ANALYTIC TREATMENT INTERRUPTION (ATI) AFTER ALLOGENEIC CCR5-D32 HSCT FOR AML IN 2013

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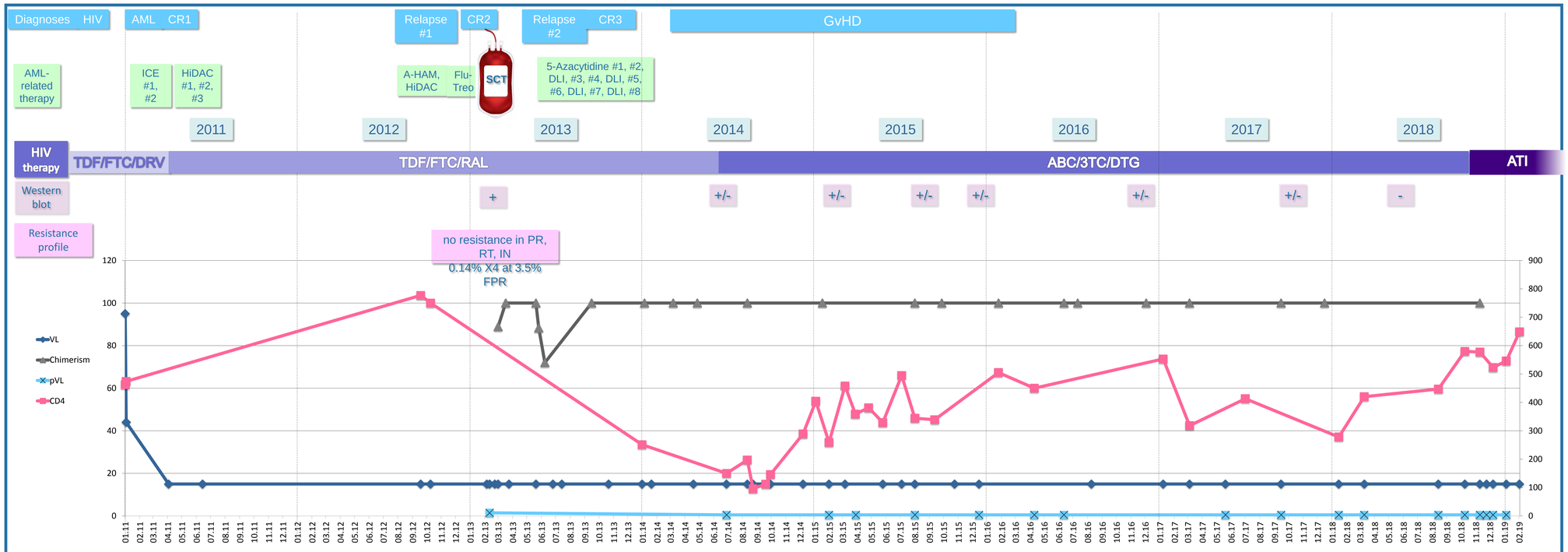
BACKGROUND

- As reported before (CROI 2016) a now 49y old HIV-infected male patient did receive unmodified HSCT from a female 10/10 CCR5-d32 DKMS-donor in February 2013 because of acute myeloid leukemia while being in 2nd complete remission (CR).
- By then proviral DNA load was 29400 cop/mL and all anticipated bands could be detected by western blot.
- At the time of HSCT coreceptor-usage was predicted as R5-tropic (Sanger: FPR 44.5%; NGS: 0.14% X4 at 3.5% FPR, geno2pheno), confirmed by phenotypic testing (TropChase).
- During HSCT and until November 2018 the patient remained on ART with undetectable viral load in plasma. He had a 2nd relapse of AML in June 2013 but after 8 courses of 5-azacytidine and 4 donor lymphocyte infusions CR was achieved and immunosuppression was stopped in October 2017.

METHODS

PBMC and tissues were analysed by ddPCR, qPCR and in situ hybridization in several laboratories as well as humeral and T-cell responses. Infectious virus was analysed on CD4+ T-cells (qVOA, MVOA). Patient was registered to IciStem as patient #19.

