Expert Opinion

Clinical overview on Lipoplatin™: a successful liposomal formulation of cisplatin

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Nanoparticle formulations for packaging existing drugs have been used to treat cancer. Lipoplatin™ is a liposomal cisplatin encapsulated into liposome nanoparticles of an average diameter of 110 nm. Lipoplatin has substantially reduced the renal toxicity, peripheral neuropathy, ototoxicity, myelotoxicity as well as nausea/vomiting and asthenia of cisplatin in Phase I, II and III clinical studies with enhanced or similar efficacy to cisplatin. During clinical development, 10- to 200-fold higher accumulation of Lipoplatin in solid tumors compared to adjacent normal tissue was found in patients. Targeting of tumor vasculature by Lipoplatin in animals suggested its antiangiogenesis potential and Lipoplatin was proposed to act like a double-sword: as chemotherapy and an antiangiogenesis drug. Lipoplatin has finished successfully one Phase III non-inferiority clinical study as first-line against NSCLC in its combination with paclitaxel showing statistically significant reduction in nephrotoxicity; two more Phase III studies are in progress, one in NSCLC with gemcitabine also showing noninferiority with reduced toxicity and another in squamous cell carcinoma of the head and neck with 5-fluorouracil. A registrational Phase II/III study against pancreatic cancer is in progress under the orphan drug status granted to Lipoplatin by the European Medicines Agency. Phase II studies are continuing in advanced breast cancer with vinorelbine and gastrointestinal cancers with radiotherapy and 5-fluorouracil. The highlights of the clinical development of Lipoplatin are reviewed.

Keywords: angiogenesis, cisplatin, Lipoplatin™, liposomes, nanoparticle, NSCLC, platinum transporters, tumor targeting

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1. Introduction

Cancer remains a devastating disease in spite of intense research for over 4 decades. Chemotherapy, surgery, radiation and patient management had major improvements. Maturation of the chemistry of chemotherapy from the 1960s to 1980s led to > 700 FDA-approved drugs. The six classes of chemotherapy drugs according to the FDA include: i) platinum compounds (cisplatin, carboplatin, oxaliplatin) (reviewed in [1]) (**Box 1**); ii) the two classes of antimicrotubule agents: vinca alkaloids (vinblastine, vinorelbine) and taxanes (paclitaxel, docetaxel) (reviewed in [2]); iii) antimetabolites (methotrexate, 5-fluorouracil (5-FU), gemcitabine); iv) antitumor antibiotics (actinomycin D, mitomycin C, bleomycin, the anthracyclines doxorubicin, daunorubicin, the podofylotoxines etoposide, teniposide, and the camptothecines irinotecan, topotecan); v) alkylating agents such as cyclophosphamide and vi) others. This last class includes natural products, monoclonal antibodies, antiangiogenesis drugs such as anti-VEGF agents [3], drugs that target signaling molecules including mTOR inhibitors [4], Bcl-2 inhibitors [5], MEK/ERK, Src, PI3K/Akt, Hedgehog and NF-κB inhibitors, anti-EGFR and

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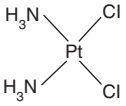
Box 1. Drug summary.

Drug name	Cisplatin, Lipoplatin™, Regulon
Phase	Phase III
Indication	Cancer
Pharmacology description	DNA crosslinker, signaling modulator
Route of administration	Intravenous
Chemical structure	
Pivotal trial(s)	Phase II with Lipoplatin + gemcitabine as first-line every 7 days in pancreatic cancer (EMEA) Phase III studies: Lipoplatin plus gemcitabine versus cisplatin plus gemcitabine as first line treatment in patients with NSCLC Phase III studies: Lipoplatin plus paclitaxel versus cisplatin plus paclitaxel as first-line treatment in NSCLC

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several tyrosine kinase inhibitors. These agents are targeted against mitogenic pathways essential to cancer cells. This list also includes heat shock protein 90 inhibitors, CDK inhibitors and proteasome inhibitors.

Emerging anticancer technologies also include those targeting epigenetic mechanisms such as histone deacetylase inhibitors [6,7], poly(ADP-ribose) polymerase inhibitors [8,9] and inhibitors of DNA methyltransferase [10,11]. Important are exotic anticancer vaccines such as the GV1001 antitelomerase vaccine, as well as immunomodulatory agents aiming at invigorating the immune system of the patient. Parallel efforts are exploiting the booming of gene discovery using genes as therapeutic molecules to induce biosynthesis of a protein by the patient's cells that could arrest tumor cell proliferation or kill tumor cells preferentially; this approach gave genesis to the field of gene therapy (reviewed in [12,13]).

Attempts to target cancer cells in the human body without damaging normal cells have also been vigorous. Effective drug delivery and tumor targeting is of paramount importance in clinical oncology, which is expected to improve the quality of life of the cancer patients. A major effort has been directed to drug delivery in parallel with discovery of new anticancer molecules. Our group has been involved in nanoparticle formulations by liposomal encapsulation of pre-existing chemotherapy drugs to achieve passive targeting of tumors; emphasis has been in reducing the side effects and enhancing targeting to both tumors and metastases. A breakthrough took place by the liposomal encapsulation of cisplatin leading to a nanoparticle liposomal formulation, Lipoplatin™ (Regulon, Inc., Mountain View, CA, USA) [14]. These nanoparticles integrate the reverse micelle technology followed by conversion into true liposomes for efficient encapsulation yields but also integrate fusogenic lipids on the surface of the nanoparticles to unlock the cell membrane barrier by promoting a direct fusion with the cell membrane.

2. Cisplatin and platinum drugs in chemotherapy

Cisplatin, since its serendipitous discovery in 1965, identification in 1969 and clinical application in the early 1970s continues to represent a cornerstone in modern chemotherapy playing an important role among cytotoxic agents in the treatment of epithelial malignancies. The drug of choice for the treatment of NSCLC is cisplatin [15]. The introduction of cisplatin has been a milestone achievement in clinical oncology and has saved the lives of testicular cancer patients [16,17]; cisplatin is recommended by the FDA for metastatic testicular, metastatic ovarian, transitional cell bladder cancer, NSCLC (in combinations with gemcitabine) and cervical cancer (in combination with radiation) whereas its off-label use has been extended to head and neck, esophageal, gastric, colorectal, hepatocellular, metastatic melanoma, and as second-line to metastatic breast, prostate and many other malignancies. However, its clinical use has been impeded by its severe toxicities, including nephrotoxicity [18], gastrointestinal toxicity, peripheral neuropathy [19], ototoxicity [20], asthenia and hematological toxicity. The significant risk of cisplatin-induced nephrotoxicity frequently hinders the use of higher doses to maximize its antineoplastic effects and hydration of the patients is used to minimize its effects.

The clinical success of cisplatin has triggered an enormous effort to discover new platinum drugs of improved efficacy and lower side effects. Out of > 3000 platinum compounds synthesized, only about 35 have exhibited adequate pharmacological advantages relative to cisplatin to justify clinical testing (reviewed in [1]). Of these, carboplatin and oxaliplatin have been registered worldwide and entered clinical practice with big success. Nedaplatin has been registered in Japan for the treatment of head and neck, testicular, lung, ovarian, cervical and NSCLC. Although the activity of heptaplatin (SKI2053R) was clearly lower than that of cisplatin in gastric cancer, its lower toxicity profile gave it registration in South Korea. Lobaplatin has been approved in China for the treatment of chronic myelogenous leukemia, inoperable, metastatic breast and small cell lung cancer after successful Phase II testing (structures are shown in **Figure 1**; reviewed in [1]).

The three drugs (cisplatin, carboplatin, oxaliplatin) differ with respect to DNA adducts, import mechanisms across the cell mebrane and toxicity profiles. The cytotoxicity of

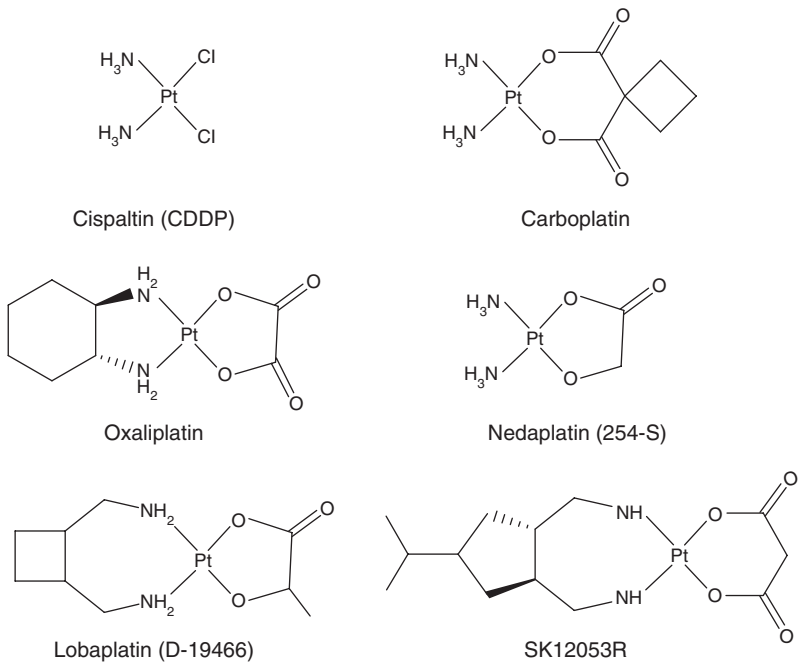

Figure 1. The structure of cisplatin, of the universally approved carboplatin and oxaliplatin and of nedaplatin, lobaplatin and hexaplatin (SKI2053R) approved in restricted Asian territories.

platinum derivatives ranked in the order: oxaliplatin > cisplatin > carboplatin in human colorectal tumor cell lines. Cellular accumulation and DNA-binding of platinum varied among the types of cells, but levels were similar on treatment with cisplatin and oxaliplatin, and lower in response to carboplatin [21].

The main objectives for the development of novel platinum drugs is the reduction in the side effects of cisplatin, the enhancement of their therapeutic index and their effectiveness against cisplatin-resistant tumors with a potential application in patients who relapse after first-line platinum-based treatment. In this respect, the clinical development of novel platinum compounds has been disappointing in spite of findings of low cross-resistance to cisplatin and superior therapeutic index in cell lines or in preclinical studies (reviewed in [1]).

3. Extravasation of Lipoplatin nanoparticles into tumors and differentiating features

3.1 Description and manufacturing of cisplatin nanoparticles

Lipoplatin is a formulation of the FDA-approved cisplatin wrapped up into tumor targeted 110 nm in diameter liposome nanoparticles using patented platform technologies.

Lipoplatin's liposomes are composed of soy phosphatidyl choline (SPC-3), cholesterol, dipalmitoyl phosphatidyl glycerol (DPPG) and methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine ($mPEG_{2000}$-DSPE). Lipoplatin is composed of 8.9% cisplatin and 91.1% lipids (w/w) (ratio ~ 1:10). Lipoplatin has an opaque appearance reflecting its liposomal nature and is being provided in 50 ml glass vials of 3 mg/ml (concentration refers to cisplatin). The concentration of 3 mg/ml of cisplatin in Lipoplatin exceeds the solubility of the free drug, cisplatin, with solubility in water or saline of 1 mg/ml. Lipoplatin is stored at 4ºC and has an expiration date of 3 years. Freeze thawing results in the formation of aggregates and should be avoided.

