Resistance to CCR5mAb RoAb3952 is Associated with a Shift in Binding from the Extracellular Loop 2 to the N-Terminus Domain of CCR5

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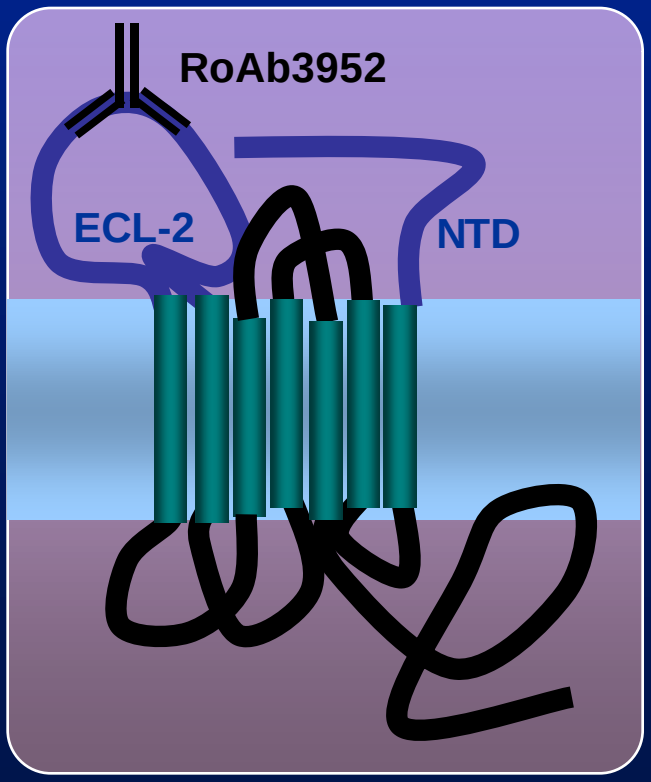
A Jekle, M Chhabra, E Chow, S Meier, A Lochner, S Sankuratri, M Brandt, N Cammack and G Heilek



XVII International HIV Drug Resistance Workshop
Sitges, Spain
June 10-14, 2008

Discovery of two mouse anti-CCR5mAbs

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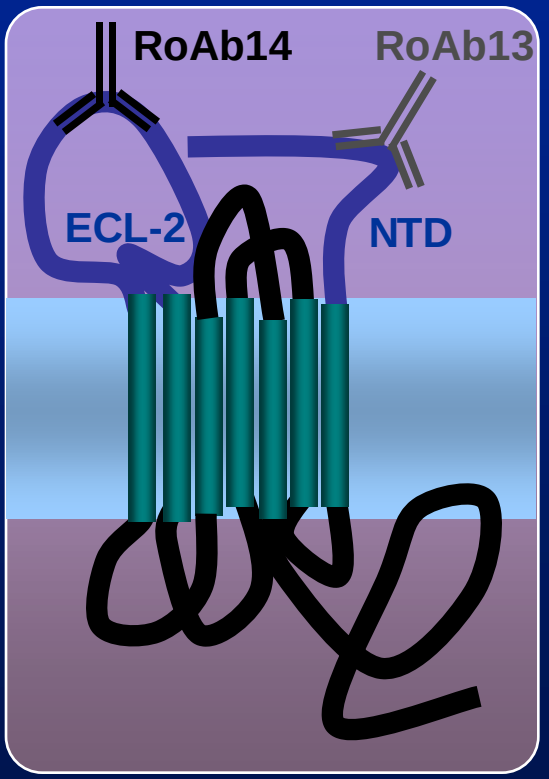
	RoAb13	RoAb14
Type	mouse	mouse
Epitope	N-terminal domain	Extracellular loop 2
IC_{50} [µg/ml] PBMC assay JRCSF	0.03 ± 0.03	0.03 ± 0.02



Ji C. et al 2007, Antiviral Res. 74: 125-137

Development of anti-CCR5Mab RoAb3952

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	RoAb13	RoAb14	RoAb3952
Type	mouse	mouse	Human/ de-immunized
Epitope	N-terminal domain	Extracellular loop 2	Extracellular loop 2
IC_{50} [µg/ml] PBMC assay JRCSF	0.03 ± 0.03	0.03 ± 0.02	0.06 ± 0.03

RoAb3952 inhibits 30 HIV strains from different clades with average IC_{50} of 0.31 µg/ml.

In-vitro passaging with anti-CCR5Mab RoAb3952

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Experimental Details:

- CD8-depleted human PBMC
- High-titer, genetically diverse virus strains
- virus strains with high RoAb3952 IC_{50}

PBMC assay	IC_{50} [µg/ml]
301567	0.07
Bal	0.26
CC1/85	0.18
NLBal	0.06
W969-7	0.07
Yu2.c	0.05

Selection of RoAb3952-resistant virus strains

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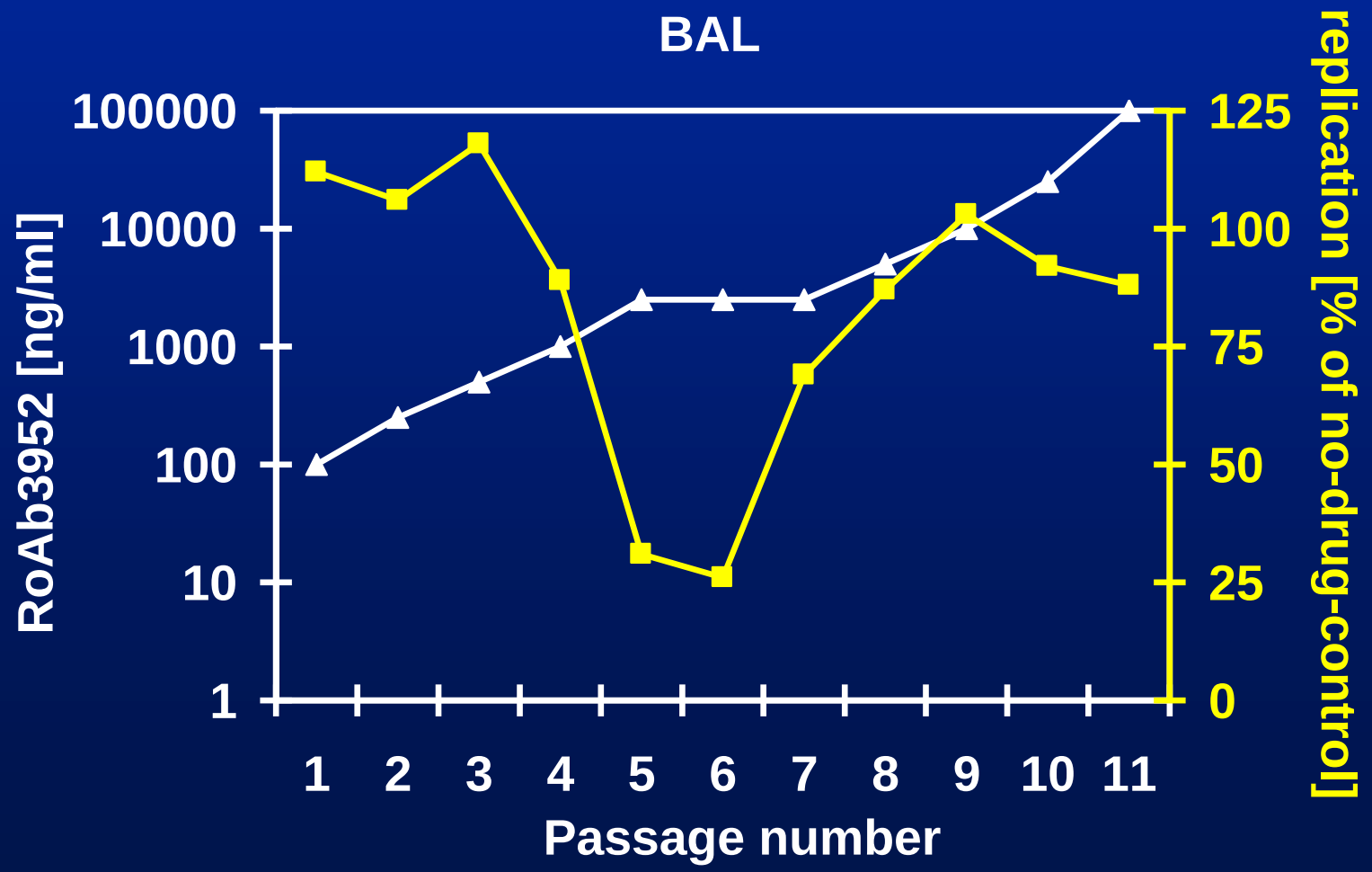

