Expert Opinion





# Review on Medusa®: a polymer-based sustained release technology for protein and peptide drugs

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The polymer-based Medusa® system (Flamel Technologies) has been designed for slow release of therapeutic proteins and peptides. The Medusa II consists of a poly L-glutamate backbone grafted with hydrophobic α-tocopherol molecules, creating a colloidal suspension of nanoparticles (10 – 50 nm) in water. The sustained drug release is based on reversible drug interactions with hydrophobic nanodomains within the nanoparticles. *In vivo*, it is suggested that the therapeutic protein is displaced by endogenous proteins present in physiological fluids, leading to a slow drug release. The peak concentration is dramatically decreased and the protein release substantially extended. The Medusa technology has been applied to subcutaneous injection for several therapeutic proteins, such as IL-2 and IFN-$\alpha_{2b}$, in animal models (rats, dogs, monkeys) and clinical trials in renal cancer (IL-2) and hepatitis C (IFN-$\alpha_{2b}$) patients.

**Keywords**: animal models, clinical trials, polymer–protein complexes, slow release

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## 1. Introduction

Drug delivery represents one of the most important areas of modern medicine development. In attempts to minimise adverse events and to obtain the maximum efficacy of drugs, it is essential, especially for peptide- and protein-based drugs, to be able to provide the therapeutic agent at a well-defined concentration and with an extended mode of action. A multitude of drug delivery systems have been developed and these have been previously reviewed in detail [1]. The most common approaches involve the application of polymers, liposomes, *in situ* depot-forming systems, implantable systems and chemical or genetic conjugations.

Liposomes have been frequently used, as they allow intravenous injection [2]. In applying this route of administration, the attachment of PEG to liposomes (pegylation) has been shown to result in extended blood circulation time, reduced uptake in liver and spleen and improved tissue distribution. Pegylated liposomes have been used for various indications, such as for infectious diseases and cancers. For instance, Doxil® (Alza Corp.), the first approved pegylated liposomal product of doxorubicin, is used for the treatment of refractory ovarian cancer and Kaposi's sarcoma [3]. Other applications of liposomes include multivesicular liposomes such as DepoFoam™ (SkyePharma), where the release of the drug is controlled by a temperature-sensitive diffusion through phospholipid bilayers [4]. The cytarabine-containing product DepoCyt® (Enzon Pharmaceuticals) has been approved for the treatment of lymphomatous meningitis, and DepoDur® (SkyePharma), a morphine sulfate, is used as a postsurgical pain relief drug. Lipid microparticles, which represent a solid, lipid-based drug delivery system, have been used for local anaesthetics, antibiotics, proteins and peptides [5,6]. The condensation of small, unilamellar

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negatively charged liposomes composed of anionic phospholipids generates cochleates, which in the presence of a cation leads to larger cigar-like structures [7]. Hydrophobic and amphiphilic drugs can be packed in cochleates. The cochleate technology has been applied for oral formulations of antifungals, such as amphotericin B [8], and as adjuvants for vaccines [9] and for delivery of genes [10].

The *in situ* depot-forming systems are characterised by their liquid or semisolid composition. *In situ* precipitation systems formed with polymers have been used for long-term delivery (up to 6 months) of leuprolide acetate (Eligard® [Sanofi Aventis] and Atrigel® [QLT, Inc.]) [11,12]. An interesting approach has been to use thermally inducible gelling systems, where the injectable is a free-flowing liquid, which at body temperature forms a gel resulting in sustained drug release [13]. OncoGel™ (Protherics) contains paclitaxel in the hydrophobic domain of a gel and the drug is released over 6 weeks and used for the treatment of solid tumours [14]. Thermoplastic semisolids such as biochronomers, a new class of polyorthoesters, are semisolid at room temperature, which makes drug incorporation without organic solvents feasible [15]. Biochronomers have been studied in relation to plasmid DNA delivery for vaccines, but clinical trials are also in progress on mepivacaine to control postsurgical pain and ganisetron delivery to control nausea. Implantable systems for subcutaneous or localised treatment have been frequently used. One of the advantages of this approach is larger volumes of drugs that can be provided in implants. Two types of implants exist. Biodegradable polyanhydrile implants (Gliadel®, Guilford Pharmaceuticals) with carmustine have been approved for the treatment of recurrent gliablastoma multiforme. Drug release occurs over a period of 5 days and the complete degradation of the co-polymer takes 6 – 8 weeks [16]. Postsurgical wound infections have been successfully evaluated in a rat model using biodegradable glycerol monostearate implants containing cefazolin and vancomycin [17]. Among the non-biodegradable implants, the contraceptive Norplant® (Wyeth) achieves effective sustained release of levonorgesterol for up to 5 years. Duros® (Alza Corp.) represents another implant for long-term release that is based on a mechanism driven by osmotic pressure. Duros implants with leuprolide have demonstrated efficacy in both animal models and clinical trials with prostate cancer patients [18].

