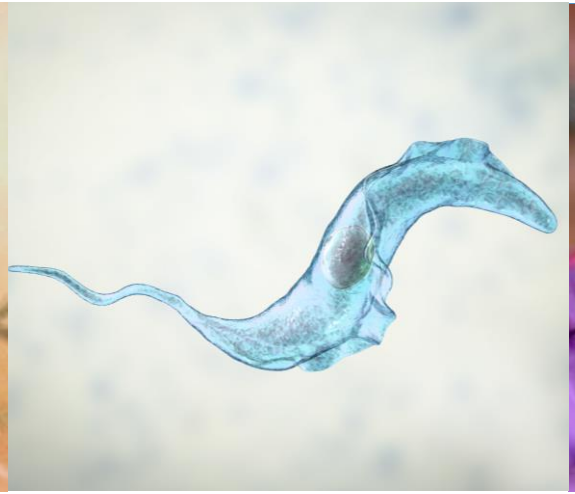
Novartis Institutes for BioMedical Research



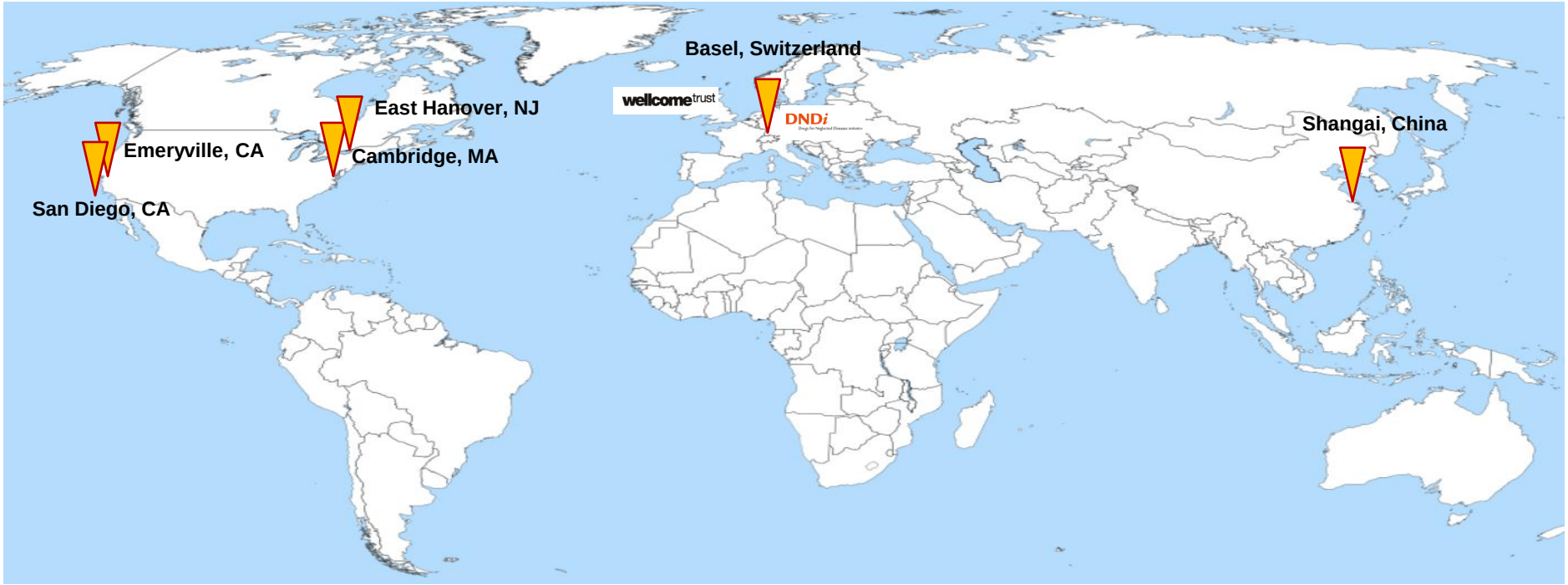
LXE408 – Towards a cure for multiple diseases

Valentina Molteni

IASOC – Ischia, September 25 2022

NOVARTIS | Reimagining Medicine

Novartis Institutes for BioMedical Research (NIBR)



NOVARTIS | Reimagining Medicine

Novartis is committed to developing world disease research

Goals

- Discover medicines for major global health challenges
- Innovate partnership models to accelerate R&D and access to medicines

Focus Areas

- Malaria
- Kinetoplastid Diseases
- Cryptosporidiosis
- Dengue

Our goal for parasitic diseases

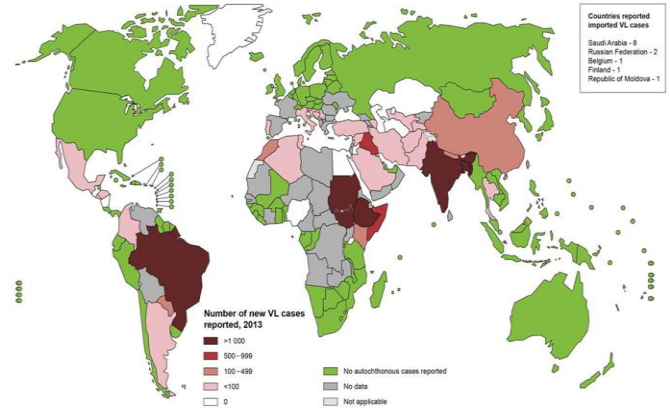
Identification of novel drug candidates to improve treatment options for infectious diseases in **low and middle income countries**

Strategy

- Prioritize diseases where **current drugs are toxic/ ineffective** and current drug discovery activities are low relative to medical burden
- Deprioritized where other approaches are likely to be more effective (vaccines, sanitation)
- Improve clinical outcomes by **reduction in burden of the infectious agent**

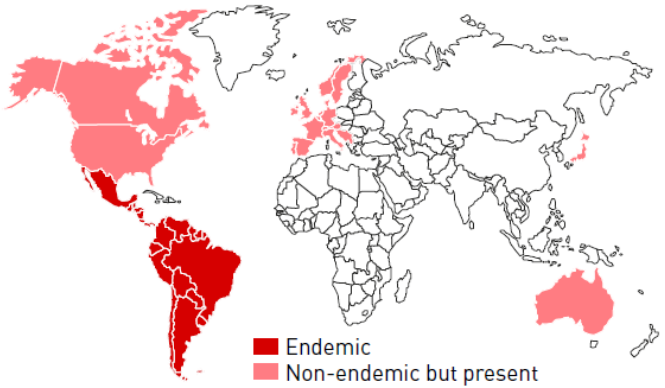
Epidemiology

Visceral Leishmaniasis (VL)



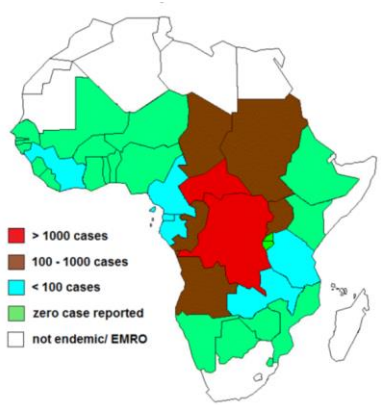
- Endemic in 98 countries, 350 million people at risk, 48,000 deaths/year
- Affects poor populations living in remote areas

Chagas



- Endemic in 21 countries, ~6 millions infected, 7,000 deaths/year
- Migration is spreading infection in non-endemic countries

Sleeping sickness (HAT)



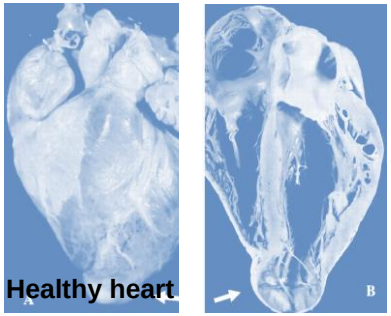
- Endemic in 36 countries in sub-Saharan Africa, 3500 deaths/year
- The number of cases of HAT has substantially decreased

Medical need

Visceral Leishmaniasis (VL)

- Fever >80%, wasting > 70%, splenomegaly > 90% (almost universal), hepatomegaly > 50%, anemia > 60%
- Non-treated disease almost always fatal within months - cause of death usually a concurrent illness

Chagas



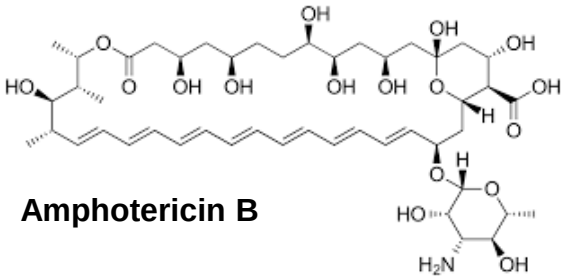
- Acute phase: 7-9 days; often unnoticed
- Indeterminate phase: ~ 70% patients asymptomatic
- Chronic phase: 10-30 years after infection in ~ 30% patients

HAT

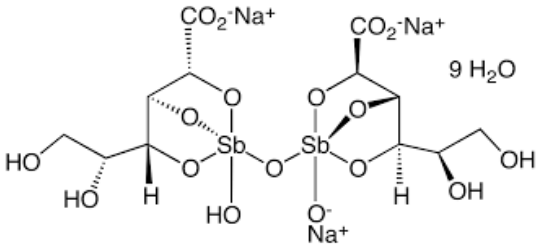
- Stage I: non-specific symptoms like headaches and fever
- Stage II of the disease: sleep cycle disruption, paralysis, progressive mental deterioration - fatal if not treated

Existing medications are toxic, difficult to administer and have suboptimal efficacy

Leishmaniasis

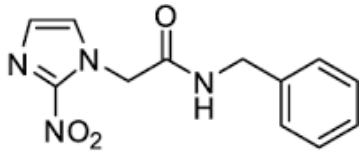


Amphotericin B

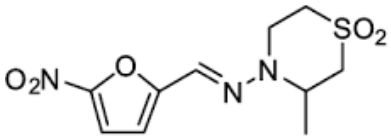


Sodium Stibogluconate

Chagas Disease

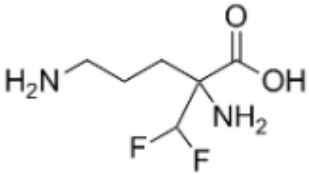


Benznidazole



Nifurtimox

HAT



Eflornithine

NECT

Vectors

Visceral Leishmaniasis (VL)