Selection of RoAb3952-resistant virus strains

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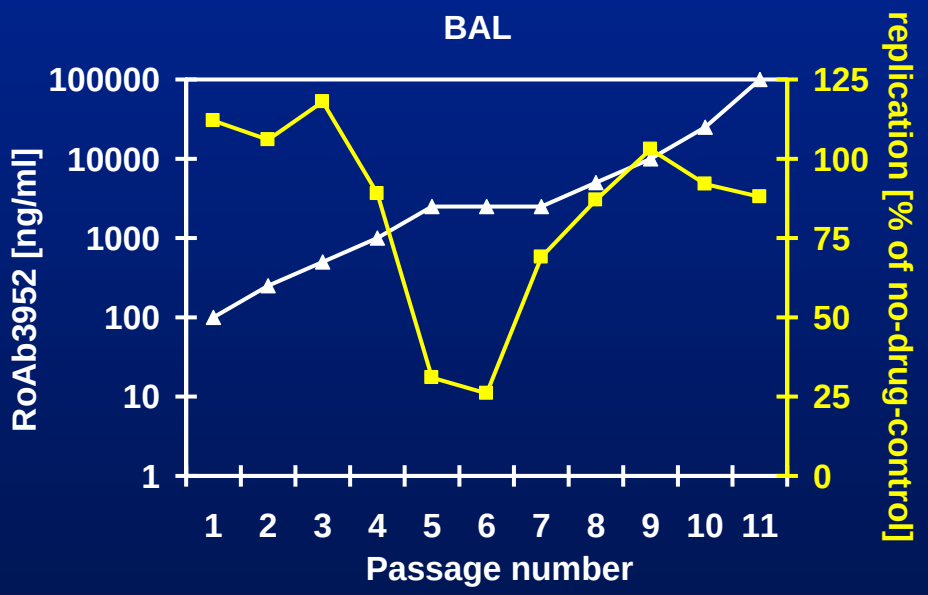

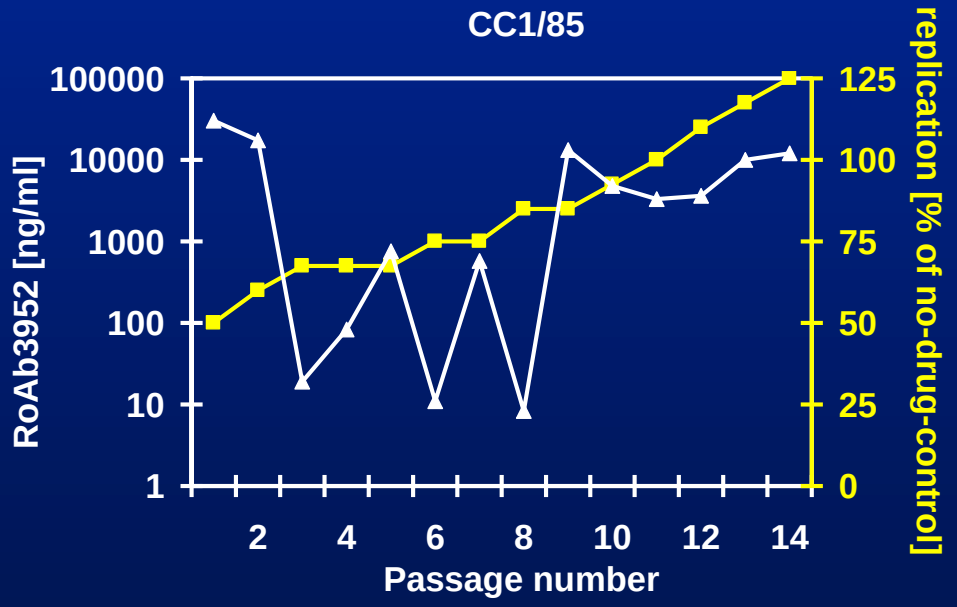

Bal_3952res can not be inhibited by RoAb3952 in the PBMC assay

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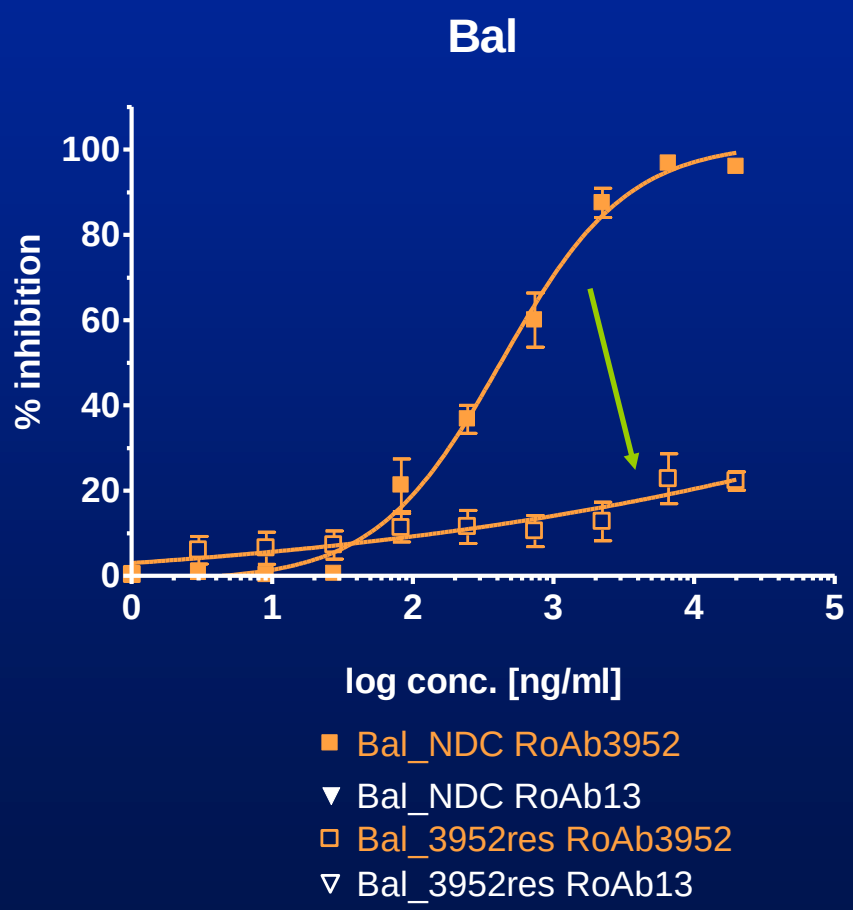

