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Abstracts

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Correspondence to:
organisation@nchiv.org

Production: Sabina Beeke, Yunka de Waart
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01

DISTINCT ANTIBODY LINEAGES WITH MULTIPLE SPECIFICITIES ACHIEVE NEUTRALIZATION BREADTH IN AN HIV-1 INFECTED ELITE NEUTRALIZER

Jelle Van Schooten[1], Anna Schorcht[1], Elinaz Farokhi[2], Gao Hongmei[3], Patricia Van der Woude[1], Judith A. Burger[1], Tom Bijl[1], Riham Ghalaiyini[1], Hannah L. Turner[2], Celia C. Labranche[3], Sok Devin[4], Ozorowski Gabriel[2], David C. Montefiori[3], Dennis R. Burton[2], Michael Seaman[3], Ian Wilson[2], Rogier W. Sanders[1], Andrew B. Ward[2], Marit J. Van Gils[1]

[1] *Amsterdam UMC, Amsterdam, the Netherlands*
[2] *The Scripps Research Institute, La Jolla, United States*
[3] *Duke University Medical Center, Durham, United States*
[4] *International AIDS Vaccine Initiative - Neutralizing Antibody Center (IAVI-NAC), La Jolla, United States*

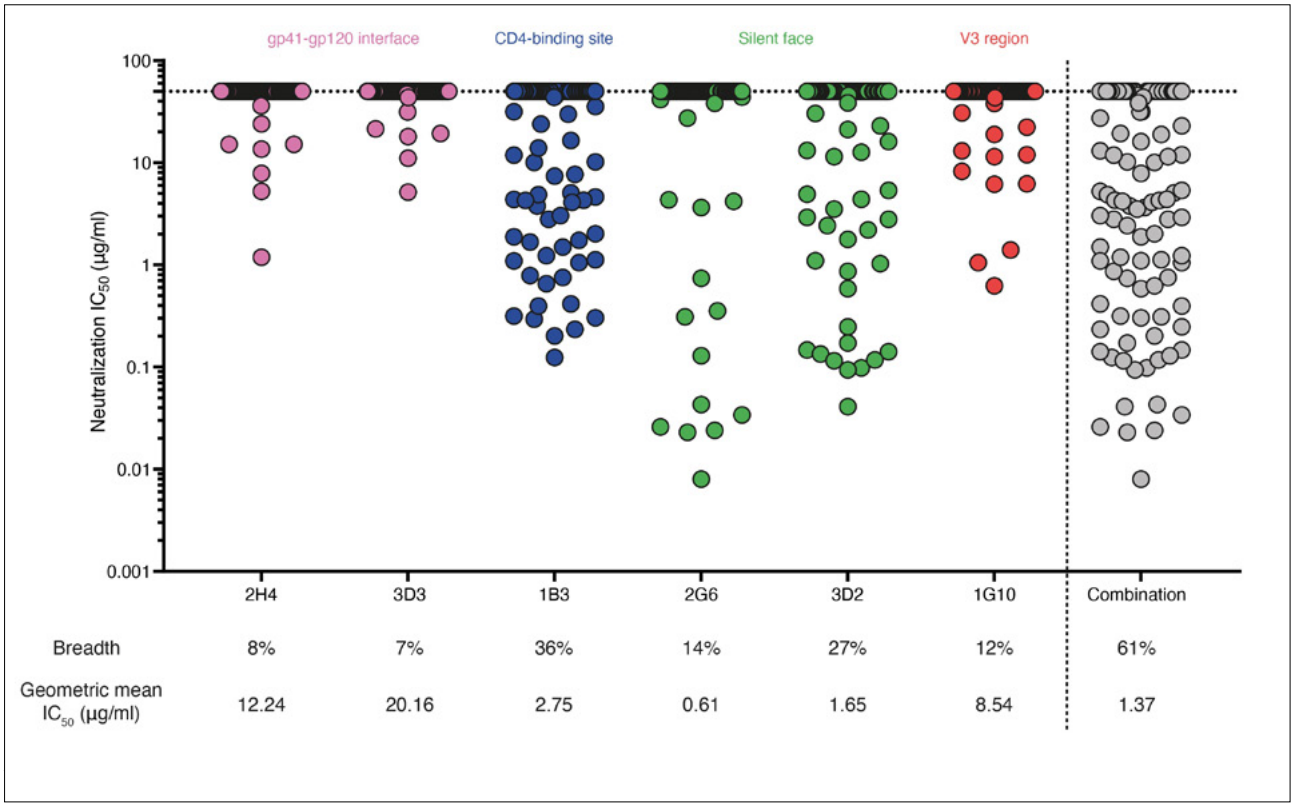
Background
Broadly neutralizing antibodies (bNAbs) have remarkable breadth and potency against most HIV-1 subtypes and are able to prevent HIV-1 infection in animal models. Defining the developmental pathways of bNAb B cell precursors towards neutralization breadth, and more specifically which HIV-1 envelope glycoproteins (Env) drove these responses, can assist in the design of strategies to elicit such responses by vaccination.

