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REVIEW

Review of the Management of Relapsed Small-Cell Lung Cancer with Amrubicin Hydrochloride

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Abstract: Lung cancer is the leading cause of cancer death, and approximately 15% of all lung cancer patients have small-cell lung cancer (SCLC). Although second-line chemotherapy can produce tumor regression, the prognosis is poor. Amrubicin hydrochloride (AMR) is a synthetic anthracycline anticancer agent and a potent topoisomerase II inhibitor. Here, we discuss the features of SCLC, the chemistry, pharmacokinetics, and pharmacodynamics of AMR, the results of *in vitro* and *in vivo* studies, and the efficacy and safety of AMR monotherapy and combination therapy in clinical trials. With its predictable and manageable toxicities, AMR is one of the most attractive agents for the treatment of chemotherapy-sensitive and -refractory relapsed SCLC. Numerous studies are ongoing to define the applicability of AMR therapy for patients with SCLC. These clinical trials, including phase III studies, will clarify the status of AMR in the treatment of SCLC.

Keywords: amrubicin, amrubicinol, topoisomerase II inhibitor, sensitive relapse, refractory relapse, second-line chemotherapy

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Small-Cell Lung Cancer

Lung cancer is the leading cause of cancer death, and approximately 15% of all patients with lung cancer are diagnosed with small-cell lung cancer (SCLC). After an apparently successful frontline therapy, most patients experience recurrence because of intrinsic or acquired resistance. At the time of recurrence, many SCLC patients are potential candidates for further therapy. Although second-line chemotherapy has been shown to cause tumor regression, many responders do not live long.[1] The median survival time (MST) is rarely more than 12 months and is usually less than 6 months after second-line therapy.[2] Treatment options for patients with recurrent SCLC include monotherapies of etoposide (VP-16),[3] oral VP-16,[4] teniposide,[5] vinorelbine,[6,7] irinotecan,[8] paclitaxel,[9] gemcitabine,[10,11] pemetrexed,[12,13] picoplatin,[14] topotecan,[15] etc.; combination therapies of VP-16 and cisplatin (CDDP),[16] doxorubicin+paclitaxel,[17] carboplatin (CBDCA)+paclitaxel,[18] CBDCA+irinotecan (CPT-11),[19] CDDP+CPT-11,[20] CPT-11+VP-16,[21] CPT-11+gemcitabine,[22] topotecan+CDDP,[23] vincristine+doxorubicin+cyclophosphamide,[24] VP-16+CDDP+ifosfamide,[25] paclitaxel+ifosfamide+CDDP,[26] CPT-11+CDDP+mitomycin,[27] etc.; or re-challenge with front-line chemotherapy.[16]

The response rate (RR) of recurrent SCLC to second-line chemotherapy, or to re-challenge with frontline chemotherapy, is highly dependent on the time between the completion of frontline chemotherapy and tumor recurrence. Patients who fail to respond to frontline chemotherapy or who relapse shortly after completion of frontline chemotherapy tend to have poor survivals, while patients who relapse 6 to 12 months after completion of frontline chemotherapy have RRs as high as 60% and better survivals.[28]

By analogy to chemo-sensitive cancers, including SCLC, two main categories of patients receiving second-line chemotherapy have been described: "chemotherapy-sensitive relapse" and "chemotherapy-refractory relapse". Chemotherapy-sensitive relapse patients have a frontline response that lasts more than 90 days after the completion of treatment. These patients receive the greatest benefit from second-line chemotherapy. In contrast, chemotherapy-refractory relapse patients comprise those who either did not respond to frontline chemotherapy, or responded initially but relapsed within 90 days of its completion.[29] New drugs are urgently needed to control SCLC more effectively, particularly for chemotherapy-refractory relapse patients.

Anthracyclines: Doxorubicin, Epirubicin, and Amrubicin

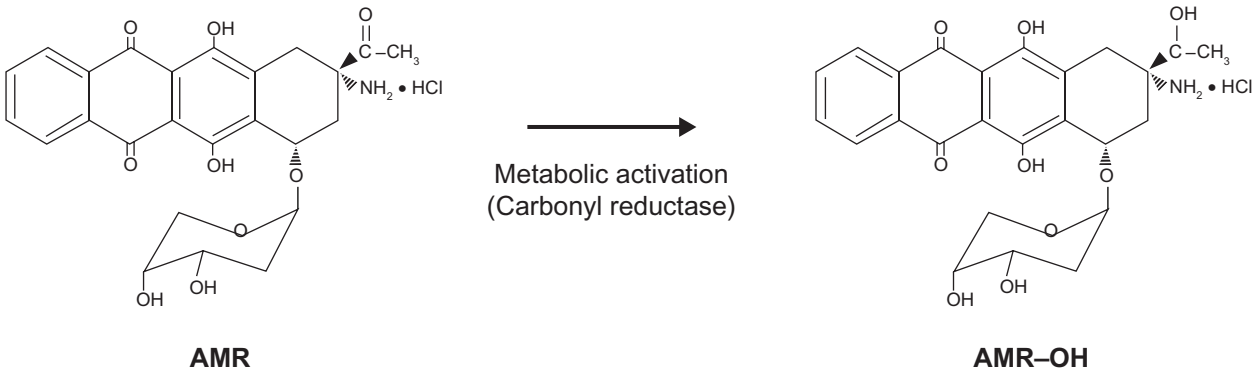
Anthracyclines, such as daunorubicin and doxorubicin, are widely used in the treatment of a variety of cancers. However, the cumulative dose-limiting cardiotoxicity of doxorubicin is a major obstacle to its use,[30] and great efforts have been made to discover means of ameliorating, preventing and delaying this side-effect.

