NANO LETTERS

pubs.acs.org/NanoLett Letter

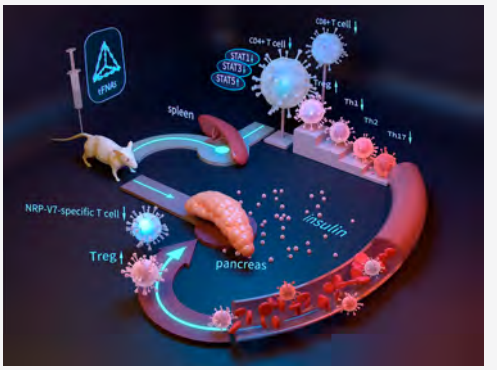
Tetrahedral Framework Nucleic Acids Induce Immune Tolerance and Prevent the Onset of Type 1 Diabetes

Shaojingya Gao,[§] Yanjing Li,[§] Dexuan Xiao, Mi Zhou, Xiaoxiao Cai, and Yunfeng Lin*

Cite This: https://doi.org/10.1021/acs.nanolett.1c01131 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A failure in immune tolerance leads to autoimmune destruction of insulin-producing β-cells, leading to type 1 diabetes (T1D). Inhibiting autoreactive T cells and inducing regulatory T cells (Tregs) to re-establish immune tolerance are promising approaches to prevent the onset of T1D. Here, we investigated the ability of tetrahedral framework nucleic acids (tFNAs) to induce immune tolerance and prevent T1D in nonobese diabetic (NOD) mice. In prediabetic NOD mice, tFNAs treatment led to maintenance of normoglycemia and reduced incidence of diabetes. Moreover, the tFNAs (250 nM) treatment preserved the mass and function of β-cells, increased the frequency of Tregs, and suppressed autoreactive T cells, leading to immune tolerance. Collectively, our results demonstrate that tFNAs treatment aids glycemic control, provides β-cell protection, and prevents the onset of T1D in NOD mice by immunomodulation. These results highlight the potential of tFNAs for the prevention of autoimmune T1D.



KEYWORDS: *Tetrahedral framework nucleic acids, Type 1 diabetes, Immune tolerance, T cells, Regulatory T cells*

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease. As a consequence of failed immune tolerance, T1D is characterized by immune-mediated attack and destruction of insulin-producing β-cells in the pancreas.[1] T1D is one of the most severe autoimmune diseases. Millions of people suffer from T1D, and disease incidence has been on the rise.[2] Faced with such a large number of patients, a cure or prevention for T1D remains elusive. For T1D patients, insulin injections and pumps are not curative, only lifesaving. Unfortunately, vaccines to prevent T1D are still in their infancy.[3] To cure or prevent T1D, immunotherapies, which can attenuate T cell responses against β cells, restore immune tolerance, replenish or maintain the β-cell mass, and halt the progress of the disease, have to be developed.[4,5]

Developing immunotherapies requires information regarding how β-cells are destroyed by immune cells. Autoreactive $CD4^+$ and $CD8^+$ T cells play a key role in T1D owing to their domination of islet infiltration and as primary drivers of β-cell loss.[6] $CD8^+$ T cells are supposed to directly target and kill β-cells, while $CD4^+$ T cells mediate the activation of $CD8^+$ T cells and promote β-cell death via cytokine secretion.[7,8] Under specific activation conditions, naive $CD4^+$ T cells can differentiate into distinct functional T helper (Th) cells, including T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17), and regulatory T cells (Tregs).[9,10] An imbalance of Th cells and Tregs is a hallmark feature of T1D. Th cells are regarded as primary effectors involved in T1D pathogenesis, while Tregs are pivotal in preventing autoimmune diseases. Tregs are considered to suppress autoreactive T cells and maintain immune homeostasis, leading to the prevention of autoimmunity.[6,10] Tregs are characterized by expression of the transcription factor forkhead box P3 (Foxp3), which is regarded as a master regulator of the development and function of $CD4^+CD25^+$Tregs.[11,12] In immunotherapies for autoimmune diseases, Tregs play an indispensable role in maintaining immune tolerance to self-antigens and in suppressing excessive immune responses.[13]

Currently, T1D immunotherapies can be categorized into nonautoantigen-specific and autoantigen-specific interventions.[5,14,15] Nonautoantigen-specific interventions suppress or eliminate autoreactive and protective immune cells without distinction, leading to an increased risk of life-threatening infections and malignancy.[16] Autoantigen-specific approaches are different. They are supposed to target T1D-related immune cells with exquisite selectivity, while preserving the systematic immune homeostasis.[14,17,18] Therefore, autoantigen-specific approaches are employed to fight against β-cell autoimmunity by targeting autoreactive T cells, inducing Tregs, and hampering the pathogenesis of T1D. By inducing Tregs, autoantigen-specific interventions are expected to achieve the ultimate goal of the treatment of autoimmune T1D to induce

