SUMMARY OF PRODUCT CHARACTERISTICS:
Lipoplatin™ / Nanoplatin™
Lipoplatin™ (Liposomal Cisplatin injection) 150mg
Nanoplatin™ (Liposomal Cisplatin injection) 150mg
www.lipoplatin.com
www.regulon.com
Updated: October 2016

Important note: Recently, several oncology centers started pre-hydration of the patient with 1 Lit saline over 1-2h prior to Lipoplatin administration. Prehydration can shorten the time of the subsequent Lipoplatin infusion from 8 h down to 2-3 hours and it reduces nephrotoxicity and bone-marrow toxicity of Lipoplatin. Lipoplatin (about 2 vials, 300 mg total in encapsulated cisplatin giving for a patient of 1.5 m² body surface area a dose of 200 mg/m²) can be administered subsequently in 1 Lit 5% dextrose or saline (dextrose is preferred to open the appetite of cancer cells for nutrients). For patients who cannot receive large volumes of saline or 5% dextrose, a slower infusion of the drug in smaller dilution (e.g., 500 ml) is recommended. A post-hydration with 1 Lit saline is also recommended. Patients should also receive as premedication 4 mg dexamethasone, antiemetics (ondansetron) and anti-histamine treatment (Benadryl, Generic Name: diphenhydramine) to reduce the chance of allergies from liposomes.

IMPORTANT: Lipoplatin monotherapy on Day 1 using 200 mg/m² Lipoplatin in combination with low-dose adjuvant radiation on Day 2 with 2 Gy dose every week for 6-9 weeks is our established treatment regimen for an optimal treatment that is void of side effects and can be taken for a long period without cumulative toxicity. This is followed by maintenance treatment once every 3-4 weeks depending on the severity and confinement of the disease. It has no side effects (only mild Grade 1) and can treat almost all cancers tested (breast, glioblastoma, lung, pancreatic, colorectal). Because of the absence of side effects it is also recommended for all other solid tumors but also for leukemias (Lipoplatin targets bone marrow) and pediatric tumors. Such a treatment will de-bulk the tumor mass and the residual disease can be treated with immunotherapy or surgery to eradicate cancer.

Table of Contents
1. NAME OF THE MEDICINAL PRODUCT
2. QUALITATIVE AND QUANTITATIVE COMPOSITION
3. PHARMACEUTICAL FORM
4. CLINICAL PARTICULARS
   4.1 Therapeutic indications
   4.2 Posology and method of administration
   4.3 Contraindications
   4.4 Special warnings and precautions for use
4.5 Interaction with other medicinal products and other forms of interaction
4.6 Pregnancy and lactation
4.7 Effects on ability to drive and use machines
4.8 Undesirable effects
4.9 Overdose

5. PHARMACOLOGICAL PROPERTIES
5.1 Pharmacodynamic properties
5.2 Pharmacokinetic properties
5.3 Preclinical safety data
5.4 Mechanism of action of Lipoplatin
5.4.1 Molecular Mechanisms of Cisplatin and Lipoplatin for Entry into Tissues and Cells
5.4.2 Molecular Mechanisms of Cisplatin and Lipoplatin-Mediated Antitumor Action
5.4.3. Achieving Targeted Cisplatin Delivery through Liposomal Encapsulation
5.4.4. Synergy with radiation for tumor cell killing.
5.4.5. Antiangiogenesis and antimetastasis properties
5.4.6. Lipoplatin monotherapy (D1,2 200 mg/m² every 14 days)
5.4.7. Combination of Lipoplatin with low-dose radiation therapy

6. PHARMACEUTICAL PARTICULARS
6.1 List of excipients
6.2 Incompatibilities
6.3 Shelf life
6.4 Special precautions for storage
6.5 Nature and contents of container
6.6 Special precautions for disposal and other handling

Administrative Data

7. MARKETING AUTHORIZATION HOLDER
8. MARKETING AUTHORIZATION NUMBER(S)
9. DATE OF FIRST AUTHORIZATION / RENEWAL OF THE AUTHORIZATION
10. DATE OF REVISION OF THE TEXT

1. NAME OF THE MEDICINAL PRODUCT
Scientific name: Liposomal Cisplatin
Commercial name: Lipoplatin™ (for pancreatic cancer) and Nanoplatin™ (for lung cancer)
Lipoplatin™ (Liposomal Cisplatin) 3 mg/ml sterile nanoparticle suspension for i.v. injection.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION
   The drug substance cisplatin has a concentration of 3mg/ml within the liposomal formulation, therefore a 50ml vial contains 150mg of cisplatin.
   Lipoplatin is a proprietary liposomally-encapsulated drug product of the FDA-approved, commercially-available cytotoxic agent cisplatin. In the Lipoplatin product, cisplatin (cis-diaminodichloroplatinum) is encapsulated in a
liposome shell composed of dipalmitoyl phosphatidyl glycerol, soy phosphatidyl choline, cholesterol and methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine lipid conjugate. The ratio of cisplatin to lipids is 8.9%-91.1% (w/w).

For the Excipients please refer to paragraph 6.1.

