

COMPOSITIONS AND METHODS FOR CLL1 MODIFICATION

RELATED APPLICATIONS

This application claims priority to U.S. Serial No.: 62/892,868 filed August 28, 2019
5 and U.S. Serial No.: 62/962,133 filed January 16, 2020, the entire contents of each of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted
10 electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 28, 2020, is named V0291_70005WO00_SL.txt and is 54,483 bytes in size.

BACKGROUND

15 When a cancer patient is administered an anti-CLL1 cancer therapy, the therapy can deplete not only CLL1+ cancer cells, but also noncancerous CLL1+ cells in an “on-target, off-tumor” effect. Since certain hematopoietic cells typically express CLL1, the loss of the noncancerous CLL1+ cells can deplete the hematopoietic system of the patient. To address this depletion, the subject can be administered rescue cells (e.g., HSCs and/or HPCs)
20 comprising a modification in the CLL1 gene. These CLL1-modified cells can be resistant to the anti-CLL1 cancer therapy, and can therefore repopulate the hematopoietic system during or after anti-CLL1 therapy.

SUMMARY OF THE INVENTION

Some aspects of this disclosure provide, e.g., novel cells having a modification (*e.g.*,
25 substitution, insertion or deletion) in the endogenous CLL1 gene. Some aspects of this disclosure also provide compositions, *e.g.*, gRNAs, that can be used to make such a modification. Some aspects of this disclosure provide methods of using the compositions provided herein, *e.g.*, methods of using certain gRNAs provided to create genetically engineered cells, *e.g.*, cells having a modification in the endogenous CLL1 gene. Some
30 aspects of this disclosure provide methods of administering genetically engineered cells provided herein, *e.g.*, cells having a modification in the endogenous CLL1 gene, to a subject in need thereof. Some aspects of this disclosure provide strategies, compositions, methods, and treatment modalities for the treatment of patients having cancer and receiving or in need of receiving an anti-CLL1 cancer therapy.

Enumerated Embodiments

1. A gRNA comprising a targeting domain which binds a target domain of Table 1 (e.g., a target domain of any of SEQ ID NOS: 1-20 or 40-43).
- 5 2. A gRNA comprising a targeting domain capable of directing cleavage or editing of a target domain of Table 1 (e.g., a target domain of any of SEQ ID NOS: 1-20 or 40-43).
3. A gRNA comprising a targeting domain which binds a target domain of any of SEQ ID NOS: 1-3 or 5-10, or SEQ ID NOS: 11-13 or 15-20.
4. A gRNA comprising a targeting domain which binds a target domain of SEQ ID NO: 4.
- 10 4.
5. A gRNA comprising a targeting domain which binds a target domain SEQ ID NO: 14, wherein the targeting domain does not comprise SEQ ID NO: 4.
6. A gRNA comprising a targeting domain which binds a target domain SEQ ID NO: 14, wherein the targeting domain is at least 21 nucleotides in length.
- 15 7. The gRNA of any of the preceding embodiments, wherein the targeting domain base pairs or is complementary with at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the target domain, or wherein the targeting domain comprises 0, 1, 2, or 3 mismatches with the target domain.
8. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 31.
- 20 8.
9. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 31, and base pairs or is complementary with at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the target domain.
- 25 9.
10. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 46.

11. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 46, and base pairs or is complementary with at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the target domain.
- 5 12. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 270.
13. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides
10 of SEQ ID NO: 270, and base pairs or is complementary with at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the target domain.
14. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 271.
- 15 15. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 271, and base pairs or is complementary with at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides of the target domain.
16. The gRNA of any of the preceding embodiments, wherein the targeting domain
20 comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 272.
17. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises at least 16 (e.g., 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) consecutive nucleotides of SEQ ID NO: 272, and base pairs or is complementary with at least 10, 11, 12, 13, 14, 15,
25 16, 17, 18, 19, or 20 nucleotides of the target domain.
18. The gRNA of any of the preceding embodiments, wherein said targeting domain is configured to provide a cleavage event (e.g., a single strand break or double strand break) within the target domain, e.g., immediately after nucleotide position 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 of the target domain.

19. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 21.
20. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 22.
- 5 21. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 23.
22. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 24.
23. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 25.
- 10 24. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 26.
25. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 27.
- 15 26. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 28.
27. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 29.
28. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 30.
- 20 29. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 44.
30. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 45.
- 25 31. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 119.

32. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 98.
33. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of Table 2 or 6.
- 5 34. A gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of Table 8 (e.g., any of SEQ ID NOS: 1-10, 40, 42, 98, or 119).
35. A gRNA comprising a targeting domain capable of directing cleavage or editing of a target domain of Table 2 (e.g., a target domain of any of SEQ ID NOS: 1-10, 40, or 42).
36. A gRNA comprising a targeting domain capable of directing cleavage or editing of a
10 target domain of Table 6 (e.g., a target domain of any of SEQ ID NOS: 67-177).
37. A gRNA comprising a targeting domain capable of directing cleavage or editing of a target domain of Table 8 (e.g., a target domain of any of SEQ ID NOS: 1-10, 40, 42, 98, or 119).
38. The gRNA of any of the preceding embodiments, wherein the target domain is in
15 exon 1, exon 2, exon 3, exon 4, exon 5, or exon 6 of the CLL1 sequence of SEQ ID NO: 31.
39. The gRNA of any of the preceding embodiments, wherein the target domain is in exon 1, exon2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the CLL1 sequence of SEQ ID NO: 46.
40. The gRNA of any of the preceding embodiments, wherein the target domain is in
20 exon 1, exon2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the CLL1 sequence of SEQ ID NO: 270.
41. The gRNA of any of the preceding embodiments, wherein the target domain is in exon 1, exon2, exon 3, exon 4, exon 5, or exon 6 of the CLL1 sequence of SEQ ID NO: 271.
42. The gRNA of any of the preceding embodiments, wherein the target domain is in
25 exon 1, exon2, exon 3, exon 4, or exon 5 of the CLL1 sequence of SEQ ID NO: 272.
43. The gRNA of any of the preceding embodiments, which is a single guide RNA (sgRNA).

44. The gRNA of any of the preceding embodiments, wherein the targeting domain is 16 nucleotides or more in length.
45. The gRNA of any of the preceding embodiments, wherein the targeting domain is 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 nucleotides in length.
- 5 46. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of any of SEQ ID NOS: 1-10, 21-30, 40, 42, 44 or 45 or the reverse complement thereof, or a sequence having at least 90% or 95% identity to any of the foregoing, or a sequence having no more than 1, 2, or 3 mutations relative to any of the foregoing.
- 10 47. The gRNA of embodiment 46, wherein the 2 mutations are not adjacent to each other.
48. The gRNA of embodiment 46, wherein none of the 3 mutations are adjacent to each other.
- 15 49. The gRNA of any of embodiments 46-48, wherein the 1, 2, or 3 mutations are substitutions.
50. The gRNA of any of embodiments 46-48, wherein one or more of the mutations is an insertion or deletion.
- 20 51. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of any of SEQ ID NOS: 1-10, 40 or 42.
52. The gRNA of any of the preceding embodiments, wherein the targeting domain
25 comprises a sequence of SEQ ID NO: 1.
53. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 2.
- 30 54. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 3.

55. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 4.
56. The gRNA of any of the preceding embodiments, wherein the targeting domain
5 comprises a sequence of SEQ ID NO: 5.
57. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 6.
- 10 58. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 7.
59. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 8.
- 15 60. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 9.
61. The gRNA of any of the preceding embodiments, wherein the targeting domain
20 comprises a sequence of SEQ ID NO: 10.
62. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 40.
- 25 63. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 42.
64. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 119.
- 30 65. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 98.

66. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of any of SEQ ID NOS: 1-10, 40, 42, 98, or 119.

5 67. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of any of SEQ ID NOS: 67-177.

68. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of any of SEQ ID NOS: 21-30 or 44-45.

10 69. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 21.

70. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 22.

15 71. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 23.

20 72. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 24.

73. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 25.

25 74. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 26.

75. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 27.

30 76. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 28.

77. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 29.
78. The gRNA of any of the preceding embodiments, wherein the targeting domain
5 comprises a sequence of SEQ ID NO: 30.
79. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 44.
- 10 80. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 45.
81. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 239.
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82. The gRNA of any of the preceding embodiments, wherein the targeting domain comprises a sequence of SEQ ID NO: 257.
83. The gRNA of any of the preceding embodiments, wherein the targeting domain
20 comprises a sequence of any of SEQ ID NOs: 216-269.
84. The gRNA of any of the preceding embodiments, which comprises one or more chemical modifications (e.g., a chemical modification to a nucleobase, sugar, or backbone portion).
25
85. The gRNA of any of the preceding embodiments, which comprises one or more 2'-methyl nucleotide, e.g., at a position described herein.
86. The gRNA of any of the preceding embodiments, which comprises one or more
30 phosphorothioate or thioPACE linkage, e.g., at a position described herein.
87. The gRNA of any of the preceding embodiments, which binds a Cas9 molecule.

88. The gRNA of any one of the preceding embodiments, wherein the targeting domain is about 18-23, e.g., 20 nucleotides in length.
89. The gRNA of any of embodiments 1-88, which binds to a tracrRNA.
90. The gRNA of any of embodiments 1-88, which comprises a scaffold sequence.
91. The gRNA of any of the preceding embodiments, which comprises one or more of (e.g., all of):
- a first complementarity domain;
 - a linking domain;
 - a second complementarity domain which is complementary to the first complementarity domain;
 - a proximal domain; and
 - a tail domain.
92. The gRNA of any of the preceding embodiments, which comprises a first complementarity domain.
93. The gRNA of any of the preceding embodiments, which comprises a linking domain.
94. The gRNA of embodiment 92 or 93, which comprises a second complementarity domain which is complementary to the first complementarity domain
95. The gRNA of any of the preceding embodiments, which comprises a proximal domain.
96. The gRNA of any of the preceding embodiments, which comprises a tail domain.
97. The gRNA of any of embodiments 91-96, wherein the targeting domain is heterologous to one or more of (e.g., all of):
- the first complementarity domain;
 - the linking domain;

the second complementarity domain which is complementary to the first complementarity domain;
the proximal domain; and
the tail domain.

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98. The gRNA of any of the preceding embodiments, wherein the gRNA has an editing frequency as measured by ICE of 70-100, e.g., 75-100, 80-100, 85-100, 90-100, or 95-100, or at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 99, or at least 100.

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99. The gRNA of any of embodiments 1-97, wherein the gRNA has an editing frequency as measured by ICE of 10-90, e.g., 20-70, 25-70, 30-70, 35-70, 40-70, 45-70, 50-70, 55-70, 60-70, or 65-70, or at least 10, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, or at least 70.

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100. The gRNA of any of the preceding embodiments, wherein the gRNA has an editing frequency as measured by ICE of at least 80.

101. The gRNA of any of the preceding embodiments, wherein the gRNA has an R^2 value of the editing frequency as measured by ICE of 0.8-1, e.g., 0.85-1, 0.9-1, 0.95-1, or at least 0.8, at least 0.85, at least 0.9, at least 0.95, at least 0.98, at least 0.99, or at least 1.

102. The gRNA of any of the preceding embodiments, wherein the gRNA has an R^2 value of the editing frequency as measured by ICE of at least 0.85.

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103. The gRNA of any of the preceding embodiments, wherein the gRNA has an editing frequency as measured by ICE of at least 80 and an R^2 value of the editing frequency as measured by ICE at least 0.85.

104. The gRNA of any of the preceding embodiments, wherein the gRNA has an editing frequency, e.g., as measured by Sanger sequencing followed by ICE or TIDE analysis, of 70-100, e.g., 75-100, 80-100, 85-100, 90-100, 95-100, or at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 99, or at least 100.

105. The gRNA of any of the preceding embodiments, wherein the gRNA has an editing frequency, e.g., as measured by Next Generation-Targeted Amplicon Sequencing (Amplicon sequencing), of 70-100, e.g., 75-100, 80-100, 85-100, 90-100, 95-100, or at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 99, or at least 100.

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106. A kit or composition comprising:

- a) a gRNA of any of embodiments 1-105, or a nucleic acid encoding the gRNA, and
- b) a second gRNA, or a nucleic acid encoding the second gRNA.

10 107. The kit or composition of embodiment 106, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4).

108. The kit or composition of embodiment 106 or 107, wherein the second gRNA targets a lineage-specific cell-surface antigen.

109. The kit or composition of any of embodiments 106-108 wherein the second gRNA targets a lineage-specific cell-surface antigen other than CLL1.

20 110. The kit or composition of any of embodiments 106-109, wherein the second gRNA targets CD33 (e.g., wherein the second gRNA comprises a targeting domain that comprises a sequence of CCCCAGGACTACTCACTCCT (SEQ ID NO: 64).

25 111. The kit or composition of any of embodiments 106-110, wherein the second gRNA targets CD123 (e.g., wherein the second gRNA comprises a targeting domain that comprises a sequence of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65) or AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66)).

30 112. The kit or composition of any of embodiments 106-111, wherein the second gRNA comprises a targeting domain that comprises a sequence of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65).

113. The kit or composition of any of embodiments 106-112, wherein the second gRNA comprises a targeting domain that comprises a sequence of AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66).
- 5 114. The kit or composition of any of embodiments 106-113, wherein the second gRNA comprises a targeting domain that comprises a sequence of Table A.
115. The kit or composition of any of embodiments 106-114, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG
10 (SEQ ID NO: 4) and the second gRNA comprises a targeting domain that comprises a sequence of CCCAGGACTACTCACTCCT (SEQ ID NO: 64).
116. The kit or composition of any of embodiments 106-115, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG
15 (SEQ ID NO: 4) and the second gRNA comprises a targeting domain that comprises a sequence of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65).
117. The kit or composition of any of embodiments 106-116, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG
20 (SEQ ID NO: 4) and the second gRNA comprises a targeting domain that comprises a sequence of AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66).
118. The kit or composition of any of embodiments 106-117, which further comprises a third gRNA, or a nucleic acid encoding the third gRNA.
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119. The kit or composition of embodiment 118, wherein the third gRNA targets a lineage-specific cell-surface antigen.
120. The kit or composition of embodiment 118, wherein the third gRNA targets CD33,
30 CLL-1, or CD123.
121. The kit or composition of any of embodiments 118-120, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4), the second gRNA comprises a targeting domain that comprises a sequence

of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65), and the third gRNA comprises a targeting domain that comprises a sequence of AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66).

5 122. The kit or composition of any of embodiments 118-120, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4), the second gRNA comprises a targeting domain that comprises a sequence of CCCAGGACTACTCACTCCT (SEQ ID NO: 64), and the third gRNA comprises a targeting domain that comprises a sequence of AGTTCCCACATCCTGGTGCG (SEQ ID
10 NO: 66).

123. The kit or composition of any of embodiments 118-120, wherein the first gRNA comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4), the second gRNA comprises a targeting domain that comprises a sequence
15 of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65), and the third gRNA comprises a targeting domain that comprises a sequence of CCCAGGACTACTCACTCCT (SEQ ID NO: 64).

124. The kit or composition of any of embodiments 118-123, which further comprises a
20 fourth gRNA, or a nucleic acid encoding the fourth gRNA.

125. The kit or composition of embodiment 124, wherein the fourth gRNA targets a lineage-specific cell-surface antigen.

25 126. The kit or composition of embodiment 124, wherein the fourth gRNA targets CD33, CLL-1, or CD123.

127. The kit or composition of any of embodiments 124-126, wherein the gRNA of (a) comprises a targeting domain that comprises a sequence of GGTGGCTATTGTTTGCAGTG
30 (SEQ ID NO: 4), the second gRNA comprises a targeting domain that comprises a sequence of TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65), the third gRNA comprises a targeting domain that comprises a sequence of AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66), and the fourth gRNA comprises a targeting domain that comprises a sequence of CCCAGGACTACTCACTCCT (SEQ ID NO: 64).

128. The kit or composition of any of embodiments 124-127, wherein the gRNA of (a), the second gRNA, the third gRNA, and the fourth gRNA are admixed.
- 5 129. The kit or composition of any of embodiments 124-127, wherein the gRNA of (a), the second gRNA, the third gRNA, and the fourth gRNA are in separate containers.
130. The kit or composition of any of embodiments 106-129, wherein (a) and (b) are admixed.
- 10 131. The kit or composition of any of embodiments 106-129, wherein (a) and (b) are in separate containers.
132. The kit or composition of any of embodiments 106-131, wherein the nucleic acid of
15 (a) and the nucleic acid of (b) are part of the same nucleic acid.
133. The kit or composition of any of embodiments 106-131, wherein the nucleic acid of (a) and the nucleic acid of (b) are separate nucleic acids.
- 20 134. A genetically engineered hematopoietic cell (e.g., hematopoietic stem or progenitor cell), which comprises:
- (a) a mutation at a target domain of Table 1 (e.g., a target domain of any of SEQ ID NOS: 1-20, or 40-43); and
- (b) a second mutation at a gene encoding a lineage-specific cell surface antigen other
25 than CLL1.
135. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 1.
- 30 136. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 2.
137. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 3.

138. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 4.
- 5 139. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 5.
140. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 6.
- 10 141. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 7.
142. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 8.
- 15 143. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 9.
- 20 144. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 10.
145. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 40.
- 25 146. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 42.
147. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 98.
- 30 148. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of SEQ ID NO: 119.

149. The genetically engineered hematopoietic cell of embodiment 134, wherein the mutation of (a) is at a target domain of any of Tables 2 or 6.

150. The genetically engineered hematopoietic cell of any of embodiments 134-149, wherein the mutation of (a) comprises an insertion, a deletion, or a substitution (e.g., a single
5 nucleotide variant).

151. The genetically engineered hematopoietic cell of any of embodiments 134-149, which comprises an insertion of 1 nt, or a deletion of 1 nt, 2 nt, or 3 nt, or 4 nt in CLL1.

10 152. The genetically engineered hematopoietic cell of any of embodiments 134-151, which comprises an indel as described herein, e.g., an indel produced by or producible by a gRNA described herein (e.g., any of gRNA D, gRNA F, gRNA G, gRNA O2, or gRNA P2).

153. The genetically engineered hematopoietic cell of any of embodiments 134-152, which
15 comprises an indel produced by or producible by a CRISPR system described herein, e.g., a method of Example 1, 2, 3, 4, or 5.

154. The genetically engineered cell of any of embodiments 150-153, wherein the deletion is fully within the target domain of any of SEQ ID NOS: 1-10.

20 155. The genetically engineered cell of embodiment 154, wherein the deletion is 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, or 17 nucleotides in length.

156. The genetically engineered cell of any of embodiments 150-153, wherein the deletion
25 has one or both endpoints outside of the target domain of any of SEQ ID NOS: 1-10.

157. The genetically engineered cell of any of embodiments 134-156, wherein the mutation results in a frameshift.

30 158. The genetically engineered hematopoietic cell of any of embodiments 134-157, wherein the second mutation comprises an insertion, a deletion, or a substitution (e.g., a single nucleotide variant).

159. Use of a gRNA of any of embodiments 1-105, or gRNAs of a composition or kit of any of embodiments 106-133, for reducing expression of CLL1 in a sample of hematopoietic cells stem or progenitor cells using a CRISPR/Cas9 system.
- 5 160. Use of a CRISPR/Cas9 system for reducing expression of CLL1 in a sample of hematopoietic cells stem or progenitor cells, wherein the gRNA of the CRISPR/Cas9 system is a gRNA of any of embodiments 1-105 or gRNAs of a composition or kit of any of embodiments 106-133.
- 10 161. A method of producing a genetically engineered cell, comprising:
(i) providing a cell (e.g., a hematopoietic stem or progenitor cell, e.g., a wild-type hematopoietic stem or progenitor cell), and
(ii) introducing into the cell (a) a guide RNA (gRNA) of any of embodiments 1-105 or gRNAs of a composition or kit of any of embodiments 106-133; and (b) an endonuclease that
15 binds the gRNA (e.g., a Cas9 molecule),
thereby producing the genetically engineered cell.
162. A method of producing a genetically engineered cell, comprising:
(i) providing a cell (e.g., a hematopoietic stem or progenitor cell, e.g., a wild-type
20 hematopoietic stem or progenitor cell), and
(ii) introducing into the cell (a) a gRNA of any of embodiments 1-105 or gRNAs of a composition or kit of any of embodiments 106-133; and (b) a Cas9 molecule that binds the gRNA,
thereby producing the genetically engineered cell.
- 25 163. The method or use of any of embodiments 159-162, which results in the genetically engineered hematopoietic stem or progenitor cell having a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
- 30 164. The method or use of any of embodiments 159-163, which results in the genetically engineered hematopoietic stem or progenitor cell having a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.

165. The method or use of any of embodiments 159-164, which is performed on a plurality of hematopoietic stem or progenitor cells.

166. The method or use of any of embodiments 159-165, which is performed on a cell
5 population comprising a plurality of hematopoietic stem cells and a plurality of hematopoietic progenitor cells.

167. The method or use of any of embodiments 159-166, which produces a cell population according to any of embodiments 285-383.

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168. The method of any of embodiments 161-167, wherein the nucleic acids of (a) and (b) are encoded on one vector, which is introduced into the cell.

169. The method of embodiment 168, wherein the vector is a viral vector.

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170. The method of embodiment 169, wherein (a) and (b) are introduced into the cell as a pre-formed ribonucleoprotein complex.

171. The method of embodiment 170, wherein the ribonucleoprotein complex is introduced
20 into the cell via electroporation.

172. The method of any of any of embodiments 161-168, wherein the endonuclease (e.g., a Cas9 molecule) is introduced into the cell by delivering into the cell a nucleic acid molecule (e.g., an mRNA molecule or a viral vector, e.g., AAV) encoding the endonuclease.

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173. The method of any of embodiments 161-172, wherein the cell (e.g., the hematopoietic stem or progenitor cell) is CD34+.

174. The method of any of embodiments 161-173, wherein cell viability of a population of
30 the cells is at least 80%, 90%, 95%, or 98% of the cell viability of control cells (e.g., mock electroporated cells) with 48 hours after introduction of the gRNA into the cells.

175. The method of any of embodiments 161-174, wherein at least 80%, 85%, 90%, 95%, or 98% of cells in the population are viable 48 hours after introduction of the gRNA into the cells.
- 5 176. The method of any of embodiments 161-175, wherein the hematopoietic stem or progenitor cell is from bone marrow cells or peripheral blood mononuclear cells (PBMCs) of a subject.
177. The method of any of embodiments 161-176, wherein the subject has a hematopoietic
10 disorder, e.g., a hematopoietic malignancy, e.g., a leukemia, e.g., AML.
178. The method of any of embodiments 161-176, wherein the subject has a hematological disorder, e.g., a precancerous condition, e.g., a myelodysplasia, a myelodysplastic syndrome (MDS), or a preleukemia.
- 15 179. The method of any of embodiments 161-177, wherein the subject has a cancer, wherein cells of the cancer express CLL1 (e.g., wherein at least a plurality of the cancer cells express CLL1).
- 20 180. The method or use of any of embodiments 159-179, which results in a mutation that causes a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
181. The method or use of any of embodiments 159-180, which results in a mutation that causes a reduced expression level of wild-type CLL1 as compared with a wild-type
25 counterpart cell.
182. The method or use of any of embodiments 159-181, which produces a genetically engineered hematopoietic stem or progenitor cell having a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
- 30 183. The method or use of any of embodiments 159-182, which produces a genetically engineered hematopoietic stem or progenitor cell having a reduced expression level of wild-type CLL1 as compared with a wild-type counterpart cell.

184. A genetically engineered hematopoietic stem or progenitor cell, which is produced by a method of any of embodiments 159-183.
185. A nucleic acid (e.g., DNA) encoding the gRNA of any of embodiments 1-105.
- 5 186. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation at a target domain of Table 1 (e.g., a target domain of any of SEQ ID NOS: 1-20), e.g., wherein the mutation is a result of the genetic engineering.
187. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation at a target domain of Table 8 (e.g., a target domain of any of SEQ ID NOS: 1-10, 40, 42, 98, or 119), e.g., wherein the mutation is a result of the genetic engineering.
- 10 188. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation at a target domain of Table 6 (e.g., a target domain of any of SEQ ID NOS: 67-177), e.g., wherein the mutation is a result of the genetic engineering.
- 15 189. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides (upstream or downstream) of a target domain of Table 1 (e.g., a target domain of any of SEQ ID NOS: 1-20 or 40-43).
190. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides (upstream or downstream) of a target domain of Table 6 (e.g., a target domain of any of SEQ ID NOS: 67-177).
- 20 191. A genetically engineered cell (e.g., a hematopoietic stem or progenitor cell), which comprises a mutation within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides (upstream or downstream) of a target domain of Table 8 (e.g., a target domain of any of SEQ ID NOS: 1-10, 40, 42, 98, or 119).
- 25 192. The genetically engineered cell of embodiment 198, wherein the mutation is within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides (upstream or downstream) of any of SEQ ID NOS: 4, 6, or 7.

193. The genetically engineered cell of embodiment 198, wherein the mutation is within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides downstream of SEQ ID NO: 4.
194. The genetically engineered cell of embodiment 198, wherein the mutation is within 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides upstream of SEQ ID NO: 4.
- 5 195. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 1.
196. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 1, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
- 10 197. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 1, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
198. A genetically engineered hematopoietic stem or progenitor cell, which comprises a
15 mutation at a target domain of SEQ ID NO: 2.
199. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 2, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
200. A genetically engineered hematopoietic stem or progenitor cell, which comprises a
20 mutation at a target domain of SEQ ID NO: 2, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
201. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 3.
- 25 202. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 3, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
203. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 3, wherein the mutation results in a reduced

expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.

204. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 4.

- 5 205. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 4, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.

206. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 4, wherein the mutation results in a reduced
10 expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.

207. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 5.

208. A genetically engineered hematopoietic stem or progenitor cell, which comprises a
15 mutation at a target domain of SEQ ID NO: 5, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.

209. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 5, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type
20 counterpart cell.

210. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 6.

211. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 6, wherein the mutation results in a reduced
25 expression level of CLL1 as compared with a wild-type counterpart cell.

212. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 6, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.

213. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 7.
214. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 7, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
215. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 7, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
216. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 8.
217. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 8, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
218. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 8, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
219. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 9.
220. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 9, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
221. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 9, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
222. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 10.

223. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 10, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
224. A genetically engineered hematopoietic stem or progenitor cell, which comprises a
5 mutation at a target domain of SEQ ID NO: 10, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
225. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 40.
- 10 226. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 40, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
227. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 40, wherein the mutation results in a reduced
15 expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
228. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 42.
229. A genetically engineered hematopoietic stem or progenitor cell, which comprises a
20 mutation at a target domain of SEQ ID NO: 42, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
230. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 42, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type
25 counterpart cell.
231. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 119.

232. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 119, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
233. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 119, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
234. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 98.
235. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 98, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell.
236. A genetically engineered hematopoietic stem or progenitor cell, which comprises a mutation at a target domain of SEQ ID NO: 98, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell.
237. The genetically engineered cell of any of embodiments 186-236, comprising a predicted off target site which does not comprise a mutation or sequence change relative to the sequence of the site prior to gene editing of CLL1.
238. The genetically engineered cell of any of embodiments 186-237, comprising two predicted off target sites which do not comprise a mutation or sequence change relative to the sequence of the site prior to gene editing of CLL1.
239. The genetically engineered cell of any of embodiments 186-238, comprising at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 predicted off target sites which do not comprise a mutation or sequence change relative to the sequence of the site prior to gene editing of CLL1.
240. The genetically engineered cell of any of the preceding embodiments, which does not comprise a mutation in any predicted off-target site, e.g., in any site in the human genome having 1, 2, 3, or 4 mismatches relative to the target domain.

241. The genetically engineered cell of any of the preceding embodiments, which does not comprise a mutation in any site in the human genome having 1 mismatch relative to the target domain.
242. The genetically engineered cell of any of the preceding embodiments, which does not
5 comprise a mutation in any site in the human genome having 1 or 2 mismatches relative to the target domain.
243. The genetically engineered cell of any of the preceding embodiments, which does not comprise a mutation in any site in the human genome having 1, 2, or 3 mismatches relative to the target domain.
- 10 244. The genetically engineered cell of any of the preceding embodiments, which does not comprise a mutation in any site in the human genome having 1, 2, 3, or 4 mismatches relative to the target domain.
245. The genetically engineered cell of any of embodiments 184 or 186-244, wherein the mutation comprises an insertion, a deletion, or a substitution (e.g., a single nucleotide
15 variant).
246. The genetically engineered cell of embodiment 245, wherein the deletion is fully within the target domain of any of SEQ ID NOS: 1-20, 40-43, 98, or 119.
247. The genetically engineered cell of embodiment 246, wherein the deletion is 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, or 17 nucleotides in length.
- 20 248. The genetically engineered cell of embodiment 167, wherein the deletion has one or both endpoints outside of the target domain of any of SEQ ID NOS: 1-20, 40-43, 98, or 119.
249. The genetically engineered cell of any of embodiments 184 or 186-248, wherein the mutation results in a frameshift.
250. The genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of any of
25 embodiments 184 or 186-249, wherein the mutation results in a reduced expression level of wild-type CLL1 as compared with a wild-type counterpart cell (e.g., less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% of the level in the wild-type counterpart cell).
251. The genetically engineered cell of any of embodiments 184 or 186-250, wherein the cell has a reduced level of wild-type CLL1 protein as compared with a wild-type counterpart

cell (e.g., less than 50%, 40%, 30%, 20%, 15%, 10%, or 5% of the level in the wild-type counterpart cell).

252. The genetically engineered cell of any of embodiments 184 or 186-251, which does not express CLL1.

- 5 253. The genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of any of the preceding embodiments, wherein the mutation results in a lack of expression of CLL1.

254. The genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of any of the preceding embodiments, which expresses less than 20% of the CLL1 expressed by a wild-type counterpart cell.

- 10 255. The genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of any of the preceding embodiments, wherein the reduced expression level of CLL1 is in a cell differentiated from (e.g., terminally differentiated from) the hematopoietic stem or progenitor cell, and the wild-type counterpart cell is a cell differentiated from (e.g., terminally differentiated from) a wild-type hematopoietic stem or progenitor cell.

- 15 256. The genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of embodiment 255, wherein the cell differentiated from the hematopoietic stem or progenitor cell is a myeloblast, monocyte, or myeloid dendritic cell.

257. The genetically engineered cell of any of embodiments 184 or 186-256, which is CD34+.

- 20 258. The genetically engineered cell of any of embodiments 184 or 186-257, which is from bone marrow cells or peripheral blood mononuclear cells of a subject.

259. The genetically engineered cell of embodiment 258, wherein the subject is a human patient having a hematopoietic malignancy, e.g., AML.

- 25 260. The genetically engineered cell of embodiment 258, wherein the subject is a human patient having a hematological disorder, e.g., a precancerous condition, e.g., a myelodysplasia, a myelodysplastic syndrome (MDS), or a preleukemia.

261. The genetically engineered cell of any one of embodiments 258-260, wherein the subject has a cancer, wherein cells of the cancer express CLL1 (e.g., wherein at least a plurality of the cancer cells express CLL1).

262. The genetically engineered cell of embodiment 258, wherein the subject is a healthy human donor (e.g., an HLA-matched donor).

263. The genetically engineered cell of any of embodiments 184 or 186-262, which further comprises a nuclease chosen from a CRISPR endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector-based nuclease (TALEN), or a meganuclease, or a nucleic acid (e.g., DNA or RNA) encoding the nuclease, wherein optionally the nuclease is specific for CLL1.

264. The genetically engineered cell of any of embodiments 184 or 186-263, which further comprises a gRNA (e.g., a single guide RNA) specific for CLL1, or a nucleic acid encoding the gRNA.

265. The genetically engineered cell of embodiment 264, wherein the gRNA is a gRNA described herein, e.g., a gRNA of any of embodiments 1-105.

266. The genetically engineered cell of any of embodiments 184 or 186-265, which was made by a process comprising contacting the cell with a nuclease chosen from a CRISPR endonuclease, a zinc finger nuclease (ZFN), a transcription activator-like effector-based nuclease (TALEN), or a meganuclease (e.g., by contacting the cell with the nuclease or a nucleic acid encoding the nuclease).

267. The genetically engineered cell of any of embodiments 184 or 186-266, which was made by a process comprising contacting the cell with a nickase or a catalytically inactive Cas9 molecule (dCas9), e.g., fused to a function domain (e.g., by contacting the cell with the nuclease or a nucleic acid encoding the nuclease).

268. The genetically engineered cell of any of embodiments 184 or 186-267, in which both copies of CLL1 are mutant.

269. The genetically engineered cell of embodiment 286, wherein both copies of CLL1 have the same mutation.

270. The genetically engineered cell of embodiment 286, wherein the copies of CLL1 have different mutations.

271. The genetically engineered cell of any of embodiments 184 or 186-186, comprising a first copy of CLL1 having a first mutation and a second copy of CLL1 having a second mutation, wherein the first and second mutations are different.
272. The genetically engineered cell of embodiment 271, wherein the first copy of CLL1
5 comprises a first deletion.
273. The genetically engineered cell of embodiment 271 or 272, wherein the second copy of CLL1 comprises a second deletion.
274. The genetically engineered cell of any of embodiments 271-273, wherein the first and second deletions overlap.
- 10 275. The genetically engineered cell of any of embodiments 271-274, wherein an endpoint of the first deletion is within the second deletion.
276. The genetically engineered cell of any of embodiments 271-275, wherein both endpoints of the first deletion are within the second deletion.
277. The genetically engineered cell of any of embodiments 271-273, wherein the first and
15 second deletion share an endpoint.
278. The genetically engineered cell of any of embodiments 184 or 186-277, wherein the first and second mutations are each independently selected from: an insertion of 1 nt, or a deletion of 1 nt, 2 nt, 3 nt, or 4 nt.
279. The genetically engineered cell of any of embodiments 184 or 186-278, which is
20 capable of forming a BFU-E colony, a CFU-G colony, a CFU-M colony, a CFU-GM colony, or a CFU-GEMM colony.
280. The genetically engineered cell of any of embodiments 184 or 186-279, which is capable of producing a cytokine, e.g., an inflammatory cytokine, e.g., IL-6, TNF- α , IL-1 β , or MIP-1 α .
281. The genetically engineered cell of any of embodiments 184 or 186-280, which is capable
25 of producing a cytokine, e.g., an inflammatory cytokine, e.g., IL-6, TNF- α , IL-1 β , or MIP-1 α , at a level comparable to an otherwise similar cell that is CLL1 wildtype.
282. The genetically engineered cell of any of embodiments 184 or 186-281, which is capable of producing a cytokine, e.g., an inflammatory cytokine, e.g., IL-6, TNF- α , IL-1 β , or MIP-1 α ,

at a level that is at least 70%, 80%, 85%, 90%, or 95% of the levels produced by an otherwise similar cell that is CLL1 wildtype.

283. The genetically engineered cell of any of embodiments 280-282, which is capable of producing the cytokine when simulated with a TLR agonist, e.g., LPS or R848, e.g., as
5 described in Example 5.

284. The genetically engineered cell of any of embodiments 280-283, which is capable of phagocytosis.

285. A cell population, comprising a plurality of the genetically engineered hematopoietic stem or progenitor cells of any embodiments 184 or 186-284 (e.g., comprising hematopoietic
10 stem cells, hematopoietic progenitor cells, or a combination thereof).

286. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 1.

287. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 1,
15 wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell population.

288. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 1,
20 wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.

289. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 2.

290. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 2,
25 wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell population.

291. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 2,

wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.

292. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 3.

- 5 293. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 3, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell population.

- 10 294. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 3, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.

295. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 4.

- 15 296. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 4, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell population.

- 20 297. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 4, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.

298. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 5.

- 25 299. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 5, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-type counterpart cell population.

300. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 5, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 5 301. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 6.
302. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 6, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
10 type counterpart cell population.
303. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 6, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 15 304. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 7.
305. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 7, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
20 type counterpart cell population.
306. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 7, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 25 307. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 8.
308. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 8, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
30 type counterpart cell population.

309. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 8, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 5 310. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 9.
311. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 9, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
10 type counterpart cell population.
312. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 9, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 15 313. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 10.
314. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 10, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
20 type counterpart cell population.
315. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 10, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 25 316. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 40.
317. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 40, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
30 type counterpart cell population.

318. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 40, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 5 319. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 42.
320. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 42, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
10 type counterpart cell population.
321. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 42, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 15 322. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 98.
323. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 98, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
20 type counterpart cell population.
324. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 98, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.
- 25 325. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 119.
326. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 119, wherein the mutation results in a reduced expression level of CLL1 as compared with a wild-
30 type counterpart cell population.

327. A cell population, comprising a plurality of genetically engineered hematopoietic stem or progenitor cells which comprise a mutation at a target domain of SEQ ID NO: 119, wherein the mutation results in a reduced expression level of CLL1 that is less than 20% of the level of CLL1 in a wild-type counterpart cell population.

- 5 328. The cell population of any of embodiments 285-218, wherein the cell population can differentiate into a cell type which expresses CLL1 at a level that is reduced with regard to the level of CLL1 expressed by the same differentiated cell type which is derived from a CLL1-wildtype hematopoietic stem or progenitor cell.

- 10 329. The cell population of any of embodiments 285-328, wherein the hematopoietic stem or progenitor cells are engineered such that a myeloid progenitor cell descended therefrom is deficient in CLL1 levels as compared with a myeloid progenitor cell descended from a CLL1-wildtype hematopoietic stem or progenitor cell.

- 15 330. The cell population of any of embodiments 285-328, wherein the hematopoietic stem or progenitor cells are engineered such that a myeloid cell (e.g., a terminally differentiated myeloid cell) descended therefrom is deficient in CLL1 levels as compared with a myeloid cell (e.g., a terminally differentiated myeloid cell) descended from a CLL1-wildtype hematopoietic stem or progenitor cell.

- 20 331. The cell population of any of embodiments 285-330, which further comprises one or more cells that comprise one or more non-engineered CLL1 genes.

332. The cell population of any of embodiments 285-331, which further comprises one or more cells that are homozygous wild-type for CLL1.

- 25 333. The cell population of any of embodiments 285-332, wherein about 0-1%, 1-2%, 2-5%, 5-10%, 10-15%, or 15-20% of cells in the population are homozygous wild-type for CLL1, e.g., are hematopoietic stem or progenitor cells that are homozygous wild-type for CLL1.

- 30 334. The cell population of any of embodiments 285-333 which further comprises one or more cells that are heterozygous for CLL1, e.g., comprise one wild-type copy of CLL1 and one mutant copy of CLL1.

335. The cell population of any of embodiments 285-334, wherein about 0-1%, 1-2%, 2-5%, 5-10%, 10-15%, or 15-20% of cells in the population are heterozygous wild-type for CLL1, e.g., are hematopoietic stem or progenitor cells that comprise one wild-type copy of CLL1 and one mutant copy of CLL1.
- 5
336. The cell population of any of embodiments 285-335, wherein at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of the copies of CLL1 in the population are mutant.
- 10
337. The cell population of any of embodiments 285-336, which comprises a plurality of different CLL1 mutations, e.g., which comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different mutations.
338. The cell population of any of embodiments 285-337, which comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different mutations.
- 15
339. The cell population of any of embodiments 285-338, which comprises at 2, 3, 4, 5, 6, 7, 8, 9, or 10 different insertions.
340. The cell population of any of embodiments 285-339, which comprises a plurality of insertions and a plurality of deletions.
- 20
341. The cell population of any of embodiments 285-340, which expresses less than 20% of the CLL1 expressed by a wild-type counterpart cell population.
- 25
342. The cell population of any of embodiments 285-341, wherein the reduced expression level of CLL1 is in a cell differentiated from (e.g., terminally differentiated from) the hematopoietic stem or progenitor cell, and the wild-type counterpart cell is a cell differentiated from (e.g., terminally differentiated from) a wild-type hematopoietic stem or progenitor cell.
- 30
343. The cell population of embodiment 342, wherein the cell differentiated from the hematopoietic stem or progenitor cell is a myeloblast, monocyte, or myeloid dendritic cell.

344. The cell population of any of embodiments 285-343, which, when administered to a subject, produces hCD45+ cells in the subject, e.g., when assayed at 16 weeks after administration.

- 5 345. The cell population of embodiment 344, which produces levels of hCD45+ cells comparable to the levels of hCD45+ cells produced with an otherwise similar cell population that is CLL1 wildtype.

346. The cell population of embodiments 344 or 345, which produces levels of hCD45+
10 cells that is at least 70%, 80%, 85%, 90%, or 95% the levels of hCD45+ cells produced by an otherwise similar cell population that is CLL1 wildtype.

347. The cell population of any of embodiments 285-346, which, when administered to a subject, produces hCD34+ cells in the subject, e.g., when assayed at 16 weeks after
15 administration.

348. The cell population of embodiment 347, which produces levels of hCD34+ cells comparable to the levels of hCD34+ cells produced with an otherwise similar cell population that is CLL1 wildtype.
20

349. The cell population of embodiments 347 or 348, which produces levels of hCD34+ cells that is at least 70%, 80%, 85%, 90%, or 95% the levels of hCD34+ cells produced by an otherwise similar cell population that is CLL1 wildtype.

- 25 350. The cell population of any of embodiments 285-349, which, when administered to a subject, produces mast cells, basophils, eosinophils, common dendric cells (cDCs), plasmacytoid dendric cells (pDCs), neutrophils, monocytes, T cells, B, cells or any combination thereof, in the subject, e.g., when assayed at 16 weeks after administration.

- 30 351. The cell population of embodiment 350, which produces levels of mast cells, basophils, eosinophils, common dendric cells (cDCs), plasmacytoid dendric cells (pDCs), neutrophils, monocytes, T cells, B, cells or any combination thereof comparable to the levels of said cell type produced with an otherwise similar cell population that is CLL1 wildtype.

352. The cell population of embodiments 350 or 351, which produces levels of mast cells, basophils, eosinophils, common dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), neutrophils, monocytes, T cells, B cells or any combination thereof that is at least 70%, 80%, 85%, 90%, or 95% the levels of said cell type produced by an otherwise similar cell population that is CLL1 wildtype.

353. The cell population of any of embodiments 350-352, wherein the produced cells are detected in a blood sample, a bone marrow sample, or a spleen sample obtained from the subject.

10

354. The cell population of any of embodiments 285-353, which, when administered to a subject, persists for at least 8, 12, or 16 weeks in the subject.

355. The cell population of any of embodiments 285-354, which, when administered to a subject, provides multilineage hematopoietic reconstitution.

15

356. The cell population of any of embodiments 285-355, which, produces CD14+ cells, e.g., when assayed at 7 or 14 days after genetic engineering.

357. The cell population of embodiment 356, which produces levels of CD14+ cells comparable to the levels of CD14+ cells produced with an otherwise similar cell population that is CLL1 wildtype.

20

358. The cell population of embodiments 356 or 357, which produces levels of CD14+ cells that is at least 70%, 80%, 85%, 90%, or 95% the levels of CD14+ cells produced by an otherwise similar cell population that is CLL1 wildtype.

25

359. The cell population of embodiments any of 356-358, wherein CD14+ levels are assayed after culturing in vitro in myeloid differentiation media.

30

360. The cell population of any of embodiments 285-359, which, produces CD11b+ cells, e.g., when assayed at 7 or 14 days after genetic engineering.

361. The cell population of embodiment 360, which produces levels of CD11b+ cells comparable to the levels of CD11b+ cells produced with an otherwise similar cell population that is CLL1 wildtype.

- 5 362. The cell population of embodiments 360 or 361, which produces levels of CD11b+ cells that is at least 70%, 80%, 85%, 90%, or 95% the levels of CD11b+ cells produced by an otherwise similar cell population that is CLL1 wildtype.

363. The cell population of embodiments any of 356-362, wherein CD15+ levels are assayed
10 after culturing in vitro in myeloid differentiation media.

364. The cell population of any of embodiments 285-362, which, produces CD15+ cells, e.g., when assayed at 7 or 14 days after genetic engineering.

- 15 365. The cell population of embodiment 364, which produces levels of CD15+ cells comparable to the levels of CD11b+ cells produced with an otherwise similar cell population that is CLL1 wildtype.

366. The cell population of embodiments 364 or 365, which produces levels of CD15+ cells
20 that is at least 70%, 80%, 85%, 90%, or 95% the levels of CD15+ cells produced by an otherwise similar cell population that is CLL1 wildtype.

367. The cell population of embodiments any of 356-366, wherein CD15+ levels are assayed
after culturing in vitro in myeloid differentiation media.
25

368. The cell population of any of embodiments 285-367, wherein the most abundant mutation in CLL1 in the cell population is a deletion, e.g., a deletion of 1 nt, 2 nt, 3 nt, or 4 nt.

- 30 369. The cell population of any of embodiments 285-367, wherein the most abundant mutation in CLL1 in the cell population is an insertion, e.g., an insertion of 1 nt.

370. The cell population of any of embodiments 285-368, wherein the most abundant mutation in the cell population within the sequence of SEQ ID NO: 16 in CLL1 is a deletion, e.g., a deletion of 1 nt.

- 5 371. The cell population of embodiment 285-370, which further comprises one or both of a 2 nt deletion or a 1 nt insertion within the sequence of SEQ ID NO: 16 in a copy of CLL1.

372. The cell population of any of embodiments 285-368, wherein the most abundant mutation in the cell population within the sequence of SEQ ID NO: 14 in CLL1 is an
10 insertion, e.g., an insertion of 1 nt.

373. The cell population of embodiment 285-368 or 224aa, which further comprises a 1 nt deletion within the sequence of SEQ ID NO: 14 in a copy of CLL1.

- 15 374. The cell population of any of embodiments 188-368, wherein the most abundant mutation in the cell population within the sequence of SEQ ID NO: 17 in CLL1 is a deletion, e.g., a deletion of 2 nt.

375. The cell population of embodiment 285-368 or 374, which further comprises a 1 nt
20 insertion within the sequence of SEQ ID NO: 17 in a copy of CLL1.

376. The cell population of any of embodiments 285-368, wherein the most abundant mutation in the cell population within the sequence of SEQ ID NO: 17 in CLL1 is an
insertion, e.g., an insertion of 1 nt.

- 25 377. The cell population of embodiment 285-368 or 376, which further comprises a 2 nt deletion within the sequence of SEQ ID NO: 40 in a copy of CLL1.

378. The cell population of any of embodiments 285-368, wherein the most abundant
30 mutation in the cell population within the sequence of SEQ ID NO: 43 in CLL1 is a deletion, e.g., a deletion of 4 nt.

379. The cell population of embodiment 285-368 or 378, which further comprises a 1 nt insertion within the sequence of SEQ ID NO: 43 in a copy of CLL1.

380. The cell population of any of embodiments 285-379, which comprises hematopoietic stem cells and hematopoietic progenitor cells.

5 381. The cell population of any of embodiments 285-380, wherein at least 80%, 85%, 90%, 95%, or 98% of cells in the population are viable.

382. The cell population of any of embodiments 285-381, wherein at least 50%, 60%, 70%, 80%, or 90% of copies of CLL1 comprise a mutation.

10

383. The cell population of any of embodiments 285-382, wherein at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of cells in the population are negative for cell surface expression of CLL1, e.g., using a flow cytometry assay for CLL1 cell surface expression.

15

384. A pharmaceutical composition comprising the genetically engineered hematopoietic stem or progenitor cell of any of embodiments 184 or 186-284.

385. A pharmaceutical composition comprising the cell population of any of embodiments
20 285-383.

386. A mixture, e.g., a reaction mixture comprising:

- 25 a) a gRNA of any of embodiments 1-105, or gRNAs of a composition or kit of any of embodiments 106-133; and
- b) a cell, e.g., a hematopoietic cell, e.g., an HSC or HPC, e.g., a genetically engineered cell of any of embodiments 184 or 186-284.

387. The mixture of embodiment 386, wherein the cell is a wild-type cell or a cell having a mutation in CLL1.

388. A kit comprising any two or more (e.g., three or all) of:

- 30 a) a gRNA of any of embodiments 1-105, or gRNAs of a composition or kit of any of embodiments 106-133;

- b) a cell, e.g., a hematopoietic cell, e.g., an HSC or HPC, e.g., a genetically engineered cell of any of embodiments 184 or 186-284;
- c) a Cas9 molecule; and
- d) agent that targets CLL1, e.g., an agent as described herein.

5 389. The kit of embodiment 388, which comprises (a) and (b), (a) and (c), (a) and d), (b) and (c), (b) and (d), or (c) and (d).

390. A method of making the genetically engineered cell (e.g., hematopoietic stem or progenitor cell) of any of embodiments 184 or 186-284, or the cell population of any of
10 embodiments 285-383, which comprises:
(i) providing a cell (e.g., a hematopoietic stem or progenitor cell, e.g., a wild-type hematopoietic stem or progenitor cell), and
(ii) introducing into the cell a nuclease (e.g., an endonuclease) that cleaves the target domain,
15 thereby producing a genetically engineered hematopoietic stem or progenitor cell.

391. The method of embodiment 390, wherein (ii) comprises introducing into the cell a gRNA that binds the target domain (e.g., a gRNA of any of embodiments 1-105 and an endonuclease that binds the gRNA).
20

392. The method of embodiment 390, wherein the endonuclease is a ZFN, TALEN, or meganuclease.

393. A method of supplying HSCs, HPCs, or HSPCs to a subject, comprising
25 administering to the subject a plurality of cells of any of embodiments 184 or 186-284, or the cell population of any of embodiments 285-383.

394. A method, comprising administering to a subject a subject in need thereof a plurality of cells of any of embodiments 184 or 186-284, or the cell population of any of embodiments
30 285-383.

395. The method of embodiment 393 or 394, wherein the subject has a cancer, wherein cells of the cancer express CLL1 (e.g., wherein at least a plurality of the cancer cells express CLL1).

- 5 396. The method of any of embodiments 393-395, which further comprises administering to the subject an effective amount of an agent that targets CLL1, and wherein the agent comprises an antigen-binding fragment that binds CLL1.

397. The method of embodiment 396, wherein the agent that targets CLL1 is an immune
10 cell expressing a chimeric antigen receptor (CAR), which comprises the antigen-binding fragment that binds CLL1.

398. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-383 for use in
15 treating a hematopoietic disorder, wherein the treating comprises administering to a subject in need thereof an effective amount of the genetically engineered hematopoietic stem or progenitor cell or the cell population, and further comprises administering to the subject an effective amount of an agent that targets CLL1, wherein the agent comprises an antigen-binding fragment that binds CLL1.

- 20 399. An agent that targets CLL1, wherein the agent comprises an antigen-binding fragment that binds CLL1, for use in treating a hematopoietic disorder, wherein the treating comprises administering to a subject in need thereof an effective amount of the agent that targets CLL1, and further comprises administering to the subject an effective amount of a genetically
25 engineered hematopoietic stem or progenitor cell of any of embodiments 184 or 186-284 or a cell population of any of embodiments 285-383.

400. A combination of a genetically engineered hematopoietic stem or progenitor cell of any of embodiments 184 or 186-284 or a cell population of any of embodiments 285-383, and
30 an agent that targets CLL1, wherein the agent comprises an antigen-binding fragment that binds CLL1, for use in treating a hematopoietic disorder, wherein the treating comprises administering to a subject in need thereof an effective amount of the genetically engineered hematopoietic stem or progenitor cell or the cell population, and the agent that binds CLL1.

401. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in
cancer immunotherapy.
- 5 402. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in
cancer immunotherapy, wherein the subject has a hematopoietic disorder.
- 10 403. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in
hematopoietic repopulation of a subject having a hematopoietic disorder.
- 15 404. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in a
method of treating a hematopoietic disorder, whereby the genetically engineered
hematopoietic stem or progenitor cell described herein or a cell population described herein
repopulate the subject.
- 20 405. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in
reducing cytotoxic effects of an agent that targets CLL1 in immunotherapy.
- 25 406. A genetically engineered hematopoietic stem or progenitor cell of any of
embodiments 184 or 186-284 or a cell population of any of embodiments 285-384 for use in
an immunotherapy method using an agent that targets CLL1, whereby the genetically
engineered hematopoietic stem or progenitor cell described herein or a cell population
described herein reduces cytotoxic effects of the agent that targets CLL1.
- 30 407. The method, cell, agent, or combination of any of embodiments 285-384, wherein the
genetically engineered hematopoietic stem or progenitor cell or the cell population is
administered concomitantly with the agent that targets CLL1.

408. The method, cell, agent, or combination of any of embodiments 393-407, wherein the genetically engineered hematopoietic stem or progenitor cell or the cell population is administered prior to the agent that targets CLL1.

5 409. The method, cell, agent, or combination of any of embodiments 393-408, wherein the agent that targets CLL1 is administered prior to the genetically engineered hematopoietic stem or progenitor cell or the cell population.