Sand fly

Chagas

Kissing bug

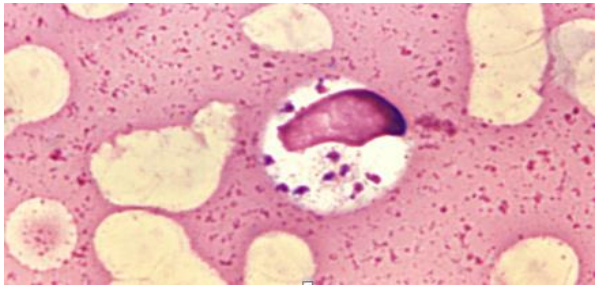
HAT

Tsetse Fly

Caused by related kinetoplastid parasites and infection of mammalian host occurs through bite of infected insect vector

Kinetoplastid parasites

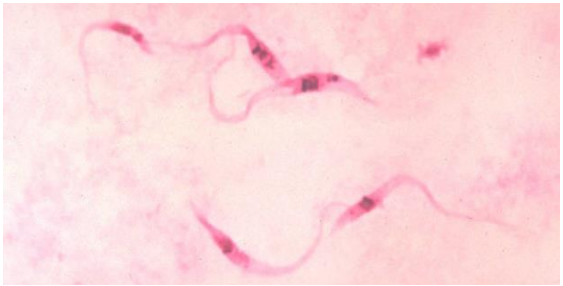
Visceral Leishmaniasis (VL)



L. donovani

Macrophages

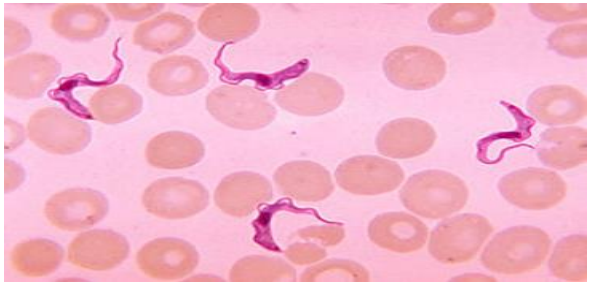
Chagas



T. cruzi

Heart, intestine,
bone marrow

HAT



T. brucei

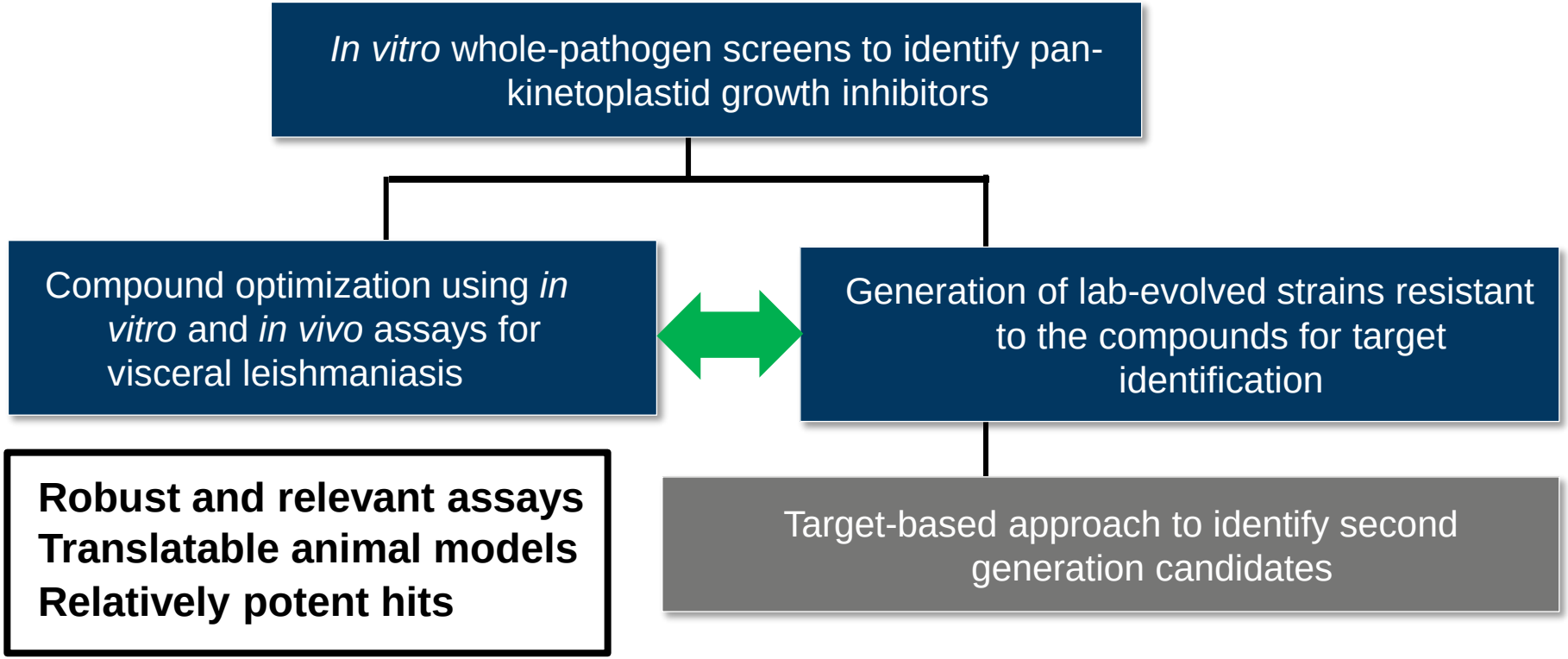
Blood, CNS

Leishmania, T. brucei and *T. cruzi* have similar biology and genomic sequence

Project objective

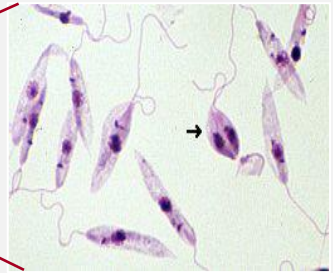
Develop potent **oral** inhibitors of *Leishmania donovani, Trypanosoma cruzi (and Trypanosoma brucei)* with a **better safety** profile than the current standard of care

Phenotypic drug discovery approach



Leishmania: dual-form Life Cycle

Sandfly stage

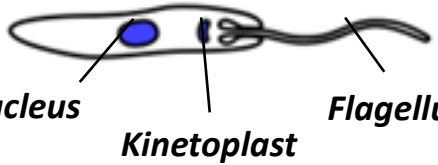


Regurgitated into bite wound

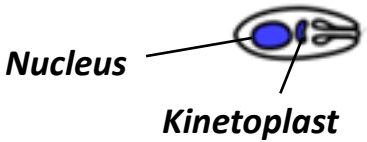
Infecting sandfly during blood meal

Mammalian host stage

Promastigote



Amastigote



In vitro macrophage infection assay

Infection with *ex vivo Leishmania* amastigotes

9-10 weeks

Infected mouse spleens

Purified *Leishmania* amastigotes

In vivo mouse VL model

Macrophage isolation

Primary peritoneal macrophages

In vitro high-content macrophage infection assay

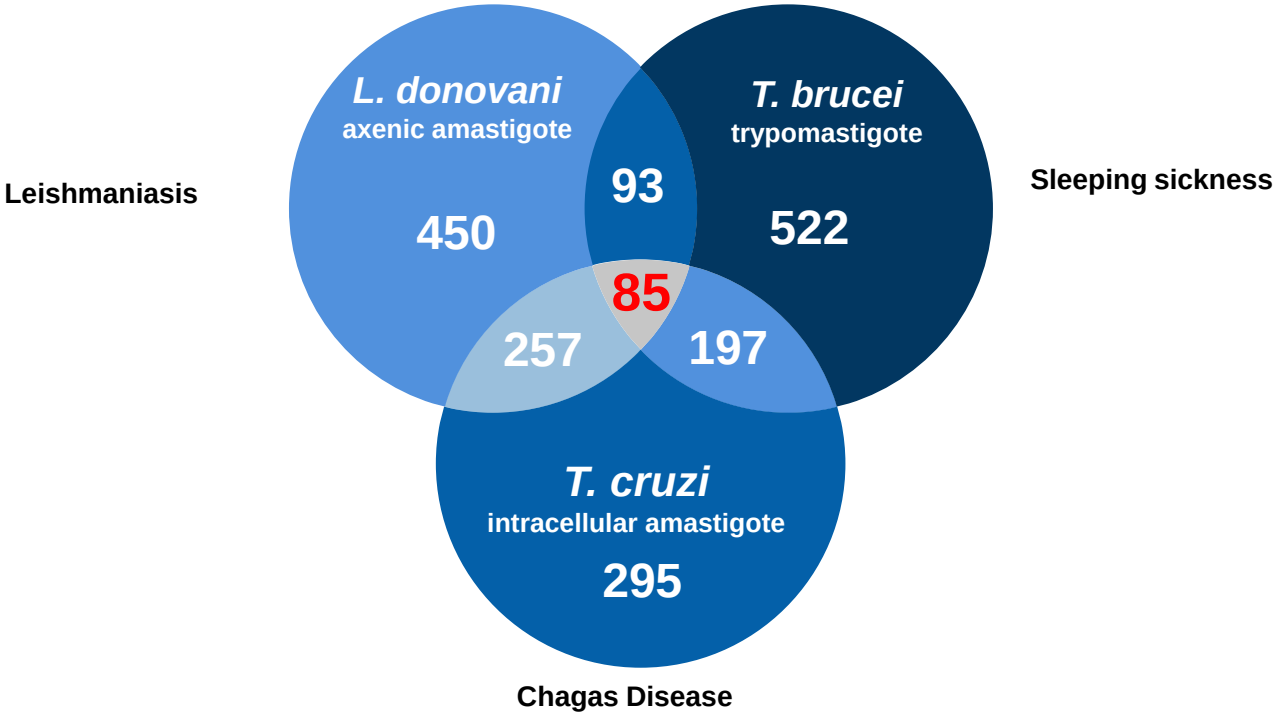
In vitro VL assay for HTS

L. donovani Axenic Amastigote Assay

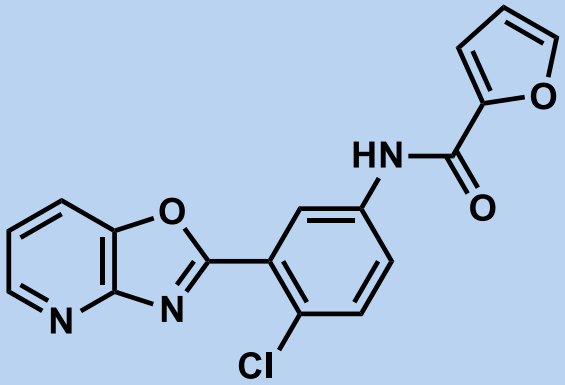
Promastigote Acidic pH and 37 ^{o}C ***Axenic Amastigote***

Axenic Amastigotes are physiologically similar to tissue-derived amastigotes but less expensive and laborious

