



When better still might not be good enough

Waldemar Debinski^{1,2}

¹Brain Tumor Center of Excellence, Thomas K Hearn, Jr. Brain Tumor Research Center, ²Department of Cancer Biology, Radiation Oncology Microbiology and Immunology, and Translational Science Institute, Wake Forest Baptist Medical Center Comprehensive Cancer Center, Winston-Salem, NC, USA

Correspondence to: Waldemar Debinski, MD, PhD. Director, Brain Tumor Center of Excellence, Thomas K. Hearn Brain Tumor Research Center, Wake Forest Baptist Medical Center Comprehensive Cancer Center, 2nd Floor Commons, Rm C210A, 1 Medical Center Boulevard, Winston-Salem, NC 27157, USA. Email: debinski@wakehealth.edu.

Comment on: Krenciute G, Prinzing BL, Yi Z, *et al.* Transgenic Expression of IL15 Improves Antiglioma Activity of IL13R α 2-CAR T Cells but Results in Antigen Loss Variants. *Cancer Immunol Res* 2017;5:571-81.

Submitted Sep 06, 2017. Accepted for publication Sep 18, 2017.

doi: 10.21037/tcr.2017.09.40

View this article at: <http://dx.doi.org/10.21037/tcr.2017.09.40>

Immunotherapy has brought, arguably, the biggest advances in cancer treatment in recent years. Cancer immunotherapy can be divided into active and passive approaches. For example, vaccines are an active immunotherapy where the patient's own immune system is mobilized to fight malignancy. On the other hand, passive immunotherapy relies on administering the effector arms of the immune system like antibodies or T cells. Antibodies, like Herceptin, are directed against tumor-associated receptors expressed on tumor cells and appear to interfere with the pro-growth signaling mediated by the targeted receptor (1). Kadcyła is an antibody-drug conjugate version of Herceptin showing significant clinical benefits; this drug is cytotoxic to cells bearing the targeted receptor (2). Newest in the line of anti-cancer antibodies are those interfering with T cell inhibitory cellular signaling, such as anti-PD1 and anti-CTLA4 antibodies (3). These antibodies have been approved for treatment of at least eight different malignancies thus far.

The engineered re-targeted T cells represent an adoptive immunotherapy approach that may potentially keep up the pace with development of other above-mentioned clinically successful immunotherapies. For example, the US Food and Drug Administration (FDA) has just approved a therapy based on chimeric antigen receptor (CAR) T cells redirected to CD19 antigen in acute lymphoblastic leukemia (ALL) (4). While very active in hematologic malignancy, the CAR T cells approach faces many obstacles in its application to the treatment of solid tumors (5), including primary brain tumors like glioblastoma (GBM).

The report by Krenciute *et al.* entitled “*Transgenic expression of IL15 improves anti-glioma activity of IL13R α 2-CAR T cells but results in antigen loss variants*” describes an attempt at developing CAR T cells therapy for GBM (6). GBM, a solid tumor, is a high-grade astrocytoma with a dismal prognosis. Its clinical management is based on invasive and toxic treatments: surgery, radiation therapy, and chemotherapy (7), with the most recent addition of TT-fields to the armamentarium (8). These authors generated CAR T cells directed to an interleukin 13 receptor alpha 2 (IL-13RA2) based on a fusion of single-chain antibody against IL-13RA2 with transmembrane region of CD28 and a CD28. ζ endodomain. The target for these CAR T cells, IL-13RA2, was discovered in GBM as the first plasma membrane receptor over-expressed in the vast majority of patients with GBM, but not normal brain (9,10). Multiple pre-clinical and clinical studies have taken advantage of the attractive properties of IL-13RA2 in designing various immunotherapeutic approaches, including vaccines, targeted cytotoxins, drug conjugates, and adoptive immunotherapy (11-14). IL-13RA2 also segregates with tumor-initiating or glioma stem-like cells (GSCs) (15). Further, IL-13RA2 is over-expressed predominantly in a mesenchymal group of GBM, and it is linked to patients' survival (16). Moreover, the GBM tumor cells that inducibly lost the IL-13RA2 exhibited lesser tumorigenic potential, supporting the idea of using aggressive approaches even when solely targeting this receptor (17).

