Introduction

Small cell lung cancer (SCLC) constitutes approximately 15% of lung cancers and is strongly associated with smoking (1). The prognosis of this cancer is dismal due to frequent disseminated disease at detection and rapid appearance of chemoresistant relapses after assumed successful first-line chemotherapy (2,3). Standard therapy consists of a combination of cisplatin/carboplatin and etoposide resulting in high initial response rates which are not durable and are followed by recurrences within approximately 1 year. The relapsing tumors are highly chemoresistant and are treated with topotecan or a CAV regimen (cyclophosphamide/adriamycin—replaced by epirubicin/vincristine) which yield low response rates of short duration at best. Patients with limited disease SCLC (LD-SCLC) have a median survival time of 16–24 months when treated with chemotheraphy but extended disease SCLC (ED-SCLC) reduces the median survival to 7–12 months (4,5). Prophylactic cranial irradiation (PCI) in chemotherapy-responsive patients decreases the risk of brain metastases and increases overall survival (OS) (6).

Unfortunately, treatment of SCLC has not changed for the last decades since a host of novel chemotherapeutics, new regimens and present-day targeted agents have uniformly been disappointing in clinical trials (4,7). Despite extensive genetic characterization, no new targets could be revealed which hold the potential to improve therapy of SCLC. The tumor suppressor proteins p53 and pRB are mutated with high frequency and additional mutations in diverse driver genes result in aggressive growth of SCLCs and early dissemination (1,8). Since the actual mechanisms of the general chemoresistance of SCLC were not clear so far, therapeutic interventions which showed activity in other malignancies, such as antiangiogenic therapy and immune checkpoint inhibition, have been studied in SCLC. Targeting of tumor neoangiogenesis aims at interruption of the vascular supply of tumors and inhibition of further growth and progression (9). Antiangiogenic therapy can be achieved by monoclonal antibodies directed to vascular endothelial growth factor (VEGF) or small molecule drugs aimed at receptor proteins and mediators, such as sunitinib and thalidomide. Bevacizumab (Avastin) is an antibody against VEGF which is commonly used in combination with platinum drugs in treatment of NSCLC, as well as in colon, kidney, ovarian and other cancers but is rarely active as single drug (10). Paradoxically, the initial anticancer activity of bevacizumab stems from its ability to normalize tumor blood vessels, such providing a window of opportunity, during which drugs can reach more of the cancer (11). In later phases, bevacizumab seems to inhibit the proangiogenic upregulation and further growth of tumor cells.

SCLC is rarely treated by surgery and, therefore, few specimens are available for a biological characterization. Tumor angiogenesis, expressed by the microvessel count (MVC), and its mediators (i.e., vascular endothelial growth factor—VEGF) significantly correlate with metastasis in surgically treated NSCLC (12). Accordingly, tumor
angiogenesis, as demonstrated by MVC and expression of VEGF, has prognostic significance in SCLC (13). Patients with SCLC express functional VEGF-receptors, VEGFR-2 and VEGFR-3, on their tumor cells and have increased levels of serum VEGF (14, 15). Combining bevacizumab with paclitaxel had therapeutic efficacy in chemoresistant, relapsed SCLC (16). The cisplatin-etoposide and bevacizumab combination as the first-line treatment for ED-SCLC could improve progression-free survival (PFS), with an acceptable toxicity profile (17). Ziv-aflibercept (VEGF trap) combined with topotecan is promising for platinum-refractory SCLC but chemotherapy combined with thalidomide (an inhibitor of angiogenesis) cannot prolong survival (18). Maintenance sunitinib (multiple RTKs inhibitor, including VEGFRs) in ED-SCLC patients following induction chemotherapy with platinum/etoposide improves median PFS by 1.6 months (19, 20). Since the effects of antiangiogenic therapy of SCLC were limited and could not be confirmed for selected active agents, such as thalidomide and sunitinib, a phase III study seemed suitable to settle the activity of bevacizumab in treatment of SCLC. Therefore, the respective study by Tiseo et al. investigated the clinical activity of bevacizumab combined with chemotherapy versus chemotherapy alone and subsequent antiangiogenic maintenance therapy in responding participants in a phase III trial in ED-SCLC patients.

