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Review article

The role of liposomes in clinical nanomedicine development. What now? Now what?

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ABSTRACT

The rapid rise in interest in 'nanomedicines' in the academic world over the last twenty years and the claims of success led to calls for reflection. The main body of text of this Commentary will be on answering the question: 'where to go with nanomedicines'? Research priorities for the future will be outlined based on experience with the most successful nanomedicines family within the broad field of nanomedicine so far: liposomes. An analysis of currently clinically tested, approved and marketed liposome-drug combinations provides these insights.

1. Introduction

One highly active sector of academic research within the field of nanomedicine has been the design of nanoparticulate pharmaceuticals. In fact, research on novel and established nanoparticle systems continues to flourish in academic drug delivery laboratories throughout the world. However, there is a growing skepticism in and outside the nanomedicine research community regarding the future clinical applicability of such nanopharmaceuticals. Especially, "big pharma" pays, in general, little attention to research on nanomedicines [1]. Discussion on the number and impact of successful nanoproducts is further complicated by multi-interpretable definitions of terms such as nanotechnology, e.g., [2] and the improper reference to medicines that were developed before the term 'nanomedicine' was introduced [3].

The rapid rise in interest in 'nanomedicines' (as shown in Fig. 1) and the underlying technologies, and the claims of success led to today's calls for reflection in literature, e.g., by Lammers et al., Danhier, Park, Witzigmann et al., Leroux, and Park [4–9]. The well-attended nanodebate sessions during the CRS Annual Meetings (2018 on 'Targeted Nanodrugs', in a Pearls of Wisdom session, and 2019 on 'Nanomedicine: BIG or NANO progress?', in a Stars Collide session) are more examples that the science community is ready for self-reflection. Here we emphasize and summarize the critical role liposomes have played in the past and will play in future academic and industrial research on nanomedicines. Research paradigms for the future will be outlined based on experience with the most successful nanomedicine family so far: liposomes.

2. Learning from the past: what would nanomedicines be without liposomes?

Nanomedicine(s) is a term that was first used in a publication 20 years ago (Scopus: TITLE-ABS-KEY (nanomedicine*; October 2019). From that time on a rapid growth occurred (Fig. 1) resulting in 18,000+ publications over the period 2001 – September 2019. Worldwide, special nanomedicine research funding sources and conferences were set up to fund and stimulate research in an area that was opening up and driven by new nanotechnologies [10]. The generation of impressively complex and smart (targeted) nanometer-range delivery systems was the result, but, how did the patient benefit from this avalanche of new research activity? In his recent article where Grondzinski takes a defensive position when discussing the outcome of all these efforts, he lists three liposome formulations (Marqibo™, Onivyde™, and Vyxeos™) and Abraxane™. However, these three liposome products are just a small fraction of the liposome products that are on the market (Table 1). Also, up until and including 2001 –at the beginning of the nanomedicines era- the term liposomes had scored already 30,000+ publications (Scopus: TITLE-ABS-KEY liposome*; Scopus: October 2019).

In Table 1 a picture of a number of key characteristics of presently *marketed* liposome products in the USA and/or Europe is given. About half of the marketed liposomes were approved before the era of nanomedicines had even begun and the term nanomedicine was coined. Thus, liposomes were already an established family of nanosized products in 2001 and the newly introduced liposome products since then basically use the same technologies. Interestingly, all active

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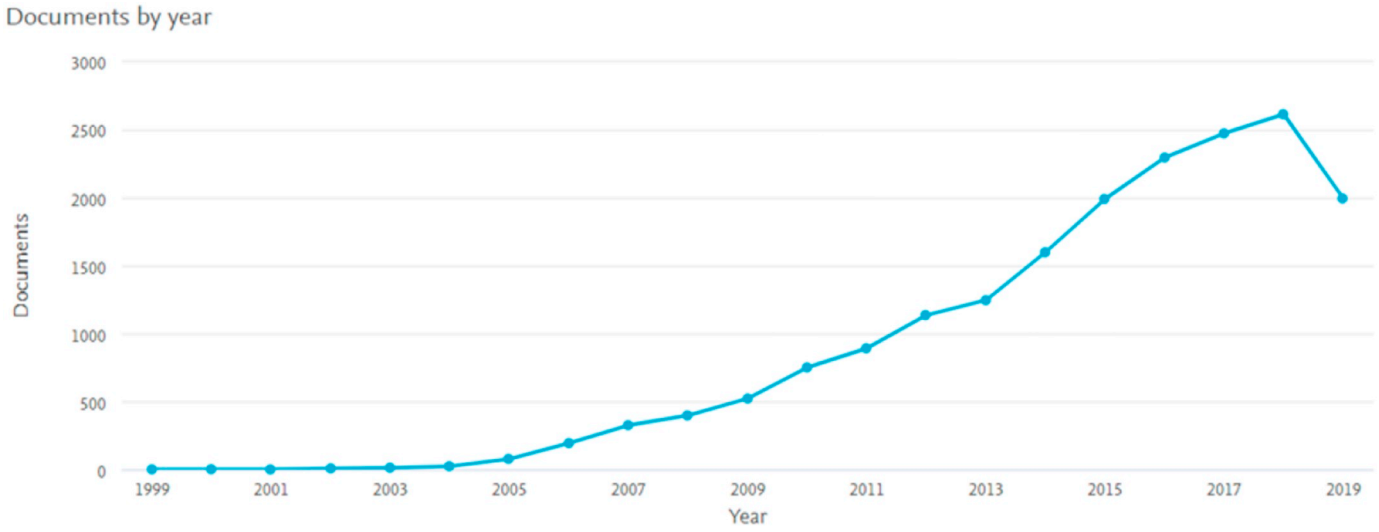

