Combinatorial CRISPR/Cas9 approaches targeting different steps in the HIV life cycle can prevent the selection of resistance

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Current cART strategies

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- Current cART is designed to efficiently control HIV replication

- The current arsenal of antiretroviral compounds can neither
- suppress viral production nor target the viral reservoir

- Alternative strategies, such as gene editing, are required to permanently disrupt the HIV genome in the cellular reservoir

- Gene editing tool: RNA-guided CRISPR/Cas9 nuclease

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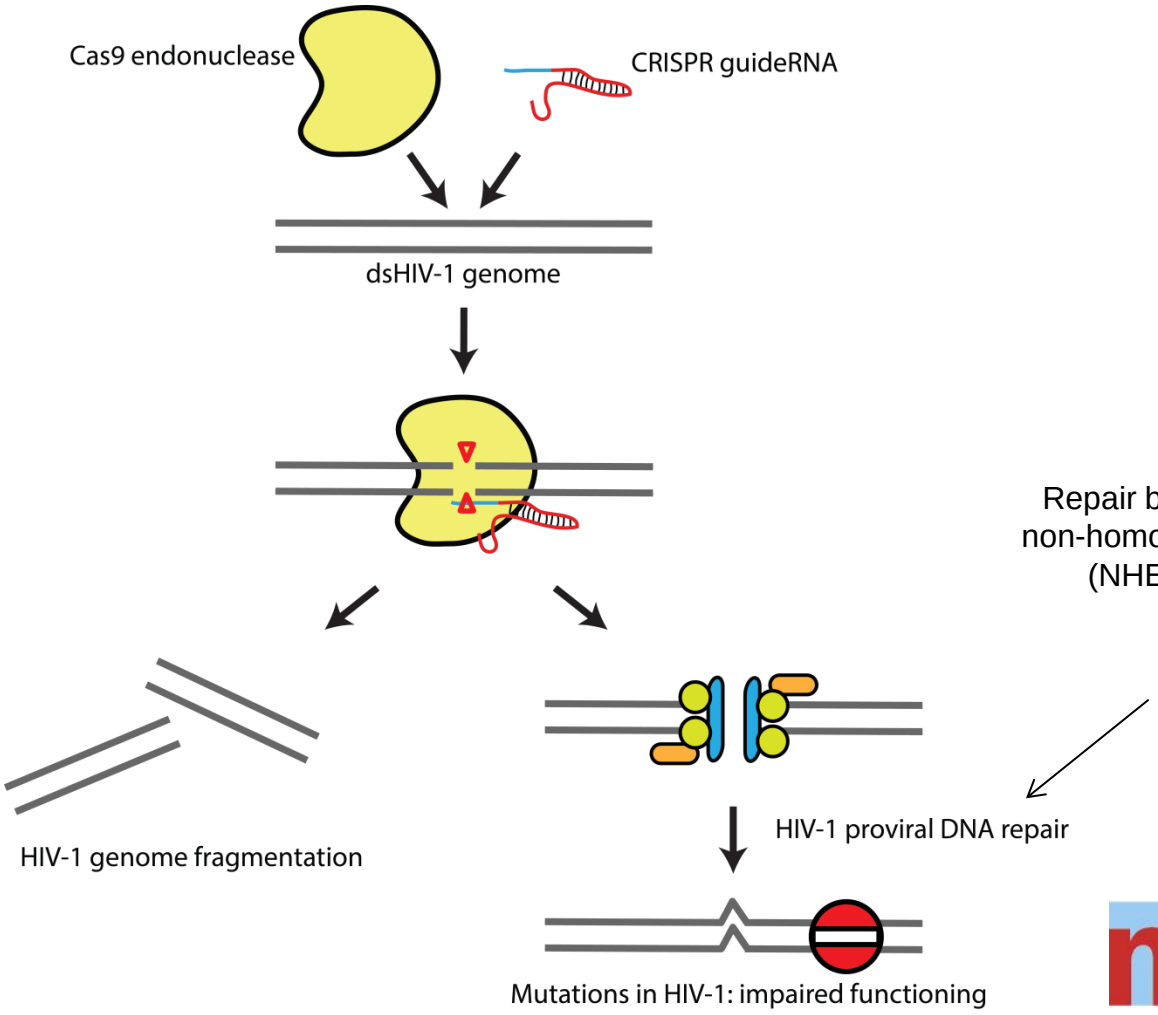
gRNA-guided CRISPR/Cas9 nuclease



Repair by the error-prone non-homologous end joining (NHEJ) machinery.

