Challenges in Developing New Therapies for AIDS

Karen Anderson
Yale University

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Structure-Function Studies

- Enzyme Mechanisms & Receptor-Ligand Interactions
  - Site-directed Mutagenesis
  - Rapid reaction Techniques
  - X-Ray Crystallography
  - Pharmacological And Cellular Assays
  - Organic Synthesis/Computational Studies/Virtual Library Screening
  - Inhibitor Design
  - Mass Spectrometry
  - NMR
  - Therapeutics
Ongoing Structure-Function Studies

Exploring Molecular Mechanism of Normal and Oncogenic Protein Signaling

Bifunctional TS-DHFR: Molecular Target for New Antiparasitic Drugs

HIV Reverse Transcriptase: Molecular Mechanisms of Inhibition, Drug Resistance, and Host Toxicity
Challenges in Developing New Therapies for AIDS

Karen S. Anderson, Ph.D.
Montclair State University
MSU Sustainability Seminar
April 9, 2019
The HIV/AIDS Epidemic: By the numbers

- HIV = Human Immunodeficiency Virus
- AIDS = Acquired Immunodeficiency Syndrome
- In 2016, 36.7 million people infected with HIV
  - Of these people, 1 million died from HIV-related causes
  - In 2016, 1.8 million new cases of HIV
- As there are currently no cure or vaccines—HIV treatment lifelong

![Image: 1.8 million people newly infected with HIV in 2016](image1.png)

![Image: 36.7 million people living with HIV by 2016](image2.png)

![Image: 20.9 million people on HIV treatment by mid-2017](image3.png)
HIV Life Cycle & Therapeutic Targets

• Current 25 drugs target key enzymes required for HIV infection (HIV Life Cycle)

1. Entry / Viral Fusion Receptors (2)
2. Reverse Transcriptase (13)
3. Integrase (3)
4. Protease (8)

• Highly Active Antiretroviral Therapy (HAART) also called combination antiretroviral therapy (cART)
  • Combination therapy administered orally
Crystal Structure of HIV-1 Reverse Transcriptase (RT)

FDA Approved RT Inhibitors & HAART

**NUCLEOSIDE RTIs (NRTIs)**

- AZT
- ddI
- ddC
- d4T
- 3TC
- Abacavir
- Tenofovir
- FTC

**NON-NUCLEOSIDE RTIs (NNRTIs)**

- TMC278 (Rilpivirine)
- TMC125 (Etravirine)
- Nevirapine
- Efavirenz
- Delavirdine
Crystal Structure of HIV-1 RT: Binding Sites for NRTIs and NNRTIs
Kinetic Methodology

• **Steady State Kinetics**
  - Time Scale of Minutes
  - Infer the Order of Binding
  - Specify Molecules at the Active Site
  - Complex Kinetic parameters:
    - $k_{\text{cat}} = \frac{k_2k_3k_4/k_2k_3}{(1+k_2/k_2 + k_2k_3/k_2k_3)}$
    - $K_M = \frac{k_{-1}/k_1}{(1+k_2/k_2 + k_2k_3/k_2k_3)}$

• **Transient Kinetics**
  - Time Scale of Milliseconds
  - Establish Pathway Directly
  - Kinetic Parameters Reflect Single Steps
  - Monitor Reactions at the Active Site

![Kinetic Reaction Diagram](image-url)
Rapid Chemical Quench

Drive Syringes

Sample Loops

Reaction Loop

Quench EDTA

Exit Loop

Substrates
- dNTP
- Metal^{2+}

Enzyme
- DNA
- Enzyme
DNA 20 mer

$$5' - *TCAGGTCCCTGTTCGGGCGC - 3'$$

$$3' - CGAAAGTCCAGGGACAAGCCCGGTGACGATCTCT - 5'$$

DNA 36 mer

40 µM dCTP
10 mM MgCl₂

100 nM HIV-1 RT
300 nM DNA/DNA

Time

0 1 sec.

