The incidence of hematological malignancies continues to rise, while the underlying biological mechanisms of tumorgenesis often remains unknown. The treatment landscape for hematological malignancies is diverse and immunotherapy is clearly entering the arena. Immune-based therapies for hematological malignancies aim at generating new agents such as monoclonal antibodies, immunotoxins, bispecific T-cell engagers, and cell therapies involving the innate and adaptive immune system. In addition, adoptive cell therapy with T/NK/NKT cells engineered with chimeric antigen receptors or T-cell receptors (TCRs) or vaccines and checkpoint inhibitors which are less toxic and might be more effective when compared with conventional chemotherapy and radiotherapy. These various approaches have shown significant promise, leading to improved patient outcomes.

Monoclonal antibodies

Monoclonal antibodies are effective in a number of hematological malignancies. Most of the currently identified targets for monoclonal antibodies are also expressed on nonmalignant cells. However, in contrast to either gene-modified T-cells (eg, CAR T-cells) or bispecific antibodies, the on-target toxicity of monoclonal antibodies on nonmalignant cells is mostly tolerable. The efficacy of the use of monoclonal antibodies (MoAbs) is highly dependent on the type of antibody (single/and/or conjugated), combinations with conventional (chemo)therapeutic strategies and depends on the underlying disease. Several modes of action have been explored among which antibody dependent cellular cytotoxicity, complement dependent cytotoxicity, and induction of apoptosis are the most well described. In addition, next to the direct effects of MoAbs on the tumor target (on-target effect), antibody-based immunotherapy may also alter the immune suppressive microenvironment by deletion of, that is, myeloid-derived suppressor cells or regulatory T and B cells by anti-CD38 as an example and hence may contribute to efficacy. The generation of bispecific antibodies, targeting the neoplastic cells and engaging CD3+ T-cells further improve efficacy in redirecting the immune system toward the tumor and tumor microenvironment. To this end, it is noteworthy that immune therapy with novel emerging strategies further focus not only on tumor target antigens but also on the complex immune system to optimize tumor-specific immunity as well as to modulate additional cellular and humoral components which either potentiate or inhibit effective immunotherapy. New strategies to improve therapy with monoclonal antibodies includes the genetically engineered structure and function of these antibodies, an approach shown to significantly improve their effectiveness.

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Vaccines

Vaccines activating the autologous immune system for prevention and treatment of infections and other diseases might also have a major impact on human healthcare. Compared to other immunotherapies such as checkpoint inhibition or adoptive T-cell therapy, most cancer vaccines to date have failed to demonstrate relevant clinical efficacy. One of the key obstacles for the development of an effective cancer vaccine is the difficulty in antigen selection and the requirement to overcome tolerance to self. In the past, most of the cancer vaccines were targeting tumor-associated antigens (TAAs), which are overexpressed in many cancers and were seen as universal targets for the treatment of patients with hematological malignancies. Unfortunately, TAAs are also expressed on normal tissues and thus central and peripheral tolerance can interfere with the efficacy of vaccination or can induce autoimmunity/autoreactivity against normal tissues. In contrast to nonmutated self-antigens, neoantigens are derived from random somatic mutations. These mutations encode for neoantigens which are present in tumor cells but not in normal cells and thus can be recognized as non-self by the host immune system and therefore are attractive targets for immunotherapies with potentially increased specificity, efficacy, and safety. Preclinical and clinical studies have demonstrated neoantigen-specific T-cells to represent the most potent tumor-reactive immune cell subpopulation. As compared to solid cancers, hematological neoplasms show a low variety and burden of somatic mutations which may hamper adequate induction of neoantigen-specific T-cells. Nevertheless, several strategies are to be explored to improve antitumor immunity.

In the context of designing vaccine approaches, tumor (leukemia)-derived (autologous) dendritic cells (DC) harboring intrinsic TAAs including neoantigens to be processed in the context of professional antigen presenting cells are promising. Alternatively, fusion of tumor cells with DC or pulsed tumor/leukemia-derived apoptotic vesicles is a potential strategy to optimize tumor-specific antigen presentation. The combined use of hypomethylating agents and checkpoint inhibitors may further potentiate tumor-specific immunity and may overcome tolerance and an immunosuppressive microenvironment.