Fig. 1. Documents published per year using TITLE-ABS-KEY (nanomedicine*) as search term (Scopus, 16 October 2019).

pharmaceutical ingredients (API) used in liposome products -with the exception of the adjuvated vaccines- were already used in the clinic.

One may wonder what the legacy will be of the enormous boost in funds to support academic nanomedicine research over the last 20 years. This has not remained unnoticed in the field and a number of articles were published in e.g., the Journal of Controlled Release, e.g. [7,8] analyzing the situation and questioning the emphasis the field puts on engineering novel complex delivery systems. What are the proceeds for mankind now and to be expected in the coming years? As a 'surrogate marker' for answering that question, one may consider the number of clinical trials in progress (filter: 'active', or 'recruiting') in Phase I, II and, III by searching the clinicaltrial.gov database. Table 2 lists the outcome of this analysis using a selection of nanomedicine-related search terms. For liposome* an additional filtering term is used, i.e., 'industry sponsored'. For 'nanomedicine' that leads to only four hits, for 'nano' to eight hits, for 'nanoparticles' to 57 hits, and for polymeric micelles to 11 hits. These are low numbers when compared with the term 'liposome*', identifying 220 registered trials 110 of which were 'industry (co-)funded'. Many trials with liposomes, including the industry (co-)funded trials (liposome*, column 2), concern cancer treatment, and they use an existing liposome product either in a comparator setting (alone or with other cytostatics) or in combination therapy with the novel (not-liposomal) API. 'Big pharma' is almost absent in the list and is not running new API-liposome combinations. The nab-paclitaxel/Abraxane® column reveals a high number of trials. These studies explore the possibility to introduce the approved nab-paclitaxel/Abraxane in new oncology treatment protocols.

In the pharma industry, the decision to develop a promising liposome-based research product into an approved and marketed medicine includes weighing the commercial forecasts and technical challenges. Industry is critically assessing the costs and added value of liposomes and of any other special drug delivery technology. It may consider combining this delivery system with a new chemical entity (NCE) a too risky development approach, as it means an extra hurdle to be taken in the approval process. The exceptions to this rule are liposome adjuvants as developed by GlaxoSmithKline (GSK) and lipid-based formulations for the successful delivery of nucleotide-based bioactives (cf. Table 1).

3. Liposomes: it is eventually patient benefit that counts, but……

What are the benefits of the approved liposome products? Is the benefit for the patient mainly a better activity, a better safety profile, or a combination of both? Meta-analyses trying to find an answer to these questions are emerging, but are still rather rare. These meta-analyses mainly focus on the U.S. Food and Drug Administration (FDA)- and EMA (European Medicines Agency)-approved products. As information about the performance of products originating and approved outside those regulatory jurisdictions in the public domain is even more difficult to obtain, this text will mainly focus on experiences with liposome products approved in the U.S. and European Union. In the following sections we will discuss clinical benefits of a number of liposome products in more detail.

4. Liposomes in oncology

4.1. Anthracycline liposomes

Petersen et al. [11] found clear evidence of prolongation of survival in mice tumor models in 11 published studies comparing liposome doxorubicin formulations with free doxorubicin (i.e., the aqueous injectable solution). They also performed a meta-analysis of eight clinical studies comparing the efficacy of anthracycline-liposome formulations and conventional anthracycline formulations in cancer patients using overall survival (OS) and progression free survival (PFS) data. No increase in OS nor in PFS of cancer patients was observed. However, the liposomal formulations enhanced tolerability by changing the side effect profile. Particularly, administration of liposomal doxorubicin (Doxil) leads to a pronounced reduction of the (non-reversible) cardiac toxicity compared to administration of conventional doxorubicin. This benefit is related to the inability of circulating liposome particles to cross the continuous endothelial linings of blood vessels in the heart. However, an increased incidence of acute infusion reactions and mucocutaneous toxicity –'hand and foot syndrome'- was observed. The skin lesions usually heal when the dose of doxorubicin-liposomes is lowered or treatment is stopped. Petersen et al. [11] and Lee [12] discuss the possible reasons for this increased therapeutic index based on an improved safety profile and similar efficacy. The only tumor-type where clinically enhanced efficacy compared to standard therapy was recorded was AIDS-related Karposi sarcoma [13]. Lammers et al. [14] see the occurrence of the enhanced permeability and retention effect (EPR) in human patients as a predictor for increased efficacy: only those patients should be selected for liposomal chemotherapy who have shown liposome accumulation at the tumor site upon administration of a diagnostic dose prior to the actual treatment.