The science of including CAR T cells into current anti-

cancer treatment modalities has been evolving during the past decades. The experience gained from multiple pre-clinical and clinical studies surmounted several important obstacles facing successful implementation of adoptive immunotherapy. Among many factors to consider are tumor heterogeneity, the immunosuppressive tumor microenvironment, barriers to delivery of the transferred T cells to tumors, and ability of T cells to proliferate/persist and exert efficient oncolytic activity (5). Noteworthy methodologic issues are related to the use of autologous T cells, their extensive engineering, and subsequent expansion for clinical use (5). Nevertheless, multiple groups have been tackling these issues with incremental success, resulting in the first adoptive CAR T cell therapy available to cancer patients (4).

IL-13RA2 has been exploited as a target for adoptive immunotherapy in GBM by several groups (6,14-16,18). Importantly, early clinical trials have already started using appropriately re-engineered T cells (19). One example is making T cells equipped with a mutated IL-13 as a ligand targeting to tumor cells; the mutated ligand has much higher affinity towards the tumor-associated IL-13RA2 than the normal tissue receptor, IL-13RA1/IL-4A (20). This change in IL-13 offered the means to specifically target GBM tumor cells, but not normal cells of the tumor micro-environment and surrounding normal tissue. The pre-clinical evaluation of such CAR T cells was very encouraging, and early-phase clinical trials have been subsequently begun. One patient with recurrent GBM who failed all other treatments experienced a complete response to CAR T cell therapy, even though the disease spread to the meninges (19). This marked and long-lasting effect in a patient who practically had no other therapeutic options provides further encouragement to develop T cells therapies for GBM.

As have other investigators working on generating CAR T cells against IL-13RA2, Krenciute *et al.* previously documented significant anti-tumor activity of single-chain variable fragment [sc(Fv)], instead of modified IL-13, IL-13RA2 targeted CAR T cells in mice bearing intracranial tumors. To boost the extent of anti-tumor effect, they equipped the engineered T cells with the gene for IL-15, so the ectopic cytokine would be released at the sites of T cell distribution and support leukocyte proliferation and survival. The rationale behind this specific approach is that IL-15 is a T cell growth factor (21). This maneuver successfully enhanced anti-tumor activity of CAR T cells transferred to mice with glioma compared to T cells

without ectopic IL-15 (as determined by Kaplan-Meier curves). This result supports the rationale behind changing T cells to produce the cytokine. Thus, the authors achieved an improvement in CAR T cell therapy of the IL-13RA2 expressing tumor in a mouse model.

However, with an improved outcome of such a modified therapy, the authors noticed an outgrowth of tumor cells lacking the receptor to various degrees. Of interest, it took the longest time for tumor recurrence in a group where tumor cells lost the IL-13RA2: more than 40 days after T cell injection. The authors attributed this phenomenon to antigen-negative immune escape. This means that IL-15 helped CAR T cells to more effectively eliminate the receptor-positive tumor cells, but either some clones of tumor cells were receptor-negative to start with and they caused the recurrence, or not all receptor-positive cells were destroyed by T cells, but could stop expressing the receptor for various reasons.

An alternative explanation is suggested here. Later tumor recurrence, which would be a desirable clinical event, maybe due to the fact that tumor cells not carrying the IL-13RA2 any more are less tumorigenic; hence, selection of such cells during T cell therapy would be a welcome outcome (17). This hypothetical scenario was not examined experimentally in the paper discussed here. Nonetheless, unfortunately, tumors recurred; either this needs to be prevented, or another round of appropriate therapy is needed in order to think about cures.

Krenciute *et al.* used mainly one cell model of GBM in their *in vitro* and *in vivo* studies, an established U-373 GBM cell line. The authors did not discuss what portion, if any, of these cells are (for example) IL-13RA2-negative or have low levels in culture or when growing tumors in immunocompromised mice, making them less susceptible to CAR T cell cytotoxicity. Also, IL-13RA2 in tumor cells from recurrent tumors was not detected directly, but only after a short-term culture. It would be useful to know how this affects the levels of the receptor of interest. Moreover, the authors did not test the therapy on GSC cells; these can be more resistant to adoptive therapy, as these cells were suggested to be against other treatment modalities.