Polymeric micro- and nanoparticulate systems are the most frequently applied methods for sustained release approaches for both subcutaneous and intramuscular administration, as well as the mucosal route of administration. The polymer carriers used are biodegradable and biocompatible. The most commonly used biodegradable polymers are, poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) [19]. Several peptide formulations for sustained release have been developed into commercial products, such as leuprolide (Lupron Depot®, TAP Pharmaceutical Products Inc.), octreotide (Sandostatin LAR®, Novartis) and triptorelin (Trelstar®, Debiopharm). For example, PLGA microspheres have been used for sustained release formulations of human growth hormone (hGH) [20,21]. PLGA nanoparticles containing vitamin E (D-α tocopherol) polyethylene glycol succinate (TPGS) have been formulated for paclitaxel delivery [22]. The application of this formulation to HT-29 cancer cells resulted in a 13-fold higher cell mortality compared with the free drug [23]. Furthermore, TPGS-based nanoparticles for doxorubicin delivery were engineered by the conjugation of folate to target folate-receptor rich tumours [24]. The uptake of nanoparticles in tumour cells was significantly enhanced, and at the same time the cell viability decreased. Polyphosphoesters, and particularly engineered polylactide-co-ethylphosphate copolymers of polyphosphoesters and PLA, have been used for paclitaxel formulations that have been evaluated in preclinical studies and Phase I clinical trials on malignancies of the central nervous system [25]. The features of this co-polymer are the rapid degradation of the labile hydrophilic phosphate groups, whereas the ester bonds are slowly degraded providing slow release of the drug. Another approach has been to apply polyanhydrides, which are biodegradable co-polymers where two fatty acids form the hydrophobic backbone. Drug release can be altered to occur over weeks to months by variation of the type of fatty acid. Polyanhydrides have been applied for local anaestethics, anticancer drugs and anti-inflammatory agents [26].

The OctoPlus system, based on crosslinked dextran forming microspheres in an aqueous environment, has been applied for protein delivery [27]. The molecular weight and concentration of dextran and PEG, as well as the degree of substitution of crosslinking groups and their concentration, influence the particle size and crosslink density of microspheres [28]. Modification of these parameters allows adjustment of the release profile of proteins from days to months [29]. Sustained IL-2 release with therapeutic activity has been demonstrated in a mouse tumour model applying dextran microspheres [30]. Furthermore, the technology has been applied to achieve the sustained release of hGH. Studies in rat models have demonstrated the excellent biocompatibility of crosslinked microspheres [31]. Microspheres carrying hGH have also displayed sustained drug release for 2 weeks in one clinical study [29]. Using a polymer based on Poly butylene terephthalate PEG PBT [32], a slow release formulation of IFN-$\alpha_{2b}$ has been developed and evaluated in a Phase I study [201]. Octoplus has announced the start of a Phase IIa trial in chronic hepatitis C patients with Locteron™, the sustained release formulation of IFN-$\alpha_{2b}$ [201].

Attempts have been made to develop sustained release products for protein delivery. For example, an hGH product, ProLease® hGH (the sustained release formulation of Genentech's hGH based on Alkermes' ProLease® injectable drug delivery system) was commercialised at the turn of the century [20,21]. However, ProLease hGH was abandoned apparently due to the high costs of production. Two other systems for sustained release of hGH have recently been developed. A once-weekly injection formulation of solid microparticles using sodium hyaluronate has been shown to cause elevated serum IGF-1 levels in cynomolgus monkeys for

6 days, indicating the release of bioactive hGH [33]. By changing the ratio of hGH and hyaluronate, a slower release of hgH was observed. In another approach, crystals of recombinant hGH were coated with a monomolecular layer of positively charged poly(arginine), which resulted in sustained release over 7 days in rats and monkeys [33]. Other problems related to the sustained release of protein therapeutics concern the process requirements, which are not compatible with proteins, and issues affecting protein stability and aggregation. For these reasons, there is a clear need for the development of efficient delivery methods for protein-based drugs.

In this review, the Medusa® II technology (Flamel Technologies) based on polyamino acid polymers is described in more detail. First, an overview of the Medusa II technology itself is given, and the chemico-physical parameters are presented. Moreover, animal toxicology studies are discussed to demonstrate the safety of the technology. Applications of the Medusa II technology for therapeutically relevant proteins such as IL-2 and IFN-$\alpha_{2b}$ in animal models for pharmacokinetic and pharmacodynamic studies are presented. Finally, the administration of slow release formulations to man in clinical trials of renal cancer (IL-2) and hepatitis C (IFN-$\alpha_{2b}$) patients is described.

## 2. The Medusa technology

Medusa I, which is based on an amphiphilic block polymer consisting of two amino acids (L-leucine and L-glutamate) is not described in this review. The second-generation polymer, Medusa II, is based on a polymer consisting of the backbone of poly-L-glutamate with hydrophobic molecules of α-tocopherol (vitamin E) randomly grafted to some of the glutamate units through a hydrolysable ester bond (**Figure 1A**) [101,102]. The polymer is made by grafting α-tocopherol from readily available poly-L-glutamic acid by a conventional esterification method in solution phase. After purification, the polymer is isolated as a ready-to-formulate solution in water. In this medium, the lateral hydrophobic vitamin E groups self-assemble into hydrophobic nanodomains, which results in the aggregation of the hydrophilic glutamate chains, forming a colloidal suspension of nanoparticles. Size exclusion chromatography, as well as static and dynamic light scattering experiments have showed that Medusa nanoparticles in water (1g/l; 0.25 M NaCl) are aggregates of 10 – 15 polymer chains, with an average molecular mass of 400 – 500 kDa and a mean hydrodynamic diameter ranging 20 – 50 nm. The critical association concentration is < 0.1 g/l and not measurable with classical pyrene fluorescence techniques. Time resolved fluorescence quenching experiments revealed that each nanoparticle contains ~ 10 hydrophobic nanodomains, each composed of 10 – 15 α-tocopherol moieties. Being anionic in nature, the surface charge is negative.