In cancer chemotherapy protocols most often combinations of cytostatics are used for treating a specific type of cancer. When analyzing the clinicaltrial.gov data bank one finds numerous clinical studies where liposomal formulations are compared, not with a placebo or free

Table 1
Liposomal formulations approved in EU and US.

Liposome	Active	Lipids	Formulation	Size range	Approval	Sales M = million	License holder/manufacturer
AmBisome	Amphotericin	HSPC: DSPG, chol 2:0.8:1 M	Freeze dried	< 100 nm (b)	EU 1990, US 11.08.1997 US	Sales 2018 (a) USA $46 M EU $229 M others $145 M Total $ 420 M	Astellas/Gilead
DaunoXome	Daunorubicin	DSPC: chol 2:1 M	Aqueous dispersion	40–80 nm	EU 1997, US 08.04.1996	~ € 6 M	Galen
DepoCyt	Cytarabine	DOPC: DPPG	Aqueous dispersion	20 μm	US 1999	Discontinued 2017 (c)	Sigma-Tau Pharmaceuticals/Pacira
DepoDur	Morphine	DOPC: DPPG	Aqueous dispersion	17–23 μm	US 18.05.2004 UK 2006	Discontinued 2014	https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=021671
Doxil/Caelyx	Doxorubicin	HSPC:chol: DSPE-PEG 56:39:5 M	Aqueous dispersion	100 nm	US 17.11.1995 EU 1996	2018 ~ $ 500 M	Janssen Products
Doxorubicin Hydrochloride Liposome	Doxorubicin	HSPC:chol: DSPE-PEG	Aqueous dispersion	100 nm	US 2017		Dr Reddy's
Exparel	Bupivacaine	DEPC: DPPG:chol: tricaprylin	Aqueous dispersion	24–31 μm	US 28.10.2011	2018 = $ 331 M (d)	Pacira
Nocita	Bupivacaine	DEPC: DPPG:chol: tricaprylin	Aqueous dispersion	25–31 μm	US 2017	2018 = $7.5 M (d)	Aratana/ Elanco (animal application
Lipodox	Doxorubicin	HSPC: chol: DSPE-PEG 65:39:5 M	Aqueous dispersion	100 nm	US 2013	2018 = $ 185 M	Sun Pharma
Marqibo	Vincristine	SPH: chol 60:40 M	Freeze dried	100 nm	US 09.08.2012	approx. $ 5 M 2018 (e)	Acrotech (f) CASI/ China
Mepact	Mifamurtide	DOPC: DOPS 3:7 M	Freeze dried	1–5 μm	EU 2009	?	Takeda IDM Pharma
Myocet	Doxorubicin	EPC: chol 55:45 M	Freeze dried	80–90 nm	EU 2000	2018 = ca. $ 10 M	Sopherion Teva
Visudyne	Verteporfin	EPG: DMPC 3:5 M	Freeze dried	18–104 nm	US 12.04.2000 EU 2000	?	Bausch + Lomp Valenta
Onivyde	Irinotecan	DSPC: chol: DSPE-PEG 3:2:0.015	Aqueous dispersion	110 nm	US 22.10.2015, EU 22.07.2016	2018 = € 109.4 mio (g)	Ipsen
Vyxeos	Daunorubicin/ cytarabine	DSPC:DSPG:chol 7:2:1	Freeze dried	107 nm	US 03.08.2017 EU 2018	2018 = $ 100 M (h)	Jazz
Arikayce	Amykacin	DPPC: chol	Aqueous dispersion	300 nm	US 28.09.2018 EMA (re) submitted (i)	2018 = $ 9.8 mio (2019 Jan.-June $ 50 M)	Insmed
Epaxal	Inactivated hepatitis A virus	DOPC:DOPE 75:25 M	Aqueous dispersion	150 nm	/EU 1999	https://www.medicines.org.uk/emc/product/4035/smpc	Janssen Cilag (j)
Shingrix	Glycoprotein E based vaccine	AS01b: MPL-L; QS-21 (n), DOPC, chol	Aqueous dispersion	50–100 nm	US 2017 EU 2018	2018: £ 784 M (k)	GSK
Mosquirix	RTS,S antigen based vaccine	AS01E: MPL-L; QS-21 (n), DOPC, chol	Aqueous dispersion	50–100 nm	positive advice EU 2015iEMA/518713/2015 EMEA/H/W/002300		GSK
Abelcet	Amphothericin	DMPC:DMPG 7:3 M	Aqueous dispersion		US 20.11.1995 EU 2009	?	Leadiant, Teva

(*continued on next page*)

Table 1 (*continued*)

Liposome	Active	Lipids	Formulation	Size range	Approval	Sales M = million	License holder/manufacturer
Onpattro	Patisiran	Dlin-MC3-DMA: PEG2000-c-DMG:DSPC	Lipid complex		US 2018	2018 = $ 12.5 M 2019 Jan-June $74.5 M	Alnylam

As compiled by A. Wendel, Sept. 2019. Strictly speaking: Abelcet and Onpattro are not vesicular lipid structures (i.e., liposomes) but lipid-based products; Exparel and Nocita are multi-vesicular liposomes with diameters in the micrometer range. For status Depocyt and Depodur: see sales-column.

