



Immunotherapy of type 1 diabetes – How to rationally prioritize combination therapies in T1D

Tobias Boettler, Matthias von Herrath*

La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

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ABSTRACT

In type 1 diabetes, insulin producing pancreatic β -cells are attacked and destroyed by autoreactive T cells, which causes major impairments of blood glucose metabolism and finally development of life-threatening complications. Currently, the treatment of this devastating disease is based on the substitution of insulin and thus can be considered palliative. Curative treatment approaches by contrast need to target the underlying causes of disease development: in this case, the autoreactive immune system and the loss of active β -cell mass. In recent years, several clinical trials have been performed studying the effects of diverse immunomodulating agents in order to halt the autoreactive immune response or finding paths to repopulate β -cell mass that could restore euglycemia. While some of the treatments showed remarkable outcomes, most of the studies failed to improve the course of disease. The reason might be that none of the candidates currently under investigation are potent enough at tolerable dosages to hold the key for the cure. Subsequently, the idea of combining defined substances has evolved in order to detect synergistic effects and improve the strength of the therapeutic potential. Observations from mouse models and clinical experience from various other diseases where combination therapies often constitute the standard treatment strongly support this hypothesis. Here, we discuss promising monotherapeutic approaches, summarize current clinical trials and propose a rationale on how to prioritize different combinations of treatments.

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1. Introduction

Type 1 diabetes (T1D) affects more than 20 million people in the United States. Complications of T1D include heart diseases and strokes, high blood pressure, renal failure and ketoacidosis (DKA) and make diabetes rank as a major leading cause of death in northern America and Europe. Type 1 diabetes develops as a consequence of an autoreactive immune system where T cells attack the β -cells in the pancreas that are responsible for insulin production and maintenance of euglycemia. The reduction of β -cell mass leads to a lack of insulin and thereby loss of blood glucose control.

Currently, the treatment of T1D is based on the replacement of insulin. The development of new forms of insulin has made remarkable progress over the last decades allowing to better simulate the fluctuations of endogenous insulin production and thus leading to a better blood glucose metabolism and improvement of long term blood glucose parameters such as Hemoglobin A1c (HbA1c). However, acute and secondary complications are still inevitable and account for major impediments on the quality of life and an increasing need for medical care as disease progresses. Moreover, maintenance of euglycemia requires close monitoring of blood glucose levels and

insulin injections, which amounts to approximately 1000 capillary punctures and just as many insulin injections per year. These numbers convey the deep impact of this disease on affected patients, especially when we take into account that more than half of the newly diagnosed patients are children. This situation underlines the need for new therapeutic approaches.

In order to develop novel strategies to treat T1D, a detailed understanding of its etiology is of critical importance. The foundation of current research is a concept where occurrence of T1D is a result of genetic predisposition and mostly unknown environmental factors. The genetic variations leading to immune mediated diabetes can be categorized into monogenic and polygenic disorders. Mutations of the transcription factors FoxP3 (forkhead box protein 3) or AIRE (autoimmune regulator gene) as monogenic causes for diabetes are rare conditions. The vast majority of cases are polygenic. Genome Wide Association Studies (GWAS) revealed over 20 genes that could be linked to disease susceptibility. Certain HLA haplotypes and mutations in the genes encoding insulin, PTPN22 (protein tyrosine phosphatase, non-receptor type 22, a molecule involved in the T cell receptor signaling pathway) and Interleukin-2 receptor are most prevalent in T1D affected individuals [1]. While knowledge of the genetic background of T1D has been multiplied in recent years, there is still no sweeping concept of the environmental factors that are involved in the development of the disease [2]. However, increasing evidence suggests that diverse microbial pathogens, particularly a

* Corresponding author.

E-mail address: matthias@liai.org (M. von Herrath).

variety of viruses, are closely linked to the pathogenesis of T1D, for good or for bad. On one hand it is well established that certain viruses, especially enteroviruses, infiltrate the pancreas and consecutively ‘unmask’ endogenous β -cells which then in some way turn immunogenic. On the other hand observations from mouse models and the ‘hygiene hypothesis’ suggest that infection with certain viruses or a higher rate of overall infections due to higher environmental exposure may be beneficial and could be considered protective [3,4].

