

Hepatocellular Carcinoma Detection by Gallium Scan and Subsequent Treatment by Gallium Maltolate: Rationale and Case Study

Lawrence R. Bernstein^{1,*}, Jacobus J.M. van der Hoeven² and Robbert O. Boer³

¹Terramatrix, 285 Willow Rd., Menlo Park, CA 94025, U.S.A. ²Department of Medical Oncology, Medisch Centrum Alkmaar, Alkmaar, the Netherlands; ³Department of Nuclear Medicine, Medisch Centrum Alkmaar, Alkmaar, the Netherlands

Abstract: Gallium is antiproliferative to many types of cancer, due primarily to its ability to act as a non-functional mimic of ferric iron (Fe^{3+}). Because Fe^{3+} is needed for ribonucleotide reductase activity—and thus DNA synthesis—gallium can inhibit DNA production and cell division. Diagnostic gallium scans have shown that hepatocellular carcinoma (HCC) is commonly avid for gallium. Furthermore, *in vitro* studies have found that gallium nitrate, and particularly gallium maltolate (GaM), have dose-dependent antiproliferative activity against HCC cell lines. Rationale thus exists to use GaM, an orally active compound that has been well tolerated in Phase I clinical trials, to treat patients whose HCC is gallium-avid in a gallium scan. Because gallium absorbed from orally administered GaM is bound predominantly to serum transferrin, which travels to all tissues in the body, GaM has the potential to treat even distant metastases. A patient with advanced HCC (20 × 10 cm primary tumor, ascites around liver and spleen, resistant to sorafenib (Nexavar[®])), whose cancer was highly gallium-avid in a ⁶⁷Ga-scan, was treated with oral gallium maltolate at 1500 mg/day q.d. After four weeks of treatment, the patient had a large reduction in pain, with greatly increased mobility and quality of life, and significantly lowered serum bilirubin and inflammation-related liver enzymes. At eight weeks, CT scans showed apparent necrosis of the tumor.

Keywords: Gallium, gallium maltolate, gallium scan, hepatocellular carcinoma.

INTRODUCTION

Cancer cells are generally more avid for gallium than healthy cells: this is the basis for the use of gallium scans in the detection and assessment of cancer. The preferential uptake of gallium by cancer cells stems mainly from gallium's close chemical similarity to ferric iron (Fe^{3+}), a metallic ion crucial for the proliferation of neoplastic cells [1]. The requirement for Fe^{3+} is due largely to its essential role in the synthesis of DNA, as Fe^{3+} is present in the active site of ribonucleotide reductase. Because gallium, unlike iron, is always trivalent under physiologic conditions, it has no redox potential; it can thus act as a non-functional mimic of Fe^{3+} [1].

The high avidity of cancer cells for Fe^{3+} provides a rationale for the use of Fe^{3+} -mimicking gallium as an anticancer therapeutic. Gallium is taken up by cancer cells in preference to healthy cells; the cancer cells are then inhibited from making DNA due to a lack of Fe^{3+} and the consequent reduction of ribonucleotide reductase activity. As hepatocellular carcinoma (HCC) is a cancer that is commonly found to be gallium-avid in gallium scans, it may be susceptible to gallium therapy.

ANTICANCER MECHANISMS OF ACTIVITY FOR GALLIUM

In order to obtain adequate amounts of Fe^{3+} , which in blood serum is nearly all bound to transferrin, most cancer cells overexpress transferrin receptor [2]. Due to the chemical similarity of Ga^{3+} and Fe^{3+} , gallium can bind to the two Fe^{3+} sites on serum transferrin (Tf), so cancer cells may thus take up Tf-bound gallium [1]. Serum Tf is usually about 33% saturated with Fe at any one time [3], leaving about 67% of metal binding sites available for Ga (about 2.7 $\mu\text{g}/\text{mL}$). Under typical physiologic conditions, the Tf binding constants for gallium are $\log K_1 = 20.3$ and $\log K_2 = 19.3$; the corresponding binding constants for Fe^{3+} are $\log K_1 = 22.8$ and $\log K_2 = 21.5$ [4]. Whereas Fe^{3+} remains bound to Tf down to a pH of about 5.5, Ga^{3+} starts dissociating from Tf at $\text{pH} < 6.8$; at $\text{pH} 6.0$ it is >50% dissociated [5]. This stability difference between the Tf complexes of Ga and Fe may provide a therapeutic advantage to Tf-bound Ga, as Ga may be released sooner than Fe in endosomes of cancer cells, increasing its availability relative to Fe. It is noted that

some cancer cells can also take up non-transferrin-bound iron and gallium [6].

