Type 1 Diabetes Pre-Clinical Testing Program (T1D-PTP)

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Overview

- Purpose:
  - To perform pre-clinical studies of potential new therapeutics for the prevention or treatment of diabetic complications (nephropathy, neuropathy, retinopathy)
- Primary objective:
  - Provide services to T1D-RAID & T1D-PTP programs
- Secondary objective:
  - Provide neuropathy phenotyping to AMDCC
- Studies performed “in house” (UCSD) or via specialist sub-contractor.

Program Management

Application to T1D RAID deemed to require additional pre-clinical studies before final consideration or direct application to T1D-PTP

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Discussions between project officer and contractor regarding T1D RAID application and its reviews. Outside experts may also be consulted

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Discussions with applicants regarding protocols for additional pre-clinical studies

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Protocol agreed by all parties (applicant, contractor, project officer)

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Identification of any sub-contractor and agreement of final budget between project officer and contractor

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Initiation of pre-clinical studies

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Monthly teleconferences between applicants, project officer and contractor during study period

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Preparation of final report with subsequent review by outside experts if required

Models and Assays Offered I

- Models (contract allows for use of existing and emerging as required)
  - Mice: STZ, Akita, db/db
  - Rats: STZ, ZDF
  - Rabbit: Alloxan

- General metabolic phenotyping
  - Glucose
  - Insulin
  - HbA1c
  - Body weight
  - Blood pressure
  - Metabolic cages for food/water uptake, urine output etc

Models and Assays Offered II

- Neuropathy
  - Pain: tactile, thermal, formalin
  - Function: NCV, NBF, RDD
  - Structure: IENF (PGP9.5/GAP43), morphometry, EM, CCM
  - Mechanistic: GC, EIA, enzyme assays, western blots, qPCR

- Nephropathy
  - GFR (inulin clearance)
  - Plasma creatinine
  - Urine albumin:creatinine
  - Glomerular histopathology (mesangial matrix expansion, arteriolar hyalinosis, glomerular basement membrane thickening, tubular interstitial fibrosis)

Models and Assays Offered III

- Retinopathy
  - Histopathology (retinal thickness, capillary cell apoptosis, capillary drop-out, neovascularization, neurodegeneration
- Wound healing
  - Blood flow, closure rate, histopathology.

Completed Neuropathy Study

- Rationale:
  - Test agent previously shown to prevent NCV slowing and ischemia in STZ-diabetic rats
- Objectives:
  - Test new formulation
  - Test dose range
  - Test in an intervention study
  - Test against indices of nerve structural damage
- Protocol:
  - Adult STZ-diabetic rats treated iv during weeks 6-12
  - MNCV, SNCV, thermal, tactile @ 0,6 & 12 wks
  - NBF, IENF, axonal caliber

Completed Neuropathy Study

- Findings:
  - Effective vs hypotension, allodynia, NCV slowing*, NBF
  - Not effective vs IENF loss, reduced axonal caliber, thermal hypoalgesia
- Follow-up:
  - *Efficacy vs NCV slowing shown to be acute and transient
  - Test agent reduced oxidative stress but not polyol pathway
- Conclusions:
  - New formulation: Effective and dose-dependent vs NCV
  - Intervention: Effective vs NCV, allodynia, NBF
  - Efficacy vs degeneration: Not effective
  - Data presented and ms in preparation highlighting transient effects and lack of efficacy on structure
  - Molecule not re-entered to RAID program

Completed Nephropathy Study

- Rationale:
  - Preliminary data of T1D-RAID applicant indicated efficacy in preventing mesangial expansion and proteinurea in the hypertensive Ren-2 model
- Objectives:
  - Test in rat models of long term type 1 (STZ) and type 2 (ZDF) diabetes
  - Examine efficacy in both prevention and reversal paradigms
- Protocol:
  - 2 studies (ZDF and STZ diabetes) with similar design
  - Up to 9 months of diabetes with twice daily treatment with test agent in prevention and reversal paradigms
  - Monthly spot or 24 hr urine collection and monthly bleeds for measurement of plasma creatinine and urine albumin:creatinine ratio by sub-contractor (Dr. Sharma)
  - Terminal histopathology of kidney (Dr. Sharma).

