Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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beti-cel & eli-cel Advisory Committee Meeting: Introduction – June 9, 2022, Afternoon

Lentiviral Vector Safety

Anne-Virginie Eggimann, MSc

Chief Regulatory Officer

bluebird bio, Inc.

bluebirdbio®

bluebird bio Uses Two Different Lentiviral Vectors (LVVs) to Manufacture Three Products

bluebird bio Uses Two Different Lentiviral Vectors (LVVs) to Manufacture Three Products

Lenti-D LVV

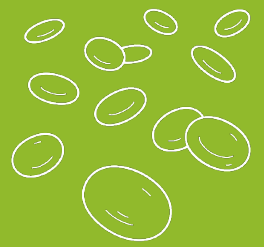
elivaldogene autotemcel (eli-cel) for early active cerebral adrenoleukodystrophy (CALD) BLA 125755

bluebird bio Uses Two Different Lentiviral Vectors (LVVs) to Manufacture Three Products

Lenti-D LVV

elivaldogene autotemcel (eli-cel) for early active cerebral adrenoleukodystrophy (CALD)
BLA 125755

BB305 LVV



betibeglogene autotemcel (beti-cel) for β-thalassemia requiring regular transfusions
BLA 125717

bluebird bio Uses Two Different Lentiviral Vectors (LVVs) to Manufacture Three Products

TODAY

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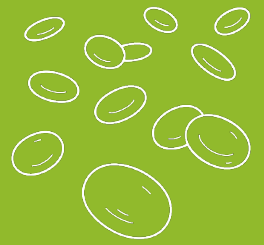
TOMORROW

BB305 LVV



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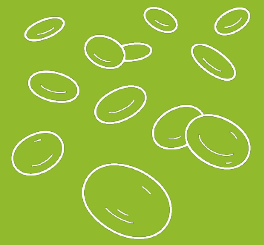

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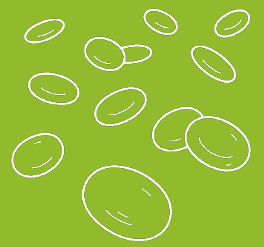
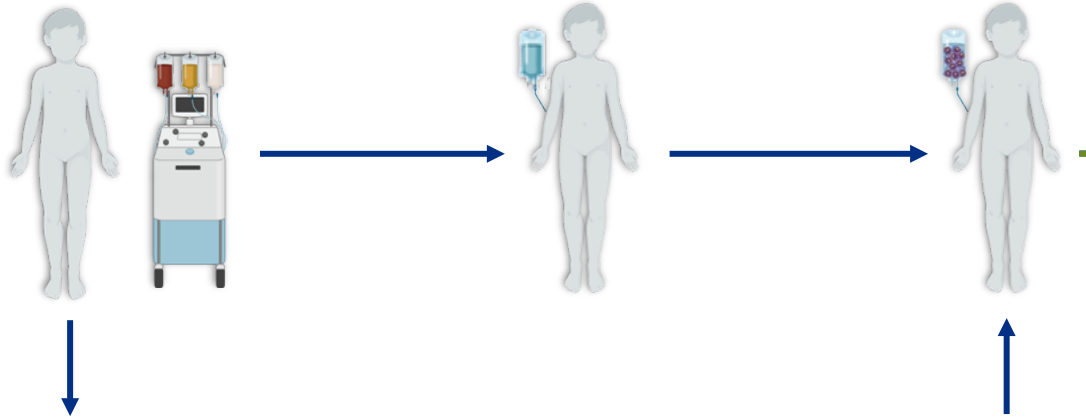


betibeglogene autotemcel (beti-cel) for β-thalassemia requiring regular transfusions
BLA 125717

lovotibeglogene autotemcel (lovo-cel) for sickle cell disease in Phase 3 clinical development

Each LVV is custom-designed for a specific mechanism of action, and each LVV has a distinct safety profile

Each Lentiviral Vector is Custom Designed to Express a Specific Protein in a Specific Cell Type

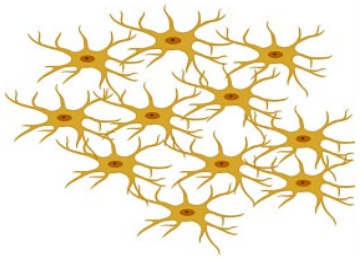


Manufacturing

- For eli-cel: **Lenti-D LVV** adds ABCD1 cDNA into the patient's CD34+ HSCs
- For beti-cel and lovo-cel, **BB305 LVV** adds the $\beta^{A\text{-}T87Q}$-globin gene into the patient's CD34+ HSCs

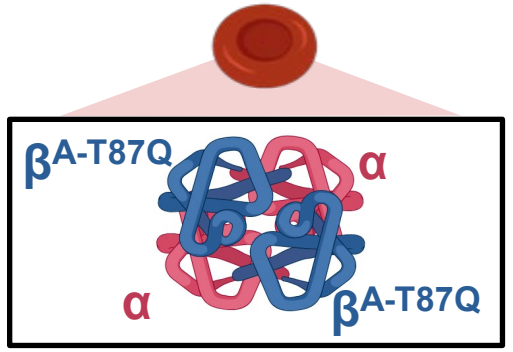
eli-cel

Transduced HSCs engraft and differentiate into cerebral microglia expressing ALD protein (ALDP)



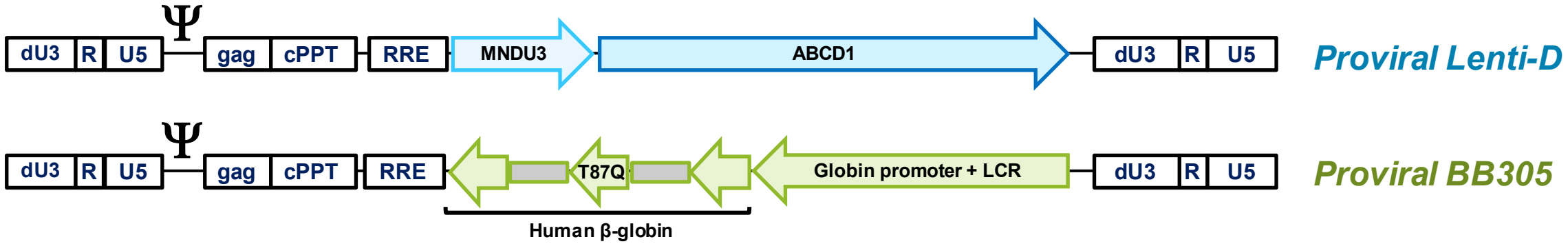
beti-cel and lovo-cel

Transduced HSCs engraft and differentiate into red blood cells (RBCs) with beti-cel derived adult hemoglobin



RBC containing beti-cel derived adult Hb, referred to as **HbA^{T87Q}**

Lenti-D and BB305 LVV Have Several Different Key Features, Including a Different Promoter



Feature	Lenti-D LVV	BB305 LVV
Transgene	*ABCD1*	$\beta^{A\text{-}T87Q}$ globin
Transcriptional control	modified viral MNDU3 promoter	human β-globin promoter and locus control region
Gene structure	cDNA	natural exon/intron
Cell type	ubiquitous; all hematopoietic lineages	specific; erythrocytes / red blood cells
Splicing	no transgene splicing	transgene undergoes splicing
PolyA signal	3'LTR	human β-globin

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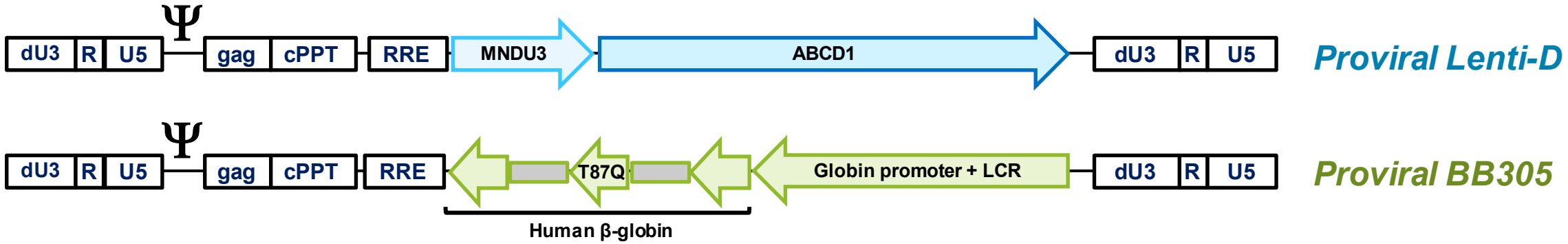

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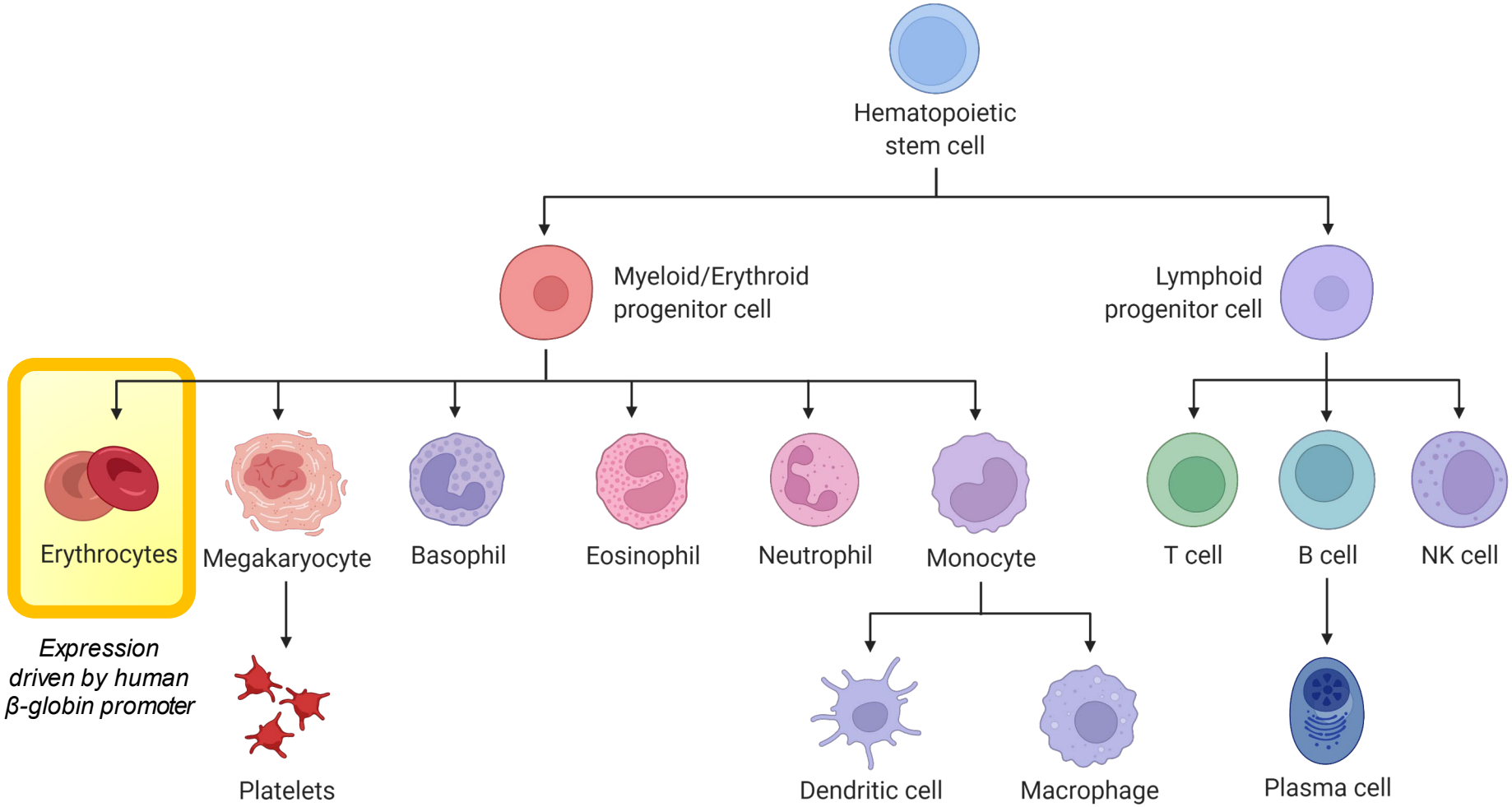
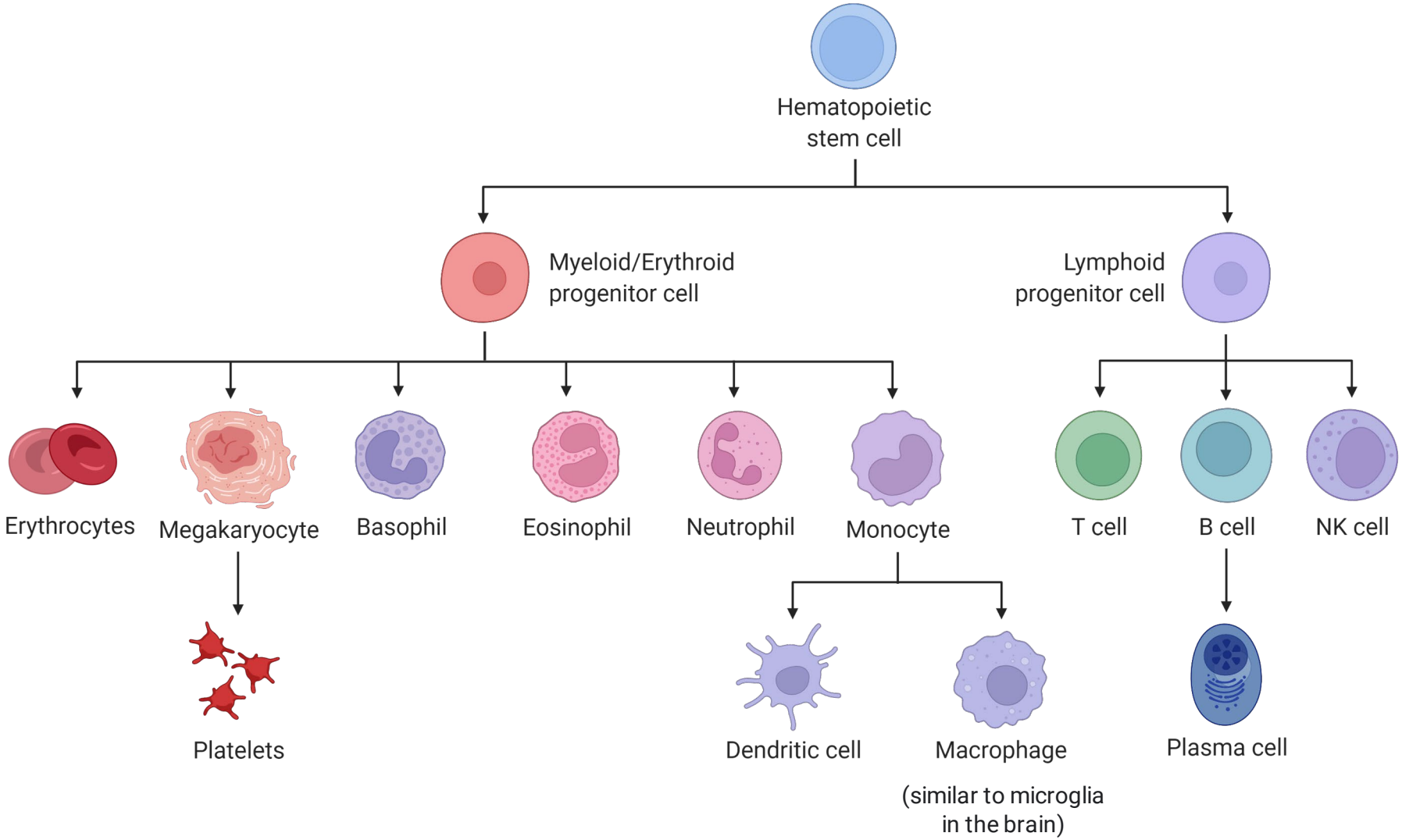
BB305 LVV uses Human β-globin Promoter which Drives High Gene Expression only in **Erythroid Lineage**