Due to gallium's binding to serum Tf, gallium becomes preferentially taken up by cancer cells that overexpress Tf-receptor. This cellular uptake, combined with gallium's release from Tf at a higher pH than iron, allows gallium to competitively inhibit ribonucleotide reductase activity by interfering with iron uptake and utilization. In addition, gallium has been found to directly inhibit ribonucleotide reductase activity [7]. Cancer cells that are unable to synthesize DNA due to ribonucleotide reductase inhibition ultimately undergo apoptosis [8].

In vitro studies with gallium nitrate and GaM in human lymphoma cells have shown that apoptosis can be also be induced *via* mitochondrial mediation, which includes the activation of pro-apoptotic Bax, loss of mitochondrial membrane potential, release of cytochrome c from the mitochondria, and the activation of caspase-3 [9]. In addition, gallium nitrate can induce generation of reactive oxygen species in human lymphoma CCRF-CEM cells, which can overwhelm the co-induced cytoprotective effects of metallothionein-2A and heme oxygenase-1.

Other antiproliferative mechanisms of action have been proposed for gallium, based on individual *in vitro* studies. These mechanisms include inhibition of protein tyrosine phosphatases [10] and of DNA polymerases [11], but their *in vivo* significance has not been substantiated to date.

Because gallium is not reducible to the divalent state under physiologic conditions, it does not become incorporated into heme, and thus does not become concentrated in healthy hematopoietic cells such as those in the bone marrow [1]. Gallium scans show that gallium also does not become concentrated at other sites of rapidly proliferating healthy cells, such as gastrointestinal mucosal cells and the transient cells of hair follicles. The reasons for this lack of concentration are not known; one hypothesis is that iron is highly conserved in these tissues, where it undergoes extensive local recycling [12]. The lack of high uptake of gallium by healthy cells, which contributes to the utility of gallium scans, also likely contributes to the relatively low toxicity observed for gallium.

GALLIUM SCANNING

Gallium scans employing ⁶⁷Ga (half-life 78.3 h) have been in use since the early 1970s to locate, stage, and assess the viability of

*Address correspondence to this author at Terramatrix, 285 Willow Rd., Menlo Park, CA 94025 U.S.A. Tel: 650-324-3344; E-mail: LawrenceRBernstein@gmail.com

cancers. The sensitivity of gallium scans, however, varies widely for different types of cancer, and even from case to case within an apparent single type of cancer. Partly for these reasons, other forms of diagnostic imaging, such as x-ray computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) using ^{18}F -fluorodeoxyglucose, have largely supplanted gallium scans for cancer imaging.

Despite the shortcomings of gallium scans, their sensitivity in detecting cancer is well correlated with transferrin receptor expression by the cancer cells [13, 14], and is thus positively correlated with increased grade and growth rate of the cancer [15, 16, 17]. Gallium scans are particularly sensitive in detecting lymphomas [18], and are still extensively used in their staging and in determining their response to therapy. Though commonly at lower sensitivity, gallium scans may also be useful in detecting several other types of cancer, including HCC [19], sarcomas [20, 21], and lung cancer [14].

PRECLINICAL AND CLINICAL ONCOLOGICAL EXPERIENCE WITH GALLIUM

In the early 1970s, following the discovery of potent anticancer activity by cisplatin (*cis*-diamminedichloroplatinum(II)), *in vitro* and *in vivo* screening of other metal compounds for anticancer activity found gallium nitrate to be particularly promising [11]. Significant efficacy was observed in several animal cancer models, including Walker 256 ascites carcinosarcoma in rats and implanted human medulloblastoma in mice. Subsequently, *in vitro* activity of gallium compounds has been observed in many cancer cell lines, including those for lymphoma [8], breast cancer [22], leukemia [10], colon cancer [10], and HCC [23].

