Mortality from non-Hodgkin’s lymphoma (NHL) is high, thus defining the need for additional therapeutic agents for this disease. Gallium nitrate is a metal compound that is presently approved for the treatment of hypercalcaemia associated with malignancy. In clinical trials first conducted over two decades ago, this drug was found to have antineoplastic activity in NHL. However, its development as an antineoplastic agent for the treatment of NHL was never rigorously pursued. Gallium has unique mechanisms of action that include its binding to transferrin in the circulation and targeting transferrin receptors present on lymphoma cells. As it shares chemical properties with iron, gallium can disrupt critical steps in iron homeostasis that are essential for tumour cell viability and growth and can inhibit the iron-dependent activity of ribonucleotide reductase. The drug may also target other cellular processes unrelated to iron. Phase I/II studies have shown that gallium nitrate displays the most efficacy and lowest toxicity in NHL when administered as a continuous intravenous infusion, producing response rates of 43% in patients with relapsed or refractory NHL. It does not suppress the white blood cells or platelets and does not share cross-resistance with other chemotherapeutic drugs. These characteristics make it particularly attractive for the treatment of myelosuppressed patients and for incorporation into combination therapy. Multi-institutional Phase II clinical trials are in progress to evaluate gallium nitrate as a single agent or in combination. These studies will help define its role in the current treatment of NHL.

Keywords: clinical trials, chemotherapy, gallium, iron, non-Hodgkin’s lymphoma, ribonucleotide reductase, transferrin receptor
Gallium nitrate

tumour models [9,10]. These studies revealed that gallium nitrate significantly inhibited the growth of a variety of murine tumours [9,10]. Following preclinical toxicology studies of gallium nitrate in animals to establish its toxicity profile and toxic dose levels [9-12], gallium nitrate was designated as an NCI investigational drug (NSC-15200) and Phase I and II clinical trials were pursued. These studies revealed the drug to have antitumour activity in urothelial malignancies and lymphoma [13-18]. The potential of gallium nitrate as a novel chemotherapeutic drug for the treatment of NHL will be the focus of this review. Other general reviews on gallium have previously been presented [12,19,20].

2. Chemistry

Gallium, with an atomic weight of 69.72 and an atomic number of 31, is a group IIIa metal that was discovered in 1875. It is a greyish metal with a greenish-blue reflection and a melting point of 29.78°C. Its coordinate chemistry is similar to that of Al3+ and In3+. Importantly, it shares certain properties with Fe3+ with respect to ionic radii and bonding; however, unlike Fe3+ that can be readily reduced to Fe2+, gallium exists as Ga3+ and is not reducible under physiological conditions. Although it binds avidly to both metal binding sites on transferrin, the iron transport protein present in the circulation, transferrin binds gallium with 300-fold less affinity than ferric ion [21]. The ability of gallium to displace iron in iron-containing proteins is linked to its antineoplastic activity, since iron is critical for cell viability and proliferation.


Although the mechanisms of cytotoxicity of gallium in NHL are only partly understood, several preclinical investigations support a model in which its antineoplastic activity can be considered to occur as a two-step process. The first involves the formation of transferrin–gallium complexes in the circulation and the targeting of these complexes to transferrin receptors present on lymphoma cells. These receptors, in turn, facilitate gallium incorporation and localisation in cells. The second step involves the trafficking of the incorporated gallium to specific intracellular targets leading to the induction of apoptosis. These steps will be discussed in detail.

3.1 Gallium transport in the circulation and cellular uptake

Investigations into the mechanisms involved in the transport and cellular uptake of radioactive 67Ga have provided clues to the transport and cellular handling of nonradioactive gallium. Early studies demonstrated that 67Ga injected into rabbits was transported in the circulation exclusively bound to transferrin [22]. Computer modelling of the speciation of nonradioactive gallium in the plasma suggested that at concentrations below which the transferrin metal binding sites would be saturated, >99.9% of gallium in the circulation will exist as transferrin–gallium. However, as transferrin binding is exceeded, more gallium would exist as gallate, Ga(OH)4-, and less as transferrin–gallium [19]. In vitro studies conducted with malignant cell lines demonstrated that transferrin enhanced the cellular uptake of 67Ga and the cytotoxicity of gallium nitrate [23-26]. Other studies showed that cellular 67Ga uptake in vitro and in vivo was mediated by transferrin receptors and could be inhibited by an antitransferrin receptor monoclonal antibody [27,28]. The initial steps in 67Ga uptake by transferrin receptor-mediated endocytosis of 67Ga–transferrin complexes appear to be similar to that of transferrin–iron complexes [29]. While these studies confirm the role of transferrin and its receptor in 67Ga uptake, it should be noted that in some cells, 67Ga might also be taken up via a transferrin-independent pathway [27,30].