Increased Sensitivity of RoAb3952-resistant Bal virus to RoAb13

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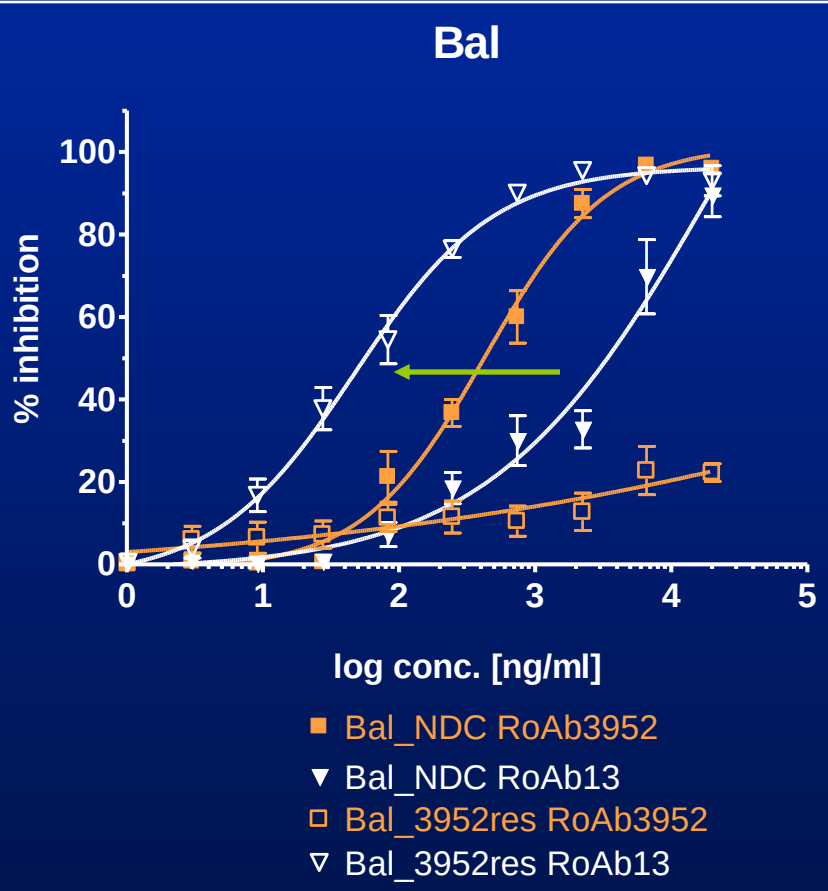

