alternating approach might be superior to a concomitant strategy, which might otherwise have antagonistic effects. In the FASTACT-2 (First-line Asian Sequential Tarceva and Chemotherapy Trial 2) study, the treatment benefit of the intercalated combination of chemotherapy and erlotinib was noted in patients with activating *EGFR* mutations in terms of PFS and OS [19]. Treatment with sequential gefitinib and cisplatin/docetaxel has been reported to have promising efficacy against *EGFR*-mutant NSCLC [20].

When EGFR-TKIs were stopped transiently and then restarted, the majority of patients had a decrease in tumor size and a reduction in tumor 18F-fluorodeoxyglucose (FDG) uptake [21]. Additionally, gefitinib and lapatinib have been reported to reduce levels of the target of pemetrexed, thymidylate synthase [22, 23], suggesting that alternating gefitinib and pemetrexed might have synergistic effects. Collectively, for comparison with the concurrent regimen arm, we used a sequential alternating regimen arm in this exploratory study.

In this study, despite the identical PFS between the groups, better OS was observed in the concurrent regimen group. The reasons for this survival outcome are not readily identifiable. The majority of patients had deep tumor regression, with seven patients having CR (Figure 3); however, some patients exhibited initial progression in the sequential alternating regimen group. Several mechanisms for *de novo* EGFR-TKI resistance have been reported, and the concurrent strategy with cytotoxic agents might circumvent such resistance.

Although almost half of the patients experienced a grade 3 or greater hematological adverse event in this study, these events were predictable and manageable. At the time this study was planned, an AUC = 6 of carboplatin was

utilized based on results of a study that had shown that a combination of pemetrexed 500 mg/m^2 plus carboplatin (AUC = 6), followed by pemetrexed maintenance therapy, had been generally tolerated in a Japanese population [24]. The incidence of hematological adverse events in this study was similar with those previously reported following carboplatin (AUC = 6) and pemetrexed treatment [25]. Additionally, interstitial lung diseases were not frequent in this study when compared with the reported incidence following gefitinib monotherapy [26, 27], and these events were reversible. Thus, the combination of gefitinib and carboplatin/pemetrexed does not appear to have additive toxicity. However, 41.5% of patients in the concurrent regimen group required dose reductions of carboplatin/pemetrexed. A lower incidence of adverse hematological events is preferred, as such, an AUC of 5 has been adopted in the NEJ009 study.

One limitation of this study is related to the nature of a phase II evaluation; specifically, this study was not designed to formally identify a difference in the efficacy and safety between the two regimens. Therefore, the findings obtained in this study should not be considered definitive.

In conclusion, this study demonstrated that both concurrent and sequentially alternating regimens with a combination of gefitinib and carboplatin/pemetrexed had promising efficacy with predictable toxicities for patients with NSCLC harboring *EGFR* mutations. The concurrent regimen was chosen as an experimental arm in an ongoing phase III NEJ009 study. The NEJ009 study will clarify whether this combinational strategy can be implemented into routine clinical practice.

ACKNOWLEDGEMENTS

We thank all participating patients, their families, and the site investigators. Authors also thank Ms. Hiromi Odagiri and Mr. Akio Kawahara in TCOG office for their technical support and Dr. Keiichi Nagao, Dr. Yushi Nakai, and Dr. Yutaka Takahashi for assistance as the Safety Monitoring Committee.

FUNDING

This study was supported by North East Japan Study Group (NEJ) and Tokyo Cooperative Oncology Group (TCOG), non-profit organizations (no grant number apply).

DISCLOSURE

SO received honoraria from AstraZeneca and Eli Lilly; AI received research support from AstraZeneca and Eli Lilly; MM received honoraria from AstraZeneca, Chugai, and Boeheringer, payment for consultant or advisory roles from AstraZeneca and Boeheringer, and research support from Boeheringer. HY received honoraria from AstraZeneca, Chugai, and Taiho, and research support from AstraZeneca and Chugai. AG received honoraria from AstraZeneca. SM received honoraria from AstraZeneca and Eli Lilly. KK received honoraria from AstraZeneca. KH received honoraria from AstraZeneca and Chugai, and payment for consultant or advisory roles from AstraZeneca. NT received honoraria from Shionogi, Kyowa, Novartis, and payment for consultant or advisory roles from Shionogi, Boehringer, and other remuneration from Sekisui. All other authors declare that they have no conflicts of interest.

REFERENCES

1. Mok TS, Wu YL, Thongprasert S et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009; 361: 947-957.

2. Maemondo M, Inoue A, Kobayashi K et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated *EGFR*. N Engl J Med 2010; 362: 2380-2388.

3. Mitsudomi T, Morita S, Yatabe Y et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11: 121-128.

4. Zhou C, Wu YL, Chen G et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced *EGFR* mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol 2011; 12: 735-742.

5. Rosell R, Carcereny E, Gervais R et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced *EGFR* mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012; 13: 239-246.