3.2 Inhibitory effects of gallium on cellular proliferation

Gallium nitrate inhibits the growth of a variety of malignant cell types, in particular myeloid and lymphoid leukaemic cell lines [25,31,32]. One mechanism responsible for the antiproliferative activity of gallium nitrate is the disruption of cellular iron homeostasis and the inhibition of ribonucleotide reductase, an iron-containing enzyme essential for deoxyribonucleotide synthesis [26,33-36]. This enzyme is composed of dimeric R1 and R2 (also termed M1 and M2) subunits [27,38]. The R2 subunit contains a binuclear iron center and a tyrosyl free radical that are essential for enzyme function [39,40]. Transferrin–gallium complexes interfere with the transferrin receptor-mediated cellular uptake of iron and its intracellular trafficking, thus inducing a state of intracellular iron deprivation [25,36]. The resultant block in iron availability to the R2 subunit leads to an inhibition in ribonucleotide reductase activity [34,41]. Interestingly, gallium nitrate also inhibits ribonucleotide reductase activity in cell lysates, indicating that it can inhibit this enzyme independently of its effect on iron transport into cells [42]. Hence, the inhibitory effects of gallium on ribonucleotide reductase activity appears to be due to indirect (cellular iron deprivation) as well as direct mechanisms.

Gallium has been shown to have additional effects on processes related to cell proliferation. Early studies found that gallium nitrate partially inhibited DNA polymerases, but the extent of inhibition was insufficient to explain the antitumour activity of gallium, and it was therefore concluded that other mechanisms were involved [43]. It has been shown to inhibit tyrosine phosphatase in Jurkat and human colon tumour (HT-29) cells [44]. However, a correlation between the inhibition of this enzyme and cell proliferation was not found, hence, the significance of this effect to the antineoplastic activity of gallium is not clear [44]. Gallium may compete with magnesium and inhibit magnesium-dependent ATPase [45]. In a cell-free assay in vitro, GaCl3 can inhibit the polymerisation of tubulin, an effect that might contribute to its antitumour activity [46].
activity [46]. Recent studies show that gallium nitrate induces apoptotic cell death in lymphoma cells through mechanisms that involve Bax (a pro-apoptotic protein), cytochrome c release from mitochondria and the activation of caspases (Chitambar et al., manuscript in preparation).

Additional studies have demonstrated that when combined with hydroxyurea, fludarabine or gemcitabine (all inhibitors of ribonucleotide reductase), gallium nitrate synergistically inhibits the growth of leukemic and lymphoma cell lines in vitro [34,47,48]. Synergism between gallium nitrate and IFN-α or paclitaxel has also been demonstrated, although the mechanism of synergy is not known [49,50].

4. Effects of gallium nitrate on other processes

While the potential role of gallium nitrate in the treatment of NHL is the subject of this review, it is important to note that gallium nitrate has significant efficacy in lowering blood calcium levels and is an approved drug for the treatment of hypercalcaemia associated with malignancy. In studies evaluating the continuous intravenous infusion of gallium nitrate for the treatment of NHL (see Section 5), two-thirds of the patients treated developed hypercalcaemia [14]. Several studies have demonstrated the antiresorptive effects of gallium nitrate in bone metastases, multiple myeloma and metabolic bone disease, including Paget’s disease and osteoporosis [51-53]. The preclinical and clinical studies of gallium nitrate in hypercalcaemia and disorders of bone metabolism have been reviewed elsewhere [53-55].

Gallium nitrate also displays immunosuppressive activity in animal models of immune disease such as adjuvant arthritis, graft versus host disease, cardiac transplant rejection and allergic autoimmune encephalomyelitis [56-59]. Gallium can suppress mitogen-activated T- and B-cell proliferation and immunoglobulin production and can inhibit the release of IL-6, TNF-α and nitric oxide from macrophages [57,60,61]. It is very likely that some of the immunomodulatory effects of gallium nitrate are due to the inhibition of mitogen-activated T- and B-cell proliferation, since these cells, once activated, display high densities of transferrin receptors thus making them targets for transferrin–gallium complexes [60-63]. The clinical significance of these immunomodulatory effects has not been evaluated in humans.

