
Engineering B cells to treat and study human disease

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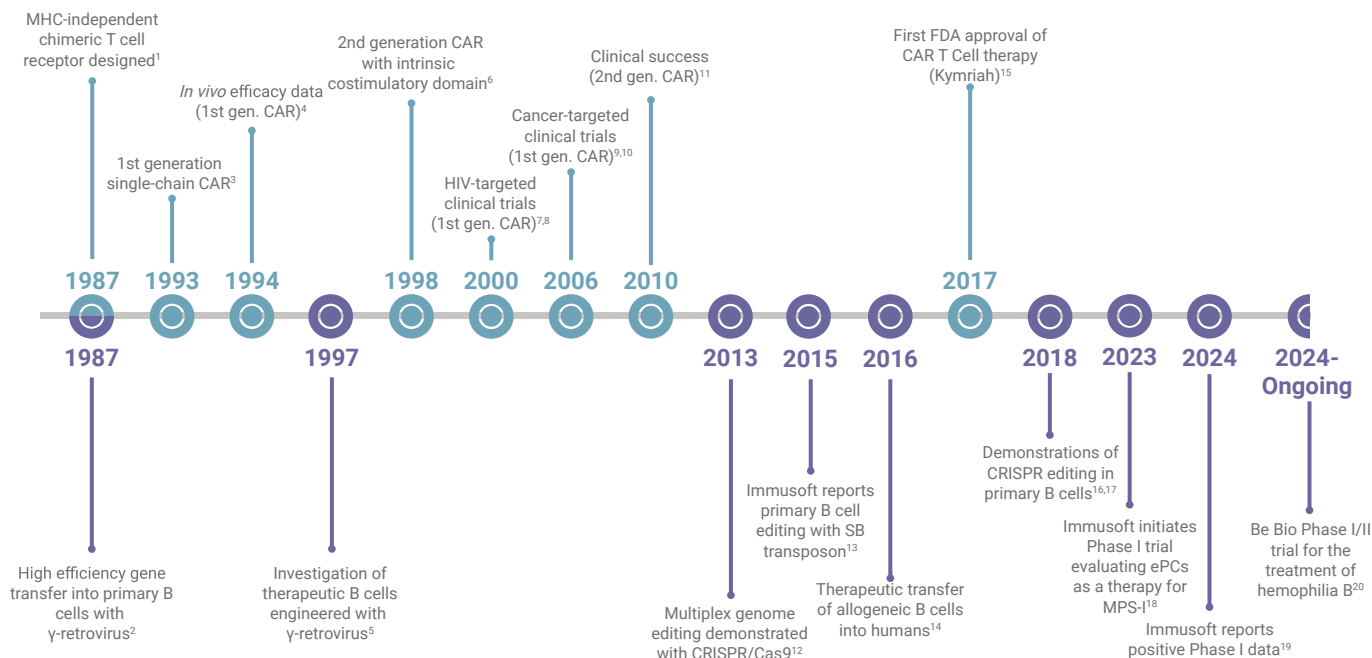
Table 1: Alternative B cell in vitro culture conditions used to generate eB cells and ePCs. eB

cells: engineered B cells; ePCs: engineered plasma cells; NHP: Non-human primate; IL: interleukin;

IFN: interferon; BAFF: B cell activating factor; APRIL: A proliferation-inducing ligand; LPS:

lipopolysaccharide; CD40L: CD40 ligand; HA: Hemagglutinin. References cited from main text.

Product	Activation and Expansion	Differentiation phase 1	Differentiation phase 2	Long-term phase	Reference
Human eB cells	anti-CD180, IL-7				40
Human eB cells	CD40L/IL-4 B cell expansion media				42
Human eB cells	StemMACS HCS Expansion Media XF, Human antibody serum, IL-4, CD40L				85
Human eB cells	anti-CD180				37,84
Human eB cells	Excellerate B cell medium, CTS Immune Cell SR, CpG, CD40L, IL-10, IL-15				89
Mouse eB cells	LPS, IL-4				40
Mouse eB cells	anti-CD180 Co-culture: anti-CD180, IL-4, NB21 feeder cells				37,44
Mouse eB cells	LPS or CpG, IL-4, anti-CD40				39
Mouse eB cells	anti-CD180 or LPS or LPS + IL-4				73
NHP eB cells	CpG, CD40L, IL-2, IL-10, BAFF				140
Human ePCs	CpG, CD40L, IL-2,IL-10, IL-15	IL-2, IL-6, IL-10, IL-15	IL-6, IL-15, IFN- α 2B		4,22,28,43,72,77, 141
Human ePCs	CpG, CD40L, IL-2,IL-10, IL-15	Insulin, transferrin, IL-2, IL-15, IL-21, NIH 3T3-CD40L feeders	Insulin, transferrin, universal type I IFN protein, IL-6, IL-15		24
Human ePCs	CpG, CD40L, IL-2,IL-10, IL-15	IL-2, IL-6, IL-10, IL-15, type I IFN, BAFF, APRIL	CpG, CD40L, IL-2, IL-10, IL-15, IL-6, type I IFN, APRIL		29
Human ePCs	CpG, CD40L, IL-2,IL-10, IL-15	IL-2, IL-6, IL-10, IL-15	IL-6, IL-15, IFN- α	IL-6, APRIL secreting feeders	23
Mouse ePCs	HA-CD40L, anti-HA, IL-4	HA-CD40L, anti-HA, IL-4, IL-21	IL-21, NIH 3T3-CD40L feeders		24
NHP ePCs	ImmunoCult-ACF Human B Cell Expansion Supplement, CpG, MCD40L, IL-2,IL-10, IL-15, IL-21	ImmunoCult-ACF Human B Cell Expansion Supplement, IL-2, IL-6, IL-10, IL-15	ImmunoCult-ACF Human B Cell Expansion Supplement, IL-6, IL-15, IFN- α 2B		142