The toxicology data in support of subcutaneous dosing in humans include single and repeated dose-toxicity studies, genotoxicity, developmental toxicity, acute local tolerance studies and immunotoxicity studies in laboratory animals. Rats given a single subcutaneous or intravenous dose of the Medusa II polymer showed no reaction at the injection site or significant signs of systemic toxicity during the 14-day observation period. No gross pathological changes were noted at the injection site, in regional lymph nodes, or in major organs/tissues at termination. Repeated-dose subcutaneous toxicity studies were conducted in rats and monkeys with doses as high as 50 mg/kg. Systemic changes were restricted to the inflammatory reaction at the injection site and/or to the degradation/elimination process by phagocytic cells. At the injection site, histopathological examinations revealed a consistent pattern of low-grade inflammation (primarily mononuclear cells, lymphocytes and/or foamy macrophages) in the cutis and/or subcutis of the injection site in treated animals and at a lower grade in control animals. These local changes were generally minimal to mild and were found in both animal groups.

The Medusa II polymer was not mutagenic or clastogenic in the Ames test, in the *in vitro* chromosomal aberration assay or in the *in vivo* (rat) micronucleus assay. Taken together, these results demonstrate the absence of genotoxic risk. The effect of the polymer on embryo-fetal development has been evaluated by subcutaneous treatment of pregnant female rats and rabbits during the period of organogenesis at a dose as high as 50 mg/kg. The polymer induced no fetal malformations, and no differences in fetal weight or changes in pre- or postimplantation loss were observed at any dose level.

The absence of undesirable immune responses against the polymer is substantiated by the administration of the polymer in repeated subcutaneous rat and monkey studies, showing an absence of specific antipolymer antibodies. In addition, clinical pathology and histopathological evaluation of the immune system and the lymphocyte subset did not present any findings indicative of adverse effects on the immune system. In conclusion, the results of the repeated-dose toxicity studies in rats and monkeys and the local tolerance studies in domestic pigs demonstrated that the Medusa II polymer has a very low order of systemic toxicity, and good local tolerance. The results of these toxicological evaluations support the use of protein formulated with the Medusa technology in humans.

Slow release formulations (lasting for 4 – 7 days) are obtained by the simple mixing of aqueous solutions of the protein or peptide with an aqueous solution of the polymer, without the use of organic solvent. Due to the hydrophobicity of the nanodomains embedded within the nanoparticles, self-association with protein will lead to a controlled release system, which has provided the excellent properties to the Medusa technology. For example, self-association of IFN-$\alpha_{2b}$ protein to Medusa II polymer, up to 100 parts of protein for 100 parts of polymer, has been demonstrated by capillary electrophoresis in 25 mM phosphate buffer. This maximum loading decreases by 25% at higher ionic strength (75 mM phosphate buffer), then remains almost constant up to 125 mM. For these loading values, the amount of free protein remaining

