Immunotherapy in Pediatric AML

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Immunotherapy may be an attractive treatment option to increase survival, and to reduce treatment-related side effects, for children with acute myeloid leukemia (AML). While immunotherapies have shown successes in many cancer types, the development and subsequent clinical implementation have proven difficult in pediatric AML. To expedite the development of immunotherapy, it will be crucial to understand which pediatric AML patients are likely to respond to immunotherapies. Emerging research in solid malignancies has shown that the number and phenotype of immune cells in the tumor microenvironment is predictive of response to several types of immunotherapies. Such a predictive model may also be applicable for AML and, thus, knowledge on the immune cells infiltrating the bone marrow environment is needed. Here, we discuss the current state of knowledge on these infiltrating immune cells in pediatric AML, as well as ongoing immunotherapy trials, and provide suggestions concerning the way forward.

Keywords: Immunotherapy ; Pediatric Acute Myeloid Leukemia

1. Immunotherapy and AML

Acute myeloid leukemia (AML) is a heterogeneous blood cancer characterized by both aberrant proliferation and arrested differentiation of immature myeloid cells in the bone marrow [1]. Due to intensified chemotherapy, risk-adapted treatment, improvement in allogeneic stem cell transplantation (allo-SCT) and optimized supportive care, survival of pediatric AML has greatly improved over the last decades [2]. Nevertheless, 20–30% of children with AML do not survive as a result of significant treatment-related toxicity and death due to relapse [3]. Furthermore, survivors often experience serious side effects and late effects due to the treatment [3]. Therefore, alternative treatment options that further improve outcome and reduce treatment-related side effects are required.

To date, therapeutic options that make use of T-cell-mediated effects to eliminate residual leukemic cells, such as allo-SCT, have shown to evoke anti-AML immunity and support the use of immunotherapy in pediatric AML [4][5][6][7]. However, allo-SCT is associated with major side effects such as chemotherapy- or irradiation-related toxicities and graft-versus-host-disease [8]. Hence, less toxic immunotherapy options that enhance anti-leukemic immune surveillance without these long-term sequelae are highly needed for this disease. Encouraged by the initial successes of immunotherapy in acute lymphoblastic leukemia (ALL) and various solid cancers, relatively new immunotherapeutic options are now available or under development for AML: immune checkpoint inhibitors (ICIs), unconjugated and bispecific antibodies, adoptive cell therapy, cytokines and other immune-modulating soluble factors, vaccines, and oncolytic viruses [6][7][9]. Antibody-drug conjugates such as gemtuzumab-ozogamicin are not considered as immunotherapy in this overview, as their main action is targeted delivery of cytotoxic agents to tumor cells.

2. Immune Cells in the TME of AML at Diagnosis

Since the prognostic and predictive potential of immune cells in the bone marrow of AML is increasingly recognized, we summarized the available information on immune cell abundance and phenotype in the TME for children with AML at diagnosis. To date, few studies have examined these parameters on a protein level. However, recent microarray- and RNA-sequencing based studies have provided valuable information [10][11]. These studies employed gene scores that either reflected the abundance of multiple immune cell types or focused specifically on T- and NK-cell abundance. An extensive study by Dufva and colleagues that used a 5-gene “cytolytic” score specific for T- and NK-cell abundance showed that children with AML (n = 273) had a median combined proportion of T- and NK-cells of nearly 25% out of all living cells in the bone marrow at diagnosis [11]. Of interest, the T- and NK-cell abundance in children did not differ from adults with AML (n = 1585) in the same study [11]. Similar results were observed in a study with 34 children and 334 adults with AML that used RNA-sequencing to estimate the abundance of multiple immune cells including antigen-presenting cells, T, B-, and NK-cells [10]. Only adults with AML ≥ 60 years (n = 102) showed a slightly higher immune cell abundance in comparison to children with AML [10]. This may be related to the increased prevalence of myelodysplastic syndrome-

related AML in older adults as this subtype is associated with relatively high levels of immune infiltration in the bone marrow [11][12].

