From immune suppression to immune modulation in type 1 diabetes patients

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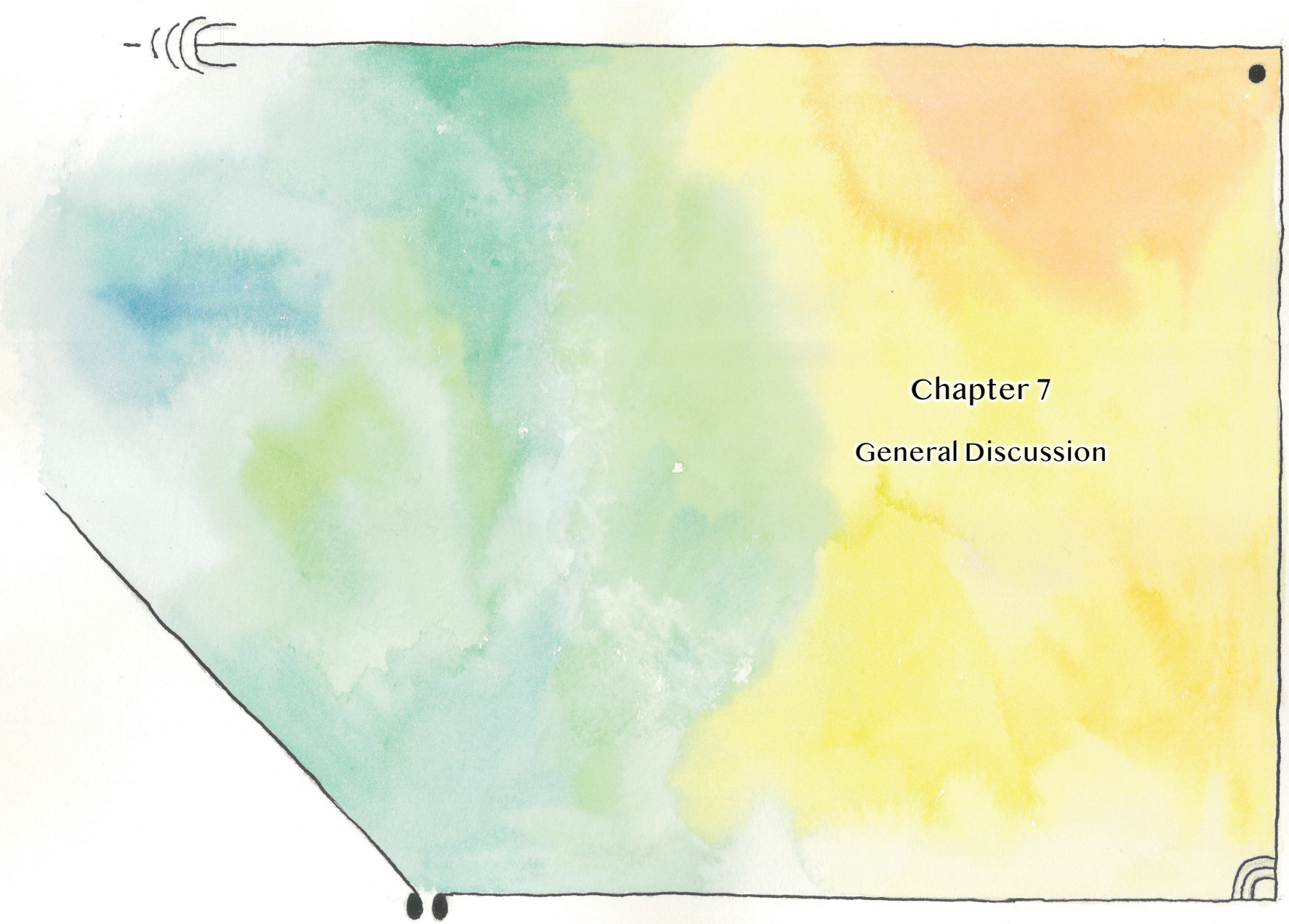
PART IV