Completed Nephropathy Study

- Findings
  - Agent prevented the increase in ACR of both STZ and ZDF rats (primary end point)
  - Agent reversed elevated ACR in STZ rats and attenuated increasing ACR in ZDF rats (primary end point)
  - Agent prevented increase in glomerular area and PAS +ve area in ZDF rats (secondary end point)
  - No efficacy against indices of neuropathy (speculative end point)

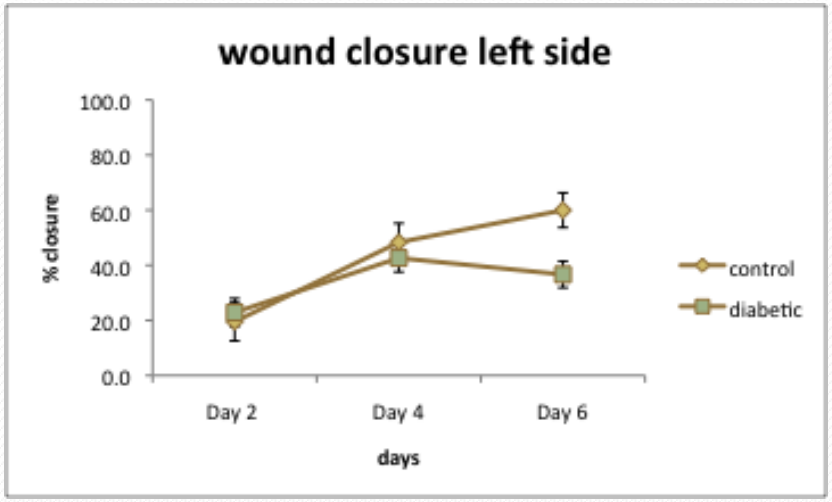
- Conclusions
  - Preliminary efficacy studies supported against primary end point in 2 models
  - Proceed to RAID……..

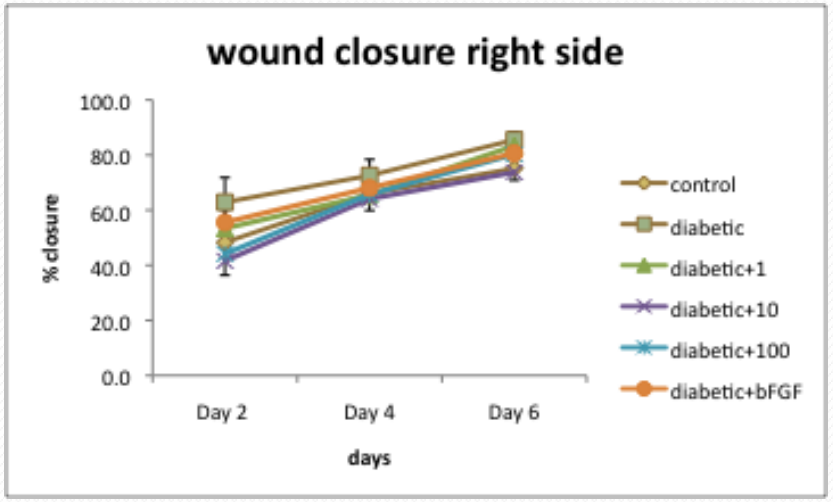
Wound Healing: A Digression in 3 Hiccups

- Rationale:
  - 2 compounds entered for RAID with preliminary data suggesting enhanced rate of wound closure in db/db mice
- Objectives:
  - Replicate preliminary data and develop dose:efficacy curve
  - Extend efficacy to type 1 mouse, type 1 rat, type 1 rabbit
  - Develop quantitative assays of skin blood flow and wound histopathology to support qualitative assessments
- Protocol:
  - Bilateral 5mm diameter full thickness wounds to the back
  - Daily topical treatment to left side, with vehicle to right side
  - Daily measurement of skin blood flow and wound diameter
  - Terminal collection of skin for histology

