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

Michael Roche

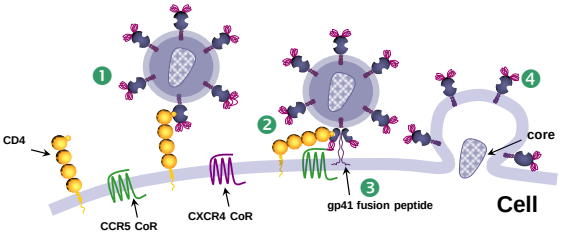
ASHM 18th September 2015

THE UNIVERSITY OF MELBOURNE

Doherty Institute

HIV Entry

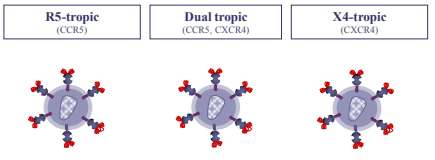
- HIV entry into target cells is mediated by the Envelope glycoprotein spikes found on the surface of the virus



Courtesy J.Sterjovski

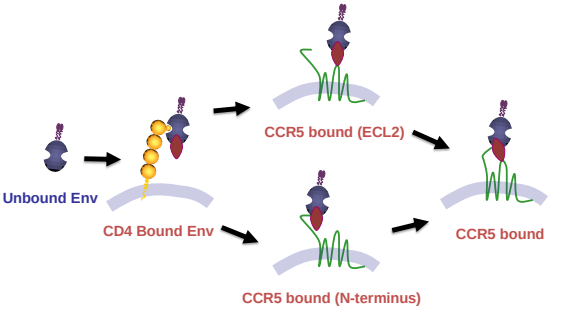
HIV Tropism

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



Courtesy J.Sterjovski

HIV and CCR5

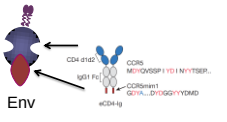


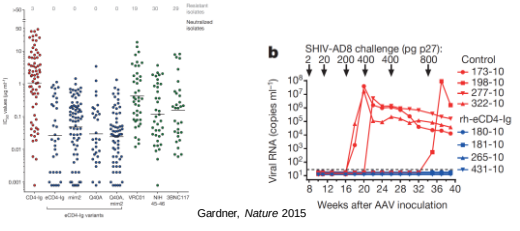
Inhibition of R5 HIV

- 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 5P12-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 then bNAB
- Protective in rhesus macaques against SHIV challenge





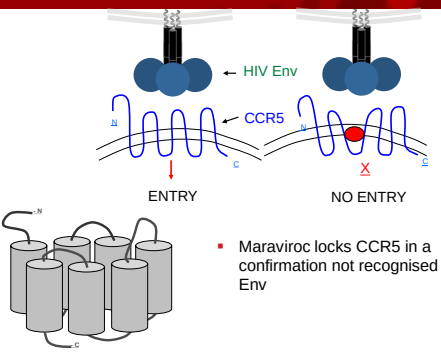
Gardner, *Nature* 2015

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

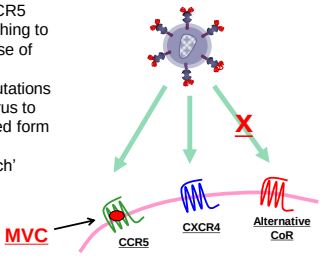
How Maraviroc Works



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

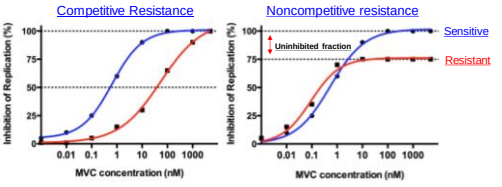
HIV becomes resistant to Maraviroc

- *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 then changes in IC_{50}
- MPI is a marker for resistance
- Non-competitive mechanism of resistance
- Resistant strains can use MVC-occupied *and* free CCR5