In contrast, a small study that used flow cytometry in children with AML (*n* = 28) revealed a significantly lower fraction of T-cells out of all mononuclear cells in the bone marrow (4%) [13]. Phenotypically, both CD4+ and CD8+ T-cells expressed higher levels of the inhibitory checkpoints LAG3 and PD-1, in comparison to healthy donors [14]. These checkpoints have been associated with exhaustion and reduced functional capacity of T-cells [15]. However, T-cells with expression of LAG3 and PD-1 still retained modest capacity for cytokine production, indicating that they were not fully exhausted [14]. Comparably, despite significant declines in granzyme, CD16, CD57, and NKG2D, NK-cells in children with AML still showed functional activity [14].

In addition, clonotype diversity of T- and BCRs can inform whether patients can generate an effective antigen-specific anti-tumor immune response, for instance after immunotherapy [15]. High levels of T- and B-cell clonal expansion indicate activation of these cells and have been associated with improved responses to immunotherapy [16]. However, a growing body of evidence suggests that many T-cells in the TME are simply ‘observers’ that are incapable of recognizing and eliminating tumor cells and accordingly present low levels of clonal expansion [17]. In a study focused on T-cell receptor (TCR) and B-cell receptor (BCR) analysis of diagnostic bone marrow transcriptomes in both adult (*n* = 151) and pediatric AML (including infant AML, up to 1 year of age; *n* = 145 in total), T-cells in infant AML presented relatively low levels of clonal expansion in comparison to pediatric and adult AML [18]. It was suggested that this might be due to limited bacterial and viral antigen exposure prior to therapy. Notably, there were no differences in clonal expansion between pediatric and adult AML. However, adult AML samples in this study had significantly more secondary immunoglobulin class switch events than pediatric AML samples. These results indicate higher levels of B-cell activation in adult AML in comparison to pediatric AML.

Taken together, microarray- and RNA-sequencing based studies have provided valuable insights into the immune cell abundance in the bone marrow of children with AML. Apart from adults with AML ≥ 60 years, children with AML appeared to have similar levels of T- and NK-cell abundance in the bone marrow at diagnosis in comparison to adults with AML. Moreover, the high proportions of T- and NK-cells in the bone marrow of children with AML in these studies look promising and anticipate that children with AML may benefit from immunotherapy in the future. Studies that evaluated the immune cell abundance and phenotype on the protein level at diagnosis are scarce in children with AML. Although the microarray- and RNA-sequencing based methods in the discussed studies have been validated and were found to robustly estimate immune cell levels as measured by protein-based assays such as flow cytometry, functional studies are needed to deepen our understanding of the phenotype of immune cells in the bone marrow in children with AML.

3. Immune Cells in the TME in Relapsed and Refractory Disease

Unfortunately, the abundance and phenotype of immune cells in the TME of relapsed and refractory pediatric AML has not been elucidated yet. In addition, no trials on immunotherapy drugs for relapsed and refractory pediatric AML have been published to date. For adults, most data on the immune cell abundance in relapsed and refractory AML stem from the studies that measured these parameters before treatment in immunotherapy trials [10][19][20]. For instance, the study that evaluated nivolumab in combination with azacytidine in relapsed and refractory adult AML showed that responders had a higher frequency of pretherapy T-cells out of all living cells in the bone marrow as measured by flow cytometry in comparison to non-responders (32.5% vs. 17.5%) [20]. Furthermore, data on the phenotype of immune cells in the TME in the relapse setting suggested that cytotoxic T-cells fail to restrain leukemia growth [10]. For instance, one study that employed RNA-sequencing reported that in comparison to the diagnostic setting, cytotoxic T-cells showed increased markers of terminal differentiation, senescence, and exhaustion at relapse [10]. Data from another study confirmed that cytotoxic T-cells showed upregulation of exhaustion markers at relapse in comparison to healthy donors, but this was also seen at diagnosis [21]. Moreover, cytotoxic T-cells in adult AML showed wide signs of impairment and exhaustion at relapse after allo-SCT [22]. Since most immunotherapy trials for AML test immunotherapeutic strategies in relapsed and refractory patients, it is key to unravel these parameters to improve the use of immunotherapies in future clinical trials.

