Tirapazamine: From Bench to Clinical Trials

Loredana Marcu[1],* and Ian Olver[2]

[1]*Royal Adelaide Hospital, Department of Medical Physics and University of Adelaide, School of Chemistry and Physics,*
[2]*Royal Adelaide Hospital, Department of Medical Oncology and University of Adelaide, Faculty of Medicine.*

Abstract: Tumour hypoxia continues to remain one of the greatest challenges in the treatment of solid tumours. An important avenue to follow with both radiotherapy and chemotherapy is the development of hypoxic cytotoxins such as tirapazamine. The present review covers the history of tirapazamine from preclinical models to clinical trials. The biochemistry as well as the pharmacokinetics of this bioreductive agent are presented. Laboratory data demonstrating the enhanced effect of radiation and cisplatin when combined with tirapazamine are also discussed. There is considerable evidence supporting the potentiation of anti-tumour effect of cisplatin by tirapazamine. Several clinical trials for various tumour sites have been testing the synergistic effect of cisplatin-tirapazamine with and without radiotherapy. These are also reviewed in the present paper.

The current literature data on tirapazamine leaves unanswered questions about its action and toxicity. While the current number of phase III trials limits comprehensive conclusions about the administration of this drug, there is a unanimous indication that further clinical studies are warranted.

Keywords: Hypoxia, Tirapazamine, Radiotherapy, Cisplatin, Clinical trials.

I. INTRODUCTION

Tumour hypoxia, both chronic and acute, continues to remain one of the greatest challenges in the treatment of solid tumours. The extent of tumour oxygenation is one of the critical factors influencing radiation response. In normoxic conditions cells are more prone to radiation damage than in hypoxic environments. The difference in radiosensitivity between hypoxic and normoxic cells is related to the capacity of hypoxic cells to repair the DNA damage created by the free radicals, damage which in an oxic environment is 'set' by the oxygen molecules making it permanent. *In vivo,* hypoxic cells are also resistant to chemotherapy [1]. One explanation for chemoresistance is that hypoxic cells proliferate at a slower rate than cells targeted by anticancer drugs [2] (chemotherapy affects mainly tissues with a high growth fraction). Furthermore, since hypoxic cells are either distant from functional blood vessels or in the vicinity of non-functional vessels, the drug perfusion to these sites is difficult.

Tumour hypoxia was initially thought to arise in cells situated at distances of at least 100-150 μm from functional blood vessels. However, more recent quantitative studies done on hypoxic tumours showed that hypoxic cells can be present within a 25-50 μm rim from blood vessels [3]. Hypoxia can be also detected close to non-functional vessels. Permanent occlusions of blood vessels create areas with low oxyhemoglobin levels, leading to chronically hypoxic cells.

Acute hypoxia is considered a more dynamic form of hypoxia because of its transient nature. Transient hypoxia occurs in tumour cells which are dependent on blood vessels that are subject to a partial decrease in functionality. This partial (or temporal) decrease in functionality limits oxygen

perfusion to the tumour, making it transiently hypoxic. To quantify acute hypoxia within a tumour is a hard task. However, experimental studies designed to monitor oxygen tension were able to quantify transient hypoxia in human tumour xenografts [4]. The studies have concluded that the changes in tumour blood flow and oxygen tension within the monitored tumours are highly heterogenous, and they occur with different magnitudes and periodicities in different regions of the same tumour. Therefore, controlling hypoxia is still a challenge in the treatment of solid tumours. Consequently, the discovery of tirapazamine, a hypoxic cell sensitizer, has positively marked the avenues to be followed in both radiotherapy and chemotherapy.

The present review covers the history of tirapazamine from preclinical models to clinical trials. The biochemistry, properties and action of this bioreductive agent are presented. Laboratory data demonstrating the enhanced effect of radiation and cisplatin when combined with tirapazamine are also discussed. There is considerable evidence supporting the potentiation of the anti-tumour effect of cisplatin by tirapazamine. Several clinical trials for various tumour sites have been testing the synergistic effect of cisplatin-tirapazamine with and without radiotherapy. These are also reviewed in the present paper.

The current literature data on tirapazamine leaves unanswered questions about its action, toxicity, and optimal administration. While the current number of phase III trials limits comprehensive conclusions about the administration of this drug, there is mounting evidence that further clinical studies are warranted.

II. TIRAPAZAMINE: PROPERTIES AND ACTION

Several strategies have been developed in the past in order to overcome tumour hypoxia: hyperbaric oxygenation during radiotherapy (increases cellular oxygen delivery), carbogen breathing (enhances the diffusion of oxygen into the tissue by increasing the arterial oxygen pressure),

*Address correspondence to this author at the Medical Physics Department, Royal Adelaide Hospital, North Terrace, Adelaide 5000, Australia; Tel: 61-8-8222 4038; Fax: 61-8-8222 5937; E-mail: lmarcu@mail.rah.sa.gov.au

vasoactive agents (nitric oxide and endothelin-1, both key molecules in controlling vascular tone), hypoxic cell radio-sensitizers (misonidazole, etanidazole). The success using these methods has been limited by either normal tissue toxicity or poor tumour response (small degree of radio-sensitization) [5-9].