DISCUSSION & SUMMARIES



Chapter 7

General Discussion

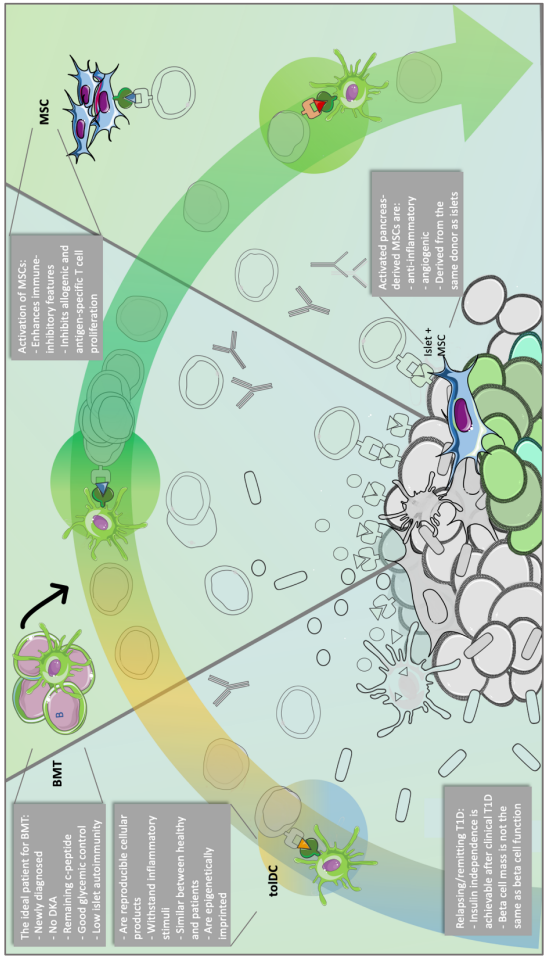
General Discussion

In this thesis, a spectrum of T1D therapies is explored: from immune suppression to immune modulation and finally therapy of the islets of Langerhans (**Figure 1**). In this discussion, five fundamental challenges regarding the development of T1D therapies will be discussed and followed by how I envision the future of these therapies.

Five challenges in discovering and implementing type 1 diabetes therapies

1. Insulin replacement is not the answer

The tragedy and blessing of T1D is that much of the general public thinks it already has a cure, namely insulin. Indeed, without insulin T1D would be a fatal disease. However, T1D Exchange data from 2014 showed that less than one in three patients over 25 years old reached their target HbA1c of 7% or less, and data from 2016 to 2018 suggest no improvements (https://t1dexchange.org/), despite the recent advances in insulin pumps and glucose sensors. Moreover, data from the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) trial demonstrated that even with intensive insulin therapy, long-term fatal complications cannot be completely prevented as small excursions out of target HbA1c range can have long term consequences, referred to as metabolic memory (1). As such, insulin does not modify the silently progressive course of T1D with its many fatal complications, such as cardiovascular disease and nephropathy. Furthermore, the longer the duration of T1D, the more prone a patient becomes to suffering from hypoglycemia unawareness with dangerous severe hypoglycemia episodes (2, 3). Therefore, when one correctly categorizes T1D as a disease with fatal risks and a loss of life-expectancy of more than ten years compared to the general population without T1D (4-6), it is hard to understand that many drugs are developed and approved for other milder auto-immune diseases, but not for T1D. The auto-immune skin disorder psoriasis, for instance, is approved for several anti-TNF-α antibodies, anti-IL-12/IL-23 and anti-IL-17A, all with various side effects including increased incidence of lymphoma (7-9). Similarly, rheumatoid arthritis (RA), an auto-immune disorder affecting the joints, is treated with TNF-α blockers, kinase inhibitors and methotrexate, of which the latter two are anti-cancer drugs (10). Although the former makes the patient more prone to infections, including tuberculosis, and in approximately 35% of patients the treatment is or becomes ineffective (11, 12), still patients and doctors are willing to take that risk. Perhaps the difference in these auto-immune disorders is that they present visible or tangible substrates of autoimmune attack (the skin for psoriasis and the inflamed joints for RA) and additionally causes pain (RA), which is an unambiguous and strong incentive for drug therapy. T1D lacks all of these



easily identifiable or measurable characteristics. Indeed, T1D lacks effective biomarkers to track disease activity, especially in terms of the amount of β cell destruction (13, 14). In a sense, after initial diagnosis, the pathogenesis and progression of T1D becomes invisible and injecting insulin becomes part of the patient's daily routine. Consequently, the urgency for the discovery of additional treatments becomes less obvious.

I propose a revision of the T1D staging system to reinstall this urgency (**Table 1**). At the moment, T1D is divided into four stages, in which stage 3 is clinical diagnosis and stage 4 is long-standing disease (15). The first three stages last up to 15 years in 80% of T1D patients diagnosed in childhood (16), though these stages could possibly last much longer in T1D patients diagnosed in adulthood (>60% of total T1D cases) (17). The last stage, however, could potentially last 50 years. In fact, there is not much information on diagnosing stage 4 T1D. Stage 4 was neither mentioned in the main paper announcing the novel staging system (15); nor in ADA's latest classification of T1D (18). Hence, this last stage begs for reconsideration. After revision, the new stage 4 could coincide with diagnosing the first microvascular complications (micro-albuminuria, non-proliferative retinopathy, peripheral neuropathy) (**Table 1**). This will include around one in three T1D patients after 10 years (19) and almost 90% of T1D patients approximately 20 years after the onset of stage 3 (20). Almost all patients still secrete c-peptide after a mixed meal tolerance test during the 20 years following clinical T1D diagnosis, indicating remaining functional β cell mass (21). Eventually this could distinguish T1D patients that could qualify for interventional immunotherapy from patients that need β cell replacement therapy. However, long-standing T1D has a much longer and divergent timeline than is appreciated in the current 4-stage system. Therefore, I propose to add two more stages.