Many gallium anticancer studies have utilized gallium nitrate as the gallium compound. Since aqueous solutions of gallium nitrate are highly acidic, citrate is usually added to chelate the gallium and bring the pH up to physiologically acceptable levels. Gallium nitrate and other simple gallium salts are not, however, well absorbed orally [24]. Furthermore, intravenously administered citrated gallium nitrate (CGN) (approved by the U.S. Food and Drug Administration in 1991 for the treatment of cancer-associated hypercalcemia) must be administered by slow infusion over several days to avoid renal toxicity [12]. This renal toxicity appears to be associated with the presence of serum gallate, $[\text{Ga}(\text{OH})_4]^-$, which forms when CGN is directly introduced into the bloodstream [24]. As a small, charged molecule, gallate is rapidly concentrated and excreted by the kidneys, where it may reach high concentrations in the renal tubules. There is evidence that these high concentrations can cause the precipitation of gallium-calcium phosphates within the renal tubules [25].

Intravenously administered CGN (Ganite[®]) has shown clinical efficacy against non-Hodgkin's lymphoma (43% response) and bladder carcinoma (40% response), with lower response levels in urothelial carcinoma, cervical carcinoma, ovarian carcinoma, squamous cell carcinoma, and metastatic prostate carcinoma [12]. Typical anticancer doses for CGN were 200 to 500 mg/m²/day by continuous infusion for ≥ 5 days, generally repeated every few weeks. CGN administered subcutaneously at 30 mg/m²/day during alternate 2-week periods, combined with a bimonthly 5-day infusion at 100 mg/m²/day, together with the M-2 chemotherapy protocol, was effective in a study of 13 patients with advanced multiple myeloma [26]. The use of CGN has, however, apparently been limited due to its renal toxicity and inconvenient mode of administration (continuous intravenous infusion over many days).

Gallium maltolate (GaM; Fig. 1) is being developed as an orally administrable form of gallium for therapeutic use. In Phase I clinical trials, this compound has shown oral bioavailability of about 27–54%, with no observed dose-limiting or other serious toxicity

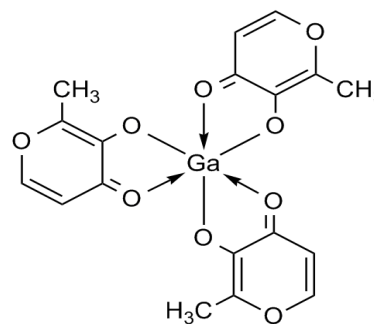


Fig. (1). Chemical structure of gallium maltolate.

[24]. At doses of up to 3,500 mg/day for repeated 28-day cycles, the most common observed toxicity was a small increase in diarrhea at doses over 2,000 mg/day [27]. Significantly, no renal toxicity has been observed with oral GaM [24]. This absence of renal toxicity is believed due to the fact that gallium from oral GaM becomes almost entirely protein-bound (mostly to Tf) in the blood. Only 2% of orally administered Ga from GaM is excreted in the urine within 72 hours of administration, compared to 49 to 94% of intravenously administered Ga from CGN within 24 hours of administration [24].

RATIONALE FOR USE OF GALLIUM MALTOLATE IN HEPATOCELLULAR CARCINOMA

When non-radioactive gallium is administered systemically to a cancer patient, the gallium follows the same uptake pattern as observed in gallium scans, and may be taken up preferentially by the cancer [1]. In sufficient concentrations, the gallium can interfere with the uptake and utilization of Fe^{3+} by the cancer cells, preventing the functioning of ribonucleotide reductase. Such cells will not be able to synthesize DNA, and will die by apoptosis [1, 8].

