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Effects of Antineoplastic Chemotherapy on Dynamics of HIV Population Genetics In Vivo

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Background

Previously we and others have demonstrated, in the absence of combination antiretroviral therapy (cART), that HIV populations change slowly and cART results in a large decrease in viraemia but not a population shift[1,2]. Antineoplastic chemotherapy (CTX) includes agents that target proliferating cells and may therefore have an effect on HIV infected CD4+ cells and HIV populations. Previously a study by Cillo et al.[3] did not detect long term effects of CTX on levels of total cell associated HIV DNA in patients on cART. However, it has been demonstrated that CTX leads to a transient increase in HIV viraemia in patients off cART[4]. We investigated the effects of chemotherapeutic agents on HIV populations in infected individuals undergoing two different CTX regimens.

Hypothesis

Administration of cytotoxic chemotherapy leads to a change in HIV populations

Participants

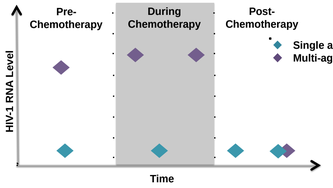
Single Agent

- Enrolled in NIH study number **140604 (N=5)**
- Provided written informed consent
- **HIV+ / Subtype B**
- **On cART**
- Comorbid **Kaposi's Sarcoma**
- Received six cycles of **Doxorubicin** OR **Paclitaxel**
- CTX was well tolerated

Multi-Agent

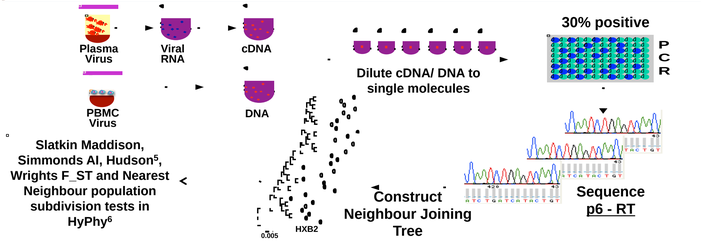
- Enrolled in NIH study number **01C0030[4] (N=6)**
- Provided written informed consent
- **HIV+ / Subtype B**
- **Off cART**
- Comorbid **B-cell lymphoma**
- Received four cycles of **Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin and Rituximab (EPOCH – R)**
- CTX was well tolerated

Analysis

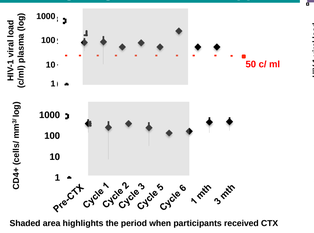


- Single Agent participants were **suppressed on cART,** viral **DNA** from **PBMC** was studied.
- Multi Agent participants were **viraemic and not on cART.** Viraemia reached a plateau before initiation of CTX. Viral **RNA** from plasma was studied.
- **Four** longitudinal time points per participant were analysed from each study.

Methods

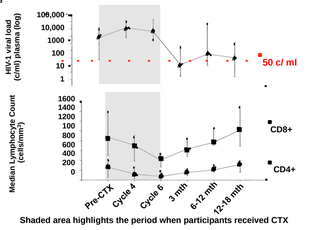


Viral Load and CD4+ Counts During Single Agent Chemotherapy



Shaded area highlights the period when participants received CTX

Viral Load and CD4+ Counts During Multi- Agent Chemotherapy[4]



Shaded area highlights the period when participants received CTX

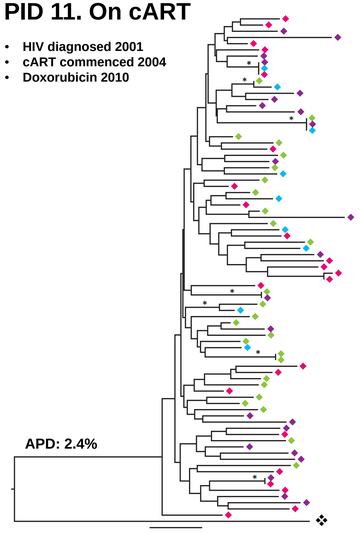
No Detectable Change in HIV Populations in PBMC After Single Agent Chemotherapy