Methods
Here, Env-specific IgG+ B cells were isolated at various time points post infection from an HIV-1 infected elite neutralizer and single cell sorted to obtain monoclonal antibodies (mAbs). MAbs were characterized using neutralization assays and high-resolution cryo-electron microscopy to specify the exact epitopes targeted by these mAbs. In addition, we obtained HIV-1 Env sequences from the same individual at subsequent time points to study the epitopes that were under selective pressure overtime.

Results
Multiple MAb lineages with moderate neutralization breadth were isolated from various time points and targeted distinct epitopes on Env such as the gp41-gp120 interface, CD4-binding site, silent face and V3 region. None of the neutralizing mAbs could match the neutralization breadth present in the serum of the HIV-1 infected elite neutralizer from which the mAbs were isolated. However, combined the mAbs could potently neutralize a broad range of viruses (Figure 1). The high-resolution cryo-electron microscopy structures of the mAbs in complex with an Env trimer isolated from the same individual revealed that the mAbs use multiple strategies to neutralize the virus; blocking the receptor binding site, binding to HIV-1 Envs N-linked glycans and disassembly of the trimer. Sequence analysis of the longitudinal HIV-1 Env sequences isolated from the same individual demonstrated that the CD4-binding site and silent face targeting mAbs drove HIV-1 Env evolution in this individual.

Conclusion
The results suggests that a diverse set of mAbs with moderate neutralization breadth can complement each other to achieve a broad and potent neutralizing response in HIV-1 infected individuals. These findings have implications for HIV-1 vaccine design strategies to induce multiple bNAbs with moderate breadth instead of a single bNAb lineage to protect against HIV-1 infection.

01: Distinct neutralizing antibodies achieve an additive effect in neutralization breadth



02

PEOPLE WITH HIV AND SUPPRESSED VIREMIA ON ART ARE NOT AT INCREASED RISK FOR ACQUIRING SARS-COV-2 INFECTION

Myrthe Verburgh[1], Anders Boyd[2], Ferdinand Wit[2], Maarten Schim van der Loeff[3], Marc van der Valk[1], Marloes Grobben[4], Margreet Bakker[5], Marit van Gils[4], Neeltje Kootstra[6], Lia van der Hoek[5], Peter Reiss[7]

[1] *Amsterdam UMC, Department of Infectious Diseases, Amsterdam, the Netherlands*
[2] *HIV Monitoring Foundation, Amsterdam, the Netherlands*
[3] *Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands*
[4] *Amsterdam UMC, Department of Medical Microbiology, Amsterdam, the Netherlands*
[5] *Amsterdam UMC, Laboratory of Experimental Virology, Amsterdam, the Netherlands*
[6] *Amsterdam UMC, Department of Experimental Immunology, Amsterdam, the Netherlands*
[7] *Amsterdam UMC, Dept. of Global Health & Infectious Diseases, Amsterdam, the Netherlands*

Background
Few studies have prospectively compared the incidence of SARS-CoV-2 infection between individuals with well-controlled HIV infection and otherwise comparable HIV-negative controls and assessed whether having HIV infection with suppressed viremia on ART is an independent risk factor for SARS-CoV-2 acquisition.
To further address this question, we designed a substudy within the ongoing AGE$_h$IV Cohort Study in Amsterdam to prospectively determine incidence and risk factors for SARS-CoV-2 infection and development of COVID-19. Moreover, we compared longitudinal changes in SARS-CoV-2 nucleocapsid (N-) antibody titres between HIV-positive and -negative participants.

