Cancer therapy using nanoformulated substances: scientific, regulatory and financial aspects

Edouard Alphandéry, Pierre Grand-Dewyse, Raphael Lefèvre, Chalani Mandawala, Mickael Durand-Dubief

Cancer therapy using nanoformulated substances:

Scientific, regulatory and financial aspects.

Edouard Alphandéry[+,*], Pierre Grand-Dewyse[+], Raphael Lefèvre[+,x], Chalani Mandawala[+,⊥], Mickael Durand-Dubief[+]

[+]Nanobacterie Inc., 36 boulevard Flandrin, 75116, Paris, France.

[⊥]Muséum National d'histoire naturelle, IMPMC, 4 place Jussieu, 75252, Paris, France.

*Université Pierre et Marie Curie, IMPMC, 4 place Jussieu, 75252, Paris, France.

[x]Institut de physique du globe de Paris, 1 rue Jussieu, 75238 Paris Cedex 05, France.

E-mail addresses:

Edouard Alphandéry: edouardalphandery@hotmail.com

Pierre Grand-Dewyse: pgranddewyse@gmail.com

Raphael Lefèvre: raphael-lefevre@hotmail.fr

Chalani Mandawala: chalani@wanadoo.fr

Mickael Durand-Dubief: durand-dubief@hotmail.fr

Abstract: Several nanoformulated anti-cancer substances are currently commercialized or under development. Pre-clinical and clinical results have revealed better properties, *i. e.* larger efficacy and lower toxicity for these substances than for conventional anti-cancer treatments. Here, we review the development of several of these substances such as **Marqibo, Myocet, Doxil, DaunoXome, MM398, MM302, Mepact, Versamune**, **Thermodox, Depocyt, Livatag, Abraxane, Eligard, Opaxio, Zinostatin Stimalamer (SMANCS), Pegasys and PegIntron, BIND-014, CRLX-101, Oncaspar, Neulasta**, **Aurimmune, Auroshell, AuNPs, Nanotherm, NanoXray**, **Magnetosome chains**, **Kadcyla (T-DM1), Ontak (DAB/IL2), Gendicine and Curcumin. We describe their specific properties such as their stability, solubility, mean of administration or targeting, distribution, metabolism and toxicity. We discuss their categorization as medical devices or drugs, their fabrication process within a regulatory environment as well as intellectual property and financial aspects that are all essential to enable their industrial development.**

Keywords: nanoformulation, nanooncology, cancer, nanoparticle, nanomedicine, nanopharmaceuticals, nanodrug, nanoformulated drug.

Introduction:

Conventional cancer treatments such as surgery, radiotherapy and chemotherapy, although under continuous improvement, still carry many drawbacks for patients, such as side effects or a lack of total efficacy. Nanoformulated therapeutic substances have been developed to overcome these drawbacks, [1, 2]. They are usually characterized by properties, which are due to their specific size, which is less than 1 μm in at least one dimension. These substances belong to a wide range of different drug types such as liposomes, virosomes, lipid based, polymeric, metallic, protein-drug, and herbal nanoformulations. They are characterized by behaviours in the organism, leading to a set of advantageous parameters such as lower recommended dose and higher maximum tolerated dose (MTD) than for their non-nanoformulated counterparts. In addition, they have been shown to efficiently target tumours through two mechanisms, passive targeting via enhanced permeability and retention (EPR) effect and/or active targeting via a ligand attached to the nanoparticle-drug complex that targets cancer cell receptors. Most of them are administered intravenously with a polyethylene glycol (PEG) coating, which enables them to avoid capture by macrophages and

to be cleared by the mononuclear phagocyte system (MPS), also called reticulum endothelial system (RES). A few of them are injected by intratumoral, intramuscular, intra-arterial or intraperitoneal routes. As a whole, nanoformulated substances have been shown to be less toxic and either equivalently or more efficient than their non nanoformulated counterparts..

I. Overview of the different existing nanodrugs

Figure 1 provides an overview of the different types of nanoformulated therapeutic substances.

I.1. Lipid based nanoformulation

Marqibo, commercialized by Spectrum pharma, previously Talon Therapeutics, is a 100 nm liposome containing vincristine sulfate, a microtubule polymerisation inhibitor (table 1). It has been approved since 2012 for treatment of acute lymphoblastic leukemia (ALL), using several intravenous injections at a dose of 2.25 mg/m^2. Preclinical studies have shown a higher circulation time of **Marqibo** compared with free vincristine, optimized delivery to target tissue, facilitated dose intensification without increased toxicity (3, 4). Clinical studies carried out on patients with ALL showed that the MTD was 3-5 mg, two times larger than that of free vincristine. When it was administered intravenously at a dose of 2.5 mg/m^2 on 13 patients, it resulted in a clearance of 345 mL/h, higher than that of 189 mL/h observed with free vincristine, a higher MTD, a superior antitumor activity, a larger amount of vincristine delivered to tumour tissues compared with free vincristine (4, 5). **Marqibo** was also tested clinically for treatment of large B-cell lymphoma (6) and non-Hodgkin Lymphoma (7), (table 2).

Myocet, commercialized by Teva Pharma, previously named Cephalon, is a non-pegylated 190 nm liposome with a membrane of phosphatidylcholine and cholesterol containing

doxorubicin, which is a toxic anthracyclic used for solid and hematologic tumour treatments (table 1). It has been approved in Europe and Canada since 2000 for first line treatment of metastatic breast cancer (MBC) using intravenous administration repeated every three weeks at a dose of 60-75 mg/m^2 in combination with 600 mg/m^2 of cyclophosphamide. Clinical assessment of this treatment on 297 patients indicates that it improves the therapeutic index of doxorubicin by significantly reducing cardio-toxicity and grade 4 neutropenia while providing comparable antitumor efficacy (8). Compared with non-liposomal doxorubicin, **Myocet** is characterized by higher EHL, AUC and lower clearance (table 3). Moreover, unwanted toxicity due to PEG (swelling on the palms of hand and soles of feet, hand-foot syndrome) or to doxorubicin (cardiac or gastrointestinal toxicity) can be avoided (9, 10, 11). However, the absence of PEG also induces undesired phagocytosis of **Myocet** by mononuclear phagocytes resulting in lower EHL, AUC and higher clearance than with the pegylated liposome formulation Doxil. **Myocet** has also been tested for a combined treatment of MBC with docetaxel and trastuzumab (12) or with gemcitabine (13), as well as for treatments of relapsed/refractory myeloma (14), several different types of lymphoma (15, 16, 17) and sarcoma (18), (table 2).

Doxil or Caelyx, commercialized by Johnson and Johnson, previously Janssen, in USA, is a long-circulating 100 nm stealth liposome composed of a phospholipid bilayer with entrapped doxorubicin and methoxypolyethylene glycol (MPEG) bound at its surface, (table 1). It has been approved since 1999 for treatments of ovarian breast cancer and AIDS-related Kaposi's Sarcoma and for treatment of Multiple Myeloma in combination with bortezomib, using several intravenous administrations at a dose of 20 mg/m^2 to 50 mg/m^2. Data related with clinical efficacy of these treatments are publically available, (19). Due to the presence of PEG, Doxil® can avoid reticuloendothelial system (RES) and is characterized by longer EHL, larger

AUC and lower clearance than with **Myocet** (table 1) (20). However, hand–foot syndrome cannot be avoided, (21). Experiments have shown that **Doxil** could be used in combination with hyperthermia for treatment of refractory ovarian cancer, (22), cyclophosphamide for solid tumour treatments (23), a nanoparticle containing an inhibitor of topoisomerase I (24). In addition, by using TNF-α the vascular leakage was increased, leading to a larger quantity of **Doxil** reaching B16BL6 melanoma mouse tumours via the EPR effect (25), (table 2).

DaunoXome, commercialized by Gilead, is a 35 to 65 nm non-pegylated liposome composed of daunorubicin, an antineoplastic anthracycline antibiotic that interacts with DNA therefore altering its replication. In this product, daunorubicin is encapsulated inside a lipid bilayer of distearoylphosphatidylcholine and cholesterol (2:1 molar ratio), (table 1). It has been approved since 1996 for the treatment of AIDS related Kaposi's sarcoma, using several intravenous administrations at a dose of 40 mg/m^2. Clinical studies carried out on patients with AIDS related Kaposi's sarcoma have shown that **Daunoxome** is efficient, yields an improved pharmacokinetic profile compared with free daunorubicin, is well tolerated and can be safely administered up to 60 mg/m^2 (26), (table 3). **Daunoxome** has also been tested for treatment of acute myeloid Leukemia (27-33), metastatic breast cancer (34), sarcoma (35) and lymphoma (36), (table 2).

MM398, under development by Merrimack, is a 100 nm liposome covered by PEG containing irinotecan, a topoisomerase I inhibitor that induces DNA breakage and cell damage when it is converted to its active form SN-98, (table 1). **MM398** has been designed to prevent early metabolism of irinotecan and increase release in the tumor. Its optimum size favors the drug diffusion in the tumor through EPR effect, to convert irinitotecan into SN-98 when the drug reaches the tumor. Intratumor administration of 0.2-0.4 mg of **MM398** in mouse glioblastoma

tumors yielded 40% of total cure when the treatment was combined with radiotherapy (38). The efficacy of **MM398** was demonstrated in a phase II clinical study carried out on patients with pancreatic cancer, which showed that intravenous injection of 120 mg/m^2 of **MM398** led to a progression free survival rate of 2 to 4 months (39). This phase II, carried out on 40 patients, showed that the percentage of patients living more than 3 months increased from 40% in the absence of treatment up to 75% in the presence of treatment (table 2).

MM302, also under development by Merrimack, is a 75 to 110 nm liposome containing doxorubicin, [40]. **MM302** has been designed to protect the heart from adverse events associated with free doxorubicin and to improve targeting using both EPR effect and an antibody that binds HER-2 receptors (table 1). Phase I clinical studies, carried out on 47 patients with advanced HER2 positive breast cancers, did not show any decline in cardiac functions for 30 to 50 mg/m^2 of **MM302** administered intravenously. Clinical anti-tumour activity of this drug is currently under clinical evaluation, (table 2).

Mepact, commercialized by Takeda, previously IDM Pharma SAS, is a less than 100 nm multi-lamellar liposome containing mifamurtide, a synthetic immunostimulant derived from muramyl dipeptide that activates monocytes, macrophages and TNF-α, IL-1β, IL-6, IL-8 and IL-12, (table 1). It has been approved since 2009 in Europe for the treatment of non-metastatic osteosarcoma following complete tumour excision, using 48 intravenous administrations at a dose of 2 mg/m^2. A phase III clinical study showed that the postoperative combination of **Mepact** with other anti-neoplastic agents such as doxorubicin, cisplatin, methotrexate and ifosfamide improves survival in patients with high grade non-metastatic resectable osteosarcoma (41), (table 2).

Versamune, under development by PDS biotechnology, is a 100-120 nm DOTAP liposome containing a human papillomavirus (HPV) antigen, which is an E7 oncoprotein, (table 1). Versamune is a therapeutic vaccine. It is designed to be administered under the skin and incorporated inside dendritic cells that present E7 peptides to T cells in order to selectively destroy HPV tumours. Preclinical studies of **Versamune** carried out on mice bearing TC-1 lung tumours showed that **Versamune** produces migration of activated dendritic cells to dendritic lymph nodes, ROS generation, anti-tumour activity due to CD8+ T lymphocyte and tumour regression (42), (table 2).

Thermodox, under development by Celsion, is a 100 nm heat sensitive liposome containing doxorubicin, a DNA intercalating agent, and coated by PEG (43, 44), (table 1). Localized heat at mild hyperthermia temperatures (greater than 39.5 °C) releases the encapsulated drug from the liposome enabling high concentrations of doxorubicin to be deposited in and around the tumour. It is currently evaluated in a phase III study for primary liver cancer and in a phase II study for recurrent chest wall breast cancer (table 2).

Depocyt, commercialized by Skypharma and Sigma-tau, is composed of lipid nanoparticles containing cytarubide (or cytosine arabinoside), a cytosine analogue with arabinose sugar that kills cancer cells by interfering with DNA synthesis. It has been approved for treatment of lymphomatous meningitis since 2001, (table 1) using 50 mg of **Depocyt** administered in the cerebrospinal fluid or lumbar pack several times. The superiority of **Depocyt** compared with non-encapsulated cytarubine was demonstrated through clinical trials carried out on 35 patients, which revealed a percentage of anti-tumor response of 72% among patients receiving **Depocyt**, higher than that of 18% observed among patients receiving non-encapsulated cytarubine, (45), (table 2).

Livatag, under development by Onxeo, previously named Bioalliance pharma, is made of 100 to 300 nm nanoparticles containing doxorubicin. It is developed for the treatment of hepatocellular carcinoma, the most common type of liver cancer. This product is able to deliver the DNA intercalating agent doxorubicin to chemoresistant cells. It was granted an orphan drug status in Europe and in the United-States. Although apparently unpublished in peer-reviewed scientific journal, a phase II study of **Livatag** carried out on patients with liver cancer seems to have led to a median survival of 32 months for patients treated with **Livatag** compared with 15 months for patients receiving current best of care (table 1). A phase III clinical study of **Livatag** is currently ongoing and first results seem to indicate a good tolerance to the treatment.

I.2. Polymeric nanoformulation:

Abraxane, commercialized by Abraxis bioscience and Astra Zeneca, is composed of chemotherapeutic human albumin nanoparticle associated with paclitaxel, (table 1). It has been approved for metastatic breast cancer treatment since 2008. The MTD of Abraxane has been shown to be 300 mg/m^2, higher than that of 175 mg/m^2 observed with free paclitaxel (46, 47). Moreover, **Abraxane®** evades hypersensitivity reactions associated with Cremophor EL, the solvent in traditional paclitaxel (48). A phase III clinical study on 454 patients with MBC has compared the efficacy of 260 mg/m^2 **Abraxane®** with that of 175 mg/m^2 cremo-paclitaxel, where both drugs were administered intravenously (49). **Abraxane** showed a higher response rate of 33% and a lower neutropenia level of 10% compared with those of 19% and 21% respectively observed with free cremo-plaxitel (table 2).