5. Pharmacokinetics

The pharmacokinetics of gallium nitrate, administered as a brief infusion or as a continuous intravenous infusion, has been examined. Excretion of gallium is primarily through the kidney. In a study by Kelsen et al. [64], plasma levels of gallium following a brief intravenous infusion (30 min) of gallium nitrate displayed a biphasic excretion pattern, with an α-phase half-life of 8.3 – 26 min and a β-phase half-life of 6.3 – 196 h. The latter phase represented gallium binding to plasma protein, presumably transferrin. A total of 69 and 91% of the administered dose was excreted in the urine by 24 and 48 h, respectively [64]. In another study, Krakoff et al. examined the pharmacokinetics of gallium following a brief intravenous infusion of gallium nitrate at three dose levels (500, 750 and 900 mg/m²). Biphasic serum gallium disappearance curves with an α-phase half-life of 87 min and β-phase half-life of 24.5 h were noted. Of the infused gallium, ~ 65% was recovered in the urine during the first 24 h following infusion, with 50% of this amount appearing within the first 4 h after injection [65]. Since higher doses of gallium produced renal toxicity (see Section 6), these investigators examined the effect of concurrent hydration and mannitol-induced diuresis on gallium pharmacokinetics. With this approach, an increase in the amount of gallium excreted in the urine occurred during the first hour following drug administration in patients receiving mannitol. However, subsequent urinary excretion of gallium (measured at ≤ 24 h) was no different from that of patients not receiving mannitol. Although mannitol diuresis did not affect the serum levels of gallium or the total amount of gallium excreted in the urine, it did alter the concentration of gallium excreted in the urine.

When administered as a continuous intravenous infusion for 7 days, mean steady-state plasma levels of gallium in two patients receiving 200 mg/m²/day were achieved by 2 – 3 days, with urinary excretion approximately matching the daily dose of the drug. Urinary excretion of gallium over 11 – 14 days ranged from 68 – 107% of the total amount administered. Mean plasma gallium concentration during steady-state infusion at this dose level ranged from 0.9 – 1.9 µg/ml and decreased to 0.45 – 0.7 µg/ml when measured 4 days after completion of the infusion. It is important to note that twice as much gallium was administered with minimal nephrotoxicity by the 7-day continuous infusion schedule than in comparison with the brief infusion schedule [64].

6. Clinical efficacy and toxicity

6.1 Phase I studies

Phase I studies have revealed that the toxicity profile and maximum tolerated dose of gallium nitrate are significantly affected by its mode of administration. Three treatment schedules have been examined: a brief intravenous infusion given over 30 – 60 min every 2 – 3 weeks, a short daily intravenous infusion for 3 consecutive days and a continuous intravenous infusion for 7 days. With the brief 30 – 60 min infusion, renal toxicity was found to be dose-limiting at doses greater than 700 mg/m² [66]. Other side effects encountered included a metallic taste, hearing loss, diarrhoea, nausea and vomiting, anaemia, leukopenia and thrombocytopenia [66]. A similar toxicity profile, with renal toxicity being dose-limiting, was observed when gallium nitrate was administered at a dose of > 300 mg/m²/day by short infusion for 3 days every 2 – 3 weeks [67]. Anaemia was also found to be dose related. Gastrointestinal toxicity (nausea, vomiting and diarrhoea) was mild with this schedule. The toxicities of gallium nitrate administered as a continuous 7-day infusion are discussed in detail with the Phase II studies.
Patient hydration status and diuresis are important in modulating the potential renal toxicity of gallium nitrate. Studies in rats have demonstrated that renal damage results from the deposition of precipitates that occlude the lumen of the renal tubules (obstructive nephropathy). Newman et al. demonstrated that in the absence of hydration and osmotic diuresis, these flocculent precipitates contained high concentrations of gallium associated with calcium and phosphorus. With isosorbide-induced diuresis these precipitates were fewer in number and were composed of calcium and phosphorus with very little gallium [11].

6.2 Phase II clinical trials of gallium nitrate in lymphoma

The efficacy of gallium nitrate therapy in lymphoma has been evaluated in five different clinical trials. In three trials it was examined as a single agent and in two trials it was used in combination with other drugs. In all these studies, clinical antineoplastic activity was demonstrated against NHL that had failed to respond to or had recurred following conventional chemotherapy. These studies and response rates are summarised in Table 1 and are discussed below.