The Lipoplatin formulation is based on the formation of reverse micelles between cisplatin and DPPG under special conditions of pH, ethanol, ionic strength and other parameters. During its manufacturing process, cisplatin–DPPG reverse micelles are subsequently converted into liposomes by interaction with neutral lipids. This process involving various steps sensitive to parameters including temperature, ethanol concentration, pH, ionic strength, type of salt, type and concentration of lipid and other sensitive variables leads to very high encapsulation efficiencies. About 15 repeated extrusions are performed using a Thermobarrel Extruder through membranes of 0.2, 0.1, 0.08 and 0.05 μm pore sizes under pressure in ultra pure nitrogen atmosphere to an average size of 110 nm.

Whereas non-PEGylated liposomes are taken up by liver macrophages and destroyed with a half-life in body fluids of 20 min, the PEGylated liposomes of Lipoplatin display a half-life of 5 days in body fluids [22]. The longevity of the nanodrug in body fluids is a prerequisite for its extravasation into tumors.

3.2 Mechanism of extravasation of nanoparticles into tumors

Tumor targeting by nanoparticles can be achieved at two levels: i) through nanoparticle formulations of drugs that extravasate and infiltrate tumors using imperfections in their vascular endothelium (passive targeting) and ii) by adding true targeting molecules on the outer surface of the nanoparticles with high affinity for proteins overexpressed in tumors.

Stealth (pegylated) liposomal doxorubicin (Doxil) has been extensively studied [23]. The vast majority of Stealth liposomes sterically inhibit both electrostatic and hydrophobic interactions of a variety of blood components at the liposome surface [24] and enter the tumor interstitium through gaps (fenestrae) in the endothelial cell walls of newly-formed vessels that feed the tumors [25]; a smaller proportion of liposomes may actually pass directly through the thin walls of the defective endothelial cells lining the neo-vessel, through a process called trancytosis [26]. Following their extravasation in the interstitial fluid surrounding the tumor, physico-chemical destabilization and subsequent breakdown of the liposomal envelope by the low pH and the presence of lipases released from dying neoplastic cells releases the drug at the extracellular space [27]. Microvascular permeability to fluorescently labeled macromolecules in human colon adenocarcinoma cells transplanted in dorsal skin chambers showed that tumor vessels were permeable to liposomes at sizes up to 400 nm in diameter [28].

Lipoplatin nanoparticles were proposed to extravasate into tumors in animal studies (**Figure 2A**) whereas human studies showed a higher concentration of total platinum in tumors and metastases of patients compared to platinum concentration in the adjacent normal tissue at about 20 h after intravenous (i.v.) administration [29]. The PEG polymer coating used on Lipoplatin was speculated to: i) give to the drug particles the ability to pass undetected by the macrophages and immune cells, ii) remain in circulation in body fluids for long periods (half-life of 116 h for total blood platinum from Phase I pharmacokinetics, see below) and iii) extravasate preferentially and infiltrate solid tumors and metastases through the altered and often compromised tumor vasculature (**Figure 2B**). Although the mechanism of entry of Lipoplatin nanoparticles into cells has not been deciphered, tumor cells were proposed to uptake more avidly Lipoplatin particles because of: i) their tendency to uptake nutrients from the environment; ii) the higher concentration of the drug into tumors; and iii) the proposed fusion of liposomes with the tumor cell membrane; the anionic lipid DPPG was proposed to give to Lipoplatin its fusogenic properties (**Figure 2C**) [29,30].

3.3 Lipoplatin administration to patients

For patient treatment, the nanoparticle suspension is diluted into 1 l 5% dextrose; the i.v. infusion is slow to reduce side effects (~ 25 mg/(m^2 h)). It is an ~ 5 h infusion for protocols using 120 mg/m^2 weekly or an 8 h infusion for protocols using 200 mg/m^2 every 14 days. Rapid infusion (1 – 2 h) results in higher nephrotoxicity and accentuates the other side effects of the nanodrug.

3.4 The differentiating features of Lipoplatin

The Lipoplatin formulation differs from another known formulation of cisplatin that was clinically tested, SPI-77, in several basic principles including loading method, type of lipids and ratio of cisplatin:lipids. Whereas the loading of cisplatin in Lipoplatin was based on reverse micelles, the mechanism of cisplatin loading in SPI-77 was passive. The Lipoplatin formulation used anionic and neutral lipids compared to SPI-77 that used only neutral lipids. The total lipid to cisplatin ratio was low (~ 10:1 mg lipid/mg cisplatin) in Lipoplatin, thus, limiting the total lipids injected to patients. For comparison, the ratio of lipids to cisplatin in the liposomal formulation SPI-77 was ~ 70:1 [31].

Finally, the two formulations differ significantly in efficacy in human clinical trials. A significant response rate of Lipoplatin plus gemcitabine in NSCLC was obtained and most importantly, its comparison with the cisplatin arm in at least one randomized Phase II and two randomized Phase III trials, all in NSCLC, have shown its non-inferiority to cisplatin (see below).

On the contrary, the promising activity of SPI-77 in animal xenograft studies was not replicated in clinical trials; for example, in a Phase II study in patients with advanced NSCLC a modest response rate of 4.5% was obtained [32]. Similarly, no objective tumor responses occurred among 24 patients in a Phase I monotherapy study [33] and 3 of 17 patients showed responses in a combination of SPI-77 and vinorelbine in a Phase I study [34]. In another Phase II study in a NSCLC population of 12 patients, 2 (17%) had stable disease and 10 (83%) had progressive disease [35]. Finally, in patients with head and neck tumors, SPI-77 was administered safely with radiation and 10 (59%) of 17 patients finishing treatment achieved initial complete response [36]; however, SPI-77 as a single agent showed disappointing results with only 2 (11%) of 18 patients showing partial response [37].

4. Molecular mechanisms of cisplatin and Lipoplatin

4.1 Import/export mechanisms of platinum drugs across the cell membrane

After infusion, cisplatin is rapidly excreted in the urine causing renal tubular damage. When it reaches normal and malignant cells, it uses the copper transporter Ctr1 for entry across the cell membrane barrier (**Figure 2C**). Two copper efflux transporters, ATP7A and ATP7B, regulate the efflux of cisplatin. Acquisition of cisplatin resistance was associated with a greatly reduced level of ATP7A [38]. hCtr1 could transport cisplatin, carboplatin and oxaliplatin [39].

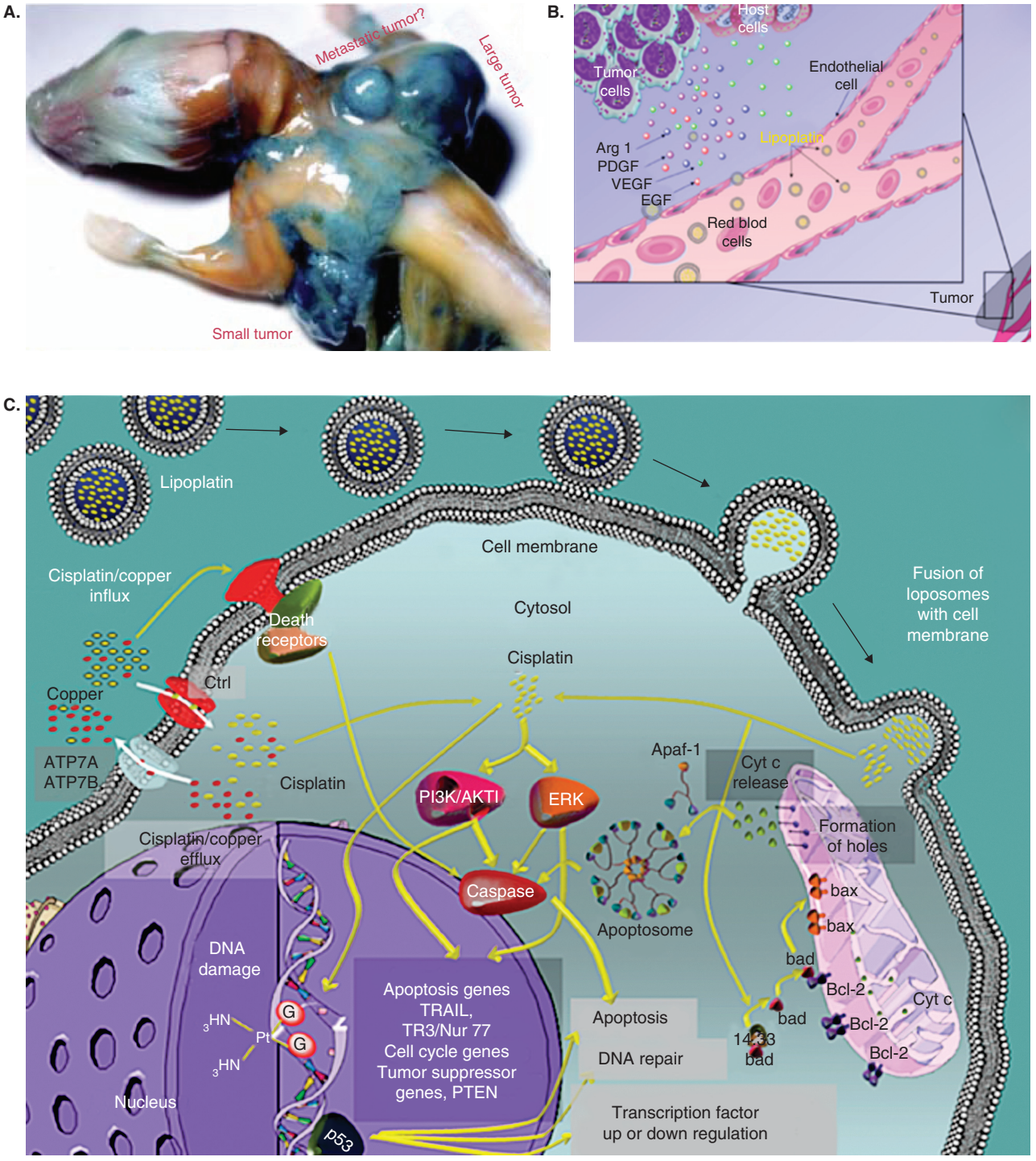

Figure 2. A. Targeting of the vasculature in SCID mice inoculated with MCF-7 cells and grown into subcutaneous solid tumors after systemic administration of liposomes containing the β-galactosidase gene (see [30] for details). **B.** Following i.v. injection, the nanoparticles extravasate through the leaky endothelium of the vasculature of the tumor which becomes compromised during the process of neoangiogenesis and concentrates preferentially into solid tumors. **C.** Fusion of the Lipoplatin nanoparticle and the cell membrane of a tumor cell (or endothelial cell of tumor vasculature) is a proposed feature, thus, bypassing cisplatin resistance at the membrane barrier. Intracellular cisplatin is activating the mitochondrial as well as other signaling pathways and inflicting DNA damage leading to apoptosis.

