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STEALTH LIPOSOMES: A REVIEW

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ABSTRACT
A Liposome is spherical, self closed vesicles of colloidal dimensions, in which phospholipids bilayer sequesters part of the solvent, in which it freely float, into its interior. Liposome technology has progressed from conventional vesicles ("first-generation liposome") to "second-generation liposome", in which long-circulating liposome are obtained by modulating the lipid composition, size, and charge of the vesicle. Liposome with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid. A significant step in the development of long-circulating liposome came with inclusion of the synthetic polymer poly-ethylene glycol (PEG) in liposome composition. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononuclear phagocyte system uptake (stealth liposome). This technology has resulted in a large number of liposome formulations encapsulating active molecules, with high target efficiency and activity. Further, by synthetic modification of the terminal PEG molecule, stealth liposome can be actively targeted with monoclonal antibodies or legends. This review focuses on stealth technology and summarizes pre-clinical and clinical data relating to the principal liposome formulations; it also discusses emerging trends of this promising technology.
KEY WORDS: Drug delivery, cancer therapy, encapsulation stealth liposome.

INTRODUCTION

A liposome is a spherical vesicle with a membrane composed of a phospholipids bilayer used to deliver drugs or genetic material into a cell liposome can be composed of naturally derived phospholipids with mixed lipid chains (like egg phosphatidyle choline) or of pure components like dioleoly phosphatidyl ethanol amine)[21]

The field of drug delivery is advancing rapidly by controlling the precise level and location of drug in the body, side effects are reduced, lower doses are often needed and new therapies are possible. Liposome have been receiving a lot of interest as a carrier for advanced drug delivery liposome were first produced in England in 1961_by Alec D. Bangham, who was studying phospholipids and blood clotting. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water solute, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends, fat-soluble medications were incorporated into the phospholipids layer.[21]Research on liposome technology has progressed from conventional vesicles ("first-generation liposome") to "second-generation liposome", in which long-circulating liposome are obtained by modulating the lipid composition, size, and charge of the vesicle. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing mononclear phagocyte system uptake (stealth liposome).

This technology has resulted in a large number of liposome formulations encapsulating active molecules, with high target efficiency and activity. Further, by synthetic modification of the terminal PEG molecule, stealth liposome can be actively targeted with monoclonal antibodies or legends. This review focuses on stealth technology and summarizes pre-clinical and clinical data relating to the principal liposome formulations; it also discusses emerging trends of this promising technology.[13]

Classification

Multilamellar liposome: Onion structure
Conventional liposome: Stabilized natural
Synthetic identical- Chain phospholipids
Glycolipid containing liposome
Specialized liposome: Antibody directed
Lipoprotein coated
Bipolar fatty acids

Stealth liposome: Polyhydroxyethyl L-asparagines coated
PEG Coating
H-PG-PEG Coated
Dope Coated

A significant step in the development of long-circulating liposomes came with inclusion of the synthetic polymer poly-ethylene glycol (PEG) in liposome composition. The presence of PEG on the surface of the liposomal carrier has been shown to extend blood-circulation time while reducing phagocyte system uptake (stealth liposome).

STEALTH LIPOSOMES Conventional liposome are remove from the circulation is too fast so long circulation time of liposome were required to take full advantage of this leaky endothelium effect. This brings us to the second important finding. Coating liposome with PEG reduces the rate of uptake by macrophages (stealth effect) and leads to a prolonged presence of liposome in the circulation and consequently provides ample time for these liposome to escape from the circulation through leaky endothelium.[11,21]

"A stealth liposome is a spherical vesicle with a membrane composed of phospholipids bilayer used to deliver drugs or genetic material into a cell. Liposome can be composed of naturally-derived phospholipids with mixed lipid chains coated or stabilized by polymers of PEG and colloidal in nature."

Stealth liposomes are achievement & development in new drug delivery and controlled release.[3] This stealth principle has been used to develop the successful doxorubicin- loaded liposome product that is presently marketed as doxil or caelyx for treatment of solid tumors recently impressive therapeutic improvements were described by using corticosteroid loaded liposome in experimental arthritic models. Regarding the application of long circulating liposome has been on their potential to escape from the blood circulation. However, long circulating liposome may also act as a reservoir for prolonged release of a therapeutic agent. Pharmacological action of vasopressin formulated in long circulating liposome.

