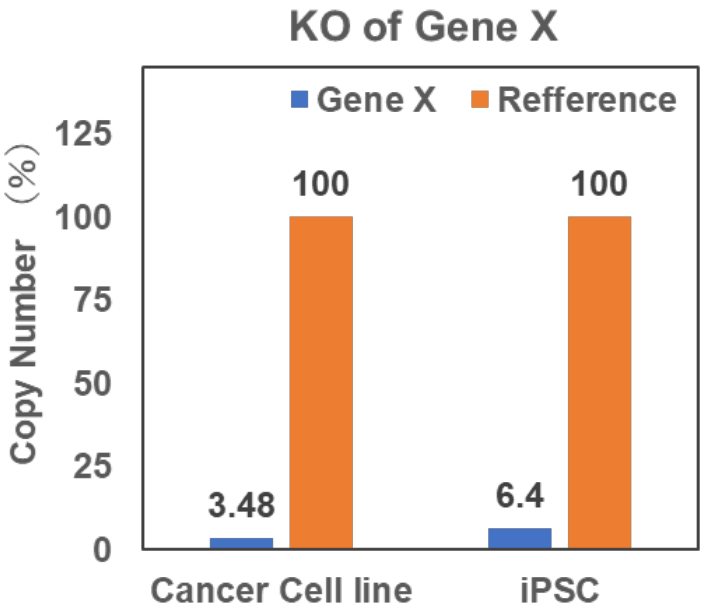
Technical Information
Genome Editing

GenAhead Bio Inc.

2018.10

PoC study with siRNA or CRISPR/Cas9

Gene Silencing using siRNA has been used to show PoC of Gene X. However, the researchers often need to screen some siRNAs to get potent silencing. Knock-out (KO) using CRISPR/Cas9 can be another option. To meet efficient gRNAs is more probable. Furthermore, after optimization with SNIPER method, mostly complete KO of the gene X were achieved both in a cancer cell line and iPSC without cloning of single cell (in the bulk culture of KO treatment).



KO% in the bulk culture of KO treatment

If KO clones are necessary, less than 20 clone pick-up would be enough to find some KO cell lines.

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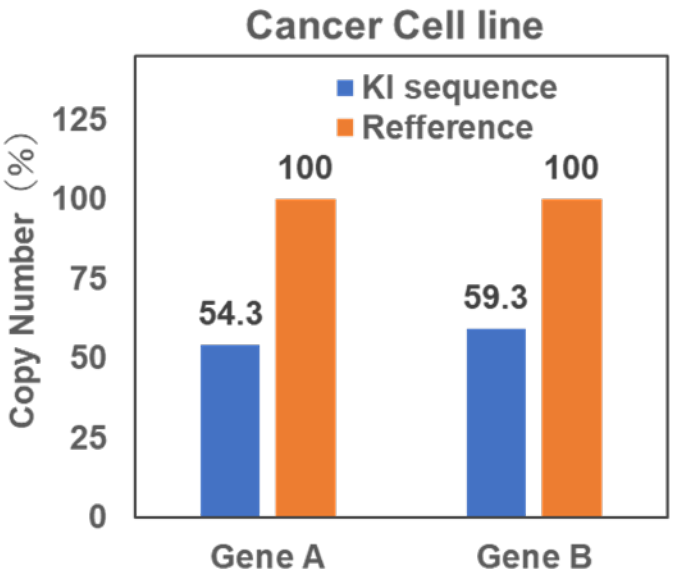
PoC study with genetic modification of the sequence

Small mutation sometimes change the gene function drastically. It is well-known that some single nucleotide polymorphism (SNP) causes genetic disease, change of drug sensitivity, or increase in odds ratio of some diseases. To investigate these cases, replacement of specific sequence at the locus (knock-in (KI)) would be useful.



The donor templates were just little-modified sequence without any antibiotics resistant genes.

On the left hands, GenAhead Bio tried to replace the original sequence to little modified sequence as SNP conversion model (blue). After SNIPER optimization, more than 50% KI were detected both in gene A and B without cloning of single cell (in the bulk culture of KI treatment).
If KI clones are necessary, less than 30 clone pick-up would be enough to find some KI cell lines.

