 (0.55-1.04)	0.77 (0.56-1.07) ; 0.76 (0.55-1.04)	35.6% ; 34.4%	37.70%
OAK	[30]	1225	0.63 (0.43-0.91)	0.73 (0.62-0.87)	30.60%	10.80%
EMPOWER-LUNG 1	[57]	710	0.54 (0.43-0.68)	0.57 (0.42-0.77)	39%	20%
ORIENT-11	[58]	397	0.31 (0.20-0.49)	NR	68.20%	39.30%
ORIENT-12	[59]	357	0.46 (0.30-0.70)	NR	NR	NR
CHECKMATE 026	[35]	541	1.15 (0.91-1.45)	1.15 (0.91-1.45)	26%	33%
CHECKMATE 9LA	[60]	1150	0.68 (0.57-0.82)	0.66 (0.55-0.80)	37.70%	25.10%
JAVELIN LUNG 200	[61]	792	1.16 (0.97-1.40)	0.90 (0.75-1.08)	15%	11%

TABLE 2B. BASELINE CHARACTERISTICS OF 18 CLINICAL TRIALS INCLUDED IN THE BAYESIAN NMA.

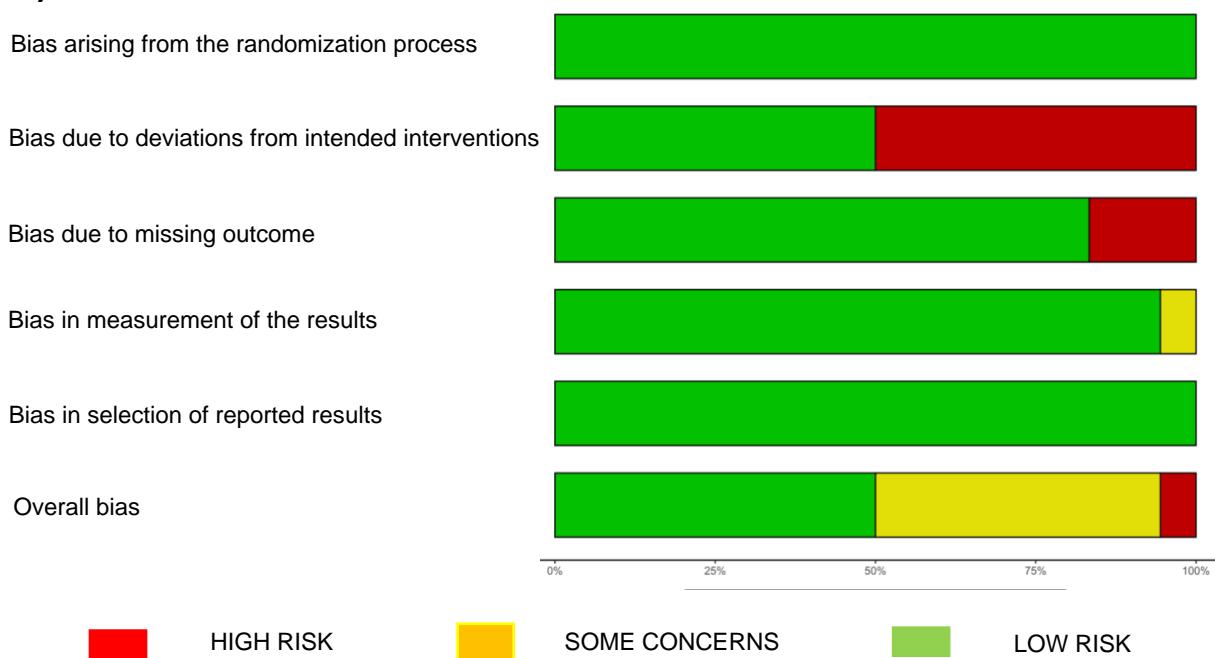
Trial breakdown according to primary and secondary outcomes. All the clinical trials were critically evaluated for efficacy of various treatment regimens for advanced NSCLC. For more details on clinical trial data, see supplementary notes, Table –

OS- Overall survival, PFS- Progression free survival, ORR- objective response rate, NR- Not reached, ICI- Immune checkpoint inhibitors

5.3 RISK OF BIAS AND METHODOLOGY ASSESSMENT

All the 18 included randomized studies were appropriately designed; hence the risk of bias was 'low' across the trials (Figure 9). It was evident that there was an adequate explanation of the mechanism for creating random sequences and concealing allocations in most cases, thereby minimizing the risk of selection bias. As a result of the widespread adoption of intention-to-treat analyses, including both quantitative and qualitative patient reports, attrition bias was sufficiently negated. As far as blinding study participants and researchers were concerned, open-labelled studies had a high risk of bias.

A)



B)

Study	Risk of bias domains					Overall
	D1	D2	D3	D4	D5	
KEYNOTE 024	+	X	+	+	+	-
KEYNOTE 042	+	X	+	+	+	+
KEYNOTE 407	+	+	+	+	+	+
KEYNOTE 189	+	+	+	+	+	+
KEYNOTE 598	+	+	+	+	+	+
KEYNOTE 021 (cohort G)	+	X	+	-	+	X
KEYNOTE 010 (a)	+	X	+	+	+	-
KEYNOTE 010 (b)	+	X	+	+	+	-
IMPOWER 110	+	X	+	+	+	-
IMPOWER 130	+	X	+	+	+	-
MYSTIC	+	+	X	+	+	-
OAK	+	+	+	+	+	+
EMPOWER-LUNG 1	+	+	+	+	+	+
ORIENT-11	+	X	+	+	+	-
ORIENT-12	+	X	+	+	+	-
CHECKMATE 026	+	+	X	+	+	+
CHECKMATE 9LA	+	+	X	+	+	+
JAVELIN LUNG 200	+	+	+	+	+	+

Judgement

X	High
-	Some concerns
+	Low
?	No information
Grey circle	Not applicable

Domains:

- D1: Bias arising from the randomization process.
- D2: Bias due to deviations from intended intervention.
- D3: Bias due to missing outcome data.
- D4: Bias in measurement of the outcome.
- D5: Bias in selection of the reported result.

FIGURE 9. RISK OF BIAS AND METHODOLOGY ASSESSMENT.

The Cochrane's Collaboration's Risk of Bias tool (ROB2) was used to assess the risk of bias across the 18 trials. A) The table shows the classification of trials into 'low', 'some concerns' and 'high'. B) The traffic-light plot.

5.4 NETWORK GRAPH

All the treatment regimens were categorized in one of the six following nodes (as discussed earlier): ICI-monotherapy, ICI-dual therapy, ICI-doublet chemotherapy, ICI dual therapy-doublet chemotherapy, ICI- and doublet chemotherapy (Figure 10). The network graph visualization represents direct and indirect evidence in the Bayesian framework.

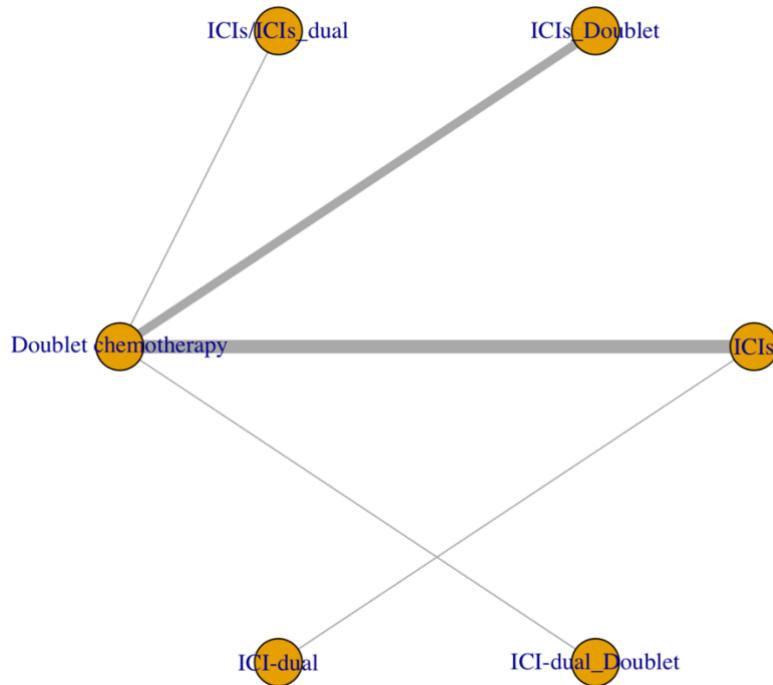


FIGURE 10. THE NETWORK GRAPH.

Network graph analysis shows the links between direct and indirect estimates between the 6 different nodes. Nodes represent the allocated treatment regimen and edges represents the associated studies.

5.5 COMPARISON OF OVERALL SURVIVAL

Bayesian NMA of overall survival was conducted with 15 treatment regimens assigned to six nodes mentioned above. Three trials (KEYNOTE 021, ORIENT 11 and ORIENT 12) did not report the HRs of their respective overall survivals, hence were excluded from the Bayesian analysis [55,58,59]. A *Fruchterman-Reingold algorithm* is also plotted to visualise better the network graph analysis (Appendix 6, Figure A). Gelman-Rubin diagnostic statistic of 1.05 and plots was used to assess the model convergence (Figure 11A and 11B). A Gelman-Rubin plot showing the *Posterior Scale Reduction-factor* (PSRF), which compares variations within each chain to in-between chain variations, was plotted to assess further the convergence (Appendix 6, Figure B).

Statistical heterogeneity was normalised once outliers were removed from the analysis and application of the random-effects model with adequate prior distributions. A Markov-chain Monte Carlo simulation calculated posterior distributions with 500/5000 burn-ins and 50,000 iterations. After every ten simulations, the autocorrelation was thinned. No publication bias was observed in Egger's test- Egger's p-value: 0.0786 (with a funnel plot) (Figure 12). The node splitting analysis did not reveal significant differences between direct and indirect estimates. Furthermore, the consistency of results was confirmed using sensitivity analysis.

There was a good fit for all treatments and studies with NMA models for OS. In most clinical trials, patients receiving anti-PD-1/PD-L1 ICIs with or without chemotherapy generally have a longer median overall survival than those receiving placebo/chemotherapy. The median overall survival was almost double in 3 studies (KEYNOTE 024, KEYNOTE 010(a) and KEYNOTE 021) in patients administered ICIs with or without chemotherapy. Patients were either administered Pembrolizumab monotherapy in KEYNOTE 024 [50] and KEYNOTE 010(a) [40] or Pembrolizumab with chemotherapy in KEYNOTE 021 [55]. Correspondingly, in KEYNOTE 189, a phase 2 multicohort open-labelled randomised trial reported a median OS of 21.4m (95% CI 16.6.-NR) in the intervention arm and 21.9m (95% CI 18-NR) in the control arm [53]. The study failed to show any superiority over chemotherapy compared to ICIs monotherapy.

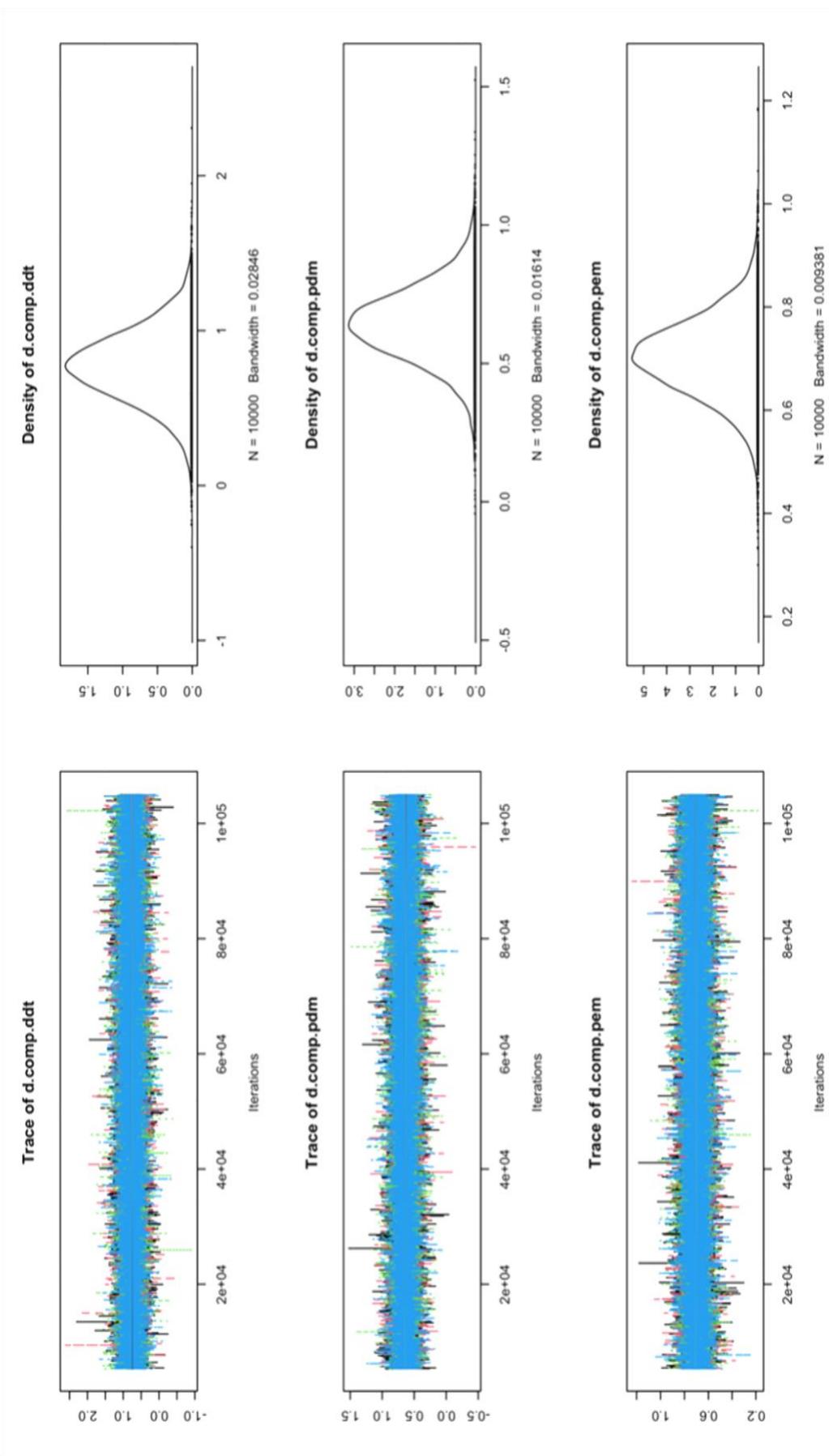


FIGURE 11 A. ASSESSING MODEL CONVERGENCE.

The trace plots (on left-hand side) display rapid up and down variations with time series. However, there's absence of any real-time long-term trend signifying the suitability of the model of Bayesian NMA. Similarly, density plots (on right-hand side) determine the posterior relative effect size estimate. We can see that the models display a normal distribution without any divergent, except for that last standard difference.

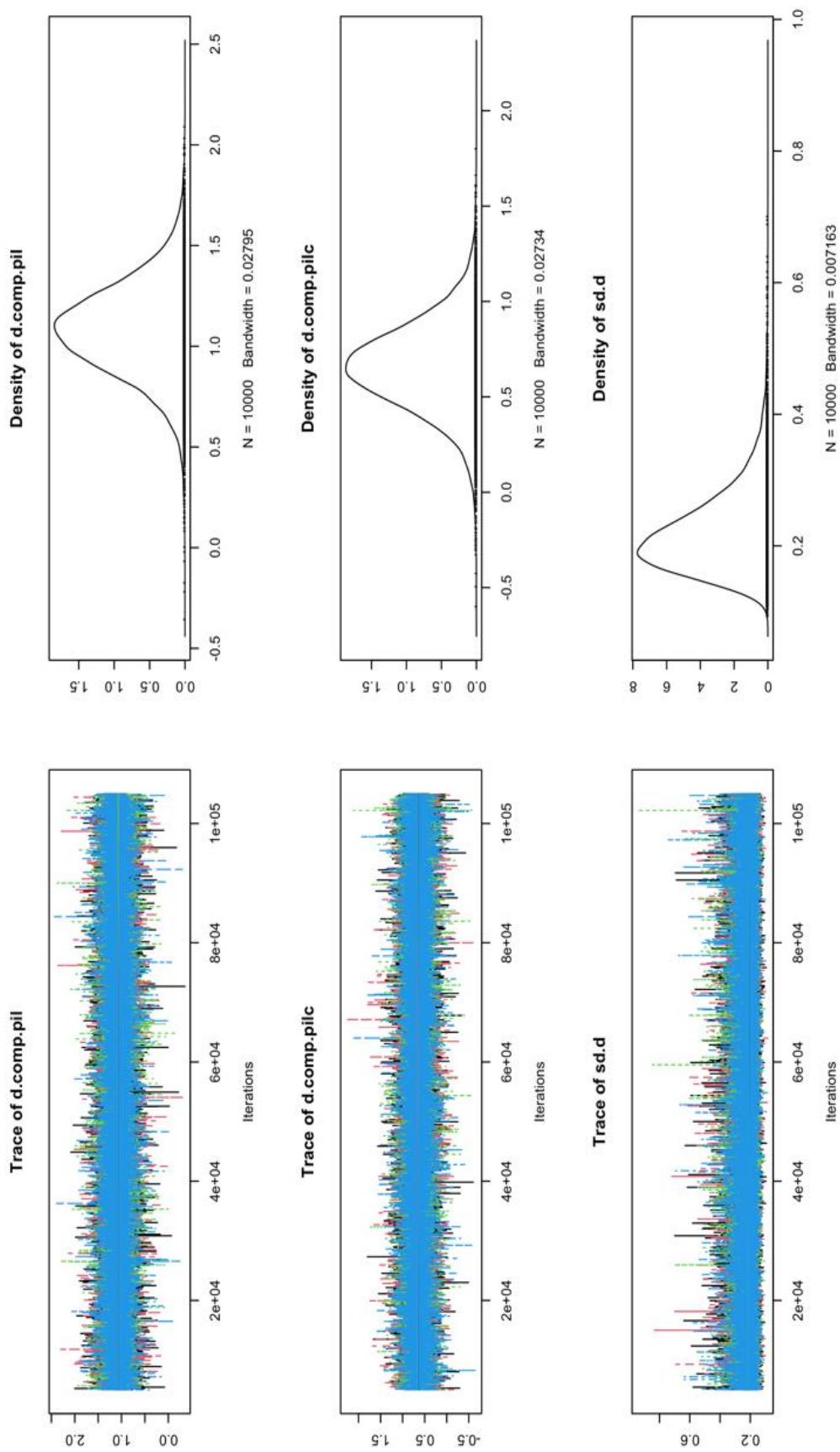


FIGURE 11 B. ASSESSING MODEL CONVERGENCE.