6. Sequist LV, Yang JC, Yamamoto N et al. Phase III study of afatinib or cisplatin

plus pemetrexed in patients with metastatic lung adenocarcinoma with *EGFR* mutations. J Clin Oncol 2013; 31: 3327-3334.

7. Oizumi S, Kobayashi K, Inoue A et al. Quality of life with gefitinib in patients with *EGFR*-mutated non-small cell lung cancer: quality of life analysis of North East Japan Study Group 002 Trial. Oncologist 2012; 17: 863-870.

8. Giaccone G, Herbst RS, Manegold C et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. J Clin Oncol 2004; 22: 777-784.

9. Herbst RS, Giaccone G, Schiller JH et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. J Clin Oncol 2004; 22: 785-794.

10. Herbst RS, Prager D, Hermann R et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2005; 23: 5892-5899.

11. Gatzemeier U, Pluzanska A, Szczesna A et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. J Clin Oncol 2007; 25: 1545-1552.

12. Janne PA, Wang X, Socinski MA et al. Randomized phase II trial of erlotinib alone or with carboplatin and paclitaxel in patients who were never or light former smokers with advanced lung adenocarcinoma: CALGB 30406 trial. J Clin Oncol 2012; 30: 2063-2069.

13. Eberhard DA, Johnson BE, Amler LC et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005; 23: 5900-5909.

14. Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011; 29: 2866-2874.

15. Inoue A, Kobayashi K, Maemondo M et al. Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naive non-small cell lung cancer with sensitive *EGFR* gene mutations (NEJ002). Ann Oncol 2013; 24: 54-59.

16. Ciuleanu T, Brodowicz T, Zielinski C et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 2009; 374:

1432-1440.

17. Paz-Ares L, de Marinis F, Dediu M et al. Maintenance therapy with pemetrexed plus best supportive care versus placebo plus best supportive care after induction therapy with pemetrexed plus cisplatin for advanced non-squamous non-small-cell lung cancer (PARAMOUNT): a double-blind, phase 3, randomised controlled trial. Lancet Oncol 2012; 13: 247-255.

18. Mahaffey CM, Davies AM, Lara PN, Jr. et al. Schedule-dependent apoptosis in K-*ras* mutant non-small-cell lung cancer cell lines treated with docetaxel and erlotinib: rationale for pharmacodynamic separation. Clin Lung Cancer 2007; 8: 548-553.

19. Wu YL, Lee JS, Thongprasert S et al. Intercalated combination of chemotherapy and erlotinib for patients with advanced stage non-small-cell lung cancer (FASTACT-2): a randomised, double-blind trial. Lancet Oncol 2013; 14: 777-786.

20. Kanda S, Ohe Y, Horinouchi H et al. Phase II study of gefitinib and inserted cisplatin plus docetaxel as a first-line treatment for advanced non-small cell lung cancer haboring an epidermal growth factor receptor activating mutation. J Clin Oncol 2013; 31, (suppl; abstr 8064)

21. Riely GJ, Kris MG, Zhao B et al. Prospective assessment of discontinuation

and reinitiation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of everolimus. Clin Cancer Res 2007; 13: 5150-5155.

22. Magne N, Fischel JL, Dubreuil A et al. ZD1839 (Iressa) modifies the activity of key enzymes linked to fluoropyrimidine activity: rational basis for a new combination therapy with capecitabine. Clin Cancer Res 2003; 9: 4735-4742.

23. Kim HP, Yoon YK, Kim JW et al. Lapatinib, a dual EGFR and HER2 tyrosine kinase inhibitor, downregulates thymidylate synthase by inhibiting the nuclear translocation of EGFR and HER2. PloS one 2009; 4: e5933.

24. Okamoto I, Takeda K, Daga H et al. Dose-escalation study of pemetrexed in combination with carboplatin followed by pemetrexed maintenance therapy for advanced non-small cell lung cancer. Lung Cancer 2010; 70: 168-173.

25. Okamoto I, Aoe K, Kato T et al. Pemetrexed and carboplatin followed by pemetrexed maintenance therapy in chemo-naive patients with advanced nonsquamous non-small-cell lung cancer. Invest New Drugs 2013; 31: 1275-1282.

26. Kudoh S, Kato H, Nishiwaki Y et al. Interstitial lung disease in Japanese patients with lung cancer - A cohort and nested case-control study. Am J Resp Crit Care 2008; 177: 1348-1357.

27. Akamatsu H, Inoue A, Mitsudomi T et al. Interstitial lung disease associated with gefitinib in Japanese patients with *EGFR*-mutated non-small-cell lung cancer: combined analysis of two Phase III trials (NEJ 002 and WJTOG 3405). Jpn J Clin Oncol 2013; 43: 664-668.

FIGURE LEGENDS

Figure 1. CONSORT diagram showing patients disposition. Adverse events causing early discontinuation of the protocol treatment are summarized in Supplementary Table S3.