A major metabolic pathway of anthracyclines is the reduction of the C-13 carbonyl group to a hydroxyl group by carbonyl reductase.[31] This step is generally regarded as an inactivation, because the 13-hydroxyl metabolites of doxorubicin, epirubicin and daunorubicin are much less cytotoxic than the corresponding parental drugs, unlike idarubicinol and idarubicin, whose metabolites are equipotent.[32]

Amrubicin hydrochloride (AMR; (+)-(7S, 9S)-9-acetyl-9-amino-7-[(2-deoxy-b-D-erythro-pentopyranosyl)-oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride) is a novel synthetic 9-aminoanthracycline derivative, with a structure similar to doxorubicin. (Fig. 1) AMR is currently approved for the treatment of SCLC and non-small-cell lung cancer (NSCLC) in Japan. Its antitumor activity was found to be superior to that of doxorubicin in experimental therapeutic models using human tumor xenografts.[33] In addition, AMR showed much less cardiotoxicity than doxorubicin in chronic experimental models using rabbits and dogs.[34,35]

Similar to other anthracyclines, AMR is metabolized to amrubicinol (AMR-OH), through reduction of its C-13 ketone group to a hydroxy group.[36] However, in contrast to other anthracyclines, the *in vitro* cytotoxic activity of AMR-OH is 18–220 times more potent than that of its parent compound, AMR.[31]

In mice experiments, Noguchi et al showed that AMR-OH has more potent antitumor activity than its parent compound, AMR.[37] The levels of AMR-OH in the tumors of these mice were higher than doxorubicin levels in doxorubicin-treated mice. In contrast, the levels of AMR and AMR-OH were lower than those of doxorubicin in several non-tumor



AMR **AMR–OH**

Figure 1. Chemical structures of amrubicin and amrubicinol.

tissues, including the heart. In addition, by measuring the concentrations of AMR-OH in seven human tumor xenografts after the administration of AMR, a good correlation was found between the level of AMR-OH in the tumor and the efficacy of AMR *in vivo*.[38] Thus, AMR appears to be a very promising antitumor agent: its potent antitumor activity is due to high levels of the active metabolite in the tumor, and its non-hematological toxicities, mainly cardiac toxicities, can be easily controlled because of the restricted distribution of the active metabolite in non-tumor tissues.

In vitro and *In vivo* Studies

Several studies have reported a comprehensive assessment of the clinical uses of AMR in combination with chemotherapeutic agents analyzed by the isobologram method[39] or by the combination index values.[40] We reported *in vitro* studies in the SCLC cell line SBC-3 and in the NSCLC cell line Ma-1—that CDDP enhanced the effect of AMR-OH, and that AMR-OH enhanced the formation of CDDP-induced DNA interstrand cross-links.[41] Another group reported the combination effects of AMR with other anticancer agents analyzed by the isobologram method in the T-cell leukemia cell line MOLT-3 and the human osteosarcoma cell line MG-63.[42] In MOLT-3 cells, AMR-OH had additive effects with bleomycin, VP-16, doxorubicin, CDDP, mitomycin-C, 4-hydroperoxy ifosfamide, 5-fluorouracil, cytarabine, and vincristine, whereas it had mainly protective (marked antagonistic) effects with methotrexate. In MG-63 cells, AMR-OH had additive effects with bleomycin, VP-16, doxorubicin, CDDP, mitomycin-C, 4-hydroperoxy ifosfamide; mainly sub-additive (mild antagonistic) effects with 5-fluorouracil and cytarabine; and mainly protective (marked antagonistic) effects with vincristine and methotrexate. Takigawa et al reported that AMR-OH was completely cross-resistant to doxorubicin and VP-16 in experiments using the doxorubicin-resistant SCLC cell line SBC-3/ADM and the VP-16-resistant SCLC cell line SBC-3/ETP.[43] Simultaneous exposure of the irinotecan (CPT-11)-resistant SCLC cell line SBC-3/SN-38 to AMR-OH and CDDP showed a synergistic effect when analyzed by the combination index values. Simultaneous exposure of the CDDP-resistant SCLC cell line SBC-3/CDDP to AMR-OH resulted in synergistic effects.[44] In addition, multi-drug combination effects have been reported for AMR-OH in combination with chemotherapeutic agents *in vitro* models when analyzed by the combination index values and in human lung cancer xenograft models.[45] In these experiments, human SCLC cell lines, NSCLC cell lines, a breast cancer cell line, and human gastric cancer cell lines were simultaneously exposed to two agents for 3 days. AMR-OH showed synergistic effects for the simultaneous use of CPT-11, CDDP, gefitinib and trastuzumab; additive effects with vinorelbine; and antagonistic interactions with gemcitabine. As for AMR, synergistic effects were found for simultaneous use with CPT-11, gefitinib and trastuzumab; and additive effects were demonstrated with CDDP and vinorelbine. In human lung cancer xenograft models, AMR administered intravenously at 25 mg/kg substantially prevented the growth of five out of six human lung cancer xenografts established in athymic nude mice. Synergistic effects were obtained for the simultaneous

