Treasures from nature

From natural antibody responses to novel immunotherapies for cancer

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Introduction 1

Cancer is one of the leading causes of death in high income countries worldwide (source: World Health Organization; WHO), and the main cause of death in the Netherlands. Thirty-one percent of deaths in the Netherlands are attributed to cancer, and for persons between the age of 40 and 80 this number even rises to 47% (source: population data of the Dutch Central Agency for Statistics (CBS, StatLine) from 2017). While the pillars of the treatment for most cancers traditionally are chemotherapy, radiotherapy, and/or surgical intervention, an increasing number of cancer patients can benefit from adding immunotherapy to their treatment. The merits of harnessing the immune system in the treatment of cancer has been long recognized in the treatment of hematologic malignancies and can lead unexpected recoveries. Exploring the successful immune responses from these cancer survivors can potentially result in new leads for the treatment of future patients.

Hematopoietic system

Hematopoiesis takes place in the bone marrow and encompasses the formation of a wide range of blood cell populations. Hematopoiesis is a hierarchical process that starts with a hematopoietic stem cell that gives rise to various differentiated and specialized cell types divided over different lineages.[1] In short, the two main lineages of the hematopoietic system are the myeloid and the lymphoid lineage. Development of the three major classes of hematopoietic cells in the myeloid lineage concerns erythropoiesis, generating erythrocytes or red blood cells, megakaryopoiesis, resulting in the production of thrombocytes or platelets, and leukopoiesis of the myeloid white blood cells, such as granulocytes and monocytes. In the lymphoid lineage lymphopoiesis concerns the development of the other white blood cells, lymphocytes, such as T cells, B cells and natural killer (NK) cells.

The various hematopoietic subsets have different characteristics and fulfill specific functions. Red blood cells are responsible for oxygen transport from the lungs through the body, and platelets are important in the coagulation cascade following tissue damage. Both entities do not contain a nucleus and are limited to the confinement of the blood vessels. White blood cells, however, are the dominant nucleated cell population in peripheral blood and lymphoid organs, and depending on the type of leukocyte, can be found in nearly every tissue in the body. The different types of myeloid and lymphoid derived white blood cells make up the immune system, a bodily defense system directed against infectious agents like bacteria, viruses and parasites, that also plays a role in protection against cancer.

Acute leukemia's are malignancies of hematopoietic precursor cells. Malignant transformation can occur both in precursors of the myeloid lineage, causing acute myeloid leukemia (AML), or in those of the lymphoid lineage, leading to acute lymphoblastic leukemia (ALL) (*Figure 1*).

Acute leukemia

While the frequency of these diseases is low in comparison to other (predominantly solid) malignancies (*Figure 2A*), the burden of leukemic disease is high, as leukemias affect patients of all ages, available therapies do not meet the specific demands of elderly/less fit patients and the clinical course of the disease is often aggressive as shown by the very poor long term survival (*Figure 2B*).