Figure 2. Kaplan-Meier curve of progression-free survival (A) and overall survival (B) for all randomly assigned patients.

Figure 3. Response to the concurrent and the sequential alternating regimens. In this waterfall plot, the bars indicate the largest percentage change in target lesions from baseline. The dashed line indicates a 30% reduction from baseline.

Figure 1

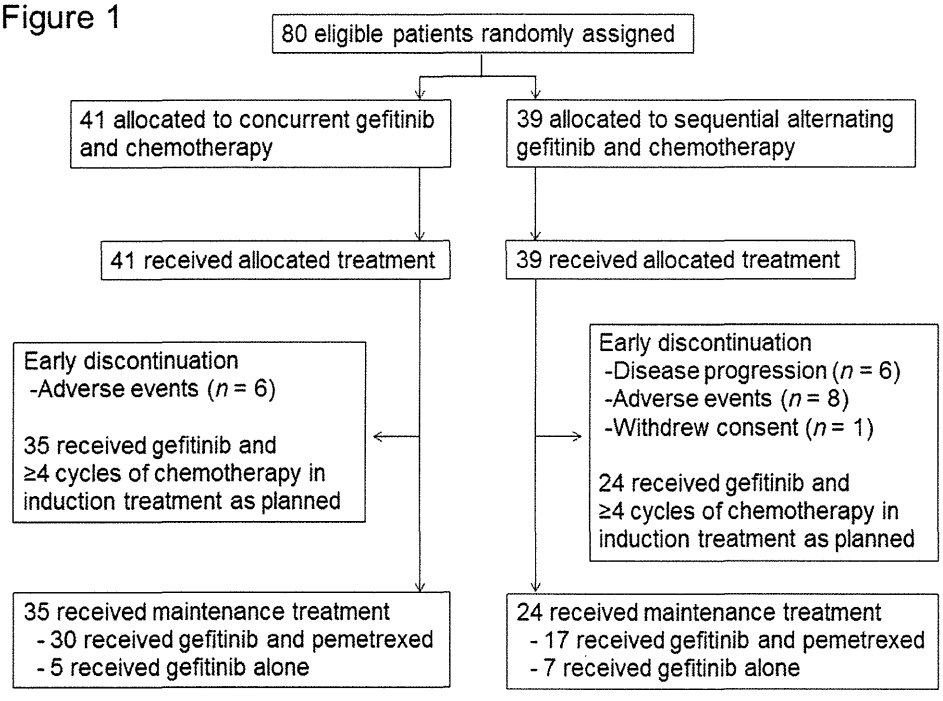
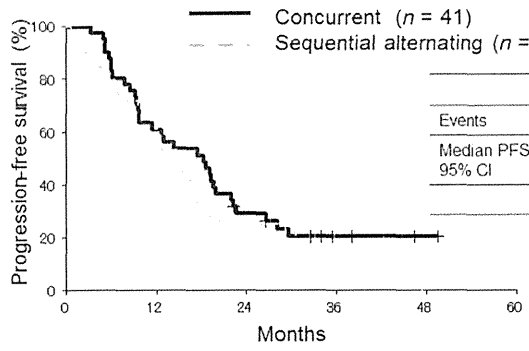


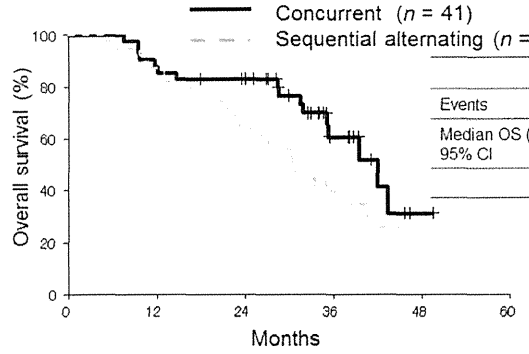
Figure 2

A



	Concurrent	Sequential
Events	32 (78.0%)	31 (79.5%)
Median PFS (months) 95% CI	18.3 9.7-21.9	15.3 11.3-17.4
	p = 0.20, HR 0.80	

B



	Concurrent	Sequential
Events	16 (39.0%)	24 (61.5%)
Median OS (months) 95% CI	41.9 35.1-NR	30.7 23.2-40.5
	p = 0.042, HR 0.55	

