

Keystone eSymposium: "Precision Engineering
of the Genome, Epigenome and Transcriptome"



In Vivo Gene Editing of Hematopoietic Stem and Progenitor Cells

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Gene editing holds great promise for treating hereditary blood disorders

Sickle cell disease (SCD)

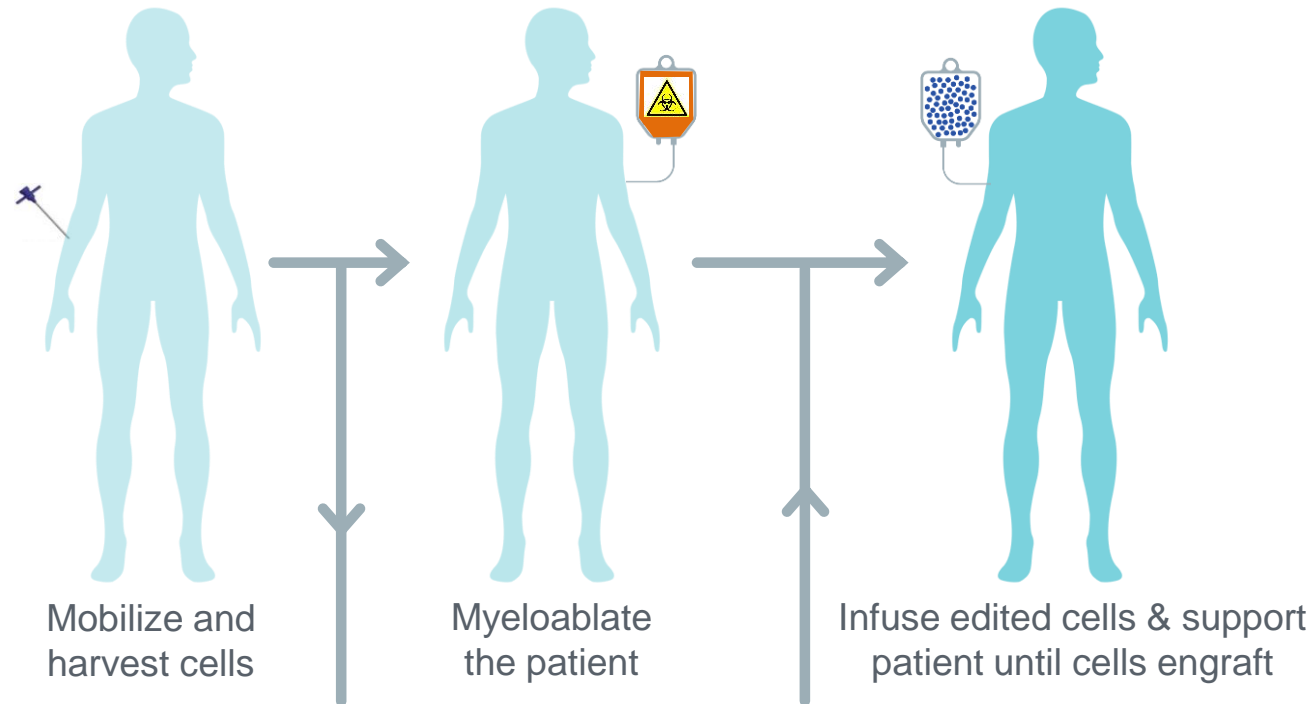
- Major global health issue impacting millions, caused by a mutation in the beta globin gene
- Characterized by severe pain and multi-organ injury with reduced life expectancy and quality of life
- Traditional treatments are limited; allogeneic hematopoietic stem cell (HSC) transplantation is reserved as a last resort for severely affected patients

→ ***Ex vivo* gene editing of autologous HSCs recently demonstrated benefit for SCD in a clinical trial**