The GOIRC-AIFA FARM6PMFJM Trial

The randomized phase III trial (EudraCT No. 2007-007949-13) tested the efficacy of bevacizumab as adjunct to first-line cisplatin plus etoposide for treatment of ED-SCLC (21). A relative reduction of the risk (hazard) of death of at least 40% with respect to the control arm (median survival of 9 months) was strived for, corresponding to a median survival of >15 months. Treatment-naive patients were randomized for a maximum of six courses of chemotherapy with and without the angiogenesis inhibitor. Patients received a combination of intravenous cisplatin (25 mg/m² on days 1 to 3), etoposide (100 mg/m² on days 1 to 3), and bevacizumab (7.5 mg/kg intravenously on day 1) administered every 3 weeks (experimental arm) or the same cisplatin and etoposide chemotherapy regimen alone given every 3 weeks (control arm). Patients responding to chemotherapy with bevacizumab continued on the inhibitor alone until disease progression or for a maximum of 18 courses. A total of 103 patients on standard chemotherapy and 101 patients in the chemotherapy/bevacizumab arm showed median OS times of 8.9 and 9.8 months and 1-year survival rates of 25% and 37% [hazard ratio (HR), 0.78; 95% CI, 0.58 to 1.06; P=0.113], respectively, at a median follow-up of 34.9 months. The response rate was 53.3% vs. 58.4% in chemotherapy alone vs. chemotherapy plus bevacizumab, respectively (odds ratio, 1.13; 95% CI, 0.65 to 1.97; P=0.657). Bevacizumab significantly prolonged OS in 41 patients who received maintenance therapy (HR, 0.60; 95% CI, 0.40 to 0.91; P=0.011). Median PFS times were 5.7 and 6.7 months for standard and bevacizumab therapy, respectively (P=0.030). It was concluded that, the addition of bevacizumab to cisplatin and etoposide in the first-line treatment of ED-SCLC has an acceptable toxicity profile and led to a statistically significant improvement in PFS, which, however, did not translate into a statistically significant increase in OS.

Dose reductions and delays were performed in 30.1% vs. 35.8% and 60.2% vs. 58.9% of patients in arm A vs. arm B/bevacizumab. Grade 3 to 5 adverse events (AEs) were reported in 64 patients (62.1%) who received only chemotherapy, compared with 52 patients (54.7%) who were also treated with bevacizumab (P=0.291). The addition of bevacizumab led to a significant survival benefit in men (HR, 0.55) and to a possible detrimental effect in women (HR, 1.55; interaction test, P=0.003). The results of this trial indicate that this combined treatment is feasible and well tolerated and leads to a small statistically significant improvement in PFS. However, the primary end point of the study (i.e., survival rate increase at 1 year from 40% to 58%) was not met. Interestingly, maintenance bevacizumab turned out to be associated with a better survival outcome. This suggests that a sequential use could be a better and safer strategy to deliver antiangiogenic drugs in SCLC. In summary, the results of this trial, together with the available knowledge in this field, overall support the conclusion that combining bevacizumab with standard platinum plus etoposide chemotherapy does not lead to meaningful survival improvement in ED-SCLC.

Significance of the GOIRC-AIFA FARM6PMFJM Trial

For lung cancer, bevacizumab obtained first-line approval in metastatic NSCLC in 2006 based on the phase III ECOG 4599 clinical trial which demonstrated an improvement of 2 months of the median OS with the addition of bevacizumab to first-line carboplatin and paclitaxel chemotherapy (22). The publication of the study
of Tiseo et al. is accompanied by an editorial which labeled angiogenesis as elusive target in SCLC (21,23). The small prolongation of the median PFS of 5.7 vs. 6.7 months and the nonsignificant improvement in the primary end point of OS (8.9 months controls vs. 9.8 months in the bevacizumab group) was regarded as a clear trend towards bevacizumab in this small phase III trial. The relatively low dose of bevacizumab (7.5 mg/kg vs. FDA–approved dose of 15 mg/kg for NSCLC) was estimated as noncritical since in the “Avastin in Lung Study” (AVAiL) trial, both doses demonstrated a similar improvement in PFS but no OS improvement (24). However, Tiseo et al. cited an increased level of angiogenesis in SCLC, as demonstrated by MCV and overexpression of VEGF (21). The improvement of the 1-year survival rate from 40% to 58%, as requested by the government funding body, was regarded to optimistic and continuation of antiangiogenic therapy for an extended period was suggested to be necessary for optimal benefit.