3. PHARMACEUTICAL FORM
Concentrate nanoparticle suspension for infusion.
150 mg in cisplatin for both Lipoplatin and Nanoplatin in a glass vial of 50-ml

4. CLINICAL PARTICULARS
4.1. Therapeutic indications
Lipoplatin is used against pancreatic cancer in combination with gemcitabine as first line treatment in a pivotal EMA study. Nanoplatin is used against non-squamous non-small cell lung cancer (ns-NSCLC) as first line treatment in combination with pemetrexed in a pivotal EMA study. A Phase II in NSCLC has been completed (Stathopoulos et al 2010 Liposomal cisplatin combined with paclitaxel versus cisplatin and paclitaxel in non-small-cell lung cancer: a randomized phase III multicenter trial. Ann Oncol 21:2227-32). Also a Phase III on non-squamous NSCLC has been completed (Stathopoulos et al 2011 Comparison of liposomal cisplatin versus cisplatin in non-squamous cell non-small-cell lung cancer. Cancer Chemother Pharmacol 68:945-950).


Lipoplatin is suggested to be used in all indications where cisplatin has applications notably in metastatic testicular tumours, non-seminomatosus germ cell carcinoma, advanced stage and refractory ovarian carcinoma, advanced stages and refractory bladder carcinoma and squamous cell carcinoma of head and neck.

4.2. Premedication, Posology and method of administration
The first vial of Lipoplatin of 150 mg (50 ml) is diluted in 1Lit 5% dextrose and is administered intravenously for 2.5 h. Each of the subsequent vials of Lipoplatin of 150 mg (50 ml) is diluted in 1 Lit saline and is administered intravenously for 2.5 h. The recommended dose of Lipoplatin is 200 mg/m² every 7 days or 200 mg/m² D1,2 every 14 days as monotherapy (giving twice the dose of Lioplatin in combination therapies). The recommended dose in combination chemotherapy (with gemcitabine, vinorelbine, Paclitaxel ot other drugs) is 200 mg/m² D1,8 in a 21-day schedule. The slow administration, especially in the beginning of the drug infusion, is of great importance to assess allergy or epigastric pain in patients. The slow administration along with high volume of 5% dextrose or saline is of great importance in
establishing the low renal and gastrointestinal toxicities of the drug.

Lipoplatin is administered in doses dependent on the calculated body surface area. Body weight measurements and appropriate dose adjustments will be made before each infusion.

Drug remaining in a vial after an infusion may be stored at 0 to 4 degrees Celsius and used in a following infusion.

Batch number of the drug used in every infusion should be recorded in patient’s hospital files.

**Premedication:** Dexamethasone and antiemetics (ondansetron)

**Posology**

Pancreatic cancer (orphan drug designation by EMA):


- Lipoplatin™ 200 mg/m² D1, D8 in combination with
- Gemzar® 1,000 mg/m² D1, D8
- in a 21-Day cycle for 3 cycles.
- Responders receive a second 3 cycle treatment

Non-squamous NSCLC:

- Nanoplatin™ 200 mg/m² D1, D8 in combination with
- Alimta® 500 mg/m² D1
- in a 21-Day cycle for 3 cycles.
- Responders receive a second 3 cycle treatment

**Method of administration**

Lipoplatin is a sterile, non-pyrogenic liposome formulation in the form of an opaque liquid nanoparticle suspension. Intravenous administration after dilution in 5% Dextrose.

Pretreatment hydration required to induce diuresis during (and after) cisplatin administration is not necessary for Lipoplatin administration.

**Preparation of Administration**

Lipoplatin 3 mg/ml Sterile Concentrate is diluted in 1 Lit 5% Dextrose for the first vial and into 1 Lit Saline for all subsequent vials. The long infusion time of 5 to 8 hours is known to decrease the gastrointestinal and renal toxicities of Lipoplatin.

**4.3. Contraindications**

Use is contraindicated in those patients with a history of allergic reaction to cisplatin or to other platinum containing compounds. Use is contraindicated in those patients (less than 1%) who develop an allergic reaction to liposomes during the infusion.

Although cisplatin induces nephrotoxicity, which is cumulative and is contraindicated in patients with renal impairment, Lipoplatin has been administered without further renal impairment to such patients. A study on 40 cancer patients with renal failure has demonstrated safety in Lipoplatin plus gemcitabine administration (Stathopoulos et al, 2011 J Clin Oncol 29: suppl; abstr 7072).

Although cisplatin has been shown to cause cumulative ototoxicity (which
is reduced with pre- and post-hydration) and is contraindicated to patients with hearing impairment, Lipoplatin has a negligible ototoxicity profile.

Although cisplatin has bone marrow toxicity (which is reduced with pre- and post-hydration) and is contraindicated in myelosuppressed patients, Lipoplatin has a milder bone marrow toxicity; nevertheless Lipoplatin should be used with caution to such patients.

4.4. Special warnings and precautions for use

Lipoplatin should only be administered under the supervision of oncologists in specialist units under conditions permitting adequate monitoring and surveillance. Supportive equipment should be available to control anaphylactic reactions.

Cisplatin reacts with metallic aluminium to form a black precipitate of platinum. Although cisplatin is protected from the lipid shell in the liposome nanoparticle in its Lipoplatin/Nanoplatin formulation, all aluminium containing i.v. sets, needles, catheters and syringes should be avoided.

The solution for infusion should not be mixed with other drugs or additives. About 12-30% of the patients experience an epigastric pain during the first minutes of Lipoplatin infusion that lasts for 5-10 min and subsides without medication (Stathopoulos et al, 2005 Oncol Rep 13, 589-595). Therefore, the drug should be infused slowly at the beginning in new patients till their sensitivity to the product is established.

