報告書のコンセプト	日本語仮訳	タイトル	文献情報	文献へのリンク
序論		Bogdanove AJ, Voytas DF. (2011). TAL effectors: customizable proteins for DNA targeting. Science. 333(6051):1843-6.		https://www.ncbi.nlm.nih.gov/pubmed/21960622
		Prashant Mali RNA-Guided Human Genome Engineering via Cas9 Science 15 Feb 2013: Vol. 339, Issue 6121, pp. 823-826		DOI: 10.1126/science.1232033
	相同組換え及び非相同組換えによる遺伝子挿入	Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells.	Takata, M, Sasaki, MS, Sonoda, E, Morrison, C, Hashimoto, M, Utsumi, H et al. (1998). EMBO J 17: 5497-5508.	
	Template依存的な相同組換えによる遺伝子挿入	The double-strandbreak repair model for recombination.	Szostak, JW, Orr-Weaver, TL, Rothstein, RJ and Stahl, FW (1983). Cell 33: 25-35.	
	Template依存的な相同組換えによる遺伝子挿入	Expression of a site-specific endonuclease stimulates homologous recombination in mammalian cells. .	Rouet, P, Smih, F and Jasin, M (1994).Proc Natl Acad Sci USA 91: 6064-6068	
	相同組換えの効率はDSBをどれだけ導入できたかに依存する	Double-strand breaks at the target locus stimulate gene targeting in embryonic stem cells.	Smih, F, Rouet, P, Romanienko, PJ and Jasin, M (1995). Nucleic Acids Res 23: 5012-5019	
	相同組換えの効率はDSBをどれだけ導入できたかに依存する	Induction of homologous recombination in mammalian chromosomes by using the I-SceI system of Saccharomyces cerevisiae.	Choulika, A, Perrin, A, Dujon, B and Nicolas, JF (1995). Mol Cell Biol 15: 1968-1973.	
１　品質特性に関する課題				
１）ゲノム編集の手法による分類				
①ZFN		Kazuki Sawamoto Gene therapy for Mucopolysaccharidoses. Mol Genet Metab. 2018 Feb; 123(2): 59-68.	Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders (LSDs) caused by a deficiency of lysosomal enzymes, leading to a wide range of various clinical symptoms depending upon the type of MPS or its	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5986190/
②TALEN		Park et al (2014) Targeted inversion and reversion of the blood coagulation factor 8 gene in human iPS cells using TALENs. PNAS		www.pnas.org/lookup/suppl/doi:10.1073/pnas.1323941111/-/DCSupplemental
		Bogdanove AJ, Voytas DF. (2011). TAL effectors: customizable proteins for DNA targeting. Science. 333(6051):1843-6.		https://www.ncbi.nlm.nih.gov/pubmed/21960622
③CRISPR/Cas９		Prashant Mali RNA-Guided Human Genome Engineering via Cas9 Science 15 Feb 2013: Vol. 339, Issue 6121, pp. 823-826		DOI: 10.1126/science.1232033
		Improving CRISPR-Cas nuclease specificity using truncated guide RNAs	Fu et al Nat Biotechnol. 2014 Mar;32(3):279-284. doi: 10.1038/nbt.2808. Epub 2014 Jan 26	https://www.nature.com/articles/nbt.2808
		Partial DNA-guided Cas9 enables genome editing with reduced off-target activity	Yin et al Nature Chemical Biology volume 14, pages 311-316 (2018) | Download Citation	https://www.nature.com/articles/nchembio.2559
２）ゲノム編集のツールによる分類				
①ウイルスベクター		E. Epstein () Combining engineered nucleases with adeno-associated viral vectors for therapeutic gene editing Adv Exp Med Biol. 2017; 1016: 29-42		https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5702533/pdf/nihms920629.pdf
②Plasmid				
③タンパク質		Silva et al (2017) CD7-edited T cells expressing a CD7-specific CAR for the threapy of T-cell malignancies. Blood. 2017 Jul 20;130(3):285-296		http://www.bloodjournal.org/content/130/3/285.long?sso-checked=true
④mRNA				
	相同組換えに８０ｂほどのDNAをテンプレートで導入	High-frequency genome editing using ssDNA oligonucleotides with zinc-finger nucleases	Chen, F, Pruett-Miller, SM, Huang, Y, Gjoka, M, Duda, K, Taunton, J et al. (2011). . Nat Methods 8: 753-755.	