Eligard, commercialized by Astellas, is a biodegradable polymer matrix depot formulation of leuprorelin, a GnRH analog. This active compound can be used in the treatment of hormone

responsive cancer by acting as a GnRH agonist and indirectly reducing estradiol and testosterone levels. The drug received marketing authorizations from U.S. Food and Drug Administration (FDA) in 2002 and from EMA in 2007 for treatment of advanced prostate cancer, using a subcutaneous administration of **Eligard** once every six month at a dose of 45 mg. The active substance (leuproreline acetate) is delivered continuously during six months. When it was tested on 243 prostate cancer patients in Belgium, it led to a 95% reduction in both median testosterone levels and prostate-specific antigen levels. Overall safety and tolerability of **Eligard** were rated as good or excellent by 90% of physicians, (50).

Opaxio, under development by Cell Therapeutics, is a chemotherapeutic product containing a polyglutamate polymer linked to paclitaxel, which is the active drug compound in Taxol®. Once inside tumour cells, polyglutamate is metabolized and releases paclitaxel that will inhibit microtubule depolymerisation. Nanoformulated paclitaxel enhances radiosensitivity of tumour cells, therefore reducing radiation toxicity to normal tissue. This in turn increases the therapeutic index of paclitaxel due to EPR effect. In combination with temozolomide and irradiation, it is intended for the treatment of newly diagnosed glioblastoma, using 6 intravenous administrations of **Opaxio** at a dose of 50 mg/m^2. Clinical evaluation of this drug is ongoing and its results are pending, (51).

Zinostatin Stimalamer (SMANCS), commercialized by Astellas Pharma in Japan, is a styrene-co-maleic acid polymer conjugated with neocarzinostatin, an antibiotic carrying anti-tumoral activity. Neocarzinostatin is a macromolecular chromoprotein with high but non-covalent affinity to DNA, which induces strong DNA damage. It has been approved in 1994 in Japan for treatment of advanced and recurrent hepatocellular carcinoma (HCC), using **SMANCS** administered in the HCC artery. The treatment leads to the deposition of SMANCS within HCC

and to the gradual release of **SMANCS** from Lipiodol into tumour tissues. MTD has been estimated as 3 mg/m^2, (52). Preclinical studies also showed that due to EPR effect, **SMANCS** reaches highest tumour to blood ratio compared with a non-nanoformulated counterpart of **SMANCS**. Clinical trials were carried out on 44 patients with HCC. **SMANCS** showed strong activity among patients treated with 3-4 mg of this drug every 3-4 weeks. Indeed, 95% of patients showed a decrease in tumour size. A multicenter phase II study involving 400 patients with primary hepatoma also reported a high response of 30-40%, (53).

Pegasys and PegIntron, receptively commercialized by F. Hoffman-La Roche and Merck, are two different types of PEGylated interferon conjugates with enhanced half-life compared with their non-nanoformulated counterparts. On the one hand, **Pegasys** contains interferon alfa-2a, which is used in chronic hepatitis C and B and is co-administered with the pro-drug ribavirin, a nucleoside analogue of guanosin, which when metabolized affects viral replication (REF). On the other hand, **PegIntron** contains interferon alfa-2b and is prescribed for a similar indication than **Pegasys**. Both products are now studied as anticancer therapeutics. The efficacy of IFNγ in the treatment of melanoma and renal-cell carcinoma is well established, but protein administration induces toxicity and interferon has a short plasma half-life ($t_{1/2}$ = 2.3 hours), necessitating a 3-times-per-week administration schedule. In a phase I/II study, PEGylated interferon was injected subcutaneously once every 12 weeks to patients with advanced solid tumours. The observed MTD was 6.0 μg per kg a week and a response rate of 14% was seen in 44 previously untreated patients with renal-cell carcinoma, (53). **Pegasys** was tested clinically on 10 patients with aids associated Kaposi sarcoma, leading to a tumour response in 9 patients and a medium survival rate of 645 days, (54). It was also tested for the treatment of Myelofibrosis on 25 patients, resulting in a complete response among 64% of patients, (55). Concerning PEG-interferon, it was used to treat 29 patients with stage IV

melanoma overexpressing basic fibroblast growth factor (FGF-2). FPG-2 decreased in 97% of patients with suppression to normal range in 35% of patients, (56). Another clinical phase II study was carried out on 21 patients with **Pegintron** to evaluate the treatment of stage 4 melanoma using Hyper Acute Melanoma (HAM) vaccine and PEG-Intron. It yielded two complete responses, one patient with stable disease and four patients with no evidence of disease after resection, (57).

BIND-014, under development by Bind therapeutics, is a 100 nm PLGA polymer conjugated to docetaxel (placlitaxel analog), PEG and A10 2'-fluoropyridine RNA aptamers that recognize PSMA, an antigen expressed on prostate cancer cells (table 1). When docetaxel and **BIND-014** were administered intravenously to mice and rats, Bind-014 showed a larger tumour accumulation of its active principle and better efficacy than docetaxel. **BIND-014** is currently evaluated in a phase II clinical trial for non-small cell lung cancer and metastatic castrate-resistant prostate cancer, (58-61), (table 2).

CRLX-101, under development by Cerulean, is a 100 nm cyclodextrin based polymer associated with camptothecin and PEG. This drug is indicated for renal cell carcinoma treatment (table 1). Preclinical studies revealed that **CRLX-101** accumulates at tumour site and releases camptothecin over a period of several days leading to tumour growth inhibition, (62). These studies also showed anti-tumour activity on gastric cells (63) and a larger efficacy than free campthotecin, (64). Clinical studies showed that **CRLX-101** could be administered intravenously up to a maximum dose of 15 mg/m^2, that it was more efficient than free campthotecin (65) but caused neutropenia (66), (table 2).

Oncaspar, commercialized by Enzon, is made of L-asparginase associated with PEG. L-asparginase catalyses the conversion of L-asparagine to aspartic acid and ammonia. Since

leukemic cells need high amount of L-asparagine, addition of L-asparginase can decrease the level of circulating asparagine and lead to cellular death. **Oncaspar®** has been approved in 1994 for treatment of acute lymphoblastic leukemia, using either one or several intravenous or intramuscular administration(s) at a dose of 2500 IU/m^2 (table 1). Pre-clinical studies, carried out on L5178Y or 6C3HED tumours, showed a higher efficacy for **Oncaspar** than for free asparaginase. Pharmacokinetic studies showed that the elimination half-life of **Oncaspar** was 6 days, 5 times longer than that of free asparaginase and that the immunogenicity of **Oncaspar** was lower than that of free asparaginase, (67). Clinical trials, carried out on 377 patients with lymphoblastic leukemia receiving intramuscularly 25 000 IU/m^2 of either **Oncaspar** or free asparaginase, showed a similar efficacy for both drugs. However lower toxicity and faster clearance of lymphoblast from bone marrow was observed with **Oncaspar,** (67), (table 2). A phase I clinical study of **Oncaspar®** showed an increased plasma half-life ($t_{1/2}$ = 357 h) and fewer hypersensitivity reactions compared with free asparaginase ($t_{1/2}$ = 20 h).

Neulasta, commercialized by Amgen, is a 2 to 4 nm PEG polymer associated with the granulocyte-colony stimulating factor (G-CSF) that improves immune system efficacy, (table 1). This drug is mainly indicated to prevent neutropenia, usually occurring during cancer treatments. It has been approved in 2002 for treatment of non-myeloid malignancies. **Neulasta** is administered subcutaneously at a dose of 6 mg and slowly releases G-CSF. It has been clinically proven that **Neulasta** has a longer half-life of $t_{1/2}$ = 33 hours compared with that of $t_{1/2}$ = 3.5 hours observed with filgrastin (Neupogen®), the non-nanoformulated counterpart of **Neulasta**, (68). A phase III clinical study compared the efficacy of **Neulasta** injected as a single dose with that of filgrastin administered daily in patients with breast cancer. It showed a level of febrile neutropenia (FN) of 9-13% with **Neulasta**, lower than that of 13-20% observed

with filgrastin, (69). Other clinical studies confirmed the enhanced activity of **Neulasta** compared with filgrastin, (70), (table 2).

I.3. Metallic nanoformulation:

Aurimmune, under development by Cytimmune, is composed of 25 nm gold nanoparticles conjugated with TNF-α and PEG, (table 1). Preclinical studies, carried out on mice bearing MC38 and LCC tumours, showed that **Aurimmune** has the same efficacy but less toxicity than free TNF-α (71). They also revealed that **Aurimmune** increases the efficacy of cryotherapy (72), thermotherapy (73, 74). When **Aurimmune** is injected intravenously, it accumulates in tumours and not in RES (75). A phase I clinical study, carried out on patients with advanced cancer, showed that **Aurimmune** efficiently targets tumours and avoids RES, that its MTD of 4 mg and plasma half-life of $t_{1/2}$ = 130 minutes are both larger than those of 0.4 mg and 28 minutes observed for free TNF-α (table 2).

Auroshell, under development by Cytimmune, is made of 130 nm silica nanoparticles covered by a 2 nm thin layer of gold nanoparticles conjugated with an anti-HER2 antibody and PEG, (table 1). **Auroshell** nanoparticles are designed to be administered intravenously and heated by a near-infrared (NIR) laser, called Aurolase, after accumulation of **Auroshell®** in the tumour. Pre-clinical studies showed that **Auroshell** illuminated by Aurolase produces mild hyperthermia ($\Delta T \sim 10$ °C) in mice with colorectal cancer, inducing antihypoxic activity. When the treatment is combined with radiation, antitumor efficacy is enhanced compared with a treatment with radiation alone, (76). The treatment could also result in tumour thermal ablation ($\Delta T \sim$ 30-40 °C) by increasing the concentration of **Auroshell** or the laser power. However, in this case, temperature increase of 5-10 °C outside of **Auroshell** area could not be avoided leading to potential side effects for healthy tissues, (77-79), (table 2).

AuNPs, under development by Nanoprobe, are 11 nm gold nanoparticles conjugated with anti-EGFR antibodies and PEG, (table 1). Preclinical studies have shown that LD50 of **AuNPs** was 3.2 g per Kg, (79). Mice bearing mammary, head and neck or glioma tumours were treated by administration of 1-4 g **AuNPs** per Kg intravenously and by exposing the mice to X-ray radiotherapy at 42-250 kVp and 30-42 Gy. This led to tumour disappearance 20-30 days following treatment and to a one year survival rate of 50-86%, higher than that of 0-20% observed for a treatment with radiation alone, (80-82). NIR heating of **AuNPs** in mice with squamous carcinoma showed tumour disappearance after three days, (83). It was also demonstrated that NIR absorption of light is 100 000 higher with **AuNPs** than with standard fluorophores, (83).

Nanotherm, commercialized by Magforce, is composed of 15 nm iron oxide nanoparticles coated with amino-silane, (table 1). They are administered inside tumours and heated under the application of an alternating magnetic field, a technique called magnetic hyperthermia. Preclinical studies were carried out on rats with RG2 glioma tumours. After administration of ~ 180 μg of Nanotherm per mm^3 of tumour, an alternating magnetic field was applied to increase the tumour temperature up to ~ 45 °C. It led to an increased survival rate of 30 days, from 10 days in the absence of treatment up to 40 days in the presence of treatment, (84). A clinical study was carried out on 60 patients with glioblastoma by administering an average of 510 mg of **Nanotherm** in tumours of average sizes ~ 17 cm^3. The application of the alternating magnetic field raised the internal tumour temperature to ~ 43-45 °C without major side effects. The treatment led to an average survival rate following first glioblastoma recurrence of ~ 13.2 months, longer than that of 6 months reached with conventional treatments, (85, 86).

NanoXray, also called NBTXR3, under development by Nanobiotix, are 50 nm hafnium oxide nanoparticles that are administered directly to tumours and trigger anti-tumour activity when exposed to X-rays, mainly through the generation of reactive oxygen species (ROS), to our understanding (table 1). Preclinical studies were carried out on mice carrying subcutaneous sarcoma tumours. **NanoXray** was administered to tumours and exposed to 8Gy radiotherapy. This treatment seems to have led to the disappearance of several tumours within 30 days following the beginning of the treatment and to an increase of the mouse survival rate (87), (table 2). It seems to be designed to increase the anti-tumour efficacy of radiotherapy while decreasing its potential side effects, such as damages to surrounding healthy tissues.

Magnetosome chains, under development by Nanobacterie, are iron oxide nanoparticles of 40 nm that are synthesized by magnetotactic bacteria and used for magnetic hyperthermia treatment of cancer (88-95), (table 1). **Magnetosome chains** are extracted from magnetotactic bacteria, purified, mixed in suspension and administered to tumours. **Magnetosome chains** are administered to tumours and heated under the application of an alternating magnetic field to trigger anti-tumor activity. Advantages of **magnetosome chains** compared with **Nanotherm** come from their larger sizes that lead to a larger quantity of heat and to their arrangement in chains that prevents aggregation and favours cellular internalization. Preclinical studies carried out on nude mice bearing MDA-MB-231 cancer cells have shown that after intra-tumour administration of 1 mg of a suspension containing **magnetosome chains** and three applications of an alternating magnetic field of average strength 20 mT and frequency 198 kHz, it was possible to completely eradicate several tumours, (88,96) (table 2).

I.4. Protein drug nanoformulation:

Kadcyla (T-DM1), commercialized by Hoffmann-La Roche, is an antibody drug conjugate composed of trastuzumab (T), also called Herceptin, connected via a stable linker to a microtubule assembly inhibitor (DM1). Trastuzumab is a monoclonal antibody that interacts with HER2/neu receptor. The latter is a member of the family of epidermal growth factor receptor (EGFR) tyrosine kinase that stimulates cell proliferation and is overexpressed in various types of cancer. Coupled to microtubule inhibitor DM1 (derivative of maytansine 1), T-DM1 delivers DM1 into cells overexpressing HER2 via receptor-mediated endocytosis. Intracellular DM1 is then released by lysosomal degradation, inhibits microtubule assembly and causes cell death. **Kadcyla** is indicated for the treatment of metastatic breast tumours overexpressing HER2 for patients that have previously received Herceptin. It uses intravenous administration of **Kadcyla** at a dose of 3.6 mg per Kg. The clearance among patients was estimated as 7 to 13 mL per day per Kg and the EHL as 3 to 4 days. Preclinical and phase 1–3 clinical data support the significant antitumor activity of T-DM1. Several randomized studies also demonstrated the higher tolerability of T-DM1 compared with conventional chemotherapeutic treatments, (97).