6.2.1 Studies of gallium nitrate as a single agent

6.2.1.1 Southwestern Oncology Group trial

In a study conducted by the Southwestern Oncology Group [13], gallium nitrate was administered by brief intravenous infusion at a dose of 700 mg/m² every 2 weeks to patients with relapsed NHL. All patients had previously been treated with chemotherapy and had received a mean of 3.3 (range 1 – 7) chemotherapeutic drug combinations. Of the 38 patients on the study, 26 had diffuse histologies (histiocytic, mixed, well differentiated or poorly differentiated), 5 had nodular histologies (poorly differentiated, mixed or histiocytic) and 7 had Hodgkin’s disease. Because of early deaths (within 4 – 13 days of starting the treatment), 33 patients could be fully evaluated for response to treatment, whereas only 5 could be partially evaluated. Responses to treatment were seen in 6 of 26 patients with diffuse histology, whereas no responses were noted in five patients with nodular histology. Interestingly, three of the patients who responded to gallium nitrate in the diffuse histology group had failed to respond to prior chemotherapy (which included cytoxan, doxorubicin, vincristine, prednisone and bleomycin), suggesting that gallium nitrate does not share cross-resistance with these drugs. One patient with Hodgkin’s disease responded to treatment as well. In this study, the overall response rate (all groups included) was 18% when the analysis was conducted on an intent-to-treat basis (36 patients) and was 21% when only those evaluated were considered (n = 33). Within the diffuse histology subgroup, the response rate was 23% (6 of 26) and the duration of response was 3 – 11 months.

In this study, five early deaths, unrelated to treatment, occurred between days 4 and 13. Three patients with diffuse NHL died, two from pneumonitis consistent with an infectious process, uncomplicated by haematological or renal toxicity and one from gastrointestinal bleeding. The other two patients who died had progressive renal failure due to lymphoma involving the renal parenchyma. The toxicities encountered in this study were haematological (low white cell and/or platelet counts) and of moderate-to-life-threatening severity in five patients. Two patients experienced diminished vision that was not accompanied by ophthalmological abnormalities and was spontaneously corrected in 3 months. Other toxicities included hypocalcaemia in three patients and mild-to-severe renal toxicity in five patients. No toxic effects were noted in 10 patients.

6.2.1.2 Southeastern Cancer Study Group trial

In a trial conducted by the Southeastern Cancer Study Group [15], 138 patients with lymphoproliferative diseases (NHL, Hodgkin’s disease and chronic lymphocytic leukaemia) were treated with gallium nitrate 700 mg/m² administered as a bolus intravenous infusion every 2 weeks. 131 patients could be evaluated for response. Treatment responses were seen in 5 of 8 (63%) patients with well-differentiated NHL, 1 of 11 (9%) patients with diffuse poorly differentiated lymphoma, 1 of 6 (17%) patients with diffuse mixed lymphoma, 1 of 5 (20%) patients with nodular histiocytic lymphoma, 1 of 36 (3%) patients with diffuse histiocytic lymphoma and 4 of 31 (13%) patients with Hodgkin’s disease. None of the 17 patients with chronic lymphocytic leukaemia responded to treatment. The overall response rate for the entire group of patients was 10% with the median duration of response lasting 3.3 months.

The toxicities encountered in this study were considered to be mild and were primarily gastrointestinal (nausea and vomiting in 18 patients, diarrhoea in 20 patients and stomatitis in 4 patients), reversible renal dysfunction (creatinine clearance was reduced by <50% in 23 patients and >50% in 4 patients) and haematological (granulocytopenia in 12 patients, anaemia in 43 patients and thrombocytopenia in 11 patients). Hypocalcaemia, associated with a rise in serum creatinine, was encountered in four patients and a metallic taste was reported by some patients.

6.2.1.3 Memorial Sloan-Kettering Cancer Center trial

An important Phase I/II clinical trial, which examined the efficacy and toxicity of a continuous intravenous infusion of gallium nitrate administered for 7 consecutive days in patients with relapsed NHL or Hodgkin’s disease, was conducted by Warrell et al. [14]. All patients had received extensive prior treatment for their disease. A total of 27 patients were entered into the Phase I portion of the study and were treated at dose levels ranging from 200 – 400 mg/m²/day for 7 days. Since renal toxicity was encountered in some patients at the highest dose level, the investigators established a dose of 300 mg/m²/day as an acceptable dose for the second phase of the study and evaluated an additional 37 patients at this dose level. Objective responses to treatment were noted in both the Phase I and II portions of the study. A total of 47 patients with measurable biddimensional disease were evaluated for a response to gallium
Table 1. Phase I - II clinical studies of gallium nitrate in lymphoma (non-Hodgkin's lymphoma and Hodgkin's disease).