410. The method, cell, agent, or combination of any of embodiments 393-409, wherein the
10 immune cell is a T cell.

411. The method, cell, agent, or combination of any of embodiments 393-410, wherein the immune cell, the genetically engineered hematopoietic stem and/or progenitor cell, or both, are allogeneic.

15 412. The method, cell, agent, or combination of any of embodiments 393-411, wherein the immune cell, the genetically engineered hematopoietic stem and/or progenitor cell, or both, are autologous.

20 413. The method, cell, agent, or combination of any of embodiments 393-412, wherein the antigen-binding fragment in the chimeric receptor is a single-chain antibody fragment (scFv) that specifically binds human CLL1.

414. The method, cell, agent, or combination of any of embodiments 393-413, wherein
25 hematopoietic disorder is a cancer, and wherein at least a plurality of cancer cells in the cancer express CLL1.

415. The method, cell, agent, or combination of any of embodiments 393-414, wherein the subject has a hematopoietic malignancy, e.g., a hematopoietic malignancy chosen from
30 Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia (e.g., acute myeloid leukemia, acute lymphoid leukemia, chronic myelogenous leukemia, acute lymphoblastic leukemia or chronic lymphoblastic leukemia, and chronic lymphoid leukemia), or multiple myeloma.

416. The method, cell, agent, or combination of any of embodiments 393-415, wherein the subject has a hematological disorder, e.g., a precancerous condition, e.g., a myelodysplasia, a myelodysplastic syndrome (MDS), or a preleukemia.

5 The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a graph showing CLL1 gRNA screening on CD34⁺ cells. Human CD34⁺ cells were electroporated with Cas9 protein and CLL1-targeting gRNAs (listed on the y-axis). Editing efficiency of *CLEC12A* locus, shown on the x-axis, was determined by Sanger sequencing and TIDE analysis.

15 **Figures 2A-2D** are a series of diagrams showing survival and differentiation of CLL1-edited CD34⁺ cells. **(A)** Schematic showing the workflow of the experiment. Human CD34⁺ cells were electroporated with Cas9 protein and CLL1-targeting gRNA, followed by analysis of editing efficiency by TIDE and a CFU assay to assess *in vitro* differentiation. **(B)** Cell viability was measured 48 hours post electroporation. No Cas9 RNP group was used as control. **(C)** Editing efficiency of *CLEC12A* locus was determined by Sanger sequencing and TIDE analysis. **(D)** Control or CLL1-edited CD34⁺ cells were plated in Methocult 2 days after electroporation and scored for colony formation after 14 days. BFU-E: burst forming unit-erythroid; CFU-GM: colony forming unit-granulocyte/macrophage; CFU-GEMM: colony forming unit of multipotential myeloid progenitor cells (generate granulocytes, erythrocytes, monocytes, and megakaryocytes). Student's t-test was used.

Figure 3 shows target expression on AML cell lines. The expression of CD33, CD123 and CLL1 in MOLM-13 and THP-1 cells and an unstained control was determined by flow cytometric analysis. The X-axis indicates the intensity of antibody staining and the Y-axis corresponds to number of cells.

30 **Figure 4** shows CD33- and CLL1-modified HL-60 cells. The expression of CD33 and CLL1 in wild-type (WT), CD33^{-/-}, CLL1^{-/-} and CD33^{-/-} CLL1^{-/-} HL-60 cells was assessed by flow cytometry. For the generation of CD33^{-/-} or CLL1^{-/-} HL-60 cells, WT HL-60 cells were electroporated with CD33- or CLL1-targeting RNP, followed by flow cytometric sorting of

CD33- or CLL1-negative cells. CD33^{-/-}CLL1^{-/-} HL-60 cells were generated by electroporating CD33^{-/-} cells with CLL1-targeting RNP and sorted for CLL1-negative population. The X-axis indicates the intensity of antibody staining and the Y-axis corresponds to number of cells.

5 **Figure 5** shows an in vitro cytotoxicity assay of CD33 and CLL1 CAR-Ts. Anti-CD33 CAR-T and anti-CLL1 CAR-T were incubated with wild-type (WT), CD33^{-/-}, CLL1^{-/-} and CD33^{-/-} CLL1^{-/-} HL-60 cells, and cytotoxicity was assessed by flow cytometry. Non-transduced T cells were used as mock CAR-T control. The CARpool group was composed of 1:1 pooled combination of anti-CD33 and anti-CLL1 CAR-T cells. Student's t test was used.

10 ns = not significant; *P < 0.05; **P < 0.01, ***P < 0.001, ****P < 0.0001. The Y-axis indicates the percentage of specific killing.

Figure 6 shows gene-editing efficiency of CD34+ cells. Human CD34+ cells were electroporated with Cas9 protein and CD33-, CD123- or CLL1- targeting gRNAs, either alone or in combination. Editing efficiency of CD33, CD123 or CLL1 locus was determined

15 by Sanger sequencing and TIDE analysis. The Y-axis indicates the editing efficiency (% by TIDE).

Figures 7A-7C shows in vitro colony formation of gene-edited CD34+ cells. Control or CD33, CD123, CLL1-modified CD34+ cells were plated in Methocult 2 days after electroporation and scored for colony formation after 14 days. BFU-E: burst forming unit-erythroid; CFU-GM: colony forming unit-granulocyte/macrophage; CFU-GEMM: colony forming unit of multipotential myeloid progenitor cells (generate granulocytes, erythrocytes, monocytes, and megakaryocytes). Student's t test was used.

20

Figure 8 shows gene editing frequency of CD34+ cells. Human CD34+ cells were electroporated with ribonucleoprotein (RNP) complexes composed of Cas9 protein and the

25 CLL1-targeting gRNAs indicated on the X-axis, the sequences of which are found in Table 8. Editing frequency of the CLL1 locus was determined by Sanger sequencing. The Y-axis indicates the editing frequency.

Figure 9 shows gene editing frequency of CD34+ cells. Human CD34+ cells were electroporated with Cas9 protein and the CLL1-targeting gRNAs indicated on the X-axis, specifically from left to right, gRNA D, F, G, O2, and P2. Editing frequency of the CLL1 locus was determined by Sanger sequencing. The Y-axis indicates the editing frequency. All gRNAs in **Figure 9** led to an editing frequency $\geq 80\%$.

30

Figure 10 shows the INDEL (insertion/deletion) distribution for human CD34+ cells edited with the CLL1-targeting gRNAs, specifically gRNA D (top left), gRNA F (middle

left), gRNA G (bottom left), gRNA O2 (top right), and gRNA P2 (middle right). The X-axis indicates the size of the INDEL and the Y-axis indicates the percentage of the specific INDEL in the mixture.

Figure 11 is a schematic and overview of the protocol and experimental
 5 procedure/timeline used for *in vivo* characterization of CLL1-edited HSPCs in NBSGW mice.

Figures 12A-12D depict long-term lineage engraftment of CLL1-edited cells in the bone marrow of mice 16 weeks post-engraftment of non-edited control cells or CLL1KO cells. **Figure 12A** shows the rates of human leukocyte chimerism calculated as percentage of human CD45+ (hCD45+) cells in the total CD45+ cell population (the sum of human and
 10 mouse CD45+ cells) in the bone marrow at week 16 post-engraftment of control cells (EP ctrl) or CLL1 KO cells edited with gRNA F. **Figure 12B** shows the percentage of hCD45+ cells that were also positive for human CD34 (hCD34+) in the bone marrow at week 16 post-engraftment of control cells (EP ctrl) or CLL1KO cells edited with gRNA F. **Figure 12C** shows the percentage of hCD45+ cells that were B-cells, T cells, monocytes, neutrophils,
 15 conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), eosinophils, basophils, and mast cells) in the bone marrow at week 16 post-engraftment of control cells (EP ctrl) or CLL1KO cells edited with gRNA F. **Figure 12D** shows the percentages of hCD45+ that were also CLL1+ quantified in the bone marrow at week 16 post-engraftment of control cells (EP ctrl) or CLL1KO cells edited with gRNA F.

Figure 13A shows cell-surface expression of CLL1 *in vitro* as measured by FACs in, from top to bottom, non-edited control cells, CLL1 KO cells edited by gRNA F (editing frequency of 79.2% as measured by TIDE), and an FMO (fluorescence minus one) control. **Figure 13B** shows the quantification granulocytes produced over time from *in vitro* culturing of non-edited control cells (EP ctrl) or CLL1 KO cells edited by gRNA F. **Figure 13C** shows
 25 the quantification monocytes produced over time from *in vitro* culture of non-edited control cells (EP ctrl) or CLL1KO cells edited by gRNA F.

Figure 14A shows the percentage of CLL1+ granulocytes (top) or monocytes (bottom) produced over time from *in vitro* culturing non-edited control cells (EP ctrl) or CLL1 KO cells edited by gRNA F. **Figure 14B** shows the percentage of CD15+ (top left) or CD11b+ positive granulocytes (top right) or the percentage of CD14+ (bottom left) or CD11b+ positive monocytes (bottom right) quantified at day 0, 7, and 14 following editing and culture of non-edited control cells or CLL1KO cells edited by gRNA F.

Figure 15 shows the percentage of phagocytosis measured in granulocytes (top) or monocytes (bottom) produced from non-edited control cells (EP ctrl) or CLL1 KO cells edited by the gRNA F.

Figure 16A shows the production of IL-6 in pg/mL (right) or TNF- α in pg/mL (left) by granulocytes produced from non-edited control cells (EP ctrl) or CLL1 KO cells edited by gRNA F, that were unstimulated, stimulated by LPS, or stimulated by R848. **Figure 16B** shows the production of IL-6 in pg/mL (right) or TNF- α in pg/mL (left) by monocytes produced from non-edited control cells (EP ctrl) or CLL1KO cells edited by gRNA F that were unstimulated, stimulated by LPS, or stimulated by R848.

Figure 17A-17B shows *in vitro* colony formation of gene-edited CD34+ cells. Control or CLL1-modified CD34+ cells were plated in after electroporation and scored for colony formation after 14 days. BFU-E: burst forming unit-erythroid; CFU-GM: colony forming unit-granulocyte/macrophage; CFU-GEMM: colony forming unit of multipotential myeloid progenitor cells (generate granulocytes, erythrocytes, monocytes, and megakaryocytes). **Figure 17A** shows colony count of BFU-E, CFU-G/M/GM, or CFU-GEMM that resulted from non-edited cells (EP ctrl) or CLL1KO cells edited by gRNA F (editing frequency of 79.2%). **Figure 17B** shows percent colony distribution of BFU-E, CFU-G/M/GM, or CFU-GEMM that resulted from non-edited cells (EP ctrl) or CLL1KO cells edited by gRNA F.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term “binds”, as used herein with reference to a gRNA interaction with a target domain, refers to the gRNA molecule and the target domain forming a complex. The complex may comprise two strands forming a duplex structure, or three or more strands forming a multi-stranded complex. The binding may constitute a step in a more extensive process, such as the cleavage of the target domain by a Cas endonuclease. In some embodiments, the gRNA binds to the target domain with perfect complementarity, and in

other embodiments, the gRNA binds to the target domain with partial complementarity, e.g., with one or more mismatches. In some embodiments, when a gRNA binds to a target domain, the full targeting domain of the gRNA base pairs with the targeting domain. In other embodiments, only a portion of the target domain and/or only a portion of the targeting domain base pairs with the other. In an embodiment, the interaction is sufficient to mediate a target domain-mediated cleavage event.

A “Cas9 molecule” as that term is used herein, refers to a molecule or polypeptide that can interact with a gRNA and, in concert with the gRNA, home or localize to a site which comprises a target domain. Cas9 molecules include naturally occurring Cas9 molecules and engineered, altered, or modified Cas9 molecules that differ, e.g., by at least one amino acid residue, from a naturally occurring Cas9 molecule.

The terms “gRNA” and “guide RNA” are used interchangeably throughout and refer to a nucleic acid that promotes the specific targeting or homing of a gRNA/Cas9 molecule complex to a target nucleic acid. A gRNA can be unimolecular (having a single RNA molecule), sometimes referred to herein as sgRNAs, or modular (comprising more than one, and typically two, separate RNA molecules). A gRNA may bind to a target domain in the genome of a host cell. The gRNA may comprise a targeting domain that may be partially or completely complementary to the target domain. The gRNA may also comprise a “scaffold sequence,” (e.g., a tracrRNA sequence), that recruits a Cas9 molecule to a target domain bound to a gRNA sequence (e.g., by the targeting domain of the gRNA sequence). The scaffold sequence may comprise at least one stem loop structure and recruits an endonuclease. Exemplary scaffold sequences can be found, for example, in Jinek, et al. Science (2012) 337(6096):816-821, Ran, et al. Nature Protocols (2013) 8:2281-2308, PCT Application No. WO2014/093694, and PCT Application No. WO2013/176772.

The term “mutation” is used herein to refer to a genetic change (e.g., insertion, deletion, or substitution) in a nucleic acid compared to a reference sequence, e.g., the corresponding wild-type nucleic acid. In some embodiments, a mutation to a gene detargetizes the protein produced by the gene. In some embodiments, a detargetized CLL1 protein is not bound by, or is bound at a lower level by, an agent that targets CLL1.

The “targeting domain” of the gRNA is complementary to the “target domain” on the target nucleic acid. The strand of the target nucleic acid comprising the nucleotide sequence complementary to the core domain of the gRNA is referred to herein as the “complementary

strand” of the target nucleic acid. Guidance on the selection of targeting domains can be found, e.g., in Fu Y et al, Nat Biotechnol 2014 (doi: 10.1038/nbt.2808) and Sternberg SH et al., Nature 2014 (doi: 10.1038/nature13011).

5 Nucleases

In some embodiments, a cell (e.g., HSC or HPC) described herein is made using a nuclease described herein. Exemplary nucleases include Cas molecules (e.g., Cas9, TALENs, ZFNs, and meganucleases. In some embodiments, a nuclease is used in combination with a CLL1 gRNA described herein (e.g., according to Table 2, 6, or 8).

10 Cas9 molecules

In some embodiments, a CLL1 gRNA described herein is complexed with a Cas9 molecule. Various Cas9 molecules can be used. In some embodiments, a Cas9 molecule is selected that has the desired PAM specificity to target the gRNA/Cas9 molecule complex to the target domain in CLL1. In some embodiments, genetically engineering a cell also
15 comprises introducing one or more (e.g., 1, 2, 3 or more) Cas9 molecules into the cell.

Cas9 molecules of a variety of species can be used in the methods and compositions described herein. In embodiments, the Cas9 molecule is of, or derived from, *S. pyogenes* (SpCas9), *S. aureus* (SaCas9) or *S. thermophilus*. Additional suitable Cas9 molecules include those of, or derived from, *Staphylococcus aureus*, *Neisseria meningitidis* (NmCas9),
20 *Acidovorax avenae*, *Actinobacillus pleuropneumoniae*, *Actinobacillus succinogenes*, *Actinobacillus suis*, *Actinomyces* sp., *Cyclophilus denitrificans*, *Aminomonas paucivorans*, *Bacillus cereus*, *Bacillus smithii*, *Bacillus thuringiensis*, *Bacteroides* sp., *Blastopirellula marina*, *Bradyrhizobium* sp., *Brevibacillus laterosporus*, *Campylobacter coli*, *Campylobacter jejuni* (CjCas9), *Campylobacter lari*, *Candidatus Puniceispirillum*, *Clostridium*
25 *cellulolyticum*, *Clostridium perfringens*, *Corynebacterium accolens*, *Corynebacterium diphtheria*, *Corynebacterium matruchotii*, *Dinoroseobacter shibae*, *Eubacterium dolichum*, *gamma proteobacterium*, *Gluconacetobacter diazotrophicus*, *Haemophilus parainfluenzae*, *Haemophilus sputorum*, *Helicobacter canadensis*, *Helicobacter cinaedi*, *Helicobacter mustelae*, *Ilyobacter polytropus*, *Kingella kingae*, *Lactobacillus crispatus*, *Listeria ivanovii*,
30 *Listeria monocytogenes*, *Listeriaceae bacterium*, *Methylocystis* sp., *Methylosinus trichosporium*, *Mobiluncus mulieris*, *Neisseria bacilliformis*, *Neisseria cinerea*, *Neisseria*

flavescens, *Neisseria lactamica*, *Neisseria meningitidis*, *Neisseria sp.*, *Neisseria wadsworthii*, *Nitrosomonas sp.*, *Parvibaculum lavamentivorans*, *Pasteurella multocida*, *Phascolarctobacterium succinatutens*, *Ralstonia syzygii*, *Rhodopseudomonas palustris*, *Rhodovulum sp.*, *Simonsiella muelleri*, *Sphingomonas sp.*, *Sporolactobacillus vineae*,
 5 *Staphylococcus lugdunensis*, *Streptococcus sp.*, *Subdoligranulum sp.*, *Tistrella mobilis*, *Treponema sp.*, or *Verminephrobacter eiseniae*.

In some embodiments, the Cas9 molecule is a naturally occurring Cas9 molecule. In some embodiments, the Cas9 molecule is an engineered, altered, or modified Cas9 molecule that differs, e.g., by at least one amino acid residue, from a reference sequence, e.g., the most
 10 similar naturally occurring Cas9 molecule or a sequence of Table 50 of WO2015157070, which is herein incorporated by reference in its entirety. In some embodiments, the Cas9 molecule comprises Cpf1 or a fragment or variant thereof.

A naturally occurring Cas9 molecule typically comprises two lobes: a recognition (REC) lobe and a nuclease (NUC) lobe; each of which further comprises domains described,
 15 e.g., in WO2015157070, e.g., in Figs. 9A-9B therein (which application is incorporated herein by reference in its entirety).

The REC lobe comprises the arginine-rich bridge helix (BH), the REC1 domain, and the REC2 domain. The REC lobe appears to be a Cas9-specific functional domain. The BH domain is a long alpha helix and arginine rich region and comprises amino acids 60-93 of the
 20 sequence of *S. pyogenes* Cas9. The REC1 domain is involved in recognition of the repeat:anti-repeat duplex, e.g., of a gRNA or a tracrRNA. The REC1 domain comprises two REC1 motifs at amino acids 94 to 179 and 308 to 717 of the sequence of *S. pyogenes* Cas9. These two REC1 domains, though separated by the REC2 domain in the linear primary structure, assemble in the tertiary structure to form the REC1 domain. The REC2 domain, or
 25 parts thereof, may also play a role in the recognition of the repeat: anti-repeat duplex. The REC2 domain comprises amino acids 180-307 of the sequence of *S. pyogenes* Cas9.

The NUC lobe comprises the RuvC domain (also referred to herein as RuvC-like domain), the HNH domain (also referred to herein as HNH-like domain), and the PAM-interacting (PI) domain. The RuvC domain shares structural similarity to retroviral integrase
 30 superfamily members and cleaves a single strand, e.g., the non-complementary strand of the target nucleic acid molecule. The RuvC domain is assembled from the three split RuvC motifs (RuvC I, RuvCII, and RuvCIII, which are often commonly referred to in the art as

RuvCI domain, or N-terminal RuvC domain, RuvCII domain, and RuvCIII domain) at amino acids 1-59, 718-769, and 909-1098, respectively, of the sequence of *S. pyogenes* Cas9.

Similar to the REC1 domain, the three RuvC motifs are linearly separated by other domains in the primary structure, however in the tertiary structure, the three RuvC motifs assemble and form the RuvC domain. The HNH domain shares structural similarity with HNH endonucleases, and cleaves a single strand, e.g., the complementary strand of the target nucleic acid molecule. The HNH domain lies between the RuvC II-III motifs and comprises amino acids 775-908 of the sequence of *S. pyogenes* Cas9. The PI domain interacts with the PAM of the target nucleic acid molecule, and comprises amino acids 1099-1368 of the sequence of *S. pyogenes* Cas9.

Crystal structures have been determined for naturally occurring bacterial Cas9 molecules (Jinek et al., *Science*, 343(6176): 1247997, 2014) and for *S. pyogenes* Cas9 with a guide RNA (e.g., a synthetic fusion of crRNA and tracrRNA) (Nishimasu et al., *Cell*, 156:935-949, 2014; and Anders et al., *Nature*, 2014, doi: 10.1038/nature13579).

In some embodiments, a Cas9 molecule described herein has nuclease activity, e.g., double strand break activity. In some embodiments, the Cas9 molecule has been modified to inactivate one of the catalytic residues of the endonuclease. In some embodiments, the Cas9 molecule is a nickase and produces a single stranded break. See, e.g., Dabrowska et al. *Frontiers in Neuroscience* (2018) 12(75). It has been shown that one or more mutations in the RuvC and HNH catalytic domains of the enzyme may improve Cas9 efficiency. See, e.g., Sarai et al. *Currently Pharma. Biotechnol.* (2017) 18(13). In some embodiments, the Cas9 molecule is fused to a second domain, e.g., a domain that modifies DNA or chromatin, e.g., a deaminase or demethylase domain. In some such embodiments, the Cas9 molecule is modified to eliminate its endonuclease activity.

In some embodiments, a Cas9 molecule described herein is administered together with a template for homology directed repair (HDR). In some embodiments, a Cas9 molecule described herein is administered without a HDR template.

In some embodiments, the Cas9 molecule is modified to enhance specificity of the enzyme (e.g., reduce off-target effects, maintain robust on-target cleavage). In some embodiments, the Cas9 molecule is an enhanced specificity Cas9 variant (e.g., eSPCas9). See, e.g., Slaymaker et al. *Science* (2016) 351 (6268): 84-88. In some embodiments, the

Cas9 molecule is a high fidelity Cas9 variant (*e.g.*, SpCas9-HF1). See, *e.g.*, Kleinstiver et al. *Nature* (2016) 529: 490-495.

Various Cas9 molecules are known in the art and may be obtained from various sources and/or engineered/modified to modulate one or more activities or specificities of the enzymes. In some embodiments, the Cas9 molecule has been engineered/modified to recognize one or more PAM sequence. In some embodiments, the Cas9 molecule has been engineered/modified to recognize one or more PAM sequence that is different than the PAM sequence the Cas9 molecule recognizes without engineering/modification. In some embodiments, the Cas9 molecule has been engineered/modified to reduce off-target activity of the enzyme.

In some embodiments, the nucleotide sequence encoding the Cas9 molecule is modified further to alter the specificity of the endonuclease activity (*e.g.*, reduce off-target cleavage, decrease the endonuclease activity or lifetime in cells, increase homology-directed recombination and reduce non-homologous end joining). See, *e.g.*, Komor et al. *Cell* (2017) 168: 20-36. In some embodiments, the nucleotide sequence encoding the Cas9 molecule is modified to alter the PAM recognition of the endonuclease. For example, the Cas9 molecule SpCas9 recognizes PAM sequence NGG, whereas relaxed variants of the SpCas9 comprising one or more modifications of the endonuclease (*e.g.*, VQR SpCas9, EQR SpCas9, VRER SpCas9) may recognize the PAM sequences NGA, NGAG, NGCG. PAM recognition of a modified Cas9 molecule is considered “relaxed” if the Cas9 molecule recognizes more potential PAM sequences as compared to the Cas9 molecule that has not been modified. For example, the Cas9 molecule SaCas9 recognizes PAM sequence NNGRRT, whereas a relaxed variant of the SaCas9 comprising one or more modifications (*e.g.*, KKH SaCas9) may recognize the PAM sequence NNNRRT. In one example, the Cas9 molecule FnCas9 recognizes PAM sequence NNG, whereas a relaxed variant of the FnCas9 comprising one or more modifications of the endonuclease (*e.g.*, RHA FnCas9) may recognize the PAM sequence YG. In one example, the Cas9 molecule is a Cpf1 endonuclease comprising substitution mutations S542R and K607R and recognize the PAM sequence TYCV. In one example, the Cas9 molecule is a Cpf1 endonuclease comprising substitution mutations S542R, K607R, and N552R and recognize the PAM sequence TATV. See, *e.g.*, Gao et al. *Nat. Biotechnol.* (2017) 35(8): 789-792.

In some embodiments, more than one (*e.g.*, 2, 3, or more) Cas9 molecules are used. In some embodiments, at least one of the Cas9 molecule is a Cas9 enzyme. In some embodiments, at least one of the Cas molecules is a Cpf1 enzyme. In some embodiments, at

least one of the Cas9 molecule is derived from *Streptococcus pyogenes*. In some embodiments, at least one of the Cas9 molecule is derived from *Streptococcus pyogenes* and at least one Cas9 molecule is derived from an organism that is not *Streptococcus pyogenes*. In some embodiments, the Cas9 molecule is a base editor. Base editor endonuclease

5 generally comprises a catalytically inactive Cas9 molecule fused to a function domain. See, *e.g.*, Eid et al. *Biochem. J.* (2018) 475(11): 1955-1964; Rees et al. *Nature Reviews Genetics* (2018) 19:770-788. In some embodiments, the catalytically inactive Cas9 molecule is dCas9. In some embodiments, the endonuclease comprises a dCas9 fused to one or more uracil glycosylase inhibitor (UGI) domains. In some embodiments, the endonuclease comprises a

10 dCas9 fused to an adenine base editor (ABE), for example an ABE evolved from the RNA adenine deaminase TadA. In some embodiments, the endonuclease comprises a dCas9 fused to cytidine deaminase enzyme (*e.g.*, APOBEC deaminase, pmCDA1, activation-induced cytidine deaminase (AID)). In some embodiments, the catalytically inactive Cas9 molecule has reduced activity and is nCas9. In some embodiments, the catalytically inactive Cas9

15 molecule (dCas9) is fused to one or more uracil glycosylase inhibitor (UGI) domains. In some embodiments, the Cas9 molecule comprises a nCas9 fused to an adenine base editor (ABE), for example an ABE evolved from the RNA adenine deaminase TadA. In some embodiments, the Cas9 molecule comprises a nCas9 fused to cytidine deaminase enzyme (*e.g.*, APOBEC deaminase, pmCDA1, activation-induced cytidine deaminase (AID)).

20 Examples of base editors include, without limitation, BE1, BE2, BE3, HF-BE3, BE4, BE4max, BE4-Gam, YE1-BE3, EE-BE3, YE2-BE3, YEE-CE3, VQR-BE3, VRER-BE3, SaBE3, SaBE4, SaBE4-Gam, Sa(KKH)-BE3, Target-AID, Target-AID-NG, xBE3, eA3A-BE3, BE-PLUS, TAM, CRISPR-X, ABE7.9, ABE7.10, ABE7.10*, xABE, ABESa, VQR-ABE, VRER-ABE, Sa(KKH)-ABE, and CRISPR-SKIP. Additional examples of base editors

25 can be found, for example, in US Publication No. 2018/0312825A1, US Publication No. 2018/0312828A1, and PCT Publication No. WO 2018/165629A1, which are incorporated by reference herein in their entireties.