The effect of single gRNAs on viral replication and escape

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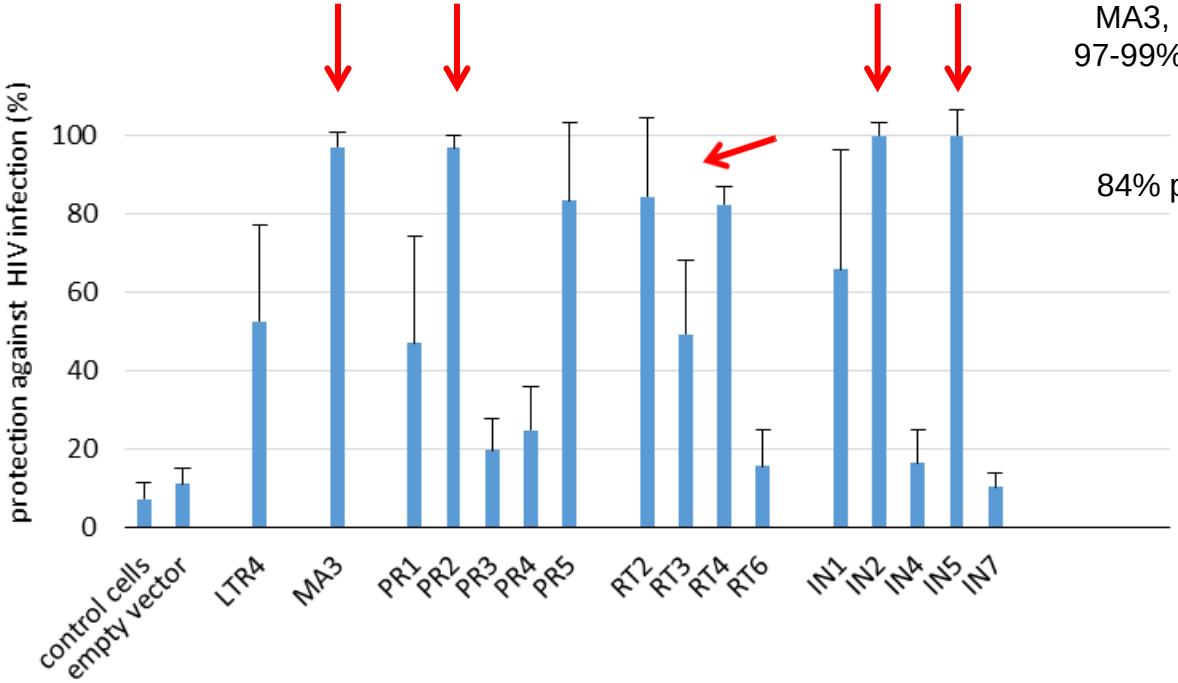
- Generated T-cells stably expressing single gRNAs and Cas9
- Infect with HIV and measure protection against infection (day 4)



MA3, PR2, IN2 and IN5 provide
97-99% protection against infection

RT2 provide
84% protection against infection

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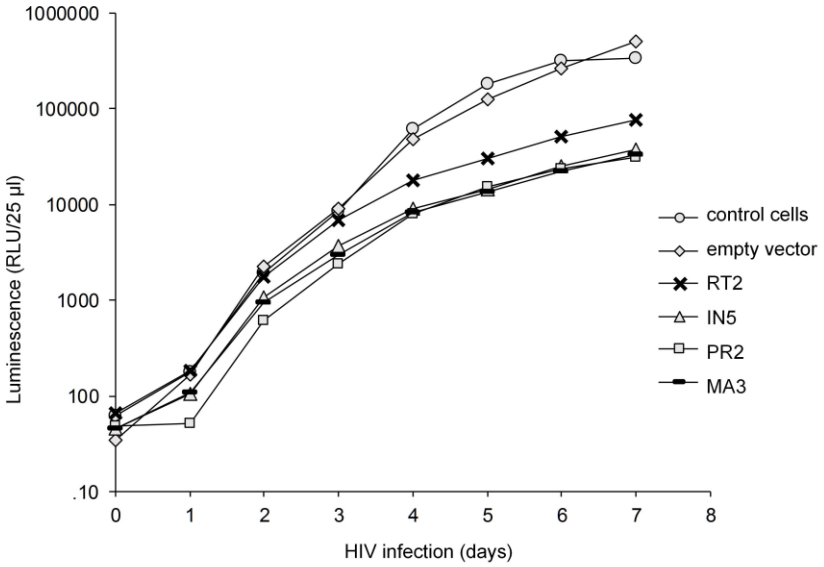
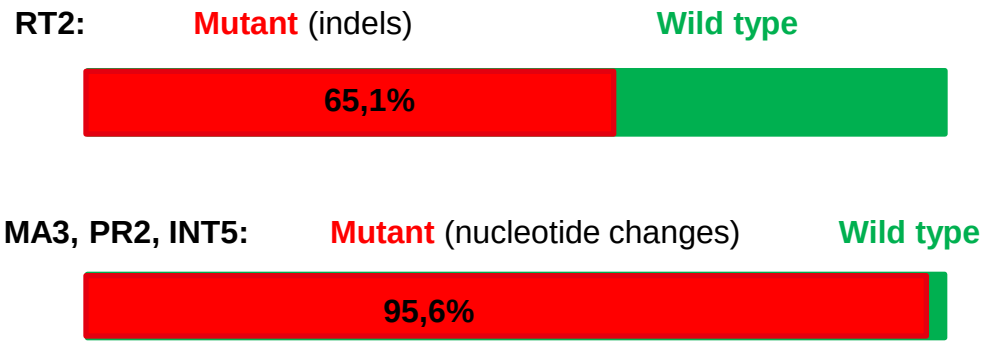
The effect of single gRNAs on viral replication and escape