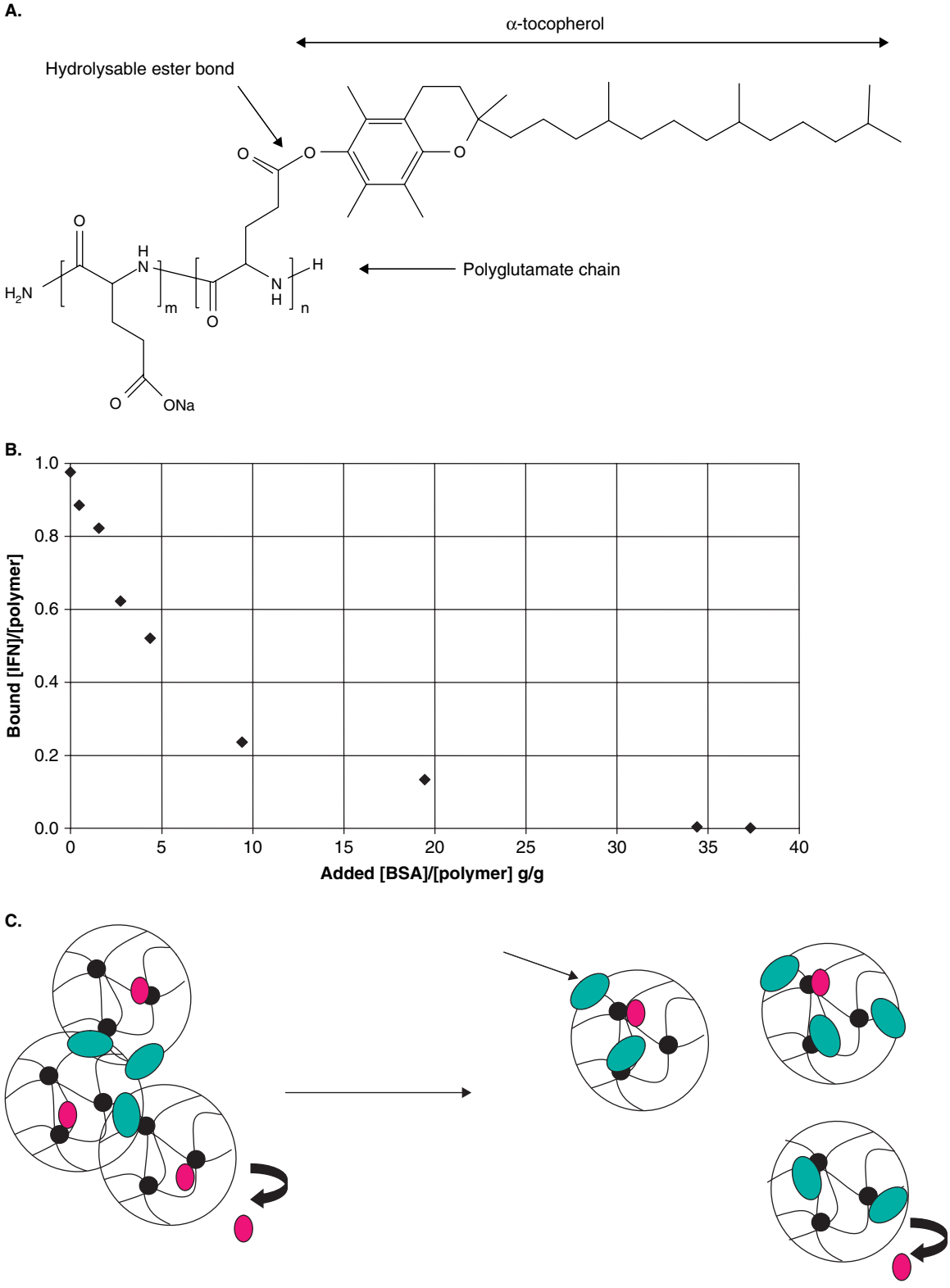



**Figure 1. A. The second-generation Medusa II technology is based on the amphiphilic polymer with a poly-L-Glu backbone on which vitamin E molecules are randomly grafted. B.** *In vitro* addition of increasing concentrations of bovine serum albumin results in release of IFN-α from the polymer–protein complex. **C.** Hypothetical release of protein from polymer–protein complexes *in vivo*. Subcutaneous delivery of polymer–protein complexes is postulated to lead to replacement of therapeutic protein (red) by endogenous protein (green).
BSA: Bovine serum albumin.

**Table 1. *In vitro* bioactivity of IL-2 XL and IFN-$\alpha_{2b}$ XL.**

| | Activity (MIU/ml) (95% CI) | % of expected activity[§] |
|---|---|---|
| IL-2 XL | 27.2 (26.0 – 28.4)[*] | 104 |
| IFN-$\alpha_{2b}$ XL | 62.5 (44.8 – 87.3)[‡] | 97 |

[*]*In vitro* measurement in the CTLL-2 cell line; proleukin was used as a reference.
[‡]*In vitro* measurement according to the European Pharmacopeia: antiviral test in the 2D9 cell line with Encephalomyocarditis virus; the International Standard for IFN-$\alpha_{2b}$ (NIBSC 95/566) was used as a reference.
[§]Expected activity calculated from the specific activity of the corresponding raw material and the concentration of the protein within the formulation.
MIU: Million international units; XL: Extended release.

in solution was not measurable, demonstrating the high affinity of the protein for the polymer. It has been demonstrated *in vitro* that the therapeutic protein is fully displaced by bovine serum albumin. For example, the IFN-$\alpha_{2b}$ in an IFN-$\alpha_{2b}$ extended release formulation is fully displaced by an excess amount of bovine serum albumin (**Figure 1B**) and the displaced protein has the same retention time as the native protein, as measured by HPLC. This indicates that the physical binding of the protein to the nanoparticles is fully reversible. *In vivo*, the therapeutic protein is believed to be displaced by endogenous proteins present in the body fluids, leading to a slow and extended release of the drug. This mechanism has not yet been clarified *in vivo* and, therefore, remains hypothetical, as illustrated in **Figure 1C**. The degradation of Medusa polymers has been extensively investigated by histological examinations in domestic pigs. Subcutaneous injection of the polymer results in depot formation. Due to its peptidic nature, Medusa polymers are degraded by proteolytic enzymes. *In vivo*, phagocytic cells such as macrophages have been found to be involved in the degradation process. The histological findings at the injection site have confirmed the biodegradation of Medusa polymers.