	相同組換えに８０ｂほどのDNAをテンプレートで導入	In vivo genome editing using a high-efficiency TALEN system.	Bedell, VM, Wang, Y, Campbell, JM, Poshusta, TL, Starker, CG, Krug, RG 2nd et al. (2012). Nature 491: 114-118.	
	相同組換えに８０ｂほどのDNAをテンプレートで導入	TALEN genome-editing system for generating human stem cell-based disease models.	Ding, Q, Lee, YK, Schaefer, EA, Peters, DT, Veres, A, Kim, K et al. (2013). Cell Stem Cell 12: 238-251.	
３）ゲノム編集の目的による分類				
	ゲノム切断とdeletionによる目的遺伝子のKO	An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level.	Bauer, DE, Kamran, SC, Lessard, S, Xu, J, Fujiwara, Y, Lin, C et al. (2013). Science 342: 253-257.	

①遺伝子破壊	ゲノム切断とdeletionによる目的遺伝子のKO	BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis.	Canver, MC, Smith, EC, Sher, F, Pinello, L, Sanjana, NE, Shalem, O et al. (2015). Nature 527: 192-197	
	目的とする遺伝子の2つにDSBを入れ目的遺伝子の大きな欠失を誘導して遺伝子をKOする	. Targeted chromosomal deletions in human cells using zinc finger nucleases	Lee, HJ, Kim, E and Kim, JS (2010) Genome Res 20: 81-89.	
	目的とする遺伝子の2つにDSBを入れ目的遺伝子の大きな欠失を誘導して遺伝子をKOする	Targeted chromosomal deletions and inversions in zebrafish.	Gupta, A, Hall, VL, Kok, FO, Shin, M, McNulty, JC, Lawson, ND et al. (2013). Genome Res 23: 1008-1017.	
	目的とする遺伝子の2つにDSBを入れ目的遺伝子の大きな欠失を誘導して遺伝子をKOする	Chromosomal deletions and inversions mediated by TALENs and CRISPR/Cas in zebrafish	Xiao, A, Wang, Z, Hu, Y, Wu, Y, Luo, Z, Yang, Z et al. (2013). . Nucleic Acids Res 41: e141	
	目的とする遺伝子の2つにDSBを入れ目的遺伝子の大きな欠失を誘導して遺伝子をKOする	Characterization of genomic deletion efficiency mediated by clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 nuclease system in mammalian cells	Canver, MC, Bauer, DE, Dass, A, Yien, YY, Chung, J, Masuda, T et al. (2014). J Biol Chem 289: 21312-21324.	
②相同組換え				
③Dead Cas9やデアミナーゼなどによる非切断改変		Targeted activation of diverse CRISPR-Cas systems for mammalian genome editing via proximal CRISPR targeting	Chen et al Nature Communications volume 8, Article number: 14958 (2017) | Download Citation	https://www.nature.com/articles/ncomms14958
④DNAメチル化＆脱メチル化				
2　非臨床試験における安全性に関する課題				
1）　ex vivo ゲノム編集				
①オフターゲット効果	The publisher's final edited version of this article is available at **Cell Stem Cell** ... However, these existing analyses of **off-target** effects and mutational load in gene-corrected**stem cells** have been restricted to genome editing has attracted wide interest for the generation of cellular models of disease using human pluripotent stem cells and other cell types. CRISPR-Cas systems and TALENs can target desired genomic sites with high efficiency in human cells, but recent	Whole-Genome Sequencing Analysis Reveals High Specificity of CRISPR/Cas9 and TALEN-Based Genome Editing in Human iPSCs	Smith et al Cell Stem Cell. Author manuscript; available in PMC 2015	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4338993/pdf/nihms658738.pdf
		Low incidence of off-target mutations in individual CRISPR-Cas9 and TALEN targeted human stem cell clones detected by whole-genome sequencing.	Veres et al Cell Stem Cell. 2014 Jul 3;15(1):27-30. doi: 10.1016/j.stem.2014.04.020	https://www.cell.com/cell-stem-cell/fulltext/S1934-5909(14)00186-6?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS1934590914001866%3Fshowall%3Dtrue