Ex vivo SCD gene editing still has significant limitations

Complex cell manufacturing process



Conditioning regimen toxicity

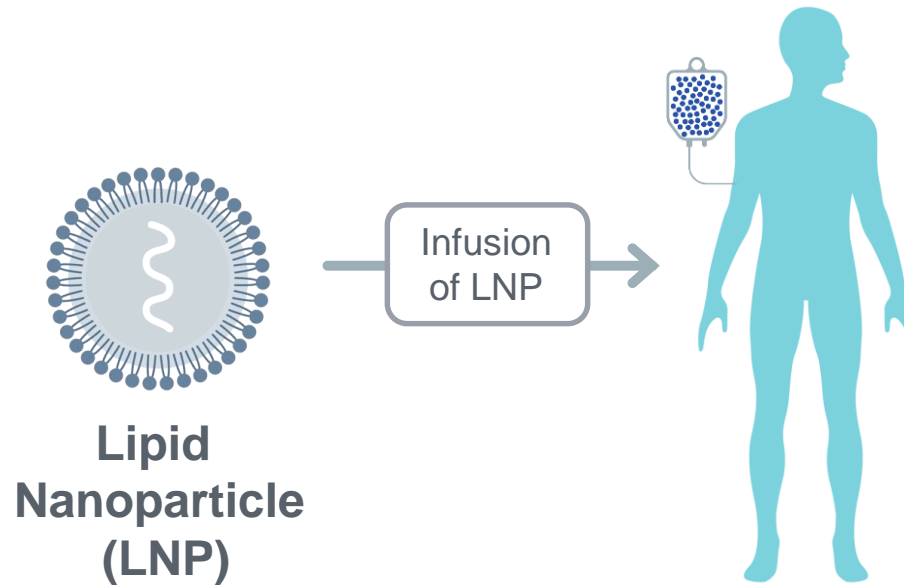
- Immunosuppression for > 1 month, predisposing to infection
- Risk of malignancy from chemotherapy drugs, especially leukemia
- Risk of infertility

Implications

- *Ex vivo* gene editing will be limited to highly selected SCD patients with severe disease
- Treatment complexity will limit access for patients in resource-poor settings

In vivo non-viral SCD gene editing could overcome these limitations

Simplified process



Improved safety and accessibility

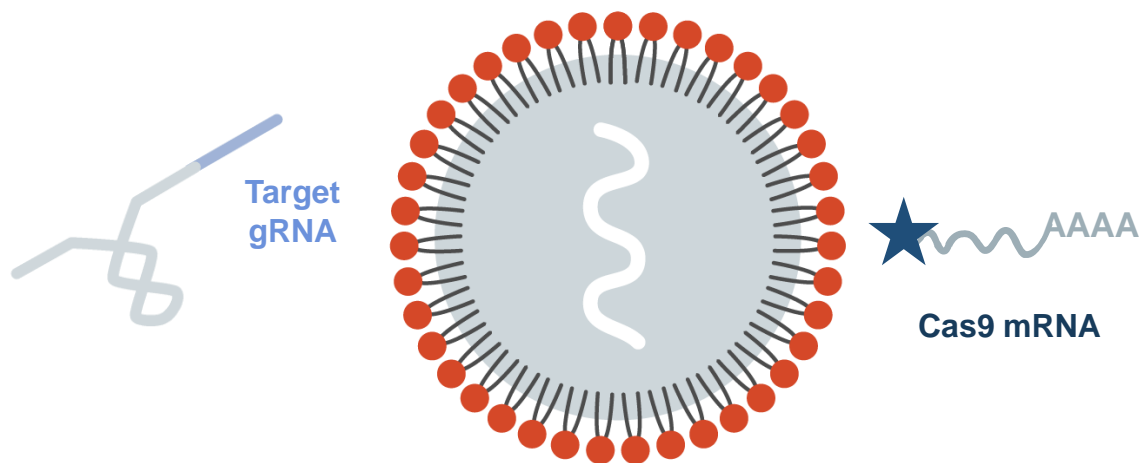
- Avoids myeloablation and associated risks of immunosuppression, malignancy, and infertility
 - Approach could become mainstream therapy for SCD
- Avoids need for complex cell manufacturing or extensive supportive care post-treatment
 - Treatment simplicity could expand access to patients in resource-poor settings

