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Technical note

siRNA/lipopolymer nanoparticles to arrest growth of chronic myeloid leukemia cells *in vitro* and *in vivo*

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ABSTRACT

Therapies for the treatment of Chronic Myeloid Leukemia and other leukemias are still limited for patients at advanced stages, which allow development of point mutations in the *BCR-ABL* fusion gene that render CML cells insensitive to therapies. An effective non-viral delivery system based on lipopolymers is described in this study to deliver specific siRNAs to CML cells for therapeutic gene silencing. The lipopolymer, based on the lipid α-linolenic acid (αLA) substitution on low molecular weight polyethyleneimine (PEI), was used to deliver siRNA against the *BCR-ABL* gene and, the resultant therapeutic effect was evaluated in *in vitro* and *in vivo* CML models. The study concluded that siRNA/PEI-αLA nanoparticles enabled silencing of the *BCR-ABL* gene and BCR-ABL protein, which consequently reduced growth on CML K562 cells *in vitro* and arrested the growth of localized tumors in a localized CML mouse model. The results from this study confirmed the potential use of lipopolymers as delivery systems and are encouraging for the future design of non-viral delivery systems for the treatment of CML and other hematological malignancies resulting from gene fusions.

1. Introduction

Chronic myeloid leukemia (CML) is a malignant neoplasm characterized by the Philadelphia (Ph) chromosome at the myeloid hematopoietic stem cell level. The *BCR-ABL* fusion gene initiates and propagates the disease that leads to uncontrolled expansion of immature myeloid cells in bone marrow and bloodstream [1,2]. Tyrosine kinase inhibitors (TKI) against ABL tyrosine kinase have shown promise in treating CML, however subsets of patients, especially those in the accelerated and in blast crisis phases, are more likely to show early relapse and develop resistance to TKI treatment [1,2]. Synthetic small interfering RNA (siRNA) molecules can be designed to bind and silence ongogenic mRNAs but the siRNA technology in clinics has been limited to solid tumors in major organs such as lung, kidney and liver [3]. Delivery of siRNA to leukemia-causing cells has yielded limited success in *in vitro* studies due to lack of functional delivery systems [3,4]. While viral delivery and electroporation have been used for siRNA delivery in exploratory studies, they cannot be translated to a clinical setting (with electroporation) or possess significant risks for translation (with viral delivery). Lipopolymer-based carriers based on low molecular weight polyethyleneimine (PEI), have been explored for efficient siRNA delivery to myeloid leukemia cells *in vitro* [5–7]. Previously, a lipopolymer derived from α-linolenic acid (αLA) substitution showed effective silencing of the Green Fluorescent Protein (GFP) in GFP-positive K562 cells in suspension or after attachment to a RGD-surface [5]. However, no studies were explored with this delivery system in a preclinical CML model. This study evaluated the PEI formulation based on αLA conjugation on 1.2 kDa PEI (PEI1.2-αLA), as siRNA carrier in human K562 CML cells *in vitro* and K562 xenograft model in mice. This animal model represents 'metastasized' CML tumors, and has been used in the literature before [8,9]. The PEI1.2-αLA was then used *in vitro* to deliver siRNA against the *BCR-ABL* fusion gene to assess changes in *BCR-ABL* mRNA and p210 BCR-ABL protein levels and proliferation of CML cells. The efficacy of BCR-ABL siRNA nanoparticles was subsequently assessed in a CML xenograft model consisting of localized tumors. The methodology for the study is included in Supplementary Material.