	We performed whole-genome sequencing to evaluate the mutational load at single-base resolution in individual gene-corrected human induced pluripotent stem cells (hiPSCs) clones in three different disease models. Single-cell clones gene correction by helper-dependent adenoviral vector (HDAdV) or Transcription Activator-Like Effector Nuclease (TALEN) exhibited few off-target effects and a low level of sequence variation, comparable to that accumulated in routine hiPSC culture.	Targeted gene correction minimally impacts whole-genome mutational load in human disease-specific induced pluripotent stem cell clones	suzuki et al Cell Stem Cell. 2014 Jul 3; 15(1): 31-36.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4144407/pdf/nihms610278.pdf
②想定外のゲノムの欠失・目的外配列の挿入、染色体の転座、逆位	ヒト細胞における染色体転座は規範的非相同末端結合により生じる	Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining	Hind Ghezraoui, Marion Piganeau, Benjamin Renouf, Jean-Baptiste Renaud, Annahita Sallmyr, Brian Ruis, Sehyun Oh, Alan E. Tomkinson, Eric A. Hendrickson, Carine Giovannangeli, Maria Jasin, Erika Brunet Vol.55(6), 2014, 829-842 Mol. Cell	https://www.sciencedirect.com/science/article/pii/S1097276514006352?via%3Dihub
	CRISPR/Cas9は予期しない大きな欠失変異や染色体再構築を引き起こす（真下先生スライド２９）	Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements	Nature Biotechnology volume 36, pages 765-771 (2018)	https://www.nature.com/articles/nbt.4192
	初代ヒト造血幹細胞および前駆細胞における患者特異的MLL転座を作り出すためのゲノム工学の使用	Use of Genome Engineering to Create Patient Specific MLL Translocations in Primary Human Hematopoietic Stem and Progenitor Cells		https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0136644
③ゲノム編集細胞のP53変異の可能性		Robert J. Ihry, (2018) p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nature Medicinevolume 24, pages939-946 (2018)		https://www.nature.com/articles/s41591-018-0050-6
		Emma Haapaniemi, (2018) CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response Nature Medicinevolume 24, pages927-930		https://www.nature.com/articles/s41591-018-0049-z
	成人造血幹細胞における遺伝子編集	Gene Editing in Adult Hematopoietic Stem Cells		https://www.intechopen.com/books/modern-tools-for-genetic-engineering/gene-editing-in-adult-hematopoietic-stem-cells
	CRISPR - Cas9：iPS細胞のゲノム編集のための有望なツール	CRISPR-Cas9: a promising tool for gene editing on induced pluripotent stem cells		http://kjim.org/journal/view.php?doi=10.3904/kjim.2016.198
	ヒトCD34 +造血細胞のゲノム編集によるMLL白血病誘発	MLL leukemia induction by genome editing of human CD34+ hematopoietic cells		http://www.bloodjournal.org/content/early/2015/08/26/blood-2015-05-646398?sso-checked=true

④その他	多機能AAV-CRISPR-Cas9とその宿主応答	A multi-functional AAV-CRISPR-Cas9 and its host response	Chew et al., Nature Methods, 2016	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5374744/
	米国人口におけるCRISPR関連ヌクレアーゼCas9に対する既存の抗体の有病率	Prevalence of Pre-existing Antibodies to CRISPR-Associated Nuclease Cas9 in the USA Population	Simhadri et al., Mol Ther Methods Clin Dev, 2018	https://www.sciencedirect.com/science/article/pii/S2329050118300603
	ヒトにおけるCas9タンパク質に対する既存の適応免疫の同定	Identification of Pre-Existing Adaptive Immunity to Cas9 Proteins in Humans	Charlesworth et al., BioRxiv, 2018	https://www.biorxiv.org/content/early/2018/01/05/243345
	Cas9免疫はCRISPR遺伝子編集療法の課題	Cas9 immunity creates challenges for CRISPR gene editing therapies		https://www.nature.com/articles/s41467-018-05843-9
