

FORUM of CLINICAL ONCOLOGY

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**The end of
Hippocratic Medicine
and a new role
for medicine**

**Bortezomib +
doxorubicin
in recurrent or
metastatic adenoid
cystic carcinoma
of the head and neck**

**Gefitinib in combination
with vinorelbine and
oxaliplatin in women
with advanced recurrent
ovarian cancer**

**Hypoxia Inducible
Factor 1 as a target
for cancer therapy**

**Side-effects of targeted treatments
for metastatic renal cell cancer**

**Cutaneous metastasis resembling herpes zoster
as an early presentation of stomach cancer**

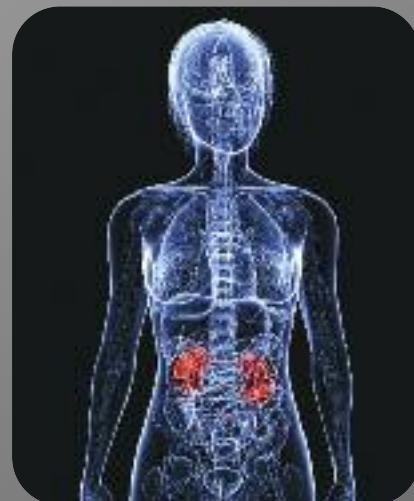


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The art of dialogue

Editorial

Vassilios Barbounis

Besides the interesting work presented in the current FCO issue, there are two elements that differentiate it from the previous ones. The first one has to do with the position article of Dr Razis which refers to the 'End of Hippocratic Medicine' and the changed character of contemporary medicine. Contemporary medicine is multifaceted and ranges from personalized treatment of an individual to one's integration to today's ecosystem and from pharmacogenomics to the cure of a deviant human culture. Dr Razis article, well-structured and full of creative ideas, will challenge established perceptions and obsolete ideas and provide food for reflection not only to inquiring but also to closed minds.

The second element relates to the "Letters to the Editor" with respect to Dr Emmanouilides article published in the previous issue, entitled "Why peer review is needed". The article dealt with the necessity of peer review assessment of research work submitted for publication. It may be postulated that this method of assessment is not unsurpassed or that it does not encourage scientific progress and innovation. Nonetheless, it is a first-rate way to assess research work amongst peers. In this way, a minimum standard in the expression of applied scientific ideas may be secured. Moreover, it constitutes a reciprocal learning process to establish uniform expression of a universally accepted scientific language. The resulted fierce reaction is indicative that our effort is on the right course.

The end of Hippocratic Medicine and a new role for medicine

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ABSTRACT

Hippocratic Medicine, throughout its long and successful development, has impressively improved our lives. We now live longer, we live better, and we suffer less. Yet, this very success has become, at the population level, clearly antibiological and is the cause of population explosion, environmental disintegration and the depletion of the planet's genetic pool. This should signal the end of Hippocratic Medicine. The alternative is the End of Man and a mass extinction of life on the planet.

Medicine in a New Role might lead the way to overcoming the deep crisis and the deadlock humanity is facing today. Medicine in its New Role should attempt to reduce and sustain human population, optimize the earth's ecosystem and integrate Homo sapiens therein the planet's ecosystem.

Medicine alone cannot heal a sick culture. Human behavior patterns, human society structures and the threats to human species' existence should be approached in a holistic way and from a multidisciplinary point of view.

Key words: Hippocratic Medicine; population explosion; human extinction; new role for medicine.

FROM PREHISTORIC TO MODERN MEDICINE

In prehistoric times, attempts by primitive man to cure disease and relieve suffering were based on instinct and experience. It seems that primitive man discovered through trial and error natural substances and plants of some medicinal value. Folk or Domestic Medicine originated in this fashion and played a part in primitive man's medicine; but it was only a part. Man at that time did not regard death and disease as natural phenomena. Serious and disabling diseases were considered of supernatural origin; thus it is not surprising that magic and religion played a large part in the Medicine of Prehistoric Man [1].

It was not until 600-400 BC that philosophers in Greece started to question the supernatural explanation of the world. They began creating a body of knowledge based on logic; when Hippocrates was born in 460 BC, Medicine had partially discarded its conceptions based on magic and religion. Hippocrates, who is rightly known as the "Father of Medicine", applied the power of observation and logical reasoning, and created the first rational and scientific medical system. He viewed disease

with the eye of a naturalist, studied the patient in his environment and developed a rigid method of medical examination. His Oath is an ethical code and an ideal which has guided the practice of Medicine for more than 2000 years. Modern Medicine is actually based on the Hippocratic Principles [1].

Even after the decline of the golden era in Greece, most of the contributions to the body of Medical Knowledge were made by Greeks. Galen, a Greek practicing in Rome, was the dominant figure in Medicine throughout the years of the Roman Empire. He followed Hippocrates' method of observation but added something new to almost every branch of medicine; designed the first experimental methods, and formulated medicine into a complete scientific system. He remained the undisputed authority from whom no one dared to differ for more than 14 centuries. Galen profoundly influences Medicine but his and the Aristotelian dogmatism hindered the progress of medical thought for centuries [1].

The Renaissance Period marked a profound change of outlook. There was an eagerness for discovery, a desire to escape from the limitations of tradition and to explore new

fields of thought and action. It was the beginning of a new era where truth was sought from experience rather than from tradition. It was the philosopher Francis Bacon who developed this method. His theories on scientific empiricism dominated the thought of scientists and researchers and prepared the rise of Scientific Medicine in the 19th Century. Scientific medical discoveries in the 19th Century multiplied and changed the entire structure of Medicine. During the 20th Century there has been such a plethora of discoveries that the face of Medicine has changed completely. The attitude has been so altered that, with the notable exception of cancer, attention is focused on morbidity rather than mortality, and the emphasis has changed from keeping people alive to keeping people fit. Life expectancy in the western world has quadrupled – from 20 years during the Roman Empire to 80 today. The fastest growing subgroups of people are the 9th and 10th Decade.

Looking back at Medical History and its long and successful evolution, it appears like a triumph of the human struggle for survival and growth in a hostile environment. From this viewpoint, man is perceived as a very rational creature [1].

The quadrupling of the life span, however, was not followed by a regulation in reproductive behavior, resulting in the population boom. There are now almost seven billion people on earth to which 30 million people are added each year. As is always the case in closed ecosystems, uncontrollable population growths result in catastrophe or even extinction of the exploding system, and to-date there have been no exceptions to this rule. Homo sapiens is approaching this point and if urgent adjustments are not made, human extinction is not only possible but imminent [2, 3, 4, 5].

From this point of view man is a very irrational creature.

THE END OF HIPPOCRATIC MEDICINE

Hippocratic Medicine is traditionally involved in our survival as individuals or groups. Living Man has been the measure of all things. Now, as in the past, Medicine has not been particularly involved in the health of future communities and our survival as a species. For Hippocratic Medicine, the reduction of human death rates is an absolute goal and concern about population growth has never been an accepted limitation on any public health measures. These policies led Hippocratic Medicine to its long and successful development. Hippocratic Medicine has impressively improved our lives. We now live much longer, we live better and we suffer less. Yet, this very success has become, at the population level, clearly antibiological and is the cause of population explosion, environmental disintegration and the depletion of the planet's gene pool.

Philosophically, these developments assert the end of Descartes' Enlightenment Ideal of a rational human nature. It should signal the end of Hippocratic Medicine as well. The alternative is the End of Man and a mass extinction of life on the planet [6, 7]. We should stress that human existence is

now threatened by overpopulation and ecological changes; it is also threatened by the interspecies wars which never stop while we become increasingly sophisticated in inventing weapons of mass destruction [8].

MEDICINE IN A NEW ROLE

Medicine in "A New Role", much more important than the traditional role of Hippocratic Medicine, might lead the way to overcoming the deep crisis and the deadlock humanity is facing today. *Medicine in its New Role should attempt to reduce and sustain human population, optimize the earth's ecosystem and integrate Homo sapiens therein. How can Medicine achieve this? The only way is by studying, trying to understand and to explain, mainly – but not exclusively – on a neurophysiological basis, the patterns of human behavior that made a mess out of our History.* These patterns have remained remarkably unchanged in eons and are basically expressed by the continuous swing during Human History from the most glorious achievements to the most terrible monstrosities. The patterns of human behavior are earmarked, at all levels (global, national and individual), by what is called "The Dichotomy of Human Mind", i.e. the mentality split between logic and belief, reason and faith, intellect and emotion. Which are the forces that shape these paranoid patterns of Human Behavior, the paranoid Human History and propel us to our own demise? Can Medicine identify these forces? [9, 10, 11, 12, 13]

Modern research in neurophysiology, with improved neuroimaging technology, seems to establish the link between areas of the brain and behavior and hence identifying potential pathways for intervention. Yet, a deep understanding of the biology and function of the human mind on human affairs and human conflicts seems still remote. Progress is being made in the study of human behavior. An approach is the study of human behavior patterns mainly but not exclusively by neurophysiology. However, even without a deep understanding of how the mind works might lead us to effectively interfere in human conflicts and human history and such precedents do exist in Medical History. The control of endemic cholera in London and the successful vaccination against small-pox were achieved without having the slightest idea about the "cause" of cholera and small-pox or about the existence of microbes. Systematic study of human behavior patterns might achieve the same goal [3, 4, 17, 18].

HEALING A SICK CULTURE

Medicine in "A New Role" can contribute in the process of healing a sick culture. But as Professor A. R. Damasio puts it [19], "It would be foolish to ask Medicine alone to heal a sick culture".

To heal a sick culture, human behavior patterns, human society structures, and the threats to Human Species' existence should be approached in a holistic way and from

a multidisciplinary point of view.

The broad goal must be contributing to the solution of the greatest issues humanity is facing today:

- Prevention of global catastrophes.

- Creation of an environment able of ensuring peace and progress for all human beings.
- Prevention of a sixth mass extinction of life on the planet, and preservation of the human species [20, 21, 22].

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A phase II study of bortezomib in combination with doxorubicin in recurrent or metastatic adenoid cystic carcinoma of the head and neck

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ABSTRACT

Background: Adenoid cystic carcinoma (ACC) of the head and neck is a salivary gland malignancy with a propensity for local and distant spread that is poorly responsive to chemotherapy. A previous phase II trial that evaluated single-agent bortezomib in advanced, incurable ACC reported high rate of disease stabilization as well as a partial response with the addition of doxorubicin to bortezomib at the time of progression. Preclinical and clinical evidence suggest increased antitumor efficacy with the combination of anthracyclines and bortezomib.

Patients & Methods: Eligible patients had incurable ACC of the head and neck, no prior chemotherapy for recurrent or metastatic disease, measurable disease by RECIST, Eastern Cooperative Oncology Group (ECOG) performance status 0-2, ejection fraction within normal limits, and adequate laboratory parameters. Treatment consisted of bortezomib 1.3 mg/m², intravenously on days 1, 4, 8 and 11, and doxorubicin 20 mg/m², intravenously on days 1 and 8, every 21 days. Dexrazoxane was added at the 8th cycle and all subsequent cycles with doxorubicin. The primary endpoint of the study was the objective response rate and the stable disease rate.

Results: From November 2007 to April 2010, 9 patients were enrolled onto the study. Three were males and 6 females. Median age was 57 years (44-75). Two patients had received prior chemotherapy as part of initial combined modality therapy, 8 had received radiotherapy; and all 9 had undergone surgery. Median number of cycles was 4 (2-10). No objective response was observed. Best response was stable disease, including 1 unconfirmed partial response. Median time of stable disease was 8 months (1-36). No grade 4 toxicity was observed. Grade 3 toxicities included neutropenia (n=2); thrombocytopenia (2); nausea (1); and constipation (2). Only 1 patient experienced grade 2 neurotoxicity. Two patients discontinued treatment due to toxicity.

Conclusions: The combination regimen of bortezomib plus doxorubicin was well tolerated but did not yield objective responses in this small sample size clinical trial in patients with ACC of the head and neck.

Key words: bortezomib; doxorubicin; adenoid cystic carcinoma; head and neck; phase II trial.

INTRODUCTION

Adenoid cystic carcinoma (ACC) is an uncommon tumor that accounts for about 25% of malignant tumors of the salivary glands [1] and 7% of head and neck cancers [2]. It predominantly arises from minor salivary glands and may demonstrate an indolent pattern of growth. However, at the time of diagnosis, even small tumors have the propensity to invade the surrounding vessels and nerves [3]. ACC is frequently associated with locoregional recurrence and/or distant metastases, which may occur late. The most frequent metastatic sites are the lungs, bones

and liver. The primary treatment of ACC is surgical. Postoperatively radiation is usually utilized in patients with perineural invasion, positive surgical margins, T2 or larger tumors and/or regional lymphadenopathy which are considered poor prognostic factors [4-8].

Combined modality treatment provides better local control but may have no impact on the development of distant metastasis. Although approximately 5% of patients have distant metastasis at original diagnosis, almost 50% of patients with ACC will develop metastatic disease during the course of their illness [9-10]. The majority of those patients will eventually

succumb to their disease within the first three years after development of metastases [11]. Patients with pulmonary disease have better survival compared to those with bone metastases or other viscera [12-13].

ACC is considered a malignancy with low or moderate sensitivity to chemotherapy. Hence, chemotherapy is usually reserved for symptomatic patients or rapidly progressive disease [13]. Several chemotherapeutic agents have been tested in ACC but have shown moderate or no activity. The response rate of cisplatin [14], gemcitabine [2], vinorelbine [15], epirubicin [16], mitoxantrone [17-18], or paclitaxel [19] monotherapy and the combination of cisplatin, doxorubicin, cyclophosphamide (CAP) [20-23] in ACC has ranged between 0-30% [13]. However, one third of patients diagnosed with ACC survive for more than 10 years, which appears to be due to the indolent nature of some of these tumors [4].

The development and evaluation of new active anti-neoplastic drugs alone and in combination with traditional chemotherapeutics in ACC should become a high priority. Bortezomib (Velcade/PS-341) is a selective inhibitor of the 26S proteasome that has shown activity against different types of tumors and is currently approved by the United States Food and Drug Administration for the treatment of multiple myeloma and mantle cell lymphoma. The combination of bortezomib with doxorubicin has been tested with promising results in patients with multiple myeloma [24-25]. Activated proteasome leads to the activation of nuclear factor- κ B (NF- κ B) and degradation of activator protein-1 (AP-1), which promote tumor growth and relate to worse prognosis in many tumors, including ACC [26-28]. The combination of NF- κ B inhibitors with 5-fluorouracil has shown antitumor activity in transformed salivary gland tumors [29]. The Eastern Cooperative Oncology Group (ECOG) conducted a phase II study of bortezomib followed by the addition of doxorubicin at progression in patients with incurable ACC (E1303) [30]. Among the 21 evaluable patients that received single-agent bortezomib, there was no objective response, however 15 patients (71%) had stable disease as best response and the median progression-free survival was 6.4 months. Of 10 evaluable patients treated with bortezomib and doxorubicin, 1 patient had a partial response and 6 had stable disease as best response. Overall, the regimen was well-tolerated with acceptable toxicities [30]. The potential activity of the combination regimen encouraged us to design and conduct this phase II study in order to investigate the efficacy and tolerability of the combination of bortezomib with doxorubicin in chemotherapy-naïve patients with recurrent or metastatic ACC of the head and neck.