Abbrevations chol: cholesterol, DEPC: 1, 2-dierucoylphosphatidylcholine, DLin-MC3-DMA ((6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraën-19-yl-4-(dimethylamino)-butanoaat), PEG2000-C-DMG (α-(3′-{[1,2-di(myristyloxy)propanoxy]carbonylamino}propyl)-ω-methoxy-polyoxyethylene), DMPC: dimyristoylphosphatidylcholine, DMPG: dimyristoylphosphatidylglycerol, DOPC: dioleylphosphatidylcholine, DOPS: dioleylphosphatidylserine, DPPC: dipalmitoylphosphatidylcholine, DPPG: dipalmitoylphosphatidylglycerol, DSPC: distearoylphosphatidylcholine, DSPE-PEG:distearoylphosphatidylcholine polyethyleneglycol, DSPG: distearoylphosphatidylglycerol, EPC: egg phosphatidylcholine, HSPC: hydrogenated soy bean phosphatidylcholine, MPL: monophosphoryl lipid A, SPH: sphingomyelin. Additional information a) Gilead Report 2018 Q4–02.04.2019, b) [11], c) On June 29, 2017, Pacira determined to discontinue all future production of DepoCyt ® (U.S. and Canada) and DepoCyte ® (European Union) due to persistent technical issues specific to the DepoCyt(e) manufacturing process. Press Release 29.06.2017, d) Pacira Reports Record Fourth Quarter and Full Year Revenues (EXPAREL net product sales expected to be in the range of $400 M to $410 M in 2019) Press Release 28.02.2019. Nocita: for veterinaty use. e) Spectrum Pharmaceuticals Reports Fourth Quarter 2018 and Full Year 2018 Financial Results and Pipeline Update (MARQIBO® (vinCRIStine sulfate LIPOSOME injection) net sales of $5.5 million) Press Release 28.02.2019, f) Spectrum Pharmaceuticals Announces the Completion of the Sale of its Marketed Portfolio (today completed the sale of its portfolio of seven FDA-approved hematology/oncology products to Acrotech Biopharma L.L.C., a step-down subsidiary of Aurobindo Pharma Limited, India) Press Release 01.03.2019, g) Ipsen delivers strong 2018 results and expects continued sales and profit growth in 2019 (Onivyde® – Sales amounted to €109.4 million. In the fourth quarter of 2018, sales were up 68.5% year-on-year and increased by 22.8% over the third quarter of 2018 driven by strong sales to ex-US partner in the fourth quarter.) Press Release 14.02.2019, h) Jazz Pharmaceuticals Annual Report 2018 (development and commercialization of exosome therapeutics to treat cancer - Vyxeos® (daunorubicin and cytarabine) liposome for injection - In 2018, Vyxeos product sales were $100.8 million, which represented 5% of our total net product sales -Vyxeos. Vyxeos is manufactured by Baxter Oncology GmbH) Report 26.02.2019, i) On 8 June 2016, Insmed Limited officially notified the Committee for Medicinal Products for Human Use (CHMP) that it wishes to withdraw its application for a marketing authorisation for Arikayce intended for the treatment of *Mycobacterium avium* complex (MAC) lung disease. j) EPAXAL - The pharmaceutical company has decided to discontinue this product. Other preparations of this medicine may still be available. This information has been left on emc for reference purposes. k) GSK Full year and fourth quarter 2018 - Shingrix sales £784 million.

cytostatic arm, but with standard cancer treatment protocols. Searching the clinicaltrial.gov data bank using the combination: 'doxorubicin AND liposomes AND combination' around 200 clinical trials were registered. A hundred of those were completed. One example is the study where Doxil together with carboplatin was compared with the standard protocol for ovarian cancer treatment, i.e., paclitaxel and carboplatin and where both the progression-free survival and therapeutic index of the 'liposome arm' was higher than for the standard treatment protocol [15].

One can conclude that anthracycline liposomes have carved out a consolidated position in cancer chemotherapy treatment routines. Remaining questions to address are: 1) how to find the proper patient group with an EPR tumor phenotype, and 2) when can the doxorubicin liposome product be introduced in existing combination chemotherapy protocols as an add-on or cytostatic replacement option? An example of such a replacement study is the assessment of efficacy and toxicity of free doxorubicin versus Doxil in a combination chemotherapy protocol for the treatment of multiple myeloma. Similar efficacy with less toxicity and supportive care were observed for the Doxil vs the free doxorubicin arm [16].