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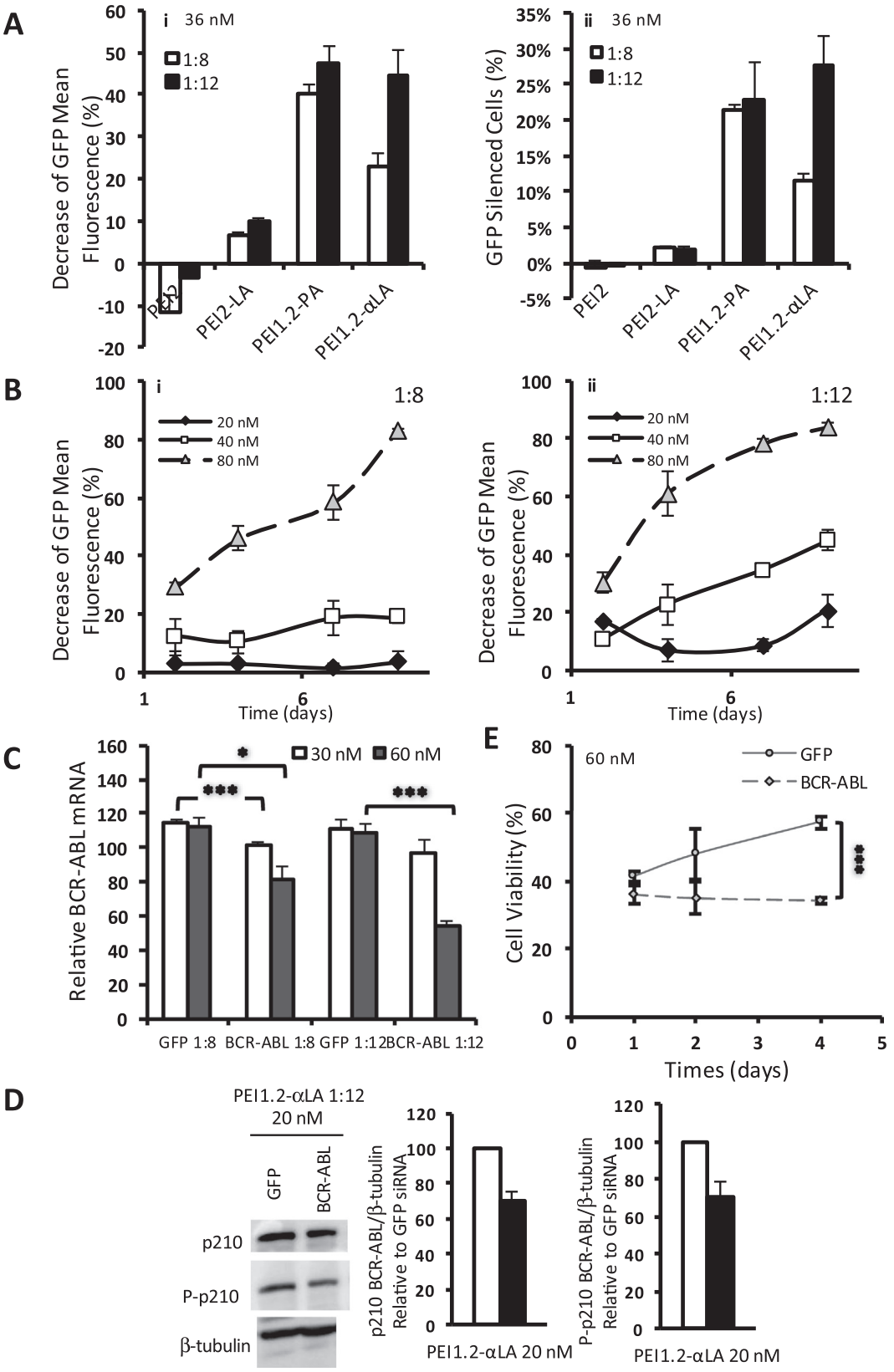