Stage 5 would start when already-diagnosed complications progress (macro-albuminuria, proliferative retinopathy, diabetic foot disease, or angina pectoris) (**Table 1**), demarcating a transition of eligibility from immunotherapy-focused intervention trials to β cell replacement trials. Within 20 years after initiation of stage 3 almost 15% of T1D patients will be included in this stage because of progression to proliferative retinopathy and 4% because of development of macro-albuminuria. Of course, these percentages are greatly dependent on glycemic control (HbA1c). To illustrate this, 51% and 23% of T1D patients

Figure 1: Take-home Messages of Parts I, II, and III. *In this thesis, five papers describing different therapies have been discussed. Firstly, the relapsing/remitting T1D case report taught us that insulin independence can be reached even after T1D diagnosis. This suggests that beta cell mass is not equal to function and that beta cells could be recovered to secrete insulin again. Secondly, autologous bone marrow transplantation (BMT) resulted in insulin independence and patient characteristics for optimal effect were identified. Furthermore, cellular therapies that were studied were tolerogenic dendritic cells (tolDCs) and mesenchymal stromal cells (MSCs). TolDCs were stable and reproducible cellular products that were similar between healthy and T1D patients, which will expedite its use in the clinic. MSCs proved to be immune inhibitory and antigen-specific. When derived from the pancreas, MSCs could be co-transplanted with islets to improve islet transplantation. Created in Biorender.com.*

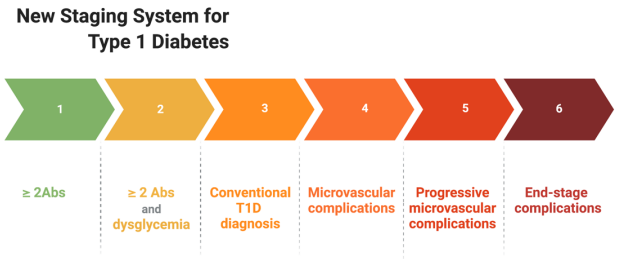


Table 1: Diagnostic Criteria for the Revised Stages of Type 1 Diabetes. *The new staging system for type 1 diabetes has 6 stages in total. The first 3 stages are identical to the staging system as brought forward by TrialNet. Stage 4 is diagnosed once patients show signs of microvascular complications, such as micro-albuminuria, non-proliferative retinopathy, and peripheral neuropathy. Stage 5 is diagnosed when these complications progress into macro-albuminuria, proliferative retinopathy, diabetic foot disease, or angina pectoris, but is not limited to these. Finally, stage 6 is diagnosed by an end-stage complication, such as end-stage renal disease, myocardial infraction, stroke, amputation due to diabetes-related foot disease, or blindness due to advanced diabetic retinopathy. In addition, hypoglycemia unawareness is a diagnostic criteria for stage 6. Created in Biorender.com.*

with a HbA1c of 9,5% and higher progressed to proliferative retinopathy and macro-albuminuria, respectively, after 20 years (20). Finally, stage 6 T1D would be demarcated by the presence of at least one of the end-stage complications such as end-stage renal disease, myocardial infarction, cerebrovascular accident, amputation due to diabetes-related foot disease, or blindness due to advanced diabetic retinopathy (**Table 1**). Besides these vascular complications, patients with hypoglycemia unawareness should be included in this stage, as it is a risk factor for potentially fatal severe hypoglycemia episodes and its incidence increases with duration of T1D (22). Hypoglycemia unawareness is currently one of the eligibility criteria for islet cell transplantation (23). In line with this, stage 6 signifies the further loss of β cell function and urgent need for β cell replacement therapy as a last resort. Up to 5% of T1D patients will have arrived at stage 6 20 years after the onset of stage 3 (20, 24, 25). Contrastingly, as an illustration of patient heterogeneity, approximately half of T1D Medalists (more than 50 years after stage 3) have yet to reach stage 5 (26), though it should be noted that these patients are the exception to the rule (**Figure 2**). More commonly, T1D patients progress through the stages and the majority of mortality is, consequently, caused by T1D related complications, as was shown in a cohort of childhood-diagnosed T1D in the United States. Approximately 90% of mortality was caused by diabetes-associated complications 30 years after the start of stage 3, by which time around 20% of T1D patients had passed away (27, 28). Together, these examples illustrate that progression through the stages can be diverse. The course of T1D might eventually be better coined as rapidly or slowly progressive, in the same vein as how multiple sclerosis is subdivided in different types. In

this way, the new staging system could therefore not only be a better tool to systematize the therapy need related to the different stages of T1D, but also help to discern different time-courses of progression. In the future, these clinical outcomes could be aligned with auto-immune signatures and β cell function, once these tests are widely available. Patients that progress rapidly would likely have less residual β cell function and higher activity of autoimmunity.

