Small Molecule HTS to Identify Inhibitors of HIV Rev Multimerization

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The goal of this proposal is to optimize a high throughput cell-based assay and test >100K compounds in order to identify lead small molecule inhibitors of Rev multimerization, essential for human immunodeficiency virus type 1 (HIV) replication. The initial inhibitors identified will serve as a Proof-of-Concept that follow on VC or other external funding to develop novel compounds with a favorable therapeutic and safety profile.

Total world-wide $$ on HIV antiviral therapeutics: $40-80B

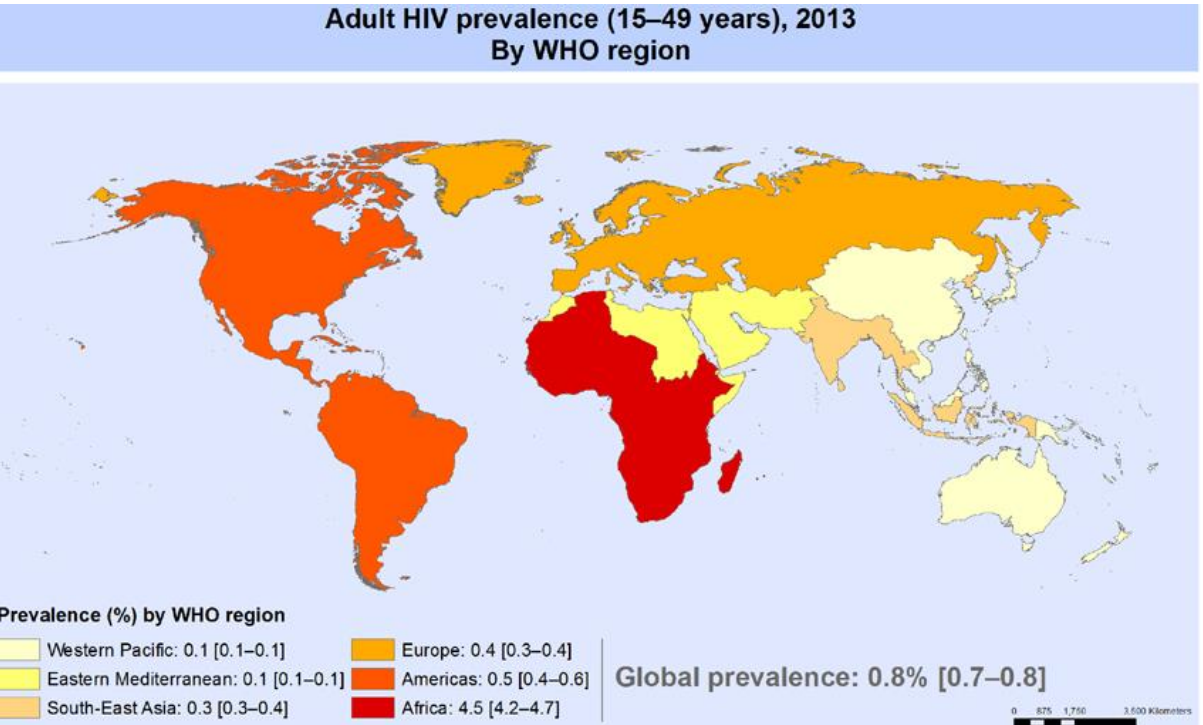
Treatment for life, without interruption