In some embodiments, the base editor has been further modified to inhibit base excision repair at the target site and induce cellular mismatch repair. Any of the Cas9

30 molecules described herein may be fused to a Gam domain (bacteriophage Mu protein) to protect the Cas9 molecule from degradation and exonuclease activity. See, *e.g.*, Eid et al. *Biochem. J.* (2018) 475(11): 1955-1964.

In some embodiments, the Cas9 molecule belongs to class 2 type V of Cas endonuclease. Class 2 type V Cas endonucleases can be further categorized as type V-A,

type V-B, type V-C, and type V-U. See, e.g., Stella et al. *Nature Structural & Molecular Biology* (2017). In some embodiments, the Cas molecule is a type V-A Cas endonuclease, such as a Cpf1 nuclease. In some embodiments, the Cas9 molecule is a type V-B Cas endonuclease, such as a C2c1 endonuclease. See, e.g., Shmakov et al. *Mol Cell* (2015) 60: 385-397. In some embodiments, the Cas molecule is Mad7. Alternatively or in addition, the Cas9 molecule is a Cpf1 nuclease or a variant thereof. As will be appreciated by one of skill in the art, the Cpf1 nuclease may also be referred to as Cas12a. See, e.g., Strohkendl et al. *Mol. Cell* (2018) 71: 1-9. In some embodiments, a composition or method described herein involves, or a host cell expresses a Cpf1 nuclease derived from *Providentia spp.* or *Francisella spp.*, *Acidaminococcus sp.* (AsCpf1), *Lachnospiraceae bacterium* (LpCpf1), or *Eubacterium rectale*. In some embodiments, the nucleotide sequence encoding the Cpf1 nuclease may be codon optimized for expression in a host cell. In some embodiments, the nucleotide sequence encoding the Cpf1 endonuclease is further modified to alter the activity of the protein.

In some embodiments, catalytically inactive variants of Cas molecules (e.g., of Cas9 or Cas12a) are used according to the methods described herein. A catalytically inactive variant of Cpf1 (Cas12a) may be referred to dCas12a. As described herein, catalytically inactive variants of Cpf1 may be fused to a function domain to form a base editor. See, e.g., Rees et al. *Nature Reviews Genetics* (2018) 19:770-788. In some embodiments, the catalytically inactive Cas9 molecule is dCas9. In some embodiments, the endonuclease comprises a dCas12a fused to one or more uracil glycosylase inhibitor (UGI) domains. In some embodiments, the Cas9 molecule comprises a dCas12a fused to an adenine base editor (ABE), for example an ABE evolved from the RNA adenine deaminase TadA. In some embodiments, the Cas molecule comprises a dCas12a fused to cytidine deaminase enzyme (e.g., APOBEC deaminase, pmCDA1, activation-induced cytidine deaminase (AID)).

Alternatively or in addition, the Cas9 molecule may be a Cas14 endonuclease or variant thereof. Cas14 endonucleases are derived from archaea and tend to be smaller in size (e.g., 400–700 amino acids). Additionally Cas14 endonucleases do not require a PAM sequence. See, e.g., Harrington et al. *Science* (2018).

Any of the Cas9 molecules described herein may be modulated to regulate levels of expression and/or activity of the Cas9 molecule at a desired time. For example, it may be advantageous to increase levels of expression and/or activity of the Cas9 molecule during particular phase(s) of the cell cycle. It has been demonstrated that levels of homology-directed repair are reduced during the G1 phase of the cell cycle, therefore increasing levels of expression and/or activity of the Cas9 molecule during the S phase, G2 phase, and/or M

phase may increase homology-directed repair following the Cas endonuclease editing. In some embodiments, levels of expression and/or activity of the Cas9 molecule are increased during the S phase, G2 phase, and/or M phase of the cell cycle. In one example, the Cas9 molecule fused to the N-terminal region of human Geminin. See, e.g., Gutschner et al. Cell Rep. (2016) 14(6): 1555-1566. In some embodiments, levels of expression and/or activity of the Cas9 molecule are reduced during the G1 phase. In one example, the Cas9 molecule is modified such that it has reduced activity during the G1 phase. See, e.g., Lomova et al. Stem Cells (2018).

Alternatively or in addition, any of the Cas9 molecules described herein may be fused to an epigenetic modifier (e.g., a chromatin-modifying enzyme, e.g., DNA methylase, histone deacetylase). See, e.g., Kungulovski et al. Trends Genet. (2016) 32(2):101-113. Cas9 molecule fused to an epigenetic modifier may be referred to as “epieffectors” and may allow for temporal and/or transient endonuclease activity. In some embodiments, the Cas9 molecule is a dCas9 fused to a chromatin-modifying enzyme.

Zinc Finger Nucleases

In some embodiments, a cell or cell population described herein is produced using zinc finger (ZFN) technology. In some embodiments, the ZFN recognizes a target domain described herein, e.g., in Table 1. In general, zinc finger mediated genomic editing involves use of a zinc finger nuclease, which typically comprises a zinc finger DNA binding domain and a nuclease domain. The zinc finger binding domain may be engineered to recognize and bind to any target domain of interest, e.g., may be designed to recognize a DNA sequence ranging from about 3 nucleotides to about 21 nucleotides in length, or from about 8 to about 19 nucleotides in length. Zinc finger binding domains typically comprise at least three zinc finger recognition regions (e.g., zinc fingers).

Restriction endonucleases (restriction enzymes) capable of sequence-specific binding to DNA (at a recognition site) and cleaving DNA at or near the site of binding are known in the art and may be used to form ZFN for use in genomic editing. For example, Type IIS restriction endonucleases cleave DNA at sites removed from the recognition site and have separable binding and cleavage domains. In one example, the DNA cleavage domain may be derived from the FokI endonuclease.

TALENs

In some embodiments, a cell or cell population described herein is produced using TALEN technology. In some embodiments, the TALEN recognizes a target domain described herein, e.g., in Table 1. In general, TALENs are engineered restriction enzymes that can specifically bind and cleave a desired target DNA molecule. A TALEN typically contains a Transcriptional Activator-Like Effector (TALE) DNA-binding domain fused to a DNA cleavage domain. The DNA binding domain may contain a highly conserved 33-34 amino acid sequence with a divergent 2 amino acid RVD (repeat variable dipeptide motif) at positions 12 and 13. The RVD motif determines binding specificity to a nucleic acid sequence and can be engineered to specifically bind a desired DNA sequence. In one example, the DNA cleavage domain may be derived from the FokI endonuclease. In some embodiments, the FokI domain functions as a dimer, using two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing.

A TALEN specific to a target gene of interest can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, a foreign DNA molecule having a desired sequence can be introduced into the cell along with the TALEN. Depending on the sequence of the foreign DNA and chromosomal sequence, this process can be used to correct a defect or introduce a DNA fragment into a target gene of interest, or introduce such a defect into the endogenous gene, thus decreasing expression of the target gene.

Some exemplary, non-limiting embodiments of endonucleases and nuclease variants suitable for use in connection with the guide RNAs and genetic engineering methods provided herein have been described above. Additional suitable nucleases and nuclease variants will be apparent to those of skill in the art based on the present disclosure and the knowledge in the art. The disclosure is not limited in this respect.

30

gRNA sequences and configurationsgRNA configuration generally

A gRNA can comprise a number of domains. In an embodiment, a unimolecular, sgRNA, or chimeric, gRNA comprises, e.g., from 5' to 3':

- 5 a targeting domain (which is complementary, or partially complementary, to a target nucleic acid sequence in a target gene, e.g., in the CLL1 gene;
- a first complementarity domain;
- a linking domain;
- 10 a second complementarity domain (which is complementary to the first complementarity domain);
- a proximal domain; and
- optionally, a tail domain.

Each of these domains is now described in more detail.

- The targeting domain may comprise a nucleotide sequence that is complementary,
- 15 e.g., at least 80, 85, 90, or 95% complementary, e.g., fully complementary, to the target sequence on the target nucleic acid. The targeting domain is part of an RNA molecule and will therefore typically comprise the base uracil (U), while any DNA encoding the gRNA molecule will comprise the base thymine (T). While not wishing to be bound by theory, in an embodiment, it is believed that the complementarity of the targeting domain with the target
- 20 sequence contributes to specificity of the interaction of the gRNA /Cas9 molecule complex with a target nucleic acid. It is understood that in a targeting domain and target sequence pair, the uracil bases in the targeting domain will pair with the adenine bases in the target sequence. In an embodiment, the target domain itself comprises in the 5' to 3' direction, an optional secondary domain, and a core domain. In an embodiment, the core domain is fully
- 25 complementary with the target sequence. In an embodiment, the targeting domain is 5 to 50 nucleotides in length. The targeting domain may be between 15-25 nucleotides, 18-22 nucleotides, or 19-21 nucleotides in length. In some embodiments, the targeting domain is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the targeting domain is between 10-30, or between 15-25, nucleotides in length.

- 30 In some embodiments, a targeting domain comprises a core domain and a secondary targeting domain, e.g., as described in International Application WO2015157070, which is

incorporated by reference in its entirety. In an embodiment, the core domain comprises about 8 to about 13 nucleotides from the 3' end of the targeting domain (e.g., the most 3' 8 to 13 nucleotides of the targeting domain). In an embodiment, the secondary domain is positioned 5' to the core domain. In many embodiments, the core domain has exact
 5 complementarity with the corresponding region of the target sequence. In other embodiments, the core domain can have 1 or more nucleotides that are not complementary with the corresponding nucleotide of the target sequence.

The first complementarity domain is complementary with the second complementarity domain, and in an embodiment, has sufficient complementarity to the
 10 second complementarity domain to form a duplexed region under at least some physiological conditions. In an embodiment, the first complementarity domain is 5 to 30 nucleotides in length. In an embodiment, the first complementarity domain comprises 3 subdomains, which, in the 5' to 3' direction are: a 5' subdomain, a central subdomain, and a 3' subdomain. In an embodiment, the 5' subdomain is 4 to 9, e.g., 4, 5, 6, 7, 8 or 9 nucleotides in length. In
 15 an embodiment, the central subdomain is 1, 2, or 3, e.g., 1, nucleotide in length. In an embodiment, the 3' subdomain is 3 to 25, e.g., 4 to 22, 4 to 18, or 4 to 10, or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. The first complementarity domain can share homology with, or be derived from, a naturally occurring first complementarity domain. In an embodiment, it has at least 50% homology with a *S.*
 20 *pyogenes*, *S. aureus* or *S. thermophilus*, first complementarity domain.

The sequence and placement of the above-mentioned domains are described in more detail in WO2015157070, which is herein incorporated by reference in its entirety, including p. 88-112 therein.

A linking domain serves to link the first complementarity domain with the second
 25 complementarity domain of a unimolecular gRNA. The linking domain can link the first and second complementarity domains covalently or non-covalently. In an embodiment, the linkage is covalent. In an embodiment, the linking domain is, or comprises, a covalent bond interposed between the first complementarity domain and the second complementarity domain. In some embodiments, the linking domain comprises one or more, e.g., 2, 3, 4, 5, 6,
 30 7, 8, 9, or 10 nucleotides. In some embodiments, the linking domain comprises at least one non-nucleotide bond, e.g., as disclosed in WO2018126176, the entire contents of which are incorporated herein by reference.

The second complementarity domain is complementary, at least in part, with the first complementarity domain, and in an embodiment, has sufficient complementarity to the

second complementarity domain to form a duplexed region under at least some physiological conditions. In an embodiment, the second complementarity domain can include a sequence that lacks complementarity with the first complementarity domain, e.g., a sequence that loops out from the duplexed region. In an embodiment, the second complementarity domain is 5 to 27 nucleotides in length. In an embodiment, the second complementarity domain is longer than the first complementarity region. In an embodiment, the complementary domain is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides in length. In an embodiment, the second complementarity domain comprises 3 subdomains, which, in the 5' to 3' direction are: a 5' subdomain, a central subdomain, and a 3' subdomain. In an embodiment, the 5' subdomain is 3 to 25, e.g., 4 to 22, 4 to 18, or 4 to 10, or 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In an embodiment, the central subdomain is 1, 2, 3, 4 or 5, e.g., 3, nucleotides in length. In an embodiment, the 3' subdomain is 4 to 9, e.g., 4, 5, 6, 7, 8 or 9 nucleotides in length. In an embodiment, the 5' subdomain and the 3' subdomain of the first complementarity domain, are respectively, complementary, e.g., fully complementary, with the 3' subdomain and the 5' subdomain of the second complementarity domain.

In an embodiment, the proximal domain is 5 to 20 nucleotides in length. In an embodiment, the proximal domain can share homology with or be derived from a naturally occurring proximal domain. In an embodiment, it has at least 50% homology with an *S. pyogenes*, *S. aureus* or *S. thermophilus*, proximal domain.

A broad spectrum of tail domains are suitable for use in gRNAs. In an embodiment, the tail domain is 0 (absent), 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In some embodiments, the tail domain nucleotides are from or share homology with a sequence from the 5' end of a naturally occurring tail domain. In an embodiment, the tail domain includes sequences that are complementary to each other and which, under at least some physiological conditions, form a duplexed region. In an embodiment, the tail domain is absent or is 1 to 50 nucleotides in length. In an embodiment, the tail domain can share homology with or be derived from a naturally occurring proximal tail domain. In an embodiment, it has at least 50% homology with an *S. pyogenes*, *S. aureus* or *S. thermophilus*, tail domain. In an embodiment, the tail domain includes nucleotides at the 3' end that are related to the method of in vitro or in vivo transcription.

In some embodiments, modular gRNA comprises:

a first strand comprising, e.g., from 5' to 3':

a targeting domain (which is complementary to a target nucleic acid in the CLL1 gene) and

a first complementarity domain; and

a second strand, comprising, preferably from 5' to 3':

5 optionally, a 5' extension domain;

a second complementarity domain;

a proximal domain; and

optionally, a tail domain.

In some embodiments, the gRNA is chemically modified. For instance, the gRNA
10 may comprise one or more modification chosen from phosphorothioate backbone
modification, 2'-O-Me-modified sugars (e.g., at one or both of the 3' and 5' termini), 2'-F-
modified sugar, replacement of the ribose sugar with the bicyclic nucleotide-cEt, 3'thioPACE
(MSP), or any combination thereof. Suitable gRNA modifications are described, e.g., in
Rahdar et al. PNAS December 22, 2015 112 (51) E7110-E7117 and Hendel et al., Nat
15 Biotechnol. 2015 Sep; 33(9): 985-989, each of which is incorporated herein by reference in
its entirety. In some embodiments, a gRNA described herein comprises one or more 2'-O-
methyl-3'-phosphorothioate nucleotides, e.g., at least 2, 3, 4, 5, or 6 2'-O-methyl-3'-
phosphorothioate nucleotides. In some embodiments, a gRNA described herein comprises
modified nucleotides (e.g., 2'-O-methyl-3'-phosphorothioate nucleotides) at the three terminal
20 positions and the 5' end and/or at the three terminal positions and the 3' end. In some
embodiments, the gRNA may comprise one or more modified nucleotides, e.g., as described
in International Applications WO/2017/214460, WO/2016/089433, and WO/2016/164356,
which are incorporated by reference their entirety.

In some embodiments, a gRNA described herein is chemically modified. For
25 example, the gRNA may comprise one or more 2'-O modified nucleotides, e.g., 2'-O-methyl
nucleotides. In some embodiments, the gRNA comprises a 2'-O modified nucleotide, e.g.,
2'-O-methyl nucleotide at the 5' end of the gRNA. In some embodiments, the gRNA
comprises a 2'-O modified nucleotide, e.g., 2'-O-methyl nucleotide at the 3' end of the
gRNA. In some embodiments, the gRNA comprises a 2'-O-modified nucleotide, e.g., 2'-O-
30 methyl nucleotide at both the 5' and 3' ends of the gRNA. In some embodiments, the gRNA

is 2'-O-modified, e.g. 2'-O-methyl-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, and the third nucleotide from the 5' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified, e.g. 2'-O-methyl-modified at the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified, e.g. 2'-O-methyl-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified, e.g. 2'-O-methyl-modified at the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and at the fourth nucleotide from the 3' end of the gRNA. In some embodiments, the nucleotide at the 3' end of the gRNA is not chemically modified. In some embodiments, the nucleotide at the 3' end of the gRNA does not have a chemically modified sugar. In some embodiments, the gRNA is 2'-O-modified, e.g. 2'-O-methyl-modified, at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA. In some embodiments, the 2'-O-methyl nucleotide comprises a phosphate linkage to an adjacent nucleotide. In some embodiments, the 2'-O-methyl nucleotide comprises a phosphorothioate linkage to an adjacent nucleotide. In some embodiments, the 2'-O-methyl nucleotide comprises a thioPACE linkage to an adjacent nucleotide.

In some embodiments, the gRNA may comprise one or more 2'-O-modified and 3'phosphorous-modified nucleotide, e.g., a 2'-O-methyl 3'phosphorothioate nucleotide. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'phosphorothioate nucleotide at the 5' end of the gRNA. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'phosphorothioate nucleotide at the 3' end of the gRNA. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'phosphorothioate nucleotide at the 5' and 3' ends of the gRNA. In some embodiments, the gRNA comprises a backbone in which one or more non-bridging oxygen atoms has been replaced with a sulfur atom. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'phosphorothioate-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, and the third

nucleotide from the 5' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'phosphorothioate-modified at the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'phosphorothioate-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'phosphorothioate-modified at the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA. In some embodiments, the nucleotide at the 3' end of the gRNA is not chemically modified. In some embodiments, the nucleotide at the 3' end of the gRNA does not have a chemically modified sugar. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'phosphorothioate-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA.

In some embodiments, the gRNA may comprise one or more 2'-O-modified and 3'-phosphorous-modified, e.g., 2'-O-methyl 3'thioPACE nucleotide. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'thioPACE nucleotide at the 5' end of the gRNA. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'thioPACE nucleotide at the 3' end of the gRNA. In some embodiments, the gRNA comprises a 2'-O-modified and 3'phosphorous-modified, e.g., 2'-O-methyl 3'thioPACE nucleotide at the 5' and 3' ends of the gRNA. In some embodiments, the gRNA comprises a backbone in which one or more non-bridging oxygen atoms have been replaced with a sulfur atom and one or more non-bridging oxygen atoms have been replaced with an acetate group. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'thioPACE-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, and the third nucleotide from the 5' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'thioPACE-modified at the nucleotide at the 3' end of the gRNA, the second nucleotide

from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'thioPACE-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the second nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'thioPACE-modified at the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA. In some embodiments, the nucleotide at the 3' end of the gRNA is not chemically modified. In some embodiments, the nucleotide at the 3' end of the gRNA does not have a chemically modified sugar. In some embodiments, the gRNA is 2'-O-modified and 3'phosphorous-modified, e.g. 2'-O-methyl 3'thioPACE-modified at the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA.

In some embodiments, the gRNA comprises a chemically modified backbone. In some embodiments, the gRNA comprises a phosphorothioate linkage. In some embodiments, one or more non-bridging oxygen atoms have been replaced with a sulfur atom. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, and the third nucleotide from the 5' end of the gRNA each comprise a phosphorothioate linkage. In some embodiments, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA each comprise a phosphorothioate linkage. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA each comprise a phosphorothioate linkage. In some embodiments, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and at the fourth nucleotide from the 3' end of the gRNA each comprise a phosphorothioate linkage. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA,

and the fourth nucleotide from the 3' end of the gRNA each comprise a phosphorothioate linkage.

In some embodiments, the gRNA comprises a thioPACE linkage. In some embodiments, the gRNA comprises a backbone in which one or more non-bridging oxygen atoms have been replaced with a sulfur atom and one or more non-bridging oxygen atoms have been replaced with an acetate group. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, and the third nucleotide from the 5' end of the gRNA each comprise a thioPACE linkage. In some embodiments, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA each comprise a thioPACE linkage. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end of the gRNA, the nucleotide at the 3' end of the gRNA, the second nucleotide from the 3' end of the gRNA, and the third nucleotide from the 3' end of the gRNA each comprise a thioPACE linkage. In some embodiments, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and at the fourth nucleotide from the 3' end of the gRNA each comprise a thioPACE linkage. In some embodiments, the nucleotide at the 5' end of the gRNA, the second nucleotide from the 5' end of the gRNA, the third nucleotide from the 5' end, the second nucleotide from the 3' end of the gRNA, the third nucleotide from the 3' end of the gRNA, and the fourth nucleotide from the 3' end of the gRNA each comprise a thioPACE linkage.

Some exemplary, non-limiting embodiments of modifications, e.g., chemical modifications, suitable for use in connection with the guide RNAs and genetic engineering methods provided herein have been described above. Additional suitable modifications, e.g., chemical modifications, will be apparent to those of skill in the art based on the present disclosure and the knowledge in the art, including, but not limited to those described in Hendel, A. et al., Nature Biotech., 2015, Vol 33, No. 9; in WO/2017/214460; in WO/2016/089433; and/or in WO/2016/164356; each one of which is herein incorporated by reference in its entirety.

gRNAs targeting CLL1

The present disclosure provides a number of useful gRNAs that can target an endonuclease to human CLL1. Table 1 below illustrates target domains in human endogenous CLL1 that can be bound by gRNAs described herein.

Table 1. Exemplary target domains of human CLL1 bound by various gRNAs are described herein. For each target domain, the first sequence represents a 20-nucleotide DNA sequence corresponding to the target domain sequence that can be targeted by a suitable gRNA, which may comprise an equivalent RNA targeting domain sequence (comprising RNA nucleotides instead of the DNA nucleotides in the sequences provided below), and the second sequence is the reverse complement thereof. Bolding indicates that the sequence is present in the human CLL1 cDNA sequence shown below as SEQ ID NO: 31.

	Target Domain Sequences
gRNA A	CCCAGAAATTGGCAAATTTG (SEQ ID NO: 1) CAAATTTGCCAATTTCTGGG (SEQ ID NO: 11)
gRNA B	ATTCCAGAACTCCAGTGAGA (SEQ ID NO: 2) TCTCACTGGAGTTCTGGAAT (SEQ ID NO: 12)
gRNA C	GAGCTATATAGCAAAGAACA (SEQ ID NO: 3) TGTTCTTTGCTATATAGCTC (SEQ ID NO: 13)
gRNA D	GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4) CACTGCAAACAATAGCCACC (SEQ ID NO: 14)
gRNA E	TTGTTTGCAGTGTGGTGGAG (SEQ ID NO: 5) CTCCACCACACTGCAAACAA (SEQ ID NO: 15)
gRNA F	TAGCTCACGACATAATTTGG (SEQ ID NO: 6) CCAAATTATGTCGTGAGCTA (SEQ ID NO: 16)
gRNA G	GGCTATTGTTTGCAGTGTGG (SEQ ID NO: 7) CCACACTGCAAACAATAGCC (SEQ ID NO: 17)
gRNA H	GGAGAGGTTTCCTGATCTTGT (SEQ ID NO: 8) ACAAGATCAGGAACCTCTCC (SEQ ID NO: 18)
gRNA I	TGAATATCTCCAACAAGATC (SEQ ID NO: 9) GATCTTGTGGAGATATTCA (SEQ ID NO: 19)
gRNA J	GTTGTAGAGAAATATTTCTC (SEQ ID NO: 10) GAGAAATATTTCTCTACAAC (SEQ ID NO: 20)
gRNA O2	ATATAATCAACTCCTCTGCC (SEQ ID NO: 40) GGCAGAGGAGTTGATTATAT (SEQ ID NO: 41)

gRNA P2	AATAGACACTTACCAGGCAG (SEQ ID NO: 42)
	CTGCCTGGTAAGTGTCTATT (SEQ ID NO: 43)

Table 2. Exemplary target domain sequences of human CLL1 bound by various gRNAs are provided herein. For each target domain, the first sequence represents a DNA target sequence adjacent to a suitable PAM in the human CLL1 genomic sequence, and the second sequence

5 represents an exemplary suitable gRNA targeting domain sequence.

	Sequences	PAM
gRNA A	CCCAGAAATTGGCAAATTTG (SEQ ID NO: 1)	GGG
	CCCAGAAAUUGGCAAAUUUG (SEQ ID NO: 21)	
gRNA B	ATTCCAGAACTCCAGTGAGA (SEQ ID NO: 2)	TGG
	AUUCCAGAACUCCAGUGAGA (SEQ ID NO: 22)	
gRNA C	GAGCTATATAGCAAAGAACA (SEQ ID NO: 3)	AGG
	GAGCUAUUAGCAAAGAACA (SEQ ID NO: 23)	
gRNA D	GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4)	TGG
	GGUGGCUAUUGUUUGCAGUG (SEQ ID NO: 24)	
gRNA E	TTGTTTGCAGTGTGGTGGAG (SEQ ID NO: 5)	AGG
	UUGUUUGCAGUGUGGUGGAG (SEQ ID NO: 25)	
gRNA F	TAGCTCACGACATAATTTGG (SEQ ID NO: 6)	TGG
	UAGCUCACGACAUAAUUUGG (SEQ ID NO: 26)	
gRNA G	GGCTATTGTTTGCAGTGTGG (SEQ ID NO: 7)	TGG
	GGCUAUUGUUUGCAGUGUGG (SEQ ID NO: 27)	
gRNA H	GGAGAGGTTCTGATCTTGT (SEQ ID NO: 8)	TGG
	GGAGAGGUUCCUGAUCUUGU (SEQ ID NO: 28)	
gRNA I	TGAATATCTCCAACAAGATC (SEQ ID NO: 9)	AGG
	UGAAUAUCUCCAACAAGAUC (SEQ ID NO: 29)	
gRNA J	GTTGTAGAGAAATATTTCTC (SEQ ID NO: 10)	TGG
	GUUGUAGAGAAAUUUUCUC (SEQ ID NO: 30)	
gRNA O2	ATATAATCAACTCCTCTGCC (SEQ ID NO: 40)	TGG
	AUAUAAUCAACUCCUCUGCC (SEQ ID NO: 44)	
gRNA P2	AATAGACACTTACCAGGCAG (SEQ ID NO: 42)	AGG
	AAUAGACACUUACCAGGCAG (SEQ ID NO: 45)	

Table 6. Exemplary target domain sequences of human CLL1 bound by various gRNAs are provided herein. For each target domain, a DNA target sequence adjacent to a suitable PAM in the human CLL1 genomic sequence is provided. A gRNA targeting a target domain provided herein may comprise an equivalent RNA sequence within its targeting domain.