In the mid 80's, through the discovery of a hypoxic cytotoxin, tirapazamine (TPZ), Brown and Lee [1] have "turned hypoxia from problem to advantage". They showed that specifically killing the hypoxic cells has greater therapeutic potential. The rationale behind this statement is that hypoxic cytotoxins target and kill cells which normally are resistant to radio/chemotherapy, therefore complementing the cytotoxic effect of the treatment. Also, hypoxic cytotoxins could target acutely hypoxic cells, which otherwise are more resistant to radiation and harder to control than chronically hypoxic cells [10]. While testing the drug which they initially called SR 4233, Brown and Lee observed that the killing potential of tirapazamine is much higher than that of any existing radiosensitizing agent (the same concentration of tirapazamine killed a much larger number of hypoxic cells than any radiosensitizing drug tested before). Furthermore, the differential toxicity to hypoxic cells presented by tirapazamine was much larger than that of any other known drug. Further studies showed that the cytotoxicity of tirapazamine resides in its ability to produce DNA double-strand breaks and chromosome breaks under hypoxic conditions [11]. While under hypoxic conditions tirapazamine is reduced to a highly reactive radical that produces strand breaks in the DNA, under aerobic conditions, the radical is back-oxidized to the non-toxic parent compound with a concomitant production of the superoxide radical, which is much less toxic than the tirapazamine radical [1].

Some preclinical models indicated that the hypoxic cytotoxicity ratio (the ratio of drug concentration under oxic:hypoxic conditions to produce equal cell kill) ranges from 50 to 300 [12]. On the other hand, Durand and Olive [13] have investigated the activity of tirapazamine in tumor-bearing mice, showing only a threefold preferential activity of the drug against hypoxic versus oxic cells, much less than the range indicated by the *in vitro* studies. They found that tirapazamine has minimal activity against cells in the centre of hypoxic tumours, suggesting that the drug might be bioreductively inactivated before reaching chronically hypoxic cells. Their conclusion that the effect of tirapazamine on tumours depends strongly on the oxygenation level was also supported by other studies [14,15]. Lartigau and Guichard [15] have studied the effect of tirapazamine on human tumour cell lines under five different oxygen concentrations, ranging from normal air concentrations (20.9% O_2) to hypoxia (0.02% O_2). Their experiments demonstrated that the cytotoxic effect of tirapazamine is highly dependent on clinically relevant partial oxygen pressure (measure of the number of oxygen molecules in a given volume). The investigation also included a dose-response study showing an increase in tirapazamine dose with increase in oxygenation for a cytotoxic effect. However, the toxic effect of tirapazamine in air for clinically relevant doses could not be seen.

The role of tirapazamine as an *in vivo* hypoxic fraction indicator has also been investigated [16]. Cells from spheroids containing about 50% hypoxic cells have been analysed after being exposed to tirapazamine. It was noticed that cells close to blood vessels presented with less DNA damage than cells distant from blood vessels. Also, repair of single-strand breaks produced under oxic conditions had a half-time of about 1 hour, while for cells under hypoxia the half-time increased to 2 hours. The study concluded that the degree of heterogeneity in DNA damage created by tirapazamine can be used to estimate the distribution of oxygen content within tumours.

III. TIRAPAZAMINE-RADIATION INTERACTION

Developed as an adjunct to radiotherapy, cisplatin has proven its role as a radiosensitizer. One of the pioneering experiments testing the efficacy of tirapazamine has compared, in a fractionated regimen, two radiation response modifiers: tirapazamine, the bioreductive cytotoxin, and nicotinamide with carbogen, a tumour oxygenation enhancer [17]. Both agents (tirapazamine and nicotinamide) have been administered before each radiation fraction, while the tumours have been exposed to carbogen immediately before and also during irradiation. The study showed that both treatment strategies improved tumour control as compared to the effect of radiation alone. However, out of the three tumour types examined, two of them showed significantly greater enhancement of tumour response after exposure to tirapazamine, while the third tumour responded equally to both treatment modalities. The authors noted that the least responsive tumour had the lowest hypoxic fraction (the ratio of hypoxic tumour cells versus total number of tumour cells), consequently the bioreduction of tirapazamine was not that efficient as in the other, more hypoxic tumours. This experiment was a demonstration of the way hypoxia can be turned from a problem to an advantage: it showed that killing hypoxic cells leads to a greater (or at least similar) enhancement of the effect of fractionated radiotherapy on tumour control than radiosensitizing (or oxygenating) the cells.

Lartigau and Guichard [15] have found the combination of tirapazamine with radiation as generating a synergistic effect on tumours. Studies on melanoma cell lines [18] have proven the same enhancement on tumour control when tirapazamine is administered, to hypoxic tumours, 1 hour before irradiation. Similar results have been achieved by Shibata *et al.* [19] after investigating the cytotoxicity and the interaction of tirapazamine with low-dose radiation (1-4 Gy) on murine SCCVII and human melanoma (G-361) cells. Their study compared the effect of tirapazamine with the sensitizing capacity of KU-2285 (hypoxic cell sensitizer) under hypoxic conditions. Cells were treated with either of the two drugs then irradiated. Tirapazamine showed superior tumour control to the hypoxic radiosensitizer at clinically relevant doses confirming, once again, its potency as a hypoxic cytotoxin.

Several studies undertaken by Masunaga *et al.* [20-22] using micronucleus assays have demonstrated that tirapazamine has a sensitizing effect (both radio- and chemo-sensitization) on the quiescent cell population in solid

tumours. Since quiescent cells are known to be radioresistant, the administration of tirapazamine before radiotherapy could explain the synergistic effect on tumours when the two treatment modalities are administered consecutively.

Some experiments were not supportive of the synergistic (more-than-additive) effect of tirapazamine-radiation on tumours, showing an additive-only response [23]. Similarly, Lambin *et al.* [23] tested the effect of tirapazamine with and without radiation on human tumor cell lines. They showed that 1 hour incubation time with tirapazamine under hypoxia followed by irradiation increases the radiosensitivity of the tumour, however the effect of the combined treatment was only additive.

