Molecular Gymnastics: Mechanisms of HIV-1 Resistance to CCR5 Antagonists and Impact on Virus Phenotypes

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HIV Entry
- HIV entry into target cells is mediated by the Envelope glycoprotein spikes found on the surface of the virus

HIV Entry

HIV Tropism
- HIV uses CD4 and a co-receptor to enter cells
- HIV is grouped depending on the co-receptor usage

- R5-tropic (CCR5)
- Dual tropic (CCR5, CXCR4)
- X4-tropic (CXCR4)

HIV and CCR5

HIV and CCR5

Inhibition of R5 HIV
- Individuals homozygous for Δ32 CCR5 do not express CCR5
- Resistant to HIV infection
- Otherwise mostly healthy
- ‘Berlin patient’ – stem cell transplant from a Δ32 CCR5 homozygous donor
- Natural ligands of CCR5 – MIP-1, MIP-1, and RANTES block HIV infection
- RANTES derivatives (AOP, PSC, and SP12-RANTES) with greater potency explored for use as topical microbicides
- Gene editing of CCR5 with Zinc Finger nucleases can protect CD4+ T cells from infection

eCD4-Ig, a one-two punch
- Combination of CD4 domain and CCR5 N-terminus mimetic
- Greater breadth and potency than bNAB
- Protective in rhesus macaques against SHIV challenge
CCR5 antagonists

- Small molecule inhibitors of CCR5
- Block binding of CCR5 ligands and HIV Env
- Maraviroc (MVC) - approved for use
- Cenicriviroc (CVC) – phase 2b complete
- Vicriviroc (VCV) – terminated
- Aplaviroc (APL) – terminated
- TAK-779, TAK-220, AD101 – preclinical

As these compounds only block R5 HIV, a tropism test is required prior to initiation of therapy with CCR5 antagonists

How Maraviroc Works

- Maraviroc locks CCR5 in a confirmation not recognised by HIV Env

HIV becomes resistant to Maraviroc

- In vitro – continued use of CCR5
- In vivo - Either through switching to CXCR4 usage or continual use of CCR5
- Continued use of CCR5 – mutations in Env allows the resistant virus to bind to the antagonist modified form of CCR5
- X4 is unlikely to be true ‘switch’ rather emergence of minority CXCR4 using

Resistance manifests in a unique way

- Represented by changes in the maximal percent inhibition (MPI) rather than changes in IC50
- MPI is a marker for resistance
- Non-competitive mechanism of resistance
- Resistant strains can use MVC-occupied and free CCR5

Questions to answer

- What determines the MPI?
- How do MVC-resistant viruses recognise and bind to the MVC-occupied receptor?
- What are the consequences of MVC-resistance? Specifically;
  - Are MVC-resistant viruses cross resistant to other entry inhibitors?
  - Do MVC-resistant viruses have changes in their tropism for CD4+ cells?
- Can we predict resistance – did MVC-resistant viruses have some intrinsic resistance prior to therapy?
- Can we inhibit MVC-resistant viruses?

Clones used in this study

<table>
<thead>
<tr>
<th>Env</th>
<th>MVC resistance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC-Sens</td>
<td>+</td>
<td>Generated from CC185 isolate in an in vitro cell culture passaging experiment</td>
</tr>
<tr>
<td>MVC-Res</td>
<td>-</td>
<td>Pre-treatment and post failure samples from two patients enrolled in MVC clinical trial</td>
</tr>
<tr>
<td>17Sens</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17Res</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>24Sens</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>24Res</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
MVC Sensitivity varies amongst resistant strains

- Reductions in MPI for resistant Envs
- MPIs vary amongst strains

V3 loop changes confer resistance but are not common

- Mutagenesis studies have mapped the resistance mutations to the variable loop 3 (V3) of gp120
- Resistance mutations are not common amongst resistant Envs and are context dependent

V3 Sequence

<table>
<thead>
<tr>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC-Sens</td>
<td>MVC-Res</td>
<td>17Res</td>
</tr>
<tr>
<td>CTLPRRSSTEFS PGDFAYTGGIDRGQAV</td>
<td>LPGSGYATGDIIDIRQAHC</td>
<td>PGRAYATGDIIGDIRQAHC</td>
</tr>
<tr>
<td>17Sens</td>
<td>17Res</td>
<td>24Res</td>
</tr>
<tr>
<td>CTLPRRSSTEFS PGDFAYTGGIDRGQAV</td>
<td>FTYAYTGGIDIDIRQAHC</td>
<td>SAGAYTGGDIIGDRQAHC</td>
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What determines the MPI?