Overall, the intent of re-staging T1D is to reinstall the urgency for T1D treatment discovery and implementation beyond insulin replacement therapy, as it becomes clear that insulin, started at stage 3, does not prevent progression towards stage 6 T1D. Hereby, insulin as a sole treatment is dismissed while more incentive is given towards treatments that prevent or delay progression to irreversible end-organ damage (stage 6). This new staging system also encourages the translation of drugs so far most used in T2D to optimize glucose control in stages 3 to 6 T1D and with this minimize complications and revitalize β cells. At the moment, there is still a stigma related to T1D complications, which are often thought of as being the T1D patient's own responsibility (29). Hopefully, this new staging system will stress that progression through the stages is actually the natural course of T1D which even optimal patient effort to manage glycemic control cannot fully prevent. Hence, a new drug is needed to stop this progression.

2. Balancing the risks and benefits of therapy

Chapter 2 shows that reversing T1D is conceptually possible if defined as being insulin independent. After autologous hematopoietic stem cell transplantation (aHSCT), all patients were insulin-free for a sustained period of time with the exception of three patients who had inadvertently received corticosteroids or had developed diabetic ketoacidosis before the therapy (30, 31). The pursuit of insulin independence came at a risk, however. Even though morbidity and mortality after aHSCT has improved to an incidence of <1% over the years, there is still a chance of serious, life-threatening complications (32). When considering new therapies, one tends to forget that T1D is still a deadly disease, with more than 70% of mortality in T1D patients in the first 10 years attributable to acute consequences of hypoglycemia or diabetic ketoacidosis and after 20 years of diagnosis due to micro- and macrovascular complications (27, 33). Yet, the risk of aHSCT has been valued to be unacceptable and therefore this therapy has not gained much interest in the T1D field. In children with acute lymphoblastic leukemia, on the other hand, allogeneic HSCT is the golden standard for therapy with a 90% 5-year survival rate (34). In this sense, there is a need for more debate on the risks that are imposed by T1D itself versus those of an effective therapy. For each patient, the risks of infrequent but acute and sometimes severe therapy-mediated morbidity would need to be weighed against the so far largely unavoidable and higher eventual T1D-related morbidity.

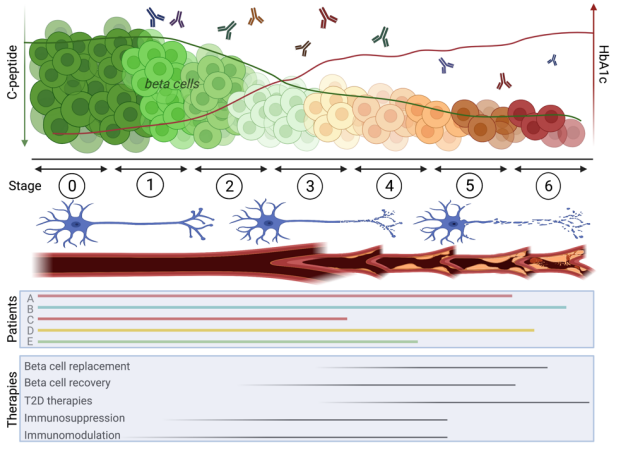


Figure 2: Case Example aided Overview of the Revised Stages of Type 1 Diabetes. *The progression of stages in type 1 diabetes is exemplified by a loss of functional beta cell mass. This is depicted in the top of the figure by a reduction in beta cell mass, a deterioration in function (green are functional beta cells and red are dysfunctional beta cells), an increase in HbA1c, and a reduction in secreted c-peptide. Type 1 diabetes is firstly diagnosed by the detection of two or more autoantibodies in stage 1. In Stage 2 dysglycemia is added, as shown by the increase in HbA1c. After stage 3 (the conventional T1D diagnosis), micro- and macrovascular complications prompt the diagnosis of stage 4 to 6 T1D, as depicted by the deteriorated blood vessels and neurons due to the progression of the disease. Under the graph, a bar graph is shown, in which every bar represents the lifespan of one patient, as an illustration of patient heterogeneity. The length of the bar corresponds to the stage of T1D and the colour suggests slowly- (green) to rapidly- (red) progressive disease. Patient A is a 40-year-old male T1D patient, who was diagnosed with stage 3 T1D at age 9 and quickly progressed to stage 6, ultimately dying of a heart attack. Patient B is a slow-progressor, being diagnosed with stage 3 T1D at age 21 and dying at age 80 due to end-stage renal disease. Patient C is another fast-progressor that sadly died at an age of 16 just after stage 3 T1D diagnosis as a consequence of diabetic ketoacidosis. Patient D diagnosed at 13 years of age with stage 3 T1D steadily progressed through the stages and died of a hospital-acquired pneumonia, when she was hospitalized for a foot amputation at age 69. Finally, patient E is a 72-year-old T1D Medalist only suffering from non-proliferative retinopathy and dying 52 years past stage 3 T1D diagnosis of a T1D-unrelated cause. Under the patient bar graph, another bar graph is shown depicting different T1D therapies. The location and length of the bars align with the stages that would be optimal for implementation of these therapies. Created in Biorender.com.*

Evidently, lowering the risks of T1D therapies like aHSCT will result in an easier choice. In addition, better patient-specific predictors for both disease course and treatment associated morbidity would make these decisions more manageable (**Chapter 2**). For example, all aHSCT patients had some period of insulin free survival, but patients with low baseline autoreactive islet-specific T-cells clearly had more benefit than patients with high frequencies of these cells (31, 35). More intensive analysis of characteristics of responders versus non-responders could give us insight into the predictors for treatment effect or

vice versa risk factors for failure. This will eventually enable risk factor-based selection of patients for a specific therapy. Evidently, this becomes increasingly important as the therapy becomes more toxic.

