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FOUND IN TRANSLATION

Adoptive cell therapy: Living drugs against cancer

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Immunotherapy has made a profound impact in the treatment of cancer in the last decade. Insights from the study of the intricate relationships between immune cells and cancer have led to the clinical development of strategies that redirect the power of the immune system to target and eliminate cancer. Today, immunotherapy represents a rapidly expanding and transformative force comprising multiple modalities.

Adoptive cell therapy (ACT) is a form of cellular immunotherapy that employs the use of ex vivo expanded or selected antigen-specific T cells, tumor-infiltrating lymphocytes (TILs) or genetically modified immune cells, including T cells or natural killer (NK) cells, expressing novel TCRs or synthetic chimeric antigen receptors (CARs; Park et al., 2011). The inherent antitumor cytotoxic potential of these cells coupled with enhancements through genetic engineering give rise to empowered lymphocytes capable of tumor recognition, sustained activation, and robust tumor-killing activity. Clinical trials worldwide have shown remarkable results in diseases that are highly resistant to conventional therapies, leading to Food and Drug Administration approval of three commercial CAR-based immunotherapies for treatment of CD19-expressing hematological cancers (Boyiadzis et al., 2018).

Although the benefits of ACT are undeniable, many challenges remain. The paucity of targetable antigens is a major hindrance to the progress of ACT. This is evident even among strategies for hematologic malignancies, for which CD19 still is the leading target (Ruella and June, 2016). Difficulties become greater when considering solid tumors. The scarce number of tumor-associated antigens and poor cell trafficking to the tumor site limit the success of ACT. Furthermore, the immunosuppressive pressures within the tumor microenvironment (TME) present significant barriers to the function and survival of immune cells. Attempts to overcome these issues have propelled the development of various innovative strategies. Here we discuss these novel approaches, describe recent technologies that support their implementation, and outline the progress and opportunities for expanding the reach of ACT.

From natural to engineered antitumor immunity

The early studies investigating the utility of ACT for cancer treatment focused on TILs. TILs are a group of lymphocytes that have naturally penetrated the TME and remain actively fighting the tumor. Many of these are T cells that are capable of recognizing tumor-specific neoantigens and, upon ex vivo expansion, can be infused back into patients where they mediate strong antitumor responses, resulting in tumor regression (Sim et al., 2014).

Despite the promising benefits of TIL therapy, there are important limitations. TILs are typically present at very low frequencies in tumors, and thus require extensive ex vivo expansion. Additionally, because TIL therapy is highly personalized, success rates of TIL expansion vary, and for some patients, cells do not reach the necessary numbers for therapeutic use (Sim et al., 2014).

To circumvent these limitations and harness the full potential of ACT, new strategies using peripheral lymphocytes have emerged. TCR T cell therapy is one such example, and it relies on the same concept of identifying neoantigen-specific T cells. However, unlike TILs, which require cell isolation and ex vivo expansion, TCR T cell therapy borrows from endogenous TCRs that can recognize tumor-specific antigens in the context of MHC molecules and translates this knowledge into the generation of patient-derived peripheral lymphocytes genetically modified to express synthetic versions of these TCRs (Park et al., 2011). TCR therapy offers many advantages over TILs, including greater yield of neoantigen-specific T cells that are more active and have higher proliferative potential compared with TILs, which may exhibit an exhausted phenotype due to repetitive stimulation (Presotto et al., 2017). A disadvantage to TCR therapy is that the targeted epitope–HLA complexes can be lost due to down-regulation of MHC class I and/or antigen expression, thus translating into suboptimal responses (Kasajima et al., 2010). CARs represent another development that has revolutionized the field of cell therapy. Initially applied in the context of T cells, CARs are synthetic membrane immune receptors that possess an antigen recognition domain and an intracellular signaling domain capable of inducing lymphocyte activation and costimulation (Ruella and June,

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2016). Unlike TCRs that engage peptides bound to MHC molecules, CARs bind to surface antigens on target cells in an antibody-like manner and independent of HLA type, thereby broadening therapeutic application. Additionally, CARs offer the flexibility of targeting not only proteins, but also lipids and carbohydrates, making them an even more attractive tool for ACT. This potent immunotherapy has led to unprecedented remissions in patients with relapsed and refractory hematologic malignancies and has been granted three Food and Drug Administration–approved products, the first one awarded in 2017 (Wall and Krueger, 2020). Though promising, the clinical data also reveal several challenges. CAR-T cells display a unique toxicity profile. Furthermore, not all responses are durable, with relapses occurring via two main mechanisms: loss of CAR-T cell population and/or antigen escape (Wall and Krueger, 2020). Additionally, the high costs associated with personalized T cell manufacturing and ancillary procedures associated with therapy administration may limit the large-scale feasibility of this approach.