## 3. Applications of the Medusa technology

The Medusa II technology has been used for two separate therapeutic proteins, which is the focus of this section. The Medusa II technology has been evaluated for the extended release of IL-2 (IL-2 XL) in comparison with the existing commercial IL-2 drug Proleukin® (Novartis). Likewise, the Medusa II technology has been applied for a slow release formulation for IFN-$\alpha_{2b}$ (IFN-$\alpha_{2b}$ XL). As with many other interleukins, IL-2 plays a pivotal role in cell-mediated immune responses. The administration of IL-2 results in various cellular responses, including the proliferation of activated T lymphocytes, the activation of B cells, the increased cytotoxicity of natural killer cells and the generation of activated killer cells. For this reason, IL-2 has been used for the treatment of various cancers and viral infections. Typically, IL-2 has been applied for the treatment of metastatic renal carcinoma and melanoma, as well as for HIV infections. Leukocytes are responsible for the secretion of IFN-α, which activates macrophages and natural lymphocytes, leading to enhancement in major histocompatibility complex glycoprotein classes I and II. As IFN-α displays strong antiviral and antiproliferative activity, it has been commonly used for treatment of viral diseases such as hepatitis C (HCV) infections and various cancers. In the treatment of chronic HCV infections, the toxicity of IFN-α has proven to be a limiting factor. The reduction of peak IFN-α concentrations and obtaining sustained levels of IFN-α may, therefore, improve efficacy and tolerability in HCV patients.

## 4. Formulation of IL-2 and IFN-α with Medusa II

In the case of IL-2, the formulation process is performed in water at room temperature in protein-friendly conditions, without using organic solvents, temperature increase or surfactants. A colloidal suspension of the Medusa polymer concentrated at 30 mg/ml is filter sterilised (pore size 0.2 µm), then mixed with a filter-sterilised water solution of IL-2 (MW = 15.5 kDa; pI = 6.8). The protein self-associates to the polymer and no free protein remains in solution. After 12 h, under gentle agitation, the formulation containing 2.5 mg/ml of IL-2 and 25 mg/ml of Medusa polymer in isotonic and neutral pH conditions is stored in vials at 5°C.

IFN-$\alpha_{2b}$ XL is formulated similarly to IL-2 XL: a sterile filtered water solution of the protein concentrated at 2.5 mg/ml is mixed with a filter-sterilised colloidal Medusa II suspension at pH 6.8, concentrated at 25 mg/ml to form an isotonic IFN-$\alpha_{2b}$ XL suspension at pH 6.5 containing 0.3 mg/ml of IFN-$\alpha_{2b}$ (MW = 19.2 kDa; pI = 5.8) and 23 mg/ml of polymer. For this protein/polymer ratio, which is well below the maximum loading capacity of the polymer, the protein is totally associated to the polymer and no free protein remains in solution.

## 5. *In vitro* bioactivity

Measurements of the *in vitro* bioactivity of IL-2 and IFN-$\alpha_{2b}$ within IL-2 XL and IFN-$\alpha_{2b}$ XL, respectively, have shown that the proteins are fully active in Medusa II formulations (**Table 1**). This clearly demonstrates that the formulation process does not degrade/denature the protein and it maintains its biological activity.

## 6. Pharmacokinetic studies on Medusa II formulations

The Medusa II-based IL-2 XL has been compared with the registered IL-2 drug Proleukin and initially was tested in several animal models. In Sprague-Dawley rats, the peak concentration of IL-2 was reduced 100-fold. The corresponding values in Beagle dogs and Cynomolgus monkeys represented a

**Table 2. A summary of pharmacokinetics results obtained after a single subcutaneous injection of IFN-$\alpha_{2b}$ XL and IFN-$\alpha_{2b}$ immediate release at a dose of 60 µg/kg IFN-$\alpha_{2b}$.**

| Species | Test item | n | $C_{max}$ ± SD (ng/ml) | Median $T_{max}$ (range) (h) | $AUC_{all}$ ± SD (ng × day/ml) | T50%$_{AUC}$ ± SD (h) | RBA (%) |
|---|---|---|---|---|---|---|---|
| Rat | IFN IR | 12 | 27.9 | 0.5 | 2.3 | 1.4 | 100 |
| | IFN-$\alpha_{2b}$ XL | 4 | 1.5 | 11.0 | 1.3 | 16.8 | 58 |
| Dog | IFN IR | 6 | 28.0 ± 4.0 | 2.0 (1.0 – 3.0) | 7.7 ± 0.5 | 4.3 ± 0.7 | 100 |
| | IFN-$\alpha_{2b}$ XL | 8 | 1.4 ± 0.6 | 24.0 (18.0 – 48.0) | 2.9 ± 0.8 | 39.0 ± 10.0 | 43 |
| Monkey | IFN IR | 3 | 59.6 ± 5.6 | 2.0 (2.0 – 2.0) | 10.4 ± 0.3 | 3.1 ± 0.3 | 100 |
| | IFN-$\alpha_{2b}$ XL | 3 | 3.4 ± 0.8 | 24.0 (24.0 – 36.0) | 5.5 ± 0.7 | 30 ± 3.5 | 53 |

The seric level of IFN-$\alpha_{2b}$ was measured using a sandwich IFN-α ELISA (IFN-α ELISA, ref. IM 3193, Immunotech, Beckman Coulter).
$AUC_{all}$ calculated by the trapezoidal rule.
For rats, n corresponds to the number of animals sampled at each time point, with different subgroups of rats being used to evaluate the entire pharmacokinetic profile. Due to this method, standard deviations are not reported for rats.
$AUC_{all}$: Area under the concentration–time curve; IR: Immediate release; XL: Extended release; RBA: Relative bioavailability; SD: Standard deviation; T50%$_{AUC}$: Time necessary to reach 50% of the $AUC_{all}$; XL: Extended release.

