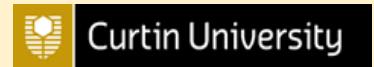


HIV-Neuropathy in patients treated without stavudine

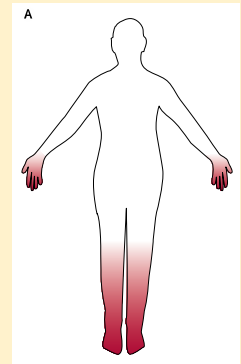
HIV-associated sensory neuropathy (HIV-SN) is a length-dependent, small fibre peripheral neuropathy, affecting up to 60% of HIV⁺ patients who take stavudine.

It is clinically identical in treated and untreated patients



Signs and symptoms are detected using the Brief Peripheral Neuropathy Screening Tool

- numbness
- burning sensation
- pain hypersensitivity
- absent deep tendon reflexes
- spontaneous pain



Assessing ankle reflex & vibration sense in a South African Clinic

Kate Cherry, Toni Wadley

Ethnicity is an independent predictor of HIV-SN

- Logistic regression modelling used factors associated with HIV-SN ($p < 0.05$) or previously associated with SN (height, AIDS).
- Following a step-wise removal procedure, factors independently associated with HIV-SN were **stavudine exposure, age, height and ethnicity**.
- Black patients had twice the risk of whites“others” had a lower risk than whites

	OR	95% CI	p
Age (years)	1.1	1.0 – 1.1	<0.0001
Stavudine use	4.27	2.8 – 6.5	<0.0001
Height (cm)	1.03	1.0 – 1.04	0.003
Black race*	2.3	1.5 – 3.6	<0.0001
Other race*	0.5	0.3 – 0.8	0.006

Kate Cherry, 2008

Logistic regression modeling provided an algorithm to predict SN in Indonesian HIV patients on stavudine

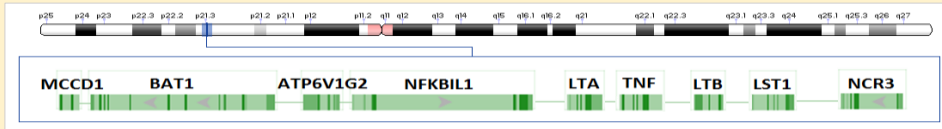
	Odds Ratio	95% CI	p
Age (years)	1.1	1.03 – 1.18	0.006
Height (cm)	1.1	1.02 – 1.17	0.01
TNFA-1031*2	3.6	1.3 – 9.8	0.01

Overall model p = 0.0009

**Factors included in the model: genotype, gender, age, height, BMI, months of HIV, months on d4T, isoniazid use, HepC, initial CD4*

Kate Cherry and Jacquita Affandi, 2007

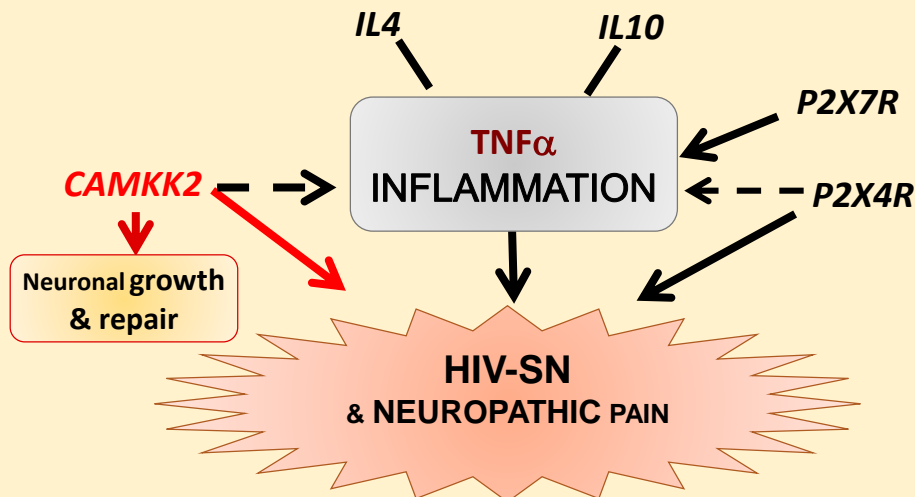
Genetic markers in *TNF* vary between ethnicities