use of AMR-OH with CDDP, CPT-11, gefitinib and trastuzumab. The combination of AMR-OH with gemcitabine was antagonistic. As just described, the combination with AMR and some chemotherapeutic agents has theoretical advantages and have proven anticancer efficacy. A clinical outcome includes both antitumor response and normal tissue toxicity from a variable drug exposure, whereas *in vitro* models represent only antitumor response. Further studies are warranted on AMR in combination with chemotherapeutic agents in clinical settings.

Mechanisms of Action of Anthracyclines and AMR

DNA topoisomerases I and II are functionally related nuclear enzymes that, in concert, catalyze the relaxation of supercoiled chromosomal DNA during DNA replication. The relaxation of DNA by topoisomerase I or II involves the transient single or double strand breakage of DNA, followed by strand passage and relegation of the DNA strand. They are extensively involved in DNA replication, transcription, and recombination, and in sister chromatin segregation, and as such are essential in maintaining cell viability.[46] Mammalian DNA topoisomerase II is the primary target of a number of antitumor agents such as doxorubicin, daunorubicin, VP-16 and amsacrine.[47] These agents interfere with the breakage–reunion reaction of DNA topoisomerase II by trapping a covalent enzyme–DNA complex, termed "the cleavable complex", in which DNA strands are broken and their 5′ termini are covalently linked to the protein. AMR and AMR-OH also stabilize the topoisomerase II-DNA complex,[36] but the mechanisms of cell killing by AMR and AMR-OH are not understood.

Combination Therapy with Topoisomerase I and II Inhibitors

Studies have shown that the use of a combination of topoisomerase I and II inhibitors completely arrests both DNA and RNA synthesis, which results in synergistic cytotoxicity. Preclinical studies have demonstrated that resistance to CPT-11, a topoisomerase I inhibitor, is often accompanied by the upregulation of topoisomerase II, causing hypersensitivity to agents that target topoisomerase II.[48–50] Consequently, the scheduling of therapy with a combination of CPT-11 and a topoisomerase II inhibitor is critical for success:[51] sequential administration of CPT-11 followed by a topoisomerase II inhibitor led to synergistic cytotoxicity, while concurrent administration led to antagonism.[52]

Clinical information on the combination of topoisomerase I and II inhibitors in the treatment of patients with SCLC is limited. Masuda et al conducted a phase II study on refractory or relapsed SCLC.[21] Twenty-five patients were treated at 4-weekly intervals with CPT-11 at a dose of 70 mg/m^2 on days 1, 8, and 15, plus VP-16 at a dose of 80 mg/m^2 on days 1–3, with granulocyte colony-stimulating factor (G-CSF) support. The overall RR was 71% and the MST was 8.9 months. Another phase II study was reported by Goto et al.[53] Forty patients with sensitive relapsed SCLC were treated with CPT-11 at a dose of 90 mg/m^2 on day 1 in weeks 2, 4, 6, and 8, CDDP at a dose of 25 mg/m^2 on day 1 weekly for 9 weeks, and VP-16 at a dose of 60 mg/m^2 on days 1–3 of weeks 1, 3, 5, 7 and 9, with G-CSF support. The overall RR was 78% and the MST was 11.8 months. Quoix et al reported a phase II study investigating the efficacy and safety of topotecan in combination with either CDDP or VP-16 in untreated extensive disease (ED)-SCLC.[54] Patients were randomized to treatment with T/C (topotecan at 1.25 mg/m^2 on days 1–5, CDDP at 50 mg/m^2 on day 5) or T/E (topotecan at 0.75 mg/m^2 on days 1–5, VP-16 at 60 mg/m^2 on days 1–5) every 21 days. The RRs were similar for the T/C (63.4%) and the T/E (61.0%) groups. The MST was 9.6 months for the T/C group and 10.1 months for the T/E group. Furthermore, Mok et al conducted a phase I–II study of the sequential administration of topotecan and oral VP-16, with alternation of the drug sequence with each consecutive cycle, and compared the hematologic toxicity between the two sequences.[55] Thirty-six patients (21 with limited disease and 15 with extensive disease) received a total of 173 courses of sequential combination chemotherapy (topotecan followed by VP-16, and VP-16 followed by topotecan). There was no significant difference in hematologic toxicity between the two sequences. The combination of topoisomerase I and II inhibitors was considered highly effective and well tolerated in the treatment of SCLC.