Pre-clinical studies involving tirapazamine and radiation have proven to be effective on hypoxic tumours. Although not controversial, the results are divided between synergistic and additive-only effects on tumour control when the two modality treatments are administered consecutively. Unanimously, the most efficient timing of tirapazamine and radiation was found to be tirapazamine prior irradiation. This result shows that the radiosensitizing property of tirapazamine is more pronounced than its cytotoxic capability.

IV. TIRAPAZAMINE-CISPLATIN INTERACTION

So far, the greatest clinical success in the history of tirapazamine has been achieved by combining the hypoxic cytotoxin with cisplatin [1].

Besides better tumour control, another advantage of combining cisplatin with tirapazamine (rather than with other anticancer agents) is the low level of added toxicity to the normal tissue. Unlike other drugs which are commonly administered together with cisplatin (taxols, 5-FU, gemcitabine) tirapazamine does not potentiate the side effects created by cisplatin [26]. As reported by the majority of clinical trials (see § V. Clinical trials), the most common side effect produced by tirapazamine was muscular cramping which, in some cases was dose limiting. Generally still, cisplatin combined with tirapazamine was well tolerated.

An added advantage in combining tirapazamine with cisplatin to the ones mentioned above consists in the ability of tirapazamine to chemo-sensitize quiescent cells. The already cited work completed by Masunaga *et al.* [20-22] has demonstrated that tirapazamine has a dual sensitizing effect

on quiescent cells, making them more responsive to both radiation and cisplatin. Since non-proliferating cells are less receptive to cisplatin (and chemotherapy in general) than cycling cells, chemo-sensitizing them to cisplatin would, expectably, enhance the effect of the platinum compound on tumour kill.

Table **1** presents the individual and also the combined effect of tirapazamine and cisplatin on tumour cells. Cisplatin (cis-diammine-dichloro-platinum) is a heavy metal complex, with a central platinum atom surrounded by 2 chlorine atoms and 2 ammonia molecules. The chemical reactivity of cisplatin is determined by the chloride groups situated in the cis-position of the platinum complex. Cisplatin has the ability to form both intrastrand and interstrand adducts with DNA [27]. Although the number of interstrand cross-links is less than 1% of the total adducts [24], it is considered that this type of adduction is responsible for the cytotoxic effect of cisplatin. Laboratory studies indicated that tirapazamine inhibits the repair of cisplatin-induced interstrand adducts [29] which might be the major pathway by which the two drugs cooperate in achieving a supra-additive effect on tumour control.

The synergistic effect on tumour cell kill caused by the combination of tirapazamine with cisplatin is highly schedule dependent [25,26]. Table **2** is a compilation of data reported by several *in vitro* as well as *in vivo* studies regarding the effect of various timings of tirapazamine and cisplatin. Although the majority of the studies indicate a synergistic effect when tirapazamine is administered a few hours before cisplatin and an additive-only when administered concurrently [26,35-37], there were some controversial results showing just the opposite [38]. Clinical trials of tirapazamine-cisplatin have adopted the a priori administration of the hypoxic cytotoxin, reporting a possible chemo-sensitizing effect of the drug on cisplatin.

V. CLINICAL TRIALS

Several Phase I clinical trials of tirapazamine with or without radiotherapy and/or chemotherapy have been designed to determine the maximum tolerated dose, toxicities, pharmacokinetics and effect on tumour control (Table **3**).

As illustrated in Table **3**, tirapazamine has been trialed on various tumour sites, in various schedules, with different outcomes. Dose escalation studies for tirapazamine resulted

Table 1.   The Individual and Combined Effect of TPZ and Cisplatin on Tumour Cells

Tirapazamine	Cisplatin	Tirapazamine + Cisplatin
Induces DNA double and single strand breaks [11]	Able to form both intrastrand and interstrand adducts with DNA [28]	TPZ inhibits/delays the repair of cisplatin-induced interstrand adducts [29]
Induces chromosome breaks [11]	Inhibits sublethal damage repair [30]	
Interrupts cell cycle progression [31]	Able to arrest cells in the G2 phase of the cell cycle [31]	TPZ chemo-sensitizes quiescent cells making them more responsive to cisplatin [20-22]
Induces apoptosis [31]	Induces oxygenation of hypoxic cells [32]	
	Suppresses tumour neovascularization [33]	

Table 2. The Effect of Various Timings of Cisplatin-Tirapazamine on Tumour Control

Reference	Type of Study	Tirapazamine-cisplatin Timing	Effect on Tumour Control
Dorie and Brown [26]	*In vivo*	TPZ 2-3 h before cisplatin	synergistic
		TPZ simultaneously with cisplatin	additive
Goldberg *et al.* [35]	*In vitro*	TPZ simultaneously with cisplatin	additive
		TPZ before cisplatin	synergistic
Wouters *et al.* [36]	*In vitro* *In vivo*	TPZ 2-3 h before cisplatin	synergistic
Siemann and Hinchman [37]	*In vivo*	TPZ 3 h before cisplatin	synergistic
		TPZ simultaneously with cisplatin	
Lartigau and Guichard [38]	*In vivo*	TPZ 3 h before cisplatin	not significant
		TPZ simultaneously with cisplatin	synergistic

in a wide range of maximum tolerated doses: from 220 mg/m^2 to 330 mg/m^2. The most commonly reported side effect and also dose-limiting toxicity was muscle cramping. Nausea and vomiting were also found as dose-limiting factors. However, compared to other chemotherapeutic agents, the toxicities produced by tirapazamine were favourable, and because they were reversible were declared as being acceptable.