Lenti-D LVV Uses a Modified Viral MNDU3 Promoter which Drives High Levels of Gene Expression in **All Lineages**



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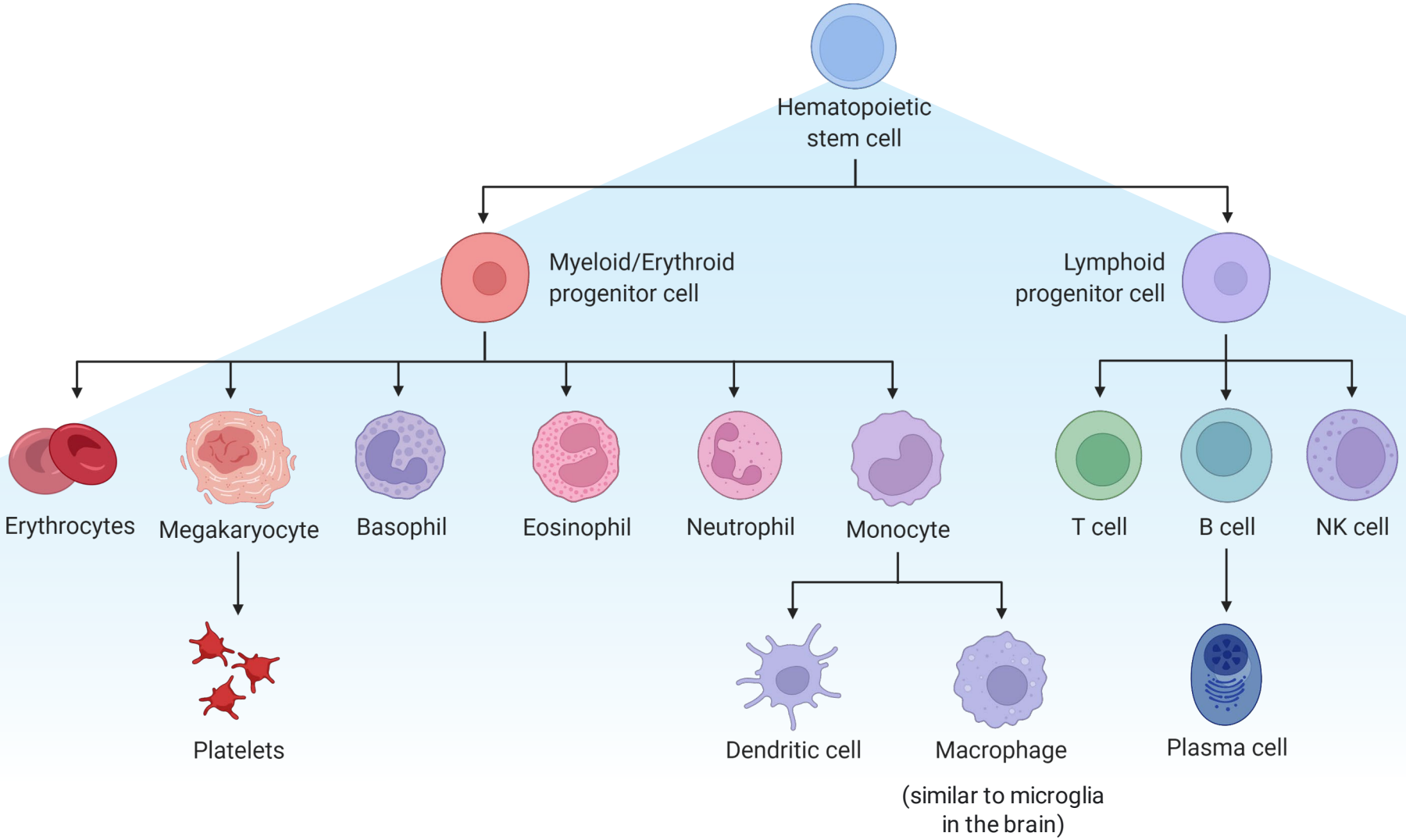

Each LVV is Designed for a Specific Purpose and Has a Unique Safety Profile

LVV safety, along with the inherent risks of the treatment process, must be weighed against:

1. **severity** of each disease
2. availability of **other treatments** and their risks
3. probability and magnitude of **life-long benefit** gene therapy could offer

beti-cel & eli-cel Advisory Committee Meeting: Lentiviral Vector Safety

Melissa Bonner, Ph.D.

Senior Vice President, Head of Research

bluebird bio, Inc.

bluebirdbio®

Vector-Related Safety Profiles Differ

eli-cel for CALD
(elivaldogene autotemcel)

Lenti-D LVV
LVV-Mediated Insertional Oncogenesis Observed

67 patients treated

3 malignancies

All 3 Lenti-D LVV mediated insertional oncogenesis

beti-cel for β-Thal
(betibeglogene autotemcel)

63 patients treated

0 malignancy

0 insertional oncogenesis

lovo-cel for SCD
(lovotibeglogene autotemcel)

BB305 LVV
No LVV-Mediated Insertional Oncogenesis has Been Observed

50 patients treated

2 malignancies

0 insertional oncogenesis

CALD: cerebral adrenoleukodystrophy; β-thal: β-thalassemia; SCD: sickle cell disease; LVV: lentiviral vector

Agenda

Background on retroviral vectors

- Safety events warranted development of safer vector designs
- Current LVVs designed to lower risk of insertional oncogenesis
- Benefits and risks of LVV
- LVV traceability and Integration Site Analysis

Lenti-D LVV used for manufacture of eli-cel

- Mechanism of action necessitates ubiquitous transgene promoter
- LVV mediated safety events

- Mechanism of action necessitates erythroid specific promoter
- No LVV mediated safety events

LVV: lentiviral vector

Retroviruses are a Family of RNA Viruses

- **Gammaretroviral vectors (GRVs) and lentiviral vectors (LVVs) are 2 distinct classes of retroviral vectors used for hematopoietic stem cell gene therapies with different clinical safety profiles**

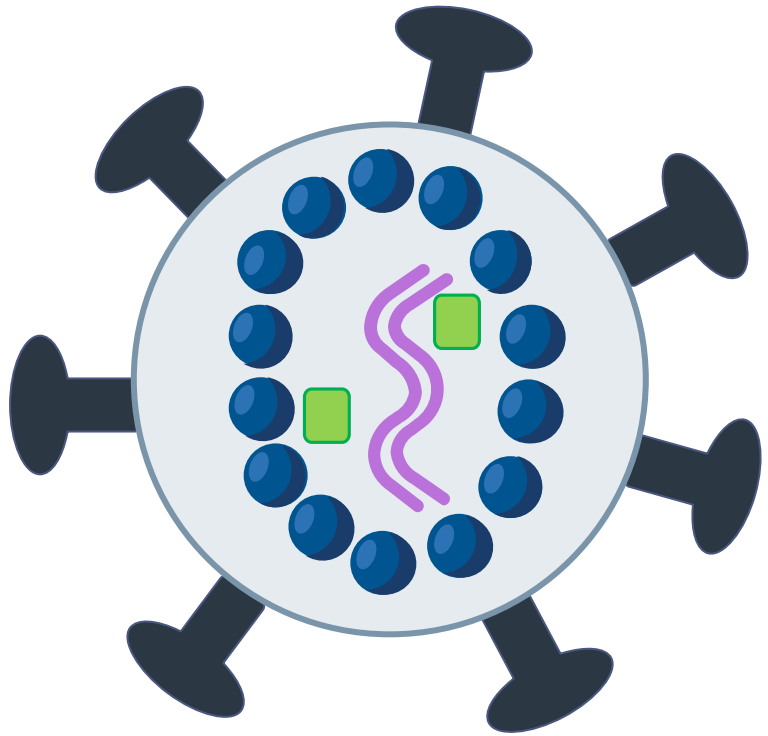

Figure adapted from https://en.wikipedia.org/wiki/Viral_vector accessed 25Apr2022