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Schedule</th>
<th>Number of patients</th>
<th>Response rate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weick et al. [13]</td>
<td>Gallium nitrate 700 mg/m² i.v. infusion over 30 min every 2 weeks</td>
<td>38 entered, 33 evaluated</td>
<td>18% (23% response in diffuse NHL)</td>
</tr>
<tr>
<td>Keller et al. [15]</td>
<td>Gallium nitrate 700 mg/m² i.v. infusion over 15 - 30 min every 2 weeks</td>
<td>138 entered, 131 evaluated</td>
<td>10%</td>
</tr>
<tr>
<td>Warrell et al. [14]</td>
<td>Gallium nitrate 200 - 400 mg/m²/day continuous i.v. for 7 days</td>
<td>27 in Phase I, 37 in Phase II, 47 evaluated for response</td>
<td>34% (43% response in NHL)</td>
</tr>
<tr>
<td>Warrell et al. [16]</td>
<td>Gallium nitrate 7-day infusion plus mitoguazone plus etoposide</td>
<td>43 treated, 42 evaluated for response</td>
<td>52%</td>
</tr>
<tr>
<td>Chitambar et al. [17]</td>
<td>Gallium nitrate 7-day infusion plus oral hydroxyurea</td>
<td>14 treated</td>
<td>43%</td>
</tr>
</tbody>
</table>

*Response rates shown are for overall responses in both NHL and Hodgkin's disease. Response rates vary with different histological subtypes.
NHL: Non-Hodgkin's lymphoma.

Gallium nitrate. Responses were seen in 6 of 15 (40%) patients with diffuse large cell lymphoma (including two complete remissions), 5 of 10 (50%) patients with diffuse poorly differentiated lymphocytic lymphoma and 2 of 5 (40%) patients with nodular poorly differentiated lymphocytic lymphoma. In addition, 3 of 17 (18%) patients with Hodgkin's lymphoma responded to treatment. The response rate for NHL patients was 43%, while the overall response rate for the entire group was 34% with a median duration of response of 2.5 months. However, some patients with NHL had responses lasting >14 months at the time of this report.

The toxicities encountered in this study were as follows. In contrast to other studies evaluating gallium nitrate as a rapid intravenous infusion, the administration of gallium nitrate by continuous intravenous infusion over a dose range of 200 – 400 mg/m²/day for 7 consecutive days every 3 – 5 weeks resulted in nausea and vomiting rather than renal dysfunction as the dose-limiting toxicities. In the Phase I evaluation, only 1 of 7 patients developed an increase in serum creatinine to >1.5 mg/dl at the 300 mg/m²/day dose level, while at the 400 mg/m²/day dose level, an increase in serum creatinine to >1.5 mg/dl was seen in 3 of 10 patients. However, it was felt that nausea and a decrease in oral fluid intake, leading to dehydration, was a significant factor in the development of renal toxicity in these patients. In general, all 37 patients treated in the Phase II evaluation (gallium nitrate 300 mg/m²/day for 7 days) tolerated this treatment very well. Hypocalcaemia occurred in two-thirds of patients whereas hypomagnesaemia occurred in one-third of patients. The haematological toxicity encountered was primarily microcytic hypochromic anaemia (median decrease in haemoglobin 1.8 g/dl). In patients with pre-existing normal white blood cell and platelet counts, only three developed leukopenia and one developed thrombocytopenia (platelets < 50,000/mm³). Additional toxicities in the entire study group (64 patients) included pulmonary complications (fever, dyspnoea, pleural effusions and lung infiltrates in 14%), nausea (6%), paresthesia (5%), diarrhoea (5%) and decreased auditory acuity (3%). Nausea and vomiting was not seen at the 300 mg/m²/day dose level.