	SEQ ID NO:	Sequence	PAM
gRNA K	67	ATGCAGAAGTCAAATTTGAA	AGG
gRNA L	68	TCAAATTTGACTTCTGCATG	TGG
gRNA M	69	TTTGACTTCTGCATGTGGAT	AGG
gRNA N	70	GGATAGGTGAGTGTCTTTTG	TGG
gRNA O	71	TTTCCATCTCACTGGAGTTC	TGG
gRNA P	72	TGGGATTTTTTCCATCTCAC	TGG
gRNA Q	73	ATGGAAAAAATCCCAGAAAT	TGG
gRNA R	74	ATCCCAGAAATTGGCAAATT	TGG
gRNA S	75	TCCCAGAAATTGGCAAATTT	GGG
gRNA T	76	CCCCAAATTTGCCAATTTCT	GGG
gRNA U	77	TCCCCAAATTTGCCAATTTT	TGG
gRNA V	78	ATTGGCAAATTTGGGGAAAA	AGG
gRNA W	79	AAAGGTAAGATTTTGAGTTA	TGG
gRNA X	80	AGAGGGAGCTGGAGGTGCTG	TGG
gRNA Y	81	CTCCAGCTCCCTCTCATGTA	TGG
gRNA Z	82	CATACATGAGAGGGAGCTGG	AGG
gRNA A1	83	CGCCATACATGAGAGGGAGC	TGG
gRNA B1	84	GCTGGACGCCATACATGAGA	GGG
gRNA C1	85	TGCTGGACGCCATACATGAG	AGG

gRNA D1	86	AGAGTCAGAAACAAGGCTGC	TGG
gRNA E1	87	GCACAGAAGAGTCAGAAACA	AGG
gRNA F1	88	CTGTGCCTTCTGTTGCTCAT	TGG
gRNA G1	89	CCTTCTGTTGCTCATTGGAT	TGG
gRNA H1	90	CTTCTGTTGCTCATTGGATT	GGG
gRNA I1	91	CCAATCCAATGAGCAACAGA	AGG
gRNA J1	92	GCTCATTGGATTGGGAGTCT	TGG
gRNA K1	93	ATATAGCTCACGACATAATT	TGG
gRNA L1	94	AAGGTAATCTTGTATTCTCT	TGG
gRNA M1	95	CAAGGCTTACATTTGTGCTC	TGG
gRNA N1	96	ACAAATGTAAGCCTTGTCCA	AGG
gRNA O1	97	GTAAGCCTTGTCCAAGGAGA	TGG
gRNA P1	98	CTTGTCCAAGGAGATGGATT	TGG
gRNA Q1	99	CAAATCCATCTCCTTGGACA	AGG
gRNA R1	100	AAGGAGATGGATTGGCATA	AGG
gRNA S1	101	TTATGCCAAATCCATCTCCT	TGG
gRNA T1	102	TAAGTGATGATGTCCAAACA	TGG
gRNA U1	103	ATGTTTGGACATCATCACTT	AGG
gRNA V1	104	TGATGATGTCCAAACATGGC	AGG
gRNA W1	105	AACATGGCAGGAGAGTAAAA	TGG
gRNA X1	106	TTTTACTCTCCTGCCATGTT	TGG
gRNA Y1	107	GCTGGCATTCTGAGCAGCAC	AGG
gRNA Z1	108	GTTGTTTATCTTCAACAGGC	TGG

gRNA A2	109	TTTTGTTGTTTATCTTCAAC	AGG
gRNA B2	110	GATAAACAACAAAAATGCAT	TGG
gRNA C2	111	AAAATGCATTGGTAAAGCCC	AGG
gRNA D2	112	AGACTATCTGTATTTCTGT	AGG
gRNA E2	113	GGGATTTTATAAATTCCTAC	AGG
gRNA F2	114	AGAGTAGATCATATGACTAT	TGG
gRNA G2	115	ATAGTCATATGATCTACTCT	GGG
gRNA H2	116	AATAGTCATATGATCTACTC	TGG
gRNA I2	117	TAGATCATATGACTATTGGC	TGG
gRNA J2	118	AGATCATATGACTATTGGCT	GGG
gRNA K2	119	CCTGAAGAAGATTCCACTCG	TGG
gRNA L2	120	CCACGAGTGGAATCTTCTTC	AGG
gRNA M2	121	TTCCACTCGTGGTATGAGAG	TGG
gRNA N2	122	ATCCACTCTCATACCACGAG	TGG
gRNA Q2	123	AACAAGAATAGACACTTACC	AGG
gRNA R2	124	AATTCTTTACTTTCTCTTTC	AGG
gRNA S2	125	ATTCTTTACTTTCTCTTTCA	GGG
gRNA T2	126	GACTTAAATAACATGTATTG	TGG
gRNA U2	127	CAATACATGTTATTTAAGTC	AGG
gRNA V2	128	AAGAATGATATGTGAGAAGA	TGG
gRNA W2	129	ATGGCCAATCCAGTGCAGCT	TGG
gRNA X2	130	AGAACCAAGCTGCACTGGAT	TGG
gRNA Y2	131	TATGTAGAACCAAGCTGCAC	TGG

gRNA Z2	132	AGCTTGGTTCTACATATTTT	AGG
gRNA A3	133	GCTTGGTTCTACATATTTTA	GGG
gRNA B3	134	TGGTTCTACATATTTTAGGG	AGG
gRNA C3	135	ACATATTTTAGGGAGGCATG	AGG
gRNA D3	136	TATTTTGAAGATCAGAGATG	TGG
gRNA E3	137	ATCTTCAAAATATCATGATT	TGG
gRNA F3	138	TCTTCAAAATATCATGATTT	GGG
gRNA G3	139	TATCATGATTTGGGTTTTCC	TGG
gRNA H3	140	TCTCTTCTTGTTTTACTTCC	AGG
gRNA I3	141	CTGTCCACCTCTTGCATTA	AGG
gRNA J3	142	CTTAATGCAAAGAGGTGGAC	AGG
gRNA K3	143	CCTCTTTGCATTAAGGTAGT	TGG
gRNA L3	144	ACTACCTTAATGCAAAGAGG	TGG
gRNA M3	145	CCAACCTACCTTAATGCAAAG	AGG
gRNA N3	146	TTAAGGTAGTTGGTCCACCT	TGG
gRNA O3	147	GAAGGCTGGCATGACCAAGG	TGG
gRNA P3	148	GAGGAAGGCTGGCATGACCA	AGG
gRNA Q3	149	TTAATGTGTGAGAGGAAGGC	TGG
gRNA R3	150	TTATTTAATGTGTGAGAGGA	AGG
gRNA S3	151	AACATTATTTAATGTGTGAG	AGG
gRNA T3	152	ATAATGTTTGTTCTCACAGA	AGG
gRNA U3	153	TGTTTGTTCTCACAGAAGGT	AGG
gRNA V3	154	GTTTGTTCTCACAGAAGGTA	GGG

gRNA W3	155	ATTGTGCTTATTTTCATAAT	GGG
gRNA X3	156	AATTGTGCTTATTTTCATAA	TGG
gRNA Y3	157	GAGTTTTTGGGAAGATGGAAA	AGG
gRNA Z3	158	AGGTTTGAGTTTTTGGGAAGA	TGG
gRNA A4	159	CCAAAACTCAAACCTGTGA	AGG
gRNA B4	160	CAAAAACTCAAACCTGTGAA	GGG
gRNA C4	161	CCTTCACAGGTTTGAGTTTT	TGG
gRNA D4	162	TATTCTAACTTTCCCTTCAC	AGG
gRNA E4	163	TCAGAAAATATGTAAGTCAC	TGG
gRNA F4	164	CTGGAAGTGGGAGGATGGCT	CGG
gRNA G4	165	GAGGTCTGGAAGTGGGAGGA	TGG
gRNA H4	166	GAATGAGGTCTGGAAGTGGG	AGG
gRNA I4	167	GTCGAATGAGGTCTGGAAGT	GGG
gRNA J4	168	AGTCGAATGAGGTCTGGAAG	TGG
gRNA K4	169	TCCAGACCTCATTCGACTTC	TGG
gRNA L4	170	GCCAGAAGTCGAATGAGGTC	TGG
gRNA M4	171	ATCTCGCCAGAAGTCGAATG	AGG
gRNA N4	172	ACTCATTTAATTCGTTGGGT	CGG
gRNA O4	173	CCAACGAATTAAATGAGTCC	TGG
gRNA P4	174	CAGGACTCATTTAATTCGTT	GGG
gRNA Q4	175	CCAGGACTCATTTAATTCGT	TGG
gRNA R4	176	AATAGGAGTACATCAAAGCC	AGG
gRNA S4	177	TGTTTATTTATGTTTACAAT	AGG

The CLL1 (NM_138337.6) cDNA sequence is provided below as SEQ ID NO: 31.

Underlining, bolding, or italics indicates the regions complementary to gRNA A, B, C, D, E, F, G, H, I, J, or O2 (or the reverse complement thereof). Bolding and italics are used where

5 there is overlap between two or more such regions.

GGCTCATTTGCAGACATATGGGTGATTGGTACAGTAGGTTTATAAACAGAAGTTTAAACTTG
 TAAGCTTAAGCTTCCGTTTATAAACAGAAGTTTAAATTATAGGTCCTGTTTAAACATTCAGC
 TCTGTTAACTCACTCATCTTTTTGTGTTTTTACACTTTGTCAAGATTTCTTTACATATTCAT
 CAATGTCTGAAGAAGTTACTTATGCAGATCTTCAATTCCAGAACTCCAGTGAGATGGAAAAA
 10 ATCCCAGAAATTGGCAAATTTGGGGAAAAAGCACCTCCAGCTCCCTCTCATGTATGGCGTCC
 AGCAGCCTTGTTTCTGACTCTTCTGTGCCTTCTGTTGCTCATTGGATTGGGAGTCTTGCGAA
 GCATGTTTCACGTAACCTTTGAAGATAGAAATGAAAAAATGAACAACTACAAAACATCAGT
 GAAGAGCTCCAGAGAAATATTTCTCTACAACCTGATGAGTAACAT**TGAATATCTCCAACAAGAT**
CAGGAACCTCTCCACCACACTGCAAACAATAGCCACCAAATTATGTCGTGAGCTATATAGCA
 15 **AAGAACAAGAGCACAAATGTAAGCCTTGCCAAGGAGATGGATTGGCATAAGGACAGCTGT**
TATTTCCCTAAGTGATGATGTCCAAACATGGCAGGAGAGTAAATGGCCTGTGCTGCTCAGAA
TGCCAGCCTGTTGAAGATAAACAACAAAAATGCATTGGAATTTATAAAATCCCAGAGTAGAT
CATATGACTATTGGCTGGGATTATCTCCTGAAGAAGATTCCACTCGTGGTATGAGAGTGGAT
AATATAATCAACTCCTCTGCCTGGGTTATAAGAAACGCACCTGACTTAAATAACATGTATTG
 20 **TGGATATATAAATAGACTATATGTTCAATATTATCACTGCACCTATAAAAAAAGAATGATAT**
GTGAGAAGATGGCCAATCCAGTGCAGCTTGGTTCTACATATTTAGGGAGGCATGAGGCATC
AATCAAATACATTTAAGGAGTGTAGGGGGTGGGGGTTCTAGGCTATAGGTAAATTTAAATAT
TTTCTGGTTGACAATTAGTTGAGTTTGTCTGAAGACCTGGGATTTTATCATGCAGATGAAAC
ATCCAGGTAGCAAGCTTCAGAGAGAATAGACTGTGAATGTTAATGCCAGAGAGGTATAATGA
 25 **AGCATGTCCACCTCCCACTTTCCATCATGGCCTGAACCTGGAGGAAGAGGAAGTCCATTC**
AGATAGTTGTGGGGGGCCTTCGAATTTTCATTTTACGTTCTTCCCCTTCTGGCCAAG
ATTTGCCAGAGGCAACATCAAAAACAGCAAATTTTAATTTTGTCCACAGCGTTGCTAGGG
TGGCATGGCTCCCATCTCGGGTCCATCCTATACTTCCATGGGACTCCCTATGGCTGAAGGC
CTTATGAGTCAAAGGACTTATAGCCAATTGATTGTTCTAGGCCAGGTAAGAATGGATATGGA
 30 **CATGCATTTATTACCTCTTAAAATTATTATTTTAAGTAAAAGCCAATAAACAAAAACGAAAA**
GGCAA (SEQ ID NO: 31)

An additional CLL1 isoform (ENST00000355690.8) cDNA is provided as:

GGAAGAACAGCCTTTCAAATTTGACTTCTGCATGTGGATAGATTTCTTTACATATTCATCAA
 35 TGTCTGAAGAAGTTACTTATGCAGATCTTCAATTCCAGAACTCCAGTGAGATGGAAAAATC
 CCAGAAATTGGCAAATTTGGGGAAAAAGCACCTCCAGCTCCCTCTCATGTATGGCGTCCAGC
 AGCCTTGTTTCTGACTCTTCTGTGCCTTCTGTTGCTCATTGGATTGGGAGTCTTGCGAAGCA
 TGTTTCACGTAACCTTTGAAGATAGAAATGAAAAAATGAACAACTACAAAACATCAGTGAA
 GAGCTCCAGAGAAATATTTCTCTACAACCTGATGAGTAACATGAATATCTCCAACAAGATCAG
 40 GAACCTCTCCACCACACTGCAAACAATAGCCACCAAATTATGTCGTGAGCTATATAGCAAAG
 AACAAGAGCACAAATGTAAGCCTTGTTCCAAGGAGATGGATTGGCATAAGGACAGCTGTTAT
 TTCCTAAGTGATGATGTCCAAACATGGCAGGAGAGTAAATGGCCTGTGCTGCTCAGAATGC
 CAGCCTGTTGAAGATAAACAACAAAAATGCATTGGAATTTATAAAATCCCAGAGTAGATCAT
 ATGACTATTGGCTGGGATTATCTCCTGAAGAAGATTCCACTCGTGGTATGAGAGTGGATAAT

ATAATCAACTCCTCTGCCTGGGTTATAAGAAACGCACCTGACTTAAATAACATGTATTGTGG
 ATATATAAATAGACTATATGTTCAATATTATCACTGCACTTATAAAAAAGAATGATATGTG
 AGAAGATGGCCAATCCAGTGCAGCTTGGTTCTACATATTTTAGGGAGGCATGAGGCATCAAT
 CAAATACATTTAAGGAGTGTAGGGGGTGGGGTTCTAGGCTATAGGTAAATTTAAATATTTT
 5 CTGGTTGACAATTAGTTGAGTTTGTCTGAAGACCTGGGATTTTATCATGCAGATGAAACATC
 CAGGTAGCAAGCTTCAGAGAGAATAGACTGTGAATGTTAATGCCAGAGAGGTATAATGAAGC
 ATGTCCCACCTCCCACCTTTCCATCATGGCCTGAACCCTGGAGGAAGAGGAAGTCCATTGAGA
 TAGTTGTGGGGGGCCTTCGAATTTTCATTTTACGTTCTTCCCCTTCTGGCCAAGATT
 TGCCAGAGGCAACATCAAAAACCAGCAAAATTTTAATTTTGTCCCACAGCGTTGCTAGGGTGG
 10 CATGGCTCCCCATCTCGGGTCCATCTATACTTCCATGGGACTCCCTATGGCTGAAGGCCTT
 ATGAGTCAAAGGACTTATAGCCAATTGATTGTTCTAGGCCAGGTAAGAATGGATATGGACAT
 GCATTTATTACCTCTTAAATTATTATTTTAAGTAAAAGCCAATAAACAAAAACGAAAAGGC
 AA (SEQ ID NO: 46)

15 An additional CLL1 isoform (NM_001207010.2) cDNA is provided as:
 CTATTTAGCATTGCTGCTGCCAGCCCCAACACATTTCTGATTGCCTAGGAAGAACAGCCTT
 TCAAATTTGACTTCTGCATGTGGATAGATTTCTTTACATATTCATCAATGTCTGAAGAAGTT
 ACTTATGCAGATCTTCAATTCCAGAACTCCAGTGAGATGGAAAAATCCCAGAAATTGGCAA
 ATTTGGGGAAAAAGCACCTCCAGCTCCCTCTCATGTATGGCGTCCAGCAGCCTTGTTTCTGA
 20 CTCTTCTGTGCCTTCTGTTGCTCATTGGATTGGGAGTCTTGGCAAGCATGTTTCACGTAAC
 TTGAAGATAGAAAATGAAAAAATGAACAACTACAAAACATCAGTGAAGAGCTCCAGAGAAA
 TATTTCTCTACAACTGATGAGTAACATGAATATCTCCAACAAGATCAGGAACCTCTCCACCA
 CACTGCAACAATAGCCACCAAATTATGTCGTGAGCTATATAGCAAAGAACAAGAGCACAAA
 TGTAAGCCTTGTTCCAAGGAGATGGATTGGCATAAGGACAGCTGTTATTTCTAAGTGATGA
 25 TGTCCAAACATGGCAGGAGAGTAAATGGCCTGTGCTGCTCAGAATGCCAGCCTGTTGAAGA
 TAAACAACAAAAATGCATTGGAATTTATAAAATCCCAGAGTAGATCATATGACTATTGGCTG
 GGATTATCTCCTGAAGAAGATTCCACTCGTGGTATGAGAGTGGATAATATAATCAACTCCTC
 TGCCTGGGTTATAAGAAACGCACCTGACTTAAATAACATGTATTGTGGATATATAAATAGAC
 TATATGTTCAATATTATCACTGCACTTATAAAAAAGAATGATATGTGAGAAGATGGCCAAT
 30 CCAGTGCAGCTTGGTTCTACATATTTTAGGGAGGCATGAGGCATCAATCAAATACATTTAAG
 GAGTGTAGGGGGTGGGGTTCTAGGCTATAGGTAAATTTAAATATTTTCTGGTTGACAATTA
 GTTGAATTTGTCTGAAGACCTGGGATTTTATCATGCAGATGAAACATCCAGGTAGCAAGCTT
 CAGAGAGAATAGACTGTGAATGTTAATGCCAGAGAGGTATAATGAAGCATGTCCCACCTCCC
 ACTTTCCATCATGGCCTGAACCCTGGAGGAAGAGGAAGTCCATTGAGATAGTTGTGGGGGGC
 35 CTTCGAATTTTCATTTTACGTTCTTCCCCTTCTGGCCAAGATTGTCAGAGGCAACA
 TCAAAAACCAGCAAATTTAATTTTGTCCCACAGCGTTGCTAGGGTGGCATGGCTCCCCATC
 TCGGTCCATCCTATACTTCCATGGGACTCCCTATGGCTGAAGGCCTTATGAGTCAAAGGAC
 TTATAGCCAATTGATTGTTCTAGGCCAGGTAAGAATGGATATGGACATGCATTTATTACCTC
 TTAATATTATTATTTAAGTAAAAGCCAATAAACAAAAACGAAAAGGCAA (SEQ ID NO:
 40 270)

An additional CLL1 isoform (NM_001300730.2) cDNA is provided as:
 GGCTCATTTGCAGACATATGGGTGATTGGTACAGTAGGTTTATAAACAGAAGTTTAACTTG
 TAAGCTTAAGCTTCCGTTTATAAACAGAAAGTTTAAATTTATAGGTCCTGTTTAAACATTCAGC
 45 TCTGTTAACTCACTCATCTTTTTGTGTTTTTACACTTTGTCAAGATTTCTTTACATATTCAT
 CAATGTCTGAAGAAGTTACTTATGCAGATCTTCAATTCCAGAACTCCAGTGAGATGGAAAAA
 ATCCCAGAAATTGGCAAATTTGGGGAAAAAGCACCTCCAGCTCCCTCTCATGTATGGCGTCC
 AGCAGCCTTGTTTCTGACTCTTCTGTGCCTTCTGTTGCTCATTGGATTGGGAGTCTTGGCAA
 GCATGTTTACGTAACCTTTGAAGATAGAAAATGAAAAAATGAACAACTACAAAACATCAGT
 50 GAAGAGCTCCAGAGAAATATTTCTCTACAACTGATGAGTAACATGAATATCTCCAACAAGAT

CAGGAACCTCTCCACCACACTGCAAAACAATAGCCACCAAATTATGTCGTGAGCTATATAGCA
 AAGAACAAGAGCACAAATGTAAGCCTTGTCCAAGGAGATGGATTGGCATAAGGACAGCTGT
 TATTTCTAAGTGATGATGTCCAAACATGGCAGGAGAGTAAATGGCCTGTGCTGCTCAGAA
 TGCCAGCCTGTTGAAGATAAACAACAAAAATGCATTGGAATTTATAAAATCCCAGAGTAGAT
 5 CATATGACTATTGGCTGGGATTATCTCCTGAAGAAGATTCCACTCGTGGTATGAGAGTGGAT
 AATATAATCAACTCCTCTGCCTGAAAAATATCAAACGAAGAAAGAAACCAGAGTCTCAACCTG
 CTGGACACTATTGGAAGTCCATCATTTAACACGTTTTTAGTATATACTTTTAGCAGGAGACA
 GCTCTGAGTCAACTGTGTTGAGGTGCCACCACAGCGAGTTTAGGCACTCAGATCCCTGCATA
 CTCATCACATTGGGCCATAATGGCAAATAGAATTTTTTGTGTTTTGTTTTGTTTTGTTTTGCTTTT
 10 TCTTTTACATAGAAATAGTAAGTGTAGGAGTGTGGGTGAGAAAGAAAGGTGGCCCTACCTC
 TGATGGTTGGCAATGATAGGATACAATGGGAGATAAGCTATCTACAAATGGAGTGGAGAAGG
 ATATATATTTCAAAGGCCTAATTTGTAGTGAAAGACTAGAGACAAAGGTAATGTGTGTGTCA
 GGAGAGAGTACAGATGGAATCTTGTGTTTGCAAACGTAGAATATGTATGTGTTGTAAATTATT
 GCAAATGGAATGGTAATCTATAATGGAATGGAACATTGTAGATATTTTCAGTTATCAAAA
 15 AGAAAAGTAAAAAGTATATAATAATTGTATGTATGATATATATATGTGTGTGTGTGTAT
 ATATATCTTCACTTTATAACTCTGTGTTGTTTTGGGGTTTTGTTTCTGAAAGGGGGTTGTAAT
 AAATGACATCTGTACTATGTCACCACAAATAAATCTCATTCTTAAACATTTAATTGATGAAC
 TTA (SEQ ID NO: 271)

20 An additional CLL1 isoform (NM_201623.4) cDNA is provided as:
 GGCTCATTTGCAGACATATGGGTGATTGGTACAGTAGGTTTATAAACAGAAGTTTAACTTG
 TAAGCTTAAGCTTCCGTTTATAAACAGAAGTTTAAATTATAGGTCCTGTTTAAACATTCAGC
 TCTGTTAACTCACTCATCTTTTTGTGTTTTTACACTTTGTCAAGATTTCTTTACATATTCAT
 CAATGTCTGAAGAAGTTACTTATGCAGATCTTCAATTCCAGAACTCCAGTGAGATGGAAAAA
 25 ATCCCAGAAATTGGCAAATTTGGGGAAAAAGTTCACGTAACCTTGAAGATAGAAATGAAAAA
 AATGAACAACTACAAAACATCAGTGAAAGAGCTCCAGAGAAATATTTCTCTACAACTGATGA
 GTAACATGAATATCTCCAACAAGATCAGGAACCTCTCCACCACACTGCAACAATAGCCACC
 AAATTATGTCGTGAGCTATATAGCAAAAGAACAAGAGCACAAATGTAAGCCTTGTCCAAGGAG
 ATGGATTTGGCATAAGGACAGCTGTTATTTCTAAGTGATGATGTCCAAACATGGCAGGAGA
 30 GTAAAATGGCCTGTGCTGCTCAGAATGCCAGCCTGTTGAAGATAAACAACAAAAATGCATTG
 GAATTTATAAAATCCCAGAGTAGATCATATGACTATTGGCTGGGATTATCTCCTGAAGAAGA
 TTCCACTCGTGGTATGAGAGTGGATAATATAATCAACTCCTCTGCCTGGGTTATAAGAAACG
 CACCTGACTTAAATAACATGTATTGTGGATATATAAATAGACTATATGTTCAATATTATCAC
 TGCACCTATAAAAAAAGAATGATATGTGAGAAGATGGCCAATCCAGTGCAGCTTGGTTCTAC
 35 ATATTTTAGGGAGGCATGAGGCATCAATCAAATACATTTAAGGAGTGTAGGGGGTGGGGGTT
 CTAGGCTATAGGTAAATTTAAATATTTTCTGTTGACAATTAGTTGAGTTTGTCTGAAGACC
 TGGGATTTTATCATGCAGATGAAACATCCAGGTAGCAAGCTTCAGAGAGAATAGACTGTGAA
 TGTTAATGCCAGAGAGGTATAATGAAGCATGTCCACCTCCCACTTTCCATCATGGCCTGAA
 CCCTGGAGGAAGAGGAAGTCCATTGAGATAGTTGTGGGGGGCCTTCGAATTTTCATTTTCAT
 40 TTACGTTCTTCCCCTTCTGGCCAAGATTTGCCAGAGGCAACATCAAAAACCAGCAAATTTTA
 ATTTTGTCCACAGCGTTGCTAGGGTGGCATGGCTCCCCATCTCGGGTCCATCCTATACTTC
 CATGGGACTCCCTATGGCTGAAGGCCTTATGAGTCAAAGGACTTATAGCCAATTGATTGTTT
 TAGGCCAGGTAAGAATGGATATGGACATGCATTTATTACCTCTTAAATTTATTATTTTAAGT
 AAAAGCCAATAAACAAAAACGAAAAGGCAA (SEQ ID NO: 272)

45

Dual gRNA compositions and uses thereof

In some embodiments, a gRNA described herein (e.g., a gRNA of Table 2, 6 or 8) can be used in combination with a second gRNA, e.g., for directing nucleases to two sites in a genome. For instance, in some embodiments it is desired to produce a hematopoietic cell that

is deficient for CLL1 and a second lineage-specific cell surface antigen, e.g., so that the cell can be resistant to two agents: an anti-CLL1 agent and an agent targeting the second lineage-specific cell surface antigen. In some embodiments, it is desirable to contact a cell with two different gRNAs that target different regions of CLL1, in order to make two cuts and create a deletion between the two cut sites. Accordingly, the disclosure provides various combinations of gRNAs.