Alternatively, mixed immunogenic peptide vaccines, either in the context of allogeneic/autologous DC-based approaches or as immunogenic peptide vaccines being explored. In contrast to autologous (DC) vaccines, allogeneic (tumor cell or cell line derived) DC cell vaccines are off-the-shelf products and more immunogenic and not impaired in antigen processing due to intrinsic patient-derived immune suppression.

The posttransplant setting, especially the period following allogeneic stem cell transplantation, is a potentially promising platform for vaccination due to cyto reduction and relative depletion of inhibitory accessory cells fostering greater immune responsiveness. Thus, another source of non-self antigens, particularly in the setting of allogeneic stem cell transplantation, are the minor histocompatibility antigens (MiHAs) derived from single nucleotide differences between patient and donor. MiHAs are recognized as non-self by the donor immune system and thus can be used as an attractive target for immunotherapy after allogeneic stem cell transplantation in the form of vaccination. In addition, currently clinical studies are ongoing with the HA-1 TCR in high-risk hematological malignancies (Leiden, Seattle).

Infusions of donor lymphocyte and antigen-specific T-cells

Donor lymphocyte infusions have been used for >30 years now to treat overt relapse, mixed chimerism or residual tumor cells following allogeneic stem cell transplantation. In addition, the adoptive transfer of antigen-specific cytotoxic T-lymphocytes and CD4+ T-helper cells has been widely used to treat viral infections including cytomegalovirus, Epstein-Barr virus, adenovirus infections and, more recently, BK and JC viruses. Currently, early phase trials are in progress to study the role of donor lymphocyte infusions in the management of refractory, invasive fungal infections. One of the greatest challenges ahead will be that of transitioning the initial promising results, mainly obtained in proof-of-concept studies, to a wider application of these therapies using standardized methodological approaches.

Checkpoint inhibitors

Checkpoint inhibitor blockade releases the brakes on tumor-specific T-cells, allowing them to persist and expand to attack malignant cells. Cancers can grow, at least in part, as a consequence of cancer-induced immunosuppression. In many individuals, immunosuppression is mediated by CTLA4 and PD-1, 2 immunomodulatory receptors expressed on T-cells. Monoclonal antibody–based therapies targeting CTLA4 or PD-1 have shown significant clinical effects in patients with hematological malignancies, especially patients with Hodgkin’s lymphoma and patients with primary mediastinal B-cell lymphoma, and are currently being explored in several other hematological malignancies and after allogeneic transplantation despite the expected risk of increasing the rate and severity of graft versus host disease. Synergistic efficacy has been shown for CTLA4 and PD-1 blocking antibodies especially in the setting of solid tumors (eg, metastatic melanoma). Furthermore, antibodies against additional checkpoint molecules like TIM3, TIGIT, LAG-3, etc., have been developed and are being tested in early clinical trials in patients with hematological malignancies.

Innate immune cells

Next to dendritic cells, other innate immune cells, especially natural killer (NK) cells, play a key role in antitumor immunity. NK cell dysfunction has been implicated in the progression of many hematological malignancies including acute myeloid leukemia (AML), MDS, and multiple myeloma (MM).

Target recognition by NK cells is dictated by the net sum of signals from an array of inhibitory and activating receptors expressed on the NK cell surface. If a net activation signal is generated, NK cells release perforin and granzymes into target cells, resulting in cell death. In addition to their role as innate cells, activated NK cells also secrete inflammatory cytokines and chemokines, which are involved in stimulating and recruiting the adaptive immune system.

Activated NK cells can also kill target cells via expression of cell surface death receptor ligands (eg, Fas ligand, TRAIL).

Unlike T-cells, NK cells do not require tumor-antigen recognition or clonal expansion before killing cancer cells. The most important inhibitory signals for NK cells are mediated via their killer immune-globulin-like receptors (KIRs) and CD94/NKG2A, which recognize as self-associated molecules the major histocompatibility complex (MHC) class I antigens HLA-A, B, C, and HLA-A-E, respectively. The expression of cell surface HLA class I molecules mediates inhibition of autologous NK cells, preventing the destruction of healthy somatic cells. However, following malignant transformation, or viral infection, cells may lose their MHC class I expression facilitating avoidance of recognition by cytotoxic T lymphocytes (CTLs). Loss of MHC class I may render them susceptible to NK cell-mediated immunosurveillance. Several non-MHC Class I binding inhibitory receptors such as PD-1, LAG-3, TIGIT, CD96, Siglec-7, and
TIM3 may also influence NK activation under different circumstances.\textsuperscript{41,46} Stressed cells, such as malignant transformed cells or virally infected cells, may express and upregulate the MHC class I chain-related (MIC) ligands MIC-A and MIC-B for the activating natural killer group 2, member D (NK2GD) receptor expressed by NK cells. Moreover, other ligands expressed by malignant cells which act to stimulate NK cell activity include the polio virus receptor (PVR/CD155) which binds to the activating receptor DNAx Accessory Molecule-1 (DNAM-1), and ligands to the natural cytotoxicity receptors, Nkp30, Nkp44, and Nkp46. Target recognition via antibody-dependent cellular cytotoxicity triggers strong NK cell activation. In this situation, CD16 (FcγRIIIa) expressed by NK cells binds to the Fc portions of antibodies, triggering NK cell cytotoxicity as well as cytokine release to stimulate the adaptive immune response. Treatment with cytokines, such as IL-2, IL-12, IL-15, IL-18, and IL-21 has been found to enhance cytotoxicity and persistence.