Because cisplatin produces cumulative nephrotoxicity which may be potentiated by aminoglycoside antibiotics, caution should be used for simultaneous treatment of a patient with Lipoplatin and aminoglycoside antibiotics. Serum creatinine, plasma urea or creatinine clearance and magnesium, sodium potassium, and calcium levels should be measured prior to initiating therapy, and prior to each subsequent course. In general, the lowering of cisplatin’s nephrotoxicity by Lipoplatin has been demonstrated in numerous studies (Stathopoulos et al, 2010 Ann Oncol, 21:2227-32. Mylonakis et al, 2010 Lung Cancer. 68:240-247). A study on 40 cancer patients with renal failure has demonstrated safety in Lipoplatin plus gemcitabine administration (Stathopoulos et al, 2011 J Clin Oncol 29: suppl; abstr 7072).

Anaphylactic-like reactions to cisplatin have been reported; these reactions have occurred within minutes of administration to patients with prior exposure to cisplatin and have been alleviated by administration of adrenaline, steroids and antihistamines. However, no reports so far show anaphylactic-like reactions to Lipoplatin.

Neurotoxicity appears to be cumulative during cisplatin administration and prior to each course, the absence of symptoms of peripheral neuropathy is recommended for cisplatin treatment. However, Lipoplatin exerts a much lower neuropathy to patients (see Undesirable Effects).

Peripheral blood counts should be monitored at 7 days after each infusion. During, and for at least three months after therapy, both male and female patients should take contraceptive measures. As is the case with all anticancer drugs, in men this drug may cause transitional or permanent sterility. Preservation of sperm may be considered for the purpose of later fatherhood (see section 4.6, Pregnancy and lactation).
4.5 Interaction with other medicinal products and other forms of interaction
Lipoplatin can be used in combination with other cytostatics that have their own pattern of side effects. The myelo- nephro-, oto- and gastrointestinal-toxicities induced by cisplatin in the Lipoplatin formulation, although much lower than those of cisplatin, will be additive to existent impairment. Neutropenia and nephrotoxicity Grade 3 are the dose limiting toxicities for Lipoplatin observed at a dose of 350 mg/m² in monotherapy whereas alopecia and neurotoxicity are the main dose limiting toxicities observed with Lipoplatin 250 mg/m² and paclitaxel 175 mg/m² (Stathopoulos et al 2010 Liposomal cisplatin dose escalation for determining the maximum tolerated dose and dose-limiting toxicity: a phase I study. Anticancer Res. 30:1317-1321).

4.6 Fertility, pregnancy and lactation
Use in Pregnancy:
Cisplatin has been shown to be mutagenic in bacteria and is teratogenic and embryotoxic in mice. Cisplatin should not be used during pregnancy unless the clinician considers the risk in an individual patient to be clinically justified. Similarly, Lipoplatin should not be used during pregnancy unless the clinician considers the risk in an individual patient to be clinically justified. As with most chemotherapy drugs, fertile female patients should have a recent negative pregnancy test prior to admission to the protocol for Lipoplatin treatment.

Male and female patients should take contraceptive measures during, and for at least three months after therapy.

Use in Lactation:
Lipoplatin should not normally be administered to mothers who are breastfeeding.

4.7 Effects on ability to drive and use machines
There are no known effects of Lipoplatin on the ability to drive or operate machinery in treated patients. However the profile of undesirable effects (central nervous system and special senses) may reduce the patients’ driving skills and abilities to operate machinery.

4.8 Undesirable effects
Phase I patients did not present any nephro-, oto- or neurotoxicity. The only reported adverse events were grade 1-2 nausea and vomiting, and myelotoxicity.

Myelotoxicity:
Myelotoxicity (usually seen as neutropenia and thrombocytopenia) is the dose-limiting toxicity when Lipoplatin is administered in combination with gemcitabine (Mylonakis et al 2010 Phase II study of liposomal cisplatin (Lipoplatin) plus gemcitabine versus cisplatin plus gemcitabine as first line treatment in inoperable (stage IIIB/IV) non-small cell lung cancer. Lung Cancer 68:240-247). Neutropenia and nephrotoxicity Grade 3 are the dose limiting toxicities for Lipoplatin observed at a dose of 350 mg/m² in
monotherapy whereas alopecia and neurotoxicity are the main dose limiting toxicities observed with Lipoplatin 250 mg/m² and paclitaxel 175 mg/m² (Stathopoulos GP, Rigatos SK, Stathopoulos J. Liposomal cisplatin dose escalation for determining the maximum tolerated dose and dose-limiting toxicity: a phase I study. Anticancer Res. 2010 30:1317-1321).

Hepatotoxicity:
A mild, transient increase in hepatic enzymes may be seen in some patients. However, there is no concern with the hepatotoxicity of Lipoplatin as monotherapy. Special precautions should be used when using a combination drug known to cause hepatotoxicity.

Nephrotoxicity:
Grade 1-2 increase in serum creatinine levels may be seen in single infusions, especially in the first cycle of treatment. No grade 3-4 increase has been reported, except in the case of rapid infusion of Lipoplatin (in 2-3 hours instead of 8 hours).

Cisplatin induces cumulative nephrotoxicity. However, the lowering of cisplatin’s nephrotoxicity by Lipoplatin has been demonstrated in numerous clinical studies even at the much higher recommended doses of Lipoplatin compared to cisplatin (Stathopoulos et al, 2010 Ann Oncol, 11:2 227-32; Mylonakis et al, Lung Cancer. 2010 68:240-247). A study on 40 cancer patients with renal failure has demonstrated safety in Lipoplatin plus gemcitabine administration (Stathopoulos et al, J Clin Oncol 29: 2011 suppl; abstr 7072).

