Review

The Position of Combination Chemotherapy with Mitomycin C and Methotrexate in the Treatment of Metastatic Breast Cancer – Especially Triple Negative Breast Cancer

Masahiko Tanabe[1 ✉], Takayo Fukuda[2], Yoshinori Ito[2]

1. Kyoundo Hospital, Department of Breast Surgical Oncology, 1-8, Kanda-Surugadai Chiyoda-ku, Tokyo, 101-0062, Japan
2. The Cancer Institute Hospital of JFCR (Japanese Foundation for Cancer Research), Department of Breast Medical Oncology, 3-8-31, Ariake, Koto-ku, Tokyo, 135-8550, Japan

✉ Corresponding author: Masahiko Tanabe mtanabe-tky@umin.ac.jp

Abstract

Complete cure of metastatic breast cancer (MBC) remains difficult, despite the development of new drugs. Triple negative (TN) breast cancer, which is defined by lack of hormone receptor (estrogen and progesterone receptors) expressions and the absence of human epidermal growth factor receptor type 2 (HER2) overexpression, is especially associated with poor long-term outcomes as compared with other breast cancer subtypes. Therefore, many patients with TN-MBC eagerly await promising novel treatments for TN-MBC. While new drugs are being developed, several conventional drugs such as mitomycin C (MMC) and methotrexate (MTX) are being used less. We reviewed our previous reports on combination chemotherapy with MMC and MTX (MMC/MTX) after multiple treatments. For the MMC/MTX regimen, MMC 8 mg/m^2 on day 1 and MTX 60mg/m^2 on days 1 and 15 were administered intravenously every 4 weeks. MMC/MTX was effective for 24% of 48 MBC patients who had been treated with anthracycline and taxane, resulting in a median time to progression (TTP) of 4.8 months. In addition, MMC/MTX was effective for 9.7 - 19.4 % of 31 patients with HER2-negative MBC after multiple treatments with anthracycline, taxane, capecitabine and vinorelbine, resulting in a median TTP of 3.9 months. Among 10 patients with TN-MBC, a partial response was observed in two (20%) and stable disease in three (30%). Thus, MMC/MTX controlled TN-MBC relatively well. Among TN-MBC patients maintaining good performance status without myelosuppression, MMC/MTX might be an option for those in whom no other treatments are effective after multiple regimens suggested in the guidelines.

Key words: metastatic breast cancer, triple negative breast cancer, combination chemotherapy with MMC and MTX

Background

Anthracycline-containing combinations (i.e. AC, EC, CAF, and FEC (A: doxorubicin/adriamycin; E: epirubicin; C: cyclophosphamide; F: 5-fluorouracil/ 5FU)) are widely recommended chemotherapeutic regimens for breast cancer in neo-adjuvant/adjuvant settings. In addition, taxanes such as docetaxel and paclitaxel are generally recommended for node-positive breast cancer. These are also key drugs

for first- or second-line treatment of metastatic breast cancer (MBC) [1-3]. Anti-microtubule agents such as eribulin and vinorelbine, and anti-metabolites such as gemcitabine, capecitabine, and S-1/TS-1, play major roles in treating MBC after the administration of anthracycline and taxanes [4]. In human epidermal growth factor receptor type 2 (HER2)-overexpressing MBC, anti-HER2 treatments such as trastuzumab, pertuzumab, and lapatinib are combined with chemotherapy, and a trastuzumab emtansine regimen is used when progressive disease is observed despite these treatments [5]. In addition, bevacizumab (anti-vascular endothelial growth factor monoclonal antibody) with paclitaxel is also considered to be a treatment option for aggressive MBC [6].

