Acute myeloid leukemia - Section 2

Current status of immunotherapy in acute myeloid leukemia

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Take Home Messages
- So far, monotherapy with Checkpoint Inhibitors has not shown any clinical benefit in r/r AML patients; in contrast, early clinical trials have demonstrated a clinical benefit for combination therapy with HMA.
- The majority of antibody and CAR T cell-based immunotherapy in acute leukaemia is targeting lineage antigens which are not leukaemia specific.
- Clinical trials utilizing bispecific T cell recruiting antibodies as well as CAR T cells are ongoing and first clinical results are expected Q4 2018 to Q3 2019.

Allogeneic hematopoietic stem cell transplantation (HSCT) and donor lymphocyte infusion for acute myeloid leukaemia (AML) can induce graft-versus-leukaemia immunity and long-term survival. Transplant-related morbidity and mortality oppose the advantage of the T cell mediated anti-leukemic immune response. In addition, age and comorbidity prevent the widespread use of HSCT in the generally elderly AML patient population. Alternative immunotherapeutic strategies seek to induce more specific anti-leukemic immune responses for patients not eligible for HSCT. The aim of immunotherapeutic intervention is increasing response rates to chemotherapy, converting MRD, bridging to HSCT and treating refractory or relapsed patients. Here, we review recent advances in immunotherapy of AML and introduce six immunotherapeutic platforms currently employed in clinical trials: i) immune checkpoint inhibitors, ii) therapeutic vaccination using dendritic cells (DCs), iii) antibody-drug conjugates, iv) T-cell recruiting antibody constructs, v) TCR-transgenic T cells and vi) chimeric-antigen-receptor T cells (CAR T cells). The usage of immunotherapy in AML is hampered by the challenge of identifying suitable intracellular and surface target antigens (Figure 1).

Current status

Checkpoint inhibitors
Monoclonal antibodies against checkpoint molecules block inhibitory signals like PD1 on T cells or PD-L1 on malignant cells, allowing for release of the “brakes” of anti-leukemic T cells. These agents have shown efficacy in several haematological (e.g. Hodgkin’s Disease) and oncological neoplasia (e.g. malignant melanoma, lung cancer). While the available data on PD-L1 expression in AML is equivocal, PD-1 seems to be up-regulated on T-cells in relapsed and to some extent in newly diagnosed AML compared to healthy donors.¹ Furthermore, hypomethylating agents (HMA) may induce the expression of PD-1 and PD-L1 in T-cells and AML cells, respectively. In agreement with these observations, monotherapy with checkpoint inhibitors have failed to demonstrate a meaningful benefit for AML patients whereas combination therapy with HMA have generated promising clinical results.¹,² Daver et al. evaluated 70 patients treated with HMA 5-azacitidine with anti-PD-1 antibody nivolumab. The overall response rate was 35%, including 21% CR/CRi that mostly lasted longer than one year without stem cell transplant. An additional 10% of patients had stable disease maintained >1 year on this combination. Currently >15 clinical trials are evaluating the combination of one or two checkpoint inhibitors with HMA in AML and MDS.³ This combination may be further supported by recent translational research that found HMAs to re-induce expression of silenced endogenous retroviruses that provoke immune responses. In addition, other combinatorial partner including Dendritic cell vaccination are being tested in clinical trials.

Dendritic cell vaccination
Vaccination strategies have the purpose to prime new or enhance pre-existing antigen-specific immune responses. Dendritic cells are professional antigen-presenting cells (APCs) and most suitable to induce strong and durable CD4 and CD8 T-cell responses. This is of particular importance for the treatment of tumour entities with low endogenous immune responses, such as AML. Published and ongoing clinical trials on therapeutic vaccination utilizing DCs vary in source of DC precursors, DC maturation protocol, target antigen, way of antigen loading route of application and interval of application. The largest phase III study is being conducted by a Belgium group utilizing WT1 mRNA-
loaded DCs for patients with AML, CML and MM. Results of 30 patients with AML at very high risk of relapse have been published demonstrating antileukemic response in 13 patients.\(^4\) Recently, another clinical trial has been published presenting 17 AML patients that were vaccinated in CR with a hybridoma of AML cells and autologous DCs.\(^5\) Significant anti-leukemic T-cell responses were induced and high relapse-free survival in this selected patient population described. Interestingly, this patient cohort is part of a larger study that is designated to analyse the combinatorial effect of PD-1 blockade with the described vaccination strategy. We have finished recruiting a phase I/II proof-of-concept study using an optimized, TLR 7/8 based maturation cocktail for generation of monocyte-derived DCs loaded with mRNA encoding WT1 and PRAME for intradermal vaccination of AML patients in CR with a non-favourable risk profile.\(^6\)