PATIENTS & METHODS

Eligibility criteria

Eligible patients were aged 18 years or older, with cytologically or histologically confirmed, locally advanced, recurrent or metastatic incurable ACC of the head and neck.

No prior chemotherapy for recurrent or metastatic ACC was allowed. However, up to 1 prior biologic/targeted therapy regimen and chemotherapy as part of initial potentially curative therapy (i.e. concurrent chemoradiotherapy) were allowed. The patients could not have had any prior therapy with anthracyclines (doxorubicin, epirubicin, daunorubicin, idarubicin, mitoxantrone) or bortezomib. Other eligibility requirements included unidimensionally measurable disease within 3 weeks before enrollment to the study according to response evaluation criteria in solid tumors (RECIST); left ventricular ejection fraction (LVEF) at or above the institutional lower limits of normal; ECOG performance status 0-2; and adequate liver, renal and bone marrow function. Pregnant women; patients with pre-existing neuropathy of grade >1 ; brain metastases; positive for human immunodeficiency virus; myocardial infarction within 6 months prior to enrollment; uncontrolled angina; and severe uncontrolled ventricular arrhythmias were excluded. The study protocol was approved by the Institutional Review Board of the University of Pittsburgh and all patients signed informed consent. The study was coordinated by the University of Pittsburgh Medical Center (UPCI protocol 06-124) and was registered in clinical-trials.gov (NCT00581360).

Treatment plan

Treatment consisted of bortezomib [provided by Millennium Pharmaceuticals, Inc. (Cambridge, Massachusetts)] 1.3 mg/m² intravenously (IV), push, over 3-5 seconds, twice weekly for 2 weeks followed by one week off treatment (i.e. days 1, 4, 8, 11), every 21 days, followed one hour later by doxorubicin at 20 mg/m² IV push over 2-5 minutes with extravasation precautions, on days 1 and 8, every 21 days. Cardioprotection with dexrazoxane (Zinecard) 200 mg/m² IV (i.e. dexrazoxane/doxorubicin ratio of 10:1) was administered prior to doxorubicin and after bortezomib on days 1 and 8 starting with the 8th cycle (doxorubicin cumulative dose of 280 mg/m²) and all subsequent cycles. Treatment continued until disease progression or intolerable toxicities to a maximum of 14 cycles, after which bortezomib could be continued alone on an once-weekly schedule (1.6 mg/m² on days 1, 8, 15, every 28 days) assuming there was no disease progression.

Baseline and treatment assessments

Clinical examination; complete blood counts (CBCs); chemistry studies; chest x-ray; Multiple Gated Acquisition Scan (MUGA) or echocardiogram; computed tomography (CT) scans or other imaging tests where indicated (i.e. ultrasonography) were included in the baseline evaluation within 3 weeks before registration. CBCs were performed before each bortezomib administration, and CBCs and chemistry tests on day 1 of each cycle. Tumor measurements were performed every 2 cycles using RECIST. When a patient achieved a response, repeat tumor measurements were required in 6 weeks (to confirm the objective response). Toxicity was assessed by the National

Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0 (NCI CTCAE 3.0).

Statistical analysis

The primary objective of this single arm phase II study was to evaluate the objective response and stable disease rate. A two-stage design was applied and permitted the trial to be stopped at the end of the first stage for lack of activity. Specifically, the study tested the null hypothesis of 5% response rate and 30% stable disease rate against the alternative that either was greater. The study was designed to provide sufficient power to detect an improvement to either a 20% response rate or a 50% stable disease rate.

In the first stage 11 patients would be accrued. All patients would be scored according to RECIST as response (complete or partial), stable disease or progressive disease. At the end of the first stage, if there were fewer than 2 responses and fewer than 5 patients with stable disease (best response after at least 2 cycles) or response, the study would terminate for lack of efficacy. If there were 2 or more patients who responded or 5 or more patients who achieved either a response or stable disease, the study would continue to the 2nd phase accruing an additional 21 patients (i.e. a total of 32). Response analysis was based on response-evaluable patients, who were defined as study-eligible patients who had received at least 1 cycle of treatment. To allow for the possibility of failure to complete 1 treatment cycle, accrual was planned for 35 patients. This design achieved a maximum type I and type II error of 10% and was optimum in the sense that the expected sample size was minimized among other designs with the same total sample size. The probability of termination at the first stage was 64%, if the null hypothesis is true. Secondary endpoints of the study were progression-free survival, overall survival and toxicity.

RESULTS

Patient characteristics and treatment

From November 2007 to April 2010, a total of 9 patients were enrolled. The study was terminated early because of slow accrual. Patient characteristics are shown in Table 1. All patients were eligible and evaluable for both response and toxicity. The majority of patients was females and had had prior treatment with surgery and radiation. Five patients had distant metastases and four locoregional relapse. All patients received at least 2 cycles of the treatment. The median number of cycles was 4 (range, 2-10). Two patients withdrew due to toxicity (one developed grade 2 neurotoxicity and another recurrent grade 3 neutropenia despite dose reductions) and a third one for personal reasons after completion of 2 cycles.

Treatment efficacy

No objective response was observed. One patient responded at both primary and metastatic site after the first 2 cycles but

Table 1.

Patient characteristics (n=9)

Median age (range)	57 years (44-75)
Sex , No.	
male	3
female	6
Performance status , No.	
0	4
1	5
Distant metastases , No.	5
Locoregional recurrence only , No.	4
Prior radiation , No.	8
Prior surgery , No.	9
Prior chemotherapy , No.	2

he recurred in the re-evaluation after the 4th cycle. All patients had stable disease as best response, with a median duration of 8 months (range, 1-36 months). No difference in the duration of disease stabilization according to prior treatment status was observed (Table 2). With a median follow up of 25 months (range, 1.2-34.5), 5 patients (56%) progressed and 2 patients died. No patient died due to treatment.

Toxicity

The regimen was well-tolerated. No grade 4 or 5 adverse events were observed. Table 3 lists the grade 2-4 toxicities. The most common grade 3 toxicities were neutropenia (n=2), thrombocytopenia (n=2) and constipation (n=2). The administration of doxorubicin and bortezomib was held in 5 and 4 patients, respectively, due to side-effects likely related to the treatment drugs; dose reduction of doxorubicin was required in 2 patients and of bortezomib in 3 patients (see Table 2).

DISCUSSION

The responsiveness of ACC to traditional chemotherapeutic agents is low or moderate. The search for novel active regimens for ACC is of major importance. Doxorubicin has been shown active in ACC [20-21]. The NF-κB is overexpressed in ACC; it is a known regulator of several adhesion molecules (i.e. E selectin) that are involved in apoptosis. A previous ECOG study showed rather limited activity of single-agent bortezomib, a proteasome inhibitor that has several targets, including NF-κB, but suggested possible activity of the combination regimen in a small number of patients who had doxorubicin added to bortezomib at the time of progression [30]. This prompted the design of the current phase II study on the combination

Table 2.

Summarized patient outcomes

Patient ID	Primary site	Recurrence site	Bortezomib doses (No.)	Bortezomib dose modifications		Doxorubicin doses (No.)	Doxorubicin dose modifications		Best response	Duration (mo)	Survival status (mo)
				Hold	Reduction		Hold	Reduction			
1	Salivary gland	Lung	31	No	No	15	Yes	No	SD	8	Alive (29.13)
2	Tongue	Locoregional	10	No	No	4	Yes	Yes	SD	23	Alive (23.40)
3	Nasopharyngeal	Lung, liver	24	Yes	Yes	17	Yes	No	SD	36	Alive (34.50)
4	Maxillary sinus	Ethmoid sinus, sphenoid sinus	16	No	No	7	No	No	SD	4	Dead (8.03)
5	Parotid	Lung, bone	7	No	No	4	No	No	SD	2	Dead (12.20)
6	Mastoid	Mastoid	28	Yes	Yes	19	Yes	No	SD	8	Alive (16.10)
7	Sublingual salivary gland	Lung, bone	15	No	Yes	8	No	Yes	SD	26	Alive (25.93)
8	Dura/cribriform plate	Ethmoid sinus, inferior orbit	23	Yes	No	12	Yes	No	SD	5	Alive (24.97)
9	Submandibular	Submandibular, lung	8	Yes	No	3	No	No	SD	1 (withdrew consent)	Alive (1.27)

of bortezomib with doxorubicin as upfront treatment of chemotherapy naïve patients with recurrent or metastatic ACC.

Our study had to be terminated early due to slow accrual. Although there was interest in this study from patients with ACC living at a long distance from the city of Pittsburgh, a major practical limitation for their participation was the need for frequent intravenous administration of the drugs. Given the small sample size of this study, our observations may be confounded by the limited power to demonstrate antitumor activity. Although we observed no objective responses, disease stabilization was uniformly seen as best response with a median duration of 8 months (range, 1-36 months), and one patient had an unconfirmed partial response. Furthermore, more than half of the patients did not progress within the first 6 months after study enrollment. We have previously suggested as null hypothesis for future studies in recurrent or metastatic ACC a duration of stable disease ≥ 6 months in 50% or a median PFS of 6 months [30].

Overexpression of several distinct receptors, including epidermal growth factor receptor (EGFR), Her-2 and c-kit, has been observed in ACC. To that end, several therapeutic

agents have been employed targeting the inhibition of activated signal transducing molecular pathways of these receptors. Gefitinib [31], cetuximab [32], lapatinib [33], and imatinib [34] have been tested in patients with recurrent or metastatic ACC. Interestingly, there has been a lack of objective responses in these studies with novel agents. An older study that evaluated single-agent epirubicin in advanced or recurrent ACC reported that 2 patients responded (10%) and 10 had disease stabilization [16]. However, differences in response criteria used between studies may have accounted for the differences in reported rates of objective response. Moreover, it remains unclear whether the prolonged stable disease achieved by many patients in the current as well as in prior studies could be attributed to treatment effect or the natural history of the disease which may progress in an indolent manner. The investigation of novel regimens like the combination of bortezomib with doxorubicin would have been more informative if conducted in the context of a randomized trial which could potentially discern the impact of treatment versus natural history of disease. However, the rarity of ACC makes this aim elusive; actually slow accrual was the reason of premature closure of the present study.

Table 3.

Toxicities (n=9)

	Gr2 (No. of patients)	Gr3 (No. of patients)	Gr4 (No. of patients)
Anemia	2	-	-
Neutropenia	4	2	-
Thrombocytopenia	1	2	-
Nausea	2	1	-
Vomiting	1	-	-
Rash	3	-	-
Fatigue	2	-	-
Neurotoxicity	1	-	-
Constipation	2	2	-

The bortezomib and doxorubicin combination was generally well-tolerated and was associated with expected side-effects. No toxic death or grade 4 toxicity was observed. Sensory neuropathy, a rather frequent adverse event of bortezomib, of grade 2 occurred in 1 patient. In regards to hematologic toxicity, 2 patients developed grade 3 neutropenia and 2 grade 3 thrombocytopenia. Despite the durable disease stabilization that was achieved with the combination of bortezomib with doxorubicin and the relatively favorable toxicity profile, we do not feel that further investigation of this regimen is of high priority. On the other hand, the development of novel agents with activity in ACC should be strongly pursued. Finally, given the rarity of ACC, cooperative groups and consortia should undertake a concerted effort to conduct studies with sufficient sample size and, hopefully, randomized in order to move forward the stagnant field of ACC treatment.

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A multicenter phase I-II trial of gefitinib in combination with vinorelbine and oxaliplatin in women with advanced recurrent ovarian cancer

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ABSTRACT

Background: The purpose of this study was to assess the tolerance and efficacy of gefitinib (Iressa) in combination with vinorelbine and oxaliplatin in pre-treated ovarian cancer (AOC) patients.

Patients & Methods: Pre-treated patients with recurrent or refractory AOC were treated in a phase I/II study with oral gefitinib 250 mg/day continuously, vinorelbine 25 mg/m² and oxaliplatin 50 mg/m² (iv) on days 1 and 8 every 3 weeks. Gefitinib was continued until disease progression.

Results: Neutropenia, febrile neutropenia and diarrhea were the Dose Limiting Toxicities (DLTs) in phase I part of the study, requiring dose reductions for vinorelbine and oxaliplatin (20 mg/m² and 40 mg/m², respectively as MTD) which were used for the subsequent phase II part of the study. Overall, 26 objective responses (CR=12 and PR=14; ORR=33.3%, 95% CI: 22.9%-43.8%) were documented; 12 (23%) and 14 (53.8%) responses occurred in patients with platinum-resistant/refractory (n=52) and platinum-sensitive (n=26) tumors, respectively. The median time to tumor progression was 3.4 and 8.2 months in patients with platinum-resistant/refractory and platinum-sensitive tumors, respectively. Grade III/IV neutropenia and grade III/IV diarrhea were observed in 30 (44%) and four (6%) patients, respectively. There were six (8.8%) episodes of febrile neutropenia but no treatment-related death.

Conclusions: The combination of vinorelbine (20 mg/m²) and oxaliplatin (40 mg/m²) gefitinib was associated with a relatively high incidence of neutropenia and offers promising activity mainly in women with platinum-sensitive AOC.

Key words: ovarian cancer; gefitinib; vinorelbine; oxaliplatin; phase I-II trial.

INTRODUCTION

Ovarian cancer is the third leading cause of cancer death among women in western countries. It is generally diagnosed late in the course of the disease, since 75% of the patients are diagnosed with advanced stages (FIGO stages III and IV). While surgery is the cornerstone of initial therapy, most patients cannot be cured by surgery alone due to residual microscopic and macroscopic peritoneal disease. First-line treatment with combination chemotherapy (including a platinum compound and paclitaxel) results in a clinical complete response rate of approximately 75% in patients with advanced ovarian cancer (AOC); however, most of the patients will relapse and there is an unmet need for the

development of salvage chemotherapy regimens. The median overall survival of patients with AOC ranges from 24 to 60 months depending on the volume of the disease at diagnosis [1]. A significant improvement in the prognosis of patients with AOC has been recorded in the past 20 years, with a 5-year survival rate in the USA increasing from 36.9% in 1974-1976 to 52.1% in 1992-1997 [2]. Despite these advances, only about 20% of patients with initial stage III disease and less than 5% of patients with stage IV disease will survive for 5 years. Patients who are refractory or resistant to platinum-containing regimens have a particularly poor prognosis.

The choice of salvage treatment in patients with a clinical relapse is based on clinical

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resistance characteristics such as quality of response to first-line treatment, the duration of progression-free interval and other clinical parameters (i.e. extension of the disease, age, residual toxicities from the first-line chemotherapy and patient performance status). Patients who achieve an initial response to platinum-based chemotherapy and who have a drug-free period of at least 6 months are generally considered to have sensitive relapse with a 40-90% chance of achieving a second response [3]. Patients who initially responded to a platinum-containing regimen but whose disease recurs less than 6 months after the completion of chemotherapy are considered to have platinum resistant tumors. Although there is anecdotal evidence that some patients may respond to platinum-based regimens [4], response rates with approved treatments such as topotecan, liposomal doxorubicin or oral etoposide do not exceed 15-20% [5-7]. Patients who progress on first-line platinum-based therapy are regarded as having platinum refractory disease and their probability to respond to salvage therapy is less than 10% [5, 6]. Therefore the development of new regimens remains a critical need.