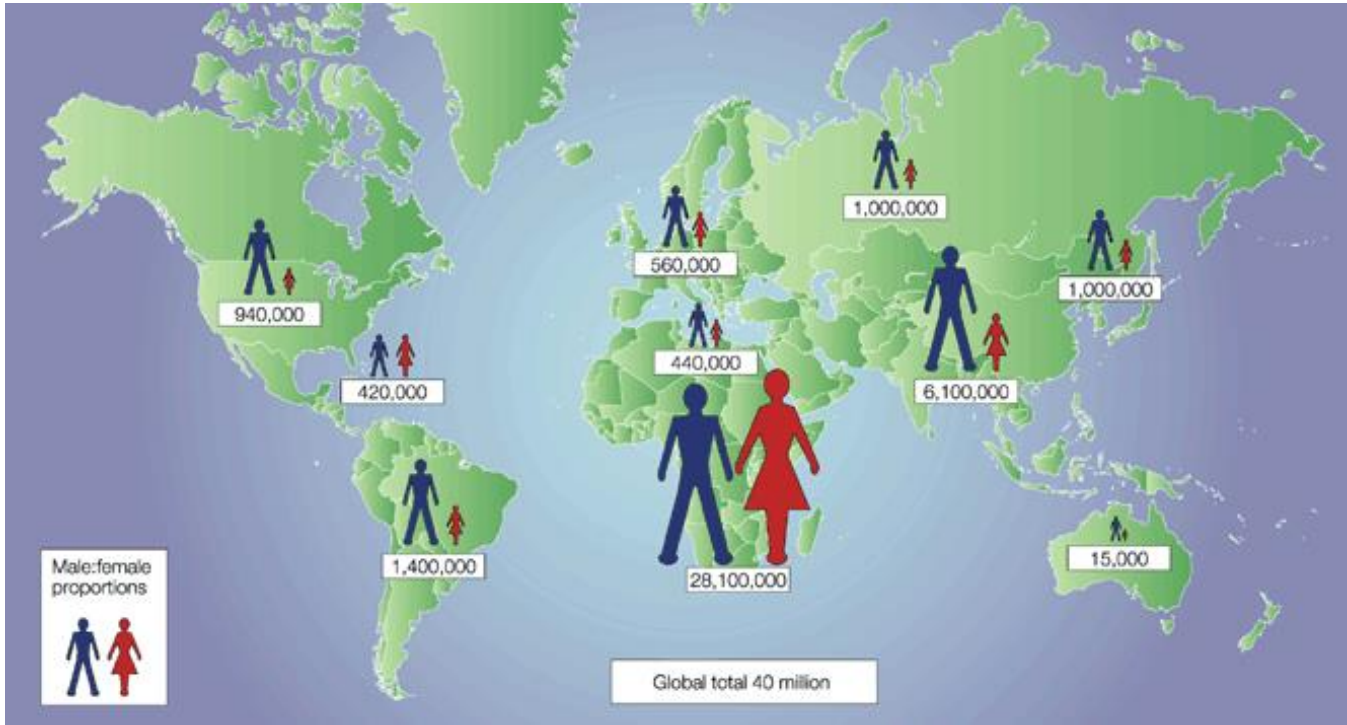
In US—yearly cost is ~$15-20K per person

No vaccine or cure in sight

A true drug ‘success’ can be worth $1-2B per year, if not more

First in class-lower barrier to FDA approval, esp. for Rx-experienced patients





Rev is V.I.P.

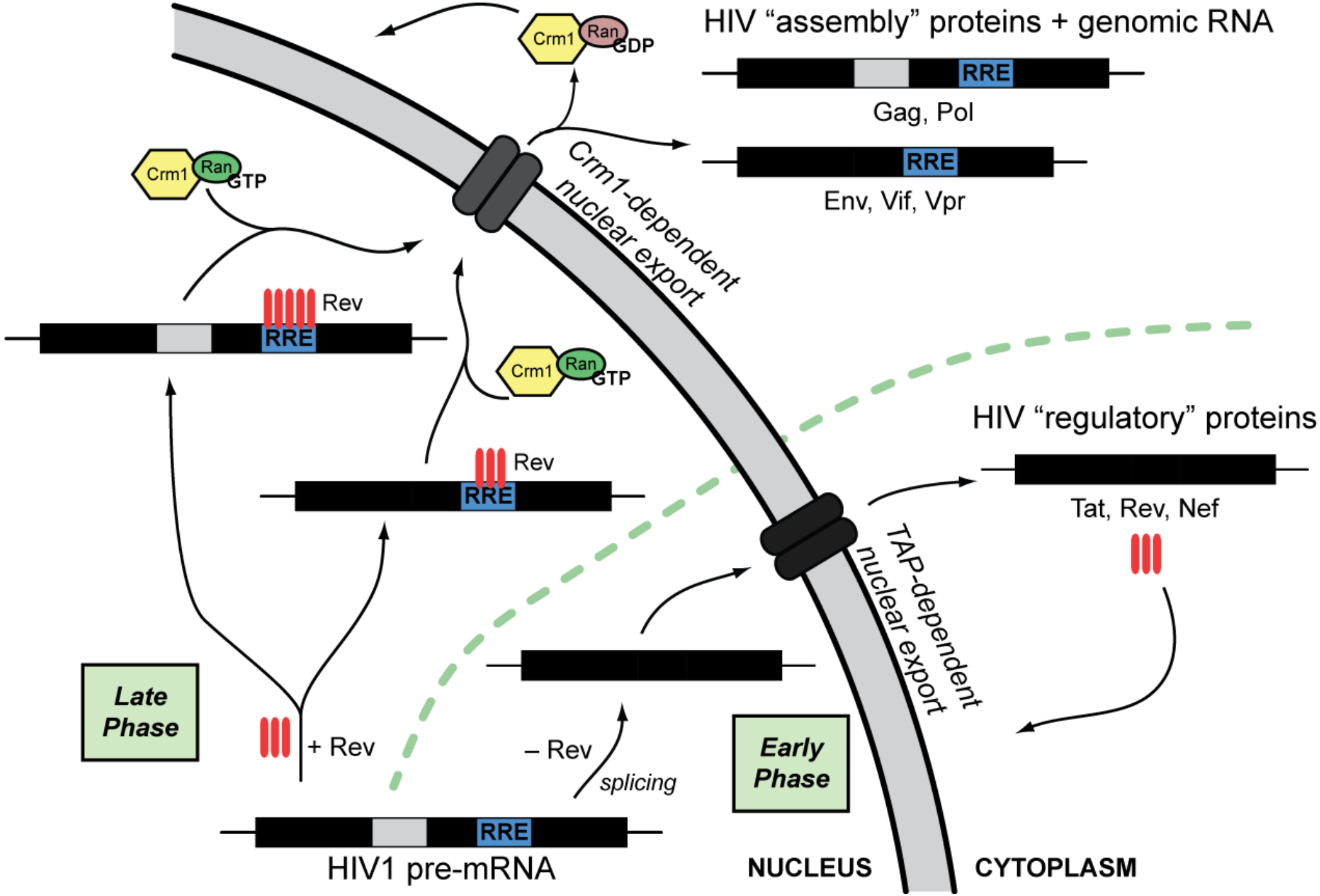
Essential viral regulatory protein, highly conserved in all HIV isolates