Ontak (DAB/IL2), commercialized by Ligand Pharma UK limited, is a fusion protein of 58 KDa (2-5 nm) composed of the catalytic and membrane translocation domains of diphtheria toxin (Met1-Thr387)-His that can inhibit protein synthesis linked to the full amino acid sequence of IL-2. IL-2 acts as a ligand that specifically targets cells expressing CD25/CD122/CD152, the high affinity part of IL-2 receptor while the truncated part of diphtheria toxin inhibits protein synthesis once internalized in REF cells. **Ontak** is therefore recommended for the treatment of patients with persistent or recurrent cutaneous T-cell lymphoma in which lymphoma cells have high affinity for IL-2. A phase II clinical trial was carried out on 60 patients with stage IV melanoma. Patients received 4 daily doses of 12 μg/kg DAB/IL2 during 21 day cycles.

Responses were partial among 17% of the treated patients, led to stable disease among 5% of patients and to mixed responses among 15% of patients. One year survival was significantly higher in partial responders (~ 80 %) than in patients with progressive disease (~ 24 %). Moreover, ~ 40 % of patients treated with DAB/IL2 were alive 1 year following the beginning of the treatment, (98).

I.5. Virosomes:

Gendicine, commercialized by Shenzhen SiBiono Gene Tech, is the first gene therapy product approved for clinical use in humans. This specific nanoparticle product is a recombinant adenovirus engineered to express the tumour suppressor gene p53**.** The restoration of wild-type p53 function in tumours is achieved by introducing an intact complementary deoxyribonucleic acid copy of the p53 gene using the adenovirus vector. Functional complementation stimulates the apoptotic pathway in tumour cells, mainly by increasing the expression of tumour suppressor genes and immune response factors. Preclinical *in vitro* and *in vivo* studies have shown that **Genedicine** triggers a dramatic tumour regression in various cancers, (99). It has been approved by the Chinese State Food and Drug Administration for the treatment of head and neck squamous cell carcinoma and various forms of cancer since 2004. A randomized trial in nasopharyngeal carcinoma has demonstrated improved locoregional control with weekly intra-tumour injection of **Gendicine** combined with radiotherapy compared with a treatment using radiation therapy alone, (100).

I.6. Herbal nanoformulation:

Compared with other nanoformulations, herbal ones appear to be less developed, specifically at an industrial level and we therefore limit our description to one example.

Curcumin, obtained from the roots and rhizones of the perennial plant *Curcuma longa*, may be used for cancer treatment due to its known cytotoxicity towards tumour cells and to its capacity to improve the anti-tumour activity of several anti-cancer drugs such as doxorubicin, tamoxifen, cisplatin, campthotecin, daunorubicin, vincristine, melphalin. However, it remains difficult to use it because of its low bio-compatibility, low solubility and extreme sensitivity to pH (101). In order to avoid these negative features, it has been suggested to use several different types of nanoformulated curcumin such as silk-fibroin nanoparticles, curcuminoligo ethyleneglycol (Cur-OEG) nanoparticles, curcumin loaded-PGLA nanoparticles (102, 103). Curcumin nanocrystals of 30 nm can be formed by mixing curcumin in a solution of alcohol and water. It was shown that nanoformulated curcumin possess a four times higher AUC and an eleven times longer residence time than free curcumin, (103).

II. Stability/solubility, administration, distribution, targeting, metabolism, elimination, toxicity of nanodrugs:

II.1. Stability/solubility

Nanoformulated substances often possess enhanced solubility and stability compared to their non-nanoformulated counterparts. For example, solubility of campthotecin and curcumin can be enhanced by factors of 25 and 10 000 respectively by encapsulation in a phospholipid micelle, (104, 105). Due to their enhanced solubility, nanoformulated substances can often be administered in a larger quantity than their non-nanoformulated counterparts.

II.2. Administration

Intravenous administration is usually chosen because it leads to nanoformulated substance distribution throughout the whole organism. Other types of parenteral administrations are also possible such as subcutaneous (for Versamune, Eligard and Neulasta) or intramuscular

(for Oncaspar). Both routes allow slow release of the nanoformulated substance from the site of injection to the blood stream and therefore do not require frequent injections. Despite its appeal in terms of patient compliance, oral administration is usually avoided due to presence of several barriers that would prevent these substances from reaching systemic circulation in this case. Such barriers are the stomach acidic environment, the protease in the gut lumen, the brush border membrane, the tightly bound intestinal epithelial cells (enterocytes) and the metabolizing liver enzymes. Several other systemic and local administration methods exist such as intratumoral (for Nanotherm, NBTXR3, chains of magnetosomes), intra-arterial (for SMANCS), intravitreal, nasal, transdermal, vaginal, pulmonary, but intravenous administration remains the gold standard, (106).

II.3. Distribution

Biodistribution properties of nanoformulated substances are mainly determined by physiological barriers they encounter before reaching cancer cells. Blood constituents such as erythrocytes, leukocytes, amino acids, hormone and lipids can release or degrade the therapeutic payload of these substances or result in their aggregation and yield embolism. PEG coating has often been used to avoid these negative interactions between blood constituents and nanoformulated substances. Liver and kidney are physiological barriers that enhance clearance of substances depending on their sizes. These barriers can be avoided for substances with sizes lying between 10 and 100 nm. The blood brain barrier (BBB) can prevent nanoformulated substances from reaching the brain. Substances indicated for brain cancer treatment need to bypass this barrier. For that, the permeability of the BBB can be increased by using vasodilators such as brady-kinin, histamine or hypertonic solution of mannitol that osmotically shrink endothelial cells. Cellular barriers are also met by nanoformulated

substances. The latter have been shown to internalize within tumour cells favourably when their size is less than 60 nm or their charge is positive. On the other hand, negatively charged nanoformulated substances don't usually internalize within tumour cells and tend to distribute well within the tumour. Three barriers, the cell membrane, the endosomes/lysosomes and the intracellular trafficking ones must be overcome by nanoformulated substances to enable intracellular therapy, (107, 108, 109), which can improve antitumor efficacy. For example, photosensitizer-nanoparticle conjugates have been internalized within HeLa cancer cells, which enhances the efficacy of photodynamic therapy, (110).

Biodistribution of nanoformulated substances usually leads to more favourable pharmacodynamic parameters than those observed for their non-nanoformulated counterparts, (111). For example, nanoformulated substances possess a longer half-life and circulation time as well as a lower clearance and a larger AUC (table 3). Nanoformulation also usually leads to an increased amount of drugs in the tumor, *e. g.* by a factor of 10 for Daunoxome.

II.4. Targeting

Targeting is either passive or active, where passive targeting is caused by the small sizes of nanoformulated, which help them to reach tumours and active targeting is due to a substance, such as an antibody that specifically recognizes cancer cell receptors. Passive targeting can usually be achieved when nanoformulated sizes are below 100-780 nm (112), the sizes of the discontinuous capillaries of tumor blood vessels. In this case, nanoformulated substances can indeed extravasate through the holes of the vessels irrigating tumours and efficiently target tumour sites, a mechanism called enhanced permeation and retention (EPR) effect. Active

targeting can involve antibodies, peptide such as RGD or CTX that recognize $\alpha_v\beta_3$ integrins or MMP-2, both overexpressed on cancer cells. In nanoformulated substances, active targeting is favoured by the large surface to volume ratio that enables the attachment a larger number of antibodies than in conventional larger or smaller drugs with less available anchoring surface.

II.5. Metatabolism and elimination

Nanoformulated substances essentially follow two routes of elimination from the organism: either excretion or cellular degradation depending on whether they are degradable or not. Biodegradation can be observed with natural nanoformulated drugs and is essentially caused by lysosomal enzymes. Elimination of non-biodegradable substances is hampered by their high molecular weight. Indeed, large nanoformulated drugs remain in tissues after cellular death or undergo exocytosis and then slowly return to the blood stream via the lymphatic circulation and are then eliminated in the kidney. The detailed knowledge of the biodistribution of a nanoformulated substance is very hard to obtain since such substance undergoes numerous transformations and modifications in the organism (opsonisation, cellular degradation), which depend on many factors (cellular types, interactions with blood, pH…) and can hardly all be apprehended.

II.6. Toxicity properties

Besides having specific biodistribution studies, nanoformulated drugs have also been shown to be less toxic than their non-nanoformulated counterparts. Nanoformulation can prevent conversion of drugs to toxic components, *e. g.* DaunoXome prevents conversion of Daunorubicin to toxic Daunorucin. It can also decrease neutropenia, *e. g.* when patients use Myocet, Abraxane, Neulasta, or induce less cardiac toxicity, *e. g.* for Myocet. Due to their

reduced toxicity, nanoformulated drugs usually possess higher MTD and LD50. MTD of Marquibo, Abraxane and Aurimmune are 2 to 10 times higher than those of vincristine, cremo-paclitaxel and TNF-α respectively. LD50 of Abraxane (~47 mg/Kg) is higher than that of free cremo-paclitaxel (30 mg/Kg). Nanoformulated drugs also usually induce less inflammatory and immune response compared with conventional drugs.

However, despite these appealing features, nanoformulated substances could induce toxicity. In particular, they may favour the production of reactive oxygen species (ROS) and free radicals, resulting in oxidative stress, inflammatory events, DNA damage, inhibition of cell division and cell death. The origin of this toxicity is not well understood and may depend on several factors such as the size, shape, surface, functionalization, reactivity of these substances, (111).

III. Biological versus chemical nanodrugs.

Nanoformulated substances are either chemically produced or biologically synthesized, by plants, bacteria or microorganism. Compared with biological nanoformulated substances, chemical ones can often more easily be produced in large quantity with a reproducible method and more simple characterization tools. On the other hand, biological nanoformulated substances often appear to be more efficient. As an example, magnetosome chains, which are produced by magnetotactic bacteria, possess better antitumor activity than their chemical counterparts. This is due on the one hand to better magnetosome magnetic properties and on the other hand to the magnetosome chain arrangement that prevents aggregation and favours cellular internalization, (88).

IV. Regulatory issues:

A clear regulatory framework for nanoformulated substances categorization seems necessary but is currently lacking.

IV.1. Are nanoformulated substances categorized as drugs or medical devices?

While most of these substances are categorized as drugs presumably due to their high level of invasiveness, a few of them, such as those developed by Magforce and Nanobiotix, have been developed as medical devices. The categorization of these substances depends on their dominant mean of action. If the latter is metabolic, pharmacological or immunological, they should be considered as drugs. Otherwise, they should be classified as medical devices. Nanoformulated substances developed by Magforce and Nanobiotix have been considered as medical devices in Europe due their physical mean of action, which is either due to heat (Magforce) or effects of X-rays on hafnium oxide nanoparticles (Nanobiotix). This classification also depends on the country where this substance is developed. Indeed, in Europe several of these substances were considered as medical devices whereas in the United-States, all types of nanoformulated substances seem to be considered as drugs. In fact, several nanoformulated substances seem to be borderline products, at the frontier between class III medical devices and drugs. This does not ease a clear categorization. In order to take into account the numerous specific properties of nanoformulated substances, a categorization, different from that of medical device and drug, should certainly be defined with an associated regulatory framework.

IV.2. Nanofabrication

Several regulatory aspects need to be considered to be able to start clinical trials with a nanoformulated substance. Firstly, its production needs to be carried out according to standards, either good manufacturing practice (GMP) standards if this substance is

categorized as a drug or ISO 13485 standard if it is classified as a medical device. Nanoformulation needs to be robust, to enable a high level of reproducibility and to enable production in large quantity. Among the critical steps of nanoformulation are: (i), coating, which should be chosen to yield high level of biocompatibility, stability and nanoformulated substance accumulation within tumours, minimization of rapid clearance by the RES following intravenous administration, prolongation of plasma circulation, improved hematocompatibility (113), (ii), nanoformulated substance sterilization, which often involves filtration with pore sizes of 220 nm to avoid possible damages caused by gamma-rays or auto-claving, (iii), nanoformulated substance characterization, including its visualization by microscopy (atomic force microscopy (AFM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM)), measurement of nanoformulated substance size and size distribution with light scattering (static and dynamic), analytical ultracentrifugation, capillary electrophoresis, and field flow fractionation, analysis of nanoformulated substance surface charge or zeta potential, examination of surface chemistry by X-ray photoelectron spectroscopy or Fourier transform infrared spectroscopy (FTIR) (114). According to the guidelines of the French regulatory agency (ANSM) on the development of nanoformulated substance, its production should enable a control of several parameters such as the sizes, size distributions, morphology, aggregation, solubility, surface, chemical composition, crystallographic structure, chemistry and charge surface of these substances.

IV.3. Toxicity:

Nanoformulated substance toxicity needs to be assessed following a pre-clinical regulatory framework. Although ISO 10993 standards have not yet been adapted for their adequate evaluation, they are usually followed to carry out regulatory toxicity tests. Toxicity tests that

need to be carry out include cytotoxicity (ISO-10993-5), drug acute systemic toxicity (ISO 10993-11), drug irritation (ISO 10993-10), drug sensitization (ISO 10993-10), drug pyrogenicity, drug toxicity evaluated 14 days after subcutaneous injection (ISO 10993-11), drug toxicity evaluated 28 days after subcutaneous injection (ISO 10993-11), drug toxicity evaluated 13 weeks after subcutaneous injection (ISO 10993-11), genotoxicity (ISO 10993-3). One of the main problems with the use of ISO 10993 standards to evaluate the toxicity of nanoformulated substance is the absence of a clear indication regarding the quantity of this substance that needs to be tested. This, of course, is a major problem since the toxicity of nanoformulated substance heavily depends on the quantity of this substance, which is tested.

IV.4. Efficacy

The efficacy of nanoformulated substance needs to be demonstrated pre-clinically, usually on mice or rats, apparently without the requirement of a specific regulatory framework or a minimum efficacy threshold (62, 87, 115).

IV.5. Risk analysis:

The development of a new nanoformulated substance can’t be undertaken without a proper risk analysis, which needs to be carried out according to ISO 13485 and/or ISO 14971. This analysis needs to address all risks associated with drug development, in particular: (i), failure to obtain the authorization for commercialization due to unsuccessful preclinical and clinical efficacy tests or to too high drug toxicity, (ii), too high costs for development and fabrication of the different components of the therapy and for clinical trials, (iii), failure to protect the nanoformulated substance against potential competitors, (iv), failure to find a structure that can produce nanoformulated substance according to GMP or ISO 13485 standards in a sufficiently large quantity and at a reasonable cost.