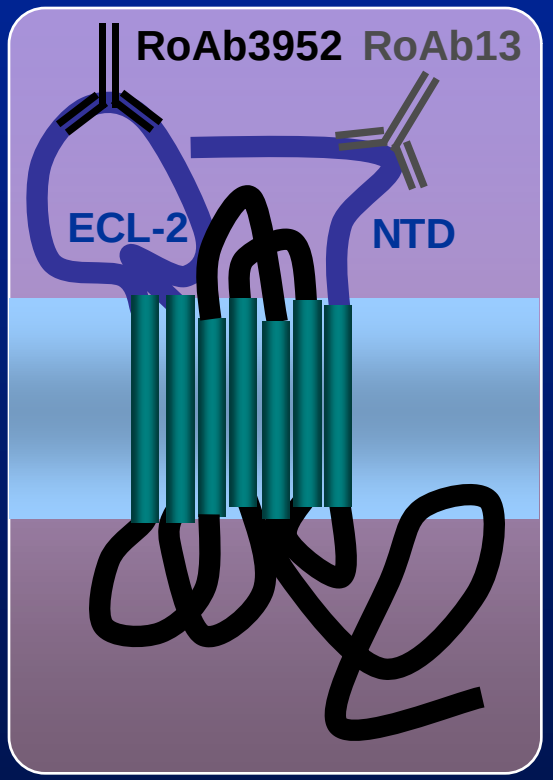

Increased Sensitivity of RoAb3952-resistant virus strains to RoAb13

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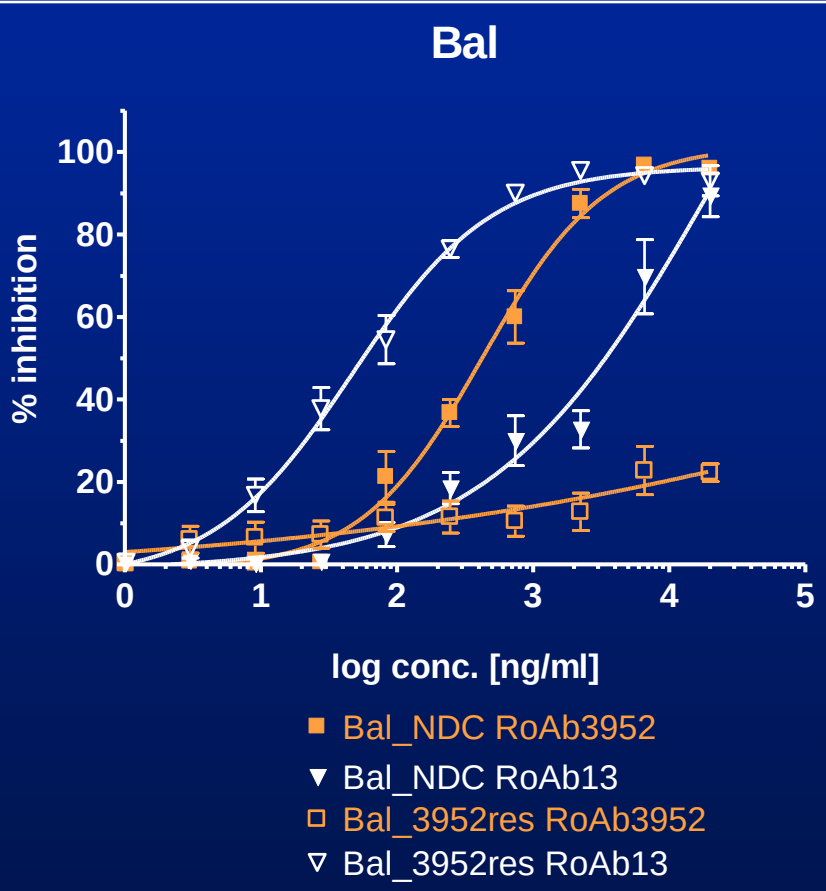
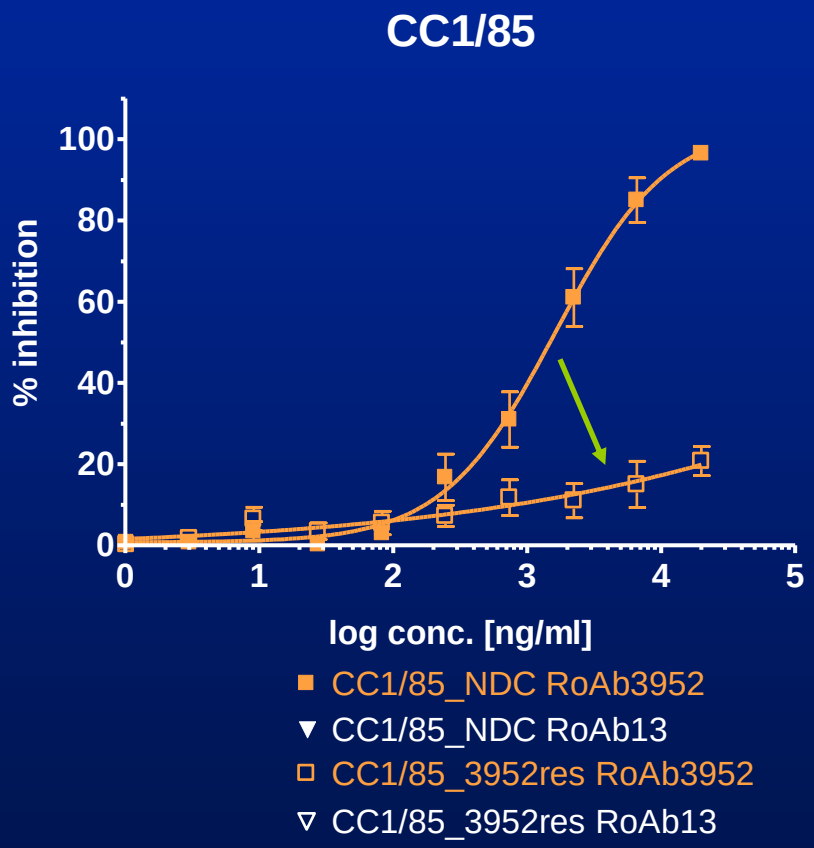
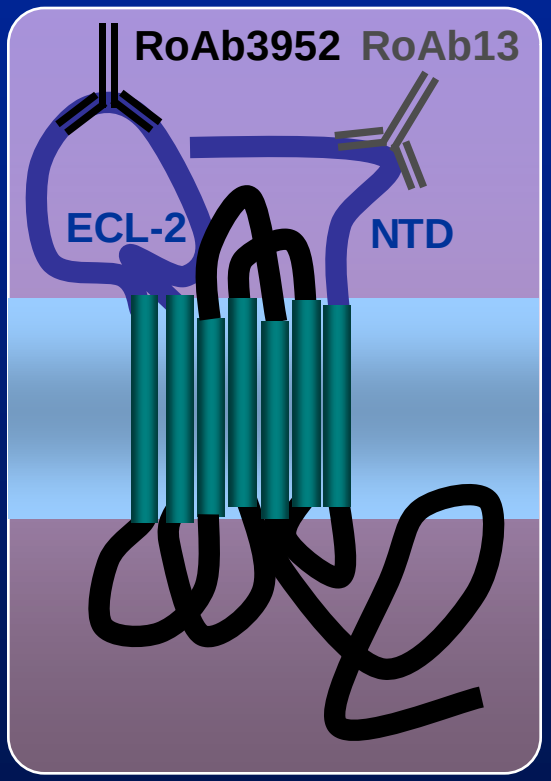
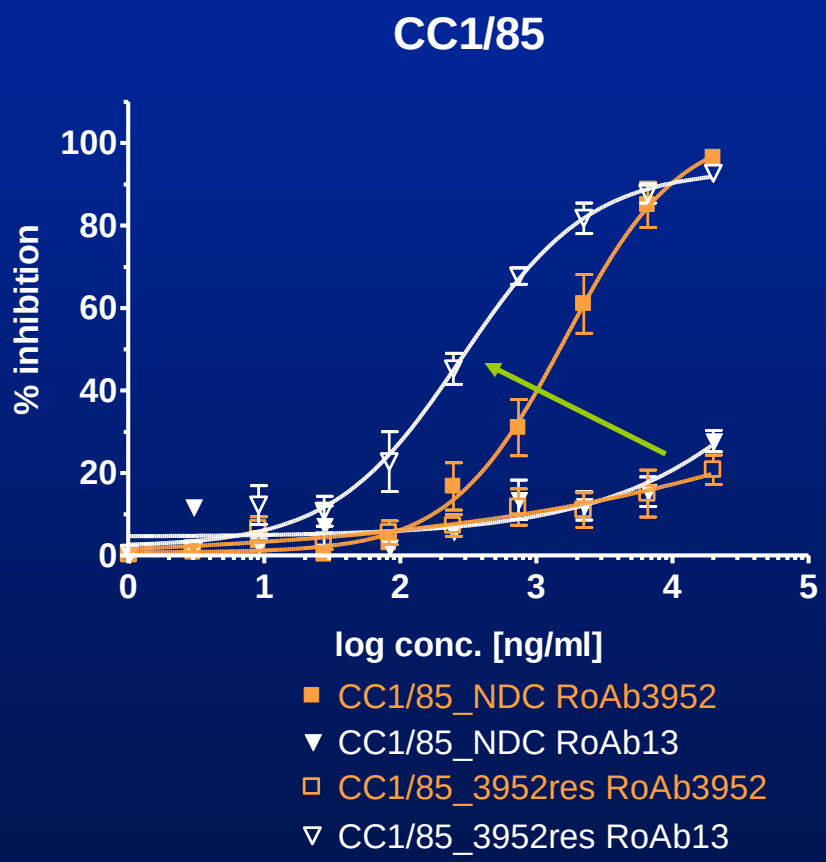




Increased Sensitivity of RoAb3952-resistant virus strains to RoAb13





Increased sensitivity of the RoAb3952-resistant virus strains to RoAb13 suggests a change in binding of the virus from the ECL-2 to the NTD of CCR5.