The results regarding tumour control reported by the Phase I trials were mixed: some have reported significant outcomes [27,38-40,42], others achieved very poor tumour responses or not at all [43,44] and some did not report on tumour effect [41,45]. However, this is not unusual for Phase I studies where only limited number of patients are treated at ideal doses in the appropriate tumour types. Yet, a unanimous conclusion was that Phase II trials are warranted.

Table 3. Phase I Clinical Trials of TPZ with or without Radiotherapy/Chemotherapy (Dose Escalation Studies)

Reference	No of Pts	Tumour Site/Type	Schedule	Maximum Tolerated Dose/Toxicity	Outcome	Conclusions
Le *et al.* 2004 [39]	30	limited-stage small cell lung cancer	RT 61 Gy Concurrent chemoradiotherapy with 2 cycles of cisplatin/etoposide TPZ 260 mg/m^2 1 h before cisplatin	260 mg/m^2 The planned 330 mg/m^2 was not reached due to toxicities: neutropenia, vomiting Overall toxicity acceptable	80% overall response rate	Favorable survival warrants further studies with TPZ for LSCLC
Craighead *et al.* 2000 [40]	15	Cervix	RT 45 Gy over 5 weeks then brachytherapy up to 85 Gy. *Dose level 1:* TPZ 190 mg/m^2 and Cisplatin 75 mg/m^2 on days 1, 15, 29 of RT TPZ alone 160 mg/m^2 on days 8, 10, 12, 22, 24, 26 of RT *Dose level 2:* TPZ 290 mg/m^2 and Cis 75 mg/m^2 on days 1, 15, 29 of RT TPZ alone 220 mg/m^2 on days 8, 10, 12, 22, 24, 26 of RT	Dose level 2 (TPZ 290 mg/m^2 on days 1, 15, 29 of RT and TPZ alone 220 mg/m^2 on days 8, 10, 12, 22, 24, 26 of RT	87% of patients had complete pelvic control of disease at 6 months.	TPZ with concurrent cisplatin and radiotherapy has acceptable toxicity, and will be tested in a phase II trial.

Reference	No of Pts	Tumour Site/Type	Schedule	Maximum Tolerated Dose/Toxicity	Outcome	Conclusions
Peters *et al.* 1999 [41]	16	Head and neck	RT 35 fractions over 7 weeks TPZ 290 mg/m^2 and cisplatin 75 mg/m^2 on day 2 of weeks 1, 4 and 7 TPZ without cisplatin 160 mg/m^2 on days 1, 3 and 5 of weeks 2, 3, 5 and 6	290 mg/m^2 on day 2 of weeks 1, 4 and 7 Febrile neutropenia was dose limiting toxicity	75% of patients disease-free survival after 21 months	Remarkable results considering the poor prognosis of T3-4 patients Phase II trial warranted.
Shulman *et al.* 1999 [42]	43	Refractory solid tumours (predominantly brain)	RT varied based on disease TPZ dose escalation from 9 mg/m^2/dose to 260 mg/m^2/dose 3 times per week (Mo, Wed, Fri) for 4 weeks	260 mg/m^2 3 times per week (Mo, Wed, Fri) for 4 weeks primary toxicity – muscle cramps	Not reported Awaiting phase II trial results	TPZ possibly has radio-sensitizing properties and it is tested in phase II trial
Aghajanian *et al.* 1997 [43]	12	Recurrent cervical carcinoma	TPZ daily over 21 days Dose levels: 195, 260, 330 and 390 mg/m^2 Cisplatin 1 h later 75 mg/m^2	330 mg/m^2 dose-limiting toxicities were nausea and vomiting	17% -major response 25%- minor response 33% - disease stabilization	Phase II testing is planned.
Johnson *et al.* 1997 [44]	13	Solid tumours (predominantly lung)	TPZ daily over 21 days Dose levels from 130 to 260 mg/m^2 Cisplatin 1 h later 75 to 100 mg/m^2	260 mg/m^2 Dose-limiting toxicity were nausea and vomiting, also fatigue and muscle cramping	15% partial response	Cisplatin in combination with TPZ is well tolerated. Phase II investigation warranted.
Senan *et al.* 1997 [45]	28	Solid tumours	TPZ every 3 weeks 50 courses of TPZ ranging from 36-450 mg/m^2	330 mg/m^2 dose-limiting toxicity were reversible deafness and tinnitus	No tumour responses were observed	TPZ alone had no effect on tumour control. The dose of 330 mg/m^2 was chosen for combined chemotherapy studies.
Doherty *et al.* 1994 [46]	44	Solid tumours (mainly brain, lung, sarcoma, rectum)	I. TPZ for 10 consecutive days 9-21mg/m^2 $^1/_2$-1h prior RT II. TPZ single doses of 18-293 mg/m^2 after RT III. TPZ without RT in single doses of 36-250 mg/m^2.	Dose escalation continues in further studies Muscle cramping was the most common side effect	Not reported	Further studies are designed to investigate maximum tolerated dose and muscle cramping.

Some Phase I trials have already suggested the possible sensitizing properties of tirapazamine on either radiation or cisplatin. Shulman *et al.* [41] has reported on radio-sensitizing effects of tirapazamine, which will be further investigated in a Phase II trial. Similarly, the 87% local control reported by Craighead *et al.* [39] after the concurrent administration of tirapazamine, radiation and cisplatin shows a potentially synergistic effect of the combined modality treatment, when compared with the 'no tumour response' reported by Senan *et al.* [44] as a result of a trial of tirapazamine as a sole therapeutic agent.