D20 D21
Pre-steady-state Kinetic Parameters

**Incorporation Efficiency** = \( \frac{k_{\text{pol}}}{K_d} \) (\( \mu \text{M}^{-1}\text{s}^{-1} \))
HIV-1 RT Molecular Mechanism

**Overall Rate Limiting Step**

- $K_d \approx 10 \text{ nM}$

**Rate Limiting Catalytic Step**

- $K_d = 2\sim 20 \text{ µM}$
- $k_{pol} = 2\sim 80 \text{ s}^{-1}$

**Overall Rate Limiting Step**

- $K_{d} = 2\sim 20 \text{ µM}$

*Kati et al. JBC, 1992*
Mechanism of NNRTIs

E • DNA\textsubscript{n} ⇄ E • DNA\textsubscript{n} • dNTP

E* • DNA\textsubscript{n} • dNTP

E • DNA\textsubscript{n+1} • PP\textsubscript{i}

E + DNA\textsubscript{n+1} + PP\textsubscript{i}


Nevirapine

Delavirdine

Efavirenz

NNRTI
New Anti-HIV Therapies Are Needed

- NRTIs and NNRTIs, major components of combination HAART (Highly Active Anti-Retroviral Therapy), improve quality of life for patients

- RT is a highly error prone polymerase

- HIV rapidly mutates producing drug resistant variants

- Toxicity issues with current therapies

- Treatment failures due to resistance and toxicity necessitates the need for new HIV therapies
HIV-1 Reverse Transcriptase: Molecular Mechanisms of Inhibition, Drug Resistance, and Host Toxicity

Molecular mechanisms of inhibition and drug resistance for NRTIs and NNRTIs

Mechanism, computational, and structure guided design of novel non-nucleoside reverse transcriptase inhibitors

Understanding molecular basis of toxicity for NRTIs due to effects on mitochondria
HIV-1 Reverse Transcriptase: Molecular Mechanisms of Inhibition, Drug Resistance, and Host Toxicity

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Current AIDS Drugs: NNRTIs

- Current NNRTIs, efavirenz and rilpivirine, components of several single tablet regimens

- Rilpivirine also a component in new long acting formulation with INSTI dosed monthly. This formulation received FDA approval Nov. 2017 in limited patient population. Use expected to increase as single tablet and extended dosing regimens become more widely used.

- Doravirine, new Merck NNRTI, FDA approval August 2018
Rilpivirine – the good, the bad and the ugly

The GOOD
• Most potent NNRTI ($EC_{50} \sim 0.7 \text{ nM}$)
• Active against common RT mutants (e.g. Y181C, K103N/Y181C)

The BAD
• High CLogP (5.75)
• Poor solubility ($<0.02 \mu\text{g/mL}$)
• Inhibition of CYP3A4
• Potentially reactive cyanovinyl group

The UGLY
• Dose limiting cardiotoxicity (inhibits HERG ion channel)
• Dose restriction limits treatment to patients with viral loads $<100,000 \text{ copies}$
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Automated Lead Design and Optimization

**Lead Generation**

- X-ray Structures of Complexes
- de novo: BOMB
- docking: Glide + ZINC
- ADME* filter: QikProp
- Synthesize
- Assay
- µM Hits

**Lead Optimization**

- FEP Scans
- ADME filter: QikProp
- Synthesize & Assay
- FEP Refinement
- Synthesize & Assay
- nM Leads

*ADME = Absorption Distribution Metabolism Excretion

Collaboration with Bill Jorgensen
Discover novel chemical scaffolds with improved pharmacological properties and resistance profiles

3D Crystallographic Information

Virtual Screen
Compound library

Evaluate virtual “hits”
in vitro and in cell culture

High Activity compounds against WT RT

Activity against NNRTI mutants

Computational Evaluations
Virtual Screening

- Chemical similarity search
  - Maybridge Library
  - Use known NNRTIs as reference structures
  - QikSim program
  - 1850 compounds selected
- Docking
  - Glide program
  - WT structure (PDB: 1rt4)
  - K103N structure (PDB: 1sv5)
  - 100 compounds selected
- Postprocessing
  - Molecular mechanics - binding energy
  - Continuum solvation - solubility
  - Flexibility
  - Conformation energies
  - Top 6 ranked compounds purchased & assayed