3. Patient heterogeneity in therapeutic response

Patient heterogeneity was previously touched upon with regard to risk assessment. The higher the risk of a treatment, the more important it becomes to select the right patient population that would benefit from the treatment. aHSCT is undoubtedly an example of that (**Chapter 2**). Patient heterogeneity is, however, a crucial point for T1D therapies in general. The extent of this heterogeneity has become clear only in recent years. The variable therapy success rates observed in subgroups of patients could be argued to reflect the heterogeneity seen in the pathophysiology of patients. Recently, this has been coined as the different 'endotypes' of T1D (36). Endotypes could be based on different T1D characteristics, such as age of onset, HLA-type, autoantibody response and response to therapy. Overall, this means that many therapies previously determined to be ineffective might indeed have had efficacy in certain subgroups of patients unidentified at that point of time, but failed to show an effect in the total study population (37). In this way, we might have inadvertently dismissed many drugs with T1D endotype specific effectiveness.

One other example of patient heterogeneity is our case of remitting T1D after intravenous immunoglobulin (IVIG) treatment (**Chapter 3**). IVIG has been investigated in a randomized controlled trial as a therapy to treat T1D in adults and children without success (38). Yet, one of our patients repeatedly experienced resolution of her T1D after IVIG treatment, exemplified by periods of insulin independence. This summons up the question whether there are more patients similar to her that could benefit from this treatment but have not yet been identified. Indeed, some smaller, older studies did find a decrease in insulin requirements after IVIG treatment in children and newly diagnosed T1D patients (39, 40). It should be noted, however, that our patient was unique with regards to several comorbidities such as chronic inflammatory demyelinating polyneuropathy (CIDP) (which was the indication of IVIG therapy) and Graves's disease, which would likely exclude her from participation in most immune intervention trials. Perhaps the reason she did respond to this treatment, though, is because her endotype is more auto-antibody driven than the 'typical' T1D patient, as she has auto-antibody driven inflammatory comorbidities. One case report showed that a child with T1D and high titers of insulin antibodies had improved glucose control after IVIG treatment, although insulin dose did not decrease (41). Reduction in autoantibody titers after IVIG was replicated by another independent case study (42). In general, the design of current trials still tends to focus on drug effectivity in more homogeneous cohorts while analysis of rare responder (e.g. with

additional comorbidities) instead gives more insight and credit to possible endotype specific effectivity of new therapies.

In summary, instead of posing a challenge, embracing patient and endotype heterogeneity in designing trials could be the savior of T1D therapies.

4. Identification of the T1D patient for preventative strategies

With the advent of the improved diagnostic criteria for T1D and new staging models, T1D patients now include persons with autoantibodies with and without dysglycemia before the conventional T1D diagnosis (15). This model was brought into being to facilitate earlier treatment of T1D in clinical trials. This is exemplified by the success of the teplimuzimab trial, which studied stage 2 T1D patients and showed the delayed onset of stage 3 T1D (43). A caveat to this staging model is that up to 20% of T1D patients test autoantibody negative at diagnosis, though more than half will seroconvert in subsequent years (44, 45). It is yet unclear whether these patients did have detectable autoantibodies at some point before the start of stage 3. Inclusion of pre-stage 3 T1D could have improved the outcomes of trials such as autologous hematopoietic stem cell transplantation that was most successful in patients treated shortly after diagnosis and with sufficient β-cell function (**Chapter 2)**. Identification of stage 1 and 2 T1D patients forms a challenge, however, as these stages are asymptomatic and requires population-based screening. In addition, screening without pre-selection is costly and difficult to defend unless the importance of this approach is justified by shown improved treatment efficacies over the traditionally studied stage 3 T1D group. On the other hand, screening could prevent hospital admissions and thereby even lower eventual health care costs. Indeed, the Fr1da study showed that autoantibody screening of the general population reduced diabetic ketoacidosis at clinical diagnosis of T1D from 16-58% to 3.2% (46-49). Currently, screening is performed by autoantibody detection, but could be preceded by pre-selection on family history; however, only approximately 15% of new cases have a family history of T1D (50). Finally, screening could be guided by HLA genotypes, as 95% of Caucasian T1D patients have the highest HLA-risk haplotype DR3/4 (51, 52). Of note, children with high genetic risk of developing T1D had detectable autoantibodies within the first three years of life so screening should start as early as three years of age (53-55). For such HLA dependent (pre-) screening, HLA typing should be generally available e.g., as part of heel-prick program for newborns.