From [1] reproduced with permission.

i.v.: Intravenous; SCID: Severe combined immunodeficient.

The body is equipped with broad-specificity transporters for the excretion and distribution of endogenous organic cations such the organic cation transporters (OCTs). Transporters also include the proton/cation antiporters MATE1, MATE2-K and MATE2-B [40]. These transporters could play predominant roles in the tissue distribution and anticancer effects and/or adverse effects of platinum agent-based chemotherapy [41].

ATP7A, XPD and SRPK1 gene expression was increased in oxaliplatin-resistant colon cancer cells; resistance was accompanied by defects in drug uptake (downregulation of the hCTR1 transporter) and enhanced DNA repair (upregulation of the XPD gene); in addition, superoxide dismutase 1 was found to play a role in oxaliplatin detoxification [42]. Human ovarian carcinoma cells exported cisplatin through lysosomes [43]. The cellular accumulation of cisplatin was dependent on levels of ATP7A mRNA whereas the cytotoxicity of oxaliplatin was affected by the levels of ATP7A and hOCT1 mRNAs in human colorectal tumor cell lines [21].

Overexpression of ATP7A and ATP7B in Me32a fibroblasts resulted in increased resistance to cisplatin, but not to carboplatin or oxaliplatin [44]. In other cell types, as for example in cisplatin-sensitive and -resistant tumor cell line pairs (ovarian A2780/A2780cis and cervical HeLa/HeLaCK cells), resistant cells expressed 1.5- to 1.8-fold lower levels of CTR1 compared to sensitive cells with a clear relationship between lower CTR1 expression, intracellular concentration, DNA platination and cytotoxicity of cisplatin [45].

Transfection of cells in culture with constructs expressing the ATP7A gene enhanced resistance not only to cisplatin but also to vincristine, paclitaxel, SN-38, etoposide, doxorubicin and CPT-11 [46]. Impaired activity in the cisplatin transporter transmembrane proteins contributed to cisplatin resistance through reduction of drug accumulation in the cell [47]. The transporters ATP7A, ATP7B, hCtr1, hOCT1 and hOCT2 were upregulated in an established cisplatin-resistant oral carcinoma cell line [48]. Oxaliplatin, but not cisplatin, was transported by human and rat OCT3/SLC22A3; expression of this molecule was important for the cytotoxic effect of oxaliplatin in colorectal cancer [49]. OCT1 and OCT2 were found to be the major determinants of the anticancer activity of oxaliplatin contributing to its antitumor specificity and the development of drugs, specifically targeted to OCTs, was proposed as a novel strategy for targeted drug therapy [50]. Thus, the import/export of platinum drugs is a complex process with many players.

4.2 Cisplatin detoxification

The S-containing tripeptide glutathione is present in cells at mM concentrations, and the formation of complexes plays an important role in the detoxification and biological activity of platinum compounds. Depletion of glutathione levels has been shown to increase the toxicity of kidney cells to cisplatin and a clinical trial demonstrated that pretreatment with glutathione reduced renal toxicity without affecting antitumor activity. Cancer cells that are resistant to cisplatin often have elevated glutathione levels. Glutathione could quench DNA-Pt monofunctional adducts before they could rearrange to toxic bifunctional adducts. High-level cisplatin resistance, attributed to human glutathione S-transferase P1, may not be due to catalysis of cisplatin conjugation but rather must be explained by other mechanisms, which may include GSTP1-mediated modulation of signaling pathways [51].

4.3 Induction of mitochondrial apoptosis by cisplatin

Cisplatin and other apoptotic stimuli trigger the release of cytochrome c from the mitochondrial intermembrane space to the cytosol, which induces the formation of the apoptosome and the activation of procaspase-9. The apoptosome is an Apaf-1 cytochrome c complex that activates procaspase-9. The 3D structure of the apoptosome has been determined at 27 A resolution to reveal a wheel-like particle with sevenfold symmetry (**Figure 2C**) [52]. Procaspase-9 molecules can bind to the inner 'hub' region of the apoptosome. This complex promotes the efficient activation of procaspase-3. Therefore, the cleavage of procaspase-9 is not required to form an active cell death complex. Cisplatin can activate the proapoptotic protein Bax resulting in cytochrome c release, caspase activation and apoptosis; Bax activation is implicated in the nephrotoxicity of cisplatin [53]. Bcl-2 plays an important role in the mitochondrial apoptotic pathway. Although the general role of Bcl-2 is antiapoptotic, Bcl-2 fragments resulting by caspase cleavage after cisplatin treatment of cells in culture could promote the apoptotic process [54].

4.4 Induction of signaling pathways by cisplatin

During signal transduction, a cell senses both the external and internal environment and converts a stimulus into an ordered sequence of phosphorylation–dephosphorylation, protease degradation, gene regulation or ion flux events across the cell membrane. There is a great number of signaling cascades including MAPK, GPCRs/MAPK, ERK/MAPK, PKC, PKA, growth factor/survival factor/mitogen, PI3K/AKT/PTEN, ceramide, proteasome, integrin, Wnt/β-catenin, insulin, cholesterol, RB/E2F, ubiquitination and cyclins/p27 regulating the cell cycle, p53/DNA damage, oxidative signaling for phosphatidylserine externalization, survival/BAD, death receptor/Bcl-2 and several others.

Cisplatin induces a number of signaling pathways (**Figure 2C**). The extracellular signal-regulated kinase pathway is activated by cisplatin. Acquisition of cisplatin resistance by ovarian carcinoma cells was associated with the loss of extracellular signal-regulated kinase activation in response to cisplatin [55]. The c-Abl nonreceptor tyrosine kinase and the c-Jun NH2-terminal kinase (JNK/stress-activated protein kinase) are activated during the injury response to cisplatin [56]. The phosphatidylinositol 3-kinase/AKT1 pathway is frequently activated in cancer cells. Downregulation of AKT1 by siRNA could significantly enhance the sensitivity of

gastric cancer cells to vincristine, adriamycin, 5-fludrouracil and cisplatin [57]. The PKC pathway may play an important role in cisplatin resistance [58].

Cisplatin can damage both extracellular protein domains and cytoplasmic signal transduction molecules. Lipoplatin is proposed to exert a different signaling effect from the cell membrane presumably taking place because of the interaction of its liposomes with the cell membrane; this mechanism might be giving access to cisplatin of functional groups in membrane molecules otherwise inaccessible to this drug; this mechanism is under investigation in MCF-7 human breast and other cells in culture looking at the up or downregulation of important signaling molecules after cisplatin versus Lipoplatin treatment (Bellimezi and Boulikas, in preparation).

5. Preclinical studies on Lipoplatin

Preclinical studies have shown the lower nephrotoxicity and other adverse effects of Lipoplatin, compared with cisplatin, in mice, rats and in severe combined immunodeficient mice [14,59]. In subsequent studies, mice and rats injected with cisplatin developed renal insufficiency with clear evidence for tubular damage, but those injected with the same dose of Lipoplatin were almost completely free of kidney injury [59]. Treatment of dogs with Lipoplatin led to the conclusion that the drug can be safely administered to clinically normal dogs at dosages of up to 150 mg/m^2 without the need for concurrent hydration protocols [60].

Independent studies have deciphered one plausible mechanism for Lipoplatin sensitivity of certain tumor cell lines [61,62]. DNA mismatch repair is a post-replicative DNA repair mechanism implicated in cell cycle control and apoptosis. Human colorectal adenocarcinoma cells lacking MLH1, one of five proteins crucial to mismatch repair function, showed a twofold resistance to Lipoplatin. Furthermore, the Lipoplatin-sensitive phenotype of MLH1-proficient cells correlated with increased apoptosis, which was found to occur through caspase-independent pathways [61]. Other studies suggested a crosstalk between Lipoplatin DNA damage signaling mediated by DNA mismatch repair and the Akt signaling pathway [62]. These studies have important implications in the treatment of colorectal cancer with Lipoplatin and Akt signaling inhibitors. Moreover, 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 was proposed as valid predictors of sensitivity or resistance to these drugs [63].

The preclinical studies set the foundation for the clinical use of Lipoplatin as an exciting new drug with lower toxicity than cisplatin, endowed with pro-apoptotic properties.

6. Lipoplatin as an antiangiogenesis factor

A major effort against cancer focuses on targeting tumor vasculature. Inhibiting tumor cells of their ability to build vasculature is known to dramatically impair the ability of the tumor for further growth by depriving of nutrients. The ability of 'lipogenes', that is, genes wrapped up in liposomes with the same shell structure as Lipoplatin, to preferentially infiltrate tumors after systemic delivery is shown in **Figure 2A**. The photograph shows a severe combined immunodeficient mouse implanted with MCF-7 human breast tumor cells that were allowed to develop into large measurable solid tumors at about 30 days post-inoculation. Following systemic injection with the reporter β-galactosidase gene, the carcass was stained with X-Gal to reveal the sites of transgene expression after relocalization of the gene vehicles from the injected peritoneal cavity to the various tissues through the arteries, veins and lymph system. Preferential staining of the tumors, especially of the vascular system around the tumors, was shown [30].

This result suggests that lipogenes (and presumably Lipoplatin nanoparticles) possess the ability to extravasate through imperfections of the leaky and often compromised endothelium of tumor vasculature and to concentrate in solid tumors; during the process, endothelial cells of tumor vasculature can be also targeted as shown by the blue staining after expression of the reporter β-galactosidase gene; in this study, biosynthesis of the β-galactosidase protein indicated that the nanoparticle had successfully crossed the cell membrane barrier and delivered the gene to the nuclei which was expressed and its RNA product was successfully translated into a functional protein detected in our assay. A similar targeting by Lipoplatin inducing apoptotic death to both endothelial cells of tumor vasculature and epithelial tumor cells was proposed [30].

7. Phase I studies

A Phase I study on 27 patients used a dose escalation from 25 to 125 mg/m$^{2.}$ All patients were at stage IV (19 pancreatic carcinomas, 6 renal cell carcinomas, 1 gastric cancer and 1 squamous cell carcinoma of the head and neck (SCCHN)). In all cases, Lipoplatin was a second- or third-line treatment and was administered when the disease was refractory to standard treatment. Lipoplatin was administered as an 8 h infusion diluted in 1 l 5% dextrose, repeated every 2 weeks. There was no need for pre- or post-hydration of the patient with Lipoplatin. This is in contrast to cisplatin chemotherapy that requires admittance of the patient the night before infusion for hydration as well as extended stay in the hospital after infusion for post hydration to reduce the nephrotoxicity of the drug. The maximum tolerated dose (MTD) was not reached even when the dose was increased up to 350 mg/m^2 in one patient as a single infusion. Because the dose of cisplatin in the Lipoplatin formulation used in the Phase I study was as high as double the dose of cisplatin (100 mg/m^2 every 21 days) and as the future plan was the combination of Lipoplatin with other cytotoxic drugs, the experimental trial ended at this point [22].