Triple negative (TN) breast cancer, which is defined by lack of hormone receptor (estrogen and progesterone receptors) expressions and the absence of HER2 overexpression, is associated with poor long-term outcomes as compared with other breast cancer subtypes, because it initially develops as a more aggressive tumor than other types of breast cancer. While TN-breast cancer lacks an effective treatment such as those targeting hormonal receptors and HER2 [7], several key drugs have been developed and are promising treatments for this subtype of breast cancer. For example, platinum analogues such as carboplatin and cisplatin, which act directly and cross-link DNA, are effective treatments for TN-breast cancer, especially in patients with germline mutations of *BRCA1/2* [8]. Capecitabine and eribulin are treatment choices for TN-MBC, based on clinical effectiveness having been reported in several subgroup analyses [9-11]. Recently, synergistic antitumor effects of S-1 with eribulin *in vitro* as well as *in vivo* for TN-breast cancer cell lines have been reported [12]. However, it is still difficult to control TN-MBC, i.e. to keep this tumor in a stable state, for long periods. Recently, several targeting therapies, including poly (ADP-ribose) polymerase inhibitors, angiogenesis inhibitors such as bevacizumab, epidermal growth factor receptor inhibitors, tyrosine kinase inhibitors, mTOR inhibitors, and statins have been advocated as new treatment options for TN-MBC [13, 14]. In addition, roles of immunotherapy for TN-breast cancers are emerging, because TN-breast cancers characteristically harbor more mutations than other breast cancer types and these mutations could serve as neoantigen targets for immunotherapy [15]. Clinical trials, currently underway, are assessing new chemotherapeutic strategies and agents, including targeted therapy and immunotherapy, and these novel approaches should be considered if they are feasible and available [14,

15]. However, TN-MBC currently remains resistant to these approaches, the efficacies of which have as yet been limited.

Treating MBC presents several challenges. First, complete cure remains difficult despite drug research advancements. The goals of systemic chemotherapy in the metastatic setting are to maximize control of symptoms, prevent serious complications, maintain quality of life and prolong the survival period [3, 16]. Second, the optimal regimen as second- or third-line therapy following anthracycline and taxane remains controversial. No gold standard regimen has yet been established for MBC, especially when treating advanced disease [16]. Nevertheless, it is difficult to conduct a clinical study to confirm that a treatment is superior to others after multiple regimens, because patients' conditions as well as their treatment histories vary markedly. While promising treatments for TN-MBC have not yet been developed, many TN-MBC patients eagerly anticipate the next effective treatment designed to maintain their quality of life as well as keep their disease under control. Therefore, it is of major importance for these patients to have additional treatment options after guideline-based recommendations have all been exhausted [1-3]. Herein, we reviewed our previous reports on MMC/MTX and focused on the possibility of this regimen serving as a treatment for TN-MBC.

Effectiveness of MMC/MTX treatment

With the development of more new drugs, two conventional drugs have come to be far less frequently used. Mitomycin C (MMC), which was isolated in Japan, is one of the most effective agents for MBC [1, 2, 17]. MMC, which is an antitumor antibiotic, bi- or tri- functionally alkylates a quinone, a urethane, and an aziridine ring of DNA [18]. Alkylating DNA at multiple targets results in the inhibition of DNA synthesis and thereby to DNA degradation [17, 18]. Recently, MMC was reported to exert an anti-proliferative effect by triggering the apoptotic signaling pathway in fibroblasts, potentially reducing fibrous scar formation effectively [19-21].

Methotrexate (MTX), which is an inhibitor of dihydrofolate reductase, is an original constituent of the CMF (cyclophosphamide/methotrexate/5FU) combination regimen. MTX enters the cell through the reduced folate carrier using an endocytic pathway activated by a folate receptor [22, 23]. After entering the cell, MTX and polyglutamated-MTX inhibit the enzyme dihydrofolate reductase, resulting in the inhibition of DNA synthesis [23].

Both MMC and MTX are now rarely used since anthracycline-containing combinations and taxanes

have become well established as neoadjuvant/adjuvant chemotherapy. However, we took a new look at these two drugs, because both have promising effects as well as adequate information on safety and toxicities. We herein review our results for combination chemotherapy with MMC and MTX (MMC/MTX) in patients who had already received multiple chemotherapeutic regimens. For the MMC/MTX regimen, MMC 8 mg/m² on day 1 and MTX 60mg/m² on days 1 and 15 were administered intravenously every 4 weeks. The MMC/MTX regimen was effective for 24% of 48 MBC patients who had been treated with anthracycline and taxanes, resulting a median time to progression (TTP) of 4.8 months [1]. In addition, this MMC/MTX regimen was effective for 9.7 % (response rate) to 19.4 % (clinical benefit rate) of 31 patients with HER2-negative MBC which had been aggressively treated with anthracycline, taxanes, capecitabine and vinorelbine, resulting in a median TTP of 3.9 months [2]. In both studies, adverse events including anemia, leucopenia, and thrombocytopenia were manageable [1, 2]. Thus, MMC/MTX for MBC yielded relatively good results even after multiple treatments. In addition, although based only on one case, aggressive MBC refractory to newly developed drugs such as eribulin and bevacizumab, after anthracycline, taxanes, capecitabine and vinorelbine, was reported to be well controlled by MMC/MTX treatment [24]. Therefore, we speculate that this MMC/MTX regimen has the potential to be a treatment option when MBC is refractory to the guideline-based therapeutic regimens.