HCC is commonly found to be gallium-avid in gallium scans [19, 28, 29]. In fact, gallium scans were commonly used in the diagnosis of HCC before the advent of CT, MRI, and PET methods. We hypothesize that gallium avidity correlates with susceptibility to gallium as a therapeutic agent.

Furthering the rationale for GaM as a potential treatment for HCC, *in vitro* studies have shown GaM to have dose-dependent antiproliferative activity in several HCC cell lines [23]. The IC₅₀ values for GaM in the four HCC cell lines studied were 25 – 35 μM , as compared to 60 – 250 μM for gallium nitrate. These studies also found that the expression of Tf-receptor 1 (CD71) and the M2 subunit (metal-binding region) of ribonucleotide reductase (RRM2) (hypothesized targets for gallium therapy) were consistently overexpressed in these cell lines. Clinical studies have also found CD71 [30] and RRM2 [31] to be consistently overexpressed by HCC cells. Furthermore, the expression of CD71 dose-dependently increased with GaM exposure (probably due to cellular sensing of iron insufficiency) [23]. This increase in CD71 could set up a self-destructive feedback loop for the cells, as the increase in CD71 could result in increased uptake of gallium.

HCC, particularly when non-resectable and when metastasized, is often resistant to treatment. When HCC is found to be gallium-avid using a gallium scan, it may be susceptible to treatment with GaM, as the active agent (gallium) is the same in both the scan and the therapy. Because gallium from oral GaM binds primarily to Tf in the blood, and because Tf reaches all body tissues (including the brain), oral GaM therapy has the potential to reach distant metastases throughout the body. The targeting of gallium to cancer cells, with healthy tissue being spared (as is seen in gallium scans), may lead to a good safety profile for GaM in patients.

These reasons, combined with the observed clinical efficacy of parenteral CGN against several types of cancer, suggest that orally administered GaM may be effective against HCC.

CASE STUDY

Based on the above rationale, we hypothesized that a patient who is found to have highly gallium-avid HCC in gallium scans would have also high gallium uptake into malignant tissue from orally administered GaM, and thus a reasonable chance for an anti-cancer response. We here report on the use of ^{67}Ga scans to determine the gallium avidity of HCC in a patient, and the subsequent treatment of the patient with oral gallium maltolate.

The subject of this study was a woman diagnosed with non-resectable HCC at the age of 69. The diagnosis was based on results of abdominal CT images and elevated serum levels of alpha-fetoprotein (200,000 ng/mL; normal < 5 ng/mL). The largest tumor, in the right lobe of the liver, was 11 cm in greatest dimension and 8 cm in smallest dimension at diagnosis; no signs of primary tumor were observed outside of the liver.

As is true in 75-90% of HCC cases [23], surgical resection was not a reasonable option at diagnosis. For this patient and in general, the therapeutic options currently available for non-resectable HCC are few and generally produce unsatisfactory results. Only one drug, sorafenib (Nexavar[®]), is currently approved in Europe and the United States for the treatment of HCC. In two placebo-controlled clinical trials, sorafenib was found to extend overall survival in advanced HCC patients by 2.3 and 2.6 months and not to extend time to symptomatic progression [32]. It is noted that 95 and 97% of the patients had a Child-Pugh score of A, representing the mildest level of liver disease. In the two clinical trials, 2% and 5% of patients treated with sorafenib had partial remissions, 55% and 70% had stable disease, and the balance had progressive disease; none of these responses to sorafenib differed significantly from those to placebo [32].

Within two weeks of diagnosis, the subject of the present study began treatment with sorafenib at a dose of 800 mg/day. The sorafenib treatment was terminated after about four months due to an apparent lack of significant efficacy and the patient experiencing severe peripheral neuropathy ("hand-foot skin reaction"), fatigue, weakness, nausea, diarrhea, abdominal pain, and anorexia. An abdominal CT scan showed progressive disease, and the alpha-fetoprotein level had risen to 250,480 ng/mL.