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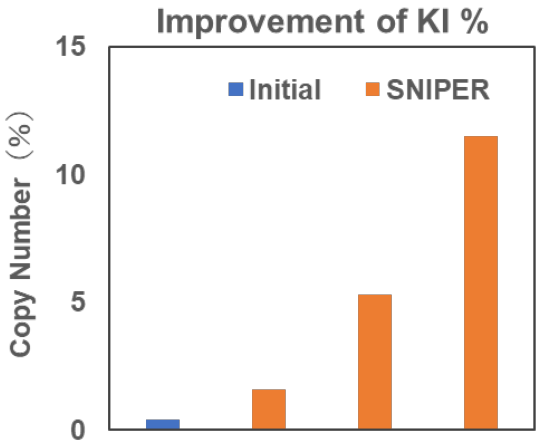
About SNIPER

Just after transfection, KI sequences are usually detectable by PCR. How many KI clones we will have after clone-pickup from the bulk culture? To answer this question, quantification of KI would be useful than PCR. GenAhead Bio has separately quantified precise KI number and random integration number by specifically designed qPCR method termed Specification of Newly Integrated Position and Exclusion of Random-integration (SNIPER), and noticed that significant increase in KI efficiencies can be achieved after simple optimization cycles by using SNIPER. Because there might be various parameters such as length of homology arm, specificity (precise KI/ random KI), amount of Donor, gRNA performance, etc., this simple method provides a merit to improve KI efficiencies expeditiously.



On the left hands, improvement of artificial large sequence (3.5 kbp) KI without antibiotics selection using SNIPER analysis was shown in the bulk culture of a cancer cell line.

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Technical Information
Nucleic Acids Delivery

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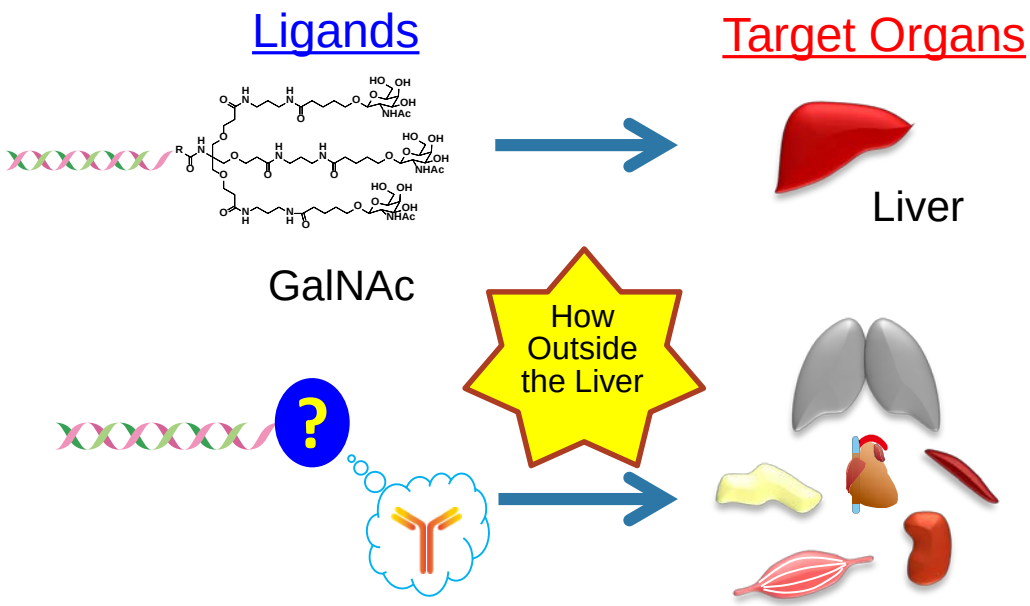
It is well known that some biopharmaceutical companies have withdrawn from siRNA research probably because the liver continues to be the only target of siRNAs. Accordingly, every venture needs to compete in a small market related to the liver. GenAhead Bio would like to introduce a novel siRNA delivery platform, which expands the target organ outside the liver.

Background

GenAhead Bio conjugated siRNAs with anti-CD71 Fab' fragment to develop a novel platform targeted to muscular organs. Using a mice model, this conjugate showed durable gene-silencing in the heart and skeletal muscle after intravenous administration. In addition, the treatment of peripheral artery disease in mice with myostatin siRNA conjugate resulted in the recovery of running performance through the significant silencing of myostatin and hypertrophy of the gastrocnemius. This result successfully demonstrates the utility of anti-CD71 antibody conjugation for nucleic acids delivery as well as reveals its therapeutic potential for muscular/cardiac diseases.