**Target antigens**

In relapsed B cell acute lymphoblastic leukaemia (B-ALL), targeting of the B-cell lineage antigen CD19 via bispecific T-cell-engaging antibody constructs (blinatumomab) and chimeric antigen receptor (CAR) T cell products has shown remarkable anti-leukemic effects and a tolerable safety profile. However, myeloid-associated lineage antigens like CD33, CD123, CLL-1 and FLT3 are also expressed within the healthy myeloid compartment and accordingly higher rates of on-target-off-leukaemia toxicity have been observed in early clinical trials. The challenge of antibody-based and CAR T cell-based immunotherapy in AML is the identification of an AML-specific surface antigen. In addition, genetic and phenotypic heterogeneity contribute to the difficulties of identifying “the CD19” of AML. The same applies for the selection of intracellular,
leukaemia-associated target antigens which are processed and their peptides presented on human leukocyte antigens (HLA) as peptide-MHC complexes on the cell surface. The intracellular antigen WT1 is the most prominent target antigen for therapeutic vaccination and TCR-transgenic T cells. Clearly, novel immunotherapeutic strategies need to target leukemic stem cells (LSCs) to impact long-term outcome in AML. It has to be considered, that choosing a suitable target antigen also depends on the applied therapeutic platform and class of effector cells. Splicing polymorphisms and affinity of the single chain variable fragment of the construct will further determine the success of the immunotherapy platform.\textsuperscript{7} AML surface data have expanded our knowledge on less known AML-associated target antigens and need to be explored further. Combinatorial targeting might increase specificity, but also the risk of immune escape variants.\textsuperscript{8}

**Antibodies**

In ALL, several antibody-based approaches have already entered standard treatment. Rituximab, an anti-CD20 directed antibody has been shown to be beneficial as an additive to conventional chemotherapeutic agents. Inotuzumab ozogamicin is a toxin-conjugated monoclonal antibody (ADC) directed against CD22 on the surface of B cells and together with blinatumomab, a bispecific T cell – recruiting antibody directed against CD19, has been approved for t/r ALL. In principle, all these treatment modalities can be translated to AML.

CD33 (SIGLEC-3) is the most commonly targeted antigen in AML. So far, no conventional antibody has achieved approval for the treatment of AML. Several clinical trials are currently running evaluating monoclonal antibodies against several AML-associated target antigens as mono – or combination therapy. Target antigens include CD157, CD47, CD123, CD135 and CLL1. The first and most prominent ADC in clinical application was gentuzumab ozogamicin (GO, Mylotarg), a humanized anti-CD33 IgG4 antibody conjugated to calicheamicin. Safety concerns and failure to verify clinical benefit in a confirmatory phase III trial enrolling patients across all cytogenetic risk groups resulted in the voluntary withdrawal of GO from the market in 2010. In recent years, both retrospective analyses and new clinical trials have been performed to unravel clinical benefits of GO in specific subgroups. A meta-analysis of five randomized controlled trials (RCTs) showed that the addition of GO to conventional chemotherapy significantly reduced the risk of relapse and resulted in an overall survival (OS) benefit mainly for cytogenetically favourable, but also for the intermediate-risk group.\textsuperscript{9} Albeit, variable treatment regimens were used and outcomes of the trials differed significantly, GO was re-approved for treatment of newly-diagnosed CD33+ AML in adults in combination therapy with chemotherapy (“7+3”) and as single-agent regimen. Approval was also granted for treatment of t/r CD33+ AML in adults and paediatric patients 2 years and older. Other ADCs directed against CD33 and alternative surface antigens like CD123 have entered clinical trials. The linker technology has been optimized with improved uniform drug loading. However, common side effects include hematotoxicity and hepatotoxicity. Clinical trials utilizing SGN-CD33A (vadastuximab talirine), an alternative ADC directed against CD33, had to be terminated.

Several T-cell recruiting antibody constructs are currently tested in early clinical trials and only few results have been presented at conferences so far. Similar to the ADCs, the optimal antigen to target is still an open question. The sister molecule of blinatumomab, AMG 330, a bispecific T-cell engager (BiTE) construct targeting CD33, is currently being tested in an international, multicentre phase I trial in t/r AML (n=50). The largest patient numbers so far have been recruited in an early trial relying on another T-cell recruiting antibody construct directed against CD123 (MGD006 by MacroGenics).\textsuperscript{10} In contrast to the BiTE technology, dual-affinity re-targeting (DART) molecules are composed of heavy and light chain variable domains of two antigen-binding specificities (A+B) on two independent polypeptide chains (VLA-VHB-VLB-VHA), which are stabilized through an additional C-terminal bridge. XmAb14045, developed by Xencor, is a structurally distinct anti-CD123 T-cell recruiting antibody construct also in early clinical development. The XmAb technology ensures structural stability and an extended serum half-life through the retention of an inactive Fc part. Recently, a bispecific CLL-1/CD3 antibody construct (MCLA-117) has been developed by Merus B.V. and a clinical phase I trial is currently recruiting t/r or elderly, previously untreated AML patients (n=50).\textsuperscript{11} At the current time point, there is no data to answer the question if the type of bispecific antibody construct influences the response rate to treatment. Independently of considerations about type of bispecific antibody construct or optimal target antigen, we are still only at the beginning of understanding the exact mechanism of action of those antibody constructs and resistance mechanisms that potentially evolve upon T-cell activation. PD-L1 upregulation on AML cells upon T-cell activation has been suggested as a potential resistance mechanism in an ex vivo system.\textsuperscript{12} Addition of a checkpoint inhibitor to T-cell recruiting antibodies might help to circumvent resistance.