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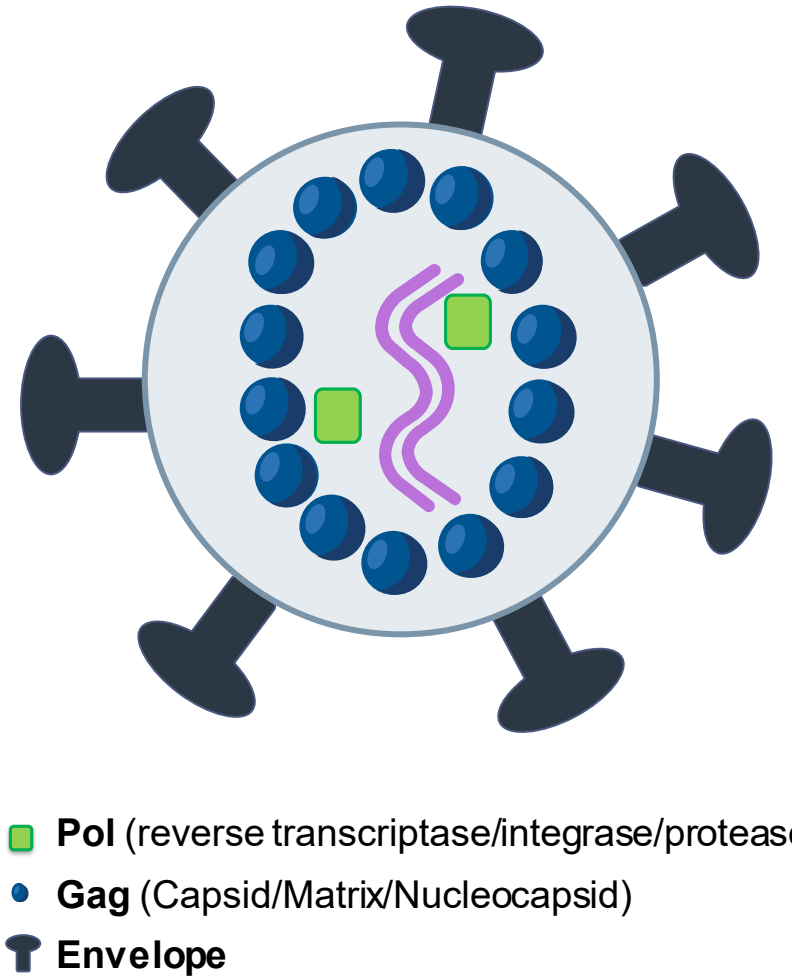
- **Retroviruses are RNA viruses that reverse transcribe viral RNA into DNA which can be integrated into genomic DNA of target cells**



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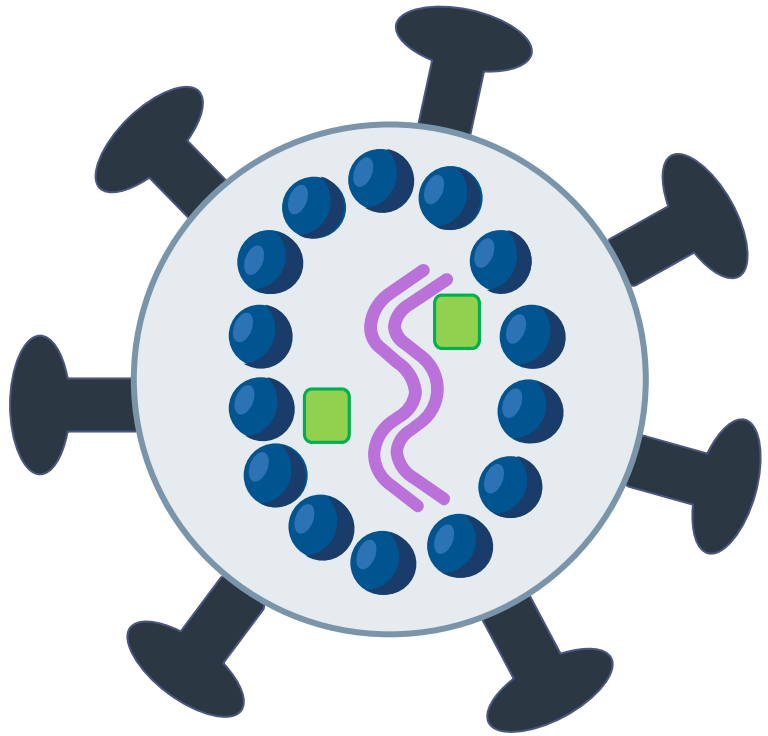
- **Retroviruses are RNA viruses that reverse transcribe viral RNA into DNA which can be integrated into genomic DNA of target cells**
- **Retroviral vectors are modified retroviruses**
  - Viral genes are replaced with a therapeutic transgene which can be delivered to target cells via a process called transduction
  - Absence of intact viral genes that encode proteins renders these vectors **replication incompetent**



Pol (reverse transcriptase/integrase/protease)
Gag (Capsid/Matrix/Nucleocapsid)
Envelope

Figure adapted from https://en.wikipedia.org/wiki/Viral_vector accessed 25Apr2022

GRVs Led to Insertional Oncogenesis in Some Gene Therapy Patients

- **Insertional oncogenesis refers to a malignancy that has directly resulted from the integration of a provirus**

- **GRVs used across a number of different diseases led to outcomes of insertional oncogenesis (occurrence 2-90%)[1]**
  - 6 cases of T-cell acute lymphoblastic leukemia out of 25 X-SCID patients treated
  - 5 cases of myeloblastic syndromes out of 15 CGD patients treated
  - 9 cases of acute leukemia out of 10 WAS patients treated
  - 1 case of lymphoid T-cell leukemia out of 68 ADA-SCID patients treated
  - *84% of insertional oncogenesis cases occurred within 5 years of treatment[1]*

GRV: gammaretroviral vector; X-SCID: X-linked severe combined immunodeficiency; CGD: chronic granulomatous disease; WAS: Wiskott-Aldrich syndrome; ADA-SCID: adenosine deaminase-severe combined immunodeficiency

[1]Tucci et al., *Nat Commun*. 2022;13(1):13

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- **These clinical trials demonstrated strong efficacy, but the serious adverse events necessitated development of a safer vector design**

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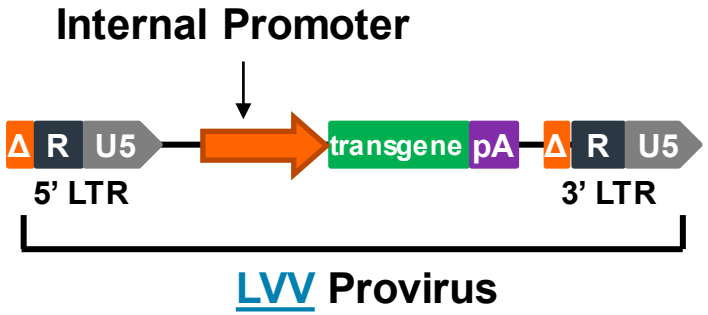
Current LVVs are Designed to Lower Risk of Insertional Oncogenesis



- **Designed to be SIN by deleting the viral promoter and enhancer from the LTRs**
  - Diminished potential for influencing the expression of nearby genes
- **Expression of the therapeutic transgene is controlled by an internal promoter which can allow for expression in the appropriate cell type**

LVV: lentiviral vector; SIN: self inactivating; LTR: long terminal repeats; HIV: human immunodeficiency virus

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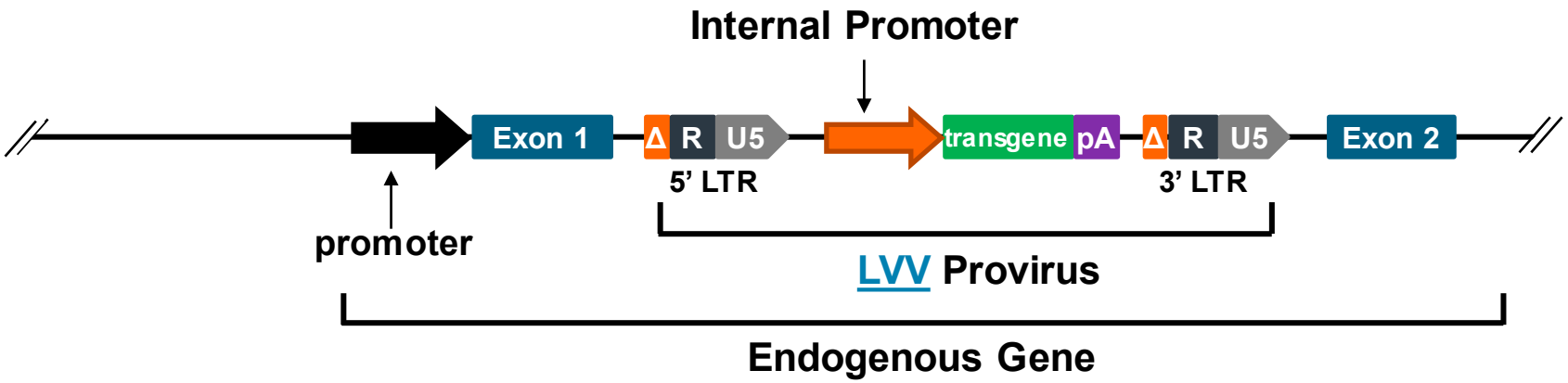

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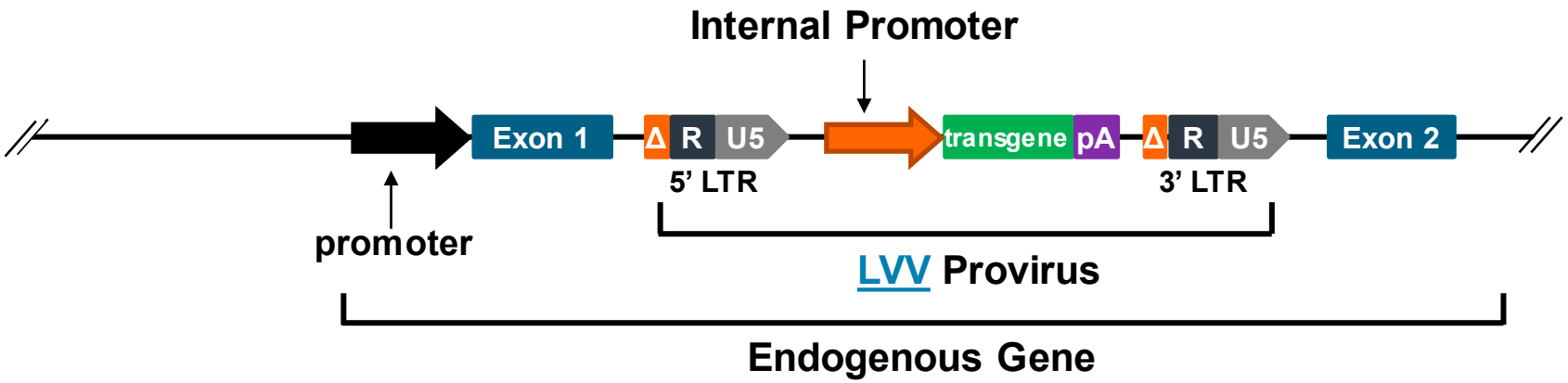

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- **Contains no intact viral genes**
  - <25% of HIV-1 genome in provirus

LTR: long terminal repeats; LVV: lentiviral vector; SIN: self inactivating; HIV: human immunodeficiency virus

LVVs have Features that are Ideal for One-Time Therapies…

Benefit

- **Therapeutic vector is stably integrated into host cell genome**
  - LVV integrate into dividing and non-dividing cells
  - Transgene is carried to all daughter cells
  - Important for hematopoietic stem cell-based gene therapy
- **Therapeutic vector is durable**
  - Therapeutic benefit expected to be life-long
- **Integrations are traceable**
  - Integration site analysis is a robust, sensitive tool

LVV: lentiviral vector
Poletti and Mavilio, *Viruses.* 2021; 13(8):1526.