In conclusion, screening programs could identify T1D patients when they still have sufficient β cell mass and before they necessitate insulin treatment. This facilitates timely enrollment in clinical trials to counter the harmful autoimmunity leading to stage 3 T1D and, concomitantly, reduce possibly fatal early complications of the disease by educating patients and caretakers (56).

5. The goal of T1D treatment

The final challenge in realizing a therapy for T1D is re-considering the goal of a treatment. A cure for T1D is obviously the ultimate goal, but this should not stand in the way of accepting therapies that would halt disease progression or prevent complications and thereby increase quality of life. Therapies that minimize symptoms and reduce the risk of complications are currently also the only immunotherapies on the market for other autoimmune diseases. T1D should not be an exception. Until there is a cure, such disease-modifying therapies could be of immense value to T1D patients. Thus, therapies that could slow the decline of c-peptide secretion by one or many years should not be withheld from T1D patients, especially since we now know that even barely detectable c-peptide levels show a clearly reduced risk of complications and less hypoglycaemic episodes in the disease course (57-60). Therefore, treating T1D rather than curing it, by chronic or intermittent therapy, should be offered.

To conclude, while we are working on finding a cure for autoimmune disease, the goal of T1D treatments at present should be to minimize progression and long-term complications rather than fast-forwarding to a cure only.

Next generation type 1 diabetes therapies

The next generation of T1D therapies would ideally take the previous points into account (**Figure 3**). Firstly, identification of different stages of T1D should guide our choices for trial inclusion and eventual treatment selection, as will be discussed in the forthcoming paragraph (**Figure 2**). In addition, the impact on complications and quality of life will be an important factor influencing which therapy is worth pursuing. Furthermore, a combination of different therapies is likely needed in order to have clinical success (61, 62). Caution should be taken, however, as combining therapies that target multiple immune pathways might pose a risk of inadvertent immunosuppression (61).

Personalized Medicine

Depending on the stage of T1D, various combinations of therapies could be suggested. In the earlier stages (1-2) with still sufficient β cell mass, the emphasis could lie on the anti-inflammatory response, whereas in later stages (3-6) β cell revival or replacement therapies could become more critical (**Figure 2**). Other important factors, besides the patient's stage of T1D need to be taken into account. For instance, patients diagnosed in childhood display a more aggressive immunophenotype than patients who are diagnosed in adulthood and are less likely to have remaining c-peptide secretion a decade after clinical diagnosis (63, 64). Thus, children in stage 1 or 2 might benefit from more aggressive immunosuppression, whereas adults in the same stage could suffice with a

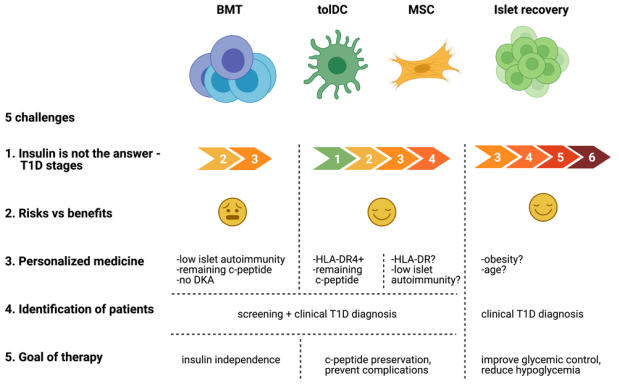


Figure 3: The Five Challenges of Implementing Type 1 Diabetes Therapies. *The preceding chapters have discussed five challenges of implementing T1D therapies. The table above summarizes each challenge as it applies to different therapies. The first column depicts autologous bone marrow transplantation (BMT), the second tolerogenic dendritic cells (tolDCs), the third mesenchymal stromal cells (MSC), and followed lastly by islet recovery therapies. In the first row, the T1D stages are depicted as was shown in Table 1. In row two, the risks versus benefits are illustrated by emoticons. The anxious emoticon in the first column represents a situation in which the risks might outweigh the benefits, whereas the smiley emoticon represents a situation in which the benefits likely outweigh the risks. Created in Biorender.com.*

milder immune inhibiting therapy. Besides the stage and age of the patient, one could select a therapy on the detection of certain autoantibodies, T-cell autoantigen reactivity, and HLA-type. To illustrate, preliminary data from the GAD-alum study suggested that its efficacy was dependent on HLA type (65). Besides, there have been indications that certain antigen therapies were more effective when patients had higher autoantibodies against the tested antigen at baseline. For instance, T1D development was delayed by oral insulin in a subgroup of patients with high insulin autoantibodies (66).

To summarize, it is important to capture the target population in a clinical trial by designing appropriate inclusion and exclusion criteria. The era of treating T1D as a singular disease is past. Patient heterogeneity needs to be embraced to unveil the potentially variously different successful treatments for different T1D endotypes.