- ✂ The gene encoding TNF is in linkage disequilibrium with 9 surrounding genes in the central MHC region – the *TNF*-block
- ✂ The TNF-block was assessed in Malay, African and European Caucasian HIV⁺ patients receiving stavudine
- ✂ Rs1799964 (TNF-1031) associates with HIV-SN in Malays and European Caucasians but not Africans
- ✂ The haplotype that includes TNF-1031 in Caucasians and Asians is not found in Africans, so TNF-1031 marks another important polymorphism

Constance Chew, Toni Wadley, Peter Kamerman 2012

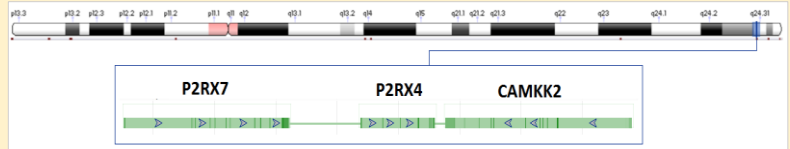
P2X7R, P2X4R, CAMKK2, IL4 & IL10 may modulate TNF and so cause neuropathy



Hayley Goulee, 2014; Jessica Gaff, ASHM 2017

The P2X-Block of genes marked susceptibility to HIV-SN in South Africans receiving stavudine

SNP	GENE	CHI ²
rs1186055	P2X7R	P=0.17
rs208307	P2X7R	P=0.15
rs10160951	P2X7R	P=0.17
rs2230912	P2X7R	P=0.08
rs2686387	P2X4R	P=0.15
rs2668252	P2X4R	P=0.13
rs1169719	P2X4R	P=0.11
rs1653587	CAMKK2	P=0.12
rs7975295	CAMKK2	P=0.007
rs2686344	CAMKK2	P=0.018
rs1560568	CAMKK2	P=0.023
rs2686367	CAMKK2	P=0.004



- Weak associations with P2X7R and P2X4R
- Strong association found with CAMKK2

Three CAMKK2 haplotypes perfectly predicted the presence (11121221112) or absence (11111111112, 11121212111) of HIV-SN, explaining HIV-SN status in 16/153 (10%) patients.

11 haplotypes plus age and height were carried forward in the logistic regression using the remaining 137 individuals.

The best model combined age and height with one haplotype of P2X7R and three of CAMKK2 ($p < 0.0001$, $R^2 = 0.18$).

So in patients given stavudine.....

Africans are more susceptible than Asians

Age, height and (different) polymorphisms around *TNF* promote SN in all ethnicities, *CaMKK2* is important in Africans

What happens without stavudine...?

Could CaMKK2 explain the greater sensitivity of Africans...?

197 patients receiving ART without stavudine for 1-12 years were recruited in Jakarta and screened for HIV-SN with the BPNS

28 patients (14%) had SN....much less than in 2007 (33%)



CAMKK2 significantly associated with HIV-SN in Indonesian HIV patients treated without stavudine

SNP	GENE	CHI ²
rs25644	<i>P2X4R</i>	0.07
rs1718158	<i>CAMKK2</i>	0.18
rs10849861	<i>CAMKK2</i>	0.046
rs1653586	<i>CAMKK2</i>	0.17
rs1653587	<i>CAMKK2</i>	0.17
rs1653587	<i>CAMKK2</i>	0.12
rs7975295	<i>CAMKK2</i>	0.011
rs1560568	<i>CAMKK2</i>	0.011
rs1132780	<i>CAMKK2</i>	0.010

4 CAMKK2 SNPs significantly associated with HIV-SN in Indonesian HIV+ patients

2 SNPs also associated in South Africans

Age and height did not affect HIV-SN in Indonesian patients treated without stavudine.

	Normal (n=169)	Neuropathy (n=28)	p
Height	166 (142 – 180)	167 (151-175)	1.0
Age	35.3 ± 5.8	37.6 ± 6.5	0.83
Last VL	0 (0 – 145,000)	(0 – 530,000)	0.21
Nadir CD4 count	121 (0-599)	65 (11-428)	0.31
Last CD4 count	468 ± 202	396 ± 180	0.74
ART duration (years)	4.1 (1 – 13)	5.4 (1 – 11)	0.33

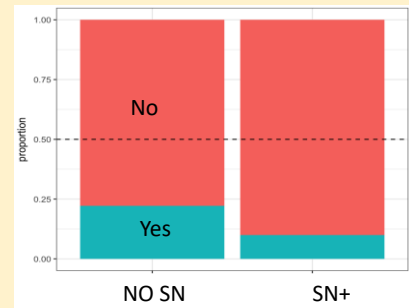
Factor affecting HIV-SN in
Indonesian patients treated without stavudine.