A divergence between the groups receiving chemotherapy and chemotherapy plus bevacizumab started after 6 months which seems to indicate that antiangiogenic therapy employing this antibody was not effective in increasing the response to chemotherapy during the initial window of opportunity. Afterwards, single agent bevacizumab may simply retard the growth of tumors, cachexia and death by reducing vascular supply, particularly in patients older than age 65 years as revealed by subgroup analysis. Furthermore, men had the most clinical benefit of antiangiogenic therapy (OS HR for women 1.55 vs. HR 0.55 for men) (21). Similarly, in the ECOG trial 4599 studying advanced-stage NSCLC treated with or without bevacizumab in combination with paclitaxel and carboplatin females did not appear to have a survival benefit that males did with addition of antiangiogenic therapy (25). How sex differences in clearance and central compartment of distribution of bevacizumab may account for the differences in outcome is not clear (26).

Since the chemotherapy—bevacizumab combination yielded a minor advancement in SCLC, the next step is to expand this regimen with immune checkpoint inhibition from a clinical point (23). The IMpower 150 phase III study (NCT02366143) addresses the role of whether bevacizumab adds to carboplatin, paclitaxel, and the PD-L1 agent atezolizumab because all patients will be treated with the three-drug regimen and half will receive bevacizumab. However, immunotherapy is difficult in SCLC patients with low expression of checkpoint inhibitors, a reduced performance state and comorbidities (27). Additionally, chemotherapy may impair immune response and antiangiogenic therapy may restrict access to tumors for chemotherapeutics as well as for immune cells resulting in antagonism, aside from the combined side effects of a triple combination.

### Cancer and anti-angiogenic therapy

The US Food and Drug Administration (FDA) approved the first angiogenesis inhibitor, namely bevacizumab, for the treatment of metastatic colorectal cancer in 2004. Resistance might be the main reason for poor improvement in OS after angiogenesis inhibitor administration in clinics (9,28). Different mechanisms of vascularization, activation of alternative signaling pathways, and increased tumor aggressiveness may account for the resistance (9). Antiangiogenic treatment of cancers aims at reducing the formation of new blood vessels in order to inhibit tumor growth (29). The anti-angiogenic therapy results in transitory improvements, in some cases increasing survival but, generally, tumors begin to grow again after a period of several months of clinical benefit. The coadministration of an anti-angiogenic with an anti-tumor drug can initially evoke a transitional normalization of tumor vessels to allow the chemotherapeutics to reach the tumor site at higher concentrations (11).

Ample evidence suggests that angiogenesis in SCLC has an important role in determining the growth rate, invasiveness and metastasis (30). However, results from clinical trials in SCLC have been disappointing and all anti-angiogenic agents failed to receive regulatory approval (30). In the phase II–III IFCT-0802 trial by Pujol et al., 147 patients received two cycles of chemotherapy with etoposide/cisplatin (EP) or PCDE cisplatin/cyclophosphamide/epirubicin/etoposide). A total of 103 patients (70.1%) with a partial response were randomized to continue with chemotherapy alone for a maximum of six cycles or chemotherapy plus bevacizumab 7.5 mg/kg on day 1 every 21 days, until a maximum of 2 years (31). Disease control rate (DCR) was similar between groups (89.2% in the chemotherapy alone vs. 91.9% in the combination group. After a median follow-up of 37.7 months, PFS did not differ significantly between groups (5.5 vs. 5.3 months, P=0.82) and median OS was also similar (13.3 vs. 11.1 months). The SALUTE (Study of Bevacizumab in Previously Untreated Extensive-Stage Small Cell Lung Cancer) phase II randomized trial enrolled a total of 102 patients with ED-SCLC to either cisplatin 75 mg/m² or carboplatin and etoposide 100 mg/m² over
3 days for four cycles alone or bevacizumab 15 mg/kg until disease progression (32). In fact, median PFS was significantly improved from 4.4 to 5.5 months (HR 0.53), whereas median OS was numerically worse in the experimental arm (10.9 and 9.4 months in control and experimental arms, respectively).