Received: March 22, 2021
Revised: May 3, 2021

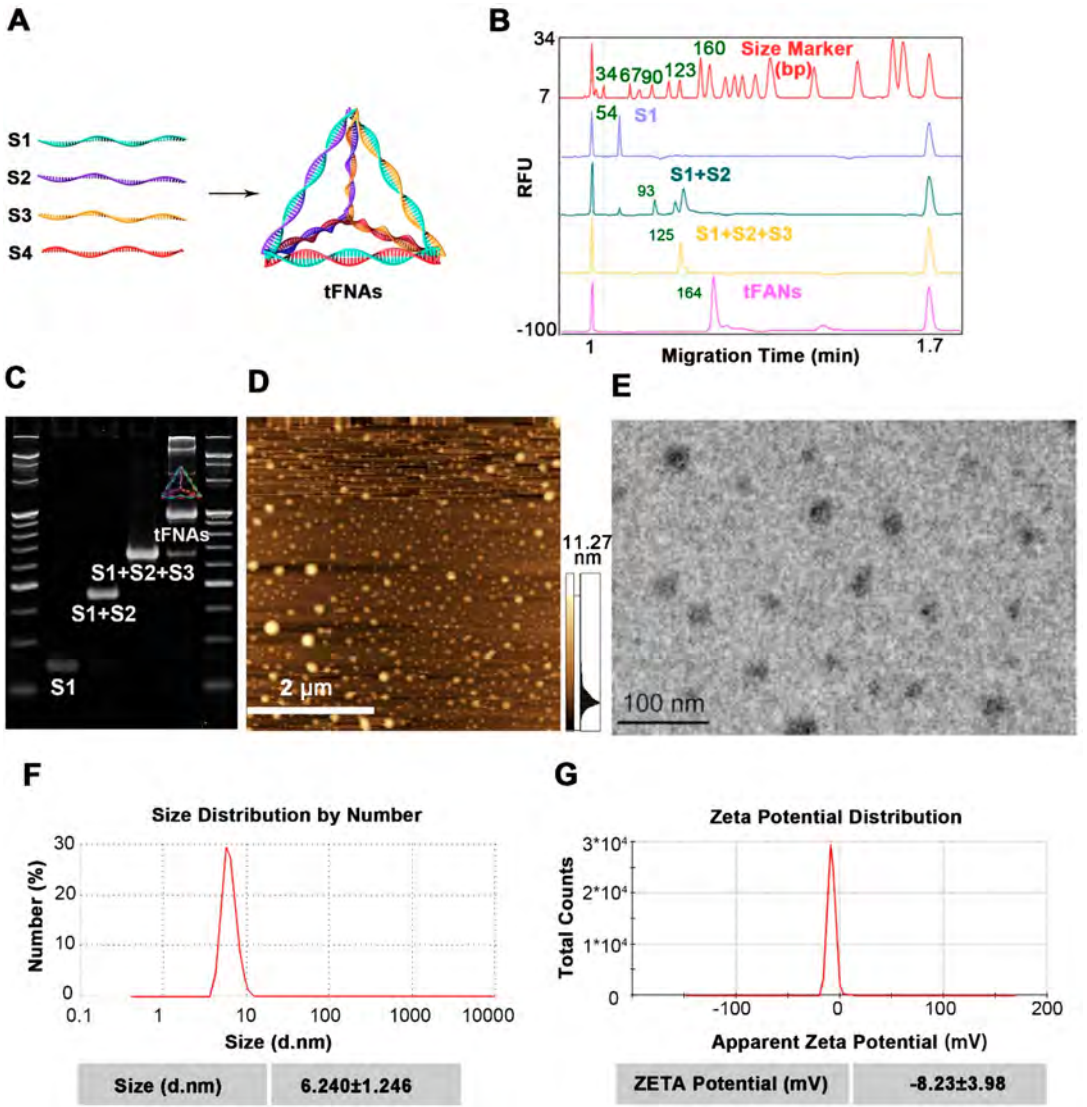


Figure 1. Synthesis and characterization of tFNAs. (A) Schematic illustration synthesis of tFNAs. (B) Molecular weight of the synthesized tFNAs detected by HPCE. Results of HPCE showing the successful synthetization of tFNAs. (C) Molecular weights of the synthesized tFNAs detected by 8% PAGE. (D, E) Synthesized tFNAs detected by AFM and TEM, respectively. (F) Size of synthesized tFNAs measured by DLS. (G) Stability of synthesized tFNAs measured by zeta potential analysis.

immune tolerance preventing tissue-specific progressive loss of β-cell function and survival.[15,19]

Limited by therapeutic materials, immunotherapies for T1D are still restricted to a small range. The characteristics of therapeutic materials, including off-target toxicity, short duration, instability, unpredictable activity, and tissue heterogeneity, contribute to their limited application. The progress of immunotherapy for T1D is built upon the premise of the breakthroughs in materials engineering.[20] The approach to induce immune tolerance is also based on advances in the field of nanomaterials.[20−22] Nanoparticles have emerged as powerful instruments to regulate immune responses because of their ability to precisely target immune cells and signals related to antigen-specific immune responses.[23−25] Tetrahedral framework nucleic acids (tFNAs), one of the most promising DNA nanoparticles, have been applied in a wide variety of biological fields owing to their unsurpassed biocompatibility.[26,27] tFNAs are self-assembling three-dimensional (3D) polyhedral, consisting of four highly specific and programmable single-stranded DNAs (ssDNA). tFNAs could participate in the regulation of macrophage activation and polarization.[28] More precisely, tFNAs can induce M1 polarization, which suggests that tFNAs are potential candidates for immunomodulation.[27,28]

Here we demonstrate the potent immunomodulation of tFNAs in preventing autoimmune diabetes in nonobese diabetic (NOD) mice, the animal model for T1D. We found that tFNAs induce immune tolerance by specifically eliminating autoreactive immune cells and up-regulating Tregs. This protects NOD mice from hyperglycemia and death, maintaining the mass and function of the islet β-cells. We also investigated the possible mechanisms of immunomodulation of tFNAs. To date, the results indicate the feasibility, safety, and validity of administering of tFNAs for T1D immunotherapy by inducing immune tolerance and halting the progression of T1D.

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

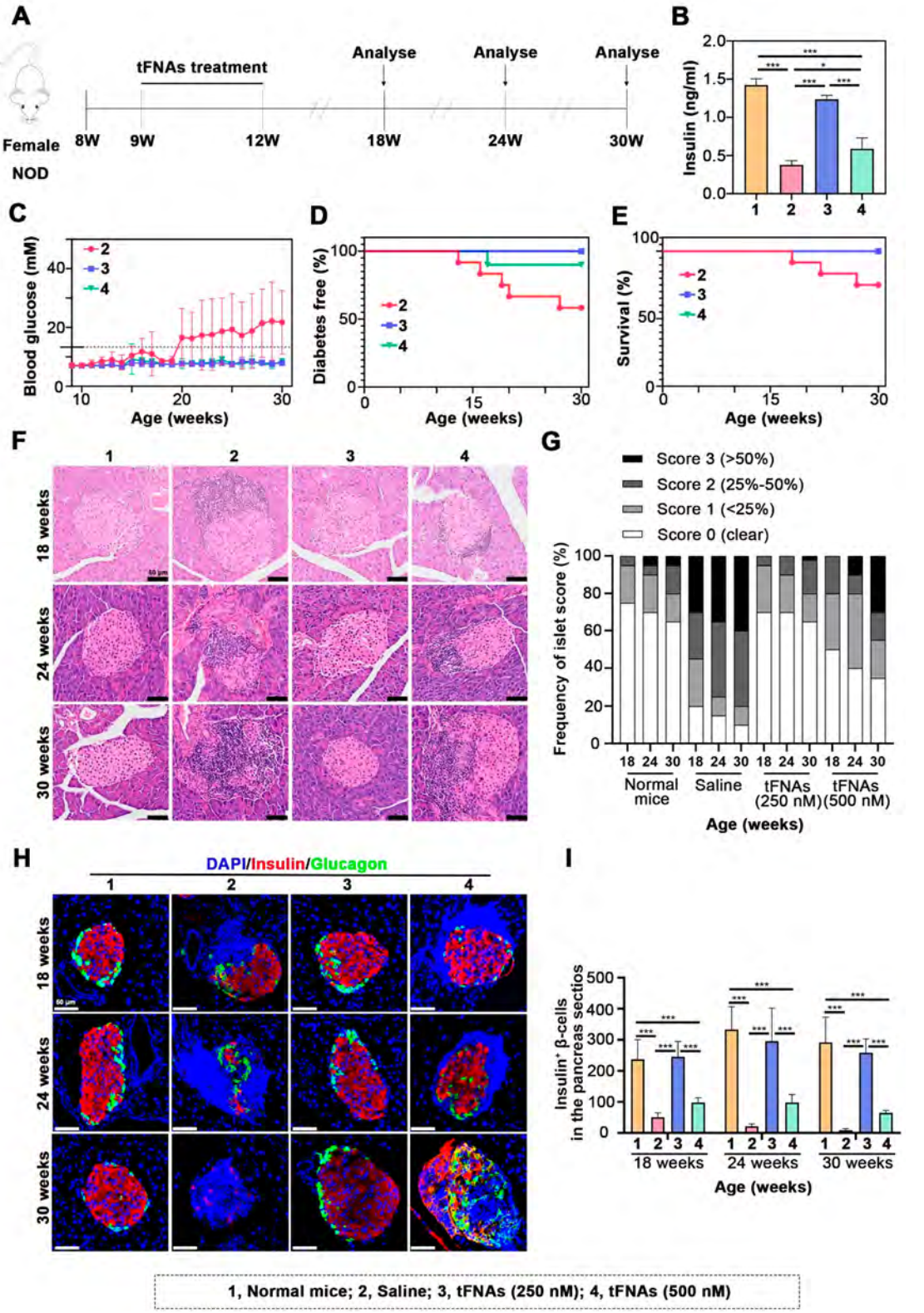


Figure 2. TFNAs protect NOD mice from T1D. (A) Experimental scheme. (B) Blood insulin concentration in the 30-week-old mice of different groups (from three or four mice per group) as indicated. (C) Weekly blood glucose values of mice treated with saline (n = 12), tFNAs (250 nM) (n = 10), tFNAs (500 nM) (n = 10). The dashed line shows the upper limit of normoglycemia. (D) Percentage of diabetes-free mice (blood glucose <13.3 mM) over the time course of the experiment. (E) Kaplan−Meier survival curve showing NOD mice treated with saline and tFNAs monitored for mortality rates. (F) Representative H&E staining (from three or four mice per group) showing mononuclear cell infiltration in pancreatic islets from 18-, 24-, 30-week-old mice. (G) Insulitis scores of pancreatic sections from F (scale bar, 50 μm). (H) Representative immunofluorescence images (from three or four mice per group) with the insets showing insulin-positive (red) and glucagon-positive (green) islets in 18-, 24-, 30-week-old mice (scale bar, 50 μm). (I) Quantification of insulin$^+$ β-cells in the pancreas sections. Throughout, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical analyses were performed using two-way ANOVA.

