Research Results Evaluating the Diamidine Class for the Treatment of HAT

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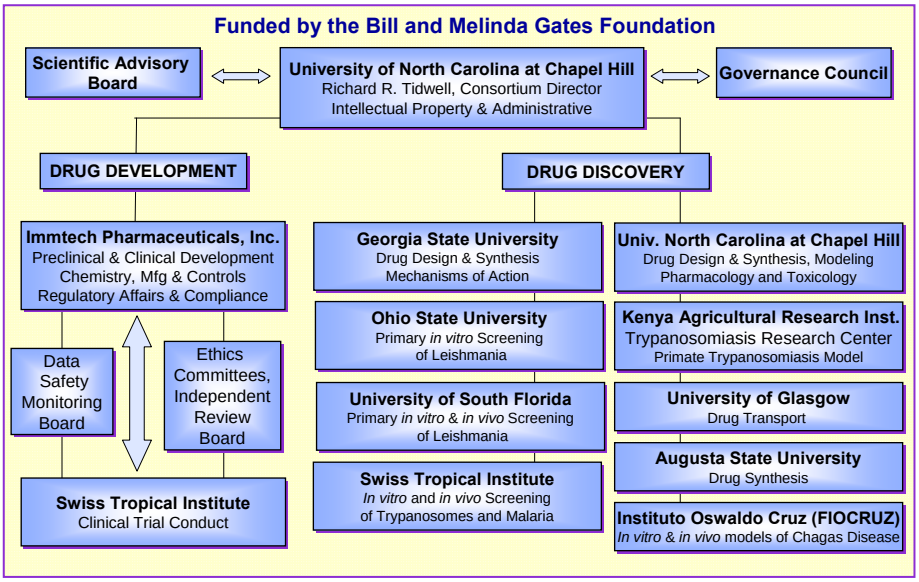
Mission Statement: Consortium for Parasitic Drug Development

The mission of the non-profit Consortium for Parasitic Drug Development (CPDD) is to discover and develop new treatments for human African trypanosomiasis (sleeping sickness) and visceral leishmaniasis. These diseases threaten nearly half the world's population and cause massive disability, death and economic loss.

Consortium for Parasitic Drug Development (CPDD)

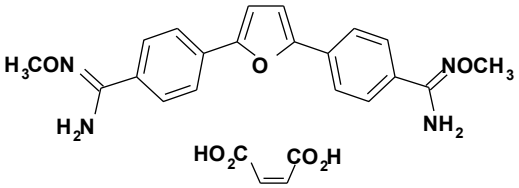


Pafuramidine Maleate

Chosen for Development in 2001

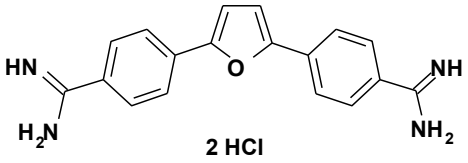
Pafuramidine (DB289)

- Orally available prodrug
- No significant activity



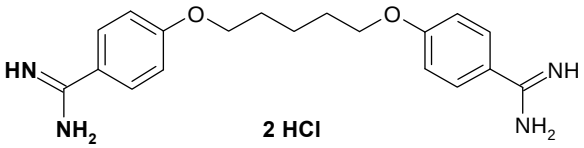
DB75 (furamidine)

- Metabolically active
- Not orally available
- Does not reach CNS

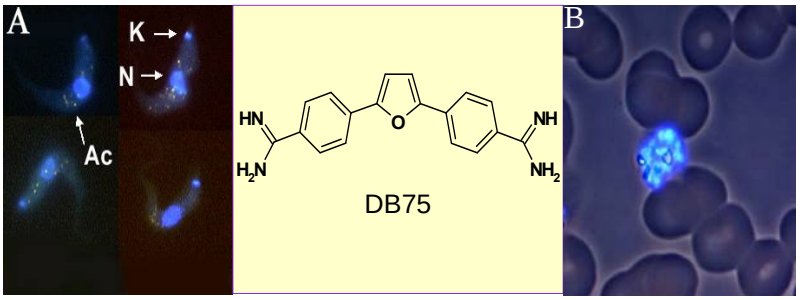


Pentamidine

- Prototype diamidine
- Standard of care for first stage HAT



Intracellular Concentration of DB75



***Trypanosoma brucei brucei* exposed to 1 µM DB 075 for 15 min (A). Early in the exposure the nucleus (N) and the kinetoplast (K) fluoresce brightly and the acidocalcisomes (Ac) only slightly. *Plasmodium falciparum* (B) exposed to 1 µM DB 075 for 4 hr.**