Fig. 1. siRNA effect in GFP-K562 cells *in vitro.* (A) GFP silencing in cells transfected with unmodified PEI and PEIs substituted with PA, LA and αLA. Decrease of mean GFP fluorescence (Ai), and GFP silenced cell population (Aii) were assessed by flow cytometry 3 days after 36 nM siRNA treatment (1:8 and 1:12 siRNA:polymer ratios) and calculated as described in the Supporting Information (n = 3). (B) Decrease of GFP fluorescence of cells transfected with PEI-αLA at 20, 40 and 80 nM as a function of time (siRNA:polymer ratios 1:8 and 1:12) (n = 3). (C and E) Cells treated with BCR-ABL siRNA/PEI-αLA at 30 nM and/or 60 nM siRNA and 1:12 siRNA:polymer ratio were analyzed by RT-qPCR on day 1 (n = 3) (C) and MTT cell viability assay (normalized to NT) on days 1, 2 and 4 (n = 4) (E) *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. (D) Western blot analysis of p210 BCR-ABL phosphorylated-BCR-ABL (P-p210 BCR-ABL) proteins from cells treated with siRNA/PEI1.2-αLA at 20 nM siRNA and 1:12 siRNA:polymer ratio for 3 days. Protein expression of p210 BCR-ABL and P-p210 BCR-ABL relative to ß-tubulin was quantified (n = 2).

The carriers used in this study were palmitic, linoleic and α-linolenic acid modified PEI (PEI1.2-PA, PEI2-LA and PEI1.2-αLA) and unmodified PEI (A scheme of the lipid substitutions on PEI is shown on Fig. S1). The synthesis and characterization of PEI-PA and PEI-LA, and PEI-αLA were described in [10] and [5,11], respectively. Complete siRNA binding to the polymers occurs at siRNA:polymer ratio of 1:2 [5], so that siRNA:polymer ratio in excess of 1:2 was used to ensure complete binding of the siRNA. The size and surface charge of siRNA/PEI-αLA nanoparticles (siRNA/polymer ratio 1:12) was 118.5 ± 13 nm and 38.8 ± 2.6 mV, respectively; in comparison to 712 ± 17.9 nm and 32 ± 0.7 mV from siRNA/PEI nanoparticles [5]. We first investigated silencing of the reporter GFP gene in K562 cells with stable GFP expression (GFP-K562) (Fig. 1A). The unmodified PEI was ineffective to silence the GFP expression. PEI1.2-PA and PEI1.2-αLA were effective in reducing mean GFP fluorescence (1:8 ratio: 40.3 ± 2.0% and 22.8 ± 3.1%; 1:12 ratio: 47.2 ± 4.0% vs. 44.6 ± 5.8% Fig. 1Ai, and Fig. 1Aii). LA-substitution on PEI was also not effective, unlike its activity in acute myeloid cell models [6]. We used PEI1.2-αLA for the remainder of the study. We further evaluated the effectiveness of PEI1.2-αLA for long term silencing (Fig. 1B). The 20 nM siRNA treatment showed a 10–20% decrease in mean GFP fluorescence, which remained constant for up to 9 days (Fig. 1B). At 40 nM, decrease in GFP fluorescence with 1:8 ratio was slightly higher than the 20 nM dose but also < 20% (Fig. 1Bi). At the 1:12 ratio, the 40 nM siRNA treatment displayed a steady increase in silencing from 11.1 ± 2.0% to 44.8 ± 3.5% over the 9 day studied period (Fig. 1Bii). At 80 nM, there was a gradual increase in GFP silencing from ~30% on day 2 to ~84% on day 9 (Fig. 1Bi, ii).

The changes in *BCR-ABL* mRNA were quantified by ddPCR one day after transfection (Fig. 1C). Treatment of cells with 30 nM siRNA yielded 12–13% *BCR-ABL* silencing while, at 60 nM, 27.3 ± 6.5% silencing (1:8 ratio, $p < 0.001$) and 49.6 ± 2.5% silencing (1:12 ratio, $p < 0.001$) was seen. Based on western blotting, treatment of cells with 20 nM siRNA for 3 days reduced the p210 BCR-ABL levels by 30%, which was also similar to the reduction of phosphorylated p210 BCR-ABL (P-p210) protein (~30%; Fig. 1D). Slightly higher reduction of p210 and P-p210 BCR-ABL proteins was found (32% and 44%, respectively) for cells treated with 30 nM siRNA (Fig. S2). To evaluate the effects of *BCR-ABL* silencing on growth (Fig. 1E), cell growth was 41.6 ± 3% and 35.9 ± 5.1% on day 1 (5.7% decrease), 48.1 ± 15.2% and 35 ± 9.8% on day 2 (13.1% decrease), and 57.5 ± 3.6% and 34.3 ± 1.6% on day 4 (23.2% decrease, $p < 0.001$) for treatment with GFP-siRNA and BCR-ABL siRNA, respectively.