Assays to Evaluate Hits and Guide Lead Optimization

- In vitro RT assays to evaluate inhibition of catalysis
- Detailed mechanistic studies to delineate mode of inhibition using transient kinetic analysis
- Fluorescence Polarization Assays to Assess Binding to WT and drug resistant forms of RT
- Structural studies to confirm and refine computational predictions
- Evaluation of physiochemical and pharmacological properties
- Evaluation of antiviral activity and cellular toxicity in HIV-1 infected human T-cells
Incubate cells with drug ± virus for 5 days
- Add MTT
- Add Stop soln after 5 hours
- Read $A_{595}$

CC$_{50}$ = cytotoxicity
EC$_{50}$ = antiviral potency
Exploring the Non-nucleoside Binding Pocket to Enhance Potency, Selectivity, and PK properties
Perform Systematic Scans:
Small Substituent Scans – we like CH₃ and Cl

30,000 nM
2,800 nM
200 nM
39 nM

and Extensive Heterocycle Scans -

>15,000 nM
3,200 nM
200 nM
6 nM

Experimental EC₅₀s from an Anti-HIV Assay Using Infected Human T-cells
Examples of FEP-Guided Lead Optimization for Anti-HIV Agents

EC$_{50}$ = 2 nM

BMCL 2006, 16, 663, 668

EC$_{50}$ = 5 nM

JACS 2006, 128, 15372

EC$_{50}$ = 2.5 nM

JACS 2011, 133, 15686

EC$_{50}$ = 310 nM

JACS 2008, 130, 9492

EC$_{50}$ = 13 nM

J Med Chem 2011, 54, 8582

EC$_{50}$ = 4800 nM

JCIM 2009, 49, 1272

EC$_{50}$ = 0.055 nM

JACS 2011, 133, 15686
Computational and Structural Design of NNRTIs

Crystal Structures → Docking Hit → Compound Synthesis

FEP Calculations → ADME filter: QikProp → JLJ494

Docking Hit
5 μM

JLJ494
55 pM

91,000-fold improvement in activity

Collaboration with William Jorgensen

Bollini et al., J Med Chem, 54, 8582-91 (2011)
FEP-Guided Lead Optimization: Steps Toward Picomolar Inhibition

A: $EC_{50} = \sim 5,000 \text{ nM}$

B: $EC_{50} = 380 \text{ nM}$

C: $EC_{50} = 14 \text{ nM}$

D: $EC_{50} = 0.055 \text{ nM}$

E: $EC_{50} = 0.320 \text{ nM}$

JLJ506

JLJ494
Protocol for RT-NNRTI Crystallography

*Develop methods for crystallizing RT with novel lead compounds*

- Expedient, repeatable method to crystallize several diverse compounds with RT
- Structural analysis
  - Assist in NNRTI compound design and development
  - Validate predictions observed in computational modeling

**Preparation of RT52A**
- Expression
- Purification
- Characterization by ESI-MS

**Crystallization**
- Broad Screening
- Nevirapine control tray
- Optimization
- Reproduce with various compounds

**Data Collection**
- Cryo-conditions
- Diffraction Data Collection
- Collection Parameters

**Structure Determination**
- Molecular Replacement
- Refinement
- Geometry Check
- Analysis

Structural Studies to Validate Binding of Catechol Diethers to RT
# Data Collection Statistics