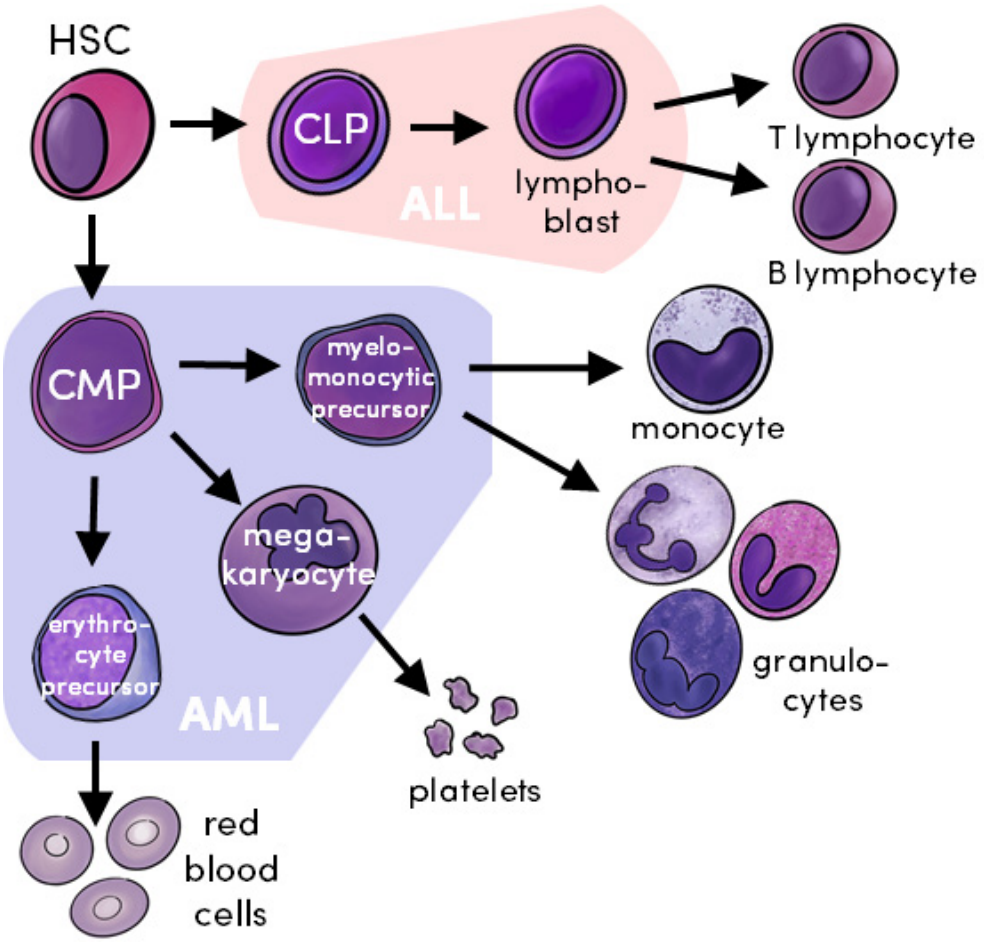

Figure 1 | Non-exhaustive, simplified schematic representation of development of certain hematopoietic subsets and their leukemic counterpart. HSC: hematopoietic stem cell; CLP: common lymphoid progenitor; CMP: common myeloid progenitor; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia.

Acute myeloid leukemia (AML)

AML is a rare, heterogenous and aggressive disease characterized by uncontrolled expansion and infiltration of leukemic blasts in the bone marrow, peripheral blood and other organs.[2] The rapid, clonal proliferation interferes with normal hematopoiesis leading to multilineage cytopenias that, depending on severity of the cytopenia, cause the typical symptoms of AML. Severe thrombocytopenia impairs hemostasis resulting in spontaneous bruising, bleeding gums or coagulation problems upon (minor) trauma. Anemia can lead to complaints of fatigue and shortness of breath and leukopenia can hamper effective immune responses resulting in increased susceptibility to infection upon encounter with microorganisms.

Malignant transformation of cells in the myeloid lineage results in distinct types of AML depending on the lineage affected, the differentiation stage where transformation occurred, and the genetic aberrations contained in the (sub)clonal malignant cell population. One of the first ways to

distinguish different subtypes of the disease was by morphology,[3] gradually complemented with immunophenotypic analysis of cell surface markers by flow cytometry and cytogenetic analysis of chromosomal abnormalities.[4] More recently molecular evaluation has been added to the diagnostic work up in classifying AML, as classification based on molecular and cytogenetic abnormalities more closely correlates with prognosis.[5–7] In addition, clinical features, for example white blood cell count and occurrence of extramedullary localizations of leukemia, are taken into account in the risk assessment of AML cases. The classification of AML and related neoplasms is described in the fourth edition of the 'WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues' dating from 2008 and extensively revised in 2016.[8,9] Currently six main categories with 22 subcategories or provisional entities are recognized.

The age-adjusted incidence of AML in the western world is 3-4 per 100.000, and while it affects people of all ages the likelihood of developing the disease increases with age.[10,11] Survival of AML is very poor, especially in the elderly who are often not fit enough to undergo the aggressive treatments necessary to achieve adequate tumor control.[11] The five-year survival in adult patients under 60 years of age has improved markedly over the past 25 years and now ranges from 35-60%. In contrast, survival of patients > 60 years of age has hardly improved in the past decades and range from only 1 to 17%.[11,12]

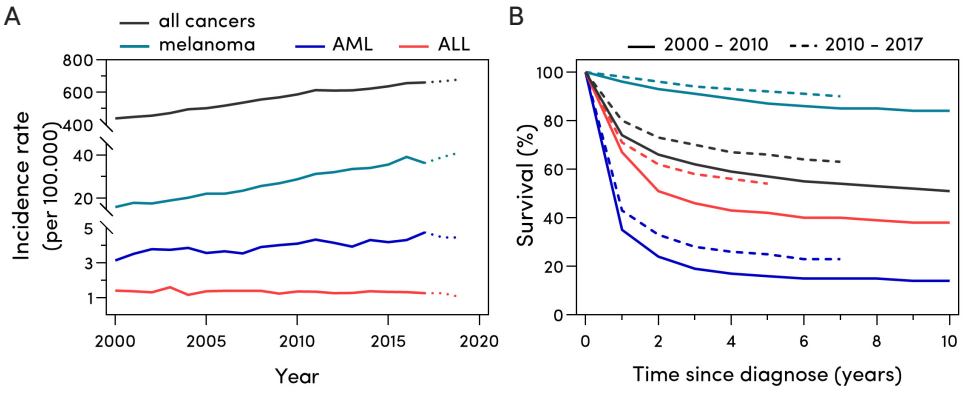

Figure 2 | Incidence and survival of acute leukemia's in the Netherlands. (A) Incidence rate per 100.000 of cases of acute lymphoblastic leukemia or acute myeloid leukemia in the Netherlands since 2000, compared to all cases of cancer and solid tumor melanoma. Dotted lines depict preliminary datasets for the years 2018 and 2019. (B) Survival curves since diagnosis for the before mentioned groups for the time period 2000-2010 and 2010-2017. Data from the Dutch Cancer Registry; www.iknl.nl.

