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Comparative Genotoxic Signatures in Cord Blood Cells From Neonates Exposed to AZT or TDF *In Utero*

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Background

Zidovudine (AZT) and tenofovir (TDF) are the most frequently used nucleos(t)ide analogues for reducing perinatal HIV transmission during pregnancy. Various genotoxicity biomarkers have been identified in neonates (1-3, review in 5) and animal models (4, review in 5) exposed to AZT *in utero*. In contrast, there are no data on TDF exposure - even though the compound is now considered by the WHO as a benchmark drug in this setting. We have previously shown that cord blood cells from neonates exposed to AZT *in utero* present a higher proportion of aneuploid cells and have a transcriptome profile that is suggestive of increased DNA alteration and repair[3].

1. Andre-Schmutz et al., *JID* 2013, **208:** 235-43.
2. Witt et al., *Environ Mol Mutagen* 2007, **48**: 322-9.
3. Senda et al., Antivir Ther 2007, **12**: 179-87.
4. Olivero et al., *JID* 2013, **208**: 244-8.
5. Afran et al., *Clin Exp Immunol* 2014, in press.

Objectives

The aim of the present study was to compare the genotoxic signatures in neonates exposed to either AZT or TDF *in utero*.

Methodology



Cord blood

- Zidovudine « **AZT** » n = 8
- Tenofovir « **TDF** » n = 9
- Unexposed « **Controls** » n = 8

Chromosomal aberration study
Karyotyping – T cells
(PHA activation / 72h culture)

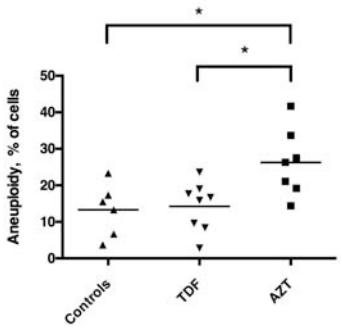
Transcriptome analysis
- CD3⁺ T cells (indirect magnetic isolation)
- CD34⁺ HSPC cells (direct magnetic isolation)



Cord blood cells from neonates exposed to either AZT or TDF *in utero* were used to enrich CD34⁺ hematopoietic stem/progenitor cells (HSPCs) or CD3⁺ T lymphocytes. The cells were then subjected to blinded karyotyping and a transcriptome (initial product of genome expression) analysis.

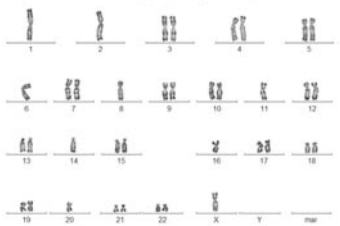
Results

Figure A. Aneuploidy Rates in Cord Blood Cells of *In Utero* Exposed Newborns to AZT or TDF Based Antiretroviral Combinations or Controls



The aneuploidy rates were determined as the percentage of all scored cells that were aneuploid (more or less than 46 chromosomes / metaphase) for each antiretroviral–exposed and control samples. Bars represent means. * : p<.05.

Figure B. Severe Hypoploidy Example



Chromosomal aberration study (Figure A and B)

Karyotyping (Figure A) revealed a higher proportion of aneuploid cells in the AZT-exposed group (n=7, mean ± SD: 26.3% ± 9.2) than in the TDF-exposed group (n=8, 14.2% ± 6.7; p<0.05) and a control group (n=6, 13.3% ± 7.2; p<0.05). All chromosomes were involved (Figure B) and the alterations were randomly distributed. Concerning AZT exposition, these results were consistent with our previous study[1].

Transcriptome analysis (Table)

Our overall analysis of transcriptome profiles revealed a distinct molecular profile in patients exposed to AZT or TDF. This signature was found both in CD34⁺ HSPCs and CD3⁺ T lymphocytes. Gene Set Enrichment Analysis revealed significant changes in the activity of genes involved in DNA repair in both the AZT and TDF groups. However, in HSPC experiments, double-strand break repair, Fanconi anaemia pathway and G2/M checkpoint gene sets were enriched in the AZT group but not in the TDF group.

A sample of genes, involved in regulating DNA damage response, chromosomal aberration and cell cycle, was subjected to further validation by quantitative PCR assay. 43% of gene alteration (seen in transcriptome analysis) in CD34⁺ cells and 72% in CD3⁺ cells was confirmed.

Table. Gene Sets Enriched in CD34+ Hematopoietic Progenitors From Cord Blood From AZT-exposed and TDF-exposed Newborns Compared to Controls (Gene Set Enrichment Analysis).

Gene sets with an FDR-q value of less than 0.25 should be considered to be significant.

	« AZT » vs « Controls »		« TDF » vs « Controls »	
Gene set name	**NES**	**FDR-q value**	**NES**	**FDR-q value**
DNA repair	1.69	0.13	1.55	0.21
Double strand break repair	1.55	0.13	-	NS
Fanconi anemia pathway	1.58	0.23	-	NS
Nucleotide excision repair	1.57	0.14	1.56	0.23
Transcription-coupled NER	1.57	0.21	1.55	0.16
G2/M checkpoints	1.55	0.12	-	NS
Chromosome maintenance	1.54	0.14	-	NS
Telomere maintenance	1.65	0.14	-	NS
Extension of telomeres	1.57	0.16	1.53	0.16
Mitochondrial protein import	1.56	0.13	1.59	0.19
DNA/RNA synthesis				
DNA strand elongation	1.57	0.17	1.66	0.13
RNA pol I transcription termination	1.56	0.14	-	NS

Data were analyzed by GSEA software with the REACTOME gene sets from the Molecular Signatures Database (gene sets could be search at http://www.broadinstitute.org/gsea/msigdb/index.jsp). Abbreviations: DNA, deoxyribonucleic acid; FDR, false discovery rate; NER, nucleotide excision repair; NES, normalized enrichment score ; NS, not significant ; RNA, ribonucleic acid.

Conclusion

Based on our cytogenetic analysis, antiretroviral combination treatments that include TDF may be less clastogenic than those including AZT. However, hematopoietic cord blood cells present transcriptional abnormalities that are suggestive of DNA damage in neonates exposed to AZT- or TDF-based ARV combination treatments *in utero*. Given the wide use of these ARV drugs during pregnancy, it is critical to further investigate their genotoxicity and potential impact on health.