Clinical Efficacy of a Petroleum-Derived Product (Larimsh™) Used as Compassionate Treatment in Patients With Terminal Prostate Cancer.

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ABSTRACT

Treatment of a light fraction of petroleum treated by metallic catalysis results in a liquid mixture of low molecular weight compounds named Larimsh™ (LR). Acute and chronic topical treatment of mice with LR (85 days with 0.1 to 1 mL/animal) indicated no signs of toxicity other than a non dose-dependent, reversible alopecia appearing by the 4th week of topical application. Alopecia completely reversed 2 weeks after treatment withdrawal. Acute oral LR (0.001 to 1 mL; the lowest doses diluted in corn oil as vehicle) gave an estimated LD50 of 21 g/kg (C.I. 95 %: 10.94 - 41.2 g/kg. LR density = 0.867 g/mL). The antineoplastic action of LR was observed using combined oral and topical treatments in mice; inoculated with a lymphoid leukemia cell line in ascitic phase (International Registry L5178Y); and in terminal patients with prostate cancer (TPCA) – who agreed to receive LR as a compassionate treatment. The survival time for mice was significantly increased when compared with non-treated inoculated mice (51±2 versus 38±2 days, p<0.05, mean ± SD, N=6 per group). In 15 patients, LR treatment for 5.5 months (C.I. 95 %: 2.9 to 8.0 months) significantly increased the expected survival time diagnosed to TPCA by their treating physicians (C.I. 95 %: 2.2 to 5.4 versus 12.6 to 41.2 months, p<0.05) which occurred concomitantly with a significant reduction of blood levels of total prostatic antigen (average 94.5%, range: 67.3 to 99.9%). A theoretical proposal is advanced as a likely explanation of LR actions.

INTRODUCTION

Ancient reports of the use of petroleum or petroleum-derived products for medicinal purposes can be found in Persian tablets dating from 400 B.C. [1]. Nowadays consensus exists regarding recognition of oil as a source of wealth and health [2]. Fractionation of crude oil is usually carried out by distillation for separating small from large molecular weight compounds. One typical fractionation, vacuum distillation, is used to produce two important fractions for oil industry: light and heavy gas oil. The former contains compounds in the molecular weight range of 200 to 1000 Daltons. Light gas oil is normally subject to hydrotreatment in order to produce naphtha and diesel with low concentrations of sulfur compounds and increased ketane number. In contrast, the heavy fraction is subject to catalytic cracking to obtain lighter fractions, i.e., ethylene, gasoline, low quality diesel and other heavy by products [3].

Early in 1985, Chem. Eng. Rodríguez-Pérez noticed a pain relieving action of the light fraction from personal use. The chemical principle orienting Rodríguez-Pérez’ work considered on one hand that oil is a natural product with a chemical composition not unlike living beings, so its potential therapeutic value could be related as in the homeopathic principle "similia similibus curentur" [4]; or due to the presence of active components as referred for the oil-derived mumie [5]. On the other hand, he also considered that if cancer is due to dehydration of nucleic acids as proposed by Robertson [6], then quick transients of high water content inside the cell could induce nucleic acids rehydration reversing thus the neoplastic state.

Under normal conditions carbohydrates as well as hydrocarbons need to be activated, i.e., glucose to glucose-6-phosphate or gasoline with the electric spark, before entering degradation pathways to generate water, carbon dioxide and energy in the form of ATP [7] or heat [8]. Artificial activation of small molecular weight hydrocarbons (SMWH) can be achieved by separating those components with the highest hydrogen content and exposing them to ultraviolet radiation in the presence of metallic catalysts [8]. These artificially activated SMWH which are very lipophilic could undergo intracellular metabolism to produce enough water molecules to induce rehydration of nucleic acids, particularly in the most rapidly proliferating tissues.

Based on these ideas, further purification of the light fraction was directed towards retaining biological activity (analgesic and/or anti-inflammatory) but improving its redox and hydration indexes, as measured by the combustion index and the ratio of oxygen and hydrogen, respectively.