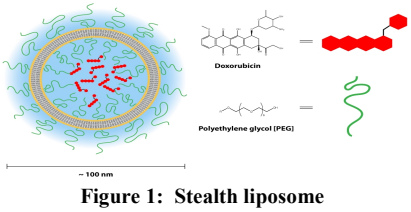

Figure 1: Stealth liposome

"Stealth liposome were introduced which can largely avoid detection by the immune system and were shown to have blood circulation time for several day (2 day as compared to minutes rather than hours of conventional liposome)".[21]

Mechanism of transportation through stealth liposomes

1. Liposome can interact with cells by different mechanisms endocytosis by phagocytic cell of the reticuloendothelial system such as macrophage sand neutrophils
2. Adsorption to the cell surface either by nonspecific weak hydrophobic or
3. Electrostatic forces or by specific interactions with cell-surface components.
4. Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm.
5. Transfer of liposomal lipids to cellular or sub cellular membranes, or vice versa, without any association of the liposome contents.
6. It often is difficult to determine what mechanism is operative and more than one may operate at the same time.[21]

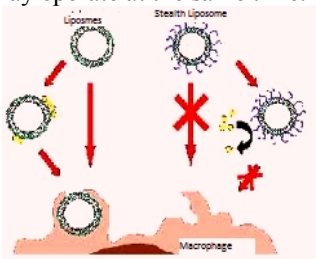

Figure 2: Fate of Liposomes and Stealth Liposomes to phagocyte cell of the reticuloendothelial system

Different mode of action of Stealth liposomes

1. Sustained release system of systemically or locally administered liposome. Examples are doxorubicin, cytosine, arabinose, cortisones, biological proteins or peptides such as vasopressin.
2. Site- avoidance mechanism: Liposome do not dispose in certain organs, such as heart, kidneys, brain and nervous system and this reduces cardio, nephro and neurotoxicity. Typical examples are reduced nephro toxicity of Amphotericin B, and reduced cardio toxicity of doxorubicin liposome.
3. Site specific targeting: In certain cases liposome with surface attached legends can bind to target cell (key and lock mechanism) or can be delivered into the target tissue by local anatomical conditions such as leaky and badly formed blood nevels, their basal lamina, and capillaries.
4. Examples include anticancer anti-infection and anti-inflammatory drugs.
5. Improved penetration in to tissues, especially in the case of dermatically applied liposomal dosage forms.
6. Examples, includes anesthetics, corticosteroids, and insulin.[1]

Characteristics of Stealth liposome

1. Stealth Liposome composed of cholesterol and phospholipids such as phosphatidylcholine and dicetylphosphate the structure, composition and proportion being practically the same as in the host cell.
2. Membranes The phospholipids possess a hydrophobic tail structure and a hydrophilic head component and organize in the following manner when dissolved in water.
3. In this way, double lipid layers are formed which seal off to form small vesicles similar to the body cells and their organelles.
4. These spheres or stealth liposome constitute small deposits that can be made to contain an antigen, an antibiotic, an allergen a drug substance or a gene as in gene therapy.
5. The stealth liposome can in turn be introduced in the body without triggering immune rejection reactions. Phospholipids Bilayers are the core structure of liposome and cell membrane formations.
6. The Stealth liposome his revered by various polymers like PGE poly ethylene glycol, poly (a-hydroxy acids , Poly (Hydroxyethyl-L-asparagine)-coated PG-PEG-modified lipids DOPE (Dioleoylphosphatidylethanolanmine)
7. The Stealth liposome is stable in nature.
8. The Stealth liposome is colloidal and uniform in nature.
9. The Stealth liposome may be a bipolar fatty acids, Antibody directed, Methyl /methylene X-linked, Lipoprotein coated, Carbohydrate coated, multiple encapsulated, Emulsion compatible.
10. Liposome encapsulated drug are delivered to varies tissue and cells and can be released when the liposome is destroyed, enabling site specific targeted drug delivery.
11. Liposome can be used for hydrophilic and lipophilic drug without any chemical modification.
12. Other tissue and cells of body are protected from the drug until it is released by liposome, decreasing to toxicity.
13. The size, charge and other characteristic can be altered depending on the drug and intended use of product.
14. They can't be taken up by reticular endothelial system and cause slow release of drug.
15. Improve Stability problem.
16. The lipid most commonly used in liposome is phospholipids, sphingolipids, glycolipids and sterols.
17. Their size ranges from 25 to 5000nm.[19]

