Combination chemotherapy for relapsed small-cell lung cancer—perspective on mechanisms of chemoresistance

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Comment on: Goto K, Ohe Y, Shibata T, *et al.* Combined chemotherapy with cisplatin, etoposide, and irinotecan versus topotecan alone as second-line treatment for patients with sensitive relapsed small-cell lung cancer (JCOG0605): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2016;17:1147-57.

Abstract: Small cell lung cancer (SCLC) has a dismal prognosis due to early dissemination and aggressive growth. Despite high response rates to initial chemotherapy, SCLC relapses fast and exhibits broad chemoresistance. The JCOG0605 Japanese trial reported increased survival for a regimen combining cisplatin with etoposide and irinotecan compared to topotecan in chemosensitive patients and proposed this treatment as standard chemotherapy. Analysis of the trial data indicates an enrichment of patients with favorable prognosis in the combination chemotherapy arm, questioning the feasibility of this highly aggressive regimen in typical SCLC patients of higher age and afflicted by comorbidities. Considering the modest prolongation of life with current therapies, quality of live should be traded against extension of survival rated in months. Circulating tumor cell (CTC) lines established from relapsed SCLC patients suggest chemoresistance due to formation of large spheroidal multicellular aggregates, termed tumorospheres, which restrict drug access and contain quiescent and hypoxic cells. With the possible exception of metformin, clinical means to eliminate such tumor spheroids are confined to experimental research with cell lines and xenografts, but this new insight into chemoresistance of SCLC discloses entirely new modes of efficient treatment of SCLC.

Keywords: Small cell lung cancer (SCLC); combination chemotherapy; quality of life; chemoresistance; metformin

Introduction

Small cell lung cancer (SCLC) comprises approximately 15% of lung cancers and is found disseminated in the great majority of patients at first presentation (1). After confirmation of the diagnosis by biopsy, chemotherapy is started and, with few examples of refractory disease, patients respond well to platinum-based combination therapy, with response rates to first-line treatment on the order of 70–90% in limited disease and 50–60% in extended disease (2). Etoposide-platinum (EP) was shown to be superior to cyclophosphamide, epirubicin, and vincristine (CEV), with significantly higher 2- and 5-year survival rates of 14% and 5% in the EP arm versus 6% and 2% in the CEV arm, respectively (3). Platinum-based chemotherapy regimens did not offer a statistically significant benefit in survival or overall tumor response but increase complete response rates, at the cost of higher adverse events (4). Trials of three- and four-drug regimens, dose-intensifying regimens, the addition of third generation cytotoxic agents (e.g., gemcitabine, taxanes, topotecan), and high-dose chemotherapy have all failed to improve outcomes (5).

However, despite high response rates to initial chemotherapy, nearly all patients with SCLC eventually relapse with relatively chemoresistant tumors which are difficult to treat and have a dismal prognosis (1,6).

Patients with "sensitive" disease, that is, who have relapsed beyond 60 or 90 days of completing first-line treatment, are regarded to benefit most from second-line treatment. Low performance status and weight loss at the time of relapse relate to a poorer prognosis. Efficacy of second-line chemotherapy is much lower than that of first-line treatment, but it can provide significant palliation and prolongation of survival for many patients (7). For patients who relapse >6 months after initial treatment, retreatment with the original regimen may be applied but for patients who relapse within 6 months, therapy is more controversial, because many patients have a poorer performance status, and the benefit of second-line chemotherapy over best supportive care was not clear (8).

The single drug approved for second-line treatment of SCLC is topotecan and an anthracycline-based regimen consisting of cyclophosphamide, adriamycin (doxorubicin or epirubicin), and vincristine (CAV/CEV) represents an alternative. Topotecan proved to result in prolonged survival compared to best supportive care (median 26 versus 14 weeks) and offered better tolerability with equal efficacy compared to the CAV scheme (9-11). However, all second line treatments resulted in poor response rates and short-lived stabilization of the disease. In general, attempts to use more aggressive regimens have resulted in larger proportions of patients achieving responses without significant prolongation of survival (12). Unfortunately, all trials to achieve better therapeutic responses with a host of alternative drugs failed so far, as well as trials employing targeted agents (13,14). The genomic makeup of SCLCs was characterized in great detail, but in the presence of a universal inactivation of the two tumor suppressor proteins p53 and retinoblastoma RB1, a range of diverse and interchangeable drivers are responsible for aggressive tumor growth (1,15). Thus, in contrast to NSCLC, where targeted agents against mutated driver proteins proved highly effective, similar kinase addictions could not be found for most SCLCs. Numerous attempts are ongoing to improve survival of these patients in order to overcome the poor progress in therapy for SCLC for the last decades. Furthermore, the definite mechanisms producing general chemoresistance to a host of unrelated drugs in relapsed SCLC has not been defined so far (16).