Desired features of *in vivo* approach

- Provides clinically meaningful, durable HSC editing
- Allows for multidosing to reach therapeutic target
- Preserves regenerative potential of edited cells
- Translatable to human HSC population

Intellia's modular *in vivo* editing platform employs non-viral LNP delivery

Lipid Nanoparticles



gRNA target site specificity
defined by 20mer at 5' end

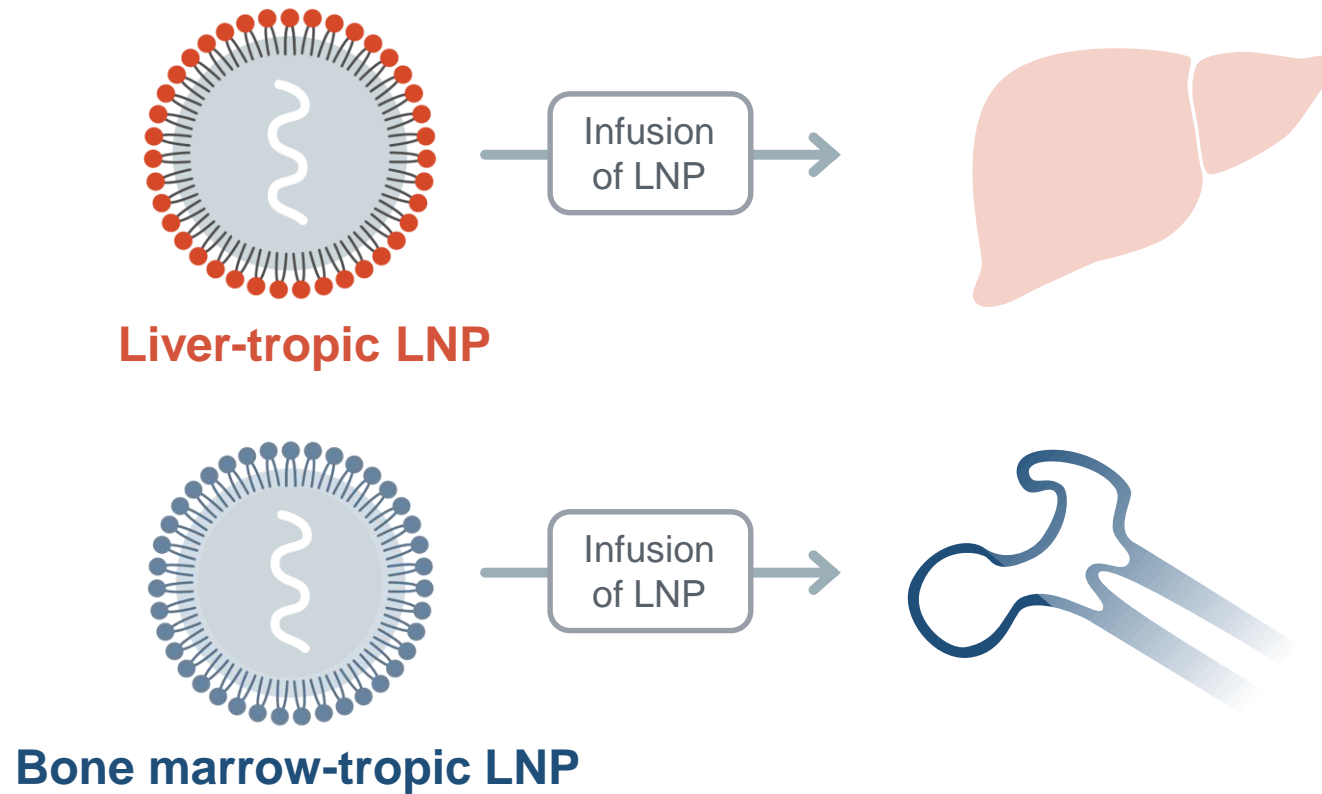
Transient Cas9
expression from mRNA

Key Advantages of LNP Delivery

- ✓ Large cargo capacity
- ✓ Biodegradable / transient expression
- ✓ Low immunogenicity
- ✓ Redosing capability
- ✓ Scalable synthetic manufacturing
- ✓ Tunable tropism

