Introduction

As a heterogeneous, aggressive myeloid malignancy, acute myeloid leukemia (AML) derives from the myeloid cells which own the ability of renewing themselves, sustaining malignant populations and producing subclones (1). Currently, AML is the most common acute leukemia in adults, accounting for more than 80% of cases in this group, and the 5-year overall survival is as poor as approximately 27% (2). Standard treatment option for AML consists of a 7-day continuous cytarabine infusion and a 3-day anthracycline treatment, which called ‘7+3’ regimen. However, it’s often difficult for most patients, especially for the older individuals to tolerate the tradition induction therapy. The aged patients are not only comprised most accounts of the AML patients, but also are associated with high relapse rates (3).

Over the past decade, immunotherapy has been a revolution in the treatment of kinds of solid tumors and liquid malignancies (4). With a better sight into the gene molecular mechanism of human immune system, immunotherapy is providing an effective and potent option for AML treatment, aiming to induce durable responses and improve survival benefit. In the following sections, we will...
review the current immunotherapeutic strategies that are potentially suitable for immunotherapy therapy in AML.

**Monoclonal antibody (mAb) therapy**

Antibodies can identify specific antigens on AML cells and then help to destroy cancer cells. To be specific, once recognize the tumor antigen, the antibody will immediately play its role to destroy cancer cell by recruiting related immune cells, binding to matched receptor or ligand or delivering particular chemotherapeutic agents to the tumor cells (5). Scientists have generated long-standing interest in applying antibody to improving the outcomes of AML patients.

Over the past few years, effort to improve the efficacy of antibody therapy has concentrated on the novel target antigen and on the exploration of new treatment strategies such as antibody-drug conjugates (ADCs). There are a series of specific antigens including CD33, CD123, CD25, CD27, CD38, CD44, CD47 express on AML cells, which have been designed for clinical applications (6).

Hematopoietic cells express surface antigens that are critical for normal immune responses and immunotherapies. Since 1973, attempts to develop antigen-specific immunotherapy to fight with tumor cells lead to the generation of mAbs, presenting as a cornerstone for the feasibility of mAb technology (7). These monoclonal antibodies play their role by activating antibody-dependent cellular toxicity (ADCC), stimulating complement-dependent cytotoxicity, inhibiting signal transduction, or directly inducing apoptosis (8). Although there are still no mAbs demonstrating enough efficacy to be listed as standard treatment, their potency and functionality with specific targeting have been widely confirmed. Overall, antigen-targeted mAb immunotherapy is regarded as a dependable novel way to improve the outcomes of AML patients.

**Anti-CD33 antibodies**

Myeloid differentiation antigen CD33 has served as the most exploited target for AML many years due to its expression on more than one leukemic blasts in most patients (9). Currently, studies to target CD33 therapeutically have focused on gemtuzumab ozogamicin (GO) and SGN-CD33A (vadastuximab talirine).

ADC is a treatment strategy which links the effector drug to specific antibody, thus providing possibility to exploit toxic molecules (10). Go is a CD33-target ADC combining calicheamicin-γ1 with a recombinant humanized antibody (IgG4) through hydrolyzable linker.

Engagement of CD33 by GO leads to immunoconjugate internalization and release of the DNA-damaging moeity in the acidic lysosomes, resulting in cell death by caspase activation and mitochondrial pathways (11). Compared to other clinical antitumor drugs, calicheamicin-γ1 is highly toxic. So, it is essential to systematically manage the selectivity of AML blasts and avoid overt toxicities when using this drug. One main adverse effects of GO are the increased hematologic and hepatic toxicity, such as the high risk of veno-occlusive disease (VOD), especially when used before allogeneic hematopoietic stem cell transplantation (HSCT) (12). On the basis of encouraging Phase II results in aged patients with relapse AML, GO was granted accelerated approval by the US FDA as the recommend treatment of CD33+ AML (13). Unfortunately, the preliminary data from a Phase III trial demonstrated an intolerable rate of fatal adverse effect and a dissatisfactory improvement in prognosis, eventually leading withdraw the drug from the US market in 2010 (14). Notwithstanding, as further studies and clinical trials are carrying on, GO’s potential when combined with other drugs is unveiled. In 2017, GO returned to the US market with a more reasonable instruction, which balances both the benefit of the risk brought by GO. It was again approved by FDA as a monotherapy for newly diagnosed CD33+ AML patients (15). However, there are still quite large a proportion of patients with CD33+ AML not benefit from GO, indicating the necessity of improving the results of CD33-target therapy.