The JCOG0605 trial of the Japan Clinical Oncology Group

The JCOG0605 trial investigated combined chemotherapy with cisplatin, etoposide, and irinotecan versus topotecan alone as second-line treatment for patients with sensitive relapsed SCLC in a multicenter (n=29), open-label and randomized phase III trial (17). This study included 180 patients and sensitive relapsed SCLC is defined as a recurrence that occurred ≥90 days after completion of first-line therapy. The term "sensitive" indicates that patients were not refractory from beginning and may be susceptible to further chemotherapy but does not suggest that the relapsing tumors are actual chemosensitive at a cellular or tumor physiological level. Randomization was done via the minimization method with biased-coin balancing for Eastern Cooperative Oncology Group (ECOG) performance status, disease stage at recruitment, and institution. Combination chemotherapy consisted of five 2-week courses of intravenous cisplatin 25 mg/m^2 on days 1 and 8, intravenous etoposide 60 mg/m^2 on days 1–3, and intravenous irinotecan 90 mg/m^2 on day 8, with granulocyte-colony stimulating factor (G-CSF) support. Topotecan therapy consisted of four courses of intravenous topotecan 1.0 mg/m^2 on days 1–5, every 3 weeks. The primary endpoint was overall survival (OS) in the intention-to-treat population, which was analyzed with a one-sided significance level of 5%, and safety was assessed in all patients who received at least one dose of medication.

This study reported a significant improvement in OS with the combination therapy in relapsed SCLC [median 18.2 months (95% CI, 15.7–20.6) with combination therapy *vs.* 12.5 months (95% CI, 10.8–14.9) with topotecan; HR, 0.67 (95% CI, 0.51–0.88); P=0.0079]. Both the proportion of patients achieving an objective response (84% *vs.* 27%; P<0.0001) and progression-free survival [5.7 months (95% CI, 5.2–6.2) *vs.* 3.6 months (95% CI, 3.0–4.4); P<0.0001] were better with combination therapy than with topotecan alone. The authors concluded that this combination chemotherapy should become the standard treatment for selected patients with sensitive relapsed SCLC.

Patient characteristics of the JCOG0605 study arms

In a critical accompanying commentary to the trial report, Kalemkerian criticized the patient selection of the JCOG0605 as severely biased (18). This study enrolled subjects who were younger and healthier than the usual population of patients with SCLC. The great majority of patients had performance status of 0–1, a very long first remission and a frequent administration of third- and fourth-line chemotherapy. Furthermore, the interval

between progression and death was unusual long and both study groups showed a much better than expected survival. In particular, patients receiving the combination therapy had a better performance status than those assigned to topotecan (58% *vs*. 44% with performance status 0) and median duration of initial response to first-line chemotherapy likewise favored the combination therapy group (181 *vs*. 148 days). Some patients in the control group received suboptimum therapy, since combination chemotherapy, rather than single-agent therapy, is regarded as the most appropriate option for patients who have a relapse more than 180 days from initial treatment (19).