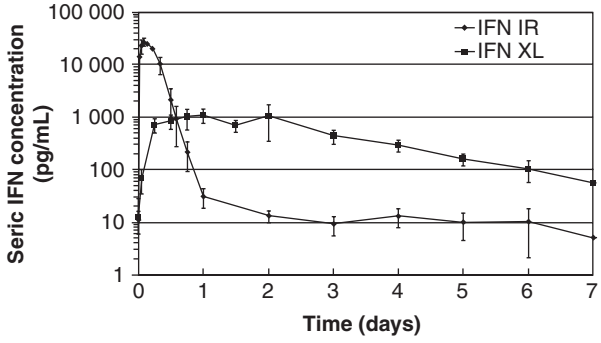



**Figure 2. The pharmacokinetic profile in Beagle dogs after a single subcutaneous administration of IFN-$\alpha_{2b}$ IR and the Medusa II formulation IFN-$\alpha_{2b}$ XL at a dose of 60 µg/kg of IFN-$\alpha_{2b}$.**
IR: Immediate release; XL: Extended release.

13- and 2-fold decrease, respectively. The release time was also extended to 3 days in rats (2 days for Proleukin), 5 days in dogs (3 days for Proleukin) and 3 – 4 days in monkeys (2 – 3 days for Proleukin). The relative bioavailability was 10% in rats, 40% in dogs and 100 – 150% in monkeys.

The Medusa II-based IFN-$\alpha_{2b}$ XL pharmacokinetics profiles were similarly investigated in three animal models (Sprague-Dawley rats, Cynomolgus monkeys and Beagle dogs). In the three species, a delayed pharmacokinetic serum profile inherent to a sustained delivery of IFN-$\alpha_{2b}$ from IFN-$\alpha_{2b}$ XL was clearly demonstrated in comparison with an IFN immediate release (IFN IR) formulation (free drug; **Table 2**). This resulted in a $C_{max}$ decrease (~ 20-fold in the three species) and an extended IFN-$\alpha_{2b}$ release time. The release was over 2 days versus 0.5 day for IFN IR in rats, over 4 days versus 1 day in monkeys, and over 6 days versus 1 day in dogs (**Figure 2**). This extended release of IFN-$\alpha_{2b}$ was concomitant with a loss of relative bioavailability of ~ 50% in the three species.

## 7. Clinical trials

A Phase I/II clinical trial has been conducted on eight renal carcinoma patients, who received a single subcutaneous injection of $10.6 \times 10^6$ IU/m$^2$ of IL-2 XL or Proleukin in a cross-over design [34]. In comparison with Proleukin, the $C_{max}$ value was 2-fold lower for IL-2. Additionally, the $T_{max}$ value was substantially extended to 48 h for IL-2 XL, whereas it was 2 – 4 h for Proleukin. Moreover, IL-2 XL administration still revealed the presence of IL-2 in the serum after 7 days, which was not the case for Proleukin (**Figure 3A**). Studies of biomarkers for IL-2 activity have demonstrated that the single administration of IL-2 XL is sufficient to significantly enhance and sustain the pharmacodynamic responses of neopterin and sCD25 (**Figure 3B**). Furthermore, IL-2 XL has been shown to induce strong cellular immune responses and an increase in the production and activation of CD4 and CD8 T cells was observed (data not shown).

A Phase I/II dose-escalating study has been conducted in 53 HCV-positive patients [35]. Single subcutaneous injections of IFN-$\alpha_{2b}$ XL were administered at 9 million international units (MIU), 18 MIU and 27 MIU (12 – 14 patients/dose), respectively. As a comparison, 14 patients received 3 subcutaneous injections of 3 MIU of Viraferon® (Schering-Plough). In the study, the safety, pharmacokinetics, biological activity and impact on the viral load were assessed. The $C_{max}$ was reduced ninefold for IFN-$\alpha_{2b}$ XL compared with Viraferon. However, in contrast to Viraferon, IFN-α was still detected in the serum 7 days after injection of IFN-$\alpha_{2b}$ XL. Keeping in mind that after intravenous injection the elimination half-life of IFN-$\alpha_{2b}$ is 1.7 – 1.9 h, a deconvolution of the extended plasma profile of IFN-$\alpha_{2b}$