In some embodiments, two or more (e.g., 3, 4, or more) gRNAs described herein are admixed. In some embodiments, each gRNA is in a separate container. In some embodiments, a kit described herein (e.g., a kit comprising one or more gRNAs according to Table 2, 6, or 8) also comprises a Cas9 molecule, or a nucleic acid encoding the Cas9 molecule.

In some embodiments, the first and second gRNAs are gRNAs according to Table 2, 6, 8 or variants thereof.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA of Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: BCMA, CD19, CD20, CD30, ROR1, B7H6, B7H3, CD23, CD38, C-type lectin like molecule-1, CS1, IL-5, L1-CAM, PSCA, PSMA, CD138, CD133, CD70, CD7, CD13, NKG2D, NKG2D ligand, CLEC12A, CD11, CD123, CD56, CD34, CD14, CD66b, CD41, CD61, CD62, CD235a, CD146, CD326, LMP2, CD22, CD52, CD10, CD3/TCR, CD79/BCR, and CD26.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen associated with a specific type of cancer, such as, without limitation, CD20, CD22 (Non-Hodgkin's lymphoma, B-cell lymphoma, chronic lymphocytic leukemia (CLL)), CD52 (B-cell CLL), CD33 (Acute myelogenous leukemia (AML)), CD10 (gp100) (Common (pre-B) acute lymphocytic leukemia and malignant melanoma), CD3/T-cell receptor (TCR) (T-cell lymphoma and leukemia), CD79/B-cell receptor (BCR) (B-cell lymphoma and leukemia), CD26 (epithelial and lymphoid malignancies), human leukocyte antigen (HLA)-DR, HLA-DP, and HLA-DQ (lymphoid malignancies), RCAS1 (gynecological carcinomas, biliary adenocarcinomas and ductal adenocarcinomas of the pancreas) as well as prostate specific membrane antigen.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-

specific cell-surface antigen chosen from: CD7, CD13, CD19, CD22, CD20, CD25, CD32, CD38, CD44, CD45, CD47, CD56, 96, CD117, CD123, CD135, CD174, CLL-1, folate receptor β , IL1RAP, MUC1, NKG2D/NKG2DL, TIM-3, or WT1.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CDw12, CD13, CD14, CD15, CD16, CD16b, CD17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32a, CD32b, CD32c, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD45RA, CD45RB, CD45RC, CD45RO, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD61, CD62E, CD62L, CD62P, CD63, CD64a, CD65, CD65s, CD66a, CD66b, CD66c, CD66F, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75S, CD77, CD79a, CD79b, CD80, CD81, CD82, CD83, CD84, CD85A, CD85C, CD85D, CD85E, CD85F, CD85G, CD85H, CD85I, CD85J, CD85K, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD99R, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120a, CD120b, CD121a, CD121b, CD121a, CD121b, CD122, CD123, CD124, CD125, CD126, CD127, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140a, CD140b, CD141, CD142, CD143, CD14, CDw145, CD146, CD147, CD148, CD150, CD152, CD152, CD153, CD154, CD155, CD156a, CD156b, CD156c, CD157, CD158b1, CD158b2, CD158d, CD158e1/e2, CD158f, CD158g, CD158h, CD158i, CD158j, CD158k, CD159a, CD159c, CD160, CD161, CD163, CD164, CD165, CD166, CD167a, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CDw198, CDw199, CD200, CD201, CD202b, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CD210a, CDw210b, CD212, CD213a1, CD213a2, CD215, CD217, CD218a, CD218b, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD236, CD236R, CD238, CD239, CD240, CD241, CD242, CD243, CD244, CD245, CD246, CD247, CD248, CD249, CD252, CD253, CD254, CD256, CD257, CD258,

CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD270, CD272, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD280, CD281, CD282, CD283, CD284, CD286, CD288, CD289, CD290, CD292, CDw293, CD294, CD295, CD296, CD297, CD298, CD299, CD300a, CD300c, CD300e, CD301, CD302, CD303, CD304, CD305, CD306, CD307a, CD307b, CD307c, CD307d, CD307e, CD309, CD312, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD324, CD325, CD326, CD327, CD328, CD329, CD331, CD332, CD333, CD334, CD335, CD336, CD337, CD338, CD339, CD340, CD344, CD349, CD350, CD351, CD352, CD353, CD354, CD355, CD357, CD358, CD359, CD360, CD361, CD362 or CD363.

- 10 In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLECL1); epidermal growth factor receptor variant III (EGFRvIII); ganglioside
- 15 G2 (CD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlep(1-1)Cer); TNF receptor family member B cell maturation (BCMA), Tn antigen ((Tn Ag) or (GalNAc.alpha.-Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA);
- 20 Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4
- 25 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor I receptor (IGF-I receptor), carbonic anhydrase IX (CAIX),
- 30 Proteasome (Prosome, Macropain) Subunit, Beta Type 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlep(1-1)Cer); transglutaminase 5 (TGS5); high molecular

weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open

5 reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6

10 complex; locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1), ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, member 1A (XAGE1); angiopoietin-binding cell surface

15 receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; protein; surviving; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints;

20 melanoma inhibitor of apoptosis (ML-1AP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-

25 Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal

30 ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxy esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like

family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2), lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

5 In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: CD11a, CD18, CD19, CD20, CD31, CD34, CD44, CD45, CD47, CD51, CD58, CD59, CD63, CD97, CD99, CD100, CD102, CD123, CD127, CD133, CD135, CD157, CD172b, CD217, CD300a, CD305, CD317, CD321, and CLL1.

10 In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: CD123, CLL1, CD38, CD135 (FLT3), CD56 (NCAM1), CD117 (c-KIT), FR β (FOLR2), CD47, CD82, TNFRSF1B (CD120B), CD191, CD96, PTPRJ (CD148), CD70, LILRB2 (CD85D), CD25 (IL2R α), CD44, CD96, NKG2D Ligand, CD45, CD7, CD15, CD19, CD20, CD22, CD37, and CD82.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets a lineage-specific cell-surface antigen chosen from: CD7, CD11a, CD15, CD18, CD19, CD20, CD22, CD25, CD31, CD34, CD37, CD38, CD44, CD45, CD47, CD51, CD56, CD58, CD59, CD63, 20 CD70, CD82, CD85D, CD96, CD97, CD99, CD100, CD102, CD117, CD120B, CD123, CD127, CD133, CD135, CD148, CD157, CD172b, CD191, CD217, CD300a, CD305, CD317, CD321, CLL1, FR β (FOLR2), or NKG2D Ligand.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets CD33. In 25 some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA targets CD123.

In some embodiments, the first gRNA is a CLL1 gRNA described herein (e.g., a gRNA according to Table 2, 6, 8 or a variant thereof) and the second gRNA comprises a sequence from Table A. In some embodiments, the first gRNA is a CLL1 gRNA comprising 30 a targeting domain, wherein the targeting domain comprises a sequence of any of SEQ ID NOs: 1-10, 40, or 42, and the second gRNA comprises a targeting domain corresponding to a sequence of Table A. In some embodiments, the first gRNA is a CLL1 gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 9, and the second gRNA comprises a targeting domain corresponding to a sequence of Table A. In

some embodiments, the first gRNA is a CLL1 gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 10, and the second gRNA comprises a targeting domain corresponding to a sequence of Table A. In some embodiments, the first gRNA is a CLL1 gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 11, and the second gRNA comprises a targeting domain corresponding to a sequence of Table A. In some embodiments, the first gRNA is a CLL1 gRNA comprising a targeting domain, wherein the targeting domain comprises a sequence of SEQ ID NO: 12, and the second gRNA comprises a targeting domain corresponding to a sequence of Table A. In some embodiments, the second gRNA is a gRNA disclosed in any of WO2017/066760, WO2019/046285, WO/2018/160768, or Borot et al. PNAS June 11, 2019 116 (24) 11978-11987, each of which is incorporated herein by reference in its entirety.

Table A. Exemplary human CD33 target sequences. Certain target sequences are followed by a PAM sequence, indicated by a space in the text. Suitable gRNAs binding the target sequences provided will typically comprise a targeting domain comprising an RNA nucleotide sequence equivalent to the respective target sequence (and excluding the PAM).

gRNA target	Target sequences	SEQ ID NO:
hCD33	ACCTGTCAGGTGAAGTTCGC TGG	178
hCD33	TGGCCGGGTTCTAGAGTGCC AGG	179
hCD33	GGCCGGGTTCTAGAGTGCCA GGG	180
hCD33	CACCGAGGAGTGAGTAGTCC TGG	181
hCD33	TCCAGCGAACTTCACCTGAC AGG	182
CD33 (in intron 1)	GCTGTGGGGAGAGGGGTTGT	183
CD33 (in intron 1)	CTGTGGGGAGAGGGGTTGTC	184
CD33 (in intron 1)	TGGGGAAACGAGGGTCAGCT	185
CD33 (in intron 1)	GGGCCCCTGTGGGGAAACGA	186
CD33 (in intron 1)	AGGGCCCCTGTGGGGAAACG	187
CD33 (in intron 1)	GCTGACCCTCGTTTCCCCAC	188
CD33 (in intron 1)	CTGACCCTCGTTTCCCCACA	189

CD33 (in intron 1)	TGACCCTCGTTTCCCCACAG	190
CD33 (in intron 1)	CCATAGCCAGGGCCCCTGTG	191
CD33 (in intron 2)	GCATGTGACAGGTGAGGCAC	192
CD33 (in intron 2)	TGAGGCACAGGCTTCAGAAG	193
CD33 (in intron 2)	AGGCTTCAGAAGTGGCCGCA	194
CD33 (in intron 2)	GGCTTCAGAAGTGGCCGCAA	195
CD33 (in intron 2)	GTACCCATGAACTCCCTTG	196
CD33 (in intron 2)	GTGGCCGCAAGGGAAGTTCA	197
CD33 (in intron 2)	TGGCCGCAAGGGAAGTTCAT	198
CD33 (in intron 2)	GGAAGTTCATGGGTACTGCA	199
CD33 (in intron 2)	TTCATGGGTACTGCAGGGCA	200
CD33 (in intron 2)	CTAAACCCCTCCCAGTACCA	201
CD33 (in intron 1)	CACTCACCTGCCCACAGCAG	202
CD33 (in intron 1)	CCCTGCTGTGGGCAGGTGAG	203
CD33 (in intron 1)	TGGGCAGGTGAGTGGCTGTG	204
CD33 (in intron 1)	GGTGAGTGGCTGTGGGGAGA	205
CD33 (in intron 1)	GTGAGTGGCTGTGGGGAGAG	206
CD33 (exon 2)	ATCCATAGCCAGGGCCCCTG	207
CD33 (exon 2)	TCCATAGCCAGGGCCCCTGT	208
CD33 (exon 2)	CCATAGCCAGGGCCCCTGTG	191
CD33 (exon 2)	TCGTTTCCCCACAGGGGCC	209
CD33 (exon 2)	TGGCTATGGATCCAAATTTC	210
CD33 (exon 2)	TGGGGAAACGAGGGTCAGCT	185
CD33 (exon 2)	GGGCCCCTGTGGGGAAACGA	186
CD33 (exon 2)	AGAAATTTGGATCCATAGCC AGG	211
CD33 (exon 3)	ATCCCTGGCACTCTAGAACC CGG	212
CD33 (exon 3)	CCTCACTAGACTTGACCCAC AGG	213

Cells comprising two or more mutations

In some embodiments, an engineered cell described herein comprises two or more mutations. In some embodiments, an engineered cell described herein comprises two mutations, the first mutation being in CLL1 and the second mutation being in a second lineage-specific cell surface antigen. Such a cell can, in some embodiments, be resistant to two agents: an anti-CLL1 agent and an agent targeting the second lineage-specific cell surface antigen. In some embodiments, such a cell can be produced using two or more gRNAs described herein, e.g., a gRNA of Table 2 and a second gRNA. In some embodiments, such a cell can be produced using two or more gRNAs described herein, e.g., a gRNA of Table 6 and a second gRNA. In some embodiments, such a cell can be produced using two or more gRNAs described herein, e.g., a gRNA of Table 8 and a second gRNA. In some embodiments, the cell can be produced using, e.g., a ZFN or TALEN. The disclosure also provides populations comprising cells described herein.

In some embodiments, the second mutation is at a gene encoding a lineage-specific cell-surface antigen, e.g., one listed in the preceding section. In some embodiments, the second mutation is at a site listed in Table A.

Typically, a mutation effected by the methods and compositions provided herein, e.g., a mutation in a target gene, such as, for example, CLL1 and/or any other target gene mentioned in this disclosure, results in a loss of function of a gene product encoded by the target gene, e.g., in the case of a mutation in the CLL1 gene, in a loss of function of a CLL1 protein. In some embodiments, the loss of function is a reduction in the level of expression of the gene product, e.g., reduction to a lower level of expression, or a complete abolishment of expression of the gene product. In some embodiments, the mutation results in the expression of a non-functional variant of the gene product. For example, in the case of the mutation generating a premature stop codon in the encoding sequence, a truncated gene product, or, in the case of the mutation generating a nonsense or missense mutation, a gene product characterized by an altered amino acid sequence, which renders the gene product non-functional. In some embodiments, the function of a gene product is binding or recognition of a binding partner. In some embodiments, the reduction in expression of the gene product, e.g., of CLL1, of the second lineage-specific cell-surface antigen, or both, is to less than or equal to 50%, less than or equal to 40%, less than or equal to 30%, less than or equal to 20%, less than or equal to 10%, less than or equal to 5%, less than or equal to 2%, or less than or equal to 1% of the level in a wild-type or non-engineered counterpart cell.

In some embodiments, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of copies of CLL1 in the population of cells generated by the methods and/or using the compositions provided herein have a mutation. In some embodiments, at least at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of copies of the second lineage-specific cell surface antigen in the population of cells have a mutation. In some embodiments, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of copies of CLL1 and of the second lineage-specific cell surface antigen in the population of cells have a mutation. In some embodiments, the population comprises one or more wild-type cells. In some embodiments, the population comprises one or more cells that comprise one wild-type copy of CLL1. In some embodiments, the population comprises one or more cells that comprise one wild-type copy of the second lineage-specific cell surface antigen.

15 Cells

In some embodiments, a cell (e.g., an HSC or HPC) having a modification of CLL1 is made using a nuclease and/or a gRNA described herein. In some embodiments, a cell (e.g., an HSC or HPC) having a modification of CLL1 and a modification of a second lineage-specific cell surface antigen is made using a nuclease and/or a gRNA described herein. It is understood that the cell can be made by contacting the cell itself with the nuclease and/or a gRNA, or the cell can be the daughter cell of a cell that was contacted with the nuclease and/or a gRNA. In some embodiments, a cell described herein (e.g., an HSC) is capable of reconstituting the hematopoietic system of a subject. In some embodiments, a cell described herein (e.g., an HSC) is capable of one or more of (e.g., all of): engrafting in a human subject, producing myeloid lineage cell, and producing and lymphoid lineage cells.

In some embodiments, a cell described herein is a human cell having a mutation in exon 2 of CLL1. In some embodiments, a cell described herein is a human cell having a mutation in exon 4 of CLL1.

In some embodiments, a population of cells described herein comprises hematopoietic stem cells (HSCs), hematopoietic progenitor cells (HPCs), or both (HSPCs). In some embodiments, the cells are CD34+.

In some embodiments, the cell comprises only one genetic modification. In some embodiments, the cell is only genetically modified at the CLL1 locus. In some embodiments,

the cell is genetically modified at a second locus. In some embodiments, the cell does not comprise a transgenic protein, e.g., does not comprise a CAR.

In some embodiments, a modified cell described herein comprises substantially no CLL1 protein. In some embodiments, a modified cell described herein comprises
5 substantially no wild-type CLL1 protein, but comprises mutant CLL1 protein. In some embodiments, the mutant CLL1 protein is not bound by an agent that targets CLL1 for therapeutic purposes.

In some embodiments, the cells are hematopoietic cells, e.g., hematopoietic stem cells. Hematopoietic stem cells (HSCs) are typically capable of giving rise to both myeloid
10 and lymphoid progenitor cells that further give rise to myeloid cells (e.g., monocytes, macrophages, neutrophils, basophils, dendritic cells, erythrocytes, platelets, etc) and lymphoid cells (e.g., T cells, B cells, NK cells), respectively. HSCs are characterized by the expression of the cell surface marker CD34 (e.g., CD34+), which can be used for the identification and/or isolation of HSCs, and absence of cell surface markers associated with
15 commitment to a cell lineage.

In some embodiments, a population of cells described herein comprises a plurality of hematopoietic stem cells; in some embodiments, a population of cells described herein comprises a plurality of hematopoietic progenitor cells; and in some embodiments, a population of cells described herein comprises a plurality of hematopoietic stem cells and a
20 plurality of hematopoietic progenitor cells.

In some embodiments, the HSCs are obtained from a subject, such as a human subject. Methods of obtaining HSCs are described, e.g., in PCT/US2016/057339, which is herein incorporated by reference in its entirety. In some embodiments, the HSCs are peripheral blood HSCs. In some embodiments, the mammalian subject is a non-human
25 primate, a rodent (e.g., mouse or rat), a bovine, a porcine, an equine, or a domestic animal. In some embodiments, the HSCs are obtained from a human subject, such as a human subject having a hematopoietic malignancy. In some embodiments, the HSCs are obtained from a healthy donor. In some embodiments, the HSCs are obtained from the subject to whom the immune cells expressing the chimeric receptors will be subsequently administered. HSCs
30 that are administered to the same subject from which the cells were obtained are referred to as autologous cells, whereas HSCs that are obtained from a subject who is not the subject to whom the cells will be administered are referred to as allogeneic cells.

In some embodiments, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of copies of CLL1 in the

population of cells have a mutation. By way of example, a population can comprise a plurality of different CLL1 mutations and each mutation of the plurality contributes to the percent of copies of CLL1 in the population of cells that have a mutation.

In some embodiments, the expression of CLL1 on the genetically engineered hematopoietic cell is compared to the expression of CLL1 on a naturally occurring hematopoietic cell (e.g., a wild-type counterpart). In some embodiments, the genetic engineering results in a reduction in the expression level of CLL1 by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% as compared to the expression of CLL1 on a naturally occurring hematopoietic cell (e.g., a wild-type counterpart). For example, in some embodiments, the genetically engineered hematopoietic cell expresses less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than or 1% of CLL1 as compared to a naturally occurring hematopoietic cell (e.g., a wild-type counterpart).

In some embodiments, the genetic engineering results in a reduction in the expression level of wild-type CLL1 by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% as compared to the expression of the level of wild-type CLL1 on a naturally occurring hematopoietic cell (e.g., a wild-type counterpart). That is, in some embodiments, the genetically engineered hematopoietic cell expresses less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of CLL1 as compared to a naturally occurring hematopoietic cell (e.g., a wild-type counterpart).

In some embodiments, the genetic engineering results in a reduction in the expression level of wild-type lineage-specific cell surface antigen (e.g., CLL1) by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% as compared to a suitable control (e.g., a cell or plurality of cells). In some embodiments, the suitable control comprises the level of the wild-type lineage-specific cell surface antigen measured or expected in a plurality of non-engineered cells from the same subject. In some embodiments,

the suitable control comprises the level of the wild-type lineage-specific cell surface antigen measured or expected in a plurality of cells from a healthy subject. In some embodiments, the suitable control comprises the level of the wild-type lineage-specific cell surface antigen measured or expected in a population of cells from a pool of healthy individuals (e.g., 10, 20, 50, or 100 individuals). In some embodiments, the suitable control comprises the level of the wild-type lineage-specific cell surface antigen measured or expected in a subject in need of a treatment described herein, e.g., an anti-CLL1 therapy, e.g., wherein the subject has a cancer, wherein cells of the cancer express CLL1

10 Methods of treatment and administration

In some embodiments, an effective number of CLL1-modified cells described herein is administered to a subject in combination with an anti-CLL1 therapy, e.g., an anti-CLL1 cancer therapy. In some embodiments, an effective number of cells comprising a modified CLL1 and a modified second lineage-specific cell surface antigen are administered in combination with an anti-CLL1 therapy, e.g., an anti-CLL1 cancer therapy. In some embodiments, the anti-CLL1 therapy comprises an antibody, a bispecific T cell engager, an ADC, or an immune cell expressing a CAR.

It is understood that when agents (e.g., CLL1-modified cells and an anti-CLL1 therapy) are administered in combination, the agent may be administered at the same time or at different times in temporal proximity. Furthermore, the agents may be admixed or in separate volumes. For example, in some embodiments, administration in combination includes administration in the same course of treatment, e.g., in the course of treating a cancer with an anti-CLL1 therapy, the subject may be administered an effective number of CLL1-modified cells concurrently or sequentially, e.g., before, during, or after the treatment, with the anti-CLL1 therapy.

In some embodiments, the agent that targets a CLL1 as described herein is an immune cell that expresses a chimeric receptor, which comprises an antigen-binding fragment (e.g., a single-chain antibody) capable of binding to CLL1. The immune cell may be, e.g., a T cell (e.g., a CD4+ or CD8+ T cell) or an NK cell.

30 A Chimeric Antigen Receptor (CAR) can comprise a recombinant polypeptide comprising at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain comprising a functional signaling domain, e.g., one derived from a stimulatory molecule. In some embodiments, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one

costimulatory molecule, such as 4-1BB (i.e., CD137), CD27 and/or CD28 or fragments of those molecules. The extracellular antigen binding domain of the CAR may comprise a CLL1-binding antibody fragment. The antibody fragment can comprise one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations of any of the foregoing.

Amino acid and nucleic acid sequences of an exemplary heavy chain variable region and light chain variable region of an anti-human CLL1 antibody are provided below. The CDR sequences are shown in boldface in the amino acid sequences.

Amino acid sequence of anti-CLL1 Heavy Chain Variable Region (SEQ ID NO: 32)

10 DIQLQESGPGLVKPSQSLTCSVT**GYSITSAYYWNWIRQ**FPGNKLEWMGYISYDGR
 NNYN**PSLKN**RISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNY**YAMDY**
 WGQGTSVTVSS

Amino acid sequence of anti-CLL1 Light Chain Variable Region (SEQ ID NO: 33)

15 ENVLTQSPAIMASAPGEKVTMT**CRASSNVISSYV**HWYQQRSGASPKLWIYSTSNLAS
 GVPARFSGSGSGTSYSLTISSVEAEDAATYYC**QQYSGYPLT**FGAGTKLEL

Additional anti-CLL1 sequences are found, e.g., in US8536310, which is incorporated herein by reference in its entirety.

20 The anti-CLL1 antibody binding fragment for use in constructing the agent that targets CLL1 as described herein may comprise the same heavy chain and/or light chain CDR regions as those in SEQ ID NO:32 and SEQ ID NO:33. Such antibodies may comprise amino acid residue variations in one or more of the framework regions. In some instances, the anti-CLL1 antibody fragment may comprise a heavy chain variable region that shares at least 70% sequence identity (e.g., 75%, 80%, 85%, 90%, 95%, or higher) with SEQ ID NO:32 and/or may comprise a light chain variable region that shares at least 70% sequence identity (e.g., 75%, 80%, 85%, 90%, 95%, or higher) with SEQ ID NO:33.

Exemplary chimeric receptor component sequences are provided in Table 3 below.

30 **Table 3: Exemplary components of a chimeric receptor**

Chimeric receptor component	Amino acid sequence
Antigen-binding fragment	Light chain- GSTSSGSGKPGSGEGSTKG (SEQ ID NO: 34)-Heavy chain

4-1BB costimulatory domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCE (SEQ ID NO: 214)
CD28 costimulatory domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP LFPGPSKPFWVLVVVGVLACYSLLVTVA FIIFWVRSKRSRLLHSDYMNMTPRRPGPTR KHYPYAPPRDFAAYRS (SEQ ID NO: 35)
ICOS costimulatory domain (boldface), ICOS transmembrane domain (italics) and a portion of the extracellular domain of ICOS (underlined)	<u>LSIFDPPPFKVTLTGGYLHIYESQLCCQLKF</u> <i>WLPIGCAAFVVVCILGCILICWLT</i> TKKKYSSS VHDPNGEYMFMR AVNTAKKSRLTDVTL (SEQ ID NO: 36)
ICOS costimulatory domain	CWLT TKKKYSSSVHDPNGEYMFMR AVNTA KKSRLTDVTL (SEQ ID NO: 37)
CD28/ICOS chimera (the ICOS portion shown in underline) including the hinge domain (italics) and transmembrane domain (bold) from CD28	<i>IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP</i> <i>FPGPSKPFWVLVVVGVLACYSLLVTVA</i> FIIFWVRSKRSRLLHSDYMFMR AVNTAKK SRLTDVTL (SEQ ID NO: 38)
CD8 α transmembrane domain (italics) and a portion of the extracellular domain of CD8 α (underlined)	<u>TTTPAPRPPTPAPT</u> <u>IASQPLSLRPEACRPAA</u> <u>GGAVHTRGLDFACDIYIWAPLAGTCGVLLLS</u> <u>LVITLYC</u> (SEQ ID NO: 215)
CD3 ζ cytoplasmic signaling domain	RVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPQRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 39)

In some embodiments, the CAR comprises a 4-1BB costimulatory domain (e.g., as shown in Table 3), a CD8 α transmembrane domain and a portion of the extracellular domain of CD8 α (e.g., as shown in Table 3), and a CD3 ζ cytoplasmic signaling domain (e.g., as shown in Table 3).

A typical number of cells, e.g., immune cells or hematopoietic cells, administered to a mammal (e.g., a human) can be, for example, in the range of one million to 100 billion cells; however, amounts below or above this exemplary range are also within the scope of the present disclosure.

5 In some embodiments, the agent that targets CLL1 is an antibody-drug conjugate (ADC). The ADC may be a molecule comprising an antibody or antigen-binding fragment thereof conjugated to a toxin or drug molecule. Binding of the antibody or fragment thereof to the corresponding antigen allows for delivery of the toxin or drug molecule to a cell that presents the antigen on the its cell surface (e.g., target cell), thereby resulting in death of the
10 target cell.

In some embodiments, the antigen-binding fragment of the antibody-drug conjugate has the same heavy chain CDRs as the heavy chain variable region provided by SEQ ID NO: 32 and the same light chain CDRs as the light chain variable region provided by SEQ ID NO: 33. In some embodiments, the antigen-bind fragment of the antibody-drug conjugate has the
15 heavy chain variable region provided by SEQ ID NO: 32 and the same light chain variable region provided by SEQ ID NO: 33.