4. Immune Cells in the TME during and after Therapy

Over the years, the use of immunotherapy during and after conventional therapy has gained interest in the field of AML due to the relatively low leukemic burden in these settings [23]. Accordingly, relatively low numbers of blasts in the bone marrow might lead to a reduction in immunosuppressive signals and more space and nutrition for immune cells [24]. Indeed, one study revealed that the RNA-sequencing based estimates of immune cell abundance in the adult AML TME were inversely associated with the leukemic burden at diagnosis [10]. Furthermore, the RNA-sequencing based estimated

immune cell abundance was significantly higher in adult AML patients in complete remission versus diagnosis [10]. Therefore, special attention has been dedicated to the potential use of immunotherapy for the eradication of minimal residual disease (MRD) in AML and other hematological cancers. For instance, in adults with B-cell precursor ALL, out of 21 MRD-positive patients, MRD conversion from positive to negative was achieved in 80% of patients after one cycle of treatment with the bispecific antibody blinatumomab [25]. Consequently, blinatumomab has been approved for the treatment of MRD in B-cell precursor ALL [25][26]. For AML, this study in combination with several other preclinical studies have added interest in the eradication of MRD with immunotherapy [27][28]. For instance, in a mouse model of AML with MRD-positivity, blocking of the immune checkpoint axis with ICIs resulted in prolonged survival in comparison to no treatment [27]. Furthermore, preliminary results from an ongoing phase II study that evaluates the anti-PD1 ICI nivolumab in adult AML patients in complete remission, showed encouraging results [28]. In particular, 71% of patients were in continuing complete remission at 12 months after treatment (ClinicalTrials.gov Identifier: NCT02532231) despite their high risk of relapse as indicated by persistent MRD or adverse prognostic factors [28].

To date, few clinical trials have assessed the role of immunotherapy as maintenance therapy for children with AML [29][30][31]. The randomized ELAM02 phase III trial evaluated whether the use of interleukin-2 after consolidation therapy would improve disease-free survival for newly diagnosed children with AML. Unfortunately, no differences in disease-free and overall survival were observed between the intervention and control arm [29]. Similarly, two phase II clinical trials reported no improvements in disease-free and overall survival with expanded NK-cell infusions after consolidation therapy for newly diagnosed pediatric AML [30][31]. Although these initial trials did not show a clinical benefit of immunotherapeutic maintenance therapy in pediatric AML, this does not preclude the potential usefulness of (combinations of) recently developed immunotherapeutic agents as consolidation therapies. Furthermore, changes in immune cell abundance and phenotype in the TME during or after therapy in pediatric AML have not been studied. Therefore, it is currently unknown how current treatment protocols affect the TME in pediatric AML and thus, which treatment time points are particularly suitable for immunotherapy intervention.

For adult AML, these data are also scarce. As mentioned above, one study revealed that the RNA-sequencing based immune cell abundance was significantly higher in complete remission in comparison to diagnosis in adults with AML (n = 22) [10]. Furthermore, CTLA4 expression was upregulated, while CD244 coinhibitory molecule was downregulated, which suggests T-cell activation after induction therapy [10]. Another study in a larger adult cohort (n = 72) reported equal results in terms of T-cell activation after induction therapy in responders, while non-responders showed relatively high levels of dysfunction in comparison to the pretreatment setting [32]. However, while some chemotherapeutic agents may indeed activate antitumor immune responses, these agents might concomitantly induce tolerogenic and immunosuppressive pathways [33]. For example, early lymphocyte recovery in 20 adult patients undergoing induction chemotherapy for newly diagnosed AML indicated that recovering T-cells in the peripheral blood were predominantly activated Tregs with suppressive activity [34][35][36].

Collectively, the use of immunotherapy during and/or after conventional treatment has the potential to support the eradication of MRD and consequently prevent relapsed disease. However, data on the immune cell abundance and phenotype is limited for both adult and pediatric AML. Since studies across a spectrum of cancers have observed plasticity of immune cell numbers and their phenotypes before- and after conventional treatment, delineating changes in bone marrow immune cell abundance and phenotype for pediatric AML will likely be important for the selection of suitable treatment time points for immunotherapy intervention [37][38][39][40][41].

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