Compared to GO, SGN-CD33A, a humanized anti-CD33 antibody, is a novel ADC successfully put advancements of conjugation and linker technology into use. In preclinical AML models, SGN-CD33A using a highly potent, synthetic DNA cross-linking pyrrolobenzodiazepine dime has demonstrated more potent than GO against tumor cell lines in vitro and xenotransplantation in vivo (16). A phase I clinical trial enrolled 131 patients evaluated the safety, pharmacokinetiics, and preliminary activity of SGN-CD33A and determined a dose of 40 μg/kg as the recommended monotherapy dose for activity and toxicity consideration (17). While the antitumor efficacy of SGN-CD33A is currently well-established, the non-hematologic adverse events caused by this drug including fatigue, nausea, and diarrhea, remain major concerns and hinder its development. In 2016, FDA decided to place a hold or partial hold on several of the trials with SGN-CD33A to further evaluate its potential risk of hepatotoxicity when
used prior or post to allogeneic HSCT. Finally, patient enrollment and treatment in all ongoing SGN-CD33A trials was suspended and the CASCADE trial permanently discontinued due to an intolerable death rate, including fatal infections, in the SGN-CD33A-containing arm of the CASCADE trial (18).

**Anti-CD123 antibodies**

The interleukin 3 (IL-3) receptor α-chain (CD123) is another attractive target-antigen which is wide displayed on AML blasts (19). Studied have reported that elevated number of CD123+ AML cell is associated with a lower CR rate after chemotherapy, contributing to the possibility of CD123 as a immunotherapy target (20).

Researchers have developed SGN-CD123A with high anti-tumor potent. Similar to SGN-CD33, the ADC drug SGN-CD123A is composed of a humanized CD123 antibody with engineered cysteines to help site-specific conjugation. It carries a pyrrolobenzodiazepine dimer (PBD) payload via dipeptide linker (21). Data from preclinical studies have showed promising anti-AML activity of SGN-CD123 with swift internalization of the antibody.

A phase 1 trial (NCT02848248) has tried to evaluate the safety and antileukemia effectivity of this drug, but this trial has been suspended early and results have not yet been publicly reported. Initial clinical products using unconjugated CD123 antibodies is CSL360, a recombinant, chimeric IgG1, anti-CD123 mAb. CSL360 bounds CD123 specifically and neutralizes IL-3, demonstrating anti-leukemic activity *in vitro*. But a clinical study in which only 1 patient achieved CR among 26, indicating CSL360 insufficient as a therapeutic strategy against relapsed/refractory AML (22).

More later efforts have focused on enhanced effector function molecules. CSL362 is another fully humanized, anti-CD123 mAb with an increased affinity for CD16 to enhance antibody-dependent cell-mediated cytolysis (ADCC) (23). For AML patients with adequate NK cell function, this drug provides a novel immunotherapy option and warrants the clinical development of CSL362 for the treatment of AML. In a phase 1 clinical trial of CSL362, 25 patients with CD123+ AML were involved. These participants were in CR but at high risk for early relapse. After using CSL362, among 20 patients evaluable for a response, 10 maintained CR with a median duration of more than 34 weeks from the start of CR, and CR was still present at the last follow-up. The drug demonstrated potent anti-tumor efficacy and help maintain CR (24). However, similar to CSL360, single agent efficacy of CSL362 is also modest. Consequently, a randomized study (NCT02472145) has been designed to evaluate the addition of CSL362 to decitabine in adults with AML qualified for intensive chemotherapy.