The trace plots (on left-hand side) display rapid up and down variations with time series. However, there's absence of any real-time long-term trend signifying the suitability of the model of Bayesian NMA. Similarly, density plots (on right-hand side) determine the posterior relative effect size estimate. We can see that the models display a normal distribution without any divergent, except for that last standard difference.

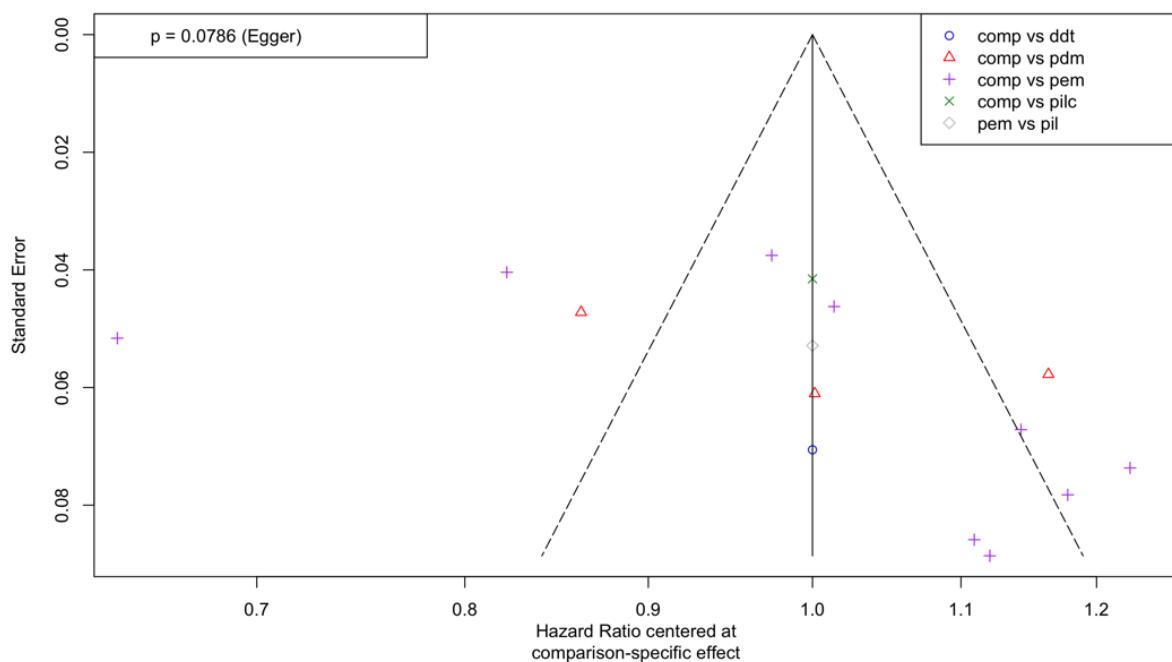


FIGURE 12. ASSESSING THE PUBLICATION BIAS.

Egger's test was performed to analyze the publication bias in the. Significant publication bias was not observed as demonstrated by a symmetrical funnel plot and a p value of 0.0786. Funnel plot was plotted with standard error on Y-axis and pooled HRs on the X-axis.

Interestingly, various combinatorial regimens of ICIs dual therapy pooled HR 0.16 (0.11-0.25) had a lower risk of death when compared with compared to ICI-monotherapy, pooled HR 0.49 (0.43-0.56) or ICI-dual therapy + doublet chemotherapy, pooled HR 0.51 (0.35-0.75). Moreover, the addition of chemotherapy in the immunotherapy regimen, ICI + doublet chemotherapy, had no survival advantage, pooled HR of 0.52(0.42-0.65) (Table 3 and 4). NMA results further confirmed the superiority of ICIs dual therapy over all other treatment regimens. Based on the SUCRA league (Figure 13A), ICI dual therapy has established its position as the best treatment regimen for overall survival. The Bayesian credible interval (Crl) (Figure 13B) is also the lowest for ICI dual therapy HR 0.17 (95% Crl 0.11-0.25). Nevertheless, all the treatment regimens, including immunotherapy, had a lower risk

of death when compared to standard chemotherapy. The Forest plot shows pooled effect size HR= 0.86 (0.81; 0.91) under the random-effects model (*Appendix 6*, Figure C), further signifying that administration of immunotherapy may yield better survival outcomes than chemotherapy alone.

comp	0.4630 [0.3119; 0.6873]	0.5265 [0.4214; 0.6578]	0.4947 [0.4342; 0.5636]	.	0.5169 [0.3539; 0.7549]
0.4630 [0.3119; 0.6873]	ddt
0.5265 [0.4214; 0.6578]	1.1372 [0.7226; 1.7895]	pdm	.	.	.
0.4947 [0.4342; 0.5636]	1.0684 [0.7049; 1.6195]	0.9395 [0.7259; 1.2161]	peM	0.3396 [0.2313; 0.4987]	.
0.1680 [0.1120; 0.2521]	0.3628 [0.2060; 0.6391]	0.3191 [0.2009; 0.5068]	0.3396 [0.2313; 0.4987]	pil	.
0.5169 [0.3539; 0.7549]	1.1163 [0.6458; 1.9295]	0.9816 [0.6326; 1.5232]	1.0448 [0.6999; 1.5596]	3.0766 [1.7661; 5.3596]	pilc

TABLE 3. LEAGUE TABLE FOR THE RELATIVE EFFECT SIZES OF THE PAIRS IN BAYESIAN NMA FOR OS.

COMP	DOUBLET CHEMOTHERAPY
PDM	ICI + DOUBLET CHEMOTHERAPY
DDT	ICI MONOTHERAPY/DUAL THERAPY
PEM	ICI MONOTHERAPY
PIL	ICI DUAL THERAPY
PILC	ICI DUAL THERAPY + DOUBLET CHEMOTHERAPY

TABLE 4. CORRESPONDING ABBREVIATIONS AND THEIR INTERPRETATIONS.

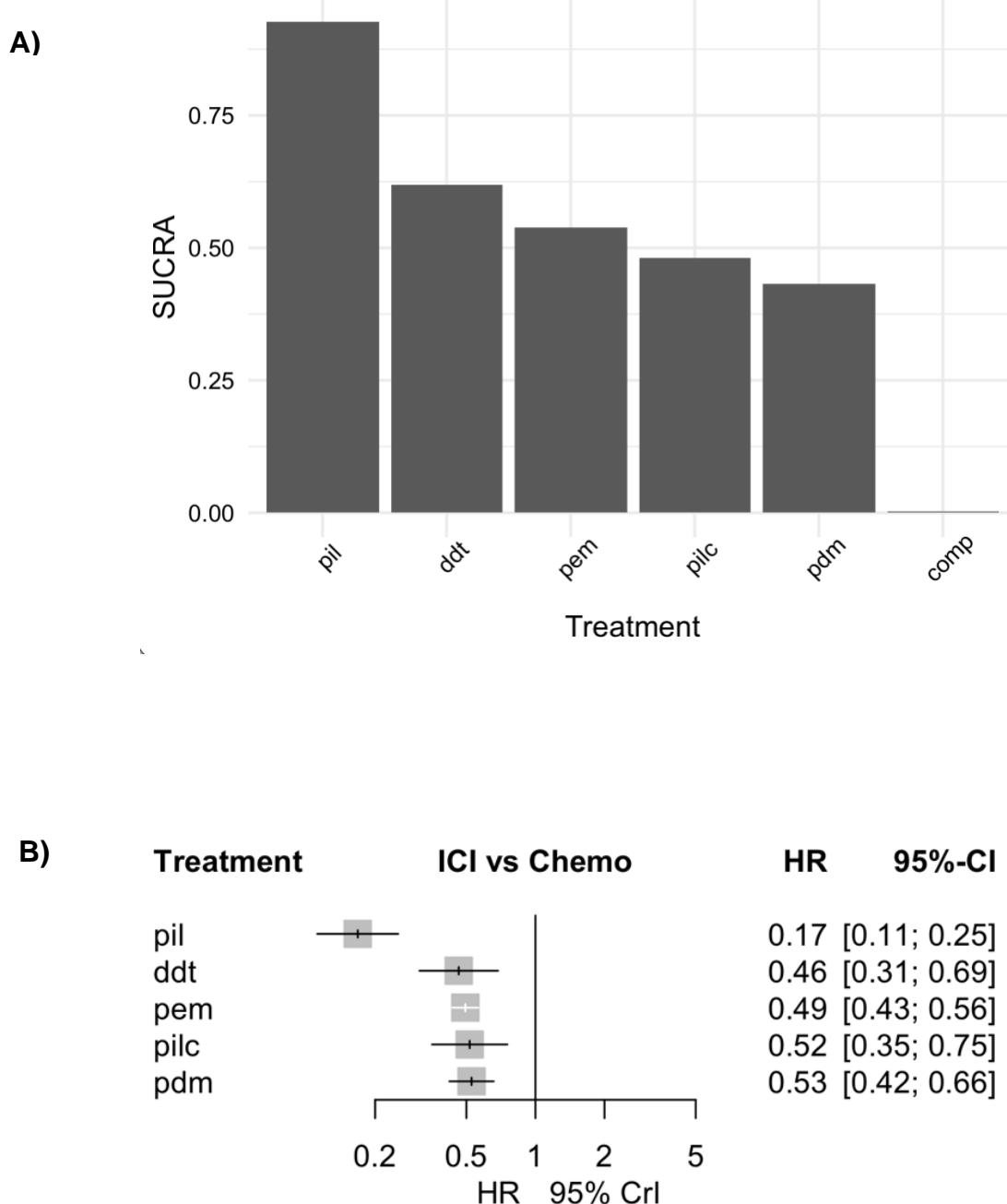


FIGURE 13. BAYESIAN NMA OF OVERALL SURVIVAL.

A) SUCRA plot confirmed the superiority of ICI dual therapy over all other treatment regimens. ICI dual therapy was ranked the best regimen among the six treatment regimens.

B) Forest plot of pairwise meta-analysis of different treatment regimens reported as HRs and their 95% CrI. ICI dual therapy has the lowest risk of disease progression or death whereas ICI + doublet chemotherapy was ranked lowest. Comp- Doublet chemotherapy, DDT- ICI single-agent/dual therapy, PEM- ICI monotherapy, PIL- ICI dual therapy, PDM- ICI + chemotherapy, PILC- ICI dual therapy + chemotherapy, HR- Hazard's ratio, CI- confidence interval

5.6 COMPARISON OF PROGRESSION-FREE SURVIVAL

Bayesian NMA of overall survival was conducted with 17 treatment regimens assigned to 6 nodes mentioned above. KEYNOTE 021 [55] did not report the HRs of the progression-free survival, hence was excluded from the Bayesian analysis. A *Fruchterman-Reingold algorithm* is also plotted to aid in better visualization of the network graph analysis (Appendix 7, Figure A). Gelman-Rubin diagnostic statistic of 1.05 and plots was used to assess the model convergence (Figure 14). A Gelman-Rubin plot showing the PSRF, which compares variations within each chain to in-between chain variations, was plotted to further assess the convergence (Appendix 7, Figure B). Statistical heterogeneity was normalized once outliers were removed from the analysis and application of the random-effects model with adequate prior distributions. A Markov-chain Monte Carlo simulation calculated posterior distributions with 500/5000 burn-ins and 50,000 iterations. At the conclusion of every ten simulations, the autocorrelation was thinned. No publication bias was observed in Egger's test- Egger's p-value: 0.0489 (with a funnel plot) (Figure 15). The node splitting analysis did not reveal significant differences between direct and indirect estimates. Furthermore, the consistency of results was confirmed using sensitivity analysis.

There was a reasonable fit for all treatments and studies with NMA models for PFS. An overarching inclination was observed in the intervention arm with the administration of anti-PDL1 therapy with or without chemotherapy. A multicohort, double-blinded clinical trial, KEYNOTE 189 [53], demonstrated significant improvement in the resultant median PFS of ICI + doublet chemotherapy arm compared to standard chemotherapy, 8.8m (95% CI 7.6-9.2) and 4.9m (95% CI 4.7-5.5) respectively. A

similar trend was observed in another phase 3 trial, KEYNOTE 024 [50]. In this trial, the participants in the intervention arm were administered Pembrolizumab as a monotherapy which yielded a PFS of 10.3m (95% CI 6.7-NR) compared to standard chemotherapy, 6m (95% CI 4.2-6.2). The resultant PFS in the intervention and control arm were 8.2m (95% CI 6.0-10.5) and 8.4m (95% CI 6.3-10.5), respectively. On the other hand, adding another ICI (ICI dual therapy) to the standard immunotherapy regimen had no clinical advantage over platinum-based chemotherapy, as seen in the KEYNOTE 598 trial [54].

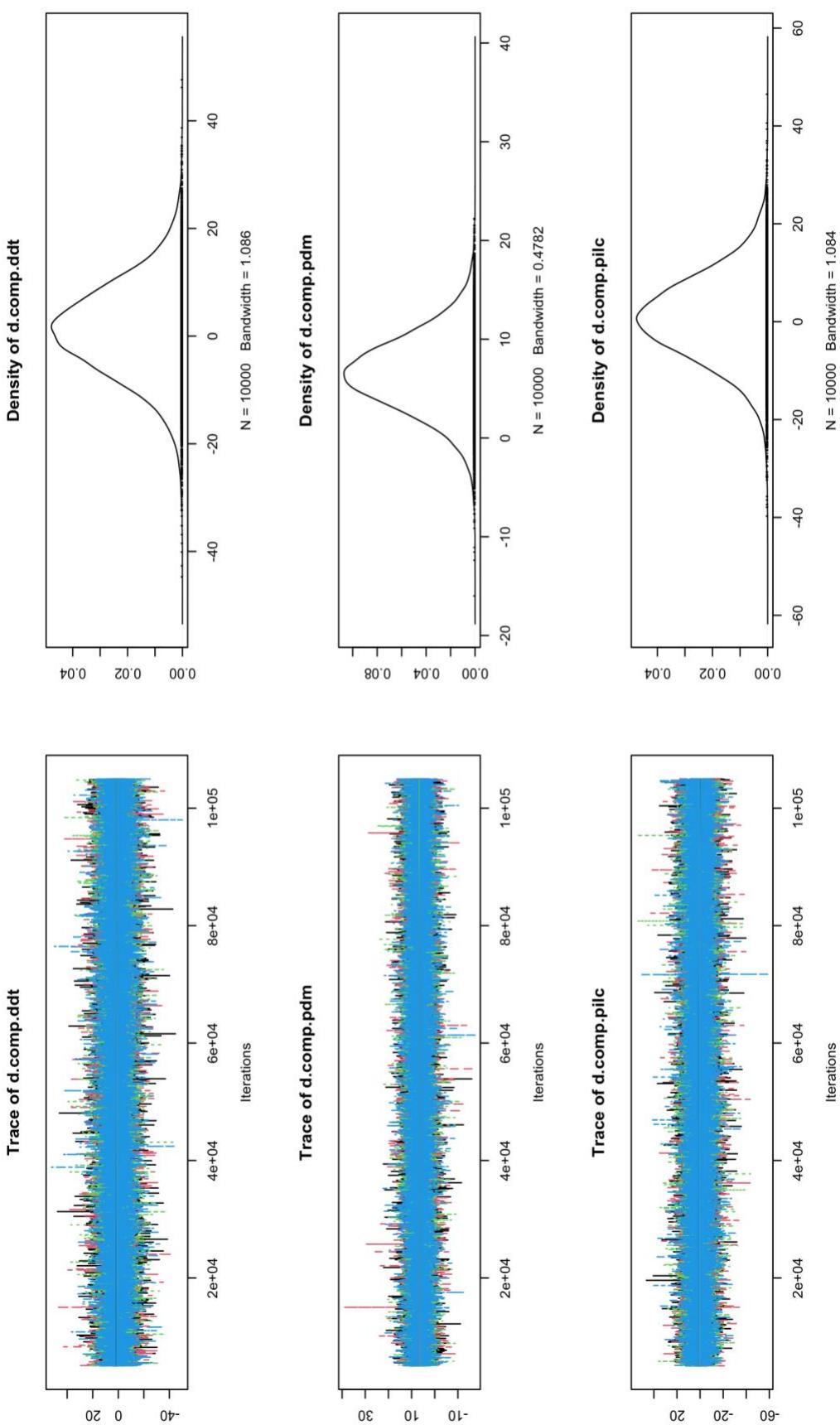


FIGURE 14 A. ASSESSING MODEL CONVERGENCE.

The trace plots (on left-hand side) display rapid up and down variations with time series. However, there's absence of any real-time long-term trend signifying the suitability of the model of Bayesian NMA. Similarly, density plots (on right-hand side) determine the posterior relative effect size estimate. We can see that the models display a normal distribution without any divergent, except for that last standard difference.