…but are Not Without Some Potential Risks

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Risk

- **All insertions are mutations**
- **Mutations may impact an endogenous gene**
  - No effect
  - Impaired gene function/knock-out
  - Altered splicing
  - Increased gene activity
- **Integration into the genome could lead to *insertional oncogenesis***

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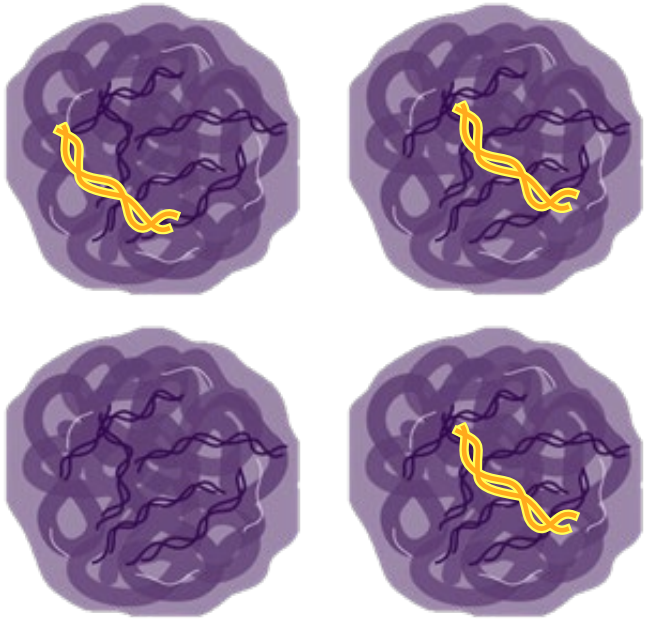
LVV Integration Creates a Unique and Traceable Genetic Barcode



Semi-random LVV integration creates unique mappable insertion sites

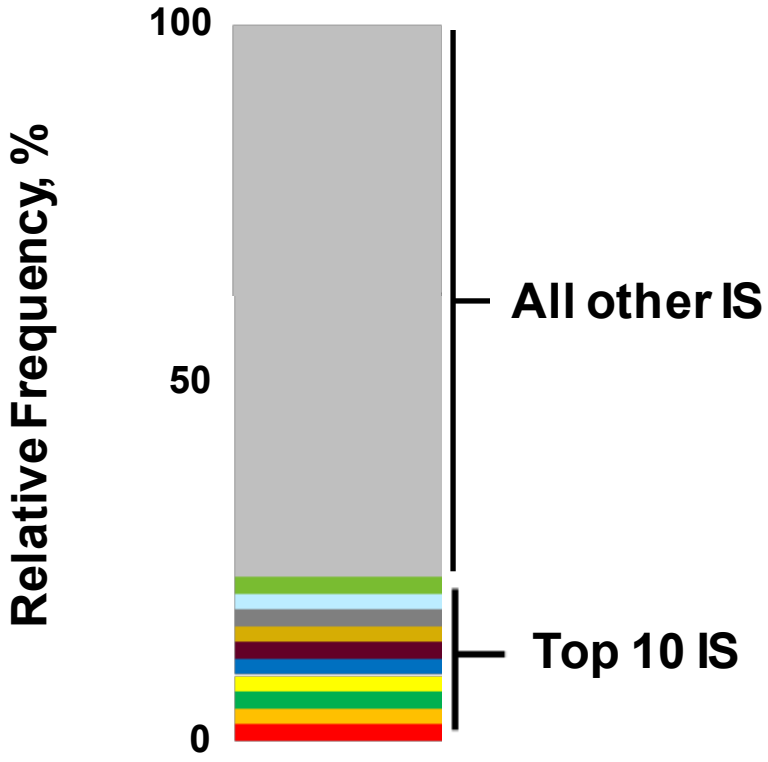
Unique locations of integrated provirus in the genome can be mapped by sequencing genomic DNA

This high throughput way of identifying vector insertion sites is called Integration Site Analysis (ISA)

LVV: lentiviral vector; ISA: integration site analysis

Integration Site Analysis (ISA) is an Important Tool for Tracking Transduced Cells

Representation of ISA Data



Identifies a population of unique mappable insertion sites[1]

Allows tracking of clonal populations

ISA enables further investigation to help determine the potential role of a specific vector insertion in gene dysregulation and oncogenesis[1,2]

Top 10 insertion sites are usually the focus of ISA and indicated by colored bars

ISA: integration site analysis; IS: insertion site

1. Biasco L et al., *Mol Ther Methods Clin Dev.* 2018; 8: 21–30. 2017; 2. Biasco L., *Hum Gene Ther*. 2017;28:1122-1129.

What ISA Can (and Cannot) Do

ISA Can

- Track clonal dynamics over time
- Nominate individual clones for further characterization
- Suggest whether >1 insertions are likely present in a single clone
- Identify oligoclonality for regulatory reporting, communications to treating physicians

ISA Cannot

- Predict which (if any) clones will become predominant
- Predict whether oligoclonality will decrease/increase over time
- Predict clinical outcomes or disease onset
- Detect oligoclonality in untransduced cells

ISA is a useful tool that allows for **traceability** of clonal populations, but it is **not predictive**

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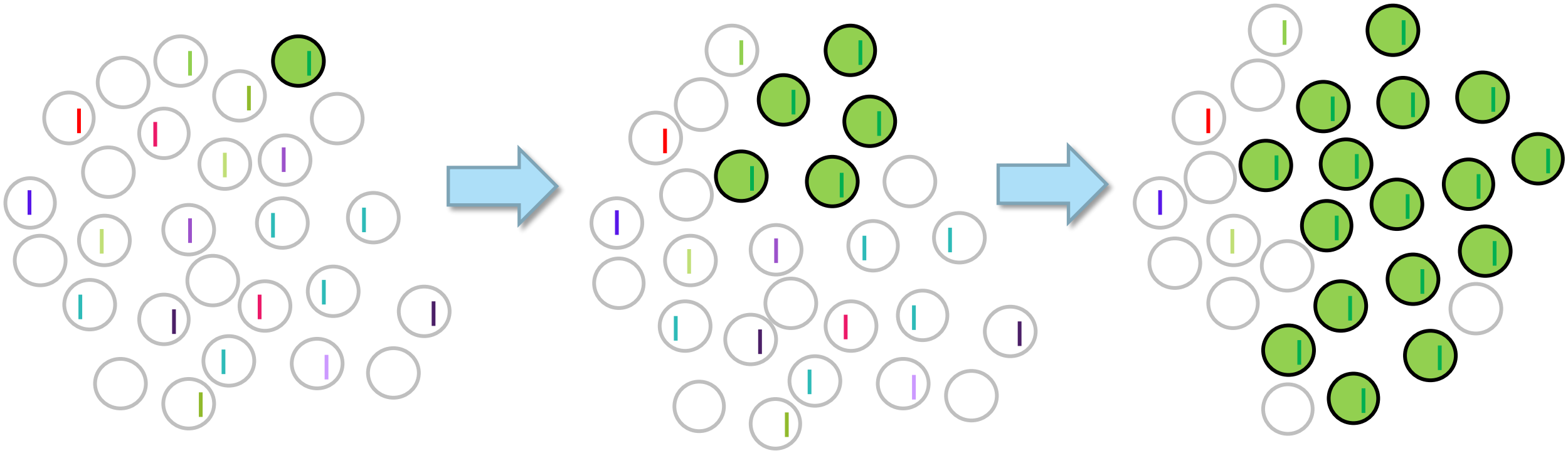
Illustrative Example ISA Tracking Over Time



KEY

Cell

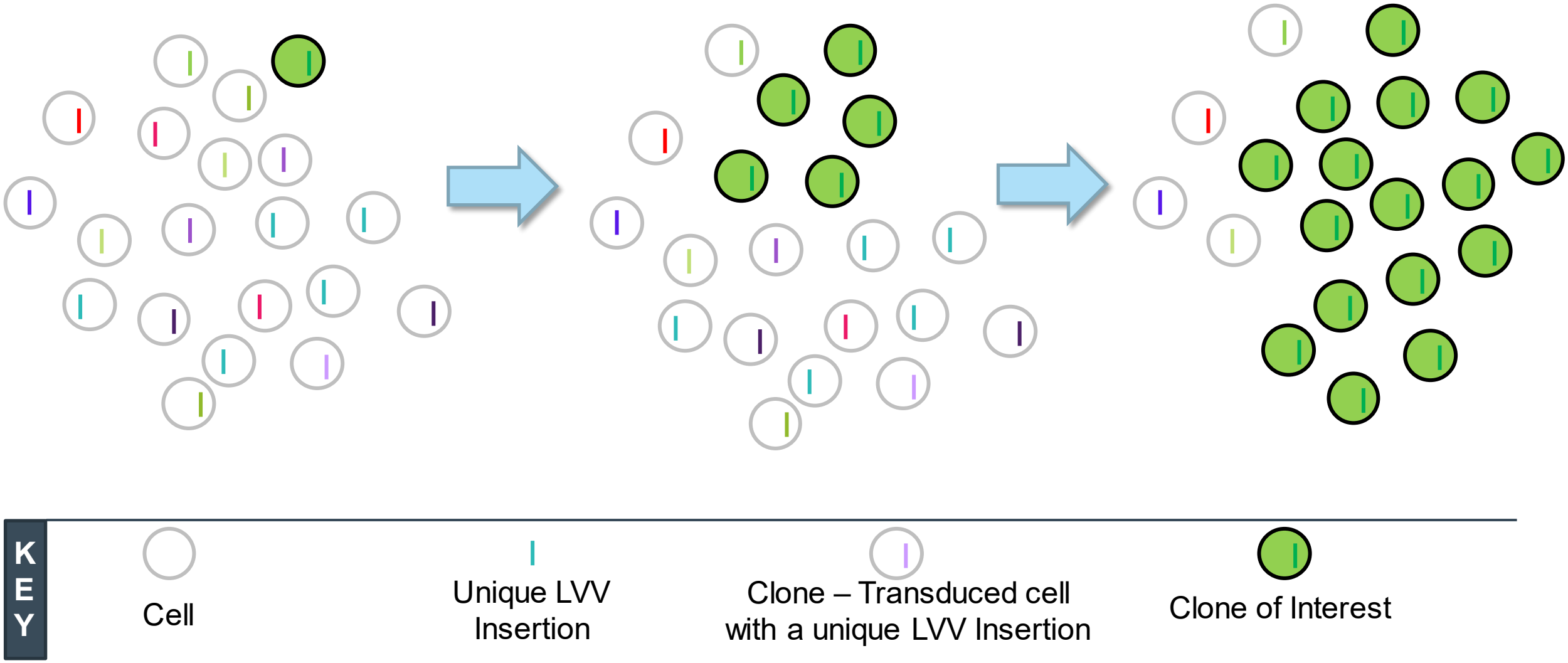
Unique LVV Insertion

Clone – Transduced cell with a unique LVV Insertion

Clone of Interest

ISA: integration site analysis; LVV: lentiviral vector; IS: insertion site

Illustrative Example ISA Tracking Over Time
ISA Only Detects Transduced Cells



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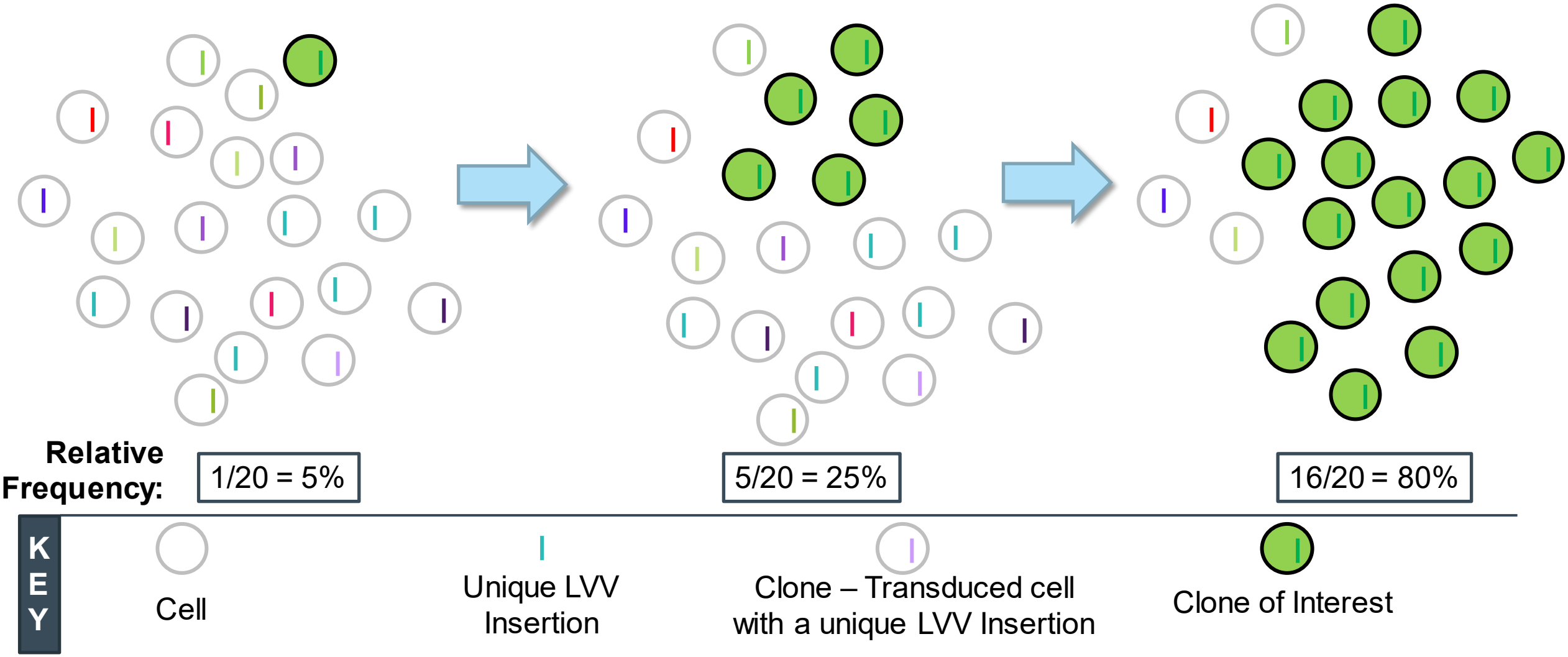