One of the most promising fractions was named Larimsh™ (LR), which is prepared using a catalytic fractionation with metals of the fourth and sixth groups of the Periodic Table followed by exposure to UV light radiation of high frequency and low
wavelength. Its patent registration was initiated in 2001 when preclinical and clinical data began to emerge indicating its safety and clinical efficacy [9].

Gas chromatographic analysis of LR shows 213 different compounds including aliphatic alkanes (hepta-, and octadekanes); alkane-isoprenoids (pristane and phytane); cyclopentanes (naphtenes of the cyclohexane and dekane series); aromatics; nitrogen, oxygen and sulfur containing compounds; and traces of metal. The average molecular weight is 250 Daltons. The production of LR is monitored by its chemical composition for a maximum of 1.5% sulfur; 85% carbon; 13% hydrogen; 0.03% nitrogen; and 0.4% oxygen. Quality control on the production of LR is carried out by gas chromatography (Fig.1).

In this work we present data supporting safety and efficacy of LR in preclinical experiments and in a group of patients with terminal prostate cancer (TPCA) who had not benefited from conventional antineoplastic therapy.

METHODS
LR is a brown translucent solution with a strong petrol-like odor and a density of 0.867 g/mL. Preparation and quality control of LR used throughout all experiments was kindly performed by Rodríguez-Pérez.

Preclinical Studies: All animals were used at the Bioterium of the School of Medicine, UNAM, and kept under a 12:12 h dark : light cycle and 22 to 24 °C for room temperature. Humidity was at around 20%. Rodent laboratory chow 5001 (PMI, Minnetonka, MN, USA) and water were available ad libitum. Male mice of eight weeks old of Balb-C and CD-1 strains were used for all experiments.

Acute Studies: Acute effects of LR were studied using 0.05, 0.1, 0.3, and 1.0 mL per oral or topical administration of the undiluted product; and in doses of 0.01, 0.005 and 0.001 mL of LR diluted with corn oil. Control groups received equivalent volumes of corn oil (Mazola™, Mexico). Three to five animals were used per group. Continuous observation of animals' behavior was done during 6 hours after treatment.

Chronic Studies: Daily administration of LR was carried out over 11 weeks per oral and topical routes using the same doses and vehicles as in acute studies. Rough estimates of food and water intake were followed by changes in the bottle of water and food weights, respectively. At the end of treatment, animals were sacrificed for gross anatomical inspection and sampling of tissues for histopathology studies. These results will be published in a separate communication. A group of CD-1 mice were further observed during three weeks to document the recovery of the LR-induced alopecia observed after topical treatment.

Lethality Tests: LR toxicity was analyzed using doses between 0.001 to 1 mL by topical or oral administration to CD-1 male mice (average body weight 44 g). The number of dead mice was monitored for over a period of 48 h.

Animal Studies: Antineoplastic action of LR was tested in male Balb-C mice using an intraperitoneal inoculation of ~1.4 x 10⁵ cells from a lymphoid leukemia cell line in ascitic phase (International Registry L5178Y). Six animals were randomly assigned to each experimental group: intact control (A); mice who received 20 applications of 0.05 mL LR orally every other day (B); inoculated untreated mice (C); inoculated mice treated with 4 topical applications of 0.2 mL of LR on days 27, 31, 33 and 35 following inoculation (D); inoculated mice treated with 4 administrations of 0.2 mL of LR orally and topically on days 27, 31, 33 and 35 following inoculation (E); and mice pretreated with 0.2 mL of LR orally 4 and 3 days before inoculation and topically plus orally on days 27, 31, 33 and 35 following inoculation (F). Body weight, food and water intake, and survival rate were observed for 90 days. The dates selected for treatment aimed at having mice at terminal stages of disease.

Human Studies: Clinical testing with LR is entirely based on its use as a compassionate treatment, i.e., all patients agreed to receive treatment aware of its experimental nature without modification of their conventional treatments.