MMC/MTX treatment for TN-MBC

We previously enrolled 10 patients with TN-MBC who had histories of treatment with anthracycline, taxanes, capecitabine and vinorelbine

in our study. Although the analysis was based on a small number of patients, a partial response (PR) was observed in two patients (20%) and stable disease (SD) in three (30%) including one with long-term SD (more than 6 months). The clinical benefit ratio (PR and long-term SD) for TN-MBC was 30% and the median TTP was 4.4 months (Table 1) [2]. These results were relatively good considering that the subtype of MBC was triple negative and that the MMC/MTX regimen was administered after multiple other treatments. Why is the MMC/MTX regimen relatively effective even after numerous aggressive treatments with anthracycline, taxanes, capecitabine, and vinorelbine? We speculate that one factor is the lack of cross resistance between MMC/MTX and the previously used anti-cancer drugs including anti-microtubule and anti-metabolite agents.

MMC for TN-breast cancer

MMC cross-links DNA, functioning in the same manner as alkylating agents like platinum analogues including cisplatin and carboplatin which bind DNA to produce DNA interstrand-cross links. The platinum analogues have been shown to be effective in patients with MBC associated with germline *BRCA1/2* mutations [25]. In addition, TN-breast cancers with acquired deficiency of BRCA1/2 function, which are referred to as 'BRCAness', also respond well to platinum analogues, because homologous recombination DNA repair is impaired in TN-breast cancer with *BRCA* mutations whether inherited or acquired [7]. Breast cancer cases with BRCAness also include some with sporadic breast cancers that share phenotypic characteristics with those associated with germline *BRCA* mutations, for example, due to low *BRCA* gene expression caused by DNA methylation of *BRCA* gene promoter regions [26, 27]. In a broad sense, double-strand DNA break repair, which requires homologous recombination, is likely to be impaired in breast cancers with BRCAness. Therefore, it is reasonable to speculate that MMC, which targets double-stranded DNA as the platinum analogues do, has potential both pharmacologically and theoretically to be effective for TN-MBC with BRCAness. Two *in vitro* studies of *BRCA1*-associated breast cancers have, in fact, shown increased sensitivity to MMC [28, 29].

We next considered the possibility of MMC exerting an immunogenic effect on breast tumors. Recently, oxaliplatin

Table 1. Comparison of response rates between TN breast cancer and non-TN breast cancer cases treated with the MMC/MTX regimen after anthracycline, taxane, capecitabine, and vinorelbine. This result is based on the study reported by Fukuda *et al.* [2].

	all		triple negative		non-triple negative	
Response	n	(%)	n	(%)	n	(%)
Complete response	0	(0)	0	(0)	0	(0)
Partial response	3	(9.7)	2	(20)	1	(4.8)
Long stable disease	3	(9.7)	1	(10)	2	(9.5)
Stable disease	6	(19)	2	(20)	4	(19)
Progressive disease	19	(61)	5	(50)	14	(67)
Total	31	(100)	10	(100)	21	(100)
Objective response	3	(9.7)	2	(20)	1	(4.8)
Clinical benefit	6	(19)	3	(30)	3	(14)

combined with cyclophosphamide was reported to be an immunogenic drug which successfully sensitized host antitumor T cell immunity [30]. In addition, anthracycline and mitoxantrone as well as MMC with bortezomib are reportedly pharmacological inducers of immunogenic cell death [31]. Although whether a single agent in the MMC regimen has an immunogenic effect has not as yet been assessed, it is possible that MMC functions as an immuno-sensitizer. It has also been speculated that MMC, which strongly inhibits fibroblast proliferation postoperatively [19-21], may change the tumor microenvironments which are surrounded by fibrosis after multiple chemotherapeutic regimens. Under such conditions, anti-cancer drugs would barely reach cancer cells. Apoptosis of fibroblasts induced by MMC would presumably lead to destruction of fibrous barriers, thereby allowing good penetration of anti-cancer drugs such as MTX.