The EGFR pathway contributes to a number of processes involved in tumor survival and growth including cell proliferation, survival, angiogenesis and metastasis, thus making it an attractive target for anticancer therapy [8]. Small molecules inhibiting the tyrosine kinase of EGFR (i.e. gefitinib, erlotinib; TKIs) as well as monoclonal antibodies against the extracellular domain of EGFR (i.e. cetuximab, panitumumab) target the EGFR pathway. Gefitinib (Iressa; Astra Zeneca) is a low molecular weight quinazoline derivative that specifically inhibits the activation of EGFR tyrosine kinase through competitive binding to the ATP-binding domain of the receptor [9]. Gefitinib is generally well-tolerated with the most prevalent toxicities being diarrhea and skin rash. Recent studies have shown that activating mutations within the tyrosine kinase domain of EGFR are associated with responses to gefitinib in patients with non-small cell lung cancer [10, 11]. As EGFR is overexpressed in 30-70% of ovarian cancers, gefitinib may have antitumor activity against this disease [12, 13]. Preclinical studies of gefitinib in ovarian cancer cell lines have shown increased growth inhibition when it is used in combination with different chemotherapeutic agents compared to its use as single agent [12]. Gefitinib has also shown antitumor activity in pre-treated patients with ovarian cancer in phase I and II studies [14, 15]. These results are in contradiction to more recently published data since gefitinib in combination with tamoxifen had minimal activity in patients with platinum and taxane resistant or refractory ovarian cancer [16]. Posadas *et al.* [17] showed a decrease in both the EGFR and p-EGFR expression in tumor samples of >50% of pre-treated patients with ovarian cancer, who received gefitinib therapy; however, this effect was not associated with a meaningful clinical benefit [17].

Vinorelbine (Navelbine; Pierre Fabre, France) is a semi-synthetic vinca alkaloid inhibiting the assembly of

microtubules; the drug has shown substantial activity in recurrent ovarian cancer producing a response rate of 15-29% [18-21]. Vinorelbine is well-tolerated, with neutropenia being the main dose-limiting toxicity [22].

Oxaliplatin, a third generation platinum derivative, has been investigated as a single agent and in combination with cisplatin, paclitaxel, gemcitabine, 5-fluouracil, leucovorin or other agents in patients with recurrent ovarian cancer. [23]. Many phase II trials have shown responses ranging from 4.3%-29% when oxaliplatin was administered as single agent in patients with recurrent AOC [24-27]. Neurotoxicity, myelosuppression and nausea/vomiting are the main toxicities of this drug [28].

The aim of the current study was to evaluate the tolerance and the efficacy of the combination of vinorelbine with oxaliplatin and gefitinib as salvage treatment in patients with recurrent AOC. The design of this trial was based on our previous phase I study which evaluated the vinorelbine plus oxaliplatin combination in pre-treated patients with advanced solid tumors. That study had demonstrated that the combination was feasible with acceptable toxicity when the drugs were given at the dose of oxaliplatin 50 mg/m² and vinorelbine 27 mg/m² [29].

PATIENTS & METHODS

Eligibility criteria

Women with histologically or cytologically confirmed advanced ovarian cancer were enrolled in the study. Patients had to have recurrent or refractory disease after at least one previous line of platinum-containing chemotherapy. Patients with non-measurable disease but with increased serum CA-125 levels were considered to have assessable disease and were included in this study. Tumor assessment based on the CA-125 was as follows: The minimum CA-125 serum level at the time of study entry had to be more than 70 U/mL. CA-125 serum levels had to have at least doubled from a baseline determination in order to be considered as evidence of disease progression from a previous treatment regimen. The CA-125 value had to be confirmed by at least two separate measurements of blood samples obtained ≥ 4 weeks apart or the patient to have other clinical evidence of progressive disease. Other eligibility criteria included: measurable, non-irradiated disease according to Response Evaluation Criteria in Solid Tumours (RECIST); age 18 to 75 years, life expectancy of at least 12 weeks, a World Health Organisation (WHO) performance status of 0-2; prior surgery and radiotherapy (to less than 25% of active bone marrow) was allowed but a treatment-free interval of at least 4 weeks was required before study entry; adequate bone marrow [absolute neutrophil count (ANC) $>1.5 \times 10^9/L$ (1500/mm³) or platelets $>100 \times 10^9/L$ (100,000/mm³)], liver [serum bilirubin $<1.25 \times$ times the upper limit of reference range (ULRR), alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $<2.5 \times$ times the ULRR if no demonstrable liver

metastases, or $<5\times$ times the ULRR in the presence of liver metastases], or renal [serum creatinine $<1.25\times$ times the ULRR] function tests; absence of any unresolved chronic toxicity greater than grade II from previous anticancer treatment (other than alopecia); absence of severe or uncontrolled systemic disease (e.g. unstable or uncompensated respiratory, cardiac, hepatic or renal disease), concomitant use of phenytoin, carbamazepine, barbiturates, rifampicin, pregnancy or breastfeeding (women of child-bearing potential); absence of known, severe hypersensitivity to gefitinib or any of the excipients of this product; absence of other co-existing malignancies with the exception of basal cell carcinoma or cervical cancer in situ. Patients with brain metastases were eligible, provided that they had received whole brain irradiation with radiological and clinical improvement. All patients gave written informed consent to participate in the study. The study was approved by the Ethics and Scientific Committees of the participating Institutions.

Treatment plan

This was a multicenter open-label one arm non-comparative phase I-II trial, involving pre-treated with platinum-based chemotherapy women with recurrent AOC. Patients were divided into two groups according to the presence of platinum-refractory/resistant (group 1) or platinum-sensitive (group 2) disease. In the phase I part of the trial, 10 patients were enrolled initially from both clinical groups in order to evaluate the safety of a fixed dose combination therapy, with gefitinib 250 mg once daily throughout the trial period together with 25 mg/m² vinorelbine administered as a 60-min intravenous infusion followed by oxaliplatin 50 mg/m² administered intravenously as a 4-hour infusion both on days 1 and 8 of each 3-week cycle. The combination therapy was administered for 6 cycles and, in the case of continuing objective response, for 3 additional cycles. Prophylactic antiemetic regimens included ondansetron (16 mg) given as a short intravenous infusion before the administration of chemotherapy. Gefitinib monotherapy was given until disease progression, unacceptable toxicity or consent withdrawal. A full safety evaluation was conducted for the initial 10 patients who received at least one chemotherapy cycle; if dose limiting-toxicities (DLT) occurred in three patients or less during the first cycle of treatment, toxicity was considered acceptable and enrolment was expanded to the planned number of patients. DLTs were defined as grade IV myelotoxicity (neutropenia or thrombocytopenia lasting >5 days), febrile neutropenia, or grade III non-hematological toxicity. If more than three patients experienced a DLT during the first cycle of treatment, toxicity was considered to be excessive and an additional 10 patients had to be treated with doses of vinorelbine and oxaliplatin reduced by 20%. If the reduced dose regimen was well-tolerated (three or less DLTs), enrolment could be expanded to the planned number of patients using the lower dose level. If the combination using

the lower dose level was not well-tolerated, the trial had to be terminated.

Patient evaluation

Baseline evaluation included patient history, physical examination, complete blood cell count (CBC) with differential and platelet count, serum chemistry and serum levels of CA-125 as well as chest X-rays, electrocardiogram, thorax and abdomen-pelvic computed tomography (CT) scans. Complete blood cell count with differential and platelet counts were performed weekly. In the case of grade III or IV hematological toxicity, complete blood cell counts with differential were performed daily until recovery. Blood chemistries, serum levels of CA-125 and a physical examination were performed before each administration of chemotherapy. Tumor response was assessed according to RECIST criteria every 2 treatment cycles by clinical evaluation and chest-abdominal-pelvic computed tomography (CT) scans. Patients with assessable disease based on CA-125 measurements were evaluated as follows: the CA-125 serum levels were determined before each cycle during treatment administration and if its levels were lower or equal than those at baseline, treatment was continued without any modification; after chemotherapy completion, CA-125 serum levels were determined every 2 months until disease progression. Partial response was defined as a serial decrease of serum CA-125 level over three samples of more than 75%. In each case the subsequent sample had to be tested more than 21 days after the previous one. Complete response was defined as the normalization of serum CA-125 level that was confirmed by a repeat measurement at least 1 month after the initial normal value. Progressive disease was defined as a serum CA-125 level of at least 70 U/mL that had at least doubled from the previous value. Stable disease was considered every value that fell between the criteria set for partial response and progressive disease. After completion of the study treatment, all patients were followed every 2 months until documented disease progression.

Statistical analysis

Patients were divided into two groups according to their response to front-line platinum-based chemotherapy: group 1 (platinum-refractory or resistant) and group 2 (platinum-sensitive).

Fleming's method was used to calculate the number of patients required in each group. A sample size of 25 patients with platinum-sensitive ovarian cancer was sufficient to give an 80% probability of rejecting a baseline response rate of 10% with an exact 5% one sided significance test when the true response is at the clinically relevant rate of 30%. The hypothesis that the response rate is equal to or less than the baseline was rejected if 6 or more responses were observed among the 25 enrolled patients. The exact size and

power of this test are 3.3% and 80.5%, respectively [30]. A sample size of 52 patients with platinum-resistant or refractory ovarian cancer was sufficient to give an 80% probability of rejecting a baseline response rate of 5% with an exact 5% one-sided significance test when the true response is at the clinically relevant rate of 15%. The hypothesis that the response rate is equal to or less than the baseline was rejected if 6 or more responses were observed among the 52 patients. The exact size and power of this test are 4.6% and 81.1%, respectively [30].

A comprehensive Statistical Analysis Plan (SAP) was prepared before database lock. The two trial patient groups were analysed separately. Platinum-sensitive patients were defined as those who relapsed more than 6 months after completion of first-line platinum-based chemotherapy, following an initial response. Platinum-refractory patients were defined as those who progressed on first line platinum-based chemotherapy. Platinum-resistant patients were defined as those with disease progression within 6 months after completion of first-line platinum-based chemotherapy. All patients who received at least one cycle

of the experimental treatment were evaluated for toxicity. For the evaluation of efficacy, the intention-to-treat (ITT) population consisted of all patients who were enrolled in the trial and received vinorelbine and oxaliplatin at the maximum tolerated doses (MTD). The standard summary statistics for continuous variables were: mean, standard deviation, median, quartiles, minimum and maximum. The standard summary statistics for discrete variables were count and proportion. Response rates and controlled disease rates were summarized by proportions, together with a 95% CI. Duration of progression-free (PFS) and overall (OS) survival were estimated using the Kaplan-Meier method.

RESULTS

Determination of the maximum tolerated dose (MTD)

From June 2002 to August 2005, the participating centres screened 81 patients and three of them failed to fulfil the eligibility criteria and were excluded from the study. Seventy-eight patients were enrolled onto the study. The patients' median age was 60 years (range, 29-75). During the phase I part of the study, 10 patients received the experimental treatment which combined gefitinib 250 mg/day with oxaliplatin at 50 mg/m² and vinorelbine at 25 mg/m². Four (40%) of these patients developed Dose-Limiting Toxicities (DLTs), since two patients experienced febrile neutropenia; one patient grade IV neutropenia lasting >5 days; and one patient grade III diarrhea. As per trial design, a second cohort of 10 patients was treated with reduced doses of vinorelbine (20 mg/m²) and oxaliplatin (40 mg/m²). Two (20%) patients presented DLTs (one patient with febrile neutropenia and one patient with grade IV neutropenia lasting >5 days). Based on this acceptable toxicity profile, the study was continued as phase II using the decreased doses of vinorelbine and oxaliplatin. The 10 women enrolled in the second cohort of the phase I part of the study plus the patients enrolled in the phase II part of the trial (n=68) were analyzed together.

Demographics of these patients are presented in Table 1. The median age was 60 years (range, 29-75) and 90% of patients had a PS of 0-1. All patients had received prior platinum-based chemotherapy; 52 (67%) patients had platinum-resistant or refractory and 26 (33%) platinum-sensitive tumors. In addition, 36 (46%) and 42 (54%) patients were taxane-resistant/refractory and taxane-sensitive, respectively. Most patients (68%) had received ≥2 lines of chemotherapy prior to study entry.

Compliance with the treatment

A total of 351 chemotherapy cycles were administered with a median 5 cycles/patient. In the phase II part of the trial, the median delivered dose intensity was 11.7 mg/m²/week for vinorelbine and 23.4 mg/m²/week for oxaliplatin. These corresponded to 88% and 87.9% of the protocol-planned

Table 1.

Patients' characteristics

	n=78 Phase I & II	N=68 Phase II only
Age		
Median (min-max)	60 (29-75)	60 (40-72)
Performance status		
0	58 (68%)	46 (67.6%)
1	17 (22%)	15 (22.1%)
2	8 (10%)	7 (10.3%)
Number of regimen		
1	25 (32%)	22 (32.4%)
2	32 (41%)	31 (45.6%)
≥3	21 (27%)	15 (22.1%)
Platinum sensitivity		
Resistant-refractory	52 (67%)	46 (67.6%)
Sensitive	26 (33%)	22 (32.4%)
Taxane sensitivity		
Resistant/refractory	36 (46%)	32 (47.1%)
Sensitive	42 (54%)	36 (52.9%)
No. of organs involved		
Ca125 only	20	18
1	38	36
2	17	13
3	1	1
4	1	-
5	1	-

doses for vinorelbine and oxaliplatin, respectively. Treatment delay occurred in 71 (20%) cycles because of hematological (n=20; 28%) or non-hematological (n=6; 8%) toxicity, and for reasons unrelated to disease or treatment [n=45 cycles; 63% (i.e. pending imaging studies for response evaluation or patients' late admission due to personal reasons)]. The median interval between cycles was 21 days (range, 21-52). Dose reductions were required in 19 (5%) cycles because of hematological (n=16; 84%) or non-hematological toxicity (n=1; 5%), as well as for other reasons not related to either treatment or the disease (n=2; 11%). Seventy five (96%) patients had discontinued treatment because of progressive disease (n=34; 45%); consent withdrawal (n=2; 3%); protocol completion (n=27; 36%); lost to follow-up (n=2; 3%); non-compliance with the protocol (n=2; 3%); and because of adverse events related to treatment (n=6; 8%: two patients experienced grade III and IV diarrhea; 1 patient hepatotoxicity grade II; 1 patient septic shock requiring hospitalization; 1 patient grade II hypersensitivity reaction with dyspnea and rash during vinorelbine administration; and 1 patient grade III neutropenia). In addition, two patients (3%) with stable disease discontinued treatment as decided by their doctors.

Toxicity

In the group of patients analyzed in the phase II part of the study (n=68), neutropenia and diarrhea were the most common adverse events associated with this chemotherapy regimen (Table 2). Grade III and IV neutropenia occurred in 16

(23.5%) and 14 (20.6%) patients, respectively; administration of Granulocyte Colony-Stimulating Factor (G-CSF; Granocyte, Sanofi-Aventis, Bridgewater, USA) was required in 25 (32%) patients. There were six patients (8.8%) who developed febrile neutropenia and required hospitalization for intravenous antibiotics and G-CSF support; all of them recovered uneventfully. Grade III anemia occurred in three (4.4%) patients and only one of them was transfused. Grade III and IV diarrhea was observed in three (4.4%) and one patients, respectively. Grade I and II neurosensory toxicity was developed in 13 (19.1%) and eight (13.2%) patients, respectively. Skin rash was observed in 30.9% of patients; it was mainly grade I but in 8.8% of patients it was of grade II. Other grade III or IV toxicities were infrequent. In addition, one patient experienced deep venous thrombosis. In total, 22 (32.4%) patients required hospitalization because of diarrhea (n=7), neutropenia (n=7), infection (n=5), and five patients were hospitalized for other reasons such as: allergic reaction during vinorelbine administration, peritonitis, pulmonary embolism, gluteal abscess and subacute bowel obstruction.