V.Intellectual property (IP)

Nanodrugs, which are not protected by patents, could hardly be developed by industries, making intellectual property issues a very important topic to address.

V.1. Magnetic hyperthermia:

In the field of magnetic hyperthermia, the different companies involved include:

- Aspen, which protected a magnetic composition that can either be heated through Néel relaxation with an alternating magnetic field (116) or that contains monodomain nanoparticles with a ligand targeting cancerous tissues (117);
- Magforce that protected a method of SPION production (118), a specific induction system that can generate an alternating magnetic field (118) and a solid or gel-like medical product, which can be heated by an alternating magnetic field (119);
- Nanobacterie, which protected the use of magnetosome chains extracted from magnetotactic bacteria for medical applications, (120), as well as a method to increase the production yield of magnetotactic bacteria by introducing iron chelating agents in the bacterial growth culture, (121).
- Nanoprobes protected a method to increase the temperature above 42 °C using gold nanoparticles irradiated by infrared light (122), a composition containing gold nanoparticles (123).

V.2. Nanoparticle exposed to X-rays:

In the area of antitumor activity triggered by nanoparticles exposed to X-ray radiations, the companies involved include:

- Nanobiotix, which protected nanoparticles able to generate free radicals or heat under X-ray (124), composite nanoparticles containing an activated seed and a

targeting molecule (125), the use of oxide nanoparticles exposed to ionizing radiation to destroy cells (126), the use of metallic nanoparticles with an atomic number larger than 25 to destroy tumour cells under ionizing beams (127), nanoparticles containing a metal covered with hafnium oxide (128);

- Nanospectra that protected the use of nanoparticles in a combined treatment with hyperthermia and X-rays (129), a method to increase the effects of radiations on a tissue or a population of cells with nanoparticles of high atomic number, (130);
- Nanoprobes, which protected a method for increasing the effects of radiation on tissues or cells using metallic nanoparticles, (131).

V.3. Liposomes

In the liposome field, examples of active companies active are:

- Celsion, which protected a thermosensitive liposome containing a therapeutic substance, (132);
- Merrimack that protected a method for delivery of a neoplastic agent such as irinotecan contained inside a liposome for tumour treatment, (133).

V.4. Polymers

In the polymer area, several companies operate such as:

- Cerulean that protected a method for fabricating campthotecin polymers containing cyclodextrin, (134), or a pharmaceutical composition containing a cyclodextrin polymer and a therapeutic agent covalently bound to cyclodextrin, (135);
- Bind Therapeutic, which protected nanoparticles containing a therapeutic substance and a PLA-PEG (136), a preparation method for therapeutic nanoparticles containing a polymer, involving an organic acid and an organic solvent, (137).

V.5. Herbal nanoformulation

Although companies working in this the field of herbal nanoformulation are unknown to us, different types of these formulations have been patented, including those derived from Moringa oleifera Lam gum, polymeric or nano-micellar vinca alkaloids (vincristine and vinblastine), liposomal curcumin, curcumin loaded nanoparticles (138).

VI. Financial issues:

Developing nanodrugs with the aim of commercializing them is a very expensive process and we present below the complex financial issues, which are stake, for companies working in this field.

VI.1. Overview of the different companies analyzed

Nanodrug development is mainly driven by start-up and small and medium companies (139). Financial aspects of several of these recently founded companies with less than 250 employees are presented (table 4). 14 companies have been selected (table 4), 10 from the United States, 3 from France and 1 from Germany. 5 of these companies are privately held while 9 of them are listed on Nasdaq or Euronext (table 4). These companies have been established between 1982 for the oldest (Celsion) and 2008 for the youngest (Nanobacterie).

VI.2. Revenue of these companies

Although these companies can't expect to rapidly generate revenues, the latter can be very significant and essentially arise from two sources: direct sale of the therapy and licensing. Examples of forecasted revenues are:

- For Magforce: $96m-$115m from direct sale of its therapy for glioblastoma treatment in Europe and $385m-$480m for prostate cancer treatment in the United-States,

(140). These forecasted revenues were calculated by multiplying the number of nanoactivators sold (25-30 in Europe and 100-125 in the United-States respectively) by the revenue of $3.8m generated by each nanoactivator, considering a market penetration of more than 10%;

- For Nanobiotix: $1200m from direct sales of its lead product, NBTXR3. This revenue was estimated by multiplying the number of patients treated by radiotherapy (250 000) by a market penetration rate of 25% times a selling price of 19 200$ per patient, (141);
- For Onxeo: $500m from direct sales of Livatag for liver cancer treatment in Europe, United-States and Asia before 2023. This estimate assumes a rather high penetration market of 30%, which could overestimate the revenue (142);
- For PharmaEngine: An unknown amount for selling MM-398 and NBTXR3 in Asia under license agreement with Merrimack and Nanobiotix, (143);
- For Bind Therapeutic: $100m-$200m through license agreements with Amgen, Pfizer and Astra Zeneca;
- For Merrimack: $10m-$650m through licence agreements with Pharmengine, Dyax and Adimab.

Most of these companies generated low revenues in 2013 (less than $4.7m). Merrimack is the only one with a high 2013 revenue of $37m.

VI.3. Accumulated losses

The revenues of these companies are much smaller than their accumulated losses, lying within the range of $30m to $447m. The companies that had the largest accumulated losses are Merrimack ($532m), which is also employing the largest number of employees (250),

Oncothyreon ($432m), Celsion ($169m). Onxeo and Magforce report accumulated losses of $141m and €32.7m respectively. For Nanobiotix, we have estimated accumulated losses since its foundation at about $38m. Accumulated losses appear less extensive for the listed European firms compared with the American ones, a phenomenon which may be due to different levels of investments and also possibly to different accounting and reporting procedures. Should this observation lead to conclude that to reach a (more or less) comparable level of development European companies as a whole spend less money than their US counterpart? If so, the reasons remain ambiguous. All companies analysed possess significant accumulated losses during the long years of nanodrugs development. It seems that profit can only be expected during the first years of nanodrug commercialization when the patents protecting these drugs are still active. With these facts in mind, one can find an explanation for the very expensive price of nanodrugs.

VI.4. Market perception of these companies

These financial figures presented in table 4 show that over a decade and substantial investments in research and development are necessary for these companies to reach commercialization and then profitably. Despite of this, market value of these companies seems to be higher than the amount of invested capital. Indeed, except for Celsion and Oncothyron, which seem to have faced difficulties, the ratio between market value and accumulated losses that we estimated to our best knowledge but without certainty lies between 1 and 14 (table 4). We consider another interesting ratio, the price to book value that shows the market's perception of a company's future profits. The higher the ratio above 1, the higher the positive perception of the market. Except for Merrimack, which at the

moment has a negative ratio (as the total assets are below the total liabilities), all the other companies for which an estimated ratio has been possible possess a ratio above 1.

Market capitalizations are retained as of October 24, 2014 with the 52 weeks High and Low also referring to this date. It should be noted the high volatility of the stock figures. Although Merrimack carries the highest accumulated losses ($532m), it nevertheless accounts by far for the highest market capitalization of $979.5m. Cerulean and Nanobiotix are valued at a quarter of Merrimack, while Bio-path Holdings and Onxeo are valued at only a fifth (table 4). This reflects the present perception of these companies by the market, which can vary by more than a factor 10 (table 4).

VII. Market:

Nanomedicine market reached $43.2b in 2010 and $50.1b in 2011. The market is expected to grow to $97b by 2016, (144). In 2012, 67 nanodevices and 33 nanotherapeutics were commercialized, 25 nanodevices were under development and 122 nanoteherapeutics were involved in 789 ongoing clinical trials (145).

Conclusion:

In conclusion, we have reviewed a series of different nanoformulated substances for cancer treatment commercialized or under development, described advantageous features of these substances compared with their non-nanoformulated counterparts, presented the way in which these substances should be developed to reach clinical trials and later on marketization.

Competing interest:

The author(s) declare that they have no competing interests.

Contributions of authors :

EA was in charge of the overall supervison of the paper and wrote most of it. Mickael Durand-Dubief, Chalani Mandawala and Raphael Lefèvre helped with the scientific aspects while Pierre Grand-Dewyse contributes on financial issues.

Expert commentary: There has recently been a large increase in the number of papers and companies working in the nanooncology field. The latter encompasses a very broad range of different types of nanoformulated drugs. Formulating a number of currently used drugs at the nanoscale enables to improve their efficacy and to reduce their toxicity. This review details the nature of these improvements. On the one hand, drugs formulated at the nanoscale are large enough to enable sufficient therapeutic payload. On the other hand, they are both smaller than the typical size of cell, enabling cellular internalization, and than the holes of the blood vessels irrigating the tumor, favoring passive tumor targeting via EPR effect. The category of nanoformulated drugs, activated or deactivated by an external source of energy (X-ray or magnetic field) also appears very interesting to the experts. Indeed, this type of therapeutic system enables to accurately control the treatment and therefore to improve its efficacy and safety. Such system can be developed using nanoparticles, which can be activated by an external source of energy. This is usually not possible with non-nanoformulated drugs.

Five-year view: Due to the large number of advantages of nanoformulated drugs pointed out in this review, the authors believe that the nanooncology field will prevail within the next five years. The lack of a well-defined regulatory environment is currently slowing down the growth of this field. Indeed, national drug agencies currently appear to disagree on the classification of this type of products, considering them either as drugs or medical devices, depending on the agency and product type. In fact, nano-formulated drugs should probably be considered as a separate category of medical product with a specific set of standards, which could be developed when enough data become available about their safety and efficacy. Another important aspect concerns the huge costs associated with the development of these drugs (a company presented in this review accumulated more than 500 million dollars deficit). One needs to understand that due to the large risk of failure associated with such development, there are only very few private investors that are ready to sufficiently invest in this type of company. This of course is very detrimental to cancer patients who can’t have access to these very promising treatments. The experts consider that a public debate, which could address these issues, is currently lacking both at a national and international level. There could be solutions to foster the development of these drugs such as: (i), changing the regulation, which can lead to very high costs without necessarily leading to improved drug safety and efficacy (it is certainly wrong to think that more expensive drugs would always be safer and more efficient), (ii), the set-up of public laboratories to assess the safety and efficacy of these drugs, enabling to reduce the financial burden for small pharmaceutical companies and, (iii), more public funding invested in companies developing these

drugs, specially the small ones that have difficulty to thrive and often end up being bought by larger pharmaceutical companies.

Key issues:

- Nanoformulated drugs are very divers, present less toxicity than their non nanoformulated counterparts and are characterized by specific biodistribution properties in the organism due to their small sizes.
- Nanoformulated drugs can usually target tumors through the EPR effect, which leads to a larger concentration of drugs in the tumor compared with their non nanoformulated counterparts.
- The activity of several of these drugs can be activated on demand by an external source of energy (magnetic field or X-ray), leading to a controlled therapy.
- Regulation is currently emerging to allow the development of these drugs.
- Small companies have recently appeared that develop these drugs.

References:

1. Thako AS, Gambhir SS. Nanooncology: The Future of Cancer Diagnosis and Therapy. CA Cancer J Clin. 2013, 63: 395-418.

2. Weissig V, Pettinger TK, Murdoch N. Nanopharmaceuticals (part I): products on the market. International Journal of Nanomedicine. 2014, 9: 4357-4373.

3. Silverman JA, Deitcher SR. Marqibo (vincristine sulfate liposome) improves the pharmacokinetics and pharmacodynamics of vincristine. Cancer Chemother. Pharmacol. 2013; 71: 555-564.

4. Harrison TS, Lyseng-Williamson KA. Vincrinstine Sulfate Liposome Injection. BioDrugs. 2013; 27: 69-74.

5. Deitcher OR, Glaspy J, Gonzalez R et al. High-dose Vincristine Sulfate Liposome Injection (Marqibo) Is Not Associated With Clinically Meaningful Hematologic Toxicity. Clin Lymphoma Myeloma Leuk. 2014; 14(3): 197-202.

6. Kaplan LD, Deitcher SR, Silverman JA, Morgan G. Phase II Study of Vincristine Sulfate Liposome Injection (Marqibo) and Rituximab for Patients With Relapsed and Refractory Diffuse Large B-Cell Lymphoma or Mantle Cell Lymphoma in Need of Palliative Therapy. Clin Lymphoma Myeloma Leuk. 2014; 14(1): 37-42.

7. Rodriguez MA, Pytlik R, Kozak T et al. Vincristine Sulfate Liposomes Injection (Marqibo) in Heavily Preatreaed Patients With Refractory Aggressive Non-Hodgkin Lymphoma: report of the pivotal phase 2 study. Cancer. 2009; 115(15): 3475-3482.

8. Batist GB, Ramakrishnan G, Rao CS et al. Reduced Cardiotoxicity and Preserved Antitumor Efficacy of Liposome-Encapsulated Doxorubicin and Cyclophosphamide Compared

with Conventional Doxorubicin and Cylophosphamide in a Randomized, Multicenter Trial of Metastatic Breast Cancer. Journal of Clinical Oncology. 2001; 19(5): 1444-1454.

9. Leonard RCF, Williams S, Tulpule A et al. Improving the therapeutic index of anthracyline chemotherapy: Focus on liposomal doxorubicin (Myocet^{TM}). The Breast. 2009; 18: 218-224.

10. Harris L, Batist G, Belt R et al. Liposome-Encapsulated Doxorubicin Compared with Conventional Doxorubicin in a Randomized Multicenter Trial as First-Line Therapy of Metastatic Breast Carcinoma. Cancer. 2002; 94(1): 25-36.

11. Mross K, Niemann B, Massing U et al. Pharmacokinetics of liposomal doxorubicin (TLC-D99; Myocet) in patients with solid tumors: an open-label, single-dose study. Cancer Chemother. Pharmacol. 2004; 54: 514-524.

12. Amadori D, Milandri C, Comella G et al. A phase I/II trial of non-pegylated liposomal doxorubin, docetaxel and trastuzumab as first-line treatment in HER-2 positive locally advanced or metastatic breast cancer. European Journal of Cancer. 2011; 47: 2091-2098.