Three weeks after sorafenib treatment was terminated, the subject received abdominal gallium scans using 134 MBq of intravenously administered ^{67}Ga citrate. Planar and SPECT images were obtained 48 hours after ^{67}Ga citrate administration. These images showed intense gallium uptake in the liver tumors, with low uptake in the surrounding liver tissue and in other organs (Fig. 2). At that time, the subject was experiencing moderate nausea and anorexia, severe fatigue and weakness, and severe pain and tenderness of the right abdomen that prevented the subject from lying on her right side. Contemporaneous CT images showed that the primary tumor had expanded to 20 cm in greatest dimension by 10 cm in smallest dimension (Fig. 3a). Some ascites was present around the liver and spleen.

Based on the high avidity of the subject's HCC for gallium, as shown by the gallium scans, treatment of the patient with orally administered GaM was initiated. The treatment was conducted under a compassionate-use, named-patient protocol at the Medisch Centrum Alkmaar, Alkmaar, the Netherlands. Under this protocol, a physician is permitted to prescribe a named experimental drug to treat a patient who cannot be adequately treated using drugs approved for use in the Netherlands, after filing a statement with the Healthcare Inspectorate of the State Supervisor of Public Health. The patient is clearly informed of the known risks associated with the experimental drug and is told that the drug has not been fully tested for safety and efficacy. The GaM used in the study was synthesized under cGMP protocols by Regis Technologies of Morton Grove, Illinois, U.S.A.

Treatment of the patient was started a week after the gallium scans were performed. GaM was administered at a dose of 1500 mg per day, taken before breakfast, in two gelatin capsules each containing 750 mg GaM with no excipients.

Four weeks after the start of GaM treatment, assays of liver condition showed improvement: serum bilirubin (total) dropped from 27.5 to 13.7 $\mu\text{mol/L}$ (normal: 2-20 $\mu\text{mol/L}$) (Fig. 4) and serum alanine aminotransferase (ALT) dropped from 47 to 22 IU/L (normal: 0-45 IU/L) (Fig. 5). The patient reported that her right abdominal pain was nearly gone, and she could lie and sleep on her right side. Her ability to engage in normal activities had substan-