HIV does not replicate in any lower animals

Phase I human clinical trials: *no* animal efficacy studies are needed

Pre-clinical data showing inhibition of viral replication in T cell lines essential

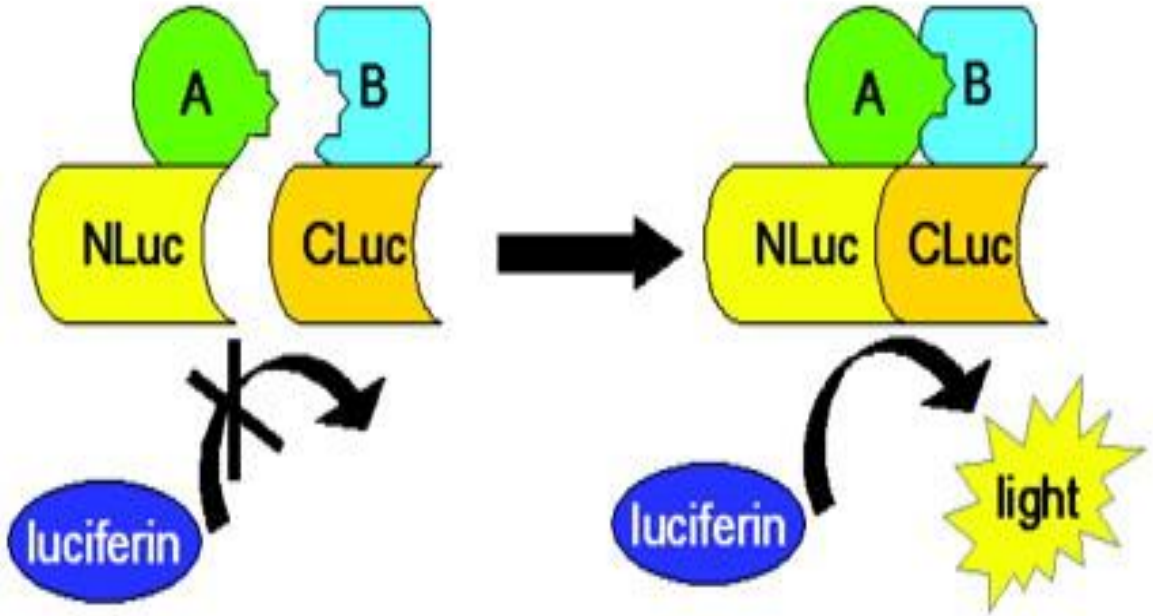
- **Replication assessed by viral loads, p24 (CA) antigen assays**
- **Sequencing resistant virus can confirm MOA of compound**
- **Also gives an idea of the 'genetic barrier' to development of resistance**
- **In vitro and in vivo toxicity studies required**
- **No known Rev inhibitors currently in development (or approved)**


HIV “assembly” proteins + genomic RNA
Crm1
Ran
GDP
RRE
Gag, Pol
RRE
Env, Vif, Vpr
Crm1
Ran
GTP
Crm1-dependent
nuclear export
Rev
RRE
Crm1
Ran
GTP
HIV “regulatory” proteins
Rev
RRE
Tat, Rev, Nef
TAP-dependent
nuclear export
Late
Phase
+ Rev
– Rev
splicing
Early
Phase
RRE
HIV1 pre-mRNA
NUCLEUS
CYTOPLASM

Target is HIV Rev-Rev interaction

Assay is based on luciferase complementation system

- **Stable, 293T-based cell line, easy to use & grow**
- **Excellent characteristics with calculated Z' >0.50 (based on 96-well format)**
- **Anticipated RLU in 384-well ~1500 (bkgrd nil)**



Research Plan:

- Migrate assay to 384-well format & Optimize
  - Work with YMCD to establish workflow logistics and timing
  - Prioritize which small molecule collections to test
  - Counterscreens of any ‘hits’
    - Cell viability
    - CMV luciferase reporter
    - Testing of Rev function in cell-based assays
    - Dose ranging studies

Other in vitro/biochemical assays to demonstrate:

- Compounds do not impact Crm1 function
- Compounds do not disrupt ability of Rev to bind RRE
- Compounds do disrupt Rev multimerization