Phase II trials with tirapazamine in combination with radiotherapy and/or cisplatin have been performed primarily in carcinomas of the head, neck and lung. The most relevant trials are collated in Table **4**. The results of the trials indicate that tirapazamine can improve outcome in advanced carcinomas when combined with cisplatin monotherapy [49-51]. Whether this combination is also superior to multiagent chemotherapy is currently under investigation. Some promising results have been reported by Rischin *et al.* [46] after trialing the combination of tirapazamine-cisplatin versus cisplatin-fluorouracil.

Table 4. **Phase II Clinical Trials of TPZ with or without Radiotherapy/Chemotherapy**

Reference	No of Pts	Tumour Site/Type	Schedule	Toxicity	Outcome	Conclusions
Rischin *et al.* 2005 [47]	122	Head and neck (stage III/IV)	RT 70 Gy in 7 weeks concurrently with cisplatin (75 mg/m^2) and TPZ (290 mg/m^2) on day 2 of weeks 1, 4, 7 and TPZ alone (160 mg/m^2) on days 1, 3, 5 of weeks 2 and 3 OR RT with cisplatin and 5-FU chemoboost	TZP/cisplatin lead to less severe toxicity than chemoboost	55% - 3year failure-free survival with TPZ/cis versus 44% with chemoboost. 84% - 3year locoregional failure-free survival with TPZ/cis versus 66% with chemoboost. (p value = 0.05)	TPZ/cisplatin had superior tumour control to chemoboost and is being evaluated in a phase III trial.
Reck *et al.* 2004 [48]	45	Non small cell lung cancer (stage III/IV)	TPZ 330 mg/m^2 (day 1), cisplatin 75 mg/m^2 (day 1) and gemcitabine 1250 mg/m^2 (day 1 and 8) every 3 weeks	Moderate toxicity: Nausea, vomiting, neutropenia	40% -6.7 months median progression free survival 35% -1 year survival	TPZ in combination with gemcitabine/ cisplatin was feasible. Results of phase III trial are awaited.
Lee *et al.* 1998 [49]	40	Head and neck (stage III/IV)	RT 70 Gy in 7 weeks concurrently with TPZ (159 mg/m^2) 3 times per week for 12 doses	Muscle cramps, nausea, vomiting RT with TPZ acceptable toxicity	64% -1 year local control 59% -2 year local control rate	Further clinical studies are warranted.
Treat *et al.* 1998 [50]	44	Non small cell lung cancer (stage III/IV)	TPZ 260 mg/m^2 1 h before cisplatin (75 mg/m^2) every 21 days for 8 cycles	Muscle cramps, fatigue, nausea, vomiting	23% partial response. Median survival (estimated) 37 weeks	TPZ with cisplatin is more active than cisplatin alone.
Miller *et al.* 1997 [51]	20	Non small cell lung cancer (stage III/IV)	TPZ 390 mg/m^2 1 h before cisplatin (75 mg/m^2) every 21 days	Temporary hearing loss (25%) Muscle cramps, skin rash, nausea, vomiting	25% major objective response 40% one-year survival	Further evaluation of TPZ with other agents in warranted.
Bedikian *et al.* 1997 [52]	48	Metastatic melanoma	TPZ 260 mg/m^2 1 h before cisplatin (75 mg/m^2) every 21 days	Muscle cramps, fatigue, nausea, vomiting	19% overall response	The TPZ-cisplatin combination is promising and warrants further studies with TPZ-other agents.

Doses of tirapazamine up to 390 mg/m^2 have been administered with or without chemotherapy, with similar side-effects reported. Moderate muscle cramping, nausea, vomiting and fatigue were the most commonly reported toxicities, with temporary hearing loss added for the highest dose [50].

Although a unanimous conclusion deriving from phase II trials was that tirapazamine is a promising agent in the treatment of advanced diseases, the number of phase III trials is very limited. Table **5** presents both closed and open phase III trials of tirapazamine and cisplatin with or without radiotherapy.

As indicated by phase II trials, combining tirapazamine with cisplatin resulted in successful outcomes for advanced disease in lung and head and neck cancers. Consequently, phase III trials were conducted on these tumour sites, for

stage III and IV cancers. CATAPULT I (Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors) [52] has studied the effect of cisplatin with and without tirapazamine showing superior results for the tirapazamine arm, therefore concluding that tirapazamine enhances the activity of cisplatin. Despite the positive results obtained by CATAPULT I, the subsequent trial CATAPULT II [53] did not support the findings. Trialing tirapazamine with cisplatin versus etoposide with cisplatin CATAPULT II reported superior outcome for the etoposide arm. These results indicate the need for further studies on the administration of tirapazamine when combined with chemotherapy.

Tirapazamine is also trialed in combination with multi-agent chemotherapy. An international trial is currently comparing vinorelbine/cisplatin with and without tirapa-

Table 5.   Phase III Clinical Trials of Tirapazamine and Cisplatin with or without Radiotherapy