Acute lymphoblastic leukemia (ALL)

Like AML, ALL is also characterized by an unwarranted expansion of leukemic cells -in this case of malignant transformed precursors in the lymphoid lineage- resulting in similar symptoms. Briefly, ALL too, is classified according to the 'WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues',[9] with an important role for recurrent chromosomal abnormalities after initial immunophenotypic subdivision based on lineage (B or T). Seventy-five percent of ALL cases develop from precursors of the B-cell lineage, while the other 25% of cases develop from T-cell precursors.[13] Based on molecular and cytogenetic abnormalities 11 (provisional) entities

are recognized for B-ALL; for T-ALL no further subdivisions are made, at present time.[9] ALL is the most frequent childhood malignancy, but it also affects adults, albeit with a lower incidence of approximately 1 per 100.000.[14]

Treatment of leukemia

Chemotherapy

The backbone of treatment for AML consists of '7+3' treatment regimens with seven days of high dose cytarabine and three days with an anthracyclin,[15] followed by consolidation therapy with allogeneic hematopoietic cell transplantation (HCT) in case of intermediate or high risk AML.[16,17] Also in ALL, multi-agent chemotherapy (vincristine, corticosteroids, an anthracycline, methotrexate and asparaginase) remains the basis of first-line therapy followed by consolidation therapy with allogeneic HCT for suitable candidates.13 In both leukemias, but especially in ALL, some progress has been made by optimizing the chemotherapeutic treatment protocols.[14,18] However, to achieve long term remission, in particularly in patients with high-risk disease, immunologic consolidation with allogeneic HCT is an essential part of the treatment.

Allogeneic hematopoietic cell transplantation

Allogeneic HCT is considered to be the strongest curative immunotherapy for, but not limited to,[19] hematologic malignancies. It is the treatment of choice in intermediate or high risk AML and ALL,[13,16,17] and in relapsed leukemia not previously treated with allogeneic HCT,[13] if the patient's condition permits. While HCT also contributes to restoring hematopoiesis after myeloablative therapy, the main purpose of allogeneic HCT is to invoke a successful immune response to the tumor (graft-versus-tumor or graft-versus-leukemia; GvL).[20]

In allogeneic HCT, CD34+ hematopoietic donor cells are infused into a preconditioned recipient (*Figure 3*). The conditioning therapy is necessary to rid the recipient of autologous lymphocytes that could otherwise cause graft rejection. Depending on the source and the preparation of the graft, there are also other, more mature, immune cell types present in addition to the stem cells.[21] Following transfusion with donor cells, the recipient is initially treated with immunosuppressive agents to prevent graft-versus-host disease (GvHD) from developing.[20] These immunosuppressive agents are gradually tapered over time, giving the newly transplanted immune system the possibility to fully develop. Different types of donors can be used as source for hematopoietic stem cells. Ideally the donor is a full HLA match with the patient, and a sibling is the most likely and preferred source with the lowest risk of GvHD. Alternatively, fully matched unrelated donors via (inter)national donor registries are an option. If also no unrelated match is available, a third option poses the use of CD34+ cells from cord blood. More recently, advances in the prevention of graft rejection and GvHD have made the use of donor stem cells obtained from HLA-haploidentical relatives increasingly possible. As such, the amount of possible suitable donors has increased, and with it the availability of allogeneic HCT for eligible patients.[20,21]

In addition to donor availability, treatment-related toxicity has provided an obstacle in widespread application of allogeneic HCT. Patients with comorbidities and/or a low performance status were considered ineligible to endure the procedure. Introduction of reduced intensity conditioning (RIC) regimens has made it possible to also treat patients that are unfit to undergo the standard

