Breakthrough of preexisting X4-tropic HIV after allogeneic stem-cell transplantation

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Background

HIV infection can effectively be treated in many HIV positive individuals.

But, sterilizing cure has only been achieved once (“Berlin patient“).

Several other attempts to replicate this success have failed.

This is the report of the “Essen patient” and what went wrong.

The “Essen patient“

1) HIV-positive (FD 2006)
   ART (2009-2010)

2) Self initated treatment interruption
   (2010-2011)

3) HIV progression,
   FD: T-cell lymphoma

4) Re-start of ART
   and chemotherapy

5) Slow decline of HIV RNA (R5-tropic)
   progression of the T-cell lymphoma

   plan for alloSCT

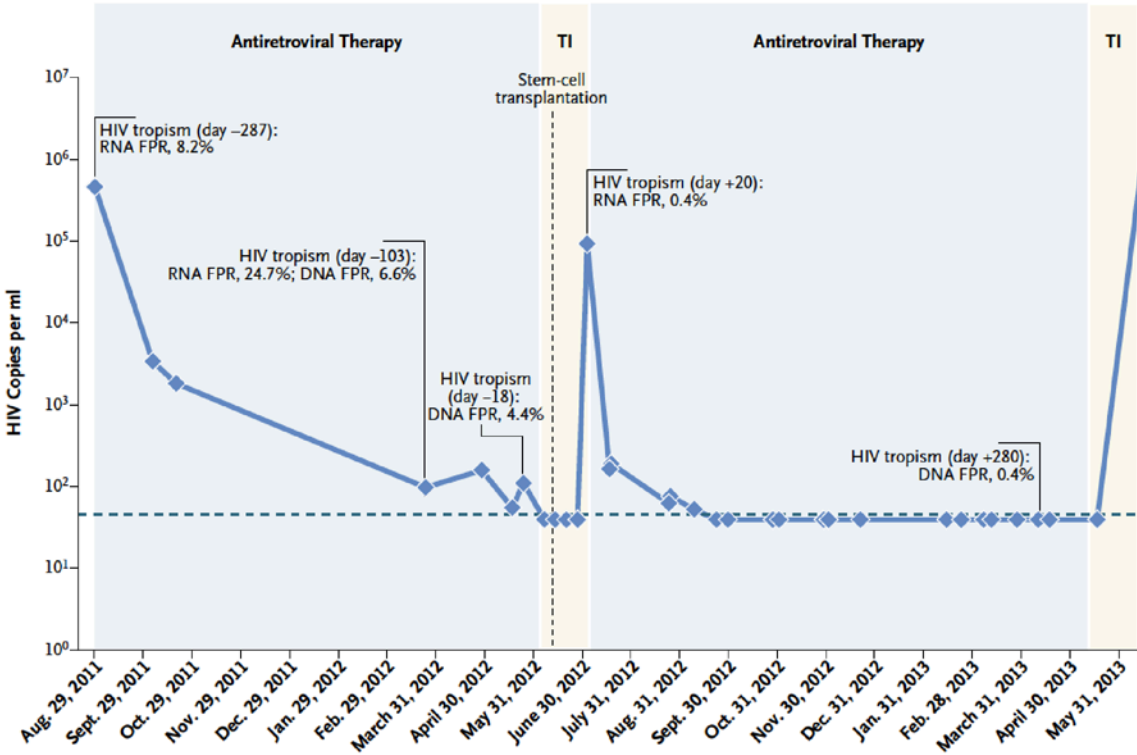
6) Stop of ART after the start of the myeloablative treatment

7) alloSCT from a CCR5 delta32 homozygous donor,

8) Rebound of HIV-RNA (X4-capable) after alloSCT (+20 days)

9) Re-start of ART

10) relapse, death due to tumor progression



HIV tropism

HIV tropism (at the time of alloSCT)

V3 (env) region was amplified and sequenced (Sanger sequencing)
Predicted coreceptor usage: geno2pheno(coreceptor) = false-positive rates (FPR)