13. Del Barco S, Colomer R, Calvo L et al. Non-pegylated doxorubicin combined with gemcitabine as first-line treatment for metastatic or locally advanced breast cancer. Final results of a phase I/II trial. Breast Cancer Res. Treat. 2009; 116: 351-358.

14. Ciolli S, Leoni F, Casini C et al. The addition of liposomal doxorubicin to borteomib, thalidomide and dexamethasone significantly improves clinical outcome of advanced multiple myeloma. British Journal of Haematology. 2008; 141: 814-819.

15. Levine AM, Tulpule A, Espina B et al. Liposome-Encapsulated Doxorubicin in Combination With Standard Agents (cyclophosphamide, vincristine, prednisone) in Patients With Newly Diagnosed AIDS-Related Non-Hodgkin's Lymphoma: Results of Therapy and Correlates of Response. J. Clin. Oncol. 2004; 22(13): 2662-2670.

16. Luminari S, Montanini A, Caballero D et al. Nonpegylated liposomal doxorubicin (Myocet™) combination (R-COMP) chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL): results from the phase II EURO18 trial. Annals of Oncology 2010; 21: 1492-1499.

17. Rigacci L, Mappa S, Nassi L et al. Liposome-encapsulated doxorubicin in combination with cyclophosphamide, vincristine, prednisone and rituximab in patients with lymphoma and concurrent cardiac diseases or pre-treated with anthracylines. Hematological Oncology 2007; 25: 198-203.

18. Stroppa E, Bertuzzi A, Di Comite G et al. Phase I study of non-pegylated liposomal doxorubicin in combination with ifosfamide in adult patients with metastatic soft tissue sarcomas. Invest. New Drugs 2010; 28: 834-838.

19. Doxil product information, manufactured for Janssen Products, LP, Horsham, PA 19044, printed in USA.

20. Barenholz Y. Doxil® - The first FDA-approved nano-drug: Lessons learned. Journal of Controlled Release 2012; 160: 117-134.

21. Leonard RCF, Williams S, Tulpule A et al. Improving the therapeutic index of anthracycline chemotherapy: Focus on liposomal doxorubicin (Myocet™). The Breast 2009; 18: 218-224.

22. Alvarez Secord A, Jones EL, Hahn CA et al. Phase I/II trial of intravenous Doxil® and whole abdomen hyperthermia in patients with refractory ovarian cancer. Int. J. Hyperthermia 2005; 21(4): 333-347.

23. El-Rayes B, Ibrahim D, Shields AF et al. Phase I study of liposomal doxorubicin (Doxil) and cyclophosphamide in solid tumors. Investgational New Drugs 2005; 23: 57-62.

24. Krauze MT, Noble CO, Kawaguchi T et al. Convection-enhanced delivery of nanoliposomal CPT-11 (irinotecan) and PEGylated liposomal doxorubicin (Doxil) in rodent intracranial brain tumor xenografts. Neuro-Oncology 2006; 9: 393-403.

25. Brouckaert P, Takahashi N, Van Tiel ST, Tumor necrosis factor-α augmented tumor response in B16BL6 melanoma-bearing mice treated with stealth liposomal doxorubicin (Doxil®) correlates with altered Doxil® pharmacokinetics. Int. J. Cancer 2004; 109: 442-448.

26. Gill PS, Espina BM, Muggia F et al. Phase I/II and Pharmacokinetic Evaluation of Liposomal Daunorubicin. Journal of clinical Oncology 1995; 13: 996-1003.

27. Fassas A, Anagnostopoulos A. The use of liposomal daunorubicin (DaunoXome) in acute myeloid leukemia. Leukemia & Lymphoma 2005; 46(6): 795-802.

28. Kapsers GJL, Zimmermann M, Reinhardt D et al. Improved Outcome in Pedriatic Relapsed Acute Myeloid Leukemia: Results of a Randomized Trial on Liposomal Daunorubicin by the International BFM Study Group. Journal of Clinical Oncology. 2013; 31(5): 599-607.

29. Camera A, Rinaldi CR, Palmieri S et al. Sequential continuous infusion of fludarabine and cytarabine associated with liposomal daunorubicin (DaunoXome®) (FLAD) in primary refractory or relapsed adult acute myeloid leukemia patients. Annal. Hematol. 2009; 88: 151-158.

30. Candoni A, Michelutti A, Simeone E et al. Efficacy of liposomal daunorubicin and cytarabine as reinduction cgemotherapy in relapsed acute lymphoblastic leukaemia despite expression of multidrug resistance-related proteins. Eur. J. Haematol, 2006; 77: 293-299.

31. Latagliata R, Breccia M, Fazi P et al. Liposomal daunorubicin versus standard daunorubicin : long term follow-up of the GIMEMA GSI 103 AMLE randomized trial in patients older than 60 years with acute myelogenous leukaemia. British journal of haematology. 2008; 143: 681-689.

32. Russo D, Piccaluga PP, Michieli M et al. Liposomal daunorubicin (DaunoXome) for treatment of poor-risk acute leukemia. Ann. Hematol. 2002; 81: 462-466.

33. Hempel G, Reinhardt D, Creutzig U et al. Population pharmacokinetics of liposomal daunorubicin in children. Clin. Pharmacol. 2003; 56: 370-377.

34. O'Byrne KJ, Thomas AL, Sharma RA et al. A phase I dose-escalating study of DaunoXome, liposomal daunorubicin, in metastatic breast cancer. British journal of Cancer. 2002; 87: 15-20.

35. McTiernan A, Whelan J, Leahy M et al. A Phase II Nonrandomized Open-Label Study of Liposomal Daunorubicin (DaunoXome) in Advanced Soft Tissue Sarcoma. Sarcoma. 2006; ID 41080: 1-5.

36. Mitchell PLR, Marlton P, Grigg A et al. A phase II study of liposomal daunorubicin, in combination with cyclophosphamide, vincristine and prednisolone, in elderly patients with previously untreated aggressive non-Hodgkin lymphoma. Leukemia & Lymphoma. 2008; 49(5): 924-931.

37. Chang H-I, Yeh M-K, Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy. International Journal of Nanomedicine. 2012; 7: 49-60.

38. Chen PY, Ozawa T, Drummond DC et al. Comparing routes of delivery for nanoliposomal irinotecan shows superior anti-tumor activity of local administration in treating intracranial glioblastoma xenografts. Neuro-Oncology. 2013; 15(2): 189-197.

39. Ko AH, Tempero MA, Shan Y-S et al. A multinational phase 2 study of nanoliposomal irinotecan sucrosofate (PEP02, MM-398) for patients with gemcitabine-refractory metastatic pancreatic cancer. British Journal of Cancer. 2013; 109: 920-925.

40. Website of the company Merrimack: www.merrimackpharma.com/pipeline/MM-302.

41. Frampton JE. Mifamurtide, A review of its Use in the Treatment of Osteosarcoma. Adis Drug Evaluation. 2010; 12(3): 141-153.

42. Chen W, Yan W, Huang L. A simple but effective cancer vaccine consisting of an antigen and a cationic lipid. 2008; 57: 517-530.

43. Poon RTP, Borys N. Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer. 2009; 10(2): 333-343.

44. Thomas MB, Jaffe D, Choti MM et al. Hepatocellular Carcinoma: Consensus Recommendations of the National Cancer Institute Clinical Trials Planning Meeting. Journal of Clinical Oncology. 2010; 28(25): 3994-4005.

45. Notice of Depocyte, http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000317/WC500035649.pdf

46. Miele E, Spinelli GP, Miele E et al. Albumin-bound formulation of paclitaxel (Abraxane® ABI-007) in the treatment of breast cancer. International Journal of Nanomedecine. 2009; 4: 99-105.

47. Bawarski WE, Chidlowsky E, Bharali DJ et al. Emerging nanopharmaceuticals. Nanomedicine. 2008, 4: 273-282.

48. Gradishar WJ, Tjulandin S, Davidson N et al. Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared With Polyethylated Castor Oil-Based Paclitaxel in Women With Breast Cancer. Journal of Clinical Oncology. 2005; 23(31): 7794-7803.

49. Braeckman J, Michielsen D. Efficacy and olerability of 1- and 3- month leuprorelin acetate depot formulation (Eligard/Depot-Eligard) for advanced prostate cancer in daily practice: a Belgian prospective non-interventional study. Arch. Med. Sci. 2014, 3: 477-483.

50. Agnihotri S, Burrell KE, Wolf A et al. Glioblastoma, a Bried Review of History, Molecular Genetics, Animal Models and Novel Therapeutic Strategies. Arch. Immunol. Ther. Exp. 2013, 61: 25-41.

51. Ishii H, Furuse J, Nagase M et al. A Phase I Study of Hepatic Arterial Infusion Chemotherapy with Zinostatin Stimalamer Alone for Hepatocellular Carcinoma. Jpn. J. Clin. Oncol. 2003, 33: 570-573.

52. Duncan R. Polymer conjugates as anticancer nanomedicines. Nature Reviews Cancer. 2006, 6: 688-701.

53. Rokx C, Van der Ende ME, Verbon A et al. Peginterferon Alfa-2a for AIDS-Associated Kaposi Sarcoma : Experience With 10 patients. HIV/AIDS. 2013, 57: 1497-1499.

54. Ianotto JC, Boyer-Perrard F, Gyan E et al. Efficacy and safety of pegylated-interferon α-2a in myelofibrosis: a study by the FIM and GEM French cooperative groups. British journal of Haematology. 2013, 162: 783-791.

55. Go RS, Lee SJ, Shin D et al. ECOG Phase II trial of graded-dose peginterferon α-2b in patients with metastatic melanoma over-expressing basic fibroblast growth factor (E2602). Clin. Cancer Res. 2013, 19: 6597-604.

56. Riker AI, Rossi GR, Masih P et al. Combination Immunotherapy for High-Risk Resected and Metastatic Melanoma Patients. The Ochsner Journal. 2014, 14: 164-174.

57. Annual report of Bind Therapeutic, http://ir.bindtherapeutics.com/annuals.cfm

58. Farokhzad OC, Cheng J, Teply BA et al. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. PNAS. 2006; 103(16): 6315-6320.

59. Gu F, Zhang L, Teply BA et al. Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. PNAS. 2008; 105(7): 2586-2591.

60. Hrkach J, Von Hoff D, Ali MM et al. Preclinical Development and Clinical Translation of a PSMA-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile. Science Translational Medicine. 2012; 4(128): 1-11.

61. Eliasof S, Lazarus D, Peters CG et al. Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle. PNAS. 2013; 110(37): 15127-15132.

62. Gaur S, Chen L, Yen T et al. Preclinical study of the cyclodextrin-polymer conjugate of camptothecin CRLX101 for treatment of gastric cancer. Nanomedicine. 2012; 8: 721-730.

63. Svenson S, Wolfgang M, Hwang J et al. Preclinical to clinical development of the novel camptothecin nanopharmaceutical CRLX101. Journal of Controlled Release. 2011; 153: 49-55.

64. Young C, Schluep T, Hwang J et al. CRLX101 (formerly IT-101)-A Novel Nanopharmaceutical of Camptothecin in clinical development. Current Bioactive Compounds. 2011; 7: 8-14.

65. Weiss GJ, Chao J, Neidhart JD et al. First-in-human phase 1/2a trial of CRLX101, a cyclodextrin-containing polymer-camptothecin nanopharmaceutical in patients with advanced solid tumor malignancies. Invest. New Drugs. 2013; 31: 986-1000.

66. Desai N, Trieu V, Yao Z et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clinical Cancer Research. 2006; 12: 1317-1324.

67. Zeidan A, Wang ES, Wetzler M, Pegasparaginase: where do we stand? Expert Opin. Biol. Ther. 2009; 9(1): 111-119.

68. Lyman GH, Pegfilgrastim: a granulocyte colony-stimulating factor with sustained duration of action. Expert Opin. Biol. Ther. 2005; 5(12): 1635-1646.

69. Willis F, Pettengell R, Pegfilgrastim. Expert Opin. Biol. Ther. 2002; 2(8): 985-992.

70. Morishita M, Leonard RC, Pegfilgrastim; a neutrophil mediated granulocyte colony stimulating factor-expanding uses in cancer chemotherapy. Expert Opin. Biol. Ther. 2008; 8(7): 993-1001.

71. Eliasof S, Lazarus D, Peters CG et al. Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticle. PNAS. 2013; 110(37): 15127-15132.

72. Goel R, Swanlund D, Coad J et al. TNF-α-based accentuation in cryoinjury-dose, delivery, and response. Mol. Cancer Ther. 2007; 6(7): 2039-2047.

73. Pedro RN, Thekke-Adiyat T, Goel R et al. Use of Tumor Necrosis Factor-alpha-coated Gold Nanoparticles to Enhance Radiofrequency Ablation in a Translational Model of Renal Tumors. Urology. 2010; 76(2): 494-498.

74. Visaria RK, Griffin RJ, Williams BW et al. Enhancement of tumor thermal therapy using gold nanoparticle-assisted tumor necrosis factor-α delivery. Mol. Cancer Ther. 2006; 5(4): 1014-1020.

75. Paciotti GF, Myer L, Weinreich et al. Colloidal Gold: A novel Nanoparticle Vector for Tumor Directed Drug Delivery. Drug Delivery. 2004; 11: 169-183.

76. Diagaradjane P, Shetty A, Wang JC et al. Modulation of in Vivo Tumor Radiation Response via Gold Nanoshell-Mediated Vascular-Focused Hyperthermia: Characterizing an Integrated Antihypoxic and Localized Vascular Disrupting Targeting Strategy. Nano. Lett. 2008; 8: 1492-1500.

77. Hirsch LR, Stafford RJ, Bankson JA et al. Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. PNAS. 2003; 100(23): 13549-13544.

78. Puvabakrishnan P, Park J. Near-infrared narrow-band imaging of gold/silica nanoshells in tumors. Journal of Biomedical Optics. 2009; 14(2): 024044-1-024044-6.

79. Stern JM, Stanfield J, Kabbani W et al. Selective Prostate Cancer Thermal Ablation With Laser Activated Gold Nanoshells. The journal of Urology. 2008; 179: 748-753.

