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A Nanoprimer To Improve the Systemic Delivery of siRNA and mRNA

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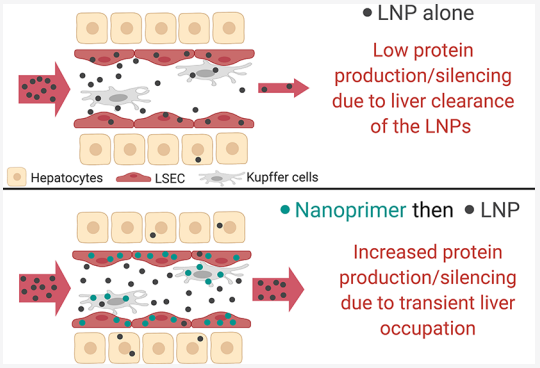
ABSTRACT: Despite tremendous interest in gene therapies, the systemic delivery of nucleic acids still faces substantial challenges. To successfully administer nucleic acids, one approach is to encapsulate them in lipid nanoparticles (LNPs). However, LNPs administered intravenously substantially accumulate in the liver where they are taken up by the reticuloendothelial system (RES). Here, we administer prior to the LNPs a liposome designed to transiently occupy liver cells, the Nanoprimer. This study demonstrates that the pretreatment of mice with the Nanoprimer decreases the LNPs' uptake by the RES. By accumulating rapidly in the liver cells, the Nanoprimer improves the bioavailability of the LNPs encapsulating human erythropoietin (hEPO) mRNA or factor VII (FVII) siRNA, leading respectively to more hEPO production (by 32%) or FVII silencing (by 49%). The use of the Nanoprimer offers a new strategy to improve the systemic delivery of RNA-based therapeutics.



KEYWORDS: *mRNA, siRNA, lipid nanoparticles, nanocarrier, nucleic acid therapy, Nanoprimer, Kupffer cells*

Myriad diseases stem from a lack, an excess, or an abnormal production of a specific protein, itself triggered by the underexpression, overexpression, or mis-expression of genes. According to the World Health Organization, monogenic diseases affect millions of people worldwide.[1] A promising approach to treat a monogenic disease is to deliver nucleic acid-based therapeutics. Instead of targeting the gene products, i.e., proteins, these drugs modulate gene expression, leading to therapeutic effects through the control of protein levels. The clinical translation of this approach has been recently achieved by the approval of the first liposomal small interfering RNA (siRNA) therapy, Patisiran (Onpattro).[2]

Despite this clinical success, nucleic acid-based treatments still face challenges, especially regarding their delivery. Rapid clearance of nucleic acids due to nuclease activity and renal filtration induces poor bioavailability following systemic administration. Moreover, their physicochemical characteristics, especially their charge, impede diffusion across cell membranes. To overcome their degradation *in vivo* and facilitate their uptake by target cells, they can be encapsulated in polymeric or lipid nanoparticles as in the case of Patisiran.[3−7] When designing a carrier, critical factors have to be taken into account,[8] such as the nucleic acids' encapsulation efficiency, the stability of the carrier, its rate of clearance by the reticuloendothelial system (RES), and the intracellular delivery of the nucleic acid. Compositions of LNPs have evolved to address these limitations: their stability is improved by using cholesterol and the rapid clearance is partly avoided by using PEG. DOPE is used as a "helper" lipid, since it enables higher transfection by promoting fusion with the endosomal membrane once inside the cells, leading to improved endosomal escape.[9] Finally, by complexing nucleic acids, cationic lipids help their condensation and encapsulation. However, since positively charged LNPs are toxic (hemolytic), ionizable lipids, such as cKK-E12, that are positively charged at low pH (during the encapsulation and inside the endosome) and neutral at physiological pH (during administration and circulation), have been developed to deliver nucleic acids inside the cells' cytoplasm.[3,10,11]

Even though these modifications in the design of LNPs have improved the efficiency of nucleic acids' delivery, low bioavailability of LNPs remains one of the main limitations for an effective systemic delivery of gene therapies. The RES, in particular Kupffer cells (KC) and liver sinusoidal endothelial

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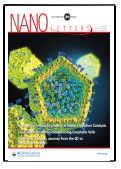