	ヒト造血幹細胞由来疾患の治療薬の将来	The Future of Therapeutics for Human Hematopoietic Stem Cell Derived Diseases		https://biotechconnectionbay.org/market-reports/the-future-of-crispr-therapeutics-for-human-hematopoietic-stem-cell-derived-diseases/
２） in vivo ゲノム編集				
①編集効率を高めるための工夫をすることにより長期にわたって編集酵素が持続発現し、より上記１）の①～③リスクが昂進する可能性	in vivo ゲノム編集による治療	In vivo genome editing of the albumin locus as a platform for protein replacement therapy	Blood. 2015 Oct 8; 126(15): 1777-1784. Prepublished online 2015 Aug 21. doi: 10.1182/blood-2014-12-615492	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4600017/
	Based on clustered regularly interspaced short palindromic repeat/Cas9 (CRISPR/Cas9)3,4 technology, here we devise a homology-independent targeted integration (HITI) strategy, which allows for robust DNA knock-in in both dividing and non-dividing cells in vitro and, more importantly, in vivo (for example, in neurons of postnatal mammals). As a proof of concept of its therapeutic potential, we demonstrate the efficacy of HITI in improving visual function using a rat model of the retinal degeneration condition retinitis pigmentosa. The HITI method presented here establishes new avenues for basic research and targeted gene therapies.	In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration.	suzuki et al Nature. 2016 Dec 1;540(7631):144-149. doi: 10.1038/nature20565. Epub 2016 Nov 16.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5331785/
②目的の組織・臓器への特異性	雌マウス生殖細胞系におけるCRISPR / Cas9により仲介されるスーパーメンデル遺伝	Super-Mendelian inheritance mediated by CRISPR/Cas9 in the female mouse germline		https://www.biorxiv.org/content/early/2018/07/04/362558
③多様なゲノム編集ツールの導入方法とその安全性				
３ 治験において留意する事項（長期フォローアップ等）				
分類？	ヘテロクロマチンはCRISPR-Cas9変異誘発を遅らせるが修復結果には影響しない	Heterochromatin delays CRISPR-Cas9 mutagenesis but does not influence repair outcome		https://www.biorxiv.org/content/early/2018/02/19/267690.full.pdf+html
その他				
スライド２６：真下先生　講演資料より		Correction of a pathogenic gene mutation in human embryos	Nature volume 548, pages 413-419 (24 August 2017)	https://www.nature.com/articles/nature23305
スライド３：三谷先生講演資料	ゲノム編集（遺伝子ノックアウト）の臨床応用：AIDSの遺伝子治療	Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV	N Engl J Med 2014; 370:901-910 DOI: 10.1056/NEJMoa1300662	https://www.nejm.org/doi/full/10.1056/NEJMoa1300662
スライド４：三谷先生講演資料	ゲノム編集の臨床応用：ユニバーサル　CAR-T	Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells	Science Translational Medicine 25 Jan 2017: Vol. 9, Issue 374, eaaj2013 DOI: 10.1126/scitranslmed.aaj2013	http://stm.sciencemag.org/content/9/374/eaaj2013.full
スライド５：三谷先生講演資料	CRISPRの臨床応用	First CRISPR clinical trial gets green light from US panel	Nature doi:10.1038/nature.2016.20137	https://www.nature.com/news/first-crispr-clinical-trial-gets-green-light-from-us-panel-1.20137
スライド９：高橋先生講演資料		TALE nucleases: tailored genome engineering made easy.	Claudio Mussolino, Toni Cathomen · Published 2012 in Current opinion in biotechnology · DOI: 10.1016/j.copbio.2012.01.013	https://www.semanticscholar.org/paper/TALE-nucleases%3A-tailored-genome-engineering-made-Mussolino-Cathomen/9a1071363d6bcdb09cf9775cdc832c218ef85baf
スライド１０：高橋先生講演資料	TALENの構造	A transcription activator-like effector toolbox for genome engineering	Nat Protoc. 2012 Jan 5;7(1):171-92. doi: 10.1038/nprot.2011.431.	https://www.ncbi.nlm.nih.gov/pubmed/22222791