NK cells have emerged as strong candidates that may provide an answer to some of these problems. NK cells are a heterogeneous population of immune cells with the ability to directly target and kill tumor cells through secretion of cytolytic granules and through activation of immune response via the release of immunomodulatory cytokines (Chiorean and Miller, 2001). These powerful cells express a diverse repertoire of activating and inhibitory receptors, and unlike T cells, cytotoxic function in NK cells is HLA independent, triggered when the combination of signals derived from these receptors upon engagement of cognate ligands on target cells favors activation.

Because NK cells in cancer patients are dysfunctional, adoptive transfer of potent, cytolytic NK cells from an allogeneic source such as umbilical cord blood (CB), peripheral blood, or induced pluripotent stem cells is an attractive strategy to induce relevant antitumor responses. Many approaches have shown encouraging results in preclinical and clinical studies. Work from our group led the field by demonstrating that allogeneic CB-derived NK cells coexpressing CD19CAR and IL-15 can induce rapid responses against relapsed or refractory lymphoid tumors in the clinical setting, with response rates reaching 73% in our patient cohort, and nearly all responders achieving complete remission (Liu et al., 2020). Notably, this potent response was not associated with cytokine release syndrome or neurotoxicity and did not induce graft-versus-host disease (GVHD).

Invariant NK T cells (NKT) have also been evaluated as potential sources for cell therapy. Recently, a phase 1 dose escalation trial revealed safe and effective antitumor responses in children with relapsed or refractory neuroblastoma who were treated with autologous NKT outfitted with a GD2-ganglioside–targeting CAR, thus demonstrating the increasing diversity of promising cell therapy strategies (Heczey et al., 2020).

These various studies have helped to establish ACT as a promising and feasible approach to treat cancer, but they have also revealed important obstacles. Targeting hematological cancers has shown great promise, but strategies for targeting solid tumors have been limited. Many factors may be responsible for this discrepancy, including poor lymphocyte trafficking to the tumor site, insufficient activation and persistence of adoptively transferred cells, and inability of immune cells to overcome the highly immunosuppressive TME. Attempts to mitigate these challenges have resulted in innovative approaches involving suppression of inhibitory signals, addition of cytokine costimulation for improved activation and persistence, and combination of ACT with adjuvant therapies.

New strategies: Expanding the potential of ACT

T and NK cells express a repertoire of surface receptors that can either activate or inhibit cell function upon contact with their cognate ligands on target cells. To evade immune surveillance, tumors often overexpress inhibitory ligands. PD-L1 is one such example, and upon binding its receptor, PD-1, on the surface of T cells, it induces a cascade of signals that decrease cell proliferation and block activation, leading to impaired antitumor responses (Kurtulus et al., 2019). Blocking immune checkpoints such as PD-1 and CTLA-4 with monoclonal antibodies has shown remarkable effect in potentiating the antitumor response in patients (Kurtulus et al., 2019), therefore demonstrating the relevance of checkpoint inhibition as a combinatorial strategy to ACT.

Recent approaches have employed CRISPR-Cas9 technology to engineer lymphocytes that are protected from immune checkpoint–induced inhibition. Cytokine-inducible SH2–containing (CIS) protein is a potentially relevant checkpoint molecule for NK cells, as it negatively regulates IL-15 signaling, an essential pathway for NK development and function (Delconte et al., 2016; Putz et al., 2017). The CIS protein is encoded by the *CISH* gene, and we demonstrated that CRISPR-mediated ablation of *CISH* enhanced CAR-NK function and metabolic fitness, and improved their antitumor response in a Raji lymphoma mouse model (Daher et al., 2020). Research into new immune checkpoints continues to expand. LAG-3, TIM-3, TIGIT, VISTA, and B7H3 are some examples of emerging candidates explored in the context of both T cells and NK cells (Lichtenegger et al., 2018). However, confirmation of their role as immune checkpoints warrants careful study, especially for some of these, such as TIM-3, which has been linked to both NK maturation and suppression of NK cytotoxic function (Ndhlovu et al., 2012).