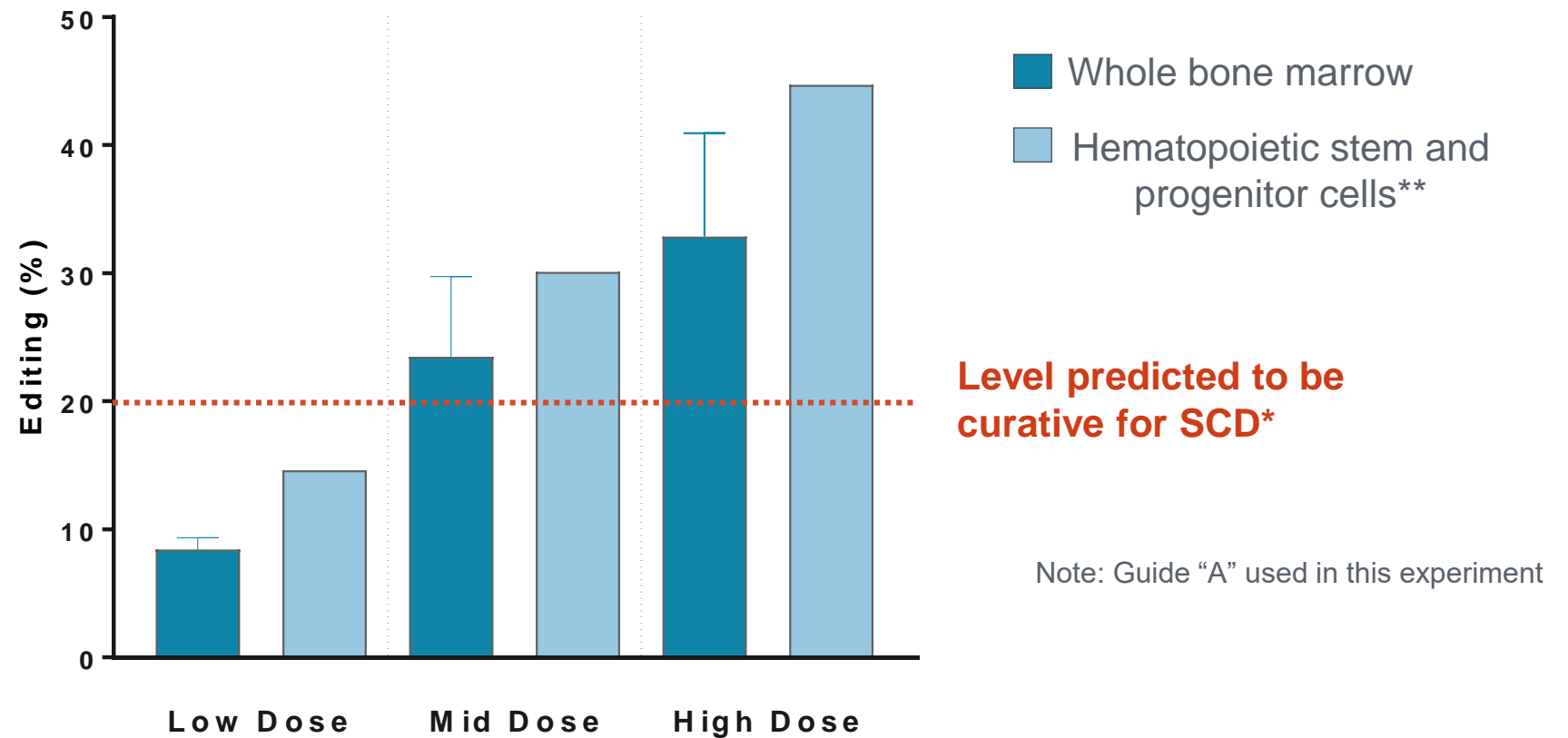
Editing HSCs *in vivo* requires LNPs with bone marrow tropism