80. Hainfeld JF, Dilmanian FA, Slatkin DN et al. Radiotherapy enhancement with gold nanoparticles. Pharmacy and Pharmacology. 2008; 60: 977-985.

81. Hainfeld JF, Dilmanian FA, Zhong Z et al. Gold nanoparticles enhance the radiation therapy of a murine squamous cell carcinoma. Physics in medicine and biology. 2010; 55: 3045-3059.

82. Hainfeld JF, Smilowitz HM, O'Connor M et al. Gold nanoparticle imaging and radiotherapy of brain tumors in mice. Future Medicine. 2013; 8(10): 1601-1609.

83. Hainfeld JF, O'Connor MJ, Lin P et al. Infrared-Transparent Gold Nanoparticles Converted by Tumors to Infrared Absorbers Cure Tumors in Mice by Photothermal Therapy. Plosone. 2014; 9(2): e88414.

84. Jordan A, Waldoefner N, Decken K, Scholz R, inventors. Nanoparticle/Active ingredient conjugates. United States patent US 20080268061. 2006 Apr. 12.

85. Maier-Hauff K, Rothe R, Scholz R et al. Intracranial thermotherapy using magnetic nanoparticles combined with external beam radiotherapy: Results of a feasibility study on patients with glioblastoma multiforme. J. Neurooncol. 2007; 81: 53-60.

86. Maier-Hauff K, Ulrich F, Nestler D et al. Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme. J. Neurooncol. 2011. 103: 317-324.

87. Maggiorella L, Barouch G, Devaux C et al. Nanoscale radiotherapy with hafnium oxide nanoparticles. Future Oncol. 2012. 8(9): 1167-1181.

88. Alphandéry E, Faure S, Seksek O et al. Chains of Magnetosomes Extracted from AMB-1 Magnetotactic Bacteria for Application in Alternative Magnetic Field Cancer Therapy. ACSNano. 2011. 5(8): 6279-6296.

89. Alphandéry E. Perspective of Breast Cancer Thermotherapies. Journal of Cancer. 2014. 5(6): 472-479.

90. Alphandéry E. Application of Magnetosomes synthesized by magnetotactic bacteria in medicine. Frontiers in bioengineering and biotechnology. 2014. 2(5): 1-6.

91. Alphandéry E, Chebbi I, Guyot F et al. Use of bacterial Magnetosomes in the magnetic hyperthermia treatment of tumours: A review. Int. J. Hyperthermia. 2013. 29(8): 801-809.

92. Alphandéry E, Lijeour L, Lalatonne Y et al. Different signatures between chemically and biologically synthesized nanoparticles in a magnetic sensor : A new technology for multiparametric detection. Sensors and Actuators B 2010. 146: 786-790.

93. Alphandéry E, Carvallo C, Menguy N. Chains of Cobalt Doped Magnetosomes Extracted from AMB-1 Magnetotactic Bacteria for Application in Alternative Magnetic Field Cancer Therapy. J. Phys. Chem. C. 2011. 115: 11920-11924.

94. Alphandéry E, Faure S, Raison L. Heat production by bacterial Magnetosomes exposed to an oscillating magnetic field. J. Phys. Chem. C 2011. 115: 18-22.

95. Alphandéry E, Amor M, Guyot F et al. The effect of iron-chelating agents on Magnetospirillum magneticum strain AMB-1 : stimulated growth and magnetosome production and improved magnetosome heating properties. Appl. Microbiol. Biotechnol. 2012. 96: 663-670.

96. Alphandéry E, Guyot F, Chebbi I. Preparation of chains of Magnetosomes from *Magnetospirillum magneticum* strain AMB-1 magnetotactic bacteria, yielding efficient

treatment of tumors using magnetic hyperthermia. Inter. J. Pharmaceutics. 2012. 434: 444-452.

97. Sadeghi S, Olevsky O, Hurvitz S. Profiling and targeting HER-2-positive breast cancer using trastuzumab emtansine. Pharmacogenomics and Personalized Medicine. 2014, 7: 329-338.

98. Ansell S M, Tang H, Kurtin P J et al. Denileukin Diftitox in Combination with Retuximab for Previously Untreated Follecular B-cell Non-Hodgkin's Lymphoma. Leukemia. 2012, 26: 1046-1052.

99. Chen GX, Zhang S, He XH. Clinical utility of recombinant adenoviral human p53 gene therapy: current perspectives. OncoTargets and Therapy. 2014, 7: 1901-1909.

100. Li J, Pan J, Zhu X et al. Recombinant adenovirus-p53 (Gendicine) sensitizes a pancreatic carcinoma cell line to radiation. Chinese Journal of Cancer Research. 2013, 25: 715-721.

101. Mathur M, Govind Vyas. Role of nanoparticles for production of smart herbal drug-An overview. Indian Journal of Natural Products and Resources. 2013, 4: 329-338.

102. Weissig V, Pettinger T, Murdock N. Nanopharmaceuticals (part I): products on the market. International Journal of Nanomedicine. 2014, 9: 4357-4373.

103. Yallapu MM, Jaggi M, Chauhan SC. Curcumin nanoformulations : a future nanomedicine for cancer. Drug. Discov. Today. 2012, 17: 71-80.

104. Koo OM, Rubinstein I, Onyuksel H. Campthotecin in sterically stabilized phospholipid micelles: a novel nanomedicine. Nanomedicine. 2005, 1: 77-84.

105. Ucisik MH, Kupcu S, Schuster B et al. Characterization of CurcuEmulsomes: nanoformulation for enhanced solubility and delivery of curcumin. Journal of Nanobiotechnology. 2013, 11:37.

106. Markovsky E, Baabur-Cohen H, Eldar-Boock A et al. Administration, distribution, metabolism and elimination of polymer therapeutics. Journal of Controlled Release. 2012, 161: 446-460.

107. Bawarski WE, Chidlowsky E, Bharali DJ et al. Emerging nanopharmaceuticals. Nanomedicine. 2008, 4: 273-282.

108. Fourmy D, Carrey J, Gigoux V, Targeted nanoscale magnetic hyperthermia: challenges and potentials of peptide-based targeting. Nanomedicine. 2015, 10: 893-896.

109. Kievit FM, Zhang M. Surface engineering of Iron Oxode Nanoparticles for targeted Cancer Therapy. Accounts of Chemical Research. 2011, 44: 853-862.

110. Wieder ME, Hone DC, Cook MJ et al. Intracellular photodynamic therapy with photosensitizer-nanoparticle conjugates: cancer therapy using a 'Trojan horse'. Photochemical and Photobiological Sciences. 2006, 5: 727-734.

111. Onoue S, Yamada S, Chan H-K, Nanodrugs: pharmacokinetics and safety. International J. of Nanomedicine 2014. 9: 1025-1037.

112. Thako AS, Gambhir SS. Nanooncology: The Future of Cancer Diagnosis and Therapy. CA Cancer J Clin. 2013, 63: 395-418.

113. Ehmann F, Sakai-Kato K, Duncan R et al. Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines. Future Medicine. 2013, 8: 849-856.

114. Desai N. Challenges in Development of Nanoparticle-Based Therapeutics. The AAPS Journal. 2012, 14: 282-295.

115. Jordan A, Scholz R, Maier6hauff K et al. The effect of thermotherapy using magnetic nanoparticles on rat malignant glioma. Journal of Neuro-Oncology 2006. 78: 7-14.

116. Schroeder Handy E, Ivkov R, Ellis-Busby D, Foreman A, inventors; Triton Biosystem Inc., assignee. Thermotherapy via targeted delivery of nanoscale magnetic particles. United States patent US 20030028071. 2003 Feb. 6.

117. Jordan A, Waldoefner N, Decken K, Scholz R, inventors. Nanoparticle/Active ingredient conjugates. United States patent US 20080268061. 2006 Apr. 12.

118. Waldoefner N, Stief K, inventors. Magnetic transducers. United States patent US 20110052609. 2010 Nov. 12.

119. Thiesen B, Jordan A, inventors; Magforce Nanotechnologies AG. Assignee. Implantable products comprising nanoparticles. United States patent US 20110223255. 2009 Feb. 11.

120. Alphandéry E, Faure S, Chebbi I; Nanobacterie, assignee. Treatment of cancer or tumors induced by the release of heat generated by various chains of Magnetosomes extracted from magnetotactic bacteria and submitted to an alternative magnetic field. United States patent US 20120302819. 2010 Nov. 18.

121. Alphandéry E, Chebbi I; UPMC and CNRS, assignee. Use of at least one iron chelate introduced in the bacterial growth medium to stimulate the growth of these bacteria. WO2012153247. 2012 15 Nov.

122. Hainfeld JF, inventor; Nanoprobes Inc., assignee; Methods and composition for increasing infrared absorptivity of a target. US 2008/0279946. 2008 13 Nov.

123. Liu W, Hainfeld JF, inventor; Nanoprobes Inc, assignee; 5 nm Nickel-NTA-Gold Nanoparticles. US 2012/0244075. 2012 27 Sep.

124. Levy L, Hochepied JF, Balencie J, Prasad P, Bergey E, Panak EA, Boussaha AK, inventors; Nanobiotix, assignee, Activatable particles, preparations and uses. W0 2005/120590 2005 9 may.

125. Lévy L, Boussaha AK, Panak EA, inventors; Nanobiotix assignee, Nanoparticles comprising an intracellular targeting element and preparation and use thereof. WO 2006/051198 2004 5 Nov.

126. Levy L, Pottier A, Rouet A, Marill J, Devaux C, Germain M, inventors; Nanobiotix, assignee, Inorganic nanoparticles, preparation and uses thereof. WO 2009147214 2008 5 June.

127. Lévy L, Pottier A, Puoi L, Maggiorella L, inventors; Nanobiotix, assignee, Metallic nanoparticles, preparation and uses thereof. WO 2011003999 2009 10 June.

128. Pottier A, Lévy L, Meyre ME, inventors ; Nanobiotix assignee, Nanoparticles comprising metallic and hafnium oxide materials, preparation and uses thereof. WO 2013087920 2012 17 Dec.

129. Krishnan S, Diagaradjane P, Schwartz JA, Wang JC, inventors; University of Texas and Nanospectra, assignee. Treatment of disease or disorders using nanoparticles for focused hyperthermia to increase therapy efficacy. US 20130197295 2013 16 Jan.

130. Krishanan S, Diagaradjane P, Goodrich G, inventors; The University of Texas and Nanospectra Inc, assignee. Enhancement of radiation therapy by targeted high-z nanoparticles. WO 2011127061 2010 5 April.

131. Hainfeld JF, Slatkin DN, inventors; NanoProbes Inc; Methods of enhancing radiation effects with metal nanoparticles. US 20090186060 2009 23 July.

132. Mei X, Qinggwei J, Yu W, inventors; Celsion Corp, assignee, Thermosensitive liposomes containing therapeutic agents. US 20110200665 2011 18 Aug.

133. For example: Hong K, Drummond DC, Kirpotin D, inventors; Merrimack Pharmaceuticals Inc, assignee. Liposome useful for drug delivery. US 20130122081 2013 16 May.

134. Cheng J, Davis ME, Khin KT, inventors; Cerulean Pharma Inc, assignee; Cyclodextrin-based polymers for therapeutic delivery. US 20130190450 2013 25 July.

135. For example Cheng J, Davis ME, Khin KT, inventors; Cerulean Pharma Inc., assignee. Cyclodextrin-based polymers for therapeutics delivery US 20130129665 2013 23 May.

136. For example Figueiredo M, Peeke E, Dewitt D et al, inventors; Bind Therapeutics Inc., assignee. Therapeutic nanoparticles comprising a therapeutic agent and methods of making and using same. US 20140178475 2014 26 June.

137. Zale SE, Troiano G, Ali MM, Hrkach J, Wright J; Bind Therapeutics Inc, assignee. Therapeutic polymeric nanoparticles with mitor inhibitors and methods of making and using same US 20140186453 2014 3 Jul.

138. Jadhav N, Powar T, Shinde S, Nadaf S. Herbal nanoparticles: A patent review. Asian journal of Pharmaceutics. 2014, 8: 58-69.

139. Bosetti R, Vereeck L. Future of Nanomedicine Obstacles and Remedies. Nanomedicine. 2011, 6: 747-755.

140. Edison investment Research. ‘’Magforce, accelerating on the US highway’’, 9 Sep. 2014.

141. Edison investment Research. ‘’Nanobiotix, Good things come in small packages’’, 3 May 2013.

142. Edison investment research. ‘’BioAlliance Pharma Awaiting Onxeo’’, 23 July (2014)

143. www.pharmaengine.com: Vision and objective.

144. Transparency Market Research. ‘’Nanomedicine Market: Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2013-2019’’, 1 August 2014, website: www.transparencymarketresearch.com

145. Werner ME, Cummings ND, Sethi M et al. Preclinical Evaluation of Genexol-PM, a Nanoparticle Formulation of Paclitaxel, as a Novel Radiosensitizer for the Treatment of Non-Small Cell Lung Cancer, Radiation Oncology. 2013. 86: 463-468.

146. Libutti SK, Paciotti GF, Byrnes AA et al. Phase I and Pharmacokinetic Studies of CYT-6091, a Novel PEGylated Colloidal Gold-rhTNF Nanomedicine, Clin. Cancer Research. 2010, 16(24): 6139-6149.

147. Schwartz JA, Price RE, Gill-Sharp KL et al. Selective Nanoparticle-Directed Ablation of the Canine Prostate, Lasers in Surgery and Medicine. 2011, 43: 213-220.

148. Johannsen M, Gneveckow U, Thiesen B et al. Thermotherapy of Prostate Cancer Using Magnetic Nanoparticles: Feasibility, Imaging, and Three-Dimensional Temperature Distribution, European Urology. 2007, 52: 1653-1662.

149. Vasievich EA, Chen W, Huang L. Enantiospecific adjuvant activity of cationic lipid DOTAP in cancer vaccine, Cancer Immun. Immunother. 2011, 60: 629-638.