2. Strategies for therapeutic intervention

Strategies to treat or prevent T1D aim to reverse autoreactivity as well as to restore β -cell mass. Each of these goals can be achieved by different means (Fig. 1). Dozens of substances have been used in human trials in order to slow down disease progression, but only a few had a measurable impact on the course of disease [5]. While most human trials have been performed with one single drug, studies in mice strongly suggest that cocktail therapies of two or more different drugs can act synergistically and thus potentially provide the cure to this devastating disease. Since vast numbers of different drugs and approaches have been analyzed, the big challenge is to prioritize which combinations are the most promising based on effects, side effects, synergy with modes of action, and observation from animal models. To answer this question, we first need to define the strategies that have shown promising results in monotherapeutic settings. It is beyond the scope of this review to summarize all potential treatment options. For a more comprehensive discussion of current approaches see Bresson and von Herrath and Rewers and Gottlieb [5,6].

2.1. Immunosuppressive drugs

Several trials have been performed that study the effects of various immunosuppressive drugs in T1D. Considering immunosuppressive therapies, however, we have to acknowledge that these drugs increase the risk of developing infections and malignancies and that some of them have been shown to inhibit beta-cell regeneration [7]. Above all, most of the patients that are being diagnosed with T1D are children. Therefore we need to seek ways to limit the duration of exposure to potentially harmful drugs. However, in human trials systemic immunomodulation has provided the most promising results. Within the multitude of immunosuppressive drugs, we are predominantly focusing on those drugs that are of particular interest when it comes to defining combination therapies, because their ability to induce tolerance goes beyond their immunosuppressive side effects and/or because they are able to induce regulatory T cells (Tregs) or regulatory dendritic cells or other APCs.

2.1.1. Anti-CD20

Since it is widely accepted that autoreactive T cells are the main players that mediate β -cell destruction, the role of B cells in the pathogenesis has been underestimated for quite some time. In NOD mice however diabetes can be prevented by selective depletion of B cells [8]. In a human phase II trial, depletion of B cells using an anti-CD20 monoclonal antibody (Rituximab) revealed a tentative preservation of C-peptide levels and insulin requirements accompanied by an improvement of HbA1C levels over one year compared to the placebo group, although B-cells reappeared to a great extent (69%) by the end of the year [9]. Side effects that appear frequently are mostly related to the administration itself; however, they decrease over the course of the therapy. Severe adverse effects are rare. This study could ultimately prove that there is a role for B cells in disease pathogenesis, which is scientifically of great interest. However, given that C-peptide decline could not be completely prevented and insulin requirements could not be decreased, B cell depletion in this setting does not appear to mediate a significant deceleration in disease progression.

2.1.2. Anti-CD3

A study by Chatenoud and Bach in 1994 suggested that use of anti-CD3 antibodies could prove to be beneficial in humans based on their observations in NOD mice [10]. Indeed, in 2002 a study was published describing remarkable results on an anti-CD3 antibody in patients with T1D in terms of C-peptide preservation and insulin requirements [11]. Additionally, sustained C-peptide levels approximately 2 years and in some cases even up to 5 years were observed [12,13]. The side effects of anti-CD3 treatment were predominantly ‘flu-like’ symptoms, such as headaches, fever and arthralgia. Moreover gastrointestinal symptoms and most importantly transient EBV-viremia with symptoms of acute Mononucleosis were observed. All patients however recovered spontaneously. Since then, modes of action of this treatment have been extensively investigated. It can be demonstrated that anti-CD3 treatment modulates the T cell receptors in a way that renders T cells blind to antigens, induces T cell anergy, blocks the IL-2 signaling pathway, and induces apoptosis [14]. Interestingly, it can also be shown that regulatory T cells are less susceptible to anti-CD3 induced apoptosis; at least when administered in low doses, thus leading to higher numbers of (antigen-unspecific) regulatory T cells. Taken together, these data have made anti-CD3 antibody treatment a very popular substance for potential combination therapies.