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

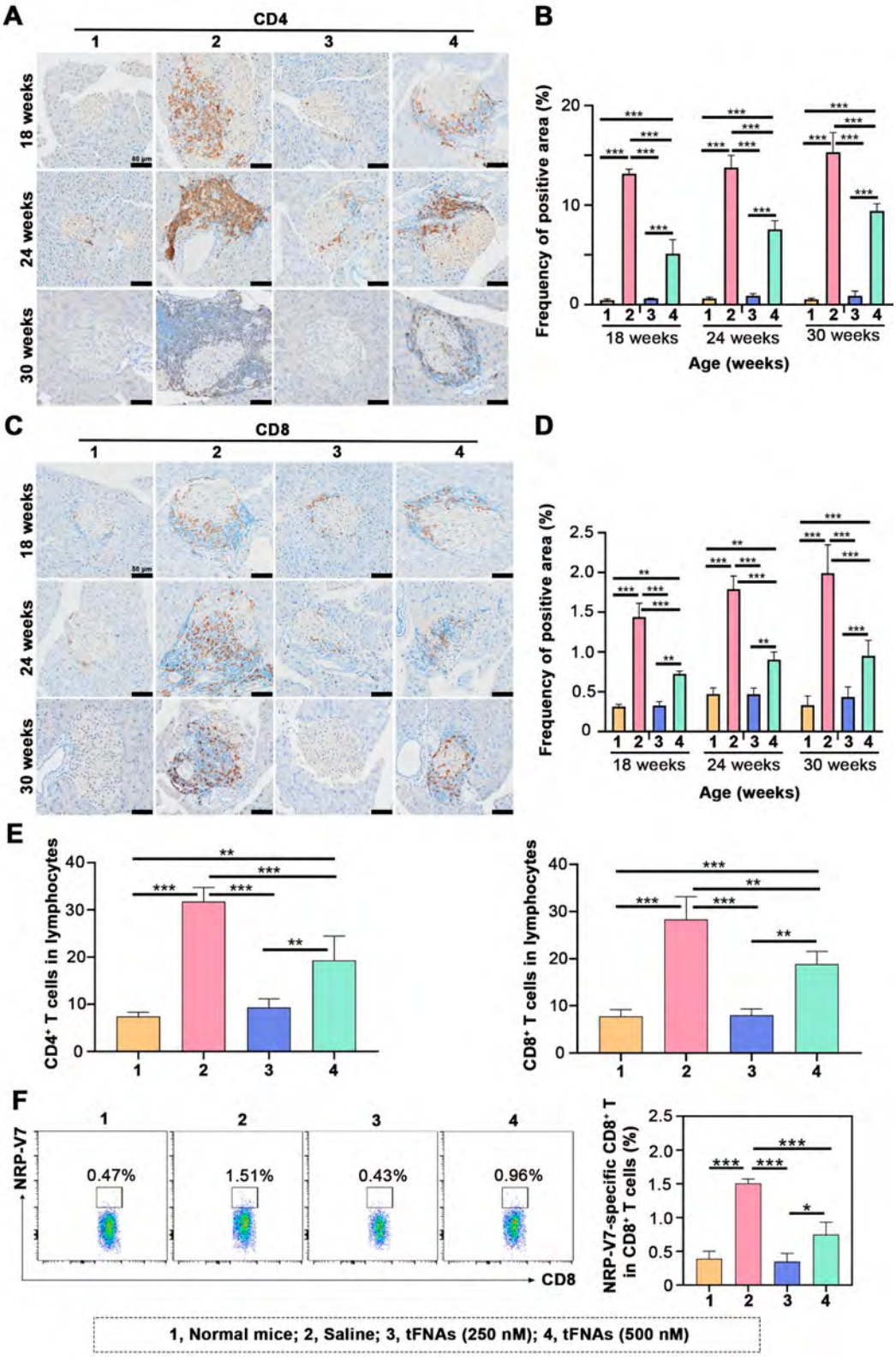


Figure 3. TFNAs altered T-cells in the pancreas. (A) Representative immunohistochemistry images (from three or four mice per group) stained for CD4 in islets of 18-, 24-, 30-week-old mice (scale bar, 50 μm). (B) Quantification of $CD4^+$ cells in the pancreas sections. (C) Representative immunohistochemistry images (from three or four mice per group) stained for CD8 in islets of 18-, 24-, 30-week-old mice (scale bar, 50 μm). (D) Quantification of $CD8^+$ cells in the pancreas sections. (E) Quantification of pancreas-infiltrated $CD4^+$ and $CD8^+$ T cells in 30-week-old mice analyzed by the flow cytometry. (F) Representative dot blots and quantification (from four or five mice per group) of flow cytometry analyses for $CD8^+NRP\text{-}V7^+$ T (antigen-specific) cells. Throughout, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical analyses were performed using two-way ANOVA.