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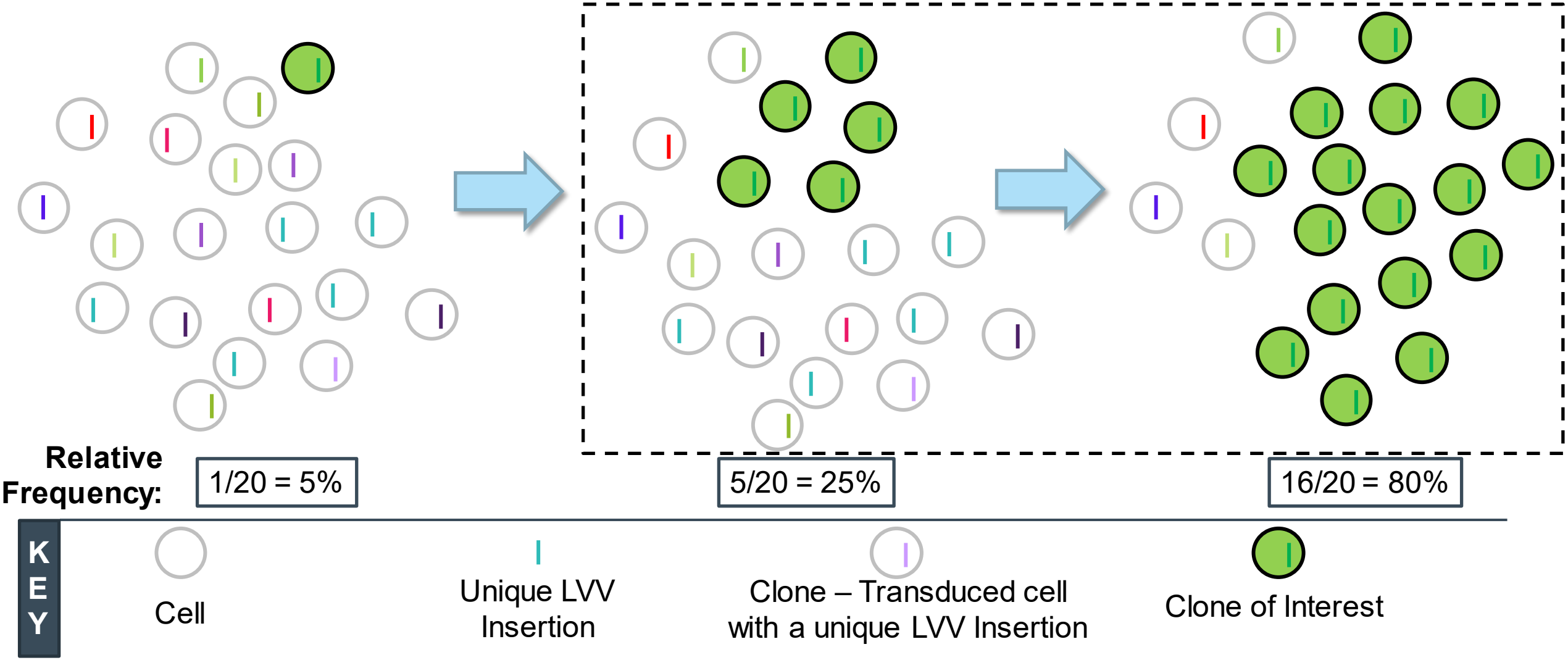

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What Does 'Oligoclonality' Mean?

Oligoclonality Can

- *Suggest* clonal hematopoiesis
- Occur in the absence of a hematological abnormality
- Satisfy regulatory guidance for post-treatment monitoring in clinical setting
- Trigger follow-up testing (i.e., CBC) out of abundance of caution

Oligoclonality Cannot

- Diagnose malignancy
- Predict development of malignancy
- Determine contribution of a clone to heterogeneous population containing untransduced cells

Oligoclonality does not equal malignancy

ISA: integration site analysis; CBC: complete blood count

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Oligoclonality Cannot

Diagnose malignancy

Predict development of malignancy

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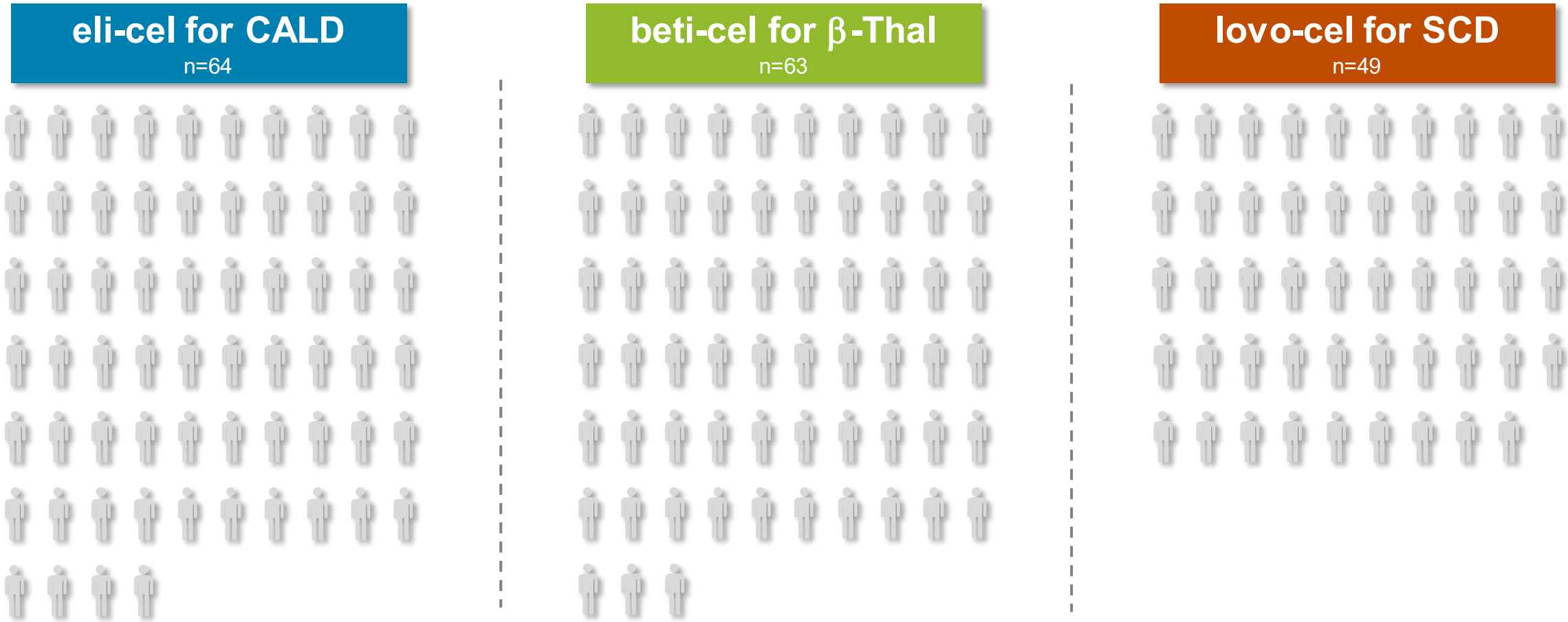
Patients who Meet Criteria for Oligoclonality

Oligoclonality defined as any IS ≥10% and PB VCN ≥ 0.1 c/dg



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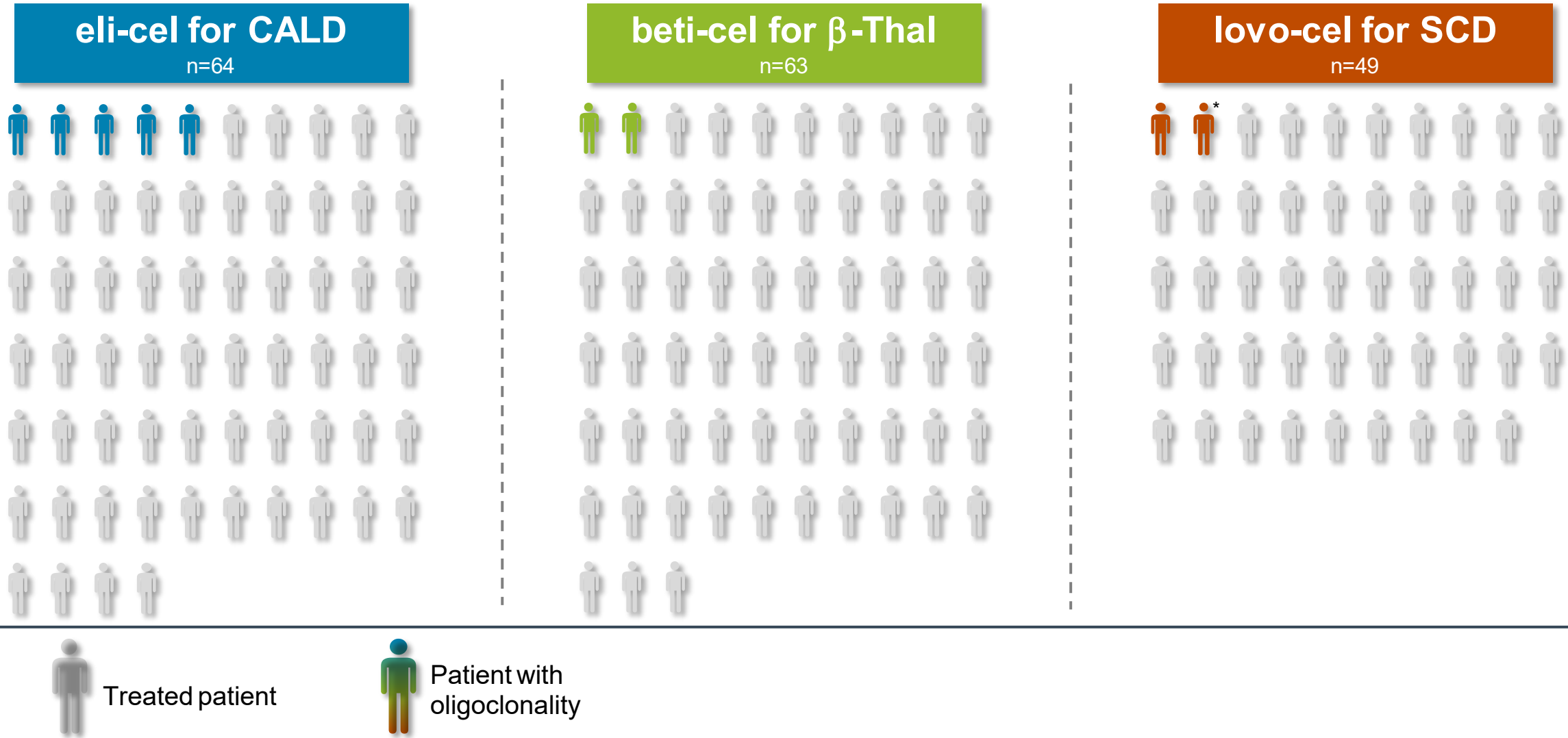
Patients who Meet Criteria for Oligoclonality