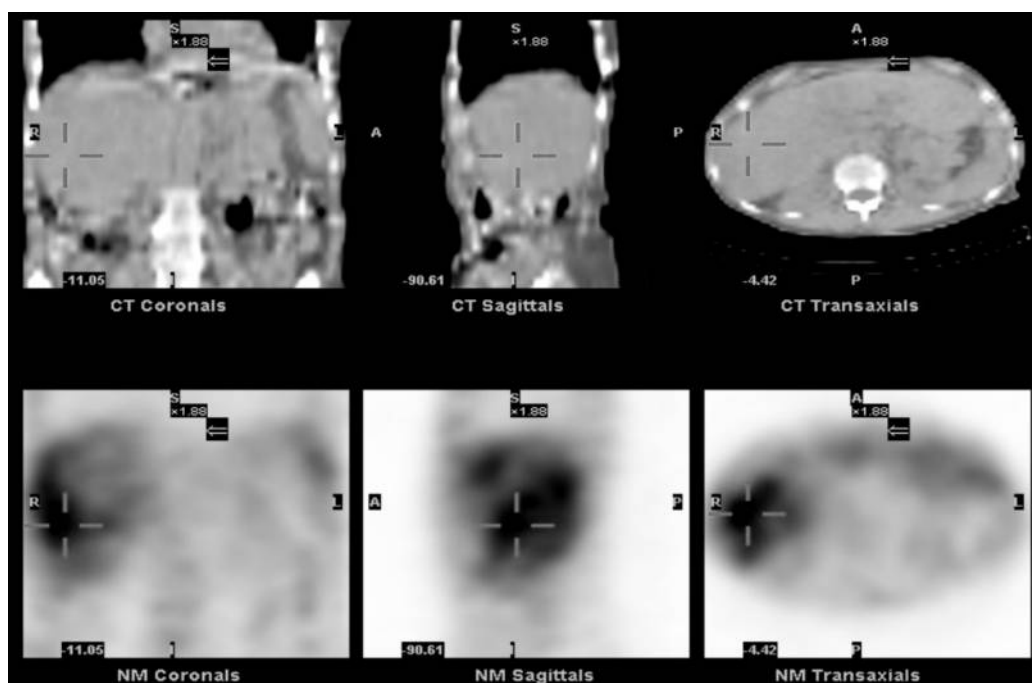


Fig. (2). Abdominal cross sections by CT (upper row) and ^{67}Ga scan (lower row) for patient with hepatocellular carcinoma. The ^{67}Ga scan showed the tumors to be highly gallium-avid (black areas represent regions of high ^{67}Ga accumulation). The patient had recently undergone four months of treatment with sorafenib (Nexavar[®]).

tially increased, to the extent that she was subsequently able to travel for several weeks at a time. At about eight weeks into the treatment, CT images were obtained; they were reviewed by expert radiologists and interpreted as showing necrosis of the primary tumor (Fig. 3b). These data indicate that the patient had at least

stable disease, and probable partial remission of disease. Her condition continued to improve over a period of about seven months. No significant side effects from the GaM treatment were reported by the patient or noted from examinations and test results.

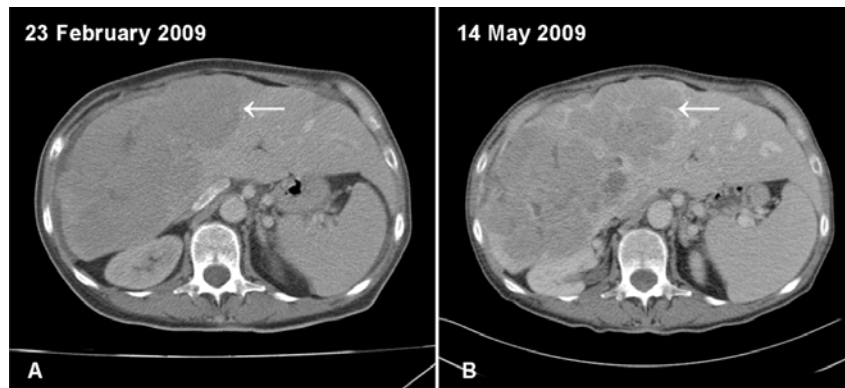


Fig. (3a). CT image taken 12 days following termination of sorafenib therapy, which had lasted for four months. Tumor is the darker area in the right lobe of the liver (to the left in the image; see arrow). **(3b).** CT image taken eight weeks after start of gallium maltolate therapy. Note evidence of tumor necrosis.

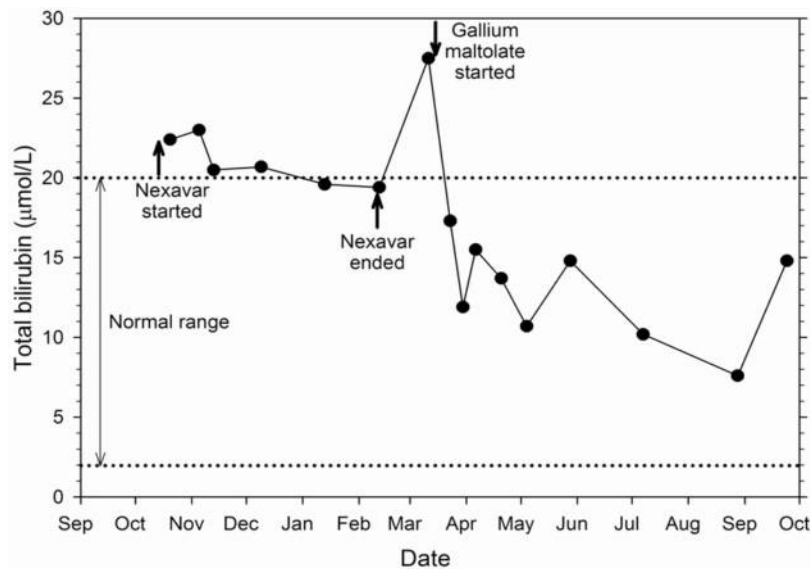


Fig. (4). Total serum bilirubin in relationship to treatments for hepatocellular carcinoma.