4.2. Other cytostatic containing liposomes: Vyxeos™ and Onivyde™

Vyxeos, approved by the U.S. FDA in 2017, is the first liposomal product containing two APIs, i.e. cytarabine and daunorubicin (an anthracycline) in a 5:1 M ratio. In the pivotal clinical phase III study in acute myeloid leukemia patients, the liposome product was compared with the standard cytarabine-daunorubicin (5:1) protocol. Vyxeos increased overall survival compared with the control ('free' cytarabine and daunorubicin) and showed a similar safety profile [17]. This is an example where in a head-to-head comparison the liposomal form indeed shows enhanced efficacy in the clinic. Twenty-four follow-up studies are registered under Vyxeos in clinicaltrial.gov; most of these are in the recruiting phase.

Onivyde is a liposome formulation containing irinotecan as an API. It is approved by the U.S. FDA [18] for the treatment of patients with metastatic adenocarcinoma of the pancreas [19]. In a phase III trial patients were randomized to receive Onivyde plus fluorouracil/leucovorin (Onivyde/5-FU/LV), Onivyde alone, or 5-FU/LV. The addition of Onivyde to 5-FU/LV improved overall survival compared to 5-FU/LV treatment, but treatment with Onivyde alone did not (FDA Prescribing information [18]). No direct head-to-head clinical comparison between irinotecan and Onivyde could be found. However, the EMA insert states: 'In the limited number of patients with prior exposure to non-liposomal irinotecan, no benefit of Onivyde has been demonstrated' [20]. The clinicaltrial.gov database lists 39 registered clinical trials, most of these are in the recruiting phase.

4.3. Lessons

Thus, liposome products as a single treatment modality can be beneficial in the treatment of cancer patients. In all cases an improved safety profile has been obtained. Efficacy is enhanced in a few clinical indications as with Doxil in the case of Karposi sarcoma and with Vyxeos in the case of acute myeloid leukemia. In other cases, the improved therapeutic index is due to an improved safety profile compared to the 'free' cytostatic agent. An important lesson is that most current chemotherapeutic treatments include combinations of oncolytics in standardized protocols and that many of the 220 clinical trials today (cf. Table 2) are set up to establish the position of approved liposome products in these protocols. Onivyde is an example of a product successfully developed to be approved for use in a treatment protocol involving such a combination of cytostatic agents.

Table 2
Analysis of clinical trial type for nanomedicines using the clinicaltrial.gov data base.

Filter: all restricted to stage: 'recruiting' and 'active-non recruiting'						
Type of nanomedicine	Liposome[a]	Liposome[a]	Nano	Nanoparticles[b]	Nab-paclitaxel/Abraxane	Polymeric micelle(s)[c]
Clinical stage[e]		Industry sponsored[f]				
Phase I	36	24[g]	2	32	108	1
Phase II or phase I and II	140	64[h]	5	23	164	10[d]
Phase III	44	22[i]	1	2	30	0

[a] Liposome, liposomes, liposomal.
[b] Corrected for nab-paclitaxel trials (next column).
[c] Hits for polymeric micelles; Genexol®, Nanoxel®, CriPec®.
[d] Included completed phase II, NK012.
[e] If a study is categorized as Phase I and II then the study is counted under the Phase II category.
[f] Most industry sponsored trials concern cancer treatment and they use the liposome formulation either in a comparator setting (alone of with other cytostatics) or in combination therapy with the novel (not- liposomal) API.
[g] Big pharma share: Pfizer 1, Novartis 1, Astra 1, Bayer 1. All using already approved liposomes.
[h] Big pharma share: Sanofi 1, Johnson and Johnson 1, Astra 6, MSD 3, Novartis 2, BMS 2, Hoffman La Roche 2, Boehringer 1, Takeda 1. All using already approved liposomes
[i] Big Pharma share: Astra 2, Pfizer 2, Hoffman La Roche 3.

5. Liposomes in other diseases

5.1. Antifungal and antibiotic liposomes

The list of approved liposomes with an antifungal API in the U.S. and European Union (EU) is short. Amphotericin B-liposomes were introduced in the early 1990s to replace the existing formulation, i.e., Fungizone®, which is based on solubilization of this hydrophobic antifungal by deoxycholate to enable administration via the intravenous route. However, the latter micelle-based formulation delivered this effective drug with serious side effects such as injection-related fever, rigor, and anemia resulting in a small therapeutic index. Several lipid-based formulations were introduced over the years as alternatives to Fungizone®. Of those only AmBisome® is a regularly used liposome product and therefore it is discussed here. The indications for AmBisome are: 1) presumed fungal infection in febrile, neutropenic patients, 2) Cryptococcal Meningitis in HIV infected patients, 3) patients with Aspergillus species, Candida species and/or Cryptococcus species infections refractory to amphotericin B deoxycholate, or in patients where renal impairment or unacceptable toxicity precludes the use of Fungizone®, and 4) patients with visceral leishmaniasis [21]. It concludes: 'AmBisome was well tolerated. AmBisome had a lower incidence of chills, hypertension, hypotension, tachycardia, hypoxia, hypokalemia, and various events related to decreased kidney function as compared to amphotericin B deoxycholate.' The indication where AmBisome stands out, not only in terms of lowering adverse effects, but also through enhanced efficacy, is the treatment of visceral Leishmania. There is a strong rationale to design anti-Leishmania APIs containing liposomes. They may be therapeutically more effective than the 'free' API as liposomes are known to accumulate in macrophages, and macrophages are the cells where the Leishmania parasites reside [22]. Cure rates of 95–100% were reported using amphotericin liposomes in field studies of Leishmania patients in India and Brazil [23,24] For example, Sundar et al. [25] found a similar clinical outcome (95% cure rate) for one injection of 10 mg/kg versus fifteen daily injections of 1 mg/kg of 'free' amphotericin. In spite of the high dosage per injection, the liposome formulation showed less severe adverse effects than the conventional formulation. Guidance documents and a reflection paper were issued to assist in the development process of generic versions of AmBisome, but until now, no generic versions have been approved, neither by the FDA nor the EMA. This hints to significant technical challenges when developing such AmBisome generics.

