HHS Public Access

Author manuscript

Cell. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Cell. 2016 October 06; 167(2): 304–306. doi:10.1016/j.cell.2016.09.033.

Customizing Functionality and Payload Delivery for Receptor-Engineered T Cells

Christopher A. Klebanoff[1,*] and **Nicholas P. Restifo**[2,3,*]

[1]Center for Cell Engineering and Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

[2]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

[3]Center for Cell-Based Therapy, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Abstract

Adoptive immunotherapy using receptor engineering to achieve specific tumor targeting by T cells holds much promise for advancing cancer therapy. Here, two studies by Boice et al. and Roybal et al. provide distinct and potentially complimentary approaches to improve the efficacy and curb potential toxicities of this approach.

Adoptive immunotherapy using T cells genetically redirected with antigen receptors enabling the recognition and destruction of cancer cells is poised to enter mainstream clinical practice. However, concerns over the safety and potency of current T cell therapies persist (Klebanoff et al., 2016). In this issue of *Cell*, Boice et al. (2016) and Roybal et al. (2016) offer distinct and potentially complimentary approaches to enhance the antitumor functions of T cells modified with chimeric antigen receptors (CARs) (Boice et al., 2016, Roybal et al., 2016). In both cases, the authors combined a mechanistic understanding of signaling networks in normal and transformed cells with creativity and ingenuity to engineer T cells capable of functioning as “micro-pharmacies.”

Somatic mutations and chromosomal deletions involving the tumor necrosis factor receptor superfamily member herpesvirus entry mediator (HVEM) are among the most common genetic lesions in human B cell lymphomas (Morin et al., 2011). However, whether loss of HVEM function directly contributes to lymphomagenesis was previously unknown. Although HVEM has several binding partners, the majority of mutations in the receptor occur throughout its extracellular domain in a region critical for binding to the co-inhibitory receptors B- and T-lymphocyte attenuator (BTLA) and CD160. Because BTLA, but not CD160, is expressed on both normal and malignant B cells, Boice et al. (2016) conjectured that disruption of the HVEMBTLA axis might contribute to B cell transformation.

*Correspondence: caklebanoff@gmail.com (C.A.K.), restifo@nih.gov (N.P.R.).

To test this hypothesis, the authors used the well-established VavP-Bcl2 murine lymphoma model. In this system, BCL2 is constitutively expressed in cells of hematopoietic origin, a phenomenon common to many B cell lymphomas in humans. Using short hairpin RNAs to attenuate HVEM expression in hematopoietic progenitor cells taken from VavP-Bcl2 transgenic mice, the authors observed a significant increase in the frequency of lymphoma formation compared with empty vector controls. Reporter expression for the hairpin construct was exclusively enriched within the B cell compartment, supporting a model whereby a B-cell-intrinsic defect in HVEM was responsible for accelerated transformation. Finally, increased mitogenic signals arising from dysregulated B cell receptor activation were found in HVEM-deficient lymphomas, a finding consistent with impaired BTLA signaling. Based on these data, Boice et al. (2016) conclude that HVEM serves as a tumor suppressor in B cells and that its loss drives B cell expansion and ultimately lymphoma formation.

Because BTLA is rarely mutated in cancer, the authors tested whether reconstitution of BTLA signaling using a recombinant soluble version of HVEM (solHVEM) could treat lymphomas harboring mutated HVEM. Although intra-tumoral injection of solHVEM caused a marked delay in xenograft lymphoma growth, systemic administration did not. To overcome this limitation, the authors designed a potentially translatable means of delivering high local concentrations of solHVEM to lymphomas in vivo. They constructed a multicistronic retroviral vector that constitutively co-expresses solHVEM in tandem with an anti-CD19 CAR currently used in late-stage clinical trials (Davila et al., 2014). With this approach, they found that CAR-mediated lymphoma destruction was measurably enhanced with incorporation of the solHVEM payload in the absence of overt toxicity to recipient mice. Importantly, although HVEM can function as a negative regulator of some T cell subsets, this potential effect did not compromise treatment efficacy. Thus, solHVEM now joins other soluble mediators, including interleukin(IL)-12 (Kerkar et al., 2010; Pegram et al., 2012) and IL-15 (Klebanoff et al., 2004), as candidate factors that might be constitutively expressed by transferred T cells to enhance antitumor immunity. However, what if the desired payload was far more noxious than solHVEM?