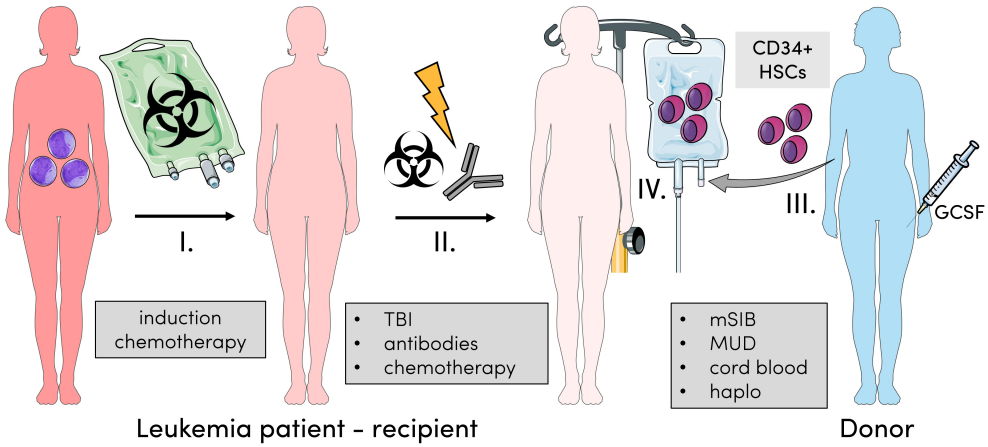

Figure 3 | Schematics of allogeneic hematopoietic cell transplantation in AML. After a patient is diagnosed with acute leukemia, she/he is treated with induction chemotherapy to attempt to achieve complete remission of the leukemia (I.). To condition the recipient for transplantation with HSCs, the patient is prepared with a combination with irradiation and/or depleting antibodies and/or chemotherapy in order to prevent graft rejection (II.). CD34+ HSCs are isolated from a healthy donor (III.). The stem cells are either collected via bone marrow aspiration under general anesthesia, or they are harvested from the peripheral blood via apheresis after GCSF stimulation. GSCF stimulates the production of CD34+ cells and mobilizes the hematopoietic stem cells from the bone marrow to the peripheral blood. Cord blood-derived stem cells are obtained from the umbilical cord postdelivery. Next, the recipient is infused with the donor stem cells (IV.) and is treated with immunosuppressive agents to prevent graft-versus-host disease from developing. GCSF: granulocyte-colony stimulation factor; haplo: haploidentical; HSC: hematopoietic stem cell; mSIB: matched sibling; MUD: matched unrelated donor; TBI: total body irradiation.

myeloablative conditioning (MAC) protocols. RIC was shown to have lower regimen-related toxicity and non-relapse mortality, resulting, despite higher relapse rates, in an overall survival (OS) comparable to MAC.[22,23] The lower treatment-associated toxicity allows elderly and less-fit patients and patients with low fitness or with comorbidities to benefit from the curative potential that is offered by allogeneic HCT.[20] While the best results are obtained when patients are transplanted in complete remission after chemotherapeutic induction treatment, allogeneic HCT is sometimes performed for chemotherapy-refractory leukemia.[24,25] In these patients, the curative effect of allogeneic HCT relies maximally on invoking a GvL response.

Relapse

Unfortunately, relapse after allogeneic HCT is common; in approximately 30-40% of patients disease relapse occurs at some point after transplantation.[26–28] There is no standard of care at this moment, and little progress has been made in the past decades in improving the outcome of leukemia relapsed after allogeneic HCT. Therapeutic options are limited, and salvage therapies consist predominantly of chemotherapy and methods to reestablish GvL.[26,27,29] Hence, relapse is a major contributor to the poor long-term survival of leukemia.

In case of relapse, the donor immune system fails to adequately recognize and successfully eliminate malignant cells from the body, the basis of the concept of allogeneic HCT. Cancer is the malignant transformation of cells in a normal tissue. It is a complex, multistep process that gradually develops over time via accumulation of mutations in a specific cell type.[30] Because tumors evolve from an

organism's own cells, the (adaptive) immune system has a hard time recognizing the wrong from the right and malignant cells can escape the attention of the immune system. While the immune system actively surveys for (pre)cancerous lesions and can recognize and eliminate tumor cells, not all nascent transformed cells exert the same level of immunogenicity.[31,32] This poses a difficulty for the immune system in exhaustively eliminating all the transformed cells in the early phases of malignant transformation. By 'overlooking' the less immunogenic malignant cells, the immune system effectively selects for a neoplasm with low immunogenicity to survive and grow out, the result of a process called immunoediting.[31] Hence, allogeneic HCT is aimed at restoring immune recognition of the malignant cells and relapse can be explained by leukemia cells escaping the control of the GvL immune response.[33]

Within the leukemic blast population in an AML patient, multiple different clones can be found.[34] These clones vary in their mutational makeup, and as such they can have different proliferative advantages or resistance features with respect to sensitivity to therapies. This can result in a different composition of the population of leukemia subclones observed at the time of relapse or in the course of refractory disease compared to subclones identified at diagnosis.[35,36] In rare instances, but especially after allogeneic HCT, AML relapse can manifests itself in extramedullary sites in the form of myeloid sarcoma.[19,37] Extramedullary relapse also occurs in so called 'sanctuary sites', like the testes or central nervous system, that are relatively shielded from chemotherapy and the donor immune system, and therefore pose an attractive environment for AML or ALL blasts.[38,39]