The effect of BCR-ABL siRNA delivery was then investigated in a CML xenograft mice model (methodology for tumor xenografts formation is described in Supporting Information). The established xenografts derived from GFP-K562 cells were injected with BCR-ABL-siRNA/PEI1.2-αLA nanoparticles subcutaneously near the tumor (SC; 3 × 10 μg siRNA, siRNA:polymer ratio 1:12) or intraperitoneally (IP; 10 μg + 3 × 15 μg siRNA, siRNA:polymer ratio 1:12). Nanoparticle injections were carried out every 3–4 days at the time points indicated in Fig. 2. Injection with RPMI alone and GFP-siRNA/PEI1.2-αLA nanoparticles served as treatment controls. Changes in the relative tumor volumes (vs. day 0) after the first injection are shown in Fig. 2 (data in Table S1). For SC-treated xenografts, GFP siRNA did not decrease tumor volumes after day 10 in comparison to RPMI group. Tumor growth with BCR-ABL siRNA showed a slower growth trend in comparison to RPMI and GFP siRNA groups (Fig. 2A). This effect was more evident on days 7 and 10, where there was a significant difference on day 7 between RPMI and BCR-ABL siRNA groups ($p < 0.05$ by *t*-test), but the rest of the groups were not significantly different. A reduction of tumor size was still appreciated on day 14 but the difference was less evident. The ddPCR analysis of extracted tumors indicated ~21% reduction of *BCR-ABL* mRNA in tumors treated with BCR-ABL siRNA in comparison with RPMI group ($p = 0.1$), where no changes in *BCR-ABL* levels with the GFP siRNA were found (Fig. S3A). Presumably, the 1-week delay between the last siRNA injection and analysis time allowed recovery of the *BCR-ABL* mRNA levels. With IP administration of siRNA, growth of tumors treated with GFP siRNA was slower in comparison with the RPMI group but there was no significant difference at any time point (Fig. 2B). BCR-ABL siRNA injection decreased the tumor volumes from day 4 up to day 15; where statistical differences were found on day 4 (between RPMI and BCR-ABL, $p < 0.05$) and on day 8 (between RPMI and BCR-ABL, and GFP and BCR-ABL, $p < 0.05$ by *t*-test). On day 8, the volume of tumors treated with BCR-ABL was 6 times less than the volume of tumors treated with GFP siRNA. Changes in volume of tumors treated with BCR-ABL siRNA on days 12 and 15 were no longer significantly different in comparison to RMPI ($p = 0.076$) and GFP siRNA ($p = 0.097$) groups (Fig. 2B). No apparent changes in the body weight were observed between treated mice and untreated mice (Fig. S3B).