Methods
Starting in September 2020, consenting HIV-positive and HIV-negative AGEhIV participants are assessed 6-monthly for incident SARS-CoV-2 infection, by combined IgA/IgM/IgG SARS-CoV-2 nucleocapsid assay (INgezim®, Eurofins Ingenasa) – values ≥6 were considered seropositive. Cumulative incidence of SARS-CoV-2 infection and associated risk factors were assessed from 27 February 2020 (date of first documented infection in the Netherlands) through April2021 by complementary log-log regression for discrete-time survival functions. In those with incident SARS-CoV-2 infection, N-antibody titres, changes in titres and factors associated with these changes were compared between groups by linear regression.

Results
241 HIV-positive and 326 HIV-negative AGE$_h$IV participants participated in this study. Median age was 60.9 years and 94.9% were Caucasian, and not significantly different between groups. In HIV-positive participants, median time since HIV-diagnosis was 21.4 years, 99.2% had undetectable HIV-viral load and baseline median CD4 count was 680 cells/mm^3. Cumulative SARS-CoV-2 incidence through October2020 was 5.5% (n=13) and 6.7% (n=21) in HIV-positive and HIV-negative participants, respectively (p=0.557) and 11.9% (n=26) and 11.6% (n=35) through April2021 (p=0.964). Younger age, African descent and being overweight were associated with incident infection *(Table 1)*. In 61 participants with incident infection, median N-antibody titre was 34.2 (HIV-positive) and 27.6 (HIV-negative). Only self-reported fever, but not HIV-status, was independently associated with N-antibody titres (coefficient +9.73 [95%CI, 3.39-16.06]; p=0.003; interaction with HIV-status p=0.868).

Of 29 participants who were N-antibody-positive in October2020, samples were available in April2021. Titres did not differ significantly between HIV-positives and -negatives at baseline and neither at follow-up. By April2021 titres had declined significantly in HIV-positive participants *(Figure 1)*. Neither HIV-status nor any other factors were significantly associated with N-antibody titre change.

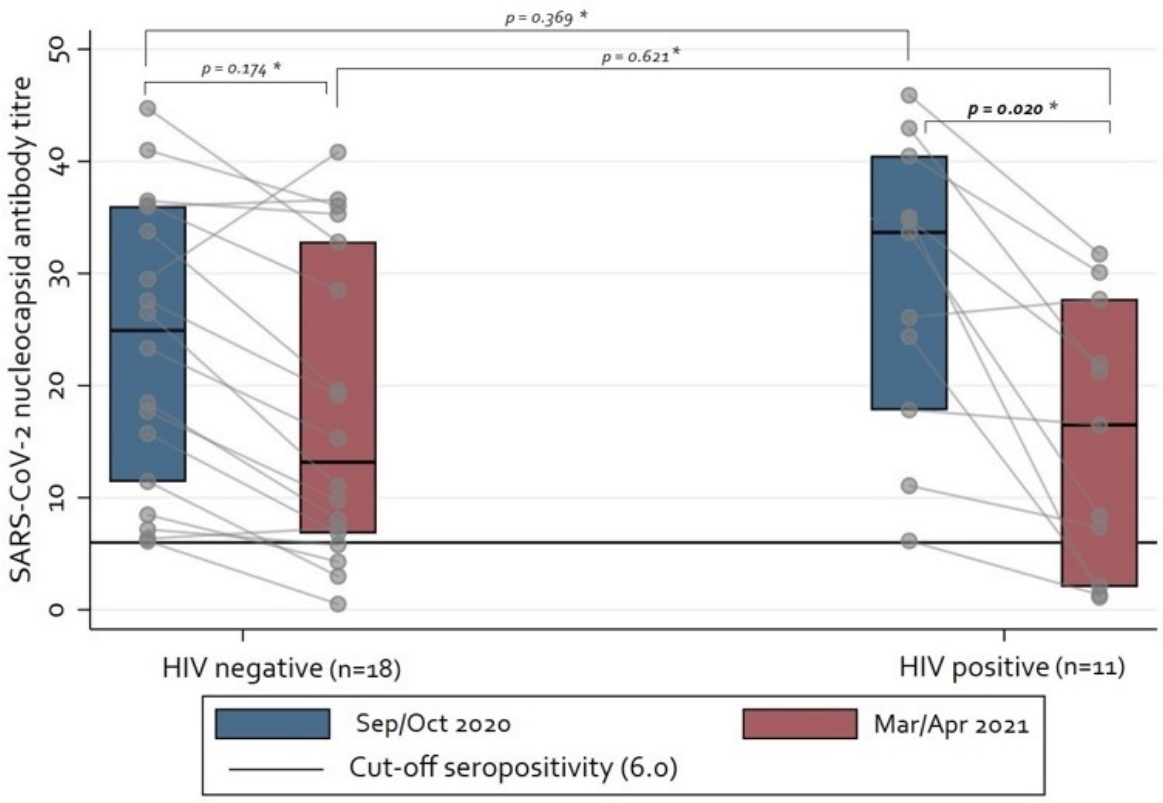
Conclusion
People with HIV and suppressed viremia with >500 CD4-cells/mm^3 on ART were not at increased risk of SARS-CoV-2 acquisition, and had comparable SARS-CoV-2 N-antibody responses in case of infection. Future analyses will address the immune responses to COVID-19 vaccines in this population.