Reference	No of Pts	Tumour Site/Type	Schedule	Toxicity	Outcome	Conclusions
International trial (Sanofi-Synthelabo)	Accruing	Head and neck (stage III/IV)	RT 70 Gy, 35 fractions and cisplatin 100mg/m^2 day 1 of weeks 1, 4, 7 of RT versus RT 70Gy, 35 fractions and TPZ 290mg/m^2 before cisplatin 75mg/m^2 day 1 of weeks 1, 4, 7 of RT and TPZ 160mg/m^2 days 1, 3, 5 of weeks 2, 3 of RT.	Open trial	Open trial	Open trial
von Pawel *et al.* 2000 [53] CATAPULT I	446	Non small cell lung tumours (stage III/IV)	TPZ 390 mg/m^2 1 h before cisplatin 75 mg/m^2 versus cisplatin 75 mg/m^2 alone every 3 weeks for 8 cycles	Generally, no significant difference between the two arms. However, nausea (86.3% versus 68.5%) and vomiting (81.7% versus 45.7%) were more significant in the TPZ arm	Median survival 34.6 versus 27.7 weeks (p value = 0.0078) Response rate 27.5% versus 13.7% (p value < 0.001)	TPZ enhances the activity of cisplatin
Shepherd *et al.* 2000 [54] CATAPULT II	539	Non small cell lung tumours (stage III/IV)	TPZ (390 mg/m^2) 1 h before cisplatin OR etoposide + cisplatin	Etoposide arm: anemia, fever, granulocytopenia. Tirapazamine arm: cramps, nausea, diarrhea, hearing decrease.	Median survival 26.7 versus 31.4 weeks (p value = 0.038) 1-year survival 21% versus 26.8%	The etoposide arm was superior to the TPZ arm

zamine and a SWOG (Southwest Oncology Group) trial evaluates the effect of paclitaxel/carboplatin with and without tirapazamine on lung cancers [25]. The optimal application of tirapazamine and the most effective combination with other therapeutic agents is the focus of ongoing clinical trials.

VI. CONCLUSIONS AND FUTURE DIRECTIONS

Regardless of the very promising results obtained by various pre-clinical studies and clinical trials of tirapazamine, this cytotoxin has not yet become part of routine practice. Although the last decade has elucidated some of the doubts regarding tirapazamine's properties, there are still unanswered questions concerning its toxicity (the cause of muscle cramping), and the exact mechanism behind the synergistic effect when combined with cisplatin. There is, however, agreement regarding several observations derived from previous investigations:

- Tirapazamine is a hypoxia-selective bioreductive agent, which is reduced to a highly reactive radical that produces strand breaks in the DNA.

- Although *in vitro* studies with tirapazamine showed that the drug is highly effective in killing cells under

hypoxic conditions, clinical trials did not achieve significant results with tirapazamine as a sole agent.

- Tirapazamine potentiates the effect of both radiation and cisplatin. When administered before chemo/radiotherapy, tirapazamine adds synergistically to the killing effect of both radiation and cisplatin.

- The majority of trials reported successful outcomes for advanced disease in head and neck, and lung cancers when combining tirapazamine with radiation/cisplatin.

- Dose escalation studies (Phase I trials) considered the 330 mg/m^2 tirapazamine as the maximum tolerated dose.

- Tirapazamine contributes only a low level of added toxicity when administered with cisplatin.

On the other hand, the lack of comprehensive data on certain aspects concerning tirapazamine as a hypoxic cytotoxin might be the reason behind the scarcity of phase III trials and the reticence in using the drug more widely. Here are some thoughts:

- Tirapazamine is a potent hypoxic cytotoxin, however some tumours might not present with a large-enough

population of hypoxic cells so the expected effect of this cytotoxic drug could be overestimated. Predictive assays on tumour oxygen status would differentiate between tumours indicating the most responsive ones to tirapazamine.

- Both pre-clinical and clinical studies have shown better treatment outcome when tirapazamine was administered in combination with other chemotherapeutic agents (especially cisplatin) than when administered as a sole agent. These results have demonstrated that tirapazamine acts more efficiently as a potentiator of chemotherapy than as an independently active cytotoxic drug. The same conclusion can be drawn from irradiated hypoxic tumours treated previously with tirapazamine. The a priori treatment with the drug has demonstrated higher efficiency in tumour control than the concurrent administration of tirapazamine and radiation. Therefore, tirapazamine has proven to be more efficient as a potentiator of radiotherapy than as an independently active cytotoxin.

- Although the majority of phase I trials have considered 330 mg/m2 tirapazamine as the maximum tolerated dose, some phase III trials (CATAPULT II) have used doses as high as 390 mg/m2 which added to chemotherapy-related toxicities, therefore reducing the expected therapeutic ratio.

- The most common anticancer drug tested in combination with tirapazamine was cisplatin. There is a lack of trials testing the efficiency of multiagent chemotherapy with tirapazamine, therefore whether tirapazamine/cisplatin is superior to multiagent therapy is still being investigated.

Tirapazamine is still advancing through clinical trials in combination with either single or multiagent therapy, therefore further progress in the study of kinetics and dynamics of the drug is expected.

REFERENCES

[1]   Brown JM. The hypoxic cell: a target for selective cancer therapy. Cancer Res 1999; 59: 5863-70.

[2]   Amellem O, Pettersen EO. Cell inactivation and cell cycle inhibition as induced by extreme hypoxia: the possible role of cell cycle arrest as a protection against hypoxia-induced lethal damage. Cell Prolif 1991; 24: 127-41.

[3]   Ljungkvist AS, Bussink J, Rijken PF, Kaanders JH, van der Kogel AJ, Denekamp J. Vascular architecture, hypoxia, and proliferation in first-generation xenografts of human head-and-neck squamous cell carcinomas. Int J Radiat Biol Oncol Phys 2002; 54: 215-28.

[4]   Bennewith KL, Durand, RE. Quantifying transient hypoxia in human tumour xenografts by flow cytometry. Cancer Res 2004; 64: 6183-9.

[5]   Henk JM. Late results of a trial of hyperbaric oxygen and radiotherapy in head and neck cancer: a rationale for hypoxic cell radiosensitizers. Int J Radiat Oncol Biol Phys 1986; 12: 1339-41.