Pharmacokinetics and Pharmacodynamics

In a pharmacokinetic study examining the time-concentration profiles of AMR and AMR-OH, the plasma concentration curves fitted a three-compartment open model.[37] AMR was metabolized to AMR-OH by human tumor cells, and substantial amounts of AMR-OH were found in cells after a five-hour incubation with AMR in several cancer cell lines.[31] AMR-OH is less susceptible than AMR to further metabolism or is retained in tissues for a longer period.[37] It was also found that the ratio of AMR-OH to AMR plasma levels was approximately 0.1, from 1 h after administration.[37] Although the plasma concentration curve of AMR exhibited a high peak in the α/β phase and a downward slope in the γ phase, that of AMR-OH exhibited a slight or low peak in the α/β phase and a continuous long plateau in the γ phase. The half-lives in the terminal phase (T1/2γ) of AMR and AMR-OH, after administration of 40 mg/m^2 AMR on day 1, were 6.2 ± 2.0 and 16.2 ± 4.66 h, respectively.[56] Another study reported the T1/2γ of AMR and AMR-OH, after administration of 30 mg/m^2 AMR on day 3, to be 2.2 ± 0.19 and 23.2 ± 18.26 h, respectively.[57]

The pharmacodynamic profiles in a phase I trial showed the relationships between the area under the concentration–time curve (AUC), the maximum drug concentration (C_{max}) of plasma AMR, and clinical efficacy. The AUC_{0-24} of AMR was significantly correlated with the AUC of AMR-OH.[56] The AUC and C_{max} of plasma AMR were related to the duration of grade 4 neutropenia.[58] Another pharmacological study reported a significant relationship between the grade of leukopenia and the AUC of AMR-OH.[59] Previously, we reported a significant relationship between hematological toxicity and the plasma trough concentration of AMR-OH.[60] Significant relationships were observed between the levels of AMR-OH on day 4 and the toxicity grades of leukopenia, neutropenia, and anemia (P = 0.018, P = 0.012, and P = 0.025, respectively). The thrombocytopenia grade exhibited a tendency towards correlation with AMR-OH levels on day 4 (P = 0.081). The plasma concentration of AMR-OH on day 4 was positively correlated with percent change in neutrophil count in the group comprising all patients, as well as in patients treated with AMR alone and in patients co-administered CDDP. The plasma concentration of AMR or AMR-OH correlated with hematological toxicity in patients treated with AMR. Such pharmacological studies might facilitate the prediction of hematological toxicity.

Clinical Trials with Amrubicin Hydrochloride Monotherapy

Phase I studies

At first, a dose escalation study of AMR given on day 1 of every 3-week period was performed in a phase I setting for 19 patients with advanced cancer.[61] Twenty-nine evaluable courses of treatment were conducted in groups, with doses increasing from 10 to 130 mg/m^2. Myelosuppression was the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) was 130 mg/m^2. The recommended dose (RD) and schedule for a phase II trial was 100 mg/m^2 every 3 weeks.

Next, as a 5-min intravenous infusion for three consecutive days, a phase I–II study was conducted on patients with previously untreated NSCLC.[62] The MTD was 50 mg/m^2/day and the DLTs were leukopenia, neutropenia, thrombocytopenia, and gastrointestinal complications. The RD in the phase II study was 45 mg/m^2 for three consecutive days every 3 weeks. A phase I study for refractory or relapsed lung cancer (NSCLC or SCLC) patients was conducted by Okamoto et al.[56] Fifteen patients were treated with AMR at doses of 30, 35, or 40 mg/m^2 on three consecutive days every 3 weeks. Grade 4 neutropenia was observed in 67% of patients, and the MTD and RD were determined as 40 mg/m^2 and 35 mg/m^2, respectively. Similarly, Igawa et al conducted a dose-escalation study of second-line and third-line settings for SCLC.[63] The RDs were determined to be 40 mg/m^2 and 35 mg/m^2, respectively.