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cells (LSEC) usually take up a significant part of LNPs administered systemically.[12,13] Avoiding this capture is a key step in order to allow the LNPs to reach the target cells. Our strategy relies on an approach that changes the way nanomedicines are biodistributed. A Nanoprimer is administered prior to the nanomedicines, here LNPs encapsulating nucleic acids. The Nanoprimer is a liposome designed with specific physicochemical properties to transiently occupy the cells responsible for limiting the bioavailability of the LNPs. Several strategies to improve the biodistribution of nanoparticles have been investigated by different groups. Examples of such attempts include the saturation of scavenger receptors with anionic compounds, such as polyinosinic acid or PEGylated polylysine peptides,[14−16] and KC depletion.[13,17,18] The priming strategy described here differs in that the Nanoprimer is engineered to prevent toxicity on KC and, more generally, to limit sensitization of the immune system, which can be triggered with PEG. Indeed, in this approach, the mechanism of action is dependent largely on the physicochemical properties of the Nanoprimer, which does not contain or encapsulate any drug nor have any moieties attached to its surface. With a comparable priming strategy, we recently showed an increased efficacy of a nanomedicine-based chemotherapy.[19−21] That work demonstrated, in a mouse model, that the combination of the Nanoprimer with irinotecan-loaded nanomedicine increases efficacy by 50%, without any additional signs of toxicity. Here, the ability of a similar approach to enhance the efficacy of nucleic acid-based treatments is evaluated. To do so, the Nanoprimer's characteristics were modified (i.e., its hydrodynamic diameter was increased) to enhance its ability to occupy specifically the KC and LSEC. Indeed, to avoid interactions with other hepatic functions, in particular with hepatocytes, the Nanoprimer was optimized to be larger than the fenestrae of the liver capillaries. This Nanoprimer's hydrodynamic diameter is over 230 nm to prevent it from passing through the Space of Disse, thus hindering its ability to interact with hepatocytes. Indeed, endothelial fenestrae measure 150−175 nm in diameter in humans[22] and about 280 nm in mice and rats.[23] Therefore, in mice, a non-null but very low interaction of the Nanoprimer with hepatocytes is expected. To evaluate this optimized Nanoprimer, it was used with LNPs encapsulating two different types of nucleic acids: human erythropoietin (hEPO) mRNA and Factor VII (FVII) siRNA.

First, the Nanoprimer's properties and its effect on the nanoparticles' uptake by the liver were studied. The Nanoprimer and its fluorescent equivalent were synthesized and characterized (Table S1). The *in vitro* toxicity of the Nanoprimer was assessed by incubating KC with increasing concentrations of Nanoprimer (up to 30 mM in total lipid concentration) followed by cell viability quantification at 4, 24, and 48 hours. For all tested concentrations, after 4 hours, limited signs of cytotoxicity were seen, with a viability above 89%. Cells incubated for 24 and 48 hours with a high concentration of Nanoprimer showed a decline in cell viability with an IC50 of 3.2 mM at 48 hours (Figure S1). This IC50 of 3.2 mM found for the Nanoprimer corresponds to 250-fold the *in vivo* situation.[24,25] At a dose corresponding to the *in vivo* situation (0.013 mM), no cytotoxicity of the Nanoprimer was observed.

Then, to confirm the Nanoprimer's accumulation in the liver, we injected a fluorescent Nanoprimer and followed its biodistribution by *in vivo* imaging in mice. Results showed that 10 min after IV injection, the Nanoprimer mostly accumulates in the liver and the spleen (Figure 1A). To prove the innocuity

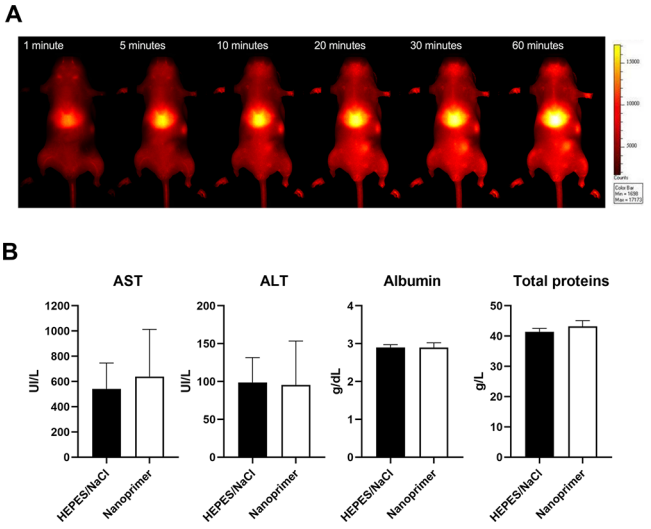

Figure 1. Nanoprimer accumulates in the liver after intravenous injection without inducing liver toxicity. (A) Images of a mouse receiving a fluorescent Nanoprimer in the tail vein at 2 mL/kg (144 mg/kg). (B) Biochemical parameters' analysis of BALB/cByJRj mice 24 hours after injection of 5 mL/kg of Nanoprimer or HEPES/NaCl. All concentrations are within the normal physiological range. Results are shown as average ± s.d. (n = 5 mice per group).

of the Nanoprimer on the liver, mice were injected with the Nanoprimer at 5 mL/kg and major biochemical parameters were analyzed 24 hours post treatment. AST, ALT, albumin, and total proteins levels showed no changes when compared to results for control mice receiving 5 mL/kg of HEPES/NaCl (Figure 1B).