Formulation of Stealth liposomes

Polymers Use in Stealth Liposome

Polymers are becoming increasingly important in the field of drug delivery. The pharmaceutical applications of polymers range from their use as binders in tablets to viscosity and flow controlling agents in liquids, suspensions and emulsions. Polymers can be used as film coatings to disguise the unpleasant taste of a drug, to enhance drug stability and to modify drug release characteristics.PEG has played a critical role in the development of stealth liposome technologies. The biocompatibility and the well-established safety profiles of PEG polymers have made them the polymer of choice for stealth liposome. However the off-patent status of these polymers makes them freely available for research in industry as well as academia. This has led to a vast number of patents covering various applications of these polymers within the drug delivery sector. Due to these issues, very limited scope remains to utilize these polymers to reformulate generic, off-patent drugs.

Another driver for novel polymer research was the increasing complexity of polymeric drug delivery systems. An ideal polymer for these applications should serve the following requirements

1. It must be biocompatible and degradable (i.e., it should degrade in vivo into smaller fragments, which can then be excreted from the body).
2. The degradation products should be nontoxic and should not create and inflammatory response.
3. Degradation should occur within a reasonable period of time as required by the application (this may vary from days to months).
4. Based on the needs of certain application, the polymer should demonstrate versatile mechanical properties.

No single polymer can match all of the above criteria. This has led companies to develop application-specific polymers and/or series of polymers that may have the structure-property variability to encompass all potential applications.

The other bio-compatible Synthetic polymers use in stealth liposome

Polyvinylpyrrolidone(PVP), polyaniline (PANI) of high stability and good processibility was prepared in acidic aqueous dispersion/solution, using the support of a water soluble polymer poly(vinyl pyrrolidone) (PVP). The high degree of dispersion and near solubility and storage stability of the stealth liposomes.

Poly (acryl amide) (PPA)

Polyacrylamide (PAM) or (PAA) is an acrylate polymer formed from acrylamide subunits that is readily cross-linked polyacrylamide is not toxic, but un-polymerized acrylamide can be present in the polymerized acrylamide. Therefore it is recommended to handle it with caution. It is highly water-absorbent, forming a soft gel used in such applications as manufacturing stealth liposomes. It is also used as a thickener and suspending agent.

Poly (2-methyl-2-oxazoline)

Poly (2-etyl-2oxazoline), poly(2-alkyl-2-oxazoline)are an important class of polymers with a wide range of potential applications such as in thermo sensitive materials, as sensors or as carrier systems for active agents in drug delivery. They all also exhibit extended blood circulation time and decreased uptake by the liver and spleen.[16]

METHOD OF PREPARATION

Stealth liposome prepared by the similar method as liposome but stealth liposome is preserved by various polymers PEG and there derivatives

Hand-Shaken Method

In order to produce liposome lipid molecules must be introduced into an aqueous environment. When dry lipoid film is hydrated the lamellae swell and grow into myelin figures. Only mechanical agitation provide by vortexing, shaking, swirling or pipetting causes myelin figures (thin lipid tubules) to break and reseal the exposed hydrophobic edges resulting in the formation of liposome. Large multilameller liposome can be made by hand-shaken method.

Sonication Method

This method is probably the most widely used method for the preparation of small unilamellar vesicles. There are two sonication techniques.

Probe Sonication

The tip of a sonicator is directly immersed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The dissipation of energy at the tip results in local overheating and therefore the vessel must be immersed into an ice/water bath, During the sonication up to one hour more than 5% of the lipids can be de-esterifies. Also, with the probe sonicator, titanium will slough off and contaminate the solution.