Gastrointestinal toxicity:
Mild nausea and vomiting is a common adverse event (69% of patients). Grade 3 nausea and vomiting is seen in less than 5% of patients. Grade 1-2 diarrhoea is seen in 20% of patients.

Neurotoxicity:
Although peripheral neuropathies and paresthesias in both upper and lower extremities have been reported with cisplatin administration that may occur after prolonged therapy or even after a single dose and may progress after stopping treatment, neuropathies from Lipoplatin treatment at doses of 120 mg/m² every 7 days for 9 weeks are mild and occur in a smaller percentage of patients (Mylonakis et al, Lung Cancer. 2010 68:240-247).

Several additional clinical studies have also established the lower neurotoxicity of LIpoplatin compared to cisplatin.

Hypersensitivity and allergic reactions:
Mild allergic reactions (erythema, mild bronchospasm, tachycardia) are reported in approximately 10% of patients, and relate to sensitivity to liposomes. In these cases, corticosteroids +/- antihistamines are administered prior to the following infusions.

Acute, severe epigastric/lumbar pain, lasting for approximately 5 minutes and spontaneously subsiding, was seen in approximately 30% of the Phase I patients. This adverse event is characteristic of liposomal drugs.
Although anaphylactic-like reactions, possibly secondary to cisplatin therapy, have been occasionally reported in patients previously exposed to cisplatin (rashes, urticaria, erythema or pruritis) such anaphylactic-like reactions have not been observed with Lipoplatin.

**Electrolyte changes:**

Electrolyte changes, mainly hypomagnesaemia may be seen in a small percentage of patients.

Less common adverse events are alopecia, neurotoxicity and metallic taste.

Toxicities are less common when the rate of infusion is slow, as dictated in the study protocol.

4.9. Overdose

Although acute overdosage of cisplatin may result in: [kidney failure, liver failure, deafness, ocular toxicity including detachment of the retina, significant myelosuppression, intractable nausea and vomiting and death from dysfunction of the respiratory centre with acid/base imbalance from penetration to the blood-brain barrier] no serious effects are anticipated with Lipoplatin overdose.

Overdosage can be expected to cause the toxic effects described above, but to an exaggerated degree. Adequate hydration and osmotic diuresis may help reduce the toxicity of cisplatin if administered promptly following overdosage.

Although the recommended dose is 200 mg/m², patients have been treated with 350 mg/m² that is defined as the MTD in Lipoplatin monotherapy. At the 350 mg/m² neutropenia Grade 3 and nephrotoxicity Grade 3 were observed in 25% of the patients; however, there was no Grade 4 toxicity of any kind (including nephrotoxicity) (Stathopoulos et al, 2010 Anticancer Res 30, 1317-1322).

Although cisplatin is administered for 4 to 6 cycles at its recommended dose and further treatments are not used because of its cumulative toxicity, Lipoplatin has been administered at up to 27 doses to a single patient without severe cumulative toxicity. Thus, Lipoplatin can be used as a maintenance therapy for extended periods.

5. PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

Class of drug: platinum drug or alkylating agent (FDA classification)

The ATC Code for cisplatin is: LO1X A01 Platinum compound.

Lipoplatin will receive its ATC code once approved for marketing authorization.

Cisplatin has biochemical properties similar to that of bifunctional alkylating agents producing inter-strand and intra-strand crosslinks in DNA. New mechanisms of action for cisplatin have recently been revealed including modulation of the mitochondrial, ERK and other signalling pathways depending on the cell type (reviewed by Boulikas 2007 Cancer Therapy 5, 349-376). Although the principal mechanism of action of cisplatin appears to be inhibition of DNA synthesis by crosslinking, other mechanisms, including enhancement of tumor immunogenicity, may be involved in its antineoplastic
activity. Cisplatin also has immunosuppressive, radiosensitising, and antimicrobial properties. Cisplatin does not appear to be cell cycle specific.

By extension Lipoplatin also acts by DNA crosslinking but also by modulating signalling pathways, enhancement of tumor immunogenicity, radiosensitising activity and antimicrobial activity; however, its mechanism of action has some additional characteristics, supposedly from its membrane penetration. For example, the signalling modulation signature profile is distinct from cisplatin’s in the same cell type. Also the cellular uptake of Lipoplatin™ and Lipoxal™, are higher in cell cultures compared to cisplatin and oxaliplatin consistent with the proposed mechanism of fusion with the cell membrane (Tippayamontri et al. 2011 Cellular uptake and cytoplasm / DNA distribution of cisplatin and oxaliplatin and their liposomal formulation in human colorectal cancer cell HCT116. Invest New Drugs 29:1321-1327).

5.2 Pharmacokinetic properties

From a Phase I study, total platinum concentrations measured in the diluted plasma samples and free plasma concentrations measured in the plasma ultrafiltrates were used for the pharmacokinetic analysis on 17 patients. During the 8 h infusion period, the maximum platinum level in the plasma, attained at 6 h for a dose of 125 mg/m², was 5.7 mg/ml and declined thereafter (Stathopoulos et al, 2005: Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): Phase I study Oncol Rep 13, 589-595).