The effect of the Nanoprimer on nanoparticles' uptake by KC was quantified using fluorescent latex nanoparticles *in vitro*. KC were first incubated with the Nanoprimer; then the nanoparticles' solution was added. Fluorescence microscopy revealed that the efficacy of the Nanoprimer to occupy KC and to prevent nanoparticles from being taken up is time dependent (Figure 2A,B). In this experimental setup, a 30 min pretreatment with the Nanoprimer induced a 2-fold decrease in KC's internalization of nanoparticles. Pretreatment for 4 hours caused a 6-fold decrease of mean fluorescence intensity per cell compared to cells treated with nanoparticles only.

To confirm that the Nanoprimer pretreatment similarly decreases KC's and LSECs' endocytic activity *in vivo* and on the basis of the *in vivo* observations (Figure 1A), the Nanoprimer was injected 10 min before fluorescent latex nanoparticles in mice. The flow cytometry analysis showed a significant decrease of the nanoparticles' accumulation in LSECs and KC, in mice treated with the Nanoprimer (Figure 2C,D). The geometric mean of fluorescence intensity in Nanoprimer-treated mice displayed a 14- and 6-fold decrease in KC and LSEC, respectively. No significant difference in the internalization by hepatocytes was observed with or without the Nanoprimer. The discrepancy in the delay taken by the Nanoprimer to be effective, from the 10 min required *in vivo* to the 4 hours *in vitro* (Figure 2), could be explained by different factors, such as the 2D conditions of cell culture vs the blood flow convection,[12] and the culture media composition affecting

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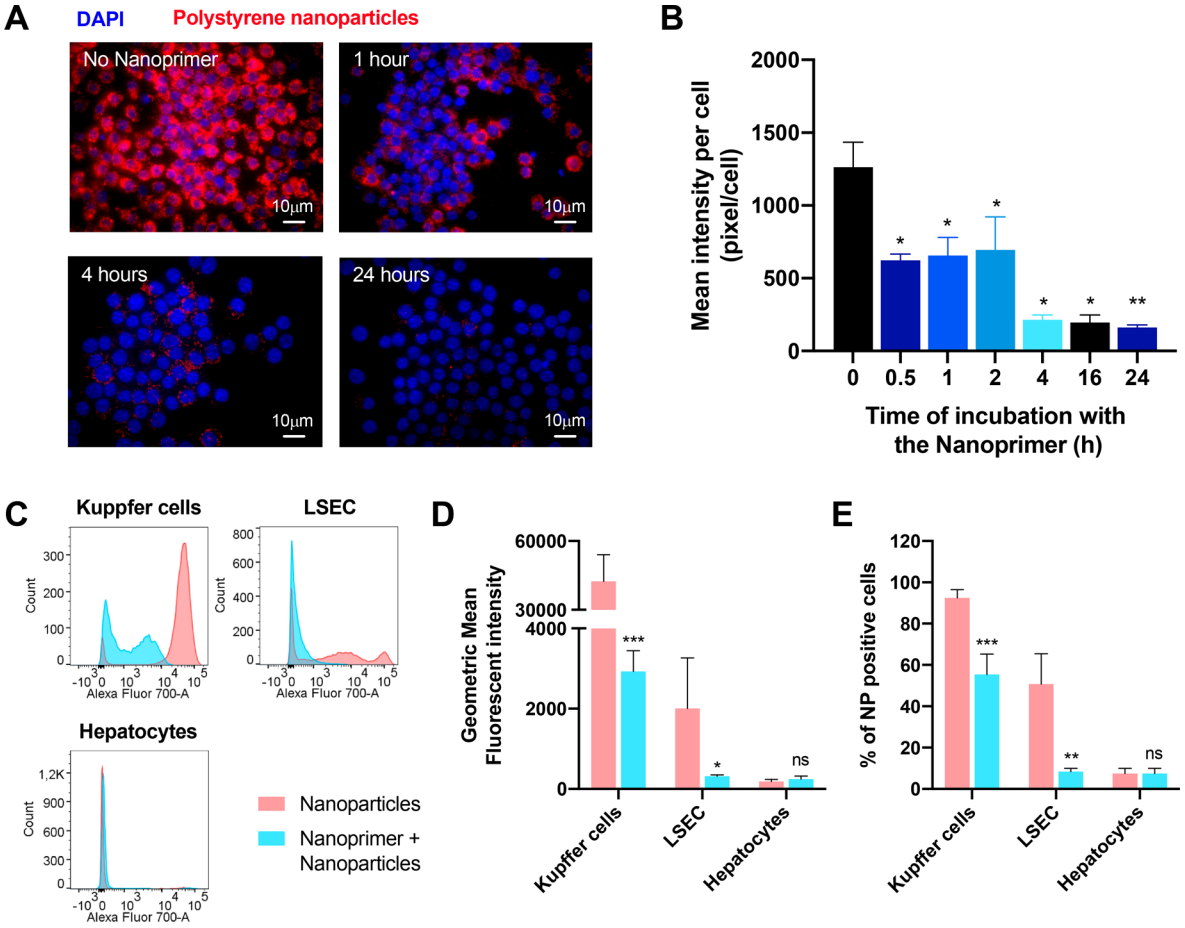