Response to treatment

All 78 patients enrolled in the phase I and II part of the study were evaluable for response. Stable disease was observed in 28 (35.9%) patients and progressive disease in 24 (30.8%). Among the patients with platinum-resistant/refractory disease (n=52), 12 patients achieved objective responses (5

Table 2.

Adverse events possibly or probably related to study treatment per patient during all cycles (n=68)

	Grade 1		Grade 2		Grade 3		Grade 4	
	N	%	N	%	N	%	N	%
Leukopenia	10	14.7	10	14.7	8	11.8	5	7.4
Neutropenia	8	11.8	7	10.3	16	23.5	14	20.6
Febrile neutropenia	-	-	-	-	3	4.4	3	4.4
Anemia	9	13.2	21	30.9	3	4.4	-	-
Thrombocytopenia	12	17.6	2	2.9	1	1.5	-	-
Diarrhea	13	19.1	8	11.8	3	4.4	1	1.5
Constipation	7	10.3	4	5.9	-	-	-	-
Nausea-vomiting	12	17.6	15	22.1	1	1.5	-	-
Fatigue	8	11.8	9	13.2	-	-	-	-
Neurotoxicity	13	19.1	8	13.2	-	-	-	-
Skin rash	15	22.1	6	8.8	-	-	-	-
SGOT elevation	3	4.4	4	5.9	-	-	-	-
SGPT elevation	2	2.9	2	2.9	2	2.9	-	-

CRs and 7 PRs; ORR: 23%; 95% CI: 11.6%-34.5%); similarly, 14 patients achieved objective responses (7 CRs and 7 PRs; ORR: 54%; 95% CI: 34.7%-73%) in the group of patients with platinum-sensitive disease (n=26). Response rate was significantly higher in platinum-sensitive patients (p=0.007). After a median follow-up duration of 7.7 months (range, 1.2-35.4) for the platinum-sensitive group and 15 months (range, 0.8-36.9) for the platinum-resistant/refractory group, 42 patients died (35 of disease progression, 2 of pulmonary embolism, 1 of post-operative sepsis and 4 of unknown reasons because they were lost to follow-up). The median TTP for patients with platinum-sensitive disease was 8.2 months (95% CI: 7.7-9.1; range, 0.8-12.8) and 3.4 months (95% CI: 2.2-4.7; range, 0.5-21) for those with platinum-resistant or -refractory disease (p=0.150; Figure 1). The median overall survival for patients with platinum-resistant or refractory disease was 8.9 months (95% CI: 6.2-11.6; range, 1.2-35.4) and for those with platinum-sensitive disease 23.8 months (95% CI: 20.2-27.5; range, 0.8-36.9). The median overall survival was significantly longer in patients with platinum-sensitive tumors (p=0.004; Figure 2). According to the protocol, 31 patients continued gefitinib as maintenance treatment after completion of the chemotherapy; nine of them remained on gefitinib for more than 3 months and three for more than 6 months.

DISCUSSION

In this phase I-II trial of salvage chemotherapy with vinorelbine/oxaliplatin/gefinitib in patients with advanced ovarian cancer we showed that the regimen was feasible, active and well-tolerated albeit at a reduced dose. In the phase I part of the study, the combination demonstrated a relatively high incidence of grade III and IV toxicities characterized as DLTs which necessitated dose reduction.

Therefore, the phase II part of the study was conducted using the reduced doses of vinorelbine (20 mg/m²), oxaliplatin (40 mg/m²) given on days 1 and 8 every 21 days and gefitinib (250 mg/d) given continuously. Using this treatment dose and schedule, the obtained overall response rate was 53.8% for patients with platinum-sensitive disease and 23% for patients with platinum-resistant/refractory disease; this difference in the response rates between the two patient groups was statistically significant. More importantly, the median TTP and the median OS were significantly better in the group of patients with platinum-sensitive, as compared to the group of patients with platinum-resistant/refractory disease.

The efficacy results obtained in the group of patients with platinum-sensitive disease are better than those achieved with either agent alone [18-21, 24-27] and similar to the results obtained with platinum re-challenge or other non-platinum-based combinations which have been used as salvage treatment in patients with platinum-sensitive relapsed disease [31, 32]. In addition, for the group of patients with platinum-resistant/refractory disease, the observed activity of the regimen is comparable to that reported for single agent vinorelbine in previous phase II trials [18-21] and higher than that achieved by oxaliplatin monotherapy [24-27]. These results are of interest since 68% of the patients enrolled in the current study were heavily pre-treated with ≥ 2 chemotherapy regimens. Moreover, 36% of the patients achieved disease stabilization and thus the disease control rate was observed in 69% of the patients. A limitation of this study is the small median follow-up duration of patients, especially for the platinum-sensitive group.

Previous studies have shown that for patients with platinum-sensitive disease a platinum-based chemotherapy combination achieves better results in terms of response

Figure 1.
Kaplan-Meier TTP curves

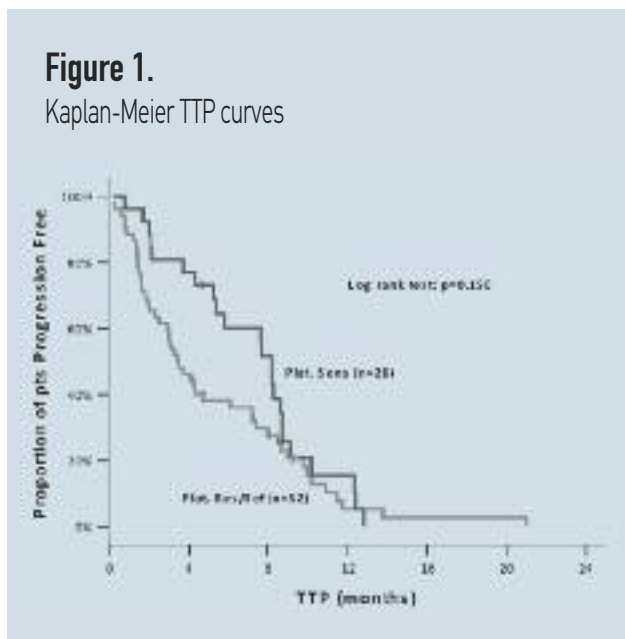
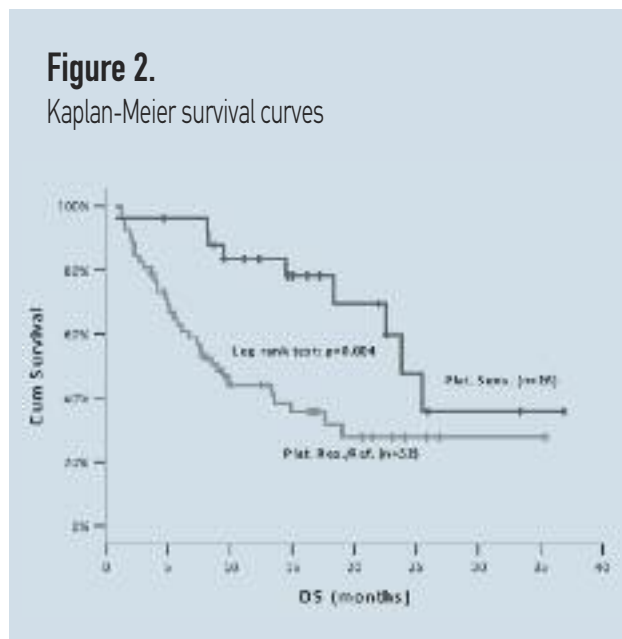


Figure 2.
Kaplan-Meier survival curves



rate, progression-free and overall survival compared to platinum single-agent [33, 34]. The ICON4/AGO-OVAR 2.2 trial investigated the paclitaxel/carboplatin combination versus carboplatin alone treatment in patients with relapsed platinum-sensitive disease. The combination regimen achieved a significantly higher overall response rate than the monotherapy (66% versus 54%) and higher median PFS (12 vs. 9 months) and overall survival (29 vs. 24 months) [33]. Similar results were also observed in another randomized trial which compared gemcitabine/carboplatin versus carboplatin alone in relapsed patients with platinum-sensitive disease in terms of response rate and PFS [34].

The combination of gefitinib with chemotherapy was based on the fact that EGFR is highly expressed in ovarian cancer cells. However, the presence of activating mutations in the tyrosine kinase domain, such as those reported in non-small cell lung cells [10, 11], is very rare [15, 17]. Nevertheless, preclinical data have shown that gefitinib is able to inhibit *in vitro* the growth of ovarian tumor cells that do not harbor any EGFR mutation. Giardelo *et al.* [12, 13] reported that gefitinib can enhance *in vitro* the cytotoxic effect of different cytotoxic agents in EGFR non-mutated ovarian cancer cells lines. Similarly, Knight *et al.* [35] also reported an additive effect of gefitinib and cytotoxic drugs in an ovarian cancer model.

Despite the encouraging preclinical data, gefitinib failed to demonstrate any substantial clinical activity in combination with tamoxifen in patients with recurrent ovarian cancer [16, 17]. Furthermore, the activity of single agent gefitinib in pretreated patients with advanced ovarian cancer has also been investigated by a GOG study which reported an objective response rate of 9% (one in eleven patients) in patients with tumors expressing EGFR; however, in that particular study, the expression of EGFR was associated with longer progression-free survival in patients treated with gefitinib [36]. On the contrary, the combination of paclitaxel/carboplatin/gefitinib, as second-line treatment in patients with ovarian cancer revealed an objective response rate of 35% in patients with platinum-resistant/refractory tumors and 73% in patients with platinum-sensitive disease [15]; in that trial no EGFR mutations were detected in the 20 ovarian tumor samples analyzed. In the present study, the vinorelbine/oxaliplatin/gefitinib regimen was given to an un-

selected patient population since the expression of EGFR on tumor cells was not required in the inclusion criteria. It is still unclear whether there are other biomarkers, besides the activating mutations of the EGFR gene already reported for non-small cell lung cancer, which could define a subgroup of patients with different solid tumors conferring sensitivity to oral tyrosine kinase inhibitors (TKIs).

The vinorelbine/oxaliplatin/gefitinib regimen was associated with substantial toxicity, since almost 48% of patients developed grade III and IV neutropenia requiring G-CSF support. Moreover, 10% of patients developed febrile neutropenia requiring hospitalization and 12 more patients required hospitalization for other reasons. This toxicity profile is overtly worse than that observed in a dose-finding study conducted by our group evaluating the combination of vinorelbine and oxaliplatin in patients with various advanced solid tumors [29]. Despite the dose reduction for vinorelbine and oxaliplatin implemented as a result of increased number of DLTs observed in the first 10 patients, the combination's toxicity profile remained substantial. This increased toxicity of the vinorelbine/oxaliplatin/gefitinib regimen should be attributed to the addition of gefitinib to the chemotherapy combination. Nevertheless, we cannot rule out the possibility that heavily pre-treated patients with advanced ovarian cancer may present a patient population with worse tolerance to the vinorelbine/oxaliplatin regimen than patients with other types of tumors.

In conclusion, the results of the present phase I-II trial indicate that the combination of vinorelbine/oxaliplatin/gefitinib is an active regimen against AOC, irrespectively of the platinum sensitivity status. The relative contribution of gefitinib to the activity of the regimen cannot be assessed by the present study. However, the toxicity profile of the combination is of concern and needs special attention. In the absence of a compelling biological rationale supporting the use of gefitinib in ovarian tumors and given the lack of a clinically relevant biomarker, the combination should not be administrated outside the context of a clinical trial. Elucidation of these questions requires additional studies both in the pre-clinical and clinical settings.

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Hypoxia Inducible Factor 1 as a target for cancer therapy

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ABSTRACT

Background: Hypoxia Inducible Factor 1 (HIF-1) is a transcription factor that controls the response of mammalian cells to oxygen deprivation. HIF-1 has been implicated in a variety of pathophysiological conditions, including development, inflammation and cancer. In the latter, constantly increasing data legitimize HIF-1 as a crucial player in cancer development and progression, and subsequently as a potential target for the development of novel treatments.

Patients & Methods: A comprehensive overview of all articles published from 1997 to date in three medical databases (PUBMED, SCOPUS and COCHRANE) was performed using the keywords "Hypoxia Inducible Factor 1" and "cancer".

Results: The influence of genetic alterations, frequently detected in human cancers, on HIF-1 α expression and function, as well as the expression of HIF-1 α in multiple cellular components that are present within the tumor microenvironment, including stromal infiltrating cells and endothelial cells, render the HIF-1 related pathway an appealing target for therapeutic intervention. We present evidence for HIF-1 implication in human cancer formation and progression, and provide preclinical data for the efficacy of the main pharmacological agents with HIF-1 inhibition properties. The main biological and translational endpoints for HIF-1 inhibition are also presented and the perspectives for early clinical development of HIF-1 inhibitors are discussed.

Conclusion: The regulation of HIF-1 subunit alpha (HIF-1 α) by hypoxia, a common feature among solid tumors, may profoundly affect tumor biology, response to treatment and patients prognosis.

Key words: Hypoxia Inducible Factor 1; solid tumors; molecular targeted agents.

INTRODUCTION

Hypoxia Inducible Factor 1 (HIF-1) is a transcription factor that controls the response of mammalian cells to oxygen deprivation. HIF-1 has been implicated in a variety of pathophysiological conditions, including development, inflammation and cancer. Thus, its role as mediator of fundamental biological processes and the potential modulation of its activity for therapeutic purposes has attracted considerable interest [1, 2]. Several aspects of the involvement of HIF-1 in human cancer should be emphasized: i) The regulation of HIF-1 α by hypoxia, a common feature of solid tumors known to profoundly affect tumor biology, response to treatment and patients prognosis; ii) The influence of genetic alterations, e.g. of p53, frequently detected in human cancers, on HIF-1 α expression and function; iii) The induction of HIF-1 α accumu-

lation by RTK signaling pathways frequently dysregulated in human cancers; and iv) The expression of HIF-1 in multiple cellular components that are present in the tumor microenvironment, including stromal infiltrating cells and endothelial cells.

These features legitimize HIF-1 as a crucial player in cancer development and progression and as a potential target for the development of novel treatments. Indeed, the interest in HIF-1 is documented by the exponentially increasing number of papers published on this topic over the last decade and by the growing number of academic groups and pharmaceutical industries actively engaged in the identification of novel strategies aimed at inhibiting HIF-1 in human cancer. However, many questions still remain unanswered regarding the distinct role of HIF in different tumor types and the best way to achieve HIF inhibition in cancer patients. It can be anti-

ated that over the next few years more inhibitors will be identified and will approach the preclinical and clinical arena for further testing. A rational plan to validate HIF-1 inhibitors in preclinical models and test them in early clinical trials is warranted, so that this exciting and promising domain for cancer therapy may yield positive results.