**Adoptive T cell therapy**

T cell adoptive immunotherapy has shown promising potential in improving multiple cancer overall survival rate. Adoptive T cell therapy embraces chimeric antigen receptor T cell (CAR-T) and TCR-T, both of which can enhance T cells’ ability to recognize and attack targeted tumor cell antigens by genetic engineering technologies. Recently, CAR-T therapy has gotten the approvals for treatment of pediatric acute lymphocytic leukemia and aggressive B-cell lymphoma in the USA, standing at the center of the promising adoptive T cell therapies and become a typical cases indicating the transition from classic immunology to synthetic cell therapy (25). In brief, CAR-T cells are defined as autologous T cells, which are genetically engineered in the laboratory thus acquire the ability to attack cancer cells (26).

A CAR consists of three domains, (I) an extracellular antigen-specific antigen-recognition domain linked to costimulatory molecules, which is derived from an antibody’s single chain variable fragment (scFv), (II) a hinge and transmembrane domain derived from CD8-α or IgG segment, an intracellular T-cell signaling domain (27). By manipulating gene expression, CAR-T cells are relocated and targeted to specific chosen antigen by their CARs, and their effector and metabolic functions will be reprogrammed eventually.

To date, more and more inspiring results on clinical efficacy has been reported. Accumulating data in B-lineage acute lymphoblastic leukemia by CD19-targeted CAR T cells, which has demonstrated response rates up to 90% (28), is suggesting that CAR-T may be a potential effective player in its use in AML (29). Following approval by the FDA, Kymriah and Yescarta, which are based on the CD19 CAR-T, have also successfully gained approval by the European Medicines Agency (EMA) for their use in the European Union (30). In the meantime, more and more clinical trials are ongoing to ensure the safety and effect of this treatment. After being treated with CD33-directed CAR T cells, a patient who had a transient decrease in marrow blasts has
gotten a successful improvement (31). Besides, there is another report demonstrating that CD19-targeted CAR T cell induced high remission rates and durable remissions in children and adults with B lymphoblastic leukemia, attesting to the potency of CART strategy (32).

One main hurdle with the development of effective broad-spectrum CART for AML is the biological heterogeneity of this myeloid malignancy based on the different myeloid progenitors from which it arises. Multiple developing targets for directed CART therapy in AML include CD123, CD33, FRβ, CLL1 or CLEC12A, FLT3, B7H6, NKG2D and Lewis Y (LeY) (33).

Although great interest has generated in applying this technology to clinical AML treatment, the lack of ideal target that is broadly expressed in AML yet dispensable has created great challenges (34). Generally, proper target antigens used for CAR-T therapy should be commonly expressed by tumor cells of most AML patients, while absent or low in their counterparts, healthy tissues, and immune cells (35). The main barrier currently preventing the clinical use of CAR-T is a lack of “expendable” antigen like CD19, which is the only known surface target unique to AML cell up to now. Other myeloid antigens on leukemic blasts are always shared by normal hematopoietic stem and progenitor cells. Once set these improper antigens as targets, prolonged myeloablation will happen, which is intolerable clinically (36). In addition, the adverse effects, such as on-target off-tumor toxicity and cytokines release syndrome, are emerging as another hurdle that obviously hampered the transition of CAR-T therapy from bench to bed. A report presented data illustrating the value of CAR-T therapy for AML still needs more time and effort to be determined. A patient with relapsed/refractory AML received CD33-targeted therapy, he experienced a dramatic cytokine release syndrome and a decrease in blasts 14 days after treatment, but then progression at 9 weeks (37).