MTX for TN-breast cancer

We next turned to the role of MTX in TN-MBC treatment. Adjuvant treatment with the CMF regimen containing MTX was recently reported to be more effective than CAF/FEC for TN-breast cancer, although the studies that obtained these results were retrospective and based on limited numbers of patients [32-34]. The difference between CMF and CAF/FEC is M(MTX) and A(doxorubicin/ adriamycin)/E(epirubicin), suggesting that MTX might be more effective for TN-breast cancer than the A of CAF and/or the E of CEF. There is one report describing the molecular mechanism of MTX actions on TN-breast cancer. According to that report, folate receptor α (FRA), via which MTX is incorporated into cells, was found to be associated with the prognosis of TN- breast cancer patients, i.e., there was a tendency for FRA expression to correlate with worse overall survival and disease-free survival, though the differences were not statistically significant [35]. In addition, FRA expression is reportedly associated with increased risk of recurrence in TN-breast cancer [36]. Based on these reports, greater FRA expression by TN-MBC might result in higher intake of MTX, possibly producing a good response to MTX in patients receiving the MMC/MTX regimen. We cannot as yet speculate as to which type of TN-breast cancer would respond well to MTX, because there are no studies using MTX as the only treatment for TN-breast cancer. Elucidating the association between FRA expression patterns and types of TN-breast cancer might provide clues for developing novel TN-MBC treatments [7, 37].

Combination of MMC and MTX

The synergistic effect of MMC and MTX was demonstrated in murine tumor systems, in which a considerable increase in activity was observed [38]. Recently, several studies on drug delivery systems have focused on the combination of MMC and MTX [39, 40]. These studies, in which MTX/MMC-PEG(polyethylene glycol)ylated chitosan nanoparticles and MMC/MTX-loaded DSPE (1,2-Distearoyl-*sn*-glycero-3-phosphoetaholamine)-PEG micelles were developed and administered to model mice, demonstrated a synergistic effect of MMC and MTX. Other nanoparticles of MMC and doxorubicin were reportedly effective for treating lung metastases of TN-breast cancer in mice [41], and various types of nanoparticles allowing simultaneous delivery of multiple anti-cancer drugs including older drugs have also been developed [42]. In the near future, good co-delivery systems for MMC and MTX are expected to become clinically available.

In humans, the combination of carboplatin plus gemcitabine was reportedly effective especially for TN-breast cancer [43, 44]. This combination is comprised of a platinum agent and an anti-metabolite analogue, a combination which is similar to that of MMC and MTX. Thus, this regimen might yield clues to resolving the difficult problem of identifying treatment targets for TN-MBC. In this review, we have sought to highlight that consideration of the molecular mechanisms underlying the actions of MMC and MTX in TN-MBC might enhance our understanding of the biological characteristics of TN-MBC, possibly providing insights useful for developing novel treatments.

Who would be most likely to benefit from MMC/MTX treatment?

Of course, not all TN-MBC patients would necessarily respond well to MMC/MTX treatment. Therefore, it is very important to select patients who would be mostly likely to benefit from this treatment strategy. Although MMC/MTX is relatively effective even in patients in poor condition who have already received multiple treatment regimens as well as in those with MBC refractory to the guideline-recommended treatments, we must be cautious in administering MMC/MTX in either first-line or earlier chemotherapeutic regimens, because one of the potential toxicities of MMC is prolonged myelosuppression. How, then, should we select suitable patients for MMC/MTX treatment? MMC/MTX could be considered a treatment option for overcoming the aforementioned difficulties in

managing MBC including TN-MBC under the following circumstances: (1) MBC patients with good performance status; (2) no myelosuppression caused by prior intensive chemotherapy; (3) no effective MBC treatment in the guidelines; (4) TN-MBC lacking effective treatment; (5) platinum analogues are not usually used due to lack of approval at the treating institution.

Conclusion

Large numbers of patients with TN-MBC are eagerly awaiting promising new treatment regimens for this cancer, even those who have already received multiple treatments. MMC/MTX controlled TN-MBC relatively well after multiple regimens of chemotherapy. For appropriately selected TN-MBC patients for whom no other effective treatments are available, MMC/MTX might be an option.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