1. HIF-1 AS A TARGET FOR CANCER THERAPY

1.1. Regulation of HIF-1 expression

HIF-1 is a basic helix-loop-helix transcription factor composed of two subunits, α and β . The β (beta) subunit, also known as aryl hydrocarbon receptor nuclear translocator (ARNT), is constitutively expressed in an oxygen independent fashion and is also involved in other transcriptional pathways, e.g., by dimerizing with the dioxin receptor, AhR (reviewed in [3, 4]). In contrast, the α (alpha) subunit, of which two, HIF-1 α and HIF-2 α , are best characterized, is rapidly degraded under normoxic conditions but accumulates under low oxygen levels. The mechanism by which the α subunit is degraded has been elegantly elucidated over the past few years. A family of enzymes, called PHDs, mediates hydroxylation of two proline residues of HIF- α in a reaction that requires O_2 , Fe^{++} and 2-oxyglutarate. Upon hydroxylation, the α subunit is recognized by the tumor suppressor gene Von-Hippel Lindau (pVHL) product, which functions as the recognition component of an E3 ligase that mediates ubiquitylation and subsequent proteasomal degradation of HIF- α (Figure 1). As mentioned above, pVHL mutations, which are frequently detected in patients with clear cell renal carcinoma, cause an accumulation of HIF- α under normoxic conditions due to degradation impairment. However, an increasing number of genetic alterations frequently implicated in human cancers have been associated with HIF- α dysregulation. In addition to gain-of-function mutations, such as v-src and ras, loss-of-function alterations, including PTEN, p53, TSC, succinate dehydrogenase, fumarate hydratase and PML, have been implicated in the accumulation of HIF- α under non-hypoxic conditions [3]. In addition, growth factor-dependent signaling pathways frequently dysregulated in human cancers, including EGF, IGF, and Her2/Neu, also have been implicated in the induction of HIF-1 α under normoxic conditions by activation of the PI3K/AKT/mTOR and MAPK pathways, further emphasizing the complexity of HIF-1 α regulation and its involvement in fundamental processes of cancer progression (Figure 1).

1.2. HIF-1 and gene expression

The list of genes and functions controlled by HIF is constantly expanding. The impact that HIF may have on human cancer is highlighted by the function of genes that are controlled by HIF and profoundly affect the behavior of cancer cells. HIF-inducible genes control tumor metabolism; angiogenesis; cell survival; migration/ invasion, all of which are hallmarks of cancer progression [5]. HIF plays a crucial role in the

induction of angiogenesis, a feature with significant therapeutic implications for HIF inhibitors. VEGF, the best characterized angiogenic factor, is transcriptionally induced by HIF via an HRE present in its promoter, although hypoxia may also control VEGF mRNA stability and/or its translation and HIF-independent pathways have also been identified [6]. A critical pathway controlled by HIF is aerobic glycolysis, a key feature of cancer cells, which have high level of glycolysis even in the presence of oxygen. Indeed, HIF-1 induces a coordinate up-regulation of genes involved in glucose metabolism and glycolysis. Finally, over the past few years, a critical role of HIF in the control of cell migration and invasion has also been elucidated, by the induction of genes such as CXCR4 [7] and lysyl oxidase [8], implicated to a different extent in invasion and metastasis. Each of these genes could represent a viable therapeutic target as well as be affected by HIF-1 targeting strategies.

1.3. HIF-1 expression in human cancer

A number of experimental models have confirmed that HIF-1 plays a critical role in tumor formation. However, controversial evidence has also been generated depending on the tumor model used, which has led to some early skepticism as to whether HIF is a good target for cancer therapy [9, 10]. HIF is over-expressed in a variety of human cancers and its expression is associated with poor prognosis and poor response to treatment [9, 11]. The pattern of HIF-1 staining that is detected in tumor tissue highlights the involvement of different pathways of HIF activation in cancer patients. Indeed, expression of HIF can be detected in perinecrotic areas of hypoxia, where the role of oxygen in its regulation is predominant. However, HIF has also been detected in well-oxygenated areas, consistent with its regulation by growth factors and genetic alterations, as well as in stromal infiltrating cells, which raises the question of the contribution of this cellular component to tumor growth and response to therapy.

2. HIF INHIBITORS: MECHANISMS OF ACTION (Figure 1)

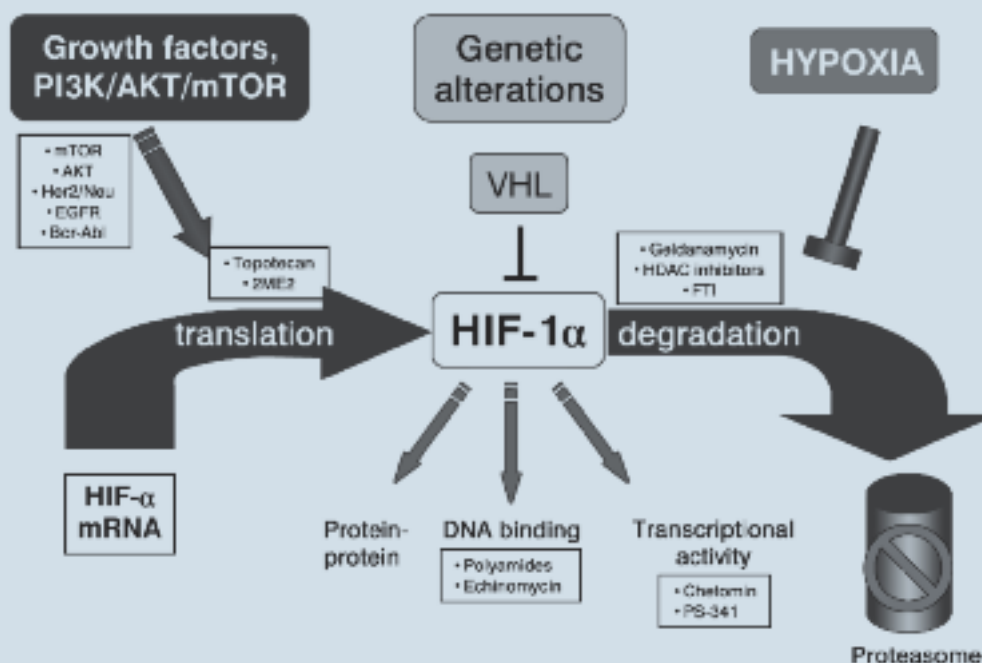
The majority of HIF-1 inhibitors identified thus far can be classified as "non-selective", as they target signaling molecules or pathways that affect multiple cellular functions [9, 10, 12]. In particular, emphasis will be placed on compounds that are relevant to the clinical setting, either because they are in clinical development or because they target pathways for which inhibitors are available.

2.1. Inhibitors of signaling pathways

Consistent with the redundant involvement of HIF-1 in multiple RTK-mediated signaling pathways that are dysregulated in human cancers, several novel inhibitors that have approached the preclinical and clinical arena also have the potential (or have indeed been shown) to inhibit HIF-1 or HIF-dependent functions. This finding raises the question as

Figure 1.

Potential mechanisms of action for HIF-1 inhibitors (see text for more details)



Abbreviations: mTOR: mammalian target of rapamycin; EGFR: Epidermal growth factor receptor; HIF: Hypoxia-inducible factor; VEGF: Vascular endothelial growth factor; 2ME2: 2-methoxyestradiol; VHL: von-hippel Lindau; HDAC: Histone deacetylase

to how, if at all, RTK inhibitors (RTKI) can be used in the clinic as HIF-1 inhibitors. There are at least two implications of HIF-1 inhibition by RTKI: The first is that inhibition of HIF-1 may play a role in the response of patients to therapy with RTKI. This possibility is supported by findings indicating that HIF-1 is downstream of a number of signaling pathways targeted by RTKI and in a cell-type specific fashion HIF-1 may be a critical mediator of these dysregulated pathways. The second implication is that inhibition of HIF-1 may become a valuable biomarker of RTKI activity, which can be validated in relevant preclinical models to be, in turn, incorporated in early clinical trials.

mTOR pathway inhibitors

The mTOR pathway has been implicated in the growth factor-dependent induction of HIF-1 α translation [13], as well as in HIF-1 β degradation [14]. It is then conceivable that mTOR inhibitors currently in clinical development might inhibit HIF-1 and impact on downstream pathways including angiogenesis. Indeed, evidence has been provided that CCI-779, a novel mTOR inhibitor in clinical development, inhibited hypoxic dependent induction of HIF-1 and vascular endothelial growth factor (VEGF) production [15] and

rhabdomyosarcoma xenograft growth by an antiangiogenic mechanism dependent on mTOR/HIF-1 α /VEGF signaling [16]. Importantly, in a mouse model of AKT1-dependent prostate intraepithelial neoplasia, HIF-1 α targets, including genes encoding most glycolytic enzymes, constituted the dominant transcriptional response to AKT activation and mTOR inhibition [17] and loss of the Von Hippel-Lindau tumor suppressor gene (VHL), sensitized kidney cancer cells to the mTOR inhibitor CCI-779 *in vitro* and in mouse models [18]. Thus, HIF-1 α might be a determinant of response in cancers in which the mTOR pathway is dysregulated and may also represent an attractive biomarker that could facilitate preclinical and early clinical development of mTOR inhibitors.

EGFR inhibitors

HIF-1 α is induced upon stimulation of the epidermal growth factor receptor pathway [13]. Accordingly, EGFR tyrosine kinase inhibitors, including Erlotinib and Gefitinib currently used in the clinic, inhibit VEGF expression by both HIF-1-dependent and -independent mechanisms [19], which may also have implications for the induction of apoptosis by these agents [20]. Cetuximab, a monoclonal antibody targeting the

epidermal growth factor receptor, also inhibits HIF-1 α levels in A431 epidermoid carcinoma cells [21].

AKT inhibitors

AKT is a critical signaling molecule, mediating RTK-dependent pathways, that may ultimately affect HIF-1 activity. Thus, AKT has been implicated in the mechanism of action of many small molecule inhibitors of HIF-1 described in the literature [22-26]. Little evidence has been provided so far that this is a feasible approach in preclinical models, but obviously AKT is an attractive target for cancer therapy and HIF-1 may represent one of many downstream targets affected by AKT inhibition.

Other signaling pathways that are frequently dysregulated in human cancers have been implicated in HIF-1 α regulation and inhibitors of these pathways may potentially block HIF-1 α accumulation. In particular, evidence has been provided that the Her2/Neu [27]; c-KIT [28; and BCR/ABL [29] pathways are implicated in the induction of HIF-1 α and VEGF expression in breast cancer; lung cancer; and leukemic cell lines, respectively.

2.2. Inhibitors of protein accumulation

Inhibition of HIF-1 α by the agents described in this section has been associated with biochemical inhibition of targets that are known to be affected by these compounds.

Topoisomerase I inhibitors

Several camptothecin analogs have been identified as HIF-1 inhibitors, and HIF-1 inhibition seems to be a common property for this class of compounds [30]. Topotecan inhibits HIF-1 α translation by a mechanism independent from DNA replication-dependent DNA damage and proteasome function [31]. Importantly, daily administration of topotecan inhibited HIF-1 α expression in xenograft experiments, which was associated with inhibition of angiogenesis and tumor growth [32]. These results have led to the implementation of a clinical trial, currently ongoing at the NCI (<http://www.clinicaltrials.gov/ct/show/NCT00182676>), in which the effect of topotecan on HIF-1 α expression in tumor tissue is being evaluated in patients with metastatic cancers. This pilot study will provide useful information regarding the potential to inhibit HIF-1 α expression in tumor tissue using classic cytotoxic agents.

Microtubule poisons

2-methoxyestradiol (2ME2), a novel antitumor and antiangiogenic agent, currently in early clinical development, was found to inhibit tumor growth and angiogenesis at concentrations that efficiently disrupt tumor cell microtubules (MTs) *in vivo* [33]. In addition, 2ME2 downregulated HIF-1 α by inhibiting its translation and blocked HIF-1-induced

transcriptional activation of VEGF expression. 2ME2/tubulin interaction was required for HIF-1 α downregulation. Interestingly, early clinical trials of this compound have shown that 2ME2 is not associated with common toxicities observed with other microtubule-targeting agents, thus inhibition of HIF-1 and angiogenesis may be an important mechanism contributing to its biological activity.

Hsp90 inhibitors

The benzoquinone ansamycin geldanamycin, an Hsp90-specific inhibitor, was found to inhibit HIF-1 α protein accumulation by a mechanism involving its degradation in a proteasome-dependent, yet VHL-independent fashion [34, 35]. HIF-1 α is one of many Hsp90 client proteins and it is unclear to what extent inhibition of Hsp90 may be associated with downregulation of HIF-1-target functions. However, geldanamycin analogs, including 17-AAG and 17-DMAG, are currently in clinical trials for cancer therapy and HIF-1 inhibition may be potentially contributing to the therapeutic activity and/or may represent a valuable biomarker of activity of these compounds. Hsp90 has also been implicated in the mechanism of action of other HIF-1 inhibitors, including radicicol [36] and the SCH66336 farnesyltransferase inhibitor [37].

Histone deacetylase (HDAC) inhibitors

Several mechanisms of action have been suggested for the activity of HDAC inhibitors, a class of compounds currently in early clinical development, on HIF-1 α degradation. Specifically, HDAC inhibitors may induce the proteasomal degradation of HIF-1 α by a mechanism that is independent of VHL and is secondary to a disruption of the HSP70/HSP90 axis function [38]. Alternatively, it has been shown that class II HDAC4 and HDAC6 were associated with HIF-1 α protein and may directly affect its degradation [39]. These results suggest that inhibitors of class II HDAC might be used to target HIF-1 α in human cancers. Whether or not HIF-1 α inhibition contributes to the therapeutic activity observed with administration of HDAC inhibitors remains to be determined.

2.3. Inhibitors of DNA binding

An attractive strategy for the inhibition of transcription factors is blocking the DNA binding within specific recognition sequences. Pioneer work in this area has been conducted by a scientific group that has designed synthetic polyamides that can specifically target consensus sequences recognized by thymidylate synthase. Indeed, a synthetic polyamide that specifically inhibits HIF-1 DNA binding has been designed and found to inhibit, as postulated, HIF-1 transcriptional activity [40]. A limitation of polyamides as therapeutic reagents may be their poor cellular permeability and diffusion in tumor tissue, although they offer significant advantages due to their high target specificity.

Echinomycin, a small molecule that binds DNA in a sequence-specific fashion, has been identified in a cell-free screen aimed to identify small molecule inhibitors of HIF-1 DNA binding. Echinomycin inhibited HIF DNA binding but not the binding of AP1 or NF- κ B to cognate DNA binding sites, suggesting a relative degree of sequence specificity [41]. Since the HRE binding site may also overlap with an E-box sequence (CACGTG), echinomycin was also found to inhibit binding of Myc to the E-box, a feature that might have potential therapeutic implications [41].

2.4. Inhibitors of HIF-1 transcriptional activity

The transcriptional activity of HIF-1 is mediated by two domains, N-TAD and C-TAD. The C-TAD binds to CBP/p300 for maximal transcriptional activity and is modulated by posttranslational modifications, including hydroxylation of Asn 803. Chetomin, a small molecule that inhibits HIF-1 binding to CBP was identified in a screen aimed to identify inhibitors of HIF-1 transcriptional activity [42]. This molecule was found to be active in *in vitro* and *in vivo* models, which provided proof of principle that HIF inhibition is a viable therapeutic strategy. However, the clinical development of chetomin for cancer therapy appears to be hampered by poor pharmacological properties [42].