Low and high molecular weight (MW) PEIs have been widely used as a non-viral vector for their ability to condense and delivery nucleic acid into the cells. PEI has been used for a range of mammalian cells, including attachment-dependent and suspension cells [12,13]. To avoid non-specific interactions and prolong plasma circulation time, cationic surface of PEI were shielded with hydrophilic polymers such as poly (ethylene glycol) (PEG), pluronic and polyacrylic acid [13]. Other PEI modifications included ligand-PEI conjugates for cell targeting such as, galactose, transferrin (Tf), and folate [14]. In the context of PEI modifications for nucleic acid delivery to leukemia cells, immune-polyplexes were constructed using high (25 kDa) MW PEI-based polyplexes attached via streptavidin bridge to biotin-labeled antibodies for targeted pDNA delivery in lymphoma cell lines [15]. Anti-CD3 and anti-CD19 immunoplexes were highly functional and selective for delivery in Jurkat T-cells (CD3+/CD19-) and Granta B-cell lines (CD3-/CD19+), respectively. Only 11% of Jurkat and 2% of Grant cells were positive with pDNA, in comparison with naked PEI that resulted in 5% of Jurkat cells transfected [15]. Another study modified linear 22 kDa PEI for pDNA delivery in CML K562 cells [16], where PEI was conjugated with PEG and/or Tf (Tf-PEG-PEI and PEI-PEG). Plasmid DNA complexes formed with a combination of native PEI and the two PEI-conjugates (PEI/Tf-PEG-PEI/PEI-PEG) gave higher transfection than complexes formed with native PEI, was equally effective to PEI/Tf-PEG-PEI and 100-fold higher than PEI/PEI-PEG. Surface charge of PEI/Tf-PEG-PEI/PEI-PEG complexes was significantly lower (between –1 and + 12 mV) in comparison with native PEI (+31 mV) and PEI/Tf-PEG-PEI (+27 mV) complexes, and similar charge to PEI/PEI-PEG (+10 mV) [16]. In this regard, our polymers represent a less toxic backbone (low MW PEI) with a membrane-compatible modification (i.e., lipid substitution), which might be amenable for further modifications, such as for cell surface targeting or anti-fouling features based on this literature. Another type of polymer used in leukemia, acute myeloid leukemia in this case, is cyclodextrin modified with PEG and a IL3Rα as targeting ligand (CD.DSPE-PEG-Fab) [17]. This delivery system with a 100 nM siRNA dose gave 40% and 50% reduction of mRNA and protein, respectively in comparison with nanoparticles with control-siRNA. An increase of siRNA dose to 200 nM induced 25% cell death in comparison with control siRNA nanoparticles. Combinatorial treatment at 200 nM with chemotherapeutic agent cytarabine gave a synergistic effect by inducing 75% cell death in comparison to 20% cell death with cytarabine treatment alone. Studies with AML patient cells gave 30% or 60% silencing of BRD4 mRNA (6 patients), 45–60% (2 patients) BRD4 protein inhibition and a significant reduction of cell viability alone (40–70%) and in combination with cytarabine (~80%). Therapeutic effect of stablished drugs or chemotherapeutic agents can be combined with siRNA therapy to induce further the anti-leukemic effect [17].

Several other studies reported BCR-ABL siRNA delivery to arrest the growth in CML K562 cells *in vitro* using commercial [3,18,19] and non-commercial agents [4,5,20–23]. The functional effects were obtained with siRNA concentrations that ranged between 70 nM and 2000 nM. In

