Supplementary Information

Targeted Delivery of Antisense Oligodeoxynucleotide by Transferrin Conjugated pH-Sensitive Lipopolyplex Nanoparticles: A Novel Oligonucleotide –Based Therapeutic Strategy in Acute Myeloid Leukemia

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Materials and Methods

Flow cytometry. Flow cytometry was performed on a Beckman Coulter EPICS XL (Beckman Coulter, Miami, FL). A minimum of 10,000 events were collected under list mode and analyzed using WinMIDI (Windows Multiple Document Interface for Flow Cytometry) analysis program.

Quantitative RT-PCR (qRT-PCR). Briefly, total RNA was extracted using Trizol reagent (Invitrogen) and cDNA was synthesized with a SuperScript III kit (Invitrogen) following manufacturer protocols. Real time PCR was performed using 2X Sybr Green kit (Applied Biosystems, Foster City, CA) in the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The following primer sets were used: (a) R2: 5'-GCCTGGCCTCACATTTTCTAAT-3' and 5'-GAACATCAGGCAAGCAAAATCA-3'; and (b) a housekeeping gene ABL: 5'-TGGAGATAACACTCTAAGCATAACTAA-AGGT-3'; and 5'- GATGTAGTTGCTTGGGACCCA-3'. *R2* mRNA was normalized to *ABL* mRNA level for each sample. Reaction conditions were 50°C x 2 min, 95°C x 10 min, 40 cycles of 95°C x 15 sec, 60°C x 30 sec, 72°C x 40 sec. The comparative cycle threshold (C_T) method, also known as delta delta C_T (DDC_T) method[1, 2], was used for relative quantitation of gene expression.

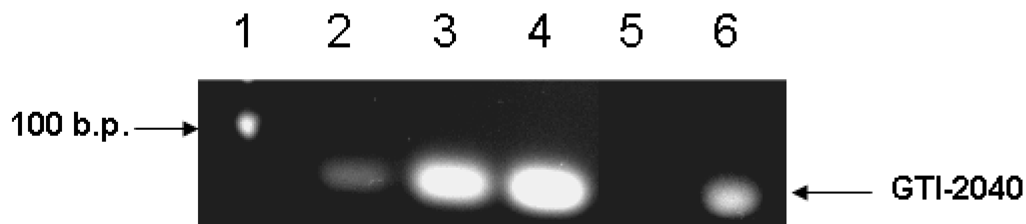

Figure S1. Agarose gel electrophoresis of GTI-2040. 1. DNA marker; 2. 1 μg GTI-2040; 3. 4 μg GTI-2040; 4. 5 μg GTI-2040; 5. LPs containing 2 μg GTI-2040; 6. LPs containing 2 μg GTI-2040, where ODNs were released from LPs after lysed with 1%SDS + 0.1%Triton. Samples were run on 1% agarose gel in 1x TAE buffer. The brightness of each ODN band after ethidium bromide staining was measured, and the amount of ODN was estimated using an established standard curve. Encapsulation efficiency was calculated based on the ratio of ODNs in LP particles (lane 6) versus the initial amount of ODNs applied (2 μg). Encapsulation efficiency was over 90%. The lack of ODN band in native LPs (lane 5) confirmed the high encapsulation efficiency.