A specific tumor mechanism to evade antiangiogenic treatment, vasculogenic mimicry (VM) was described recently for SCLC (33). It was demonstrated that SCLC patients (37/38) have rare CTC subpopulations which coexpress vascular endothelial-cadherin (VE-cadherin or CDH5/CD144) and cytokeratins consistent with VM. During this process tumor cells form “endothelial-like” vessels by trans-differentiation of aggressive tumor cells into an endothelial cell type which effects de novo generation of vascular networks and a micro-circulation that is independent of non-cancer host cells. First reported in uveal melanoma, VM is described for other tumors, such as breast cancer, glioblastoma, colorectal cancer and others (34). VEGF-A blockade in melanomas was associated with HIF1α expression and an adaptive increase in CD144-positive VM, leading to formation of channels displaying Tie-1 and MMP-2 upregulation which were resistant to antiangiogenic treatment (35). New data show that the angiogenic factor YKL-40 (chitinase-3-like 1/CHI3L1) acts on glioblastoma-stem like cells (GSCs) to drive two major forms of tumor vascularization: angiogenesis and VM (36). GSCs can to transdifferentiate into vascular pericytes or smooth muscle cells (PC/SMCs) that either coordinate with endothelial cells (ECs) to facilitate angiogenesis or assemble in the absence of ECs to form blood-perfused channels via VM. We have shown previously that circulating tumor cell (CTC) lines established from blood samples of patients with ED-SCLC show marked release of CHI3L1/YKL40 (37).

Chemoresistance of relapsed SCLC

Although the camptothecin topotecan is approved for the monotherapy of relapsed SCLC, its low response rates and the short duration of tumor control are disappointing (38). Chemoresistance of relapsed SCLC proved to be universal and the underlying mechanisms in relapsed SCLCs was not elucidated until now. Antiangiogenic therapy seems not to improve the prognosis of SCLC over standard therapy to a major extent, in line with all attempts of a host of diverse drugs employed in clinical trials during the last decades (5). In essence, either SCLC cells have an as yet undetected universal mechanism of chemoresistance or all drugs fail to reach some tumor cells at effective concentrations. Inhibition of apoptosis in SCLC cells seems not to be involved as shown by several unsuccessful trials of bcl-2 antagonists (39). CTC counts in SCLC patients may exceed more the 400 cells in 7.5 mL blood and are responsible for the generation of dormant disseminated tumor cells (DTCs) and induction of secondary lesions at distal sites (40). The high CTC count allowed us to set up 7 permanent CTC SCLC lines from blood samples of patients with ED-SCLC and to study their cell biologic characteristics. These lines seem to represent the extremely small fraction of the CTCs capable of forming metastases, since they were isolated from relapsed SCLC and have converged at a similar phenotype as shown by gene and protein expression analysis (manuscript submitted).

Dissemination of tumors is supposed to be effected by surviving chemoresistant cells after chemotherapy and, therefore, CTCs were assumed to retain a resistant phenotype. However, the SCLC CTCs tested as single cells proved to be chemosensitive to second-line chemotherapeutics topotecan and epirubicin, but all seven lines form spontaneously large multicellular spheroidal structures, termed tumorospheres (Figure 1), which reach diameters of 1–2 millimeters (41-43). Correspondingly, analysis of paraffin-embedded sections of tumorospheres showed hypoxic-necrotic cores and low labeling with the proliferation marker Ki67 of cells in the inner layers of the clusters. Similar organized 3D-structures are not found in
cell lines cultured from SCLC tumors which exhibit loosely attached cell agglomerations at most. Chemoresistance of tumorspheres is caused by limited penetration of drugs, low proliferative activity, cell-cell contact-mediated resistance and resistance to irradiation due to lack of oxygen radical formation. 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 (44).

Unfortunately, at present most means to eliminate tumor spheroids are in early preclinical development. The current 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 and the investigations dealing with drug delivery to multicellular tumor aggregates are lagging behind (45). To be most effective anticancer drugs must penetrate tissue efficiently, reaching all the cancer cells in a concentration sufficient to exert a therapeutic effect (46). By current means the chemotherapy of SCLC is expected to lack major progress and the actual mechanism of drug resistance must be addressed to improve survival of patients with relapsed SCLC to a considerable extent.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References


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