In 2018, the FDA approved the first antibiotic containing liposome formulation: the aminoglycoside amikacin in a liposome dispersion for pulmonary delivery to patients with persistent *Mycobacterium avium* infections. From the FDA label text one can read: 'This indication is approved under accelerated approval based on achieving sputum culture conversion (defined as 3 consecutive negative monthly sputum cultures) by month 6. Clinical benefit has not yet been established' [26]. The EMA has not yet approved this formulation that has received orphan drug designation [27].

5.2. Liposomal bupivacaine

In 2011, the FDA approved bupivacaine liposomes 'for single-dose infiltration in adults to produce postsurgical local analgesia and as an interscalene brachial plexus nerve block to produce postsurgical regional analgesia'. Considering its particle size, this liposome product does not qualify as nanomedicine. After local injection of these large (25 μm range), multivesicular liposomes local anesthetic effects last - for 96–120 h, depending on the injection site. The duration of the anesthetic effect of 'free' bupivacaine injections is much shorter. The FDA prescribing information (updated FDA 2018 [28]) mentions a number of successful clinical trials, but also a number of non-successful ones. The clinicaltrail.gov data bank reports 226 studies, 38 of those completed 'with results', but only a few with outcomes reported in the public domain. One of the reported outcomes of clinical trials was the reduction in opioid use compared to the alternative treatment. Taking together: mixed results were reported and the exact position of bupivacaine liposomes in therapy still has to be established [29–31].

5.3. Liposomal adjuvant AS01

Many adjuvant systems have been designed to enhance or guide the immune response in vaccines [32–35]. Aluminum salts were the preferred adjuvant system in human vaccines in the past, but nowadays liposome-based systems are introduced in new vaccines. Two vaccines with liposome adjuvant systems (AS01) containing the immunostimulants monophosphoryl lipid A (MPL) and QS-21 (a saponin) are approved (Table 1): Shingrix, as a shingles vaccine against varicella zoster virus), and Mosquirix against malaria. Interestingly, Mosquirix is approved by the EMA, but will not be used in the EU [36]. In the clinicaltrial.gov databank 66 studies on AS01 were registered and 38 were completed. Considering the need for powerful and safe adjuvants for new vaccines and the positive experience with AS01 and other liposomal adjuvants in on-going clinical trials, this particular use of liposomes in clinical practice will grow in the years to come.

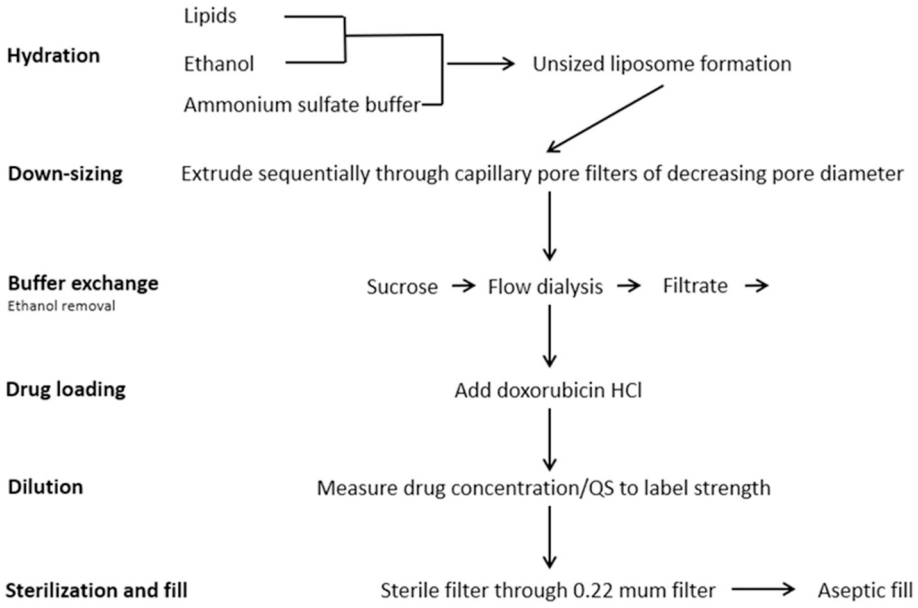

Fig. 2. Manufacturing process of Doxil/Caelyx. From [37].