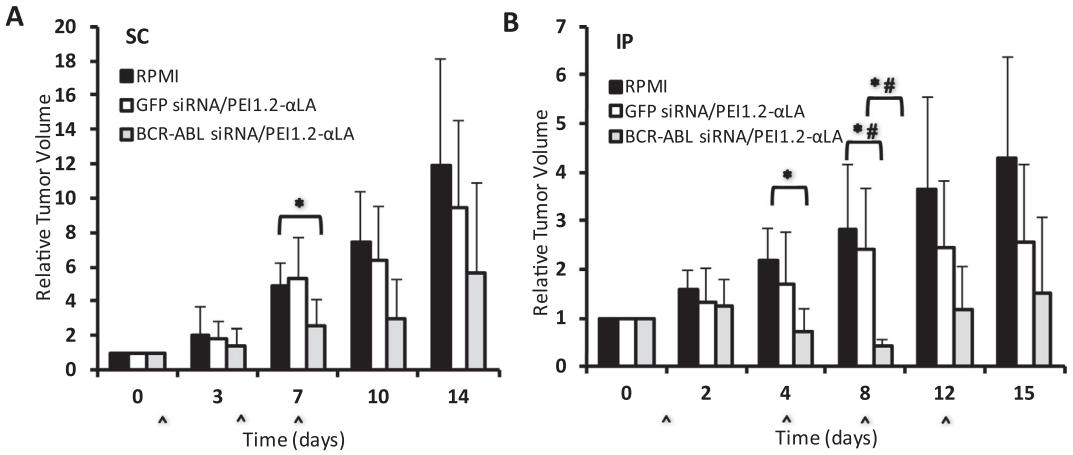

Fig. 2. BCR-ABL siRNA treatment in CML xenografts. Established tumors were treated (A) SC injection of siRNA (3 × 10 μg siRNA, siRNA:polymer ratio 1:12), and (B) IP injection of siRNA (1 × 10 μg siRNA, followed by 3 × 15 μg siRNA, siRNA:polymer ratio 1:12). * ($p > 0.05$) by T-test and # ($0.05 > p < 0.10$) by ANOVA. '^' on the horizontal axis indicates injections days: 1, 4, 7 days for SC treatment (A), and 1, 4, 8 and 12 for IP treatment (B).

this study, we observed silencing effects at 20–60 nM, doses considerably lower in comparison with the dose range used in the reported studies.

We further explored the feasibility of BCR-ABL siRNA delivery in nude mice bearing CML solid tumors. We demonstrated that *BCR-ABL* gene was effectively silenced by repeated administration of BCR-ABL siRNA/PEI1.2-αLA via SC route, inhibiting the growth of xeno-transplanted tumors: The mice weights after the IP treatment showed no significant changes between untreated mice and mice treated with the nanoparticles, indicating no gross adverse effects. A previous animal study performed from our research group, [24] evaluated the effect of siRNA delivery with a lipopolymer (linoleic acid substituted PEI) in a breast cancer tumor model [24]. The IP treatment, which consisted of 4 injections of 10 μg of siRNA and siRNA:polymer w/w ratio 8:1 every 48 h with a total 40 μg of siRNA (~0.5 mg/kg/day), showed similarly significant tumor volume reduction with siRNA nanoparticle treatment [24]. This study performed functional marker analysis on the kidney (serum creatinine and urea) and liver (alanine aminotransferase and gamma-glutamyl transpeptidase) of mice treated by IP injections; these results did not show significant changes/difference in comparison with untreated mice, suggesting no signs of nephrotoxicity or hepatotoxicity after systemic siRNA/lipopolymer treatment [24]. In the present study, we performed IP treatment with 4 injections of 1 × 10 μg and 3 × 15 μg siRNA and siRNA:polymer w/w ratio 1:12 every 48 h (a total of 55 μg of siRNA, giving ~0.69 mg/kg/day), with slightly higher siRNA amount and siRNA:polymer ratio than the study by Aliabadi, et al. However, given that the lipopolymers used in both studies are of similar nature (i.e., lipid-substitution of low molecular PEI), we expected to find no signs of toxicity in liver and kidney of mice from our study.

We are aware of only one *in vivo* study that evaluated the therapeutic effect of oligonucleotides in CML model [25]. Zhang et at., delivered the G3139 antisense oligonucleotide (ASO) against *Bcl-2* gene with transferrin receptor (TfR) conjugated lipoplexes (LP) to K562 (which over-express Tf-R) xenografts in mice. Although Tf-LP G3139 delivery suppressed tumor growth for increased survival, it was found that this effect was due to *Bcl-2* silencing (expected) and immune cells through tool-like receptor (TLR9) activation (unexpected). This suggests that not only an effective delivery vehicle is needed but also a careful design of the silencing agent so that the secondary effects are optimized and the intended effect takes place. To our knowledge, this is the first time that siRNA administration was demonstrated in CML *in vivo* models. This may reflect the limited *in vivo* success with the currently used siRNA delivery systems. We recognize that future studies will be needed with a more physiologically-relevant *in vivo* model, i.e., grafting of cells derived from CML patients in NOD/SCID/γc (non-obese diabetic/severe combined immunodeficiency/mutation of IL-2 receptor γ–chain deficient) mice [26] as well as a more careful analysis of toxicities upon systemic siRNA injection with our delivery system.