2.1.3. Anti-T-Lymphocyte Globulin (ATG)

ATG is a very potent immunosuppressive drug. It depletes almost the entire T cell population in treated patients and is primarily used in the induction period after solid organ transplantation or in acute rejection settings in transplant patients. Since ATG is a polyclonal non-

Reverse autoreactivity			Restoration of β -cell mass
Ag-specific vaccines / Induction of tolerance	Immuno-suppressive drugs	Anti-inflammatory drugs	Induction of β -cell regeneration / β -cell Transplantation
<ul style="list-style-type: none"> • GAD65 • Insulin • HSP60* 	<ul style="list-style-type: none"> • Anti-CD20 • Anti-T-Lymphocyte Globulin(ATG) • Anti-CD3 • Anti-CTLA4 * 	<ul style="list-style-type: none"> • Anti-TNF-α • Anti-IL1 • α-1 anti-Trypsin* • Oral IFN-α* 	<ul style="list-style-type: none"> • GLP-1 analogues • Islet Neogenesis Associated Protein (INGAP-) peptide* • Pioglitazone* • Various transplantation strategies*
• Bone marrow transplantation*			

* Not discussed in this review

Fig. 1. Strategies of therapeutic intervention.

human protein common side effects include fever and serum sickness including arthralgia, rashes and lymphadenopathy. Moreover, anaphylaxis can occur. Administration over a longer period increases the risk for immunoproliferative disorders, which is why only short term treatments should be considered. A study from Prague in 2004 suggested that short term ATG treatment in T1D patients had remarkable effects on C-peptide preservation [15]. In fact, two subjects from the study did not require exogenous insulin over several months after study entry. Additional studies are being performed to confirm and elaborate on those findings (NCT00515099 and NCT00190502).

2.2. Anti-inflammatory treatments

2.2.1. TNF- α modulation

Tumor Necrosis Factor- α (TNF- α) is a proinflammatory cytokine that is related to various autoimmune diseases. Blockade of the TNF- α signaling pathway is a powerful strategy in the treatment of several auto-inflammatory disorders, such as Rheumatoid Arthritis and Inflammatory Bowel Disease. In the development of T1D however, the role of TNF- α is somewhat ambiguous. Whereas the presence of TNF- α seems to accelerate the pace of disease development early in the process, it has been shown to effectively diminish the numbers of autoreactive T cells after establishment of the disease. Thus, both modes of action could eventually be targeted therapeutically.

The beneficial site of TNF- α signaling is currently under investigation in a phase I trial using Bacillus Calmette-Guerin (BCG) to induce systemic TNF expression (NCT00607230). An earlier study with a single dose of BCG did not show significant effects [16], however, recent in vitro data underline the hypothesis that TNF- α is able to reduce the number of autoreactive T cells [17].

Recently, promising data were derived in a clinical trial with Etanercept, a soluble TNF- α receptor fusion protein, in children with new onset T1D [18]. In patients that received TNF- α blockade treatment, C-peptide levels showed a remarkable increase whereas insulin doses decreased from baseline to week 24 in the absence of severe side effects. In the placebo group C-peptide levels decreased and insulin doses increased in the study period. The small cohort (18 subjects included) and the short follow up period (24 weeks) are the restrictions of this study and additional trials need to confirm and expand the observations.

2.2.2. Anti-interleukin 1

Anakinra has been shown to block the signaling pathways of both isoforms IL1 α and IL1 β via IL1 receptor blockade and was initially approved for the treatment of Rheumatoid Arthritis. IL1 has been reported to cause β -cell dysfunction [19]; consequently therapeutic IL1 receptor blockade has been successfully performed in humans with type 2 diabetes [20]. However, preliminary data from a short term treatment pilot study in patients with T1D did not reveal an effect on C-peptide levels [21].

