Biomarkers in chimeric antigen receptor T-cell therapy

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Immunotherapy based on the adoptive transfer of T cells genetically reprogrammed to express chimeric antigen receptors (CARs) targeting tumors has the exciting potential to transform the treatment of cancer. CAR T-cell therapy has resulted in high response rates as well as durable complete remissions in patients with B-cell malignancies, and this approach is currently being investigated in several other hematologic and solid tumor indications. As CAR T-cell therapies progress into later phase clinical trials and become commercially available to patients, the identification of relevant biomarkers will be required to predict treatment outcomes and guide the development of cellular products with optimal clinical potency.

CAR T cells represent a breakthrough in personalized cancer therapy. In this approach, T cells are genetically modified to encode an artificial receptor that binds a tumor antigen, endowing the T cells with the ability to recognize and kill antigen-expressing cells. Until recently, CAR T cells were shown to be safe, but had disappointing clinical activity in a number of pilot trials [1–3]. The central issue in these studies was that the first-generation CAR T cells did not expand and persist in patients. Efforts to overcome this challenge with dose escalation were not successful. Based on these findings, our group at the University of Pennsylvania developed and extensively optimized novel T-cell culture systems [4] as well as a potent second-generation anti-CD19 CAR signaling through 4-1BB/CD3 ζ (CTL019). Preclinical studies had led to the identification of the 4-1BB co-stimulatory endodomain as a critical determinant of the survival and proliferation of CAR T cells in mice engrafted with pre-B acute lymphoblastic leukemia (ALL) and carcinoma cells [5,6]. We then conducted the first clinical trial testing a CAR with 4-1BB signaling delivered by lentiviral vector transduction and found that autologous CAR T cells are capable of eliciting significant on-target antitumor activity in chronic lymphocytic leukemia (CLL) [7,8]. In these studies, the CAR T cells proliferated 10³- to 10⁴-fold in the patients and have persisted at least beyond 7 years in the first two subjects treated [PORTER ET AL. UNPUBLISHED DATA, 2017]. CD19-directed CAR T cells have also proven effective in inducing long-term remissions in ALL, with reproducibly high rates of complete responses [9–12]. This ultimately led to the approval of the first CAR T-cell therapy (Kymriah[™] or tisagenlecleucel, formerly CTL019) by the US FDA in 2017.

Can biomarkers predict CAR T-cell success & failure?

Despite their clinical potential, second-generation CAR T cells do not expand and durably persist in some patients, including subsets of individuals with ALL or CLL, while in others robust expansion of CAR T cells occurs. A certain level of expansion and persistence of these cells is necessary for clinical benefit, but the predictive phenotypes and mechanism(s) associated with significant proliferation, persistence and clinical responses remain unclear. A key question currently facing the field is how to identify patient- or disease-specific factors and T-lymphocyte intrinsic

qualities that determine why some patients, but not others have such dramatic responses to CAR T cells. To date, this has not been systematically addressed. Thus, such biomarker studies have the potential to yield important biological insight into the predictive correlates and mechanistic underpinnings of response to CAR T-cell therapy that will propel the field forward.

Traditionally, biomarkers are defined as biological parameters measured in patients that provide information about treatment impact. In the context of CAR T cells in which therapeutic success is dependent on a viable self-replicating ‘drug’, the definition of biomarkers is extended to include the phenotypic and functional properties of T cells prior to engineering as well as the final cellular product. Other than monitoring the bioactivity of these genetically redirected T cells *in vivo* and establishing potential surrogate end points for treatment, biomarkers within pre-infusion and/or premanufactured patient samples could serve as powerful indicators of treatment efficacy.

Currently, we and others have not been able to identify consistent patient- or disease-specific characteristics that predict which subjects will respond best to CAR T-cell therapy or why. Our group at the University of Pennsylvania has almost 7 years of experience using CTL019 cells to treat B-cell cancers, and the longest involvement is with treating CLL. In the setting of this malignancy, response is not related to patient age, prior therapy, genetic risk profile, disease burden, cell dose or other typical risk factors [13,14]. We have shown that CTL019 cells can expand dramatically *in vivo*, eliminate high volume and bulky disease and persist [7,8,13]. Long-term B-cell aplasia accompanies these responses, implying that the persisting CAR T cells remain functional [13]. In CLL, we identified the following key biological correlates of response: patients responding to CTL019 display dramatic *in vivo* expansion of CAR T cells in the first 2 weeks following infusion, followed by lognormal decay with the complete and persistent eradication of the tumor cells; the infused CAR T cells persist long term in the responding patients; and persistent CAR T cells are polyfunctional. Regardless of the disease treated, type of CAR-triggered co-stimulatory signaling (i.e., 4-1BB or CD28) or T-cell subset enriched in infusion products, it appears that all CD19-directed CAR T cells require *in vivo* proliferation to be effective. Collectively, these results indicate that some patients possess T cells that, when engineered with a CAR directed against a tumor-associated antigen, display potent effector and memory functions following adoptive transfer. Given the intensive and somewhat costly manufacturing process required to generate autologous CAR T cells, the potential toxicity, and possible new alternatives for some patients, the ability to use biomarkers to identify which patients are most likely to respond would have tremendous benefit for these subjects and lead to an optimal personalized approach to therapy. Furthermore, once we understand the characteristics of an ideal and functional T-cell product, we may be able to intervene to generate maximally active CAR T cells for most, if not all, future patients.