6.2.2.2 Combination gallium nitrate and hydroxyurea
In a second study of combination chemotherapy, a continuous intravenous infusion of gallium nitrate was administered along with oral hydroxyurea to patients with relapsed
Gallium nitrate

low-grade and intermediate-grade (diffuse large cell) NHL [17]. The rationale for combining these drugs was based on preclinical studies that demonstrated synergy between gallium and hydroxyurea in leukaemic cell lines [34]. This synergy appears to result from a combined inhibition of the R2 subunit of ribonucleotide reductase by action on different sites of the subunit. Gallium interferes with the incorporation of iron into R2 protein, whereas hydroxyurea destroys the tyrosyl free radical of R2 [34,41]. In this study, patients with stage III or IV NHL that had relapsed after treatment with conventional chemotherapy were treated at four different dose levels of continuous intravenous infusion gallium nitrate (200, 250, 300 or 350 mg/m²/day for 7 days), with a minimum of three patients treated at each dose level. The three patients treated with 200 mg/m²/day gallium nitrate dose level also received hydroxyurea 500 mg/day orally for 7 days, whereas patients treated at the higher dose levels of gallium nitrate received hydroxyurea 1000 mg/day for 7 days. Treatment cycles were repeated every 3 - 4 weeks. Of the 14 patients treated, one attained a complete remission, one attained a near-complete remission and four attained a partial response (> 50% shrinkage in the size of measurable lymphoma). Four additional patients had minor responses (stable disease) while other patients had progression of their disease during treatment. Responses to treatment were seen at all dose levels of gallium nitrate resulting in an overall response rate of 43% (complete plus partial responses). The median duration of response to treatment was 7 weeks (range 3 - 38 weeks).

The toxicities of treatment in this study were anaemia (grade 2 in two patients) and reversible renal dysfunction (grades 2 and 3 in two patients and one patient, respectively). Asymptomatic hypocalcaemia, consistent with the known effects of gallium nitrate on bone metabolism, occurred in 10 patients. Other toxicities encountered were relatively minor (grade 1) and were consistent with the known individual side effects of each drug. These included neutropaenia, nausea, vomiting, skin rash, mucositis and diarrhoea (all grade 1).

6.3 Current, ongoing clinical trials with gallium nitrate
A multi-institutional Phase II clinical trial to confirm the efficacy of gallium nitrate in NHL is in progress in the U.S. This study, sponsored by Genta Inc. (Berkeley Heights, N.J., USA) the manufacturer of gallium nitrate (Ganite™ ), is evaluating the clinical activity and toxicity of gallium nitrate in patients with NHL whose disease has relapsed after treatment or is refractory to treatment. Prior studies indicated that higher response rates and lower toxicity were seen with the continuous infusion schedule; the present study, therefore, is evaluating the clinical activity of gallium nitrate administered at 300 mg/m²/day or 200 mg/m²/day by continuous intravenous infusion for 7 consecutive days. It is anticipated that the results of this study will be available later this year.

7. Safety and tolerability
Gallium nitrate, administered as a 7-day continuous intravenous infusion, is generally well-tolerated, even by elderly patients. With the 300 mg/m²/day 7-day schedule, attention needs to be paid to renal function prior to starting the drug and to fluid intake during treatment in order to maintain adequate hydration and urine output. Concurrent administration of potentially nephrotoxic drugs such as aminoglycosides may increase the risk for developing renal insufficiency and should therefore be avoided. If such drugs need to be administered, the renal function should be closely monitored and gallium nitrate should be discontinued if a rise in the serum creatinine level occurs. In studies evaluating gallium nitrate in patients with hypercalcaemia, renal toxicity (as evidenced by a rising blood urea nitrogen and serum creatinine levels) was noted in 12.5% of patients treated at the lower dose of gallium nitrate (200 mg/m²/day for 5 days) recommended for the treatment of hypercalcaemia of malignancy. Since these patients had various underlying medical conditions, the relationship of renal dysfunction to the drug was not clear. Hence, renal toxicity does not appear to be a major concern as long as adequate fluid intake and urine output are maintained. According to the manufacturer's recommendations, serum creatinine levels should be monitored during treatment for hypercalcaemia and the drug should be discontinued if the serum creatinine levels exceed 2.5 mg/dl. Visual and auditory toxicities were reported in < 1% of patients receiving gallium nitrate during Phase II trials in various malignancies. The development of anaemia has been noted in some patients treated with gallium nitrate; however, suppression of white blood cells or platelets (which occurs with other chemotherapeutic drugs) is not usually seen. The development of hypocalcaemia in patients who receive gallium nitrate for the treatment of NHL can usually be managed by supplementation with oral calcium carbonate. Although the administration of gallium nitrate by a continuous intravenous infusion schedule may seem inconvenient, it can be efficiently managed in the outpatient setting using a portable, lightweight, programmable infusion pump. With this pump, the entire dose of gallium nitrate can be administered over 1 week without the need for refilling or changing the pump reservoir.