All patients included in the study were previously diagnosed for TPCA by a health professional or medical institution independent of our group. Patient recruitment was done by personal recommendation from patients themselves. Initial interviews were carried out by one of the clinicians of our group at a practitioner's office (DLRVM or LBH) or directly at the Hospital of Mother Teresa of Calcutta, both located in Mexico City. In all cases written informed consent was given by the patients themselves; only in 2 out of 15 cases consent was given by their legal sponsor or relative due to the severe condition of the patient. Average age of patients was 70.5 years (C.I. 95%: 66.2 to 74.8 years); the time since diagnosis was an average of 3.2 years (C.I. 95 %: 2.0 to 4.4); and the expected survival time diagnosed by their treating physicians was of 3.8 months (C.I. 95%: 2.2 to 5.4 months). For more details about patients see Table I.

Figure 1. Quality control of LR production is carried out by gas chromatography using a 100 m capillary column (Agilent 19091Z-530 HP-1) at 350 °C and 1 mL/min of helium as carrier gas. Front and back detectors used were FID at 300 °C and TCD at 250 °C, respectively. Diethylstilbestrol (DES) is used as internal standard; in this chromatograph the signal corresponds to the injection of one μL of a solution containing 0.0841 g of DES in 5 mL methanol. LR contains 213 components of which 33 are the most abundant. The largest component plus orally on days 27, 31, 33 and 35 following inoculation (E); and mice pretreated with 4 administrations of 0.2 mL of LR orally and topically on days 27, 31, 33 and 35 following inoculation (F).

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Treatment with LR was provided free of charge and given either topically in the inguinal region and/or orally contained in gel capsules. The route of administration was selected considering individual condition of patients. Initial treatment included three capsules per day containing 0.65 mL of LR each (2 to 4 mL/day), and topical application embedded in a paper pad (1 to 3 mL/day). Duration of treatment was continued as long as necessary and as a function of clinical outcome and the results of laboratory tests. Measurements of blood serum concentration of total prostate-
specific antigen (PSA) and bone gammagrams were carried out in established clinical laboratories independent from our group; their cost was paid by patients.

RESULTS

Preclinical Data; Acute and Chronic Toxicity: Chronic administration of LR induced a significant decrease in body weight (P<0.05) without regard to the route of administration; whereas food intake was decreased with oral treatment (P<0.05) and water intake was increased only with topical application of LR (P<0.05) when compared with control mice treated with corn oil as vehicle (Fig. 2A, B and C). Chronic administration of topical LR in mice causes significant alopecia; over 90% of body surface area by day 30, that resumes completely within two weeks when treatment was discontinued. Lethality was not observed following topical treatment (Fig. 2D) either acutely or chronically whereas chronic oral administration of diluted LR (1:1000 and 1:100 in corn oil) caused earlier (by 3rd week, only 1:1000 dilution) and larger lethality than vehicle (80 and 55 versus 30%, P<0.05, for 1:1000, 1:100 and vehicle, respectively). Dose response following acute administration of undiluted LR (Fig. 2E) gave an estimated LD₅₀ in CD-1 mice of 21 g/kg (C.I. 95% :10.94±1.2 g/kg for observations up to 48 hr).

Table I. Clinical data of patients with terminal prostate cancer who agreed to receive Laminar as compassionate treatment (LR Tx)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age at LR Tx (yr)</th>
<th>Date of LR Tx (mo, yr)</th>
<th>Time of LR Tx (mo)</th>
<th>Dose of LR (ml)</th>
<th>Expected survival (days)</th>
<th>Observed survival (days)</th>
<th>Previous Tx and severity of disease before LR Tx</th>
<th>Effect of LR on patient's SPA (ng/ml)</th>
<th>Clinical status up to the date indicated (mo, yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>6</td>
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<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
<tr>
<td>11</td>
<td>65</td>
<td>5, 2001</td>
<td>4</td>
<td>1:100</td>
<td>60</td>
<td>30</td>
<td>None</td>
<td>113.9 ng/ml</td>
<td>-Decreased 0.2, 2001</td>
</tr>
</tbody>
</table>