The previous phase II trial of the cisplatin, etoposide, and irinotecan combination led by the same group, in which sensitive relapse was defined as more than 56 days after the end of treatment (rather than ≥90 days), reported a much shorter median survival than did JCOG0605 (11.8 *vs*. 18.2 months), despite a similar objective response rate (78% *vs*. 84%) suggesting an important impact of the long median duration of initial response (20). Another Japanese study reported that re-induction with the first-line combination regimen yielded a favorable median OS of 15.7 months in patients who had relapse beyond 180 days (21). In JCOG0605, a lower-than-standard dose of topotecan was used, but attenuated-dose topotecan is commonly used in practice (22). Finally, 50% of patients treated with the combination therapy required dose-reductions and 22% stopped treatment because of adverse events consisting of grade 3–4 neutropenia and anaemia in more than 80% of patients, and febrile neutropenia occurred in 31% of patients, raising serious concerns about the tolerability of this regimen. Unfortunately, quality of life was not analyzed. In these patients, with limited survival expectations, symptom palliation, quality of life, and convenience of therapy are especially important end points. Moreover, symptom palliation correlates well with QoL and survival duration, providing further rationale for therapy selection based on these parameters (23). The survival reported in JCOG0605 is encouraging for the highly selected patients enrolled in the trial, but previous experience suggests that promising initial results might not be reproducible in other populations (24,25). Especially, these study participants do not represent the average patient with SCLC in the USA including elderly people who smoke and have impaired performance status due to comorbidities and the aggressiveness of the disease. Further study is needed before the cisplatin, etoposide, and irinotecan combination can be accepted as the standard treatment for patients with relapsed SCLC.

Chemoresistance of relapsed SCLC

Although topotecan has been approved by many countries for the monotherapy of relapsed SCLC, its low response rate and short median survival time is disappointing. Compared with topotecan, irinotecan and etoposide did not show any advantages as single agents (26). However, the combination of cisplatin with etoposide and irinotecan represents a potentiation of the cytotoxicity of the DNA-damaging agent cisplatin and the inhibition of the subsequent startup for DNA repair by both topoisomerases I and II by irinotecan and etoposide, respectively. In this manner, basic processes of every cell in the body are affected, such taking into account severe side effects in the hope of a small differential impact on malignant versus normal tissues. The combination of several agents with high toxicity is of course thus contrary to the aim of targeted therapy to avoid chemotherapeutics with poor specificity and to develop agents against key proteins of the tumors which are indispensable for tumor growth and progression. However, SCLC exhibits no oncogene addiction which can be suppressed for broader subpopulations of the patients and, consequently, all attempts to apply precision medicine failed so far (1,13). Furthermore, the mechanisms behind chemoradioresistance in relapsed SCLCs were not elucidated so far and, therefore, specific agents to resensitize the tumor cells could not be formulated. Chemoresistance of relapsed SCLC proved to be universal and new camptothecins, platinums and other drugs with novel targets failed (27). Moreover, research investigated SCLC was hampered by scarcity of tumor material, since after drawing of a small biopsy therapy is initiated by chemotherapy without any further invasive procedure.

A unique feature of SCLC, namely the occurrence of excessive numbers of circulating tumor cells (CTCs) provided an opportunity to study tumor dissemination and evolution of chemoresistance. In contrast to breast, colon and prostate patients who have a negative prognosis with a CTC count of several cells/7.5 mL of blood as detected with the CellSearch system, CTC counts in SCLC patients may exceed more the 400 cells in the same volume of blood (28,29). CTCs are shed by tumors and are responsible for induction of secondary lesions at distal sites (30). The high CTC counts of SCLC recurrences allowed us to set up permanent CTC SCLC lines and to study their cell biologic characteristics (31,32). The CTCs as single cells proved to be chemosensitive to second-line chemotherapeutics topotecan and epirubicin (33). However,

all six lines established from relapsed SCLC patients so far formed large multicellular spheroidal structures, termed tumorospheres, which exhibited marked resistance to a range of chemotherapeutic drugs *in vitro* (34). The tumorospheres reach diameters of 1–2 millimeters and they assemble spontaneously in tissue culture (35). Such structures are known to contain interior layers of quiescent cells and hypoxic core regions. Chemoresistance is caused by limited penetration of drugs, low proliferative activity, cell-cell contact-mediated resistance and resistance to irradiation by lack of oxygen radical formation (36). Cell death in response to chemotherapeutics only occurs in outer spheroid regions, as a viable multicellular tumor spheroids (MCTS) core could be isolated after recovery from cytostatic treatment and removal of the dead cell layer. Such protection from cytotoxic drugs in form of a physical barrier which limits access of agents, nutrients and oxygen leaves a host of unrelated compounds ineffective without referring to individual cellular pathways of drug inactivation (28,37).