Toxins or drugs compatible for use in antibody-drug conjugates are known in the art and will be evident to one of ordinary skill in the art. See, e.g., Peters et al. *Biosci. Rep.*(2015) 35(4): e00225; Beck et al. *Nature Reviews Drug Discovery* (2017) 16:315-337;
20 Marin-Acevedo et al. *J. Hematol. Oncol.*(2018)11: 8; Elgundi et al. *Advanced Drug Delivery Reviews* (2017) 122: 2-19.

In some embodiments, the antibody-drug conjugate may further comprise a linker (e.g., a peptide linker, such as a cleavable linker) attaching the antibody and drug molecule.

Examples of antibody-drug conjugates include, without limitation, brentuximab
25 vedotin, glembatumumab vedotin/CDX-011, depatuxizumab mafodotin/ABT-414, PSMA ADC, polatuzumab vedotin/RG7596/DCDS4501A, denintuzumab mafodotin/SGN-CD19A, AGS-16C3F, CDX-014, RG7841/DLYE5953A, RG7882/DMUC406A, RG7986/DCDS0780A, SGN-LIV1A, enfortumab vedotin/ASG-22ME, AG-15ME, AGS67E, telisotuzumab vedotin/ABBV-399, ABBV-221, ABBV-085, GSK-2857916, tisotumab
30 vedotin/HuMax-TF-ADC, HuMax-Ax1-ADC, pinatuzumab vedotin/RG7593/DCDT2980S, lifastuzumab vedotin/RG7599/DNIB0600A, indusatumab vedotin/MLN-0264/TAK-264, vandortuzumab vedotin/RG7450/DSTP3086S, sofituzumab vedotin/RG7458/DMUC5754A, RG7600/DMOT4039A, RG7336/DEDN6526A, ME1547, PF-06263507/ADC 5T4, trastuzumab emtansine/T-DM1, mirvetuximab soravtansine/ IMGN853, coltuximab

- ravtansine/SAR3419, naratuximab emtansine/IMGN529, indatuximab ravtansine/BT-062, anetumab ravtansine/BAY 94-9343, SAR408701, SAR428926, AMG 224, PCA062, HKT288, LY3076226, SAR566658, lorvotuzumab mertansine/IMGN901, cantuzumab mertansine/SB-408075, cantuzumab ravtansine/IMGN242, laprituximab
- 5 emtansine/IMGN289, IMGN388, bivatuzumab mertansine, AVE9633, BIIB015, MLN2704, AMG 172, AMG 595, LOP 628, vadastuximab talirine/SGN-CLL1A, SGN-CD70A, SGN-CD19B, SGN-CD123A, SGN-CD352A, rovalpituzumab tesirine/SC16LD6.5, SC-002, SC-003, ADCT-301/HuMax-TAC-PBD, ADCT-402, MEDI3726/ADC-401, IMGN779, IMGN632, gemtuzumab ozogamicin, inotuzumab ozogamicin/ CMC-544, PF-06647263,
- 10 CMD-193, CMB-401, trastuzumab duocarmazine/SYD985, BMS-936561/MDX-1203, sacituzumab govitecan/IMMU-132, labetuzumab govitecan/IMMU-130, DS-8201a, U3-1402, milatuzumab doxorubicin/IMMU-110/hLL1-DOX, BMS-986148, RC48-ADC/hertuzumab-vc-MMAE, PF-06647020, PF-06650808, PF-06664178/RN927C, lupartumab amadotin/ BAY1129980, aprutumab ixadotin/BAY1187982, ARX788,
- 15 AGS62P1, XMT-1522, AbGn-107, MEDI4276, DSTA4637S/RG7861. In one example, the antibody-drug conjugate is gemtuzumab ozogamicin.

- In some embodiments, binding of the antibody-drug conjugate to the epitope of the cell-surface lineage-specific protein induces internalization of the antibody-drug conjugate, and the drug (or toxin) may be released intracellularly. In some embodiments, binding of the
- 20 antibody-drug conjugate to the epitope of a cell-surface lineage-specific protein induces internalization of the toxin or drug, which allows the toxin or drug to kill the cells expressing the lineage-specific protein (target cells). In some embodiments, binding of the antibody-drug conjugate to the epitope of a cell-surface lineage-specific protein induces internalization of the toxin or drug, which may regulate the activity of the cell expressing the lineage-
- 25 specific protein (target cells). The type of toxin or drug used in the antibody-drug conjugates described herein is not limited to any specific type.

CLL1 Associated Diseases and/or Disorders

- The present disclosure provides, among other things, compositions and methods for
- 30 treating a disease associated with expression of CLL1 or a condition associated with cells expressing CLL1, including, e.g., a proliferative disease such as a cancer or malignancy (e.g., a hematopoietic malignancy), or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia.

In some embodiments, the hematopoietic malignancy or a hematological disorder is associated with CLL1 expression. A hematopoietic malignancy has been described as a malignant abnormality involving hematopoietic cells (*e.g.*, blood cells, including progenitor and stem cells). Examples of hematopoietic malignancies include, without limitation,

5 Hodgkin lymphoma, non-Hodgkin lymphoma, leukemia, or multiple myeloma. Exemplary leukemias include, without limitation, acute myeloid leukemia, acute lymphoid leukemia, chronic myelogenous leukemia, acute lymphoblastic leukemia or chronic lymphoblastic leukemia, and chronic lymphoid leukemia.

In some embodiments, cells involved in the hematopoietic malignancy are resistant to

10 conventional or standard therapeutics used to treat the malignancy. For example, the cells (*e.g.*, cancer cells) may be resistant to a chemotherapeutic agent and/or CAR T cells used to treat the malignancy.

In some embodiments, the leukemia is acute myeloid leukemia (AML). AML is characterized as a heterogeneous, clonal, neoplastic disease that originates from transformed

15 cells that have progressively acquired critical genetic changes that disrupt key differentiation and growth-regulatory pathways. (Dohner et al., *NEJM*, (2015) 373:1136). Without wishing to be bound by theory, it is believed in some embodiments, that CLL1 is expressed on myeloid leukemia cells as well as on normal myeloid and monocytic precursors and is an attractive target for AML therapy.

In some cases, a subject may initially respond to a therapy (*e.g.*, for a hematopoietic malignancy) and subsequently experience relapse. Any of the methods or populations of genetically engineered hematopoietic cells described herein may be used to reduce or prevent relapse of a hematopoietic malignancy. Alternatively or in addition, any of the methods described herein may involve administering any of the populations of genetically engineered

20 hematopoietic cells described herein and an immunotherapeutic agent (*e.g.*, cytotoxic agent) that targets cells associated with the hematopoietic malignancy and further administering one or more additional immunotherapeutic agents when the hematopoietic malignancy relapses. In some embodiments, the subject has or is susceptible to relapse of a hematopoietic malignancy (*e.g.*, AML) following administration of one or more previous therapies. In some

25 30 embodiments, the methods described herein reduce the subject's risk of relapse or the severity of relapse.

In some embodiments, the hematopoietic malignancy or hematological disorder associated with CLL1 is a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia. Myelodysplastic syndromes (MDS) are

hematological medical conditions characterized by disorderly and ineffective hematopoiesis, or blood production. Thus, the number and quality of blood-forming cells decline irreversibly. Some patients with MDS can develop severe anemia, while others are asymptomatic. The classification scheme for MDS is known in the art, with criteria designating the ratio or frequency of particular blood cell types, e.g., myeloblasts, monocytes, and red cell precursors. MDS includes refractory anemia, refractory anemia with ring sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, chronic myelomonocytic leukemia (CML). In some embodiments, MDS can progress to an acute myeloid leukemia (AML).

10

EXAMPLES

Example 1: Genetic editing of CLL1 in human cells

Design of sgRNA constructs

The sgRNAs indicated in Table 4 were designed by manual inspection for the SpCas9 PAM (5'-NGG-3') with close proximity to the target region and prioritized according to predicted specificity by minimizing potential off-target sites in the human genome with an online search algorithm (e.g., the Benchling algorithm, Doench et al 2016, Hsu et al 2013). All designed synthetic sgRNAs were produced with chemically modified nucleotides at the three terminal positions at both the 5' and 3' ends. Modified nucleotides contained 2'-O-methyl-3'-phosphorothioate (abbreviated as "ms") and the ms-sgRNAs were HPLC-purified. Cas9 protein was purchased from Aldervon.

20

Table 4: Sequences of target domains of human CLL1 that can be bound by suitable gRNAs. A corresponding gRNA will typically comprise a targeting domain that may comprise an equivalent RNA sequence.

25

gRNA Name	Sequence	PAM	Exon
gRNA A	CCCAGAAATTGGCAAATTTG (SEQ ID NO: 1)	GGG	Exon 2
gRNA B	ATTCCAGAACTCCAGTGAGA (SEQ ID NO: 2)	TGG	Exon 2
gRNA C	GAGCTATATAGCAAAGAACA (SEQ ID NO: 3)	AGG	Exon 4
gRNA D	GGTGGCTATTGTTTGCAGTG (SEQ ID NO: 4)	TGG	Exon 4

gRNA E	TTGTTTGCAGTGTGGTGGAG (SEQ ID NO: 5)	AGG	Exon 4
gRNA F	TAGCTCACGACATAATTTGG (SEQ ID NO: 6)	TGG	Exon 4
gRNA G	GGCTATTGTTTGCAGTGTGG (SEQ ID NO: 7)	TGG	Exon 4
gRNA H	GGAGAGGTTCTGATCTTGT (SEQ ID NO: 8)	TGG	Exon 4
gRNA I	TGAATATCTCCAACAAGATC (SEQ ID NO: 9)	AGG	Exon 4
gRNA J	GTTGTAGAGAAATATTTCTC (SEQ ID NO: 10)	TGG	Exon 4

Human CD34+ Cell Culture and Electroporation

Cryopreserved human CD34+ cells were purchased from Hemacare and thawed according to manufacturer's instructions. Human CD34+ cells were cultured for 2 days in
5 GMP SCGM media (CellGenix), supplemented with human cytokines (Flt3, SCF, and TPO, all purchased from Peprotech). Cas9 protein and ms-sgRNA (at a 1:1 weight ratio) were mixed and incubated at room temperature for 10 minutes prior to electroporation. CD34+ cells were electroporated with the Cas9 ribonucleoprotein complex (RNP) using Lonza 4D-Nucleofector and P3 Primary Cell Kit (Program CA-137). Cells were cultured at 37°C until
10 analysis. Cell viability was measured by Cellometer and ViaStain AOPI Staining (Nexcelom Biosciences).

Genomic DNA analysis

Genomic DNA was extracted from cells 2 days post electroporation using the prepGEM
15 DNA extraction kit (ZyGEM). The genomic region of interest was amplified by PCR.

PCR amplicons were analyzed by Sanger sequencing (Genewiz) and allele modification frequency was calculated using TIDE (Tracking of Indels by Decomposition).

In vitro colony-forming unit (CFU) assay

20 Two days after electroporation, 500 CD34+ cells were plated in 1.1 mL of methylcellulose (MethoCult H4034 Optimum, Stem Cell Technologies) on 6 well plates in duplicates and cultured for two weeks. Colonies were then counted and scored using StemVision (Stem Cell Technologies).

Results

Human CD34+ cells were electroporated with Cas9 protein and the indicated CLL1-targeting gRNA as described above.

The percentage editing was determined by % INDEL as assessed by TIDE (Figures 1 and 2C).

As shown in Figure 1, gRNAs D, F, and G showed a high proportion of indels, in the range of approximately 80-100% of cells. In comparison, gRNAs A and H gave much lower proportions of indels, in the range of approximately 10-20% of cells. gRNAs B, C, E, and I showed an intermediate proportion of indels, in the range of approximately 30-60% of cells.

CLL1 gRNA D was further assessed for cell viability and *in vitro* differentiation (Figure 2A). As shown in Figure 2B, cells electroporated with gRNA D showed comparable viability to negative control cells 48 hours after electroporation. These cells also showed strong editing efficiency of the *CLL1/CLEC12A* locus, with an indel percentage of approximately 70% (Figure 2C). Furthermore, as shown in Figure 2D, cells electroporated with gRNA D were able to differentiate *in vitro*. In particular, substantial numbers of BFU-E and CFU-G/M/GM colonies formed from cells receiving gRNA D. Lower levels of CFU-GEMM colony formation was observed in gRNA D-electroporated cells as well.

Example 2: Generation and evaluation of cells edited for two cell surface antigens

Results

Cell surface levels of CD33, CD123 and CLL1 (CLEC12A) were measured in unedited MOLM-13 cells and THP-1 cells (both human AML cell lines) by flow cytometry. MOLM-13 cells had high levels of CD33 and CD123, and moderate-to-low levels of CLL1. HL-60 cells had high levels of CD33 and CLL1, and low levels of CD123 (FIGURE 3).

CD33 and CLL1 were mutated in HL-60 using gRNAs and Cas9 as described herein, CD33 and CLL1-modified cells were purified by flow cytometric sorting, and the cell surface levels of CD33 and CLL1 were measured. CD33 and CLL1 levels were high in wild-type HL-60 cells; editing of CD33 only resulted in low CD33 levels; editing of CLL1 only resulted in low CLL1 levels, and editing of both CD33 and CLL1 resulted in low levels of both CD33 and CLL1 (FIGURE 4). The edited cells were then tested for resistance to CART effector cells using an *in vitro* cytotoxicity assay as described herein. All four cell types (wild-type, CD33^{-/-}, CLL1^{-/-}, and CD33^{-/-} CLL1^{-/-}) experienced low levels of specific killing in mock CAR control conditions (FIGURE 5 leftmost set of bars). CD33 CAR cells effectively killed wild-type and CLL1^{-/-} cells, while CD33^{-/-} and CD33^{-/-} CLL1^{-/-} cells showed

a statistically significant resistance to CD33 CAR (FIGURE 5, second set of bars). CLL1 CAR cells effectively killed wild-type and CD33^{-/-} cells, while CLL1^{-/-} and CD33^{-/-} CLL1^{-/-} cells showed a statistically significant resistance to CLL1 CAR (FIGURE 5, third set of bars). A pool of CD33 CAR and CLL1 CAR cells effectively killed wild-type cells, CD33^{-/-} cells, and CLL1^{-/-} cells, while CD33^{-/-} CLL1^{-/-} cells showed a statistically significant resistance to the pool of CAR cells (FIGURE 5, rightmost set of bars). This experiment demonstrates that knockout of two antigens (CD33 and CLL1) protected the cells against CAR cells targeting both antigens. Furthermore, the population of edited cells contained a high enough proportion of cells that were edited at both alleles of both antigens, and had sufficiently low cell surface levels of cell surface antigens, that a statistically significant resistance to both types of CAR cells was achieved.

The efficiency of gene editing in human CD34⁺ cells was quantified using TIDE analysis as described herein. At the endogenous CD33 locus, editing efficiency of between about 70-90% was observed when CD33 was targeted alone or in combination with CD123 or CLL1 (FIGURE 6, left graph). At the endogenous CD123 locus, editing efficiency of about 60% was observed when CD123 was targeted alone or in combination with CD33 or CLL1 (FIGURE 6, center graph). At the endogenous CLL1 locus, editing efficiency of between about 40-70% was observed when CLL1 was targeted alone or in combination with CD33 or CD123 (FIGURE 6, right graph). This experiment illustrates that human CD34⁺ cells can be edited at a high frequency at two cell surface antigen loci.

The differentiation potential of gene-edited human CD34⁺ cells as measured by colony formation assay as described herein. Cells edited for CD33, CD123, or CLL1, individually or in all pairwise combinations, produced BFU-E colonies, showing that the cells retain significant differentiation potential in this assay (FIGURE 7A). The edited cells also produced CFU-G/M/GM colonies, showing that the cells retain differentiation potential in this assay that is statistically indistinguishable from the non-edited control (FIGURE 7B). The edited cells also produced detectable CFU-GEMM colonies (FIGURE 7C). Colony forming unit (CFU)-G/M/GM colonies refer to CFU-G (granulocyte), CFU-M (macrophage), and CFU-GM (granulocyte/macrophage) colonies. CFU-GEMM (granulocyte/erythroid/macrophage/megakaryocyte) colonies arise from a less differentiated cell that is a precursor to the cells that give rise to CFU-GM colonies. Taken together, the differentiation assays indicate that human CD34⁺ cells edited at two loci retain the capacity to differentiate into variety of cell types.

*Materials and Methods**AML cell lines*

Human AML cell line HL-60 was obtained from the American Type Culture Collection (ATCC). HL-60 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM, Gibco) supplemented with 20% heat-inactivated HyClone Fetal Bovine Serum (GE Healthcare). Human AML cell line MOLM-13 was obtained from AddexBio Technologies. MOLM-13 cells were cultured in RPMI-1640 media (ATCC) supplemented with 10% heat-inactivated HyClone Fetal Bovine Serum (GE Healthcare).

10 *Guide RNA design*

All sgRNAs were designed by manual inspection for the SpCas9 PAM (5'-NGG-3') with close proximity to the target region and prioritized according to predicted specificity by minimizing potential off-target sites in the human genome with an online search algorithm (Benchling, Doench et al 2016, Hsu et al 2013). All designed synthetic sgRNAs were purchased from Synthego with chemically modified nucleotides at the three terminal positions at both the 5' and 3' ends. Modified nucleotides contained 2'-O-methyl-3'-phosphorothioate (abbreviated as "ms") and the ms-sgRNAs were HPLC-purified. Cas9 protein was purchased from Aldevron. Typically, the gRNAs described in the Examples herein are sgRNAs comprising a 20 nucleotide (nt) targeting domain sequence, 12 nt of the crRNA repeat sequence, a 4 nt tetraloop sequence, and 64 nt of tracrRNA sequence.

Table 5: Sequences of target domains of human CD33, CD123, or CLL-1 that can be bound by suitable gRNAs. The adjacent PAM sequences are also provided. A suitable gRNA typically comprises a targeting domain that may comprise an RNA sequence equivalent to the target domain sequence.

Target gene	Sequence	PAM	Target location
CD33	CCCCAGGACTACTCACTCCT (SEQ ID NO: 64)	CGG	CD33 exon 3
CD123	TTTCTTGAGCTGCAGCTGGG (SEQ ID NO: 65)	CGG	CD123 exon 5
	AGTTCCCACATCCTGGTGCG (SEQ ID NO: 66)	GGG	CD123 exon 6
CLL1	GGTGGCTATTGTTTGCAGTG	TGG	CLL1 exon 4

	(SEQ ID NO: 4)		
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AML cell line electroporation

Cas9 protein and ms-sgRNA (at a 1:1 weight ratio) were mixed and incubated at room temperature for 10 minutes prior to electroporation. MOLM-13 and HL-60 cells were
 5 electroporated with the Cas9 ribonucleoprotein complex (RNP) using the MaxCyte ATx Electroporator System with program THP-1 and Opt-3, respectively. Cells were incubated at 37°C for 5-7 days until flow cytometric sorting.

Human CD34+ cell culture and electroporation

10 Cryopreserved human CD34+ cells were purchased from Hemacare and thawed according to manufacturer's instructions. Human CD34+ cells were cultured for 2 days in GMP SCGM media (CellGenix) supplemented with human cytokines (Flt3, SCF, and TPO, all purchased from Peprotech). CD34+ cells were electroporated with the Cas9 RNP (Cas9 protein and ms-sgRNA at a 1:1 weight ratio) using Lonza 4D-Nucleofector and P3 Primary
 15 Cell Kit (Program CA-137). For electroporation with dual ms-sgRNAs, equal amount of each ms-sgRNA was added. Cells were cultured at 37°C until analysis.

Genomic DNA analysis

Genomic DNA was extracted from cells 2 days post electroporation using prepGEM
 20 DNA extraction kit (ZyGEM). Genomic region of interest was amplified by PCR. PCR amplicons were analyzed by Sanger sequencing (Genewiz) and allele modification frequency was calculated using TIDE (Tracking of Indels by Decomposition) software available on the World Wide Web at tide.deskgen.com.

In vitro colony-forming unit (CFU) assay

25 Two days after electroporation, 500 CD34+ cells were plated in 1.1 mL of methylcellulose (MethoCult H4034 Optimum, Stem Cell Technologies) on 6 well plates in duplicates and cultured for two weeks. Colonies were then counted and scored using StemVision (Stem Cell Technologies).

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Flow cytometric analysis and sorting

Fluorescence-conjugated antibodies against human CD33 (P67.6), CD123 (9F5), and CLL1 (REA431) were purchased from Biolegend, BD Biosciences and Miltenyi Biotec, respectively. All antibodies were tested with their respective isotype controls. Cell surface staining was performed by incubating cells with specific antibodies for 30 min on ice in the presence of human TruStain FcX. For all stains, dead cells were excluded from analysis by DAPI (Biolegend) stain. All samples were acquired and analyzed with Attune NxT flow cytometer (ThermoFisher Scientific) and FlowJo software (TreeStar).

For flow cytometric sorting, cells were stained with fluorescence-conjugated antibodies followed by sorting with MoFlo Astrios Cell Sorter (Beckman Coulter).

CAR constructs and lentiviral production

Second-generation CARs were constructed to target CD33 and CLL-1, with the exception of the anti-CD33 CAR-T used in CD33/CLL-1 multiplex cytotoxicity experiment. Each CAR consisted of an extracellular scFv antigen-binding domain, using CD8 α signal peptide, CD8 α hinge and transmembrane regions, the 4-1BB costimulatory domain, and the CD3 ξ signaling domain. The anti-CD33 scFv sequence was obtained from clone P67.6 (Mylotarg) and the CLL-1 scFv sequence from clone 1075.7. The anti-CD33 uses a heavy-to-light orientation of the scFv and the anti-CLL1 CAR construct uses a light-to-heavy orientation. The heavy and light chains were connected by (GGGS)₃ linker (SEQ ID NO: 63). CAR cDNA sequences for each target were sub-cloned into the multiple cloning site of the pCDH-EF1 α -MCS-T2A-GFP expression vector, and lentivirus was generated following the manufacturer's protocol (System Biosciences). Lentivirus can be generated by transient transfection of 293TN cells (System Biosciences) using Lipofectamine 3000 (ThermoFisher). The CAR construct was generated by cloning the light and heavy chain of anti-CD33 scFv (clone My96), to the CD8 α hinge domain, the ICOS transmembrane domain, the ICOS signaling domain, the 4-1BB signaling domain and the CD3 ξ signaling domain into the lentiviral plasmid pHIV-ZsGreen.

CAR transduction and expansion

Human primary T cells were isolated from Leuko Pak (Stem Cell Technologies) by magnetic bead separation using anti-CD4 and anti-CD8 microbeads according to the manufacturer's protocol (Stem Cell Technologies). Purified CD4⁺ and CD8⁺ T cells were

mixed 1:1, and activated using anti-CD3/CD28 coupled Dynabeads (Thermo Fisher) at a 1:1 bead to cell ratio. T cell culture media used was CTS Optimizer T cell expansion media supplemented with immune cell serum replacement, L-Glutamine and GlutaMAX (all purchased from Thermo Fisher) and 100 IU/mL of IL-2 (Peprotech). T cell transduction was performed 24 hours post activation by spinoculation in the presence of polybrene (Sigma). CAR-T cells were cultured for 9 days prior to cryopreservation. Prior to all experiments, T cells were thawed and rested at 37°C for 4-6 hours.

Flow Cytometry based CAR-T cytotoxicity assay

The cytotoxicity of target cells was measured by comparing survival of target cells relative to the survival of negative control cells. For CD33/CLL1 multiplex cytotoxicity assays, wildtype and CRISPR/Cas9 edited HL60 cells were used as target cells for CD33/CLL-1 multiplex cytotoxicity assays. Wildtype Raji cell lines (ATCC) were used as negative control for both experiments. Target cells and negative control cells were stained with CellTrace Violet (CTV) and CFSE (Thermo Fisher), respectively, according to the manufacturer's instructions. After staining, target cells and negative control cells were mixed at 1:1.

Anti-CD33 or CLL1 CAR-T cells were used as effector T cells. Non-transduced T cells (mock CAR-T) were used as control. For the CARpool groups, appropriate CAR-T cells were mixed at 1:1. The effector T cells were co-cultured with the target cell/negative control cell mixture at a 1:1 effector to target ratio in duplicate. A group of target cell/negative control cell mixture alone without effector T cells was included as control. Cells were incubated at 37°C for 24 hours before flow cytometric analysis. Propidium iodide (ThermoFisher) was used as a viability dye. For the calculation of specific cell lysis, the fraction of live target cell to live negative control cell (termed target fraction) was used. Specific cell lysis was calculated as $((\text{target fraction without effector cells} - \text{target fraction with effector cells}) / (\text{target fraction without effectors})) \times 100\%$.

Example 3: Design and Screening of gRNAs for editing CLL1 in human cells

Design of sgRNA constructs

The gRNAs investigated in this Example were designed by inspection of the SpCas9 PAM with close proximity to the target region. All the 20bp sequences in the coding region

with an SpCas9 PAM (5'-NGG-3') at the 3' end were extracted. Using these methods, 123 total gRNAs targeting the target domains of human CLL1 as described in Tables 2 and 6 were designed.

5 Screening of gRNAs in THP-1 cells

The 123 gRNAs were filtered according to an off-target prediction algorithm (based on number of mismatches), which identified 66 gRNAs for further investigation in THP-1 cells. Human AML cell line THP-1 was obtained from the American Type Culture Collection (ATCC). THP-1 cells were cultured and electroporated with the ribonucleoprotein RNP complexes composed of Cas9 protein and gRNA (mixed at a 1:1 weight ratio). Genomic DNA was extracted from cells and the genomic region of interest was amplified by PCR for all 66 of gRNAs investigated. PCR amplicons were then analyzed by Sanger sequencing to calculate editing frequency (ICE, or interference of CRISPR edits) in two replicates, which is shown in Table 7. In the first replicate, the editing frequency was obtained for all gRNAs that were amplified and sequenced. In the second replicate, the editing frequency was obtained for 42/66 gRNAs, and the results for each gRNA were comparable across the two replicates. As depicted in Table 7, 20 of the gRNAs investigated had an ICE value or editing frequency ≥ 80 .

