**SUMMARY (cutting edge) 7/17/25**

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Source

* Degroote, L., Martens, P.-J., Viaene, M., et al. (2025). Verapamil and low-dose anti-mouse thymocyte globulin combination therapy stably reverses recent-onset type 1 diabetes in NOD mice by acting on the beta cell and immune axes. *Diabetologia*. https://doi.org/10.1007/s00125-025-06490-8Executive Summary

This research investigates the synergistic potential of combining verapamil, a calcium channel blocker, with low-dose anti-thymocyte globulin (ATG) to treat recent-onset (stage 3) type 1 diabetes (T1D) in Non-Obese Diabetic (NOD) mice. The study demonstrates that this combination therapy significantly outperforms either monotherapy in achieving durable diabetes reversal by targeting both beta cell protection and immune modulation. Key findings include improved beta cell function, increased pancreatic insulin content and beta cell volume, reduced severe insulitis, and a favorable shift in regulatory T cell (Treg) to effector memory T cell (TEM) ratios in pancreatic draining lymph nodes (PLNs). These results highlight a promising strategy for T1D reversal in humans, particularly with early intervention.

Main Themes and Key Findings

1. Superior and Sustained Diabetes Reversal with Combination Therapy

* **Combination Efficacy:** The combination of verapamil and low-dose rabbit-anti-mouse ATG (mATG) demonstrated the highest and most durable diabetes reversal rates in NOD mice. By day 56 after therapy initiation, 45% (9 out of 20 mice) of combination-treated mice achieved durable diabetes reversal.
* **Monotherapy Limitations:**Verapamil monotherapy achieved stable reversal in 20% (3 out of 15) of mice by day 56.
* Low-dose mATG monotherapy showed initial efficacy (39% reversal by day 7) but waned to 17% (3 out of 18) by day 56, indicating a temporary effect.
* **Synergistic Effect:** The study suggests a synergistic interaction where "mATG rapidly modulates the immune response, allowing verapamil to preserve beta cell function more effectively." This prevents the loss of therapeutic efficacy observed with mATG alone.
* **Early Intervention Importance:** Combination therapy was "most efficacious in mice with mild hyperglycaemia at therapy start," emphasizing "the importance of early intervention either at clinical diagnosis (stage 3 type 1 diabetes) or prior to the acute decline in beta cell function (stage 2 type 1 diabetes)."

2. Beta Cell Protection and Function Preservation

* **Improved Beta Cell Function:** Only combination therapy-treated mice showed "significantly better preservation of C-peptide secretion compared with untreated controls" by day 14. C-peptide is a marker of endogenous insulin production.
* **Increased Insulin Content and Volume:** The enhanced beta cell function in combination-treated mice was associated with "an increased pancreatic insulin content" and "greater insulin-positive volume relative to pancreatic volume" compared with untreated controls.
* **TXNIP Downregulation:** Verapamil, both alone and in combination, reduced the expression of thioredoxin-interacting protein (Txnip) in pancreatic beta cells of reversed mice. Txnip is a "key regulator of beta cell apoptosis and dysfunction," and its reduction suggests "improved beta cell survival and function." The study noted that "This upregulation [of Txnip] was absent in mice becoming normoglycaemic by day 14 after therapy start, regardless of treatment regimen."
* **Verapamil's Mechanism:** Verapamil protects beta cells by:
* Reducing TXNIP expression, which is induced under hyperglycemic conditions and promotes beta cell apoptosis.
* "Preventing the pro-apoptotic pathways activated by hyperglycaemia."
* Mitigating oxidative stress by reducing TXNIP's inhibition of thioredoxin.
* Upregulating genes (Txnrd1, Srxn1) that maintain cellular redox potential.

3. Immune Modulation and Reduced Insulitis

* **Reduced Insulitis Severity:** Combination therapy "exhibited a modest increase in the proportion of insulitis-free islets and a reduced proportion of heavily infiltrated islets" compared to untreated controls. Insulitis refers to immune cell infiltration in pancreatic islets, which contributes to beta cell destruction in T1D.
* **Transient Lymphocyte Depletion by mATG:** Low-dose mATG, alone or in combination, led to a "marked decrease in WBC, lymphocyte and monocyte counts by day 3 after therapy start," which largely recovered by day 14. This initial depletion limits the activation of new self-reactive T cells.
* **T Cell Phenotype Shifts in Peripheral Blood:** mATG treatment (alone or combined) caused:
* An initial increase in the CD4+:CD8+ T cell ratio, normalizing by day 14.
* Reduced frequency of naive T cells (CD44LowCD62L+) and increased proportion of effector memory T cells (TEMs, CD44HiCD62L−) in peripheral blood.
* An initial decrease in FoxP3+CD4+ regulatory T cells (Tregs) in peripheral blood at day 3, followed by "higher frequencies... at day 14."
* **Favorable Immune Regulation in Pancreatic Draining Lymph Nodes (PLNs):**Only in combination-treated mice was "a higher CD4+ regulatory T cell to CD8+ effector memory T cell ratio observed in the pancreatic draining lymph nodes."
* Combination therapy resulted in a "notable expansion of FoxP3+CD4+ Tregs, both CD25+ and CD25−, in the PLNs of combination-treated mice." This is significant because PLNs are "a key site for T cell proliferation and activation."
* **Cytokine Release:** mATG treatment (alone or combined) triggered "significant increases in plasma values of IFN-γ, IL-2 and IL-10 on day 3 after therapy start," which partially or completely recovered by day 14.

4. Clinical Relevance and Future Directions

* **Complementary Mechanisms:** The study highlights that verapamil and ATG have "complementary mechanisms of action and distinct targets," making their combination a promising strategy for "more durable therapeutic outcomes."
* **Current Clinical Trials:** The findings are timely as clinical trials for verapamil monotherapy (VER-A-T1D; NCT04545151) and low-dose ATG (MELD-ATG; NCT04509791) are soon to read out, and combination therapy trials are being initiated (ISRCTN45965456; NCT06455319). This preclinical study provides strong evidence supporting these ongoing and future human trials.
* **Limitations:** The study acknowledged that it did not confirm the previously observed downregulation of proinflammatory cytokine IL-21 with verapamil therapy, as plasma concentrations were below the detection limit in their assay.

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