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

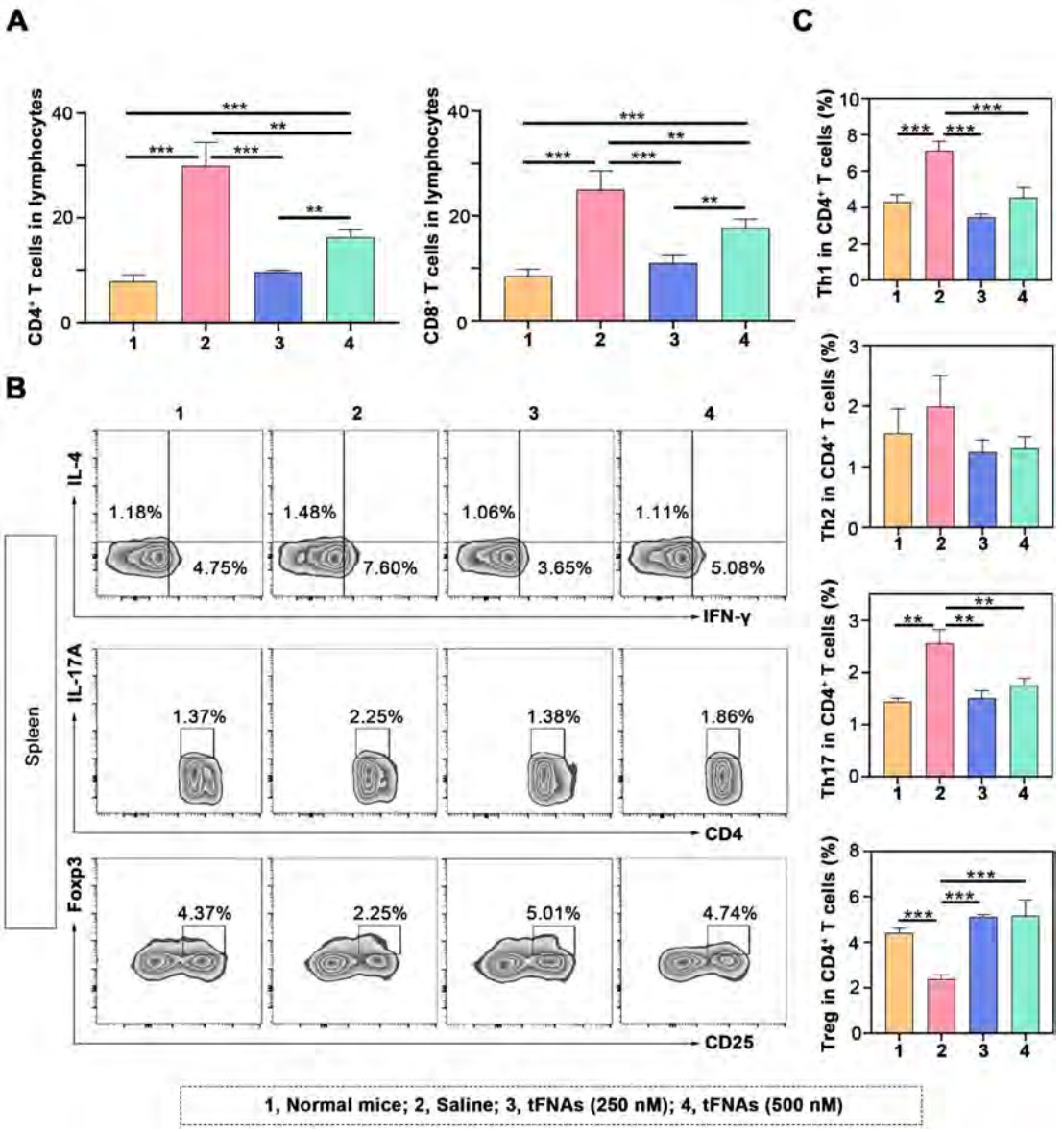


Figure 4. TFNAs treatment mediated immune regulation. (A) Quantification of $CD4^+$ and $CD8^+$ T cells of 30-week-old mice (from four or five mice per group) in spleen analyzed by the flow cytometry. (B) Representative plots of flow cytometry analyses for $CD4^+IFN\gamma^+$ T cells (Th1), $CD4^+IL4^+$ T cells (Th2), $CD4^+IL17^+$ T cells (Th17), and $CD4^+CD25^+Foxp3^+$ T cells (Treg) obtained from the spleen of 30-week-old mice, respectively. (C) Percentage of Th1, Th2, Th17, and Treg cells shown in B. Throughout, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical analyses were performed using one-way ANOVA.

SYNTHESIS AND CHARACTERIZATION OF TFNAS

tFNAs were composed of four ssDNAs constructs owing to the specific base pairing principle (Figure 1A). The high-performance capillary electrophoresis (HPCE) was used to confirm tFNAs were successfully synthesized by four ssDNAs molecules (Figure 1B). In 8% polyacrylamide gel electrophoresis (PAGE), tFNAs were 200 bp according to the theoretical DNA markers, which agreed with the previously reported value (Figure 1C).[29−33] Atomic force microscopy (AFM) and transmission electron microscopy (TEM) were utilized to measure the three-dimensional structure of tFNAs; triangular structures were detected (Figure 1D,E). To determine the diameter of tFNAs, the average size was detected by dynamic light scattering (Figure1F). Based on the zeta potential, tFNAs had a charged surface of −8.23 ± 3.98 mV (Figure 1G).

PROTECTION OF NOD MICE FROM DIABETES DEVELOPMENT BY TFNAS

In vivo bioluminescence imaging was employed to detect the biodistribution of tFNAs. Notably, tFNAs accumulated in the major organs, including the thymus, spleen, pancreas, and kidneys, of prediabetic NOD mice (Figure S1). To investigate whether tFNAs could delay the onset of T1D and find the optimal dose for tFNAs treatment, NOD mice were divided into three groups: saline group, tFNAs (250 nM) group, and tFNAs (500 nM) group. We investigated the prevention effects of tFNAs therapy in 9-week-old prediabetic NOD female mice. We used a 4-week course of tFNAs and followed the effects for 30 weeks age of NOD mice (Figure 2A). As expected, after the experiment the serum insulin and C-peptide levels of the mice were 3-fold higher in tFNAs (250 nM) treated mice compared to the saline group at the end of experiment (Figure 2B, Figure

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

S2). The results confirmed that tFNAs treatment controlled the hyperglycemic level and maintained normoglycemia (blood level <14 mmol/L) in the NOD mice (Figure 2C). All the mice treated with 250 nM tFNAs remained diabetes-free throughout the experiment compared with 50% in the saline group. Most of the saline group mice developed T1D between 15 and 25 weeks of age (Figure 2D). Moreover, all the 250 and 500 nM group mice surprisingly survived through the follow-up period (Figure 2E).

The inflammatory profile of the islets revealed invasive insulitis, regarded as T1D hallmarks. In the saline group, islet infiltration and β-cell destruction progressed over time, leaving almost no functional islets behind. In contrast, the mice receiving tFNAs showed a reduced degree of insulitis. Most of the islets of the NOD mice treated with 250 nM tFNAs showed no infiltration (score 0) and maintained this up to 30 weeks of age, reaching a similar status to that of normal mice. Similarly, the NOD mice treated with 500 nM tFNAs experienced a delay in islet destruction and decreased insulitis (Figure 2F, G).

To further examine the insulin-producing β-cells, the pancreases of the mice from the different groups were collected and analyzed by immunofluorescence costaining with insulin and glucagon. As shown in Figure 1H, I, the insulin-producing β-cells were almost intact in the NOD mice treated with 250 nM tFNAs, while most of the β-cells were lost in the saline group. The loss of β-cells could not be completely prevented in the NOD mice treated with 500 nM tFNAs. Notably, according to the immunofluorescence analysis, there was no difference between the normal mice group and tFNAs (250 nM) group. These results were highly consistent with the results of the pancreas hematoxylin and eosin (H&E) staining and serum insulin level results (Figure 2B,F,G).

To determine whether tFNAs could protect β-cells from death, we performed costaining for insulin and transferase dUTP nick end labeling (TUNEL) assay. Through experimental follow-up, it was established that 250 nM tFNAs treatment significantly decreased the β-cell death (Figure S3A,B). Costaining with insulin and Ki67 was performed to detect β-cell proliferation. Significantly enhanced β-cell mitosis was detected at all ages in the tFNAs (250 nM) group (Figure S3C,D). Compared with the 250 nM group, the immunofluorescence consequences of the 500 nM group were not satisfactory (Figure S3).

TFNAS INDUCED REDUCTION IN THE FREQUENCY OF ISLET-SPECIFIC T CELLS IN THE PANCREAS OF THE NOD MICE

T1D is an autoimmune disease in which T cells infiltrate the pancreas and attack the insulin-producing β-cells. To investigate the reason why tFNAs delayed the onset of T1D and whether long-term therapeutic effects were achieved, the frequency of T cells infiltrating pancreas were detected by immunohistochemistry and flow cytometry. A large number of T cells were observed to have infiltrated the pancreases in the saline group. The 250 nM tFNAs treatment affected the T cells in the pancreas and reduced the local frequency of both $CD4^+$ and $CD8^+$ T cells. This indicated a decrease in the overall inflammation of the pancreas. Whereas the number of $CD8^+$ and $CD4^+$ T cells increased over time in the tFNAs (500 nM) group, and the difference in the number of pancreas-infiltrating T cells between the 500 nM and saline groups were not apparent (Figure 3A–E). A significant decrease in Treg frequency was observed in the saline group using the flow cytometry and immunofluorescence analyses. Treatment with 250 nM tFNAs restored the pancreas-infiltrating Tregs remarkably (Figure S4). Importantly, in the NOD model, islet antigen peptide mimotope NRP-V7-specific T cells were regarded as antigen-specific T cells for T1D.[34,35] After the experiment, the frequency of the islet antigen-specific $CD8^+$ T cells was diminished by approximately 30% in the pancreases of the 250 nM tFNAs treated mice compared with the pancreases of the saline treated mice. The levels of islet antigen-specific $CD8^+$ T cells in the tFNAs (250 nM) treated mice were similar to those in the normal mice (Figure 3F).