Platinum concentrations were also determined in surgical specimens at ~20h postinfusion in patients undergoing prescheduled surgery. Platinum levels were the highest in the stomach tumors (40-200 µg/gr tissue) compared to 4 µg/gr in normal stomach tissue. Liver tumors displayed 30-100 µg/gr tissue compared to 3-4 µg/gr in normal liver tissue. Finally colon tumor specimens displayed 6-7 µg/gr tissue compared to 0.02 µg/gr in normal colon tissue. In muscle tissue it was zero and in fat tissue15-30 µg/gr tissue. A colon metastasis from hepatocellular carcinoma displayed ~2 µg/gr tissue total platinum compared to 0.08 µg/gr in normal colon tissue from the same patient (Boulikas et al, 2005. Systemic Lipoplatin infusion results in preferential tumor uptake in human studies. Anticancer Res, 25:3031-3040). Currently there are no data available on platinum levels attained in lung, breast or other tumors but their platinum uptake has been proposed to correlate to their degree of vascularization and maturation of the wall of tumor vasculature.

Studies on mice and rats have shown that the highest concentration of platinum after bolus tail vein Lipoplatin or intraperitoneal injection is in the kidney tissue but without causing nephrotoxicity to the animals. After several days (also pronounced after repetitive administrations) there is accumulation in liver and spleen again without impairment in the hepatic function of the animals (Boulikas 2004 Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts. Oncol Rep 12: 3-12).

After cisplatin intravenous administration, the clearance of total platinum from plasma is rapid during the first four hours, but then proceeds more slowly because of covalent binding to serum proteins. Levels of unbound platinum fall with a half-life of 20 minutes to 1 hour depending on the rate of drug infusion. On the contrary, after Lipoplatin intravenous administration the half
life is 50-116 hours establishing the long circulation properties of the Lipoplatin nanoparticles. The long circulation property of Lipoplatin nanoparticles is essential for their extravasation to tumors through the compromised endothelium of their vasculature.

The elimination of intact cisplatin and various platinum-containing biotransformation products is via the urine. About 15-25% of administered platinum is rapidly excreted in the first 2-4 hours after administration of cisplatin. This early excretion is mostly of intact cisplatin. In the first 24 hours after administration, 20-80% is excreted, the remainder representing drug bound to tissues or plasma protein.

On the contrary, after Lipoplatin intravenous administration, 9.1% was excreted in the urine during the 8 h of i.v. infusion, 16.8% during the following 16 h and 10% during the following 24 h. During the third day, an additional 4.8% was excreted in the urine. Therefore, during the 3 days from the start of the Lipoplatin infusion about 40.7% of total platinum is excreted in the urine (Stathopoulos et al, 2005: Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): Phase I study Oncol Rep 13, 589-595).

5.3 Preclinical safety data
Cisplatin has been shown to be mutagenic and may also have an anti-fertility effect. The mutagenic and anti-fertility effects of Lipoplatin have not been studied. However, animals can be administered with doses that are 5-7 times higher than Cisplatin in order to start producing the same side effects. Preclinical studies have shown the lower nephrotoxicity and other adverse effects of Lipoplatin, compared to cisplatin, in mice, rats, and in SCID mice (Boulkas 2004 Low toxicity and anticancer activity of a novel liposomal cisplatin (Lipoplatin) in mouse xenografts. Oncol Rep 12: 3-12. Devarajan et al 2004: Low renal toxicity of Lipoplatin compared to cisplatin in animals. Anticancer Res, 24, 2193-2200) and dogs with applications in veterinary oncology (Marr et al 2004 Preclinical evaluation of a liposome-encapsulated formulation of cisplatin in clinically normal dogs. Am J Vet Res 65, 1474-1478). Studies in cell lines have deciphered one plausible mechanism for the sensitivity of certain tumor cell lines to Lipoplatin (Fedier et al 2006 MLH1-deficient tumor cells are resistant to lipoplatin, but retain sensitivity to lipoxal. Anticancer Drugs 17:315-23). Analysis of molecular markers known to be related to cisplatin resistance showed a direct correlation between cisplatin and lipoplatin resistance and ERCC1 and LRP expression and were proposed as valid predictors of sensitivity or resistance to these drugs (Arienti et al 2008 Activity of lipoplatin in tumor and in normal cells in vitro. Anticancer Drugs. 19:983-90).

A study in rats has shown that Lipoplatin 12 mg/Kg has a lower neurotoxicity to cisplatin 4 mg/Kg after intraperitoneal administration once weekly for 4 weeks. The onset of peripheral neurotoxicity was assessed by measuring tail nerve conduction velocity (NCV), morphological and morphometric analysis of dorsal root ganglia (DRG), and morphological analysis of the sciatic nerve (Canta et al. 2011 Cancer Chemother Pharmacol. 68:1001-8).

5.4 Mechanism of action of Lipoplatin
5.4.1 Molecular Mechanisms of Cisplatin and Lipoplatin for Entry into
Tissues and Cells

Cisplatin (also carboplatin and oxaliplatin) are actively imported by cells using the Ctr1 (Copper transport protein 1) first identified in Saccharomyces cerevisiae and later across species. Ctr1 actively mediates influx and intracellular accumulation of platinum in human cells. ATP7B actively outfluxes platinum drugs from the cytoplasm to the extracellular space. A major mechanism of resistance of tumor cells to platinum drugs is associated with down regulation of Ctr1 or upregulation of ATP7B. An independent mechanism to platinum drug resistance includes upregulation of DNA repair mechanisms in resistant cells. Additional mechanisms are upregulation of glutathione or metallothionein levels for cisplatin detoxification.

Lipoplatin on the other hand enters cells rapidly either via phagocytosis / endosomal engulfment or by the direct fusion of the Lipoplatin nanoparticles with the cell membrane because of the presence of the fusogenic lipid DPPG on its shell. Lipoplatin accumulates into cancer tissues supposedly by the extravasation of the nanoparticles through the compromised endothelium sprouted during neoangiogenesis. Thus, Lipoplatin concentration in normal tissues is minimized explaining its low toxicity. A higher concentration of Lipoplatin in primary or metastatic tissue compared to the adjacent normal tissue has been shown in surgical specimens in patients at 20h from Lipoplatin infusion (Boulikas et al, 2005 Anticancer Res. 25: 3031-3040).