O2 (Table 1): Factors associated with incident SARS-CoV-2 infection in participants of the AGEhIV COVID-19 substudy

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
HIV status				
HIV negative	REF	.964	REF	.640
HIV positive	1.01 (0.61-1.68)		1.13 (0.67-1.92)	
Age ~				
53 – 59 years	3.58 (1.28-10.01)	**.009**	3.68 (1.26-10.73)	**.009**
60 – 64 years	2.40 (0.80-7.21)		2.60 (0.82-8.21)	
65 – 69 years	0.94 (0.24-3.77)		0.93 (0.23-3.77)	
70 years and older	REF		REF	
Ethnic origin				
Caucasian	REF	**.006**	REF	**.005**
African	3.86 (1.69-8.79)		3.35 (1.60-7.05)	
Asian	1.12 (0.17-7.51)		1.71 (0.34-8.73)	
BMI #				
Underweight (<18.5 kg/m^2)	-	.083	-	**.038**
Normal weight (18.5-24.9 kg/m^2)	REF		REF	
Overweight (25.0-29.9 kg/m^2)	1.65 (0.98-2.77)		1.74 (1.02-2.96)	
Obese (≥30.0 kg/m^2)	0.70 (0.25-1.98)		0.63 (0.21-1.86)	
Total number of comorbidities #				
0	REF	**.049**		
1-2	0.50 (0.28-0.90)			
3 or more	0.52 (0.16-1.65)			
Self-reported compliance with social distancing measures ‡				
(Very) poorly	1.46 (0.53-3.99)	.062		
Neutral	REF			
(Very) well	0.59 (0.33-1.04)			
CD4 cell count (per 100 cells/mm^3 decrease)	1.05 (0.97-1.15)	.228		
**CD4 nadir $** (per 100 cells/mm^3 decrease)	1.07 (0.94-1.23)	.297		

Values represent hazard ratios (HR) with 95% confidence interval (CI). Values in bold indicate a *P* value <0.05.
Factors which were assessed univariably were age, gender, ethnicity, BMI, total number of comorbidities, presence of COVID-19 related symptoms , substance use (tobacco, alcohol, recreational drugs), number of sexual contacts, number of household contacts, self-reported compliance with the social distancing measures, baseline CD4 and CD8 cell counts and CD4/8 ratio, use of PrEP (in HIV-negative participants) and (in HIV-positive participants) HIV-specific parameters (nadir CD4 count, years since HIV diagnosis and years since start of antiretroviral treatment (ART)). The multivariable model was built using a step-wise backward selection procedure, assessing all variables associated with a p <0.20 in univariable analyses.
Abbreviations: BMI, body mass index; REF, reference group.
~ At moment of SARS-CoV-2 N-antibody test
# Last available data prior to baseline (defined as February 27, 2020)
‡ In the six months prior to the SARS-CoV-2 N-antibody test
$ Only in HIV positive (n=241)

O2 (Figure 1): Change in SARS-CoV-2 N-antibody titres in 29 (11 HIV-positive and 18 HIV-negative) participants with positive SARS-CoV-2 antibodies and with a second available measurement



Boxplots represent median and interquartile range. Grey dots represent individual measurements; grey lines represent individual changes in SARS-CoV-2 N-antibody titres. The black line represents the cut-off value for seropositivity of the combined IgA/IgM/IgG SARS-CoV-2 nucleocapsid assay by INgezim® (sensitivity 100%, specificity 98.2% [ref: Hoste et al. Diagn Microbiol Infect Dis. 2020])
* Wilcoxon rank-sum test