Supplemental figure 1. Developmental timelines of engineered T and B cell therapies. Although the first efforts to engineer T (top) and B (bottom) cells emerged simultaneously, B cells have lagged owing to the relative difficulty of accomplishing gene transfer without using oncogenic γ -retroviruses. Developments in transposon and CRISPR gene editing technologies have accelerated recent progress in the B cell engineering field, with the first clinical trials beginning within the last two years. Icons were created with BioRender.com.

1. Kuwana, Y. *et al.* Expression of chimeric receptor composed of immunoglobulin-derived V regions and T-cell receptor-derived C regions. *Biochem. Biophys. Res. Commun.* **149**, 960–968 (1987).
2. Wu, D.-D., Huszar, D., Dick, J. E., Bernstein, A. & Phillips, R. A. High efficiency gene transfer and expression in normal murine B lymphocytes. *J. Immunol. Methods* **101**, 279–285 (1987).
3. Eshhar, Z., Waks, T., Gross, G. & Schindler, D. G. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 720–724 (1993).
4. Moritz, D., Wels, W., Mattern, J. & Groner, B. Cytotoxic T lymphocytes with a grafted recognition specificity for ERBB2-expressing tumor cells. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4318–4322 (1994).
5. Zambidis, E. T., Kurup, A. & Scott, D. W. Genetically transferred central and peripheral immune tolerance via retroviral-mediated expression of immunogenic epitopes in hematopoietic progenitors or peripheral B lymphocytes. *Mol. Med.* **3**, 212–224 (1997).
6. Krause, A. *et al.* Antigen-dependent CD28 signaling selectively enhances survival and proliferation in genetically modified activated human primary T lymphocytes. *J. Exp. Med.* **188**, 619–626 (1998).
7. Walker, R. E. *et al.* Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. *Blood* **96**, 467–474 (2000).

8. Mitsuyasu, R. T. *et al.* Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. *Blood* **96**, 785–793 (2000).
9. Lamers, C. H. J. *et al.* Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. *J. Clin. Oncol.* **24**, e20–2 (2006).
10. Kershaw, M. H. *et al.* A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin. Cancer Res.* **12**, 6106–6115 (2006).
11. Kochenderfer, J. N. *et al.* Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* **116**, 4099–4102 (2010).
12. Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
13. Herbig, E. J. *et al.* 373. Expression of human iduronidase from sleeping beauty engineered human B lymphocytes as a cellular therapy for mucopolysaccharidosis type I. *Mol. Ther.* **23**, S148 (2015).
14. Winkler, J. *et al.* Adoptive Transfer of Purified Donor-B-Lymphocytes after Allogeneic Stem Cell Transplantation: Results from a Phase I/IIa Clinical Trial. *Blood* **128**, 502–502 (2016).
15. Maude, S. L. *et al.* Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* **378**, 439–448 (2018).
16. Hung, K. L. *et al.* Engineering Protein-Secreting Plasma Cells by Homology-Directed Repair in Primary Human B Cells. *Mol. Ther.* **26**, 456–467 (2018).
17. Johnson, M. J., Laoharawee, K., Lahr, W. S., Webber, B. R. & Moriarity, B. S. Engineering of Primary Human B cells with CRISPR/Cas9 Targeted Nuclease. *Sci. Rep.* **8**, 12144 (2018).
18. ISP-001: Sleeping Beauty Transposon-Engineered B Cells for MPS I.
<https://www.clinicaltrials.gov/study/NCT05682144>.

19. Immusoft to Announce Positive Phase 1 Data for First Engineered B Cell Therapy in a Clinical Trial. *Immusoft* <https://www.immusoft.com/2024/09/23/immusoft-to-announce-positive-phase-1-data-for-first-engineered-b-cell-therapy-in-a-clinical-trial/> (2024).
20. BeCoMe-9: a Clinical Study of BE-101 for the Treatment of Adults with Moderately Severe or Severe Hemophilia B. <https://clinicaltrials.gov/study/NCT06611436?term=NCT06611436&rank=1>
<https://clinicaltrials.gov/search?term=NCT06611436>.