	Normal (n=169)	Neuropathy (N=28)	p
Male/female	121/48	19/9	0.66
Malay/other ethnicity	160/9	27/1	0.84
Alcohol yes/no	33/136	11/17	0.023
Isoniazid yes/no	72/97	17/11	0.057

Do we believe alcohol is critical?

Maybe, but....

- Only assessed as a questionnaire
- Indonesia is a muslim country
- Did not affect nerve conduction
- Some patients reported use of methanol



*Alcohol is not important in South Africa
where there is no stigma to its use*

Now checking ...

Does declared alcohol use in Indonesia mark other risk behaviours?

Phone survey to determine nature and quantity of alcohol consumed

Do we believe isoniazid is critical?

-95% received pyridoxine (vitamin B6) supplements

-not a significant effect in 2007 in patients receiving stavudine

- were the supplements sufficient? 10-25mg/day

Van der Watt et al. 2015. INT J TUB LUNG DIS 19:1312–9

South African study

SN was present in 16% of individuals pre-ART and associated with previous/current TB. Others developed SN on ART (initially stavudine) ...this was not linked with TB

53% were pyridoxine deficient, despite all receiving 4 mg/day pyridoxine & 42% of patients receiving 25mg pyridoxine (mostly a recent prescription to patients with neuropathy).

Those with a history of TB and pre-ART DSP were more likely to be pyridoxine deficient.

More severe HIV disease may promote HIV-SN in Indonesian patients treated without stavudine.

	Normal (n=169)	Neuropathy (N=28)	p
Male/female	121/48	19/9	0.66
Malay/other ethnicity	160/9	27/1	0.84
Isoniazid yes/no	72/97	17/11	0.057
Alcohol yes/no	33/136	11/17	0.023
HIV RNA >500 copies yes/no	7/162	5/23	0.016
Nadir CD4 <200 cells/ul yes/no	109/60	23/5	0.048

And in South Africa without stavudine?

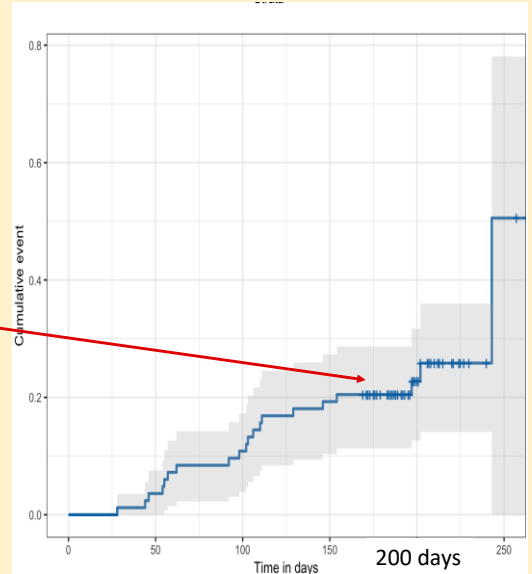
Hot off the press from South Africa

From the current study: 9 / 151 (6%) ART naive people screened had pre-existing SN. This is lower than expected (perhaps the test-and-treat policy).

Of the 83 neuropathy-free patients followed up for about six months, 20 developed SN (24% cumulative incidence in six months).