Oligoclonality defined as any IS ≥10% and PB VCN ≥ 0.1 c/dg



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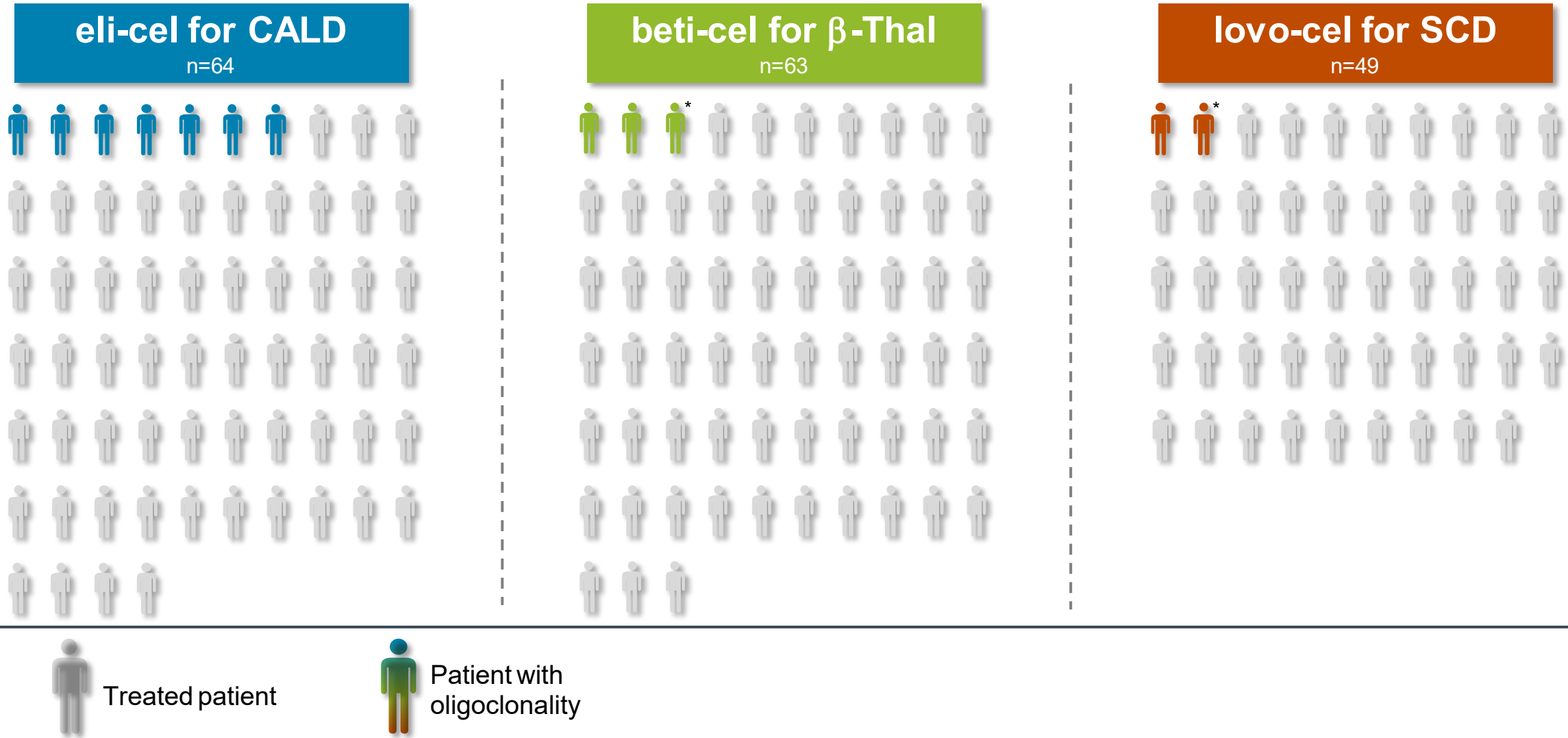

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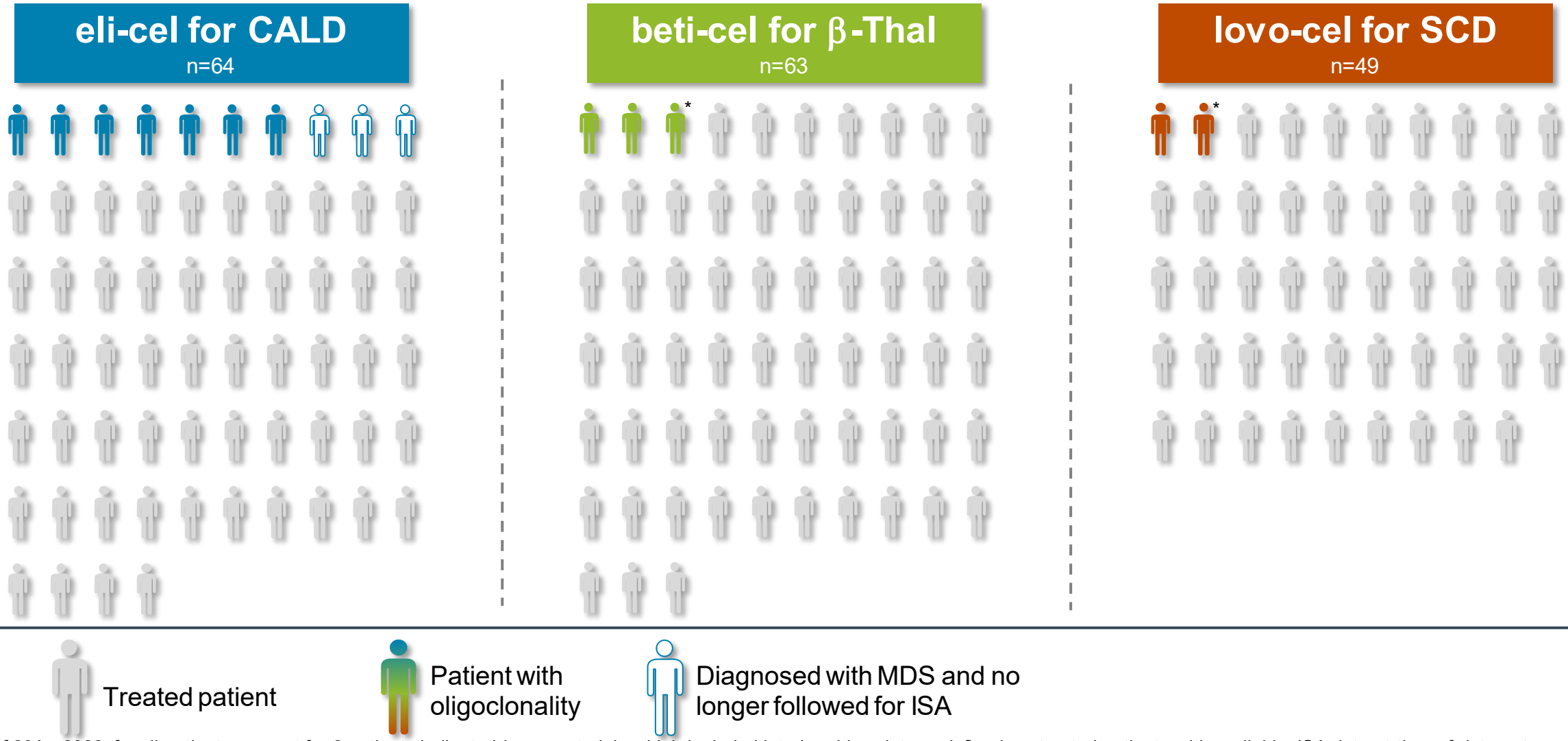

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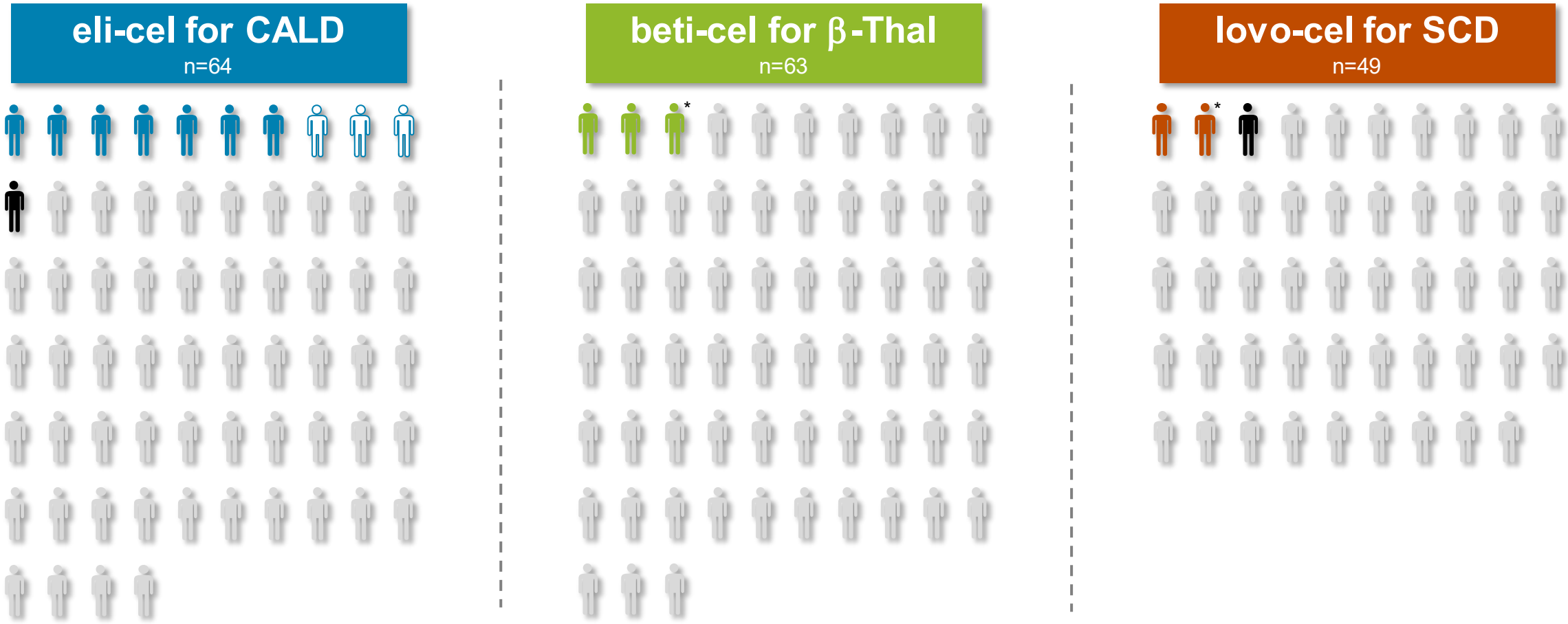

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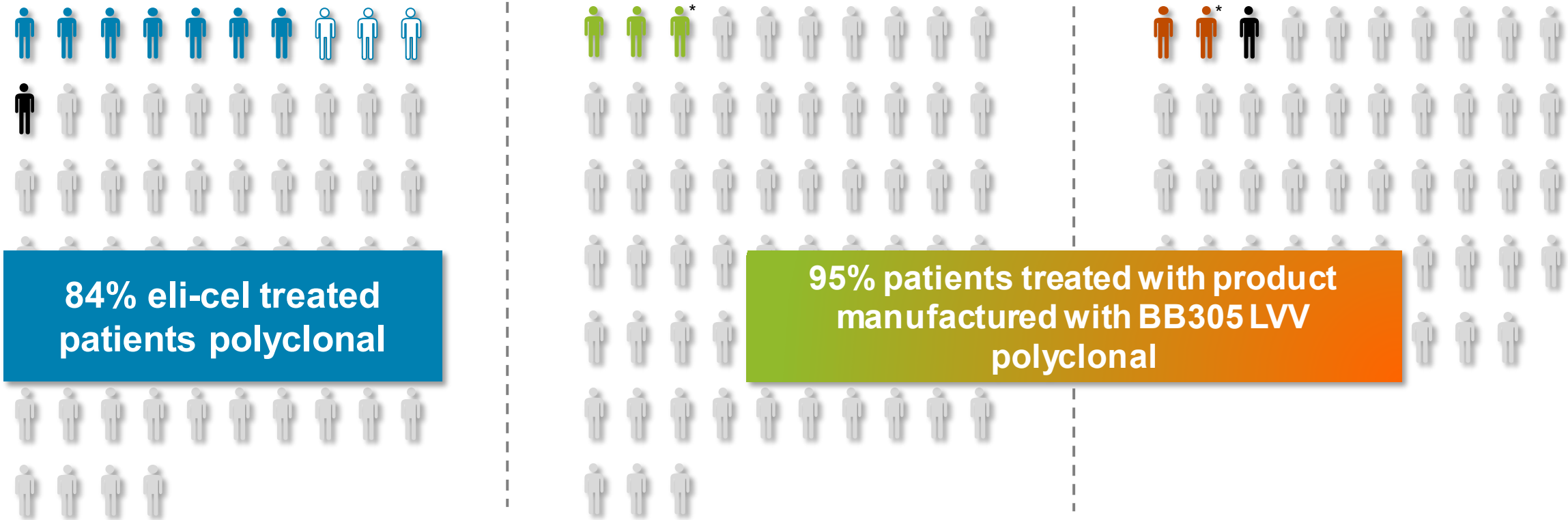
>90% of Patients Currently Have Polyclonal ISA Profiles

eli-cel for CALD
n=64

beti-cel for β-Thal
n=63

lovo-cel for SCD
n=49



84% eli-cel treated patients polyclonal

95% patients treated with product manufactured with BB305 LVV polyclonal

KEY

- Treated patient
- Patient with oligoclonality
- Diagnosed with MDS and no longer followed for ISA
- Patient had oligoclonality but is no longer being followed for ISA

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Agenda

Background on retroviral vectors

- Safety events warranted development of safer vector designs
- Current LVVs designed to lower risk of insertional oncogenesis
- Benefits and risks of LVV
- LVV traceability and Integration Site Analysis

Lenti-D LVV used for manufacture of eli-cel

- Mechanism of action necessitates ubiquitous transgene promoter
- LVV mediated safety events