- LNPs designed, formulated, and tested *in vivo* to identify compositions with enhanced delivery to bone marrow and HSCs



Bone marrow-tropic LNP enables editing of mouse bone marrow as well as hematopoietic stem and progenitor cells (HSPCs)

- Dose dependent editing at 1 week post dosing in whole bone marrow and HSPC populations
- Levels predicted to have therapeutic benefit if achieved in patients with SCD

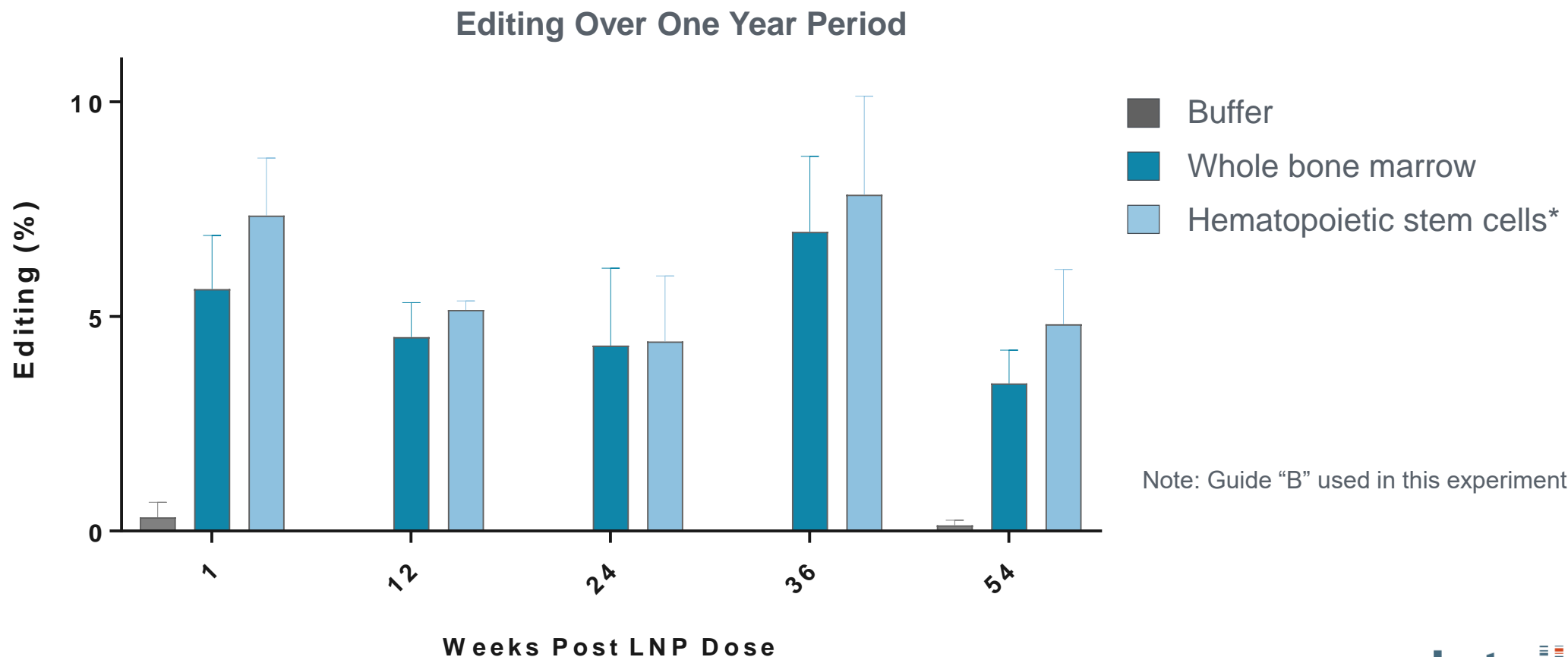


* *Blood*. 2017;130(17):1946-1948.