➔ total prevalence 6% + (24% of 94%) = 28.6%

...but when 100% received stavudine: prevalence = 58%



So we now know that

The incidence of HIV-SN has halved with the removal of stavudine but still develops on ART

The key factors of age and height are replaced by control of HIV replication

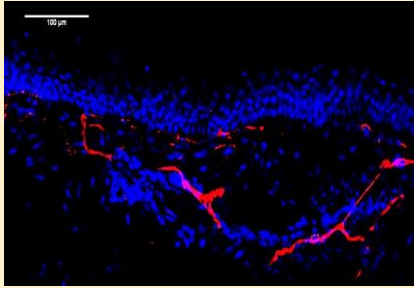
The greater susceptibility of Africans is retained without stavudine

Link with CAMKK2 alleles is retained in Indonesian patients and patients treated without stavudine

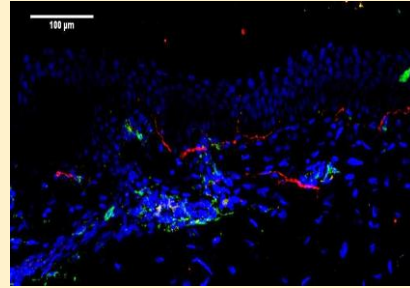
Studies are underway in Jakarta and Johannesburg to address....

1. Critical genetic profiles
2. Pathways involving CaMKK2 (Jessica Gaff)
3. The role of CMV
4. The role of chemokines (Jenjira Mountford)

Clinical and histological profiles of HIV-SN



Healthy control: **Robust, continuous nerves, no CD14⁺ macrophages**



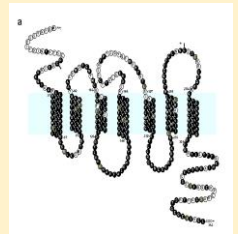
HIV-SN: **Fragmented nerves with perivascular CD14⁺ macrophages – some near damaged nerves**

We have now extended the study to identify chemokine receptors expressed on infiltrating cells

What are chemokines and why could they mediate HIV-SN?

Chemoattractant cytokines or chemokines are small disulphide-linked polypeptides

Chemokines mediate their effects on cells via specific cell surface receptors that contain seven transmembrane spanning regions (TM7 receptors).



Chemokines are produced at sites of inflammation and bind to the endothelium – this traps circulating inflammatory cells expressing the cognate receptor

CC chemokines

- Produced by activated T cells
- Affect T cells & monocytes

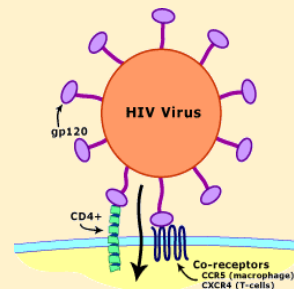
CXC chemokines

- produced by macrophage & tissue cells
- affect neutrophils and hence acute inflammation