In a separate manuscript, Roybal et al. (2016) offer a potential solution to the challenge of safely delivering toxic pay-loads or targeting high-risk antigens using a modular synthetic receptor based on the Notch receptor. Whereas a second-generation CAR design common to most receptors in clinical development co-opts endogenous signaling pathways associated with T cell activation, the syn-Notch receptor does not (Figure 1). syn-Notch activation leads to the intracellular liberation of a synthetic transcription factor that works entirely independently of the normal transcriptional machinery that is present within a T cell. This is accomplished by repurposing a transcription factor network present in yeast to selectively drive gene expression. In this manner, a user can have complete control over when and how a T cell responds to given stimuli, including reprogramming a T cell to produce antibodies like B cells.

Despite its elegance, the synNotch receptor, as presently configured, has certain limitations that could complicate its clinical development. For example, the construct makes use of a Notch core region taken from mice and a yeast-derived transcriptional regulator. Even in

immunocompromised patients, expression of transgenes encoding novel proteins can be immunogenic, leading to T-cell-mediated rejection and loss of persistence of the modified cells (Riddell et al., 1996). Indeed, the cell therapy field is rapidly moving to employ only human or humanized segments into CAR designs, precisely because of immunogenicity concerns (Klebanoff et al., 2016). Exchange of the murine-derived regions for the corresponding human sequence is straightforward. However, replacement of the transcriptional output to a human sequence will be more challenging because of the requirement that it function orthogonally to any other transcriptional network operative in T cells. One solution could be incorporation of a wholly synthetic transcription factor system based on artificial zinc fingers derived in part from human sequences and that bind to modular DNA-binding domains (Khalil et al., 2012). Alternatively, a fully human transcription factor network exclusive to other human cell types, such as neurons or endocrine cells, but absent in T cells might be used. In either case, confirmation that these new transcription factor networks function orthogonally and are not immunogenic will be essential. Overriding all of these concerns is the essential problem challenging the entire field, which is the scarcity of truly tumor-specific targets on most solid cancers for which CAR T cells might be safely redirected (Klebanoff et al., 2016).

Regardless, both papers make important inroads and further expand the toolkit available to the practicing cell therapist. Next-generation immunotherapies incorporating conditional receptors and new payload delivery systems are on the horizon with the exciting promise to enhance potency while simultaneously improving safety.

REFERENCES

Boice M, Salloum D, Mourcin F, Sanghvi V, Amin R, Oricchio E, Jiang M, Mottok A, Denis-Lagache N, Ciriello G, et al. (2016). Cell 167, this issue, 405–418. [PubMed: 27693350]

Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, Chung SS, Stefanski J, Borquez-Ojeda O, Olszewska M, et al. (2014). Sci. Transl. Med 6, 224–225.

Kerkar SP, Muranski P, Kaiser A, Boni A, Sanchez-Perez L, Yu Z, Palmer DC, Reger RN, Borman ZA, Zhang L, et al. (2010). Cancer Res. 70, 6725–6734. [PubMed: 20647327]

Khalil AS, Lu TK, Bashor CJ, Ramirez CL, Pyenson NC, Joung JK, and Collins JJ (2012). Cell 150, 647–658. [PubMed: 22863014]

Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, Grewal N, Spiess PJ, Antony PA, Palmer DC, et al. (2004). Proc. Natl. Acad. Sci. USA 101, 1969–1974. [PubMed: 14762166]

Klebanoff CA, Rosenberg SA, and Restifo NP (2016). Nat. Med 22, 26–36. [PubMed: 26735408]

Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, et al. (2011). Nature 476, 298–303. [PubMed: 21796119]

Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, and Brentjens RJ (2012). Blood 119, 4133–4141. [PubMed: 22354001]

Riddell SR, Elliott M, Lewinsohn DA, Gilbert MJ, Wilson L, Manley SA, Lupton SD, Overell RW, Reynolds TC, Corey L, and Greenberg PD (1996). Nat. Med 2, 216–223. [PubMed: 8574968]