03

STRONG REDUCTION IN INCIDENCE OF COVID-19-RELATED HOSPITALIZATION AND DEATH AMONG PEOPLE LIVING WITH HIV IN THE NETHERLANDS FOLLOWING THE IMPLEMENTATION OF THE NATIONAL SARS-COV-2 VACCINATION PROGRAM

Ferdinand Wit[1], Peter Reiss[1], Bart Rijnders[2], Casper Rokx[2], Anna Roukens[3], Kees Brinkman[4], Marc van der Valk[1]

[1] *Stichting HIV Monitoring, Amsterdam, the Netherlands*
[2] *Erasmus UMC, Rotterdam, the Netherlands*
[3] *Leiden UMC, Leiden, the Netherlands*
[4] *Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands*

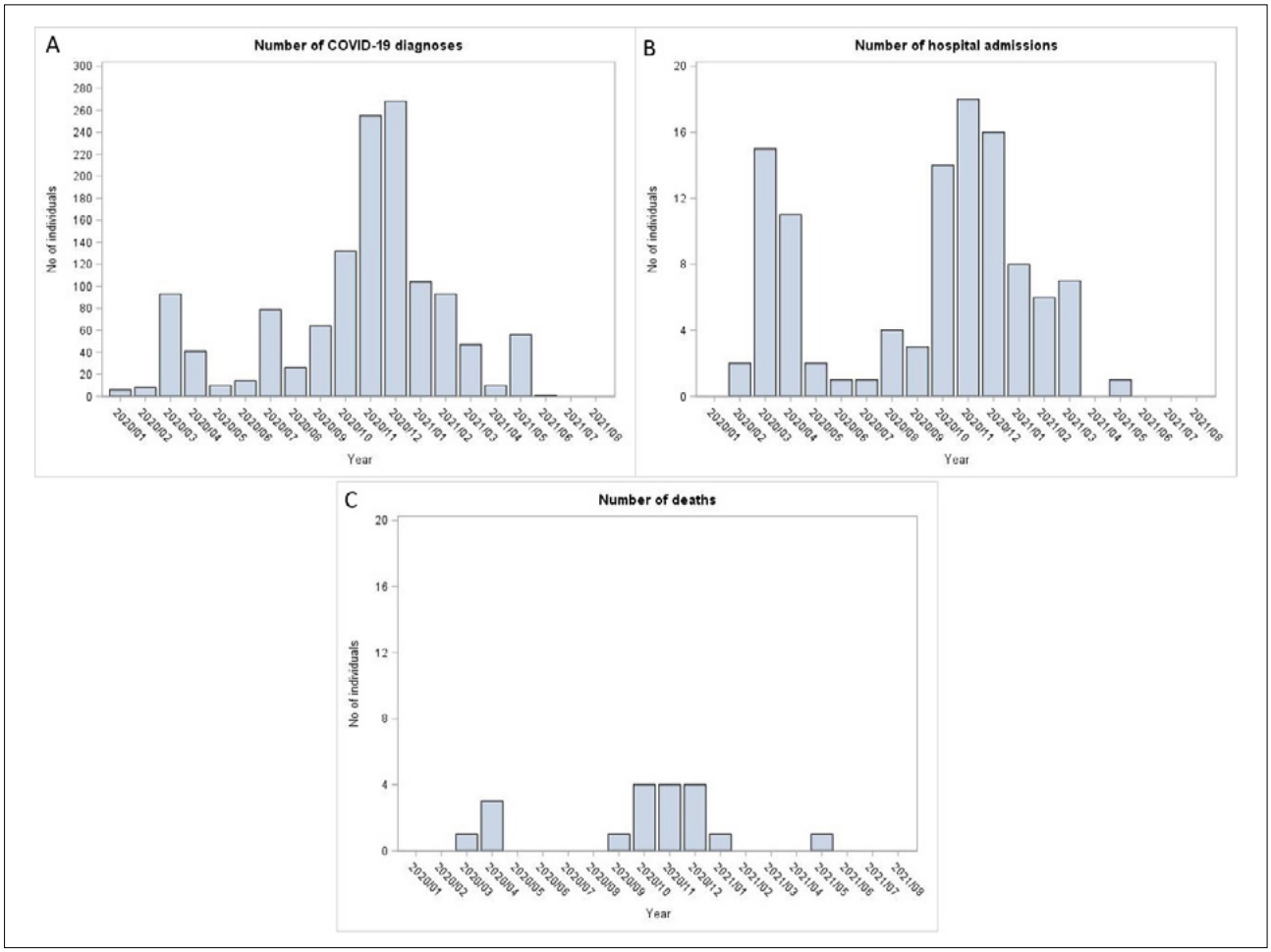
Background
By September 2021 82.1% of the Dutch general adult population, and an even higher proportion of vulnerable, including older individuals had been fully vaccinated against SARS-CoV-2. Vaccine uptake has resulted in a strong decline of severe COVID-19 cases. Currently, hospitalizations for COVID-19 are mainly among partially or unvaccinated individuals, or those poorly responding to vaccination. Whether vaccinated adult people living with HIV (PLHIV) are adequately protected from severe COVID-19 remains to be adequately documented.