Acute leukemias are known to produce an immunosuppressive milieu via a wide range of mechanisms, and various mechanisms of immune escape of leukemic cells have been observed. Blasts from relapsed AML patients were shown to downregulate MHC class II, evading recognition by donor T cells. Exposing blasts to interferon gamma can restore MHC expression and, subsequently, T cell-mediated cytotoxicity.[40] Alternatively, AML cells can suppress the activity and maturation of NK cells by inducing microRNA (miR)–29b expression via the aryl carbon hydroreceptor (AHR),[41] or can escape the donor immune system in case of insufficient killer-cell immunoglobulin-like receptors (KIRs) mismatch.[42] Additionally leukemia blasts can upregulate expression of inhibitory checkpoint molecules such as protein 1 ligand 1 (PD-L1) and others, but also molecules like CD200 that suppress macrophages and NK cells.[43,44] AML cells can further induce immune cell dysfunction and increase regulatory T-cell populations via metabolic mechanisms, like (enhanced) expression of amino-acid catabolizing enzymes arginase-2 (ARG2) and indoleamine 2,3-dioxygenase-1 (IDO1).[45,46]

Therapeutic strategies for AML relapsed after allogeneic HCT therefore are for the most part aimed at restoring the allogeneic immune response. Possible approaches to enhance the GvL response are rapid tapering of immunosuppressive drugs that are still in play, infusion of donor lymphocytes (DLI) or a second allogeneic HCT.[19,26,27] Lastly, hypomethylating agents (HAs; e.g. azacytidine, decitabine) or histone deacetylase (HDAC) inhibitors are epigenetic modifying drugs and induce upregulation of (silenced) leukemia-related genes.[29] In this way these agents can potentially reinvigorate the immune response against leukemia. Advancing knowledge on the immune escape mechanisms of AML, paves the way for new therapeutic interventions aimed at reversing the mechanisms underlying immune escape.

Clinical advances in targeted (immuno)therapy

While effective, allogeneic HCT is a crude therapy, and treatment-related morbidity varies between 10-30%.[47] In attempts to improve outcome and decrease the necessity for allogeneic HCT, research focuses on identifying new and other ways in targeting leukemia, such as small molecules and immunotherapeutic agents. Since its introduction, no major breakthroughs have been made in reducing the need for allogeneic HCT or in improving outcome of AML relapsed after allogeneic HCT. Advances have been made and are ongoing for subtypes of leukemia, but there is still a long way to go. Most non-chemotherapeutic treatments focus on specific ways of redirecting or reactivating the immune system or on targeting specific mutations in tumor cells. Many of these therapies rely on a mutation in or the membrane expression of a specific protein that is overexpressed or limited to the tumor cells. Some of these therapies do not have a place in first-line treatment (yet) and are predominantly used in relapsed or refractory disease and/or used in the context of clinical trials. T*able 1* provides an overview of the protein inhibiting drugs that are currently part of the treatment of AML or ALL or soon will be, and *Table 2* lists immunotherapeutic targets used or currently under investigation.

Registered therapies in ALL

In B-ALL it is standard practice to include rituximab in the subgroups of patients with lymphoblasts that express CD20, where it has resulted in a modest improvement of outcome.[48] Additionally, the kinase inhibitor imatinib is included in first line treatment of Philadelphia chromosome positive (Ph+) B-ALL.[49] Imatinib improved OS, in part by facilitating allogeneic HCT,[49] however, development of resistance is common, and the use is limited to Ph+ ALL.[13] More recently, improvement in the outcome of relapsed ALL has been made by introduction of CD19-targeting immunotherapies. Patients, mainly pediatric, with relapsed B-ALL have been successfully treated with chimeric antigen receptor (CAR) T cells, autologous T cells engineered to express a single-chain variable fragment aimed at an antigen expressed by the tumor cells, in this case CD19.[50] Alternatively, antibodies have been modified into bispecific T-cell engagers (bTCE) to redirect T cells towards tumors cells. Blinatumumab is a CD19-targeting bTCE that is presently used in relapsed B-ALL. Benefit was demonstrated in Ph- B-ALL as single agent compared to chemotherapy, with OS of 7.7 month versus 4 months, respectively.51 Blinatumumab is currently tested as first-line therapy in a prospective clinical trial (HOVON 146; EudraCT# 2017-000766-30). While promising, resistance, via down regulation of CD19 by lymphoblasts, and vanishing of CAR T cells poses a problem and negatively affect the long-term benefit of these therapies.[52,53]