FPR <5	=> X4-capable (X4-tropic or dual-tropic)
FPR 5-15	=> intermediate
FPR >15	=> R5-tropic

HIV tropism (retrospective using the Illumina MiSeq platform)

V3 (env) region was amplified and sequenced (Illumina MiSeq platform)
NGS => clonal information about the V3-sequence

FPRs for each V3 sequence was determined

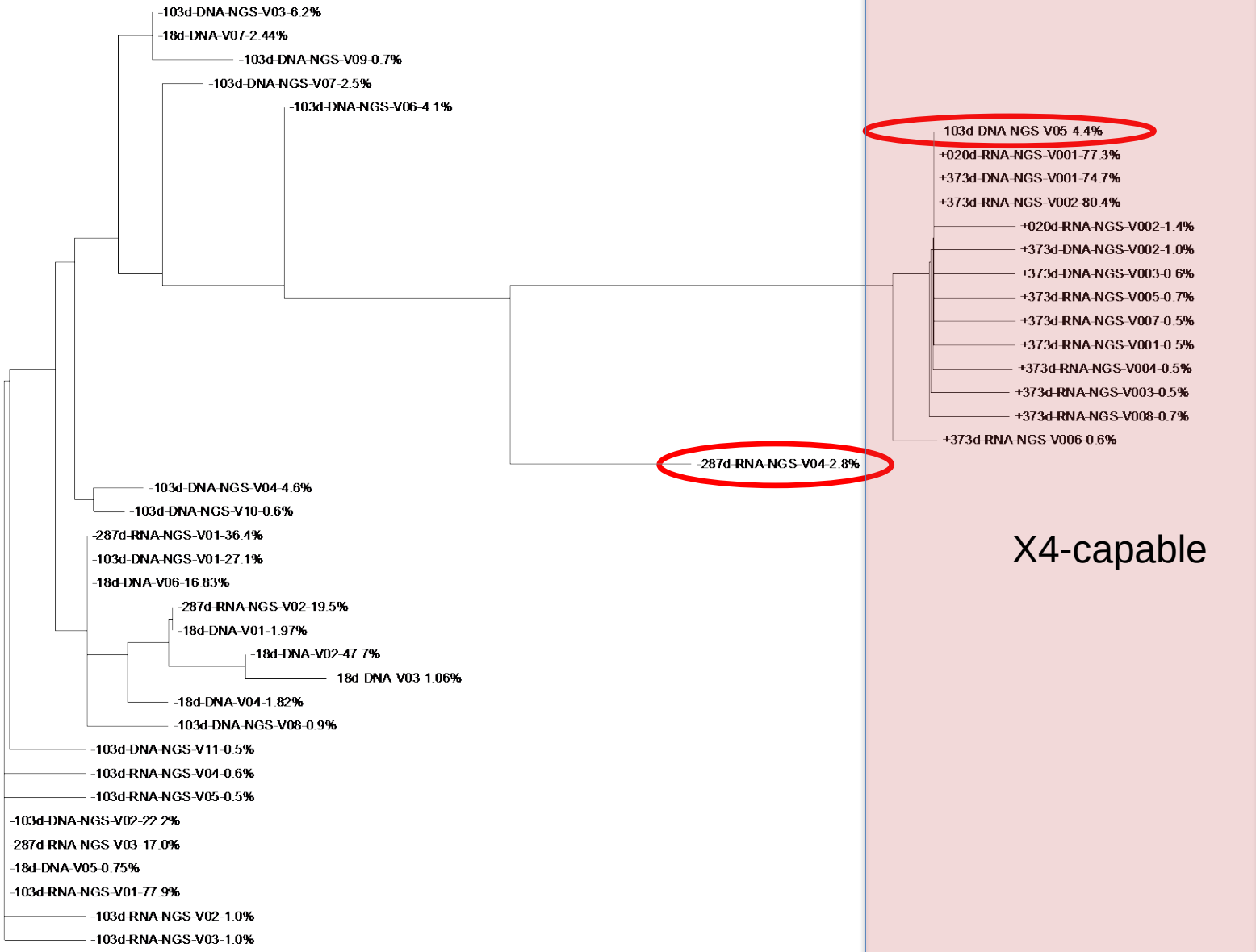
Samples available for retrospective analysis using the Illumina platform

days before/after alloSCT	HIV RNA copies/ml	CD4+ cell count number of cells	material
287 days **before** alloSCT	462,500	415	RNA
103 days **before** alloSCT	471	475	RNA/DNA
18 days **before** alloSCT	55	314	DNA
20 days **after** alloSCT	93,380	13	RNA
373 days **after** alloSCT	7,582,496	101	RNA/DNA