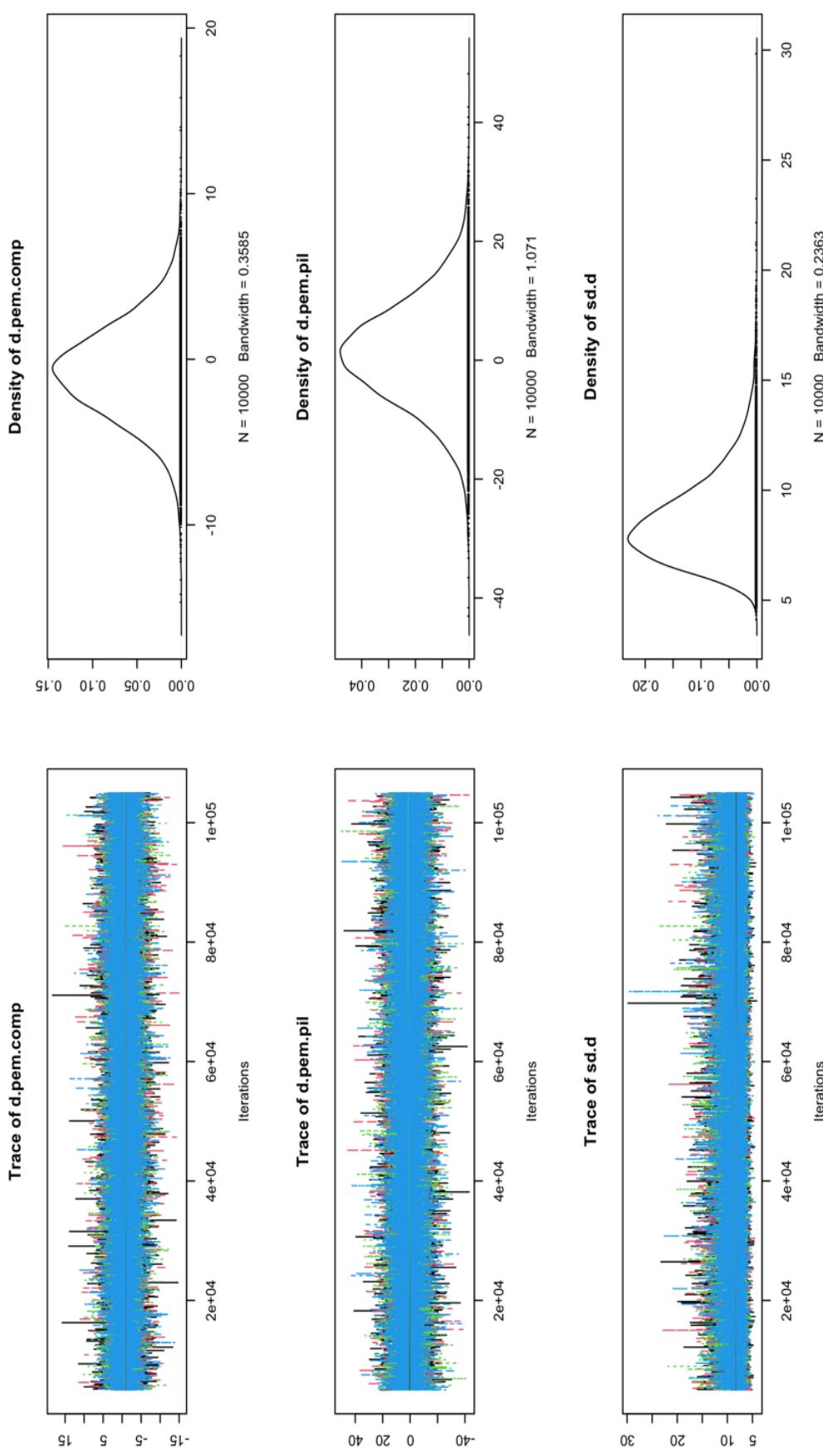


FIGURE 14 B. ASSESSING MODEL CONVERGENCE.

The trace plots (on left-hand side) display rapid up and down variations with time series. However, there's absence of any real-time long-term trend signifying the suitability of the model of Bayesian NMA. Similarly, density plots (on right-hand side) determine the posterior relative effect size estimate. We can see that the models display a classic bell curve indication a normal distribution without any divergent, except for that last standard difference.

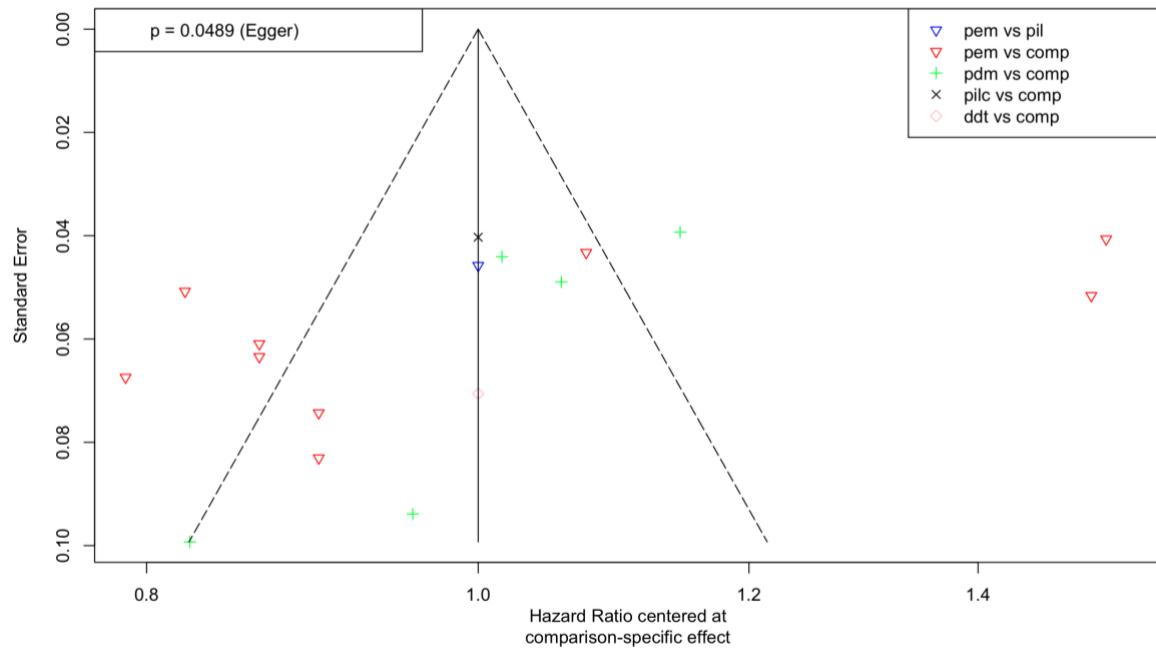


FIGURE 15. ASSESSING THE PUBLICATION BIAS.

Egger's test was performed to analyze the publication bias in the. Significant publication bias was not observed as demonstrated by a symmetrical funnel plot and a p value of 0.0489. Funnel plot was plotted with standard error on Y-axis and pooled HRs on the X-axis.

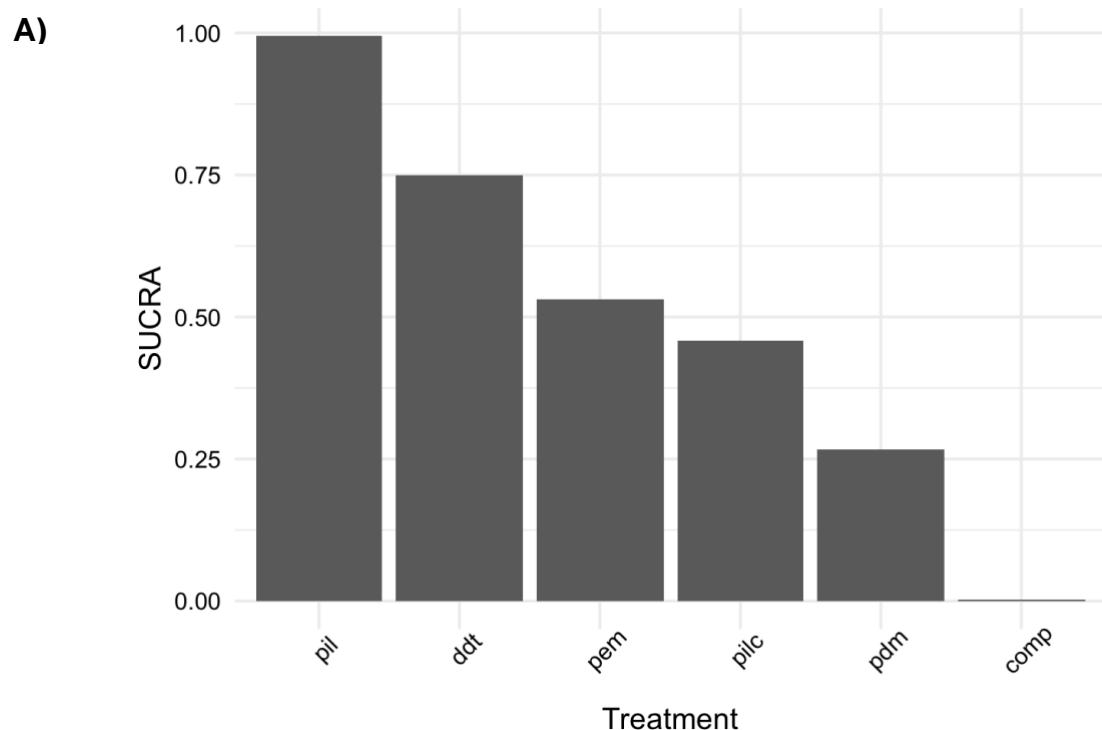
Another striking feature of the 17 clinical trials included in the Bayesian NMA was that Pembrolizumab was most likely the best ICI when compared to another single-agent immunotherapy regimen like Nivolumab or Atezolizumab. For instance, studies administering Avelumab (Javelin Lung 200), Nivolumab (CHECKMATE 026), and Atezolizumab (OAK), all had a significant decrease in the PFS when compared with chemotherapy regimen (Table 5) [30,32,61].

A similar trend was also observed in the Bayesian analysis, which is in agreement with the NMA of overall survival. ICI dual therapy has the lowest risk of death or disease progression, pooled HR of 0.16 (0.10-0.2) amongst all the other combinatorial regimens, followed by ICI plus chemotherapy, pooled HR of 0.34 (0.21-0.55) (Table 4). Both forest plot and SUCRA league have demonstrated that ICI dual therapy is

most likely to be the best treatment regimen in terms of PFS with the lowest HR 0.17 (95% CrI 0.10-0.27) (Figure 16A and 16B). Relative effects of all the included trials in the pairwise meta-analysis have successfully demonstrated the superiority of immunotherapy with or without chemotherapy over standard doublet chemotherapy. The Forest plot shows pooled effect size HR=0.85 (95% CI 0.78-0.91) under the random-effects model (*Appendix 7*, Figure C), further signifying that administration of immunotherapy may yield better survival outcomes than chemotherapy alone.

comp	0.3499 [0.2188; 0.5597]	0.6041 [0.4899; 0.7448]	0.4784 [0.4098; 0.5585]	.	0.5066 [0.3212; 0.7990]
0.3499 [0.2188; 0.5597]	ddt
0.6041 [0.4899; 0.7448]	1.7263 [1.0323; 2.8867]	pdm	.	.	.
0.4784 [0.4098; 0.5585]	1.3671 [0.8339; 2.2414]	0.7919 [0.6104; 1.0275]	pem	0.3465 [0.2192; 0.5475]	.
0.1657 [0.1022; 0.2687]	0.4736 [0.2415; 0.9290]	0.2744 [0.1621; 0.4645]	0.3465 [0.2192; 0.5475]	pil	.
0.5066 [0.3212; 0.7990]	1.4477 [0.7526; 2.7851]	0.8386 [0.5079; 1.3847]	1.0590 [0.6545; 1.7134]	3.0566 [1.5735; 5.9377]	pilc

TABLE 5. LEAGUE TABLE FOR THE RELATIVE EFFECT SIZES OF THE PAIRS IN BAYESIAN NMA FOR PFS.



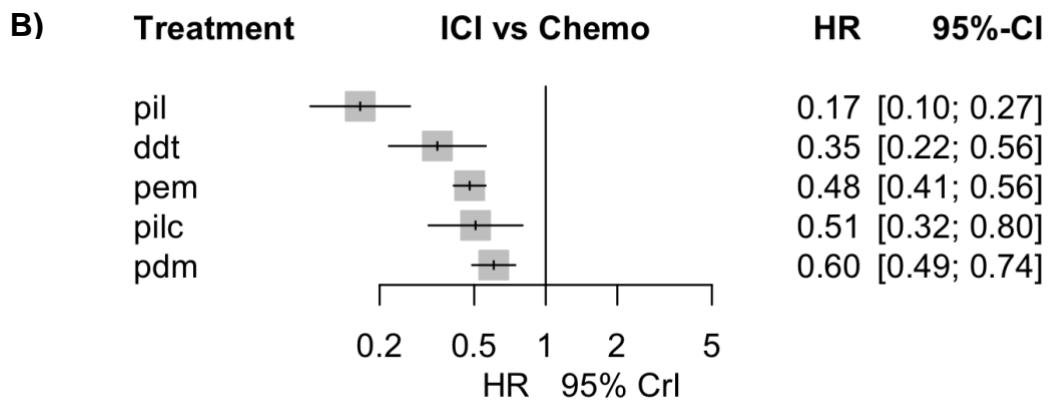


FIGURE 16. BAYESIAN NMA OF PROGRESSION FREE SURVIVAL.

A)-SUCRA plot confirmed the superiority of ICI dual therapy over all other treatment regimens.
 B) Forest plot of pairwise meta-analysis of different treatment regimens reported as HRs and their 95% Crl. ICI dual therapy has the lowest risk of disease progression or death whereas ICI + doublet chemotherapy was ranked lowest. Comp- Doublet chemotherapy, DDT- ICI single-agent/dual therapy, PEM- ICI monotherapy, PIL- ICI dual therapy, PDM- ICI + chemotherapy, PILC- ICI dual therapy + chemotherapy, HR- Hazard's ratio, CI- confidence interval

5.7 COMPARISON OF OVERALL RESPONSE RATE

All 18 trials were incorporated into the NMA of ORR. A similar trend was observed in ORR analysis as PFS and OS Bayesian NMA. Immunotherapy with or without chemotherapy had considerably higher response rates when compared with standard doublet chemotherapy regimens in the treatment of advanced or metastatic NSCLC. OAK study reported a 3-fold increase in the ORR with ICI monotherapy vs chemotherapy, 30.6% and 10.8%, respectively [30]. Significant rises in response rates were observed in multiple studies like KEYNOTE 024, KEYNOTE 189, and KEYNOTE 010(a) with an ORR of 44.8% vs 27.8%, 47.6% vs 18.9%, 30% vs 8%, respectively [40,50,53].

Figure 17 shows an overview of the comparative analysis of ORR in various phase II and phase III trials. The initial analysis suggested that Pembrolizumab, with or without chemotherapy, is probably the best regimen compared to other single-agent ICIs and ICI dual therapy. However, adding another immunotherapy agent (ICI dual therapy) yielded no added advantage and demonstrated similar ORRs in KEYNOTE 598 study, 45.4% vs 45.4% [54]. Moreover, CHECKMATE 026 reported a considerable decrease in the ORR, 26% vs 33%, indicating that it is unlikely to be the best single-agent ICI therapy [32].

A *Fruchterman-Reingold algorithm* is also plotted to aid in better visualization of the network graph analysis (*Appendix 8*, Figure A). Network meta-analysis of ORR (Figure 18) yielded similar results as PFS and OS Bayesian NMA. The pooled odd's ratio calculated for each pairwise model with six nodes is shown in figure 8 and is compared with doublet chemotherapy. ICI-dual therapy, pooled OR 0.02 (95% CI 0-0.3), was regarded as the best treatment regimen regarding response rates, followed by ICI + doublet chemotherapy, pooled OR 0.06 (95% CI 0.02-0.16). A pooled effect size OR= 2.03 (*Appendix 8*, Figure B) signifies that combinatorial regimens involving ICIs have better survival outcomes than chemotherapy alone.