Bath Sonication

The liposome dispersion in a tube is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method compare to sonication the dispersion directly using the tip. Material being sonicated can be kept in a sterile container, unlike the probe units, or under an inert atmosphere. The lipid bilayer of the liposome can fuse with other bilayers (e.g.,cell membrane), thus delivering the liposome contents. By making liposome in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be delivered past the lipid bilayer.

Reverse-Phase Evaporation Method

Historically this method provided a breakthrough in liposome technology. Reverse-phase evaporation is based on the formation of inverted micelles. These inverted micellers are formed upon sonication. These inverted miceller are formed upon sonication of a mixture of a buffered aqueous phase, which contains the water soluble molecules to be encapsulated into the liposome and an organic phase in which the amphiphilic molecules are solubilized. The slow removal of the organic solvent leads to transformation of these inverted micelles into a gel-like and viscous state. At a critical point in this procedure, the gel state collapses and some of the inverted micelles disintegrate. The excess of phospholipids in the environment contributes to the formation of a complete bilayer around the remaining micelles, which results in formation of liposomes.

Freeze- Dried Rehydration Method

Freeze-dried liposomes are formed from performed liposomes. Very high encapsulation efficiencies even for macromolecules can be achieved using freeze dried hydration method. During the dehydration the lipid bilayers and the materials to be encapsulated into the liposomes are brought into close contact. Upon reswelling the chances for encapsulation of the adhered molecules are much higher. The rehydration is a very important step and in should be done very carefully. The aqueous phase should be added in very small portions with a micropipette to the dried materials. After each addition the tube should be vortexed thoroughly. As a general rule, the total volume used for rehydration must be smaller than the starting volume of the liposome dispersion.

Detergent Depletion Method

The detergent depletion method is used for preparation of a variety of liposomes and proteoliposome formulations. Detergents can be depleted from a mixed detergent-lipid miceller by various techniques which leads to the formation of very homogeneous liposome. In practice all lipids below their phase transition temperature can be used with this preparation method. Not all detergents are suited for this method and only a few detergents can be used for detergent depletion method. The most popular detergent are sodium cholate, alkyl (thio) glucoside and alkyloxy polyethylenes. Mixed micelles are prepared by adding the concentrated detergent solution to multilamellar liposomes (the final concentration of the detergent should be well above the critical micelle concentration (CMC) of the detergent).

Storage of Stealth liposomes

Once lipid particles have been formed, maintaining the physical properties of the particles can be difficult. Size distribution can change on storage due to degradation of the components. Permeability of the membrane can lead to leakage of encapsulated material.

Stability issues due to hydrolytic degradation are a general problem with lipid products. Aqueous formulations of drug products tend to be less stable since the presence of excess or bulk water leads to rapid hydrolytic degradation in lipid preparations.After the sizing process is complete, lipid suspensions should be stored at close to pH 7.

Lipids containing ester linked hydrocarbon chains are susceptible to acid and base hydrolysis. Hydrolysis rate is dramatically affected by temperature therefore lipid suspensions should be kept refrigerated during storage. Lipid suspensions should not be frozen as the freezing process could fracture or rupture the vesicles leading to a change in size distribution and loss of internal contents. The use of cryoprotactants such as dextrose, sucrose and trehalose may increase stability from hydrolysis.

Also, samples may experience oxidation upon storage. The addition of small amounts of antioxidants during processing may stabilize the suspension and limit oxidation of the product. Hydrolysis of the lipid begins to occur immediately resulting in monoacyl derivatives (Lyso lipids) which act as detergents and disrupt the membrane, thus permeabilizing the membrane. After 5-7 days at 4-8°C the internal contents will begin to leak indicating hydrolytic degradation of the lipid If membrane structure is not a critical parameter in yours experiments, vesicles may be stored for 1-2 months with minimal (<10%) hydrolytic degradation.

Storage time depends on a number of factors including temperature, pH, medium, etc. Liposomes stored in a buffer at pH 7.4 and at 4°C

did not display membrane structural changes for 5-7 day as demonstrated by retention of a trapped fluorescent marker. Beyond that time the fluorescent marker began to leak out of the liposome indicating the presence of membrane destabilizing component.