Sequence variability of the V3 loop

Days before (-)/ after (+) alloSCT	RNA/ DNA	ID-percent of reads	V3 Sequence CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHC	FPR	
-287 days	RNA	V01-36.4%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10.5	
		V02-19.5%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10.5	
		V03-17.0%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAYC	8.5	
		V04-2.8%	CTRPNNNTRKGITLGPGRVFFAT-EIIGDIRKAYC	6.9	
-103days	RNA	V01-27.1%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10.5	
		V02-22.2%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAYC	8.5	
		V03-6.2%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10.5	
		V04-4.6%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRKAYC	6.8	
		V05-4.4%	CTRPNNKTRKAITLGPGRVYYTK-EIIGDIRKAYC	0.4	X4-capable
	DNA	V06-4.1%	CTRPNNNTRKGIHLGPGRVFYAT-EIIGDIRKAYC	5.7	
		V07-2.5%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRKAYC	6.8	
		V08-0.9%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10.5	
		V09-0.7%	CTRPNNNTRRGIHLGPGKVFYAT-EVIGDIRQAYC	7.8	
		V10-0.6%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRKAYC	6.0	
		V11-0.5%	CTRPNNNTRKGIHLGPGRVFYAT-EIIGDIRQAYC	7.0	
	RNA	V01-77.9%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAYC	8,5	
		V02-1.0%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAFC	6,8	
		V03-1.0%	CTRPNNSTRRGIHLGPGRVFYAT-EIIGDIRQAYC	4,2	
		V04-0.6%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAYC	8,5	
		V05-0.5%	CTRPNNNTRRGIHLGPWRVFYAT-EIIGDIRQAYC	11,7	
-18 days	DNA	V01-70.8%	CTRPNNNTRRGIHLGPGKVFYAT-EIIGDIRQAYC	10,5	
		V02-0.8%	CTRPNNNTRRGIHLGPGRVFYAT-EIIGDIRQAYC	8,5	
		V03-1.1%	CTRPNNNTRRGIHLGPGKVFYAA-EIIGDIRQAYC	7,4	
+20 days	RNA	V01-77.3%	CTRPNNKTRKAITLGPGRVYYTK-EIIGDIRKAYC	0,4	X4-capable
		V02-1.4%	CTRPNNKTRKAITLGPGRIYYTK-EIIGDIRKAYC	0,5	

Phylogenetic analysis of the V3 sequences



Coreceptor usage

CXCR4-predicted minority viruses were also present prior to transplantation in the case of the “Berlin patient”.

NGS-RNA during treatment interruption: 2.9% with FPR 2.7 – 9.3

All of these HIV variants were highly dependent on CCR5 for replication and had a high genetic barrier toward CXCR4 usage.

Symons et al., CID 2014: 59, 596

Therefore, HIV-V3 sequences obtained from the “Essen patient” were tested using the same assays.