150. Notice of Mepact, http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000802/WC500026565.pdf.

151. 2014 annual report of Merrimack, http://investors.merrimackpharma.com/secfiling.cfm?filingID=1193125-14-82294&CIK=1274792

152. Notice of Marqibo, http://www.drugs.com/cdi/marqibo-injection.html

153. Notice of Depocyt, http://www.ema.europa.eu/docs/fr_FR/document_library/EPAR_-_Product_Information/human/000317/WC500035649.pdf

154. 2013 annual report of Bioalliance Pharma, http://www.onxeo.com/site/pdf/RA-2013_BioAlliancePharma-EN.pdf

155. Farma JM, Puhlmann M, Soriano PA et al. Direct evidence for rapid and selective induction of tumor neovascular permeability by tumor necrosis factor and a novel derivative, colloidal gold bound tumor necrosis factor, Int. J. Cancer. 2007, 120: 2474-2480.

156. Bardhan R, Chen W, Bartels M et al. Tracking of Multimodal Therapeutic Nanocomplexes Targeting Breast Cancer in Vivo, NANO Letters. 2011, 10: 4920-4928.

157. Young C, Schluep T, Hwang J et al. CRLX101 (formerly IT-101)-A Novel Nanopharmaceutical of Campthotecin in Clinical Development, Current Bioactive Compounds. 2011, 7: 8-14.

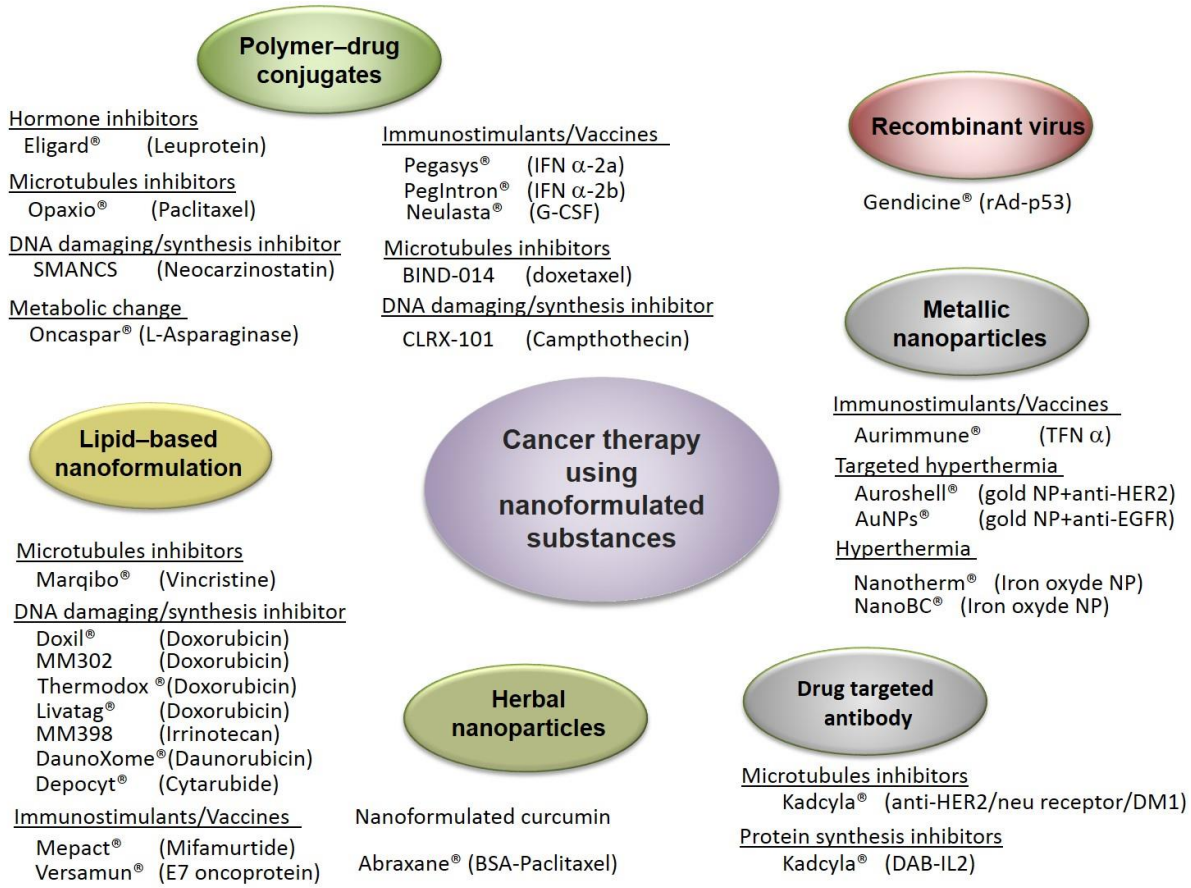
158. Hainfeld JF, Smilowitz HM, O'Connor M et al. Gold nanoparticle imaging and radiotherapy of brain tumors in mice. Future Medicine. 2013; 8(10): 1601-1609.Frenkel V, Etherington A, Greene M et al. Delivery of Liposomal Doxorubicin (Doxil) in a Breast Cancer Tumor Model: Investigation of Potential Enhancement by Pulsed-High Intensity Focused Ultrasound Exposure. Academic radiology. 2006, 13(4): 469-479.

159. Rose PG, Blessing JA, Lele S et al. Evaluation of pegylated doxorubicin (Doxil) as second-line chemotherapy of squamous cell carcinoma of the cervix: A phase II study of the Gynecologic Oncology Group. Genecologie Oncology. 2006, 102: 210-213.

160. Kaspers GJL, Zimmermann M, Reinhardt D et al. Improved Outcome in Pedriatic Relapsed Acute Myeloid Leukemia: Results of a Randomized Trial on Liposomal Daunorubicin by the International BFM Stdy Group. Journal of Clinical Oncology. 2013, 31: 599-607.

161. Willis F and Pettengell R. Pegfilgrastim. Expert. Opinion Biol. Ther. 2002, 2: 985-992.

1.


Cancer therapy using nanoformulated substances
Polymer–drug conjugates
Hormone inhibitors
Eligard® (Leuprotein)
Microtubules inhibitors
Opaxio® (Paclitaxel)
DNA damaging/synthesis inhibitor
SMANCS (Neocarzinostatin)
Metabolic change
Oncaspar® (L-Asparaginase)
Immunostimulants/Vaccines
Pegasys® (IFN α-2a)
PegIntron® (IFN α-2b)
Neulasta® (G-CSF)
Microtubules inhibitors
BIND-014 (doxetaxel)
DNA damaging/synthesis inhibitor
CLRX-101 (Campthothecin)
Recombinant virus
Gendicine® (rAd-p53)
Metallic nanoparticles
Immunostimulants/Vaccines
Aurimmune® (TFN α)
Targeted hyperthermia
Auroshell® (gold NP+anti-HER2)
AuNPs® (gold NP+anti-EGFR)
Hyperthermia
Nanotherm® (Iron oxyde NP)
NanoBC® (Iron oxyde NP)
Lipid–based nanoformulation
Microtubules inhibitors
Marqibo® (Vincristine)
DNA damaging/synthesis inhibitor
Doxil® (Doxorubicin)
MM302 (Doxorubicin)
Thermodox ®(Doxorubicin)
Livatag® (Doxorubicin)
MM398 (Irrinotecan)
DaunoXome®(Daunorubicin)
Depocyt® (Cytarubide)
Immunostimulants/Vaccines
Mepact® (Mifamurtide)
Versamun® (E7 oncoprotein)
Herbal nanoparticles
Nanoformulated curcumin
Abraxane® (BSA-Paclitaxel)
Drug targeted antibody
Microtubules inhibitors
Kadcyla® (anti-HER2/neu receptor/DM1)
Protein synthesis inhibitors
Kadcyla® (DAB-IL2)

Drug name	Company	Composition	Coating	Surface Charge	Size (nm)	Active principle	Mode of action	Mode of admin.	Targeting	Cancer	Ref
Accurin (Bind-014)	Bind	Polymer (PLGA) + docetaxel/PEG	PEG	-8 mV	100	docetaxel	Pharmaceutical	iv	Prostate antigen (PSMA) + EPR	NSC lung Prostate	46
CRLX101	Cerulean	Cyclodextrin-based polymer + camptothecin/PEG	PEG	-6 mV	20-30	camptothecin	Pharmaceutical	NA	NA	Renal cell carcinoma	47
CRLX301	Cerulean	Docetaxel-based polymer	NA	NA	NA	docetaxel	Tubulin inhibitor	NA	NA	NA	NA
Abraxane	Abraxis bioscience Astra Zeneca	Paclitaxel in a human albumin polymer	None	NA	130	Paclitaxel	Pharmacol-ogical	iv	EPR + Albumin-gp60	Metastatic breast NSC lung Pancreatic Melanoma	53
Genexol-PM (Cynvilog)	Lupin Laboratories	PEG copolymer + paclitaxel	PEG	-8 mV	24	Paclitaxel	Inhibition of mitosis	iv	EPR	Metastatic breast tumor	112
Aurimmune CYT-6091 PT-cAau-TNF	Cytimmune	Gold nanoparticles + TNF-α/PEG	TNF-α THIOL-PEG	-2 mV	25	nanoparicle	Radiofrequency ablation / cryoablation / Chemiotherapy	iv	TNF-α + EPR	Advanced cancer	61, 113
Auroshell Aurolase	Nanospectra	Gold / silica nanoparticles + Anti-HER2/PEG	PEG + anti-HER2	N.A.	130 (silica) 2 (gold)	nanoparticle	Heat NIR radiation	iv	EPR + Anti-HER2	Prostate	114
AuNPs (cat No: 1115)	Nanoprobes	Gold nanoparticles + Anti-EGFR/PEG	PEG + Anti-EGFR	N.A.	11	nanoparticles	Radiotherapy Heat therapy	iv	EPR + Anti-EGFR	Glioma, head and neck, breast cancer	69
Magnetosome	Nanobacterie	Iron oxide nanoparticles	Lipids + proteins	-24 mV	40-60	nanoparticles	Heat Magnetic hyperthermia	it	NA	Solid tumors	74
NBTXR3 (NanoXray)	Nanobiotix	Hafnium oxide nanoparticles	None	-50 mV	50	nanoparticles	Radiotherapy Gerneration of ROS	It	None	Solid tumors Soft tissue sarcoma	83
Nanotherm	Magforce	Iron oxide nanoparticles	Amino-silicane	NA	15	nanoparticles	Heat Magnetic hyperthermia	it	None	Glioblastoma Prostate pancreatic carcinoma	115, 85

Table 1: Characteristics of nanodrugs

Drug name	Company	Composition	Coating	Surface Charge	Size (nm)	Active principle	Mode of action	Mode admin.[1]	Targeting	Main indications	Ref
Versamune PDS0101	PDS biotech-nology	HPV oncoprotein E7 in DOTAP liposomes	NA	45-55 mV	100-120	Antigen	Immunologic	NA	NA	Cervix cancer	116
Doxil	Janssen products	Doxorubicin in Liposomes	PEG	NA	100	Doxorubicin	Pharmacol-ogical	iv	EPR	Metastatic breast cancer	35
Daunoxome	Gilead	Daunorubicin in liposome (phospahtidylcholine + cholesterol)	None	NA	35-65	Daunorubicin	Pharmacol-ogical	iv	EPR	Leukemia Sarcoma Breast cancer	35
Myocet	Elan Pharma	Doxorubicin in liposomes	None	NA	190	Doxorubicin chlorohydrate	Pharmacol-ogical	iv	EPR	Metastatic breast cancer	35
Mepact	IDM Pharma SAS	Mifamurtide in multilamellar liposomes			<100	Mifamurtide	Immunological	iv	Macropha ge RES	Osteosarcoma	118
MM-398	Merrimack	Irinotecan encapsulated in a liposome	PEG	NA	100	Irinotecan	DNA breakage and cell death	Iv it	NA	Pancreatic cancer Colorectal cancer Glioma	117
MM-302	Merrimack	HER-2-targeted liposome containing doxorubicin	PEG-DSPE	NA	75-110	Doxorubicin	Pharmacological	iv	Anti-HER-2 antibody	Advanced HER-2 breast cancer	117
Thermodox	Celsion Corp	Heat sensitive liposome containing doxorubicin	PEG	NA	100	Doxorubicin	Doxorubicin release d by heat	iv	EPR	Liver cancer Breast cancer	35
Marqibo	Talon Therapeutics	Liposomes containing vincristine sulfate	none	NA	100	Vincristine sulfate from periwinkle	Inhibition of mitosis	iv	EPR	Acute lyphoblastic leukemia Non-Hodgkin's lymphoma	119
Depocyt	Skypharma	Cytarubide encapsulated in lipid nanoparticles	none	NA	$2\ 10^4$	Cytarubide	antineoplastic	Intrath e-cally	None	Lymphomatous meningitis Neoplastic meningitis	120
Livatag	Bioalliance pharma	Doxorubicin in nanoparticles	NA	NA	100-300	Doxorubicin	Pharmacological	paren.	NA	Liver cancer	121
Oncaspar	Enzon	PEG-L asparginase	PEG	NA	NA	L-asparaginase from E-Coli or from Erwina.	Selective killing of leukemic cells due to depletion of plasma asparagine	im iv	NA	Acute lymphoblastic leukemia	55
Neulasta (pegfilgrasti m)	Amgen	G-CSF bound PEG	PEG	NA	~ 2-4	G-CSF	Increase the number of immune cells	sc	None	Protects against neutropenia due to cancer treatments	56

Table 1: Characteristics of nanodrugs iv: intravenous; im: intramuscular; it: intratumoral; sc: subcutaneous

Drug formulation	Preclinical studies	Clinical studies	AMM
Accurin (Bind-014)	Blood half life circulation ~ 20 hours in rats Minimal liver accumulation in rats Prolonged tumor growth suppression compared to solvent-based DTXL formulation, (46).	Patients with advanced solid tumors show tumor shrinkage for a dose of Bind-014 below that of solvent-based DTXL formulation (46)	No
CRLX101	Accumulation of CRLX101 into solid tumors and release of CPT over a period of several days to give inhibition of tumor (47). Gastric cancer cell lines showed high in vitro cytotoxicity for CRLX101 and also strong antitumor activity in vivo (48). CRLX101 more efficient than Camptothecin alone (49)	CRLX101 was administered intravenously. Maximum tolerated dose is 15 mg/m2. adverse effect: neutropenia (51). CRLX101 enhances efficacy of CPT (124).	No
Aurimmune CYT-6091 PT-cAu-TNF	For MC38 and LCC bearing mice: same efficacy but less toxicity than free TNF, (122). Increase the efficacy of cryotherapy, (60). Increase the eficacy of thermotherapy, (61, 62). After iv injection, accumulation in tumor and not in RES.	Pahse I of advanced cancers: Maximum acceptable dose ~ 4 mg compared with 0.4 mg for free TNF, (113). Targeting of tumor and avoidance of RES. Half life 130 minutes compared with 28 minutes for free TNF.	No
Auroshell Aurolase	- Imaging: Nanoparticles used for FOI and MRI and NBI, (123). - Mild hyperthermia ($\Delta T \sim 10$ °C) in mice with colorectal cancer: anti hypoxic activity and increased efficacy of radiation, no tumor ablation (64). - Thermal ablation ($\Delta T \sim 30$-40 °C) in mice and dogs with prostate cancer: ablation of tumor, but increase of temperature ($\Delta T \sim 5$-10 °C) in regions without nanoparticles (65, 66, 67).	NA	No