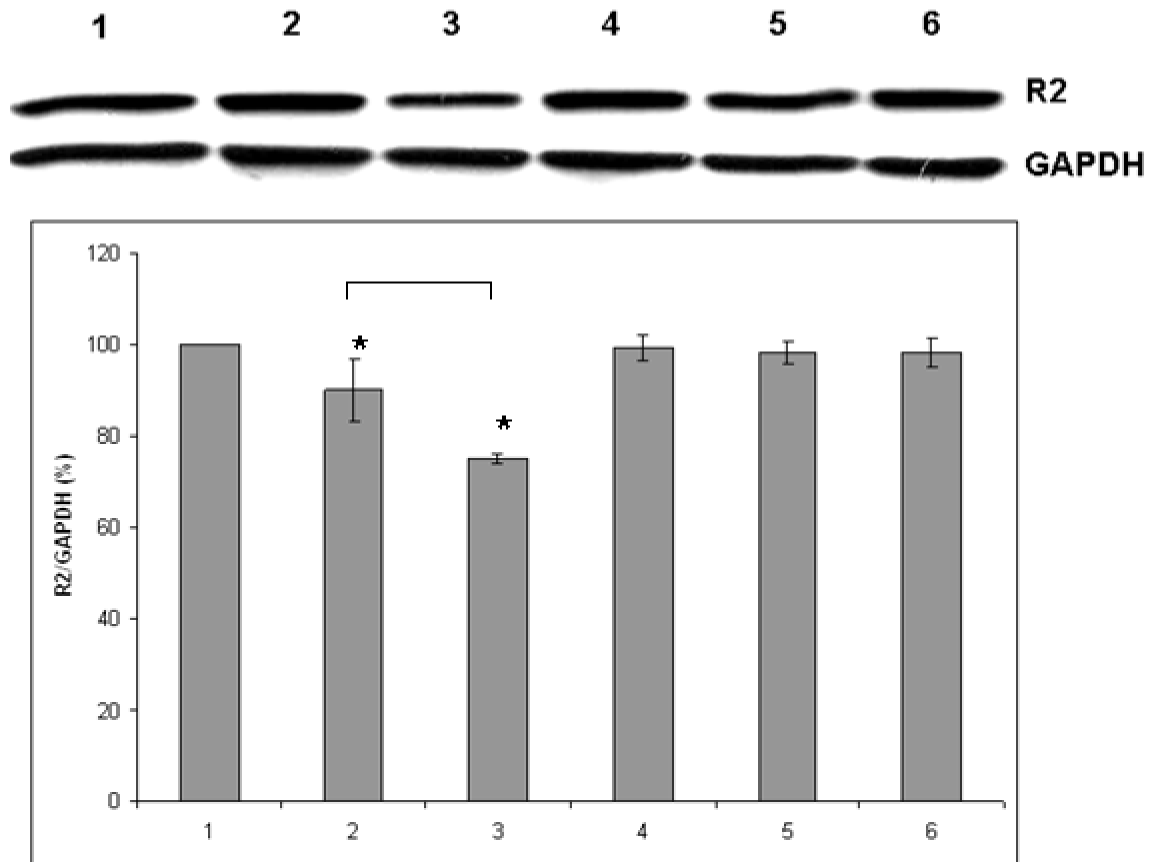

Figure S2. Western blot result for R2 down regulation in Kasumi-1 AML cells under various conditions after 48 hr incubation. (1) Mock, (2) 1 μM LPs (GTI-2040), (3) 1 μM Tf-LPs (GTI-2040), (4) 1 μM free GTI-2040, (5) 1 μM LPs (scrambled), and (6) 1 μM Tf-LPs (scrambled). Upper panel shows representative western blot image. Lower panel shows the densitometry data. Each column reflects the average of at least three independent experiments. The standard deviation is elucidated with an error bar. * indicates these data are statistically different from each other.