**Adoptive T-cell therapy**

Genetically modified T cells take the platform of T-cell recruiting antibody constructs one step further and ameliorate T-cell exhaustion, anergy and senescence as a cause of initial treatment failure. Chimeric Antigen Receptor (CAR) are genetically engineered cell membrane-bound receptors that combine extracellular antibody binding and intracellular effector cell signalling. Since the first generation of CARs in 1989, the introduction of costimulatory domains (mainly CD28 or 4-1BB) in so-called second-generation CAR constructs greatly improved their anti-tumour effector function and paved their way from clinical trials to approved “living drugs” in t/r ALL and advanced diffuse large cell B-cell lymphoma. In AML only, a limited number of early phase I trials have been initiated. The LeY-CAR T cell trial was the first to evaluate CAR T cell therapy in AML. None of the 4 treated patients developed grade 3 or 4 toxicity, and infused CAR T cells persisted for up to 10 months. However, although one patient responded with transient reduction in blast count, all patients relapsed 28 days to 23 months after adoptive CAR T cell transfer.\textsuperscript{13} One significant concern is that most antigens used in CAR T cell therapy will also impact normal haematopoiesis. Of the first 6 refractory AML patients post allogeneic HSCT who received CD123-CAR T cells (costimulation domain: CD28), 1/2 responded at the first dose level program and 1/4 at the second dose level with another 2 with blast count reduction. Lymphodepleting regimen was done with fludarabine and cyclophosphamide. Importantly, all expected complications including cytokine release syndrome were reversible and manageable.\textsuperscript{14} Another phase I trial utilizing allogeneic “off-the-shelf” anti-CD123 CAR T cells (UCART123) was recently...
reported to be under development but has not been registered yet. And finally, a trial applying CAR T cells directed at NKG2D ligands to patients with r/r AML, MDS, and multiple myeloma is estimated to be completed in near future, but results are still pending. The trials vary in production steps, choice of target antigen, antibody clone, costimulatory domain and effector cells. Fine-tuning the development process of CAR T cells might be able to provide differential recognition of target antigens on leukemic vs. healthy cells. At least in preclinical models, a dual-targeting approach recognizing two independent leukaemia-associated antigens was shown to provide increased specificity accompanied by reduced off-leukaemia toxicity and to prevent antigen escape mechanisms. T-cell receptor gene-transduced (TCRtg) lymphocytes target a degraded, processed and presented antigen in the context of HLA molecules on the surface of the malignant cell. Again, similar to the vaccine approach, WT1 is the dominant antigen for TCRtg based immunotherapeutic strategies. Similar to the production of CAR T cells, this is based on an ex vivo generation of TCR-gene transduced T-cells using a retroviral vector. Only very limited data has been published on adoptive transfer of TCRtg cells in AML patients. Recently, Tawara et al published a study in which a TCR that specifically reacts with WT1 peptide in the context of HLA-A24.02 was applied. Interestingly, after the second transfer, sequential WT1 peptide vaccines were given to support persistence of the adoptively transferred T-cells. Eight patients were treated and 2/8 patients showed a transient decrease in blast counts in the bone marrow. Further trial recrural, later trial phases and long-term follow up is needed to address the question of efficacy.

**Conclusion / Future**

Remarkable progress has been made in immunotherapy of AML in the recent years. Multiple immunotherapy platforms have developed and are currently exploited in clinical trials. Immunotherapy in AML is complicated by different characteristics including lack of an AML-specific target antigen, low mutational burden resulting in low endogenous immune responses and intrinsic resistance mechanisms. Hence, dual targeting strategies as well as combinatorial approaches will be needed to battle the complexity and heterogeneity of AML. Preclinical examples demonstrate the additive cytotoxicity on AML cells by combining a T cell recruiting antibody construct with a tyrosine kinase inhibitor. Albeit exciting progress has been made in immunotherapy of AML, many further steps have to be taken before the vision of an individualized immunotherapy approach will be applicable for each AML patient. Importantly, the optimal time point for immunotherapeutic intervention is poorly defined. Likely, immunotherapy in AML will induce the best treatment responses for MRD eradication after induction chemotherapy. Understanding the biology of the disease and mode of action and resistance of the different immunotherapy platforms will pave the way for developing future treatment concepts in AML.

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