Figure 3

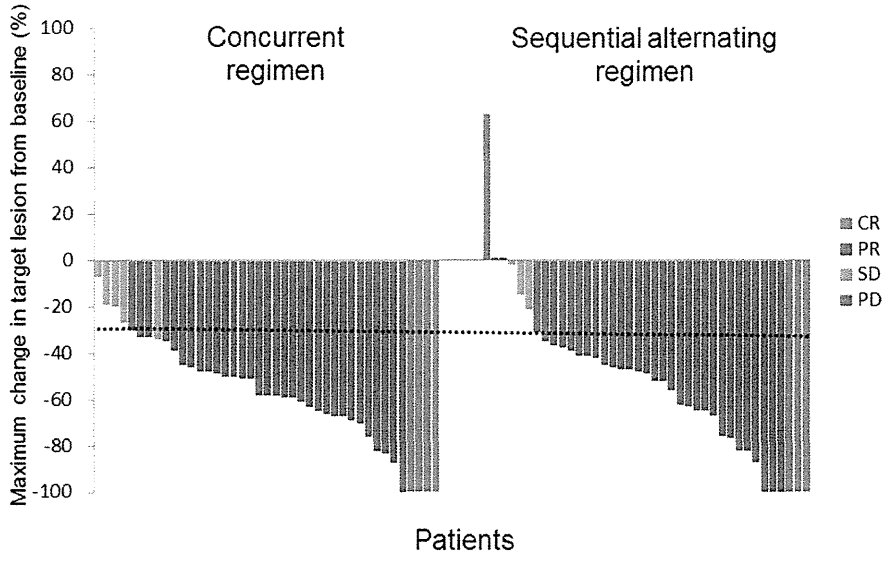


Table 1. Baseline characteristics of patients

	Concurrent regimen $n = 41$	Sequential alternating regimen $n = 39$
Characteristics	No. of patients (%)	No. of patients (%)
Gender		
Male	15 (36.6%)	13 (33.3%)
Female	26 (63.4%)	26 (66.7%)
Age (years)		
Median	62	61
Range	41-75	39-75
Smoking status		
Never smoked	22 (53.7%)	22 (56.4%)
Previous or current smoker	19 (46.3%)	17 (43.6%)
ECOG performance status score		
0	21 (51.2%)	17 (43.6%)
1	19 (43.9%)	22 (56.4%)
2	1 (2.4%)	0 (0%)
Histologic diagnosis		
Adenocarcinoma	41 (100.0%)	39 (100.0%)
Clinical stage		
IIIB	2 (4.9%)	1 (2.6%)
IV	37 (90.2%)	36 (92.3%)
Postoperative relapse	2 (4.9%)	2 (5.1%)
Type of *EGFR* mutation		
Exon 19 deletion	24 (58.5%)	17 (43.6%)
L858R	17 (41.5%)	20 (51.3%)
Others	0 (0%)	2 (5.1%)

ECOG: Eastern Cooperative Oncology Group; *EGFR*: epidermal growth factor receptor.

Table 2. Most commonly reported adverse events

Toxicity	Concurrent regimen (n = 41)					Sequential alternating regimen (n = 39)					
Grade*	1	2	3	4	≥ Grade 3	1	2	3	4	≥ Grade 3	p value for ≥ Grade 3
Vomiting	8	4	1	0	2.4%	4	2	0	0	0.0%	1.0
Appetite loss	16	11	3	0	7.3%	10	10	0	0	0.0%	0.24
Fatigue	12	10	1	0	2.4%	12	3	0	0	0.0%	1.0
Rash	20	12	1	0	2.4%	20	10	0	0	0.0%	1.0
Diarrhea	14	6	4	0	9.8%	14	2	0	0	0.0%	0.12
Stomatitis	13	6	2	0	4.9%	7	1	0	0	0.0%	0.49
Paronychia	4	5	1	0	2.4%	4	3	1	0	2.6%	1.0
Pneumonitis	1	1	0	0	0.0%	0	1	0	1	2.6%	0.49
AST/ALT elevation	16	9	4	0	9.8%	14	9	8	0	20.5%	0.22
Neutropenia	1	11	15	5	48.8%	1	5	13	5	46.2%	0.83
Febrile neutropenia	0	0	1	0	2.4%	1	0	1	1	5.1%	0.61
Anemia	11	5	9	5	34.1%	14	8	5	0	12.8%	0.035
Thrombocytopenia	8	6	6	11	41.5%	9	8	8	3	28.2%	0.25

*Grade of National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

REVIEW

The molecular biology of lung cancer brain metastasis : an overview of current comprehensions and future perspectives

Masaki Hanibuchi[1], Sun-Jin Kim[2], Isaiah J. Fidler[2], and Yasuhiko Nishioka[1]

[1]*Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan,* [2]*Department of Cancer Biology, The University of Texas M. D. Anderson Cancer Center, Houston, USA*

Abstract : Brain metastases occur in 20-40% of patients with advanced malignancies and lung cancer is one of the most common causes of brain metastases. The occurrence of brain metastases is associated with poor prognosis and high morbidity in patients with advanced lung cancer, even after intensive multimodal therapy. Progress in treating brain metastases has been hampered by a lack of model systems, a lack of human tissue samples, and the exclusion of brain metastatic patients from many clinical trials. While the biology of brain metastasis is still poorly understood, it is encouraging to see more efforts are beginning to be directed toward the study of brain metastasis. During the multi-step process of metastasis, functional significance of gene expressions, changes in brain vasculature, abnormal secretion of soluble factors and activation of autocrine/paracrine signaling are considered to contribute to the brain metastasis development. A better understanding of the mechanism of this disease will help us to identify the appropriate therapeutic strategies, which leads to circumvent brain metastases. Recent findings on the biology of lung cancer brain metastases and translational leads identified by molecular studies are discussed in this review. J. Med. Invest. 61 : 241-253, August, 2014