8. Regulatory affairs
Gallium nitrate (Ganite™ ) is presently being developed and marketed by Genta Inc. It was first approved by the FDA for treatment of hypercalcaemia of malignancy in 1991 and was initially marketed by another pharmaceutical company. However, the ownership of the drug changed hands and manufacturing of the drug ceased in the late 1990s. After the ownership of gallium nitrate transferred to Genta Inc., there was a renewed interest in its development and the drug was approved again by the FDA in October 2003 for the treatment of hypercalcaemia of malignancy.
Gallium nitrate is now available on the market as a prescription drug for the treatment of hypercalcaemia of malignancy, and it is being further developed as an antineoplastic agent and is undergoing clinical evaluation for the treatment of lymphoma and other malignancies.

9. Conclusion

The high mortality rate in NHL highlights the need to develop novel drugs for this disease and to further investigate drugs that have previously shown therapeutic promise. Phase II studies in NHL conducted independently, by different investigators, all demonstrated responses to gallium nitrate as a single agent and in combination with other drugs. Responses to the treatment of brief intravenous infusions of gallium nitrate were seen in patients with recurrent NHL as well as in patients with NHL refractory to prior treatment. However, the continuous infusion schedule described by Warrell et al. appears to have the greatest efficacy, with a 43% response rate in patients with relapsed or refractory NHL.[14]

The novelty of this infusional approach is due to the fact that it alters the pharmacokinetics of gallium, allowing the attainment of steady-state gallium levels in the blood, as well as its binding to transferrin and its targeting to transferrin receptors on the surface of NHL cells. In addition, twice as much gallium can be delivered by this schedule than by brief infusion without increasing nephrotoxicity. Fluid intake and hydration, to maintain adequate urine output, are important factors in reducing the adverse effects of gallium on the kidney. The drug is generally well-tolerated, with many patients experiencing no adverse effects. An important property of gallium nitrate is that it is not myelosuppressive and thus may be administered to patients with low white blood cell and platelet counts.

Gallium is clearly active in NHL and warrants further evaluation. The Phase II multi-institutional clinical trials currently in progress will be important in confirming the results of earlier clinical studies and in providing information regarding the activity of gallium nitrate in different subtypes of NHL based on the newer histological classification of lymphoma.

10. Expert opinion

Gallium nitrate and additional gallium compounds in development represent an important class of metals with therapeutic potential in NHL and other disorders. Since gallium shares properties with ferric iron, certain iron-containing proteins important for tumour cell viability and function may incorporate gallium instead of iron, thereby leading to cell death. Other mechanisms of cytotoxicity may also be involved. In this regard, gallium could be considered an analogue of iron that kills cells through perturbation of critical iron-dependent processes. The selective activity of gallium in NHL appears to be related, at least in part, to the ability of transferrin-gallium complexes to target the transferrin receptors present on the surface of lymphoma cells. Transferrin receptors are present on lymphoma cells in vivo and their density appears to correlate with the biological aggressiveness of this malignancy.[58,69]. Gallium can therefore be considered a form of "targeted therapy" in which a drug (gallium nitrate) utilizes an endogenous ligand (transferrin) to home in on specific cell-surface targets (transferrin receptors).

Although the clinical antitumour activity of gallium nitrate in NHL was recognised almost two decades ago, its development as an antineoplastic agent in this disease was never rigorously pursued. An explanation for this appears to be the fact that two-thirds of the patients treated with a continuous infusion of gallium nitrate in the pivotal study by Warrell et al. developed hypocalcaemia.[14] This effect of gallium nitrate apparently eclipsed its antilymphoma activity and led to a shift in the focus of these investigations to basic and clinical investigations of the effects of gallium on calcium and bone metabolism. Whereas this avenue of research led to FDA approval of gallium nitrate for the treatment of hypercalcaemia of malignancy, the activity of gallium nitrate in NHL appeared to have been all but forgotten. However, a clinical trial by Chitambar et al., published almost a decade after the last report on gallium nitrate in NHL, confirmed its activity in this disease.[17]

The initial Phase II studies of gallium nitrate in NHL were conducted prior to the availability of some of the therapeutic strategies, such as purine analogue drugs, monoclonal antibodies and high-dose therapy with stem cell transplantation (HDT-SCT), which are currently employed for the treatment of NHL. Hence, it is important to attempt to define where gallium nitrate might be best used in the present-day treatment of NHL and how it compares to other chemotherapeutic drugs. Although there are no studies directly comparing the efficacy of gallium nitrate to other agents, a review of early Phase II studies suggests that the 43% response rate seen with the continuous infusion of gallium nitrate in relapsed NHL is comparable to that reported for many other drugs. For example, responses in NHL to etoposide, doxorubicin, cisplatin, ifosfamide, methotrexate and high-dose cytarabine (Ara-C) as single agents range from 28 - 48%.[70-74] whereas responses to the purine analogues fludarabine and 2-chlorodeoxyadenosine are 50 - 60% and 43%, respectively.[75-78.] With rituximab, the response rate is 46% in relapsed NHL.[79] Hence, it appears reasonable to conclude that in NHL, the response to gallium nitrate is within the range seen with these agents.