Lipoplatin has been shown to bypass resistance of cells, presumably at the cell membrane level; indeed, previously cisplatin–treated patients with NSCLC who relapsed, responded to Lipoplatin treatment with a partial response of about 25% (Froudarakis et al, manuscript in preparation). This study shows that Lipoplatin can treat tumors resistant to platinum drugs.

The scheme shows a blood vessel in tumor tissue. Lipoplatin nanoparticles of 100nm in diameter are depicted as spheres with the yellow toxic payload of cisplatin inside them. In normal tissue, blood vessels are impenetrable by small nanoparticles. On the contrary, tumor blood vessels
have imperfections (tiny holes) in their walls (called endothelium); tumor blood vessels are established during the process of neo-angiogenesis (meaning sprouting of new blood vessels by a tumor cell mass during its growth phase). Lipoplatin nanoparticles take advantage of these tiny holes to pass through and extravasate inside the tumor reaching a concentration that can be 10- to 200-fold higher compared to the adjacent normal tissue.

5.4.2 Molecular Mechanisms of Cisplatin and Lipoplatin-Mediated Antitumor Action

Efficient internalization of cisplatin in mammalian cells has been shown to elicit generation of reactive oxygen species, which in turn modulate signal transduction pathways that ultimately lead to cell death by either apoptosis or necrosis. The two chloride ligands of cisplatin play a pivotal role in commencing cisplatin’s toxic cascade. Indeed, due to the lower chloride ion content of the cytoplasm, upon intracellular entry of cisplatin, both its chloride groups exchange with water to yield diaquo (hydroxo) cisplatin species. These intermediates are highly reactive against nucleophilic groups on either DNA or proteins. For example, diaquo-cisplatin attacks on DNA may lead to the formation of intra- or interstrand DNA crosslinks, or to the formation of DNA monoadducts. The major product is 1,2-interstrand d(GpG) crosslinks, although d(ApG) crosslinks may also be observed. In other cases, only one cisplatin hydroxo species crosslinks DNA, while the other attacks nucleophilic groups (thiols, amino, hydroxyl) of proteins of various sizes, thereby forcing them to crosslink on the DNA.

Efficient access of hydroxo-cisplatin intermediates to DNA depends on the structure and state of activation of chromatin. In quiescent cells chromatin is highly condensed. In contrast, chromatin structure opens up locally during either DNA replication or transcription. Relaxation of chromatin structure leads to local melting of the nucleosome structure, which in turn renders the linker regions of the nucleosome octamers free to interact with factors from solution, such as promoter-binding transcription factors as well as toxic hydroxo-cisplatin intermediates. Cancer cells divide faster, replicate mutated DNA much more frequently and relax key gene chromatin structure more often than normal cells; this renders them significantly more susceptible to cisplatin and hence Lipoplatin than normal cells.

Formation of DNA adducts in cisplatin-treated cells alters the pattern and/or binding affinity for many DNA-interacting protein complexes, thereby triggering mechanisms of DNA repair, cell cycle arrest, apoptosis and necrosis. Although the spatial and temporal orchestration of these events is poorly understood, several putative effectors have been identified and proposed to mediate downstream signaling in response to cisplatin-generated DNA damage. For example, the activity of the high-mobility group box protein HMG1 may modulate the efficiency of nucleotide excision, mismatch repair and chromatin remodeling in response to cisplatin. Moreover, the tumor suppressor protein p53 may in turn regulate a plethora of downstream genes and effectors in response to cisplatin treatment. Finally, other effectors, such as the AKT and c-abl kinases, as well as members of the Mitogen Activated Protein kinase superfamily including the stress-responsive JNK and p38 kinases have also been implicated in the signal transduction of cisplatin.
cytotoxicity. Lipoplatin has similar molecular effects on cells. However, because of its tumor accumulation and rapid entrance through the cell membrane to cytoplasm and nucleoplasm its effects on tumor cells including signalling cascades are amplified while minimizing damage to normal tissue.

After cisplatin damage cells have two options: either to repair the damage or undergo apoptosis. Tumor cells have developed mechanisms to evade apoptotic death; one mechanism is accumulation of mutations in their DNA allowing for clonal selection and expansion of those cells that have survival advantages in a platinum drug environment forming resistant tumors. Lipoplatin can bypass these mechanisms and exert a more effective killing of tumor cells.

Delivery of cisplatin “payload” directly to tumor cells facilitated by DPPG fusion circumventing the need for Ctr1-receptor mediated transportation required by naked cisplatin. After concentrating in tumors and metastases DPPG promotes the fusion of Lipoplatin with the cell membrane. Once they reach the tumor target Lipoplatin nanoparticles have the advantage, unique to Regulon’s technology, to fuse with the cell membrane of the tumor cell and empty their toxic payload inside the cytoplasm. Liposomes developed by others (e.g. Doxil of SPI-77 of Alza/J&J) are unable to do the fusion process; thus the toxic drug is emptied outside the tumor cell and is less effective.

5.4.3. Achieving Targeted Cisplatin Delivery through Liposomal Encapsulation
Import of platins into the cytosol of target cells is highly regulated to reach energy- and signal (transporter)-dependent biochemical equilibrium. Shifting this equilibrium in favour of higher intracellular platinum concentration by means of a suitable drug delivery system should therefore result in increased apoptosis of target cancer cells.