2. Author contributions

JVS, HMA, XJ, and HU conceived and designed the experiments. AM synthesized the polymers for this study. JVS, HMA, MM performed the experiments. JVS, HMA, MM, XJ and HU analyzed the data. HMA, XJ and HU contributed reagents/materials/analysis tools. JVS, XJ, HU wrote the manuscript. All authors have given approval to the final version of the manuscript.

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Conflict of interest

JVS and HU are founders of RJH Biosciences Inc., which is commercializing the described biomaterials. XJ, MM and HMA declare no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ejpb.2018.06.018.

References

[1] T. O'Hare, M.S. Zabriskie, A.M. Eiring, M.W. Deininger, Pushing the limits of targeted therapy in chronic myeloid leukaemia, Nat. Rev. Cancer 12 (2012) 513–526.

[2] E. Jabbour, H. Kantarjian, Chronic myeloid leukemia 2014 update on diagnosis, monitoring, and management, Am. J. Hematol. 89 (2014) 547–556.

[3] P. Resnier, T. Montier, V. Mathieu, J.P. Benoit, C. Passirani, A review of the current status of siRNA nanomedicines in the treatment of cancer, Biomaterials 34 (2013) 6429–6443.

[4] B. Landry, J. Valencia-Serna, H. Gül-Uludag, X. Jiang, A. Janowska-Wieczorek, J. Brandwein, et al., Progress in RNAi-mediated molecular therapy of acute and chronic myeloid leukemia, Mol. Ther. Nucleic. Acids 4 (2015) e240.

[5] J. Valencia-Serna, P. Chevallier, R.B. Kc, G. Laroche, H. Uludag, Fibronectin-modified surfaces for evaluating the influence of cell adhesion on sensitivity of leukemic cells to siRNA nanoparticles, Nanomedicine 11 (2016) 1123–1138.

[6] B. Landry, H.M. Aliabadi, A. Samuel, H. Gül-Uludag, X. Jiang, O. Kutsch, et al., Effective non-viral delivery of siRNA to acute myeloid leukemia cells with lipid-substituted polyethylenimines, Plos One 7 (2012) e44197.

[7] H. Gül-Uludağ, J. Valencia-Serna, C. Kucharski, L.A. Marquez-Curtis, X. Jiang, L. Larratt, et al., Polymeric nanoparticle-mediated silencing of CD44 receptor in CD34+ acute myeloid leukemia cells, Leuk. Res. 38 (2014) 1299–1308.

[8] L.L. Zhou, Y. Zhao, A. Ringrose, D. DeGeer, E. Kennah, A.E.J. Lin, et al., AHI-1 interacts with BCR-ABL and modulates BCR-ABL transforming activity and imatinib response of CML stem/progenitor cells, J. Exp. Med. 205 (2008) 2657–2671.

[9] J.M. Golas, K. Arndt, C. Etienne, J. Lucas, D. Nardin, J. Gibbons, et al., SKI-606, a 4-Anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice, Cancer Res. 63 (2003) 375–381.

[10] J. Valencia-Serna, H. Gül-Uludag, P. Mahdipoor, X. Jiang, H. Uludag, Investigating siRNA delivery to chronic myeloid leukemia K562 cells with lipophilic polymers for therapeutic BCR-ABL down-regulation, J. Cont. Release 172 (2013) 495–503.

[11] B. Thapa, R. Bahadur Kc, H. Uludag, Novel targets for sensitizing breast cancer cells to TRAIL-induced apoptosis with siRNA delivery, Int. J. Cancer 142 (2018) 597–606.

[12] K. Kunath, A. von Harpe, D. Fischer, H. Petersen, U. Bickel, K. Voigt, et al., Low-molecular-weight polyethylenimine as a non-viral vector for DNA delivery: comparison of physicochemical properties, transfection efficiency and in vivo distribution with high-molecular-weight polyethylenimine, J. Contr. Release 89 (2003) 113–125.

[13] U. Lungwitz, M. Breunig, T. Blunk, A. Göpferich, Polyethylenimine-based non-viral gene delivery systems, Eur. J. Pharm. Biopharm. 60 (2005) 247–266.