[6]   Brown JM. Clinical trials of radiosensitizers: what should we expect? Int J Radiat Oncol Biol Phys 1984; 10:425-9.

[7]   Dische S. Chemical sensitizers for hypoxic cells: a decade of experience in clinical radiotherapy. Radiother Oncol 1985; 3: 97-115.

[8]   Lee DJ, Pajak TF, Marcia VA, et al.. Results of an RTOG Phase III trail (RTOG 85-27) comparing radiotherapy plus etanidazole with radiotherapy alone for locally advanced head and neck carcinomas. Int J Radiat Oncol Biol Phys 1995; 32: 567-76.

[9]   Peters LJ. Targeting hypoxia in head and neck cancer. Acta Oncol 2001; 40: 937-40.

[10]   Denekamp J, Daşu A. Inducible repair and the two forms of tumour hypoxia-time for a paradigm shift. Acta Oncol 1999; 38: 903-18.

[11]   Wang J, Biedermann KA, Brown JM. Repair of DNA and chromosome breaks in cells exposed to SR 4233 under hypoxia or to ionizing radiation. Cancer Res 1992; 52: 4473-7.

[12]   Zeman EM, Brown JM, Lemmon MJ, Hirst VK, Lee WW. SR-4233: A new bioreductive agent with high selective toxicity for hypoxic mammalian cells. Int J Radiat Oncol Biol Phys 1986; 12: 1239-42.

[13]   Durand RE, Olive PL. Physiologic and cytotoxic effects of tirapazamine in tumor-bearing mice. Radiat Oncol Investig 1997; 5: 213-9.

[14]   Siim BG, Menke DR, Dorie MJ, Brown JM. Tirapazamine-induced cytotoxicity and DNA damage in transplanted tumors: relationship to tumor hypoxia. Cancer Res 1997; 57: 2922-8.

[15]   Lartigau E, Guichard M. Does tirapazamine (SR-4233) have any cytotoxic or sensitizing effect on three human tumour cell lines at clinically relevant partial oxygen pressure? Int J Radiat Biol 1995; 67: 211-6.

[16]   Olive PL. Detection of hypoxia by measurement of DNA damage in individual cells from spheroids and murine tumours exposed to bioreductive drugs. I. Tirapazamine. Br J Cancer 1995; 71: 529-36.

[17]   Dorie MJ, Menke D, Brown JM. Comparison of the enhancement of tumor responses to fractionated irradiation by SR 4233 (tirapazamine) and by nicotinamide with carbogen. Int J Radiat Oncol Biol Phys 1994; 28: 145-50.

[18]   Zhang M, Stevens G. Effect of radiation and tirapazamine (SR-4233) on three melanoma cell line. Melanoma Res 1998; 8: 510-5.

[19]   Shibata T, Shibamoto Y, Sasai K, et al. Tirapazamine: hypoxic cytotoxicity and interaction with radiation as assessed by the micronucleus assay. Br J Cancer 1996; 27: S61-4.

[20]   Masunaga S, Ono K, Hori H, et al. Effects of bioreductive agents, tirapazamine and mitomycin C, on quiescent cell populations in solid tumors, evaluated by micronucleus assay. Jpn J Cancer Res 1997; 88: 907-14.

[21]   Masunaga S, Ono K, Hori H, et al. Change in oxygenation status in intratumour total and quiescent cells following gamma-ray irradiation, tirapazamine administration, cisplatin injection and bleomycin treatment. Br J Radiol 2000; 73: 978-86.

[22]   Masunaga S, Ono K, Hori H, et al. Potentially lethal damage repair by total and quiescent tumor cells following various DNA-damaging treatments. Radiat Med 1999 ;17: 259-64.

[23]   Lambin P, Guichard M, Chavaudra N, Malaise EP. The effect of the hypoxic cell drug SR-4233 alone or combined with the ionizing radiations on two human tumor cell lines having different radiosensitivity. Radiother Oncol 1992; 24: 201-4.

[24]   Reed E, Kohn KW. Platinum analogues, in Chabner B, Collins JM (ed): Cancer chemotherapy-principles and practice. Philadelphia: Lippincott, 1990.

[25]   Gandara DR, Lara PN, Goldberg Z, et al. Tirapazamine: prototype for a novel class of therapeutic agents targeting tumour hypoxia. Sem Oncol 2002; 29: 102-9.

[26]   Dorie MJ, Brown JM. Tumour-specific, schedule-dependent interaction between tirapazamine (SR 4233) and cisplatin. Cancer Res 1993; 53: 4633-36.

[27]   Rischin D, Peters L, Hicks R, et al. Phase I trial of concurrent tirapazamine, cisplatin, and radiotherapy in patients with advanced head and neck cancer. J Clin Oncol 2001; 19: 35-42.

[28]   Prestayko AW, Crooke ST, Carter SK. Cisplatin – current status and new developments. Academic Press 1980.

[29]   Kovacs MS, Hocking DJ, Evans JW, Siim BG, Wouters BG, Brown JM. Cisplatin anti-tumour potentiation by tirapazamine results from hypoxia-dependent cellular sensitization to cisplatin. Br J Cancer 1999; 80: 1245-51.

[30]   Coughlin CT, Richmond RC. Biologic and clinical developments of cisplatin combined with radiation: concepts, utility, projections for new trials, and the emergence of carboplatin. Semin Oncol 1989; 16: 31S-43S.

[31]   Lin PS, Ho KC, Yang SJ. Tirapazamine (SR 4233) interrupts cell cycle progression and induces apoptosis. Cancer Lett 1996; 105: 249-55.