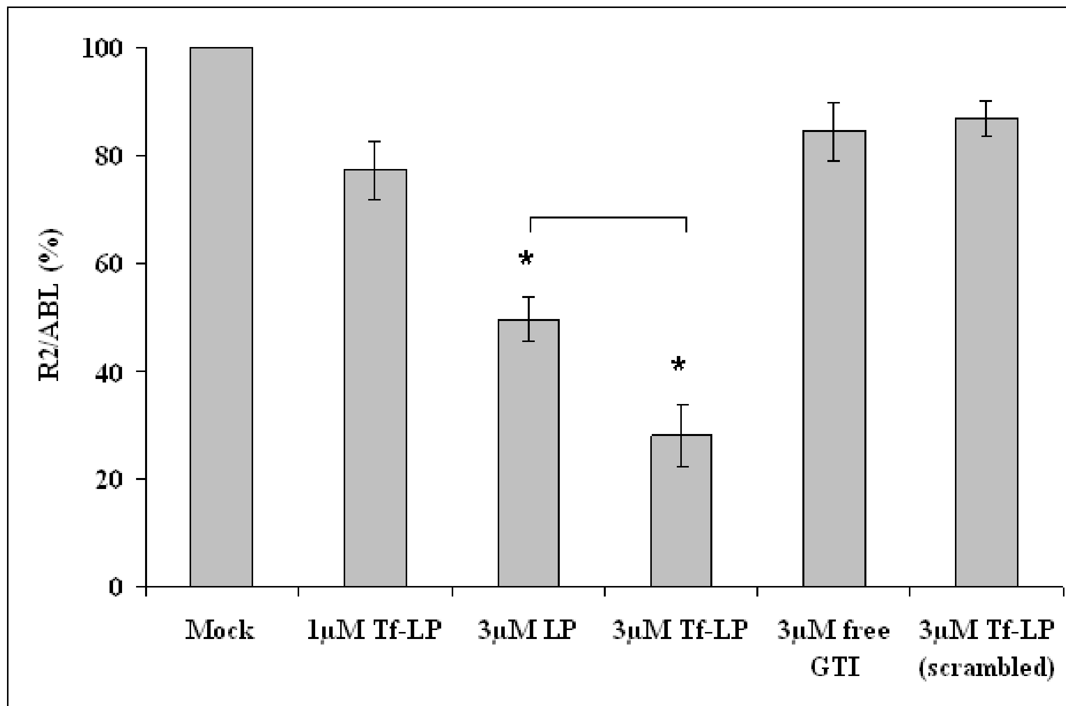

Figure S3. qRT-PCR results of *R2* mRNA downregulation in Kasumi-1 AML cells under various conditions after 48hr. Every sample was compared with Mock. Each column reflects the average of at least three independent experiments. The standard deviation is elucidated with an error bar. * indicates these data are statistically different from each other.

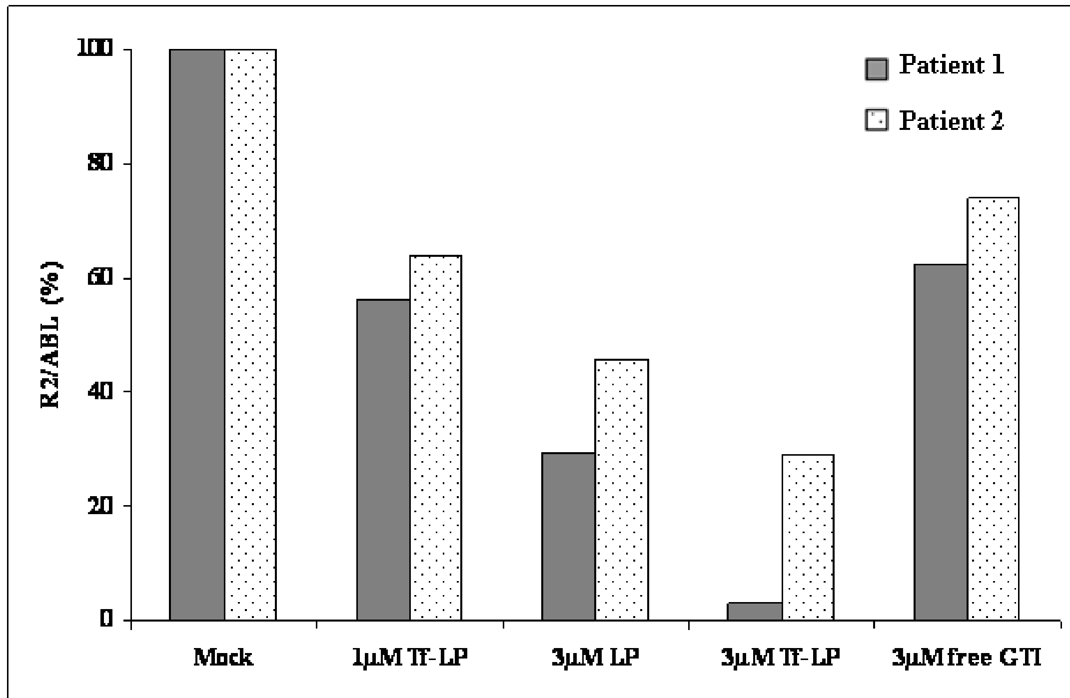

Figure S4. qRT-PCR results for *R2* mRNA downregulation in AML patient primary cells after 48hr. Every sample was compared with Mock.