Methods
Stichting HIV Monitoring (SHM) prospectively collects relevant HIV- and ART-related data, including diagnosis of and hospitalizations for COVID-19, on all consenting PLHIV in the Netherlands. SHM uses electronic queries of hospital medical records to quickly identify diagnoses of and hospitalizations for COVID-19, including after the national SARS-CoV-2 vaccine program was initiated in January 2021. Details regarding diagnosis, disease severity, hospitalizations and outcomes of COVID-19 are also prospectively collected. Risk factors for severe COVID-19 were investigated using multivariable logistic regression.

Results
Up to September 2021, SHM collected data on 1320 SARS-CoV-2 infections (mostly symptomatic) in adult PLHIV (see Table), 109 of which resulted in hospitalization, and 18 of those in ICU admission. Independent risk factors for hospitalization were age (odds ratio 1.66 per 10 years increase), African (OR 2.7) and Latin American (OR 1.2) origin, obesity (OR 2.2), chronic kidney disease (OR 4.1), current CD4 count below 500/mm^3 (CD4 0-199: OR 4.4; CD4 200-499: OR 2.1), prior AIDS diagnosis (OR 1.9).
In total, 19 (1.4%) out of these 1320 PLHIV diagnosed with SARS-CoV-2 infection died as a direct result of COVID-19.
SARS-CoV-2 vaccine uptake was rapid and high among PLHIV in the Netherlands, once becoming eligible from April 2021 onwards. The Figure shows that the number of diagnoses (panel A), hospitalizations (panel B) and deaths (panel C) followed the same pattern as observed in the general Dutch population for the first and second COVID-19 waves. However, from April 2021 onwards the number of incident diagnoses, hospitalizations and COVID-19-related deaths among PLHIV declined strongly. Notably, no increase in incidence was apparent since the start of the third wave in the Netherlands in July 2021.

Conclusion
These findings, pending data on individual vaccination status and without being able to rule out potential delayed reporting of some COVID-19 diagnoses and hospitalizations to SHM, strongly suggest that implementing SARS-CoV-2 vaccination for PLHIV in the Netherlands has resulted in marked protection against the development of and hospitalization for severe COVID-19.

03 (Figure 1)


A
Number of COVID-19 diagnoses
No of individuals
Year
B
Number of hospital admissions
No of individuals
Year
C
Number of deaths
No of individuals
Year

O3 (Table 1)

	Hospitalized	Emergency room visit, but not hospitalized	Not hospitalized
N	109	37	1174
Age, years	57.0 (51.2-65.2)	53.2 (46.6-58.7)	49.1 (40.1-56.9)
Male sex	75.9%	70.3%	81.7%
HIV transmission category			
- MSM	42.6%	37.8%	67.4%
- Other men	33.3%	32.4%	14.3%
- Women	24.1%	29.7%	18.3%
Region of origin			
- Netherlands / Europe / North America	50.5%	59.3%	67.2%
- Sub-Saharan Africa	24.7%	18.5%	12.4%
- Latin America / Caribbean	24.7%	22.2%	20.4%
Years known HIV positive	15.8 (9.9-22.5)	13.2 (9.6-19.6)	11.9 (6.9-17.2)
On ART	99.0%	100%	99.3%
HIV viral load >200 cp/mL	5 (4.7%)	3 (8.3%)	22 (1.9%)
Viral load (when detectable) in cps/mL	40,000 (6,049-62,743)	3,935 (557-43,000)	1,046 (588-52,488)
Current CD4 count, mm^3	559 (396-821)	600 (490-780)	718 (539-890)
Nadir CD4 count, mm^3	176 (60-275)	240 (74-353)	266 (140-398)
Prior AIDS diagnosis	38.0%	21.6%	18.1%

Legend: N (%) or median (IQR), as appropriate.