Of the various systems that may achieve acceptable drug delivery in various tissues, liposomal nanoparticles are gaining ground in targeted cancer therapy, as they help reducing toxicity of contained therapeutics. The prototype medicinal product candidate emanating from these efforts is Lipoplatin. Clinical trial results suggest that PEG coating confers Lipoplatin particles an average circulation half-life of 40 hours in the plasma of human patients, as opposed to a mere 6 hours for cisplatin. Moreover, once infused into the patient, Lipoplatin preferentially targets its cytotoxic activity to the primary and secondary lesions, while leaving normal tissue essentially unaffected. Extravasation of Lipoplatin is on average 40 times (2 to 200 times depending on tumor type and vascularisation) more pronounced in tumors than it is in normal tissues. Moreover, the fusogenic lipid DPPG commands a 5-fold higher fusion of the Lipoplatin particles to the leaky membrane of the neo-vascularising tumor cells, as compared to normal cells.

5.4.4. Synergy with radiation for tumor cell killing. Lipoplatin is the only nanoparticle drug available that contains a heavy metal inside a liposome. Platinum can uptake high energy from external sources such as laser or gamma rays that can burst the nanoparticle to release the toxic drug or to heat up the surrounding cytoplasm. These exciting properties are under current investigation to explore the full potential of this exciting nanoparticle.

5.4.5. Antiangiogenesis and antimetastasis properties. Lipoplatin nanoparticles are endowed with antiangiogenesis and antimetastasis properties.

The antiangiogenesis property of Lipoplatin has been suggested from the encapsulation of the beta-galactosidase gene into a liposome of the same composition as the Lipoplatin liposome; after systemic delivery to SCID mice with human tumors (Figure 5) the foreign “blue” gene stained preferentially the vasculature that the tumors under the skin of the animals developed to supply the tumor with nutrients. This shows that Regulon’s liposomes can target preferentially the vascular endothelial cells; in case of Lipoplatin, targeting of these cells with toxic cisplatin instead of the “blue” gene would cause their destruction. Thus, Lipoplatin limits tumor vascularization by attacking their endothelial cells in addition to the known property of cisplatin to attack the epithelial cell of the tumor.

The antimetastasis potential of Lipoplatin was shown recently at the “Reference Oncology Center, Italian National Cancer Institute” in Aviano (http://www.ncbi.nlm.nih.gov/pubmed/24029417). Lipoplatin inhibited both migration and invasion of cervical cancer cells supporting its antimetastasis potential. This is a very important feature of Lipoplatin because migration and invasion are essential steps used by cancers to mediate their metastases.

5.4.6. Lipoplatin monotherapy (D1,2 200 mg/m² every 14 days)
A monotherapy study has shown significant response rate of Lipoplatin in NSCLC mostly applied as second- and third-line. A partial response of 38% with 43% SD as second-line chemotherapy is considered significant, rarely seen with other drugs.

A total of 21 patients (2 patients 1\textsuperscript{st}-line, 10 as 2\textsuperscript{nd}-line and 9 as 3\textsuperscript{rd}-line) All 21 patients were evaluable for toxicity. Grade 1 myelotoxicity was observed in two (9.52%) patients Grade 1 nausea and vomiting in 4 (19.05%) patients. Grade 1 fatigue and peripheral neuropathy in 3 (14.29%) patients. No alopecia was noted.

During the time of the drug infusion, temporary myalgia was observed in 5 patients, but it lasted for only 5-10 min.

Cisplatin monotherapy induces to patients much higher toxicities (Grade 3, 4) compared to 0% Grade 3, 4 after Lipoplatin treatment. In addition, cisplatin displays a much lower efficacy than Lipoplatin in monotherapy studies (11.1% for cisplatin vs 38.1% for Lipoplatin). Cisplatin is considered to be the best drug or the “Queen of Chemotherapy” among approximately 1,000 oncology products approved by FDA and EMA. Cisplatin has also a very broad spectrum against the vast majority of human cancers of epithelial origin (About 80-90% of cancers are epithelial malignancies). The fact that Lipoplatin displays such a big difference in efficacy compared to cisplatin advocates for the value of this drug in cancer management.

<table>
<thead>
<tr>
<th>Toxicities and response</th>
<th>Cisplatin monotherapy</th>
<th>Lipoplatin monotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grades 3/4 hematologic toxicities</td>
<td>0.8%</td>
<td>0%</td>
</tr>
<tr>
<td>Grades 3/4 Neutopenia and thrombocytopenia</td>
<td>4.5% and 3.6%</td>
<td>0%</td>
</tr>
<tr>
<td>Grades 3/4 Anemia (low hematocrit)</td>
<td>6.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Grade and Toxicity</td>
<td>Grades 3/4</td>
<td>Grades 0%</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>0.8%</td>
<td>0%</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>21% and 19%</td>
<td>0%</td>
</tr>
<tr>
<td>Renal toxicity</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>8.6%</td>
<td>0%</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>11.1%</td>
<td>38.1%</td>
</tr>
</tbody>
</table>

**Publications**


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### 5.4.7. Combination of Lipoplatin with low-dose radiation therapy

Combination of D1,2 Lipoplatin with low-dose radiation therapy (1 Gy on Day 2) enhances 14 times the radiosensitizing potential of Lipoplatin as shown in preclinical studies in Canada. Indeed, Lipoplatin was shown to have the best radiosensitizing potential among all platinum compounds. In these studies, Lipoplatin™ and other platinum drugs were tested on the F98 glioma cells for their ability to improve the cell uptake and increase the synergic effect when combined with ionizing radiation. The cytotoxicity and synergetic effect of platinum compounds were assessed by colony formation assay, while the cellular uptake was measured by Inductively Coupled Plasma Mass Spectrometer (ICP-MS). After 4 h exposure with platinum compounds, cells were irradiated (1.5 to 6.6 Gy) with a 60Co source. Lipoplatin compared to cisplatin improved the cell uptake by 3-fold because of its liposomal nature, and its radiosensitizing potential was enhanced by 14-fold ([Charest et al, 2010](#)).