In vivo Efficacy of Pafuramidine (DB289) in Animal Models of HAT

- Mice infected acutely with *T. b. brucei* were cleared of infection with a DB75 (single IV dose) or DB289 (single IV or oral dose)
  - DB289 had an excellent dose response, with an ED_{50} of 2.7 mg/kg
  - No recrudescence 30 days post treatment ≅ cure
- Mice chronically infected with *T. b. brucei* were cleared of parasites when treated with DB289 9.5 mg/kg/d for 3 days
  - No parasites were detected 30 days post treatment
  - Brain extracts were still infectious, indicating that DB289 or DB75 was not effective against CNS disease
- Vervet monkeys infected with *T. b. rhodesiense* were cleared of parasites when treated with DB289 6.7 mg/kg/d for 5 days
  - All three animals remained free of infection in the blood and CSF 90 days post treatment
- No overt toxicity or adverse events were observed

Pafuramidine (DB289) and DB75 Preclinical Safety Data

- Completed standard protocols for:
  - Safety pharmacology (neurological, cardiac, renal effects)
  - Assays for genetic toxicity
  - Animal toxicology
    - Acute to 3 month administration
    - Juvenile study
  - Pharmacokinetics
  - Animal and *in vitro* metabolism
  - Protein binding and tissue accumulation
  - Milk secretion in rats
  - Effects on embryo and fetal development, pre- and post-natal development, fertility
- Only significant safety finding was liver toxicity at higher doses of DB289 or DB75, and minimal safety margin based on calculated animal and human exposure

Pafuramidine Phase 1 Clinical Summary
Safety and Pharmacokinetics

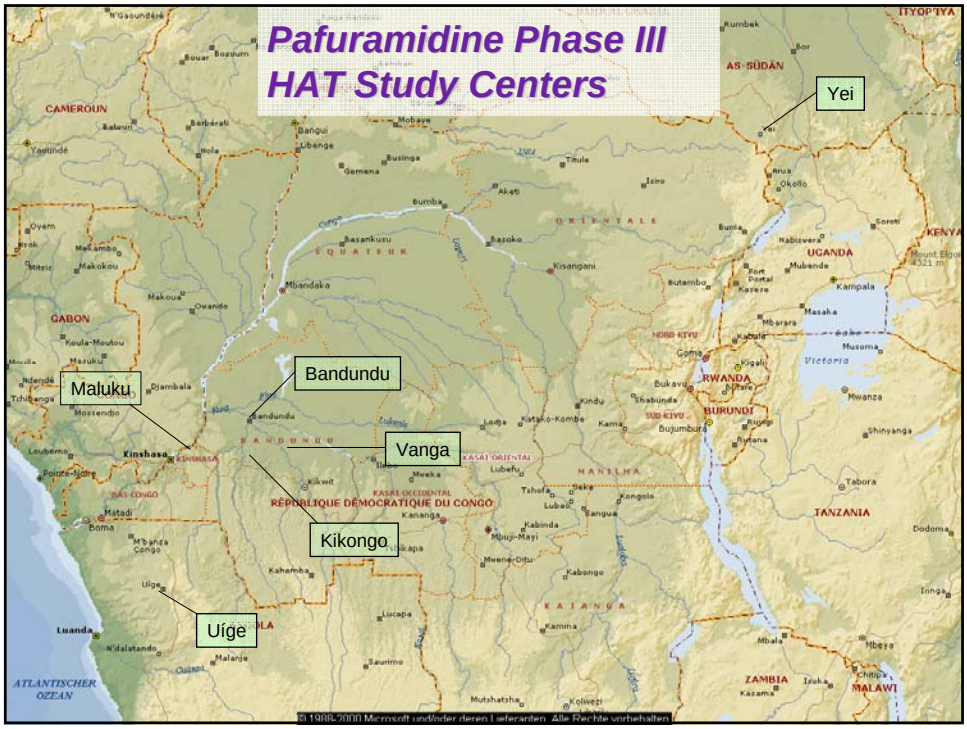
- Phase 1 clinical studies with single and multiple oral doses of pafuramidine in healthy volunteers
  - Pafuramidine was well tolerated
  - One subject had a serious adverse event of hepatitis, presumed to be due to DB289
- DB289 and DB75 pharmacokinetics
  - Pafuramidine was absorbed and converted to DB75
  - Plasma levels correlated with *in vivo* efficacy in animal models of trypanosomiasis
  - Plasma levels were above the *in vitro* IC_{50} for *P. falciparum* (malaria)