Keywords **:** *brain metastasis, lung cancer, molecular biology*

I. INTRODUCTION

Brain metastases constitute the most frequent malignant disease in the central nervous system (CNS), outnumbering primary brain tumor cases 10-fold (1). Up to 20-40% of patients with adult systemic malignancies will develop brain metastases in the course of their disease (2). A variety of systemic malignancies can metastasize to the CNS, although the majority of the lesions come from lung cancer

Abbreviation used : CNS, central nervous system ; QOL, quality of life ; NSCLC, non-small cell lung cancer ; SCLC, small cell lung cancer ; WBRT, whole brain radiation therapy ; BBB, blood-brain barrier ; TJ, tight junction ; ECM, extracellular matrix ; ICAM, intercellular adhesion molecule ; VCAM, vascular-cell adhesion molecule ; PECAM, platelet-endothelial-cell adhesion molecule ; EMT, epithelial-mesenchymal transition ; VEGF, vascular endothelial growth factor ; PlGF, placental growth factor ; MMP, matrix metalloproteinase ; ADAM, a disintegrin and metalloprotease ; EGFR, epidermal growth factor receptor ; ALK, anaplastic lymphoma kinase ; ROCK, Rho kinase ; HGF, hepatocyte growth factor ; PI3K, phosphoinositide 3-kinase ; ET, endothelin ; miRNA, microRNA ; SNP, single nucleotide polymorphism ; TGF-β, transforming growth factor-β ; CEA, carcinoembronic antigen ; ProGRP, pro-gastrin-releasing peptide ; PCI, prophylactic cranial irradiation ; IL, interleukin ; TNF-α, tumor necrosis factor-α ; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor ; CSF, cerebrospinal fluid ; EML4, microtubule-associated protein-like 4

Received for publication May 27, 2014 ; accepted June 23, 2014.

Address correspondence and reprint requests to Yasuhiko Nishioka, Department of Respiratory Medicine and Rheumatology, Institute of Health Biosciences, the University of Tokushima Graduate School, 3-18-15 Kuramoto-cho Tokushima, 770-8503, Japan and Fax : +81-88-633-2134.

(40-50%) followed by breast cancer (20-30%), melanoma (5-10%), lymphoma, and various other primary sites like the gastrointestinal tract (4-6%) and prostate (3, 4). Brain metastasis represents a significant healthcare problem and has an adverse impact on patient morbidity and outcome (5). In addition, tumors in the CNS strongly affect patients' quality of life (QOL), impairing sensory and motor neural functions and inducing headache, nausea, vomiting, and seizures (6). While conventional treatment regimens provide marginal survival benefits (7), the prognosis for patients with brain metastases is dismal. With an increasing incidence (8), and a frequent occurrence in patients whose extracranial cancer has been controlled, brain metastasis is becoming a major limiting factor for cancer patients' QOL and survival.

Lung cancer, including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), is the leading cause of malignancy-related death worldwide. Lung cancer is the malignancy that most commonly gives rise to brain metastases. Approximately 10-25% of lung cancer patients have brain metastases at initial diagnosis and about 40-50% of them develop brain metastases during the course of disease (9). Treatment options are limited and standard of care is generally whole brain radiation therapy (WBRT) with corticosteroids to alleviate edema and overall survival is 3-6 months after diagnosis of CNS disease. Favorable prognostic factors that affect survival include Karnofsky's performance status, patient age (< 65 years), control of primary tumor, and absence of extracranial metastatic disease (10).

To overcome lung cancer brain metastases, an understanding of the molecular biology of brain metastasis is crucial. The current review gives a broad overview about the accumulating recent knowledge of lung cancer brain metastasis and the latest developments in the field.

II. BIOLOGY OF BRAIN METASTASIS

Similar to the metastatic process in other organs, brain metastasis formation is a highly selective, multi-step process, involving complex interactions between tumor and host cells, which is clearly explained by the "seed-and-soil" hypothesis (11, 12).

The brain is considered as a "sanctuary" site for metastatic cancer cells because many of the therapeutic agents can not cross the blood-brain barrier (BBB) (13). The BBB is a network composed of both endothelial cells and supporting components that protects the CNS microenvironment. In contrast to the periphery, the endothelium of brain micro-vessels is characterized by continuous tight junctions (TJs), decreased pinocytosis activity, very low pinocytic activity, and overexpressed efflux pumps (14) (Figure 1). With the reinforcement of the surrounding extracellular matrix (ECM), basal membrane, pericytes, and the end-feet of astrocytes, the BBB effectively prevents the free exchange of substances between the blood and the interstitial fluid of the brain (15). Therefore, the brain forms a special challenge for tumor cells because of BBB and all other steps have to be successfully completed