RoAb3952-resistance is associated with multiple mutations throughout gp160

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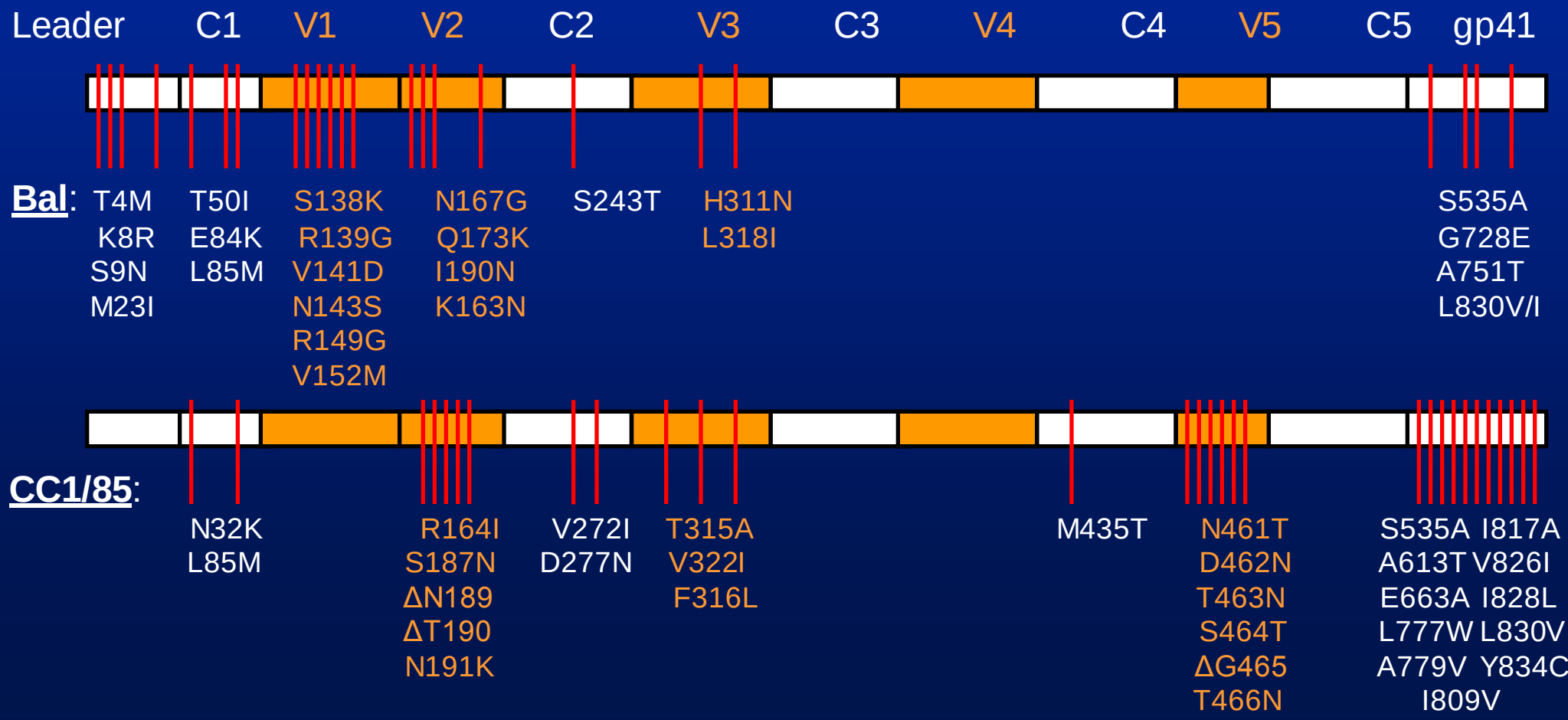

RoAb3952-resistance is associated with multiple mutations throughout gp160

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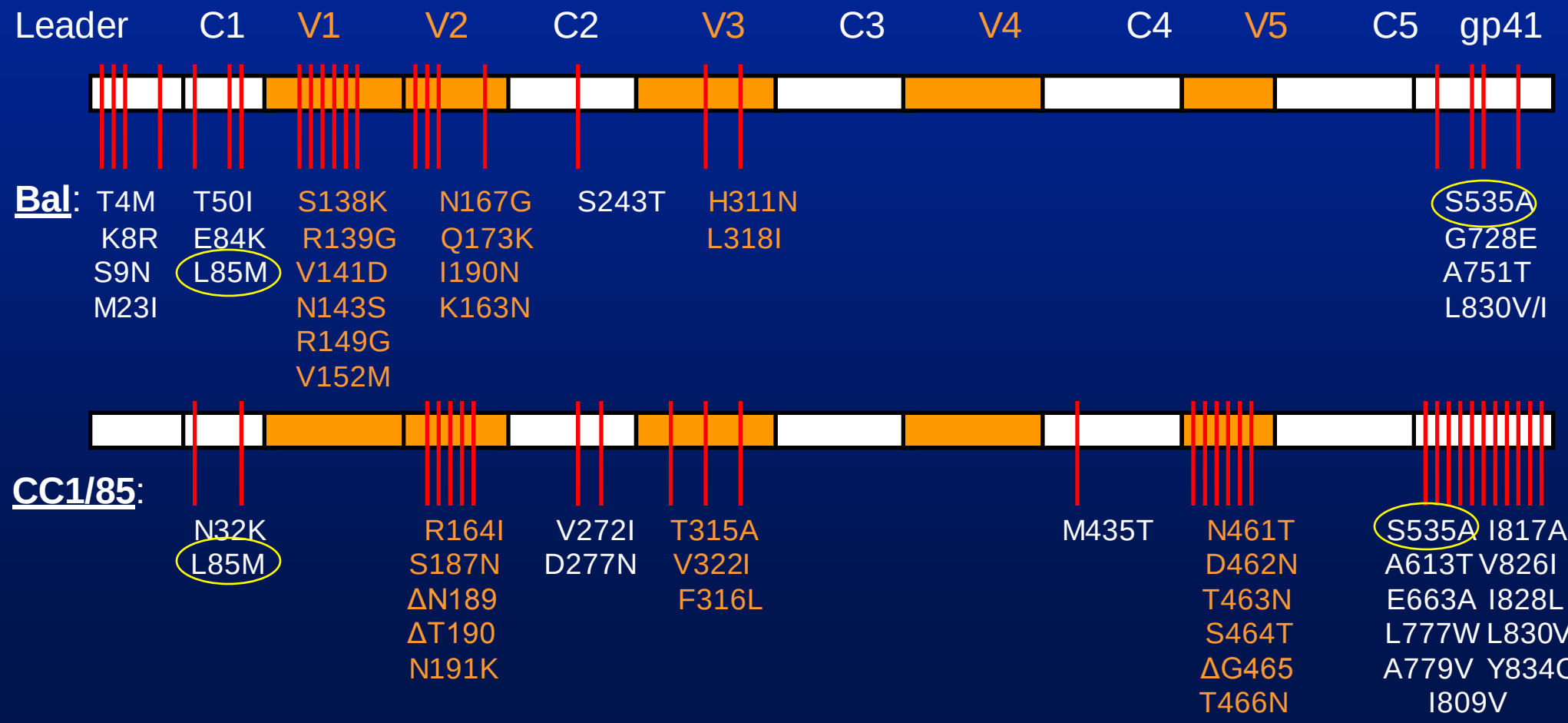