<table>
<thead>
<tr>
<th>Complex</th>
<th>JLJ494</th>
<th>JLJ506</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength, Å</td>
<td>1.075</td>
<td>1.075</td>
</tr>
<tr>
<td>Space Group</td>
<td>C2</td>
<td>C2</td>
</tr>
<tr>
<td># Molecules in Asymmetric Unit</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unit Cell, a,b,c in Å</td>
<td>a=225.13, b=69.21, c=104.14</td>
<td>a=225.45, b=69.58, c=104.40</td>
</tr>
<tr>
<td>Resolution Range, Å</td>
<td>50 – 2.85</td>
<td>50 – 2.90</td>
</tr>
<tr>
<td>Last Shell, Å</td>
<td>2.90 – 2.85</td>
<td>2.95 – 2.90</td>
</tr>
<tr>
<td>$R_{sym}$ (last shell)</td>
<td>0.097 (0.546)</td>
<td>0.073 (0.596)</td>
</tr>
<tr>
<td>Completeness, % (last shell)</td>
<td>98.5 (98.4)</td>
<td>99.8 (98.8)</td>
</tr>
<tr>
<td>No. Unique Reflections</td>
<td>35716</td>
<td>34776</td>
</tr>
<tr>
<td>Redundancy (last shell)</td>
<td>3.8 (3.7)</td>
<td>3.8 (3.7)</td>
</tr>
<tr>
<td>Avg. I/σ</td>
<td>16.6 (2.7)</td>
<td>16.2 (2.6)</td>
</tr>
</tbody>
</table>
Three-dimensional Structure vs. Computational Model

Frey et al. JACS, 134, 19501-3 (2012)
Picomolar Inhibitor Featuring Bicyclic Replacement of Cyanovinylphenyl Group

JLJ506

JLJ555

**EC$_{50}$ values (nM) for each drug against WT and NNRTI-resistant virus**

<table>
<thead>
<tr>
<th>VIRUSES</th>
<th>FDA approved Drugs</th>
<th>Catechol Diether NNRTIs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EFV</td>
<td>RPV</td>
</tr>
<tr>
<td>WT X4</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>K101P</td>
<td>870</td>
<td>1142</td>
</tr>
<tr>
<td>K103N</td>
<td>806</td>
<td>13</td>
</tr>
<tr>
<td>Y181C</td>
<td>41</td>
<td>51</td>
</tr>
<tr>
<td>G190S</td>
<td>1212</td>
<td>2</td>
</tr>
<tr>
<td>Sol. µg/ml</td>
<td>68</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CC$_{50}$µM</td>
<td>15</td>
<td>8</td>
</tr>
</tbody>
</table>

Collaboration with R. Silicano

Advantages of New NNRTIs

- Resistance profile of new NNRTI compounds is different from currently available NNRTIs.

- K101P virus is resistant to *all* approved NNRTIs but *none* of the three test compounds.

- Improved solubility, especially relative to rilpivirine
FEP-Guided Lead Optimization

Lee, Chan et al. ACS Med Chem Let, 7, 1156-60 (2016)
In vitro pharmacological profiling of efavirenz, cmpd I (JLJ636), and cmpd II (JLJ532) against targets for adverse drug reactions

Rows represent compounds tested and columns represent targets. Percentage inhibition at 10 µM concentration of the compounds is color-coded

Selection of Compounds for Preliminary PK analysis in BALB/c mice

- Among all the tested compounds, JLL-532 and JLJ-636 showed promise in terms of potency and solubility, against a panel of HIV strains containing RT variants with K101P, K103N, E138K, Y181C, E138K/M184V, Y181C/K103N mutations.
- Low Toxicity (Therapeutic Index >200,000) and no significant inhibition against a panel of 35 metabolic enzymes, ion channels, and receptors including major CYP450 isoforms and HERG ion channel.