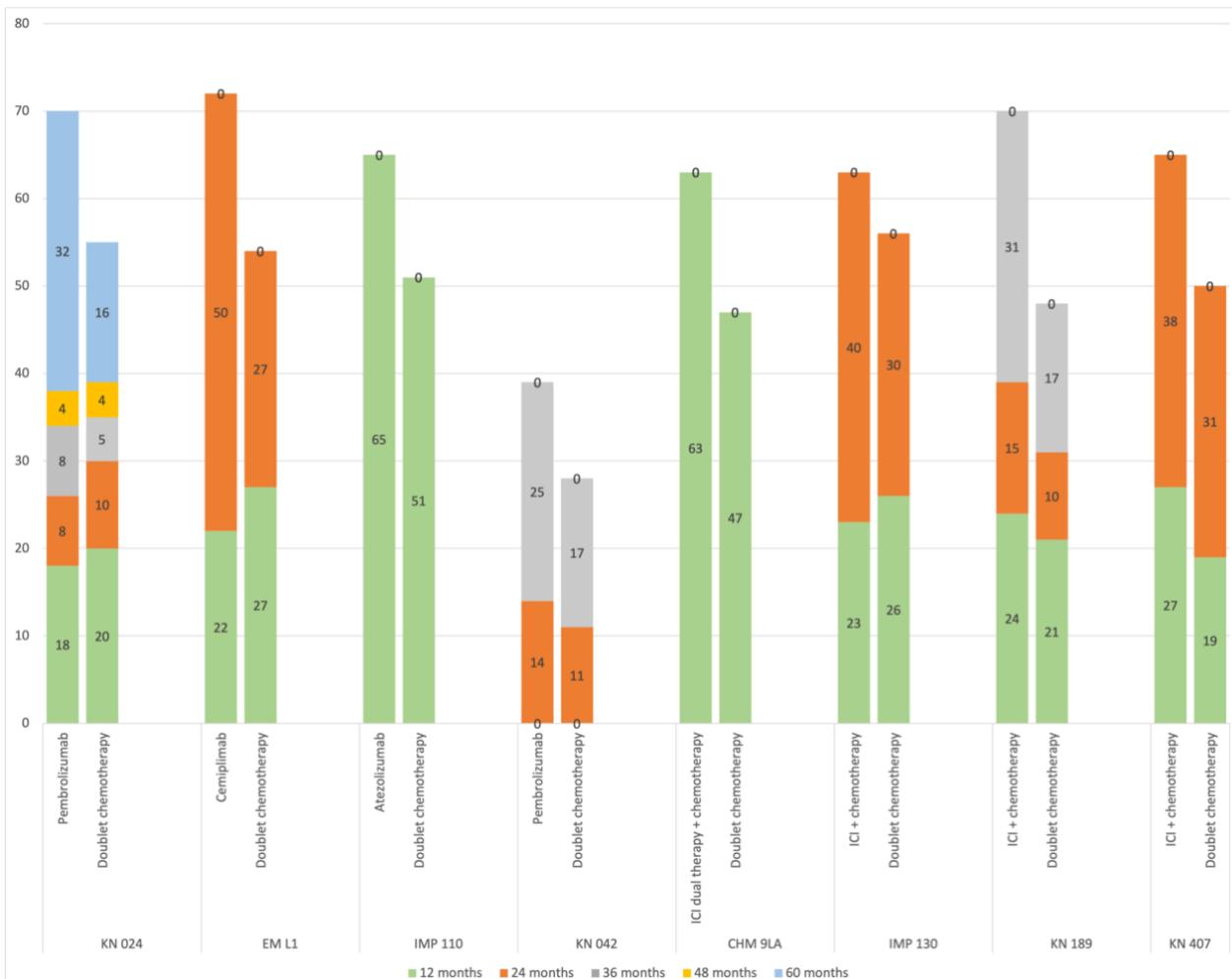


FIGURE 17. A COMPARATIVE ANALYSIS OF ORR IN THE FORM OF A STACKED BAR CHART.

Several datasets from phase II and III trials evaluating immunotherapy-based regimens (12, 24, 36, 48, and 60 months) are presented in the table below for a comparison between the experimental arm (immunotherapy) and control arm (chemotherapy). The bar heights depict survival rates. KN- Keynote, EM L- Empower lung, CHM- Checkmate, IMP- IM power.

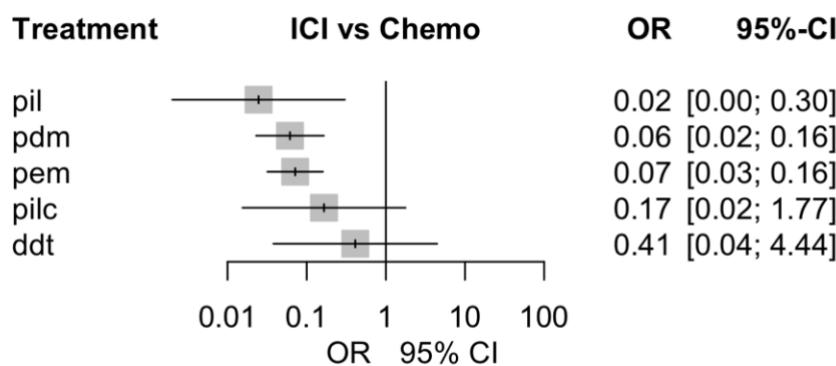


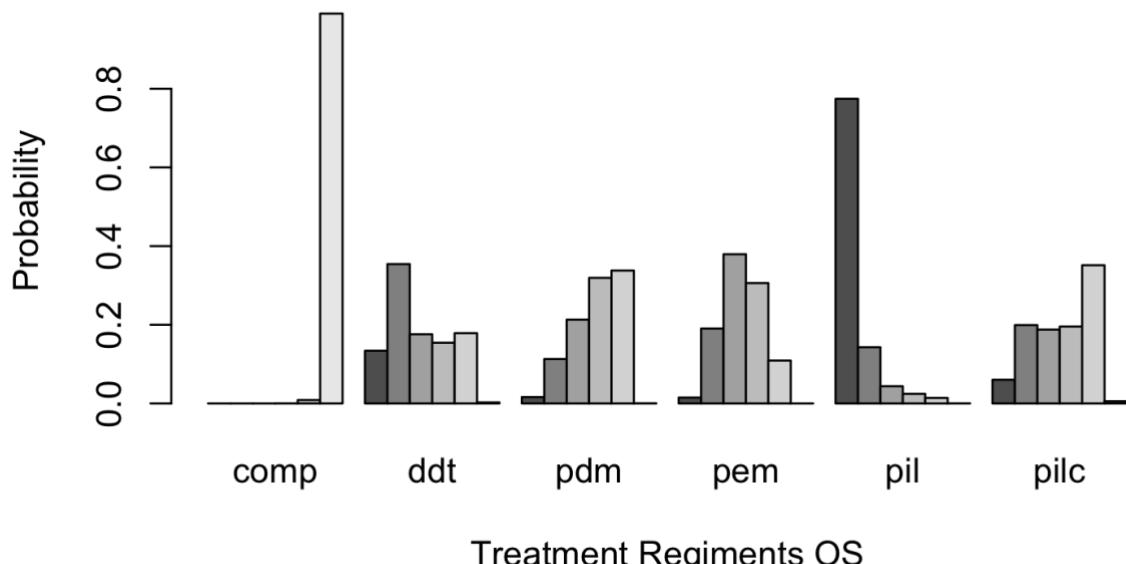
FIGURE 18. FOREST PLOT DEPICTING POOLED ORS OF 18 CLINICAL TRIALS.

Network graphs displays comparison between each treatment nodes wherein ICI dual therapy yielded the best results followed by ICI + doublet chemotherapy, ICI monotherapy. Comp- Doublet chemotherapy, DDT- ICI single-agent/dual therapy, PEM- ICI monotherapy, PIL- ICI dual therapy, PDM- ICI + chemotherapy, PILC- ICI dual therapy + chemotherapy, OR- Odd's ratio, CI- confidence interval

5.8 BAYESIAN RANKINGS

Bayesian probability ranking (BPR) analysis was performed based on Bayesian NMA of OS, PFS and ORR to produce three different *rankograms* (Figure 19). The *rankograms* successfully demonstrated the superiority of ICI dual therapy over all the different treatment regimens included in this Bayesian NMA. Results of *rankograms* were coherent with that of the individual Bayesian NMA of OS, PFS and ORR, as well as the SUCRA scores. The cumulative probability of ICI dual therapy was maximum for both OS and PFS (92.6% and 99.4%, respectively), followed by ICI monotherapy which had a probability of 53.7% and 53.2% for OS and PFS, respectively (SUCRA score are tabulated in *Appendix 10*)

A)



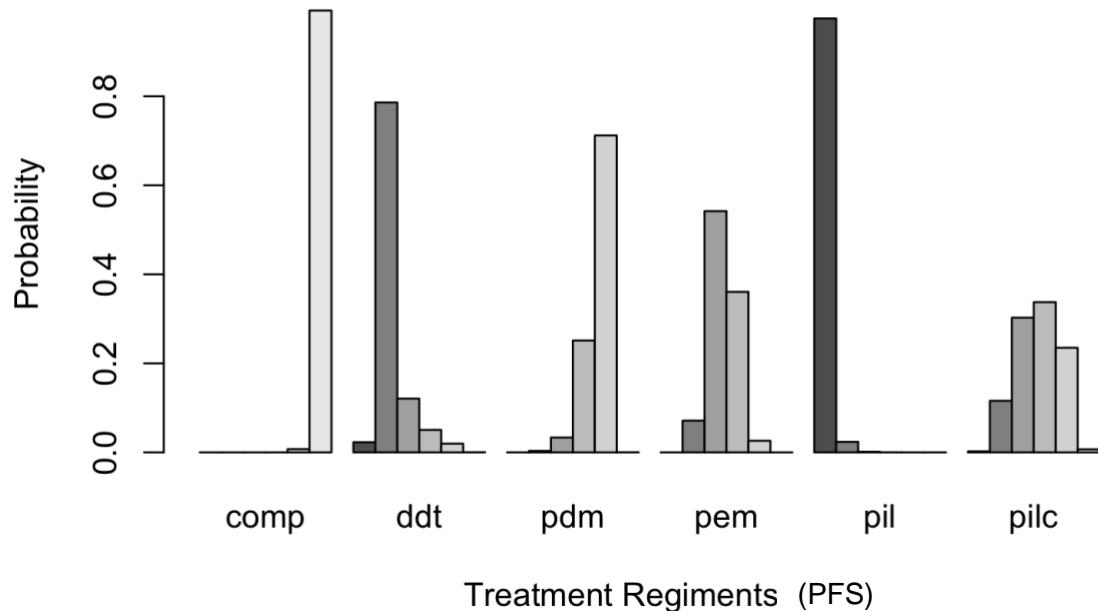
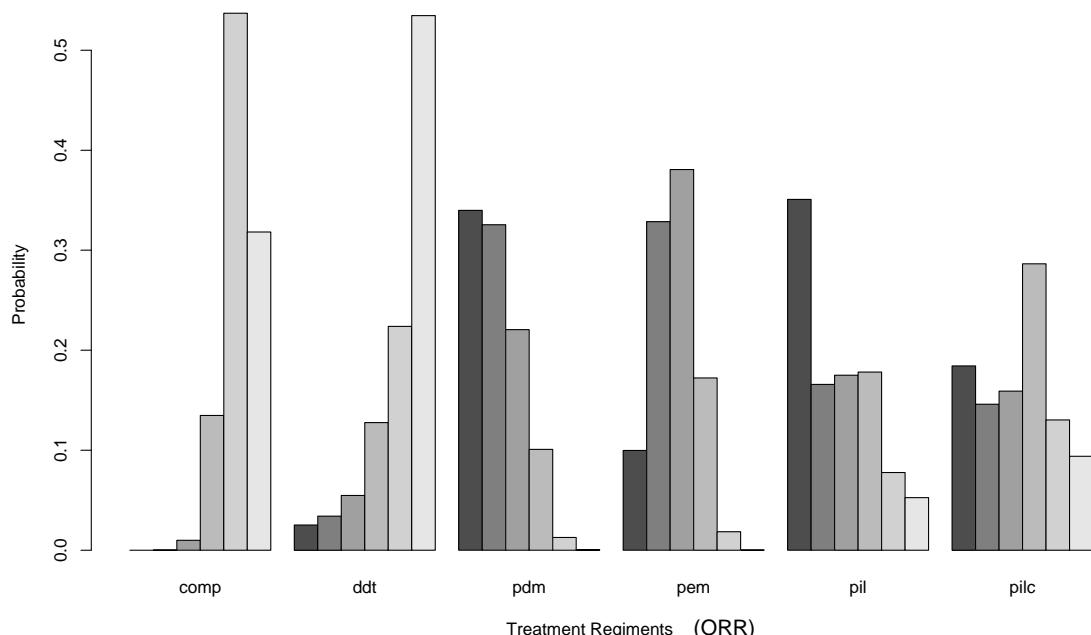
B)**C)**

FIGURE 19. RANKING PROFILES IN THE BAYESIAN NETWORK ANALYSIS FOR A) OS AND B) PFS C) ORR.

A *rankogram* is plotted using the cumulative probabilities of different treatment regimens as plotted on Y-axis which ranks them as first best option, second option and so on. ICI dual therapy is probably the best treatment option in terms of both of OS and PFS as the first bar which signifies the first rank is the highest followed by ICI single-agent therapy. However, in terms of ORR, ICI + chemotherapy was ranked second. Comp- Doublet chemotherapy, DDT- ICI single-agent/dual therapy, PEM- ICI monotherapy, PIL- ICI dual therapy, PDM- ICI + chemotherapy, PILC- ICI dual therapy + chemotherapy

5.9 HETEROGENEITY AND TRANSITIVITY ASSESSMENT

Significant heterogeneity was observed across the clinical trials (Higgin's I² statistic= ~92%). However, performing *Baujat* plot analysis identified outliers and their corresponding contribution to heterogeneity (*Appendix 9*). Following the removal of outliers, there was a drop in the ℓ^2 value noted. Assessment of model convergence with Brooks-Gelman-Rubin diagnostics (discussed in 3.5 and 3.6) resulted in the generation of satisfactory models for the Bayesian NMA using Markov-chain-Monte-Carlo simulations. In light of the results of this study, it appears that the included trials favour transitivity and consistency and that direct or indirect comparisons between them are possible.

Further analysis of the three survival outcomes was conducted via a frequentist approach to calculate SUCRA scores. It verified the findings of the Bayesian approach and demonstrated a similar trend in SUCRA scores (*Appendix 10*).

6. DISCUSSION

6.1 PRINCIPAL FINDINGS

This Bayesian network meta-analysis incorporated a total of 7521 patients from 18 randomised phase II and III trials. The Bayesian NMA shows comprehensive results from the most appropriate statistical methods and recent clinical outcomes measured in terms of overall survival, progression-free survival, and overall response rates. It is one of the few studies incorporating 18 clinical trials investigating the efficacy of immunotherapy regimens as the first-line treatment option for patients in advanced or metastatic NSCLC. The Bayesian framework mainly focused on NSCLC patients with high PD-L1 levels.

With a pragmatically designed Bayesian hierarchical modelling, this NMA provided evidence for clinical practice and includes the following key results: -

- i. All the five treatment regimens incorporating immunotherapy with or without chemotherapy yielded a better survival outcome than standard doublet chemotherapy in patients with high PD-L1 levels.
- ii. ICI-dual therapy demonstrated superiority over all the five nodes in terms of OS, PFS and ORR.
- iii. The addition of chemotherapy to ICI monotherapy significantly improves the cumulative probability of survival and is ranked second after ICI-dual therapy in terms of ORR.
- iv. Pembrolizumab is most likely the best single-agent immunotherapy compared to other ICI monotherapies (Nivolumab, Cemiplimab and Avelumab).

The Bayesian NMA revealed that ICI dual therapy is associated with significantly improved survival outcomes in terms of OS and PFS with similar pooled HR 0.16 (0.11-0.25) and 0.16 (0.10-0.2), respectively. A tremendous enthusiasm is seen around combining ICIs, especially PD-L1 and CTL4 inhibitors. As demonstrated in preclinical studies [62], combined immunotherapies have superior outcomes over single agents, and the Nivolumab plus Ipilimumab regimen is approved (accelerated FDA approval) to treat melanoma patients following promising results demonstrated by CHECKMATE 069 [63]. Compared with a response rate of 11% with ICI monotherapy, ICI dual therapy (Nivolumab plus Ipilimumab) had an objective response rate of 61% in patients with BRAF-wild melanoma [63].

T-cell activation in the host requires two critical signals (Figure 20). First, binding TCR to MHC via antigen-presenting cells activates various downstream molecules generating the first signal [64]. T cells engage with APCs via CD80/B7-1) or CD86/B7-2) in one such pathway. Secondary costimulatory signals are generated from various mechanisms that modulate T-cell activation. CD28, expressed on T cells, is a homolog of CTLA-4. CTLA-4 predominantly influences T cell activity during the priming phase. Due to CTLA-4 binding to B7 on APCs, CD28 engagement with B7 inhibits costimulatory signals, diminishing T cell immunity . CD28-B7 binding and TCR-MHC binding are prevented from being stimulated by CTLA-4 expression on CD8+ and CD4+ T cells [65]. Regulatory T cells, in contrast, express CTLA-4 constitutively. CTLA-4 is critical for regulating self-tolerance, as evidenced by mice lacking CTLA-4 showing reduced immunosuppressive activity. CTLA-4 and PD-1 are similar molecules. This B7-CD28 family member is expressed by various myeloid cells, B and T cells. It inhibits T cell proliferation and cytokine production, which are pro-inflammatory events. It is known that T cells express PD-1, which binds to APCs that

express PD-L1. Studies have indicated that PD-1/PD-L1 interactions enable tumour cells to escape immune surveillance [66]. Induced anergy and apoptosis in activated T cells by PD-1/PD-L1, tumour resistance to cytotoxic T cell responses, and Fox3p plus CD4+ regular T cell differentiation are associated with this phenomenon [67,68].