RoAb3952-resistance is associated with multiple mutations throughout gp160

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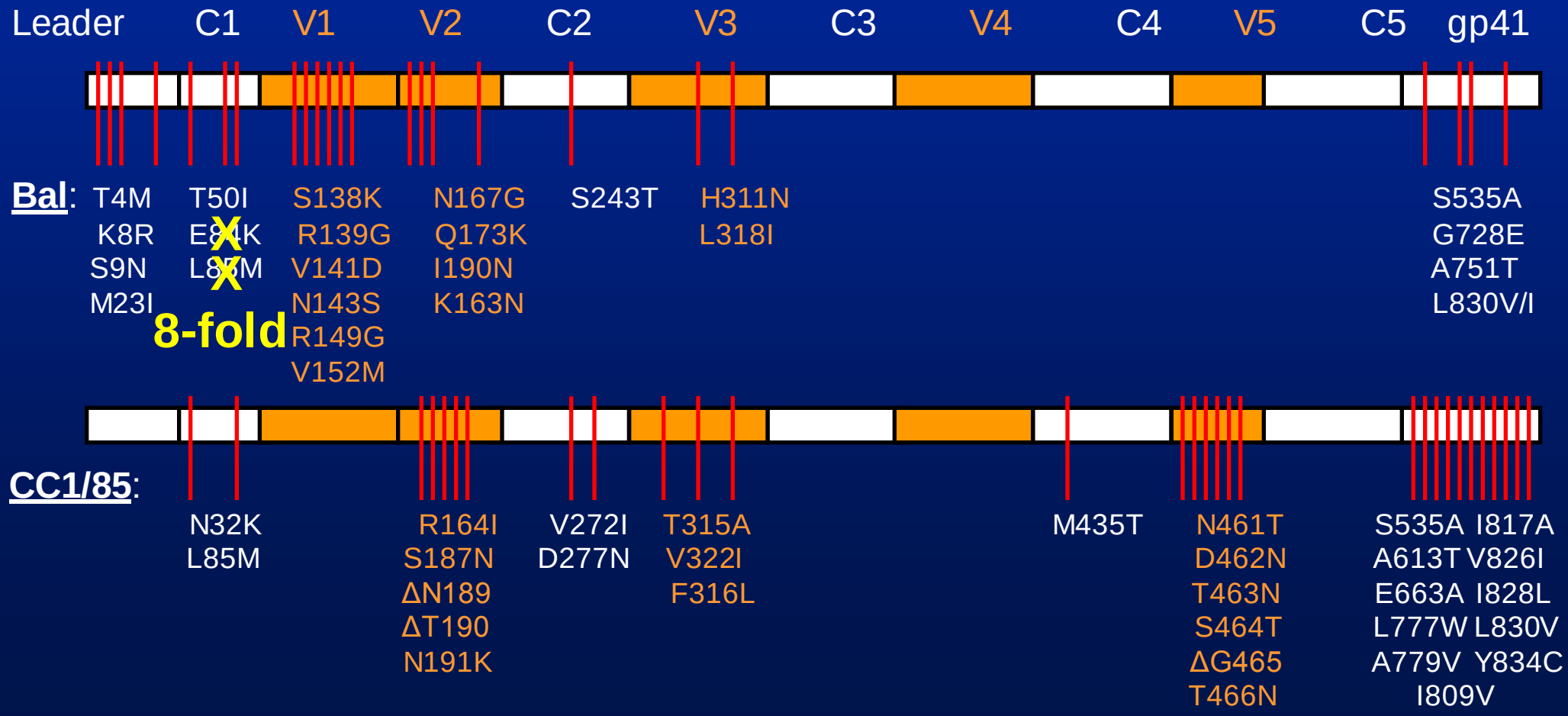

RoAb3952-resistance is associated with multiple mutations throughout gp160

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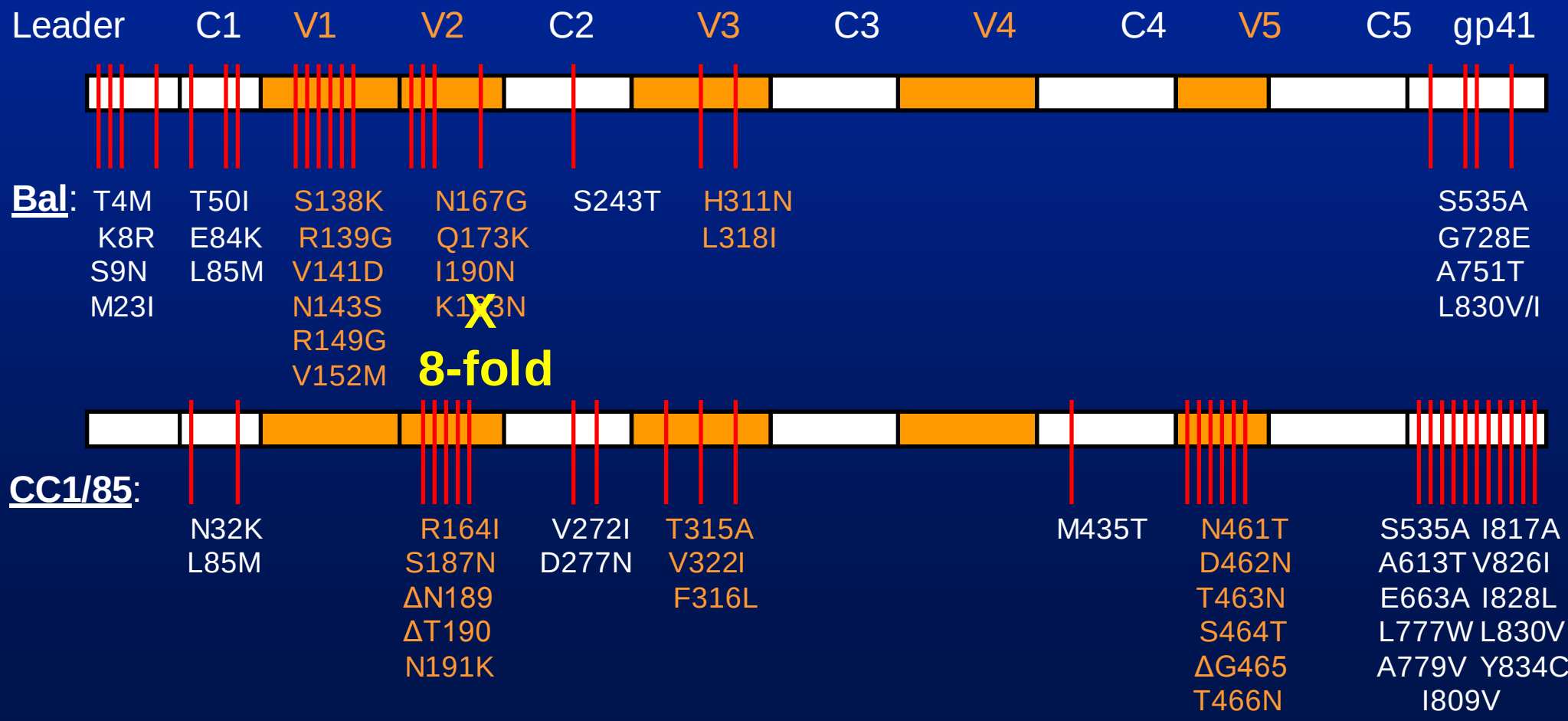