2.3. Antigen-specific vaccines and induction of regulatory T cells

Establishment of a vaccination that results in the emergence of antigen-specific regulatory T cells and the induction of tolerance to autoantigens is a much desirable goal. It would ultimately lead to an abortion of autoreactivity in the absence of major side effects that are observed in immunosuppressive treatments. Moreover, individuals at risk could be treated prior to significant destruction of β -cell mass and clinical signs of disease. Much effort has been performed in order to edge closer to that goal. However, the risk of boosting autoreactivity should never be underestimated. Several autoantigens have been described in T1D; insulin and the 65-kD isoform of glutamic acid decarboxylase (GAD65) are believed to be the major autoantigens that drive the emergence of autoreactivity and consequently have

been studied most intensively in terms of inducing tolerance in humans.

2.3.1. Insulin

Insulin-derived molecules have attracted much attention in search of a potent vaccination strategy. While those strategies have been shown to mediate a powerful effect in mice [22–24], the clinical trials that have been completed did not observe such effects in humans [25,26]. However, several trials are still ongoing and might bring more promising results (NCT00057499 and NCT00453375).

2.3.2. GAD65

The most encouraging results on the induction of tolerance have been obtained in a phase II clinical trial with GAD65 injected into patients in an alum-formulation [27]. In patients with newly diagnosed T1D (within 6 months after onset) that have been treated with GAD-alum C-peptide levels decreased significantly slower than in patients treated with placebo. The study also underlined the need for a rapid treatment after onset of disease since the beneficial effects of the vaccination have not been observed in patients that have been diagnosed earlier, between 6 and 18 months prior to GAD-alum treatment. Currently additional phase III trials are being performed that include patients within the first 3 months after onset (NCT00751842 and NCT00723411).

2.4. Restoration of β -cell mass

Obviously, it is of great advantage to treat patients with T1D at recent onset when there is still some β -cell mass left to help control the glucose metabolism. As discussed earlier, the faster therapy starts after disease onset, the stronger its effect [13,27]. In the majority of patients however, there is no β -cell mass left and thus, all treatments that tackle the autoreactive immune system would not obviate the need for insulin injections, as potent as they might be. This underlines the need for strategies to repopulate insulin producing cells. This can be achieved by either inducing regeneration of endogenous β -cells or by transplantation of β -cells from a donor. However, it has to be kept in mind that if the autoreactive immune cells and especially the memory cells are still present, they will immediately start to attack these 'fresh' target cells.

2.4.1. Induction of β -cell regeneration

Human β -cells are lazy proliferators; their ability to expand however becomes evident during pregnancy or obesity when higher amounts of insulin are needed [28,29]. The Glucagon-like peptide-1 (GLP-1) receptor agonist Exenatide is capable of inducing β -cell expansion as well as insulin secretion. While the stimulation of β -cell growth has only been certified in rodents [30], decrease of glucose levels has been observed in humans with type 2 diabetes [31]. These effects still need to be confirmed in patients with T1D (NCT00456300). In additional studies the effects of Islet Neogenesis Associated Protein (INGAP-) peptide (NCT00071409) and Pioglitazone (NCT00545857) on islet regeneration are being characterized.

2.4.2. Transplantation of β -cells

In addition to presenting islet autoantigens, allo- or xenogeneic β -cell transplants present non-self antigens and thus constitute a potential target for autoreactive as well as alloreactive immune cells. Due to immunosuppressive treatment, which is required to prevent graft rejection, and procedure related side effects, such β -cell transplantations cannot be performed in children. Moreover, several immunosuppressants used to prevent graft rejection in solid organ transplantations have been shown to impair β -cell mediated blood glucose control by inhibiting β -cell replication and/or insulin transcription and translation [32]. In order to obviate the need for immunosuppressive treatments in β -cell transplantation an encapsulation device

has been created that is not immunogenic and protects the transplanted cells within the device from autoreactive as well alloreactive immune attacks via a special membrane. In mice and Rhesus Macaques, this device has been shown to protect transplanted encapsulated allogeneic cells from being destroyed by the host's immune system [33,34].