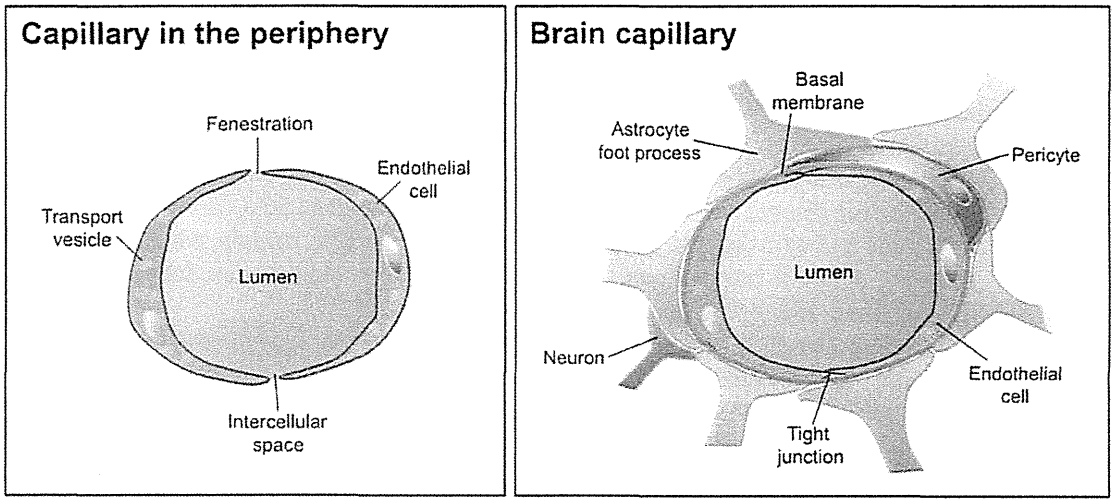


Figure 1. Schematic comparison of a brain capillary with a capillary in the periphery. Schematic structure of the blood-brain barrier. Cerebral endothelial cells, coming in contact with pericytes and astrocytes, form the morphological basis of the blood-brain barrier. Endothelial cells are interconnected by a continuous line of tight junctions (Cited from reference number 14 and revised).

for tumor cells to invade through BBB and survive.

When metastatic cancer cells enter the brain circulation, they might arrest in sites of slow flow within the capillary bed at vascular branch points. Then, interactions between cancer cells and brain endothelial cells or transendothelial migration are mediated by interaction of tumor cell-surface receptors and endothelial cell adhesion molecules (16). After overcoming the BBB, tumor cells are confronted with components of the local microenvironment including the extracellular matrix (ECM), resident brain parenchymal cells (astrocytes, microglia), and paracrine signaling molecules including cytokines and growth factors (16). Survival and successful tumor growth require adaption to and interaction with these factors. The brain also establishes an adequate blood supply via angiogenesis, angiectasia, vasculogenesis, vasculogenic mimicry, making brain metastases grow and proliferate better (17). During the above process, functional significance of gene expressions, changes in brain vasculature, abnormal secretion of soluble factors and activation of autocrine/paracrine signaling contribute to the brain metastasis development.

III. MOLECULAR PATHWAYS MEDIATING LUNG CANCER BRAIN METASTASIS

1. Gene expression analyses

In order to identify pathways specific to the development of brain metastasis and novel molecular targets/translational leads, several groups have undertaken gene expression studies in animal models and human tissue cohorts.

Kikuchi *et al.* compared gene expression of primary lung adenocarcinoma with brain metastases originating from these tumors. Of 23,040 genes tested, 244 showed a different expression level, including genes coding for plasma membrane proteins, cellular antigens and cytoskeletal proteins, which may modulate cell-cell interactions (18). Brain metastases of lung adenocarcinoma were evaluated in another study as well, by comparing the gene expression profile of metastatic brain tumors originating from lung adenocarcinoma with that of healthy lung. Zohrabian *et al.* have found 1,561 differently expressed genes by using cDNA microarray. The overexpression of genes associated with invasion and metastasis, adhesion, angiogenesis and cell migration was validated by real-time PCR (19).

In a cohort of primary tumors from lung cancer patients who developed brain metastases, Grinberg-Rashi *et al.* found the expression levels of three genes, CDH2, KIFC1, and FALZ, was highly predictive of brain metastases (20). N-cadherin, which is coded by CDH2 gene, is involved in multiple processes including inducing invasion, migration, promoting survival of cancer cells, regulating adhesion and neurite outgrowth (21). Clinically, N-cadherin overexpression has been shown to be associated with decreased survival in poorly differentiated SCLCs (22).

DCUN1D1, a squamous cell carcinoma-related oncogene, is associated with tumor progression and poor outcomes in NSCLC. Recently, the overexpression of DCUN1D1 has been found to be useful in identifying patients who are at higher risk for brain metastases. Yoo *et al.* showed that 14 of 16 DCUN1D1-positive NSCLC patients (87.5%) resulted in brain metastases (23).