Previous trials using unmodified allogeneic NK cells have shown promising clinical activity with good tolerability, primarily in patients with AML and MM. Given the success of CAR-T approaches, a logical next step was to develop CAR-NK cells to further enhance cytotoxicity and targeting of NK cells.\textsuperscript{7,48} CAR-NK cells may have several advantages over current autologous CAR-T approaches. NK cells do not rely solely on the CAR for tumor recognition, retaining the capacity to kill cancer cells that lose target antigen via recognition of stress induced ligands. Since allogeneic NK cells do not cause graft versus host disease, they hold potential for development as standardized off-the-shelf therapeutics for adoptive cancer immunotherapy, greatly increasing accessibility and reducing cost. They may also be safer than CAR T-cells; to date, there are no reported cases of CRS or neurotoxicity with CD19 CAR-NK cell therapy. Potential sources of allogeneic NK cells include donor NK cells, cord-derived NK cells, induced pluripotent stem cell (iPS)-derived NK cells, and NK cell lines.\textsuperscript{44,48}

Recently, \( \gamma^6 \) T-cells, a subset of T-cells expressing \( \gamma^6 \beta^8 \)TCRs rather than the conventional \( \alpha^\beta \)TCR, have been used for immunotherapy of hematological malignancies.\textsuperscript{49} Donor-derived \( \gamma^6 \) T-cells\textsuperscript{49–53} selected after depletion of \( \beta^8 \)TCR T-cells and infused following lymphodepletion were found to induce partial or even complete responses in patients with acute myeloid leukemia and MM. In addition, infusions of NKT cells have been used in the treatment of hematological malignancies.\textsuperscript{52}

### Bispecific antibodies: T-cell redirecting antibodies

Bispecific antibodies that recruit and redirect T-cells to attack tumor cells have tremendous potential for the treatment of hematological malignancies. These antibody constructs promote tumor cell killing by crosslinking a CD3 component of the T-cell receptor complex with the tumor-associated antigen on the surface of the tumor cell. Importantly, this mode of action does not rely on a cognate interaction between the T-cell receptor and a peptide/HLA complex. Thus, this strategy is not dependent on HLA restriction or on HLA expression representing a significant advantage since HLA class I and II molecules may be downregulated in malignancies. Therefore, bispecific antibodies may find a key role in hematological malignancies with low neoantigen burden and low inflammation.\textsuperscript{54–55} These novel immunotherapeutics may productively be combined with checkpoint inhibitors.

Extensive optimization and process development have progressed a large number of bispecific/trispecific antibodies into clinical trials for a wide range of indications, with promising signs of therapeutic activity. As an example, Blinatumomab has already been approved for the treatment of refractory, relapsed, BCP-ALL and also for patients with molecularly resistant disease following intensive chemotherapy.\textsuperscript{53–55} However, T-cell activation and consecutive cytokine release as well as inflammation-induced alterations of the blood–brain barrier are associated with T-cell engaging antibodies, especially targeting CD19, inducing cytokine-release syndrome and neurotoxicities (ICANS) which can rarely be life-threatening. Very promising efficacy data for bispecific antibodies were also demonstrated\textsuperscript{53–55} in the treatment of AML and, especially advanced MM.\textsuperscript{44} Novel formats allow to target antigen signatures on the tumor cell thus to increase specificity of the approach and by reducing nonspecific T-cell activation also to reduce CRS and neurotoxicity.\textsuperscript{46–54}

### Gene-modified T-cells

The last decade has witnessed technological advances, which have allowed genetic modifications of T-cells providing personalized cellular therapies, that target specific tumor-associated antigens. A major advantage of gene-modified T-cells is that they are a living drug which can expand and proliferate in the patient. Persistence over time of these gene-modified T-cells has been demonstrated in responding patients.