Phase II studies

Yana et al conducted a phase II study on previously untreated ED-SCLC patients.[64] AMR was administered intravenously at a dose of 45 mg/m^2/day on three consecutive days every 3 weeks. Of the 33 patients, the overall RR and MST were 75.8% and 11.7 months, respectively. The 1-year and 2-year survival rates were 48.5% and 20.2%, respectively; however, hematologic toxicities were severe: grade

3/4 neutropenia, anemia, and thrombocytopenia were observed in 84.8%, 78.8%, and 39.4% of patients, respectively.

The efficacy and safety of AMR in patients with previously treated SCLC have been demonstrated in several phase II studies (Table 1). In Japan, five phase II studies have been conducted at different doses of AMR for relapsed SCLC. In the first three studies described below, AMR was administered as a single agent at 40 mg/m^2 for three consecutive days.[65–67] In the first study, conducted by our group, 19 patients were treated with AMR. The RRs in 7 sensitive and 12 refractory relapse patients were 43%, and 33%, respectively.[65] In the second study, conducted by Onoda et al, the RR and MST in sensitive relapse and refractory relapse patients were 52% and 11.6 months, and 50% and 10.3 months, respectively.[66] In the third study, a randomized phase II trial comparing topotecan and AMR was conducted.[67] Sixty patients were randomly assigned to either AMR or topotecan, and 59 (36 sensitive relapse and 23 refractory relapse patients) were evaluable. For AMR treatment, the RRs of overall, sensitive relapse, and refractory relapse patients were 38%, 53%, and 17%, respectively. The median progression-free survival time (PFS) and MST were 3.5 months and 8.1 months, respectively. In the fourth study, conducted by Kato et al, 45 mg/m^2 of AMR was administered on days 1–3, every 3 weeks.[68] Thirty-four patients were treated with AMR, and there were four complete responses (CRs) and 14 partial responses (PRs), with an RR of 53%. The RR and MST among sensitive relapse and refractory relapse patients were 50% and 10.4 months, and 60% and 6.8 months, respectively. The fifth study was conducted by Kaira et al, in which 35 mg/m^2 of AMR was administered to both SCLC and NSCLC patients.[28] In this study, 29 relapsed SCLC patients were enrolled, and the RR and MST among sensitive relapse and refractory relapse patients were 60% and 12.0 months, and 37% and 11.0 months, respectively. These five studies resulted in an RR of the sensitive relapse patients in this fifth report of 50.0%–53.0%, and that of refractory relapse patients of 17.0%–60.0%. AMR is a promising therapeutic for chemotherapy-sensitive relapse patients as well as for chemotherapy-refractory relapse patients. To support the efficacy for chemotherapy-refractory relapse patients, a phase II study of AMR in patients with SCLC that is refractory or relapsed within 90 days of completing previous treatment is ongoing in Japan.

Table 1. Phase II studies of amrubicin monotherapy for recurrent SCLC.

Authors	Dose (mg/m^2)	n	RR (%)	PFS (months)	MST (months)
Sensitive relapse					
Kudoh et al[65]	40	7	42.8	NA	NA
Onoda et al[66]	40	44	52	4.2	11.6
Inoue et al[67]	40	17	53	3.9	9.9
Kato et al[68]	45	24	50	NA	10.4
Kaira et al[28]	35	10	60	4	12
Jotte et al[69]	40	50	44	4.6	9.3
Refractory relapse					
Kudoh et al[65]	40	12	33.3	4	8.3
Onoda et al[66]	40	16	50	2.6	10.3
Inoue et al[67]	40	12	17	2.6	5.3
Kato et al[68]	45	10	60	NA	6.8
Kaira et al[28]	35	19	36.8	4	11
Ettinger et al[29]	40	75	21	3.2	6

Abbreviations: RR, response rate; PFS, progression free survival; MST, median survival time.

Two phase II studies have been conducted outside Japan. In the first study, conducted in the USA, 76 sensitive relapse patients were randomly assigned to either AMR or topotecan.[69] The RR, the median PFS and MST for AMR were 36%, 4.3 months and 9.3 months, respectively. In the second study, 75 refractory relapse patients were treated with 40 mg/m^2 AMR on three consecutive days every 3 weeks.[29] The RR and MST were 21% and 6.0 months, respectively. The RRs and MSTs in these two studies conducted outside Japan were considerably lower than those of the Japanese phase II studies.