04

CASCADE OF CARE FOR PEOPLE LIVING WITH HIV IN ARUBA AND CURAÇAO

Marlous Rodriguez[1], Jaclyn De Kort[2], Diederik Van de Wetering[3], Ashley Duits[4], Bert Rodenburg[2], Athalia Alberto[3], Karina Kelly[2], Monique Nijhuis[1], Esther Rooijakkers[3], Andert Rosingh[5], Ard Van Sighem[6], John De Wit[7], Annemarie Wensing[1], Marije Hofstra[1]

[1] *Translational Virology, UMC Utrecht, Utrecht, the Netherlands*
[2] *Department of Infectious Diseases, Dr. Horacio E. Oduber Hospital, Oranjestad, Aruba*
[3] *Department of Infectious Diseases, Curaçao Medical Center, Willemstad, Curaçao*
[4] *Red Cross Blood Bank Foundation, Willemstad, Curaçao*
[5] *Department of Medical Microbiology, Lab HOH, Oranjestad, Aruba*
[6] *Stichting HIV Monitoring, Amsterdam, the Netherlands*
[7] *Interdisciplinary Social Sciences, University Utrecht, Utrecht, the Netherlands*

Background
The HIV epidemic on the Dutch Caribbean islands is growing. Insight in the cascade of care is urgently needed. We compared characteristics of people living with HIV (PLHIV) in the two largest islands, and identified existing barriers for curbing the HIV epidemic.

Methods
Clinical data was collected of all PLHIV in care in Aruba in the past decade to determine the cascade of care. For Curaçao, clinical data was collected by Stichting HIV Monitoring. A subset of patients completed an additional questionnaire (Aruba, n=91 (newly diagnosed 2018-2020 n=30); Curaçao n=117 (newly diagnosed 2018-2020 n=36)).

Results
In Aruba and Curaçao, 376 and 1283 individuals were diagnosed with HIV-1 (Table 1). The average number of patients yearly entering care has remained around 50 in Curaçao, compared to an increase from 18 (2010-2012) to 34 (2017-2019) in Aruba. HIV-1 positive migrants in Aruba (43% of population) mainly originate from Colombia (16%) and Venezuela (10%), whereas in Curaçao migrants (23%) mainly originate from Dominican Republic (7%) and Haiti (7%). The most reported route of transmission is MSM in Aruba (65%) and heterosexual contact in Curaçao (61%). Women represent only 14% of the known HIV-1 population in Aruba compared to 38% in Curaçao. Of those diagnosed in Aruba, 52% presented late (baseline CD4 <350 and/or AIDS-defining illness) and the delay to entry in care after the first positive test was median 16 days (IQR:2-68). In Curaçao, 59% presented late between 2000-2017, which remained high (64%) among patients newly diagnosed in 2018 and onwards.

Although only 20% of newly diagnosed individuals perceived themselves at high risk for HIV before diagnosis, 70% indicated they had postponed HIV testing because they were afraid of the result. 65% and 56% indicated they had never heard of PEP or PrEP respectively. More than half of individuals in care did not inform family (46%) or close friends (63%) about their status, mostly due to stigma and fear of rejection.

Early 2021, 312 and 669 individuals were still in care in Aruba and Curaçao respectively, and the majority (Aruba: 80.8%; Curaçao 88.6%) was virologically suppressed on ART (Table 1).

Conclusion
While the characteristics of PLHIV differ between the islands, the gaps in the cascade of HIV care are similar. Earlier diagnosis, linkage to care and subsequent retention in care are essential to curb the increasing HIV incidence yet this is challenged by unawareness of HIV risks and prevention, migration(laws) and stigma.