Focus on pan-kinetoplastid inhibitor



Pan-kinetoplastid oxazolopyridine hit



GNF-5343

Kinetoplastid activities

L. donovani EC_{50} = 930 nM (axenic mastigotes)

T. cruzi EC_{50} = 75 nM

T. brucei EC_{50} = 150 nM

L. donovani EC_{50} = 7.3 μM (mouse macrophages)*

Macrophage CC_{50} > 50 μM

*SAR driving assay

- Commercial compound with very poor solubility, low metabolic stability and no oral bioavailability

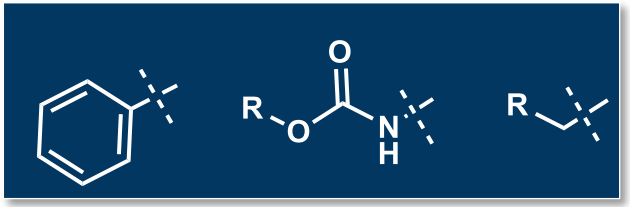
Imidazolopyridines - an alternative core

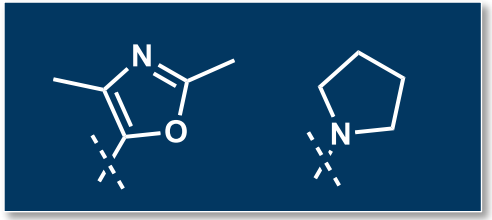
Potency:
NH > O

NH needed

R_2: Modulates potency and physicochemical properties

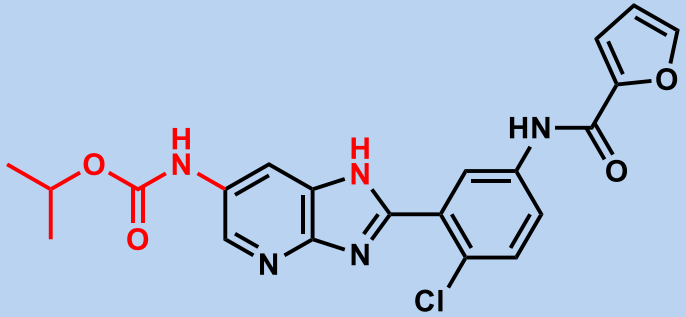
R_1: Steep SAR





Potency:
Cl > F > Me >>H

GNF-3934 has acceptable PK



GNF-3934

Kinetoplastid parasite activities

L. donovani EC_{50} = 260 nM

T. cruzi EC_{50} = 169 nM

T. brucei EC_{50} = 27 nM

Macrophage CC_{50} = 10 μM

Mouse PK
PO dose: 100 mg/kg in MC/Tween suspension
IV dose: 5 mg/kg in PEG300/D5W (3:1)

Route	Parameters	Units	GNF-3934
p.o.	C_{max}	μM	27.6
	AUC_{inf}	μM*h	159
	$T_{1/2}$	h	2.02
	F	%	74.5
i.v.	V_{ss}	L/kg	0.49
	CL	mL/min/kg	17.69
	$T_{1/2}$	h	0.63

Mouse model of visceral leishmaniasis

Efficacy group 

Dosing

Liver burden determination

Tail vein Injection $4X10^7$

Day

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

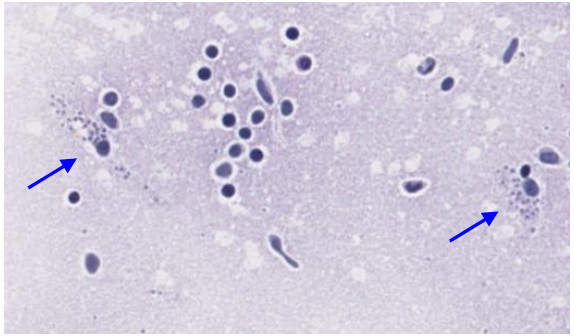
Tail vein Injection $4X10^7$

day 1 PK

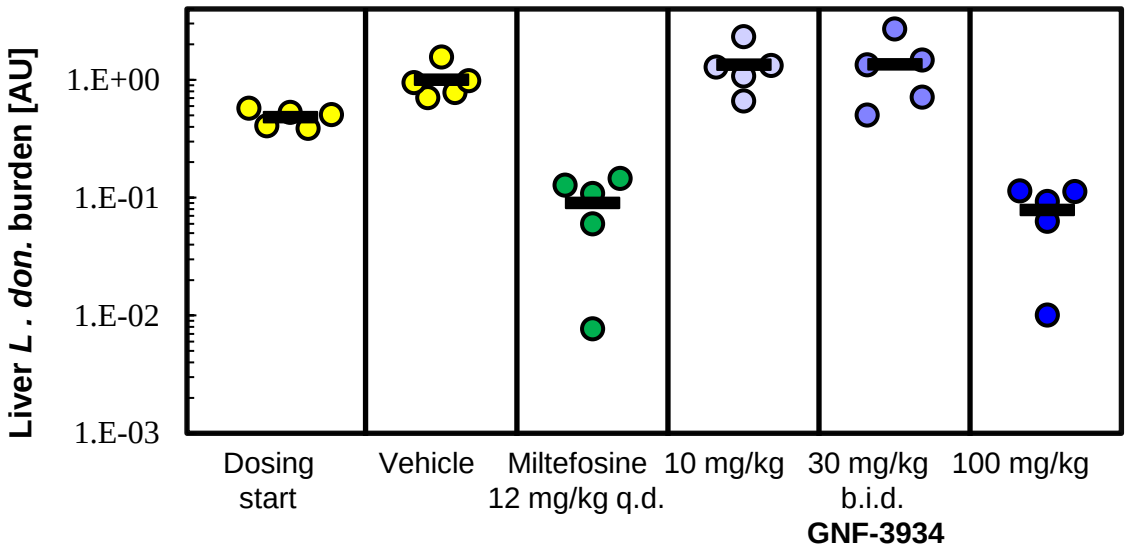
day 8 PK

PK group 

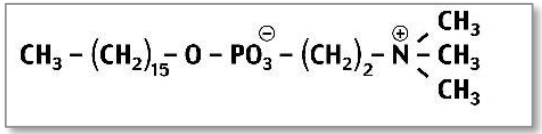
L. donovani -infected macrophages in mouse liver (arrows)



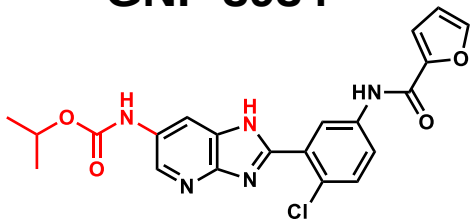
GNF-3934 achieves efficacy at high doses



miltefosine

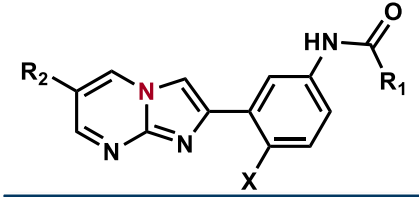


GNF-3934



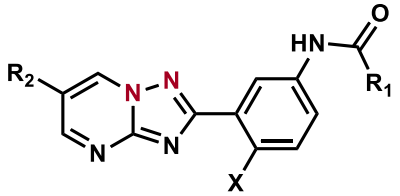
Need to increase potency and in vivo exposure

Core structure changes



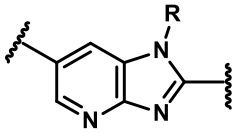
Imidazolopyrimidines

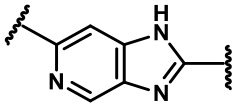
Imidazolopyridines



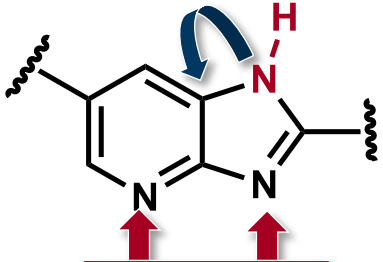
Triazolopyrimidines

N-Alkyl Imidazolopyridines

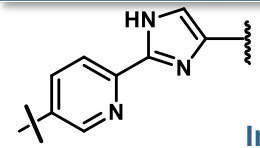




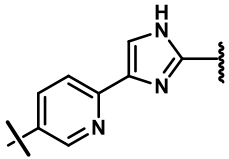
Imidazolopyridines

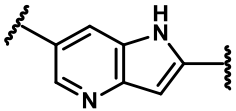


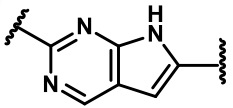
Needed



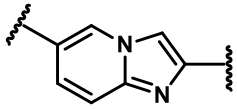
Imidazolylpyridines



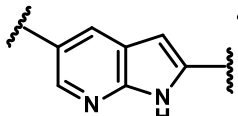




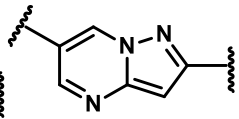
Pyrrolopyrimidines



Aza-indoles



Pyrrolopyridines



Pyrazolopyrimidines

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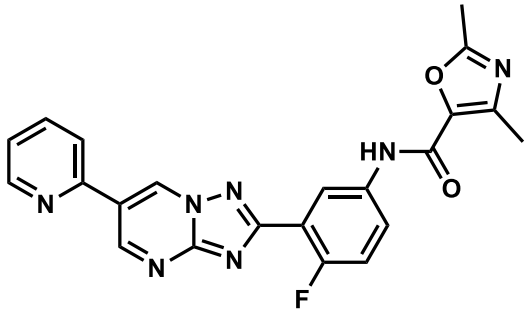
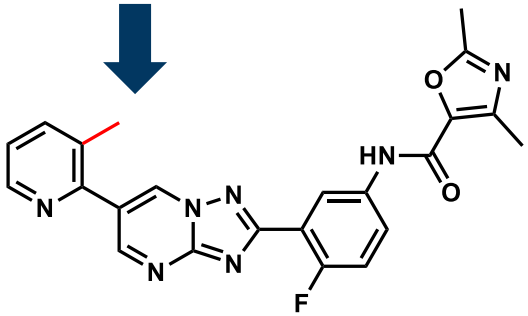
Two very similar front-runner compounds