** Lin⁻Sca-1⁺c-Kit⁺ (LSK) cell population

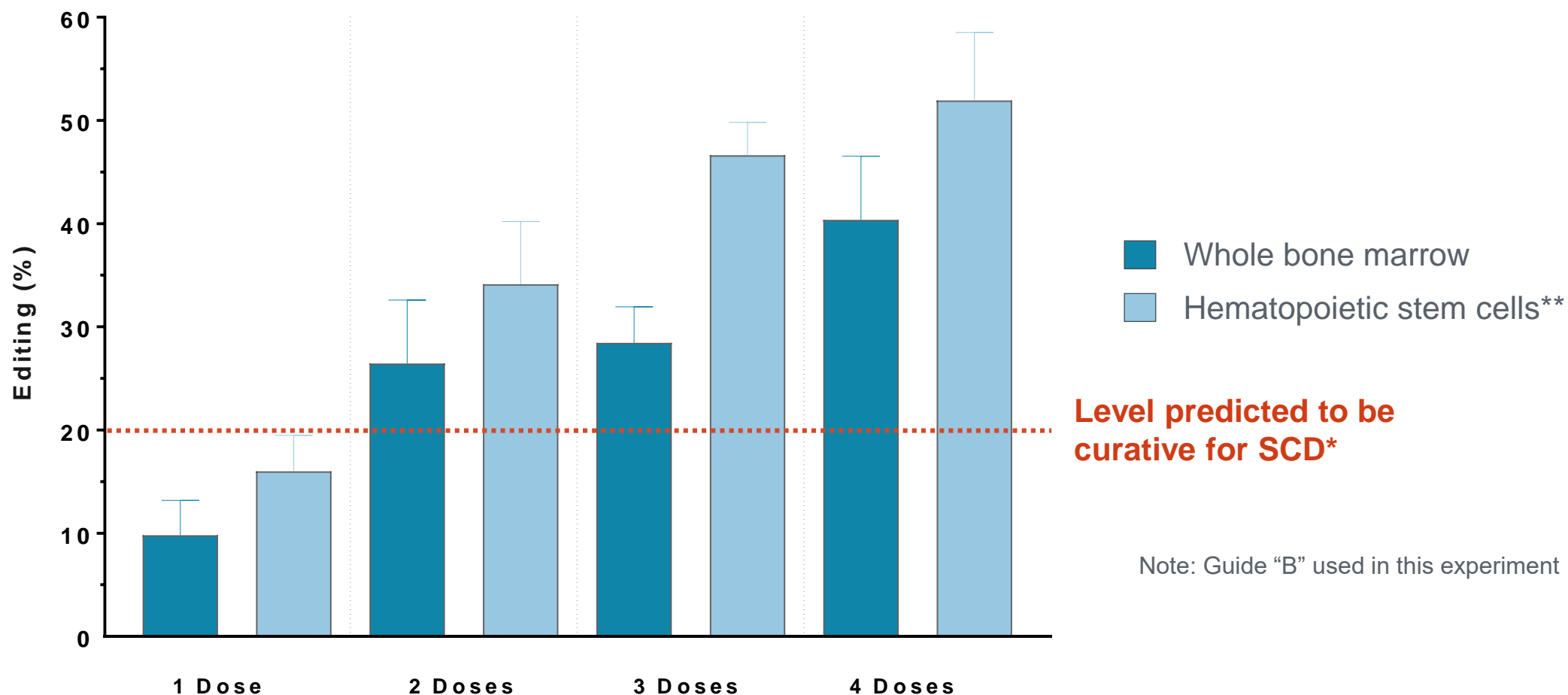
Editing of mouse bone marrow and HSCs is durable through at least one year

- Editing was similar across all time points assessed, in both whole bone marrow and HSCs populations
- Results highlight the potential for a single-course, long-lasting therapy



Editing of mouse bone marrow and HSCs increases with multidosing

- Non-immunogenic LNP delivery platform may enable stepwise “treat-to-target” approach



* *Blood*. 2017;130(17):1946-1948.