There is also considerable interest in enhancing lymphocyte activation through costimulation. Targeting costimulatory pathways such as 4-1BB, OX40, CD27, and CD40 with agonist monoclonal antibodies or through engineering (via incorporation of 4-1BB signaling domain in CARs, for instance) has led to increased T cell survival, proliferation, cytokine production, and memory generation in clinical studies of CAR-T therapy (Yeku and Brentjens, 2016), thus highlighting the validity of this approach. It is important to note that some of these targets are also expressed on NK cells, and therefore these strategies should be fully explored in the context of NK cell immunotherapies.

The cytokine milieu has also been extensively investigated in the context of ACT. As key factors influencing T and NK cell biology, cytokines can be regulated to promote and enhance cell trafficking, proliferation, activation, and function. Efforts toward evaluating the contribution of cytokine modulation resulted in the generation of “armored” T and NK cells designed to overexpress relevant cytokines such as IL-15, IL-12, and IL-18, among others. This has

been achieved by various mechanisms, including addition of cytokine costimulatory receptor, and engineering cells to express membrane-tethered or secreted cytokine molecules (Yeku and Brentjens, 2016). Our group has reported compelling preclinical and clinical data demonstrating the superior performance of CAR-engineered NK cells when supported by IL-15, thus validating the utility of combining cytokine modulation with CAR-mediated tumor targeting for ACT (Liu et al., 2020). This is particularly important as we transition into ACT for solid tumors, as engineering immune cells capable of effectively functioning amid the multitude of challenges within the TME will likely require a multipronged approach.

From individualized to "off-the-shelf" therapies

The exponential growth in ACT strategies for cancer treatment points to the need for platforms that allow large-scale manufacturing of diverse cell products. Currently, CAR-T cell therapies typically require collection and modification of the patient's own (autologous) T cells. This highly individualized process is time-consuming, limits scalability, and it may not be applicable to all patients, as some are lympho-depleted and thus unable to provide sufficient cells of suitable quality for this application. Establishing well-characterized cell banks of healthy donor–derived (allogeneic) cells that are available "off-the-shelf" is an attractive approach to address these limitations and provide high-potency products at a reduced cost. Allogeneic T cells are not ideal candidates, as they have a high risk of inducing GVHD even after HLA matching. A few studies have reported that disrupting the native TCR in adoptively transferred T cells or using virus-specific T cells (not reactive to human proteins) are potential strategies to minimize GVHD (Kagoya et al., 2020). However, this requires an extra gene-editing step, further adding to the complexity of cell manufacturing. Moreover, alloimmune rejection by host lymphocytes may affect the persistence of infused cells.

Allogeneic NK cells, in contrast, are a suitable option for ACT, as they do not express polymorphic, antigen-specific receptors and do not induce GVHD (Liu et al., 2020). Moreover, donor-derived NK cells have been shown to eliminate alloreactive T cells in the transplant setting, suggesting a mechanism by which CAR NK cells may be at lower risk of being rejected (Ruggeri et al., 2002). Indeed, we observed persistence of HLA-mismatched CAR NK cells in the peripheral blood of patients for up to a year after infusion (Liu et al., 2020). Because NK cells recognize tumor cells through their native receptors, CAR-NK cells could presumably remain active against the tumor even if the CAR-specific antigen is down-regulated during tumor progression. Several sources of allogeneic NK cells have been used in the development of off-the-shelf therapies, ranging from human NK cell lines to pluripotent stem cell–derived NK cells, and have shown promising results in preclinical and clinical studies targeting hematological and solid malignancies. CB is an attractive source of NK cells as they are immediately available for use, respond well to in vitro expansion, can be transduced to efficiently express CAR and costimulatory molecules, and are capable of strong antitumor response in vitro and in vivo. In the clinical setting, CB-derived CAR-NK showed excellent safety profile, were well tolerated, and induced complete remissions in a subset of patients (Liu et al., 2020). In sum, CB offers the benefit of unlimited manufacturing potential and the possibility of developing a readily accessible cryopreserved bank of NK cells especially designed for each indication.

Technological developments to support ACT

As of 2020, cell therapies represent the largest group of agents in development for immunotherapeutic applications (Yu et al., 2020). With each emerging strategy comes the need to evaluate immune cell phenotype and function, and to monitor progress and survival. Furthermore, as we seek to identify new targets by examining tumor cells and the TME, it becomes evident that technological advancements are essential to support these demands.