Intrinsic T-cell fitness: a predictive correlate of CAR T-cell potency?

Because typical patient- and disease-associated characteristics do not explain the relatively low response rates to CAR T-cell therapy observed in certain cancers such as CLL [13,14], the exploration of other factors that might influence the clinical efficacy of these cells is critical. Indeed, ‘immunological fitness’, dictated by the baseline T-cell state would be an important determinant of functional CAR T cells. In this regard, molecules involved in antigen receptor-mediated input signaling, differentiation potential, survival, effector function and clonality should be examined at the time of T-cell collection and in the final postmanufactured CAR T-cell product to allow for a dissection of pathways leading to optimal proliferative capacity and antitumor activity.

The various stages of T-cell differentiation can be identified by flow cytometry using a defined panel of surface markers. The earliest identifiable memory T-cell stage, stem cell memory (T_{SCM}), may be the population giving rise to the successive stages: central memory (T_{CM}), effector memory (T_{EM}), terminally differentiated effector memory RA (T_{EMRA}) and effector (T_E) cells [15]. Whether the memory T-cell pool is maintained by T_{SCM} or by T_{CM} cells – or both – has yet to be defined, but both subsets have been shown to expand and differentiate into effector cells *in vivo* [15,16]. One study has clearly demonstrated that single T_{CM} cells can be serially transplanted to a new murine host a number of times and reconstitutes the T-cell repertoire [16]. In patients with B-cell malignancies who were treated with anti-CD19 CAR T cells, the magnitude of expansion of these transferred lymphocytes directly correlated with the frequency of cells with an early memory phenotype ($CD8^+CD45RA^+CCR7^+$) measured in the infused product [17]. We recently demonstrated that CLL patients who have high proportions of $CD27^+CD45RO^-CD8^+$ T cells at the premanufacturing stage of CAR T-cell generation have a high probability of responding to CTL019 therapy [14]. This is critical because patients can be selected based on a baseline differentiation phenotype before their products need to be made.

Markers & mechanisms of CAR T-cell dysfunction

Profound T-cell dysfunction and exhaustion, originally described in mouse models and human diseases of chronic, uncontrolled viral infections is now well described [18,19]. Similarly, in some cancers, T cells display deficiencies in proliferative capacity, response to homeostatic cytokines and disease control. It is becoming increasingly clear that T-cell dysfunction is regulated in part by co-expression of multiple negative checkpoint regulators (NCRs), including PD-1. Interactions of NCRs with their cognate ligands may play a role in suppressing CAR T-cell function *in vivo* through termination of proliferation and effector functions. Although the inhibitory functions of these receptors are thought to be nonredundant, it is now recognized that they may synergize with each other to cause immune cell dysfunction. In the context of biomarker assessment, we recently found high frequencies of CTL019 cells from patients not responding to this therapy co-expressing multiple NCRs, including PD-1, TIM-3 and LAG-3 in the infusion product when compared with complete responders [20]. Thus, inhibitory molecule upregulation on postmanufactured CAR T cells may not only serve as a predictive indicator of poor clinical outcomes, but also represent a tractable point of intervention with checkpoint blockade manipulation. This may be particularly relevant in the setting of solid tumors where CAR T cells must function in a strongly immunosuppressive microenvironment.

In our first-in-human trial of CAR T cells directed to the glioma tumor-specific antigen, EGFRvIII, we were able to confirm that EGFRvIII CAR T cells engraft in the peripheral blood, traffic to the brain and exert antigen-directed activity [21]. However, we observed that the inhibitory tumor microenvironment ultimately hampers clinical efficacy. Several key molecules upregulated in tumors included PD-L1, indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) [21]. Accordingly, CAR T-cell-based therapeutic approaches will also need to address how to increase both persistence and antitumor efficacy as well as how to predict robust on-target activity in the strongly suppressive tumor bed.

Biomarkers of unique CAR T-cell toxicity

Analysis of biospecimens is an integral component of clinical trials and often is the primary mechanism through which insights are obtained about the biological activity of the treatment regimen. In addition to identifying key correlates of CAR T-cell potency by examining premanufacturing T cells and the cellular infusion product, blood-based biomarkers have served as predictive indicators of toxicity in many CAR T-cell trials, including our own. The most significant and unique toxicity is cytokine release syndrome (CRS). Our group has been at the forefront of defining CRS, understanding the biology of this reaction and developing effective strategies for management [13,22–24]. The incidence and severity of CRS appear to be lower in some indications such as CLL, compared with others (e.g., ALL), despite CLL patients having very-high tumor burdens for reasons that are not well defined. CRS has been reversible with the IL-6 receptor-targeting monoclonal antibody tocilizumab with or without additional therapies. We have also seen toxicity from tumor lysis syndrome that can be both prevented and managed with careful observation and support [7]. Recent work on examining biomarkers associated with these on-target toxicities has revealed mechanisms which informed successful and life-saving interventions. For example, our predictive model of CRS, built on analyses of 51 pediatric and adult ALL patients showed that soluble factors (i.e., IFN- γ , IL1RA and soluble gp130) produced in the first 3 days following CAR T-cell infusion could be used to predict severe CRS in almost all patients [25].