- Generated T-cells stably expressing single gRNAs and Cas9





- Two recent studies suggest that NHEJ repair machinery may facilitate HIV escape[1,2]

[1]Callaway, Nature News, 2016; [2]Liang et al, Retrovirology, 2016.

The effect of single gRNAs on viral replication and escape

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Nucleotide substitution patterns in HIV escape variants

HIV target gene	PR	RT	IN	MA	Totaal
Samples (n)	14	21	14	18	67
target sequence	ATTAGT	AAGGAA	GTGCTA	GATCGA	
A-->T	4	5	0	1	10
A-->C	2	13	0	0	15
A-->G	10	10	7	10	37
T-->A	0	0*	5	5	10
T-->C	13	0*	5	13	31
T-->G	1	0*	5	18	24
C-->A	0*	0*	1	1	2
C-->T	0*	0*	1	1	2
C-->G	0*	0*	11	0	11
G-->A	2	1	1	1	5
G-->T	1	2	3	1	7
G-->C	1	15	1	1	18
	34	46	40	52	172

* Nucleotide is not present in target site

Accelerating viral escape

The cellular error-prone non-homologous end joining (NHEJ) machinery

HIV-RT and APOBEC3G

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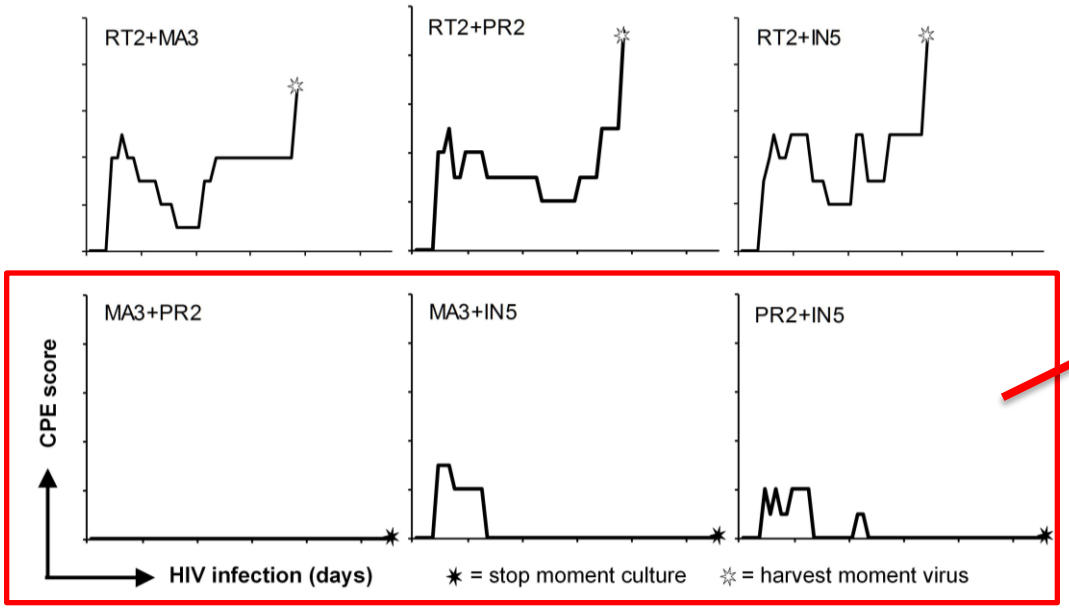
The effect of two gRNAs on viral replication and escape