Almost ten years ago, it was said that “*Just about everything that relates to GBM pathobiology and its clinical course invites thinking about specific targeting of more than one tumor compartment/target and more than one mechanism controlling pathobiology of GBM, hence its maintenance and progression. This truly prompts consideration of rational combinatorial therapy/cocktail of drugs*” (22). The mostly negative experiences with

implementation of novel therapies in GBM, usually tested as individual treatments, only accentuated the validity of this statement. Thus, considering the complex and heterogenous nature of GBM, Krenciute *et al.* correctly suggest that we need targets in addition to IL-13RA2 to avoid antigen loss and to impact more of the tumor microenvironment, and more patients with transfer of re-engineered autologous T cells. Fortunately, there is no shortage of good candidates to supplement IL-13RA2 targeting. What may not be an optimal target is the deletion variant of the epidermal growth factor receptor (EGFRvIII), expression of which is very heterogenous and present in a fraction of GBM patients (23) although it was interesting to see that the systemically administered T cells targeting EGFRvIII localized to some extent to intracranial tumors (23).

Among potentially other candidates for re-targeting T cells to GBM are, for example, Eph receptor A2 and A3. The Eph receptors belong to the largest mammalian family of the protein receptor tyrosine kinases (TRK) that play important roles in development. In adulthood, they appear to be expressed and functional in various malignancies. The EphA2 receptor is over-expressed in up to 60% of patients with GBM. It is linked to survival and present in various GBM compartments, such as differentiated tumor cells, tumor-initiating cells or GSCs, neovasculature, and tumor cells infiltrating normal brain (24). This receptor is also a good partner with IL-13RA2 for T cell targeting, since more than 90% of patients with GBM over-express the two receptors due to the only partial overlap in their expression. The EphA3 receptor is also an attractive partner to add to GBM targeting based on IL-13RA2 or combined IL-13RA2/EphA2 targeting. The EphA3 receptor has many features of the EphA2 receptor, as it is present in more than 50% of patients with GBM, is linked to survival in the mesenchymal GBM subgroup, and is important for the function of GSCs (25,26). But the EphA3 receptor, unlike the EphA2 receptor, is also expressed in tumor-associated macrophages that play a permissive role in GBM progression (25). EphA3 receptor-positive cells also frequently localize to the perivascular niche. Thus, IL-13RA2, EphA2, and EphA3 receptors are over-expressed in close to 100% of patients with GBM, and are present in various compartments responsible for disease progression and resistance to treatment. It is thus expected that targeting of these three receptors with appropriately re-engineered T cells will be less prone to the antigen loss variants, as observed with targeting of the IL-13RA2 alone in immunocompromised mice (although the IL-13RA2

antigen loss may have a beneficial effect on its own). A similar multi-targeted approach has been recently proposed using targeted cytotoxic agents (25). In this way, various GBM compartments are targeted one at a time, such as differentiated tumor cells, tumor-initiating cells, tumor-associated neovasculature, and tumor cells infiltrating normal brain. Only such concerted efforts will provide further significant progress in the management of GBM independently of the type of drugs used.

Acknowledgments

Funding: This work was supported by National Cancer Institute grants R01 CA74145 and P01 CA207206.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Xian-Xin Qiu (Shanghai Proton and Heavy Ion Center (SPHIC), a.k.a. the Proton and Heavy Ion Center of Fudan University Shanghai Cancer Center (FUSCC), Shanghai, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2017.09.40>). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N*