RoAb3952-resistance is associated with multiple mutations throughout gp160

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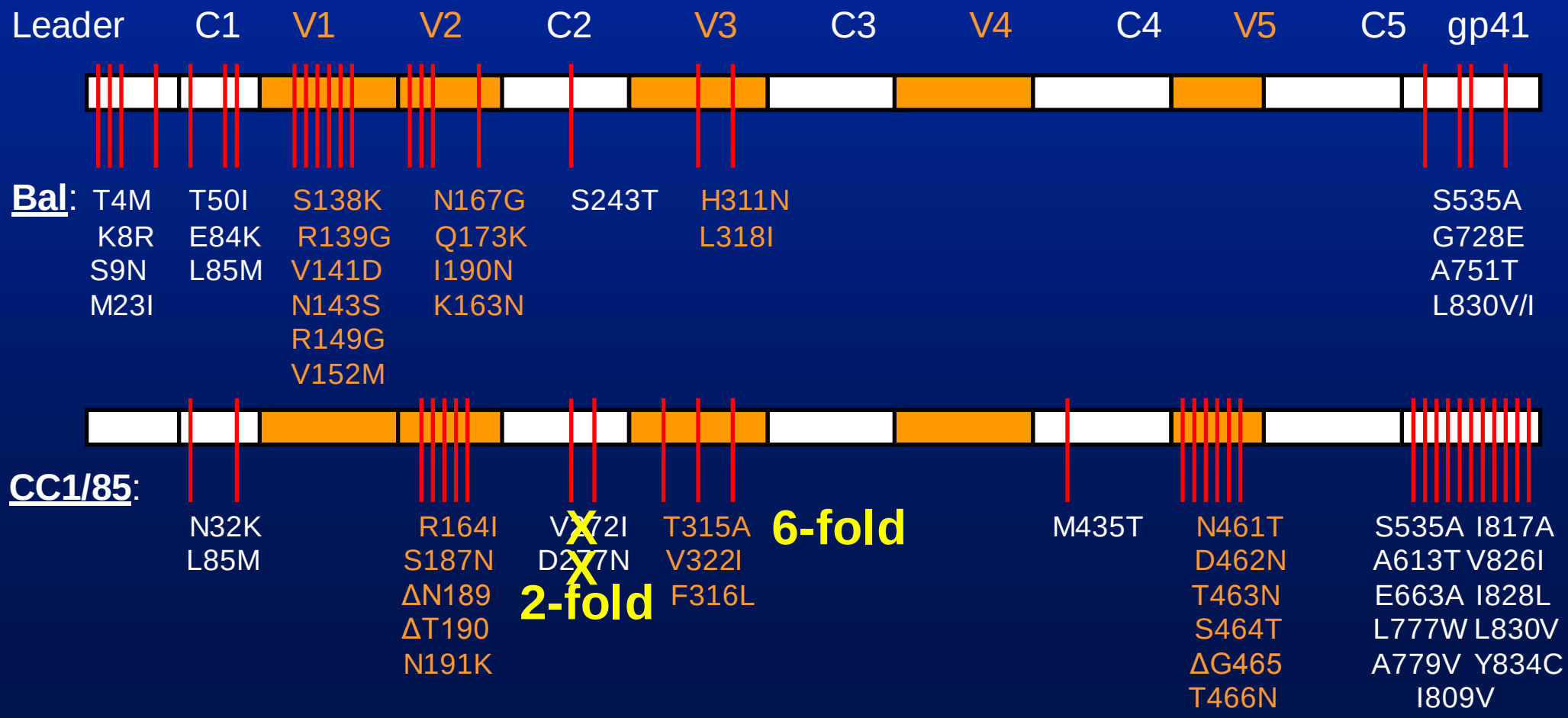

Multiple mutations in gp160 contribute to RoAb3952-resistance:

Clone-dependent phenotype of S535A mutation

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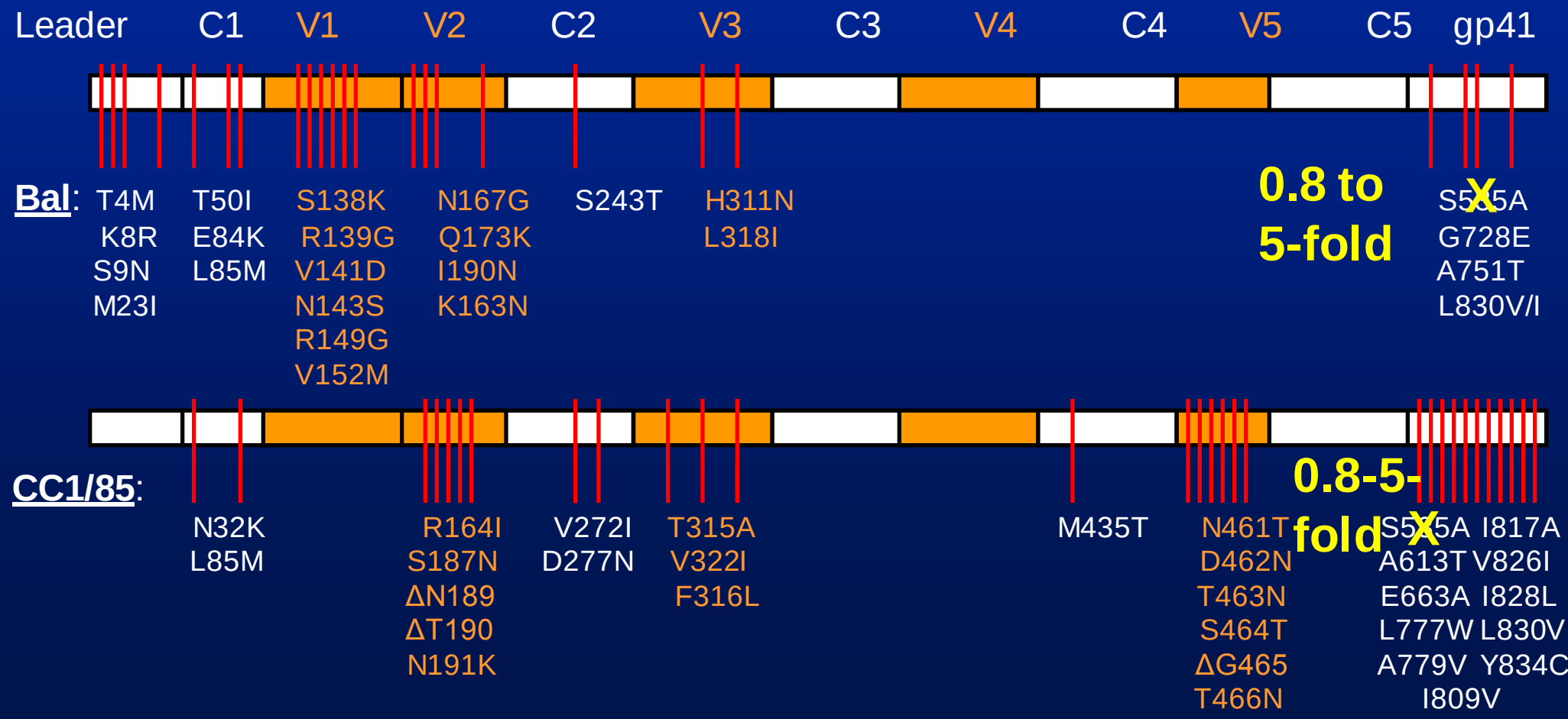