Interestingly, there were two phase II studies comparing topotecan and AMR conducted in Japan and USA.[67,69] In the Japanese study, topotecan was administered at a dose of 1.0 mg/m^2 on days 1–5, every 3 weeks. For topotecan treatment, the RRs of overall, sensitive relapse and refractory relapse patients were 13%, 21%, and 0%, respectively. The median PFS and MST were 2.2 months and 8.4 months, respectively. AMR had significantly better overall RR rates than topotecan ($P = 0.039$). However, the hematologic and nonhematologic toxicities worse than grade 3 were more frequent in the AMR arm. In terms

of overall survival, there was no statistical difference between topotecan and AMR. However, a significant difference in overall survival was observed between patients treated with AMR and those without AMR ($P < 0.001$). The USA study was conducted only for sensitive relapse patients, and topotecan was administered at a dose of 1.5 mg/m^2 on days 1–5, every 3 weeks. The RR, the median PFS and MST for topotecan were 8%, 3.5 months, and 8.9 months, respectively. AMR gave significantly better overall RR rates than topotecan ($P < 0.012$). The most common grade ≥3 adverse events with AMR vs. topotecan were neutropenia (53% vs. 74%), thrombocytopenia (31% vs. 52%) and leukopenia (27% vs. 30%). Statistical analyses in terms of overall survival between topotecan and AMR were not reported. As a result, AMR had better overall RR rates than topotecan. There is no difference between topotecan and AMR in the terms of overall survival. However, considering subsequent chemotherapy after the enrollment in these studies, AMR may have more influence than topotecan on overall survival.

Side Effects

The most frequent toxicity was myelosuppression. Previous phase II studies of AMR monotherapy for treated SCLC found that treatment was associated with a high incidence of bone marrow suppression or grade 3 or 4 hematologic toxicity.[29,66,67] These toxicities comprised neutropenia (83%), thrombocytopenia (20%), and anemia (33%) in Onoda et al's report; neutropenia (93%), thrombocytopenia (28%), and anemia (21%) in Inoue et al's report; and neutropenia (66.7%), thrombocytopenia (40.6%), and anemia (30.4%) in Ettinger et al's report. Consistent with these results, the major adverse events in our own study were grade 3 or 4 hematologic toxicities including neutropenia (85%), leukopenia (85%), thrombocytopenia (32%), and anemia (42%).[65]

Non-hematologic toxicities were generally mild, except for grade 3 febrile neutropenia. Onoda et al described the most frequent grade 3 or 4 non-hematologic toxicities as anorexia (15%), asthenia (15%), hyponatremia (8%), nausea (5%), and febrile neutropenia (5%).[66] In Inoue et al's report, the most frequent grade 3 or 4 non-hematologic toxicities were fatigue (17%), febrile neutropenia (14%), infection (10%), anorexia (7%), stomatitis (3%), and nausea (3%).[67] According to Ettinger et al, the most common grade 3 or 4 non-hematologic toxicity was fatigue (21.7%).[29] Grade 3 or 4 febrile neutropenia was seen in 11.6%. No cardiotoxicity, except for one transient atrial fibrillation, was observed among these three reports. No treatment deaths occurred in our study,[65] or in that of Onoda et al.[66] However, there was one treatment-related death, resulting from neutropenic infection, in the AMR arm of Inoue et al's study,[70] and there was one patient death each of pulmonary hemorrhage, acute myocardial infarction, and interstitial lung disease in the Ettinger et al study.[29]

Clinical Trials with Amrubicin-based Combination Therapy

Rationale for combination therapy

As shown in Table 2, AMR has been used in clinical trials in double combination regimens. There is a clear need for non-cross-resistant therapeutic options. *In vitro* antitumor synergy with many chemotherapeutic agents may indicate AMR as an ideal candidate for use in combination therapy.

Topoisomerase I Inhibitors and Amrubicin

CPT-11 and AMR have been used in three phase I studies of patients with advanced NSCLC.[57,71,72] In the first study,[57] both drugs were administered on days 1 and 8, and the MTDs of CPT-11 and AMR were 100 and 45 mg/m^2, respectively. This level had 3 of 4 patients with DLTs (persistence of grade 4 neutropenia and grade 4 leukopenia, persistence of grade 4 neutropenia, and grade 3 febrile netropenia). The RDs of CPT-11 and AMR were 100 and 40 mg/m^2, respectively. In the second study,[71] patients were treated at 3-weekly intervals with dose-escalated AMR (days 1–3) plus a fixed dose of 60 mg/m^2 CPT-11 (days 1 and 8). The 30 mg/m^2 AMR dose was one dose level above the MTD, since diarrhea and leukopenia were the DLTs. The RDs are 60 mg/m^2 of CPT-11 (days 1 and 8) and 25 mg/m^2 of AMR (days 1–3), administered every 3 weeks. The third study was a dose escalation study of AMR in combination with fixed-dose CPT-11 in patients with

Table 2. Clinical trials with amrubicin-based combination therapy.