Current efforts are dedicated to overcoming difficulties such as finding the suitable antigen to minimize “off-tumor” toxicity. At the same time, multicenter Phase II trials are carrying out to confirm the clinical efficacy and manage unknown toxicities. Some other attempts such as suicidal control of CAR-T cells, temporary expression of the CARs, and improvement of the affinity of the CARs, have also been made to (38).

Overall, CAR-T therapy has the potential to be a powerful weapon against AML in the near future, which still require explore to make full understanding of it.

**Immune checkpoint inhibitors (ICIs)**

The regulation of immune homeostasis relies on the balance between the stimulatory and inhibitory signals expression that mediate the T-cell activation, in which immune checkpoints (ICPs) play a significant role (39). Based on evidences observed in preclinical mouse model, ICP inhibition to treat late relapse after transplantation seems a rational attempt. Data from early ongoing clinical trials has also shown promising results of PD-1 inhibitor nivolumab with HMAs and single CTLA-4 inhibitor ipilimumab (40). At this moment, ICPs inhibition therapy is no doubt a key breakthrough in the immunotherapy of AML, following its success in significantly improving the overall survival rate of solid tumor patients.

**Anti-PD-1/PD-L1**

PD-1 is a co-inhibitory molecule expressed on immune cell including T cell, B cells, and myeloid cells. In antitumor immune response, binding of PD-1 to its ligand PD-L1 on tumor cells leads to downregulation of proliferation and immune response of T cell, which is called the “exhaustion” state of T cell. Activation of PD-1/PD-L1 signal pathway serves as a major mechanism of immune evasion by tumor cells (41). More and more studies have shown a higher expression of PD-L1 in tumor cells of some AML patients, which is also closely related to disease relapse rate (42). Currently, there are several ongoing clinical trials on PD-1/ PD-L1 inhibitors, including pidilizumab, nivolumab, pembrolizumab, durvalumab, and atezolizumab.

Nivolumab is used for treatment for metastatic melanoma, squamous non-small cell lung cancer as well as renal cell carcinoma (43). As a human IgG4 anti-PD-1 mAb, it got the approval from FDA for the treatment for relapsed or progressed patients with classical Hodgkin’s lymphoma after stem cell transplantation.

Pembrolizumab is another PD-1 blockade drug, which was first approved by FDA in treating metastatic melanoma and then unresectable or metastatic solid tumor with specific genetic anomalies (44). Pidilizumab is a humanized IgG1 mAb which interacts with PD-1 to activate antitumor immune response of T cell. Instead of single agent approaches, scientists tend to find out novel therapeutic combinations of ICIs with other drugs to achieve better clinical efficacy. For instance, epigenetic drugs could regulate the expression of PD-1 molecules on tumor-infiltrate lymphocytes and tumor cells, thus modulating the
methylation state of PD-1 genes to enhance T cell function may be a promising treatment direction (45).

Anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4)

CTLA-4 is a CD28 homologue expressed on the surface of T lymphocytes with higher affinity for CD80/CD86. When CTLA-4 competitively binds to CD80/CD86, signal required for T cell activation reduces, which eventually leads to T cell anergy rather than stimulation, or activation (46). In AML murine models, preclinical studies have described that longstanding leukemic cells were generally more resistant to lysis by tumor-specific cytotoxic T cells and showed higher expression of PD-1 and CTLA-4/CD80. Blockade of CTLA-4/CD80 interaction could enhance the activity and quantity of tumor-reactive T cells in vitro and prolonged murine survival time in vivo (47).

Currently, FDA has approved ipilimumab, a human lgG1 mAb, for treating melanoma. It works by antagonizes CTLA-4 and has also been explored in solid tumors, such as lung cancer and bladder cancer. Encouraging results were noted in a clinical trial evaluating ipilimumab enrolled 29 patients with recurrent or progressive hematological malignancies. All participants underwent allogeneic HSCT, but relapsed more than 3 months after last transplantation (48).