Conclusion

Biomarkers play a significant role in the development of novel therapeutic agents and can provide critical insights that allow for the rational and data-driven clinical development of the candidate product. Identification of reliable predictive indicators of response to CAR T-cell therapies and associated toxicities will lead to strategies for improving the immunological health of patients as well as the development and testing of approaches that will permit most subjects to benefit from treatment. In addition, the ability to select patients most likely to respond to CAR T cells would have tremendous clinical impact, as this therapy would be applied only to patients most likely to achieve remission. This highly promising biomarker-driven concept could be extended beyond cancer to other diseases treated by adoptive T-cell transfer.

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References

1. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood* 116(7), 1035–1044 (2010).
2. Lamers CH, Sleijfer S, Vulto AG *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(13), e20–e22 (2006).
3. Kershaw MH, Westwood JA, Parker LL *et al.* A Phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin. Cancer Res.* 12(20 Pt 1), 6106–6115 (2006).
4. Levine BL, Cotte J, Small CC *et al.* Large-scale production of CD4⁺ T cells from HIV-1-infected donors after CD3/CD28 costimulation. *J. Hematother.* 7(5), 437–448 (1998).
5. Milone MC, Fish JD, Carpenito C *et al.* Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy *in vivo*. *Mol. Ther.* 17(8), 1453–1464 (2009).
6. Carpenito C, Milone MC, Hassan R *et al.* Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc. Natl Acad. Sci. USA* 106(9), 3360–3365 (2009).
7. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* 365(8), 725–733 (2011).
8. Kalos M, Levine BL, Porter DL *et al.* T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci. Transl. Med.* 3(95), 95ra73 (2011).
9. Maude SL, Frey N, Shaw PA *et al.* Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* 371(16), 1507–1517 (2014).
10. Lee DW, Kochenderfer JN, Stetler-Stevenson M *et al.* T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukemia in children and young adults: a Phase I dose-escalation trial. *Lancet* 385(9967), 517–528 (2015).
11. Maude SL, Laetsch TW, Buechner J *et al.* Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N. Engl. J. Med.* 378(5), 439–448 (2018).
12. Park JH, Riviere I, Gonen M *et al.* Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N. Engl. J. Med.* 378(5), 449–459 (2018).
13. Porter DL, Hwang WT, Frey NV *et al.* Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci. Transl. Med.* 7(303), 303ra139 (2015).
14. Fraietta JA, Lacey SF, Wilcox N *et al.* Biomarkers of response to anti-CD19 chimeric antigen receptor (CAR) T-cell therapy in patients with chronic lymphocytic leukemia. *Blood* 128(57), (2016).
15. Gattinoni L, Lugli E, Ji Y *et al.* A human memory T-cell subset with stem cell-like properties. *Nat. Med.* 17(10), 1290–1297 (2011).
16. Graef P, Buchholz Veit R, Stemberger C *et al.* Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8⁺ central memory T cells. *Immunity* 41(1), 116–126 (2014).
17. Xu Y, Zhang M, Ramos CA *et al.* Closely related T-memory stem cells correlate with *in vivo* expansion of CAR-CD19-T cells and are preserved by IL-7 and IL-15. *Blood* 123(24), 3750–3759 (2014).
18. Barber DL, Wherry EJ, Masopust D *et al.* Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439(7077), 682–687 (2006).
19. Wherry EJ, Kurachi M. Molecular and cellular insights into T-cell exhaustion. *Nat. Rev. Immunol.* 15(8), 486–499 (2015).
20. Fraietta JA, Lacey SF, Wilcox NS *et al.* Biomarkers of response to anti-CD19 chimeric antigen receptor (CAR) T-cell therapy in patients with chronic lymphocytic leukemia. *Blood* 128(22), 57 (2016).
21. 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.* 9(399), doi:10.1126/scitranslmed.aaa0984 (2017).
22. Grupp SA, Kalos M, Barrett D *et al.* Chimeric antigen receptor modified T cells for acute lymphoid leukemia. *N. Engl. J. Med.* 368(16), 1509–1518 (2013).
23. Lee DW, Gardner R, Porter DL *et al.* Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 124(2), 188–195 (2014).
24. Maude S, Frey N, Shaw P *et al.* Sustained remissions with chimeric antigen receptor T cells for leukemia. *N. Engl. J. Med.* 371, 1507–1517 (2014).
25. Teachey DT, Lacey SF, Shaw PA *et al.* Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Cancer Discov.* 6(6), 664–679 (2016).