Translational challenges in visceral leishmaniasis drug development: different models, different drugs' mechanism of action, different predictive value: towards an emerging answer?

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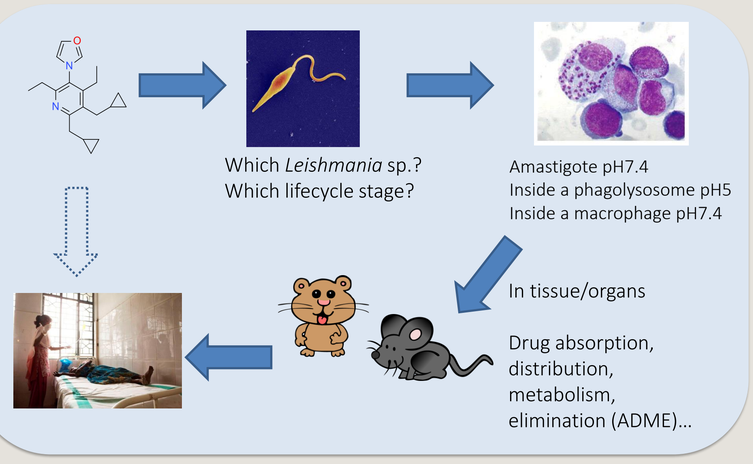
BACKGROUND

New safe, low cost, and field-adapted drugs for visceral leishmaniasis (VL) are urgently needed. Despite substantial screening and lead optimization efforts during the last five years within various organizations, few new chemical entities have successfully entered into the clinic. Whether the right tools or the correct decision-making processes are being used to progress compounds should therefore be reviewed. E.g. VL animal models need to be standardized, and variability linked to the animal or *Leishmania* species used, as well as readouts, should be assessed in a careful and systematic way.

THE CHALLENGE



Leishmania:

complexity & challenges

HOW CAN WE DESIGN & SELECT NEW VL DRUG CANDIDATES?

- ❑ General lack of systematic data generated
- ❑ Lack of targets/target validation and translation into whole cell activity
- ❑ Determination of cidality/rate of kill *in vitro* is not well described
- ❑ Lack of cross-validated and standardized *in vitro* and *in vivo* models (species, treatment regimen/ duration, readouts, …)
- ❑ IVIVC difficult
- ❑ Lack of translational understanding/human prediction, clinically validated targets
- ❑ MoA or PK/PD of current/new treatments is not well understood
- ❑ Chemical structures or PK profile of current treatment are unusual.
- ❑ Regional treatment sensitivity differences (East Africa vs India): Host genetic background, *Leishmania* strains

QUESTION 1: what about *in vitro* parameters ?

- ❑ Five compounds, miltefosine, tafenoquine, DNDI-0690, DNDI-6148 and DNDI-1047, belonging to different chemical series, alkyl phospholipid, aminoquinoline, nitroimidazole, oxaborole and aminopyrazole respectively, were selected for this analysis, as all showed efficacy in VL animal models (>95% parasite reduction in the liver).

	IC_{50} (µM)		IC_{90} (µM)		A_{max} (%)	
	L. donovani	*L. infantum*	*L. donovani*	*L. infantum*	*L. donovani*	*L. infantum*
Miltefosine	6.9 ±3.6 (n=21)	10.1 ±4.2 (n=160)	12.3 ±5.5 (n=21)	17.9 ±8.7 (n=160)	100	nd
Tafenoquine	2.03 ±0.00 (n=2)	0.86 ±0.59 (n=2)	3.57 ±0.00 (n=2)	2.1 ±1.7 (n=2)	nd	nd
DNDI-0690	0.03 ±0.00 (n=2)	0.10 ±0.07 (n=4)	0.06 ±0.00 (n=2)	0.56 ±0.37 (n=4)	100	100
DNDI-6148	1.54 ±0.94 (n=5)	2.18 ±1.42 (n=28)	27.4 ±28.8 (n=5)	20.4 ±25.9 (n=21)	62	nd
DNDI-1047	0.32 ±0.00 (n=2)	0.13 ±0.09 (n=10)	0.85±0.05 (n=2)	0.44 ±0.29 (n=10)	95	nd

- ❑ Range of IC_{50} measured *in vitro* (intramacrophage assays) against both *L, donovani* and *L. infantum*, is wide and ranges from 3 nM to 12 µM.
- ❑ While most of these compounds showed about 100% of parasites killed, the oxaborole DNDI-6148 only reached 60% activity. This correlated with its high IC_{90} value of > 20 µM.
- ❑ No more than a 3-fold difference was calculated between *donovani* and *infantum* data, irrespective of the compound.