GNF-6702

L. donovani EC_{50} = 20 nM

VL model ED_{95} = 0.5 mg/kg bid



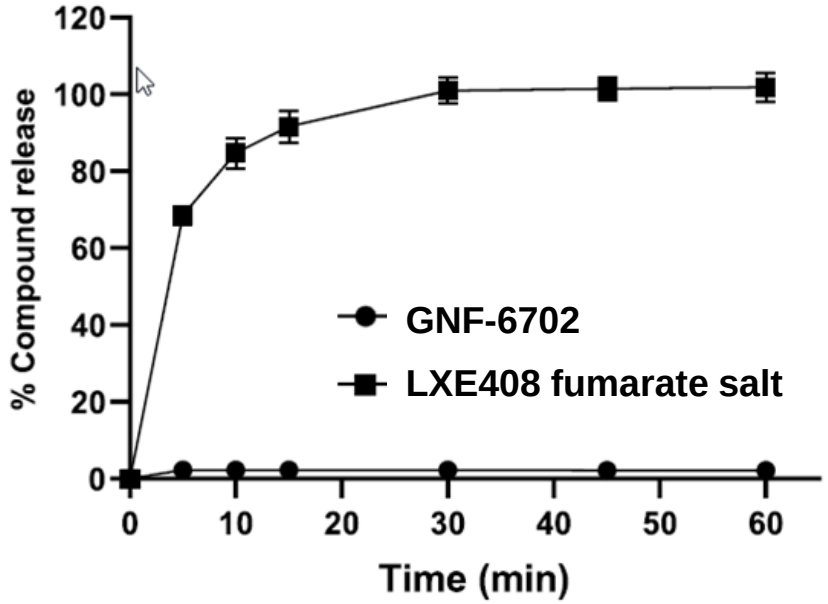
LXE408

L. donovani EC_{50} = 40 nM

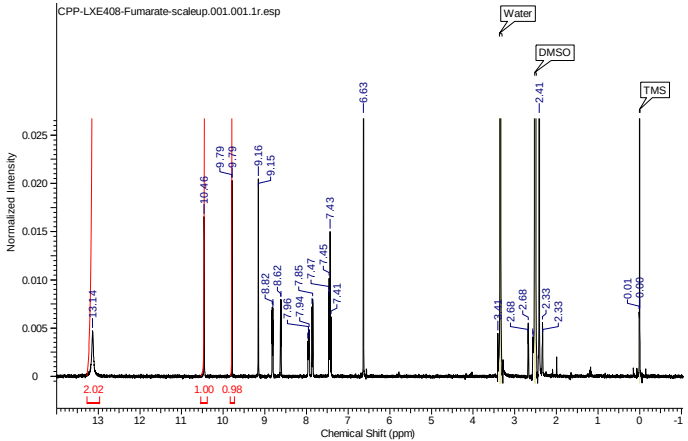
VL model ED_{95} = 1 mg/kg bid

Poor water solubility
Developability issues
(*e.g.* low oral absorption, under-proportional PK, inter-subject variability)

Time course dissolution of GNF-6702 vs LXE408

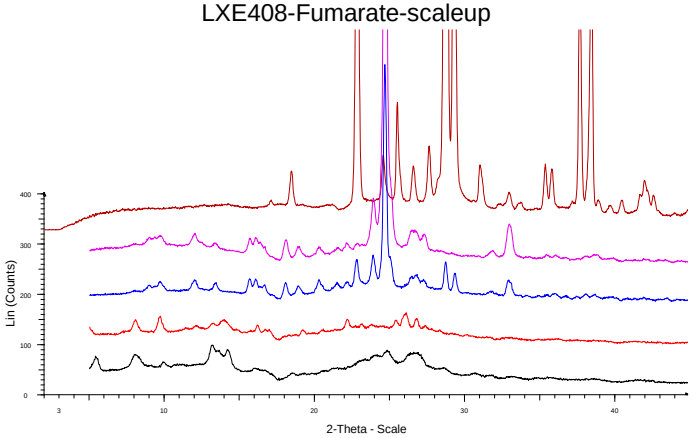


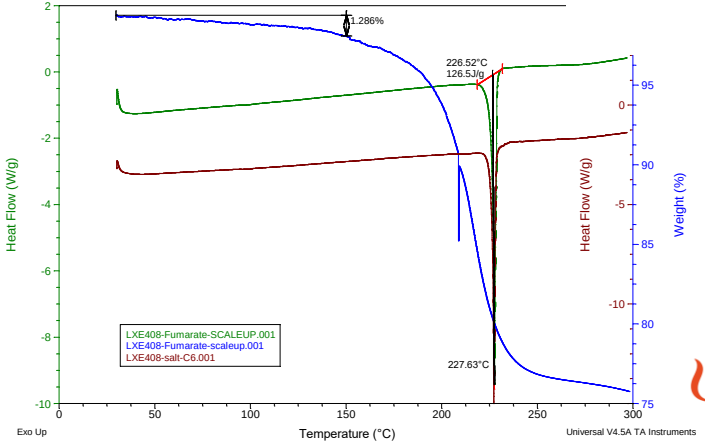
Properties of LXE408 fumarate



NMR

counterion/co-former1:1





X-Ray Powder Diffraction (XRPD)

Strongly crystalline

Thermogravimetric analysis (TGA)

/Differential scanning calorimetry (DSC)

m.p 227 ^{o}C

1.2% weight loss before decomposition

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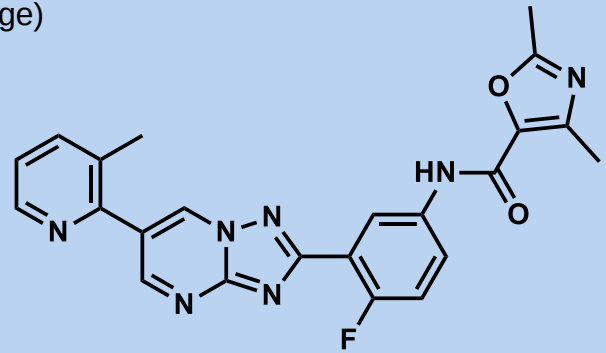
LXE408 is active on all *Leishmania* species and strains tested

Parasite growth inhibition:
L. donovani HU3 EC_{50} (Ethiopia) = 0.040 µM (mouse macrophage)
L. donovani HU3 EC_{50} (Ethiopia) = 0.042 µM (human macrophage)
L. donovani 9515 EC_{50} (India) = 0.041 µM
L. infantum ITMAP263 EC_{50} (Morocco) = 0.140 µM
T. cruzi EC_{50} = 0.017 µM
T. brucei EC_{50} = 0.023 µM

Cytotoxicity:
Macrophage CC_{50} > 50 µM
3T3 CC_{50} > 20 µM

Proteasome chymotrypsin-like activity inhibition:
L. donovani proteasome IC_{50} = 0.057 µM
T. cruzi proteasome IC_{50} = 0.056 µM
T. brucei proteasome IC_{50} = 0.065 µM



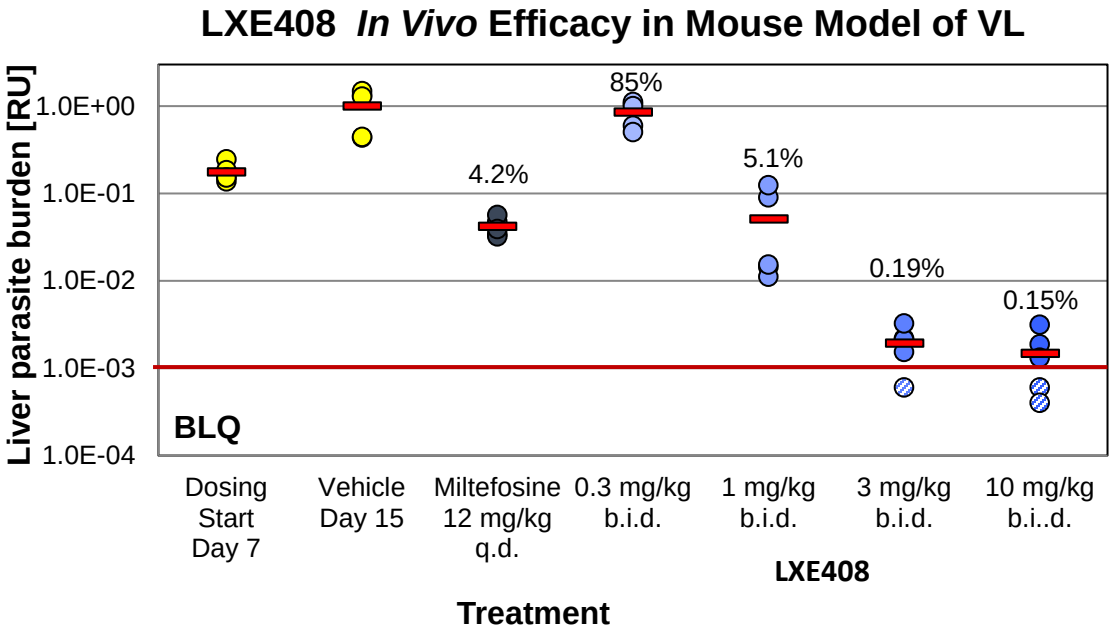
LXE408

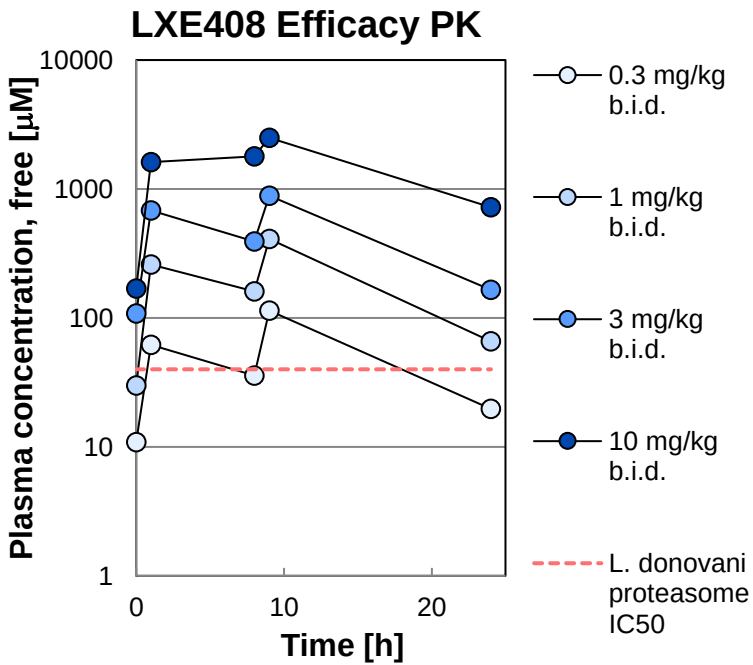
In vitro potency of VL drugs vs LXE408 on intramacrophage *L. donovani*

	Published Data*	In-house Data
Amphotericin B EC_{50} [µM]	0.03 – 0.06	0.13
Miltefosine EC_{50} [µM]	5.7 – 7.7	3.5
Paromomycin EC_{50} [µM]	75 - 202	n.d.
Sodium stibogluconate EC_{50} [µg/ml]	22 - 28	n.d.
LXE408 EC_{50} [µM]	n.a.	0.040