Gene transfer into human T-cells can be accomplished by several means. Long-term culture of the genetically modified T-cells is often required to reach meaningful clinical doses, with the functional impact of prolonged ex vivo expansion potentially adversely affecting subsequent long-term persistence in vivo. Gene delivery (or transfer) is mainly achieved through the use of viral (retroviral or lentiviral) vectors. These vectors can be manufactured to clinical grade on a large-scale producing stable integration into the genome of the T-cell and its progeny. Adverse consequences due to insertional mutagenesis in T-cells have not yet been reported and are unexpected given the mature differentiation status of the T-cells at the time of exogenous gene integration.

However, lentiviral vectors are particularly attractive when less differentiated T-cell subsets are targeted for modification, as they have the unique ability to infect T-cells even upon minimal activation, a property lacking in retroviral vectors. Novel nonviral systems (eg, Sleeping Beauty and PiggyBac) allow larger fragments of DNA to be inserted when compared to viral vectors. These novel strategies of nonviral gene transduction are likely to reduce the cost of genetic modification of immune cells in the future by avoiding the need for a large-scale manufacture of clinical grade viral vectors. When combined with CRISPR/Cas or other gene-editing technologies, site directed insertion of the gene encoding the TCR or the CAR is achievable. Genetic modification of T-cells for targeting tumor cells can either be performed in the form of CAR T-cells or TCR gene-modified T-cells.

### CAR T-cells

T-cells redirected to specific surface antigens on malignant cells by engineered CARs are emerging as powerful therapies for hematological malignancies. In contrast to bispecific antibodies, which link the activated T-cells to the tumor cells by a small molecule with binding domains for both CD3 and a surface antigen expressed on the tumor cell, here T-cells are engineered to express a new antigen recognition receptor, which targets the antigen on the tumor cell surface.

An increasing number of clinical trials using CAR T-cells for the treatment of hematological malignancies have been reported, especially targeting B-cell lymphoma, BCP-ALL and MM. Two cell products have been approved in 2017/2018 for clinical use Kymriah (Tisagenlecleucel) and Yescarta (axicabtagene ciloleucel) which received FDA and European marketing authorization for the treatment of relapsed or refractory CD19+ BCP-ALL and for the treatment of diffuse...
large B-cell lymphoma. These approvals were based on impressive responses observed in patients with BCP-ALL, including high-risk patients.\textsuperscript{59} The overall response rate and the role of complete remission in B-cell lymphoma is lower than in ALL, but long-term remissions without further therapy have been reported for up to 40% of patients, enough to encourage the approval and speed up the ongoing research.\textsuperscript{59–61} In addition in 2020 FDA has approved liso-cel (Breyanzi) another CD19- CAR T-cell product for the treatment of adult patients with relapsed refractory large B-cell lymphoma after ≥2 lines of systemic. In 2020, a first CAR T-cell product brexucabtagene autoleucel (Tecartus) was approved for patients with mantle cell lymphoma, who do not respond to other treatment or have recurrent therapy.\textsuperscript{62} Promising initial results have been also reported in the treatment of patients with MM, where CAR T-cells target the B-cell membrane antigen.\textsuperscript{63,64} These results\textsuperscript{64} have also led to the approval of ABECMA (Iso-Cel), a B-cell membrane antigen targeting CAR T-cell product for patients with relapsed refractory MM by the FDA and EMA in 2021.

**TCR gene-modified T-cells**

TCR-modified T-cells are a novel alternative of adoptive cell therapy designed to treat hematological malignancies and solid tumors.