Cellular Immunotherapies

Cell therapies are almost by definition personalized. As the majority of this thesis includes cellular immunotherapies, these will now be discussed in more detail. Tolerogenic dendritic cells and mesenchymal stromal cells are of interest as they by themselves

already combine two treatment modalities, namely as antigen-presenting cells but additionally as cells with general anti-inflammatory properties (**Chapter 4 & 5**). Conceivably, antigen-specific therapy would be most beneficial when started earlier in the disease process (stages 1 or 2) before considerable antigen spread occurs (**Figure 3**). This is substantiated by the GAD-alum trial, which showed a slowed decrease in fasting c-peptide four years after cessation of therapy only in T1D patients in the first 6 months of diagnosis, but not in longer standing T1D (67). This suggests that earlier enrollment (stages 1 or 2) could result in improved outcomes.

Tolerogenic Dendritic Cells

Thus far, tolerogenic dendritic cells were shown to be safe and feasible in a phase one trial in primarily stage 4 T1D patients (68). Currently a phase two trial is being planned which will include T1D patients with remaining c-peptide secretion, mimicking a pre-stage 3 situation. Indeed, the best target population for tolDC therapy will likely be stage 1 or 2 T1D patients and stage 3 or 4 T1D with remaining c-peptide secretion (**Figure 3**). TolDC therapy could be followed by anti-CD3 antibody therapy in patients with high auto-autoimmune signatures after a sufficient amount of time, as to not intervene with the beneficial effect of tolDC therapy on Tregs.

Important and indispensable for clinical translation, tolerogenic dendritic cells proved to be stable cellular products in terms of their phenotype and function (**Chapter 4**). The clinical background of the donor, either healthy or with type 1 diabetes, did not change the phenotype, transcriptome, or methylome of tolDCs. Furthermore, mature tolDCs remarkably resembled immature tolDCs with regards to their epigenetic profile, substantiating the claim that tolDCs are locked in a semi-mature state. As methylation is seen as a stability marker, our findings provide confidence that the use of these tolDCs as a cellular therapy constitutes a low risk of their conversion into an inflammatory phenotype. Besides immune-related genes, several T1D risk genes showed to be changed in vitamin D3-(VD3) treated tolDCs when compared to inflammatory DCs, both on a transcriptional and epigenetic level. This could give insights why VD3 supplementation early in life was shown to decrease the chance of developing T1D, as it might offset the T1D genetic risk profile (69, 70). Vitamin D is a pleiotropic hormone, having roles in calcium homeostasis, bone metabolism, and immunity. Immune cells, especially DCs, express VD receptors and the enzyme 1α-hydroxylase that converts vitamin D to its active form VD3, signifying an important role of VD3 in DCs specifically (71). As it is known that VD3 is decreased in T1D patients (72), tolDC therapy may be seen as a specific supplementation of VD3 to one of the cells it acts upon.

Many of the advantages of using tolDCs as a cellular therapy could simultaneously be seen as disadvantages and thus are two sides of the same coin. Several of these need to be discussed.

Firstly, tolDCs pulsed with a diabetogenic peptide is an antigen-specific therapy. In this way, the therapy provides a more targeted immune modulation compared to classical more general immune suppression. On the one hand, this reduces the risk of infection and of cancer compared to general immune suppression, but on the other hand, this targeted immune intervention might not be effective enough to counter the multi auto-antigen directed auto-immune process leading to T1D. Consequently, tolDCs might have to be combined with other immunomodulatory therapies, as proposed previously, for instance, with concomitant Treg infusion (73). The functionality of tolDCs, however, might be altered if produced after the administration of another immunosuppressive therapy. Furthermore, the C19-A3 peptide with which we pulsed tolDCs is a peptide epitope of proinsulin that was found to be well-tolerated and safe, both as a peptide therapy and when presented by tolDCs (68, 74, 75). It has currently only been tested in HLA-DR4 patients, however, thus limiting the number of patients that could benefit from this therapy. Therefore, other peptides need to be examined in order to broaden the patient population eligible for such therapies. Any autoantigen should always be tested with caution, as it might result in antigen-dependent immune activation, but especially these with post-translational modifications, such as defective ribosomal products and hybrid insulin peptides (76, 77). Indeed, treatment of multiple sclerosis (MS) patients with a myelin altered peptide ligand caused exacerbation of MS (78).

Secondly, tolDCs are the patient's own cells, which is advantageous as there is no risk of rejection. However, the functionality of these cells could be affected by suboptimal glycemic control. Our tolDCs from T1D patients did not differ from tolDCs from healthy controls, but our T1D patients were selected to have an HbA1c of less than 8%. A different group, on the other hand, found that tolDCs produced from T1D patients with poor glycemic control (mean HbA1c 10.2%) were less tolerogenic, albeit their tolDCs were produced with vitamin D2 as opposed to VD3 (79, 80). If these results would hold true for our tolDCs, this would limit the target group of tolDCs to patients that are successful in managing their blood glucose, which is approximately 30% of adult T1D patients in the United States and less in adolescents and children (https://t1dexchange.org/). This problem would be solved, however, if indeed the target population would be patients with stage 1 or 2 T1D, as by definition these patients would have a HbA1c of < 7%.