Questions to answer

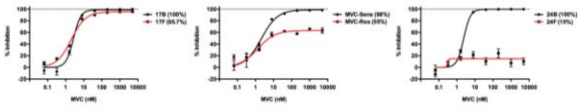
- What determines the MPI?
- How do MVC-resistant viruses recognise and bind to the MVC-occupied receptor?
- What are the consequences of MVC-resistance? Specifically;
  - Are MVC-resistant viruses cross resistant to other entry inhibitors?
  - Do MVC-resistant viruses have changes in their tropism for 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

Env	MVC resistance	Description
MVC-Sens	-	Generated from CC1/85 isolate in an *in vitro* cell culture passaging experiment
MVC-Res	+	
17Sens	-	Pre-treatment and post failure samples from two patients enrolled in MVC clinical trial
17Res	+	
24Sens	-	
24Res	+	

MVC Sensitivity varies amongst resistant strains

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



17Res 'Weak' | MVC-Res 'Moderate' | 24Res 'Strong'

Roche, *Retrovirology* 2013

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 (10 20 30)
MVC-Sens	CTRPNNNTRKSIHIG PGRAFYATGDIIGDIRQAHC
MVC-Res	T V
17Sens	CTRPGNNTRKSIHMG PGSSIYATGAIIGDIRQAHC
17Res	F DV
24Sens	CTRPNNNTRKSIPIG-PGRAFYATGDIIGDIRQAHC
24Res	S A

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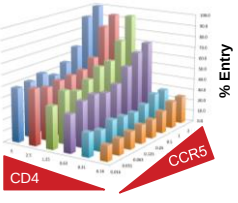
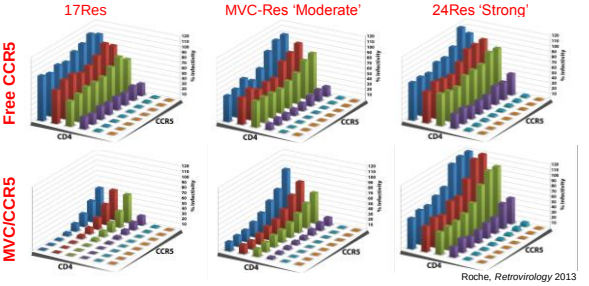
What determines the MPI?

- 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

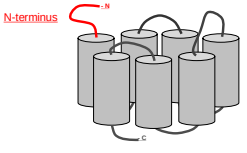
- Only strongly resistant 24Res is unaffected by changes in CCR5 expression in the presence of MVC

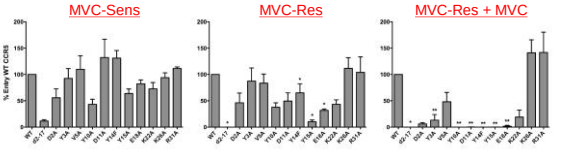


Roche, *Retrovirology* 2013

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

- When forced to use the MVC-occupied receptor, the MVC-res Env becomes critically reliant on the N-terminus
- Represents a shift to a region of CCR5 not modified by MVC





Roche, *J Virol* 2011

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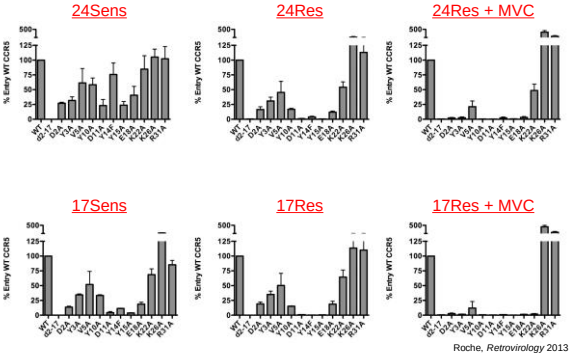
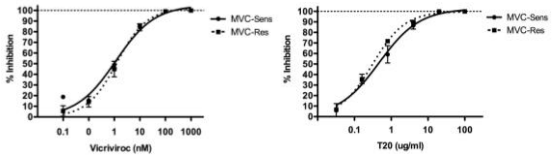


Roche, *Retrovirology* 2013

Does MVC resistance lead to cross resistance?