Table 2. Effect of LR on Balb-C mice inoculated with lymphoid leukemia cells (L5178Y)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Initial (final) body weight (g)</th>
<th>Survival (days)</th>
<th>Survival (%)</th>
<th>Comparison with inoculated mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control intact</td>
<td>26±1.5 (26±1.5)</td>
<td>=&gt;90</td>
<td>100</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>B. Control treated with LR (0.2 ml/PO/q4d)</td>
<td>25±1.5 (27±1.5)</td>
<td>=&gt;90</td>
<td>100</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C. Inoculated L5178Y</td>
<td>26±1.5 (38±1.5)</td>
<td>14% by 10th day</td>
<td>16% by 20th day</td>
<td>14% by 20th day</td>
</tr>
</tbody>
</table>

Antineoplastic Effects: The survival rate for Balb-C male mice inoculated with lymphoid leukemia cells is shown in Table II. Survival in days and percent of survival were significantly increased only when LR was given simultaneously per oral and topical treatment with LR given one week before the expected death date, i.e. at a terminal state of the disease, increased significantly survival in days but not survival rate (38±2 versus 51 ± 2, P<0.05) when compared with the inoculated untreated mice. Oral administration of 0.2 mL of undiluted LR every other day during 60 days caused no lethality in Balb-C mice.

These results indicate that LR is a mixture of components which may include novel or non-novel chemical structures, but all of them are necessary to obtain the therapeutic effect with minor adverse reactions; and, b) dilution and / or systemic metabolism of LR appear to segregate an unknown number of components which are protective against major toxicity but retain a very potent anabolic that prevent body weight loss despite the significant decrease in food intake. It remains to be settled whether these components are cytotoxic and responsible for halting neoplastic tissue proliferation.

Clinical Data: Patient data (Table I) indicate that treatment with LR per oral and topical caused a significant seven fold increase (P<0.05 at least) of the expected survival time diagnosed by their treating physicians: 3.8 months [IC 95% 2.2 to 5.4, median=2.5, N=15] versus 26.9 months [IC 95% 12.6 to 41.2, median=14. N=15].

The increase in survival observed was very similar whether patients died at some time after receiving treatment or if they are still alive after receiving an average of 5.5 months of treatment [IC 95 % 2.9 to 8.0, median=3. N=15]. Clinical response to treatment included a consistent report in all patients of a generalized sense of well being, no pain in the inguinal region, better sleeping time and a decrease in urinary incontinence. Also shown in Table I, LR treatment significantly decreased patient's PSA (P<0.05 at least) from 94.0 ng/mL (IC95 % 5.2 to 183.4, median=24. N=13) to 4.6 ng/mL (IC95 % 2.1 to 7.0, median=2.8. N=13) when comparing the registered data before and after treatment. As shown...
in Table 1 three out of 15 patients decided to stop treatment while feeling recovered and no further follow up was possible. Data shown in Figure 3 depicts only those patients when more than two PSA measurements could be obtained. Due to the high cost of the bone gammagraphs, only one patient agreed to take two after treatment in the same laboratory where he had taken the first one. Figure 4 illustrates a reduction in the size and the progressive reduction in the number of metastasis.

![Figure 2](image2.png)

**Figure 2.** LR was given to CD-1 male mice (41.6 ± 4.5 g initial body weight) for 11 weeks. Each group included 5 to 10 mice. Data from daily measurements of body weight (A), food (B), water intake (C), and lethality (D) were aggregated weekly. Water and food intake were measured per cage hence data reported are expressed per g of body weight. Food intake measurements do not correct for powder released into the cage. Control mice (—) were treated with corn oil either topically or orally; lethality was observed only in the group treated with corn oil orally. Volume for treatments corresponded to 1/100 of body weight. Shaded areas depict values for a group of five intact mice with 95% confidence intervals. Statistical analysis was done with ANOVA followed by Student t test using SPSS V10 with a P value <0.05 for significant differences. When compared with CTL (——), LR decreased body weight; Topical LR (--) increased water intake (P<0.05), an effect that occurred 9 weeks after treatment. Corn oil dilutions of LR at 1:100 (▓) or 1:1000 (░) given daily orally decreased water and food intake (P<0.05). Greater lethality was observed with 1:1000 than with 1:100 dilution of LR (80 vs. 59%, P<0.05). Only mice treated with Topical LR exhibited alopecia on 90% of body surface by the 4th week of treatment that reversed completely within two weeks after cessation of treatment. Unquantified locomotor hyperactivity was noticed only after Topical LR after the 2nd week and thereafter until treatment withdrawal. Acute lethality monitored at 24 (X) and 48 hours (♦) gave an estimate of 21 g/kg (C.I. 95%:10.94-41.2 g/kg. LR density = 0.867 g/mL), as shown in panel E.