Interestingly, recent evidence has been provided that inhibition of the proteasome function, which blocks HIF-1 α protein degradation, is also associated with inhibition of HIF-1 transcriptional activity by a mechanism that involves the TAD domain of HIF-1 [43]. Bortezomib, an inhibitor of proteasome function, which is approved for the treatment of multiple myeloma, is currently being tested in several tumor types and it would be interesting to see whether HIF-1 inhibition may be part of its therapeutic activity in tumors over-expressing HIF-1 α .

2.5. Miscellaneous

HIF-1 inhibitors are constantly discovered and reported in the literature. However, in many cases a clear mechanism of action of HIF-1 inhibition has neither been reported nor identified. PX-478 is a potent antitumor agent found to be active in many xenograft models [44]. PX-478 inhibits HIF-1 α protein accumulation by an unknown mode of action. Interestingly, its activity in tumor xenografts appears to be associated with HIF-1 α levels [44]. Inhibition of HIF-1 α protein expression following treatment with PX-478 has been demonstrated in animal models and this agent will soon be tested in clinical trials as HIF-1 inhibitor in solid tumors [44].

YC-1 is a cyclic GMP inhibitor, known for its anti-platelet and vasodilatory effects. YC-1 inhibits HIF-1 α protein accumulation by a mechanism that appears to be independent from the inhibition of cGMP [45]. YC-1 was active in animal models and inhibited HIF-1 α expression in tumor tissue, thus it may soon be tested as anticancer agent.

Many different agents have been implicated in HIF-1 inhibition. Among these, thioredoxin inhibitors were originally found to inhibit HIF-1 protein accumulation [46], although recent evidence indicates that these agents may also inhibit transcriptional activity [47]. Curcumin, a component of the yellow spice turmeric, inhibits HIF-1 α protein accumulation by several mechanisms including degradation of HIF-1 α , which may be potentially associated with inhibition of HIF-1 activity [48].

2.6. Gene therapy

Genetic approaches to target HIF-1 α expression and function are an attractive strategy to inhibit HIF-1 in human cancers and have been tested in animal models with promising results. Specifically, expression of therapeutic genes under the control of adenoviruses engineered to be expressed under hypoxic conditions, anti-sense and siRNA approaches have all been tested and found to be somewhat active in different tumor models [49-54]. Although targeting the hypoxic tumor is an appealing therapeutic strategy, the issue of delivery is still largely unresolved and currently hampers the potential application of this approach.

2.7. Natural products

Many natural products have been identified and found to inhibit HIF-1 protein expression and function [55]. In most instances, the exact mechanism of action of these compounds has not been elucidated, the activity has only been shown in cell culture and not been confirmed *in vivo*. Natural products may have novel and interesting mechanisms of action in inhibiting HIF-1. Further studies will be required to determine whether any of the agents identified thus far has the potential to be used as therapeutic agent for cancer therapy.

3. PRECLINICAL DEVELOPMENT AND TRANSLATIONAL ENDPOINTS

The development of molecular targeted agents requires a rationally designed plan so as to validate the activity on the intended target, as well as to implicate this effect in a meaningful therapeutic activity. Unlike cytotoxic agents, whose development has been largely based on efficacy studies in multiple xenograft models, the development of molecular targeted agents requires preclinical models tailored to the specific agent under investigation.

Several approaches have been described to validate the activity of HIF-1 inhibitors. Human cancer cell lines engineered to express the luciferase reporter gene under control of hypoxia response elements have been established [32]. These cell lines have been used in xenograft and orthotopic models to monitor the activity of HIF-1 inhibitors on their target. The advantage of this approach is that luciferase can be measured in a non-invasive fashion giving the opportunity to serially monitor the effect of an agent on a functional

Table 1.

Main categories of agents with HIF-1 inhibition properties

Category	Agent	Mechanism of action	Phase of development	References
mTOR inhibitors	CCI-779	Inhibition of mTOR/HIF-1 α /VEGF signaling	II-IV	[15-18]
EGFR inhibitors	Gefitinib Erlotinib Cetuximab	HIF-1 α -dependent VEGF inhibition	II-IV	[13], [19-21]
Akt inhibitors	RO112267	RTK-dependent inhibition of HIF-1 α	II	[22-26]
Topoisomerase I inhibitors	Topotecan	Inhibition of angiogenesis	II-IV	[30-32]
Microtubule inhibitors	2ME2	Inhibition of HIF-1 α transcriptional activity	I-II	[33]
HSP90 inhibitors	Geldanamycin 17-AAG 17-DMAG	VHL-independent but proteasome- dependent HIF-1 degradation	II-III	[34-37]
HDAC inhibitors	HDAC4i HDAC6i	VHL-independent but proteasome- dependent HIF-1 degradation. Disruption of HSP90/HSP60 axis	II-III	[38-39]
Proteasome inhibitors	Chetomin Bortezomib	Inhibition of HIF-1 transcriptional activity	II-IV	[42-43]
Inhibitors of DNA binding	Synthetic polyamides Echinomycin	Inhibition of HIF-1 transcriptional activity	I-II	[40-41]
Natural products	Miscellaneous	Unknown	I	[55]
Miscellaneous	PX-478 YC-1 Thioredoxin inhibitors Curcumin	Unknown cGMP inhibition Inhibition of HIF-1 protein accumulation HIF-1 α degradation	Preclinical Preclinical I I	[44] [45] [46-47] [48]
Gene therapy	Adenovirus vectors engineered to be expressed under hypoxic conditions	Anti-sense or siRNA approaches	I-II	[49-54]

Abbreviations: mTOR: mammalian target of rapamycin; EGFR: Epidermal growth factor receptor; HIF: Hypoxia-inducible factor; VEGF: Vascular endothelial growth factor; 2ME2: 2-methoxyestradiol; HSP: Heat-shock protein; VHL: von-hippel Lindau; HDAC: Histone deacetylase; cGMP: cyclic guanine monophosphate; siRNA: RNA silencing

basis. More elegant models based on non-invasive imaging of reporter genes may be anticipated in the future, and they should provide a valuable tool for validating the activity of HIF- inhibitors [50, 52, 54].

Since inhibition of HIF-1 may be associated with inhibition of angiogenesis and tumor metabolism, tissue endpoints reflecting these activities have been developed to monitor the effect of HIF inhibitors. Evaluation of tissue endpoints documenting the functional inhibition of the HIF pathway is

essential to validate the activity of HIF inhibitors and to better understand how to use these agents in the clinic. Tissue endpoints can easily be measured in animal models and careful analysis of these endpoints should be encouraged and warranted for the development of HIF inhibitors. Analysis of HIF-1 α protein levels by immunohistochemistry (IHC) or Western Blott; mRNA expression of HIF-1 target genes by real-time PCR and surrogate markers such as MVD or CAIX, have all been proposed and applied [32,44,45]. A potential

limitation of translating these pharmacodynamic endpoints to the clinic is that fresh-frozen tissue must be acquired from patients, which is not always feasible or applicable.

An alternative approach that could overcome these limitations and find broader application is the use of imaging techniques assessing functional inhibition of HIF-1. Two main strategies have been used so far: 18FDG-PET, which provides an indication of tumor metabolism, and DCE-MRI, which reflects blood flow and angiogenesis [56-58]. The rationale for using tumor metabolism as a readout of HIF-1 activity is based on the coordinate transcriptional regulation of glycolytic enzymes by HIF-1, which is consistent with the possibility that inhibition of HIF be associated with a decrease of PET signal [56]. The application of DCE-MRI relies on the assumption that inhibition of HIF may be associated with meaningful inhibition of angiogenesis [58]. Again, this is largely supported by the direct induction of a number of angiogenic factors by HIF, including but not limited to VEGF. However, these techniques are not widely available for application in preclinical models and further studies are required to fully elucidate the association between inhibition of HIF and functional results.

4. EARLY CLINICAL DEVELOPMENT: PERSPECTIVES

Many agents have been shown to inhibit HIF-1 α protein expression and function in cell culture models. Several of the agents identified have also been shown to inhibit HIF-1 in animal models, which has been in turn associated with antitumor and antiangiogenic activities. However, many questions remain to be answered as to the potential application of these results to the clinical setting. As discussed in the previous paragraph, validation of these agents in

relevant preclinical models is essential for further clinical development. As therapeutic efficacy cannot be used as a reliable readout of HIF inhibition, more appropriate translational endpoints should be defined and used in both preclinical and early clinical trials in order to validate the activity of HIF-1 inhibitors. Indeed, early clinical trials of these molecules should emphasize the activity of the investigational agent on HIF-1 expression and/or function according to the known or proposed mechanism of action of the compound. Imaging techniques should be also developed to measure inhibition of HIF-1 or HIF-1-target functions, including but not limited to angiogenesis and tumor metabolism. Studies on tumor tissue are warranted to validate the activity of HIF-1 inhibitors on meaningful biological endpoints that may be then correlated with clinical benefit.

Although the use of HIF-1 inhibitors in the clinical setting is still in its early phase of development, a strong scientific rationale has been provided for testing these agents in clinical trials aimed to validate the activity on HIF-1 and HIF-1-target functions. However, it is plausible that HIF-1 inhibitors may have limited activity when used as single agents [59]. Importantly, evidence has been provided that HIF-1 may contribute to resistance to chemotherapy [60] and radiation therapy [61, 62], further suggesting that HIF-1 inhibitors may find a valuable application in combination with currently available therapeutic strategies. Indeed, combination therapies should be tested in preclinical models and rapidly translated to relevant clinical models. The rational development of combination strategies with conventional therapeutic approaches, i.e., chemotherapy and radiation therapy, as well as with novel molecular targeted therapies is warranted to fully exploit the potential of this novel and exciting area of developmental therapeutics.

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Side-effects of targeted treatments for metastatic renal cell cancer

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ABSTRACT

With advances in surgical approach and the wide variety of systemic treatment options available for metastatic renal cell carcinoma (mRCC), the management of patients in clinical practice is changing. The ability to control drug-related toxicity is an essential component of cancer care. Targeted therapies are associated with specific adverse events, although they are well-tolerated considering the benefits they provide. Most adverse events can be managed without pharmacological measures. Most of these events should be controlled before they advance to grade 3 severity. For example, tyrosine kinase inhibitors (TKIs), such as sunitinib and sorafenib which target the VEGF pathway, can be associated with cardiac toxicity, whereas the mammalian target of rapamycin (mTOR) inhibitors everolimus and temsirolimus can be associated with pneumonitis. Clinical trials demonstrating the efficacy and tolerability of therapy in different patient groups provide valuable data on overall treatment tolerability as well as toxicities in these patients. In particular, assessing the severity of adverse events as well as treatment discontinuation and dose reduction, large trials demonstrate the tolerability of an agent in every day clinical practice.

Key words: side-effects; targeted treatment; renal cell cancer; TKIs; VEGF; mTOR.

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 3% of adult malignancies, with a male to female ratio of 2:1 [1]. It is characterized by a lack of early warning signs, diverse clinical manifestations, and resistance to radiation and chemotherapy. Increasingly, renal cell cancers are diagnosed at an earlier stage, and nephron-sparing surgery and thermal ablation are gaining acceptance as a treatment of choice for smaller tumors [1, 2]. Renal cell carcinoma (RCC) is most commonly seen between the ages of 50 and 70 years. During the early 1990s, studies demonstrated that the Von Hippel-Lindau (VHL) tumor suppressor gene was inactivated in a majority of patients with sporadic clear cell renal carcinomas through mutation or methylation [3]. Functional VHL encodes a protein that targets hypoxia inducible factor (HIF) for degradation [3]. Inactivation of VHL leads to upregulation of HIF-1H, and as a result, it leads to transcription of multiple genes involved in tumorigenesis, including vascular endothelial growth factor (VEGF); platelet-derived growth factor (PDGF); and fibroblast growth factor (FGF) [3]. Subsequent studies demonstrated

that the mTOR protein (mammalian target of rapamycin) also plays an important role in promoting the translation of HIF-1H mRNA [4]. Based on this work, several novel targets emerged for anticancer therapy in renal carcinoma. Patients with localized tumors can usually be cured surgically. However, one-third of patients present with metastatic disease and another 30% to 40% will eventually develop distant metastases. These patients have a median survival of 12 months, with 5% to 10% having a five-year survival [5]. This may be changing because of earlier diagnosis and novel therapies that have reached the clinic [5]. Radical nephrectomy is the standard for larger and central tumors. Recent clinical trials have established the role of targeted therapy as the first line of therapy in patients with metastatic disease [6-8]. While the optimal treatment strategy continues to evolve, four agents that target angiogenesis (sunitinib, bevacizumab, sorafenib and pazopanib) and mTOR-targeted therapy (everolimus, temsirolimus) have been approved as front or second-line agents. These drugs, designed to target specific pathways involved in mRCC pathogenesis, have largely replaced cytokines (immunotherapy)

in treatment-naïve patients and have the potential to revolutionize mRCC treatment. Current clinical trials are testing newer agents, combinations of approved agents, and the optimal sequencing thereof. In addition to these approved agents, a number of new tyrosine kinase inhibitors (TKIs) are emerging, as well as a number of therapies designed for novel targets such as mesenchymal-epithelial transition factor (c-met) and tumor necrosis factor alpha (TNF- α) [9-11].

On the other hand, targeted therapies have distinct mechanisms of action and consequently exhibit distinct patterns of specific toxicities. However, these agents are generally well-tolerated, given the benefits they provide and toxicities can be managed in most cases with medical treatment or supportive measures. It has been suggested that certain adverse events (AEs) could have a greater impact in specific patient subgroups, such as the elderly and/or patients with comorbidities, and that these patients may require further support. Early intervention and prophylaxis are the keys to successful toxicity management. AEs should be managed or treatment withdrawn, before toxicities advance to grade 3; this will also help minimize the length of any dose interruption.

CUTANEOUS ADVERSE EVENTS

The most important side-effect is hand-foot syndrome (HFS) because hand-foot skin reactions can have a negative impact on patients' daily activities and quality of life (QoL). This situation includes symptoms like numbness, tingling, burning, redness, erythema, swelling, moist desquamation, ulceration, blistering and severe pain of hands and/or feet [12]. All patients do not react the same way; severity level classification is as follows: grade 1 – minimal skin changes or dermatitis (e.g. erythema) without pain; grade 2 – skin changes (e.g. peeling, blisters, bleeding, edema) or pain, not interfering with function; and grade 3 – ulcerative dermatitis or skin changes with pain, interfering with function. If reported early enough, HFS is easily reversible [12].

Moreover, it is well established that there are differences between tyrosine kinase inhibitors (TKIs) HFS and classical HFS. TKIs-related HFS manifests with hyperkeratotic plaques (callus-like); peripheral erythema (often non-tender) of the lateral-flexor surface digits; and the histology type

involves thickened stratum corneum; abnormal sweat glands; epidermal hyperplasia; and few granulocytes [13]. On the other hand, classical HFS includes erythema, tenderness, paresthesia, edema, advanced ulceration and desquamation of the fat pads, digits; and the histology type involves mild spongiosis, necrotic keratinocytes and dilated dermal vasculature. Table 1 summarizes the major differences of HFS manifestation of the two groups.