Unfortunately, at present most means to eliminate tumor spheroids are in early preclinical development. The efforts to improve cancer therapy largely rested upon massive work to fully characterize the genome of cancer cell and decipher their transcriptomes. However, tumors have been described as "organs" with three-dimensional structures and specific microenvironmental characteristics (38). To be most effective anticancer drugs must penetrate tissue efficiently, reaching all the cancer cells in a concentration sufficient to exert a therapeutic effect. Most research into the resistance of cancers to chemotherapy has concentrated on molecular mechanisms of resistance, whereas the role of limited drug distribution within tumors or spheroids has remained largely unattended (39). Around 95% of new anticancer drugs eventually fail in clinical trial, despite robust indications of activity in existing *in vitro* preclinical models (40). Innovative models are required that better capture tumor biology, instead of reductionist 2D-culture or artificial cluster models. Techniques to grow 3D-cultures include aggregating cells at the bottom of a drop, different methods to prevent cell from attaching to substrates or growing cells in stirred culture systems. 3D-spheroid closely resembled avascular tumor nodules, micrometastases, and inter-vascular regions of large solid tumors (41). Resistance to cytotoxic agents is due to insufficient distribution of the drugs, non-proliferative and hypoxic cells in the core of the spheroid, cell-cell interactions mediated by E-cadherin, and production of extracellular matrix (ECM) proteins. Comparison of 3D- with 2D-cultures suggested up-regulation of E-cadherin, downregulation of vimentin, decreased expression of the proliferation marker Ki-67 and increased expression of the apoptotic marker caspase-3 in spheroids (42).

Several approaches may be promising to target multicellular tumor structures. Drug formulations with lipids or nanomaterials which accumulate at tumors or penetrate cellular aggregates are in development. Junction openers are investigated in order to open cell-cell connections in order to improve drug diffusion. Furthermore, ECM components can be attacked enzymatically but most enzymes are rapidly inactivated in the circulation. Special formulations like in the case of pegylated recombinant hyaluronidase (PEGPH20) overcome this limitation and seem to have a therapeutic benefit in patients with hyaluronic acid-rich pancreatic ductal adenocarcinomas (43). Treatment led to re-expansion of the tumor vasculature, reduction in tumor hypoxia, and increased penetration of drugs into the tumor as well as reduced signaling via CD44 (44).

Nine substances that specifically target cells in inner MCTS core regions were identified in a screen of drugs in 3D-cell cultures (45). These compounds act as inhibitors of the respiratory chain in dependence of extracellular glucose concentrations and showed synergistic cytotoxicity with chemotherapeutics against spheroids. Outer MCTS cells (or cells cultured in 2D), with direct access to glucose resort to glycolysis while cells in inner MCTS regions with lower glucose levels become sensitive to inhibitors or uncouplers of the respiratory chain. Sequential treatment with chemotherapeutics and metformin targeted the dormant cell population in the MCTS core (45). The reported cancer-protective effect of metformin could be induced, in addition to other mechanisms, by a combination complex I respiratory chain inhibition and concomitant lowering of blood glucose levels. The beneficial effect of metformin medication in diabetic patients for treatment of SCLC has been documented in several studies. A trial in 259 SCLC patients showed that the use of metformin decreased SCLC recurrence rate (46). Median OS and DFS were significantly better in the metformin group (OS 19.0 *vs*. 11.5 months, DFS 10.5 *vs*. 7.0 months). In another study with 79 diabetic patients, median OS and DFS were again significantly better in the metformin group (OS 18.0 *vs*. 11.5 months, DFS 10.8 *vs*. 6.5 months) (47). Metformin might be considered a potential useful anticancer drug in treating SCLC patients. Metformin could enhance CP treatment in SCLC cells, likely through promoting further

IGF-1R down-regulation (48). Trials of metformin in combination with (radio)chemotherapy are ongoing for NSCLC (49,50).

Conclusions

This study confirms the previous finding that a higher dose intensity of chemotherapy can be delivered to SCLC patients with a good performance status which the typical patient with lower performance status is unable to tolerate. Chemoresistance in SCLC seems to be related to CTC-derived tumorospheres which resemble highly organized multicellular structures which differ from most spheroidal cell aggregates induced by prevention of cellular attachment. This type of physiological resistance requires completely new strategies to eliminate tumor cells and to prolong survival of SCLC patients.

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Footnote

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