![Figure 3](image3.png)

**Figure 3.** TPCA patients treated with LR had a persistent decrease of serum concentrations of total prostate-specific antigen (PSA) measured in clinical laboratories independent from our research group. The number of the patient in each panel corresponds to data shown in Table 1.

![Figure 4](image4.png)

**Figure 4.** Bone gammagrams (BG's) are shown with authorization of Patient number 2, who agreed to release his name. The dates of examination are shown on the top of each record. BG A and BG B were taken before starting LR treatment on May, 2001 (as shown in Table 1). This patient received 2mL orally plus 1 mL topical LR daily, for 6 months. BG C was taken 3 months after completing treatment with LR. Mr. D. is still alive, asymptomatic and his last measurement of PSA on November 2006 gave a value of 2.0 ng/mL (updated April, 2007 with a value of 1.6 ng/mL PSA). His PSA value before treatment with LR was 113.9 ng/mL (May, 2001). BG's and PSA measurements were carried out at Grupo Los Angeles, a well known private health center in Mexico city. Interpretation of BG's were done by specialized personnel that do not belong to our research team. All clinical analysis were paid by the patient.
Usual representations of nucleotides and DNA base pairs do not take into consideration the presence of hydration spheres as shown in panel A. The estimated number of water molecules per base pair is 12 to 24 and they may or not be ordered and display different densities along the double helix; shaded circles are an approximate representation following data reported by several authors [26,29,37]. Water related hydrogen bonds like HOH...O= and H2O...HN- contribute 46% as linkers between amino acids and nucleotide bases at the protein-DNA interface, while the remaining 54% are largely involved in screening unfavorable electrostatic contacts [33].

During DNA replication, dehydration occurs [36] and quick interconversion of tautomerized bases, i.e., G $\rightarrow$ G*, is greatly decreased originating point mutations like CG $\rightarrow$ AT [37]. Dehydration may also trigger the endogenous formation of polyphosphoric acids, which are by themselves strong dehydrators [6]. When DNA repair mechanisms cannot cope with these DNA abnormalities [25], a cell may enter into the S phase and produce mutated or antineoplastic resistant cell populations [42].

The management of PCA has long been a challenge; surgery, radiation, and hormonal therapy are the mainstays of treatment [12,13]; however, for terminal stages when refractoriness occurs or metastasis appears, clinical outcome and quality of life evolve with bad prognosis due to the long-term morbidity with palliative treatments [14].

In this work we report preclinical data obtained with LR indicating low toxicity following either acute or chronic administration (see Fig. 2), and antitumor effectiveness in a model of murine neoplasia (see Table II).

A potential antineoplastic action of LR was observed in 15 patients with TPCA who agreed to participate in this study fully aware that this is a promising but not yet fully studied or approved therapy, i.e., as a compassionate treatment. In those TPCA patients with more than one measurement of PSA, clinical improvement appears to be related to a decrease in PSA (see Table I; Fig. 3); an observation consistent with the usefulness of PSA measurements as indicators of malignancy progression [15].

Cancer therapies based on non-toxic natural products are known collectively as alternative therapies [16]. Their value is usually questioned from the ethical, legal and scientific points of view [17]. Some authors even perceive Mexico as a “Mecca” for testing protocols of non-allowed or difficult to get treatments [16]; and sound criticisms against the use of most natural products rely on the fact of their unknown composition [18]. These criticisms could be partly overcome by the simultaneous report of preclinical and clinical data gathered with LR as a compassionate treatment. The authors are fully aware that the chemical identification of the 213 components awaits elucidation as well as further in vitro testing using specific PCA human cell lines; but meanwhile, the usefulness of LR could benefit TPCA who otherwise are just sent home without any medical therapy [19]. In the group of 15 TPCA patients, five had metastasis and 12 had undergone different forms of treatment (see Table I). This observation is consistent with reports from the literature indicating that most PCA patients become refractory to treatment within 18 to 24 months after medical or surgical castration [20]. Following LR treatment, survival was significantly enhanced well beyond the time predicted by the physicians who discharge them home (see Table I); and so far, seven patients have survived well beyond the expected survival of $\sim$12 months reported in the literature [21].
With data presented here we cannot exclude or determine the contribution to clinical improvement of other treatments received by TPCA patients. However, the significant decrease in PSA observed in 13 out of the 15 patients studied (see Table 1 and Fig. 3) and the change in the sequential bone gammagraph done in only one patient (see Fig. 4) after LR treatment are promising results that may encourage other investigators with more resources to pursue further research on LR.