Management of HFS

General considerations should be described in all patients receiving therapy. First line directions must include decreasing pressure on skin, soft shoes, stable body temperature, keeping the extremities moist, use of urea-based creams and petroleum-lanolin ointments, as well as the use of aloe vera, and finally prescribing anti-inflammatory drugs and pyridoxine 100 mg are some solutions so as to avoid drug interruption [14]. For grade 1 (pain, changes), emollients are usually prescribed; for grade 2 (redness, edema, pain), the choice is between dose interruption (less advisable) and dose reduction in case of no improvement and/or multiple episodes; for grade 3 (painful, ulcers, ADLs), dose interruption is recommended, dose reduction or discontinuation after 2 episodes [14].

GI ADVERSE EVENTS

Stomatitis

Even low-grade oral mucositis can have a negative effect on everyday activities and patient QoL. The symptoms of this condition are firstly pain and burning sensation; feeding difficulties; sense of taste deterioration; communication-elocution problems; dehydration-sticky saliva secretions; halitosis; a raw feeling in the throat; swollen buccal tissues; and superinfection, which can lead to septicemia (Candida) [15, 16]. According to NCI, the levels of severity are: grade 0 – No symptoms; grade 1 – sore mouth, no ulcers; grade 2 – sore mouth with ulcers, but able to eat normally; grade 3 – liquid diet only; grade 4 – unable to eat or drink. The only clinical management in this situation is patient education [15]. Physicians should warn patients of the possibility of developing

Table 1.

Major differences between classical Hand-Foot Syndrome (HFS) and HFS associated with TKIs

TKI HFS	Classical HFS
<ul style="list-style-type: none"> ■ Hyperkeratotic plaques, callus-like ■ Peripheral erythema, often non-tender ■ Lateral, flexor surface digits ■ Histology: thickened stratum corneum, abnormal sweat glands, epidermal hyperplasia, few granulocytes 	<ul style="list-style-type: none"> ■ Erythema, tenderness, paresthesia, edema ■ Advanced ulceration, desquamation ■ Fat pads, digits ■ Histology: mild spongiosis, necrotic keratinocytes, dilated dermal vasculature

mouth ulcers; stomatitis; and oral mucositis and encourage proper oral hygiene [16]. Non-alcoholic mouthwashes, such as saltwater, and the use of mild toothpaste are recommended. Patients also have to avoid agents containing hydrogen peroxide, alcohol, iodine, and thyme derivatives.

Diarrhea

With appropriate management, Grade 3 diarrhea does not occur. In case of diarrhea, patients should wait until the pulp is brown and could take as prevention 2 grated apples/day and loperamide 2 mg/ diphenoxylate after each unformed stool. On the other hand, if diarrhea is systematic, its management focuses on electrolytic disorders, avoidance of dairy products, empiric antibiotics such as fluoroquinolone, oral loperamide (4 mg), oral diphenoxylate/atropine 5 mg and, for hospitalized patients, iv fluids and octreotide 100µg SC tid. If diarrhea persists, drug dose delay and/or reduction could not be avoided.

Gastrointestinal perforation

Risk factors for gastrointestinal perforation include the following: peptic ulcer disease; tumor necrosis; GI obstruction; diverticulosis; colitis; abdominal carcinomatosis; prior abdominal or pelvic radiotherapy; prolonged (>1 month) NSAID use; recent endoscopy; and bevacizumab therapy. GI perforations have been reported in <1% of patients with NSCLC, in <2% of patients with colorectal cancer and in ~1% of patients with RCC [17]. The hypothesis is that VEGF inhibition on capillary beds of intestinal villi causes regression of normal blood vessels in the GI tract. In the case of diffuse abdominal carcinomatosis the increased pressure on weakened bowel areas creates microperforations. Also VEGF inhibition could reduce blood flow to the splanchnic vasculature by thrombosis or vasoconstriction and lead to bowel infarction and perforation [18].

HAEMATOLOGIC ADVERSE EVENTS

Neutropenia

Sunitinib and sorafenib were associated with a decreased risk of high-grade, an increased risk of all-grade and high-grade neutropenia, thrombocytopenia and anemia, and high-grade lymphopenia. Stratified analysis by the presence or not of concomitant chemotherapy demonstrated similar risks [19, 20]. The standard treatment of FN had been inpatient management with broad-spectrum i.v. antibiotics for all patients.

Thrombocytopenia

We have to mention that grade 3 or higher thrombocytopenia is rare. With sunitinib 50 mg dosing, thrombocytopenia always recovers in weeks off and at grade 3, dose reduction to 37.5 mg is indeed necessary. In a large expanded-access trial with respect to sunitinib 50 mg use in mRCC, the

incidence of thrombocytopenia was 8% with incidences of grade 3-4 adverse events similar across subgroups [21]. Temsirolimus rarely causes high grade thrombocytopenia (grade 3 or 4).

Bleeding

The mechanism of action of all anti-VEGF agents may contribute to bleeding. Inhibiting VEGF, the key mediator of angiogenesis, decreases the renewal capacity of the endothelial cell in response to trauma and causes endothelial dysfunction and defects in the internal vascular lining [22]. The most common adverse event is epistaxis. It should be mentioned, that patients with hemoptysis due to lung metastases should not be considered for first-line bevacizumab treatment. In a large meta-analysis study with respect to bleeding, the overall bleeding incidence and incidence of high-grade bleeding among patients receiving bevacizumab was 30.4% and 2.8% respectively [23, 24]. Higher relative risks were observed in patients with non-small-cell lung cancer [relative risk (RR) 3.41], renal cell carcinoma (RR 6.37), and colorectal cancer (RR 9.11), receiving bevacizumab at 5 mg/kg/week [23]. In patients with grade 3-4 bleeding, bevacizumab should be permanently discontinued. With respect to the role of aspirin, studies show that the concomitant use of bevacizumab with chemotherapy and aspirin did not substantially increase the risk of serious bleeding. Medical oncologists should be cautious in patients with bleeding diathesis, acquired coagulopathy and in patients receiving full-dose anticoagulation, before starting anti-angiogenesis therapy.

Thromboembolic events

Inhibition of VEGF-A prevents endothelial cell renewal, production of platelet inhibitors and promotes thrombogenic activity. Moreover, endothelium dysfunction can lead to compromised blood vessel integrity and exposure of subendothelial collagen [25]. This releases tissue factors and activates the coagulation cascade. Risk factors for thromboembolism are: cancer (especially ovarian, pancreatic, bone or brain); major surgery; indwelling venous catheter; advanced age; prolonged immobility; prior atherosclerotic events; bevacizumab; cardiac or respiratory failure; cytotoxic chemotherapy; estrogen therapy; diabetes; hypercholesterolemia; hypertension; and myocardial infarction. So, in patients at high risk for atherosclerotic events, primary prevention with aspirin (<325 mg/day) is recommended [25]. There is no evidence of an increased risk of bleeding with concomitant aspirin and bevacizumab. However, patients should be monitored carefully due to risk of hemorrhage with both agents.

Among the different placebo-controlled trials comparing the combined treatment of bevacizumab and chemotherapy vs. chemotherapy alone, several have demonstrated trends for increased risk of arterial thromboembolic complications [26-

28]. However, the event rate was relatively low and these trends did not reach statistical significance. Most of these trials excluded patients with history of stroke or myocardial infarction, unstable angina, serious cardiac arrhythmias and clinically significant congestive heart failure or peripheral vascular disease within 6-12 months [25]. Use of sunitinib and sorafenib has associated arterial thromboembolic events, although the overall risk remains unclear. A systematic review and meta-analysis focused to determine the incidence and relative risk (RR) associated with the use of sunitinib and sorafenib showed RR=3.03 compared to control patients [29]. The analysis was also stratified for the underlying malignancy (renal cell cancer vs. non-renal cell cancer) and TKI (sunitinib vs. sorafenib), but no significant differences in incidence or RR were observed [29].

WOUND-HEALING COMPLICATIONS

Angiogenesis is an essential process in wound healing. New blood vessels deliver oxygen and nutrients (and drugs) to the damaged endothelium. VEGF-A regulates normal and pathological angiogenesis. So inhibition of VEGF-A inhibits normal as well as pathological angiogenesis, potentially impairing wound healing. Impaired wound healing presents as wound dehiscence, bruising or bleeding. For this situation potential risk factors include: radiotherapy, anastomotic leaks, infection, tumor involvement at the surgery site, history of diabetes requiring medication and obesity. Compared with published reports, we observed less hemorrhagic and wound healing issues but a significant increase in incidence and

severity of intraoperative adhesions [30]. Potential reasons for lower complication rate could include increased time from TKI discontinuation to surgery; longer time to postoperative TKI re-initiation; increased use of preoperative angioembolization; and the lack of preoperative bevacizumab administration [30]. In the absence of current recommendations, it is advised to stop bevacizumab at least four to five weeks before a surgical intervention and to resume 4 weeks later [31]. For the tyrosine kinase inhibitors, treatment can be stopped 24-48 hours before surgery and resume 3-4 weeks later [30]. Finally, for the mTOR inhibitors, it is advisable to stop the treatment 7-10 days before and to resume at least 3 weeks later [31].

CARDIOVASCULAR SIDE-EFFECTS

Hypertension

Hypertension is one of the most frequent side-effects of systemic inhibition of vascular endothelial growth factor (VEGF) signaling. Its incidence and severity are dependent on the type of drugs, dose, and schedule used. Recognition of this side-effect is indeed an important issue, as poorly controlled hypertension could lead to serious cardiovascular events.

Inhibition of VEGF may lead to hypertension via different mechanisms such as nitric oxide; vascular stiffness; thyroid dysfunction; effect on vascular smooth muscle cell; and stimulation of neurohumoral pathways. Pre-existing hypertension should be properly controlled before starting bevacizumab treatment, but prophylactic antihypertensive therapy is not recommended. Generally, hypertension is

Table 2.

Hypertension with sunitinib and bevacizumab: most commonly observed at grades 2 or 3

Grade	Sunitinib	Bevacizumab
2	Antihypertensive (mono) therapy may be indicated. 18% continue sunitinib at the same dose level.	Temporarily discontinue bevacizumab until BP <160/100 mmHg or return to baseline. Administer antihypertensive therapy.
	Requiring more than one antihypertensive drug or more intense treatment. 12% continue sunitinib at the same dose level. Physicians tend to interrupt or discontinue treatment at grade 3 events, but this is not necessary if another antihypertensive therapy is added.	Generally hypertension is adequately controlled with oral antihypertensives (ACE inhibitors, diuretics, calcium channel blockers). ACE inhibitors preferred for patients with proteinuria.
4	Life-threatening consequences (hypertensive crisis). Medically significant/uncontrolled hypertension, hypertensive crisis or hypertensive encephalopathy. Permanently discontinue sunitinib.	Permanently discontinue bevacizumab.

BP: Blood pressure; ACE: Angiotensin converting enzyme

adequately controlled with oral antihypertensive agents (angiotensin conversion enzyme -ACE- inhibitors, diuretics, calcium channel blockers) [32-34]. The ACE inhibitors are preferred for patients with proteinuria. The optimal anti-hypertensive agent for managing bevacizumab-induced hypertension has not yet been established. Data varies on hypertension outcomes after discontinuing bevacizumab: There are two cases: 1) decreased blood pressure after discontinuation and 2) persisting hypertension for up to 6 months after discontinuation [32]. Data on hypertension by other anti-VEGF molecules has not been studied (sunitinib hypertension) [32]. Table 2 summarizes the hypertension treatment regime associated with sunitinib and bevacizumab.

Disruption of VEGF-VEGFR signaling in the heart

Inhibition of VEGF-VEGFR signaling in the heart is relevant to patients with poorly controlled hypertension. Disruption of VEGF-VEGFR signaling during imposition of pressure load leads to capillary density reduction and is associated with contractile dysfunction, fibrosis and heart failure. The risk factors for cardiovascular toxicity are: history of coronary arterial disease; coronary heart failure; hypertension; smoking; obesity; increased cholesterol; and triglyceride levels. The major cardiac side-effects are the following: reduction of left ventricular ejection fraction (EF drop ~10% with sunitinib, clinical CHF Gr 3 or 4: 2% with sunitinib, 1% with bevacizumab); conduction disturbances; ST-segment or T-wave changes; elevated cardiac serum markers (T_{pl}, CK-MB, BNP, pro-BNP); significant clinical symptoms (angina, dyspnea, dizziness); and myocardial infarction.

INFECTIONS

In this case, side-effects are mostly caused by everolimus. Everolimus has immunosuppressive properties and may predispose patients to infections, especially opportunistic infections. Localized and systemic infections, including pneumonia, other bacterial infections, and invasive fungal infections have been reported in 37% of patients treated with everolimus, but the incidence of grade 3/4 infections was low (10%) [35]. Infections can be severe (leading to respiratory failure) and occasionally fatal. Most frequent infections were nasopharyngitis; pneumonia; urinary tract infection; bronchitis; and sinusitis [35]. Patients with fungal infections should be comprehensively treated prior to initiation of everolimus therapy. If diagnosis of invasive systemic fungal infection is made during treatment with everolimus, permanently discontinue everolimus and treat with appropriate antifungal therapy.

Non-infectious pneumonitis

Non-infectious pneumonitis, a potentially serious adverse event associated with rapamycin and rapamycin derivative treatment, is also seen with everolimus. It comprises one of a number of typical radiographic appearances with or

without symptoms (dry cough, breathlessness, malaise) and signs (pleural effusion, crepitations, hypoxemia) in the absence of a non-drug-related cause [36]. The Phase II study showed grade 1 or 2 pneumonitis in 31% of patients, while in phase III studies grade III pneumonitis was reported in 19% [37]. None of the patients required steroids and pneumonitis was resolved with cessation of the drug [37].

In a large study of 242 patients receiving everolimus evaluating non-infectious pneumonitis, 13.5% of patients presented with this adverse reaction. Grade 3 pneumonitis was present in 3.6% (interfering with daily living or oxygen was indicated). No grade 4 (life-threatening) pneumonitis was observed [38]. Out of the 37 cases reported, 54% were reversible within the follow-up period; resolution followed dose reduction for 20 patients and treatment discontinuation in 10 patients [38]. Corticosteroid therapy was initiated in 16 cases.

Pneumonitis (interstitial-like lung disease) has been noted with temsirolimus and oral EGFR inhibitors (gefitinib, erlotinib). Its incidence is about 1% but can be fatal. Symptoms include: dyspnea, cough, low-grade fever. Risk factors are: tobacco history and pre-morbid pulmonary fibrosis. Non-infectious pneumonitis caused by everolimus typically occurs within 2 to 6 months of initiating therapy. A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory symptoms and in whom infectious, neoplastic and other non-medicinal causes have been excluded, by means of appropriate investigation [39]. The majority of cases were medically manageable and reversible with drug interruption and/or reduction; half received a brief course of steroids. But, for severe cases, everolimus should be discontinued and the use of corticosteroids, O₂, bronchodilators, antibiotics, may be indicated until clinical symptoms resolve [39].