TFNAS SPECIFICALLY INDUCED TREGS WHILE SUPPRESSING AUTOREACTIVE CELLS IN THE BLOOD AND SPLEEN

To confirm the exact root cause of the prevention of T1D, the frequencies of the T cells and their subgroups were analyzed at 6 and 18 weeks after the tFNAs injections. With the treatment of the 250 nM tFNAs treatment, the frequencies of $CD4^+$ and $CD8^+$ T cells in spleen were significantly reduced. In contrast, the 500 nM tFNAs treatment had a limited effect on preventing T-cell infiltration (Figure 4A).

For $CD4^+$ T cell subgroups, in the saline group, the Treg cell frequency was remarkably reduced by approximately 50%. A decrease in the number of Tregs could lead to T1D.[36] Under the treatment of tFNAs, the frequency of Tregs in the blood and spleen was restored by the follow-up period (Figure 4B,C, Figures S5–S7). Tregs have a critical effect on re-establishing immune tolerance in autoimmune diseases; thus, it is highly likely that an increase in the percentage of Tregs contributed to the prevention of T1D.

The blood and spleen of the saline group showed a significant 2- to 3-fold elevation in the number of Th1 and Th17 cells compared with the normal mice (Figure 4B,C, Figures S5–S7), which promoted the development of T1D. Under the tFNAs treatment, it was established that the Th1 and Th17 cells were accurately suppressed in the blood and spleen, similar to those in the normal mice group, at 18 weeks after treatment (Figure 4B,C, Figure S6). However, to some extent, the effect of the 500 nM tFNAs treatment was not permanent. At 18 weeks after treatment, the frequency of Th1 cells rebounded in the blood and spleen (Figure 4B,C, Figure S7). The effect of the 250 nM tFNAs treatment was stable throughout the experiment and was similar to the normal standard (Figure 4B,C, Figures S5–S7). The number of Th2 cells remained stable during the experiment. In short, these results collectively revealed that the tFNAs injection induced immune tolerance by favoring Treg differentiation while inhibiting responses from the Th1 and Th17 cells' responses in the immune system.

A number of approaches aiming to induce immune tolerance against T1D have been tried, however, with limited success. Tregs are crucial for controlling autoimmunity and inducing immune tolerance. Repairing Tregs to reverse or prevent T1D is a highly promising approach to T1D immunotherapy.[37] Instead of infusing Tregs directly, inducing antigen-specific Tregs to abrogate islet β-cell destruction by making use of a nanomaterial or biomaterial treatment is more favored.[21,23,24] Tregs can be generated as a mature T cell subset in the thymus and can also differentiate from naive $CD4^+$ T cells in the periphery. In peripheral organs, T-cell activation requires TCR signal (signal 1), CD28/CD80 costimulation (signal 2), and

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

the help of various cytokines (signal 3).[38] During T-cell activation, the absence of signal 2 or 3 results in the development of clonal anergy.[39,40] Meanwhile, various cytokines dictate the different differentiation programs of naïve CD4$^+$ T cells in the periphery.[41] tFNAs can promote the expression of immunosuppressive cytokines, which agrees with the previous results from studies of our research group.[28,42] After tFNAs treatment, the microenvironment of the periphery gets altered, and the immunosuppressive cytokines, especially TGF-β, promote the differentiation of naive CD4$^+$ T cells to Tregs (Figure S8).[43]

In many reports, tFNAs are considered to be great carriers.[44−47] In the development of DNA nanostructures, four ssDNAs combine to form three-dimensional (3D) tetrahedral DNA nanostructures, so-called tFNAs.[48] Based on their capability of traveling through cellular membranes, tFNAs are regarded as excellent drug delivery carriers with good biocompatibility and biodegradability (Figure S9).[44−47] In our study, we demonstrate that tFNAs had exclusively immunomodulatory effects, and in consideration of their excellent drug loading capabilities, tFNAs may provide a general choice for the alteration of autoimmune diseases.

Various materials used in immunotherapy were listed in the recent review.[49] Macromaterials, micromaterials, and especially nanomaterials were introduced and discussed in detail; however, framework nucleic acids were not mentioned in the research. Our study thus suggests a new kind of nanomaterial for immunotherapy. Based on the immunomodulation and drug carrier capabilities of tFNAs, they may be able to participate in T1D vaccine research as adjuvants like the other nanomaterials mentioned in the review.

TFNAS MODULATE THE DIFFERENTIATION OF THE CD4+ T CELLS BY SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION (STAT) SIGNALS

The mechanism of induction of immune tolerance remains elusive. Our study attempted to shed light on the possible mechanism by which tFNAs induce immune tolerance. Activation of the STAT protein family is believed to be involved in coordinating the immune system, especially modulating the differentiation of CD4$^+$ T cells.[50,51] The phosphorylation of the STAT family is essential for the dimerization and activation of the STAT family. To investigate the possible mechanism of CD4$^+$ T cell differentiation, the levels of phosphorylation of STAT1 (p-STAT1), STAT3 (p-STAT3), STAT5 (p-STAT5) in lymphocyte and CD4$^+$ T cells were detected by Phosflow analysis (Figure 5A−D). In the saline group, compared with the normal mice group, the p-STAT1 and p-STAT3 levels were higher, while the level of p-STAT5 was inhibited (Figure 5A−D). Under the tFNAs treatment, the p-STAT5 levels were remarkably up-regulated, while the p-STAT1 and p-STAT3 levels were significantly down-regulated, compared with the saline group (Figure 5A−D). In the tFNAs treatment group, the changes in the p-STAT levels in mice treated with 500 nM were not dramatic, and the p-STAT levels in tFNAs (250 nM) were very close to those of normal mice (Figure 5A−D).

Among members of the STAT family, STAT1, STAT3, and STAT5 are strongly associated with T1D.[52,53] The balance between the Th cells and Tregs was often found altered in T1D patients influenced by STAT phosphorylation.[53] More specifically, STAT1 is indispensable for IFN-γ signaling, and IFN-γ is the hallmark cytokine of Th1 cells.[54] Differentiation of