Figure 2. The Nanoprimer decreases the nanoparticles' uptake in KC and LSEC *in vitro* and *in vivo*. (A, B) Immortalized rat KC were treated with a solution of Nanoprimer (0.5 mg/mL). Fluorescent latex nanoparticles were subsequently incubated on cells for 2 hours. (A) Cells observed under the microscope. (B) Nanoparticles' fluorescence intensity quantification with ImageJ. (C−E) Mice treated either with the Nanoprimer 10 min before the injection of fluorescent latex nanoparticles or with the fluorescent latex nanoparticles alone. The liver was harvested after 1 hour; hepatocytes, KC (F4/80 microbeads), and LSEC (CD146 microbeads) were isolated and analyzed by flow cytometry. (C) Flow cytometry read. Histograms are representative of three independent experiments. (D) Mean fluorescent intensity per cell. (E) Number of nanoparticle-positive cells. Results shown are averages of three measurements ± s.d., *p*-values are shown compared to the group without Nanoprimer. ns: not significant, * *p*-value <0.05, ** *p*-value <0.01, *** *p*-value <0.001.

the Nanoprimer's interaction with cells. The timing of the injections of the Nanoprimer and the LNPs was chosen in order to maximize its efficacy, while considering the practical aspects of such a treatment. On the one hand, to be inserted easily in the medical practice, the Nanoprimer needs to be administered during the same treatment session as the nanomedicine. Even with such short delay between the two injections, a direct interaction between the Nanoprimer and the LNPs is unlikely for two reasons. First, the Nanoprimer accumulates in the liver within the first minutes after injection (Figure 1); thus, most of the Nanoprimer is occupying KC when the LNPs are injected. Second, both types of nanoparticles have a negatively charged surface, and because LNPs are PEGylated, they should not tend to aggregate or fuse. On the other hand, the Nanoprimer must reduce LNPs' uptake by the RES within minutes after injection to be relevant for medical use.

Altogether, these data demonstrate that the pretreatment of mice with the Nanoprimer specifically reduces KC's and LSECs' clearance activity without impacting hepatocytes' uptake capacity.

Then, LNPs with different formulations, encapsulating siRNA or mRNA, were synthesized, characterized (Table S2), and tested *in vitro* (Figure S2). The formulation B5 yielded the best transfection or silencing efficacy *in vitro* for siRNA and mRNA and was subsequently used for the rest of the experiments.

The administration of LNPs encapsulating fluorescent nucleic acids allowed the evaluation of the Nanoprimer's impact on their biodistribution. For both mRNA and siRNA, a diminution of the fluorescence in the liver up to 43% was measured *ex vivo*, 1 hour after the injection of the Nanoprimer at 5 mL/kg (360 mg/kg) followed by the LNPs (Figure 3A,B, Figure S3). This reduction in LNPs' entrapment by the liver was associated with an increase in the quantity of LNPs measured in the serum 1 hour after injection, between 8- and 16-fold with 5 mL/kg of the Nanoprimer (Figure 3C,D). Different doses of the Nanoprimer—3, 4, and 5 mL/kg (216, 288, and 360 mg/kg)—were tested in combination with the administration of siRNA LNPs. A significant dose-effect was observed, which corroborates the hypothesis that the Nanoprimer is responsible for the biodistribution improvement. Liver occupancy by the Nanoprimer leads to an increased

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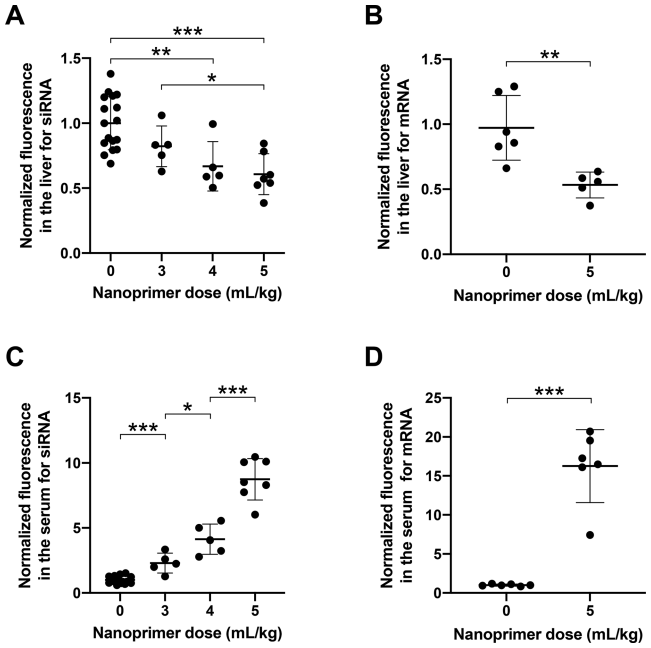