At this point, it would appear that the most fruitful area for further development of gallium nitrate is in the treatment of diffuse large-cell or transformed (aggressive) lymphoma, rather than indolent follicular lymphoma. Although gallium does have activity in low-grade follicular NHL, the natural history of this disease is one of long survival (years) even without treatment and the high efficacy of alkylating agents, purine analogues and monoclonal antibodies (including radioimmunoconjugates) in this histological subtype make treatment with gallium nitrate an attractive option.
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them the treatments of choice. Hence, clinical trials of gallium nitrate in follicular NHL are less likely to flourish. In contrast, purine analogues and monoclonal antibodies have limited single-agent activity in the more aggressive diffuse large-cell lymphomas. Patients with this histological subtype are usually treated with additional chemotherapeutic regimens (with or without rituximab) or with HDT-SCT. Since the success of these approaches varies and relapses are frequent, there is great potential for the development of gallium nitrate (as a single agent or in combination with other drugs) for the treatment of patients in relapsed large cell lymphoma (including relapse after HDT-SCT).

Since diffuse large-cell lymphoma is treated with combinations of different drugs, it is worth speculating how gallium nitrate might be logically incorporated into combination chemotherapy protocols. Preclinical studies have demonstrated that gallium nitrate is synergistic with hydroxyurea, fludarabine, gemcitabine, IFN-α and paclitaxel. Hence, studies in NHL could be designed utilising ribonucleotide reductase inhibitors and other drugs in combination with gallium nitrate. As discussed earlier, the combination of gallium nitrate and hydroxyurea has been examined in a clinical trial and has shown efficacy with minimal toxicity [17]. An attractive aspect of combining gallium nitrate with other agents is the lack of overlapping toxicities. In particular, since gallium nitrate does not suppress white blood cell and platelet counts, combining it with myelosuppressive drugs would not be expected to result in an increase in haematological toxicity. Another important point regarding combination chemotherapy is that gallium nitrate does not share cross-resistance with other antineoplastic drugs, as evidenced by the fact that NHL unresponsive to chemotherapeutic drugs may still respond to gallium nitrate, and by the observation that gallium-resistant cell lines remain sensitive to other chemotherapeutic agents in vitro [80]. Furthermore, in elderly NHL patients or patients who are severely myelosuppressed and are unable to tolerate conventional chemotherapy, a combination of gallium nitrate and other nonmyelosuppressive agents such as rituximab and decitabron would be a reasonable therapeutic strategy.

In summary, gallium nitrate is a unique drug with meaningful clinical activity in the treatment of NHL. Although the drug was nearly ‘lost’, a renewed interest in its potential has spawned new clinical trials that will advance our understanding of how it should be used optimally to treat NHL and other malignancies. In parallel with these clinical trials, further research into the cellular and molecular processes that determine tumour responsiveness to gallium is warranted.

Bibliography

Papers of special note have been highlighted as either of interest (+) or of considerable interest (++) to readers.


**Clinical study of brief infusion gallium nitrate in NHL, Hodgkin’s disease and chronic lymphocytic leukaemia.**


**Clinical study using gallium nitrate in combination with other investigational drugs for the treatment of relapsed NHL.** High response rates, including complete remissions, were noted in this study.


**Clinical study of the combination of gallium nitrate and hydroxyurea based on preclinical studies of mechanisms of action. This study confirmed the response rates reported previously by Warrell et al. [24].**


**Review of gallium nitrate in the treatment of bladder cancer.**


**General review of gallium nitrate.**


**Review of gallium compounds focusing on cancer therapeutics.**


Mechanism of action: investigation identifying ribonucleotide reductase as a target for the antineoplastic action of gallium.


Mechanism of action: investigation showing that the R2 subunit of ribonucleotide reductase is a target for the antiproliferative action of gallium.


Mechanism of action: investigation showing that gallium nitrate also inhibits ribonucleotide reductase enzyme activity independent of action on cellular iron homeostasis.

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