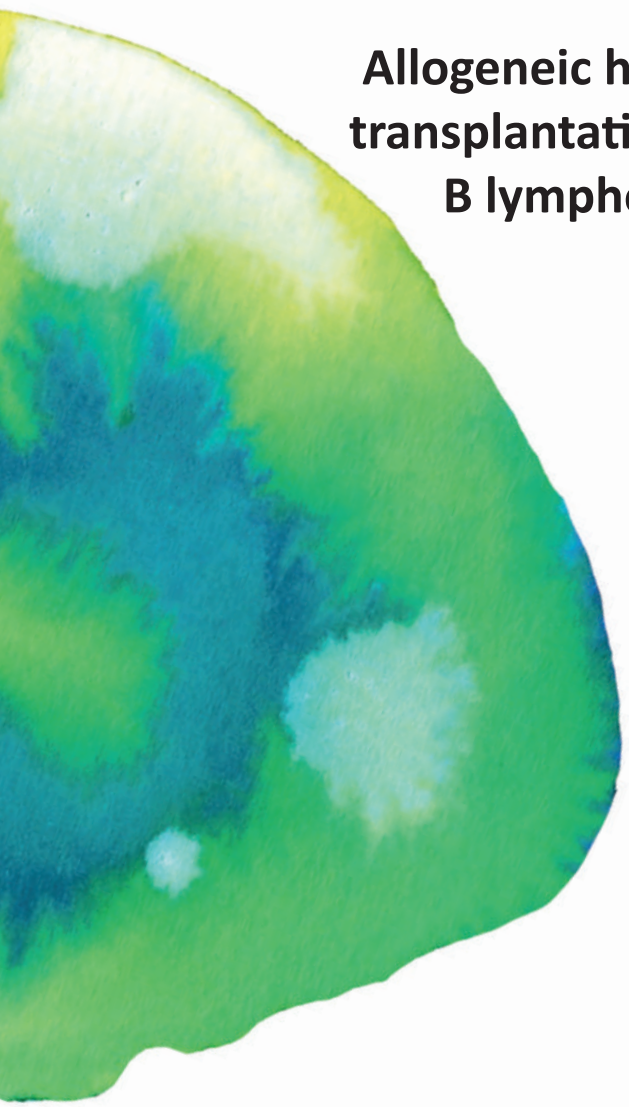
Allogeneic hematopoietic stem cell transplantation as immunotherapy

Gillissen, M.A.

CHAPTER 7

English summary and epilogue:

Allogeneic hematopoietic stem cell transplantation as immunotherapy: B lymphocytes versus leukemia



M.A. Gillissen

Research described in this thesis focuses on the role of B lymphocytes in graft versus leukemia responses following allogeneic hematopoietic stem cell transplantation (HSCT) as treatment of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). **Chapter 1** provides an overview on the current knowledge of AML, MDS, the immune system and allogeneic immune responses. AML and MDS are myeloid malignancies with a poor prognosis. Treatment involves chemotherapy, in fit patients followed by an allogeneic HSCT. The purpose of allogeneic HSCT is to induce an immune response against residual malignant cells, referred to as graft versus leukemia (GvL) response.

In **chapter 2** the intricate relationship between donor B lymphocytes and the GvL response is exemplified by a case report of a patient who developed AML relapse after he had been treated with rituximab, a B cell depleting agent. This patient had an early relapse of AML, less than 3 months after allogeneic HSCT. Rapid tapering of immunosuppressants re-induced complete remission of AML via a potent GvL response but caused severe graft versus host disease (GvHD) of the skin that was steroid-refractory. Upon depletion of B cells with rituximab, GvHD resolved as did the GvL response. Blood was drawn at the time of maximal GvL response, where we demonstrate the presence of AML specific antibody-producing B cells. This anti-tumor B cell effect was nullified by rituximab treatment, and these data suggest an important contribution of B cells in the GvL responses that developed in this patient.

Following up on this finding we searched the B cell repertoire of five high-risk AML patients with durable remission after allogeneic HSCT, as described in **chapter 3.** For this we isolated B cells from the peripheral blood of patients, 1-2 years after allogeneic HSCT. B cells were immortalized by transfecting the cells with Bcl-xL and Bcl-6, generating plasmablast-like cells that express the antigen receptor and produce antibodies in the supernatant.[1] Screening of supernatants of these B cell clones for binding to AML cell lines and absence of binding to non-hematopoietic cells and tissues allowed us to identify 16 patient derived clonal B cell lines producing antibodies that recognized antigens expressed on the cell surface of AML cells, but not on normal hematopoietic and non-hematopoietic cells. Target identification revealed the U5 snRNP200 complex as the target of 7 out of these 16 antibodies. The U5 snRNP200 complex is a large multi-protein that is a component of the spliceosome. In normal cells the U5 snRNP200 complex is expressed in the cytoplasm and nucleus, but in AML it is exposed on the cell membrane of cells. Strikingly, U5 snRNP200 complex specific antibodies were cytotoxic: they induced death of AML cells, in the absence of cytotoxic leukocytes or of complement. Cell death occurred in a non-apoptotic way, via a Fc-tail dependent mechanism that could be blocked by stabilization of the actin skeleton using cytochalasin D. U5 snRNP200 complex

antibodies were cytotoxic in vitro and in vivo; treatment of mice with AML (growing as a solid tumor under the skin) with U5 snRNP200 complex-specific antibodies led to significant tumor growth inhibition. U5 snRNP200 complex specific antibodies were detected in four out of five AML patients with durable remission after allogeneic HSCT tested, but not in multiple myeloma patients who received an allogeneic HSCT or in healthy individuals. Thus, donor derived U5 snRNP200 complex-recognizing AML-specific antibodies may contribute to GvL responses.