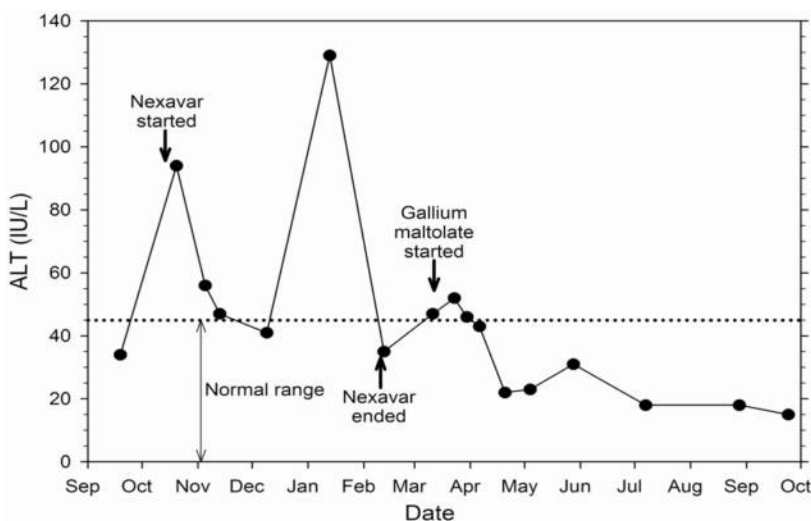


Fig. (5). Serum ALT in relationship to treatments for hepatocellular carcinoma.

Serum gallium (measured by inductively coupled plasma mass spectrometry) at eight weeks into GaM treatment was 375 ng/mL, which was less than our target of >700 ng/mL; at 15 weeks serum gallium was measured at 765 ng/mL. At four months into the treatment, the patient stopped taking GaM for two weeks; she reported that this hiatus was inadvertent.

Eight months after the initiation of GaM treatment, the patient was admitted with progressive abdominal discomfort. Her liver was enlarged and ascites were present as signs of progressive disease. The administration of GaM was stopped and palliative care was offered. The patient died 15 months after the diagnosis of HCC and ten months after ascites and a 20 cm tumor were present, when GaM treatment was started. The patient had responded to GaM treatment with probable partial remission of disease, near elimination of severe right abdominal pain, and a large improvement in her quality of life and daily functioning.

CONCLUSIONS

Significant rationale for the use of orally administered gallium maltolate in the treatment of HCC is provided by: (1) the known clinical efficacy of parenteral gallium nitrate in the treatment of lymphoma, multiple myeloma, urothelial carcinoma, and other cancers; (2) the *in vitro* antiproliferative activity of gallium maltolate against HCC cell lines at concentrations that are physiologically reasonable (and that are substantially lower than those for gallium nitrate; this antiproliferative activity is due primarily to gallium acting as a non-functional mimic of Fe³⁺, which inhibits ribonucleotide reductase activity and thus DNA synthesis and cell division); (3) the preferential accumulation of gallium by many HCC tumors, as is known from decades of gallium scan results; (4) the lack of high gallium accumulation by healthy tissues, also known from gallium scans, which contributes to a good safety profile for gallium; and (5) the possibility that treatment could reach metastases throughout the body (including the central nervous system), because gallium absorbed from GaM becomes bound to serum transferrin, which travels to all body tissues.

In most HCC patients, including the subject reported here, the disease is too advanced at diagnosis for surgery (which is the only treatment currently available used with curative intent). Sorafenib (Nexavar[®]) appears to be of limited benefit to some patients for a short period.

For all these reasons, plus the encouraging results obtained using oral GaM to treat a patient with sorafenib-resistant, gallium-avid, advanced HCC, further studies using GaM are warranted in patients with non-resectable HCC that is gallium-avid in a gallium scan.

ABBREVIATIONS

CGN	=	Citrated gallium nitrate
GaM	=	Gallium maltolate
HCC	=	Hepatocellular carcinoma
Tf	=	Transferrin

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