The usefulness of LR to help TPCA is also suggested by the verbal report from all patients as a "sense of well being". This observation is consistent with our findings of the analgesic action of LR in chronic arthritic patients [22], and it may also be related to the hyperactivity observed in mice following topical application of LR (see legend, Fig. 2). The possible presence of psycho-stimulant and antineoplastic components in LR could be related to chemicals with affinity for some specific drug receptors, such as the opioid receptors. The right combination of high dose of morphine plus low doses naloxone have been proposed as a therapeutic option that combines favorable tumor suppression with reduced neuronal toxicity as the result of the simultaneous activation of PI3K/PKB/Akt and Ras/Raf/MEK/Erk pathways [23]. This issue will require additional work.

Possible toxicity of LR in humans has been followed up with daily ingestion of 0.5 mL for almost 15 years by two volunteer healthy subjects (RRP and MGM). Up to date, there is no indication of hepatic, cardiac or neurologic damage when monitored by conventional clinical and laboratory measurements (unpublished data). Minor flatulence at the beginning of treatment is the only adverse reaction reported by patients and volunteers.

Trans-epithelial absorption of LR occurs within seconds when placed onto the skin or given orally (unpublished data); although it remains to be determined what percentage or which components of LR reach the intracellular space or remain on the cell surface, including those that vaporize at body temperature. Alopecia is one of the most discomforting side effects of cancer therapy and estrogens appear to stimulate rapid hair re-growth [24]. Chronic LR treatment induces a reversible alopecia in mice only when given topically; but it does not occur when mice receive oral treatment. TPCA patients and volunteers receiving topical plus oral LR chronic treatment do not have any noticeable alopecia. It remains to be seen whether the anabolic component that appears to be present in LR, as suggested by data on food and water intake in mice (see Fig. 2) has estrogenic activity; although initial chromatographic analysis indicates that LR does not contain diethylstilbestrol (Fig. 1).

The beneficial effects of LR in TPCA patients could be explained considering the presence of several biologically active compounds contained in a combination at optimal proportions. In this respect, it is worth recognizing that therapy of patients with cancer usually requires the use of combinations of antineoplastic drugs, analgesic drugs and/or the application of surgery or radiotherapy [13]. The quality of life and cost of treatment of TPCA patients tends to be unbalanced by age and a bad prognosis [14]. In this study, nine of the 15 TPCA patients treated with LR remained asymptomatic throughout the observation period reported here (Table 1) so we conclude that they did not develop refractoriness to therapy, as usually occurs with other forms of cancer therapy that trigger DNA repair [25]. Hence a possible explanation for the beneficial effects of LR could be based on the re-hydration hypotheses: "if cancer occurs as a result of a dehydration of the cell’s DNA, then a rehydration process would be useful to correct or redirect the progression of normalÆhyperplasticÆdysplasticÆneoplastic tissues, which occur as a result of varying degrees of dehydration".

DNA rehydration as a pharmacodynamic principle of LR action is presented here as a hypotheses in as much as it can be tested experimentally. The key issue of LR induced transients of high intracellular water concentration may occur either via the rapid metabolism of the SMWH-AA or due to the transfer of large hydration spheres carried by the very lipophilic SMWH-AA components of LR. The rehydration hypothesis is based on arguments 1 to 4 described as follows:

1. **Water plays a significant role in structure and function of DNA.**

The sensitivity of DNA structure and conformational stability to hydration and salt effects has been well established since 1984 [26]. The estimated amount of water carried by DNA is around was 0.43 to 0.56 g of H2O/g of DNA; and water molecules may be strongly attached to the DNA surface or diffuse isotropically in a sphere of radius of approximately 2.8 Angstroms [27,28].