6. Liposome manufacture

Production techniques for liposomes have been successfully scaled up from lab scale to industrial scale. For example, the manufacture of Doxil liposomes is a multistep process (Fig. 2) carried out under aseptic conditions and with sterile filtration as a last step before filling and finishing.

The reproducibility -batch to batch variation- of the drug-containing liposome product depends heavily on details in the manufacturing process. Early in the development stage the critical product attributes and the design space should be established [37]. A number of examples of actions by regulatory bodies underline the importance of having a full understanding and control over the manufacturing conditions and a validated quality control procedure in place. Manufacturing flaws (of undisclosed nature) in the production plant led to a supply stoppage and shortage of Doxil™ in the US/Caelyx™ in the rest of the world [38,39]. Other, single case examples of manufacturing problems were reported for Depocyte™ [40] and for AmBisome™ [41].

7. Follow-on liposome products: generics and similars

It is not a trivial task to develop generic versions of the innovator products. The patents protecting Doxil/Caelix and AmBisome have long expired and in the US only two generic versions of Doxil are on the market (Lipodox, Sun Pharma, and Doxorubicin Hydrochloride Liposomes, Dr. Reddy's). Neither of the two passed the EMA because of inequivalence of the non-liposome-bound ('free') doxorubicin pharmacokinetic profiles in bioequivalence trials [42,43]. Is the -difficult to quantitatively assess- 'free' doxorubicin pharmacokinetic profile indeed an essential parameter to assess bioequivalence of a test, generic, product with the reference product: Doxil/Caelix? This question is still debated. Hsu and Huang [44] analyzed the most critical bioequivalence parameters in population pharmacokinetic simulation studies for generic liposomal products and argued that a liposome classification system might be considered where bioequivalence testing depends on the extent of reticuloendothelial system uptake and in vivo release rates of the liposome associated bioactive.

No generic versions for other liposomal products have been approved in the US or Europe so far. A list of ongoing efforts on Doxil follow-on products to enter the US and European market was recently published [45]. From this list, one can derive that three pegylated doxorubicin liposome formulations are commercially available in China.

Besides the above-mentioned hurdles regarding bioequivalence and manufacturing complexity, generic companies may hesitate to develop such products in specific markets (EU and US), because the expected sales (number of patients) and margins may not entirely outbalance the development and production costs.

Globally many amphotericin and doxorubicin liposome products are marketed. It is not always clear whether these products are designed following the 'sameness' principle, i.e. to be a generic version of the originator product, or that they are different and thus basically innovator products. Adler-Moore et al. [46] list marketed amphotericin-liposomes outside the US and EU: "AmBbisome (AHPL), Ambihope (Abbott Healthcare Pvt. Ltd.), Ambilip, Amphotin, and Amphotin-LIP (United Biotech), Amfy and Amfitas (Intas), Amfocan (Dabur), Ampholip and Amphotret (Bharat Serum), Amfotex (Alkem/Cytomed), Amfocare (Criticare), Lambin (Sun Pharmaceutical Industries Ltd.), Lypholyn (Lyka Labs Ltd.), Mycol (VHB/ Cytocare), Phoricin (Chandra Bhagat Pharma), Phosome (Cipla), and AmBiL (Taiwan Liposome Comp.)". Benefit-cost ratios for the industrial development of such products may be higher in countries where these generics are registered than in the EU and the US, because of different regulatory regimens for approval.

Some of these products have the same chemical composition as AmBisome, but even then, differences in in vitro performance are reported. Clinical comparisons with AmBisome could not be found in the public domain. Lifecare India developed Fungisome™. These are amphotericin liposomes containing soy phosphatidylcholine/cholesterol which are different (phospho)lipids than those used in AmBisome. A special feature of Fungisome™ is that it needs to be sonicated before administration, limiting its use in the clinic [47]. Anfogen™ is an amphotericin liposome product with a similar lipid composition as AmBisome, but it is produced with a different manufacturing process. Anfogen was approved by the authorities in Argentina. Olson et al. [48] compared the physicochemical properties, antifungal and toxicity properties in vitro and in animals. Their study showed that Anfogen and Ambisome differ in their physicochemical properties. Therefore, Anfogen should not be considered to be a generic version of AmBisome. Anfogen was later withdrawn because of 'toxicity concerns' [46].

In conclusion, only two follow-on doxorubicin-liposome products made it to the U.S. market and none to the EU. For the many products

available outside these two regions, little or no clinical reference performance data is available in the English language in the public domain.

8. Targeting ligands: do they do their job?

Using ligands presented on the surface of liposomes for the targeted delivery of the bioactive at the site of action has been a longstanding ambition for liposome scientists. In animal models positive results have been reported, but, so far, no positive effects on efficacy in patients were published for such ligand-targeted liposomes. When looking in the clinicaltrial.gov data bank a number of studies pop up [49]. Three studies from a Swiss, Basel-based group: A phase I study targeting advanced tumors (2007–2010), a phase II study in breast cancer patients starting in 2018 and still recruiting now and a pharmacokinetic study in glioblastoma patients (starting 2018 and recruiting). The companies Merrimack Pharmaceuticals, SynerGene Therapeutics and Mebiopharm also submitted clinical trial proposals, but no clinical results were posted yet.