Figure 3. Change in the biodistribution and the bioavailability of the LNPs with the Nanoprimer. (A, B) Fluorescence detected in the livers *ex vivo*, after injection of (A) siRNA LNPs and (B) mRNA LNPs, 1 hour after the injection of the Nanoprimer followed by the injection of LNPs encapsulating the Cy5.5-labeled nucleic acids. (C, D) Fluorescence detected in the blood, after injection of (C) siRNA LNPs and (D) mRNA LNPs, 1 hour after the injection of the Nanoprimer followed by the injection of LNPs encapsulating Cy5.5-labeled nucleic acids. All values are normalized by the fluorescent intensity average without Nanoprimer. Data shown are the average of the measurements ± s.d. * *p*-value <0.05, ** *p*-value <0.01, *** *p*-value <0.001.

blood bioavailability of LNPs that favors subsequent accumulation in the target tissue.

One application of nucleic acid treatments is to use the patient's own cells to produce a secreted protein of interest from administered mRNA. Here, the production of hEPO secreted in the blood after the injection of LNPs encapsulating hEPO mRNA, was significantly increased by 32% for primed animals (Figure 4A). Another application of nucleic acids is to

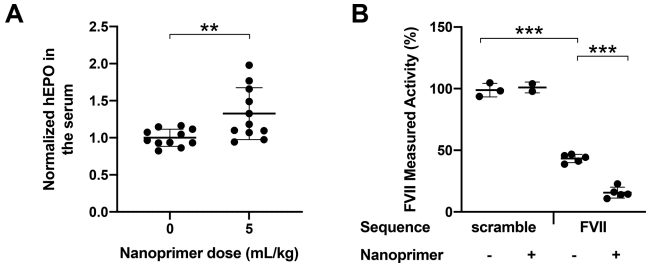

Figure 4. Increase in the efficiency of treatments using nucleic acids to modulate secreted proteins expression. (A) hEPO measured in the blood 24 hours after the injection of the Nanoprimer or PBS followed by the injection of LNPs encapsulating hEPO mRNA. All values are normalized by the average without Nanoprimer of the corresponding experiment. (B) FVII measured activity in the blood (normalized by the value without treatment) 48 hours after the injection of the Nanoprimer followed by the injection of LNPs encapsulating anti-FVII or scrambled siRNA. Data shown are the average of the measurements ± s.d., ** *p*-value <0.01, *** *p*-value <0.001.

shut down the expression of an overexpressed or mis-expressed gene, with a siRNA for instance. Here, the expression of FVII secreted in the blood after the injection of LNPs encapsulating FVII-specific siRNA was significantly decreased by 49% for primed animals (Figure 4B). The quantity of LNPs available in the blood 1 hour after the injection of 5 mL/kg (360 mg/kg) of Nanoprimer, was about 8-fold higher for siRNA encapsulating LNPs, while the shutdown efficiency was improved by 49%. For the LNPs encapsulating mRNA, the availability in the blood 1 hour after injection of 5 mL/kg (360 mg/kg) of Nanoprimer was about 16-fold higher, while the protein production was increased by 32%. This discrepancy between the increased circulation and the increase in protein expression (or respectively silencing) can be explained by the fact that even if LNPs circulate longer in the blood, the internalization by targeted cells and the transfection (or respectively silencing) are not 100% effective.

To investigate the localization of the Nanoprimer within KC and better understand its mechanism of action, confocal microscopy experiments were performed. Those experiments showed that the Nanoprimer was internalized by KC within the endolysosomal organelles (Figure S4). Further work will focus on explaining whether the LNPs' uptake inhibition by the Nanoprimer is due to receptor saturation or to a limited endocytosis capacity of the KC. A hypothesis is that carboxylic groups on the surface of the Nanoprimer interact with scavenger receptors, which are able to recognize COOH-nanoparticles that mimic phosphatidylserine on the surface of apoptotic cells.[15,26,27] The Nanoprimer's interaction with scavenger receptors may lead to their saturation, preventing the internalization of the therapeutic agents injected afterward. Another possibility is that the Nanoprimer's accumulation in cellular organelles saturates the endocytic pathway, transiently decreasing the internalization ability of cells.