V16 – FPR: 0.2

V13 – FPR: 0.4

V12 – FPR: 0.5

V11 – FPR: 4.2

V10 – FPR: 5.7

V07 – FPR: 6.8

V06 – FPR: 6.8

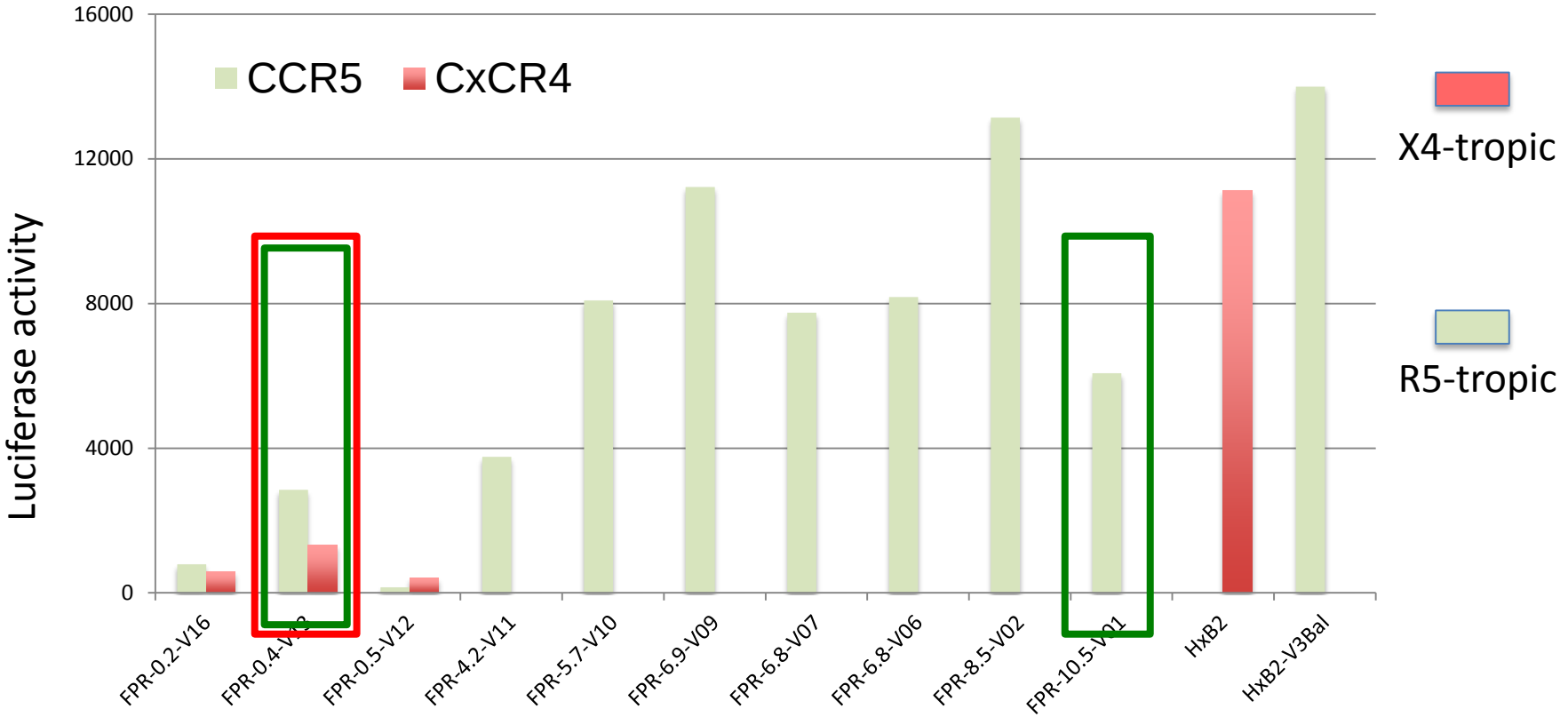
V09 – FPR: 6.9

V02 – FPR: 8.5

V01 – FPR: 10.5

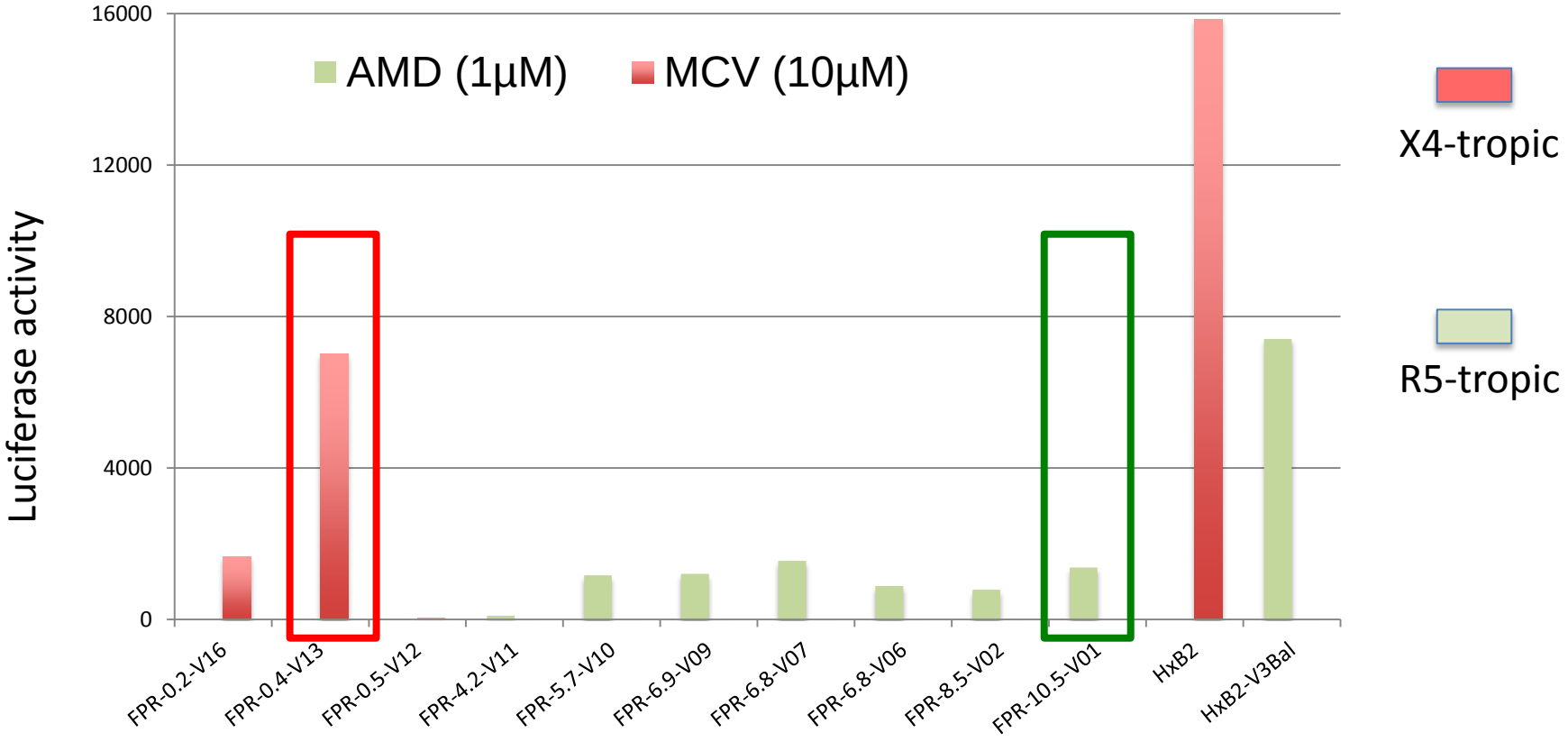
Coreceptor usage

Chimeric HIV isolates were analyzed in cell culture using the U373-MAGI-CCR5E and U373-MAGI-CXCR4$_{CEM}$ cell lines.



Coreceptor usage

Chimeric HIV isolates were grown in PBMCs in the presence of either AMD (CXCR4-blocker) or MCV (CCR5-blocker):



Conclusions

The selective pressure exerted by the transplantation of allogeneic stem-cells homozygous for the CCR5 delta32 mutation resulted in the **selection of an already preexisting X4-tropic HIV variant.**

In contrast to the “Berlin patient” we observed highly replicative competent **X4-tropic viruses**.

Therefore even the presence of minor X4-capable and replicative competent HIV variants can be responsible for the **lack of control of HIV replication** from therapy regimens aiming at the functional knock-out of the cellular CCR5 co-receptor of immune cells.

Even a tiny fraction can make all the difference if they have the might.

Thank you

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