Table 2: Precinical and clinical results obtained with nanodrugs

Drug formulation	Preclinical studies	Clinical studies	AMM
AuNPs (cat No: 1115)	LD50: 3.2 g AuNPs/Kg Radiotherapy with 42-250 kVp ; 30-42 Gy X-rays and 1.35 g AuNPs/Kg in mice with mammary, head and neck and glioma tumors: - Disappearance of tumor in 20-30 days (68, 69) - One year survival 50-86 % compared with 0-20 % for radiation only, (68, 125). NIR heating of AuNPs in mice with squamous carcinmoa: - Disappearance of tumor in three days (71).	NA	No
Magneto-somes	Magnetic hyperthermia treatment of mice with xeno-grafted MDA-MB231 breast tumors: - It Administration of 1 mg of a suspension of magnetosomes. - Several tumors completely desappear. - Magnetosomes more efficient than chemical nanoparticles.	NA	
NBTXR3 (NanoXray)	Radiotherapy with 8Gy and NBTXR3 in mice with sarcoma tumors: - Disappearance of tumor in 30 days and increased survival rate, (83).	NA	No
Nanotherm	Magnetic hyperthermia with nanotherm in rats with RG2 glioma tumors (85): - Quantity of Nanotherm administered is 180 µg per mm³ of tumor, field of 100 kHz and maximum strength of 22 mT applied during 30 minutes. - Average heating temperature is ~ 45 °C. - Increase of survival by ~ 4. Survival following tumor administration: 10 days without treatment, 40 days with magnetic hyperthermia.	Magnetic hyperthermia with Nanotherm for treatment of prostate cancer (Phase I), (115): - Average quantity of Nanotherm administered in tumor is ~ 110 mg. - 6 therapies of 60 minutes with one week separating each cession. Magnetic field of 100 kHz, 6 mT. - Temperature reached during the treatment 41-55 °C. Magnetic hyperthermia plus radiotherapy with Nanotherm for treatment of glioblastoma on 14 patients (72): - Average quantity of Nanotherm administered in tumor is 10-80 µg per mm³ of tumor. - 4 to 10 therapies of 60 minutes with at least 48 hours separating each cession. Magnetic field of 100 kHz, 22 mT maximum strength. - Temperature reached during the treatment 42-50 °C. Magnetic hyperthermia plus radiotherapy with Nanotherm for treatment of glioblastoma on 60 patients (73): - Average quantity of Nanotherm administered in tumor is 30 µg per mm³ of tumor. Average tumor size ~ 17 cm². - 6 therapies of 60 minutes. Magnetic field of 100 kHz, 18 mT maximum strength. - Temperature reached during the treatment 51 °C. - Average survival following first recurrence: 13,2 months compared with 6 months with conventional treatments.	Yes

Table 2: Precinical and clinical results obtained with nanodrugs

Drug formulation	Preclinical studies	Clinical studies	AMM
Versamune PDS0101	Immunotherapy for TC-1 lung tumor bearing mice, (39): - DOTAP/E7 produces migration of activated DC to DLN. - Generation of ROS due to cationic DOTAP in dendritic lymph nodes activates dendritic cells. - Anti-tumor activity due to $CD8^+$ T lymphocyte. - Tumor regression.	NA	No
Abraxane ABI-007	LD50 in mice : 47 mg/kg for abraxane compared with 30 mg/Kg for cremo-paclitaxel After 24h iv injection in mice with tumor, 33% more tumor accumulation for abraxane than for cremo-paclitaxel	Phase III study on 454 patients with metastactic breast cancer, comparison between ABI-007 (iv, 260 mg/m2) and cremo-paclitaxel (175 mg/m²), (53): - Overal response rate: 33% for abraxane and 19% for cremo-paclitaxel. - Neutropania: 10% for abraxane versus 21% for cremo-paclitaxel. Phase III for NSC lung cancer and phase II for pancreatic cancer and melanoma. MTD is 300 mg/m² for abraxane versus 175 mg/m² for cremo-paclitaxel, (54).	Yes
Doxil	TNF-α increases the efficacy of Doxil in mice with melanoma (23). Increase of the delivery of Doxyl in breast tumors by using ultrasound (126).	AMM for treatment of ovarian cancer: 50 mg/m2 iv administration. AMM for treatment of Kaposi's sarcoma: 20 mg/m2 iv administration. Phase I of Doxil (30-50 mg/m2) plus cyclophosphamide for treatment of solid tumors, (21). Phase II of Doxil as a second line treatment for squamous cell carcinoma (127).	Yes
Daunoxome	NA	Treatment of acute myeloid leukemia, (25, 128): - Reduce the conversion of daunorubicin to toxic daunorucinol. - MTD = 150 mg/m2. - Increased efficacy and increased tumor exposure by 10 compared with free daunorubicin. - Phase III, complete remission of 64%. Treatment of sarcoma (33): - MTD= 60 mg/m2 Kaposis sarcoma - Response rate 3% for treatment of soft tissue sarcoma (100 mg/m2) Treatment of metastatic breast cancer (32): - MTD = 120 mg/m2 - Manageable toxicity and efficacy.	Yes
Myocet	NA	Treatment of metastatic breast cancer with: - Myocet alone (6): 60 mg/m2 of Myocet in 297 patients. Myocet improves the therapeutic index of free doxorubicin. Less neutropenia. - Myocet alone (8): Phase III on 224 patients. Comparaison of Myocet with free doxorubicin. Myocet (75 mg/m2) and free doxorubicin (75 mg/m2): less cardiac toxicity and similar efficacy between Myocet and free doxorubicin. - Myocet + docetaxel + trastuzumab: 50 mg/m2 of Myocet. Overall response rate 56%, complete response rate: 8% Treatment of multiple myeloma (12): - Myocet + VTD (bortezamib, thalidomide and dexamethasone): Myocet (50 g/m2) treatment of 70 patients. Response rate of 81% for Myocet + VTD compared with 50% for VTD. Treatment of Aids non Hodgkin's lymphoma (13): - 24 patients treated with Myocet (80 mg/m2) + cyclophosphamide + prednisome : 88 response rate. Biodistribution of Myocet (75 mg/m2) administered in 18 patients: longer half life with less free drug available for tissue biodistribution than conventional doxorubicin.	Yes

Table 2: Precinical and clinical results obtained with nanodrugs

Drug name	Preclinical studies	Clinical studies	AMM
Depocyt	NA	NA	
Oncaspar	Teatment of L5178Y lymphosarcoma or 6C3HED tumors xenogratfted on mice, (55): - More efficacy of PEG-Asp compared with free aspariginase.	Pharmakokinetics, (55): - Elimination half life of PEG-Asp is 6 days, 5 times longer than that of free aspariginase. - One dose of PEG-Asp is equivalent to 6 doses of free asparaginase. - Immunogenicity of PEG-Asp is less than that of free asparaginase. Treatment of acute lymphoblastic leukemia (ALL), (55): - Trial DFCI 90-01 on 377 patients with PEG-Asp (2500 IU/m2) or free asparaginase (25 000 IU/m2), IM: Similar 5 years event free survival of 80 %, but less toxicity for PEG-Asp than for free asparaginase. - Trial CCG-1962 on 118 patients with PEG-Asp (2500 IU/m2) or free asparaginase (25 000 IU/m2), IM: Similar 5 years event free survival of 80 %, but more rapid clearance of lymphoblast from bone marrow with PEG-Asp.	Yes
Marquibo	Xeno-grafted studies on mice (2): - Higher circulation time of Marquibo compared with free vincristine - Optimized delivery to target tissue. - Facilitate dose intensification without increasing toxicity.	Treatment of acute lymphoblastic leukemia, (2): - Use of a dose of 2.5 mg/m2 - Injectable dose (3-5 mg) two times more than free vincristine sulfate. - Trial Rally on 13 patients: Clearance of 345 mL/h compared with 189 mL/h for free vincristine sulfate. - Higher maximum tolerated dose, superior antitumor activity, higher amount delivered to target tissue compared to free vincristine. Note: SM/Chol liposomes: less expensive than liposomes with PEG.	Yes
Genexol-PM (Cynvilog)	NA	NA	
Mepact	IV administration of Mepact for treatment of rodents with lung and liver metastasis, (38): - Inhibit tumor growth and increase survival. IV administration of Mepact after resection of primary tumor for treatment of dogs with osteosarcoma, (38): - Improves survival.	Phase I on 28 patients with metastactic breast cancer, (38): - Use of 0.05-12 mg/m2 Phase II on 16 patients with osteosarcoma: - Macrophage activation. Increase in levels of circulating TNF-α and IL-6. Study on 14 patients with advanced metastatic cancer: - No accumulation and rapid clearance. Phase III of Mepact administered with chemotherapy for treatment of osteosarcoma - Increase of 6 year survival rate from 70% to 80% - Recommended dose of 2 mg/m2.	Yes

Table 2: Precinical and clinical results obtained with nanodrugs

Drug name	Preclinical studies	Clinical studies	AMM
Neulasta (pegfilgrastim)		Half life of Neulasta 33 hours compared with 3,5 hours for filgrastin. Phase III study comparing a single dose of Neulasta with daily injection of filgrastin in patients with breast cancer, (56): - 9-13% febrile neutropenia (FN) with Neulasta compared with 13-20% FN with filgrastin. AMM in 2002 for treatment of patients with non myeloid malignancies, (Willis 2002): recommended dose : 6 mg.	Yes
Cyclosert	NA	NA	
Livatag	NA	NA	
MM-398	Treatment of mice with glioblastoma, (36): - it administration of 0.2-0.4 mg of MM-398. 40 % of mice totally cured when combined with radiotherapy.	Phase II study for patients with pancreatic cancer, (37): - Iv injection of 120 mg/m2 every three weeks. Progression free survival of 2-4 months. Phase II study for patients with metastatic gastric or gastro-oesophageal adenocarcinoma: - Comparison between treatments with MM-398 (120 mg/m2), irinotecan (300 mg/m2), docetaxel (75 mg/m2). - Similar efficacy between the three treatments.	No
MM-302	NA	NA	

Table 2: Precinical and clinical results obtained with nanodrugs

	Free Doxorubicin	Myocet	Doxil	Free Daunorubicin	Daunoxome
Elimination half life (h)	0.2 (35)	2.5 (35)	55 (35)	0.8 (24)	5 (24)
Area under the curve ($\mu g.h.ML^{-1}$)	4 (35)	45 (35)	900 (35)	10 (24)	375 (24)
Clearance (l/h)	47 (7)	5 (7)	0.004 (7)	14 (24)	0.4 (24)

Table 3: Pharmacodynamic parameters of a few drugs.

Companies	Location	Year funded	IPO Date	Amount raised	Price per share	Company valuation	Rev. 2013	Losses 2013	Accumul. losses	Assets 2013	Share price Feb 7 2015	52 weeks low/high	Mkt Cap Oct 2014	Nbr employe	R&D	G&A	Marc Cap/ Acc. Loss	Price to book
Celsion Corp	US	1982	2001	$6.25m	$0.50		$0.5m	$12.9m	$169.3m	45.7	$2.28	$2.15/4.03	$45.6m	13	$9.4m	$6.5m	0.31	1.68
Nanoprobe	US	1991	PH				est $2m	n.a.	n.a.	n.a.	n.a			19				
Merrimack	US	1993	2012	$100,1m	$7.00	$747m	$46.2m	$131m	$572m	65	$8.87	$4.13/12	$939.2m	250	$147.1m	$21.2m	1.71	-12.7
Magforce	GE	1997	2013	$43m	$2.3	$55m	$6.9m	$2m	$50.2m	22.7	$7.2	$4.83/8.49	$173.23m	20	n.a.	n.a.	3.60	8.6
Onxeo	FR	1997	2005	$38.4m	$17	$38.4m	$0.5m	$19.6m	$141m	5.1	$7.19	$5.44/12.67	€291.36m	52	est $12.4m	n.a.	1.88	18.2
Cytimmune	US	1998	PH	NA	NA				n.a.									
Nanospectra	US	2002	PH	NA	NA		$1.7m	$2.6m	$5m	n.a.	0.0			10				
Nanobiotix	FR	2003	2012	$18,2m	$7.7.00	$82m	$0.1m	$10.5m	est$38.4m	6.4	$21.56	$7.25/34.26	$303.13m		est €7.7m	est €4.7m	est8.2	76.9
Cerulean	US	2005	2014	$75m	$7.00	$133m	0,0	$5.6m	$101.4m	6.8	$8.24	$3.35/8.25	$165.8m	22			2.69	2.9
Bind Therapeutics	US	2006	2013	$70.5m	$15.00	$237	$10.9m	$31.4m	$104.7m	88.4	$6.02	$5.13/14.75	$99.6m	59	$24.4m	$13.4m	1.22	2.4
Nanobacterie	FR	2008	PH	NA	NA		0,0	$0.18m	n.a.		0.0			5				
PDS Biotechology	US	2005	PH	NA	NA		2.4							est 4				
Oncothyreon	US	2007		NA	NA		0.0	$38.8m	$432.1m	$77.7m	$1.5	$1.48/4.08	$137.3m		$33.2m	$8.0m	0.34	2.2
Bio-Path Holdinds	US	2007	2014	$15m	NA		0.0	$3.3m	$15.4m	$5.1m	$2.44	$1.90/4.2	$210.6m	2	$1.5m	$1.6m	13.8	70.4

Table 4: Financial Figures