Wound Healing

- Study #1: STZ-diabetic C57 Bl/6 mice
  - Left (vehicle) side replicated slower closure of diabetics, but right side closed faster than left in all groups (C, D+vehicle, D+drug)

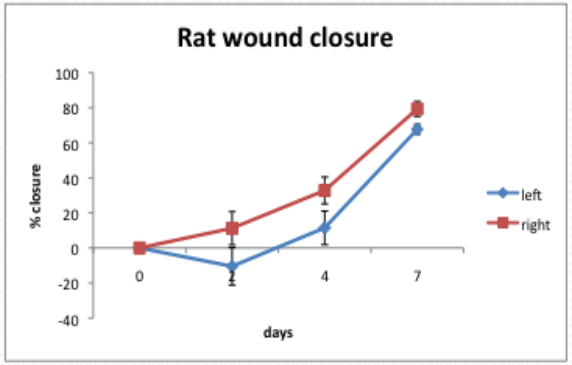


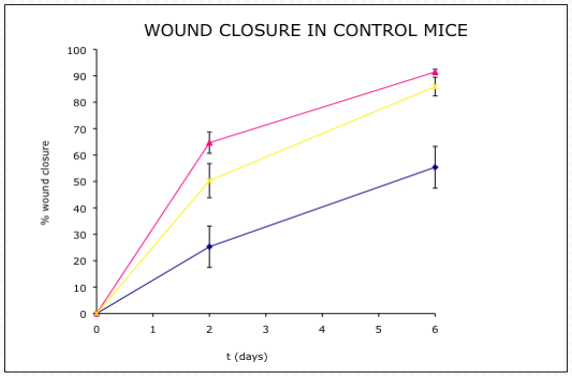


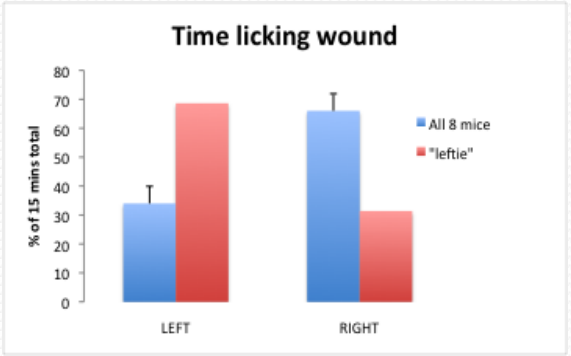
  - Diabetes increased blood flow and both drug and bFGF increased flow further on the treated side

More Wound Healing

- Normal rats and mice
  - Right side closes faster than left, irrespective of which side is wounded first
  - Right side also closes faster than left in rats
  - A single central wound closes like the right side
  - A single left wound closes like the right side after bilateral wounding
  - Mice preferentially lick the right wound of a pair
- Modifications to protocol
  - Treat only left side and use the right wound as decoy.
  - May not apply to db/db mice as they are too fat or lazy to lick their backs…