Different liver cell types, mainly LSEC, KC, and hepatocytes, can be transfected by LNPs and could be responsible for the enhanced production of hEPO. Blocking the liver's RES may impede the production of hEPO by the cells of the RES. However, it is unlikely that the KC plays an important role in the production of hEPO. Indeed, when incubated *in vitro* in the same conditions, with LNPs encapsulating mRNA coding for mCherry, only 6% of macrophages, compared to 75% of hepatocytes, express mCherry (Figure S5). In addition, instead of entering the KC by endocytosis, the LNPs are phagocytosed and cannot deliver as efficiently the encapsulated RNA in the cytoplasm. Thus, blocking the RES in the liver enables an increase in the production of hEPO likely because LNPs are more easily taken up by hepatocytes. Furthermore, the data presented here show that the Nanoprimer dramatically decreases the LNPs' internalization by LSEC and KC, by 84% and 40%, respectively (Figure 2E). Since FVII is a secreted protein produced exclusively by hepatocytes,[7] the increased internalization of LNPs in hepatocytes after injection of the Nanoprimer is responsible for the silencing of FVII. Because the composition of LNPs encapsulating mRNA and siRNA is similar, LNPs encapsulating hEPO mRNA are likely to be internalized in hepatocytes as well.

The choice of composition of LNPs was based on previously published observations[3] as well as on *in vitro* experiments (Figure S2). This formulation contains a PEG-lipid, mostly to avoid recognition by the RES. However, PEG can trigger an immune reaction known as the complement activation-related pseudoallergy,[28] which can lead to life threatening situations.

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Furthermore, at the second administration, an accelerated blood clearance phenomenon can occur,[29] which results in total rapid clearance of the pegylated object. Besides immune reactions, PEG has been shown to decrease transfection efficiency.[30] Further work on the design of the LNPs could improve their uptake by the target cells. For example, the PEG amount in the formulation could be decreased, since the Nanoprimer occupies the RES transiently, or the LNPs surface could be functionalized with targeting agents.

The mRNA or siRNA treatments, combined or not with the Nanoprimer, were injected up to three times, at 4 days interval, and were well tolerated *in vivo*: the measured weight loss was below 5% at every time point with no clinical signs of suffering (Figure S6). Furthermore, when the Nanoprimer was injected alone, no change in the concentration of the major biochemical parameters were noticed (Figure 1B), which is promising regarding its systemic toxicity.

To conclude, this work shows that the priming strategy improves the efficacy of nucleic acid-based treatments by avoiding the clearance of LNPs by occupying safely and only transiently the RES. Furthermore, along with previous results obtained on chemotherapy nanomedicines,[19] these experiments demonstrate the generalizability of this approach as the Nanoprimer can improve treatment outcomes of different therapeutics, extending from oncology to monogenic diseases. The separation of the functions ensuring the efficacy of a treatment into two distinct objects offers new prospects for designing novel therapeutic agents and shifting the therapeutic paradigm. Indeed, the effect of the Nanoprimer allows us to focus on other LNP functions (for example, cellular uptake by decreasing the amount of PEG-lipid or using an active targeting) that will decrease the compromise between the required functions for an efficient delivery of nucleic acid-based treatments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.0c00752.

> Characterization of the Nanoprimer and LNPs, *in vitro* cytotoxicity of the Nanoprimer, transfection efficiency and cytotoxicity of several LNP formulations, biodistribution of LNPs, *in vitro* localization of the Nanoprimer after uptake by Kupffer cells, *in vitro* efficiency of transfection of LNPs in hepatocytes and macrophages, *in vivo* tolerance of the treatments, and materials and experimental methods details (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Author Contributions

#N.R.M.S. and M.S.P. are co-first authors and contributed equally.

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Notes

The authors declare the following competing financial interest(s): Pr. Langer is an advisor for Nanobiotix. Laurence Poul, Julie Devalliere, Francis Mpambani, Audrey Darmon, Maxime Bergere, Ocane Jibault and Matthieu Germain are employees of Curadigm, a subsidiary company of Nanobiotix.

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■ ABBREVIATIONS

CHOL, cholesterol; DLS, dynamic light scattering; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; EPO, erythropoietin; ESA, EPO stimulating agents; FVII, factor VII; hEPO, human erythropoietin; IVIS, in vivo imaging system; KC, Kupffer cells; LNPs, lipid nanoparticles; LSEC, liver sinusoidal endothelial cells; Luc, luciferase; mRNA, mRNA; PdI, polydispersity index; PEG, polyethylene glycol; RES, reticuloendothelial system; RNA, ribonucleic acid; siRNA, small interfering RNA; TTR, transthyretin

■ REFERENCES

(1) WHO. Genes and human diseases. https://www.who.int/genomics/public/geneticdiseases/en/index2.html, accessed May 1, 2020.