Authors	Histology	Phase	Patient selecion	Drugs	Schedule (day)	Interval (weeks)	MTD (mg/m^2)	RD (mg/m^2)
Topoisomerase I inhibitors								
Hotta et al[71]	NSCLC	I	untreated or treated	AMR CPT-11	1, 8 1, 8	3	45 100	40 100
Yanaihara et al[57]	NSCLC	I	untreated	AMR CPT-11	1–3 1, 8	3	30> 60	25 60
Oshita et al[72]	NSCLC	I	untreated	AMR CPT-11	1–3 1	2	40 60	35 60
Shibayama et al[58]	SCLC	I	untreated or treated	AMR Topotean	3–5 1–5	4	40 0.75	35 0.75
Nogami et al[73]	SCLC	II	untreated or treated	AMR Topotean	3–5 1–5	3	– –	35 0.75
Platinum agents								
Yoshimura et al[74]	NSCLC	I	untreated	AMR CDDP	1–3 1	3	30 80	30 80
Ikeda et al[75]	NSCLC	I	treated	AMR CDDP	1–3 1–3	3–4	30 20	25 20
Ohe et al[76]	SCLC	I/II	untreated	AMR CDDP	1–3 1	3	45 60	40 60
Fukuda et al[77]	SCLC	I	untreated	AMR CBDCA	1–3 1	3	40 AUC5	35 AUC5
Inoue et al[70]	Elderly SCLC	I	untreated	AMR CBDCA	1–3 1	3	40 AUC4	35 AUC4

Abbreviations: MTD, maximum tolerated dose; RD, recommended dose; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; AMR, amrubicin; CPT-11, irinotecan; CDDP, cisplatin; CBDCA, carboplatin; AUC, area under curve.

ED-SCLC reported by Oshita et al.[72] Previously untreated patients with ED-SCLC were treated with CPT-11 at 60 mg/m^2 on day 1 and dose-escalated AMR on days 1–3, with prophylactic subcutaneous G-CSF on days 5–9 every 2–3 weeks. At 40 mg/m^2 AMR, DLTs such as grade 4 neutropenic fever were observed, and therefore this dose level was defined as the MTD, with an overall RR of 100%.

A phase I study of combination topotecan and AMR therapy in SCLC patients with relapsed or ED-SCLC was reported by Shibayama et al.[58] Topotecan and AMR were administered on days 1–5 and on days 3–5 every 4 weeks, respectively. DLTs (grade 4 neutropenia lasting for more than 4 days, grade 3 febrile neutropenia, or grade 4 thrombocytopenia) were observed at 0.75 mg/m^2 topotecan and 40 mg/m^2 AMR, and thus these were determined to be the MTDs. An objective response was observed in six patients (67%). The phase II study of the same regimen every 3 weeks for chemo-naïve or relapsed SCLC was reported.[73] The RRs were obtained in 23 (74%) of the 31 chemo-naïve and 12 (43%) of the 28 relapsed patients. Myelosuppresion was the principal toxixity with grade 4 leukopenia, neutropenia, thrombocytopenia and anemia of 46%, 80%, 25% and 7%, respectively. Grade 3–4 febrile neutropenia was observed in 41% of the patients, of whom one patient further developed Grade 5 septic shock. Other grade 3 or greater non-hematological toxicities included diarrhea, pneumonitis, vomiting, fatigue and hyponatremia in 2%, 3%, 5%, 9% and 2%, respectively. One patient each developed fatal diarrhea and pneumonitis. At the time of data analysis with a median follow-up time of 43.2 months, MST and median PFS were 14.9 and 5.3 months in the chemo-naïve patients and 10.2 and 5.1 months in the relapsed patients, respectively. Other ongoing studies include a phase II study of CPT-11 plus CDDP followed by AMR in patients with ED-SCLC, and a phase I–II study of AMR and CPT-11 in patients with advanced SCLC. The combination of topoisomerase I Inhibitors and AMR seemed effective for SCLC, despite the severe toxicity profiles. Their preliminary findings contradict the preclinical evidence from *in vitro* studies that showed

a lack of synergism with concurrent exposure to topoisomerase I and II inhibitors.