- Generated T-cells stably expressing two gRNAs and Cas9
- Long-term infection with HIV reporter virus (luciferase) (n=4)



combination of gRNAs	Viral breakthrough (MOI 0.003)				Viral breakthrough (MOI 0.006)			
RT2+MA3	23	34*	38*	-	38*	13	48*	30
RT2+PR2	-	-			38*	-		
RT2+IN5	-	-	-	23	55*	30*	34*	21*
MA3+PR2	-	-			-	-		
MA3+IN5	-	-	-	-	-	-	-	-
PR2+IN5	-	-			-	-		

- = no viral breakthrough

* = used for deep-sequence analysis of the CRISPR target site

Concluding remarks

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- gRNAs differ in potency to inhibit HIV replication
- Rapid viral escape is observed to all single gRNAs
- Error-prone NHEJ repair mechanism is accelerating viral escape
- A combination of two potent gRNAs can successfully prevent viral replication and escape

Gene-editing may provide a future alternative for control of HIV-infection.

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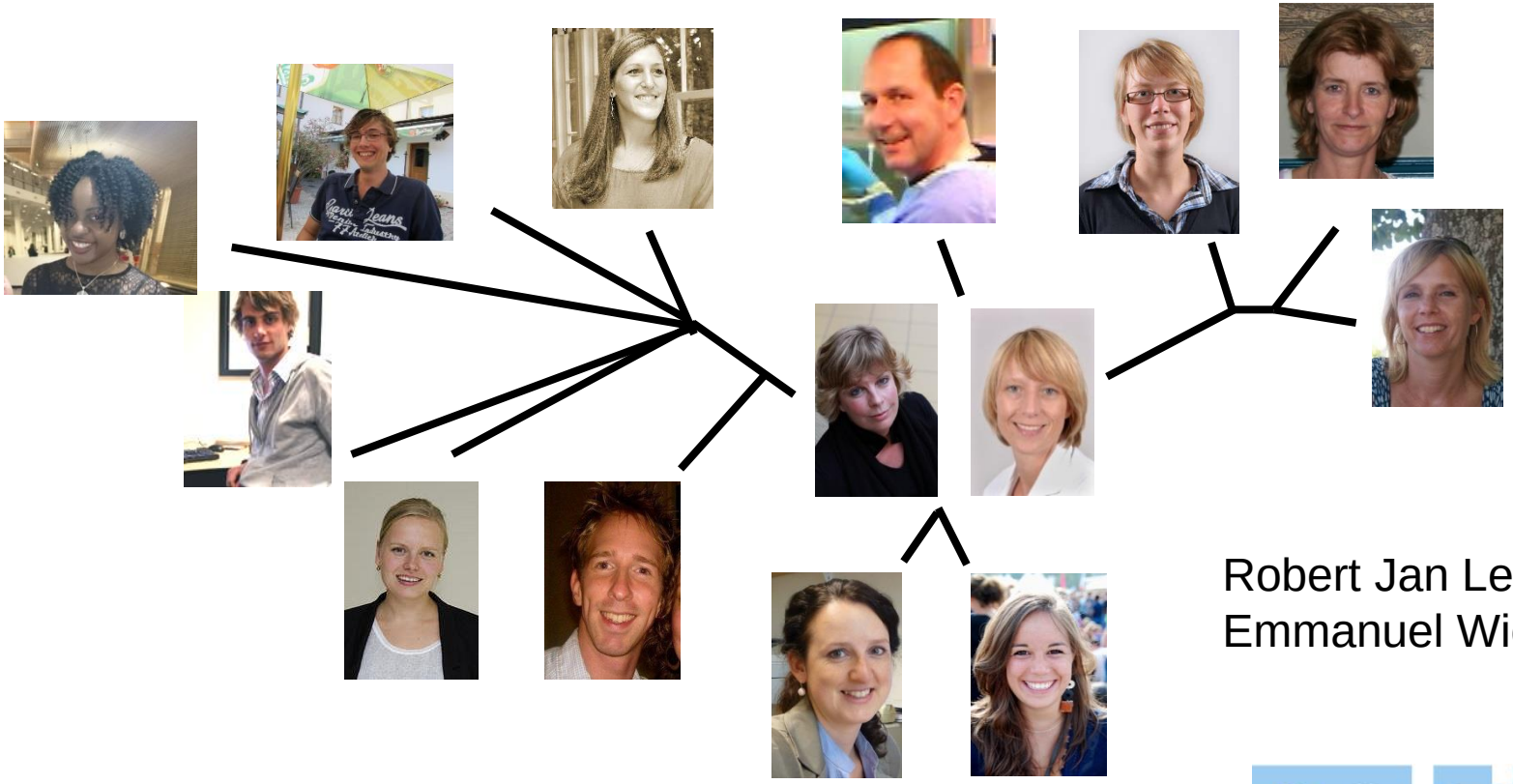
Acknowledgment



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Disclosure of speaker's interests

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No conflict of interest

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