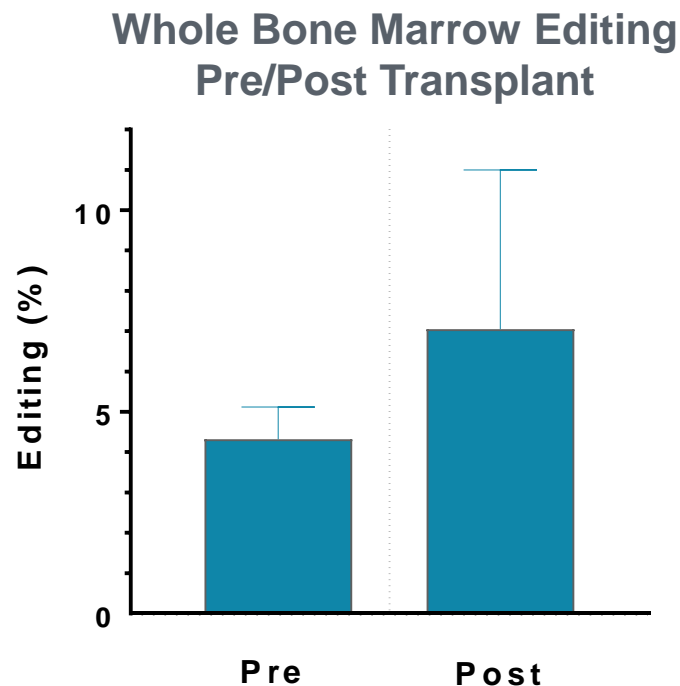
** Lin⁻Sca-1⁺c-Kit⁺CD34⁺Flk2⁻ cell population

Edited HSCs retain their capacity to reconstitute primary hematopoietic lineages

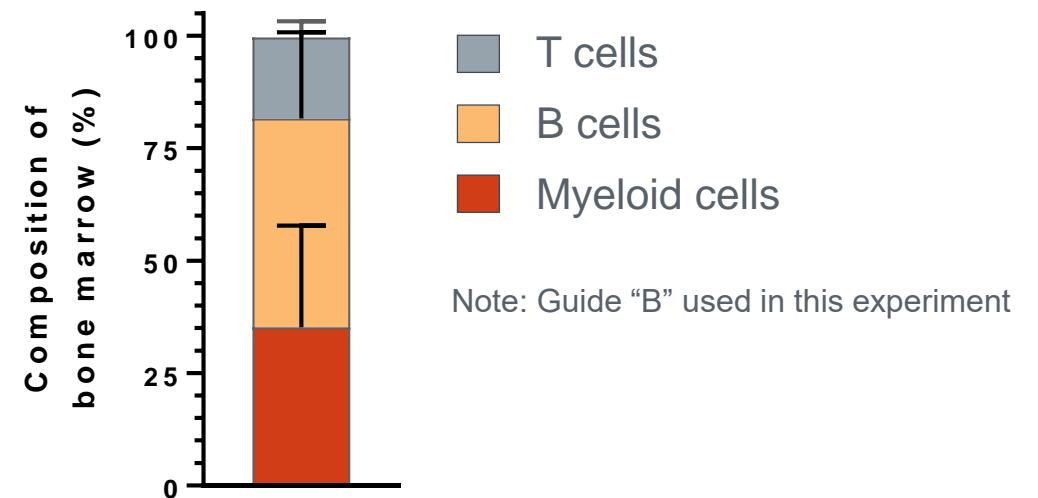
In a competitive transplant model using mice treated with a single dose of LNP, edited cells:

- ✓ Remained stable as a percentage of the bone marrow and HSC populations over a 14-week period
- ✓ Reconstituted healthy bone marrow with normal production of lymphoid and myeloid lineages