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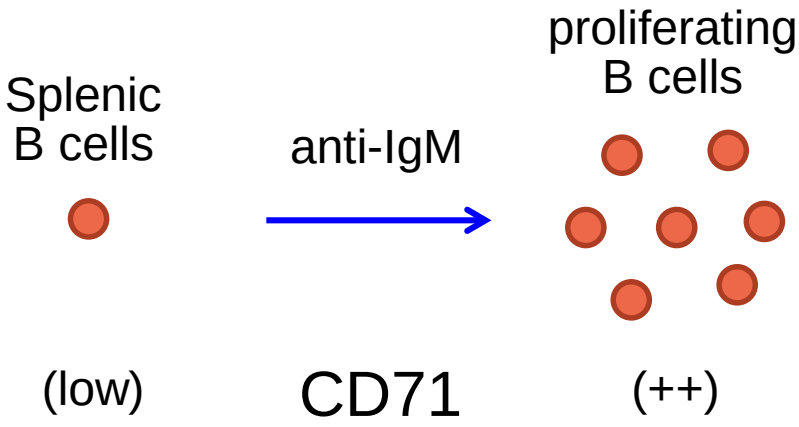
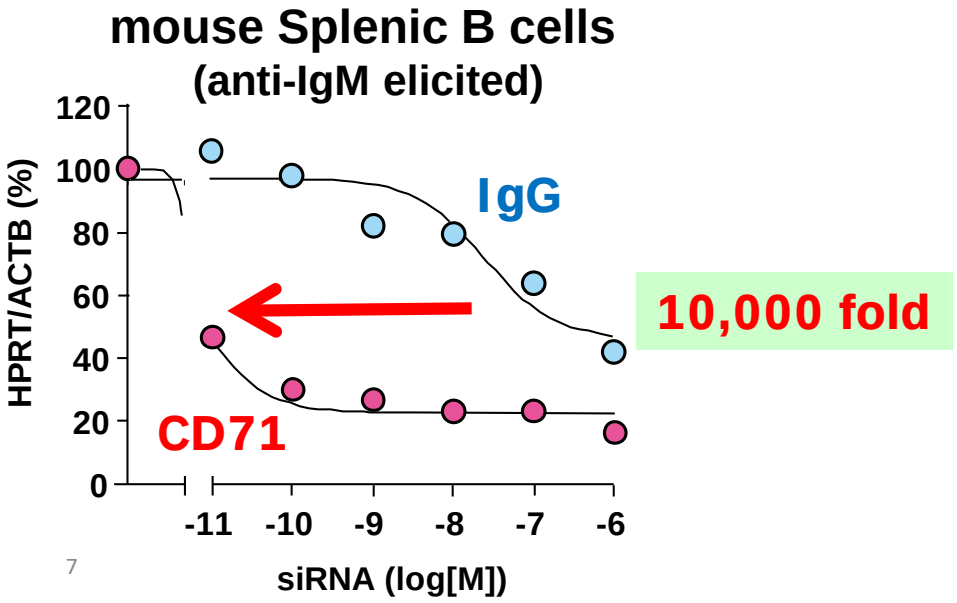
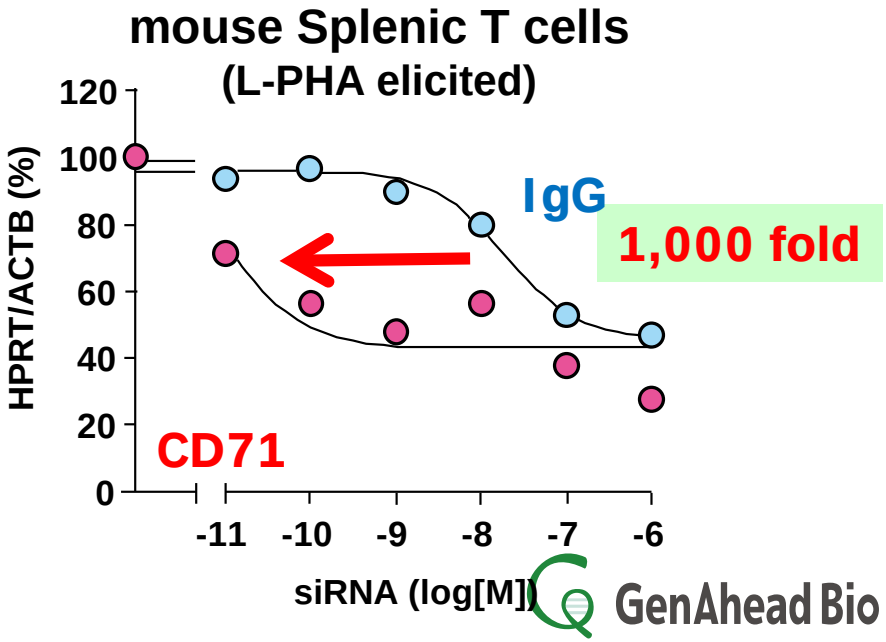
In vitro knock-down activities against CD71-positive cells



(* CD71 is highly expressed on growing immune cells but not on resting cells .)