JLL532 (Cmpd II)

JLJ636 (Cmpd I)

Serum concentration versus time for JLJ-636 after 20 mg/kg/ip injection into mice
## Comparison of PK parameters in BALB/c mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>JLJ-532</th>
<th>JLJ-636</th>
<th>Efavirenz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µM)</td>
<td>1.33</td>
<td>27.9</td>
<td>5.7</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$\text{AUC}_{0-24}$ (µM h)</td>
<td>21.89</td>
<td>443.3</td>
<td>64.6(0-72h)</td>
</tr>
<tr>
<td>Solubility (µg/mL)</td>
<td>510</td>
<td>82.9</td>
<td>68</td>
</tr>
<tr>
<td>$\text{EC}_{50}$ (µM)-WT RT</td>
<td>0.00031</td>
<td>0.0019</td>
<td>0.002</td>
</tr>
<tr>
<td>$\text{CC}_{50}$ (µM)</td>
<td>18</td>
<td>&gt;100</td>
<td>15</td>
</tr>
</tbody>
</table>
Resolution Limit (Å) | 2.85
---|---
X-Ray Source | APS 24ID-E
Wavelength, Å | 0.97915
Space group | C2
No. molecules in asymmetric unit | 1
Unit cell, a,b,c in Å (α,β,γ, in °) | a=224.4, b=69.5, c=104.5, α=90, β=106.0, γ=90
Resolution range, Å | 50.0-2.85
Last Shell, Å | 2.90-2.85
R-sym (last shell) | 0.069 (0.510)
Completeness, % (last shell, %) | 99.4 (99.0)
No. of Reflections (Unique Reflections) | 137490 (36173)
Redundancy (last shell) | 3.8 (3.8)
Avg. I/σ (last shell) | 24.2 (3.3)
R-free, R-factor | 0.2725, 0.2270
RMS deviation bond lengths (Å), angles (°) | 0.003, 0.631
Avg. B-factor: Protein/Inhibitor | 69.4, 53.9, 54.1, 87.3
Ramachandran Favored, Allowed, Outliers (%) \{MolProbity\} | 96.62, 3.38, 0

Omit map σ = 1.0

Many hydrophobic contacts

H-bond(s): C=O K103
sc NH K102

π-π interaction: Y181, Y188
W229

VDW interactions: P95, V108,
L100, L234
Important Selection Criteria for Preclinical Candidate

- Synergy with other AIDs drugs for combination therapy
- In vivo evaluation of pharmacokinetic properties in mice
- Evaluate efficacy of candidates with optimal properties in Hu-mouse AIDS model
- Feasibility for developing a long-acting sustained release formulation
Synergy Studies for JLJ636 with Other AIDS Drugs

Conclusions from PK and Cellular Studies

- The peak concentrations were found for JLJ532 and JLJ636 in the serum comparable to or > than Efavirenz.

- Drug concentrations are maintained up to 24 hrs (JLJ532) and 48 hrs (JLJ636). These concentrations are > 3000 fold above the potencies and much lower than cytotoxicity.

- These data suggests that JLJ532 and JLJ636 can be administered every 24 hr in humanized mice infected with HIV to conduct the efficacy studies without concerns for drug toxicity.

- Potent Synergy with current approved NRTIs and ones in clinical evaluation suggest JLJ636 could be a useful component of combination therapy.
Preclinical Studies for JLJ636

• Optimized lead compound with activity in 1-2 nM range maintaining activity on drug-resistant mutants
• Excellent ADME-Tox and physiological properties
• In vitro pharmacological studies show no off targets
• Pharmacokinetic studies show excellent in vivo bioavailability in mice

Testing JLJ636 in Humanized Mice Infected with HIV

A

1) Serum Drug Levels
2) CD4+ cells
3) PVLs (RNA copies)

Hu PBL
30,000 pfu
HIV-1 JRCSF

Days
-7
-1 0
8
16
21
28
32

Free compound I 100 mg/kg/day for 32 days
OR
190 mg/kg compound I-NP single dose at day -1

*Free compound I withdrawal at day 19

4 groups (3 mice per group)
1) Control (infected and PBS injected)
2) Continuous group (100 mg/kg/day free compound I for 32 days)
3) Withdrawal group (100 mg/kg/day free compound I withdrawn at day 19)
4) Compound I-NP (190 mg/kg single dose)

B

Serum compound I (µg/ml)