BB305 LVV used for manufacture of beti-cel and lovo-cel

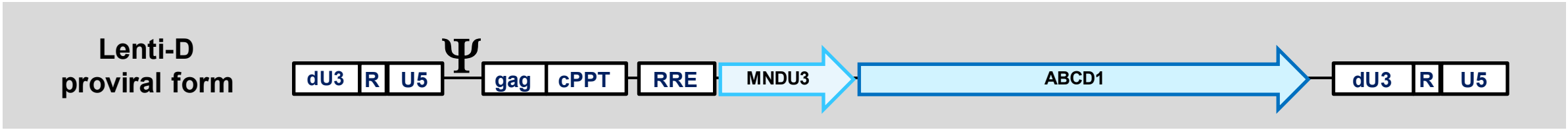
- Mechanism of action necessitates erythroid specific promoter
- No LVV mediated safety events

LVV: lentiviral vector

Lenti-D Lentiviral Vector Was Designed to Treat Patients with CALD

The Lenti-D LVV is designed to deliver intact copies of *ABCD1* to autologous HSCs to enable production of functional copies of ALDP[1]

- The ubiquitous **MNDU3 promoter** determined to be an appropriate promoter for use in Lenti-D LVV for expression of functional ALDP in engrafted hematopoietic-derived cells in the central nervous system to stabilize disease progression
- Suitability of MNDU3 promoter for gene therapy for CALD was first demonstrated by Cartier et al, 2009[2]
- Nonclinical assessments using Lenti-D LVV-transduced CD34+ HSPCs have not identified oncogenesis (tumorigenicity) as a quantifiable hazard



1. Eichler F, et al. *N Engl J Med.* 2017;377(17):1630-8. 2. Cartier N, et al. *Science* 2009;326(5954):818-23.
CALD: cerebral adrenoleukodystrophy; HSPC: hematopoietic stem and progenitor cell; LTR: long terminal repeat; ALDP: adrenoleukodystrophy protein

3 Cases of MDS Considered to be Insertional Oncogenesis Following eli-cel Treatment

		104-18[1]	104-08[1]	102-03[2]
CBC Finding	**Finding**	Thrombocytopenia	Pancytopenia/Thrombocytopenia	Thrombocytopenia
	First Seen	Persistently post-infusion; PE >D100	Persistently post-infusion; PE >D100	At MDS diagnosis, Month 92
Persistent Oligoclonality	**First Met Criterion**	Month 12	Month 12	Month 92*
	Insertion Sites	***MECOM***, *SLC6A16*	***MECOM***, *ACTR3, RAP2C-AS1, ST3GAL6-AS1*	***PRDM16***, *GAB3, CAMK2A, TYK2, SNX12, MIR106A*
Bone marrow evaluation at diagnosis	**Morphology**	**Megakaryocyte dysplasia**		**Multilineage dysplasia**
	Blasts	<5%		**15-20%**
	Karyotype	46XY, Chr. 14 aberration, germline	46XY, normal	46XY, normal
	NGS	*CDKN2A* c.168C>G, germline**	None Detected	*KRAS* c.35G>C, 14% VAF *NRAS* c.35G>C, 3% VAF
Diagnosis		**MDS-SLD**	**MDS-SLD**	**MDS-EB-2**
Time of Diagnosis		Month 14	Month 26	Month 92
Insertional Oncogenesis		**Likely Insertional Oncogenesis**	**Likely Insertional Oncogenesis**	**Likely Insertional Oncogenesis**

PE >D100, platelet engraftment after relative day 100; MDS-SLD, myelodysplastic syndrome with single lineage dysplasia; MDS-EB-2, myelodysplastic syndrome with excess blasts 2; VAF, variant allele frequency; 1. Data as of Aug 2021; 2. Data as of Nov 2021; ongoing studies with an open database

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** reported as variant of uncertain significance at 44% VAF subsequently confirmed as germline

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3 Cases of MDS Considered to be Insertional Oncogenesis Following eli-cel Treatment

LVV Exoneration Criteria

1. **Classical driver alterations consistent with MDS/AML**
2. **Transcriptional profile consistent with properties of known MDS/AML driver alterations**
3. **Insertion site(s) unremarkable with respect to cancer-associated genes**
4. **Insertion site(s) does not disrupt genomic elements**
5. **Insertion site(s) found in other patients without sequelae**
6. **No substantial change in gene expression around insertion site**
7. **Vector is NOT transcriptionally active in tumor cells**

MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; *MECOM*: MDS1 and EVI1 Complex Locus; PRDM16: PR/SET Domain 16

3 Cases of MDS Considered to be Insertional Oncogenesis Following eli-cel Treatment

LVV Exoneration Criteria	Criteria Met?	Findings
1. Classical driver alterations consistent with MDS/AML	X	No classic MDS/AML driver mutations identified (2/3 patients)
2. Transcriptional profile consistent with properties of known MDS/AML driver alterations	?	No definitive known MDS/AML driver alterations
3. Insertion site(s) unremarkable with respect to cancer-associated genes		
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2. **Transcriptional profile consistent with properties of known MDS/AML driver alterations**	?	No definitive known MDS/AML driver alterations
3. **Insertion site(s) unremarkable with respect to cancer-associated genes**	X	*MECOM* and *PRDM16* known proto-oncogenes
4. **Insertion site(s) does not disrupt genomic elements**		
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3 Cases of MDS Considered to be Insertional Oncogenesis Following eli-cel Treatment

LVV Exoneration Criteria	Criteria Met?	Findings
1. **Classical driver alterations consistent with MDS/AML**	✗	No classic MDS/AML driver mutations identified (2/3 patients)
2. **Transcriptional profile consistent with properties of known MDS/AML driver alterations**	?	No definitive known MDS/AML driver alterations
3. **Insertion site(s) unremarkable with respect to cancer-associated genes**	✗	*MECOM* and *PRDM16* known proto-oncogenes
4. **Insertion site(s) does not disrupt genomic elements**	✓	Insertion sites do not disrupt mapped genomic features
5. **Insertion site(s) found in other patients without sequelae**	✓	*MECOM* and *PRDM16* are common insertion sites
6. **No substantial change in gene expression around insertion site**		
7. **Vector is NOT transcriptionally active in tumor cells**		

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3. **Insertion site(s) unremarkable with respect to cancer-associated genes**	✗	*MECOM* and *PRDM16* known proto-oncogenes
4. **Insertion site(s) does not disrupt genomic elements**	✓	Insertion sites do not disrupt mapped genomic features
5. **Insertion site(s) found in other patients without sequelae**	✓	*MECOM* and *PRDM16* are common insertion sites
6. **No substantial change in gene expression around insertion site**	✗	Aberrant gene expression found in all 3 patients
7. **Vector is NOT transcriptionally active in tumor cells**	?	No blasts in 2 patients; aberrant gene expression in all 3

MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; *MECOM*: MDS1 and EVI1 Complex Locus; PRDM16: PR/SET Domain 16

Lenti-D LVV-Mediated Insertional Oncogenesis Likely Multifactorial

- **Gene expression signature changes seen in all genes analyzed, including known proto-oncogenes, in 3 eli-cel patients diagnosed with MDS**
  - Clones all contain between 2 and 6 insertion sites
- **No clear alternative driver MDS/AML mutations in 2 patients**
- **The presence of vector insertion sites in proto-oncogenes are common, and the vast majority of clones do not expand**
  - In a non-bluebird clinical trial for ADA-SCID using an MND-containing retrovirus MECOM was the most common insertion sites and there were no clonal expansions and no malignancy[1, 2]

LVV, lentiviral vector; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; ADA-SCID, adenosine deaminase-deficient severe combined immunodeficiency; *MECOM*: MDS1 and EVI1 Complex Locus;
1. Cooper et al., *Blood* 2017; 129(19):2624-2635.; 2. Reinhardt et al., *Blood* 2021; 138(15):1304-1316.

Agenda

Background on retroviral vectors

- Safety events warranted development of safer vector designs
- Current LVVs designed to lower risk of insertional oncogenesis
- Benefits and risks of LVV
- LVV traceability and Integration Site Analysis

Lenti-D LVV used for manufacture of eli-cel

- Mechanism of action necessitates ubiquitous transgene promoter
- LVV mediated safety events

BB305 LVV used for manufacture of beti-cel and lovo-cel

- Mechanism of action necessitates erythroid specific promoter
- No LVV mediated safety events

LVV: lentiviral vector

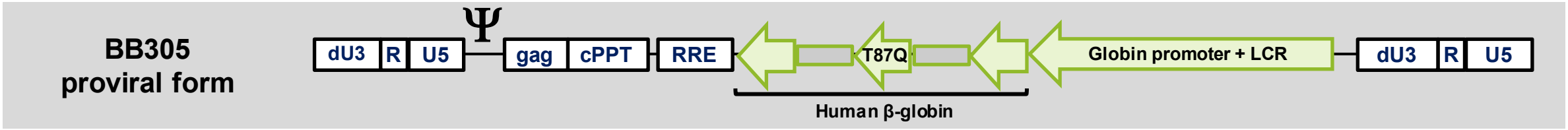
beti-cel: No Cases of Malignancy or Insertional Oncogenesis

No malignancy, no insertional oncogenesis

beti-cel: No Cases of Malignancy or Insertional Oncogenesis

No malignancy, no insertional oncogenesis

- **β-globin promoter** selected for appropriate and optimized expression transgenic $\beta^{A\text{-}T87Q}$ in erythroid lineage cells
  - SIN LVV design with internal cell-type specific (erythroid) promoter limits potential for aberrant enhancer activity affecting expression of nearby genes
- Nonclinical assessments using BB305 LVV-transduced CD34+ HSPCs have not identified oncogenesis (tumorigenicity) as a quantifiable hazard
- No evidence of baseline increased risk of hematologic malignancy in patients with β-thalassemia in literature



SIN: self inactivating; LVV: lentiviral vector; HSPC: hematopoietic stem and progenitor cell

lovo-cel for sickle cell disease: 2 Malignancies; No Insertional Oncogenesis

2 malignancies, **no insertional oncogenesis**

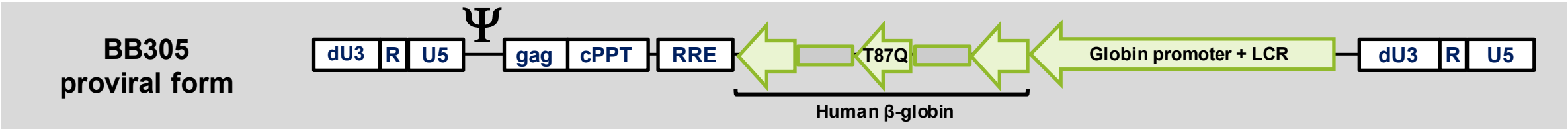
lovo-cel for sickle cell disease: 2 Malignancies; No Insertional Oncogenesis

2 malignancies, **no insertional oncogenesis**

- **β-globin promoter** selected for appropriate and optimized expression transgenic βA-T87Q in erythroid lineage cells
  - SIN LVV design with internal cell-type specific (erythroid) promoter limits potential for aberrant enhancer activity affecting expression of nearby genes

- Nonclinical assessments using BB305 LVV-transduced CD34+ HSPCs have not identified oncogenesis (tumorigenicity) as a quantifiable hazard

- **Evidence of baseline increased risk of hematologic malignancy in patients with SCD in literature**



SIN: self inactivating; LVV: lentiviral vector; HSPC: hematopoietic stem and progenitor cell

Malignancy, but NOT Insertional Oncogenesis, Observed in 2 Group A lovo-cel Patients

		206-A-2	206-A-1
CBC Finding	**Finding**	Abnormal CBC and Peripheral Blasts (3%)	Abnormal CBC and Peripheral Blasts (9%)
	First Seen	Month 36	Month 66
Persistent Oligoclonality[1]	**First Met Criterion**	N/A	Month 60
	Insertion Sites	N/A	*VAMP4*
Bone Marrow Evaluation at Diagnosis	**Blasts**	10%	22 – 50%
	Karyotype & Molecular Diagnostics	• **Monosomy 7** • Abnormal 19p • ***RUNX1*** (NP_001745.2:p.Asp198Gly), • ***PTPN11*** (NP_002825.3:p.Phe71Leu), • ***KRAS*** NP_203524.1:p.Gly12Ala	• **Monosomy 7** • Partial loss of 11p • ***RUNX1*** Exon 5 stop gained p.A149*fs • ***PTPN11*** Exon 3 missense: p.A72V
Diagnosis		**MDS/AML**	**AML**
Insertional Oncogenesis		**No** – no LVV	**No** – *VAMP4* insertion exonerated