20 **Table 7.** Editing frequency of gRNAs designed to target human CLL1 in THP-1 cells

gRNA	SEQ ID NO:	Sequence	Replicate 1		Replicate 2	
			ICE	R ²	ICE	R ²
gRNA B	22	AUCCAGAACUCCAGUGAGA	62	0.94	71	0.95
gRNA Q	216	AUGGAAAAAAUCCCAGAAAU	37	0.97	36	0.98
gRNA R	217	AUCCCAGAAAUUGGCAAAUU	0	1	4	0.99
gRNA S	218	UCCCAGAAAUUGGCAAAUUU	21	0.98	21	0.98
gRNA A	21	CCCAGAAAUUGGCAAAUUUG	16	0.98	17	0.99
gRNA V	219	AUUGGCAAAUUUGGGGAAAA	27	0.98	28	0.98
gRNA U	220	UCCCCAAAUUUGCCAAUUUC	0	1	0	1
gRNA P	221	UGGGAUUUUUCCAUCUCAC	71	0.93	-	-
gRNA O	222	UUUCCAUCUCACUGGAGUUC	79	0.97	80	0.96
gRNA Y	223	CUCCAGCUCCCUCUCAUGUA	95	0.95	96	0.96
gRNA F1	224	CUGUGCCUUCUGUUGCUCAU	28	0.97	42	0.96

gRNA G1	225	CCUUCUGUUGCUCUUGGAU	76	0.95	75	0.97
gRNA H1	226	CUUCUGUUGCUCUUGGAUU	73	0.96	71	0.94
gRNA J1	227	GCUCUUGGAUUGGGAGUCU	91	0.93	94	0.94
gRNA I1	228	CCAAUCCAAUGAGCAACAGA	66	0.94	74	0.94
gRNA E1	229	GCACAGAAGAGUCAGAAACA	89	0.97	91	0.97
gRNA D1	230	AGAGUCAGAAACAAGGCUGC	28	0.98	29	0.97
gRNA C1	231	UGCUGGACGCCAUACAUGAG	88	0.94	90	0.94
gRNA B1	232	GCUGGACGCCAUACAUGAGA	81	0.94	84	0.93
gRNA A1	233	CGCCAUACAUGAGAGGGAGC	56	0.95	63	0.96
gRNA Z	234	CAUACAUGAGAGGGAGCUGG	80	0.94	83	0.94
gRNA X	235	AGAGGGAGCUGGAGGUGCUG	96	0.96	95	0.96
gRNA I	29	UGAAUAUCUCCAACAAGAUC	78	0.95	76	0.95
gRNA C	23	GAGCUAUUAUGCAAAGAACA	81	0.95	85	0.96
gRNA K1	236	AUAUAGCUCACGACAUAAUU	55	0.98	53	0.97
gRNA F	26	UAGCUCACGACAUAAUUUGG	94	0.97	95	0.98
gRNA D	24	GGUGGCUAUUGUUUGCAGUG	86	0.95	85	0.92
gRNA G	27	GGCUAUUGUUUGCAGUGUGG	94	0.97	95	0.96
gRNA E	25	UUGUUUGCAGUGUGGUGGAG	50	0.97	51	0.97
gRNA H	28	GGAGAGGUUCCUGAUCUUGU	83	0.95	82	0.95
gRNA J	30	GUUGUAGAGAAAUAUUUCUC	84	0.96	88	0.96
gRNA N1	237	ACAAAUGUAAGCCUUGUCCA	42	0.96	86	0.96
gRNA O1	238	GUAAGCCUUGUCCAAGGAGA	77	0.97	58	0.96
gRNA P1	239	CUUGUCCAAGGAGAUGGAUU	93	0.96	94	0.94
gRNA R1	240	AAGGAGAUGGAUUUGGCAUA	57	0.92	59	0.93
gRNA T1	241	UAAGUGAUGAUGUCCAAACA	36	0.97	41	0.97
gRNA V1	242	UGAUGAUGUCCAAACAUGGC	60	0.96	59	0.96
gRNA W1	243	AACAUGGCAGGAGAGUAAAA	58	0.96	66	0.97
gRNA B2	244	GAUAAACAACAAAAAUGCAU	0	1	0	1
gRNA C2	245	AAA AUGCAUUGGUAAAGCCC	85	0.93	92	0.96
gRNA A2	246	UUUUGUUGUUUAUCUUC AAC	0	1	0	1
gRNA Z1	247	GUUGUUUAUCUUC AACAGGC	0	1	3	0.99
gRNA Y1	248	GCUGGCAUUCUGAGCAGCAC	69	0.97	72	0.95

gRNA X1	249	UUUUACUCUCCUGCCAUGUU	55	0.95	-	-
gRNA U1	250	AUGUUUGGACAUCAUCACUU	72	0.95	-	-
gRNA S1	251	UUAUGCCAAAUCCAUCUCCU	77	0.97	-	-
gRNA Q1	252	CAAAUCCAUCUCCUUGGACA	42	0.96	-	-
gRNA M1	253	CAAGGCUUACAUUUGUGCUC	37	0.94	-	-
gRNA F2	254	AGAGUAGAUAUAUGACUAU	56	0.96	-	-
gRNA I2	255	UAGAUCUAUAUGACUAUUGGC	6	0.99	-	-
gRNA J2	256	AGAUCUAUAUGACUAUUGGCU	82	0.96	-	-
gRNA K2	257	CCUGAAGAAGAUUCCACUCG	85	0.95	-	-
gRNA M2	258	UUCCACUCGUGGUAUGAGAG	54	0.94	-	-
gRNA O2	44	AUAUAAUCAACUCCUCUGCC	88	0.97	-	-
gRNA P2	45	AAUAGACACUUACCAGGCAG	96	0.96	-	-
gRNA N2	259	AUCCACUCUCAUACCACGAG	35	0.96	-	-
gRNA L2	260	CCACGAGUGGAAUCUUCUUC	36	0.96	-	-
gRNA H2	261	AAUAGUCAUAUGAUCUACUC	10	0.99	-	-
gRNA G2	262	AUAGUCAUAUGAUCUACUCU	28	0.98	-	-
gRNA E2	263	GGGAUUUUUAUAAUCCUAC	0	1	-	-
gRNA T2	264	GACUUAAAUAACAUGUAUUG	50	0.96	-	-
gRNA V2	265	AAGAAUGAUUAUGUGAGAAGA	0	1	-	-
gRNA W2	266	AUGGCCAAUCCAGUGCAGCU	88	0.96	-	-
gRNA Y2	267	UAUGUAGAACCAAGCUGCAC	60	0.97	-	-
gRNA X2	268	AGAACCAAGCUGCACUGGAU	79	0.97	-	-
gRNA U2	269	CAAUACAUGUUAUUUAAGUC	37	0.97	-	-

Screening of gRNAs in primary CD34+ human stem and progenitor cells (HSPCs)

- Primary human CD34+ HSPCs were cultured and electroporated with ribonucleoprotein RNP complex composed of the Cas9 protein and one of the 14 gRNAs listed in Table 8. These 14 gRNAs screened include those that were selected from screening performed in the THP-1 cells and/or those gRNAs that had a favorable off-target profile.

Table 8. Sequences of target domains of CLL1 gRNAs screened in human CD34+ cells. The corresponding gRNAs comprised a targeting domain consisting of the equivalent RNA

sequence. The target region nomenclature is based on the CLL1 isoform ENST00000355690.8.

gRNA	SEQ ID NO:	Sequence	PAM	Target Region
gRNA A	1	CCCAGAAATTGGCAAATTTG	GGG	Exon 2
gRNA B	2	ATTCCAGAACTCCAGTGAGA	TGG	Exon 2
gRNA C	3	GAGCTATATAGCAAAGAACA	AGG	Exon 4
gRNA D	4	GGTGGCTATTGTTTGCAGTG	TGG	Exon 4
gRNA E	5	TTGTTTGCAGTGTGGTGGAG	AGG	Exon 4
gRNA F	6	TAGCTCACGACATAATTTGG	TGG	Exon 4
gRNA G	7	GGCTATTGTTTGCAGTGTGG	TGG	Exon 4
gRNA H	8	GGAGAGGTTCTGATCTTGT	TGG	Exon 4
gRNA I	9	TGAATATCTCCAACAAGATC	AGG	Exon 4
gRNA J	10	GTTGTAGAGAAATATTTCTC	TGG	Exon 4
gRNA K2	119	CCTGAAGAAGATTCCACTCG	TGG	Exon 6
gRNA O2	40	ATATAATCAACTCCTCTGCC	TGG	Exon 6
gRNA P1	98	CTTGTCGAAGGAGATGGATT	TGG	Exon 5
gRNA P2	42	AATAGACACTTACCAGGCAG	AGG	Exon 6

The editing frequency of these gRNAs in primary human CD34+ HSPCs was calculated and is depicted in Figure 8 and Figure 9. Of the 14 gRNAs tested, 5 demonstrated an editing efficiency above 80% (Figure 8 and Figure 9). These gRNAs included gRNA D, gRNA F, gRNA G, gRNA O2, and gRNA P2 and their calculated mean editing efficiencies are shown in Table 9.

Table 9. Mean editing efficiencies of gRNAs screened in primary human CD34+ HSPCs

Guide	Mean Editing Frequency
gRNA D	86.9
gRNA F	85.7
gRNA G	85.1
gRNA O2	90.7
gRNA P2	86.7

The INDEL (insertion/deletion) distributions for gRNA D, gRNA F, gRNA G, gRNA O2, and gRNA P2, as evaluated in the primary human CD34+ cells was quantified and are shown in Figure 10. Each gRNA led to INDELs ranging from -21 to +1. gRNA D resulted in INDELs of varying sizes including -21, -9, -7, -5, -1, 0, and +1, with an INDEL of +1 occurring at the highest frequency. gRNA F resulted in INDELs of varying sizes including -3, -2, -1, 0, and +1, with an INDEL of -1 occurring at the highest frequency. gRNA G resulted in INDELs of varying sizes including -10, -7, -5, -2, -1, and +1, with an INDEL of -2 occurring at the highest frequency. gRNA O2 resulted in INDELs of varying sizes including -6, 0, and +1, with an INDEL of +1 occurring at the highest frequency. gRNA P2 resulted in INDELs of varying sizes including -6, -4, -3, -2, -1, 0, and +1, with an INDEL of -4 occurring at the highest frequency.

The off-target effects of gRNA D, gRNA F, gRNA G, gRNA O2, and gRNA P2 were also predicted, as shown in Table 10. gRNAs were prioritized based on minimizing off-target effects. These off-target predictions were based on sequence complementarity with up to 1 nucleotide mismatch allowed between the PAM and the target or up to 3 nucleotide mismatch or gap between the guide and the target.

Table 10. Off-target predictions for gRNAs targeting human CLL1

Guide	No mismatch/gap in PAM		1 mismatch/gap in PAM	
	# of mismatch/gap in guide		# of mismatch/gap in guide	
	2	3	2	3
gRNA G	8	224	11	319
gRNA D	4	214	8	364
gRNA F	1	98	5	217
gRNA O2	2	168	20	441
gRNA P2	8	349	18	478

Among other gRNAs targeting human CLL1 investigated in this Example, three gRNAs (gRNA D, gRNA F, and gRNA O2) demonstrated particularly efficient on-target editing in primary human CD34+ HSPCs, low level of predicted off-target effects, and a desirable INDEL distribution.

Example 4: Evaluation of CLL1 KO CD34+ cells *in vivo*

Editing in CD34+ human HSPCs

gRNAs (Synthego) were designed as described in Example 1 and Example 3. The human CD34+ HSPCs were then edited via CRISPR/Cas9 as described in Example 1 using the CLL1 targeting guide RNA F. Non-edited, electroporated control (EP Ctrl) HPSCs were also generated.

After *ex vivo* editing, the genomic DNA was harvested from cells, PCR amplified with primers flanking the target region, purified, and analyzed, in order to determine their editing efficiency in the CD34+ HSPCs. As shown in Table 11, gRNA F had high editing efficiencies, specifically 75.4%.

Table 11. Gene editing efficiency of CLL1 gRNA

CD34 ⁺ HSPCs	Editing efficiency
EP only control	N/A
CLL gRNA F-edited	75.4% (TIDE)

Investigating engraftment efficiency and persistence of CLL1 KO CD34+ HSPCs in vivo

Female nonirradiated NOD.B6.SCID Il2 γ ^{-/-} Kit(W41/W41) (NBSGW) mice (n=15) were engrafted with the CLL1 KO HSPCs edited with gRNA F, or non-edited (EP Ctrl) (Figure 11). At weeks 8 and 12 following engraftment, peripheral blood was collected from each mouse for analysis by FACS for measuring engraftment. At week 16, following engraftment, mice were sacrificed, and blood, spleens, and bone marrow were collected for FACS analysis for multilineage differentiation (Figure 11).

Results from cell samples obtained from the bone marrow of engrafted animals

At week 16 following engraftment, rates of human leukocyte chimerism in mice were calculated as percentage of human CD45+ (hCD45+) cells in the total CD45+ cell population (the sum of human and mouse CD45+ cells) in the two groups of mice (n=15 mice/group) that received the non-edited control cells (EP Ctrl) or the CLL1 KO cells edited by gRNA F (Figure 12A). As shown in Figure 12A, the bone marrow chimerism was equivalent across control and CLL1 KO group, indicating no loss of nucleated bone marrow frequency.

Additionally, at week 16 post-engraftment, the percentage of hCD45+ cells that were also positive for human CD34 (hCD34+) in the bone marrow was quantified (Figure 12B). As shown in Figure 12B, the percentage of hCD45+ cells also expressing hCD34+ was equivalent across control or the CLL1 KO group.

5 At week 16 post engraftment, the percentage of hCD45+ cells that were B-cells, T cells, monocytes, neutrophils, conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), eosinophils, basophils, and mast cells were quantified in the bone marrow (Figure 12C). The percentages of these various immune cell subtypes were equivalent between the control and CLL1 KO groups. These data indicate multilineage human
10 hematopoietic reconstitution from the edited CLL1 KO cells in the mice.

The percentages of CLL1 KO cells that were hCD45+ were quantified in the bone marrow of control and CLL1 KO cell engrafted mice at week 16 post-engraftment (Figure 12D). The percentage of hCLL1+ hCD45+ cells was significantly lower in the CLL1 KO cells edited with gRNA F compared to the control group, indicating loss of CLL1 from
15 nucleated blood cells in these groups. These data also demonstrate the long term persistence of CLL1 KO HSCs in the bone marrow of NBSGW mice.

Example 5: Evaluation of CLL1 KO CD34+ cells *in vitro*

20 *Editing in CD34+ human HSPCs*

gRNAs (Synthego) were designed as described in Example 1 and Example 3. The human CD34+ HSPCs were then edited via CRISPR/Cas9 as described in Example 1 using the CLL1-targeting guide RNA F, as well as a non-edited, electroporated control (EP Ctrl).

After *ex vivo* editing, the genomic DNA was harvested from cells, PCR amplified
25 with primers flanking the target region, purified, and analyzed by TIDE to determine the editing frequency in the CD34+ HSPCs. As shown in Figure 13A, gRNA F showed an editing frequency of 79.2%. Cell surface expression of CLL1 was also quantified by FACs in the CLL1 KO cells edited by gRNA F, the non-edited control (EP ctrl), or the FMO (fluorescent minus one) control. CD34+ HSPCs edited by gRNA F exhibited lower
30 expression of CLL1 compared to the non-edited control (EP ctrl) (Figure 13A).

Non-edited control cells (EP ctrl) or CLL1 KO cells edited by gRNA F were cultured with myeloid differentiation media, inducing either granulocytic (Figure 13B) or monocytic (Figure 13C) lineages, and the cell numbers were quantified over time. The CLL1 KO cells

demonstrated comparable cell growth to the non-edited control cells in both granulocytic (Figure 13B) and monocytic (Figure 13C) differentiation culture.

Additionally, the percentage of cells that were CLL+ in granulocytic differentiation (Figure 14A, top) or cells that were CLL1+ in monocytic differentiation (Figure 14A, bottom), were quantified at day 0, 7, and 14 following editing and culture of non-edited control cells or CLL1 KO cells edited by gRNA F. The granulocytes and monocytes generated from CLL1 KO cells exhibited sustained, loss of CLL1 expression over time, as compared to the non-edited control cells (Figure 14A). The ability of the CLL1 KO cells to differentiate into myeloid cells *in vitro* was also evaluated. The percentage of CD15+ (Figure 14B, top left) or CD11b+ positive granulocytes (Figure 14B, top right) was quantified at day 0, 7, and 14 following editing and culture of non-edited control cells or CLL1KO cells edited by gRNA F. Expression of these granulocyte markers were not affected by loss of CLL1. The percentage of CD14+ (Figure 14B, bottom left) or CD11b+ positive monocytes (Figure 14B, bottom right) was also quantified at day 0, 7, and 14 following editing and culture of non-edited control cells or CLL1 KO cells edited by gRNA F. Similar to the granulocyte markers, expression of these monocyte markers were not affected by loss of CLL1. Expression of CD33 (marker for myeloid cells) and HLA-DR (antigen presentation) were also unaltered by CLL1 disruption. These data indicate the loss of CLL1 did not affect *in vitro* myeloid differentiation.

The function of CLL1 KO cells was also evaluated *in vitro*. The percentage of phagocytosis performed by granulocytes (Figure 15, top) and monocytes (Figure 15, bottom) was quantified in the control cell population and the CLL1 KO cell populations. Phagocytosis activity was equivalent between the control and CLL1 KO cells for both granulocytes and monocytes, demonstrating the CLL1 KO cells retained phagocytosis activity (Figure 15). The ability of CLL1 KO cells to produce inflammatory cytokines upon stimulation was also evaluated. Granulocytes (Figure 16A) and monocytes (Figure 16B) produced from non-edited control cells or CLL1 KO cells edited by gRNA F were unstimulated or stimulated with LPS or R848. The levels of IL-6 (Figure 16A or 16B, left) and TNF- α (Figure 16A or 16B, right) were subsequently quantified. CLL1 KO granulocytes and monocytes exhibited intact inflammatory cytokine production upon TLR agonist stimulation and cytokine production was equivalent to non-edited control cells. Production of other cytokines, including IL-1 β and MIP-1 α was also not altered by CLL1 disruption. Taken together, these data demonstrate that loss of CLL1 did not affect *in vitro* myeloid cell function.

The differentiation potential of the gene-edited CD34+ CLL1 KO cells edited by gRNA F was also measured by a colony formulation assay. Following electroporation, CD34+ edited cells were plated and cultured for two weeks. Colonies were then counted and scored using StemVision (Stem Cell Technologies). Cells edited for CLL1 by gRNA F (editing frequency of 79.2%) produced fewer BFU-E, CFU-G/M/GM, and CFU-GEMM colonies compared to non-edited control cells (Figure 17A). However, cells edited for CLL1 produced similar distributions and percentages of BFU-E colonies (Burst forming unit-erythroid), CFU-G/M/GM colonies, and CFU-GEMM colonies, as non-edited control cells, showing that the CLL1 edited cells retain significant differentiation potential in this assay (Figure 17B). Colony forming unit (CFU)-G/M/GM colonies refer to CFU-G (granulocyte), CFU-M (macrophage), and CFU-GM (granulocyte/macrophage) colonies. CFU-GEMM (granulocyte/erythroid/macrophage/megakaryocyte) colonies arise from a less differentiated cell that is a precursor to the cells that give rise to CFU-GM colonies. Taken together, the differentiation assays indicate that human CD34+ cells edited at the CLL1 locus retain the capacity to differentiate into variety of cell types.

Example 6 Evaluation for resistance of CLL1 edited cells to CART effector cells

This Example describes evaluation of resistance of CLL1 edited cell to CART effector cells targeting CLL1. CLL1 KO cells that lack CLL1 expression are resistant to CLL1 CAR killing, compared to wild-type CLL1+ cells, as measured by the assays described herein.

Editing in CD34+ human HSPCs

gRNAs (Synthego) are designed as described in Example 3. The human CD34+ HSPCs are then edited via CRISPR/Cas9 as described in Example 1 using the CLL1 targeting gRNAs, e.g., a CLL1 targeting gRNA of Table 2, 6, or 8.

CAR constructs and lentiviral production

Second-generation CARs are constructed to target CLL1. The CAR consists of an extracellular scFv antigen-binding domain, using a CD8 α signal peptide, a CD8 α hinge and transmembrane region, a 4-1BB or CD28 costimulatory domain, and a CD3 ξ signaling domain. The anti-CLL1 CAR uses the CLL-1 scFv sequence from clone 1075.7 in a light-to-heavy chain orientation. The heavy and light chains are connected by (GGGS)₃ linker (SEQ

ID NO: 63). The CLL1 CAR cDNA sequence is sub-cloned into the multiple cloning site of the pCDH-EF1 α -MCS-T2A-GFP expression vector, and lentivirus is generated following the manufacturer's protocol (System Biosciences). Lentivirus can be generated by transient transfection of 293TN cells (System Biosciences) using Lipofectamine 3000 (ThermoFisher).

5

CAR transduction and expansion

Human primary T cells are isolated from Leuko Pak (Stem Cell Technologies) by magnetic bead separation using anti-CD4 and anti-CD8 microbeads according to the manufacturer's protocol (Stem Cell Technologies). Purified CD4+ and CD8+ T cells are
10 mixed 1:1, and activated using anti-CD3/CD28 coupled Dynabeads (Thermo Fisher) at a 1:1 bead to cell ratio. The T cell culture media is CTS Optimizer T cell expansion media supplemented with immune cell serum replacement, L-Glutamine and GlutaMAX (all purchased from Thermo Fisher) and 100 IU/mL of IL-2 (PeproTech). T cell transduction is performed 24 hours post activation by spinoculation in the presence of polybrene (Sigma).
15 CAR-T cells are cultured for 9 days prior to cryopreservation. Prior to all experiments, T cells are thawed and rested at 37°C for 4-6 hours.

Flow Cytometry based CAR-T cytotoxicity assay

The cytotoxicity of target cells is measured by comparing survival of target cells
20 relative to the survival of negative control cells. For CLL1 assays, wildtype and CRISPR/Cas9 edited human CD34+ HSPCs are used as target cells. Wildtype Raji cell lines (ATCC) are used as a negative control. Target cells and negative control cells are stained with CellTrace Violet (CTV) and CFSE (Thermo Fisher), respectively, according to the manufacturer's instructions. After staining, target cells and negative control cells are mixed at
25 1:1.

Anti-CLL1 CAR-T cells are used as effector T cells. Non-transduced T cells (mock CAR-T) are used as control. The effector T cells are co-cultured with the target cell/negative control cell mixture at a 1:1 effector to target ratio in duplicate. A group of target cell/negative control cell mixture alone without effector T cells is included as control. Cells
30 are incubated at 37°C for 24 hours before flow cytometric analysis. Propidium iodide (ThermoFisher) is used as a viability dye. For the calculation of specific cell lysis, the fraction of live target cell to live negative control cell (termed target fraction) is used.

Specific cell lysis is calculated as $((\text{target fraction without effector cells} - \text{target fraction with effector cells}) / (\text{target fraction without effectors})) \times 100\%$.

The analysis described above shows that CLL1 KO HPSCs (and their progeny) are resistant to anti-CLL1 CAR-T-mediated killing, while non-edited control HPSCs (and their progeny) are susceptible to anti-CLL1 CAR-T-mediated killing.

Example 7: Treatment of Hematologic Disease

An exemplary treatment regimen using the methods, cells, and agents described herein for acute myeloid leukemia or MDS is provided. Briefly, a subject having AML or MDS that is a candidate for receiving a hematopoietic stem cell transplant (HSCT) is identified. A suitable HSC donor, e.g., an HLA-matched donor, is identified and HSCs are obtained from the donor, or, if suitable, autologous HSCs from the subject are obtained.

The HSCs so obtained are edited according to the protocols and using the strategies and compositions provided herein, e.g., a suitable guide RNA targeting a CLL1 target domain described in any of Tables 2, 6, or 8. In an exemplary embodiment, the editing is effected using a gRNA comprising a targeting domain described herein for gRNA D, gRNA F, or gRNA O2. Briefly, a targeted modification (deletion, truncation, substitution) of CLL1 is introduced via CRISPR gene editing using a suitable guide RNA and a suitable RNA-guided nuclease, e.g., a Cas9 nuclease, resulting in a loss of CLL1 expression in at least 80% of the edited HSC population.

The subject having AML or MDS may be preconditioned according to a clinical standard of care, which may include, for example, infusion of chemotherapy agents (e.g., etoposide, cyclophosphamide) and/or irradiation. Depending on the health status of the subject and the status of disease progression in the subject, such pre-conditioning may be omitted, however.

A CLL1-targeted immunotherapy, e.g., a CAR-T cell therapy targeting CLL1 is administered to the subject. The edited HSCs from the donor or the edited HSCs from the subject are administered to the subject, and engraftment, survival, and/or differentiation of the HSCs into mature cells of the hematopoietic lineages in the subject are monitored. The CLL1-targeted immunotherapy selectively targets and kills CLL1 expressing malignant or pre-malignant cells, and may also target some healthy cells expressing CLL1 in the subject,

but does not target the edited HSCs or their progeny in the subject, as these cells are resistant to targeting and killing by a CLL1-targeted immunotherapy.

The health status and disease progression in the subject is monitored regularly after administration of the immunotherapy and edited HSCs to confirm a reduction in the burden
5 of CLL1-expressing malignant or pre-malignant cells, and to confirm successful engraftment of the edited HSCs and their progeny.

EQUIVALENTS AND SCOPE

Those skilled in the art will recognize, or be able to ascertain using no more than routine
10 experimentation, many equivalents of the exemplary embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description.

Articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between two or more members of a group are considered satisfied if one, more than one, or
15 all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes “or” between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in which more than one members of the group are present, and embodiments in which all of the group members are present. For purposes of brevity those embodiments have
20 not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

It is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitation, element, clause, or descriptive term, from one or more of the claims or from one or more relevant portion of the description, is introduced
25 into another claim. For example, a claim that is dependent on another claim can be modified to include one or more of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included,
30 unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

Where elements are presented as lists, it is to be understood that every possible individual element or subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term “comprising” is

intended to be open and permits the inclusion of additional elements, features, or steps. It should be understood that, in general, where an embodiment, is referred to as comprising particular elements, features, or steps, embodiments, that consist, or consist essentially of, such elements, features, or steps, are provided as well. For purposes of brevity those
5 embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific
10 value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context
15 and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

All publications, patent applications, patents, and other references (e.g., sequence
20 database reference numbers) mentioned herein are incorporated by reference in their entirety. For example, all GenBank, Unigene, and Entrez sequences referred to herein, e.g., in any Table herein, are incorporated by reference. Unless otherwise specified, the sequence accession numbers specified herein, including in any Table herein, refer to the database entries current as of August 28, 2019. When one gene or protein references a plurality of
25 sequence accession numbers, all of the sequence variants are encompassed.

In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. For purposes of brevity, all of the embodiments in which one or more elements, features,
30 purposes, or aspects is excluded are not set forth explicitly herein.