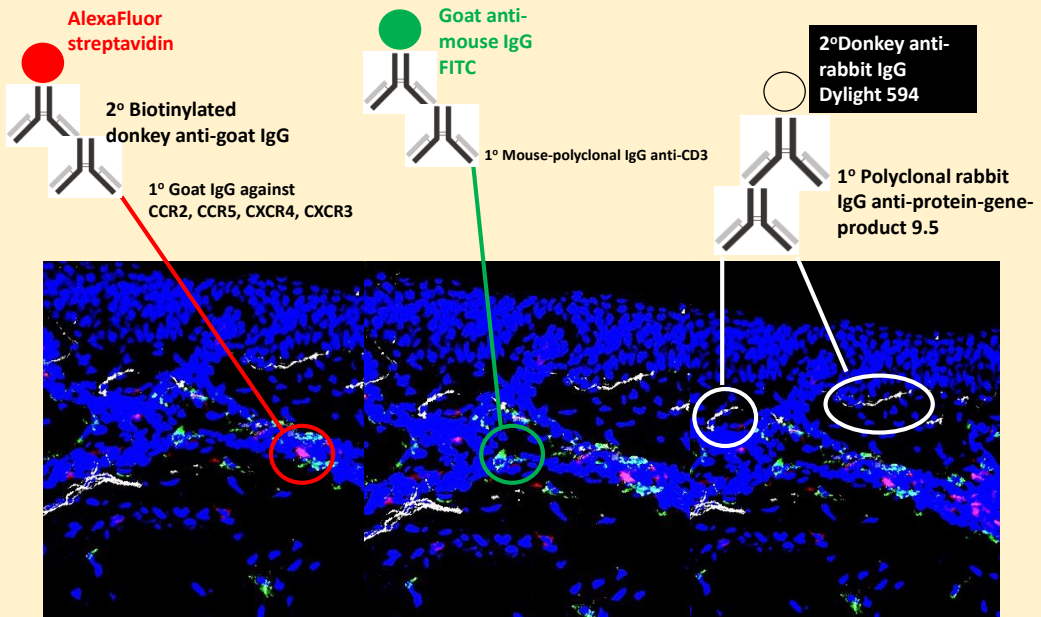
CX3CL1 = fractalkine

- implicated in inflammation mediated by macrophage

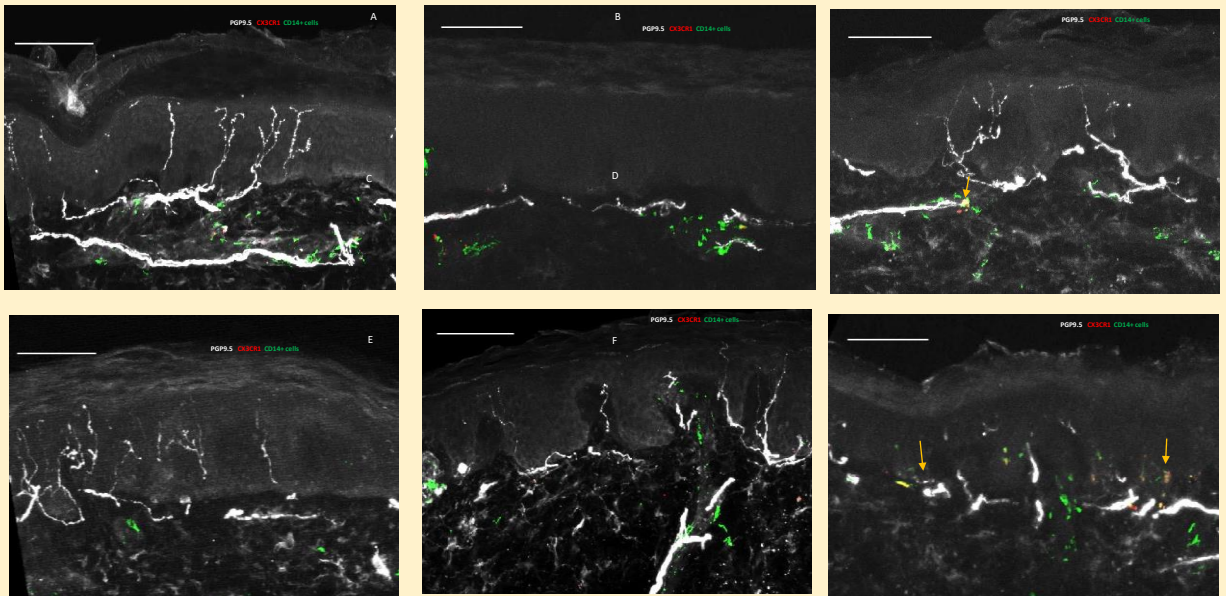
Chemokine receptors are co-receptors for HIV...so if expressed on nerves may allow direct viral damage



To identify chemokine receptors on CD3⁺ cells



CX3CR1 (fractalkine) is expressed on a subset of CD14⁺ macrophage near damaged nerves