Key: APD = Average pairwise distance I * Bootstrap ≥ 80 (no branch was >95%) I ❖ HXB2 outgroup
Scale 0.005 nt substitutions/site I ◆ Pre CTX I ◆ Cycle 1 I ◆ 1mth Post I ◆ 2 mth Post

One representative tree is shown (one out of five participants)
Hypermutants removed

PID 11. On cART

- **HIV diagnosed 2001**
- **cART commenced 2004**
- **Doxorubicin 2010**



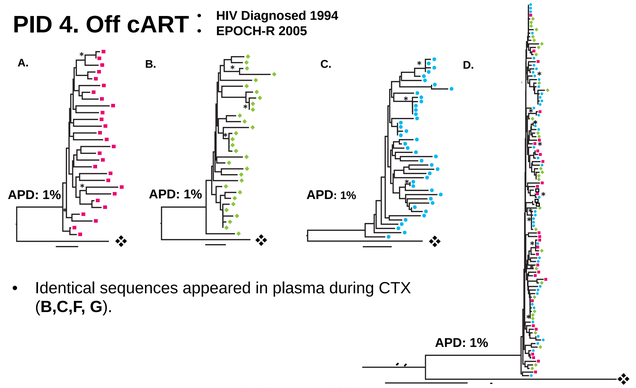
- Trees constructed from HIV sequences obtained from PBMC from all participants studied on a regimen of single agent CTX were similar.
- No significant change in hypermutant frequency observed in any of the participants (5/5) during CTX.
- No evidence of a HIV population shift was seen in any of the participants (0/5).
- Evidence for the persistence of identical sequences (5/5) and therefore surviving cellular clones during CTX was observed.
- A change in CD4+ T-cell numbers was not seen and we did not identify an effect on HIV structure using phylogenetics.

Multi-Agent Chemotherapy During Untreated HIV-1 May Result in Changes to HIV-1 Population Structure

Key: APD = Average pairwise distance I * Bootstrap ≥ 80 (no branch was >95% I ❖ HXB2 outgroup
Scale 0.005 nt substitutions/site I ■ Pre CTX I ◆ Cycle 2 I ● End CTX I □ Post CTX

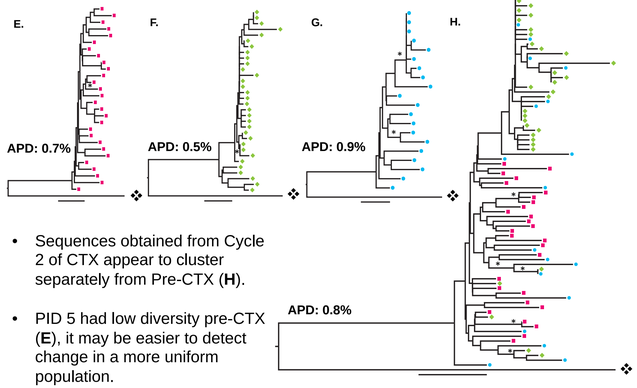
Two trees are shown where a change in phylogenetic topology was seen

PID 4. Off cART : HIV Diagnosed 1994 EPOCH-R 2005



- Identical sequences appeared in plasma during CTX (**B,C,F, G**).

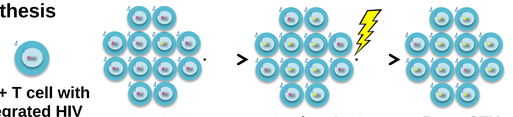
PID 5. cART naive : HIV Diagnosed 2001 EPOCH-R 2006



- Sequences obtained from Cycle 2 of CTX appear to cluster separately from Pre-CTX (**H**).
- PID 5 had low diversity pre-CTX (**E**), it may be easier to detect change in a more uniform population.