Regulon proposes a combination of Lipoplatin monotherapy with low dose radiation (1-2 Gy). This low dose of radiation should not cause any side effects to the patients such as burns and tissue damage. However it will enhance the anticancer effect of Lipoplatin 14 times. Thus, the already suggested 38:11=3.4-fold higher efficacy of Lipoplatin monotherapy over cisplatin monotherapy is expected to become 3.4x14 or about 50 fold higher. This will represent a tremendous medical breakthrough and the success of such a protocol can be assessed in all human cancers starting with ns-NSCLC, breast, prostate and stomach cancers. Because it is not
the proper radiation therapy, this low dose can be applied to not only locally advanced but metastatic cancers. The total radiation dose in our proposed protocol is 6-15 Gy for 6 cycles of 14 days plus 9 months maintenance therapy (once every month) for the first year. Maintenance therapy for the 2nd and 3rd years can be every 2 and 3 months (6 Gy for the 2nd year; 4 Gy for 3rd year). Thus, the patient can be treated with no side effects and an efficacy better than any other treatment. This could become a universal protocol for all cancers.

6. PHARMACEUTICAL PARTICULARS

6.1. List of lipid excipients
Soy phosphatidyl choline (SPC-3)
Cholesterol
mPEG-DSPE
DPPG

6.2. Incompatibilities
There is a total loss of cisplatin in 30 minutes at room temperature when mixed with metoclopramide and sodium metabisulphite in concentrations equivalent to those that would be found on mixing with a commercial formulation of metoclopramide. Cisplatin and sodium bisulphite have been known to react chemically. Such antioxidants might inactivate cisplatin before administration if they are present in intravenous fluids.
The same precaution should be taken for Lipoplatin.

6.3. Shelf life
Prior to first use: 24 months
In use: 3 months
After dilution: 7 days under refrigeration

6.4. Special precautions for storage
Lipoplatin is stored at 2 to 8º C. The vial should never be frozen. Repetitive freezing and thawing increases the size of the nanoparticles causing formation of aggregates. Lipoplatin can be shipped at room temperature; there are no changes in the particle size and other characteristics by storage at room temperature for up to 20 days. It should be stored at 2 to 8º C upon arrival to the hospital pharmacy.

6.5. Nature and contents of container
150 mg/50 ml
The 50 ml clear glass vials of 3 mg/ml (concentration refers to cisplatin) are closed with rubber closures, and packed as single vials in a small Styrofoam box.
Packaging:
Lipoplatin vials will be packaged in cardboard boxes and transported in room temperature. Upon arrival it should be stored 0º to 4ºC.

6.6 Special precautions for disposal and other handling
Refer to local cytotoxic handling guidelines.
Preparation (Guidelines):

1. Chemotherapeutic agents should be prepared for administration only by professionals who have been trained in the safe use of the preparation.
2. Operations such as reconstitution, dilution and transfer to syringes should be carried out only in the designated area.
3. The personnel carrying out these procedures should be adequately protected with clothing, gloves and eye shield.
4. Pregnant personnel are advised not to handle chemotherapeutic agents.

Contamination:

(a) In the event of contact with the skin or eyes, the affected area should be washed with copious amounts of water or normal saline. A bland cream may be used to treat the transient stinging of skin. Medical advice should be sought if the eyes are affected.
(b) In the event of spillage, operators should put on gloves and mop up the spilled material with a sponge kept in the area for that purpose. Rinse the area twice with water. Put all solutions and sponges into a plastic bag and seal it.

Disposal:
Syringes, container, absorbent materials, solution and any other contaminated material should be placed in a thick plastic bag or other impervious container and incinerated.

Administrative Data

7. MARKETING AUTHORISATION HOLDER
Not available currently. Regulon, Inc. (USA) is the owner of the patent and clinical results.
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www.regulon.org
Licensing and distribution rights have been assigned to pharmaceutical partners for specific countries

8. MARKETING AUTHORISATION NUMBER(S)
Will become available after filing with EMA, London, UK according to the centralized procedure for the 28 EU countries. Simultaneous or subsequent applications are expected for US FDA, Taiwanese FDA, Brazilian FDA, Chinese SFDA, Canadian FDA, Australian FDA. Lipoplatin is under registration in Turkey (2016), Russian Federation (2016), Iran (2016), Libya (2016), while EMA registration is expected in 2017.

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION
A request for Conditional Marketing authorization to the CHMP at EMA was first filed in 2012 for NSCLC (Nanoplatin) and pancreatic cancer (Lipoplatin). Both filings have been accepted and Rapporteur and co-Rapporteur have been assigned for the filings by EMA. Meetings between the regulatory team of Regulon and EMA as well as the Rapporteur took place. Additional data are to be submitted in 2016 for the final marketing authorisation.

10. DATE OF REVISION OF THE TEXT
   June 2016

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