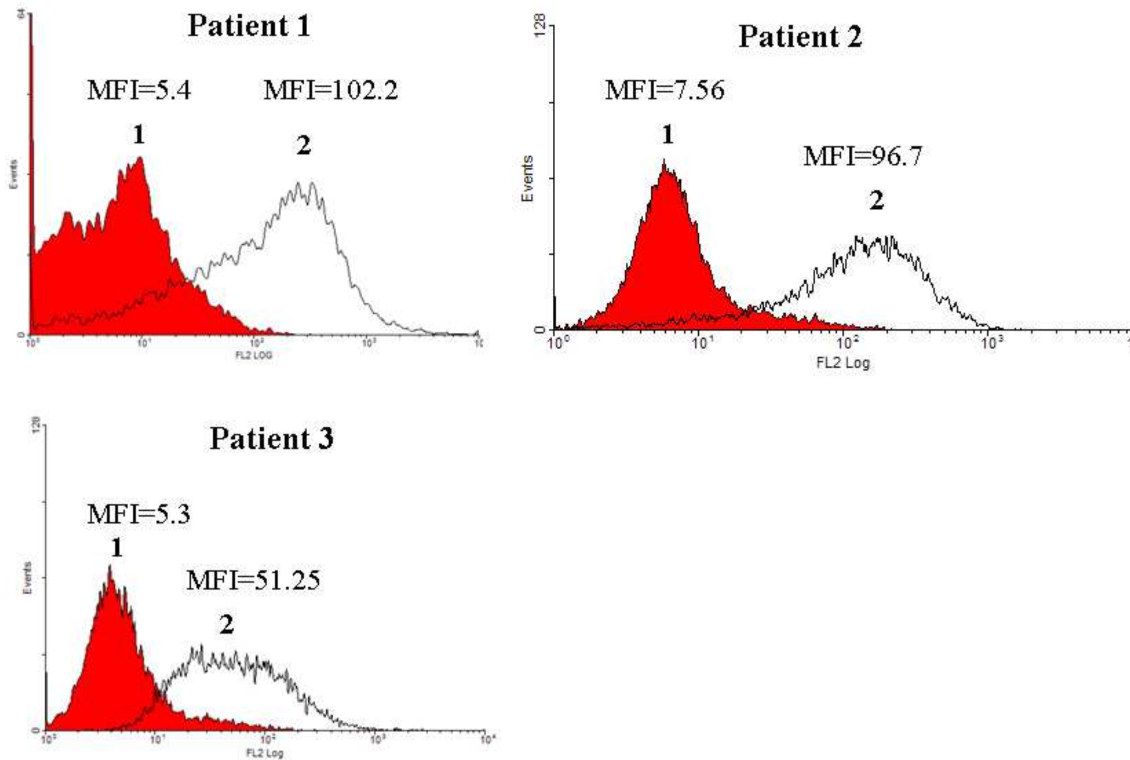

Figure S5. Flow cytometry study of TfR expression level on the patient cell surface. 1. cells stained with PE-isotype; 2. cells stained with PE-anti-TfR; Mean fluorescence intensity (MFI) was labeled for each sample.

References

1. User Bulletin #2. *ABI PRISM 7700 Sequence Detection System*.
2. Yalcin, A. Quantification of thioredoxin mRNA expression in the rat hippocampus by real-time PCR following oxidative stress. *Acta Biochim. Pol.* **2004,** *51*, 1059-1065.