However, as previously reported in other primary and metastatic clinical or experimental tumors (24-26), the genetic heterogeneity would limit the translation of the gene profiles into the clinical trials.

2. Cell surface molecules

1) Integrins

Integrins are a family of cell surface receptors that mediate cell adhesion and signal transduction. Integrins interact with ECM components such as collagen, laminin, and fibronectin and play crucial roles in cell migration. Integrins can also activate signaling cascades to mediate cell survival (27). The expression of integrin $\alpha3\beta1$ has been associated with lung cancer brain metastasis. Compared with their parental cell line and bone-metastasizing counterparts, tumor cells that preferably metastasize to the brain highly expressed $\alpha3\beta1$ integrin (28). Moreover, inhibiting $\alpha3\beta1$ integrin function decreased brain metastases formation in nude mice (28). It has been posited that the interaction of the $\alpha3\beta1$ integrin with laminin, which promotes tumor cell migration and invasion, may be critical to this effect (28).

2) Immunoglobulin superfamily of cell adhesion molecules

Endothelial cells express several adhesion molecules belonging to the immunoglobulin superfamily, including members of the intercellular adhesion molecules (ICAMs), vascular-cell adhesion molecule (VCAM) and platelet-endothelial-cell adhesion molecule (PECAM) (29). These are essential in immune

response and inflammation, but some of them were also shown to be involved in the interaction of vascular endothelial and tumor cells and formation of metastases. Recently, ICAM-1 and VCAM-1 were shown to play a crucial role in polychlorinated biphenyl-mediated enhancement of brain metastasis formation of lung carcinoma cells (30).

3) Cadherins

Cadherin dysfunction is involved in tumor progression and metastasis formation. Loss of expression of E-cadherin induces epithelial-mesenchymal transition (EMT) in carcinoma cells, which initiates an increase in cell motility and metastasis formation. In metastatic lesions, a re-expression of E-cadherin has been observed, which plays an important role in the proliferation of tumor cells at the metastatic site. Correspondingly, metastatic brain tumors were shown to express high levels of E-cadherin (31), while low expression of E-cadherin in primary NSCLC was shown to correlate with increased risk for the development of brain metastasis (32). In addition, the expression level of N-cadherin was observed to be highly predictive of brain metastasis formation in NSCLC (20).

3. Soluble factors

1) Vascular endothelial growth factor (VEGF)

Angiogenesis is an important aspect of tumor metastasis. Recent studies have examined the roles of VEGF, which influences both angiogenesis and blood vessel permeability. VEGF signaling and function in brain metastasis has also been extensively characterized in preclinical models. Measurement of VEGF levels in the culturing media of cells growing *in vitro* has shown that VEGF is secreted by tumor cells with high brain metastatic activity (33). Increased VEGF secretion has also been detected in brain metastasis xenografts in nude mouse models (33). Antisense VEGF transfectants of PC14-PE6 lung adenocarcinoma cells exhibited a decreased incidence of experimental brain metastases, suggesting that VEGF is necessary for brain tumor initiation and growth (33). Jubb *et al.* compared VEGF expression, proliferation, microvessel density, vascular pattern and vascular maturity in matched primary NSCLC and brain metastases (34). They found that brain metastases are characterized by a significantly higher proliferation rate and vascular maturity than their matched primaries. These findings are important because if the vasculature of brain metastasis is mature, then patients with cerebral secondaries may be less likely to respond to anti-VEGF treatment, even though patients with primary NSCLC might benefit from such therapy (34).

2) Placental growth factor (PlGF)

Li *et al.* (35) recently found PlGF, a member of VEGF subfamily, may be associated with SCLC brain metastasis. PlGF in the serum of SCLC patients with brain metastases was significantly higher than that without brain metastases and normal specimens. In addition, SCLC cell-derived PlGF activates the VEGFR1/Rho/ERK signaling pathway in cerebral endothelial cells, resulting in the disassembly of TJs and promoting transendothelial migration (35). Moreover, the down-regulation of PlGF suppressed SCLC cell metastasis to the brain in an experimental brain metastasis model.

3) Chemokines

Chemokines play an important role in cell migration, invasion and tumor angiogenesis (36). Emerging data support the putative involvement of the CXCR4/CXCL12 signaling axis in NSCLC brain metastasis (37). High CXCL12 expression within sites of lung cancer metastasis has been demonstrated in mouse xenograft studies (38). Hartmann *et al.* showed that the CXCR4 chemokine receptor closely co-operates with integrins to promote adhesion and chemoresistance of SCLC cells (39). Overexpression of CXCR4 and CXCL12 has been described in histolopathological specimens of brain metastases and correlated with brain-specific metastasis in a cohort of NSCLC patients (40). In a recent study, Paratore *et al.* (41) have investigated the expression of CXCR4/CXCL12 in primary NSCLC specimens of patients with and without brain metastases. CXCL12 and CXCR4 immunoreactivities in metastatic NSCLC samples were significantly higher than that in paired non-metastatic NSCLC.