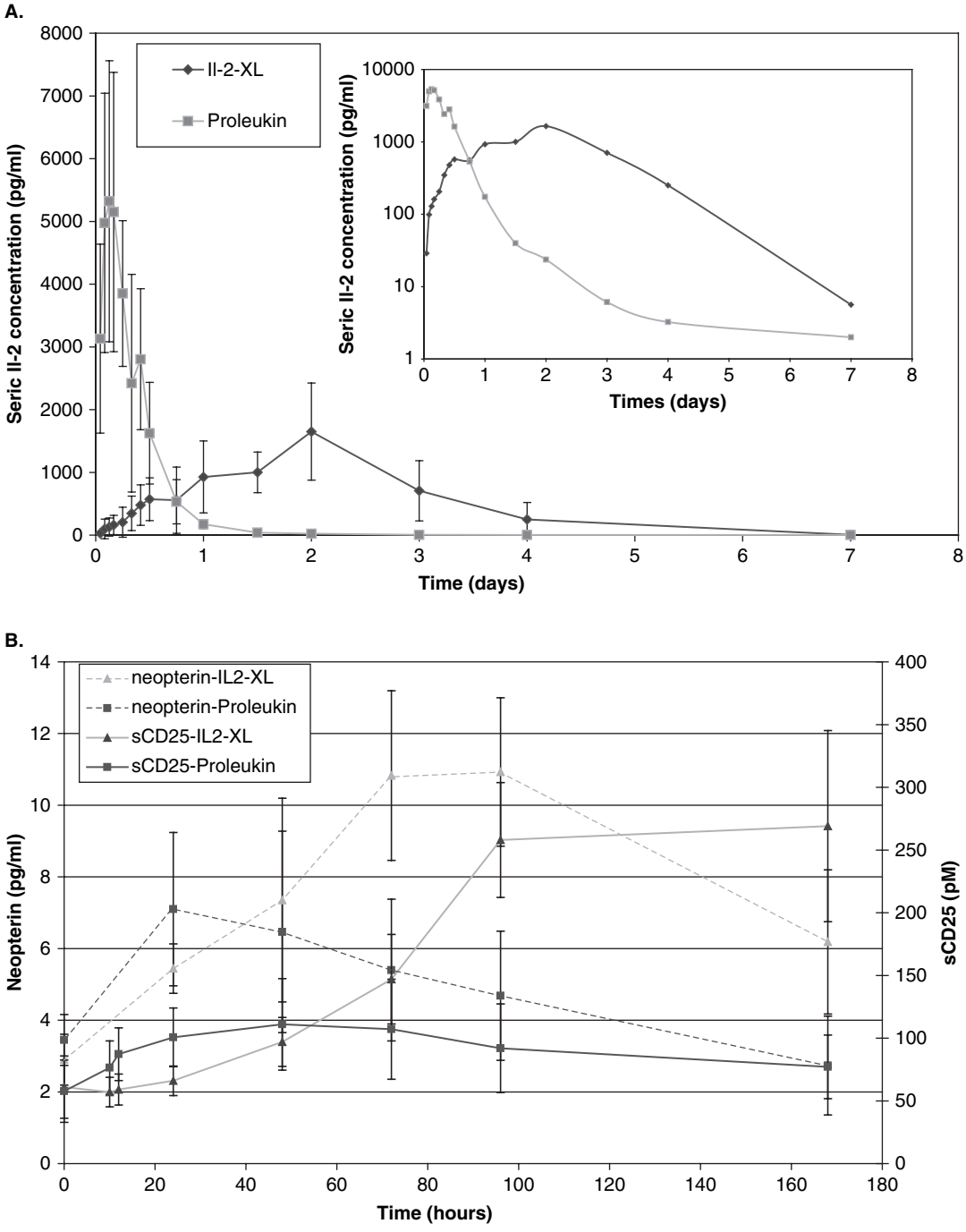



**Figure 3. A. The pharmacokinetic profile of IL-2 after a single subcutaneous administration of IL-2 XL and Proleukin in renal carcinoma patients on a linear scale (insert in logarithmic scale). B.** The biomarkers' response (neopterin and sCD25) after a single subcutaneous administration of IL-2 XL and Proleukin in renal carcinoma patients.

XL: Extended release.

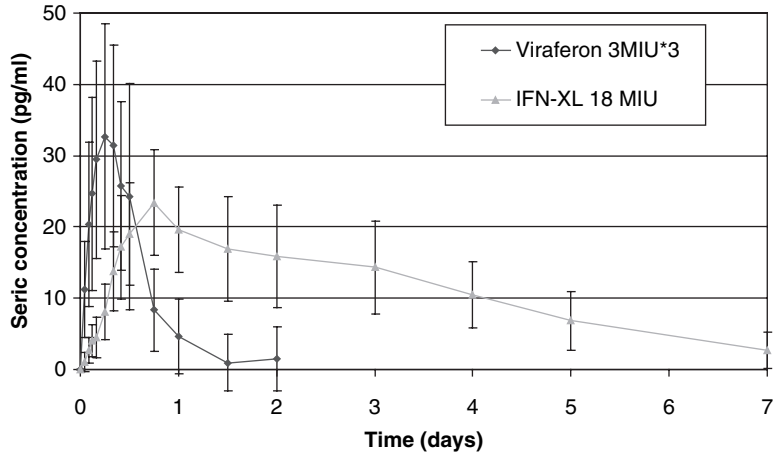
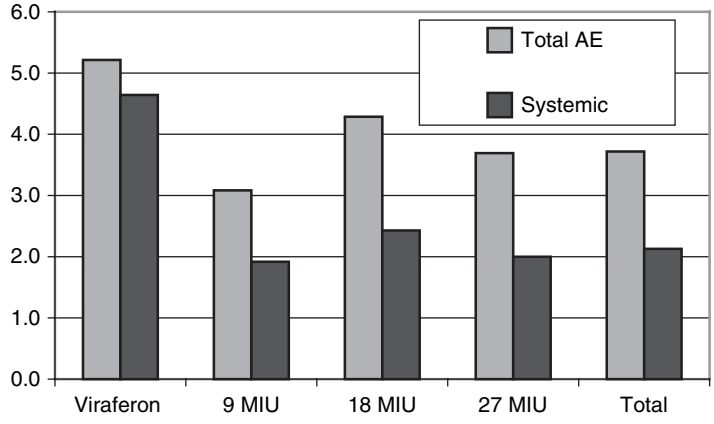



**Figure 4. The pharmacokinetic profile in humans after a single subcutaneous injection of IFN-$\alpha_{2b}$ XL and three injections of Viraferon (for the Viraferon group, only the pharmacokinetic profile after a single administration on day 1 is presented).**
XL: Extended release; MIU: Million international units.