Pafuramidine (DB289) Phase II HAT Studies: Efficacy at Primary Endpoint

- Phase IIa study – N =32
  - Uncontrolled proof of concept trial at 2 sites (Angola and DRC)
  - DB289 100 mg BID x 5 days
  - Primary efficacy endpoint at end of treatment
    - 93% (27/29)
- Phase IIb study – N = 111
  - Controlled, randomized trial at 2 sites (DRC)
  - DB289 100 mg BID x 5 days vs pentamidine 4 mg/kg/d x 7 days
  - Interrupted after 81 subjects due to efficacy concerns
  - Revised protocol (Phase IIb-2) – DB289 10 days open label
  - Primary efficacy endpoint at 3 months post treatment (per protocol)
    - Pafuramidine 5 d =85% (33/39)
    - Pentamidine 7 d = 100% (40/40)
    - Pafuramidine 10 d = 93% (26/28)

Pafuramidine (DB289) Phase II HAT Studies: Efficacy at 24 months Post Treatment

	Phase IIa	Phase IIb		Phase IIb-2
	DB289 100 mg BID 5 days	DB289 100 mg BID 5 days	Pentamidine 4 mg/kg 7 days	DB289 100 mg BID 10 days
Treatment Completed	29	40	41	30
Treatment Failures	2	5	0	0
Relapses	4	1	1	4
Fatalities during Follow-up	0	1	0	0
Cure Rate (per protocol)	79% (23/29)	82% (32/39)	98% (39/40)	87% (24/28)



Phase III HAT Program
Single Pivotal Study for Registration

- Objectives
  - Demonstrate efficacy of pafuramidine similar to pentamidine
  - Demonstrate better safety and tolerability of pafuramidine compared to pentamidine
- Design
  - Multi-center, multi-country, randomized, open-label comparative trial
  - Pafuramidine 100 mg BID x 10 days vs pentamidine 4 mg/kg IM x 7 days (standard of care)
  - Primary endpoint: 12 months post treatment
- Enrollment and treatment completed in March 2007
- N = 273 patients

Pafuramidine (DB289) Phase 3 Study: Efficacy in Per Protocol Population

	Pafuramidine	Pentamidine
Interim Analysis* – August 2007		
Overall efficacy (per protocol)	86% (61/71)	94% (63/67)
Primary Analysis – 12 months post treatment		
Overall efficacy (per protocol)	89% (109/122)	96% (110/115)
Overall Efficacy (intent-to-treat)	80% (109/136)	80% (110/137)

* Interim analysis performed when ½ of enrolled subjects completed the 12 months follow up evaluation

Phase 3 HAT Study
Liver Safety

Lab Test	Toxicity Grade	Pafuramidine	Pentamidine
ALT		n =123	n= 124
	Grade 0	97%	35%
	Grade 1	3%	64%
	Grade 2	----	2%
AST		n= 117	n= 122
	Grade 0	87%	12%
	Grade 1	13%	25%
	Grade 2	----	43%
	Grade 3	----	20%
Total Bilirubin		n= 108	n= 107
	Grade 0	92%	95%
	Grade 2	8%	5%

Supportive Phase 1 Clinical Trials for Registration of Pafuramidine Maleate

- Additional studies needed to provide adequate safety data
  - Only small numbers of sleeping sickness patients at sites qualified to conduct clinical trials
  - Diseases under study cause significant adverse events and laboratory abnormalities; therefore, healthy volunteers better to assess drug effects on laboratory parameters (liver, kidney, cardiac, etc)