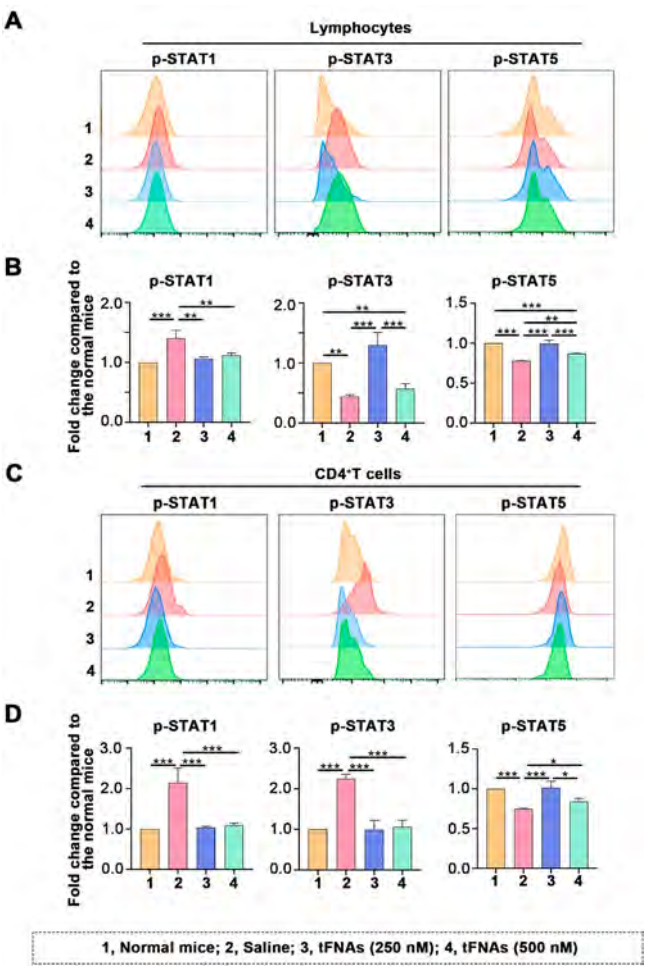


Figure 5. TFNAs mediated phosphorylation of STATs in spleen. (A,B) The levels of p-STAT1, p-STAT3 and p-STAT5 in lymphocytes of 30-week-old mice from different group (four or five mice per group) were determined by Phosflow analysis. Representative plot (A) and histogram analysis (B) were shown. (C,D) The levels of p-STAT1, p-STAT3 and p-STAT5 in CD4$^+$ T cells from different group were determined by Phosflow analysis. Representative plots (C) and histogram analysis (D) were shown. Throughout, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Statistical analyses were performed using one-way ANOVA.

the Th2 cells demands the assistance of STAT3 and STAT5.[55,56] Interestingly, STAT3 and STAT5 are both double-edged swords for T cell differentiation.[53] To be more specific, STAT5 is an important negative regulator for Th17 cells, and gene promoter for Th1 and Tregs.[55,57] In T1D treatment, the unique position of STAT5 is highlighted.[58] The incidence of T1D is significantly decreased in STAT5 transgenic NOD mice, in which STAT5 is overexpressed. This paper reveals that STAT5 provides protection against T1D by up-regulating Tregs. Apart from the Th2 cells, STAT3 plays an important role in increasing the amount of Th17 cells and decreasing the number of Tregs.[59] It can be deduced that STAT3 activation closely related to T1D by impairing the regulatory T cells and activating the Th17 cells.[60,61] Th17 cell expansion, activation, and dysregulation are thought to have critical roles in T1D. Our results suggest that the mechanism by which tFNAs induces immune tolerance includes the preferential promotion of p-STAT5 signaling in lymphocytes

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

and CD4$^+$ T cells, while inhibiting p-STAT1 and p-STAT3 signaling.

Control over the autoimmune diseases by inducing endogenous modulatory mechanisms is a desired and challenging goal.[62] In the present study, we verified the immunomodulation capability of tFNAs, which selectively suppressed the diabetogenic T cells and up-regulated immune regulatory T cells to induce immune tolerance and prevent the onset of autoimmune T1D. The experimental results indicate that tFNAs may be an effective option for people predisposed to T1D, in which diabetogenic immune cells exist and pancreatic β-cells are close to being destroyed. According to our knowledge, this is the first example of the immunomodulation functions of tFNAs. In this study, tFNAs exclusively participated in the T1D therapies as an immune regulator, even though tFNAs are excellent drug delivery carriers. In the future, these materials could provide unlimited possibilities for the interventions of autoimmune diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.1c01131.

Materials and Methods, Figures S1−S9, and Table S1 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Yunfeng Lin − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology and College of Biomedical Engineering, Sichuan University, Chengdu, Sichuan 610041, China;* orcid.org/0000-0003-1224-6561; Phone: 86-28-85503487; Email: yunfenglin@scu.edu.cn

Authors

Shaojingya Gao − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China*

Yanjing Li − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China*

Dexuan Xiao − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China*

Mi Zhou − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China*

Xiaoxiao Cai − *State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China;* orcid.org/0000-0002-5654-7414

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.nanolett.1c01131

Author Contributions

[§]S.G. and Y.L. contributed equally to this work. Y.L., S.G., Y.L., and X.C. conceived this project. S.G. and Y.L.designed the project and collected the data. S.G. analyzed the data and wrote the manuscript. D.X. and M.Z. provided help during data collection, and D.X. provided writing assistance and helped during proof-reading of the article.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by This study was supported by National Key R&D Program of China (2019YFA0110600) and National Natural Science Foundation of China (81970916, 81671031).

■ ABBREVIATIONS

T1D, type 1 diabetes; Treg, regulatory T cells; tFNAs, tetrahedral framework nucleic acids; NOD, nonobese diabetic; Th, functional T helper; Foxp3, forkhead box P3; 3D, three-dimensional; ssDNA, single-stranded DNAs; PAGE, polyacrylamide gel electrophoresis; HPCE, high-performance capillary electrophoresis; AFM, atomic force microscopy; TEM, transmission electron microscopy; H&E, hematoxylin and eosin; TUNEL, transferase dUTP nick end labeling; STAT, signal transducer and activator of transcription; PD-L1, programmed death-ligand; PD-L1 platelets, overexpressed PD-L1 on platelets

■ REFERENCES

(1) DiMeglio, L. A.; Evans-Molina, C.; Oram, R. A. Type 1 diabetes. *Lancet* **2018**, *391* (10138), 2449−2462.

(2) Stabler, C. L.; Li, Y.; Stewart, J. M.; Keselowsky, B. G. Engineering immunomodulatory biomaterials for type 1 diabetes. *Nat. Rev. Mater.* **2019**, *4* (6), 429−450.

(3) Serr, I.; Furst, R. W.; Achenbach, P.; Scherm, M. G.; Gokmen, F.; Haupt, F.; Sedlmeier, E. M.; Knopff, A.; Shultz, L.; Willis, R. A.; Ziegler, A. G.; Daniel, C. Type 1 diabetes vaccine candidates promote human Foxp3(+)Treg induction in humanized mice. *Nat. Commun.* **2016**, *7*, 10991.

(4) Martinov, T.; Fife, B. T. Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance. *Ann. N. Y. Acad. Sci.* **2020**, *1461* (1), 73−103.

(5) Ni, Q.; Pham, N. B.; Meng, W. S.; Zhu, G.; Chen, X. Advances in immunotherapy of type I diabetes. *Adv. Drug Delivery Rev.* **2019**, *139*, 83−91.

(6) Ilonen, J.; Lempainen, J.; Veijola, R. The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nat. Rev. Endocrinol.* **2019**, *15* (11), 635−650.

(7) Burrack, A. L.; Martinov, T.; Fife, B. T. T Cell-Mediated Beta Cell Destruction: Autoimmunity and Alloimmunity in the Context of Type 1 Diabetes. *Front. Endocrinol. (Lausanne, Switz.)* **2017**, *8*, 343.

(8) Walker, L. S.; von Herrath, M. CD4 T cell differentiation in type 1 diabetes. *Clin. Exp. Immunol.* **2016**, *183* (1), 16−29.

(9) Zhu, J.; Yamane, H.; Paul, W. E. Differentiation of effector CD4 T cell populations (*). *Annu. Rev. Immunol.* **2010**, *28*, 445−89.

(10) Zhou, L.; Chong, M. M.; Littman, D. R. Plasticity of CD4+ T cell lineage differentiation. *Immunity* **2009**, *30* (5), 646−55.

(11) Fontenot, J. D.; Gavin, M. A.; Rudensky, A. Y. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* **2003**, *4* (4), 330−6.

(12) Khattri, R.; Cox, T.; Yasayko, S. A.; Ramsdell, F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* **2003**, *4* (4), 337−42.