List of adverse events

A. General safety:
Headache, stiffness, pyrexia, lumbar pain, flu-like, chills, myalgia, nausea, asthenia, irritability, sweating

B. Local tolerance:
Erythema, swelling

C. Hematology:
Neutropenia, lymphopenia

**Figure 5. Adverse events in patients after a single subcutaneous injection of IFN-$\alpha_{2b}$ XL and three injections of Viraferon, respectively.**
AE: Adverse events; XL: Extended release.

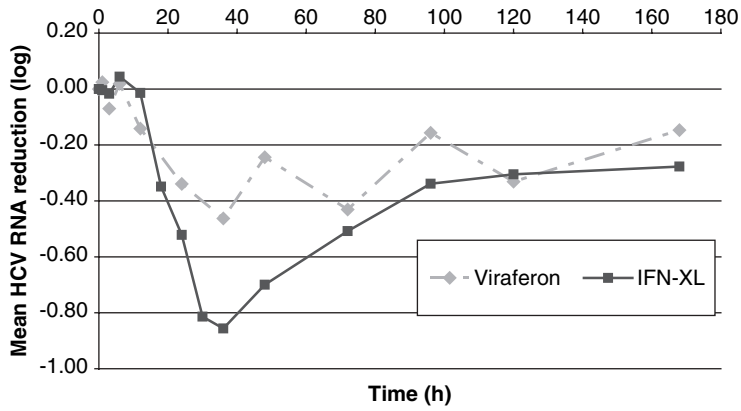



**Figure 6. Viral response in HCV (genotype 1 non-responder) patients after a single subcutaneous injection of IFN-$\alpha_{2b}$ XL and three injections of Viraferon.**

HCV: Hepatitis C virus; XL: Extended release.

XL indicates that the Medusa II technology prolongs the bioabsorption time to > 7 days (with a terminal half-absorption time of 38 – 57 h), compared with 1 day for Viraferon (**Figure 4**). The number of adverse events was significantly lower for IFN-α XL (3.7 per patient) compared with Viraferon (5.2 per patient; **Figure 5**). The reduction in the mean viral load was also higher after administration of IFN-$\alpha_{2b}$ XL (1.27 log) than for Viraferon (0.97 log), including patients with the non-responding genotype I. Furthermore, injection of IFN-α XL induced a robust antiviral response (0.98 log versus 0.57 log) in patients previously resistant to IFN-α therapy (**Figure 6**).

## 8. Conclusions

The Medusa technology described in this review has been applied to several therapeutically relevant drugs. For example, a single administration of the IL-2 XL formulation demonstrated reduced $C_{max}$ values and extended release in rats, dogs and primates, compared with Proleukin. The local tolerance and toxicity profiles were also shown to be favourable. Moreover, the IL-2 release was substantially extended in renal carcinoma patients and the single subcutaneous injection enhanced the pharmacodynamics of biomarkers and induced the production of T cells. Animal studies suggested the superiority of IFN-$\alpha_{2b}$ delivery by the Medusa-based IFN-$\alpha_{2b}$ XL formulation over the immediate release formulation IFN-$\alpha_{2b}$-IR. A clinical trial in HCV patients also confirmed extended release and lower viral load after IFN-$\alpha_{2b}$ XL administration, compared with Viraferon. Most importantly, positive responses were observed in previously non-responding patients with genotype I.

## 9. Expert opinion

Drug delivery today still represents one of the key issues for the development of more efficient and specific new medicines. This issue has been addressed by development of various delivery systems based on pegylated liposomes (doxorubicin), multivesicular liposomes (DepoFoam), and anionic phospholipids (antifungals). Moreover, *in situ* depot forming systems (Eligard and Atrigel) have been used for long-term drug delivery. Many biodegradable polymers, typically PLA and PLGA have provided the basis for commercial formulations of sustained-release peptides (Lupron Depot, Sandostatin LAR and Trelstar).

Concerning protein based drugs, the OctoPlus system, based on crosslinked dextran forming microspheres and PEG PBT, has been evaluated for IL-2, hGH and IFN-$\alpha_{2b}$. In addition, the ProLease technology has been applied to hGH, which reached the market, but was later withdrawn due to high production costs and lack of efficacy. Other critical issues are the initial high peak concentration after drug administration and the relatively short time of therapeutic action. The Medusa technology has been shown to provide both delivery of lower drug concentrations and substantially extended drug release. Previously, the application of the Medusa I pBLE amphiphilic block polymer (L-leucine and L-glutamate) [36], showed for insulin a favourable profile in Phase I and IIa pharmacokinetic and pharmacodynamic studies in healthy or Type 1 diabetes mellitus volunteers in comparison to the registered diabetes drug Lantus® (Sanofi Aventis). Most importantly, the application of the Medusa technology has demonstrated a high safety profile in humans. Its manufacture is also based on a simple and fast polymerisation process, which is also cost effective.

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