Evaluation parameters of Stealth liposome

Size Distribution of Liposomes: Liposome size distribution was determined by photon correlation spectroscopy. Which ensure the uniformity in size of stealth liposomes (Mean liposome size of 100nm).[9,10]

Zeta Potential determination: The Zeta potential was evaluated by the determination of electrophoretic mobility at the 90º angle. The measurements were performed in triplicate using the 3000 HS zeta-sizer equipment. The samples were diluted with a suitable diluents for the zeta potential determination.[7]

Lipid Quantification and Chemical Stability: Phospholipid concentration & Cholesterol concentration and purity were determined by HPLC or enzymatically by cholesterol oxidase. Purity of phospholipids as raw materials, and the extent of their hydrolysis during various steps of liposome preparation and liposome storage, were assessed by TLC and enzymatic determination of the increase in level of non-esterified fatty acids.[10]

Drug Quantification: Drug concentration was determined spectrophotometricall y. The drug quantification was confirmed by HPLC. Purity of drug and its degree of degradation during the processes of liposome preparation and liposome storage were determined by a combination of HPLC.[10]

Level of Free Drug: Two approaches were used : the selective adsorption of free drug to dowexcation exchanges either in polycarbonate tips of pipetors (range 0.1-1.0ml) or in small glass columns (ii) small gel-exclusion chromatography)[10]

Liposome stablility: Stealth liposome diluted with 10-fold in 0.9% Nacl, pH 6.5 or mouse plasma pH 7.4 and incubated at 37ºC for 30min. The drug release from liposome was separated and determined by ultracentrifugation at 150,000g 10ºC for 60 min followed graphite furnace atomic absorption spectrometry[10]

Drug release determination: In-*vitro* drug release assays of drug from both bare liposomes and stealth liposomes were performed using a dialysis method. Briefly, bare liposomal were pre-dialyzed in buffered saline using dialysis tubings to remove any free drug. The volume of 2ml bare liposomal or stealth liposomal was mixed with 2ml blank marine plasma and the mixture was then placed into the same kind of dialysis tubing. Bothe bare and stealth liposomes-loaded dialysis bobbing were placed into two beakers containing 50ml HBS, respectively. The beakers were incubated with water bath at 37ºC. At various time points including 0min, 5min, 15min, 30min, 1hr, 4hr, 6hr, 10hr, 24hr and 48 hrs, aliquots of samples were carefully withdrawn from the beakers and then replaced with equal volume of HBS, respectively.

Afterwards, concentrations of drug were measured using fluorospectrophotometry method and HPLC method, respectively. The release rate (%) was calculated using the following formula the release rate for drug = $(W_n/W)x100\%$, where W_n (µg) was the released amount drug or the release medium and w (µg) was the gross amount of drug added in the liposomes.

Polydispersity index: Polydispersity index of liposome dispersion were determined by dynamic light scattering using a Malvern. The lipid content of liposomes disper sion was accessed by phospholipid quantification according to rouscret radio activity of the liposomes dispersion was assayed in a ultima gold liquid scintillation counter.[17]

Pharmaceutical application of Stealth liposomes

Stealth liposomes in cancer therapy

Stealth liposomes are used in cancer therapy due to their property of long circulation time and targeted drug delivery. PEGylated liposomal doxorubicin was the first and is still the only stealth liposome formulation to be approved in both the USA and Europe for treatment of Kaposi's sarcoma and recurrent ovarian cancer DOXIL/Calyx is now undergoing trials for treatment of other malignancies such as multiple myeloma, breast cancer. Another stealth liposome formulation is in which cisplatin is encapsulated in the aqueous core of sterically stabilized liposomes. The stealth behavior of these compounds is evident from their apparent half-life of approximately 60-100 hours. Phase I/II clinical trials have been run to treat head and neck cancer and lung cancer.[8,13]

Stealth Liposomes for vaccines

Genetic vaccination encoding antigens from bacteria, virus, and cancer has shown promise in protecting humoral and cellular immunity. The success of liposomes-based vaccines has been demonstrated in clinical trials and further human trials are also in progress.[13]

Stealth Liposomes in gene transfection

Formulation of stealth liposomes are most suitable transfecting vectors in cationic form. Gene encapsulation in liposomal vesicles allows condensation of DNA plasmid into a highly organized structure, and protects DNA against degradation during storage and in the systemic circulation of the gene encoding a therapeutic protein. Moreover, structural organization of the gene-delivery system must bypass the cell membrane and facilitate endosomal escape, avoiding DNA degradation in the lyposomal compartment.[13]