EC_{50} values were determined on *L. donovani* proliferating in mouse peritoneal macrophages
n.a. – not applicable
n.d. – not determined
* Seifert K, Escobar P, Croft SL. In vitro activity of anti-leishmanial drugs against Leishmania donovani is host cell dependent. J Antimicrob Chemother. 2010;65(3):508-11

LXE408 is highly potent in vivo





Efficacious dose (ED_{95})	$AUC_{0\text{-}24h}$ [h*µM]	C_{max} [µM]
1.0 mg/kg b.i.d.	32	2.4

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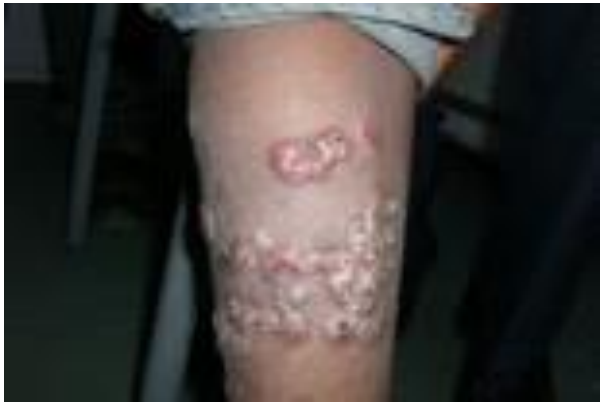
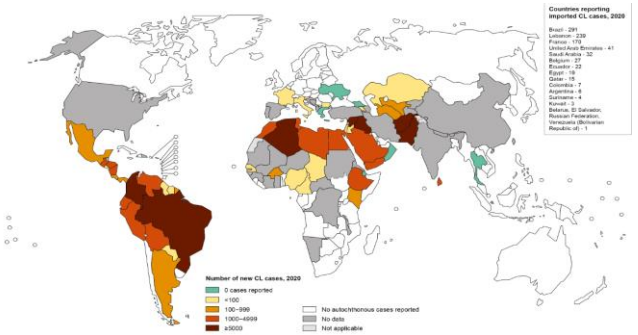
Mouse Model of Cutaneous Leishmaniasis

- *Leishmania major* metacyclic promastigotes injected into footpads of BALB/c mice
- Parasite burden is quantified indirectly through measurement of footpad swelling
- BALB/c mice are unable to control *L. major* infection and die if not treated
- The same assay can be run in C57/BL6 mice where it has self – healing course (similar to human infections)





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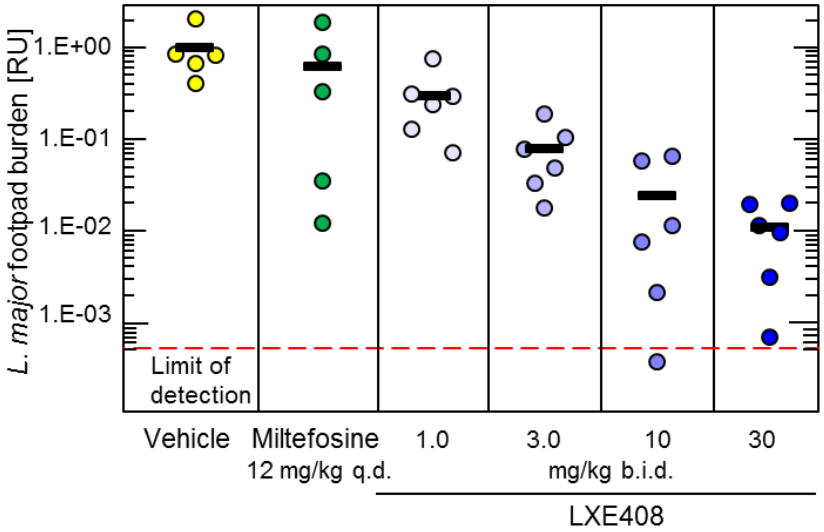
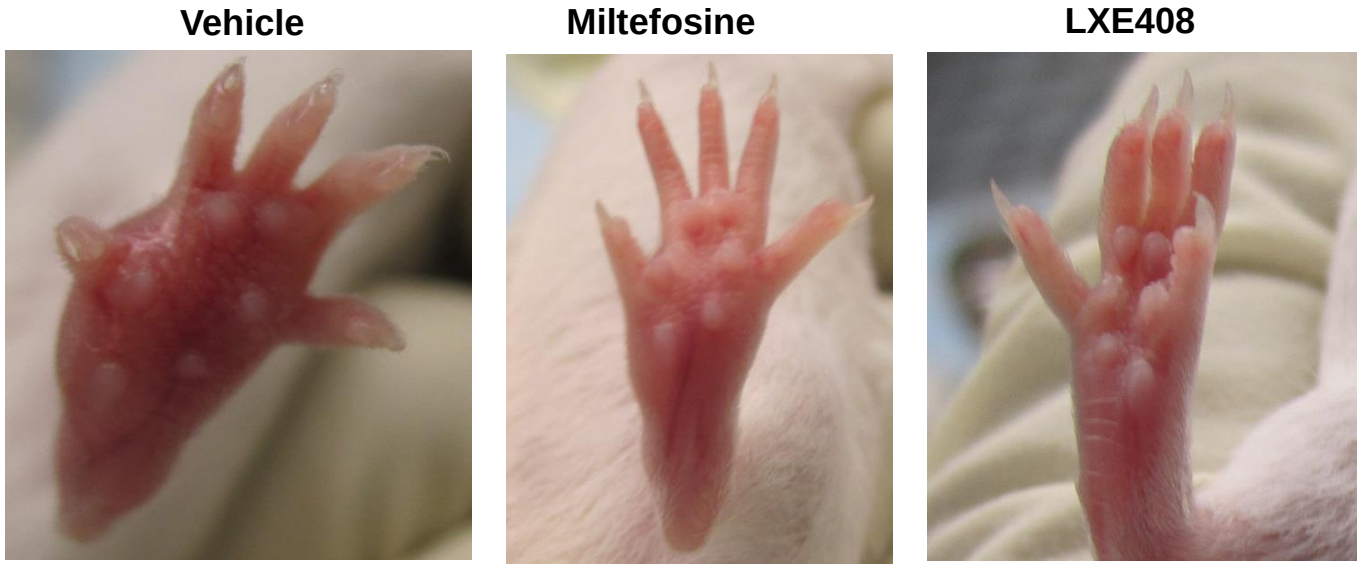
LXE408 is highly efficacious

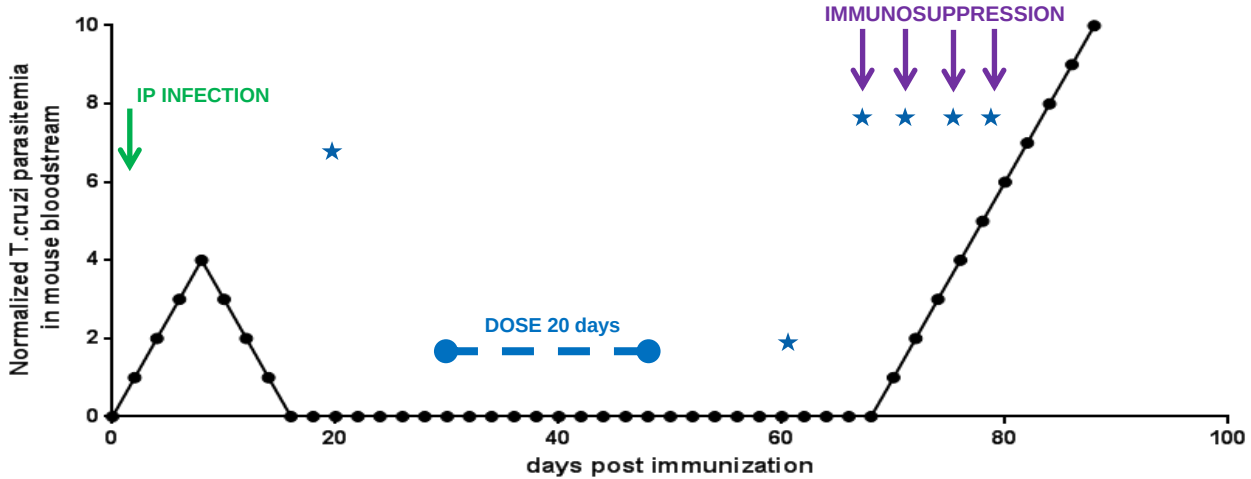


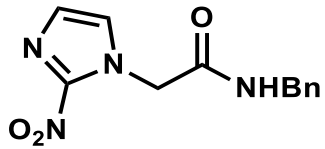
- Treatment from day 8 until day 15
- Miltefosine doesn't show reduction of parasites
- LXE408 decreases parasite burden in a dose specific manner