To quantify non-apoptotic cell death induced by AML antibodies we needed a reliable and fast method. We developed a novel high throughput method, which we describe in **chapter 4**. In this assay the number of living cells instead of dead cells are quantified by adding a fixed number of unlabeled calibration beads to the analysis. Using this method, we found EC50 values to be highly reproducible and considerably lower compared to EC50 values obtained by conventional assays, demonstrating the high sensitivity of this assay**.**

In **chapter 5** we describe the characterization and target identification of one other of the 16 antibodies identified in chapter 3. AT1413 is an antibody obtained from a patient with high-risk AML who developed a lasting GvL response after allogeneic HSCT. The target recognized by AT1413 is a novel, sialylated epitope on the membrane protein CD43, that we named CD43s. Strikingly, we found that CD43s is overexpressed by all WHO 2008 types of AML and MDS. Over 60 newly diagnosed AML and MDS patient samples were tested and AT1413 bound to all of them. When we incubated AML cells with AT1413, leukemic cells were killed by antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Moreover, AT1413 was highly efficacious against malignant cells in an AML mouse model. Mice with a human immune system (HIS mouse) were inoculated with human AML, and treated with AT1413 or control antibody. AT1413 completely abolished AML outgrowth, without affecting healthy human hematopoietic cells. Given the broad expression of CD43s, and the efficacy of AT1413 in vivo we consider AT1413 as having high potential as a therapeutic antibody.

In **chapter 6** we placed the role of B cells in GvL responses after allogeneic HSCT in the broader perspective of tumor immunology. Historically, attention has been focused on the role of T cells in anti-tumor immune responses. T cells recognize antigens in a human leukocyte antigen (HLA)-restricted way, and manipulation of T cells to enhance tumor responses is either non-specific, by releasing immune-inhibitory mechanisms for example by blocking checkpoint inhibitor proteins such as PD-1, or very laborious, via ex vivo isolation, manipulation and cloning of tumor specific T cells that have to be tailor made for each individual patient. We argue for more attention to the contribution of B cells and antibodies in tumor immunology. The

major advantage of B cells is that they respond to antigens in a non-HLA restricted manner. Our studies demonstrate that thorough screening of B cells and their antibody responses against tumors can help identify novel tumor targets and highly specific antibodies that can then be further developed into novel therapies that are off-the-shelve available. In addition, by identifying AML-specific antibodies that do not bind to non-malignant cells we demonstrated that GvL responses are distinct from GvH responses. This is important as it answers to one of the oldest questions in the field of allogeneic HSCT immunology.

Epilogue

While allogeneic HSCT can be highly effective against aggressive malignancies such as AML, it is a non-specific approach that is associated with high morbidity and mortality due to GvHD and opportunistic infections. Novel therapies, more specifically aimed at the malignant cell and with significantly fewer side-effects are highly anticipated. Thus far, the most effective and least expensive immune modulating therapy that is off-the-shelve and readily available to patients independent of HLA-type are monoclonal antibodies. Targeting lymphoma's expressing CD20 with the 'naked' (non-conjugated) antibody rituximab,[2] multiple myeloma with the CD38 antibody daratumumab,[3] or lymphoma's expressing CD30 with the antibody-drug conjugate brentuximab-vedotin[4] have proved very successful. Crucial for the success of antibody therapy is the specificity of the target antigen and for many tumors such antigens have not been identified yet. Our search for AML-specific antibodies has revealed unanticipated AML-specific targets and antibodies that have high potential as therapeutic antibodies. The future will learn whether these antibodies will prove as effective as the above-mentioned antibodies in the treatment of acute myeloid leukemia.

References

1. Kwakkenbos, M. J. *et al.* Generation of stable monoclonal antibody–producing B cell receptor–positive human memory B cells by genetic programming. *Nat Med* **16,** 123–128 (2010).
2. Maloney, D. G., Grillo-López, A. J., White, C. A. & Bodkin, D. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* **90,** 2188–2195 (1997).
3. Lokhorst, H. M. *et al.* Targeting CD38 with Daratumumab Monotherapy in Multiple Myeloma. *N. Engl. J. Med.* 150826140037005 (2015). doi:10.1056/NEJMoa1506348
4. Younes, A. *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N. Engl. J. Med.* **363,** 1812–1821 (2010).