Usual representations of nucleotides and DNA base pairs do not take into consideration the presence of hydration spheres (Fig. 5A). The estimated number of water molecules per base pair is 12 to 24; they may
or may not be ordered and display different densities along the double helix. The illustration we present (Fig. 5) is an approximation of data published by Schneider [29], where dark circles of different sizes indicate the likely distribution of water molecules.

In aqueous solutions DNA displays a preferential form (B), whereas in partly dehydrated solutions, i.e., in the presence of ethanol, DNA will be in the ‘A’ form. Water-related hydrogen bonds like HOH\cdots\cdot= and H_2O\cdots\cdotHN- (where O= and HN- are functional groups on the nucleotides chemical structure) contribute significantly to the presentation of the ‘B’ form of DNA rather than the ‘A’ form [30].

The contribution of the water molecules of the hydration shell represents 73% of hydrogen bonds whereas the Watson-Crick pairs do so in 27% of the total energy of stabilization of the helical B-form DNA [31]. One group of water molecules can accommodate in the major groove linking successive charged phosphate oxygens along the polynucleotide chains. The second is associated with bases in the major groove and forms a central core of density along the helix axis. These two families of water molecules provide a layer of hydration lining the interior wall of the major groove leaving a central channel to accomodate cations [32].

Hydration effects on DNA double helix stability modulates ligand binding to natural DNA in response to changes in water activity. Furthermore, 46% of the bridging water molecules act as linkers between amino acids and nucleotide bases at the protein-DNA interface, while the remaining 54% are largely involved in screening unfavorable electrostatic contacts [33]. When the amount of available water molecules is modified, DNA double helix displays a differential affinity in specific regions for low molecular weight chemicals, such as urea, ethylene glycol or N-methylated glycine [34]. Water is more tightly bound around guanine-cytosine (GC) base pairs in the double helix and maximum hydration occurs at 0 and 100% GC content and with a minimum hydration at 50% GC content [35]. These observations are consistent with the idea that adenine-thymine (AT)-rich double stranded DNA is more unstable and sensitive to changes in the hydration equilibrium and to the presence of small molecular weight compounds [34].

2. **Dehydration is a natural process that contributes to the regulation of DNA replication.**

When a cell enters into the S-phase, it is estimated that nucleic acids undergo an approximate 40% dehydration in order to account for polymerase insertion and proofreading fidelity [36]. Dehydration during DNA replication appears to be essential for preventing nucleotids tautomeration (keto to enol forms), which in the absence of water occurs very slowly (approximately 73 hr for reaching a 90% equilibrium for G \leftrightarrow G*) [37]. Nevertheless, during DNA replication genetic errors may be introduced despite the tight enzyme control involved; hence eukaryotic cells use of at least six DNA repair mechanisms which focus mainly on nucleotide alterations, in as much as these are targets of antineoplastic drug action [25]. Less attention is given to the possible contribution of deoxyribose catabolism as a cellular defense mechanism [38] by producing water, carbon dioxide and metabolic energy in the presence of foreign DNA [39]. Double strand breaks of DNA due to interstrand crosslinking may result from a dehydration reaction between base pairs and this, in turn, may trigger deoxyribose catabolism as a compensating mechanism. Furthermore, the extent of damage would depend on the resulting production of polyphosphoric acids which are potent intracellular dehydrators [6] producing disruption of base pairing (Fig. 5B). Intracellular flow of metabolic energy, surrounding pH and most importantly the relative densities of hydration spheres inside and surrounding the structure of nucleic acids will very likely determine the progression of the cell cycle.