THREE QUESTIONS asked,

FIVE COMPOUNDS as an answer

QUESTION 2: *in vivo* or the endless battle: mouse vs hamster & *donovani* vs *infantum*

Results expressed as reduction of parasite load compared to non-treated/placebo group in the 3 target organs (liver/spleen/bone-marrow), when possible		Acute, self- curing model Read out limited to liver, possibly spleen		Chronic, lethal model Read out in 3 target organs	
		L. donovani	*L. infantum*	*L. donovani*	*L. infantum*
Miltefosine	40 mg/kg qd – 5 days	96.7 / 97.7 / -	99.1 / 97.1 / 98.4	98.3 / 99.6 / 99.0	97.8 / 99.3 / 96.4***
	20 mg/kg bid – 5 days	-	-	-	98.4 / 99.5 / 97.4
	20 mg/kg qd – 5 days	-	-	81.1 /95.7 / 93.0	80.0 / 94.5 / 76.9
	12 mg/kg qd – 5 days	69.8 / - / - ***	-	-	-
	10 mg/kg qd – 5 days	58.5 / - / -**	-	-	18.9 / 54.8 / 48.1
Tafenoquine	5 mg/kg qd – 5 days	99.3 / 45.1 / -	98.4 / 62.7 / 57. 8	-	59.2 / 18.1 / 0
	2.5 mg/kg qd – 5 days	95.4 / 49.1 / -	97.5 / 36.2 / 17.3	-	7.2 / 0 / 0
	5 mg/kg bid – 5 days	-	-	-	94.0 / 52.9 / 0
	10 mg/kg qd – 5 days	-	-	-	68.6 / 40.6 / 0
	5 mg/kg qd – 3 days	99.8 / 51.6 / -	-	-	68.9 / 43. 6 / 2.3
DNDI-0690	6.25 mg/kg qd – 5 days	99.8 / - / -*	92.1 / - / -	-	-
	6.25 mg/kg bid – 5 days	-	-	-	91.8 / 89.0 / 80.0*
	12.5 mg/kg qd – 5 days	99.8 / - / -	98.5 / - / -	-	-
	12.5 mg/kg bid – 5 days	-	-	99.8 / 99.9 / 100	98.0 / 98.5 / 96.7*
	25 mg/kg bid – 5 days	-	-	99.9 /100 / 99.9	-
	6.25 mg/kg bid – 10 days	-	-	-	100 / 99.96 / 99.77
DNDI-6148	25 mg/kg bid – 5 days	96.1 / - / -	97.1 / - / -	93.2 / 90.3 / 92.8	98.3 / 98.3 / 93.6
	50 mg/kg bid – 5 days	98.9 / - / -	99.7 / - / -	99.3 / 98.6 / 83.5	100 / 99.9 / 99.4
	25 mg/kg bid – 10 days	-	-	99.9 / 99.8 / 99.6	100 / 99.96 / 99.4
DNDI-1047	25 mg/kg bid – 5 days	-	-	95.6 / 97.5 / 81.5	99.0 / 98.7 / 97.7
	50 mg/kg bid – 5 days	99.0 / - / -	-	-	98.7 / 99.2 / 71.6
	25 mg/kg bid – 10 days	-	-	-	99.9 / 99.8 / 99.2