LXE408 leads to complete regression of swelling



Mouse efficacy model of chronic Chagas

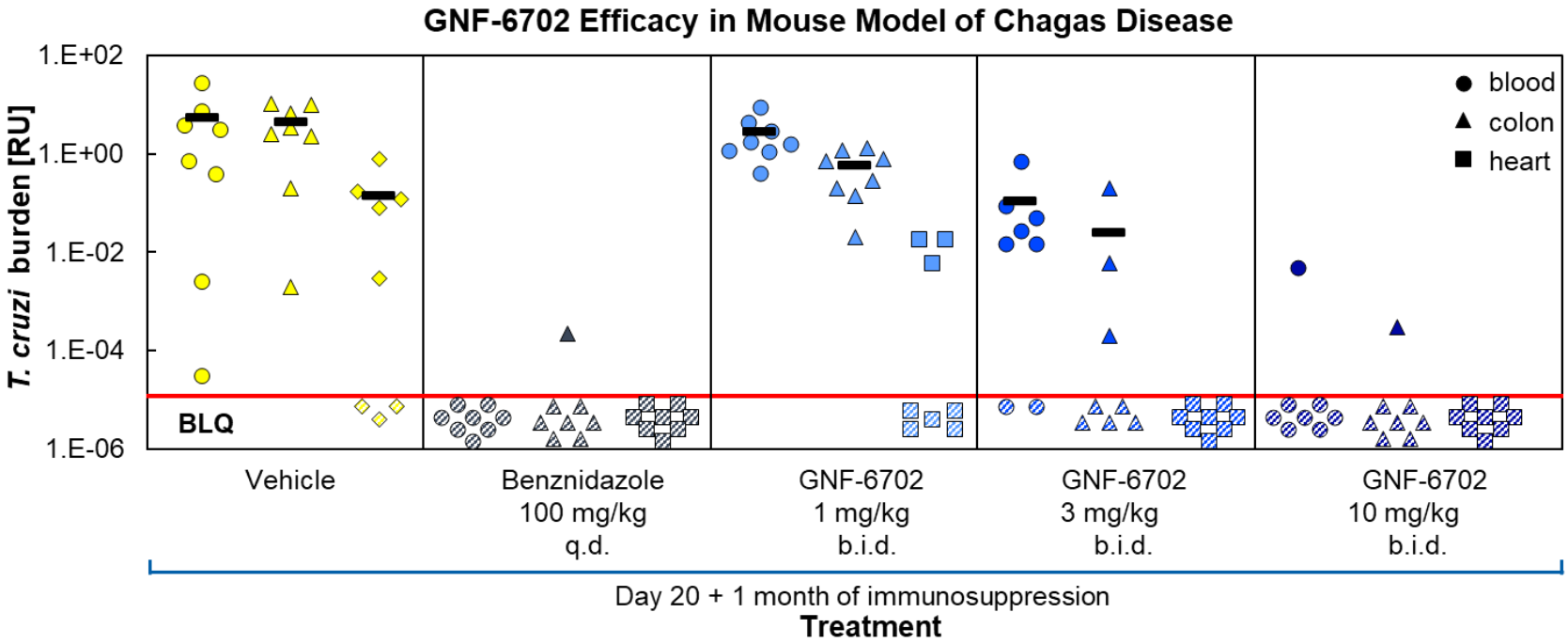




benznidazole

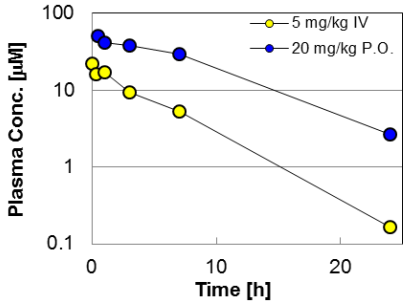
- Treatment initiated in indeterminate stage of Chagas disease
- Highly sensitive parasite detection by PCR after 1 month of immunosuppression
- Good correlation with clinical trial results

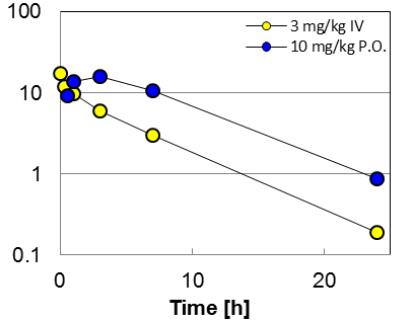
GNF-6702 clears *T. cruzi* from Infected Mice

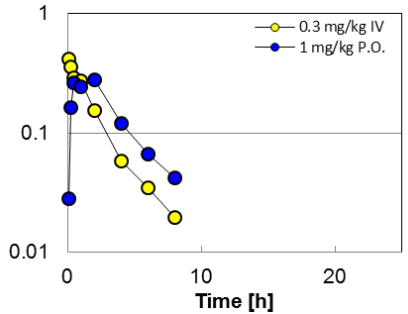


GNF-6702 clears T. cruzi in 7 out 8 treated mice, similar to benznidazole

LXE408 shows higher clearance in dog







	Mouse	Rat	Dog
IV Formulation	75% PEG300, 25% D5W	75% PEG300, 25% D5W	75% PEG300, 25% D5W
CL (mL/min/kg)	3.0 ± 0.5	2.1 ± 0.02	12 ± 4.5
V_{ss} (L/kg)	0.63 ± 0.04	0.53 ± 0.03	2.1 ± 0.30
$t_{1/2\ term.}$ (h)	3.1 ± 0.2	3.8 ± 0.13	3.8 ± 1.9
DN* AUC (µM•h) i.v.	13 ± 2.2	18.2 ± 0.66	3.5 ± 1.1
PO FormulatioN	0.5% MC/Tween80	0.5% MC/Tween80	0.5% MC/Tween80
DN* AUC (µM•h) p.o.	6.9 ± 1	12± 1.9	1.5 ± 0.83
DN* C_{max} (µM) p.o.	0.77 ± 0.16	1 ± 0.8	0.33 ± 0.086
T_{max} p.o. (h)	3.2 ± 0.6	5.7 ± 2.3	3.9 ± 7.0
Oral BA (%F)	65± 9	67 ± 10	44 ± 24

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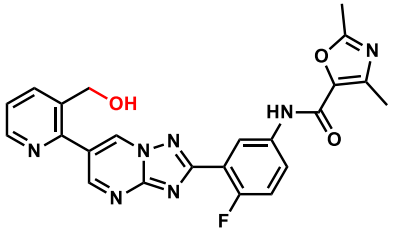
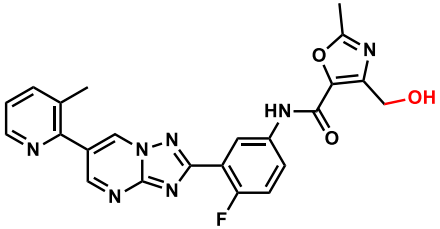
*DN = dose normalized (1 mg/kg)

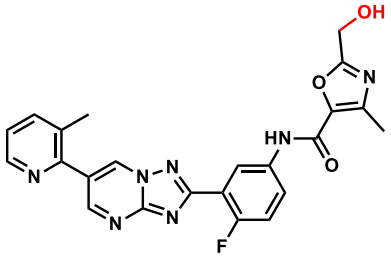
Metabolism of LXE408 is different in dog hepatocytes



MAT447
Present in all species



MAT448
Predominant in mouse, rat, monkey and human



MAN519
Predominant in dog

Hepatocytes	MAT447	MAT448	MAN519
Human	+	++	-
Monkey	+	++	-
Dog	+	+	++
Rat	+	++	-
Mouse	+	++	-

+: Detectable; ++: Predominant -: Barely detectable

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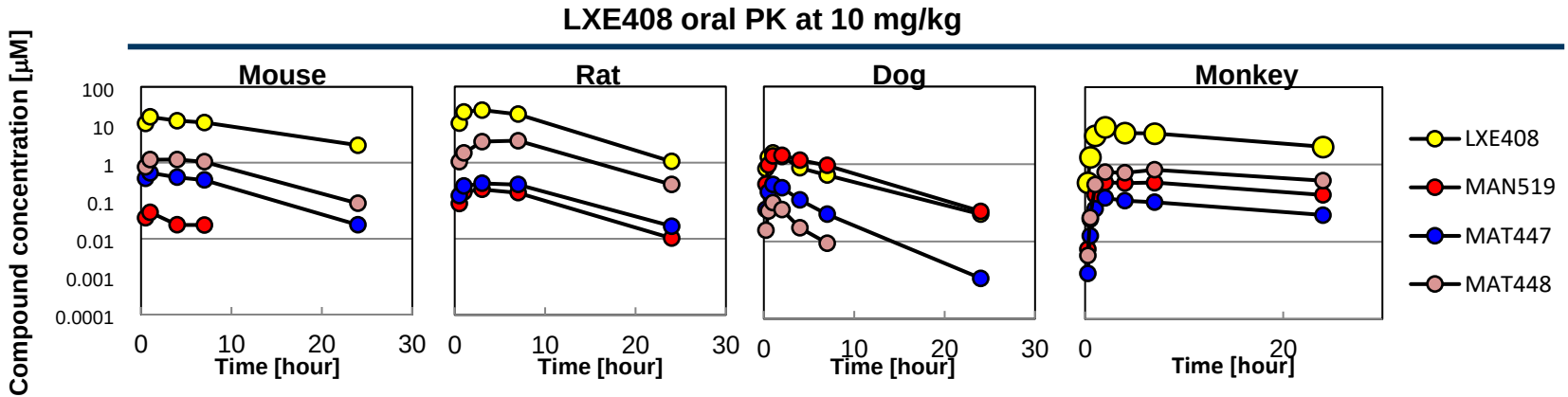
MAN519 is the predominant metabolite in dog

Dog is an outlier in terms of nature and extent of metabolism



	Metabolite AUC relative to LXE408 AUC			
	Mouse	**Rat**	**Dog**	**Monkey**
MAT447	1.5%	1.5%	9.1%	1.7%
MAT448	**4.5%**	**17%**	2.3%	**11%**
MAN519	0.14%	1.5%	**120%**	5.1%

CYP2D15 is the main CYP involved in the metabolism of LXE408 in the dog

Metabolism of LXE408 in Recombinant Dog CYP Enzyme Incubations

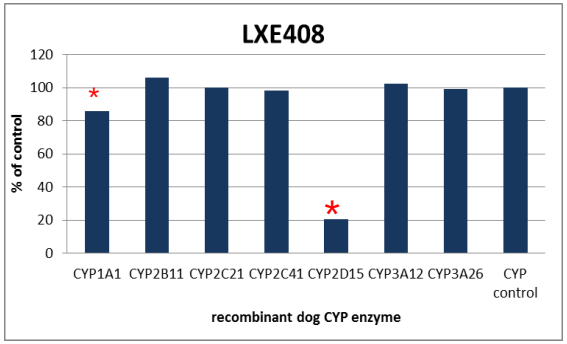


Fig 1. % of LXE408 (1 uM) remaining compared to Dog control incubation

	LXE408	MAN519	MAT448	MAT447
CYP1A1	7.50	0.45	--	0.14
CYP2D15	1.79	2.41	--	--
control	8.03			

Peak Area Ratio of parent and metabolites observed at 30 min incubation in Dog

- In dog, CYP2D15 appears to be responsible for most of the formation of MAN519, CYP1A1 is involved to a lesser extent
- Dog CYP2D15 is an ortholog of human CYP2D6
- Polymorphism exists in genes encoding canine CYP2D15 that has the potential to impact the metabolism of drugs

CYP2D6 is not involved in the metabolism of LXE408 in human

Metabolism of LXE408 in Recombinant Human CYP Enzyme Incubations

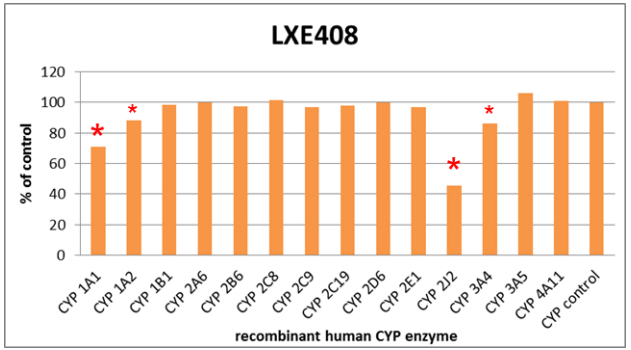


Fig 3. % of LXE408 (1 uM) remaining compared to Human control incubation

	LXE408	MAN519	MAT448	MAT447
CYP 1A1	5.62	0.65	--	0.16
CYP 1A2	6.97	0.36	--	--
CYP 3A4	6.82	0.08	0.13	--
CYP 2J2	3.58	0.59	0.47	--
control	7.61			

Peak Area Ratio of parent and metabolites observed at 30 min incubation in Human

- In Human, CYP1A2 and CYP3A4 appear to be the enzymes mostly involved in LXE408 metabolism. There is no genetic polymorphism for CYP1A2 and CYP3A4. CYP1A2 can be induced by cigarette smoking.
- Extrahepatic CYP1A1 and CYP2J2 may be involved in the metabolism of LXE408. The role of extra-hepatic CYP2J2 enzymes in the clearance of drugs does not seem to be established.