3. **Sustained dehydration may lead to tumor cell development.**

Those cells approaching the G1 to S boundary that carry a detected DNA damage that could not be corrected will undergo apoptosis, i.e., via caspase-mediated action [40]. However, if DNA enters S-phase holding tautomerized nucleotides, i.e., guanine or cytosine in the enol state (G*); the dehydration that occurs during DNA replication [36] will prevent G* to G conversion because the time to reach 90% equilibrium for G \leftrightarrow G* changes from 10^8 sec to 73 hr in the absence of water. Hence G* will likely be misread and cause the point mutation GC \rightarrow G*T \rightarrow AT [37,41]. Whether these point mutations for guanine and cytosine may result in altered expression of p16 and/or p53 proteins is an issue that remains to be established; but DNA dehydration per se will certainly alter DNA protein interaction [35] and this could affect not only p16 or p53 but also the interaction of cyclins with DNA. Absence or mutation of these checkpoints may allow cells to enter S-phase, survive DNA damage and produce mutated or antineoplastic-resistant cell populations as proposed by Lane et al. [42].
In summary, hydration ↔ dehydration ↔ rehydration equilibrium may significantly contribute to the control of cell populations undergoing apoptosis and cell death; but those cell populations with DNA damage will likely become hyperplasic ↔ dysplastic ↔ neoplastic tissues.

4. Intracellular rehydration resulting from the cold combustion of artificially activated small molecular weight hydrocarbons (SMWH-AA) may help to control tumor cell development.

Carbohydrate and hydrocarbon combustion may be total or partial. In the former only water and carbon dioxide are formed; while during the latter, carbon monoxide and nitric oxide among others, can be produced [7,8]. Highly reactive hydrocarbons produce exothermic reactions in the presence of oxygen, such as ether. In clear contrast, high energy content hydrocarbons (HEHC) are not reactive in the presence of oxygen and must be activated; as in glucose when transformed into glucose-6-phosphate or car’s gasoline that needs an electric spark. Under both circumstances oxygen is needed to complete combustion [7,8]. The energy potential of HEHC is measured by their hydrogen content and during their metabolism they release unstable intermediates like hydroxyl free radicals readily neutralized in the presence of oxygen to produce water and carbon dioxide [7,8]. Activated carbohydrates and hydrocarbons enter into cold combustion (constant temperature) as it occurs inside the cell with the production of ATP or other high energy compounds (endoergic processes). The extent of temperature control depends on oxygen supply. In biological systems this is a way to produce metabolic energy and maintaining a redox equilibrium [7].

Artificial activation of hydrocarbons is usually done in the oil and mining industry by increasing temperature [1,8]. This is one way in which petroleum, mumie and other soil derived enriched sources of drugs are thought to be produced in the Earth’s crust [1,5]. This activation process can be accelerated using metallic catalysis and radiation with ultraviolet light, as done with LR and described in its patent registration [8,9].

LR was produced as an enriched fraction of SMWH with the highest possible hydrogen content, and artificially activated for entering into quick cold combustion (SMWH-AA) by means of a metallic catalytic process under UV radiation [9]. Cellular metabolism via oxidation and/or peroxidation could aid cold combustion to produce high intracellular concentrations of water, a process that is currently under investigation by our group.

The rehydration hypotheses proposed could be tested experimentally using microcalorimetric testing [28]; high angle neutron fiber diffraction analysis [29]; or by in vitro mutagenicity assays induced by nucleotide tautomerization [37].

Rehydration of nucleic acids occurring as a result of LR intracellular cold combustion would retrieve spheres of water hydration with four important consequences: i) enhancing DNA–protein interaction [35]; ii) preventing the occurrence of point mutations due to tautomerization of nucleotides [37]; iii) prevention of nucleic acid bending [33], and nucleic distortion [6]; and iv) attenuating the production of polyphosphoric acids and the synthesis of purines and pyrimidines from small molecular weight precursors [6].

The rehydration hypotheses could also explain the pleiotropic effects reported for mumie and maybe of some other plant extracts [5] due to the effect of water derived from their intracellular metabolism leading to functional recovery of DNA.

In conclusion, preclinical and clinical data presented here indicate that oral and topical administration of LR may be a useful compassionate alternative treatment for the management of TPCA patients, and LR chemical characterization and further elucidation on its mechanism (s) of action deserves further research.

REFERENCES
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