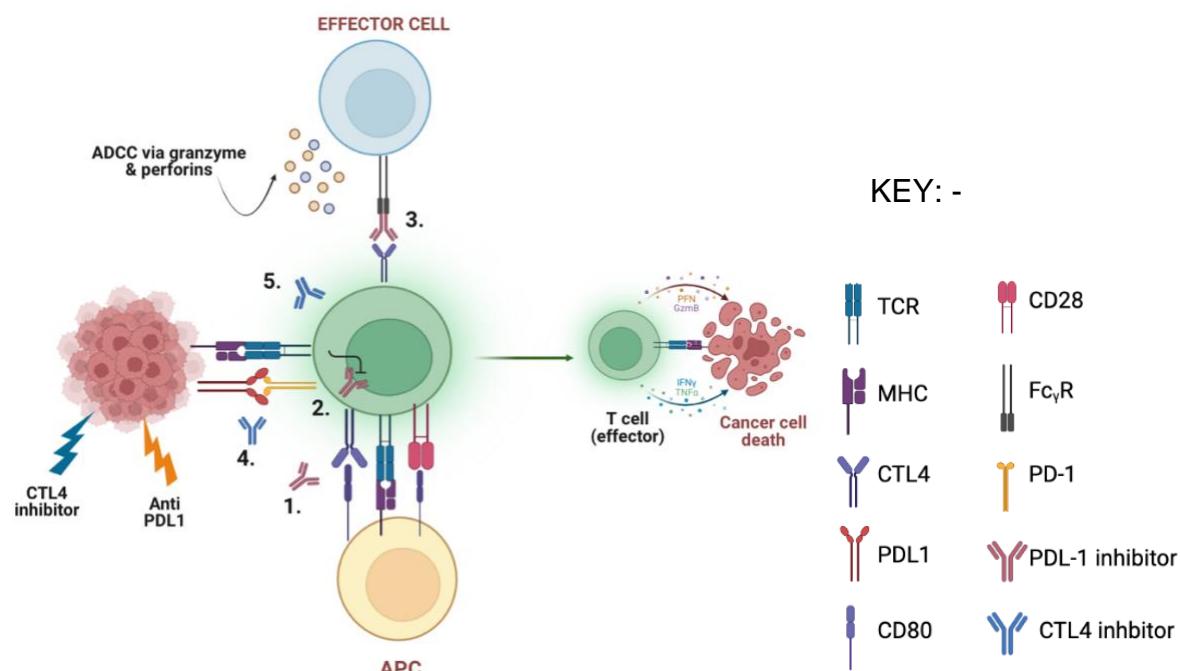


FIGURE 20. SYNERGISTIC MECHANISM OF ACTION OF ICI DUAL THERAPY

(1) T-cell activation restored by CTL4-i as it binds competitively with CD80 and inhibits interaction between CD80 and CTL4 on the antigen presenting cell. (2) Inhibition of transendocytosis, CD28 ligands CD80 concurrently mediated by CTL4. (3) Ipilimumab (IgG1 antibody) is a CTL4-i that binds to the F_c end of F_{cy} receptor on various immune effector cells which leads to antibody dependent cellular (ADCC) toxicity and depletion of subsets of T cells. (4) Through the inhibition of PDL-1 and PD-1 interactions, a PDL-1-i restores T cell activity. Moreover, PDL-1 is expressed by cancerous cells. (5) Above mentioned action is achieved by interaction between PDL-1 and CD28.

APC- Antigen presenting cell, ADCC- Antibody-dependent cellular cytotoxicity, MHC- Major Histocompatibility complex, TCR- toll like receptor.

CHEKMMATE 9LA, a multicentre, open-labelled phase 3 trial comparing ICI dual therapy with standard chemotherapy, demonstrated a significant improvement in the intervention arm's survival outcomes and response rates [60]. As initially planned,

during the interim analysis, the OS was 14.1m, 95% CI 13.2–16.2 vs 10.7m, 95% CI 9.5–12: HR 0.69, 95% CI 0.55–0.87. With 3.5 months longer median follow-up (median 13.2 months), median OS was 15.6m (95% CI 13.9–20.0) in the intervention arm vs 10.9 months (9.5–12.6) in the comparator arm, HR 0.66, 95% CI 0.55–0.80. An ORR of 38% was observed in the patients administered ICI-dual therapy vs 24.9% in the control arm. On the other hand, trials like NEPTUNE [69] and ARTIC [70] failed to show any clinical advantage in the intervention arm (Pembrolizumab plus Ipilimumab). Although AstraZeneca is yet to publish the results of the combination strategy, it has not yet been determined whether it will improve survival in NEPTUNE since the study did not meet the endpoints. Researchers have debated whether "less is more" or "more is better" in ICI dual therapy studies. Compared to monotherapy, nivolumab and ipilimumab had better outcomes, while pembrolizumab and ipilimumab had objectionable toxicity profiles. These conflicting outcomes warrant further investigation of various combinatorial regimens, including ICI dual therapy.

Immune checkpoint inhibitors have revolutionised cancer research and patient treatment with unprecedented clinical advantages. Before the advent of immunotherapy, platinum-based chemotherapy was the standard of care in NSCLC patients with no targetable mutations (like EGFR, ALK and BRAF). However, the median OS in chemotherapy was approximately 8-12m. Obtaining 5-year overall survival data was nearly impossible, and it limited oncologists' clinical decision-making capabilities in practice for the longest time. Recently, in 2020, the very first 5-year OS data of the KEYNOTE 024 trial was published by ESMO [50]. It revealed a significant increase in the overall survival of patients administered Pembrolizumab monotherapy compared with standard doublet chemotherapy (31.9% and 16.3%, respectively). This

principle finding of KEYNOTE 024 suggested that Pembrolizumab could prolong the survival in advanced NSCLC patients and should be recommended as first-line treatment in patients with no targetable mutations. This Bayesian NMA also suggested that ICI + doublet chemotherapy could further prolong overall survival rates than standard chemotherapy, as seen in Table 2B with pooled HR 0.52 (0.42-0.65).

Several studies have successfully demonstrated a synergistic mechanism of action between ICIs and cytotoxic agents (Figure 21). Chemotherapy has immunomodulatory properties due to its antitumor effect [71]. Tumour cell lysis increases tumour antigens and alters the tumour microenvironment, resulting in positive immunomodulatory effects. Cytotoxic agents increase the antitumour immunity and tumour mutational burden [72]. Moreover, some chemotherapeutic agents upregulate MHC class 1, thus increasing resultant antigen presentation. It also promotes dendritic cell maturation along with enhancing T-cell activation.

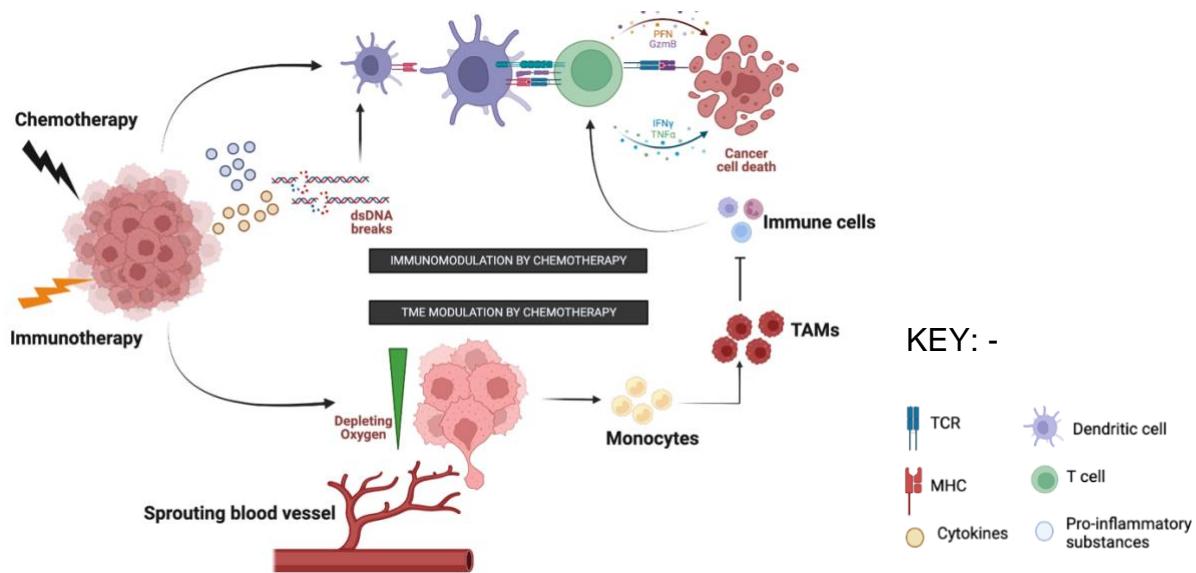


FIGURE 21. SYNERGISTIC MECHANISM OF ACTION OF ICI + CHEMOTHERAPY

ICI + Chemotherapy can modulate both immune system and the tumor microenvironment (TME). Firstly, due the cytotoxicity of chemotherapy, ds DNA breaks leads to production of pro-inflammatory substances that attenuate multiple survival pathways (for example, STAT 6). Downregulation of such critical pathways leads to apoptosis and depletion of PDLs and DCs on cancerous cells. In turn it triggers T cell activation along with upregulated activity of APCs via TCR-MHC interactions. On the other hand, increased apoptosis also modulates the TME. Secreted DAMPs (not shown in the figure) are sensed by TCR which in turn recruits immature DCs and further modulates the antigenicity of cancer cells.

TCR- toll like receptors, TAMs- tumor associated macrophages, DC- dendritic cell, IFN- interferons, GzmB- GranzymeB, TNF- tumor necrosis factor.

A multicentre, phase I study, KEYNOTE 021 (not included in this Bayesian NMA), evaluated different CIT regimens [73]. Patients were administered one of the following regimens: Pembrolizumab plus carboplatin and paclitaxel or Pembrolizumab plus carboplatin and paclitaxel plus Bevacizumab or Pembrolizumab plus carboplatin and pemetrexed. ORR across the three treatment regimens were 52%, 48% and 71%, respectively, which led to the phase II trial. KEYNOTE 021 (cohort G), a phase II randomised trial, evaluated different survival outcomes in Pembrolizumab plus carboplatin and pemetrexed with standard doublet chemotherapy in 123 patients [55]. Significant improvement was observed in the PFS and ORR of the intervention arm.

A higher response rate was demonstrated in the CIT arm, 55%, than in the control arm, 29%, with PFS of 13m and 6m, respectively. The findings of this Bayesian NMA were on par with these published data, and in terms of ORR, ICI plus doublet chemotherapy was ranked just below the ICI dual therapy. According to KEYNOTE-021, patients who receive CIT treatment with PD-L1 expression $\geq 50\%$, $\geq 1-49\%$, $\geq 1\%$ and $<1\%$ had an overall response rate of 80%, 26%, 54% and 57%, respectively. Higher levels of PD-L1 expression were associated with greater levels of response. According to the first interim analysis of KEYNOTE 407, CIT was associated with significant improvements in survival and response rate regardless of PD-L1 levels: the median OS was 15.9 vs 11.3m (HR 0.64, 95% CI [0.49, 0.85]; $p = 0.0008$), PFS was 6.4 vs 4.8m (HR 0.56, 95% CI [0.45, 0.70]; $p = 0.0001$), and ORR was 58.4% vs 35.0% [52]. Hence, CIT was established as an alternative first-line treatment option over pembrolizumab monotherapy for non-squamous NSCLC patients with PD-L1 $\geq 50\%$ (without any targetable mutations).

In this Bayesian NMA, 18 RCTs with a total of 7521 patients compared the survival outcomes in terms of OS, PFS and ORR of different treatment nodes in the treatment of advanced NSCLC (ICI monotherapy, ICI dual therapy, ICI + chemotherapy, ICI dual therapy + chemotherapy and standard doublet chemotherapy). ICI dual therapy and ICI plus chemotherapy had considerably lower HR than the other four treatment regimens. These results should be cautiously adopted (limitations mentioned in section 6.4). The addition of chemotherapy leads to increased toxicity but finding the right balance between benefit and risk is the key to determining the best treatment regimen for each patient.

In terms of OS and PFS, all the treatment nodes demonstrated superiority over standard chemotherapy. According to the *rankogram*, which estimated probabilities of different regimens based on SUCRA values, ICI dual therapy had the highest probability of being the most effective regimen (92.6% and 99.4%), followed by ICI monotherapy (53% and 53.2%). Surprisingly, although ICI plus chemotherapy ranked second in the league table, it was ranked third in the *rankogram*. However, this does not contradict the findings of the league table, as a *rankogram* is a mere representation of cumulative probabilities. A *rankogram* plots the cumulative probabilities as a first best option, second best option and so on. It incorporates the differences in the SUCRA values and gives an overall description of various regimens.

Moreover, analysis by both Bayesian and Frequentist methods yielded very similar SUCRA scores in terms of OS, PFS and ORR. Combination regimens, including immunotherapy, were established to yield better survival outcomes when compared to standard chemotherapy. ICI dual therapy was ranked first by both the approaches in terms of OS and PFS, followed by ICI single-agent therapy. Frequentist analysis also verified ORR results, and ICI plus chemotherapy was ranked second by both analyses. In practice, it is helpful to use both strategies since it increases our confidence in the results.

Despite the small number of patients included (12 clinical trials), a significant improvement in PFS and ORR was observed in ICI dual therapy treatment node. These results are in accordance with a meta-analysis conducted by Peng et al. [74]. This Bayesian NMA is one of the few that have reported the superiority of ICI dual therapy to be the best treatment regimen in the current clinical practice. However,

more trials are warranted to gather concrete evidence for the same. Moreover, the total number of studies included in each pairwise comparison should be considered before interpreting these results.

6.2 APPLICABILITY OF INCLUDED STUDIES

To facilitate a comprehensive analysis, it was decided to limit inclusion criteria rather than perform a broad overview that would not allow for a comprehensive analysis. In the first instance, targeted therapies such as TKIs and crizotinib are excluded, as are early-stage treatments (stage I and II). Therefore, applying this meta-analysis's results to a wide range of situations is impossible. In addition, the statistical analysis relies on weak clinical trials. Since one-armed clinical trials lack a comparator arm, only two-arm phase III trials were analysed. This lack of information does not reflect the entire dataset.

6.3 BAYESIAN NMA HETEROGENEITY

The heterogeneity of a network meta-analysis refers to the differences in outcomes between studies and is inevitable. Heterogeneity results from various clinical factors vary across the studies, like the type of intervention or patient characteristics and the intervention effect. Different studies will have different 'true' intervention effects. This Bayesian NMA had considerable heterogeneity despite having strict study protocols. All the 18 clinical trials had slight variations in their study designs. For instance, the demographics of the trials, co-existing diseases or liver and brain metastases, sample size variations, and immunohistochemical assays to stratify patients based on the PD-L1 levels may have contributed to considerable heterogeneity.

Moreover, some trials reported massive crossovers to the intervention arm, which may have confounded the survival analysis. Some studies included patients with PD-L1 levels as low as 1%, and some included patients having PD-L1 levels of at least 50%. This heterogeneity is most likely a result of the presence of these factors, and a *Baujat* plot and influence analysis identified three outliers in this Bayesian NMA (Appendix 9).

6.4 STRENGTHS AND LIMITATIONS OF THE ANALYSIS

This Bayesian NMA reported a well-designed and comprehensive analysis of direct and indirect evidence from 18 different randomised trials that have proved the superiority of ICI dual therapy as the best treatment regimen followed by ICI + chemotherapy for advanced NSCLC. Moreover, it was also established that Pembrolizumab is most likely to be the best ICI monotherapy. Both qualitative and quantitative analyses were performed based on primary and secondary outcomes (PFS, OS, and ORR), which assessed the quality of included studies. Furthermore, the findings of Bayesian analyses were also confirmed by Frequentist approach which increases the confidence in the results. All the 18 RCTs were well-designed and included more than 10,000 patients. Large numbers of patients favour the reduction of statistic errors and are critical for any statistical analysis.

Albeit the merits of the analysis mentioned above, there are several limitations. The entire dataset was extracted from various published studies, not from the individual patient. Therefore, patient factors (for example, TMB) were not incorporated in the analysis, which may have resulted in different opinions about the best treatment regimen. Another demerit was that one phase II trial was also included, and such

datasets are not as reliable as data from phase III studies. However, its impact on the principal findings is debatable since only one phase II trial was included. Patients were not stratified based on histology due to the limited number of trials available for each type which also limited the capacity to perform subgroup analysis. Finally, platinum-doublet chemotherapy is typically used to treat advanced NSCLC with no genetic alterations. During this NMA, both pemetrexed-based and non-pemetrexed-based chemotherapies were analysed. There is a higher success rate with pemetrexed than with other third-generation cytotoxic agents for non-squamous NSCLC patients. As a result, different chemotherapy regimens combined with immunotherapy may exert different synergistic effects. Since the evidence for the regimens included in this review is new and combinations have yet to be evaluated, the prior statement is tentative.

6.5 FUTURE RESEARCH IMPLICATIONS

By integrating RCTs evidence (direct and indirect comparisons), this analysis aims to provide clinicians with a reference resource for evaluating the merits and demerits of multiple promising combinatorial regimens. This systematic review and NMA have established state-of-the-art care for advanced NSCLC based on the evidence supporting the efficacy of ICI treatments as a first-line treatment. ICI dual therapy and ICI combined with doublet chemotherapy are more effective and safer treatments for advanced NSCLC. Antiangiogenic drugs such as bevacizumab, administered concurrently with ICIs plus doublet chemotherapy, exacerbate the toxic effects. However, there is generally less observed toxicity when compared to chemotherapy. A combination of chemotherapy and ICI is superior to chemotherapy alone. Combining an ICI with a chemotherapy backbone is preferred in place of standard doublet

chemotherapy. These results can complement current guidelines and treatment protocol in the management of advanced NSCLC.

Prospective randomised trials are warranted comparing ICI dual therapy with ICI dual therapy plus doublet chemotherapy and single-agent ICI with ICI dual therapy. In principle, ICI dual therapy could target more checkpoints. However, it may also increase the adverse effects. These head-to-head clinical trials would further answer unanswered questions and enable us to understand the fine distinctions between biomarker development and patient selections. It may also be possible to generate credible nomograms by analysing biomarkers for specific patients.

7. CONCLUSION

In conclusion, combining direct and indirect evidence-based networks from various published randomized clinical trials has shed some light on the best combinatorial regimens for treating previously untreated advanced NSCLC. Based on this Bayesian network meta-analysis, the immunotherapy regimens have exhibited potentially better treatment outcomes than standard chemotherapy. It could be stated that ICI-dual therapy may show better survival outcomes in the treatment of advanced NSCLC, shifting the current paradigm from chemotherapy to newer immunotherapeutic agents. Moreover, the addition of chemotherapy (CIT regimens- specially carboplatin and pemetrexed) is likely to improve the overall benefit-to-risk ratio.