T-cells recognize antigens through a unique antigen-specific TCR promoting the elimination of a given target and amplifying the attack through the recruitment of other components of the immune response. T-cells can target peptides derived from both intracellular and extracellular proteins, including those encoded by genes mutated in cancer cells. T-cells can actively distribute within tissues and in the tumor environment having the potential for in vivo expansion and self-maintenance, as they can establish a memory compartment. Typically, the genes encoding the alpha and beta chains of the TCR are cloned into retroviral or lentiviral vectors for gene transfer into autologous T-cells. Novel nonviral transduction technologies, which are increasingly developed and established, are also being optimized for clinical grade TCR transfer. TCR-modified T-cells can mediate antitumor efficacy and have been used to target several antigens like NYESO-1, MAGE-A3 and PRAME, MAGE-A10, and WT1. Clinical trials have been performed to treat patients with MM and AML.\textsuperscript{65}

**European research contributions**

The whole development program of Blinatumomab from the pilot trial until the final approval by the FDA and the EMA for relapsed refractory B-cell precursor ALL was performed under the leadership of European scientists. In 2018, the FDA granted accelerated approval for the treatment of adult and pediatric patients with B-cell precursor ALL in the first or second complete remission with minimal residual disease ≥0.1% based on a European trial. Also in Europe, most new indications for treatment with bispecific antibodies are now being tested in the clinic. Promising data on the use of bispecific antibodies also in MM and partially in AML have been generated in European centers.\textsuperscript{4} New formats of bispecific antibodies are being developed by European scientists and European biotech companies.

Technologies of gene modification of T-cells including CAR and TCR gene-transfer have been intensively developed in Europe. Patients in the United Kingdom and other European countries have been among the first in the world to receive these therapies outside the United States. European scientists and clinicians are leading the development of gene-modified neoantigen-specific T-cells, which are predicted to have a major impact in the management of hematological and non-hematological tumors. Other major contributions are the development and validation of novel strategies for immune cell selection.

Despite our major contributions, Europe is currently lagging behind the large number of clinical trials initiated in the United States and China, especially in the CAR T-cell field. Currently, European patients are queuing up to receive treatment with CAR T-cells for hematological malignancies in the US and Chinese centers due to the lack of open clinical trials in Europe. Presently, >400 clinical trials are listed as investigating CAR T-cell therapy in the treatment of hematological or solid cancer. About 87% of new reagents are produced in the United States and China, with only a minor activity in Europe. European biotech companies are also moving to the United States for a much more rapid transfer of CAR-T approaches into the clinic and access to larger numbers of patients and a more lucrative market. In addition, a lack of early clinical trials and the time-consuming regulatory processes for trials using ATMP are clear hurdles for clinical trial activities with novel immunotherapeutic agents in Europe.

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**Summary box: Main research & policy priorities**

1. The treatment landscape for hematological malignancies is diverse and immunotherapy is clearly entering the arena. Immune-based therapies for hematological malignancies aim at generating new agents such as monoclonal antibodies, vaccines, immunotoxins, bispecific T-cell engagers, and cell therapies involving the innate and adaptive immune system using unmodified and gene-modified immune cells.

2. Compared to other immunotherapies such as checkpoint inhibition or adoptive T-cell therapy, most cancer vaccines to date have failed to demonstrate relevant clinical efficacy. Thus, another source of non-self antigens, particularly in the setting of allogeneic stem cell transplantation, the minor histocompatibility antigens (MiHA) derived from single nucleotide differences between the patient and donor is currently explored.

3. Monoclonal antibody–based therapies targeting CTLA4 or PD-1 have shown significant clinical effects in patients with hematological malignancies, especially patients with Hodgkin’s lymphoma, patients with primary mediastinal B-cell lymphoma and are being tested in early clinical trials in patients with other hematological malignancies (B-NHL, MM, etc.)

4. Extensive optimization and process development have progressed a large number of bispecific/trispecific antibodies into clinical trials for a wide range of indications, with promising signs of therapeutic activity—been approved for treatment of refractory, relapsed, BCP-ALL—and are currently evaluated in other hematological malignancies (B-NHL, MM, and AML).

5. T-cells redirected to specific surface antigens on malignant cells by engineered CARs are emerging as powerful therapies for hematological malignancies. Following the approval for BCP-ALL, diffuse large B-cell lymphoma, mantle cell lymphoma, and MM, a wide range of other tumor entities are currently targeted with CAR-T/CAR-NK cells in ongoing clinical trials.

6. TCR gene-modified T-cells are increasingly explored in hematological malignancies and currently clinical studies are ongoing with the HA-1 TCR in high-risk hematological malignancies but also TCRs targeting various cancer tests and differentiation antigens.
Conclusion

Thus, immune therapies are increasingly being explored in hematological malignancies. The most promising ones are currently clearly T-cell-based therapies with CAR T-cells and T-cell engaging antibodies, which have induced deep and long-lasting remissions in some hematological malignancies and are increasingly explored for all other hematological malignancies but also solid tumors.

Disclosures

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