Stealth liposomes in targeted drug delivery

Targeted stealth liposomes offer various advantages over individual drug targeted by means of polymers or antibodies. One of the most compelling advance is the dramatic increase in drug amount that con be delivered to the targeted. Furthermore, the number of ligand molecules exposed on the liposome surface can be increased, improving ligand avidity and degree of uptake immune liposomes also provide a “by stander killing effect, because the drug molecules can diffuse in to adjoining tumor cells.

Stealth Liposomes in diagnostic imaging

Stealth liposomes are use in diagnostic imaging as vesicles to carry different type of compound in bilayer or in the aqueous compartment makes them suitable for contrast procedures, including gamma scintillation, magnetic resonance imaging (MRI), computed tomography imaging (CTG), and sonography. Using stealth liposomes in diagnostic imaging lead to several advantages owing to their capability to incorporate multiple contrast moieties, to specifically deliver the agent to the target area, and to enhance the contrasting signal in order to diagnostic agent (Tc, Mn, Gd) in stealth liposomes, metals can be complexed with a soluble chelating agent that will be encapsulated in the aqueous core of vesical.[4,13]

Stealth Liposomes in inflammations

Stealth liposomes composed of lipids with long and saturated hydrocarbon chains in mixtures with cholesterol were shown to accumulate at the sites of inflammations. Such liposomes were used for diagnostic purposes. Liposomes containing corticosteroids were injected also directly into the sites of inflammations, especially into arthritic joints where they acted as a sustained release system.[13]

Stealth Liposomes in deliver drugs into the lung infections

Stealth liposomes can be used also to deliver drugs into the lung. This is most often done by inhalation of liposome aerosol. This can be used either for the treatment of various lung disorders, infections, asthma, or using lungs as a drug depot for the systemic delivery. By tailoring lipid composition a variety of release kinetics can be obtained. One of the possible applications of these aerosols is in the asthma relief in which the dosing frequency can be substantially reduced and single inhalation can last overnight.[13]

Other Pharmaceutical uses of stealth liposomes

Liposome-encapsulated hemoglobin is being developed as an oxygen therapeutic. The spatial isolation of hemoglobin by a lipid bilayer potentially minimizes the cardiovascular hemodynamic effects associated with other modified forms of hemoglobin,

forms of hemoglobin. The circulation half-life is 65 hours for this PEG-LEH formulation the results demonstrate that LEH circulates for a prolonged time after administration and that the animals tolerate at least 25% of blood exchange without any distress.

Intragastric administration, however, shows that liposomes enhance the systemic bioavailability of certain water insoluble drugs and vitamins. Several designs to stabilize liposomes in low pH, degradative enzyme, and bile salts containing environments are being studied. They include liposomes composed from many bilayers with different chemical stability and with programmable degradation kinetics, liposome encapsulated in biodegradable gels or capsules, polymer coated liposomes, and similar. Liposomes can be applied also as a thick cream, gel, or tincture. In addition to subcutaneous or intramuscular drug depot these formulations can be applied topically.

CONCLUSION

Stealth liposome can play a vital role in drug delivery, more efficiently and on a target based approach target. However the off-patent status of these polymers makes them freely available for research in industry as well as academia. This has led to a vast number of patents covering various applications of these polymers within the drug delivery sector .

Table-1- Formulations of stealth liposomal drug delivery in different stage

Company Name	Brand Name	Targeted Disease	Position
Neo pharm	LE-SN38 LEM LE-AON LEP	Various solid tumors Prostate Cancer Various Solid tumors Various solid tumors	Phase I/II Phase I/II Phase I/II Phase I/II
Celsion	ThermaDox	Prostate cancer	Phase I
Antigenics Inc.	Aroplatin ATRA-IV	Colorectal cancer , Acute promyelocytic Leukemia	Phase II
Ghead Sciences	AmBisome DaunoXome	Fungal infection, Karposi's sarcoma	Marketed
ALZA	Doxi	Ovarian Cancer	Marketed

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