*, **, ***: Average of several experiments results: 2, 4 and 12, respectively

- ❑ All 5 compounds are active after 5 days dosing in both models, mouse and hamster, and against both VL strains.
- ❑ When efficacity is borderline (90-95%), doubling the dose or extending the duration to 10 days ensures a very high level of parasite reduction. The exception is DNDI-1047 for which efficacy was higher at 25 mg/kg bid than at 50. This may happen when the safety margin is too narrow, and the effect of toxicity starts to compromise efficacy in weakened animals.
- ❑ For all compounds, except for tafenoquine, for a given dose the same level of efficacy was observed in all organs. Tafenoquine is known to accumulate in the liver and this could explain why in this case parasitaemia is marginally reduced or even enhanced in spleen and bone marrow (hamster *L. infantum* model), while parasite burden reduction can reach 99% in the liver.
- ❑ In only two cases, for the same animal model, the dose needed for efficacy (>95%), was different when animals were infected with *donovani* or *infantum*. Indeed, 25 mg/kg of DNDI-6148 given bid for 5 days was sufficient in the hamster/*infantum* model, but not in the hamster/*donovani* model. Similarly, sufficient efficacy was achieved with 6.25 mg/kg DNDI-0690 given qd in mouse/*donovani* model, not in the mouse/*infantum* model. Neither parasite load, compound source, nor virulence as checked by reference control drugs can explain the first case, but the second discrepancy observed for DNDI-0690 may be due to a compound batch difference or higher parasite burden as measured in non-treated groups (30 to 500% higher in hamster infected by *donovani*). However, this parasite load difference did not affect results for the reference drug miltefosine.

QUESTION 3: which PK parameter(s)?

PK parameters measured at the efficacious dose and at steady state, in non-infected satellite animals	Efficacious dose (mg/mL)	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (h*µg/mL)	C_{min} (µg/mL)	Efficacious dose (mg/mL)	C_{max} (µg/mL)	$AUC_{0\text{-}24h}$ (h*µg/mL)	C_{min} (µg/mL)
Miltefosine	40 qd	58.1 (0.85)	998.8 (14.7)	15.6 (0.23)	40 qd	34.6 (0.49)	676.65 (9.68)	10.63 (0.15)
Tafenoquine*	2.5 qd	0.042 (0.0006)	0.91 (0.014)	0.031 (0.0005)	5 bid	0.27 (0.0012)	3.52 (0.016)	0.09 (0.0004)
DNDI-0690	12.5 qd	6.9 (0.26)	75.6 (2.69)	0.1 (0.004)	12.5 bid	0.98 (0.01)	8.14 (0.10)	0.084 (0.001)
DNDI-6148	25 bid	15.5 (1.02)	111.3 (7.3)	0.16 (0.01)	25 bid	3.3 (0.41)	36.0 (4.47)	0.082 (0.01)
DNDI-1047	nd	nd	nd	nd	25 bid	2.75 (0.07)	34.45 (0.92)	0.47 (0.013)

*: Tafenoquine showed acceptable efficacy in liver only. Criteria of >95% was never reached in spleen. In brackets: free concentration obtained by correcting total drug concentration with unbound fraction, measured in PPB assay

- ❑ For all compounds, while hamster is known to be a fast metabolizer, exposure is substantially higher in the mouse (1.5 to 9-fold as per $AUC_{0\text{-}24h}$ at ss). This could reflect the fact we are comparing an acute and a chronic model, where parasitaemia and parasites sites are not similar.
- ❑ This difference in exposure is reduced when PK parameters are corrected with free fraction values and is almost negligible when only C_{min} is considered, as opposed to the usual AUC value.

CONCLUSIONS AND PERSPECTIVES

Even if mouse and hamster models are different in several aspects, the first being notably more appropriate during Lead Optimization and the second being closer to human clinicopathology, this analysis shows that they both lead to the same outcome, irrespective of the class of compound. However, and when applicable, it remains appropriate to test any promising compounds in both models, as this would provide some reassurance that the results are relevant for humans, not just mice or hamsters. Considering the particular case of tafenoquine which accumulates in the liver, or miltefosine that needs at least 5 days to reach a steady state and still circulates at high concentration 2 days after end of dosing, several questions remain to be answered, and tools need to be developed in order to avoid mistakes during compound progression: What about the parasite burden/tropism in the different models as it relates to the tropism of the compounds themselves? Can we solve this issue with bioluminescence models? Is it essential to measure the volume of distribution? Do we need to target 100% activity in *in vitro* assays and sterile cure in *in vivo* models? Is an IV, IP or co-ABT dosing acceptable for a Proof-of-Concept? Can we better understand what is needed by doing animal POP-PK analysis? And finally, do these animal models translate to the situation in man?
Hopefully, these questions will be answered in the near future when clinical trial data starts to be generated for the promising NCEs currently in preclinical development.

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