The highlights of this study were that Lipoplatin had mild hematological and gastrointestinal toxicity, did not show any

nephro-, neuro- and oto-toxicity, did not cause hair loss and was void of most other side effects characteristic of cisplatin treatment. Grade 1 and 2 myelotoxicity (neutropenia) and grade 1 and 2 GI tract toxicity (vomiting) were observed only at the dose of 125 mg/m^2 (**Table 1**). No other toxicity was observed even with repeated doses. At the beginning of the infusion, 8 (29.6%) of 27 patients described acute severe epigastric and back pain that lasted for about 5 min and subsided spontaneously without analgesic administration. This pain is characteristic of other liposomal drugs as well. Patients with mild renal insufficiency and with plasma creatinine of 1.5 – 2.2, treated with a dose of Lipoplatin 100 mg/m^2, showed no increase in plasma creatinine [22].

A further finding was the long circulation of Lipoplatin, a property necessary for its preferential extravasation through the leaky vasculature of tumors. Indeed, the half-life of total platinum in human plasma was determined to be 60 – 117 h depending on the dose. At the dose of 100 mg/m^2, the half-life was 117 h (about 5 days) compared to ~ 6 h for cisplatin [22]. Although measurement of the response rate was not a primary goal of the study, 3 (11.1%) of 27 patients were recorded to have achieved a partial response; of the remaining 24 patients, 14 (51.9%) achieved stable disease and clinical benefit in a follow-up of 2 – 5 months [22]. Provided that all patients had failed previous chemotherapy, that they all were at stage IV of their disease and had a rather poor performance status, this finding is very encouraging.

In a different Phase I study, Lipoplatin, dose-escalated at 100 mg/m^2 by increments of 10% on days 1 and 8, was combined with gemcitabine 1000 mg/m^2 days 1 and 8, repeated every 21 days in patients with refractory or resistant NSCLC with PS ≤ 2. The dose of 120 mg/m^2 of Lipoplatin was defined as the MTD in its combination with gemcitabine. A disease control rate of 3 (23%) of 13 was found; the median overall survival was 29 weeks (range 4 – 52) and the median time to progression 12 weeks (range 3 – 36) [64]. The drug was also successfully used for mesothelioma by the same group [65].

8. Phase II studies

8.1 Pilot Phase II with Lipoplatin + gemcitabine as second-line every 14 days

A pilot Phase II study using Lipoplatin at dose levels of 75, 100 and 125 mg/m^2 every 14 days in a combination with gemcitabine 1 g/m^2 every 14 days was tested on 26 patients (19 patients with pancreatic cancer and 7 with NSCLC), of a PS 1 – 2. All patients were resistant to previous first- or second-line chemotherapy and Lipoplatin + gemcitabine was given as a third-line treatment. No renal toxicity, neuropathy, ototoxicity, hepatotoxicity, cardiotoxicity or allergic reactions were observed. Nausea and vomiting grade I – II was seen in 4 (15.3%) patients and myelotoxicity of grade III was seen in 1 patient and of grade I – II in 15 (57.6%) patients. Mild asthenia was common. Lipoplatin at 125 mg/m^2 and 1 g/m^2 gemcitabine induced grade III and IV neutropenia and grade III nausea and vomiting. Six (23%) patients showed partial response. Stable disease was seen in 65.3% and clinical benefit in 42.3% of the patients [66].

8.2 Phase II with Lipoplatin + gemcitabine as second-line every 14 days in pancreatic cancer

The standard cytotoxic treatment of advanced or metastatic pancreatic cancer is single agent gemcitabine. The addition of cisplatin, irinotecan, oxaliplatin and taxanes, in combination with gemcitabine, has shown higher response rates but overall survival has not significantly increased. The horizon has been broadened by erlotinib (EGFR inhibitor) when combined with gemcitabine [67].

A Phase I – II cohort, dose escalation trial of Lipoplatin and gemcitabine was conducted on advanced-stage pretreated pancreatic cancer patients who were refractory to previous chemotherapy. Twenty-four patients (11 male, 13 female; median age 66 years, range 47 – 80 years) with histologically or cytologically confirmed adenocarcinoma of the pancreas and bidimensionally measurable disease, had a life expectancy of at least 3 months. WHO performance status was 0 in 4.2% of the patients, 1 in 45.8% and 2 in 50%. The vast majority of patients were at stage IV (79.2%). All patients had undergone previous chemotherapy: 11 patients with gemcitabine as a single agent treatment and 13 with gemcitabine combined with irinotecan.

The gemcitabine dose was kept standard at 1000 mg/m^2 given as a 60 min i.v. infusion and the Lipoplatin was escalated from 25 to 125 mg/m^2 administered as an 8 h i.v. infusion on days 1 and 15 and cycles were repeated every 4 weeks (28 days). Lipoplatin 125 mg/m^2 was defined as the dose limiting toxicity and 100 mg/m^2 as the MTD in this combination treatment. Standard ondansetron antiemetic treatment was administered to all patients whereas prophylactic administration of recombinant human G-CSF was not allowed.

Temporary abdominal pain which lasted for 2 – 4 min, and which righted itself, was observed in 10/24 patients at the beginning of the Lipoplatin infusion. Grade 3 myelotoxicity was observed in two out of four patients at the fifth dosage level. No febrile neutropenia was seen. No neurotoxicity or renal toxicity was observed. The non-hematological toxicities are summarized in **Table 2**.

Partial response (PR) was defined as > 50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions compared with pretreatment measurements, lasting for at least 4 weeks, during which time no new lesions appeared and no existing lesions enlarged. Stable disease (SD) was defined as 50% reduction to a 25% increase in the sum of the products of the two perpendicular diameters of all measurable lesions and the appearance of no new lesions for 8 weeks.

Preliminary objective response rate data showed a PR in 2 (8.3%) of 24 patients, disease stability in 14 (58.3%) patients

Table 1. Adverse effects of Lipoplatin monotherapy at a dose escalation up to 125 mg/m^2 every 14 days.

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	19	10	10	0
Thrombocytopenia	5	10	5	0
Anemia (hemoglobin)	19	10	0	0
Renal (creatinine)	0	0	0	0
Hepatic	0	0	0	0
Nausea/vomiting	5	5	10	0
Neuropathy	0	0	0	0
Allergy	0	0	0	0
Cardiotoxicity	0	0	0	0
Ototoxicity	0	0	0	0
Hair loss	0	0	0	0

Numbers indicate percentage of patients from a total of 27 patients included in this study.

Table 2. Non-hematological toxicities using 1 g/m^2 gemcitabine and Lipoplatin dose escalation from 25 to 125 mg/m^2 every 14 days.

	Grade 1 n (%)	Grade 2 n (%)	Grade 2 n (%)	Grade 4 n (%)
Nausea	5 (20.8)	0	0	0
Vomiting	2 (8.3)	0	0	0
Alopecia	14 (58.3)	0	0	0
Fatigue	8 (33.3)	0	0	0
Diarrhea	2 (8.3)	0	0	0
Cardiotoxicity	0	0	0	0
Neurotoxicity	3 (12.5)	0	0	0
Nephrotoxicity	0	0	0	0
Thrombotic episodes	4 (16.7)	0	0	0

Data are based on 24 patients and are taken from [68].

for a median duration of 3 months (range 2 – 7 months) and clinical benefit in 8 (33.3%) patients. At the end of the study, seven (29.2%) patients were still alive. Median survival from the beginning of second-line treatment was 4 months (range 2 – 8+ months). The 14-day administration schedule of the combination was very well tolerated up to the dose of 100 mg/m^2 of Lipoplatin when gemcitabine was maintained at 1000 mg/m^2. Taking into account that all of the patients were refractory or in disease progression while on a previous treatment including gemcitabine, the response rate produced were attributed to the addition of Lipoplatin [68].

In subsequent studies, the schedule of Lipoplatin was changed from biweekly to weekly or was increased to 200 mg/m^2 every 14 days (see Phase III) to allow administration of a higher total dose of Lipoplatin and enhance its efficacy.

8.3 Lipoplatin orphan drug registrational EMEA study in pancreatic cancer

Pancreatic adenocarcinoma, the malignant tumor of pancreatic gland, constitutes a major unresolved health problem, affecting > 230,000 people worldwide each year. The term 'pancreatic cancer' usually refers to adenocarcinomas of the pancreatic duct, that is, of the exocrine part of the pancreas, which constitutes > 90% of the diagnosed pancreatic cancer cases. Pancreatic cancers are very hard to diagnose because they grow in the absence of alarming symptoms; about 85% of the patients are usually diagnosed at an advanced stage and have bad prognosis. Indeed, being the tenth most common cancer, pancreatic adenocarcinoma is the sixth leading cause of cancer-related deaths.

Lipoplatin received the orphan drug status by the European Medicines Agency (EMEA) [69]. A multi-center Phase II/III registrational clinical study is in progress using

Lipoplatin plus gemcitabine as first-line treatment in inoperable, locally advanced or metastatic pancreatic cancer with the involvement of 20 oncology centers of excellence in various EU countries. Inclusion criteria are: adult male or female, 18 – 70 years of age with histologically or cytologically confirmed diagnosis of locally advanced or metastatic pancreatic adenocarcinoma; patients should have at least one bidimensionally measurable lesion, no previous chemotherapy or radiotherapy, a performance status 0 – 1, a life expectancy > 3 months and adequate hematologic/hepatic/renal functions. During Phase II, 61 patients will receive i.v. Lipoplatin 120 mg/m^2 (days 1, 8, 15 in a 21-day cycle) plus gemcitabine 1000 mg/m^2 (days 1, 8 in a 21-day cycle) for three cycles. Patients with absence of disease progression at response evaluation will continue with maintenance therapy (Lipoplatin 120 mg/m^2 and gemcitabine 1000 mg/m^2 days 1, 15 in a 28-day cycle), until disease progression or unacceptable toxicity. During Phase III, 328 patients will be randomized (164 in each arm) to compare the same schedule of Lipoplatin plus gemcitabine as in Phase II with i.v. gemcitabine 1000 mg/m^2 weekly for 7 weeks, followed by a 1-week break. The sample size calculation is based on a target 1-year survival rate of 30% versus an 18% rate for the gemcitabine-only arm.

It is worth noting that Tarceva, a small molecule targeting EGFR (Genentech/Roche Holding AG) was approved in 2005 for pancreatic cancer in combination with gemcitabine based on a 24% 1 year survival compared to 18% of patients receiving gemcitabine plus placebo.

8.4 Weekly Lipoplatin + gemcitabine as first-line in NSCLC

A recently completed Phase II study used up to six 21-day cycles of Lipoplatin 120 mg/m^2 (days 1, 8 and 15) and gemcitabine 1000 mg/m^2 (days 1 and 8) (Arm A or LipoGem) versus cisplatin 100 mg/m^2 (day 1) and gemcitabine 1000 mg/m^2 days 1 and 8 (Arm B or CisGem) on 88 patients. The LipoGem treatment was better tolerated, with myelotoxicity as the main side effect. There was a significant reduction in nephrotoxicity in the LipoGem versus the CisGem arm (0 versus 5% Grade III, respectively, p value < 0.001). The ORR across all histological subtypes of NSCLC was 31.7% in the LipoGem arm versus 25.6% in the CisGem arm but not statistically significant (p value = 0.411). However, a preliminary efficacy of Lipoplatin/Gem versus cisplatin/Gem in the adenocarcinoma histological subtype of NSCLC showed 83.3 versus 54.2% response/stabilization rates [70]. This was an exciting finding proposed to be investigated further in a Phase III on non-squamous NSCLC.