Cumulative rankings (Figure 19) of the treatment regimens have demonstrated the superiority of ICI-dual therapy and ICI monotherapy over standard chemotherapy. Based on the SUCRA values, ICI dual therapy might be the best treatment option for OS and PFS, followed by ICI plus doublet chemotherapy in terms of ORR. ICI monotherapy may also be considered an appropriate treatment for advanced NSCLC patients with higher PD-L1 levels and no targetable mutations.

The patient should discuss with their doctor the benefits, costs, and risks of each treatment option. Due to the lack of prospective studies of direct comparisons, the treatment choice must be made based on a discussion about each option's benefits, costs, and risks. More randomized trials comparing ICI dual therapy with ICI dual therapy plus chemotherapy is warranted to identify the most effective treatment strategy for advanced NSCLC bearing high PD-L1 expression.

8. REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021 Feb 4;71(3):209–49.
2. Early Diagnosis [Internet]. Shinyapps.io. 2022 [cited 2022 Jul 19]. Available from: <https://crukancerintelligence.shinyapps.io/EarlyDiagnosis>
3. Chen Z, Fillmore CM, Hammerman PS, Kim CF, Wong K-K. Non-small-cell lung cancers: a heterogeneous set of diseases. *Nature Reviews Cancer*. 2014 Jul 24;14(8):535–46.
4. Minna JD, Roth JA, Gazdar AF. Focus on lung cancer. *Cancer Cell*. 2002 Feb [cited 2021 Jan 25];1(1):49–52.
5. Westra WH. Early glandular neoplasia of the lung. *Respiratory Research*. 2000 Nov 17;1(3).
6. Auerbach O, Stout AP, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. *The New England Journal of Medicine*. 1961 Aug 10;265:253–67.
7. Travis WD, Garg K, Franklin WA, Wistuba II, Sabloff B, Noguchi M, et al. Bronchioloalveolar Carcinoma and Lung Adenocarcinoma: The Clinical Importance and Research Relevance of the 2004 World Health Organization Pathologic Criteria. *Journal of Thoracic Oncology*. 2006 Nov;1(Supplement):S13–9.
8. Raso MG, Wistuba II. Molecular Pathogenesis of Early-Stage Non-small Cell Lung Cancer and a Proposal for Tissue Banking to Facilitate Identification of New Biomarkers. *Journal of Thoracic Oncology*. 2007 Jul;2(7):S128–35.
9. Howington JA, Blum MG, Chang AC, Balekian AA, Murthy SC. Treatment of stage I and II non-small cell lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013 143(5 Suppl):e278Se313S.

10. Ball D, Mai GT, Vinod S, Babington S, Ruben J, Kron T, et al. Stereotactic ablative radiotherapy versus standard radiotherapy in stage 1 non-small-cell lung cancer (TROG 09.02 CHISEL): a phase 3, open-label, randomised controlled trial. *The Lancet Oncology*. 2019 Apr 1;20(4):494–503.
11. Faivre-Finn C, Le Pechoux C, Lunt C. 170 Lung ART: Phase III study comparing post-operative conformal radiotherapy to no post-operative radiotherapy in patients with completely resected non-small cell lung cancer and mediastinal N2 involvement. *Lung Cancer*. 2013 Jan;79:S58.
12. Ramalingam S, Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. *The oncologist*. 2008 13 Suppl 1:5–13.
13. Scagliotti GV, De Marinis F, Rinaldi M, Crinò L, Gridelli C, Ricci S, et al. Phase III Randomized Trial Comparing Three Platinum-Based Doublets in Advanced Non-Small-Cell Lung Cancer. *Journal of Clinical Oncology*. 2002 Nov 1;20(21):4285–91.
14. Fossella F, Pereira JR, von Pawel J, Pluzanska A, Gorbounova V, Kaukel E, et al. Randomized, Multinational, Phase III Study of Docetaxel Plus Platinum Combinations Versus Vinorelbine Plus Cisplatin for Advanced Non-Small-Cell Lung Cancer: The TAX 326 Study Group. *Journal of Clinical Oncology*. 2003 Aug 15;21(16):3016–24.
15. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of Four Chemotherapy Regimens for Advanced Non-Small-Cell Lung Cancer. *New England Journal of Medicine*. 2002 Jan 10;346(2):92–8.
16. Gridelli C, Ardizzone A, Le Chevalier T, Manegold C, Perrone F, Thatcher N, et al. Treatment of advanced non-small-cell lung cancer patients with ECOG performance status 2: results of an European Experts Panel. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2004;15(3):419–26.
17. Maira S-M, Pecchi S, Huang A, Burger M, Knapp M, Sterker D, et al. Identification and Characterization of NVP-BKM120, an Orally Available Pan-Class I PI3-Kinase Inhibitor. *Molecular Cancer Therapeutics*. 2011 Dec 21;11(2):317–28.

18. Rodon J, Bendell J, Razak ARA, De Jonge MJA, Eskens F, Di Tomaso E, et al. A Phase I Dose Escalation and Expansion Trial of BKM120, An Oral Pan-PI3K Inhibitor, in Patients with Advanced Solid Tumors: Analysis of Pharmacodynamic Biomarker Data. *Annals of Oncology*. 2012 Sep;23: ix158–9.
19. J. Shuttleworth S, A. Silva F, R.L. Cecil A, D. Tomassi C, J. Hill T, I. Raynaud F, et al. Progress in the Preclinical Discovery and Clinical Development of Class I and Dual Class I/IV Phosphoinositide 3-Kinase (PI3K) Inhibitors. *Current Medicinal Chemistry*. 2011 Jun 1;18(18):2686–714.
20. Weill D, Mack M, Roth J, Swisher S, Proksch S, Merritt J, et al. Adenoviral-Mediated p53 Gene Transfer to Non-small Cell Lung Cancer Through Endobronchial Injection. *Chest*. 2000 Oct;118(4):966–70.
21. Howlader N, Forjaz G, Mooradian MJ, Meza R, Kong CY, Cronin KA, et al. The Effect of Advances in Lung-Cancer Treatment on Population Mortality. *New England Journal of Medicine*. 2020 Aug 13;383(7):640–9.
22. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. *Science*. 2011 Mar 24;331(6024):1565–70.
23. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nature Reviews Immunology*. 2020 May 20; 20:1–18.
24. Oiseth SJ, Aziz MS. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *Journal of Cancer Metastasis and Treatment*. 2017 Oct 31;3(10):250.
25. National Center for Biotechnology Information. How does the immune system work?. Nih.gov. Institute for Quality and Efficiency in Health Care (IQWiG); 2016.
26. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immuno surveillance to tumor escape. *Nature Immunology*. 2002 Nov;3(11):991–8.

27. Coughlin CM, Salhany KE, Gee MS, LaTemple DC, Kotenko S, Ma X, et al. Tumor Cell Responses to IFNy Affect Tumorigenicity and Response to IL-12 Therapy and Antiangiogenesis. *Immunity*. 1998 Jul;9(1):25–34.
28. Groh V, Wu J, Yee C, Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature*. 2002 Oct;419(6908):734–8.
29. Reiman JM, Kmiecik M, Manjili MH, Knutson KL. Tumor immunoediting and immunosculpting pathways to cancer progression. *Seminars in Cancer Biology*. 2007 Aug;17(4):275–87.
30. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* (London, England). 2017 [cited 2019 May 26];389(10066):255–65.
31. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. *New England Journal of Medicine*. 2015 Jul 9;373(2):123–35.
32. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. *New England Journal of Medicine*. 2017 Jun 22;376(25):2415–26.
33. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non-Small-Cell Lung Cancer. *New England Journal of Medicine*. 2016 Nov 10;375(19):1823–33.
34. Castro G de, Kudaba I, Wu Y-L, Lopes G, Kowalski DM, Turna HZ, et al. 363 KEYNOTE-042 5-year survival update: pembrolizumab versus chemotherapy in patients with previously untreated, PD-L1–positive, locally advanced or metastatic non–small-cell lung cancer. *Journal for ImmunoTherapy of Cancer*. 2021 Nov;9(Suppl 2):A390–0.
35. Carbone DP. First-Line Nivolumab in Stage IV or Recurrent Non-Small Cell Lung Cancer. *Oncology Times*. 2017 Sep;39(17):28–9.

36. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, et al. Pembrolizumab plus Chemotherapy for Squamous Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 2018 Nov 22;379(21):2040–51.
37. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 2018 May 31;378(22):2078–92.
38. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *New England Journal of Medicine*. 2018 Jun 14;378(24):2288–301.
39. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 2015 Oct 22;373(17):1627–39.
40. Herbst RS, Baas P, Kim D-W, Felip E, Pérez-Gracia JL, Han J-Y, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* (London, England) . 2016;387(10027):1540–50.
41. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. *New England Journal of Medicine*. 2018 Dec 13 17;379(24):2342–50.
42. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. *Science*. 2015 Mar 12 ;348(6230):124–8.
43. Rizvi NA, Cho BC, Reinmuth N, Lee KH, Luft A, Ahn M-J, et al. Durvalumab With or Without Tremelimumab vs Standard Chemotherapy in First-line Treatment of Metastatic Non–Small Cell Lung Cancer. *JAMA Oncology*. 2020 May 1;6(5):661.
44. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nature Medicine*. 2018 Aug 6;24(9):1441–8.

45. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim S-W, Carcereny Costa E, et al. Nivolumab plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer. *New England Journal of Medicine*. 2019 Sep 28;
46. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JP, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG, Moher D. The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations. *Ann Intern Med*. 2015;162(11):777-784.
47. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019 Aug 28;366:i4898.
48. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Statistics in medicine*. 2002;21(11):1539–58.
49. West H, McCleod M, Hussein M, Morabito A, Rittmeyer A, Conter HJ, et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *The Lancet Oncology*. 2019 Jul;20(7):924–37.
50. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. *Journal of Clinical Oncology*. 2019 Mar;37(7):537–46.
51. Mok TSK, Wu Y-L, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet (London, England)*. 2019;393(10183):1819–30.

52. Paz-Ares L, Vicente D, Tafreshi A, Robinson A, Soto Parra H, Mazières J, et al. A Randomized, Placebo-Controlled Trial of Pembrolizumab Plus Chemotherapy in Patients With Metastatic Squamous NSCLC: Protocol-Specified Final Analysis of KEYNOTE-407. *Journal of Thoracic Oncology*. 2020 Jun;
53. Rodríguez-Abreu D, Powell SF, Hochmair MJ, Gadgeel S, Esteban E, Felip E, et al. Pemetrexed plus platinum with or without pembrolizumab in patients with previously untreated metastatic nonsquamous NSCLC: protocol-specified final analysis from KEYNOTE-189. *Annals of Oncology*. 2021 Jul 1;32(7):881–95.
54. Boyer M, Şendur MAN, Rodríguez-Abreu D, Park K, Lee DH, Çiçin I, et al. Pembrolizumab Plus Ipilimumab or Placebo for Metastatic Non–Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score $\geq 50\%$: Randomized, Double-Blind Phase III KEYNOTE-598 Study. *Journal of Clinical Oncology*. 2021 Jan 29;JCO.20.03579.
55. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *The Lancet Oncology*. 2016 Nov;17(11):1497–508.
56. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for First-Line Treatment of PD-L1–Selected Patients with NSCLC. *New England Journal of Medicine*. 2020 Oct 1;383(14):1328–39.
57. Sezer A, Kilickap S, Gümüş M, Bondarenko I, Özgüroğlu M, Gogishvili M, et al. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. *The Lancet*. 2021 Feb;397(10274):592–604.
58. Yang Y, Wang Z, Fang J, Yu Q, Han B, Cang S, et al. Efficacy and Safety of Sintilimab Plus Pemetrexed and Platinum as First-Line Treatment for Locally Advanced or Metastatic Nonsquamous NSCLC: a Randomized, Double-Blind, Phase 3 Study (Oncology pRogram by InnovENT anti-PD-1-11). *Journal of Thoracic Oncology*. 2020 Oct 1;15(10):1636–46.

59. Zhou C, Wu L, Fan Y, Wang Z, Liu L, Chen G, et al. Sintilimab Plus Platinum and Gemcitabine as First-Line Treatment for Advanced or Metastatic Squamous NSCLC: Results From a Randomized, Double-Blind, Phase 3 Trial (ORIENT-12). *Journal of Thoracic Oncology*. 2021 Sep 1;16(9):1501–11.
60. Paz-Ares L, Ciuleanu T-E, Cobo M, Schenker M, Zurawski B, Menezes J, et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. *The Lancet Oncology*. 2021 Feb 1;22(2):198–211.
61. Barlesi F, Vansteenkiste J, Spigel D, Ishii H, Garassino M, de Marinis F, et al. Avelumab versus docetaxel in patients with platinum-treated advanced non-small-cell lung cancer (JAVELIN Lung 200): an open-label, randomised, phase 3 study. *The Lancet Oncology*. 2018 Nov;19(11):1468–79.
62. Das R, Verma R, Sznol M, Boddupalli CS, Gettinger SN, Kluger H, et al. Combination Therapy with Anti-CTLA-4 and Anti-PD-1 Leads to Distinct Immunologic Changes In Vivo. *The Journal of Immunology*. 2014 Dec 24;194(3):950–9.
63. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. *New England Journal of Medicine*. 2015 May 21;372(21):2006–17.
64. Cantrell D. T CELL ANTIGEN RECEPTOR SIGNAL TRANSDUCTION PATHWAYS. *Annual Review of Immunology*. 1996 Apr;14(1):259–74.
65. Veillette A, Bookman MA, Horak EM, Bolen JB. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. *Cell*. 1988 Oct;55(2):301–8.
66. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nature Reviews Drug Discovery*. 2015 Jul 31;14(8):561–84.
67. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-

- associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*. 2002 Jun 24;8(8):793–800.
68. Tsushima F, Yao S, Shin T, Flies A, Flies S, Xu H, et al. Interaction between B7-H1 and PD-1 determines initiation and reversal of T-cell anergy. *Blood*. 2007 Feb 8;110(1):180–5.
69. AstraZeneca. Update on the Phase III NEPTUNE Trial of Imfinzi Plus Tremelimumab in Stage IV Non-small-cell Lung Cancer. 2019 Available from: <https://www.astrazeneca.com/media-centre/press-releases/2019/update-on-the-phase-iii-neptune-trial-of-imfinzi-plus-tremelimumab-in-stage-iv-non-small-cell-lung-cancer-21082019.html>.
70. Kowalski DM, Reinmuth N, Orlov SV, Fischer JR, Sugawara S, Mandziuk S, et al. ARCTIC: Durvalumab + tremelimumab and durvalumab monotherapy vs SoC in ≥ 3L advanced NSCLC treatment. *Annals of Oncology*. 2018 Oct;29:viii493–4.
71. Ramakrishnan R, Gabrilovich DI. Mechanism of synergistic effect of chemotherapy and immunotherapy of cancer. *Cancer Immunology, Immunotherapy*. 2010 Oct 26;60(3):419–23.
72. Champiat S, Ileana E, Giaccone G, Besse B, Mountzios G, Eggermont A, et al. Incorporating Immune-Checkpoint Inhibitors into Systemic Therapy of NSCLC. *Journal of Thoracic Oncology*. 2014 Feb;9(2):144–53.
73. Cummings AL, Santoso KM, Goldman JW. KEYNOTE-021 cohorts D and H suggest modest benefit in combining ipilimumab with pembrolizumab in second-line or later advanced non-small cell lung cancer treatment. *Translational Lung Cancer Research*. 2019 Oct;8(5):706–9.
74. Peng M, Li X, Lei G, Weng YM, Hu MX, Song QB. The efficacy and safety of immune checkpoint inhibitor combination therapy in lung cancer: a systematic review and meta-analysis. *OncoTargets and Therapy*. 2018 Oct;Volume 11:7369–83.