Available therapies in AML

Fms-like tyrosine kinase 3 (FLT3) is frequently mutated in AML -either an internal tandem duplication (ITD) (25%) or a point mutation in the tyrosine kinase domain (5%)- and associated with high relapse rate and short survival.[54] The FLT3-targeting small molecule midostaurin was the first new therapy approved by the FDA for the treatment of AML since 2000, and is now part of first line treatment in FLT3-mutated AML. Also approved in 2017, and currently under evaluation for addition in first-line treatment of AML, is enasidenib, an agent inhibiting isocitrate dehydrogenase (IDH) 2.[55] With IDH1 or 2 mutations occurring in 20% of AML cases,[56] they are common and inhibitors of these enzymes are soon expected to take a place in the treatment of IDH-mutated AML. In addition, there is an array of small molecule inhibitors of various proteins being clinically investigated that will have

to prove their merit and place in the treatment of AML.[57] The B-cell lymphoma 2 (BCL-2) inhibitor venetoclax is registered as a therapeutic option in unfit or elderly AML patients in combination with low dose cytarabine and a HA.[58,59] HAs are used in AML patients ineligible for standard induction chemotherapy or in relapsed disease. It has been suggested that combination of epigenetic modifying drugs with checkpoint inhibitors (CPIs) could result in a synergistic effect in reactivating the immune system.[60]

Table 1 | Small molecule protein inhibitors in AML or ALL

Target	Therapeutic	Indication/application
BCR-ABL/t(9;22)	Imatinib (PKI)	Ph+ B-ALL, or as monotherapy in R/R disease[61]*
	Dasatinib (PKI)	Ph+ B-ALL resistance for previous agent, Ph+ B-ALL in children*
BCL-2	Venetoclax (protein inhibitor)	AML patients not eligle for high-dose chemotherapy/allogeneic HCT[62]
FLT3	Midostaurin (PKI) (other FLT3 PKIs under evalauation: gilteritinib, quizartinib)	FLT3-mutated AML[62]*
IDH1 and IDH2	Ivosidenib (IDH1 inhibitor) or enasidenib (IDH2 inhibitor)[55]	Multiple trials in first-line treatment for AML population with IDH1 and/or 2 mutation (HOVON150/NCT03839771, and NCT0317324863)

FLT3: Fms-like tyrosine kinase 3; IDH: isocitrate dehydrogenase; Ph+: Philadelphia-chromosome positive; PKI: protein kinase inhibitor; R/R: relapsed/refractory. *Farmacotherapeutisch Kompas[64] list registered agents and their indications in the Netherlands. NB: Registered trials at clinicaltrials.gov are depicted by 'NCT' numbers.

Checkpoint inhibitors

Antibody-based immunomodulatory agents like CPIs (see BOX for background) have currently no place in the treatment of (relapsed) acute leukemia but are subject of evaluation for these indications. In combination with a HA, the CPIs nivolumab (anti-PD-1) (NCT03092674) and durvalumab (anti-PD-L1) (NCT02775903) are evaluated for their effectivity in newly diagnosed elderly or unfit AML patients not eligible for high-intensity induction chemotherapy and allogeneic HCT. A variety of trials with different combinations and similar indications (relapsed/refractory AML and/or patients unfit for intensive treatment) are scheduled or ongoing. First results from a non-randomized open label trial in relapsed/refractory AML patients treated with nivolumab in combination with azacytidine were encouraging.[65] A literature analysis of CPI treatment before or after allogeneic HCT for various hematologic malignancies, suggests checkpoint inhibition can be efficacious, but comes with an increased risk of (lethal) GvHD, especially when used before allogeneic HCT.[66] How and if CPIs in AML in the context of allogeneic HCT should be applied, warrants further research and phase I trials for feasibility of CPIs in AML relapsed after allogeneic HCT have been initiated (e.g. NCT03600155 and NCT03286114).

Antibody-based therapies under evaluation

The CD38-targeting antibody daratumumab, registered for multiple myeloma, is currently being evaluated for relapsed B and T ALL (NCT03384654) as well as refractory/relapsed AML (NCT03067571 and NCT03537599). Some case reports have already reported on the (off label) use of daratumumab in relapsed ALL.[67,68] In B-ALL antibodies or antibody-derived therapies like antibody-drug conjugates

(ADC) targeting CD22 have been developed. In AML, a number of antibodies and antibody-derived agents targeting molecules that are overexpressed by leukemia blasts, such as CD33, CD123 and CLEC12A, are under investigation (see *Table 2*).[69] The benefit of these agents is not fully established yet, and past results were not always unequivocal. For example, for CD33-targeting agents the road has not been without bumps, thus far preventing widespread application in the treatment of AML.[69] The clinical results of unconjugated anti-CD33 antibody lintuzumab have had mixed results,[70] as was the case for the CD33-based ADC gemtuzumab ozogamycine. Reevaluation of the results in meta-analysis has shown a marked survival benefit for the latter.[69] Lintuzumab is now being developed as radiolabeled antibody with first promising results.[71] While antibody-based immunotherapies targeting AML-associated antigens have not found their way into first-line treatment of AML yet, clinical evaluation is ongoing, and available results show value of this approach.