8.5 Weekly Lipoplatin + vinorelbine as first-line in advanced breast cancer

The frequent use of anthracyclins and taxanes in the adjuvant setting, leading to the development of drug resistance and cardiac insufficiency, raised the need for development of new agents against advanced or metastatic breast cancer. The cisplatin–vinorelbine combination has been studied recently and an overall response rate of 64% was obtained. Nevertheless, the use of cisplatin was limited by the frequently induced nausea, vomiting and nephrotoxicity. The aim of a Phase II study was to evaluate the efficacy and safety of the Lipoplatin–vinorelbine combination as first-line treatment in advanced breast cancer patients. Twenty of thirty-four programmed patients with advanced or metastatic breast cancer with no previous treatment, PS 0 – 2, HER2/neu negative, and at least one measurable lesion were enrolled from August 2007 to April 2008 in a Phase II study. Treatment included vinorelbine 30 mg/m^2 i.v. days 1 and 8, and Lipoplatin 120 mg/m^2 days 1, 8 and 15. Cycles were repeated every 3 weeks for a total of 6 cycles. The primary objectives were response rate and time to treatment failure. In all, 45% of patients had one metastatic site, 30% had two and 25% had three or more. A total of 74 cycles were administered with a median number of 4 per patient. At the time of the analysis, 16 patients were evaluable for response. An objective tumor response was achieved in eight (50%) patients, with complete response in two (13%) patients. Six (38%) patients had SD. All patients (20) were evaluable for toxicity. Most adverse events were mild to moderate. No WHO grade 3 – 4 nephrotoxicity, asthenia or neuropathy was noted. Three (15%) patients developed hypomagnesemia; however, it was of no clinical significance. One (5%) patient presented grade 3 anemia and seven (35%) patients grade 3 – 4 neutropenia with only one episode of febrile neutropenia. The new combination of Lipoplatin and vinorelbine showed promising activity and good tolerance as first-line treatment for HER2/neu negative advanced or metastatic breast cancer [71].

8.6 Lipoplatin-gemcitabine in cisplatin-treated NSCLC patients

A Phase II trial is evaluating response and toxicity in advanced NSCLC patients who underwent previously cisplatin-based chemotherapy; thus, this trial is addressing the efficacy of Lipoplatin plus gemcitabine in patients whose disease is refractory to classical cisplatin chemotherapy. Patients were treated with Lipoplatin 120 mg/m^2 days 1 and 8 plus gemcitabine 1000 mg/m^2 days 1 and 8 every 3 weeks; the study is in progress as of April 2009. Twenty-seven (77.8%) patients (21 males) were assessable for response and toxicity according to the WHO criteria of a median age of 70 years (41 – 78). Twenty-two (81.5%) patients were at stage IV at diagnosis; 14 (51.8%) patients had adenocarcinoma and 13 (48.2%) had squamous-cell carcinoma in histological type.

PR was observed in 6 (22.2%), SD in 5 (18.5%) and progressive disease in 16 (59.2%) patients.

With respect to hematological toxicity grade 3 – 4 neutropenia was observed in six (22.2%) patients, grade 3

thrombocytopenia in one (3.7%) patient and grade 3 anemia in one (3.7%) patient. Other toxicities included grade 3 – 4 nausea/emesis in nine (33.3%) patients, grade 3 fever in nine (33.3%) patients and grade 3 nephrotoxicity in one (3.7%) patient. Further toxicities such as rush, constipation and peripheral neuropathy were rare and/or mild. Median overall time to tumor progression was 14 weeks (3 – 50). The preliminary results of this continuing Phase II trial were encouraging in terms of response rate and toxicity [72]. Especially important is the fact that Lipoplatin seems to have activity in cisplatin-resistant tumors, something predicted previously from the liposomal nature of the drug; Lipoplatin was proposed to be able to treat cisplatin-resistant tumors with resistance arising at the cell membrane level and not at the level of DNA repair [12]. It will be interesting to examine the gene expression profile of Ctr1, ATP7A, ATP7B cisplatin transporters as well as for ERCC1 and other DNA repair genes in white blood cells or in tumor specimens in the group of patients with PR, SD and progressive disease.

8.7 Lipoplatin, 5-FU and radiotherapy for locally advanced gastrointestinal adenocarcinoma

The objective of a Phase II study was to investigate the toxicity, response rates and overall survival of Lipoplatin radio-chemotherapy in locally advanced gastric adeno-carcinomas, in those unable to undergo surgery and to test the radiosensitizing ability of Lipoplatin because of the concentration of its nanoparticles in tumors. Patients with locally advanced gastric cancer or gastric cancer inoperable for medical reasons or recurrent carcinomas of a performance status of 0 – 2 were recruited. Patients with previous radiotherapy, with an extensive metastatic disease or with uncontrolled brain metastasis were excluded.

Lipoplatin was given at a dose of 120 mg/m^2, 5-FU at 400 mg/m^2 (day 1), while radiotherapy was given through 3.5 Gy fractions on days 2, 3 and 4 in a 7-day schedule. Two groups of six patients received 4 and 5 consecutive cycles, respectively. Twelve of twenty planned patients in this study have completed treatment. No WHO grade 3 or 4 nephrotoxicity, anemia, asthenia or neuropathy were noted, except of grade III neutropenia in 1 (8%) of 12 patients. A net improvement of the performance status (from a median of 1 – 0) was recorded at 2 months after the end of therapy. The response rates assessed with CT-scan, endoscopy and biopsies confirmed 33% (2/6) complete remission and 3 (50%) of 6 PR in patients treated with four cycles and 4 (80%) of 5 complete remission in patients treated with five cycles [73].

Concurrent hypofractionated radiotherapy (4 – 5 Gy/fraction, 2 fractions a week) and 5-FU bolus 1 h before RT at doses of 300 mg/m^2 in patients suffering from recurrent or locally advanced inoperable colorectal cancer was an established scheme in this center [74].

9. Tumor targeting in human studies

Intravenous infusion of Lipoplatin to four patients (one with hepatocellular adenocarcinoma, two with gastric cancer, and one with colon cancer, **Table 3**) followed by a prescheduled surgery ~ 20 h later was used to show the accumulation of the drug in the lesion. During this study, tumor specimens were obtained during surgery but also adjacent noncancerous tissue; the specimens were first extracted in saline solution, a mild method that preserves cellular integrity, and the platinum that was solubilized was related to platinum trapped in tissues ('Trapped' in **Table 3**). Saline-insoluble material from tumor specimens was subsequently extracted in sodium dodecyl sulfate that dissolved membranes, nuclei, denaturing protein assemblies, RNA and DNA from chromatin; the sodium dodecyl sulfate-soluble fraction of the specimens revealed the amount of platinum that was bound to macromolecules ('Reacted' in **Table 3**). The ratio of platinum in tumor specimens versus platinum in the adjacent normal tissue revealed the concentration-fold of the nanoparticles in the cancer over normal tissue (**Table 3**).

Direct measurement of platinum levels by atomic absorption in the extracts from specimens from the excised tumor and the adjacent normal tissue as well as metastases (colon metastasis from a liver tumor, liver metastasis from a gastric cancer) showed that total platinum levels that reacted with macromolecules and caused damage to tissue were on the average 10 – 171 times higher in malignant tissue compared to the adjacent normal tissue; most effective targeting was observed in colon cancer with an accumulation up to 200-fold higher in colon tumors compared to normal colon tissue. Gastric tumor specimens accumulated the highest levels of drug than any other tissue and, thus, Lipoplatin may prove effective against stomach cancers in future clinical studies (**Table 3**) [29].

In conclusion, Lipoplatin was preferentially concentrated in the primary tumor and the metastases in human patients undergoing chemotherapy. High tumor levels were seen at about 20 h from infusion of the drug under conditions in which blood levels of Lipoplatin had dropped to below 1 mg/ml from Phase I study [22]. Targeting was proposed to take place at two levels: i) after i.v. injection, Lipoplatin was preferentially (40-times) concentrated into tumors by extravasation through the leaky tumor vasculature. To achieve this result, the nanoparticles of Lipoplatin are coated with PEG for long circulation and low clearance by macrophages. ii) Once inside the tumor, Lipoplatin nanoparticles were proposed to diffuse to the extracellular space and to be taken up more avidly by the cell membrane of the tumor cell compared to normal cell (five times more). This is supposed to arise from the avidity of tumor cells for nutrients (the lipid shell of Lipoplatin composed of lipids is mistaken as a nutrient) as well as by an enhanced diffusion of the nanoparticles with the cell membrane; to enhance uptake the nanoparticles the fusogenic lipid, DPPG, was used during formulation. These two mechanisms together contribute to an up to 200-fold higher

Table 3. Summary of human targeting by Lipoplatin.

Patient no. and specimen	Trapped (µgPt/g tissue)	Tumor Pt/ normal tissue Pt	Reacted (µgPt/g tissue)	Tumor Pt/ normal tissue Pt
No. 1 Liver tumor	5.18	0.31	33.18	10.50
No. 1 Normal liver tissue	16.45		3.16	
No. 1 Colon metastasis	4.44	74.00	2.17	27.12
No. 1 Normal colon tissue	0.06		0.08	
No. 2 Liver metastasis	34.51	2.04	96.64	24.16
No. 2 Normal liver tissue	16.94		4.00	
No. 3 Stomach tumor 1	44.17	16.86	220.45	55.53
No. 3 Stomach tumor 2	28.46	10.86	37.92	9.55
No. 3 Normal stomach tissue	2.62		3.97	
No. 4 Colon tumor 1	4.42	221.00	6.85	171.25
No. 4 Colon tumor 2	1.86	93.00	5.83	145.75
No. 4 Normal colon tissue	0.02		0.04	

Values in the column 'Trapped' or 'Reacted' are expressed in µg platinum (Pt)/g tissue measured by atomic absorption. The other two columns show the ratio (total platinum in tumor) versus (total platinum in the corresponding normal tissue).
Adapted from [29].

damage to cancer tissue compared to normal tissue and may contribute to the low side effects of the drug.