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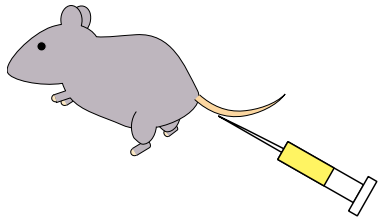
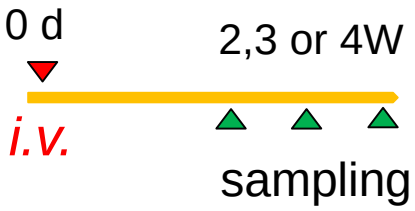
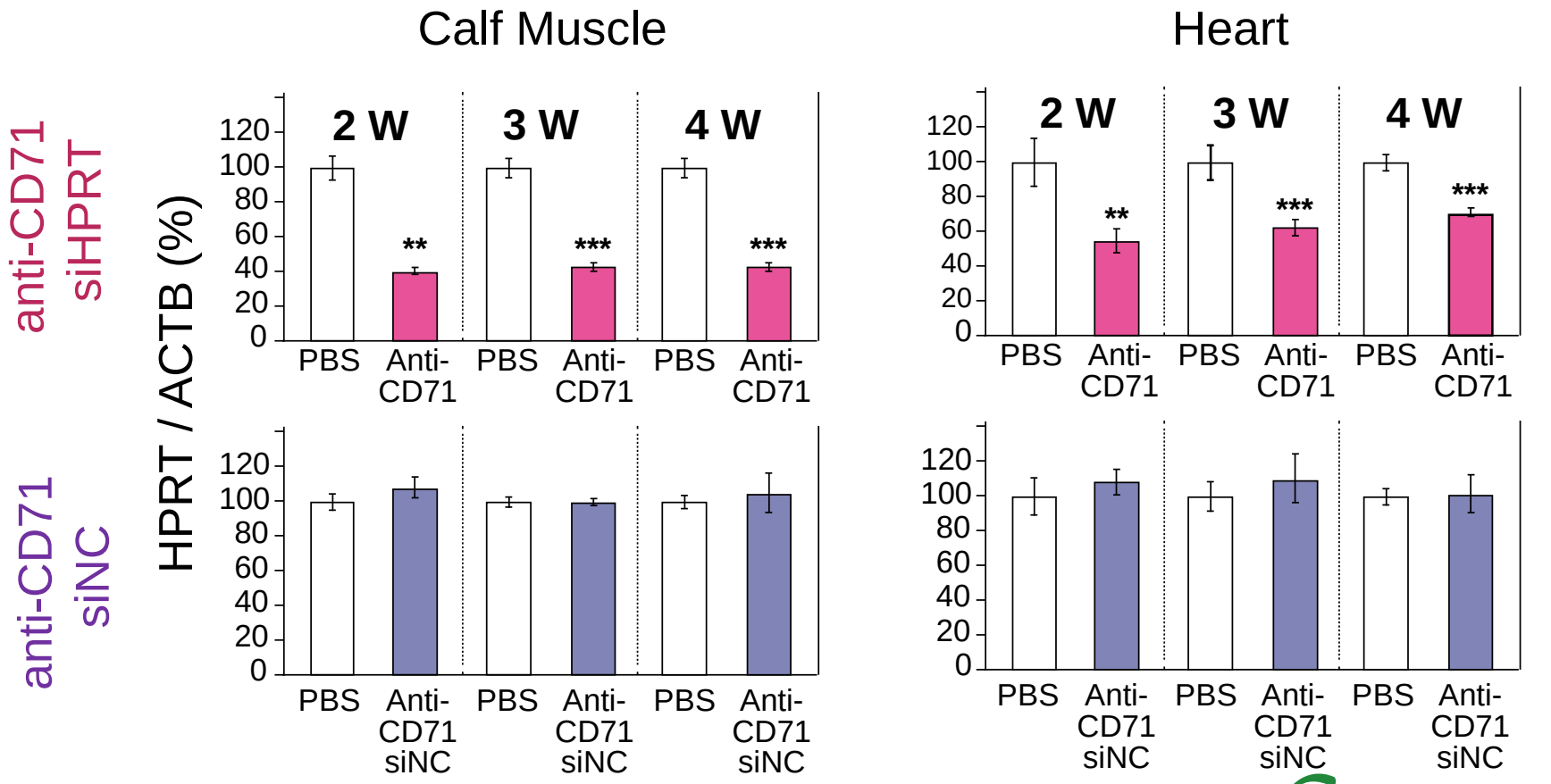
1 month silencing with Single *i.v.* Injection