- Engl J Med 2001;344:783-92.
2. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012;367:1783-91.
 3. Couzin-Frankel J. Breakthrough of the year 2013. Cancer immunotherapy. *Science* 2013;342:1432-3.
 4. Turtle CJ, Hay KA, Hanafi LA, et al. Durable Molecular Remissions in Chronic Lymphocytic Leukemia Treated With CD19-Specific Chimeric Antigen Receptor-Modified T Cells After Failure of Ibrutinib. *J Clin Oncol* 2017;35:3010-20.
 5. Yang JC, Rosenberg SA. Adoptive T-Cell Therapy for Cancer. *Adv Immunol* 2016;130:279-94.
 6. Krenciute G, Prinzing BL, Yi Z, et al. Transgenic Expression of IL15 Improves Antiglioma Activity of IL13R α 2-CAR T Cells but Results in Antigen Loss Variants. *Cancer Immunol Res* 2017;5:571-81.
 7. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009;10:459-66.
 8. Stupp R, Taillibert S, Kanner AA, et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. *JAMA* 2015;314:2535-43.
 9. Debinski W. An immune regulatory cytokine receptor and glioblastoma multiforme: an unexpected link. *Crit Rev Oncog* 1998;9:255-68.
 10. Debinski W, Gibo DM, Hulet SW, et al. Receptor for interleukin 13 is a marker and therapeutic target for human high-grade gliomas. *Clin Cancer Res* 1999;5:985-90.
 11. Okano F, Storkus WJ, Chambers WH, et al. Identification of a novel HLA-A*0201-restricted, cytotoxic T lymphocyte epitope in a human glioma-associated antigen, interleukin 13 receptor alpha2 chain. *Clin Cancer Res* 2002;8:2851-5.
 12. Mintz A, Gibo DM, Madhankumar AB, et al. Molecular targeting with recombinant cytotoxins of interleukin-13 receptor alpha2-expressing glioma. *J Neurooncol* 2003;64:117-23.
 13. Sonawane P, Choi YA, Pandya H, et al. Novel Molecular Multilevel Targeted Antitumor Agents. *Cancer Transl Med* 2017;3:69-79.
 14. Kahlon KS, Brown C, Cooper LJ, et al. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. *Cancer Res* 2004;64:9160-6.
 15. Brown CE, Starr R, Aguilar B, et al. Stem-like tumor-initiating cells isolated from IL13R α 2 expressing gliomas are targeted and killed by IL13-zetakine-redirected T Cells. *Clin Cancer Res* 2012;18:2199-209.
 16. Brown CE, Warden CD, Starr R, et al. Glioma IL13R α 2 is associated with mesenchymal signature gene expression and poor patient prognosis. *PLoS One* 2013;8:e77769.
 17. Nguyen V, Conyers JM, Zhu D, et al. IL-13R α 2-Targeted Therapy Escapes: Biologic and Therapeutic Implications. *Transl Oncol* 2011;4:390-400.
 18. Kong S, Sengupta S, Tyler B, et al. Suppression of human glioma xenografts with second-generation IL13R-specific chimeric antigen receptor-modified T cells. *Clin Cancer Res* 2012;18:5949-60.
 19. Brown CE, Alizadeh D, Starr R, et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. *N Engl J Med* 2016;375:2561-9.
 20. Thompson JP, Debinski W. Mutants of interleukin 13 with altered reactivity toward interleukin 13 receptors. *J Biol Chem* 1999;274:29944-50.
 21. Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and its therapeutic implications in cancer. *Trends Pharmacol Sci* 2012;33:35-41.
 22. Debinski W. Drug cocktails for effective treatment of glioblastoma multiforme. *Expert Rev Neurother* 2008;8:515-7.
 23. O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med* 2017;9.
 24. Wykosky J, Gibo DM, Stanton C, et al. EphA2 as a novel molecular marker and target in glioblastoma multiforme. *Mol Cancer Res* 2005;3:541-51.
 25. Ferluga S, Tomé CM, Herpai DM, et al. Simultaneous targeting of Eph receptors in glioblastoma. *Oncotarget* 2016;7:59860-76.
 26. Day BW, Stringer BW, Al-Ejeh F, et al. EphA3 maintains tumorigenicity and is a therapeutic target in glioblastoma multiforme. *Cancer Cell* 2013;23:238-48.

Cite this article as: Debinski W. When better still might not be good enough. *Transl Cancer Res* 2017;6(Suppl 7):S1244-S1247. doi: 10.21037/tcr.2017.09.40