→ **Nonviral approach preserves normal stem and progenitor cell function in mice**

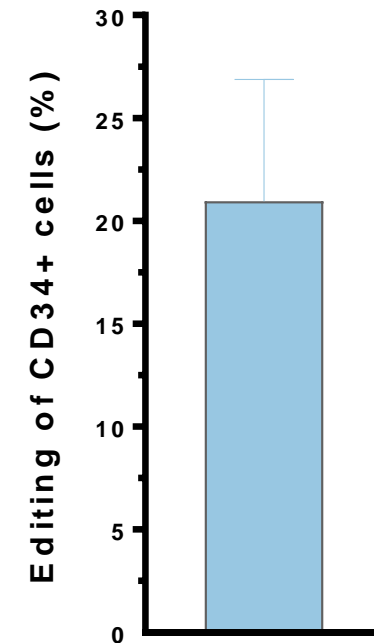
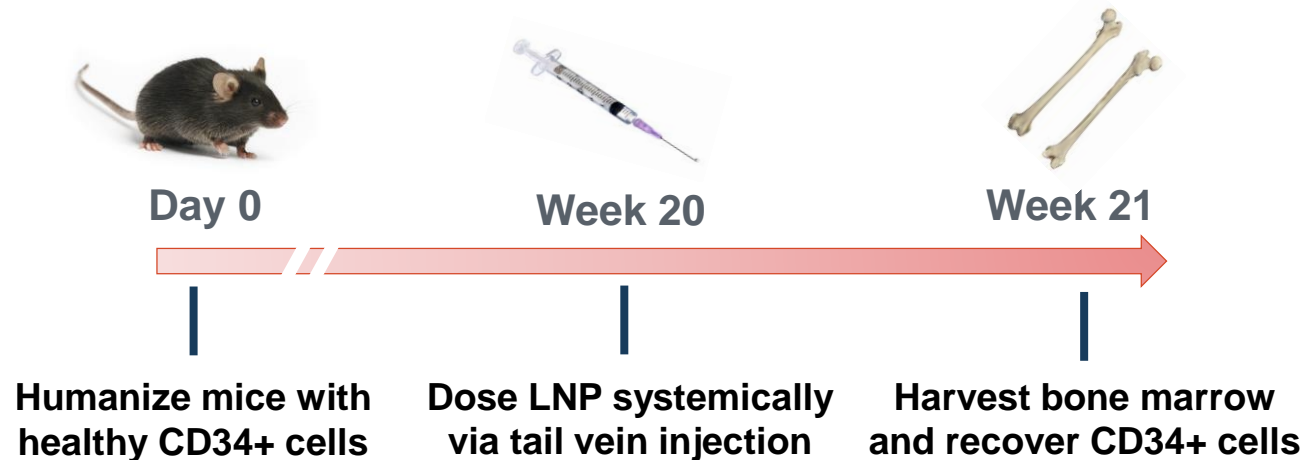


Normal Lineage Distribution



Nonviral CRISPR/Cas9 delivery supports editing of human HSPCs in mice

- Single *in vivo* dose of LNP achieved clinically significant level of editing in human CD34+ cells
- Results suggest cross-species relevance of LNP platform for genome engineering of HSPCs



Note: guide "C" used in this experiment

Key Takeaways

- LNPs well suited to deliver CRISPR/Cas9 to hematopoietic cells for durable *in vivo* gene editing
- Achieved clinically relevant levels of HSC editing in wild-type mice
- Multidosing of LNPs increased editing in stepwise manner, potentially enabling “treat-to-target” therapeutic approach
- Edited cells retain regenerative potential and reconstitute primary hematopoietic lineages
- Achieved therapeutically relevant levels of editing of human CD34+ cells in a xenotransplant mouse model, highlighting cross-species translation

Next step: Demonstrate relevance to future clinical application by confirming activity in nonhuman primate model

Intellia

THERAPEUTICS