(13) Sakaguchi, S.; Yamaguchi, T.; Nomura, T.; Ono, M. Regulatory T cells and immune tolerance. *Cell* **2008**, *133* (5), 775−87.

(14) Frumento, D.; Ben Nasr, M.; El Essawy, B.; D'Addio, F.; Zuccotti, G. V.; Fiorina, P. Immunotherapy for type 1 diabetes. *J. Endocrinol Invest* **2017**, *40* (8), 803−814.

(15) Roep, B. O.; Wheeler, D. C. S.; Peakman, M. Antigen-based immune modulation therapy for type 1 diabetes: the era of precision medicine. *Lancet Diabetes Endocrinol.* **2019**, *7* (1), 65−74.

(16) Rosenblum, M. D.; Gratz, I. K.; Paw, J. S.; Abbas, A. K. Treating Human Autoimmunity: Current Practice and Future Prospects. *Sci. Transl. Med.* **2012**, *4* (125), 125sr1−125sr1.
(17) Henry, R. A.; Kendall, P. L.; Thomas, J. W. Autoantigen-specific B-cell depletion overcomes failed immune tolerance in type 1 diabetes. *Diabetes* **2012**, *61* (8), 2037−44.
(18) Miller, S. D.; Turley, D. M.; Podojil, J. R. Antigen-specific tolerance strategies for the prevention and treatment of autoimmune disease. *Nat. Rev. Immunol.* **2007**, *7* (9), 665−77.
(19) Garber, K. Immunology: A tolerant approach. *Nature* **2014**, *507*, 418−20.
(20) Eppler, H. B.; Jewell, C. M. Biomaterials as Tools to Decode Immunity. *Adv. Mater.* **2020**, *32* (13), 1903367.
(21) McCarthy, D. P.; Yap, J. W.; Harp, C. T.; Song, W. K.; Chen, J.; Pearson, R. M.; Miller, S. D.; Shea, L. D. An antigen-encapsulating nanoparticle platform for TH1/17 immune tolerance therapy. *Nanomedicine* **2017**, *13* (1), 191−200.
(22) Singha, S.; Shao, K.; Ellestad, K. K.; Yang, Y.; Santamaria, P. Nanoparticles for Immune Stimulation Against Infection, Cancer, and Autoimmunity. *ACS Nano* **2018**, *12* (11), 10621−10635.
(23) Kishimoto, T. K.; Maldonado, R. A. Nanoparticles for the Induction of Antigen-Specific Immunological Tolerance. *Front. Immunol.* **2018**, *9*, 230.
(24) Thorp, E. B.; Boada, C.; Jarbath, C.; Luo, X. Nanoparticle Platforms for Antigen-Specific Immune Tolerance. *Front. Immunol.* **2020**, *11*, 945.
(25) Liu, Q.; Wang, X.; Liu, X.; Kumar, S.; Gochman, G.; Ji, Y.; Liao, Y. P.; Chang, C. H.; Situ, W.; Lu, J.; Jiang, J.; Mei, K. C.; Meng, H.; Xia, T.; Nel, A. E. Use of Polymeric Nanoparticle Platform Targeting the Liver To Induce Treg-Mediated Antigen-Specific Immune Tolerance in a Pulmonary Allergen Sensitization Model. *ACS Nano* **2019**, *13* (4), 4778−4794.
(26) Zhang, T.; Tian, T.; Zhou, R.; Li, S.; Ma, W.; Zhang, Y.; Liu, N.; Shi, S.; Li, Q.; Xie, X.; Ge, Y.; Liu, M.; Zhang, Q.; Lin, S.; Cai, X.; Lin, Y. Design, fabrication and applications of tetrahedral DNA nanostructure-based multifunctional complexes in drug delivery and biomedical treatment. *Nat. Protoc.* **2020**, *15* (8), 2728−2757.
(27) Li, S.; Tian, T.; Zhang, T.; Cai, X.; Lin, Y. Advances in biological applications of self-assembled DNA tetrahedral nanostructures. *Mater. Today* **2019**, *24*, 57−68.
(28) Zhang, Q.; Lin, S.; Shi, S.; Zhang, T.; Ma, Q.; Tian, T.; Zhou, T.; Cai, X.; Lin, Y. Anti-inflammatory and Antioxidative Effects of Tetrahedral DNA Nanostructures via the Modulation of Macrophage Responses. *ACS Appl. Mater. Interfaces* **2018**, *10* (4), 3421−3430.
(29) Cui, W.; Zhan, Y.; Shao, X.; Fu, W.; Xiao, D.; Zhu, J.; Qin, X.; Zhang, T.; Zhang, M.; Zhou, Y.; Lin, Y. Neuroprotective and Neurotherapeutic Effects of Tetrahedral Framework Nucleic Acids on Parkinson's Disease in Vitro. *ACS Appl. Mater. Interfaces* **2019**, *11* (36), 32787−32797.
(30) Qin, X.; Li, N.; Zhang, M.; Lin, S.; Zhu, J.; Xiao, D.; Cui, W.; Zhang, T.; Lin, Y.; Cai, X. Tetrahedral framework nucleic acids prevent retina ischemia-reperfusion injury from oxidative stress via activating the Akt/Nrf2 pathway. *Nanoscale* **2019**, *11* (43), 20667−20675.
(31) Zhu, J.; Zhang, M.; Gao, Y.; Qin, X.; Zhang, T.; Cui, W.; Mao, C.; Xiao, D.; Lin, Y. Tetrahedral framework nucleic acids promote scarless healing of cutaneous wounds via the AKT-signaling pathway. *Signal Transduct Target Ther* **2020**, *5* (1), 120.
(32) Zhang, Y.; Ma, W.; Zhu, Y.; Shi, S.; Li, Q.; Mao, C.; Zhao, D.; Zhan, Y.; Shi, J.; Li, W.; Wang, L.; Fan, C.; Lin, Y. Inhibiting Methicillin-Resistant Staphylococcus aureus by Tetrahedral DNA Nanostructure-Enabled Antisense Peptide Nucleic Acid Delivery. *Nano Lett.* **2018**, *18* (9), 5652−5659.
(33) Liu, Y.; Sun, Y.; Li, S.; Liu, M.; Qin, X.; Chen, X.; Lin, Y. Tetrahedral Framework Nucleic Acids Deliver Antimicrobial Peptides with Improved Effects and Less Susceptibility to Bacterial Degradation. *Nano Lett.* **2020**, *20* (5), 3602−3610.
(34) Trudeau, J. D.; Kelly-Smith, C.; Verchere, C. B.; Elliott, J. F.; Dutz, J. P.; Finegood, D. T.; Santamaria, P.; Tan, R. Prediction of spontaneous autoimmune diabetes in NOD mice by quantification of autoreactive T cells in peripheral blood. *J. Clin. Invest.* **2003**, *111* (2), 217−23.
(35) Lieberman, S. M.; Evans, A. M.; Han, B.; Takaki, T.; Vinnitskaya, Y.; Caldwell, J. A.; Serreze, D. V.; Shabanowitz, J.; Hunt, D. F.; Nathenson, S. G.; Santamaria, P.; DiLorenzo, T. P. Identification of the beta cell antigen targeted by a prevalent population of pathogenic CD8+ T cells in autoimmune diabetes. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100* (14), 8384−8.
(36) Sheehy, D. F.; Quinnell, S. P.; Vegas, A. J. Targeting Type 1 Diabetes: Selective Approaches for New Therapies. *Biochemistry* **2019**, *58* (4), 214−233.
(37) Bluestone, J. A.; Buckner, J. H.; Fitch, M.; Gitelman, S. E.; Gupta, S.; Hellerstein, M. K.; Herold, K. C.; Lares, A.; Lee, M. R.; Li, K.; Liu, W.; Long, S. A.; Masiello, L. M.; Nguyen, V.; Putnam, A. L.; Rieck, M.; Sayre, P. H.; Tang, Q. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl. Med.* **2015**, *7* (315), 315ra189.
(38) Curtsinger, J. M.; Lins, D. C.; Mescher, M. F. Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. *J. Exp. Med.* **2003**, *197* (9), 1141−51.
(39) Xing, Y.; Hogquist, K. A. T-cell tolerance: central and peripheral. *Cold Spring Harbor Perspect. Biol.* **2012**, *4* (6), a006957.
(40) Mescher, M. F.; Popescu, F. E.; Gerner, M.; Hammerbeck, C. D.; Curtsinger, J. M. Activation-induced non-responsiveness (anergy) limits CD8 T cell responses to tumors. *Semin. Cancer Biol.* **2007**, *17* (4), 299−308.
(41) Zhu, J. T Helper Cell Differentiation, Heterogeneity, and Plasticity. *Cold Spring Harbor Perspect. Biol.* **2018**, *10* (10), a030338.
(42) Zhao, D.; Cui, W.; Liu, M.; Li, J.; Sun, Y.; Shi, S.; Lin, S.; Lin, Y. Tetrahedral Framework Nucleic Acid Promotes the Treatment of Bisphosphonate-Related Osteonecrosis of the Jaws by Promoting Angiogenesis and M2 Polarization. *ACS Appl. Mater. Interfaces* **2020**, *12* (40), 44508−44522.
(43) Kalekar, L. A.; Mueller, D. L. Relationship between CD4 Regulatory T Cells and Anergy In Vivo. *J. Immunol.* **2017**, *198* (7), 2527−2533.
(44) Sirong, S.; Yang, C.; Taoran, T.; Songhang, L.; Shiyu, L.; Yuxin, Z.; Xiaoru, S.; Tao, Z.; Yunfeng, L.; Xiaoxiao, C. Effects of tetrahedral framework nucleic acid/wogonin complexes on osteoarthritis. *Bone Res.* **2020**, *8*, 6.
(45) Fu, W.; You, C.; Ma, L.; Li, H.; Ju, Y.; Guo, X.; Shi, S.; Zhang, T.; Zhou, R.; Lin, Y. Enhanced Efficacy of Temozolomide Loaded by a Tetrahedral Framework DNA Nanoparticle in the Therapy for Glioblastoma. *ACS Appl. Mater. Interfaces* **2019**, *11* (43), 39525−39533.
(46) Li, S.; Sun, Y.; Tian, T.; Qin, X.; Lin, S.; Zhang, T.; Zhang, Q.; Zhou, M.; Zhang, X.; Zhou, Y.; Zhao, H.; Zhu, B.; Cai, X. MicroRNA-214−3p modified tetrahedral framework nucleic acids target survivin to induce tumour cell apoptosis. *Cell Proliferation* **2020**, *53* (1), e12708.
(47) Sun, Y.; Li, S.; Zhang, Y.; Li, Q.; Xie, X.; Zhao, D.; Tian, T.; Shi, S.; Meng, L.; Lin, Y. Tetrahedral Framework Nucleic Acids Loading Ampicillin Improve the Drug Susceptibility against Methicillin-Resistant Staphylococcus aureus. *ACS Appl. Mater. Interfaces* **2020**, *12* (33), 36957−36966.
(48) Goodman, R. P.; S, I. A. T.; Tardin, C. F.; Erben, C. M.; Berry, R. M.; Schmidt, C. F.; Turberfield, A. J. Rapid Chiral Assembly of Rigid DNA Building Blocks for Molecular Nanofabrication. *Science* **2005**, *310*, 1661.
(49) Shields, C. W. t.; Wang, L. L.; Evans, M. A.; Mitragotri, S. Materials for Immunotherapy. *Adv. Mater.* **2020**, *32* (13), 1901633.
(50) Seif, F.; Khoshmirsafa, M.; Aazami, H.; Mohsenzadegan, M.; Sedighi, G.; Bahar, M. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Commun. Signaling* **2017**, *15* (1), 23.