LXE408 tox data supported IND filing

100 mg/kg

10 mg/kg
NOAEL

100 mg/kg

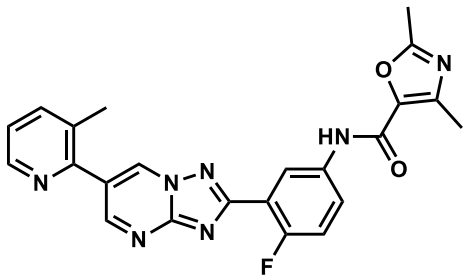
30 mg/kg

10 mg/kg
NOAEL

20x

9x

Human predicted ED_{95}
AUC = 7 h*µg*ml^{-1}



LXE408

The road to patients

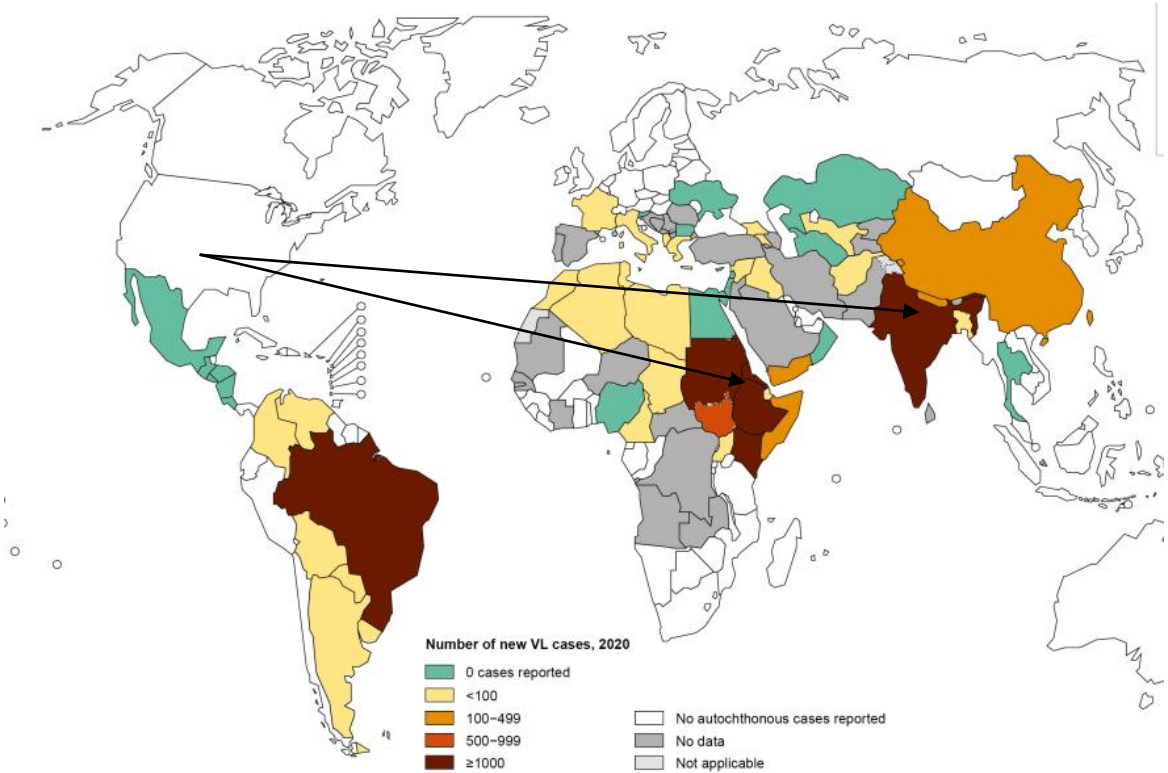
Toxicology

Healthy Volunteers

Adults

Children

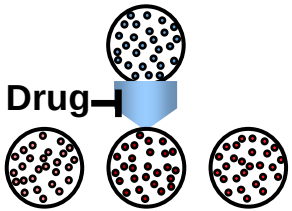
LXE408 is ready for VL patient studies

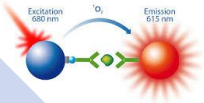


Goals of therapy

- Oral
- Short
- Effective
- Safe

Identifying new drug targets from HTS hits





Generation of Laboratory-Evolved Drug Resistant Mutants

Identification of Resistance-Conferring Mutations

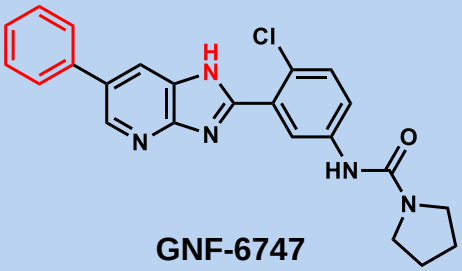
Mechanism of Action Hypothesis

Validation of Molecular Target Hypothesis

NEW DRUG TARGET

Selection of *T. cruzi* mutant resistant strains

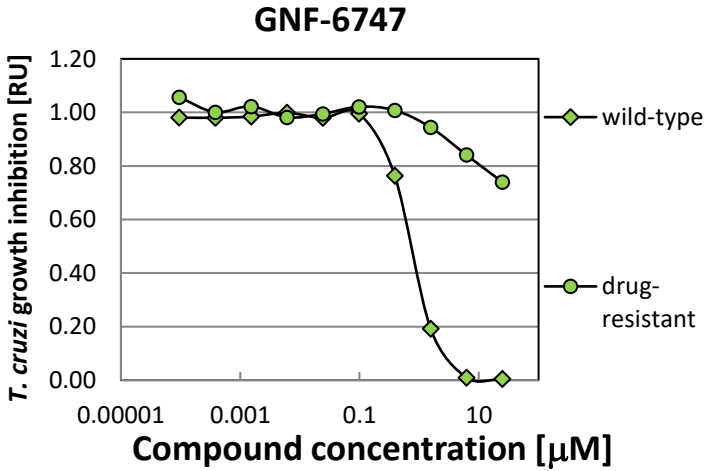
- Three GNF-3934 resistant *T. cruzi* strains evolved during a 4 month selection
- One GNF-3934 resistant strain characterized further - evolved strain shows resistance to all tested analogues
- Evolved strain is equally sensitive to unrelated compounds (e.g. nifurtimox)



GNF-6747

Wild-type *T. cruzi*
EC_{50}= 0.73 µM

Drug-resistant *T. cruzi*
EC_{50}> 25 µM



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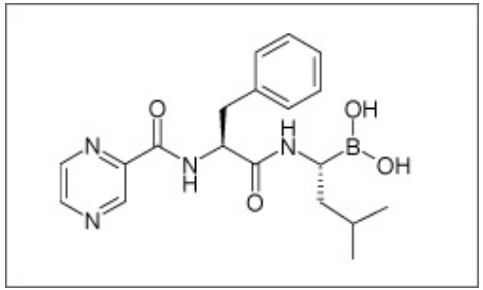
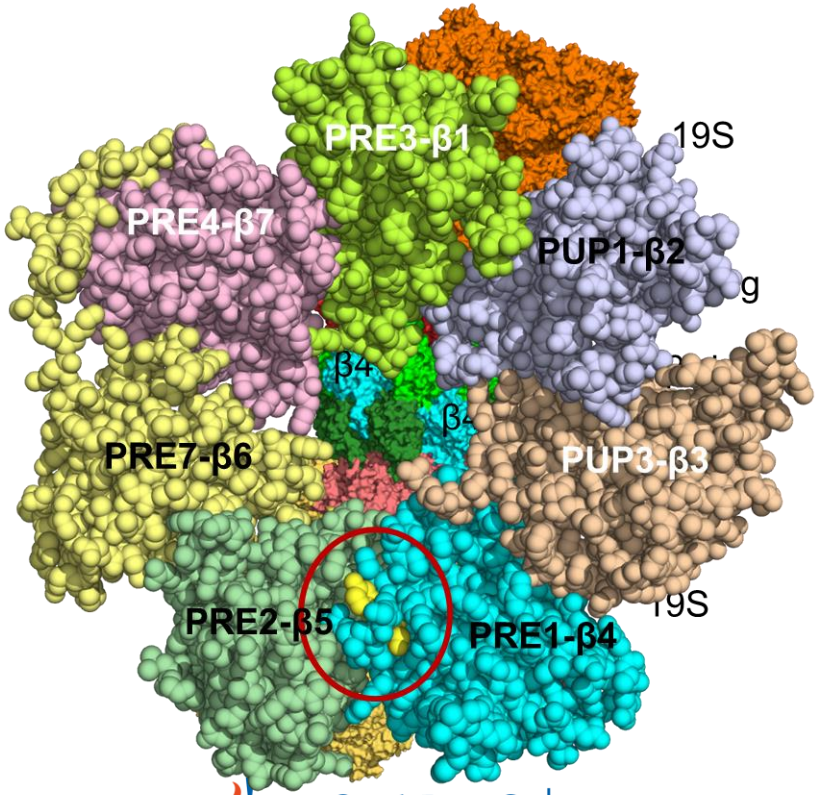
Mutations conferring resistance are at interface of b4 and b5 proteasome subunits

- Two resistance mutations identified in the *T. cruzi* β4 subunit – I29M (GNF-3934) and F24L (GNF-8000)
- Mutations are outside the β5 subunit chymotrypsin active site
- Human proteasome inhibitor bortezomib binds in β5 subunit