PROTEINURIA

There is a relationship between VEGF molecule and proteinuria (urinary protein excretion of >300 mg/day). Inhibition of VEGF-A alters the integrity of the glomerular vascular endothelium in kidneys and also increases glomerular permeability and intraglomerular pressure [40]. As a result, protein escapes in urine. The VEGF molecule in the nephron cells is required for maintaining podocyte function and survival; development and repair of glomerular vessels; maintenance of renal filtration system; formation of fenestrations; and filtration barrier creation [40]. There are risk factors for proteinuria such as: underlying renal disease; nephrectomy; uncontrolled hypertension; diabetes mellitus; and immunosuppression treatment. Bevacizumab, sunitinib and other anti-VEGF drugs are frequently complicated by mild proteinuria and hypertension [40, 41]. Proteinuria decreases after treatment ends and serious renal function impairment is rare [41]. Moreover, full dose ACE Inhibitors may reduce the severity of proteinuria. Renal effects, such as high-grade proteinuria and acute kidney injury, have been described. The most common histo-

pathological kidney lesion is thrombotic microangiopathy, with other glomerular lesions and interstitial nephritis occurring less frequently [41]. Glomerular injury may develop from loss of VEGF effect on maintaining the filtration barrier.

FATIGUE

Fatigue can have a significant negative effect on everyday activities and patient QoL. Symptoms are the following: altered energy levels; attention deficits; sleep disturbance; reduced endurance; listlessness; sluggishness; dizziness; apathy; exhaustion; anxiety; and depression. Severity levels are as follows: grade 0 – No symptoms; grade 1 – Mild symptoms; grade 2 – Moderate or causing difficulty with daily life activities; grade 3 – Severe, interfering with daily life activities; grade 4 – Disabling symptoms. Of course fatigue is not a dangerous side-effect and patients should be informed that accepting this adverse event may allow dramatic tumor remission which *per se* may reduce fatigue (typically observed in patients responding to treatment).

Physicians should consider that several fatigue-inducing factors are treatable, such as hypophosphatemia; anemia; depression; pain; reduced activity level; hypothyroidism. Hypothyroidism may be an underlying cause of fatigue in TKI-treated patients and may be a biomarker for response to treatment [42]. First of all, thyroid function should be controlled prior to treatment and at regular intervals followed by replacement therapy upon TSH increase [43].

In a retrospective medical review analysis of 145 patients, the most common any grade AE for sunitinib was fatigue/asthenia (81.2%), followed by mucositis/stomatitis (58.8%) and decreased taste sensation (42.4%), while for sorafenib the respective findings were fatigue/asthenia (43.3%) followed by hand-foot syndrome (38.3%) and diarrhea (31.7%) [42]. Treatment discontinuation, interruption, and dose reduction due to AE in patients receiving sunitinib and sorafenib is a common management procedure in balancing favorable treatment outcome and quality of life [44, 45].

PATIENT INFORMATION AND COMMUNICATION

The patients' benefit of any therapy depends on their adherence to and acceptance of the drug regimen. Patients who feel in control are more likely to adhere to their treatment schedules. Therefore, patient empowerment is an important part of the treatment. The new oral targeted therapies have the advantage of allowing patients to control their own treatment; patients are treated at home and may have fewer hospital visits. Consequently, strong patient communication and education is essential and an open discussion at scheduled times avoids further complication of adverse therapy reactions. Recording of symptoms is vital for the clinician to distinguish between drug overdose and disease progression. These discussions are important to build individual relationships and help patients cope with emotional stress, as well as physical problems associated with cancer.

Through communication and by understanding their disease, they are actively involved in their own treatment.

NEW TARGETED THERAPIES IN CLINICAL TRIALS

There are several targeted therapies under investigation for use in metastatic renal cell carcinoma. New targeted therapies against VEGF include axitinib, cediranib and tivozanib. Axitinib is tested in Phase II trials in patients with cytokine-refractory metastatic renal cell carcinoma (after prior use of sunitinib, sorafenib or cytokines) [47]. There was an overall progression-free survival of 8 months and phase III trials were designed [47]. Similar PFS rates around 9 months were observed with cediranib in refractory metastatic RCC. Tivozanib, a potent inhibitor of VEGFR 1-3, c-kit and PDGFR, was evaluated in metastatic RCC and despite the high incidence of adverse reactions, one third of the patients benefited from therapy [48].

New therapies targeting the mTOR pathway are under investigation as well. Akt, that functions upstream of mTOR, is a potential therapeutic target due to its vital role in a number of cellular processes, including apoptosis and cell cycle progression. Perifosine, an Akt inhibitor, was tested in a phase II trial, in second-line setting with mRCC, with low response rates [49]. Ridaforolimus, an mTOR inhibitor, is promising in non-small cell lung cancer, sarcomas and RCC. PDK-1, a kinase inhibitor of the mTOR pathway, has *in vitro* pre-apoptotic activity and is under clinical evaluation.

The last molecules in trials for mRCC include inhibitors of the *c-Met* proto-oncogene involved in papillary RCC; the inhibitors of the integrin α V β 3 involved in angiogenesis through mediation of extracellular matrix signals have been tested in combination with bevacizumab. Bevacizumab has been tested in combination with the histone deacetylase inhibitor HDACI in a phase II trial, since repression of the tumor suppressor gene transcription causes cancer. Infliximab, a TNF- α monoclonal antibody, has also been included in an mRCC phase II trial, since TNF- α is secreted by renal tumors and is involved in tumorigenesis [51].

CONCLUSION

Although considerable advances are being made in the treatment of metastatic renal cell carcinoma in terms of clinical benefit, it is also essential to consider the associated toxicities and the patient's perspective. Early intervention and prophylaxis is the key factor to successful management of toxicities. AEs should be managed or treatment withdrawn before toxicities advance to grade 3, which will also help minimize the length of any dose intervention. New targeted agents have relatively favorable safety profiles, with generally manageable toxicities. Effective management of AEs through patient education and appropriate medical and supportive strategies is vital in the provision of cancer care. Through education of patients and healthcare professionals, AEs can be identified and treated earlier and their severity minimized.

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Cutaneous metastasis resembling herpes zoster as an early presentation of stomach cancer

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ABSTRACT

Cutaneous metastasis from gastric adenocarcinoma is an infrequent disease entity. When present, it typically signifies disseminated disease with a poor prognosis. Only two cases with zosteriform metastasis due to gastric adenocarcinoma have been reported so far. We report a case of a 58-year-old male patient whose skin lesion was misdiagnosed as a herpetic infection and was ultimately the first sign of gastric adenocarcinoma.

Key words: gastric adenocarcinoma; early cutaneous metastasis; distant metastasis; herpetic lesions.

INTRODUCTION

Metastases to the skin from visceral carcinoma are relatively rare with a reported incidence of 0.7-9.0% [1, 2]. Cutaneous metastases from gastric adenocarcinoma are an infrequent disease entity [3]. When present, they typically signify disseminated disease and carry a poor prognosis. We report a case of a 58-year-old male patient whose skin lesion, initially misdiagnosed as a Herpes zoster infection, was finally the first sign of gastric adenocarcinoma.

CASE REPORT

A 58 year-old man visited an internist for skin lesions in the abdominal, right and left thoracic wall characterized by a mild burning sensation (Figure 1). The physician diagnosed him with Herpes zoster infection and prescribed the pertinent medication. One month later the lesion spread to the left thoracic wall (Figure 2). At that time, the patient complained for abdominal discomfort and visited another doctor who diagnosed him with gastritis and prescribed antihistamines. Due to further exacerbation of the skin lesions, the patient visited a dermatologist who performed a biopsy. The biopsy revealed invasion of the dermis and serosa by adenocarcinoma cells. He was subsequently admitted to the oncology unit to investigate the primary tumor site location. From the laboratory exams, his hematocrit was 32%, his white blood cell count

was 8,500 cells/cm³ (60% polymorphonuclear cells) and his platelet count was 250,000 cells/cm³. His CA19-9 levels were 230 U/ml while the rest of the tumor markers and biochemical results were unremarkable. A chest and abdominal computerized scan showed multiple hypodense metastatic lesions in the liver, enlarged lymph nodes in the lesser omentum and thickened wall in the pyloric antrum. The upper gastrointestinal endoscopic finding was a circular mass in the pyloric antrum and lesser curvature. The stomach biopsy findings were positive for adenocarcinoma. He started receiving chemotherapy but finally died 4 months later.

DISCUSSION

A cutaneous metastasis may be the first sign of visceral malignancy, or the first sight of tumor recurrence. The incidence of cutaneous metastases is not known. Some studies report an incidence of 3-4% while a recent meta-analysis demonstrated an incidence of 5.3% [4]. The most common primaries associated with cutaneous metastases are lung and colon cancers in males; and breast, colon and ovary cancers in females [2, 5].

A cutaneous metastasis as the first manifestation of gastric carcinoma is very rare. In a retrospective study 1,287 patients with internal malignancies were evaluated using physical and dermatological examinations. Only 212 had gastrointestinal system malignancy.

Cutaneous metastases were observed in only 15 patients (1.2%). None of them was a metastasis from gastric cancer [7]. Cutaneous metastases from the upper digestive tract have been reported in less than 1% [1]. Metastases to the skin appear mainly as a nodule, or as a cellulitis-like lesion. The erysipelas-like and zosteriform presentations are very rare; only three cases with erysipelatoides carcinoma [8] and two cases with zosteriform metastasis due to gastric adenocarcinoma have been reported so far [9, 10]. Skin metastases may be solitary or in multiple sites. Skin involvement usually occurs in the vicinity of the primary tumour as a direct spreading to the abdominal wall [11]. Cutaneous metastases usually appear late in the course of the disease, but may also

constitute the presenting sign [12]. Due to lack of data, the survival of patients with gastric cancer plus skin metastases is not known. Still, the prognosis is very poor as suggested in one report including 7 patients with only 1.2 month median survival [13]. However, the contribution of systemic therapy and the extent of the disease in the patients included in this study were not clear [13].

In summary, skin metastases from stomach cancer as well as other systemic malignancies seem to be an ominous sign while appropriate treatment in such cases is not well-established. That was the case of our patient who survived only four months despite aggressive chemotherapy.

Figure 1.

Skin lesions in right thoracic wall



Figure 2.

Skin lesions in right and left thoracic wall and in the abdominal region



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On the article “Why Peer Review is Needed”

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Thank you for giving me the opportunity to participate in the Open Peer Commentary that your respectable Journal has adopted. Open Peer Commentary, consisting of solicited expert commentaries on published articles to which the authors are encouraged to respond, represents, in fact, an extension of Peer Review and can be considered most appropriate when directed at an article addressing the subject of peer review necessity. Dr Christos Emmanouilides has recently published an article in FCO entitled “Why Peer Review is Needed”. His choice to write his views on the subject is undoubtedly commendable but the way they are presented in the aforementioned article leaves the well-intentioned reader skeptical for several reasons. Dr Emmanouilides poses the indirect question “Why Peer Review is Needed”. The logical assumption is that either he, himself, doubts the necessity of peer review or poses a rhetorical question in order to build up a strong case for the necessity thereof. In either case, the reader would expect a vivid argument. Instead, he/she reads a list of seemingly unquestionable qualities and advantages of the process of peer review. Dr Emmanouilides appears to suggest that peer review is a uniquely infallible recipe which can be successfully used by any reviewer as long as he/she learns to perform it correctly. Unfortunately, this is not the case. In fact, the literature of the past 20 years is replete of publications ardently debating the role of peer review in evaluating articles prior to publication while, on the other hand, an international congress is held every 4 years since 1986, devoted on peer review (International Congress on Peer Review and Biomedical Publication). There is considerable skepticism and criticism of peer review as a mechanism ensuring quality of biomedical research and, in our opinion, this literature ought to be critically reviewed in Dr Emmanouilides' article and the argumentation persuasively rebutted before the author's conclusion that “peer review is necessary for assuring a satisfactory level of relevance of the submitted research, for improving the manuscript to its full capacity” is reached. If his conclusive statement were right, then the

following citations from the literature and quotations of eminent editors of high-prestige journals would be absolutely inappropriate. More specifically:

1. The authors of a Cochrane Database Systematic Review on the subject concluded that “...little empirical evidence is available to support the use of editorial peer review as a mechanism to ensure the quality of biomedical research”.
2. Several contributors to JAMA's issue on peer review illustrated “a worrying number of biases by which peer review is beset including nationality bias, language bias, specialty bias, even gender bias as well as bias towards the publication of positive results”.
3. Drummond Rennie, deputy editor of the Journal of American Medical Association (JAMA), concluding the 5th International Congress on Peer Review and Biomedical Publication has remarked that “There seems to be no study too fragmented, no hypothesis too trivial, no literature too biased or too egotistical, ... no conclusions too trifling or too unjustified ... for a paper to end up in print”.
4. Richard Horton, Editor of The Lancet has said that “the mistake, of course, is to have thought that peer review was any more than a crude means of discovering the acceptability -not the validity- of a new finding. Editors and scientists alike insist on the pivotal importance of peer review. We portray peer review to the public as a quasi-sacred process that helps to make science our most objective truth teller. But we know that the system of peer review is biased, unjust, unaccountable, incomplete, easily fixed, often insulting, usually ignorant, occasionally foolish, and frequently wrong”.
5. Another drawback of peer review is the alleged built-in bias “by academics against highly innovative work in part as a result of the fact that they have vested interests in maintaining the status quo after having spent many years or decades supporting it”.
6. More radical is the approach of Fiona Godlee from BioMed Central who suggested abandoning systematic prepublication

peer review altogether. *“Editors could then focus their energies on encouraging higher quality research at the outlet, coupling this with an efficient system for postpublication criticism and rating of articles”.*

Finally, another well-known disadvantage of peer review are the so called peer review failures. This refers to the situation

where a peer-reviewed article contains obvious errors that undermine at least one of its main conclusions. In this context, the article by Dr Emmanouilides, published in FCO, should be considered as a peer review failure. Many journals have no procedure to deal with peer review failures beyond publishing letters to the Editor.

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On “Peer Review”

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As FCO is maturing as a journal, it is interesting to see an editorial and an article on the value of peer review, right after an award winning article on scientific misconduct.

There is no question as to the need for strict and well-thought out review of all submitted material. It is the only road leading to a successful journal.

That being said, I would like to point out that “peer review” is named this way since it is

supposed to come from (professional) peers.

This means that the reviewers should strive for professional and fair reviews written in a courteous manner without personal empathy, otherwise the journal risks becoming yet another forum for personal conflict.

As a member of the editorial board, I am sorry to have witnessed such inappropriate behavior.

Hopefully, we will see to it that such unprofessional conduct subsides.

Why peer review is needed

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Peer review is necessary before study publication.

Health workers and medical doctors are in particular need to be kept informed about medical achievements. Medicine is constantly evolving, with changes occurring over the years; there is also new technology and new drugs. Disease management becomes different and the treatment of patients, specifically those with cancer, may improve.

How do medical doctors gain access to research achievements? -Through publications

and conferences. Reviewers of papers submitted for publication have the responsibility to decide whether the study in question is worth addressing medical workers, the people responsible for the health of others. A second and a third opinion of experts on the subject does help and improves studies submitted for publication. There are certain regulations and criteria that define what a proper manuscript is, so as to best educate the readers. Originality, clarity, scientific and clinical value are all important paper criteria, necessary for them to reach the readership.

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Key Words: 5-10, for indexing purposes.

Introduction: Provides a context or background for the study (that is, the nature of the problem and its significance) and states the specific purpose or research objective of, or hypothesis tested by, the study or observation.

Patients & Methods: This section should include only information that was available at the time the plan or protocol for the study was being written; all information obtained during the study belongs in the Results section.

Results: This section presents results in logical sequence in the text, tables, and illustrations, giving the main or most

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