10. Phase III studies

10.1 Lipoplatin plus gemcitabine versus cisplatin plus gemcitabine as first-line treatment in patients with NSCLC

A randomized multi-center Phase III non-inferiority clinical study compares Lipoplatin 120 mg/m^2 on days 1, 8 and 15 plus gemcitabine 1 g/m^2 on days 1 and 8 in a 21-day cycle (Arm A or Lipo/gem) with cisplatin 100 mg/m^2 on day 1 plus gemcitabine 1 g/m^2 on days 1 and 8 in a 21-day cycle (Arm B or *Cis*/gem) as first-line treatment in patients with NSCLC. Patients have disease evaluation after three and six cycles and the planned number of patients is 200 in each treatment arm. The primary end points are overall survival. Secondary end points are toxicity, overall response rates, progression-free survival and quality of life. Adverse events are assessed using the WHO Common Toxicity Criteria (CTC). Eligibility criteria included confirmed diagnosis of inoperable or metastatic NSCLC, no previous chemotherapy, WHO PS 0 – 1, and adequate end-organ function. Lipoplatin was administered without hydration as a 6 h infusion in 1 l 5% dextrose compared to patients receiving cisplatin who were admitted to the hospital the day before treatment from pre-hydration and had an extended stay for post hydration to minimize adverse effects and enhance renal excretion of cisplatin.

In a preliminary report on this non-inferiority Phase III trial presented to ASCO [75], 59 patients were included of whom 33 received the Lipo/gem and 26 the *Cis*/gem regimen. There were no grade 4 toxicities. Grade 3 toxicities were observed in < 5% of the patients and were comparable in the two groups, with the exception of neutropenia (3% for Lipo/gem and 15% for *Cis*/gem) (**Table 4**). Grade 2 nephrotoxicity was reported for 6% of Lipo/gem patients versus 19% of *Cis*/gem patients. Neurotoxicity was also markedly less in the Lipo/gem arm. Particularly important might be the significantly lower neuro- and nephro-toxicity of the Lipoplatin arm and its administration on an outpatient basis with clear pharmacoeconomic benefits; Lipoplatin was administered without pre- and post-hydration as a 6-h infusion.

An interim analysis of this trial on 101 patients of whom 60 received the Lipo/gem and 41 the *Cis*/gem regimen, with a stratification for histological subtypes of NSCLC, showed there was a significant reduction in nephrotoxicity, nausea/vomiting, neurotoxicity and asthenia in the Lipo/gem compared to *Cis*/gem treatment arms [76]. This study has recruited > 280 patients and is expected to lead to a pivotal EMEA study in the non-squamous histological subtypes of NSCLC in 2009.

10.2 Lipoplatin plus paclitaxel versus cisplatin plus paclitaxel as first-line treatment in NSCLC

The use of a taxane in combination with a platinum compound has become an acceptable standard as first-line treatment for patients with advanced or metastatic NSCLC [77-79].

This randomized Phase III used 200 mg/m^2 Lipoplatin plus 135 mg/m^2 paclitaxel administered on day 1 repeated every 2 weeks (Lipo-Taxol or Arm A). Lipoplatin was infused for 8 h in 1 l 5% dextrose. Arm B (*Cis*-Taxol) was 75 mg/m^2

Table 4. Preliminary toxicity data from a randomized non-inferiority Phase III study.

	Lipoplatin arm (33 patients)	Cisplatin arm (26 patients)
Nephrotoxicity grade II	6.0	19.0
Nephrotoxicity grade III	0	4.5
Nausea and vomiting	0.0	6.8
Asthenia and anorexia	1.8	11.4
Anemia	5.3	2.3
Leucopenia	14.0	9.1
Neutropenia*	3.0	15.0
Thrombocytopenia	10.5	13.6
Neurotoxicity	+	+++

Numbers indicate percentage of patients.
Data were taken from [75].
*Neutrophiles are more important that leucocytes for fighting infections; the fact that Lipoplatin does not cause neutropenia to the extent of cisplatin is a positive virtue of the drug.

Table 5. Summary of the preliminary toxicity results of the Phase III Lipoplatin plus paclitaxel versus cisplatin plus paclitaxel study.

	Toxicity	
	Arm A: Lipo-taxol	Arm B: Cis-taxol
Renal toxicity	3.70%	25.92%
Neurotoxicity grade I – II	Grade I – II only: 25.92%	Grade I – III: 44.44%
Nausea-vomiting	18.52%	25.92%
Myelotoxicity	Grade I – II only: 37.04%	Grade I – III: 62.96%

Data were taken from [80].

cisplatin (hydration of 2 l) and 135 mg/m^2 paclitaxel, administered every 2 weeks. One cycle was 14 days and the plan was to give nine cycles (treatments) per patient unless disease progression was detected before the ninth cycle.

The main objective of the study was to show that Lipoplatin was not inferior to cisplatin when combined with paclitaxel as first-line treatment as assessed by overall survival in a randomized group of patients with NSCLC at stage IIIB/IV (with locally advanced or metastatic disease) but that patients in the Lipoplatin/paclitaxel arm (Arm A) had a better toxicity profile and showed a better quality of life (EORTC questionnaire) compared to patients in the cisplatin/paclitaxel arm (Arm B). Secondary objectives of the study were to compare the time to tumor progression, 1-year survival and response rate between the two arms.

In a preliminary report of the study [80], 61 chemonaive patients were recruited as of December 2006 and 54 patients were evaluable for response and toxicity, 27 in each arm. The median age was 65 (42 – 80). The toxicity data are summarized in **Table 5**.

In the Lipo-Taxol arm, renal toxicity was observed in 1 (3.70%) patient, neurotoxicity grade I – II in 7 (25.92%) patients nausea-vomiting in 5 (18.52%) patients and myelotoxicity grade I – II in 10 (37.04%) patients. In the *Cis*-Taxol Arm, renal toxicity was observed in 7 (25.92%) patients, neurotoxicity grade I – III in 12 (44.44%) patients nausea-vomit in 7 (25.92%) patients and myelotoxicity grade I – III in 17 (62.96%) patients. Thus, the toxicity differences were very important between the two arms. In particular, the renal toxicity seemed to be sevenfold lower in the Lipoplatin arm. Also significantly lower was the neurotoxicity and myelotoxicity of grade III (totally absent in the Lipoplatin arm). It was concluded that the response rate was similar but toxicity and in particular nephrotoxicity, neurotoxicity and myelotoxicity were significantly lower in the Lipoplatin arm [80].

This Phase III was terminated successfully after treating 236 patients (of whom 229 were evaluable), 114 in arm A (Lipo-Taxol) and 115 in arm B (*Cis*-Taxol), respectively; the data showed the non-inferiority of the Lipoplatin–paclitaxel combination compared to cisplatin–paclitaxel in the schedule described above but with statistically significant lower toxicities in the Lipoplatin–paclitaxel arm for nephrotoxicity, grade 3 and 4 leukopenia, grade 2 and 3 neuropathy, asthenia (fatigue) and gastrointestinal toxicity (nausea/vomiting). There was no significant difference in median and overall survival as well as time to tumor progression between the two arms [81]. Patient cases from this study before and after treatment with Lipoplatin plus paclitaxel are shown in **Figure 3**.

10.3 Lipoplatin plus 5-FU versus cisplatin plus 5-FU against SCCHN

Cisplatin remains the reference drug in the induction chemotherapy setting for SCCHN when used in combination with 5-FU. However, its clinical use is limited by its peripheral neuropathy, as well as renal and hematological toxicity, manifesting at increasing cumulative doses.

A randomized, multi-center Phase III trial against SCCHN was designed, in which conventional cisplatin or Lipoplatin were used in combination with 5-FU, to compare efficacy and safety profiles of both treatment arms. A pharmacokinetics study from this trial was published [82]. Inclusion criteria were: patients with histologically confirmed SCCHN (primary metastatic or patients with relapsed/progressive disease) with at least one measurable bidimensional lesion, between the age of 18 – 75 years, a performance status of at least Eastern Cooperative Oncology Group 3, an adequate bone marrow function (a peripheral absolute leukocyte count of at least 2500/mm^3 and platelet count of at least 100,000/mm^3) and an adequate liver function, with a sufficient

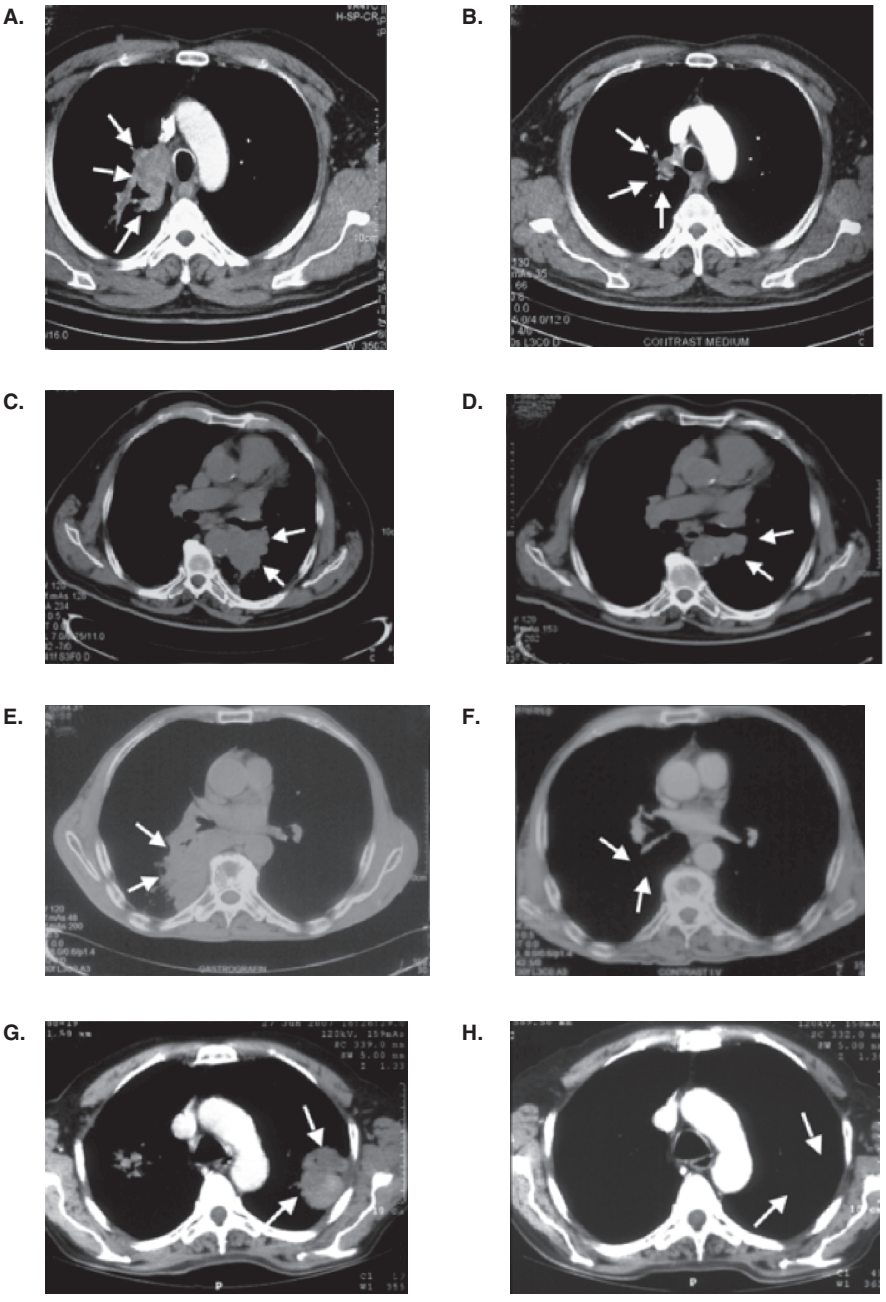

Figure 3. Patient cases from the Phase III study comparing Lipoplatin to cisplatin in combination with the same dose of paclitaxel documented with CT slices (with mediastinal window settings) before and after treatment. A. A low differentiated adenocarcinoma in the right upper lobe is present. **B.** The lesion was recorded as a partial response after four cycles of Lipoplatin–paclitaxel chemotherapy. **C.** A large adenocarcinoma cell tumor (arrows) in the left lower lobe. **D.** Follow-up scan taken after five cycles of Lipoplatin–paclitaxel treatment show reduction of volume of the lesion. **E.** CT section demonstrating a large tumor mass in the right lobe. **F.** Follow-up scan after nine cycles of Lipoplatin–paclitaxel treatment demonstrated a remarkable mass reduction. **G.** CT section demonstrating a large tumor mass in the left lobe. **H.** Follow-up scan after nine cycles of Lipoplatin–paclitaxel treatment showed mass reduction.