[1]Assessment based on ISA algorithm in use for relevant protocol revision

Malignancy, but NOT Insertional Oncogenesis, Observed in 2 Group A lovo-cel Patients

		206-A-2	206-A-1
CBC Finding	**Finding**	Abnormal CBC and Peripheral Blasts (3%)	Abnormal CBC and Peripheral Blasts (9%)
	First Seen	Month 36	Month 66
Persistent Oligoclonality[1]	**First Met Criterion**	N/A	Month 60
	Insertion Sites	N/A	*VAMP4*
Bone Marrow Evaluation at Diagnosis	**Blasts**	10%	22 – 50%
	Karyotype & Molecular Diagnostics	• **Monosomy 7** • Abnormal 19p • ***RUNX1*** (NP_001745.2:p.Asp198Gly), • ***PTPN11*** (NP_002825.3:p.Phe71Leu), • ***KRAS*** NP_203524.1:p.Gly12Ala	• **Monosomy 7** • Partial loss of 11p • ***RUNX1*** Exon 5 stop gained p.A149*fs • ***PTPN11*** Exon 3 missense: p.A72V
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Insertional Oncogenesis		**No** – no LVV	**No** – *VAMP4* insertion exonerated

[1]Assessment based on ISA algorithm in use for relevant protocol revision

HGB-206 Group A 2021 AML Case: Not Insertional Oncogenesis

LVV Exoneration Criteria	Findings
1. **Classical driver alterations consistent with MDS/AML**	Monosomy 7, partial loss of 11p, *RUNX1, PTPN11*
2. **Transcriptional profile consistent with properties of known MDS/AML driver alterations**	RNAseq data consistent with monosomy 7 and contains *PTPN11* and *RUNX1* mutations
3. **Insertion site(s) unremarkable with respect to cancer-associated genes**	*VAMP4* has no known association with cellular proliferation or oncogenesis
4. **Insertion site(s) does not disrupt genomic elements**	*VAMP4* insertion does not disrupt mapped genomic features
5. **Insertion site(s) found in other patients without sequelae**	*VAMP4* IS common and this patient is the only one with *VAMP4* IS >0.05% at any point
6. **No substantial change in gene expression around insertion site**	No remarkable expression changes in 10 MB region around *VAMP4* IS
7. **Vector is NOT transcriptionally active in tumor cells**	Very low level HBB detected in CD34+ cells

MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; *VAMP4*, Vesicle-Associated Membrane Protein 4; IS, insertion site; HBB, Hemoglobin Subunit Beta; *RUNX1*, RUNX Family Transcription Factor 1; *PTPN11*, Protein Tyrosine Phosphatase Non-Receptor Type 11.

Goyal, S et al. *N Engl J Med*. 2022; 386:138-147.

Vector-Related Safety Profiles Differ

eli-cel for CALD
(elivaldogene autotemcel)

Lenti-D LVV
LVV-Mediated Insertional Oncogenesis Observed

67 patients treated

3 malignancies

All 3 Lenti-D LVV mediated insertional oncogenesis

beti-cel for β-Thal
(betibeglogene autotemcel)

BB305 LVV
No LVV-Mediated Insertional Oncogenesis has Been Observed

63 patients treated

0 malignancy

0 insertional oncogenesis

lovo-cel for SCD
(lovotibeglogene autotemcel)

50 patients treated

2 malignancies

0 insertional oncogenesis

β-thal: β-thalassemia; SCD: sickle cell disease; CALD: cerebral adrenoleukodystrophy; LVV: lentiviral vector

Summary of LVV Safety and Insertional Oncogenesis

- **LVVs used for gene therapy have both inherent (insertion biases) and engineered (SIN and replication incompetent) properties to limit risk of insertional mutagenesis leading to endogenous gene dysregulation**

LVV: lentiviral vector; SIN: self inactivating; ISA: integration site analysis; CBC: complete blood counts

Summary of LVV Safety and Insertional Oncogenesis

- **LVVs used for gene therapy have both inherent (insertion biases) and engineered (SIN and replication incompetent) properties to limit risk of insertional mutagenesis leading to endogenous gene dysregulation**
- **ISA is a useful tool that allows for traceability of clonal populations, but it is not predictive**
- **Regular CBC analyses for all patients treated with novel, one-time therapies is recommended**

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- **Regular CBC analyses for all patients treated with novel, one-time therapies is recommended**
- **Different severe genetic diseases have unique disease-specific and treatment-related risks of malignancy**
  - **eli-cel:** malignancy, likely mediated by Lenti-D LVV insertion and considered to be insertional oncogenesis
  - **beti-cel:** to-date **no malignancy, no insertional oncogenesis**

LVV: lentiviral vector; SIN: self inactivating; ISA: integration site analysis; CBC: complete blood counts

Vector Related Safety Profiles Differ

TODAY

Lenti-D LVV
used for manufacture of elivaldogene autotemcel (eli-cel) for cerebral adrenoleukodystrophy (CALD)

LVV-Mediated Insertional Oncogenesis Observed

67 patients treated
3 malignancies
3 insertional oncogenesis

LVV: lentiviral vector

Vector Related Safety Profiles Differ

TODAY

Lenti-D LVV
used for manufacture of elivaldogene autotemcel (eli-cel) for cerebral adrenoleukodystrophy (CALD)

LVV-Mediated Insertional Oncogenesis Observed

67 patients treated
3 malignancies
3 insertional oncogenesis

TOMORROW

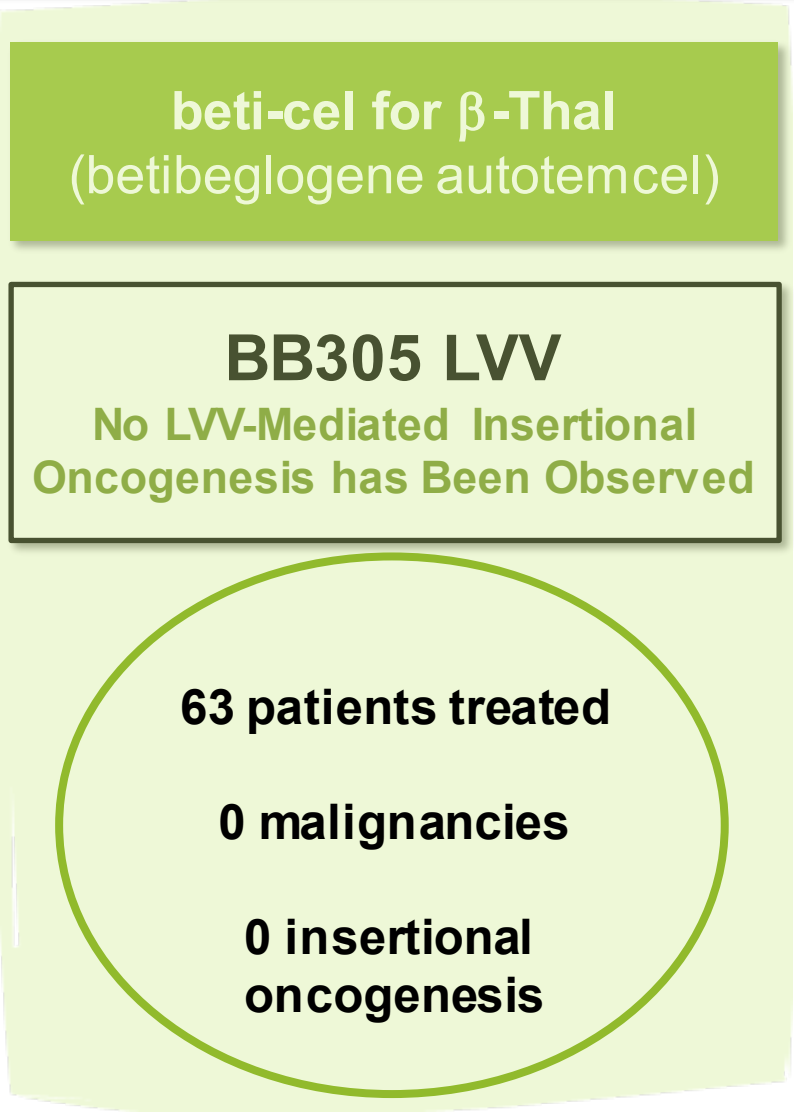
BB305 LVV
used for manufacture of betibeglogene autotemcel (beti-cel) for β-thalassemia requiring regular transfusions

No LVV-Mediated Insertional Oncogenesis Observed

63 patients treated
0 malignancies
0 insertional oncogenesis

LVV: lentiviral vector

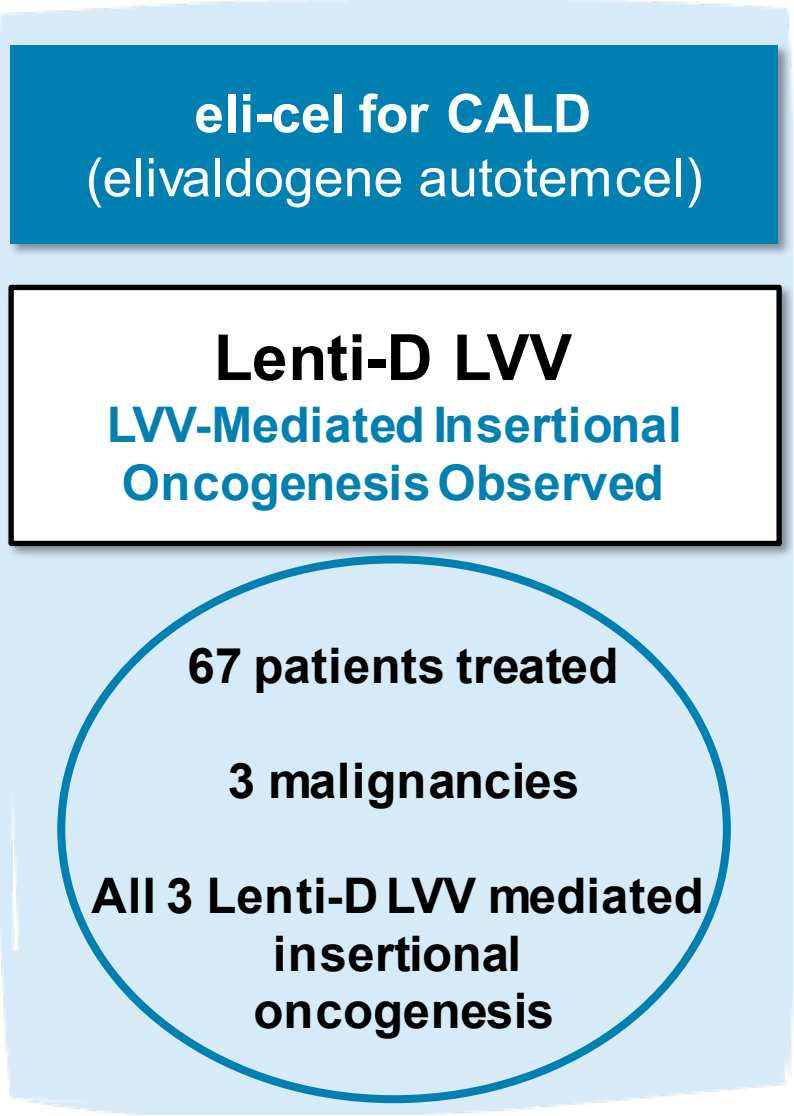
beti-cel Benefit/Risk Positive



- In β-thalassemia patients treated with beti-cel, the **great majority of patients achieved transfusion independence, across all phases of clinical studies, all ages, and all genotypes**
- **Durable transfusion independence** up to 7 years post-treatment
- The safety profile largely reflects known side effects of mobilization and conditioning agents

β-thal: β-thalassemia; LVV: lentiviral vector

eli-cel Benefit/Risk Positive Despite Insertional Oncogenesis



- **eli-cel is an essential life-saving therapy for patients with mismatched donors,** and a meaningful option for those with a MUD
- Treatment with eli-cel allows for the possibility that a fatal neurodegenerative **disease can be stabilized**, with preservation of physical and intellectual function in the majority of patients
- eli-cel treated patients are more likely to achieve both **overall and event free survival** than allo-HSCT patients treated with an NMSD graft

CALD: cerebral adrenoleukodystrophy; LVV: lentiviral vector; HSCT: hematopoietic stem cell transplant; MUD: matched unrelated donor; NMSD: non-matched sibling donor

Each LVV has a unique and distinct safety profile

Each LVV gene therapy safety profile, along with the inherent risk of autologous transplant, must be weighed against :

1. **severity** of each disease
2. availability of **other treatments** and their risks
3. probability and magnitude of **life-long benefit** gene therapy could offer