100 mg/kg/day free compound I
100 mg/kg/day free compound I (withdrawn at day 19)
190 mg/kg compound I-NP single dose at day-1

Days
0 4 8 12 16 20 24 28 32

* - drug withdrawn at day 19

Collaboration with Priti Kumar and Mark Saltzman
Testing JLJ636 in Humanized Mice Infected with HIV

A
Hu PBL
-7

HIV-1 JRCSF
30,000 pfu

-1 0

8 16 21 28 32

Days

Free compound I 100 mg/kg/day for 32 days

OR

190 mg/kg compound I-NP single dose at day -1

1) Serum Drug Levels
2) CD4+ cells
3) PVLs (RNA copies)

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4) Compound I-NP (190 mg/kg single dose)

B

Serum compound I (µg/ml)

100 mg/kg/day free compound I
100 mg/kg/day free compound I (withdrawn at day 19)
190 mg/kg compound I-NP single dose at day-1

Days

0 4 8 12 16 20 24 28 32

* - drug withdrawn at day 19

Collaboration with Priti Kumar
Unique Features for JLJ636, Pre-clinical Lead Candidate


### A

<table>
<thead>
<tr>
<th></th>
<th>D0</th>
<th>D8</th>
<th>D16</th>
<th>D25</th>
<th>D32</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 + No treatment</td>
<td>79.7</td>
<td>0.46</td>
<td>61.6</td>
<td>1.40</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>0.073</td>
<td>19.8</td>
<td>8.39</td>
<td>20.6</td>
<td>2.28</td>
</tr>
<tr>
<td>HIV-1 + Compound I-NP</td>
<td>78.9</td>
<td>1.01</td>
<td>71.0</td>
<td>0.73</td>
<td>71.5</td>
</tr>
<tr>
<td></td>
<td>0.058</td>
<td>20.0</td>
<td>7.87</td>
<td>27.5</td>
<td>4.29</td>
</tr>
<tr>
<td>HIV-1 + Free compound I</td>
<td>81.6</td>
<td>7.55</td>
<td>71.0</td>
<td>2.64</td>
<td>69.6</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>10.1</td>
<td>0.71</td>
<td>25.7</td>
<td>2.89</td>
</tr>
</tbody>
</table>

### B

- Control
- 100 mg/kg/day free compound I
- 100 mg/kg/day free compound I (withdrawn at day 19)
- 190 mg/kg/day compound I-NP single dose at day -1

### C

- Control
- 100 mg/kg free compound I
- 100 mg/kg/day free compound I (withdrawn at day 19)
- 190 mg/kg/day compound I-NP single dose at day -1

Top 10 List: Unique Features for JLJ636, Pre-clinical Lead Candidate

1. Optimized lead JLJ636 anti-HIV activity 1-2 nM, maintains activity on common drug-resistant mutants including K101P, while current NNRTIs, including rilpivirine are ineffective
2. Excellent ADME-Tox and physiological properties
3. In vitro pharmacological studies show no off targets including HERG and CYP3A
4. Pharmacokinetic studies show excellent in vivo bioavailability in mice
5. Efficacy in humanized AIDS mouse model infected with HIV (CD4+; viral load undetectable)
6. Long-acting nanoparticle formulation showing sustained concentrations of JLJ636 and efficacy for almost 1 month after single dose
7. Marked synergistic antiviral activity in HIV-infected cells current FDA-approved NRTIs and INSTIs as well as the new Merck NRTI (EFdA), in early phase trials
8. Excellent candidate for combination therapy regimens
9. Potential Agent for Pre-Exposure Prophylaxis (PrEP)
10. US Patent Appl. 9914709
11. HIV drug implant studies underway; PK shows sustained drug levels >3 months, in vivo efficacy studies ongoing.