From [109] with permission from Gene Therapy Press.

renal function (defined as creatinine clearance > 50 ml/min). Exclusion criteria included progression during 100 mg/(m^2 day) cisplatin-based chemotherapy, no progressive disease after chemotherapy or radiochemotherapy, < 3 weeks since previous surgery, pregnancy, active/unstable ischemic heart disease, Hepatitis B or C and use of nonstudy cancer therapy. Stratification criteria were primary metastatic disease, recurrent or progressive SCCHN, previous chemotherapy/no previous chemotherapy, previous cisplatin-based chemo-therapy/previous non cisplatin-based chemotherapy and center.

This study is using treatment with 100 mg/(m^2 day) Lipoplatin as a 4 h i.v. infusion (days 1, 8, 15) plus 1000 mg/(m^2 day) 5-FU (days 1 – 5 continuous infusion) every 21 days (one cycle) for six cycles (Arm A). The comparative arm (Arm B) uses 100 mg/(m^2 day) cisplatin with pre- and post-hydration (day 1) plus 1000 mg/(m^2 day) 5-FU (days 1 – 5 continuous infusion) every 21 days (one cycle) for six cycles.

A dose reduction of cisplatin occurred from 100 to 70 mg/m^2 when the creatinine clearance fell between 99 and 70 ml/min, leukopenia < 500/µl during the last cycle, neutropenic fever/infection during last cycle or thrombopenia < 50,000/µl during the last treatment cycle. Cisplatin was reduced to 50 mg/m^2 when the creatinine clearance fell between 69 and 50 ml/min or mucositis CTC grade 4 occurred. 5-FU was reduced in dose from 1000 to 500 mg/(m^2 day) when severe hand and foot syndrome or mucositis CTC grade 4 occurred. No dose reductions of Lipoplatin were performed.

An interim analysis was reported [83] on 46 evaluable patients, 25 in the Lipoplatin/5-FU and 21 in the cisplatin/5-FU arm, respectively, after at least two cycles in both arms. The main end points for this interim analysis were hemato- and nephro-toxicity.

Toxicity: Seven patients had to stop cisplatin therapy due to severe toxicity as compared to one patient in the Lipoplatin treatment arm. Severe hematotoxicity was more frequent in the cisplatin arm, with grade III and IV toxicity occurring in 31.7% of the patients treated with the cisplatin-based regimen versus 12% in the Lipoplatin-based regimen (**Table 6**). Grade IV leucopenia occurred in 22.2% of the patients treated with cisplatin/5-FU, whereas in the Lipoplatin/5-FU arm, 0% grade IV leukopenia occurred.

One of most debilitating toxic side effects and a great impingement on the quality of life of cisplatin-based chemotherapies is neuropathy. Lipoplatin seems to reduce neurotoxicity profoundly. A total of 67% of the patients treated with the cisplatin regimen experienced grade I and II neuropathy compared to 27% in the Lipoplatin arm. More patients developed severe mucositis in the cisplatin-based regimen than in the Lipoplatin regimen: 33.3% of the patients treated with cisplatin suffered grade III or IV mucositis and mostly hospitalization was required, compared to only 8% in the Lipoplatin treatment arm. The renal toxicity profile of both drugs also showed marked differences: 23.8% of the treated patients suffered a significant reduction in kidney function, with a decrease in creatinine clearance below 50 ml/min in the cisplatin arm; furthermore, three patients suffered acute renal insufficiency in the cisplatin arm. In contrast, no grade III or IV renal toxicity occurred in patients treated with Lipoplatin. This continuing study has shown so far that the Lipoplatin formulation reduces both the hematological and non-hematological toxicity profiles of cisplatin to a clinically relevant extent when combined with 5-FU.

The efficacy results showed 38.8% objective partial remission in the Lipoplatin arm versus 19% in the Lipoplatin arm. However, 64% of the patients achieved SD while being treated with Lipoplatin/5-FU, compared to 50% of the patients in the cisplatin/5-FU regimen. A total of 24% of the patients progressed while being treated with Lipoplatin/5-FU versus 14.3% of these treated with cisplatin/5-FU. A high rate of SD was observed in the Lipoplatin versus cisplatin arms (64 versus 50%); also the clinical benefit rate (SD + partial remission) was similar for the cisplatin (88.5%) and Lipoplatin combinations (83%), although there were more objective responses seen in the cisplatin arm. Because patients with advanced SCCHN have an increased risk of renal toxicity due to poor hydration, the observed reduction of side effects with cisplatin can help to preserve the dose density of chemotherapy, and thereby efficacy, and to improve the quality of life of these patients [83].

Increasing the dose of Lipoplatin to its weekly recommended schedule of 120 mg/m^2 and further reducing its infusion rate to reduce toxicities might improve the efficacy results. The overall Lipoplatin dose in the LipoFU study is 300 mg/m^2 every 21 days compared to 360 mg/m^2 every 21 days in the LipoGEM study (see above section 10.1). Also, the LipoFU trial recruits both chemonaive and previously treated patients compared to LipoGEM that recruits only chemonaive patients. Both 5-FU and gemcitabine belong to the class of antimetabolites according to the FDA classification.

11. Discussion

11.1 Clinical benefit of Lipoplatin in NSCLC

Lung cancer is the most common cause of cancer-related death in men and the second most common in women, while it is responsible for 1.3 million deaths worldwide annually and ~ 300,000 new cases in the EU. Approximately 80% of lung cancer cases are NSCLC and in > 70% of these cases, disease is diagnosed at a late stage, when already locally advanced or metastatic. NSCLC is a slow spreading malignancy that consists of three major subtypes, adenocarcinoma, squamous cell carcinoma and undifferentiated large cell carcinoma, with frequencies 50, 30 and 5%, respectively [84]. A preferred regimen for first-line treatment against NSCLC include gemcitabine and cisplatin in EU and carboplatin-paclitaxel in the US [85,86]. Cisplatin–gemcitabine–bevacizumab [87], vinorelbine–platinum [88] and cisplatin–pemetrexed [89] have also been tested as front line. In the second-line setting of NSCLC, docetaxel, pemetrexed and erlotinib are widely used [90] although further experimental

Table 6. Hematological and non-hematological toxicities for cisplatin/5-FU regimen (n = 21) versus Lipoplatin/ 5-FU regimen (n = 25).

Grade	I		II		III		IV	
% patients	Cisplatin	Lipoplatin	Cisplatin	Lipoplatin	Cisplatin	Lipoplatin	Cisplatin	Lipoplatin
WBC	33.3	16.0	22.2	7.0	9.5	0.0	22.2	0.0
Platelets	19.0	8.0	14.3	0.0	4.8	0.0	0.0	0.0
Hemoglobin	33.3	20.0	38.9	16.0	9.5	16.0	0.0	0.0
Nausea	4.8	8.0	27.8	16.0	28.6	8.0	9.5	0.0
Mucositis	4.8	8.0	22.0	4.0	19.0	4.0	14.3	4.0
Diarrhea	9.5	0.0	9.5	4.0	19.0	4.0	0.0	0.0
Infection	0.0	0.0	9.5	12.0	28.6	28.0	14.3	0.0
Allergic reaction	0.0	0.0	0.0	8.0	0.0	0.0	0.0	4.0
Renal	9.5	12.0	28.6	40.0	23.8	0.0	0.0	0.0
Neuropathy	33.3	27.0	33.3	0.0	19.0	0.0	0.0	4.0

Data were taken from [83].
5-FU: 5-Fluorouracil; WBC: White blood cell.

second-line treatments have been explored including gemcitabine–irinotecan [91]. Response rates of 20 – 40% can now be expected, with a median survival of 8 – 11 months and a 1-year survival rate of 30 – 40% [86,92].

In the quest for new treatments the combination of bevacizumab, a humanized anti-VEGF monoclonal antibody, with chemotherapy was shown to produce better outcomes than chemotherapy alone in chemotherapy-naive, advanced, non-squamous NSCLC patients. Indeed, The Eastern Cooperative Oncology Group study E4599 [86] on 878 patients comparing paclitaxel/carboplatin with or without bevacizumab was the first Phase III randomized trial to show a survival benefit for carboplatin–paclitaxel plus bevacizumab over chemotherapy alone; the results of this study led the FDA to approve this novel combination for first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous NSCLC. The median survival was 12.3 months in the group assigned to chemotherapy plus bevacizumab, as compared with 10.3 months in the chemotherapy-alone group.

In all preclinical and clinical studies described here, Lipoplatin displayed low renal toxicity. The mechanism of severe nephrotoxicity caused by cisplatin, but not carboplatin, oxaliplatin and nedaplatin, is not fully understood. Emerging data showed that the nephrotoxicity of platinum agents was closely associated with their renal accumulation, which was determined by the substrate specificity of the OCT and MATE families; indeed, a luminal H^+/organic cation antiporter, rMATE1 (multidrug and toxin extrusion) as well as human MATE1 and hMATE2-K, stimulated the H^+-gradient-dependent antiport of oxaliplatin, but not of cisplatin in rat kidneys [93].

A number of agents have been shown to ameliorate experimental cisplatin nephrotoxicity; these include antioxidants (e.g., melatonin, vitamin E, selenium and many others), modulators of nitric oxide (e.g., zinc histidine complex), agents interfering with metabolic pathways of cisplatin (e.g., procaine HCl), diuretics (e.g., furosemide and mannitol), and cytoprotective and antiapoptotic agents (e.g., amifostine and erythropoietin). On the contrary, nitric oxide synthase inhibitors, spironolactone and gemcitabine, augment cisplatin nephrotoxicity (reviewed in [94]).