[14] A. Kichler, Gene transfer with modified polyethylenimines, J. Gene. Med. 6 (2004) S3–S10.

[15] V.M. Guillem, M. Tormo, F. Revert, I. Benet, J. García-Conde, A. Crespo, et al., Polyethyleneimine-based immunopolyplex for targeted gene transfer in human lymphoma cell lines, J. Gene Med. 4 (2002) 170–182.

[16] M. Kursa, G.F. Walker, V. Roessler, M. Ogris, W. Roedl, R. Kircheis, et al., Novel shielded transferrin-polyethylene glycol-polyethylenimine/DNA complexes for systemic tumor-targeted gene transfer, Bioconjug. Chem. 14 (2003) 222–231.

[17] J. Guo, E.G. Russell, R. Darcy, T.G. Cotter, S.L. McKenna, M.R. Cahill, et al., Antibody-targeted cyclodextrin-based nanoparticles for siRNA delivery in the treatment of acute myeloid leukemia: physicochemical characteristics, in vitromechanistic studies, and ex vivopatient derived therapeutic efficacy, Mol. Pharmaceutics. 14 (2017) 940–952.

[18] M. Wilda, U. Fuchs, W. Wössmann, A. Borkhardt, Killing of leukemic cells with a BCR/ABL fusion gene by RNA interference (RNAi), Oncogene 21 (2002) 5716–5724.

[19] Z. Zhelev, R. Bakalova, H. Ohba, A. Ewis, M. Ishikawa, Y. Shinohara, et al., Suppression of bcr-abl synthesis by siRNAs or tyrosine kinase activity by Glivec alters different oncogenes, apoptotic/antiapoptotic genes and cell proliferation factors (microarray study), FEBS Lett. 570 (2004) 195–204.

[20] Y. Arthanari, A. Pluen, R. Rajendran, H. Aojula, C. Demonacos, Delivery of therapeutic shRNA and siRNA by Tat fusion peptide targeting BCR-ABL fusion gene in chronic myeloid leukemia cells, J. Contr. Release 145 (2010) 272–280.

[21] L.S. Mendonça, F. Firmino, J.N. Moreira, M.C. Pedroso de Lima, S. Simoes, Transferrin receptor-targeted liposomes encapsulating anti-BCR-ABL siRNA or asODN for chronic myeloid leukemia treatment, Bioconjug. Chem. 21 (2010) 157–168.

[22] A.C.N. Oliveira, K. Raemdonck, T. Martens, K. Rombouts, R. Simón-Vázquez, C. Botelho, et al., Stealth monoolein-based nanocarriers for delivery of siRNA to cancer cells, Acta Biomaterialia 25 (2015) 216–229.

[23] C. Yang, N. Panwar, Y. Wang, B. Zhang, M. Liu, H. Toh, et al., Biodegradable charged polyester-based vectors (BCPVs) as an efficient non-viral transfection nanoagent for gene knockdown of the BCR–ABL hybrid oncogene in a human chronic myeloid leukemia cell line, Nanoscale 8 (2016) 9405–9416.

[24] H.M. Aliabadi, R. Maranchuk, C. Kucharski, P. Mahdipoor, J. Hugh, H. Uludag, Effective response of doxorubicin-sensitive and -resistant breast cancer cells to combinational siRNA therapy, J. Contr. Release 172 (2013) 219–228.

[25] X. Zhang, C.G. Koh, B. Yu, S. Liu, L. Piao, G. Marcucci, et al., Transferrin receptor targeted lipopolyplexes for delivery of antisense oligonucleotide G3139 in a murine K562 Xenograft Model, Pharm. Res. 26 (2009) 1516–1524.

[26] L. Caldemeyer, M. Dugan, J. Edwards, L. Akard, Long-term side effects of tyrosine kinase inhibitors in chronic myeloid leukemia, Curr. Hematol. Malig. Rep. 11 (2016) 71–79.