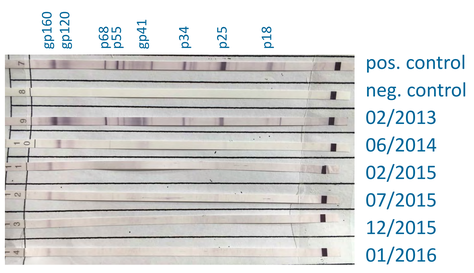


TropChase

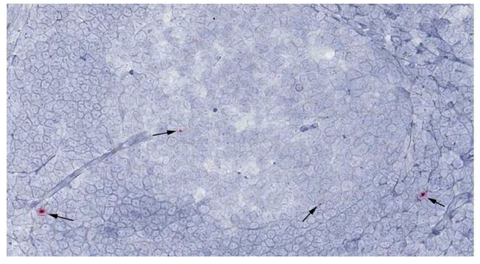
Clone	gp120-V3 amino acid sequence	# sequencing reads	genotypic prediction FPR (%)	phenotypic analysis in Tcells Magi (R5/X4)	MT2 (X4)
D1	CTRPNNNTREGIHIGPGRAFFTTGEIIGNIREASC	4	95,78	R5	R5
D2	CTRPNNNTRKSIHIGPGRAFFTTGEIIGNIKEAYC	2	95,64	R5	R5
D3	CTRPNNNTRKSIHIGPGRAFFTTGEIIGNIGEAYC	2	95,64	R5	R5
D4	CTRPNNNTRKGIHIGPGRAFFTTGEIIGNIREASC	2062	77,33	R5	R5
D5	CTRPNNNTRKGITIGPGRAFFTTGEIIGDIRQAHC	4886	30,67	R5	R5
D6	CTRPNNNTRKGIHIGSRKAFFTTGGIIGDIRQAYC	2	10,61	R5/X4	X4
D7	CTRPHTNTRKRIHIGPGRAFFTTGEIIGDIRQAYC	7	1,74	R5/X4	X4
D8	CTKPNNNTRKRIHIGPGRAFFTTGEIIGNIRQASC	2	1,74	R5	R5
D9	CTRPNNNIRKRIHIGPGRAFFTTGEIIGNIREAYC	3	1,16	R5/X4	X4
HxB2V3Bal		control R5	51,8	R5	R5
HxB2		control X4	0	X4	X4

Monique Nijhuis, Utrecht, ICISTEM

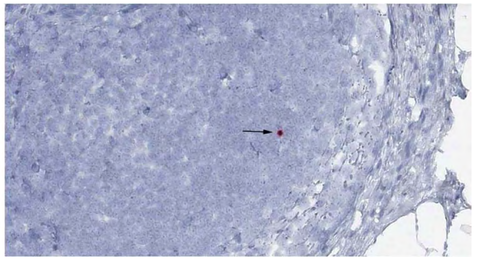
WESTERN BLOT



DNAscope



RNAscope



POST-TRANSPLANTATION

- Concerning HIV, all PBMC samples were negative for proviral DNA by conventional and digital droplet PCR in different labs at multiple time points.
- Liquor (July 2014), rectum (April 2015, March 2016), ileum (March 2016) and bone marrow (August 2015) showed also negative test results.
- Further testing with 0.1 Mio cells from the ileum showed 1/4 replicates positive with LTR-, but negative with gag-primers. There were also 2 positive signals in T-cell subsets (T_{CM} 0.2 Mio cells: ddPCR 6.7 cop/10^6cells, qPCR neg., T_{EM} 0.36 Mio cells: qPCR 5 cop/10^6cells, ddPCR neg.) with all other subsets negative in ddPCR and qPCR.
- No HIV-DNA could be detected by PCR in lymph nodes collected 05/17, but via in situ hybridization assays (RNAscope, DNAscope) few positive signals were detected.
- Viral outgrowth assays (VOA) were negative February 2016, March 2016 and May 2016 (23 Mio CD4+ Tcells, IUPM <0.031/10^6 CD4 T cells).
- Mouse viral outgrowth assays (mVOA, April 2016 Rag2-/-γc-/-, April 2017 NOD-SCID IL2gR-/-) also showed negative test results.
- CTL-assays showed a strong response against HLA-A2-epitope YV9 (RT) and HLA-B7-epitope YL9 (Gag-P6), which was not present in cells from the stem-cell donor.
- The Western blot shows an incomplete pattern (gp160 slightly positive, others negative).

SUMMARY & CONCLUSION

- Despite low signals in ultrasensitive assays no virus could be detected in qVOA/mVOA in the Duesseldorf patient. Taking into account the homozygous CCR5-d32 status we consider a viral rebound to be unlikely. Since the functional relevance is unclear an ATI is the only way to find out whether HIV has been eradicated by allogeneic CCR5-d32 HSCT.
- Therefore ART was stopped in November 2018 after thorough discussion with the patient.
- Plasma viral load and proviral DNA is measured twice weekly since stopping cART, immunological follow-up is performed monthly.
- Despite all plasma samples being negative after ATI longer surveillance is essential.

We are grateful to the patient for his participation and commitment.