9. APPENDICES

1. APPENDIX 1

PRISMA 2020 CHECKLIST

Taken from - The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations (Hutton *et al.*, 2015)

PRISMA NMA Checklist of Items to Include When Reporting A Systematic Review Involving a Network Meta-analysis

Section/Topic	Item #	Checklist Item	Reported on Page #
TITLE			
Title	1	Identify the report as a systematic review incorporating a network meta-analysis (or related form of meta-analysis).	Pg. 1
ABSTRACT			Pg. 6,7
Structured summary	2	<p>Provide a structured summary including, as applicable:</p> <p>Background: main objectives</p> <p>Methods: data sources; study eligibility criteria, participants, and interventions; study appraisal; and synthesis methods, such as network meta-analysis.</p> <p>Results: number of studies and participants identified; summary estimates with corresponding confidence/credible intervals; treatment rankings may also be discussed. Authors may choose to summarize pairwise comparisons against a chosen treatment included in their analyses for brevity.</p> <p>Discussion/Conclusions: limitations; conclusions and implications of findings.</p>	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known, including mention of why a network meta-analysis has been conducted.	Pg. 25

Objectives	4	Provide an explicit statement of questions being addressed, with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 25
METHODS			
Eligibility criteria	5	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. <i>Clearly describe eligible treatments included in the treatment network and note whether any have been clustered or merged into the same node (with justification).</i>	Pg. 27, Appendix 3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 27
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 27, Appendix 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 27 Appendix 1
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 27 Appendix 4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Appendix 4
Geometry of the network	S1	Describe methods used to explore the geometry of the treatment network under study and potential biases related to it. This should include how the evidence base has been graphically summarized for presentation, and what characteristics were compiled and used to describe the evidence base to readers.	Pg. 37

Risk of bias within individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 28
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). <i>Also describe the use of additional summary measures assessed, such as treatment rankings and surface under the cumulative ranking curve (SUCRA) values, as well as modified approaches used to present summary findings from meta-analyses.</i>	Pg. 28
Planned methods of analysis	14	<p>Describe the methods of handling data and combining results of studies for each network meta-analysis. This should include, but not be limited to:</p> <ul style="list-style-type: none"> • <i>Handling of multi-arm trials;</i> • <i>Selection of variance structure;</i> • <i>Selection of prior distributions in Bayesian analyses; and</i> • <i>Assessment of model fit.</i> 	Pg. 28
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pg. 28
Additional analyses	16	<p>Describe methods of additional analyses if done, indicating which were pre-specified. This may include, but not be limited to, the following:</p> <ul style="list-style-type: none"> • Sensitivity or subgroup analyses; • Meta-regression analyses; • <i>Alternative formulations of the treatment network; and</i> • <i>Use of alternative prior distributions for Bayesian analyses (if applicable).</i> 	Pg. 28 28-29
RESULTS			

Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 33
Presentation of network structure	S3	Provide a network graph of the included studies to enable visualization of the geometry of the treatment network.	Pg. 38
Summary of network geometry	S4	Provide a brief overview of characteristics of the treatment network. This may include commentary on the abundance of trials and randomized patients for the different interventions and pairwise comparisons in the network, gaps of evidence in the treatment network, and potential biases reflected by the network structure.	Pg. 38-39
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 33-35
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment.	Pg. 42 & 49
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: 1) simple summary data for each intervention group, and 2) effect estimates and confidence intervals. <i>Modified approaches may be needed to deal with information from larger networks.</i>	Pg. 37-39
Synthesis of results	21	Present results of each meta-analysis done, including confidence/credible intervals. <i>In larger networks, authors may focus on comparisons versus a particular comparator (e.g. placebo or standard care), with full findings presented in an appendix. League tables and forest plots may be considered to summarize pairwise comparisons.</i> If additional summary measures were explored (such as treatment rankings), these should also be presented.	Pg. 37-56

Exploration for inconsistency	S5	Describe results from investigations of inconsistency. This may include such information as measures of model fit to compare consistency and inconsistency models, <i>P</i> values from statistical tests, or summary of inconsistency estimates from different parts of the treatment network.	Pg. 45 & 52
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies for the evidence base being studied.	Pg. 36
Results of additional analyses	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression analyses, <i>alternative network geometries studied, alternative choice of prior distributions for Bayesian analyses</i> , and so forth).	Pg. 56
DISCUSSION			
Summary of evidence	24	Summarize the main findings, including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policymakers).	Pg. 57
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias). <i>Comment on the validity of the assumptions, such as transitivity and consistency. Comment on any concerns regarding network geometry (e.g., avoidance of certain comparisons).</i>	Pg. 66
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 69

TABLE 1. PRISMA GUIDELINES

2. APPENDIX 2

SEARCH STRING FOR SYSTEMATIC LITERATURE REVIEW

```
((((((((clinical trial[Title&Abstract]) OR (trial[Title&Abstract])) OR (randomized controlled trial[Title&Abstract])) OR (phase[Title&Abstract])) AND (non-small cell lung cancer[Title]))) OR (advanced non-small-cell lung cancer[Title])) OR (non-small-cell lung cancer[Title])) OR (non-small cell lung carcinoma[Title])) OR (NSCLC[Title])) AND (immune checkpoint inhibitor[Title])) OR (PD-1[Title])) OR (PD-L1[Title])) OR (CTLA-4[Title])) OR (pembrolizumab[Title])) OR (atezolizumab[Title])) OR (nivolumab[Title])) OR (ipilimumab[Title])) OR (durvalumab[Title])) OR (sintilimab[Title]))))))))))))
```

Institutes of Health Ongoing Trials Register - “www.clinicaltrials.gov”

3. APPENDIX 3

INCLUSION CRITERIA: -

- Only advanced NSCLC patients with high PD-L1 levels
- Studies including ICI and chemotherapy or included ICI + chemotherapy and chemotherapy.
- Only previously untreated cases.
- Studies that reported OS, PFS and ORR (their HRs and 95% CIs).
- Only RCTs.

EXCLUSION CRITERIA: -

- Previously treated cases.
- Low grade NSCLC.
- Patients with driver mutations (EGFR and ALK)
- Ongoing randomized studies.
- Case-control, retrospective, cohort, and case reports.
- Ineligible comparator

4. APPENDIX 4

DATA EXTRACTION MANUAL

DATA ITEM	DESCRIPTION
1. Clinical Trial Name/ Registration	List clinical trial's name and registration number.
2. Author Name	List surname and initials of the lead author.
3. Year of publication	List Official date the clinical trial study was published.
4. Trial Design	The phase (II or III) of the clinical trial, description of whether the clinical trial was randomised or non-randomised, open-label or double-blind and single or multicentre (multicohort).
5. Participants Information	Brief description of the number of participants enrolled, which intervention arm they were assigned to, and histology of NSCLC they had.
6. Intervention(s) and Comparator (s) Dose Administration	The treatment interventions, dose and schedule administered in the experimental arm and comparator arm.
7. Progression-Free Survival (PFS)	The median PFS (%) for the experimental and comparator arms. Confidence intervals (95%), p-values and hazard ratios for each PFS value also noted.
8. Overall Survival (OS)	The median OS (m) for the experimental and comparator arms. Confidence intervals (95%), p-values and hazard ratios for each OS value also noted.
9. Overall Response Rate (ORR)	The median ORR (%) for the experimental and comparator arms. Confidence intervals (95%), p-values and hazard ratios for each ORR value also noted.

TABLE 2. DATA EXTRACTION GUIDELINES

5. APPENDIX 5

ROB 2 ASSESSMENT FOR QUALITY – Taken from ‘The Cochrane collaborative tool for assessing risk of bias (Higgins and Green, 2008)’

BIAS DOMAIN	SOURCE OF BIAS	SUPPORT FOR JUDGMENT	REVIEW AUTHORS' JUDGMENT (ASSESS AS LOW,
			UNCLEAR OR HIGH RISK OF BIAS)
Selection bias	Random sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence
	Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrolment	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations before assignment
Performance bias	Blinding of participants and personnel*	Describe all measures used, if any, to blind trial participants and Performance bias due to knowledge of the researchers from knowledge of which intervention a participant allocated interventions by participants and received. Provide any information relating to whether the intended personnel during the study blinding was effective	
Detection bias	Blinding of outcome assessment	Describe all measures used, if any, to blind outcome assessment Detection bias due to knowledge of the allocated from knowledge of which intervention a participant received. interventions by outcome assessment	
		Provide any information relating to whether the intended blinding was effective	
Attrition bias	Incomplete outcome data*	Describe the completeness of outcome data for each main Attrition bias due to amount, nature, or handling outcome, including attrition and exclusions from the analysis. of incomplete outcome data	
		State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomised participants), reasons for attrition or exclusions where reported, and any preinclusion in analyses for the review	
Reporting bias	Selective reporting	State how selective outcome reporting was examined and what Reporting bias due to selective outcome	
		was found reporting	
Other bias	Anything else, ideally prespecified	State any important concerns about bias not covered in the other Bias due to problems not covered elsewhere domains in the tool	

TABLE 3. ROB2 ASSESSMENT GUIDELINES

BAYESIAN ANALYSIS OF OS: -

A)

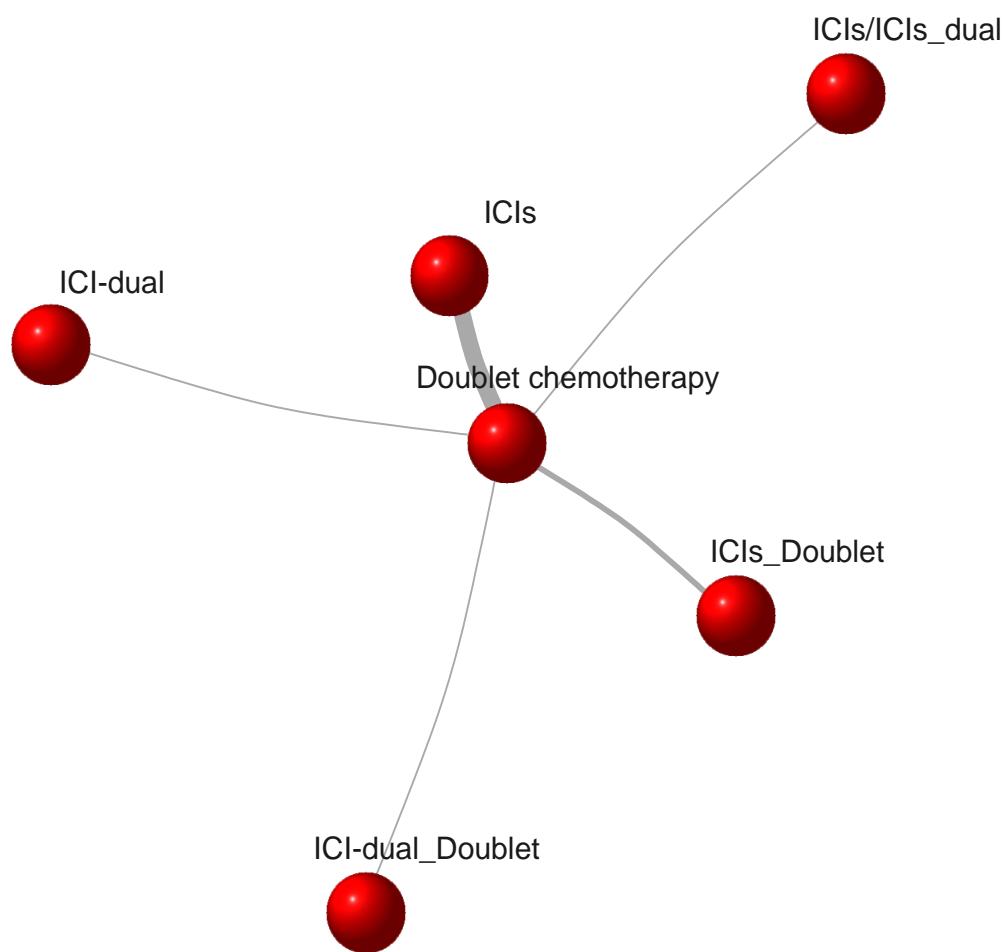


FIGURE A. FRUCHTERMAN-REINGOLD ALGORITHM FOR NETWORK GRAPH ANALYSIS-OS

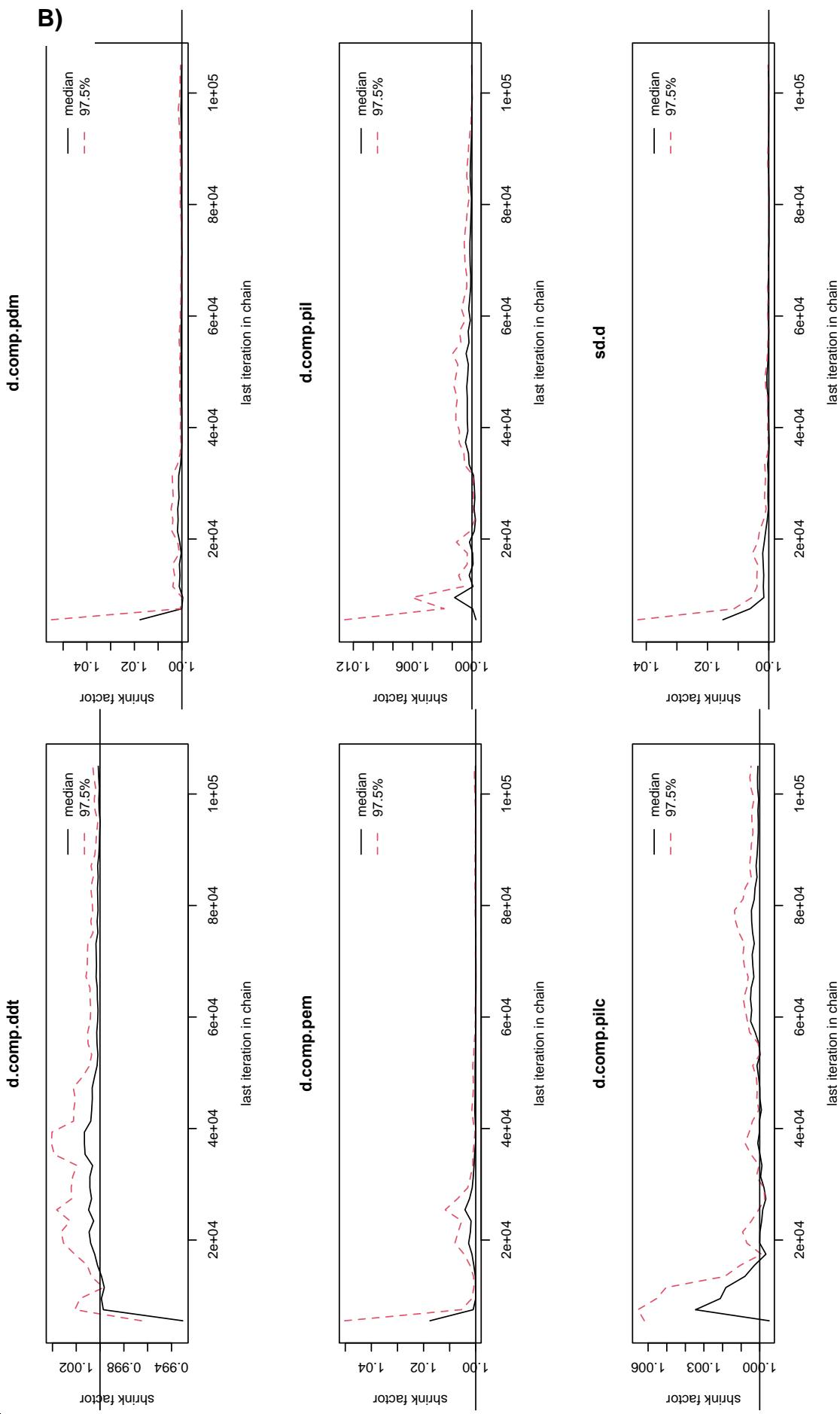


FIGURE B. GELMAN-RUBIN PLOT FOR BAYESIAN NMA-OS (MODEL CONVERGENCE)

The plot shows the PSRF comparing variations within the chains to in-between variations. PSRF should gradually shrink to 0 with increase in the number of iterations. However, the cut off value is 1.05. Various pairwise analysis plot is shown with all of them converging to 0 towards the infinity. Comp- Doublet chemotherapy, DDT- ICI single-agent/dual therapy, PEM- ICI monotherapy, PIL- ICI dual therapy, PDM- ICI + chemotherapy, SD- standard deviation. PSRF- Posterior scale reduction factor

C)

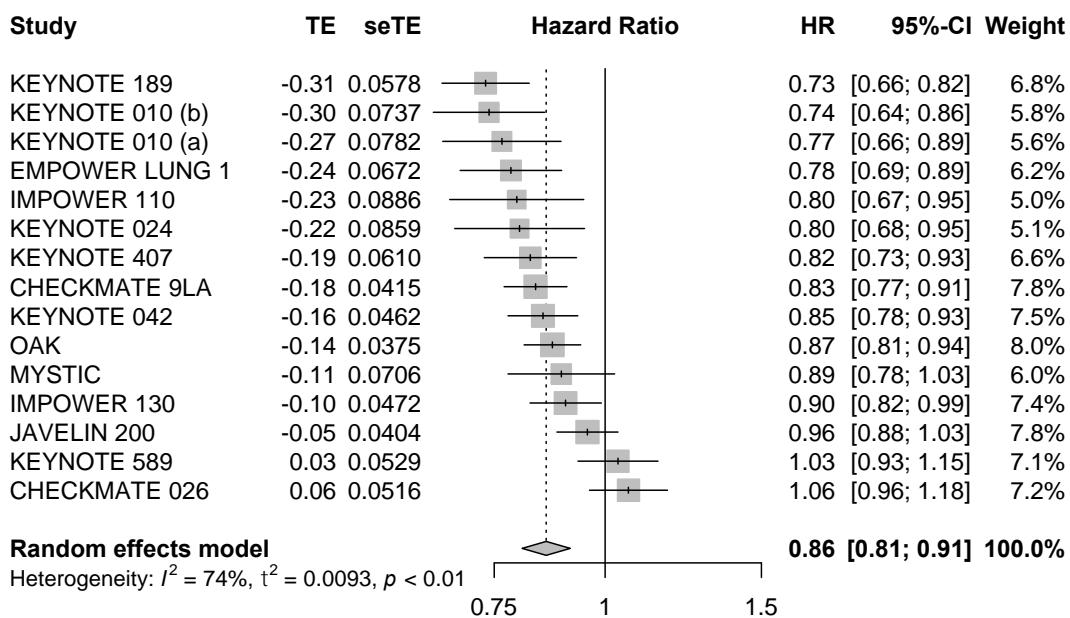


FIGURE C. FOREST PLOT- POOLED EFFECT SIZES UNDER RANDOM EFFECTS MODEL OS

HR of every study was calculated along with standard error and their 95% CI along with weight of each study (percentage that random effect model attributed to each study) is shown. A pooled HR of all the studies (diamond shape) 0.86 (0.81;0.91) was observed when comparing various studies and treatment nodes for OS. Significant heterogeneity ($I^2=74\%$) was present with $\tau^2 = 0.0093$.
 HR- Hazard's ratio, CI- Confidence Interval, OS- Overall survival, I^2 – Higgin's heterogeneity statistic, τ^2 – Heterogeneity variance.