Alternative therapeutic approaches that focus on the immune system that are currently under evaluation are for example administration of ex vivo-generated allogeneic NK cells (NCT04347616) and dendritic cell vaccines (NCT01373515).

Table 2 | Targeted immunotherapies for AML and ALL

Target	Therapeutic	Indication/application
CD5	CAR T cells	Under investigation for R/R T-cell malignancies including T-ALL (NCT03081910/MAGENTA)[72]
CD19	Blinatumomab (bTCE, CD3 x CD19)	R/R B-ALL[61]*
	CAR T cells	R/R B-ALL
CD19	Blinatumomab (bTCE, CD3 x CD19)	R/R B-ALL[61]*
CD20	Rituximab (mAb)	B-ALL with ≥ 20% of blasts CD20+[61]
CD22	Inotuzumab ozogamicine (ADC)	CD22+ R/R B-ALL[61]*
	Epratuzumab	Under investigation for CD22+ (R/R) ALL[73,74]
CD33	Gemtuzumab ozogamicin (ADC) vadastuximab talirine[75] (ADC) IMGN77969 (ADC)	Specific cases of CD33+ AML or CD33+ relapsed AML[62]
	AMG 33076 (bTCE, CD3 x CD33)	Trial in R/R AML (NCT02520427)
	Lintuzumab-Ac-225 (radiolabeled mAb)	Tested in phase I trial for R/R AML[71]
CD38	Daratumumab (mAb)	Under evaluation for ALL and AML
CD123	SL-401 (mAb)	Currently under evaluation for AML
	Flotetuzumab (bTCE, CD3 x CD123), and others[76]	Trial in R/R AML[63] (NCT02152956)
	CAR T cells	Trials in AML; clinicaltrials.gov
CLEC12A	MCLA-11776 (bTCE, CD3 x CLEC12A)	Trial in R/R AML or in first-line treatment of unfit elderly[63] (NCT03038230)

ADC: antibody-drug conjugate; bTCE: bispecific T-cell engager; CAR: chimeric antigen receptor; CLEC12A: C-type lectin domain family 12 member A; mAb: monoclonal antibody; R/R: relapsed/refractory. *Farmaco-therapeutisch Kompas[64] list registered agents and their indications in the Netherlands. NB: Registered trials at clinicaltrials.gov are depicted by 'NCT' numbers.

BOX | Melanoma as poster child of therapeutic advances in solid tumor immunology

Especially in the field of solid malignancies, new approaches of reinvigorating the immune response against cancer have been discovered in the past two decades. Early results and pioneering work on this subject came in particular from studies in melanoma. T cells were found to play an important role in effective anti-tumor immune responses. Tumor-reactive T cells from peripheral blood or T cells isolated from tumor lesions of patients have been expanded ex vivo and reinfused, in a number of cases resulting in complete eradication of the tumor and metastases.[77] However, the effectivity of anti-tumor immunity is hampered by immune suppressive escape mechanisms employed by the tumor cells. Tumor cells were found to express or upregulate ligands of checkpoint receptors, immune regulatory molecules crucial to self-tolerance present on T cells.[78] This abrogated T-cell activation, resulting in the inability of T cells to kill tumor cells or adequately penetrate the tumor. By blocking immunologic checkpoint pathways, anti-tumor T-cell functions could be restored. Checkpoint inhibitors (CPIs) are blocking antibodies directed against molecules involved in immunologic checkpoint pathways.[78] Examples of such molecules to which blocking antibodies have been developed are programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein (CTLA-4) , T-cell immunoglobulin, mucin-domain containing-3 (TIM-3) and lymphocyte activation gene 3 protein (LAG3). As a consequence the prognosis of (metastasized) melanoma has improved markedly over the past years.[79] This finding was awarded the Nobel Prize for Medicine in 2018. Meanwhile, these principles have be shown to be applicable in many more (solid) tumor types.