(2) Hoy, S. M. Patisiran: First Global Approval. *Drugs* **2018**, *78* (15), 1625−1631.
(3) Oberli, M. A.; Reichmuth, A. M.; Dorkin, J. R.; Mitchell, M. J.; Fenton, O. S.; Jaklenec, A.; Anderson, D. G.; Langer, R.; Blankschtein, D. Lipid Nanoparticle Assisted MRNA Delivery for Potent Cancer Immunotherapy. *Nano Lett.* **2017**, *17* (3), 1326−1335.
(4) Reichmuth, A. M.; Oberli, M. A.; Jaklenec, A.; Langer, R.; Blankschtein, D. MRNA Vaccine Delivery Using Lipid Nanoparticles. *Ther. Delivery* **2016**, *7*, 319−334.
(5) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an Emerging Platform for Cancer Therapy. *Nat. Nanotechnol.* **2007**, *2* (12), 751−760.
(6) Wang, A.; Langer, R. S.; Farokhzad, O. Nanoparticle Delivery of Cancer Drugs. *Annu. Rev. Med.* **2012**, *63* (1), 185−198.
(7) Whitehead, K. A.; Dorkin, J. R.; Vegas, A. J.; Chang, P. H.; Veiseh, O.; Matthews, J.; Fenton, O. S.; Zhang, Y.; Olejnik, K. T.; Yesilyurt, V.; Chen, D.; Barros, S.; Klebanov, B.; Novobrantseva, T.; Langer, R.; Anderson, D. G. Degradable Lipid Nanoparticles with Predictable in Vivo SiRNA Delivery Activity. *Nat. Commun.* **2014**, *5*, 1−10.
(8) Allen, T. M.; Cullis, P. R. Liposomal Drug Delivery Systems: From Concept to Clinical Applications. *Adv. Drug Delivery Rev.* **2013**, *65* (1), 36−48.
(9) Du, Z.; Munye, M. M.; Tagalakis, A. D.; Manunta, M. D. I.; Hart, S. L. The Role of the Helper Lipid on the DNA Transfection Efficiency of Lipopolyplex Formulations. *Sci. Rep.* **2015**, *4*, 4−9.
(10) Dong, Y.; Love, K. T.; Dorkin, J. R.; Sirirungruang, S.; Zhang, Y.; Chen, D.; Bogorad, R. L.; Yin, H.; Chen, Y.; Vegas, A. J.; Alabi, C. A.; Sahay, G.; Olejnik, K. T.; Wang, W.; Schroeder, A.; Lytton-Jean, A. K. R.; Siegwart, D. J.; Akinc, A.; Barnes, C.; Barros, S. A.; Carioto, M.; Fitzgerald, K.; Hettinger, J.; Kumar, V.; Novobrantseva, T. I.; Qin, J.; Querbes, W.; Koteliansky, V.; Langer, R.; Anderson, D. G. Lipopeptide Nanoparticles for Potent and Selective SiRNA Delivery in Rodents and Nonhuman Primates. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (11), 3955−3960.
(11) Fenton, O. S.; Kauffman, K. J.; McClellan, R. L.; Appel, E. A.; Dorkin, J. R.; Tibbitt, M. W.; Heartlein, M. W.; De Rosa, F.; Langer, R.; Anderson, D. G. Bioinspired Alkenyl Amino Alcohol Ionizable Lipid Materials for Highly Potent in Vivo MRNA Delivery. *Adv. Mater.* **2016**, *28* (15), 2939−2943.
(12) Tsoi, K. M.; MacParland, S. A.; Ma, X.-Z.; Spetzler, V. N.; Echeverri, J.; Ouyang, B.; Fadel, S. M.; Sykes, E. A.; Goldaracena, N.; Kaths, J. M.; Conneely, J. B.; Alman, B. A.; Selzner, M.; Ostrowski, M. A.; Adeyi, O. A.; Zilman, A.; McGilvray, I. D.; Chan, W. C. W. Mechanism of Hard-Nanomaterial Clearance by the Liver. *Nat. Mater.* **2016**, *15*, 1212−1221.
(13) Zhang, Y.-N.; Poon, W.; Besla, R.; Robbins, C.; Dai, Q.; Li, A.; Zheng, G.; Tavares, A. J.; Chan, W. C. W.; Chen, J.; Ding, D.; Ouyang, B. Effect of Removing Kupffer Cells on Nanoparticle Tumor Delivery. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (51), E10871−E10880.
(14) Xu, Z.; Tian, J.; Smith, J. S.; Byrnes, A. P. Clearance of Adenovirus by Kupffer Cells Is Mediated by Scavenger Receptors, Natural Antibodies, and Complement. *J. Virol.* **2008**, *82* (23), 11705−11713.
(15) Baumhover, N. J.; Duskey, J. T.; Khargharia, S.; White, C. W.; Crowley, S. T.; Allen, R. J.; Rice, K. G. Structure-Activity Relationship of PEGylated Polylysine Peptides as Scavenger Receptor Inhibitors for Non-Viral Gene Delivery. *Mol. Pharmaceutics* **2015**, *12* (12), 4321−4328.