11.2 Clinical benefit of Lipoplatin in other tumors

The Phase II studies on pancreatic, breast and gastric cancers are expected to promote Lipoplatin as an important drug to the arsenal of chemotherapeutics. Two groups of advantages of the drug are expected to help its promotion in the clinic: i) lower nephrotoxicity and administration benefits (without hydration); and ii) concentration into tumors. Important data are expected to be obtained from tumors of high vascularization (e.g., gastric cancer) because of the proposed ability of Lipoplatin nanoparticles to use the vascular system of the tumor for extravasation. These studies will also provide information on the combination drug that gives optimal anticancer results with Lipoplatin against a certain indication. Gemcitabine, 5-FU, paclitaxel, vinorelbine and radiation have been under evaluation so far. Of these, gemcitabine and 5-FU are from the antimetabolite class; paclitaxel and vinorelbine are antimicrotubule agents; and radiation can inflict double strand breaks on the DNA. Each drug has also different toxicity profile with gemcitabine, for example, displaying myelotoxicity and paclitxel neurotoxicity.

The preliminary Phase II and III studies of Lipoplatin reviewed here, as well as further planned studies, are expected to establish Lipoplatin as an important chemotherapy drug with a broad range of activity against epithelial malignancies,

tumor targeting (see below), lower side effects and with an improved quality of life and overall survival. Especially important, a breakthrough in the chemotherapy field using nanotechnologies is anticipated to be the efficacy of Lipoplatin, compared to cisplatin in randomized trials against the non-squamous histological types of NSCLC, as well as in pancreatic, breast and gastrointestinal cancers. A preliminary efficacy in the adenocarcinoma histological subtype of NSCLC showed 83% response/stabilization rate with Lipoplatin + gemcitabine compared to 54% in the cisplatin + gemcitabine treatment arm in a recently completed Phase II study.

Also eminent is its significant radiosensitizing ability, especially in brain metastases from NSCLC after concurrent radiation (Angel, Theageneion Anticancer Hospital, Thessaloniki, Greece, in preparation). Finally, pharmacoeconomic benefits arise from its i.v. infusion without pre- or post-hydration on an outpatient basis, with less use of hematopoietic factors and no hospitalization costs from chemotherapy complications. Furthermore, it has allowed administration of a higher total dose of cisplatin due to a highly reduced cumulative toxicity.

12. Conclusions and prospects

12.1 Liposomes and other nanomaterials in cancer

Liposomes can be used as carriers of peptide, protein and antigen-encoding DNA vaccines [95]. Liposomes may be effective vehicles to improve the delivery of antisense oligonucleotides to the liver for the therapy of hepatotropic viruses [96]. Phospholipid liposomes and charged nanoparticles can be mixed together using sonication to yield particle-stabilized liposomes that repel one another and do not fuse [97]. A nanoliposomal CPT-11 (irinotecan) formulation has been described with unprecedented drug loading efficiency and *in vivo* drug retention using a modified gradient loading method [98]. Drugs of poor water-solubility and high toxicity, such as camptothecin, can also benefit from nanotechnology formulations.

Sterically stabilized liposomes have been used for various applications by others; such liposomes prevent opsonization and reticular endothelial system uptake. PEGylation is known to greatly enhance the longevity of proteins, liposomes and other molecules in blood circulation [99]. Naturally occurring polymers of N-acetylneuraminic acid (polysialic acids) are biodegradable, exhibit long half-lifes in the blood circulation and have, therefore, been proposed as carriers of short-lived drugs and small peptides [100]. Poly-(lactide), poly-(lactide-co-glycolide) and poly-(lactide-co-caprolactone) microspheres have also been used for the encapsulation of 5-FU by spray drying and slow release for inhalation delivery system for adjuvant therapy of lung cancer [101]. Upgrading these promising technologies and products to successful clinical studies remains a difficult task.

12.2 Possibilities of our technology

Cisplatin, one of the most widely used and most effective cytotoxic agents in the treatment of epithelial malignancies was encapsulated into 100 nm in diameter liposomes in a stable formulation, Lipoplatin. The present article reviews the clinical data using Lipoplatin and discusses the mechanisms of the liposomal formulation. One important issue contributing to the therapeutic efficacy of Lipoplatin results from its ability to target primary tumors and metastases and to cause a greater damage to tumor tissue compared to normal tissue. Tumor uptake of the Lipoplatin nanoparticles (**Table 1**, **Figure 2**) results from their preferential extravasation through the leaky vasculature of tumors. Furthermore, a higher uptake of Lipoplatin nanoparticles by tumors takes place presumably arising from a more avid phagocytosis by tumor cells compared to adjacent normal tissue in human studies. The two mechanisms result to an overall 10- to 400-fold higher intracellular uptake of total platinum in tumor cells compared to cells in normal tissue. Lipoplatin is currently under several Phase III evaluations.

Antisense VEGF oligodeoxynucleotides formulated in cationic liposomes could downregulate the expression of VEGF and could inhibit the growth of tumors [102]; our liposomes as carriers of antisense VEGF could also combined with Lipoplatin nanoparticles to test efficacy in animal studies. Antiangiogenic agents alone cannot eradicate tumors completely and are combined with other therapy to enhance their effects. Flk-1, a soluble VEGF receptor, is a potent inhibitor of angiogenesis. Flk-1 gene therapy combined with cisplatin improved antitumor efficacy in animals [103]. Phage display peptide libraries led to the identification of peptides (for example, CTKNSYLMC) with affinity for gastric cancer vascular endothelial cells [104]. Peptides are proposed here to be attached at the end of PEG in Lipoplatin to target specific types of cancer vascular endothelial cells as second-generation Lipoplatin nanoparticles, thus, enhancing the antiangiogenesis potential of the drug.

12.3 The pharmacoeconomics of Lipoplatin

Lipoplatin is being administered on an outpatient basis without pre- or post-hydration and with clear pharmacoeconomic benefits over cisplatin that requires admittance of the patient to the hospital a day before and a day after treatment for pre- and post-hydration. Hospitalization costs are usually $1000/day in most Western countries. Although a 6 – 8 h infusion is recommended to minimize adverse reactions, a 4 h infusion is being used in the Phase III SCCHN study to deliver a total dose of 100 mg/m^2 [83] and a 3 h infusion to deliver a total dose of 120 mg/m^2 [73]. In addition, there is less healthcare requirements for the recovery of patients from adverse reactions, especially nephro- and neuro-toxicity as well as less use of the expensive hematopoietic factors GM-CSF after administration of Lipoplatin compared to cisplatin. The expected increase in overall survival and improvement in the quality of life suggested from preliminary results (e.g., [70,75,76,80]) are also considered important benefits. Although, the pricing of Caelyx/Doxil over doxorubicin is about 20 – 27 times higher per mg on

the basis of the active pharmaceutical ingredient. Although Lipoplatin has not received marketing authorization yet, its pricing takes into consideration its affordability for establishing it as a drug able to replace cisplatin in all world markets.

13. Expert opinion

The present article reviews the features and possible clinical applications of a nanotechnology formulation for cisplatin. The advantages of the platform encapsulation technology for Lipoplatin are described; its proposed ability to cross the cell membrane barrier and to deliver its payload to the interior of the cell suggest a property close to that of a magic bullet. The same technology was also applied to a liposome formulation of oxaliplatin (Lipoxal™) that has completed successfully a Phase I study [105].

A similar technology has been applied to liposomal encapsulation of plasmids carrying therapeutic genes for gene therapy applications (**Figure 2**). So far, the human IL-12 has been tested in human trials expressed from a liposomally-encapsulated Semliki Forest virus; the completed Phase I study has proven safety, has determined the MTD and has shown that repeated administration of the therapeutic lipovirus is feasible without immune reactions to the patient [106]. Obviously, regimens integrating combination Lipoplatin chemotherapy with liposomal gene therapy would have the advantage of targeting both nanoparticles classes to similar tissues *in vivo*, especially to primary solid tumors and metastases; a more potent anticancer effect is expected with the proposed nanoparticle combinations than using the drugs separately or in a nonliposomal form.

A putative antiangiogenic activity of Lipoplatin has been shown in animal studies (**Figure 2**). This implies that Lipoplatin particles are primarily targeted to tumors and tumor vasculature. However, mechanisms of cellular uptake of the Lipoplatin particles by tumors and normal tissue await further elucidation. To demonstrate fusion between Lipoplatin and the cell membrane, we are using fluorescent lipids to label nanoparticles and show transfer of the label to membrane lipids in cells in culture with confocal microscopy. Continuing studies in our group are also using gene expression profile in patients from comparative Phase III studies before, during and at the end of treatment to assess mechanisms in responding versus non-responding patients. Studies can also be undertaken to determine the extent and nature of damage by Lipoplatin versus cisplatin at the DNA and other macromolecules.

The dose-dense Lipoplatin administration is important for efficacy. A weekly schedule of 120 mg/m^2 allows a higher total dose to be administered, now that the low cumulative toxicity of the drug has been established from Phase III studies. In a monotherapy study using 100 mg/m^2 every 14 days against NSCLC, the efficacy was low and the side effects were negligible [107]; the total dose administered was 100 mg/m^2 every 2 weeks as monotherapy compared to 240 mg/m^2 every 2 weeks as combination therapy in Phase II [70] and Phase III [76]. A dose-dense monotherapy study in advanced breast cancer is starting with dose escalation from a weekly 120 mg/m^2.

Lipoplatin could be tested with drugs that have a mechanism of action complementing or synergizing its own. For example, ionizing radiation eliciting DNA strand breaks or taxanes stabilizing tubulin polymers might show a synergistic effect with Lipoplatin even higher to that of cisplatin. Furthermore, Lipoplatin could be combined with a higher number of other chemotherapy regimens to explore reduction in the overall toxicity of the combination therapy.

The advent of taxanes (paclitaxel, docetaxel) stabilizing tubulin, molecules that can inhibit signaling and a number of new approaches such as those targeting apoptosis or DNA topoisomerases is revolutionizing cancer chemotherapy. A plethora of clinical trials in progress optimizes the different ways drugs can be administered; for example, the addition of cisplatin or carboplatin to paclitaxel results in higher response rates than for each of the drugs as single agents [108]. One could foresee application of nanotechnology and the extension of the Lipoplatin and Lipoxal formulations to taxanes and other molecules with tumor targeting abilities. Such an achievement and its promotion to the clinic would increase the efficacy of chemotherapy while reducing the side effects. The end goal of an effective anticancer regimen should always be the improvement in the quality of life of the patient and an extension in overall survival.

Acquired resistance to chemotherapy is a major hurdle. The major factor of resistance seems to be linked with transport of the chemotherapy drug across the cell membrane barrier. Lipoplatin was proposed to enter by direct fusion bypassing the Ctr1 copper transporter and other resistance mechanisms at the cell membrane level; in such a case, the drug could find applications in cisplatin resistant tumors, also suggested from a Phase II study [72] (see section 8.6).

Lipoplatin is anticipated to successfully complete several Phase III studies and become an important addition to the arsenal of anticancer drugs. It is hoped that chemotherapy regimens integrating Lipoplatin will allow higher overall survival of patients suffering with non-small cell lung, pancreatic, head and neck, gastric and other cancers with lower side effects and improvement in quality of life compared to cisplatin regimens.

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