7. APPENDIX 7

BAYESIAN ANALYSIS OF PFS: -

A)

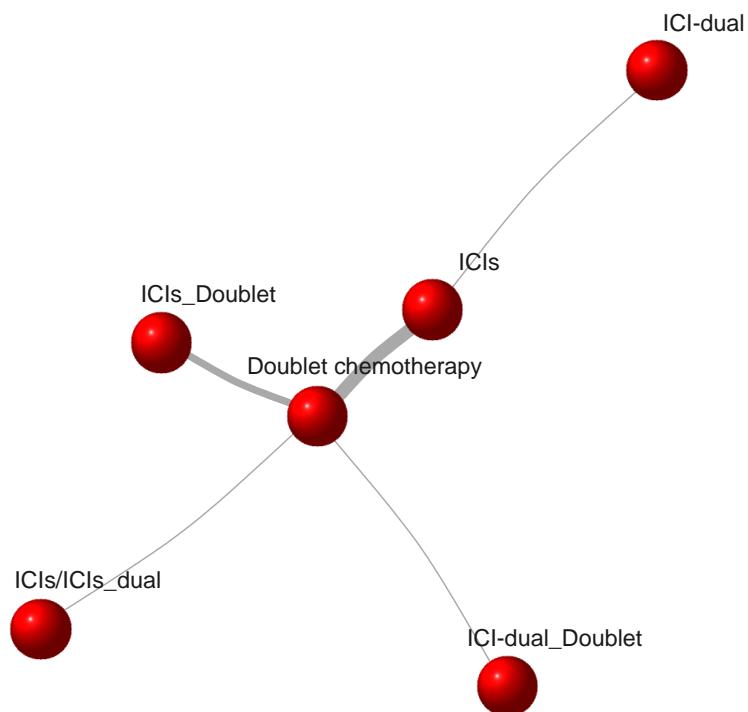


FIGURE A. FRUCHTERMAN-REINGOLD ALGORITHM FOR NETWORK GRAPH ANALYSIS- PFS

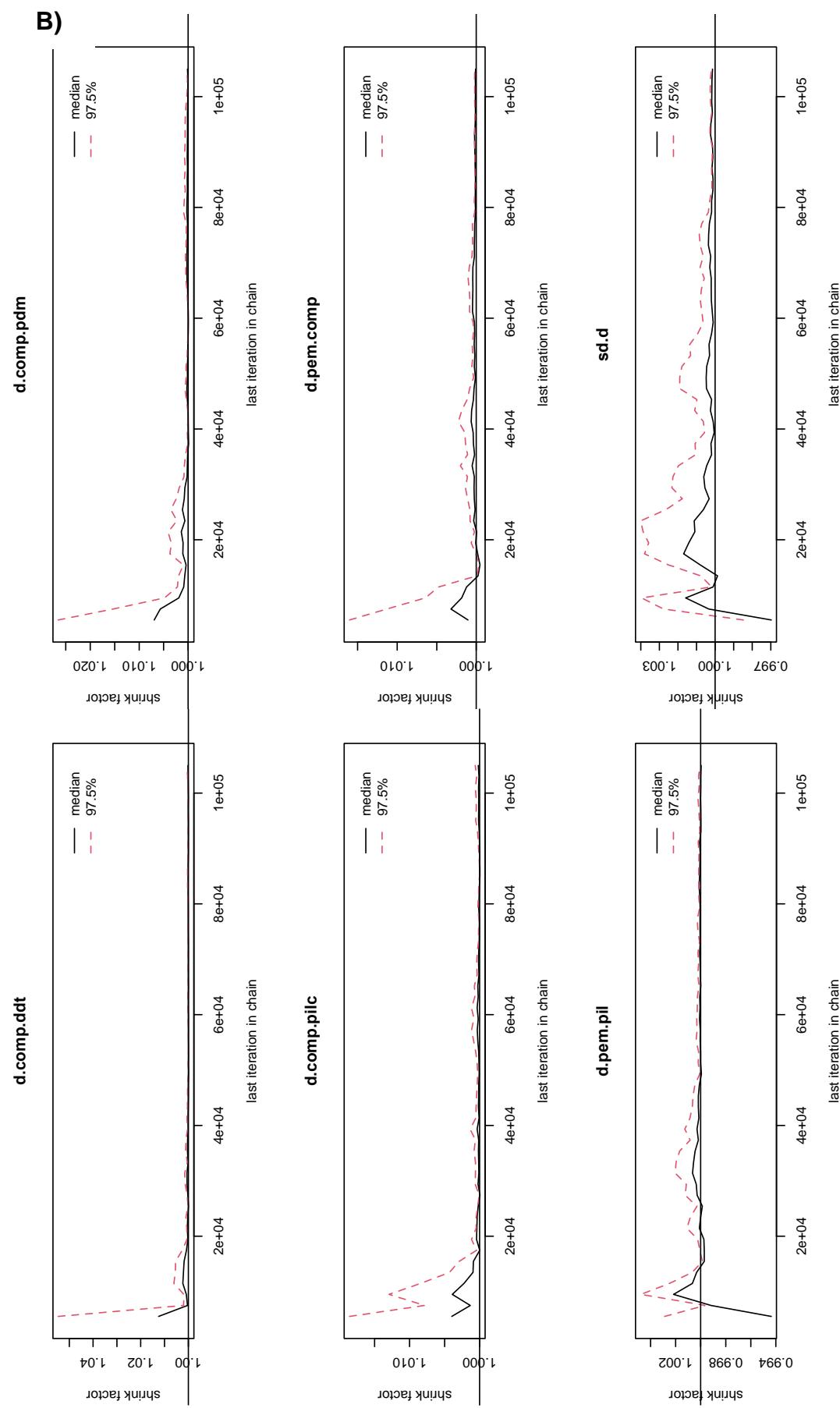


FIGURE B. GELMAN-RUBIN PLOT FOR BAYESIAN NMA-PFS (MODEL CONVERGENCE)

The plot shows the PSRF comparing variations within the chains to in-between variations. PSRF should gradually shrink to 0 with increase in the number of iterations. However, the cut off value is 1.05. Various pairwise analysis plot is shown with all of them converging to 0 towards the infinity. Comp- Doublet chemotherapy, DDT- ICI dual therapy, PEM- ICI monotherapy, PIL- ICI single-agent/dual therapy, PDM- standard deviation. PSRF- Posterior scale reduction factor

C)

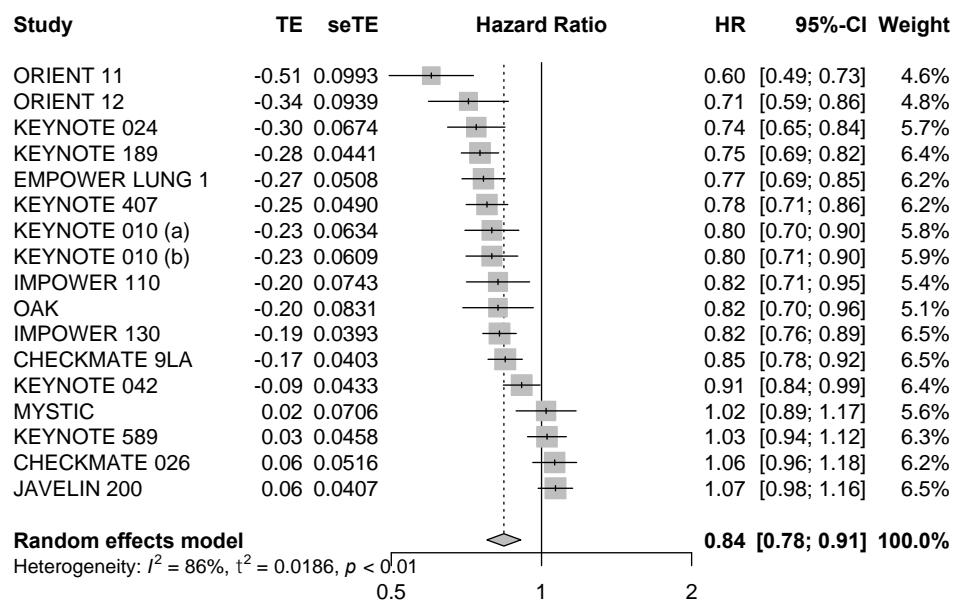


FIGURE C. FOREST PLOT- POOLED EFFECT SIZES UNDER RANDOM EFFECTS MODEL PFS

HR of every study was calculated along with standard error and their 95% CI along with weight of each study (percentage that random effect model attributed to each study) is shown. A pooled HR of all the studies (diamond shape) 0.84 (0.78;0.91) was observed when comparing various studies and treatment nodes for OS. Significant heterogeneity ($I^2=86\%$) was present with $\tau^2 = 0.0186$.
 HR- Hazard's ratio, CI- Confidence Interval, OS- Overall survival, I^2 – Higgin's heterogeneity statistic, τ^2 – Heterogeneity variance.

8. APPENDIX 8

BAYESIAN ANALYSIS OF ORR: -

A)

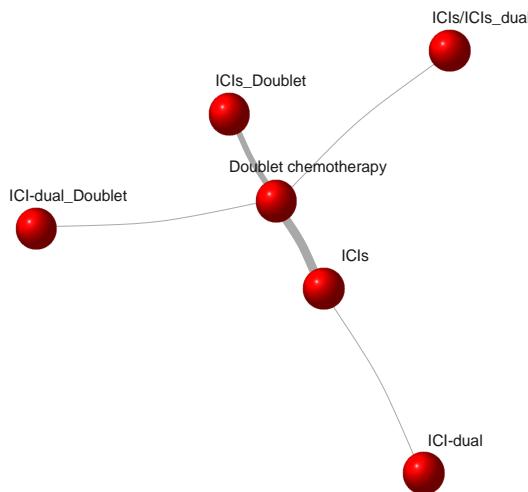


FIGURE A. FRUCHTERMAN-REINGOLD ALGORITHM FOR NETWORK GRAPH ANALYSIS- ORR

B)

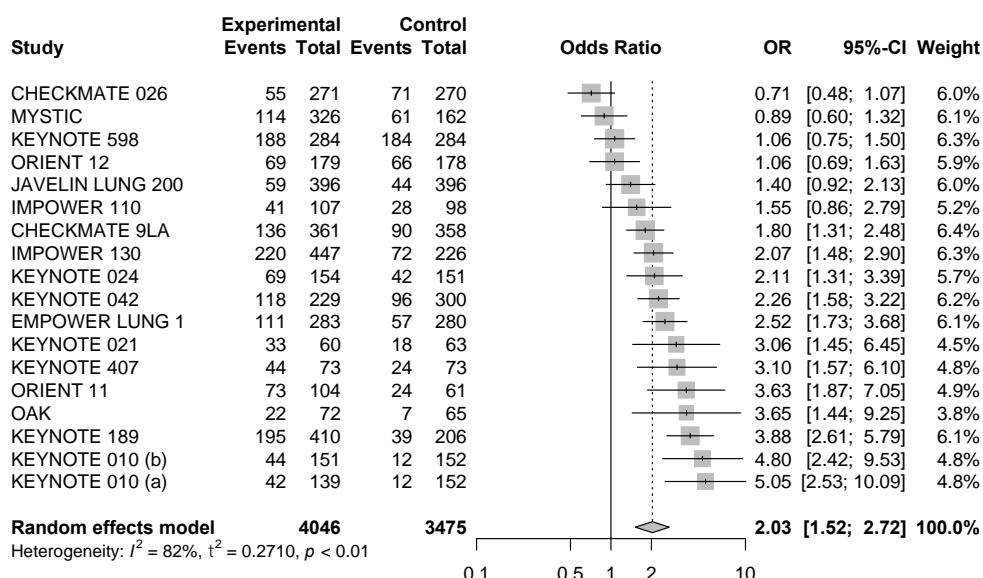
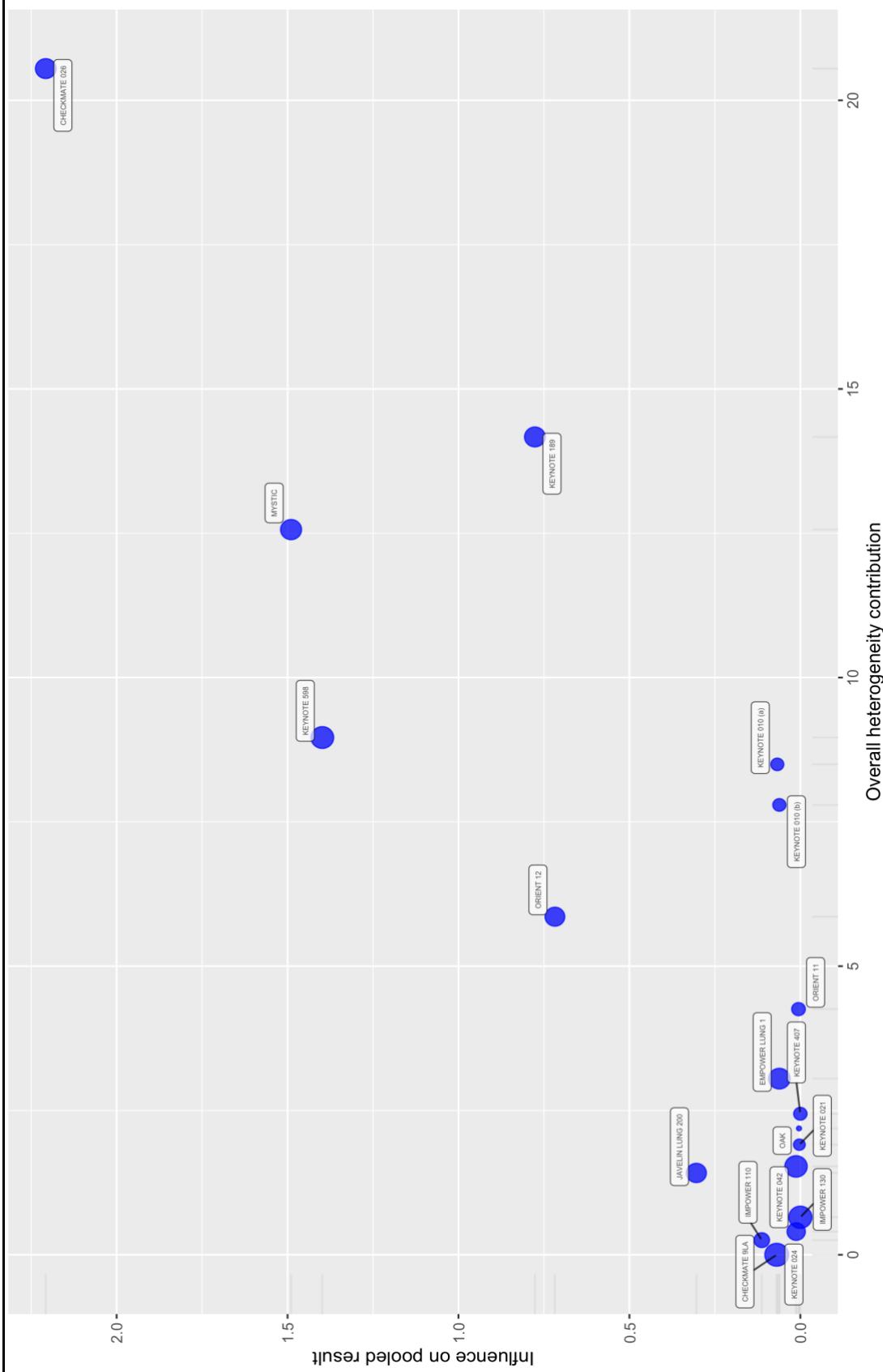


FIGURE B. FOREST PLOT- POOLED EFFECT SIZES UNDER RANDOM EFFECTS MODEL ORR

OR of every study was calculated along with standard error and their 95% CI along with weight of each study (percentage that random effect model attributed to each study) is shown. A pooled HR of all the studies (diamond shape) 2.03 (1.52;2.72) was observed when comparing various studies and treatment nodes for OS. Significant heterogeneity ($I^2=82\%$) was present with $\tau^2 = 0.2710$.
 OR- Odd's ratio, CI- Confidence Interval, OS- Overall survival, I^2 – Higgins's heterogeneity statistic, τ^2 – Heterogeneity variance.

9. APPENDIX 9

DIAGNOSITC PLOT FOR ASSESSING THE HETEROGENEITY



BAUJAT PLOT-

A diagnostic plot that identifies the outliers in terms of heterogeneity and contribute overly towards it. The horizontal axis shows the contribution made by each study to the overall effect size (based on Cochran's Q measure of heterogeneity) and vertical axis shows the influence. Moreover, it shows how this affects the size of pooled effect. CHECKMATE 026, MYSTIC and KEYNOTE 189 are three most influential studies in term of heterogeneity.

10. APPENDIX 10

SUCRA SCORES FOR OS, PFS AND ORR

TREATMENT NODE	BAYESIAN APPROACH			FREQUENTIST APPROACH		
	OS	PFS	ORR	OS	PFS	ORR
ICI dual therapy	0.926	0.994	0.67525	0.9999	0.9969	0.872
ICI monotherapy	0.537	0.532	0.663615	0.5289	0.5321	0.6511
ICI monotherapy/dual therapy	0.62	0.748	0.181085	0.5973	0.7509	0.2565
ICI dual therapy + chemotherapy	0.483	0.458	0.537165	0.4589	0.4589	0.4509
ICI + chemotherapy	0.432	0.266	0.77552	0.4148	0.2608	0.7091
Standard chemotherapy	0.002	0.001	0.167365	0.0001	0.0003	0.0604

TABLE 4. CALCULATED SUCRA VALUES FOR OS, PFS & ORR (BAYESIAN AND FREQUENTIST APPROACH)

Analysis by both Bayesian approach and Frequentist method yielded very similar results in terms of OS, PFS and ORR. Combination regimens including immunotherapy were established to yield better survival outcomes when compared to standard chemotherapy. ICI dual therapy was ranked first by both the approaches in terms of OS and PFS followed by ICI single-agent therapy. ORR results were also verified by Frequentist analysis and ICI plus chemotherapy was ranked second by both the analyses.

OS- Overall survival, PFS- Progression-free survival, ORR- overall response rate, SUCRA- Surface under the cumulative ranking, ICI- Immune checkpoint inhibitor

Bold indicates statistically significant scores.