B cells in tumor immunology

Since the introduction of CPI therapy for solid tumors, the knowledge on tumor immunology has expanded vastly. The contribution of other immune cell types as well as the importance of the tumor microenvironment has been recognized in both pro- and anti-tumor immune reactions. Recently, more attention has been generated for the role of B cells in tumor immunology and response to immunotherapy. In a number of solid tumors such as colon and ovarium carcinoma, infiltration of B cells strongly correlated with infiltration of T cells, and colocalization of the two was associated with a good prognosis.[80–82] In melanoma patients, B-cell depleting therapy lead to a decrease of CD8+ T cells in the tumor sites.[83] Ongoing research is providing new insights on the presence of tertiary lymphoid structures (TLS), lymphocyte infiltrates in tumors consisting of B and T cells organized in lymph node-like structures, and their relevance in tumor immune responses.[81,84] The presence of B cells in TLS has been found to be an important positive predictor for response to CPIs.[85–87] Data on a first comprehensive dissection of the tumor microenvironment of AML was recently published, distinguishing also in AML immune-excluded and immune-infiltrated profiles.[88] While the analysis focused predominantly on T cells, colocalization of T and B cells was reported in the immune-infiltrated leukemias, in contrast to the immune-excluded subtypes.[88] Whether the presence of B cells here has prognostic value or is modulated upon therapy, like is seen for solid malignancies, remains to be determined.

In hematologic malignancies there are findings suggestive of a contribution of B cells to the anti-tumor immune response prompted by allogeneic HCT and DLI. In patients with multiple

myeloma, chronic myeloid leukemia, or AML antibodies specifically reacting with antigens expressed by their respective tumor type have been detected.[89–91] Antibodies against BCMA, identified in the serum of multiple myeloma patients have paved the way for development of therapies targeting BCMA.[89] Similarly, in three AML patients donor-derived B cells targeting snRNP200 were discovered, antibodies derived from these B cells had anti-leukemia functionality in that they were capable of inducing death of AML blasts.[91]

In short

Advances have been made in recent years and a number of promising (immune)therapeutic agents for AML are under investigation, but generally there is a paucity of new targets for the treatment of AML. A major obstacle is the fact that most targets are also expressed by non-malignant hematopoietic progenitor or mature hematopoietic cells. The substantial non-relapse mortality of allogeneic HCT, and the high relapse rate combined with the absence of real therapeutic options for relapsed leukemia, warrant the need for additional, more effective and less toxic therapeutic options. Examining the B-cell immune response of patients with high risk AML that have responded well to therapy could open up a way to identify new targets for (immuno)therapy.

Outline of this thesis

The scope of this thesis includes how B cell responses of patients who have successfully been cured of their cancer can be applied as source for new immune therapies. In patients who experienced a potent antitumor response, as indicated by their survival after immunotherapy despite slim odds, B cells may have contributed to the immune response that controlled the tumor. Identifying said B cells might prove useful in identifying novel tumor-associated targets for development of future therapies. The thesis focusses on, but is not limited to, the place B cells and antibody responses take in antitumor immunology in the context of allogeneic HCT and the application of patient-derived antibodies in acute leukemia. In addition, this thesis touches upon whether expression of tumor-associated antigens recognized by such antibodies are restricted to the tumor type of the patient the B cell was discovered in.

This chapter, **chapter 1**, provided a brief introduction in the biology of acute leukemia's, the treatment thereof, in particular allogeneic HCT, and the current state of available immunotherapy and other targeted therapies. In **chapter 2** we have examined the outcome of patients treated in the Amsterdam UMC that experienced a relapse of AML or myelodysplastic syndrome after allogeneic HCT. We confirmed the observation that relapse after allogeneic HCT has a dismal prognosis and describe that in individual cases tapering of immunosuppressing therapy can invoke a graft versus leukemia immune response. **Chapter 3** describes the case of an AML patient with severe GvHD. Upon treatment with the B-cell depleting agent rituximab, his GvHD is resolved, however, this happens at the expense of relapse of the leukemia. An AML-reactive B cell is found when examining B cells of the patient prior to rituximab treatment and AML relapse. **Chapter 4** reports on the identification of an antibody directed against a sialylated variant of CD43 (CD43s) by searching the B cells of a patient in long term remission of AML following allogeneic HCT. We show that CD43s is widely expressed on AML blasts and in **chapter 5** we discuss an additional way the CD43s-targeting antibody can be used to target AML by engineering it into a bispecific T-cell engager. CD43s-expression is not limited to AML, but also present in melanoma, and as such can also serve as a new way to target melanoma, as we describe in **chapter 6**. In **chapter 7** we show how an antibody, derived from a patient cured

of stage IV melanoma, can safely target CD9 expressed on precursor B-ALL in the absence of CD9-mediated platelet aggregation. **Chapter 8** is a discussion of antibody and B-cell responses observed in patients after allogeneic HCT. It considers how this can contribute to a better understanding of graft-versus-leukemia effect and in the identification of (new) targets for immunotherapy of cancer. **Chapter 9** includes a summary of the thesis followed by an epilogue containing reflections on findings presented in this thesis, as well as contemplations on related subjects.

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