

## Optimization of pharmaceutical properties of a lead compound targeting human African trypanosomiasis by nanoformulations

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### ABSTRACT

Human African trypanosomiasis (HAT) is a neglected tropical disease caused by the protozoan parasite *Trypanosoma brucei*. HAT is present in 36 countries in sub-Saharan Africa, has 70 million people at risk, and causes 10,000 new cases each year. Current drug therapies need improvement in efficacy, reduced toxicity, and bioavailability.<sup>1</sup> As a result, new research is being conducted in the pursuit of small molecule chemotherapeutics that target HAT.

Using a drug target repurposing approach, we discovered that lapatinib, a human epidermal growth factor receptor (EGFR) inhibitor, exhibited modest potency with an EC<sub>50</sub> of 1.54 micromolar against *T. brucei*. Through structure-activity relationship (SAR) studies, a lead compound, NEU-617, was synthesized and shown to have an EC<sub>50</sub> of 42 nM as well as excellent selectivity over human cells. Despite these promising results, NEU-617 has poor physicochemical properties for targeting the blood-brain barrier (BBB): high molecular weight (541), high lipophilicity (logP = 7.1) and high binding to plasma proteins (>99%).<sup>2</sup> Indeed, we do not see significant levels of the drug in the central nervous system, which is critical for HAT therapeutics.

Nanoformulations have been shown to avoid first pass metabolism, generating prolonged exposures to a drug. Also, nanoformulations rich in fatty acids can aid in BBB penetration.<sup>3</sup> We describe here our ongoing studies of applying nanoformulation technology to NEU-617 to improve its applicability as a potential HAT therapeutic.

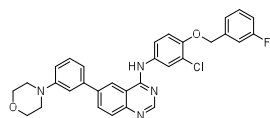
### BACKGROUND

Human African trypanosomiasis (HAT)

- 60 million people at risk, affects 36 countries in sub-Saharan Africa
- 100% fatal if not treated
- Two *Trypanosoma brucei* subspecies: *T.b. gambiense* and *T.b. rhodesiense*

Current medicine for HAT:

- Only 4 drugs, half of which do not cross into the blood-brain barrier (Stage 2)
- Sub-optimal: toxic, show resistance, and not orally bioavailable<sup>4</sup>
- Cyclodextrin complexes encapsulating melarsoprol improved pharmaceutical properties<sup>5</sup>



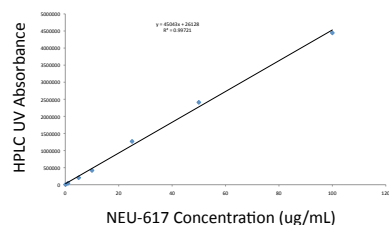
**NEU-617**  
*T.b. brucei* EC<sub>50</sub> = 0.042 μM  
HepG2 IC<sub>50</sub> = >20 μM  
Plasma protein bound 99.6%  
CNS exposure < 5%

### NANOEMULSION CHARACTERISTICS

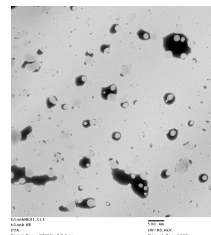
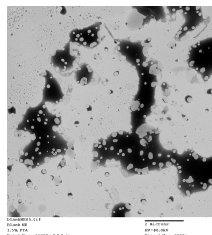


Batch	Size	Charge
Blank	Avg: 137.9 nm PDI: 0.066	Zeta: -52.3 mV Dev: 10.2 mV
NEU-617 (2mg/mL)	Avg: 157.4 nm PDI: 0.039	Zeta: -11.3 mV Dev: 20.3 mV
Rhodamine-123 (0.02%)	Avg: 157.7 nm PDI: 0.043	Zeta: -22.0 mV Dev: 5.14 mV

Through known concentrations of NEU-617 standards, the final concentration of the NEU-617 nanoemulsion was determined to be 1.38 mg/mL



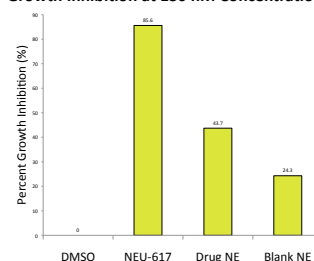
### NANOEMULSION CHARACTERISTICS CONT.



Blank nanoparticles stained in 1.5% phosphotungstic acid show particles in the 100 nm range

### BIOLOGICAL SCREENING

#### Growth Inhibition at 150 nM Concentrations



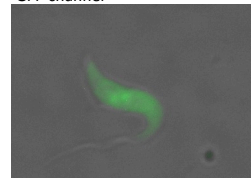
•At 1.5 μM concentrations, both NEU-617 and nanoemulsions show 100% growth inhibition

•At 0.003% (or 70 nM) the blank nanoemulsion shows no growth inhibition

DIC



GFP channel



Trypanosome after 35 minute incubation in Rhodamine-123 nanoemulsion with fluorescence excitation at 470 nm and emission at 525 nm

### FUTURE DIRECTIONS

This study shows that NEU-617 can be encapsulated in nanoemulsions and screened for growth inhibition against the *Trypanosoma brucei* parasite. Further studies are needed to determine if other nanoparticles like liposomes or polymer based nanoparticles like poly(lactic-co-glycolic) acid (PLGA), or poly(epsilon-caprolactone) (PCL) show the same inhibition profiles. Also, encapsulation of the commercial drug lapatinib may show improved encapsulation efficiencies and inhibition over NEU-617.

### 6. REFERENCES AND ACKNOWLEDGEMENTS

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