HIV-1 Reverse Transcriptase: Molecular Mechanisms of Inhibition, Drug Resistance, and Host Toxicity

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HIV-1 Reverse Transcriptase: Molecular Mechanisms of Inhibition, Drug Resistance, and Host Toxicity

Mechanism, computational, and structure guided design of novel non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibitors

Design of a novel class of covalent NNRTI inhibitors for drug resistant HIV
Destroying Drug-Resistant HIV with Novel Chemical Warheads
FEP-Guided Lead Optimization

cyanovinyl

indolizine

1-naphthyl

2-naphthyl
**Challenges with Drug-Resistant HIV**

- Common RT resistant mutations are Y181C and K103N/Y181C
- Inhibition of K103N/Y181C RT is often >10x less potent

<table>
<thead>
<tr>
<th>Compound</th>
<th>WT</th>
<th>K103N/Y181C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz</td>
<td>0.002</td>
<td>0.03</td>
</tr>
<tr>
<td>Rilpivirine</td>
<td>0.00067</td>
<td>0.002</td>
</tr>
<tr>
<td>JLJ532</td>
<td>0.00031</td>
<td>0.024</td>
</tr>
<tr>
<td>JLJ555</td>
<td>0.00038</td>
<td>0.011</td>
</tr>
<tr>
<td>JLJ636</td>
<td>0.0019</td>
<td>0.021</td>
</tr>
<tr>
<td>JLJ651</td>
<td>0.0062</td>
<td>0.280</td>
</tr>
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</table>
Structures with Non-Covalent NNRTIs Offer A Starting Point

- C-Cl bond points toward Y/C181
- Cl – S distance 4.6 Å
**Covalent NNRTIs Candidates with Chemical Warheads**

1. SN2
   - 2: $X = F$
   - 3: $X = Cl$

2. Michael Addition
   - 4: $X = H$
   - 5: $X = CH_3$
Enzymatic Assays to Test Covalent Binding: Time-dependent IC$_{50}$
Confirmation of Covalent Modification via ESI-TOF Mass Spectrometry

- No change to p51
- No change to p66 with Cmpd 2 or Cmpd 4
- ~500 Da addition to p66 with Cmpd 3 and Cmpd 5

1 molecule of Cmpd 3 or Cmpd 5 covalently bound to the p66 subunit of RT
Enzymatic Crystal Structures Confirm the Presence of Covalent Bond with Cmpd 3 and Cmpd 5

Chan et al. PNAS, 114, 9725-30 (2017)
# Cellular Data Support Covalent Inhibition

- Cmpds 1, 2, 4: >10x ↑ EC$_{50}$
  - Non-covalent
- Cmpds 3, 5: slight ↓ EC$_{50}$
  - Covalent
- Also Cmpds 3 & 5 are less cytotoxic (higher CC$_{50}$) than Efiv/Rpv

<table>
<thead>
<tr>
<th>Compound</th>
<th>WT (µM)</th>
<th>K103N/Y181C (µM)</th>
<th>CC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz</td>
<td>0.002</td>
<td>0.03</td>
<td>15</td>
</tr>
<tr>
<td>Rilpivirine</td>
<td>0.0007</td>
<td>0.002</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>0.0062</td>
<td>0.280</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2</td>
<td>0.890</td>
<td>15</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3</td>
<td>0.580</td>
<td>0.570</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>1.6</td>
<td>&gt;12</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>0.560</td>
<td>0.500</td>
<td>32</td>
</tr>
</tbody>
</table>

*Chan et al. PNAS, 114, 9725-30 (2017)*
Summary and Future Directions

- Computationally and structure-guided potent NNRTI lead candidate developed with improved PK and ADME/Tox properties compared with current FDA approved drugs

- Preclinical animal studies demonstrated efficacy of free compound and long-acting formulation in HIV-infected Hu-mouse AIDS model

- HIV drug implant studies underway; in vivo efficacy ongoing

- First covalent NNRTIs targeting HIV-1 RT that destroys Y181C drug resistant mutant

- Structural & Mechanistic Characterization of Newer Covalent NNRTIs Underway that Target WT HIV-1 RT
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