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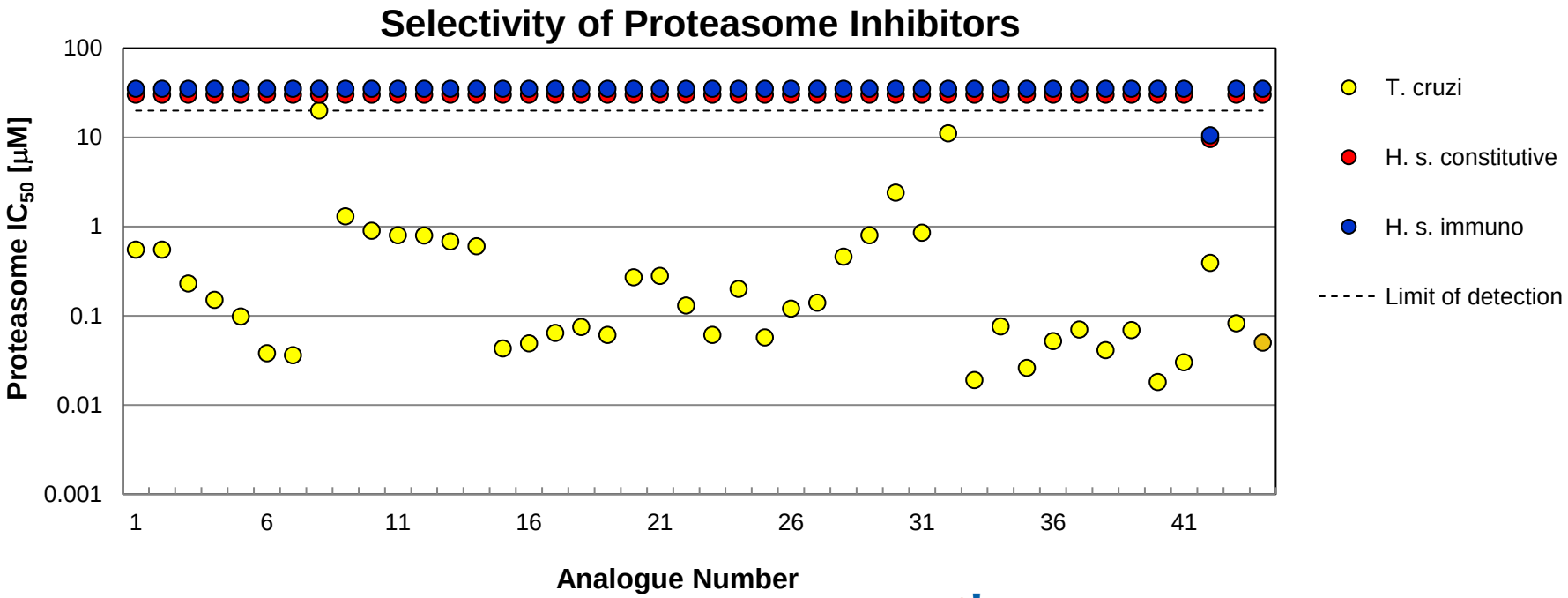
LXE408 analogs do not inhibit human proteasome



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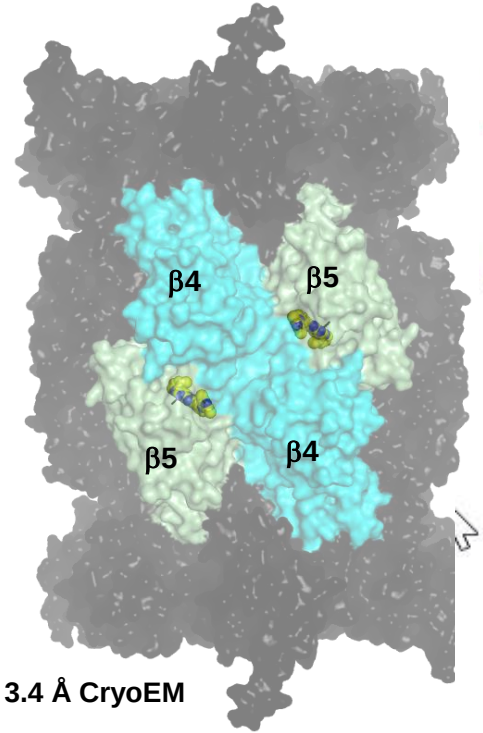
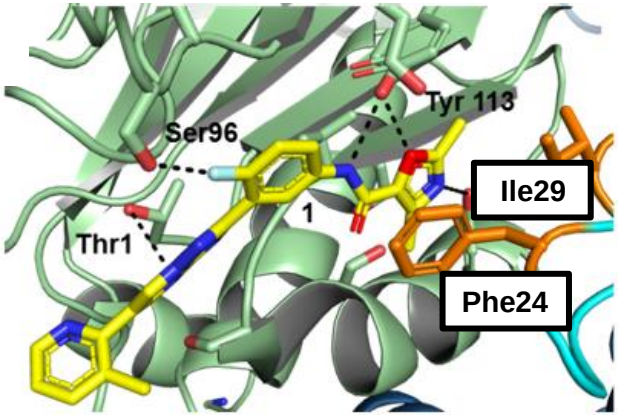
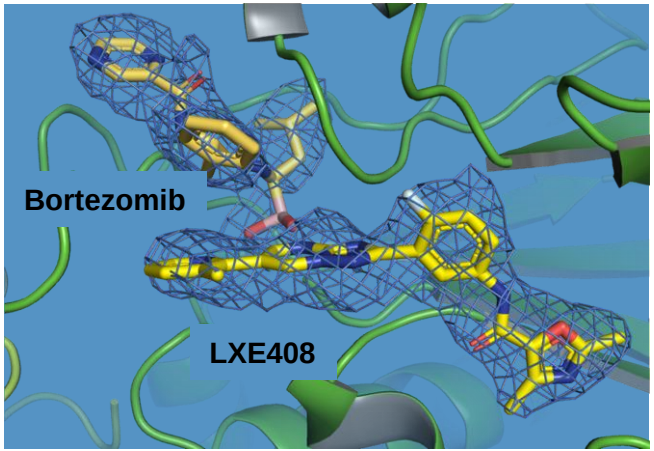
LXE408 binds in beta 5 active site pocket



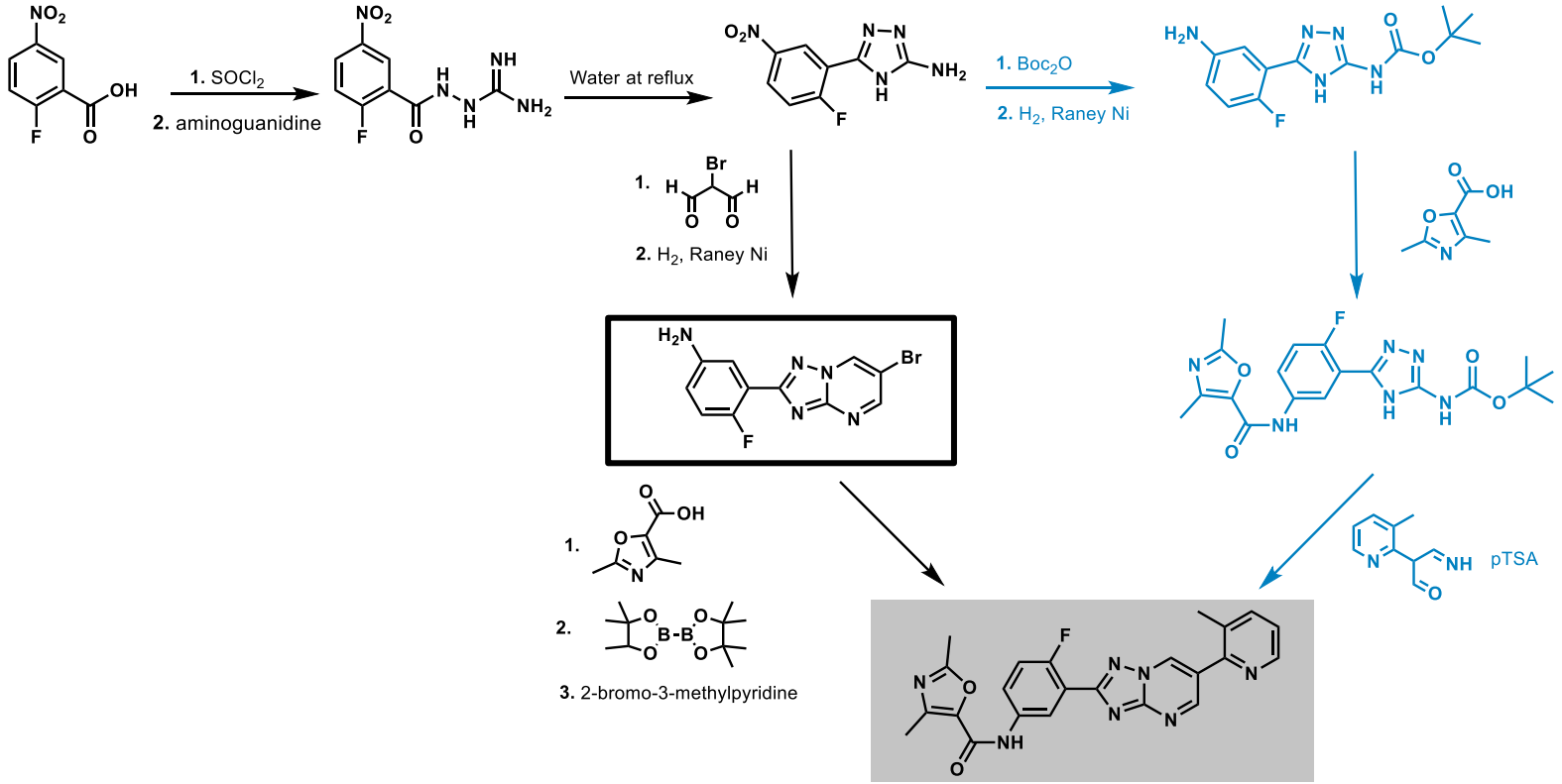
3.4 Å CryoEM





		24	29
L. donovani	1	MAETAIAFRCQDYVMVAAAGLNAFYYIKITDAEDKITQLDTHQLIACTGE	
T. cruzi	1	MSETTIAFRCNSFVLVAAAGLNAFYYIKIMDTEDKVTQLDSHKVVACAGE	
T. brucei	1	MAETTIGFRCQDFVLVAAAGLNAFYYIKITDTEDKITELDSHKVVACAGE	
H.sapiens	1	MEYLIGIQGPDYVLVASDRVAASNIVQMKDDHDKMFKMSEKILLLCVGE	
Lab evolved *T.cruzi* mutants			**L M**

Synthesis of LXE408



Conclusions

- Whole-pathogen *in vitro* assays identified a pan-kinetoplastid inhibitor growth inhibitor series
- The parasite proteasome has been identified as a new drug target for the treatment of kinetoplastid infections
- Parasite-selective proteasome inhibitor LXE408 is entering Phase 2 clinical studies for VL

Hare et. al. *Nature* (2016) **537**: 229

Nagle et. al. *Journal of Medicinal Chemistry* (2020) **63**: 10773

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