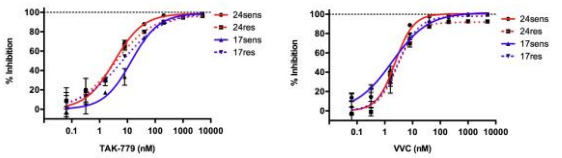
- For moderately resistant MVC-Res
  - No reduction in MPI to VCV
  - No increase in T-20 IC_{50}



Roche, *J Virol* 2011

Does MVC resistance lead to cross resistance?

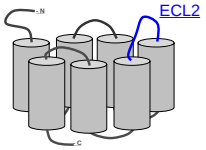
- Both weakly and strongly MVC resistant Envs retain sensitivity to TAK-779
- Strongly MVC resistant Env displays weak cross-resistance to VCV
- Cross resistance does not appear to occur with MVC resistance

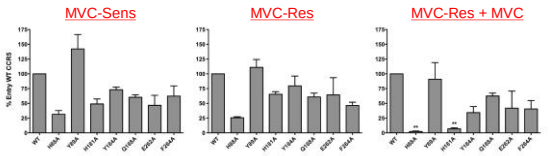


Roche, *Retrovirology* 2013

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





Roche, *J Virol* 2011

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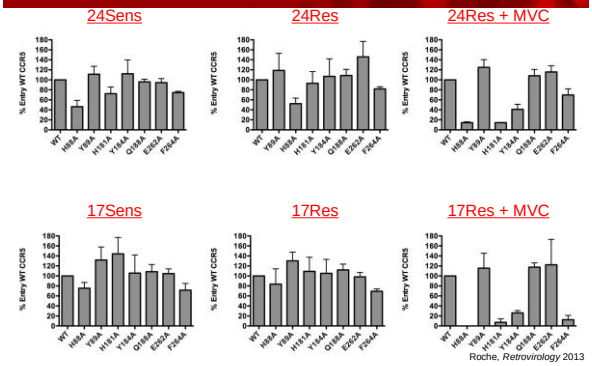
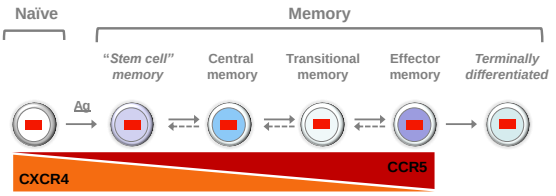


Roche, *Retrovirology* 2013

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

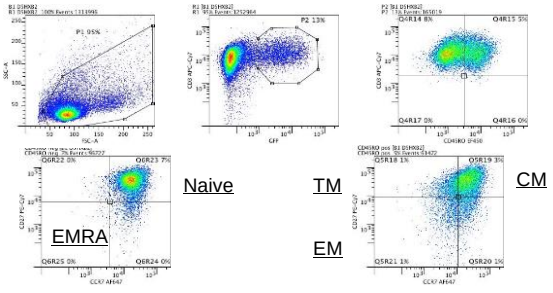


- Coreceptor expression varies amongst CD4+ T cell subsets
- Do changes in CCR5 affinity change infection of different subsets?

Bleul et al. PNAS 1997, Lee et al. PNAS 1999, Gorry et al. Curr HIV/AIDS rep 2011

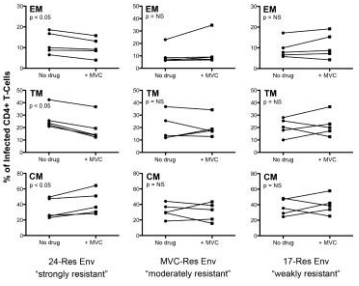
Do MVC-resistant Envs have altered T-cell tropism?