A more relative disadvantage of tolDCs is that patients need to undergo a lengthy leukapheresis process (3-4 hours) in order to retrieve blood cells needed for production of this cellular therapy, which changed the T-cell responses requiring some cases several

months to recover to baseline levels (68). The complete production procedure does not only hamper a speedy implementation of therapy, but also necessitates a laboratory that is equipped and specialized to GMP produce such cells. However, the logistic and clinical burden of tolDCs shows to be moderate, at most, compared to other immunotherapies, such as anti-CD3, which requires at least one set of two weeks of intravenous treatment (43, 81). Finally, an inherent problem with cellular therapies is that the cells have a limited life span and likely also a time limited effect necessitating repeated administration. TolDCs, however, have shown to confer a legacy effect by infectious tolerance and linked suppression, thereby possibly circumventing this problem (82).

In conclusion, tolerogenic dendritic cells are attractive as antigen-specific therapy and have proven to be safe and feasible in T1D patients. Next, a phase two clinical trial should investigate whether C19-A3 pulsed tolDCs are also effective in preserving c-peptide. In the future, other peptides should be tested, in addition to combinations with other immunotherapies, with the best effectivity expected in early stage T1D patients.

Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) can be used as an antigen-specific therapy, similarly to tolDCs (**Chapter 5**). We showed that MSCs can be safely activated by pro-inflammatory cytokines to express HLA class II and thereby present antigens (**Figure 3**). This is advantageous as MSCs were already investigated as a treatment for T1D with regards to their combined anti-inflammatory and regenerative potential. MSC therapy preserved c-peptide secretion in recent-onset T1D patients (83). Furthermore, MSCs could be used as an off-the-shelf allogeneic therapy, as risk of rejection is limited because of their hypo-immunogenic nature (84). This facilitates quicker usage, although batch-to-batch variability could be problematic and universal quality control criteria should be implemented.

Similar to tolDCs, disadvantages of MSCs are their costs and the complicated logistics of production and administration. Therapies with living cells in this respect remain intrinsically variable and have to be produced by trained personnel and used in a timely fashion. In the foreseeable future this intrinsic complexity will limit their implementation to the more developed countries. Alternatives for cellular therapies could be nanoparticles or extracellular vesicles. These have less variability as they are not complete cells, but can still relay antigen-specificity (85, 86).

Islets as target of type 1 diabetes therapies

The final report in this thesis touches upon the importance of also engaging the islets of Langerhans in our efforts to treat T1D (**Chapter 6**) (**Figure 3**). Mesenchymal stromal cells can be used simultaneously as immune- and islets supporting agents. In the context of

islet transplantation, these could reduce the amount of islet transplantations necessary by improving the function of the transplanted islets. Besides improving islets for transplantation, islets within a T1D patient could benefit from MSCs or other β cell therapies as well (83). After all, β cell mass is not always reflected into function, as was seen in our case report of relapsing / remitting T1D (**Chapter 3**). This case of T1D alternated functional β cell sufficiency with phases of insulin dependency. Currently, we merely have measures of functioning β cells, namely c-peptide secretion, but as illustrated by our case report, remaining β cell mass might be sufficient, if insulin secretion can be stimulated again. One study showed that 73% of long-standing T1D patients still secreted low levels of c-peptide after a mixed meal stimulation test (21) and another study showed that 58% of T1D patients had residual β cells at autopsy (87). These patients could in theory benefit from approaches that revive these β cells to produce insulin again (21, 88). Most of these so called β cell recovery strategies at the moment target the GLP-1 pathway. Liraglutide, for instance, is a GLP-1 analogue and was shown to significantly reduce HbA1c rates in T1D patients compared to placebo, when combined with insulin (89-92). In addition, it significantly reduced mean body weight by several kilograms depending on the dose (91). Subgroup analysis showed that patients with residual c-peptide secretion had a better clinical outcome than patients with no c-peptide secretion left, suggesting that T1D with endotypes in stage 3 and further with residual c-peptide secretion that are also overweight would benefit most from this therapy. Once c-peptide levels are no longer detectable, strategies to replace β cells or revive the existing dormant β cells could be advised, though clinical translation of this idea is still challenging (93).

A liaison between islet, immune and stromal cell therapies

This thesis illustrates a journey from general immune suppressive therapies towards more islet-specific immunomodulation in T1D. This journey does not occur on a one-way street, however. The take-home message of this thesis, then, is neither that immune suppression per se is flawed nor that antigen-specific immunomodulation is the sole answer to cure all T1D patients. Rather, optimal therapy might likely be a combination of controlled immune suppression and functional antigen-specific immunomodulation capable to protect β cells. Dissecting the endotypes in T1D will help us guide which end of the therapy spectrum is the best fit for each specific patient. The need and type of islet-targeted therapy will furthermore also be determined by the stage of T1D. Above all, the T1D field will benefit from acknowledging that apart from finding a cure, therapies that successfully halt or slow down T1D progression and minimize its long-term complications are additionally worthy to pursue.

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