Development of Immunotherapies for Type 1 Diabetes
Value and Limitations of Mouse Models

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The use of animal models
• It’s very valuable to use animal models, if they are used in a correct fashion
• It’s very important in the design of the experiment to know the limitations of animal models
• Disclaimer: no Novo Nordisk drugs or confidential aspects will be discussed here

Strategy T1D – a prioritization process from mice to humans
1. Prioritize existing therapies in animal models
   - Efficacy versus side effects
   - Biomarkers that identify responders
   - Combinability with other immunotherapeutics (i.e., Ag+αCD3)
   - Ease of use, route and formulation
   - Licensing aspects
The non-obese diabetic (NOD) mouse: Some key translational successes and failures

- Approximately 195 published methods prevent or delay the development of T1D in NOD mice

- Not all treatments prevent disease, treatment dose and timing strongly influence efficacy, and several therapies have successfully treated overtly diabetic mice
  Shoda et al. (Immunity, 2005)

- Modest, temporary trial effects of CTLA-4, B cell and alpha-1 anti-trypsin pathway targeting were predictable from data in the NOD model

- C-peptide preservation was correctly predicted in recently diagnosed patients with anti-CD3

The non-obese diabetic (NOD) mouse – incidence can vary depending on site/colony

La Jolla, Florida, Colorado-T1D incidence over time in NOD colony

- Many key characteristics of human T1D apply, such as genetic dependence on MHC class II, heterogeneous albeit stronger infiltrate, but some don’t, for example no CD4 cells or regulatory cells in human islets

Thus there is a strong need to address reproducibility in the NOD model

Immune Tolerance Network (ITN) together with JDRF has developed a Type 1 Diabetes Preclinical Consortium

- Multi-center network of geographically diverse research laboratories
- Use standardized SOPs to assess data reproducibility across sites
- To generate high-quality, FDA-acceptable preclinical data to support combination therapy studies
Reproducibility in the NOD model: a case study

- There can be a synergy, in this case with anti-CD3 and IL1 blockers, in reverting diabetes
- It might be more complicated to implement a combination therapy when the synergy is geographically-dependant
- Animal models are depending on a multitude of environmental factors in addition to genetics
- There are factors we can’t control, therefore repetition in various geographical locations while using with the best common SOPs is very important

Outcome: strong inter-site variability, but no obvious added benefit from IL-1β blockade

Anti-CD3 mono (1)   Anti-CD3/anti-IL-1β (1)
Anti-CD3 mono (2)   Anti-CD3/anti-IL-1β (2)

Reproducibility in the NOD model:
The Novo Nordisk experience using 2 sites and dose response kinetics to detect optimal effect (prevention ex)

The model is too variable in conjunction with a certain immune intervention
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Increasing stringency level options in the NOD mouse model
We can set the stringency of the test ourselves

Spontaneous NOD model > Transfer NOD model > Spontaneous NOD model

PREVENTION

The stringency of the testing platform matters a lot

Predictive potential of biomarkers in NOD mice: autoantibodies

Preexisting Autoantibodies Predict Efficacy of Oral Insulin to Cure Autoimmune Diabetes in Combination With Anti-CD3

New data

Combination therapy with anti-CD3 and p277 offers synergistic protection

Human trial is still required and it has to have a positive outcome

Predictive potential of biomarkers in NOD mice: CD8+ T cells

Fewer IGRP-specific CD8 T cells correlate with euglycemia

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Alternate models: The RIP-LCMV mouse

- Well-characterized CD8 T cell-driven model, mimics the infiltration of islets by CD8 T cells observed in humans but not genetics of diabetes – short clinical course (practical and high-stringency model)
- Because of the aspect of resembling some of pathogenetic features of human type I diabetes, this model was used often.

Intravital imaging of CTLs killing islet cells in RIP-LCMV mice

- Once CD8 cells enter mouse pancreas, they have no directionality
- They travel randomly until they encounter antigens
- We have different animal models with different levels of stringencies and mechanisms. Each might illuminate certain aspects of human disease

Stringent testing of combination therapies in RIP-LCMV mice after diabetes onset

- Anti-CD3 + nasal proinsulin combination therapy

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Ongoing general challenges: 60-80% of published preclinical data are not reproducible

- Mouse numbers are usually too low in academic labs
- Negative results rarely published
- Experimental "noise" often overlooked
- Wrong statistical tests applied
- One single animal model used
- Single site experimentation
- Randomization issues

Mitigation actions & consequences

- Increase mouse numbers to adequately power studies
- Provide a strong incentive to publish negative data
- Use all appropriate control groups
- Bolster expertise in statistics
- Use of both spontaneous & induced T1D animal models
- Multi-site collaborations
- Randomization where appropriate & feasible

Recommendations, moving forward

For all T1D studies
- Use GraphPad StatMate™ to calculate statistical power for all experiments.
  To do so, we need:
  ▶ Desired effect size to be discussed
  ▶ Historical/Hypothesized T1D incidence baseline for control group(s)
- This will define how many mice to enroll in each desired group
  ▶ Exception: allow lower power (e.g., 60-80%) for pilot experiments (n=16/group)

For T1D prevention studies
- Display T1D incidence increasing with age
- Follow mice up to 30 weeks of age
- Track in-house T1D incidence approx. every quarter (more closely if construction on site)

For T1D intervention studies
- Display T1D incidence decreasing with time post-treatment onset
- Follow mice up to 4-5 wks post-onset at least
- Analyze data from multiple angles
- Individual & mean BGs,
  ▶ Transient vs. permanent cure
  ▶ Mild vs. severe hyperglycemia (endpoints)

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Sample power calculations

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For T1D prevention studies
- Experience of a ~75% baseline incidence in NOD mice [2013]

For T1D intervention studies
- Experience of a 20% baseline remission in liraglutide-treated NOD mice
- Aim for a given combination: > 50-70% remission (hopefully even more)

Sample size of 16 mice per group has an 80% power to detect a significant (P<0.05) decrease of 48% in T1D upon Treatment X

Sample size of 20 mice per group has an 80% power to detect a significant (P<0.05) increase in cured mice of 45.7% upon lira/agent X combination (compared to our lira baseline)

Summary of ongoing effort toward standardizing statistical methods to employ for T1D studies

Conclusions and recommendations
- Mouse models for type 1 diabetes offer value if used and interpreted appropriately
- Need for careful protocol design (cage randomization, threshold definition, etc.)
- Proper use of statistics
- Ultimately, validation at geographically different sites
- Rationale for clinical trials in the past sometimes questionable based on animal data

Many thanks
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