Advanced mass cytometry has vastly increased our ability to profile multiple cell populations within a single sample. By interrogating >40 proteins in a single cell, we have gained insights into the heterogeneity of tumors, the plasticity of immune cells, and the alterations in function and phenotype that occur when lymphocytes encounter their targets (Fox et al., 2019).

Recently, the possibility of simultaneously interrogating protein and gene expression patterns as well as epigenetic signatures at the single-cell level has revolutionized the field. This novel multi-omics approach can be a powerful tool for understanding key biological processes and mechanisms underlying the antitumor potential of adoptively transferred cells. Further advancements in spatial transcriptomics promise to deepen our understanding by allowing the visualization of transcriptome data directly on tissue sections, providing an innovative approach to analysis of tumor heterogeneity and immune infiltration based on morphology and gene expression patterns (Maynard et al., 2020).

Concluding remarks

Immunotherapy is revolutionizing the treatment of cancer by delivering unprecedented responses in patients with poor prognoses and for whom conventional therapies offer limited benefit. Cell-based immunotherapies have emerged as powerful tools that vastly expand treatment possibilities. Recent developments in single-cell profiling allow for in-depth characterization of the intricate interactions between the immune system and cancer. Coupled with advanced gene editing techniques, this knowledge will likely translate into a new generation of custom-designed therapies specific for the unique challenges of each cancer. Indeed, the ideal approach is one that offers a safe and efficacious product that is available to patients upon need. Recent clinical data established CB-derived CAR-NK cells as a viable off-the-shelf strategy, and other approaches are currently being explored. Though still in the early stages, we look forward to a promising future for ACT with the hope that the successes seen in hematological malignancies will be realized in the treatment of solid tumors.

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References

Boyiadzis, M.M., et al. 2018. *J. Immunother. Cancer.* https://doi.org/10.1186/s40425-018-0460-5

Chiorean, E.G., and J.S. Miller. 2001. *J. Hematother. Stem Cell Res.* https://doi.org/10.1089/15258160152509073

Daher, M., et al. 2020. *Blood.* https://doi.org/10.1182/blood.2020007748

Delconte, R.B., et al. 2016. *Nat. Immunol.* https://doi.org/10.1038/ni.3470

Fox, J.J., et al. 2019. *Am. J. Clin. Exp. Urol.*

Heczey, A., et al. 2020. *Nat. Med.* https://doi.org/10.1038/s41591-020-1074-2

Kagoya, Y., et al. 2020. *Cancer Immunol. Res.* https://doi.org/10.1158/2326-6066.CIR-18-0508

Kasajima, A., et al. 2010. *Hum. Pathol.* https://doi.org/10.1016/j.humpath.2010.05.014

Kurtulus, S., et al. 2019. *Immunity.* https://doi.org/10.1016/j.immuni.2018.11.014

Lichtenegger, F.S., et al. 2018. *Front. Immunol.* https://doi.org/10.3389/fimmu.2018.00385

Liu, E., et al. 2020. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa1910607

Maynard, K.R., et al. 2020. *Neuropsychopharmacology.* https://doi.org/10.1038/s41386-019-0484-7

Ndhlovu, L.C., et al. 2012. *Blood.* https://doi.org/10.1182/blood-2011-11-392951

Park, T.S., et al. 2011. *Trends Biotechnol.* https://doi.org/10.1016/j.tibtech.2011.04.009

Presotto, D., et al. 2017. *Front. Immunol.* https://doi.org/10.3389/fimmu.2017.01564

Putz, E.M., et al. 2017. *Oncoimmunology* https://doi.org/10.1080/2162402x.2016.1267892

Ruella, M., and C.H. June. 2016. *Curr. Hematol. Malig. Rep.* https://doi.org/10.1007/s11899-016-0336-z

Ruggeri, L., et al. 2002. *Science.* https://doi.org/10.1126/science.1068440

Sim, G.C., et al. 2014. *BioDrugs.* https://doi.org/10.1007/s40259-014-0097-y

Wall, D.A., and J. Krueger. 2020. *Curr. Oncol.* https://doi.org/10.3747/co.27.5283

Yeku, O.O., and R.J. Brentjens. 2016. *Biochem. Soc. Trans.* https://doi.org/10.1042/BST20150291

Yu, J.X., et al. 2020. *Nat. Rev. Drug Discov.* https://doi.org/10.1038/d41573-020-00099-9