- 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

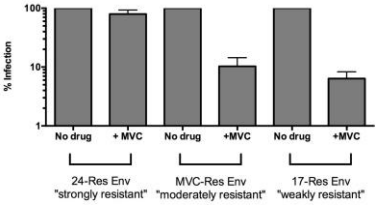
- In the presence of MVC, strongly resistant 24-Res Env has a shift in T-cell tropism towards increased infection of central memory cells and reduced infection of effector memory and transitional memory cells



Flynn, *Virology* 2013

MVC-resistant Envs have attenuated M-tropism

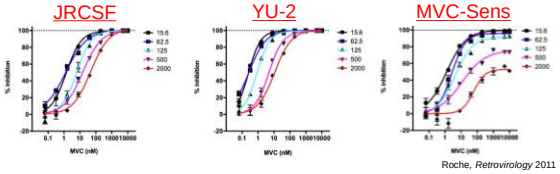
- Matched MVC-sensitive and MVC-resistant Envs display similar levels of Macrophage entry
- The presence of MVC attenuates or abolishes entry by moderately or weakly MVC-resistant Envs
- An altered interaction with CCR5 appears important for Macrophage tropism



Flynn, *Virology* 2013

Are some viruses pre-triggered to escape MVC?

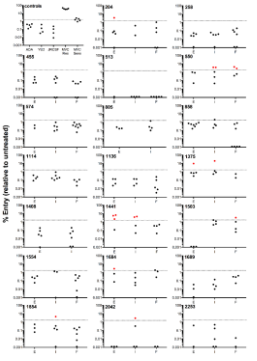
- The CC1/85 isolate is unique in its ability to evolve CCR5 antagonist resistance *in vitro* relatively easily
- The MVC-sens Env is sensitive to MVC in most assays
- When the CCR5 levels are increased a partial level of resistance is observed
- Perhaps this explains why this isolate can evolve resistance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



Roche, *Retrovirology* 2011

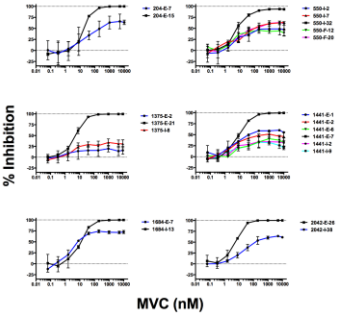
Baseline MVC-resistance in a therapy naïve subtype C cohort

- 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



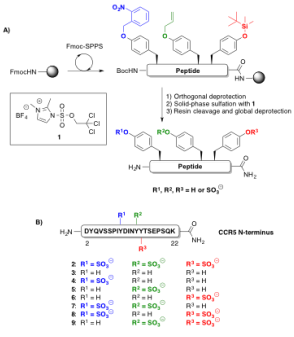
Baseline MVC-resistance in a therapy naïve clade C cohort

- Varying MPIs observed for selected clade C Envs when infecting CCR5^{high} cells
- Are these Envs more likely to evolve genuine resistance to MVC?
- Implications for MVC as a microbicide or PrEP



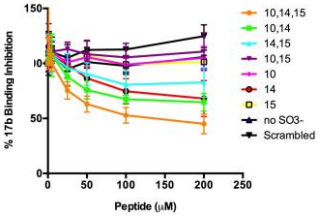
Inhibiting the MVC-resistant viruses

- 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

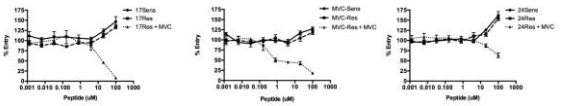
- Single sulfated variants display little binding to souble gp120
- Sulfation required at tyrosine 10, 14 and 15 for maximal peptide binding to gp120



Liu, *ACS Chem Biol* 2014

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^{high} cells – can we predict the capacity of virus to evolve resistance?

Acknowledgments

Burnet Institute
Paul Gorry
Melissa Churchill
Lachlan Gray
Hamid Salimi
Jacqueline Flynn
Renee White
Katharina Borm
Andy Poumbourious

Pfizer/ViiV
Mike Westby
Becky Jubb

University of Melbourne
Damian Purcell

University of Sydney
Richard Payne
Johnny Lui

Peter Doherty Institute
Sharon Lewin

Australian Government
National Health and Medical Research Council

Burnet Institute
Medical Research. Practical Action.

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