4. Proteases

1) Matrix metalloproteinases (MMPs)

Different proteolytic enzymes have been implicated in brain metastasis formation and migration of tumor cells through BBB endothelial cells. MMPs might have special importance in the process of transendothelial migration of tumor cells through the BBB, because TJ proteins can be targets of MMP degradation. MMP-induced disruption of TJs was shown to promote invasion of tumor cells into the CNS (42). MMP-9 was reported to be overexpressed by brain metastatic lung adenocarcinoma cells (43).

2) A disintegrin and metalloprotease 9 (ADAM9)

ADAM-9 is a membrane-tethered protease and

belongs to a member of the "a disintegrin and metalloprotease" family. ADAM9 is overexpressed in brain metastatic lung cancer cells. Shintani *et al.* found that the expression of ADAM9 up-regulated integrin α3β1 and facilitated brain metastasis formation (44).

5. Driver mutations

1) Epidermal growth factor receptor (EGFR) mutations

It is found that driver mutations in NSCLC, at least in part, would be associated with brain metastases. In East Asian patients, Matsumoto *et al.* (45) and Gow *et al.* (46) have found EGFR mutations in 63 and 44% of brain metastases, respectively. This prevalence is similar to that reported in primary tumors of the same population, varying from 30 to 50% (47, 48). Among the 110 patients enrolled, Li *et al.* found the frequencies of EGFR mutations were 64% and 31% in the patients with and without brain metastases, respectively (49). Eichler *et al.* showed that the numbers of brain metastases were significantly higher in patients with EGFR-mutated NSCLC compared to those with wild-type (50). Sekine *et al.* demonstrated that NSCLC patients with the exon 19 deletions had more multiple and smaller brain metastases with smaller peritumoral brain edema than those without any mutations (51). Moreover, Heron *et al.* (52) reported that tumors with exon 19 deletions showed a higher incidence of CNS involvement as compared with tumors bearing a L858R mutation (21% vs. 3%). While published data are limited to draw any definite conclusions, brain metastases would be more frequent in NSCLC patients with EGFR mutations.

2) Anaplastic lymphoma kinase (ALK) translocations

More recently, ALK translocations have been found to be another "druggable" alteration besides activating EGFR mutations in NSCLC (53). ALK translocations appear to be constant between primary tumors and brain metastases (54), and ALK-positive tumors may predispose to brain metastasis formation (55). In contrast, Doebele *et al.* found patients with ALK translocations were predisposed to liver, but not adrenal, bone, or brain metastases, compared to ALK-negative cohort (56). Further studies should be required to clarify the significance of ALK translocations in NSCLC patients with brain metastases.

6. Growth factors and signaling pathways

1) Wnt signaling

The activation of canonical Wnt/TCF pathway has also been identified as playing a role in lung cancer spread to the brain. Treatment of a brain-seeking lung cancer cell line H2030-BrM3 with Wnt3a significantly increased the expressions of the WNT/TCF target genes, LEF1 and HOXB9. Confirming that LEF1 and HOXB9 are involved in metastasis, overexpression of the two genes led to an increase in brain metastases whereas knockdown of each gene decreased metastatic incidence (57). Supporting the hypothesis that TCF4 may play an important role in lung cancer development, Xu *et al.* found increased TCF4 overexpression in lung cancer patients with advanced stages (stage III-IV) compared with early stages (stage I-II) (58). Furthermore, expression of Wnt3a in a four-gene signature predicted increased mortality rates in lung cancer patients (59).

2) Rho/Rho kinase (ROCK) signaling

During invasion of tissues and migration through vessel walls and ECM components, metastasizing tumor cells require increased motility, which is dependent on the remodeling of the cytoskeleton. Rho/ROCK pathways had been proposed to be involved in the regulation of paracellular permeability and junctional dynamics in endothelial cells (60). While little is known about the behavior of tumor cells during transmigration through the BBB, it has been shown that inhibition of ROCK decreases the migration of SCLC cells through the brain endothelium (61).

3) Hepatocyte growth factor (HGF)/Met signaling

The receptor tyrosine kinase Met and its ligand HGF promoted metastatic spread to the lung, liver and brain in an experimental model using the NCI-H460 lung cancer cell line (62). A study of matched lung cancer brain metastases and primary tumors from the same patients identified increased expression of total and phosphorylated c-Met in the brain metastases. The expression and activation of c-Met in the primary lung tumors also correlated with the development of brain metastases (63).

4) Phosphoinositide 3-kinase (PI3K)-Akt signaling

The PI3K-Akt pathway is a crucial regulator of cell survival and proliferation, and increased PI3K activity has been reported in several cancer types. Recently, a PI3K inhibitor was found to effectively control metastatic growth of HER2-positive breast cancer cells in multiple organs, including brain metastases (64). However, inhibition of PI3K had no effect on the transmigration of SCLC cells through brain endothelial cells (61).