Phase 1 Study	Population and Location	Treatments to be Administered	Objective/ Primary Endpoint
C05-013 (Safety Study) Initiated Nov 2007	Healthy men & women South Africa 175 subjects	DB289 100 mg BID x 14 days or Placebo	Safety and tolerability
C06-018 (Thorough QT Study)	Healthy men & women US 144 subjects	DB289 100 mg BID x 5 days or Placebo or Moxifloxacin	ECG parameters including QT intervals vs. controls Safety and tolerability

New Challenges to the Registration Program: Safety Study (December 2007)

- Liver function test abnormalities occurred approximately 5 days post dosing in healthy volunteers who received pafuramidine
- FDA was consulted
  - All clinical activities were put on hold
  - All sites that were actively enrolling patients in studies were provided details and instructions for additional follow up of subjects
- Prior studies were reviewed with particular focus on liver toxicity
  - No other study showed evidence of liver toxicity in this time frame relative to drug administration
  - No additional concerns related to the liver were identified
- A plan was developed to provide additional safety data and to address the FDA questions in order that the clinical hold could be lifted

New Challenges to the Registration Program: Safety Study (February 2008)

- 5 healthy volunteers who received pafuramidine in the safety study were hospitalized with acute renal insufficiency approximately 8 weeks after the first dose of study drug
- Kidney biopsy from 2 subjects showed changes consistent with a drug-induced hypersensitivity reaction, most likely due to pafuramidine or one of its metabolites
- These abnormalities were classified as Serious Adverse Events and led to another review of prior studies for any evidence of renal toxicity
- These subjects have resolved most of the kidney function abnormalities, but still have protein in their urine and continue to be followed by a nephrologist

HAT Subjects in Phase 3 Study Who Received Pafuramidine and Had Renal Adverse Events

- 2 cases of glomerulonephritis approximately 6-8 weeks after last dose of pafuramidine initially attributed to post-Streptococcal immune response by the investigators
  - 1 subject recovered with corticosteroid and diuretic therapy
    - Brother also had same disease and died
    - Likely post-Streptococcal disease and probably not related to pafuramidine
  - 1 subject required IV corticosteroids and had normal renal function at 3 month follow up
    - Event could have possibly been related to pafuramidine
- 1 case of nephropathy and pneumopathy 2 months after last dose of pafuramidine
  - Subject hospitalized for medical care; diuretics, amoxicillin
  - Recovered after addition of corticosteroids to medical regimen
    - Investigator initially ascribed this event to lung infection
    - Event could have possibly been related to pafuramidine

Decision to Discontinue Pafuramidine Development

- Shared data regarding liver and renal serious adverse events with investigators, FDA, CPDD collaborators, Data Safety Monitoring Board, CPDD Governance Council and Steering Committee for Pneumocysitis pneumonia study (ongoing Phase 3)
- Discussed potential risk to subjects versus potential benefit from oral drug for HAT and Pneumocysitis pneumonia in HIV/AIDS
- Received recommendations from oversight groups
- Final decision was that the program should be discontinued in February 2008

Positive Outcomes and Lessons Learned

- Many patients screened (> 100,000) and treated (> 400)
- Increased awareness of HAT in communities at risk
- Improved infrastructure for HAT centers
- Personnel trained in better diagnostic methods and GCP; these personnel become trainers go on to train new HAT teams
  - STI team
  - Local HAT teams
- Increased understanding of how to conduct clinical trials for this disease (clinical staff and regulatory agencies)
- Strong bonds of trust between research teams, HAT teams and stakeholders

General Rule
Set Backs are Normal

Where do we go from here?

- CPDD continues to evaluate new compounds for activity versus trypanosomes
  - Lead candidate for late (CNS) stage disease has been selected
    - Diamidine that is not a prodrug
    - Early safety and efficacy data suggest this drug candidate has an appropriate profile to be an IV agent
    - Formulation work is underway
    - Clinical trials could begin as early as 4Q2009
  - Additional discovery work is continuing on diamidines and also other classes of potential drugs