Stem Cell Reports, Volume *18*

Supplemental Information

Universal cell donor lines: A review of the current research

Ariel Simpson, Alex W. Hewitt, and Kirsten A. Fairfax

Supplementary Table 1. Progress in the development of universal cell donor lines

Study Year	Gene/s Deleted	Gene/s Induced	Model Tested	Conclusion
1990	β2M		Mouse	Mice homozygous for β2M deletion had no detectable class I antigens and were deficient in CD8+ T cells responsible for cell mediated cytotoxicity (Koller et al., 2010; Zijlstra et al., 2010)
1992	TAP1		Mouse	Mice with TAP1 deficiency have reduced MHC class I surface expression due to impaired molecular assembly and transportation leading to impaired cytotoxic immune response (Van Kaer et al., 1992)
1996	CIITA		Mouse	No expression of conventional MHC class II on cell surface of CIITA-deficient mice shown by reduced CD4 T cell numbers in the periphery leading to impaired MHC class II mediated responses (Chang et al., 1996)
2000	TAPBP		Mouse	Defective MHC class I assembly and expression and CD8+ T cell development (Grandea et al., 2000)
2013	β2M		Human ESCs	HLA class I-negative cells targeted by homozygous disruption of β2M demonstrated reduced CD8+ T cell responses upon differentiation in embryoid bodies (Riolobos et al., 2013)
2014		CTLA-4, PD-L1	Human ESCs, humanised mice	Knock in hESCs expressing CTLA-Ig and PD-L1 confers immune protection in humanised mice. Allogeneic hESC-derived teratomas, fibroblasts and cardiomyocytes avoided immune rejection; cells from parental hESCs were rejected (Rong et al., 2014)
2015	β2M		Human ESCs	Deletion of exons 2 and 3 of β2M alleles in hESCs led to HLA-I deficiency on the surface of cells and resistance to CD8+ T cell-mediated responses (D. Wang et al., 2015)
2016	TAP1, TAPBP		Human ESCs	TAP1- and TAPBP-deificent hESC lines demonstrated MHC class I deficiency on cell surface with a two-fold reduction in accumulation of T lymphocytes and NK cells. Cell lines maintained karyotypes, pluripotency and differentiation ability (Cui et al., 2016)

2017	β2M	HLA-E	Human iPSCs	Knock-in HLA-E at the β2M locus in human iPSCs confers regulated expression of HLA-E. Edited cells and differentiated derivatives are not recognised as allogeneic by CD8+ T cells and are resistant to NK mediated lysis (Gornalusse et al., 2017)
2017		HLA-G	Human ESCs	hESCs over-expressing HLA-G1 and differentiated neural progenitor cells retain pluripotency characteristics of stem cells with enhanced immunological tolerance (Zhao et al., 2017)
2018	β2M, CIITA		Human iPSCs	Knock-out of β2M and CIITA in hiPSCs does not alter the electrical properties of differentiated cardiomyocytes while inducing minimal in human immune cells (Mattapally et al., 2018)
2019	β2M, CIITA	CD47	Mouse & human iPSCs	Both mouse and human iPSCs lose immunogenicity when MHC class I and II genes are inactivated and CD47 is overexpressed while retaining pluripotency. Differentiated cells derived from the iPSC lines evade immune rejection in MHC-mismatched allogeneic recipients without immunosuppression (Deuse et al., 2019)
2019	HLA-A, HLA-B, HLA-C, CIITA	PD-L1, HLA-G, CD47	Human iPSCs	Ablation of HLA-A/B/C and HLA class I molecules in hiPSCs and expression of immunomodulatory factors blunted T cell responses, NK killing and macrophage engulfment (Han et al., 2019)
2019		CC21, PD-L1, CD47, CD200 Serpinb9, H2-M3, FasL, MFGE8	Mouse and mouse ESCs	Expression of eight immunomodulatory transgenes in mESCs and derivatives prevents immune rejection in immunocompetent, allogeneic recipients. The cells generated ectopic tissues that maintained high expression of immunomodulatory transgenes (Harding et al., 2019.)
2021	B2M, CIITA, CD155	HLA-E	Mouse	In CD20-expressing leukaemia/lymphoma mouse models, CD20 CAR T cells evaded recognition by NKG2A+ and DNAM1+ NK cells as well as CD4 and CD8 T cell responses (Wang et al., 2021)
2023	B2M, CIITA		Mouse	OPCs were developed from healthy donor cells that resulted in rescue of the major phenotypic features of Canavan disease in disease-specific mouse model (Feng et al., 2023)

Supplementary Table 2. Overview of cell safety switches in cell-based therapies

Switch	Type of Switch	Mechanism	Considerations	Reference/s
FITC	On	Formation of pseudoimmunological synapse by combining with antigen receptors on CAR-T cell surface.		(Cao et al., 2016; Lee et al., 2019; Ma et al., 2016)
Rapamycin	On	Assembles antigen binding and intracellular signalling subunits into complete construct.		(Bayle et al., 2006; Duong et al., 2019)
Dasatinib	Off	Directly prevents CAR-T cell activation.		(Weber et al., 2019)
HSV-TK	Off	Upon administration of ganciclovir, kills any dividing cell co-expressing CDK and HSV-TK gene system.	Cell death can take up to several days Virally derived protein has higher risk of immunogenicity	(Tiberghien et al., 2001)
Inducible Caspase9 (iCasp9)	Off	Activation of caspase-9 apoptotic pathways upon administration of chemical inducer of dimerisation (CID) AP1903.	Human derived thus, lower risk of immunogenicity	(Di Stasi et al., 2011; Sahillioglu & Schumacher, 2022; Straathof et al., 2005)