They received ipilimumab as single infusion at dose cohorts between 0.1 and 3 mg/kg. Exacerbate clinical graft-versus-host disease (GVHD) wasn’t observed in this study, but organ-specific immune adverse events (IAEs) and malignancy regression were obvious induced by ipilimumab. Organ-specific IAEs were seen in four patients, including arthritis, hyperthyroidism and pneumonitis. These outcomes were very promising in patients with post-allo-SCT relapsed AML, indicating it a valuable direction to explore in the near future.

Vaccines

Vaccines against AML are designed to actively boost the immunity to recognize and destroy AML cells via the introduction of tumor-associated antigens (TAAs) (49). The current researches of AML vaccination are based on the specific identification of TAA and increasing understanding of antigen presentation by dendritic cells (DCs). Active immune system function through vaccination has been explored broadly, especially for patients who are very likely to relapse after traditional therapy with or without HSCT. So far, the main types of AML-target vaccines are peptide vaccines and DC vaccines.

Peptide vaccines

Peptide vaccination has focused on the TAA Wilm’s tumor 1 (WT1), a zinc finger antigen overexpressed in AML cell and particularly in leukemic blasts. WT1 can induce cytotoxic T lymphocytes (CTLs) to destroy WT1-expressing AML cells, demonstrating to be the best choice in peptide vaccine preparation (50). In a Phase II trial, a more antigenic peptide was created through modifying the TCR binding region of the HLA A2-restricted WT1 peptide epitope 126. Twenty-two high-risk leukemia patients in remission received the treatment. The final result indicated that the multivalent WT1 vaccine was well tolerated, stimulated a specific immune response, and was associated with 5-year-survival rate in this cohort of participants (51).

DC vaccines

As the most powerful antigen-presenting cell population, DCs play an essential role in inducing antigen-specific immune response in vivo, thus becoming the ideal cell type for vaccination purpose (52). This is also why scientists has generated keen interest in the use of DC vaccine immunotherapy since the mid-1990s (53). These specific DCs usually come from patient’s monocytes or leukemic cell lines. Host-derived monocytes need to undergo differentiation ex-vivo through transduction of TAA mRNA, tumor lysate or AML blast fusion (54). WT1 mRNA transduction method has proved to induce immense molecular responses, presenting as an optimal treatment option for patients in partial remission (50). DC grown from AML cell lines has been used in some clinical trials, which demonstrate dependable safety safe but show limited antitumor effect in a small part of patients (55). In a recent trial, DCs transduced with RNA encoding the TAA telomerase hTERT were used to treat 19 high-risk patients with AML in CR. They encouraging result that hTERT-DCs can be well tolerated in most patients, 11 patients (58%) were free of relapse during follow-up visit, indicating hTERT-DCs to be safe and associated with recurrence-free survival (56). In spite of the therapeutic excitement of DC-tumor vaccines against AML, further refinements in vaccine strategies are clearly needed to develop this promising area of investigation into a clinically meaningful therapy.
Conclusions

In the past decades, our increasing understanding about human immune system has led to paradigm shifts in the clinical management of solid and hematologic malignancies. More and more studies in immunotherapy have progressed and show encouraging potential to become a therapeutic option of AML. One critical fact that has surfaced is that AML is a heterogeneous and aggressive malignancy, so it’s hardly to handle it with any single agent. Based on the fact that many novel treatment strategies are under development, there is no doubt that more potent target therapies will be soon available for the clinical treatment of AML. Increasing data from preclinical and clinical trials has demonstrated the greatly enhanced antitumor efficacy when combine different immune approaches with chemoradiotherapy, which indicating the promising future of immunotherapies. Over the next decade, more improved efforts will be devoted to applying immunotherapeutic agents to clinical use. Besides, personalized immunotherapy approaches combined with traditional chemoradiotherapy hold promise to become the gold standard of AML treatment. We envisage that such combine strategies would provide a safe and effective option for patients with AML in the near future.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob-19-67). The authors have no conflicts of interest to declare.

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