2.5. Combination therapies

Combinations of different treatment strategies comprise obvious advantages such as targeting multiple pathways and allowing reduction in drug toxicities by modulating treatment doses. Moreover, studies in animals have shown remarkable results using different combination therapies. Recently, the Diabetes Combination Therapies Assessment Group was launched by the Immune tolerance Network (ITN) and the Juvenile Diabetes Research Foundation (JDRF) to provide strategies on the development of combination therapies for human trials.

To be suitable for combination therapies, a defined substance needs to meet several requirements. Some of these requirements pertain to all substances and each drug class has additional requirements to meet. As drafted in Fig. 2, general requirements for substances are (i) promising results in monotherapeutic settings in humans, (ii) in the absence of major side effects and (iii) a well-defined safety profile after having acquired many treatment years. It is important to mention however, that those requirements cannot be mandatory since it would not be wise to exclude a substance that has for example shown promising results in monotherapeutic trials but did not acquire a lot of treatment years due to its recent approval.

Drug availability might yet be another challenge. Drugs that have recently been approved or are still under development are strongly protected by pharmaceutical companies that have spent large amounts of money on their development. While it is of great interest

for those companies to find new applications for their product, the risk of discovering previously unknown adverse effects in a combination setting can never be underestimated. This has been observed for example in combination trials of Natalizumab with immunomodulatory drugs in patients with multiple sclerosis [35]. Even if those adverse effects are only related to the use of the specific drug in a combination setting, it is very likely to have a strong impact on the future development of this substance in terms of approval, introduction to the market, searching for new applications and last but not least, the image.

Due to well-defined safety profiles but also to drug availability issues, most of the currently ongoing combination trials include substances that have been on the market for quite some time. Those trials include the use of IL2 (Proleukin) and Sirolimus (Rapamune) (NCT00525889), GAD-alum (Diamyd), Sitagliptin and Lansoprazole (NCT00837759), Epidermal growth factor and Gastrin (NCT00239148), Mycophenolate Mofetil and anti-CD25 antibody (Daclizumab) (NCT00100178) or ATG and Pegfilgrastim (Neulasta) (sponsored by the Helmsley Trust). Apart from β -cell transplants and hematopoietic stem cell transplantation that have been performed in combination with immunosuppressive drugs, only one clinical trial has published results on a combination therapy in patients with T1D. The outcome of this study is somewhat disappointing. Patients with long-standing T1D have been treated with Exenatide and an anti-CD25 antibody without significant success [36]. However, none of the substances have shown promising results in monotherapeutic settings and patients have not been treated at recent onset making this specific approach a high-risk endeavor.

In order to increase the chances of designing more successful combination therapies we propose the algorithm drafted in Fig. 2. Since the most promising data have been acquired with immunosuppressive or anti-inflammatory drugs and most of them are already approved or have even been used for several years, potential combination therapies could be based on these substances.