Wang et al. [49] and Belfiore [50] review the obstacles these ligand-targeted liposomes encounter upon injection. To access solid tumors, the EPR effect should occur and even after passing through the endothelium the targeted liposomes should be able to contact the target tumor cells embedded in an extracellular matrix containing a variety of other, non-tumor cells. One may wonder whether ligand-based targeting for solid tumors will ever work. Wouldn't it be more logical to try to reach endothelial cells, other target cells present in the bloodstream or in close contact with the blood through sinusoids? Another hurdle to be addressed early on is the GMP production of these complex ligand-exposing systems. Considering the above-mentioned shortages of supply of liposomes without targeting ligands, the challenges offered by attachment of a ligand on the liposome surface in a reproducible manner under GMP conditions and meeting stability requirements should not be underestimated.

9. Now what? The new paradigms for the future: the KISS principle and the IBS principle

Keep it simple, stupid (KISS). Here, the recent commentaries by Leroux [8] and Witzigmann et al. [7] enter the discussion. Research funding and top publication-bias favor the design of complex, new carrier systems, while industry favors the KISS principle.

And then the IBS principle: *It's biology, stupid* (IBS). There is an urgent need for research aiming to understand and appreciate the challenges and chances the (patho)biological environment offers. Fifty years of research and clinical experience have taught us a lot about the fate of different types of liposomes in the body. The effects of surface PEGylation, particle size, surface charge and site of injection on in vivo performance are well documented. The barriers that can't be overcome under healthy and pathological conditions will not disappear by denying their existence.

Approaches that may further the success of liposomes as a drug carrier system should focus on: 1) identifying -prior to patient treatment- tumors with an EPR characteristic by non-invasive imaging of a pre-dose of imaging probe-labelled liposomes. Only in case of a positive image, treatment with drug laden liposomes should be considered [14], 2) exploring the possibilities for enhancing the EPR effect by pharmacological and physical means [51], 3) using liposomes to reduce drug access to organs which can cause dose limiting toxicity of the bioactive (such as cardiac tissue in 'the Doxil case'), 4) exploiting targets with a natural tendency to take up liposomes upon injection such as Kupffer cells ('the AmBisome case'), hepatocytes, and splenic macrophages, 5) exploring the possibilities of physically triggering drug release (e.g. via focused ultrasound technology) from liposomes which are present in the targeted area, 6) exploiting combination treatment regimens, e.g. Vyxeos (liposomal cytarabine/daunorubicin 5/1) and Onivyde (liposomal irinotecan), or inclusion of hyperthermia, radio-, immunotherapy and other cytostatics, 7) exploring not only 'old' but also 'new' drug molecules where a carrier system is essential (incl. biopharmaceuticals, nucleotide based bioactives), 8) continuing the exploration of possibilities to use liposomes for (local) sustained release (e.g. Exparel), and 9), last but not least, developing validated animal models with better predictability for the performance in patients. For instance, by using spontaneous and metastatic tumors, also in companion animals, or patient-derived xenografts or genetically engineered mouse models.

10. And what about an industrial drive from A to Z?

The present liposomal product design technologies may need further improvements, but the 'old' issues such as poor phospholipid quality, low encapsulation efficiency, lack of sizing technologies, complex manufacturing procedures and poor stability have been successfully addressed. In addition, the present easier access to large amounts of pharmaceutical grade phospholipids (the main building blocks of liposomes) and the awareness that therapeutic liposomes are biocompatible, biodegradable and toxicologically safe facilitates the development of future liposome products.

University spin-off companies have played a pivotal role in the early development stages of the present generation of liposome products. 'Big pharma' was absent in the early stage (with the exception of GSK developing liposomal adjuvants). An illustrative example is the bumpy road for Doxil. The first steps for Doxil were taken by Liposome Technology Inc. (LTI) in Menlo Park with links to University of California San Francisco. LTI became Sequus, which was acquired by ALZA, which was then acquired by Johnson & Johnson. Will the time come that 'big pharma' or 'medium sized pharma' takes the lead in an early development stage and takes the product all the way to launch? The delivery issues of nucleotide-based bioactives might be the trigger to such a paradigm shift. These new bioactives need lipids to form complexes in the nanometer range for a successful performance (admittedly, not liposome-structured) and medium sized companies are working on those from the start (e.g. Alnylam, Moderna, BioNTech).

11. Conclusion

Over the years, the nanomedicine field has brought new drugs to the patient. Liposomes played a leading role in achieving this. One may argue that the field has somehow been disappointing in the case of other families of nano-sized drug delivery vehicles, such as emulsions, drug-polymer conjugates, dendrimers, polymeric nanoparticles, and nanobubbles [52]. While clinical trials with quite a number of the latter nanosystems are currently ongoing (Table 2), the pace of clinical drug development is generally slow and the process is associated with a high attrition rate and often hampered by limited access to financial resources.

In conclusion, the field can learn from the 'liposome experience' and should seriously take into account the challenges and opportunities which biology brings to the table of nanomedicine designers.

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