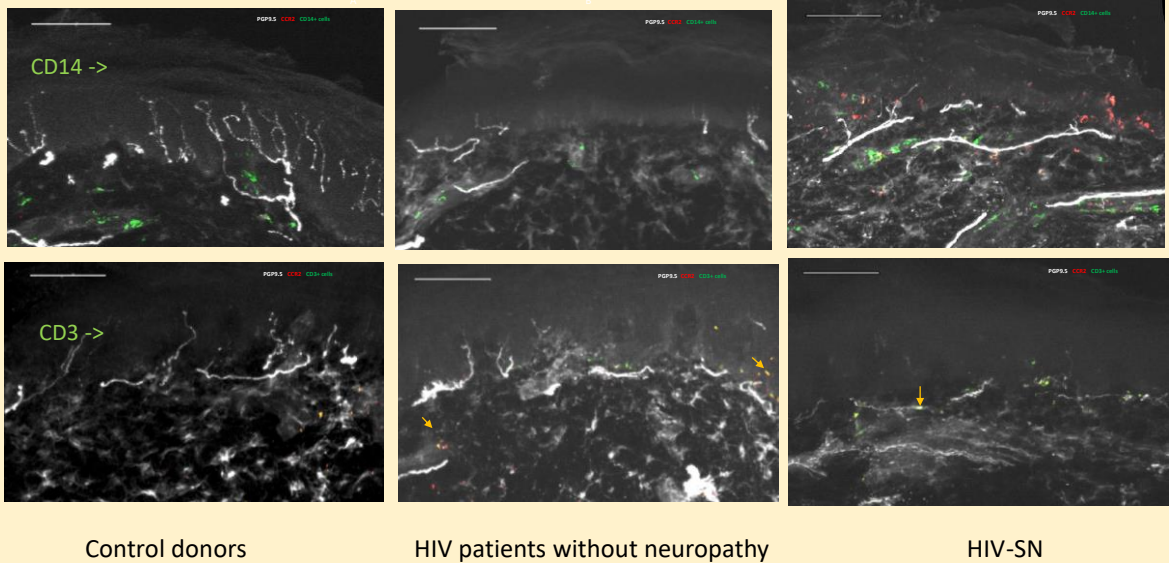


Control donors

HIV patients without neuropathy

HIV-SN

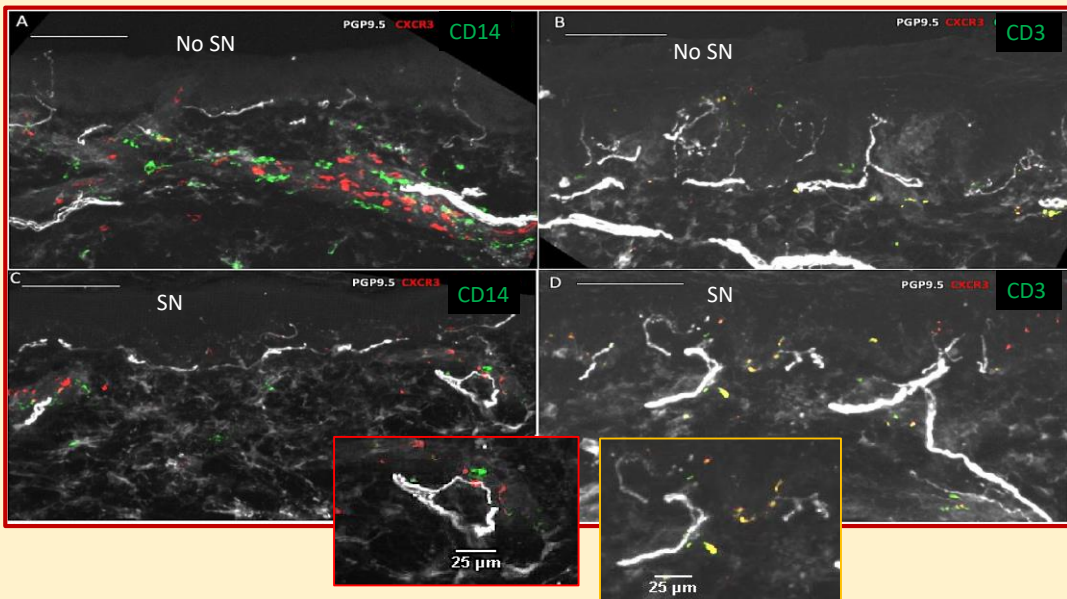
CCR2 is expressed on CD3⁺ T-cells near to damaged nerves



CXCR3 is a ligand for CXCL10 (IP10) which is unregulated by HIV infection (not SN).

CXCR3 was expressed in sections from all HIV patients.

CXCR3⁺ cells located close to CD14⁺ cells (red box) but CXCR3 co-localised with CD3 (yellow box).



Acknowledgments

UNIVERSITY OF INDONESIA

Dr Fitri Octaviana
 Dr Riwanti Estiasari
 Dr Yanuar Ahmed
 Mr Ibnu Ariyanto
 Dr Dennis Dewanto



CURTIN UNIVERSITY

Patricia Price
 Silvia Lee
 Jenjira Mountford
 Jessica Gaff
 Connie Jackaman



Kate Cherry (Monash)

Constance Chew & Hayley Goullee (UWA)

UNIVERSITY OF THE WITWATERSRAND

Peter Kamerman
 Toni Wadley

And...

Gaëlle Ngassa Mbenda
 Prinisha Pillay