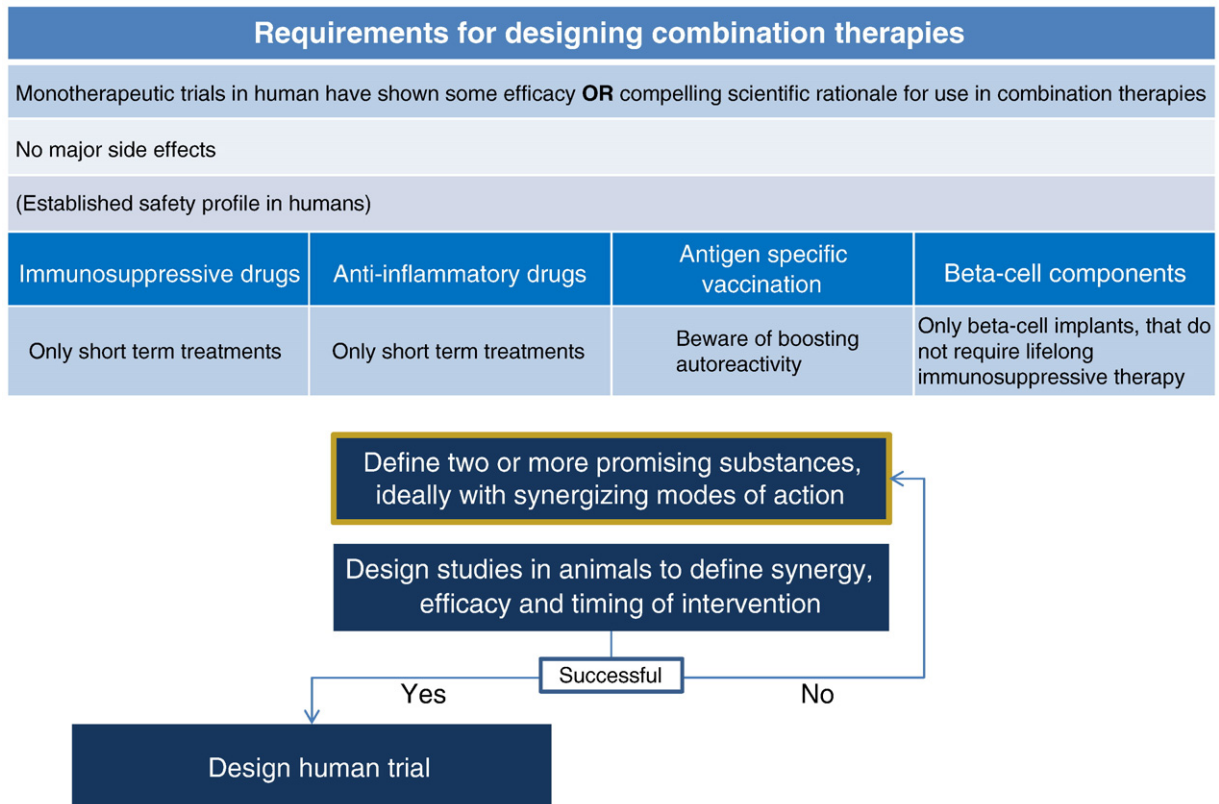


Fig. 2. Algorithm of designing combination therapies for T1D.

2.5.1. Immunosuppressive or anti-inflammatory drugs in combination with antigen-specific vaccines

This combination is of particular interest, since studies in animals have already been performed and have shown very promising results. In two studies, anti-CD3 treatment has been performed in combination with GAD [37] or in combination with intranasal Proinsulin [38]. These types of combination therapies would be excellent candidates for clinical studies in humans; particularly the combination of anti-CD3 and GAD therapy meets almost all prerequisite requirements proposed in Fig. 2: Both substances have been studied in monotherapeutic trials with quite some success and have shown no major side effects in humans. Moreover, GAD-treatment did not augment the autoreactive immune response (even though GAD antibody titers increased in treated patients) [27] and anti-CD3 treatment was administered only for a short term treatment [11,13], thereby both substances meeting their drug-class specific requirements.

2.5.2. Immunosuppressive or anti-inflammatory drugs in combination with β -cell components

Since β -cell mass decreases over time, patients with long-standing disease are very likely to have lost most of their insulin producing cells. Thus, treatments that only target the autoreactive immune system would not obviate the need for insulin injections in these patients. This has to be addressed in combination trials by adding β -cell components. Unfortunately, none of the β -cell components have shown great success in human trials up to date. This applies to the induction of β -cell regeneration as well as the transplantation of pancreatic islets. However, some promising candidates are studied in clinical trials right now and a study in mice already showed remarkable results using anti-CD3 treatment in combination with a GLP-1 analogue [39], making this combination another promising approach.

We strongly believe that the combination of different drugs provides great opportunities in the treatment of T1D as combination therapies have considerably improved the treatment of various other diseases, such as chronic viral infections (HCV and HIV) or diverse malignancies. This approach may not necessarily be a cure for this disease, but even to halt the disease progress, preserve β -cell function or significantly decrease the amounts of insulin needed would be great improvements.

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