References

1. Tanabe M, Ito Y, Tokudome N, Sugihara T, Miura H, Takahashi S, et al. Possible use of combination chemotherapy with mitomycin C and methotrexate for metastatic breast cancer pretreated with anthracycline and taxanes. Breast Cancer. 2009; 16: 301-6.
2. Fukuda T, Tanabe M, Kobayashi K, Fukada I, Takahashi S, Iwase T, et al. Combination chemotherapy with mitomycin C and methotrexate is active against metastatic HER2-negative breast cancer even after treatment with anthracycline, taxane, capecitabine, and vinorelbine. Springerplus. 2015; 4: 376.
3. Crown J, Dieras V, Kaufmann M, von Minckwitz G, Kaye S, Leonard R, et al. Chemotherapy for metastatic breast cancer-report of a European expert panel. Lancet Oncol. 2002; 3: 719-27.
4. Andreopoulou E, Sparano JA. Chemotherapy in Patients with Anthracycline- and Taxane-Pretreated Metastatic Breast Cancer: An Overview. Curr Breast Cancer Rep. 2013; 5: 42-50.
5. Mendes D, Alves C, Afonso N, Cardoso F, Passos-Coelho JL, Costa L, et al. The benefit of HER2-targeted therapies on overall survival of patients with metastatic HER2-positive breast cancer--a systematic review. Breast Cancer Res. 2015; 17: 140.
6. Gray R, Bhattacharya S, Bowden C, Miller K, Comis RL. Independent review of E2100: a phase III trial of bevacizumab plus paclitaxel versus paclitaxel in women with metastatic breast cancer. J Clin Oncol. 2009; 27: 4966-72.
7. Sharma P. Biology and Management of Patients With Triple-Negative Breast Cancer. Oncologist. 2016; 21: 1050-62.
8. Joensuu H, Gligorov J. Adjuvant treatments for triple-negative breast cancers. Ann Oncol. 2012; 23 Suppl 6: vi40-5.
9. Kotsori AA, Dolly S, Sheri A, Parton M, Shaunak N, Ashley S, et al. Is capecitabine efficacious in triple negative metastatic breast cancer? Oncology. 2010; 79: 331-6.
10. Cortes J, Hudgens S, Twelves C, Perez EA, Awada A, Yelle L, et al. Health-related quality of life in patients with locally advanced or metastatic breast cancer treated with eribulin mesylate or capecitabine in an open-label randomized phase 3 trial. Breast Cancer Res Treat. 2015; 154: 509-20.
11. Twelves C, Awada A, Cortes J, Yelle L, Velikova G, Olivo MS, et al. Subgroup Analyses from a Phase 3, Open-Label, Randomized Study of Eribulin Mesylate Versus Capecitabine in Pretreated Patients with Advanced or Metastatic Breast Cancer. Breast Cancer (Auckl). 2016; 10: 77-84.
12. Terashima M, Sakai K, Togashi Y, Hayashi H, De Velasco MA, Tsurutani J, et al. Synergistic antitumor effects of S-1 with eribulin in vitro and in vivo for triple-negative breast cancer cell lines. Springerplus. 2014; 3: 417.
13. Yao H, He G, Yan S, Chen C, Song L, Rosol TJ, et al. Triple-negative breast cancer: is there a treatment on the horizon? Oncotarget. 2016.
14. Zeichner SB, Terawaki H, Gogineni K. A Review of Systemic Treatment in Metastatic Triple-Negative Breast Cancer. Breast Cancer (Auckl). 2016; 10: 25-36.
15. Gadi VK, Davidson NE. Practical Approach to Triple-Negative Breast Cancer. J Oncol Pract. 2017; 13: 293-300.
16. Moreno-Aspitia A, Perez EA. Anthracycline- and/or taxane-resistant breast cancer: results of a literature review to determine the clinical challenges and current treatment trends. Clin Ther. 2009; 31: 1619-40.
17. Hortobagyi GN. Mitomycin-C in breast cancer. Semin Oncol. 1985; 12: 65-70.
18. Garewal HS. Mitomycin C in the chemotherapy of advanced breast cancer. Semin Oncol. 1988; 15: 74-9.
19. Sun Y, Ge Y, Fu Y, Yan L, Cai J, Shi K, et al. Mitomycin C induces fibroblasts apoptosis and reduces epidural fibrosis by regulating miR-200b and its targeting of RhoE. Eur J Pharmacol. 2015; 765: 198-208.
20. Nava MB, Rocco N, Catanuto G, Frangou J, Rispoli C, Ottolenghi J, et al. Role of Mitomycin C in Preventing Capsular Contracture in Implant-Based Reconstructive Breast Surgery: A Randomized Controlled Trial. Plast Reconstr Surg. 2017; 139: 819-26.
21. Sui T, Ge DW, Yang L, Tang J, Cao XJ, Ge YB. Mitomycin C induces apoptosis in human epidural scar fibroblasts after surgical decompression for spinal cord injury. Neural Regen Res. 2017; 12: 644-53.
22. Matherly LH, Hou Z, Deng Y. Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. Cancer Metastasis Rev. 2007; 26: 111-28.
23. Chabner BA, Roberts TG, Jr. Timeline: Chemotherapy and the war on cancer. Nat Rev Cancer. 2005; 5: 65-72.
24. Tanabe M. Combination Chemotherapy of Mitomycin C and Methotrexate Was Effective on Metastatic Breast Cancer Resistant to Eribulin, Vinorelbine, and Bevacizumab after Anthracycline, Taxane, and Capecitabine. Case Rep Oncol. 2016; 9: 422-6.
25. Narod SA. BRCA mutations in the management of breast cancer: the state of the art. Nat Rev Clin Oncol. 2010; 7: 702-7.
26. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer. 2004; 4: 814-9.
27. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016; 16: 110-20.
28. Sirohi B, Arnedos M, Popat S, Ashley S, Nerurkar A, Walsh G, et al. Platinum-based chemotherapy in triple-negative breast cancer. Ann Oncol. 2008; 19: 1847-52.
29. Moynahan ME, Cui TY, Jasin M. Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. Cancer Res. 2001; 61: 4842-50.
30. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity. 2016; 44: 343-54.
31. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013; 31: 51-72.
32. Wang S, Shi Y, Yuan Z, Wang X, Liu D, Peng R, et al. Classical CMF regimen as adjuvant chemotherapy for triple-negative breast cancer may be more effective compared with anthracycline or taxane-based regimens. Med Oncol. 2012; 29: 547-53.
33. Colleoni M, Cole BF, Viale G, Regan MM, Price KN, Maiorano E, et al. Classical cyclophosphamide, methotrexate, and fluorouracil chemotherapy is more effective in triple-negative, node-negative breast cancer: results from two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Clin Oncol. 2010; 28: 2966-73.
34. Cheang MC, Voduc KD, Tu D, Jiang S, Leung S, Chia SK, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline

substitution in the NCIC.CTG MA.5 randomized trial. Clin Cancer Res. 2012; 18: 2402-12.

35. Zhang Z, Wang J, Tacha DE, Li P, Bremer RE, Chen H, et al. Folate receptor alpha associated with triple-negative breast cancer and poor prognosis. Arch Pathol Lab Med. 2014; 138: 890-5.

36. Ginter PS, McIntire PJ, Cui X, Irshaid L, Liu Y, Chen Z, et al. Folate Receptor Alpha Expression Is Associated With Increased Risk of Recurrence in Triple-negative Breast Cancer. Clin Breast Cancer. 2017.

37. Lehmann BD, Jovanovic B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One. 2016; 11: e0157368.

38. Mabel JA, Wodinsky I. Mitomycin C combination therapy against murine tumor systems. Effectiveness with cyclophosphamide and methotrexate. Cancer. 1983; 51: 600-5.

39. Jia M, Li Y, Yang X, Huang Y, Wu H, Huang Y, et al. Development of both methotrexate and mitomycin C loaded PEGylated chitosan nanoparticles for targeted drug codelivery and synergistic anticancer effect. ACS Appl Mater Interfaces. 2014; 6: 11413-23.

40. Li Y, Lin J, Wu H, Chang Y, Yuan C, Liu C, et al. Orthogonally functionalized nanoscale micelles for active targeted codelivery of methotrexate and mitomycin C with synergistic anticancer effect. Mol Pharm. 2015; 12: 769-82.

41. Zhang T, Prasad P, Cai P, He C, Shan D, Rauth AM, et al. Dual-targeted hybrid nanoparticles of synergistic drugs for treating lung metastases of triple negative breast cancer in mice. Acta Pharmacol Sin. 2017; 38: 835-47.

42. Lee MS, Dees EC, Wang AZ. Nanoparticle-Delivered Chemotherapy: Old Drugs in New Packages. Oncology (Williston Park). 2017; 31: 198-208.

43. Maisano R, Zavettieri M, Azzarello D, Raffaele M, Maisano M, Bottari M, et al. Carboplatin and gemcitabine combination in metastatic triple-negative anthracycline- and taxane-pretreated breast cancer patients: a phase II study. J Chemother. 2011; 23: 40-3.

44. Ithimakin S, Ratanawichitrasin A, Veerasarn V, Akewanlop C, Soparattanapaisarn N, Rojananin S, et al. A phase II study of the combination of gemcitabine plus carboplatin as the neoadjuvant treatment in locally advanced breast cancer. J Med Assoc Thai. 2013; 96 (Suppl 2): S67-74.