Platinum Agents and Amrubicin

We have identified five studies of AMR and platinum agents. The combination of first three studies was AMR and CDDP, and that of the last two studies was AMR and carboplatin (CBDCA). The first study, reported by our group, was a phase I study of AMR and CDDP in patients with previously untreated advanced NSCLC.[74] AMR was administered on days 1–3, and CDDP was administered at a fixed dose of 80 mg/m^2 on days 1, every 3 weeks. The MTD and recommended dose (RD) for AMR were determined to be at 30 mg/m^2. The second was a phase I study of AMR and CDDP in patients with previously treated NSCLC.[75] AMR was administered on days 1–3, and CDDP was administered at a fixed dose of 20 mg/m^2 on days 1–3, every 3 or 4 weeks. The MTD was determined to be at 30 mg/m^2 for AMR . The recommended dose was determined to be 25 mg/m^2 for AMR. The third was a phase I–II study of AMR and CDDP in previously untreated patients with ED-SCLC.[76] AMR was administered on days 1–3 and CDDP on day 1, every 3 weeks. The MTD was determined to be at 45 mg/m^2 for AMR and 60 mg/m^2 for CDDP. The RD was determined to be 40 mg/m^2 for AMR and 60 mg/m^2 for CDDP. The RR at the recommended dose was 87.8% (36/41 patients). The MST was 13.6 months and the 1-year survival rate was 56.1%. Grade 3/4 neutropenia and leukopenia occurred in 95.1% and 65.9% of patients, respectively. The forth was a phase I trial of AMR and CBDCA in previously untreated patients with ED-SCLC.[77] AMR and CBDCA were administered by intravenous infusion on days 1, 2, and 3, and on day 1, respectively. The MTDs of AMR and CBDCA were determined to be 40 mg/m^2 and the AUC was 5. A dose of 35 mg/m^2 AMR and CBDCA at AUC 5 was recommended in this regimen. The DLTs included neutropenia, leukopenia, thrombocytopenia, febrile neutropenia, and liver dysfunction. Evaluation of the responses revealed two patients with CR, nine with PR (RR 73%), and the MST was 13.6 months. The fifth was a phase I trial of AMR combined with CBDCA for elderly patients with SCLC,[70] and is described in the "Amrubicin Therapy for Elderly SCLC Patients" section.

In our pharmacological study, we established the relationships between AMR-OH and hematological toxicity during treatment with AMR alone, as well as during co-administration with CDDP, using a sigmoid E_{max} model for pharmacodynamic analysis.[60] The sigmoid curve for co-administration with CDDP was shifted to the left compared with that for AMR alone. This shift may indicate that patients treated with AMR and CDDP developed neutropenia more often than would be expected if they were treated with AMR alone. This mild additive effect in hematological toxicity is in agreement with clinical observations noted in many previous reports: patients receiving combined treatment with AMR and CDDP experienced more profound myelotoxicity than those treated with AMR alone, and the dose of AMR for combined treatment with CDDP was less than that used for AMR monotherapy.[74-76]

Phase III Studies

To our knowledge, AMR is currently undergoing phase III clinical studies in one monotherapy trial and two double combination regimen trials. The monotherapy trial involves patients with SCLC, after failure of first-line chemotherapy, comparing AMR with topotecan. The combination regimen trials comprise a randomized, multicenter study comparing CPT-11 with CDDP versus AMR with CDDP in the treatment of ED-SCLC; and a study of AMR with CDDP versus VP-16 with CDDP in ED-SCLC patients.

Amrubicin Therapy for Elderly SCLC Patients

In a first-line setting, AMR monotherapy for treating elderly and high-risk patients with SCLC has been reported.[78] A dose of 40 mg/m^2 on days 1–3 every 3 weeks was feasible, and had a favorable anticancer effect with an RR of 73%. Another phase I study used a combination therapy of AMR and CBDCA in previously untreated elderly SCLC patients.[70] DLTs were observed in all three patients at level 1 (AMR at 40 mg/m^2 and CBDCA at AUC 4.0) with grade 4 neutropenia or thrombocytopenia, or grade 3 diarrhea. The MTD of this combination therapy was AMR at 40 mg/m^2 and CBDCA at AUC 4.0, and the recommended dose for a phase II trial is AMR at 35 mg/m^2

and CBDCA at AUC 4.0. There are no reports of a second-line setting for AMR treatment of elderly patients with SCLC.

Future Approaches

Combination regimens that comprise agents with different mechanisms of action can result in synergistic antitumor activity and may overcome resistance to chemotherapy. In SCLC, combination chemotherapy generally yields higher overall RRs than does single agent therapy. However, care must be taken in the selection of agents to avoid overlapping toxicities that may adversely affect quality of life, especially in patients with extensive SCLC. To our knowledge, AMR is undergoing phase I or I–II clinical trials in combination regimens, including AMR plus TS-1 (tegafur, gimeracil and oteracil potassium), AMR plus nedaplatin, and AMR after concurrent VP-16 and CDDP plus accelerated hyperfractionated thoracic radiotherapy.

Unfortunately, it has been impossible in this review to cite all the references referring to the use of AMR in SCLC; likewise, we have not discussed the clinical trials of AMR performed in NSCLC patients. We have not discussed the downstream metabolites of AMR-OH. The detailed molecular mechanisms of how AMR induces apoptosis in cancer cells are unclear. Finally, most of the clinical trials with AMR have been performed in Japan: more trials conducted outside Japan are warranted.

Conclusions

It is clear that AMR, with its predictable and manageable toxicities, is one of the most attractive agents for the treatment of chemotherapy-sensitive and -refractory relapsed SCLC. Numerous studies are ongoing in an attempt to define the applicability of AMR as a single agent or in combination chemotherapy for patients with SCLC. These clinical trials, including phase III studies, will clarify the status of AMR in the treatment of SCLC.

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Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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