Supplementary References

Cao, Y., Rodgers, D. T., Du, J., Ahmad, I., Hampton, E. N., Ma, J. S. Y., Mazagova, M., Choi, S.-H., Yun, H. Y., Xiao, H., Yang, P., Luo, X., Lim, R. K. V., Pugh, H. M., Wang, F., Kazane, S. A., Wright, T. M., Kim, C. H., Schultz, P. G., & Young, T. S. (2016). Design of Switchable Chimeric Antigen Receptor T Cells Targeting Breast Cancer. Angew Chem *55*, 7520–7524. 0.1002/anie.201601902.

Cui, D., Wang, J., Zeng, Y., Rao, L., Chen, H., Li, W., Li, Y., Li, H., Cui, C., & Xiao, L. (2016). Generating hESCs with reduced immunogenicity by disrupting TAP1 or TAPBP. Biosci. Biotechnol. Biochem. *80*, 1484–1491. 10.1080/09168451.2016.1165601.

Duong, M. T., Collinson-Pautz, M. R., Morschl, E., Lu, A., Szymanski, S. P., Zhang, M., Brandt, M. E., Chang, W.-C., Sharp, K. L., Toler, S. M., Slawin, K. M., Foster, A. E., Spencer, D. M., & Bayle, J. H. (2019). Two-Dimensional Regulation of CAR-T Cell Therapy with Orthogonal Switches. Mol. Ther. Oncolytics *12*, 124–137. 10.1016/j.omto.2018.12.009.

Grandea, A. G., 3rd, Golovina, T. N., Hamilton, S. E., Sriram, V., Spies, T., Brutkiewicz, R. R., Harty, J. T., Eisenlohr, L. C., & Van Kaer, L. (2000). Impaired assembly yet normal trafficking of MHC class I molecules in Tapasin mutant mice. Immun. *13*, 213–222. 10.1016/s1074-7613(00)00021-2.

Harding, J., Vintersten-Nagy, K., Shutova, M., Yang, H., Tang, J. K., Massumi, M., Izaidfar, M., Izadifar, Z., Zhang, P., Li, C., & Nagy, A. (2019). Induction of long-term allogeneic cell acceptance and formation of immune privileged tissue in immunocompetent hosts. bioRxiv. 10.1101/716571

Lee, Y. G., Marks, I., Srinivasarao, M., Kanduluru, A. K., Mahalingam, S. M., Liu, X., Chu, H., & Low, P. S. (2019). Use of a Single CAR T Cell and Several Bispecific Adapters Facilitates

Eradication of Multiple Antigenically Different Solid Tumors. Cancer Res. *79*, 387–396. 10.1158/0008-5472.can-18-1834

Ma, J. S. Y., Kim, J. Y., Kazane, S. A., Choi, S.-H., Yun, H. Y., Kim, M. S., Rodgers, D. T., Pugh, H. M., Singer, O., Sun, S. B., Fonslow, B. R., Kochenderfer, J. N., Wright, T. M., Schultz, P. G., Young, T. S., Kim, C. H., & Cao, Y. (2016). Versatile strategy for controlling the specificity and activity of engineered T cells. Proc. Natl Acad. Sci. U.S.A *113*, 450–458. 10.1073/pnas.1524193113.

Tiberghien, P., Ferrand, C., Lioure, B., Milpied, N., Angonin, R., Deconinck, E., Certoux, J. M., Robinet, E., Saas, P., Petracca, et al (2001). Administration of herpes simplex-thymidine kinase-expressing donor T cells with a T-cell-depleted allogeneic marrow graft. Blood *97*, 63–72. doi: 10.1182/blood.v97.1.63.

Van Kaer, L., Ashton-Rickardt, P. G., Ploegh, H. L., & Tonegawa, S. (1992). TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4−8 T cells. Cell *71*, 1205–1214. 10.1016/s0092-8674(05)80068-6

Wang, B., Iriguchi, S., Waseda, M., Ueda, N., Ueda, T., Xu, H., Minagawa, A., Ishikawa, A., Yano, H., Ishi, T., Ito, R., Goto, M., Takahashi, R., Uemura, Y., Hotta, A., & Kaneko, S. (2021). Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. Nat. Biomed. Eng *5*, 429-440. 10.1038/s41551-021-00730-z.

Weber, E. W., Lynn, R. C., Sotillo, E., Lattin, J., Xu, P., & Mackall, C. L. (2019). Pharmacologic control of CAR-T cell function using dasatinib. Blood Adv. *3*, 711–717. 10.1182/bloodadvances.2018028720

Zhao, H.-X., Jiang, F., Zhu, Y.-J., Wang, L., Li, K., Li, Y., Wang, X.-H., Li, L.-S., & Yao, Y.-Q. (2017). Enhanced Immunological Tolerance by HLA-G1 from Neural Progenitor Cells (NPCs) Derived from Human Embryonic Stem Cells (hESCs). Cell. Physiol. Biochem. *44*, 1435–1444. 10.1159/000485539.