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX

(51) Renauld, J. C. Class II cytokine receptors and their ligands: key antiviral and inflammatory modulators. *Nat. Rev. Immunol.* **2003**, *3* (8), 667−76.
(52) Darnell, J. E., Jr. STATs and gene regulation. *Science* **1997**, *277* (5332), 1630−5.
(53) Fabbri, M.; Frixou, M.; Degano, M.; Fousteri, G. Type 1 Diabetes in STAT Protein Family Mutations: Regulating the Th17/Treg Equilibrium and Beyond. *Diabetes* **2019**, *68* (2), 258−265.
(54) O'Shea, J. J.; Murray, P. J. Cytokine signaling modules in inflammatory responses. *Immunity* **2008**, *28* (4), 477−87.
(55) O'Shea, J. J.; Plenge, R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity* **2012**, *36* (4), 542−50.
(56) Banerjee, S.; Biehl, A.; Gadina, M.; Hasni, S.; Schwartz, D. M. JAK-STAT Signaling as a Target for Inflammatory and Autoimmune Diseases: Current and Future Prospects. *Drugs* **2017**, *77* (5), 521−546.
(57) Laurence, A.; Tato, C. M.; Davidson, T. S.; Kanno, Y.; Chen, Z.; Yao, Z.; Blank, R. B.; Meylan, F.; Siegel, R.; Hennighausen, L.; Shevach, E. M.; O'Shea, J. J. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* **2007**, *26* (3), 371−381.
(58) Jin, Y.; Purohit, S.; Chen, X.; Yi, B.; She, J. X. Over-expression of Stat5b confers protection against diabetes in the non-obese diabetic (NOD) mice via up-regulation of CD4(+)CD25(+) regulatory T cells. *Biochem. Biophys. Res. Commun.* **2012**, *424* (4), 669−74.
(59) Chen, Z.; Laurence, A.; O'Shea, J. J. Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. *Semin. Immunol.* **2007**, *19* (6), 400−8.
(60) Flanagan, S. E.; Haapaniemi, E.; Russell, M. A.; Caswell, R.; Allen, H. L.; De Franco, E.; McDonald, T. J.; Rajala, H.; Ramelius, A.; Barton, J.; Heiskanen, K.; Heiskanen-Kosma, T.; Kajosaari, M.; Murphy, N. P.; Milenkovic, T.; Seppänen, M.; Lernmark, Å.; Mustjoki, S.; Otonkoski, T.; Kere, J.; Morgan, N. G.; Ellard, S.; Hattersley, A. T. Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat. Genet.* **2014**, *46* (8), 812−814.
(61) Haapaniemi, E. M.; Kaustio, M.; Rajala, H. L. M.; van Adrichem, A. J.; Kainulainen, L.; Glumoff, V.; Doffinger, R.; Kuusanmäki, H.; Heiskanen-Kosma, T.; Trotta, L.; Chiang, S.; Kulmala, P.; Eldfors, S.; Katainen, R.; Siitonen, S.; Karjalainen-Lindsberg, M.-L.; Kovanen, P. E.; Otonkoski, T.; Porkka, K.; Heiskanen, K.; Hänninen, A.; Bryceson, Y. T.; Uusitalo-Seppälä, R.; Saarela, J.; Seppänen, M.; Mustjoki, S.; Kere, J. Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. *Blood* **2015**, *125* (4), 639−648.
(62) Kretschmer, K.; Apostolou, I.; Hawiger, D.; Khazaie, K.; Nussenzweig, M. C.; von Boehmer, H. Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* **2005**, *6* (12), 1219−27.

https://doi.org/10.1021/acs.nanolett.1c01131
Nano Lett. XXXX, XXX, XXX−XXX