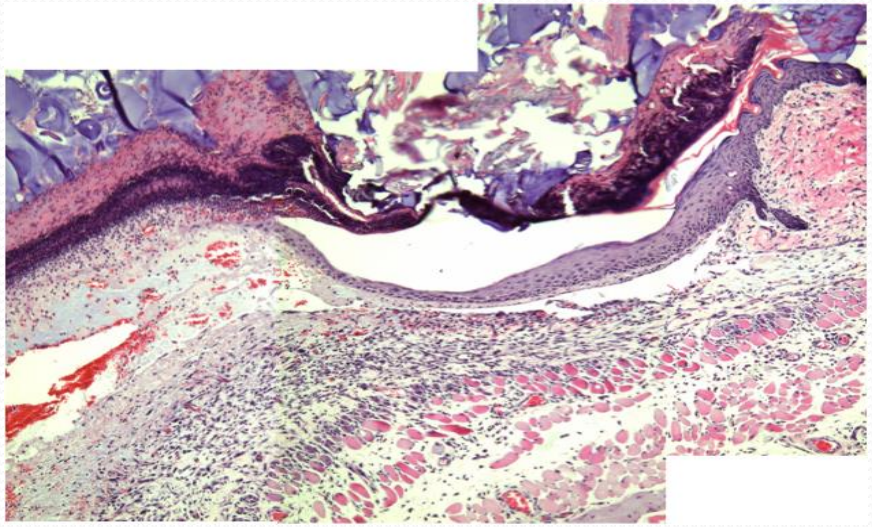


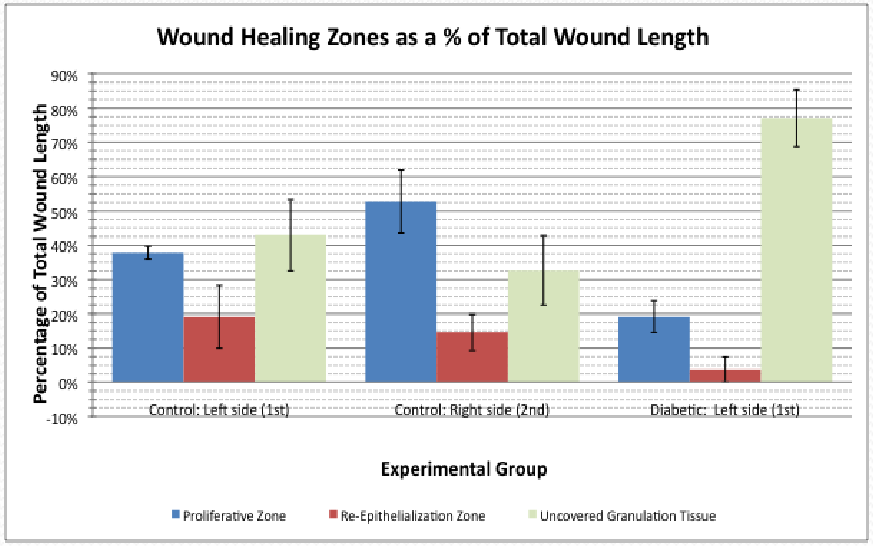




Histopathology:quantification

- Observer measures length of each of 3 zones in coded tissue
- Method identifies faster closure of right vs left wound in control mice as an increase in the proliferation zone
- Slower closure of left wound in diabetic mice identified in decreased proliferation and re-epithelialization zones and an increase in the uncovered granulation tissue





Programmatic Problems…..

- Technical:
  - None encountered to date with neuropathy (in house) or nephropathy (in house or sub-contractors)
  - Wound healing digression….

- Demand:
  - Contract designed to support up to 5 studies/year
  - Initial low numbers of projects, although early projects involved long term studies
  - Recent increase in demand:
    - 2009 projects:
      - Nephropathy: 4 studies (2 rat models, prevention and intervention)
      - AMDCC neuropathy phenotyping
      - Wound healing: 1 project, with up to 4 studies (2 x mouse, rat, rabbit)
    - 2010 projects:
      - Neuropathy: 1 project with up to 3 studies (2 mouse models, 1 rat model)
      - AMDCC neuropathy phenotyping
      - Wound healing: 1 project, with up to 4 studies (2 x mouse, rat, rabbit)

Users to date

- ACADEMICS
  - **PRO's**: Provides resources to develop compounds beyond observations of efficacy, does "non-publishable" work, familiar with application process,
  - **CON's:** No $ to the applicant, frequently have poor business plan, protective
- SMALL BIOTECHs
  - **PRO's:** Resources, data validation, prepares folio for sale/partnership
  - **CON's:** IP issues
- BIOTECHS
  - **PRO's:** Inexpensive route to advance molecule/project leader within company
  - **CON's:** Time to write application, review time, keeping management interested, inclined to keep control or program by paying CRO
- PHARMA
  - **PRO's:** Inexpensive route to advance molecule/project leader within company
  - **CON's:** More likely to be done in house