Tests for Population Subdivision Demonstrate Changes in Viral Population Structure During Chemotherapy

Hypothesis



			Tree Based				Distance Based			
			Simmonds AI		Slatkin Maddison		Hudson	Wrights	Nearest Neighbour	Change in Population Structure
PID	Sampling Time Point	Association Index	Bootstrap Significance	Migration Events	Probability	p(K*s)	F_ST	Snn	During CTX
Single CTX 10	Pre - During	1.03	0.33	19	0.45	0.220	0.65	0.92	No
	Pre - Post (1 m)	0.58	0.76	2	0.07	0.480	0.05	0.40	No
	During - Post (1 m)	0.67	0.65	2	0.06	0.940	0.11	0.26	No
	During - Post (3 m)	1	0.34	6	1.00	0.120	0.05	0.61	No
	Pre - Post (3 m)	0.98	0.37	21	1.00	0.310	0.62	0.87	No
Single CTX 11	Pre - During	1.02	0.33	16	0.23	0.660	0.71	0.37	No
	Pre - Post (1 m)	0.88	0.63	14	0.00	0.070	0.79	0.56	Yes (1/5)
	Pre - Post (2 m)	0.83	0.63	10	1.00	0.930	0.77	0.18	No
	During - Post (1 m)	1.12	0.08	20	0.70	0.340	0.37	0.78	No
	During - Post (2 m)	1.15	0.11	10	1.00	0.990	0.04	0.23	No
Single CTX 12	During - Post (3 m)	0.81	0.61	4	0.23	0.530	0.55	0.13	No
Single CTX 13	Pre -During	1.3	0.13	8	1.00	0.100	0.26	0.32	No
	Pre -Post (1 m)	0.76	0.71	6	0.41	0.660	0.65	0.39	No
Single CTX 14	Pre - During (3 m)	0.93	0.41	8	1	0.670	0.65	0.03	Yes (1/5)
Multi-Agent CTX 1	During - End	1.07	0.25	18	0.89	0.220	0.44	0.41	No
Multi-Agent CTX 4	Pre - During	0.79	0.85	14	0.02	0.170	0.04	0.07	Yes (2/5)
	During - Post	0.32	0.97	2	0.05	0.090	0.31	0	Yes (2/5)
	During - End	0.91	0.54	19	0.19	0.340	0.17	0	Yes (1/5)
	Pre - End	1.02	0.32	22	0.79	0.178	0.00	0.46	Yes (1/5)
	End - Post	0.10	1	2	0.06	0.010	0.10	0.00	Yes (1/5)
	Pre - Post	0.53	0.88	2	0.08	0.106	0.56	0.01	Yes (1/5)
Multi-Agent CTX 5	During - End	0.87	0.61	11	0.03	0.000	0.00	0.49	Yes (3/5)
	Pre - End	0.60	0.98	9	0.00	0.00	0	0	Yes (4/5)
	During - Pre	0.26	1	5	0.00	0.000	0	0	Yes (4/5)
Multi-Agent CTX 6	Pre - End	0.67	0.85	6	0.06	0.237	0.23	0.00	Yes (1/5)
Multi-Agent CTX 7	Pre - During	0.80	0.78	15	0.26	0.012	0.02	0.01	Yes (2/5)
Multi-Agent CTX 8	Pre - During	Too few	Too few	Too few	Too few	Too few	Too few	Too few	
Significance threshold		< 0.1	>0.95		< 0.05	< 0.009	< 0.05	< 0.05	

Tests for Population Subdivision confirmed a HIV population shift in PID 5 that occurred between pre-CTX and C2 (4/5 tests).

Summary

- **Multi-agent CTX during untreated HIV-1 infection may result in changes to HIV-1 populations in patients with relatively low genetic diversity, in whom changes may be more easily identified.**
- **The use of Doxorubicin or Paclitaxel alone has little detectable effect on cell associated HIV-1 populations.**

Acknowledgments

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