[32]   Sorenson CM, Barry MA, Eastman A. Analysis of events associated with cell cycle arrest at G2 phase and cell death induced by cisplatin. J Natl Cancer Inst 1990; 82: 749-54.

[33]   Yan R, Durand RE. The response of hypoxic cells in SCCVII murine tumors to treatment with cisplatin and X-rays. Int J Radiat Oncol Biol Phys 1991; 20: 271-4.

[34]   Yoshikawa A, Saura R, Matsubara T. A mechanism of cisplatin action: antineoplastic effect through inhibition of neovascularization. Kobe J Med Sci 1997; 43: 109-20.

[35]   Goldberg Z, Evans J, Birrell G, Brown JM. An investigation of the molecular basis for the synergistic interaction of tirapazamine and cisplatin. Int J Radiat Oncol Biol Phys 2001; 49: 175-82.

[36]   Wouters BG, Wang LH, Brown JM. Tirapazamine: a new drug producing tumor specific enhancement of platinum-based chemotherapy in non-small-cell lung cancer. Ann Oncol 1999; 10: S29-33.

[37]   Siemann DW, Hinchman CA. Potentiation of cisplatin activity by the bioreductive agent tirapazamine. Radiother Oncol 1998; 47: 215-20.

[38]   Lartigau E, Guichard M. The effect of tirapazamine (SR-4233) alone or combined with chemotherapeutic agents on xenografted human tumours. Br J Cancer 1996; 73: 1480-5.

[39]   Le QT, McCoy J, Williamson S, *et al*. Phase I study of tirapazamine plus cisplatin/etoposide and concurrent thoracic radiotherapy in limited-stage small cell lung cancer (S0004): a Southwest Oncology Group study. Clin Cancer Res 2004; 10: 5418-24.

[40]   Craighead PS, Pearcey R, Stuart G. A phase I/II evaluation of tirapazamine administered intravenously concurrent with cisplatin and radiotherapy in women with locally advanced cervical cancer. Int J Radiat Oncol Biol Phys 2000; 48: 791-5.

[41]   Peters LJ, Rischin D, Hicks RJ, Hughes PG, Sizeland AM. Extraordinary tumor control in phase I trial of concurrent tirapazamine cisplatin and radiotherapy for far advanced head and neck cancer. Int J Radiat Oncol Biol Phys 1999; 45: 148-9.

[42]   Shulman LN, Buswell L, Riese N, *et al*. Phase I trial of the hypoxic cell cytotoxin tirapazamine with concurrent radiation therapy in the treatment of refractory solid tumours. Int J Radiat Oncol Biol Phys 1999; 44: 349-53.

[43]   Aghajanian C, Brown C, O'flaherty C, *et al*. Phase I study of tirapazamine and cisplatin in patients with recurrent cervical cancer. Gynecol Oncol 1997; 67: 127-30.

[44]   Johnson CA, Kilpatrick D, von Roemeling R, *et al*. Phase I trial of tirapazamine in combination with cisplatin in a single dose every 3 weeks in patients with solid tumors. J Clin Oncol 1997; 15: 773-80.

[45]   Senan S, Rampling R, Graham MA, *et al*. Phase I and pharmacokinetic study of tirapazamine (SR 4233) administered every three weeks. Clin Cancer Res 1997; 3: 31-8.

[46]   Doherty N, Hancock SL, Kaye S, *et al*. Muscle cramping in phase I clinical trials of tirapazamine (SR 4233) with and without radiation. Int J Radiat Oncol Biol Phys 1994; 29: 379-82.

[47]   Rischin D, Peters L, Fisher R, *et al*. Tirapazamine, Cisplatin, and Radiation versus Fluorouracil, Cisplatin, and Radiation in patients with locally advanced head and neck cancer: a randomized phase II trial of the Trans-Tasman Radiation Oncology Group (TROG 98.02). J Clin Oncol 2005; 23: 79-87.

[48]   Reck M, von Pawel J, Nimmermann C, Groth G, Gatzemeier U. Phase II-trial of tirapazamine in combination with cisplatin and gemcitabine in patients with advanced non-small-cell-lung-cancer (NSCLC). Pneumologie 2004; 58: 845-9.

[49]   Lee DJ, Trotti A, Spencer S, *et al*. Concurrent tirapazamine and radiotherapy for advanced head and neck carcinomas: a Phase II study. Int J Radiat Oncol Biol Phys 1998; 42: 811-5.

[50]   Treat J, Johnson E, Langer C, *et al*. Tirapazamine with cisplatin in patients with advanced non-small-cell lung cancer: a phase II study. J Clin Oncol 1998;16: 3524-7.

[51]   Miller VA, Ng KK, Grant SC, *et al*. Phase II study of the combination of the novel bioreductive agent, tirapazamine, with cisplatin in patients with advanced non-small-cell lung cancer. Ann Oncol 1997; 8: 1269-71.

[52]   Bedikian AY, Legha SS, Eton O, *et al*. Phase II trial of tirapazamine combined with cisplatin in chemotherapy of advanced malignant melanoma. Ann Oncol 1997; 8: 363-7.

[53]   von Pawel J, von Roemeling R, Gatzemeier U, *et al*. Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATAPULT I study group. Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors. J Clin Oncol 2000; 18: 1351-9.

[54]   Shepherd F, Koschel G, von Pawel J, *et al*. Comparison of Tirazone (Tirapazamine) and cisplatin *vs*. etoposide and cisplatin in advanced non-small cell lung cancer (NSCLC): Final results of the international Phase III CATAPULT II Trial. Lung Cancer 2000; 29: S28 (abstract only).