HPRT silencing

10 mg/kg (*i.v.*)







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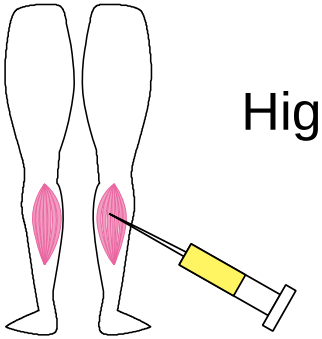
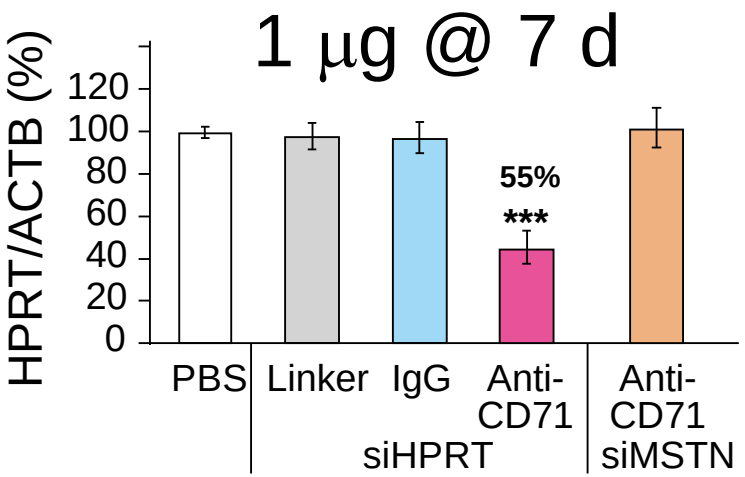
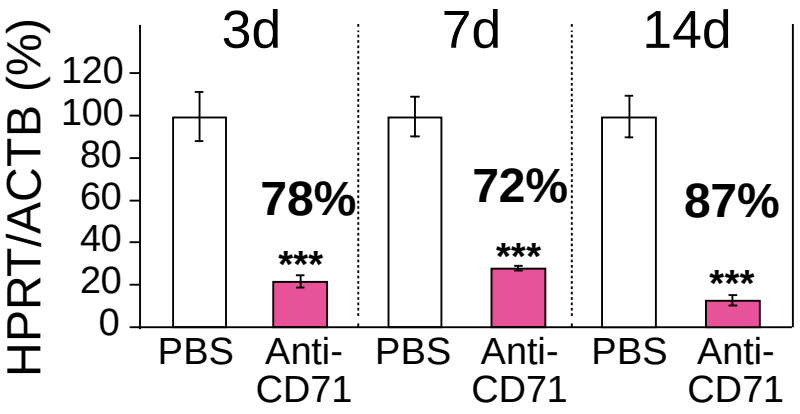
Direct Application: Intramuscular Injection

HPRT silencing

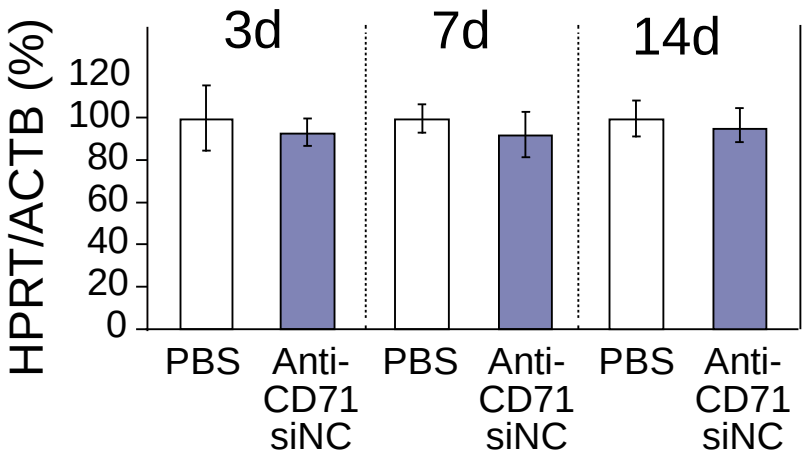


Highest CD71 Expression
~ Good Bioavailability





40 μg anti-CD71 siHPRT



40 μg anti-CD71 siNC

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- ✓ About therapeutic effects in disease model, please feel free to contact us directly.
- ✓ Gene silencing in cardiac/muscular tissues using CD71 was first demonstrated by us in J. Controlled Release 237, 1-13, (2016) as proprietary technologies.
- ✓ GenAhead Bio is looking forward to having a future of research and development partnership with pharmaceutical companies in both fields.

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