- Changes in receptor affinity investigated using 293-Affinofile affinity profiling system
- CD4 and CCR5 expression is controlled by separate inducible promoters
- 48 cell populations with varying CD4/CCR5 levels are created

Affinity for the MVC-CCR5 complex determines the MPI

- Only strongly resistant 24Res is unaffected by changes in CCR5 expression in the presence of MVC

MVC-resistant Envs become critically dependent on the CCR5 N-terminus

- When forced to use the MVC-occupied receptor, the MVC-resistant Env becomes critically reliant on the N-terminus
- Represents a shift to a region of CCR5 not modified by MVC
Does MVC resistance lead to cross resistance?

- For moderately resistant MVC-Res
  - No reduction in MPI to VCV
  - No increase in T20 IC50

- Both weakly and strongly MVC resistant Envs retain sensitivity to TAK-779
- Strongly MVC resistant Env displays weak cross-resistance to VCV
- Cross resistance does not appear to occur with MVC resistance

MVC-resistant Envs still require interaction with the CCR5 ECLS

- MVC-resistant Env sensitive to mutations in ECL 1 and 2
- Some interaction with CCR5 ECLS still required
- CCR5 antagonists modify CCR5 differently

Do changes in the engagement and affinity for CCR5 alter tropism?

- Coreceptor expression varies amongst CD4+ T cell subsets
- Do changes in CCR5 affinity change infection of different subsets?

Do MVC-resistant Envs have altered T-cell tropism?

- Using GFP reporter viruses and a series of T-cell maturation markers we investigated the distribution of infection amongst a T-cell population
MVC resistant Envs have alterations in T-cell tropism

- In the presence of MVC, strongly resistant 24-Res Env has a shift in T-cell tropism towards increased infection of central memory cells and reduced infection of effector memory and transitional memory cells.

![Graph showing T-cell tropism alterations](image)

MVC-resistant Envs have attenuated M-tropism

- Matched MVC-sensitive and MVC-resistant Envs display similar levels of Macrophage entry.
- The presence of MVC attenuates or abolishes entry by moderately or weakly MVC-resistant Envs.
- An altered interaction with CCR5 appears important for Macrophage tropism.

![Graph showing M-tropism attenuation](image)

Are some viruses pre-triggered to escape MVC?

- The CC1/85 isolate is unique in its ability to evolve CCR5 antagonist resistance in vitro relatively easily.
- The MVC-sens Env is sensitive to MVC in most assays.
- When the CCR5 levels are increased a partial level of resistance is observed.
- Perhaps this explains why this isolate can evolve resistance.
- Can this be used to prescreen patients before commencement of MVC therapy?

![Graph showing pre-triggered resistance detection](image)

Baseline MVC-resistance in a therapy naïve subtype C cohort

- MVC sensitivity assessed in a panel of Envs from a subtype C cohort of individuals with progressive disease.
- Residual viral entry in the presence of MVC in 16/244 Envs (8 patients).
- No genetic correlates.

![Graph showing baseline MVC-resistance](image)

Baseline MVC-resistance in a therapy naïve clade C cohort

- Varying MPIs observed for selected clade C Envs when infecting CCR5<sup>hi</sup> cells.
- Are these Envs more likely to evolve genuine resistance to MVC?
- Implications for MVC as a microbicide or PrEP.

![Graph showing varying MPIs](image)

Inhibiting the MVC-resistant viruses

- Increased dependence on CCR5 N-terminus appears to be a hallmark of MVC-resistant strains.
- Can we inhibit this interaction?
- Peptide representing aa 2-22 of the CCR5 N-terminus.
- Chemical sulfation of tyrosine residues at position 10, 14, and 15.
Sulfation at three residues critical for peptide binding to gp120

- Single sulfated variants display little binding to soluble gp120
- Sulfation required at tyrosine 10, 14 and 15 for maximal peptide binding to gp120

Sulfated mimetic of CCR5 N-terminus inhibits MVC-resistant strains

- Sulfated CCR5 N-terminus mimetic displays minimal activity in the absence of MVC
- In the presence of MVC, peptide is capable of inhibiting entry of all MVC-resistant strains tested

Conclusions

- MVC-resistant strains escape MVC by binding to CCR5 N-terminus – common to all resistant Envs studied to date
- A sulfated peptide mimic of the CCR5 N-terminus can block this interaction
- MVC-resistant Envs display little or no cross-resistance to other CCR5 antagonists
- Efficient MVC/CCR5 use by resistant strains can lead to increased infection in CD4+ central memory T cells
- Weak MVC/CCR5 use by resistant strains can lead to attenuation of macrophage infectivity
- Baseline resistance to MVC can be detected when using CCR5<sup>high</sup> cells – can we predict the capacity of virus to evolve resistance?

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