Roybal KT, Williams JZ, Morsut L, Rupp LJ, Kolinko I, Choe JH, Walker WJ, and Lim WA (2016). Cell 167, this issue, 419–432. [PubMed: 27693353]

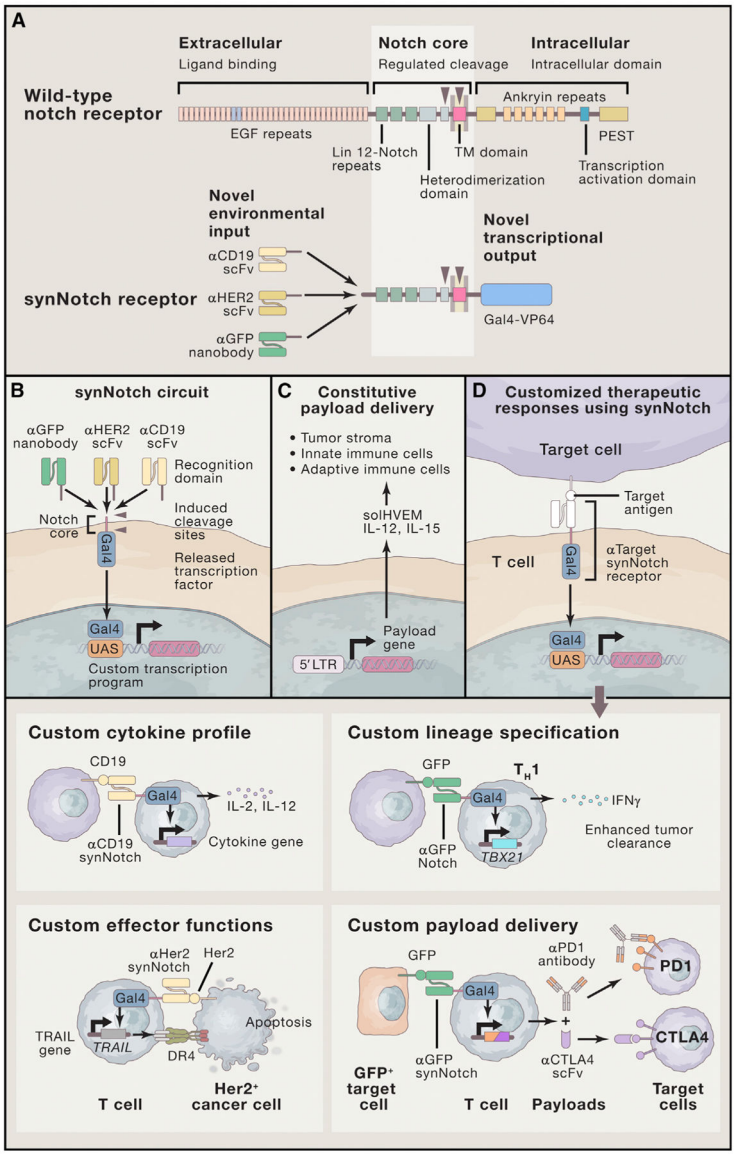

Figure 1. Customized Sensing and Transcriptional Responses Using the synNotch Receptor in T Cells

(A) The synthetic Notch (synNotch) receptor is built upon a central core derived from the wild-type Notch receptor. This includes a transmembrane (TM) domain, two regulated proteolytic cleavage domains, and a series of Lin 12-Notch repeats and heterodimerization domains that prevent spontaneous receptor activation.

(B) Both the extra- and intra-cellular domains of Notch can be replaced in a synNotch receptor with diverse antigen recognition domains, such as the single-chain variable fragments (scFvs) of an antibody and an artificial transcription factor, such as Gal4-VP64.

(C and D) (C) In contrast with constitutive expression of transgenes using the 5’-long terminal repeat (LTR) of a retrovirus, (D) the synNotch receptor allows for customized therapeutic responses triggered in a ligand-dependent manner. This could include the release of specific cytokines, activation of lineage-specific transcription factors, triggering of defined effector mechanisms, or secretion of large macromolecules such as antibodies.

CTLA4, cytotoxic T-lymphocyte associated protein 4; DR4, death receptor 4; PD1, programmed cell death 1; PEST, peptide sequence rich in proline, glutamate, serine, and threonine; UAS, upstream activation sequence.