Summary

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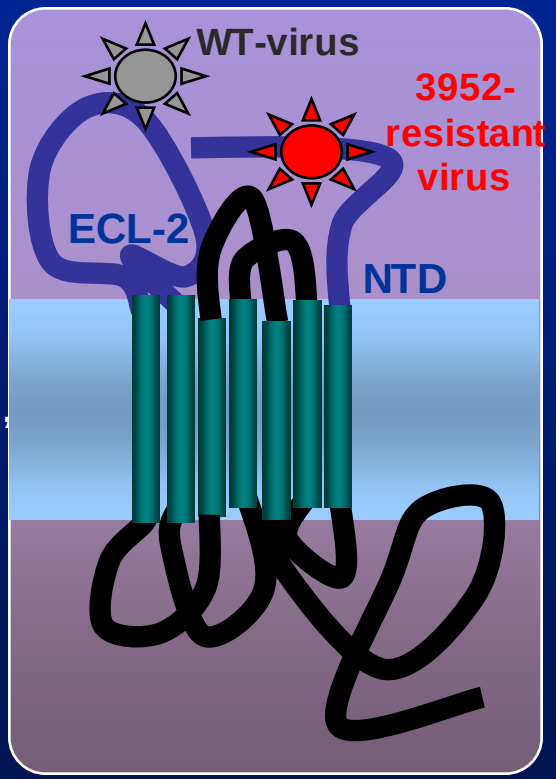
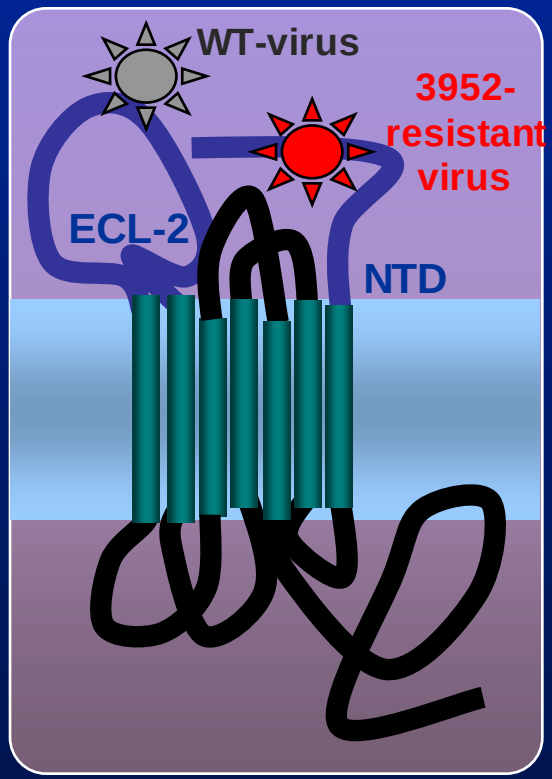
- Using high titer, genetically diverse primary HIV strains and CD8-depleted human PBMC, it is possible to select *in vitro* virus variants resistant to the CCR5mAb RoAb3952.
- RoAb3952-resistant HIV variants are still R5-tropic.
- RoAb3952-resistance is associated with mutations throughout gp160.
- RoAb3952-resistant virus strains are more sensitive to RoAb13, suggesting a shift of binding from ECL-2 to NTD of CCR5.
- RoAb3952-resistant virus strains are cross-resistant to 2D7.



Acknowledgements

- Roche Palo Alto:
  - Milloni Chhabra
  - Sonja Meier
  - Eugene Chow
  - Adriane Lochner
  - Gabrielle Heilek
  - Surya Sankuratri
  - Nick Cammack
- Roche Penzberg:
  - Michael Brandt



Back-up slides

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RoAb3952-resistant envelope clones are cross-resistant to 2D7

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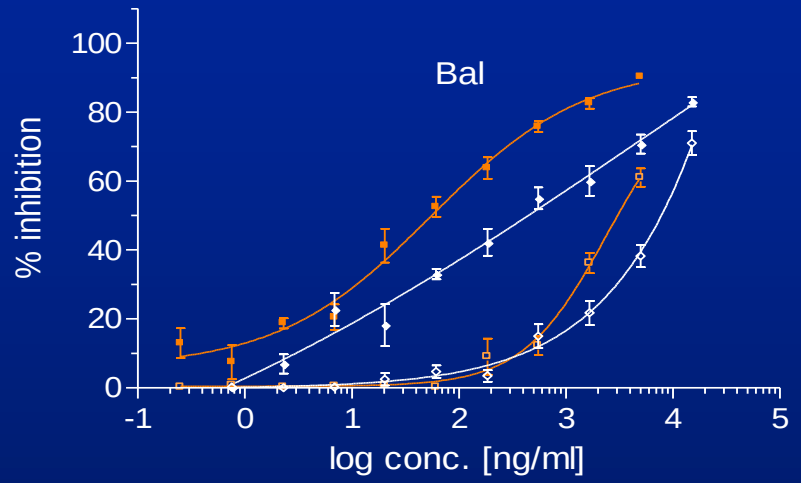
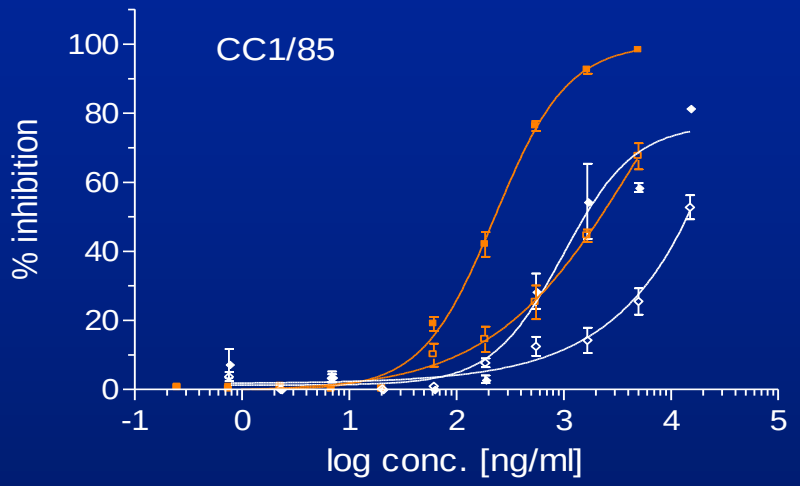


- Bal_NDC #18 RoAb3952
- Bal_NDC #18 2D7
- Bal_3952res #22 RoAb3952
- Bal_3952res #22 2D7



- CC1/85_NDC #103 RoAb3952
- CC1/85_NDC #103 2D7
- CC1/85_3952res #19 RoAb3952
- CC1/85_3952res #19 2D7

- RoAb3952-resistant clones are cross-resistant to 2D7 (CCR5mAb binding to ECL-2).
- RoAb13 is inactive in single cycle assay.