(16) Haisma, H. J.; Kamps, J. A. A. M.; Kamps, G. K.; Plantinga, J. A.; Rots, M. G.; Bellu, A. R. Polyinosinic Acid Enhances Delivery of Adenovirus Vectors in Vivo by Preventing Sequestration in Liver Macrophages. *J. Gen. Virol.* **2008**, *89* (5), 1097−1105.
(17) Ohara, Y.; Oda, T.; Yamada, K.; Hashimoto, S.; Akashi, Y.; Miyamoto, R.; Kobayashi, A.; Fukunaga, K.; Sasaki, R.; Ohkohchi, N. Effective Delivery of Chemotherapeutic Nanoparticles by Depleting Host Kupffer Cells. *Int. J. Cancer* **2012**, *131* (10), 2402−2410.
(18) Nizzero, S.; Blanco, E.; Ferrari, M.; Shen, H.; Li, F.; Wolfram, J.; Zhang, G.; Liu, H.; Li, Z. A Chloroquine-Induced Macrophage-Preconditioning Strategy for Improved Nanodelivery. *Sci. Rep.* **2017**, *7* (1), 1−13.
(19) Germain, M.; Meyre, M. E.; Poul, L.; Paolini, M.; Berjaud, C.; Mpambani, F.; Bergere, M.; Levy, L.; Pottier, A. Priming the Body to Receive the Therapeutic Agent to Redefine Treatment Benefit/Risk Profile. *Sci. Rep.* **2018**, *8* (1), 1−11.
(20) Poul, L.; Devalliere, J.; Paolini, M.; Darmon, A.; Bergere, M.; Jibault, O.; Mpambani, F.; Germain, M. Abstract 3613: Mononuclear Phagocytic System Occupancy to Increase Nanomedicines Based Treatment Efficacy. In *Proceedings: AACR Annual Meeting*; AACR, American Association for Cancer Research, 2019; Vol. *79*, pp 3613−3613. DOI: 10.1158/1538-7445.AM2019-3613.
(21) Germain, M.; Poul, L.; Meyre, M.-E.; Paolini, M.; Mpambani, F.; Bergere, M.; Pottier, A.; Levy, L. Abstract LB-072: Redefine Nanomedicine Products Bioavailability to Improve Anti-Tumor Efficacy. In *Proceedings: AACR Annual Meeting*; AACR, American Association for Cancer Research, 2018; Vol. *78*, pp LB-072−LB-072. DOI: 10.1158/1538-7445.AM2018-LB-072.
(22) Braet, F.; Wisse, E. Structural and Functional Aspects of Liver Sinusoidal Endothelial Cell Fenestrae: A Review. *Comp. Hepatol.* **2002**, *1*, 1.
(23) Sarin, H. Physiologic Upper Limits of Pore Size of Different Blood Capillary Types and Another Perspective on the Dual Pore Theory of Microvascular Permeability. *J. Angiog. Res.* **2010**, *2* (1), 14.
(24) Lopez, B. G.; Tsai, M. S.; Baratta, J. L.; Longmuir, K. J.; Robertson, R. T. Characterization of Kupffer Cells in Livers of Developing Mice. *Comp. Hepatol.* **2011**, *10* (1), 2.
(25) Sohlenius-Sternbeck, A. K. Determination of the Hepatocellularity Number for Human, Dog, Rabbit, Rat and Mouse Livers from Protein Concentration Measurements. *Toxicol. In Vitro* **2006**, *20* (8), 1582−1586.
(26) Zhang, L. W.; Bäumer, W.; Monteiro-Riviere, N. A. Cellular Uptake Mechanisms and Toxicity of Quantum Dots in Dendritic Cells. *Nanomedicine* **2011**, *6* (5), 777−791.
(27) Maiseyeu, A.; Bagalkot, V. In Vitro Uptake of Apoptotic Body Mimicking Phosphatidylserine-Quantum Dot Micelles by Monocytic Cell Line. *Nanoscale Res. Lett.* **2014**, *9* (1), 1−6.
(28) Neun, B. W.; Barenholz, Y.; Szebeni, J.; Dobrovolskaia, M. A. Understanding the Role of Anti-PEG Antibodies in the Complement Activation by Doxil in Vitro. *Molecules* **2018**, *23* (7), 1700.
(29) Abu Lila, A. S.; Kiwada, H.; Ishida, T. The Accelerated Blood Clearance (ABC) Phenomenon: Clinical Challenge and Approaches to Manage. *J. Controlled Release* **2013**, *172* (1), 38−47.
(30) Xu, L.; Anchordoquy, T. Molecular Nanomedicine Towards Cancer: *J. Pharm. Sci.* **2011**, *100* (1), 38−52.

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