04 (Table 1): Cascade of care for PLHIV in Aruba (2010-2020) and Curaçao (<1999-2020)

	Aruba, N (%)	Curaçao, N (%)
Diagnosed and linked to care	376	1283
Deceased	18 (4.8)	194 (15.1)
Migrated	27 (7.2)	140 (10.9)
Retention in care		
Retained in care	312/331 (94.3)	669/949 (70.5)
Lost to care	19 /331 (5.7)	280/949* (29.5)
On antiretroviral therapy	308/ 312 (98.4)	663/669 (99.1)
Viral load follow up		
Viral load < 50	252 (80.8)	574 (86.6)
Viral load 50 – 500	30 (9.6)	36 (5.4)
Viral load > 500	12 (3.8)	15 (2.3)
No laboratory results available	14 (4.5)	38 (5.7)

* 86/280 were lost to care before 2010, and are unlikely to be living in Curaçao without needing care or antiretroviral treatment. 194 were lost to care after 2010; 63 (32%) were born outside of former Netherlands Antilles and have potentially migrated to their country of origin.

05

ESTIMATING THE PREVENTION POTENTIAL OF HIV INFECTIONS IN AMSTERDAM MSM, HETEROSEXUALS, AND MEN AND WOMEN WITH A MIGRATION BACKGROUND

Alexandra Blenkinsop[1,3], Nikos Pantazis[2], Melodie Monod[3], Ard Van Sighem[4], Daniela Bezemer[4], Eline Op de Coul[5], Thijs Van de Laar[6], Maria Prins[7], Peter Reiss[7], Godelieve De Bree[7], Oliver Ratmann[3]

[1] *AIGHD, Amsterdam, the Netherlands*
[2] *University of Athens, Athens, Greece*
[3] *Imperial College London, London, United Kingdom*
[4] *Stichting HIV Monitoring, Amsterdam, the Netherlands*
[5] *RIVM, Bilthoven, the Netherlands*
[6] *Sanquin, Amsterdam, the Netherlands*
[7] *Amsterdam UMC, Amsterdam, the Netherlands*

Background
Amsterdam and other UNAIDS Fast-Track cities aim for zero new HIV infections. Our primary aims are to estimate the proportion of undiagnosed infections and the proportion of locally acquired HIV infections in Amsterdam in 2014-2019, i.e. infections that could have been locally averted, both in MSM and heterosexual individuals, and Dutch-born and foreign-born individuals.

Methods
We located diagnosed HIV infections in Amsterdam using postcode data (PC4) at time of registration in ATHENA, and estimated their infection times using clinical data (Pantazis, 2019). We then inferred the proportion of undiagnosed from the estimated times to diagnosis. To determine the sources of Amsterdam infections, we used HIV sequences of Amsterdam patients to phylogenetically reconstruct transmission chains, and tabulate their growth in 2014-2019. Frequent late diagnoses indicate that more recent phylogenetically observed chains are increasingly incomplete, and we use a Bayesian model similar to (Bezemer, 2021), to estimate the actual growth of Amsterdam transmission chains, and the proportion of locally acquired infections.

Results
846 patients were diagnosed in Amsterdam in 2014-2019. Of these, an estimated 537 were infected since 2014, and 36% were in Dutch MSM, 50% in non-Dutch MSM, 4% in Dutch heterosexuals and 10% in non-Dutch heterosexuals. Concomitantly, an estimated 13% of new infections in 2014-2019 in Dutch MSM from Amsterdam remained undiagnosed by 2019, and 18% in non-Dutch MSM, 27% in Dutch heterosexuals and 42% in non-Dutch heterosexuals. Figure 1 illustrates the phylogenetically reconstructed Amsterdam transmission chains. Adjusting for undiagnosed and unsequenced individuals, we estimate there were 421 ongoing transmission chains with at least one not virally suppressed individual by 2014, of which 70% had no new infection in 2014-2019 and 30% had at least one new infection since 2014. In 2014-2019, we estimate there were in Amsterdam MSM 183 emergent chains and 550 new infections, while in heterosexuals, there were 60 emergent chains and 117 new infections. Since 2014, in Amsterdam MSM, the estimated proportion of locally acquired infections was 67% [59-74%], with no substantial differences in migrant groups. In Amsterdam heterosexuals, this was 55% [38-70%] overall, highest in heterosexuals born in South America and the Caribbean (64% [44-81%]), and lowest in heterosexuals born in Sub-Saharan Africa (44% [20-66%]).

Conclusion
The data indicate substantial potential to further curb local transmission, in both MSM and heterosexual Amsterdam residents. Foreign-born MSM would likely benefit most from intensified interventions, as in 2014-2019 most local transmissions in Amsterdam occurred in this group.

O5 (Figure 1): Phylogenetically reconstructed Amsterdam transmission chains A

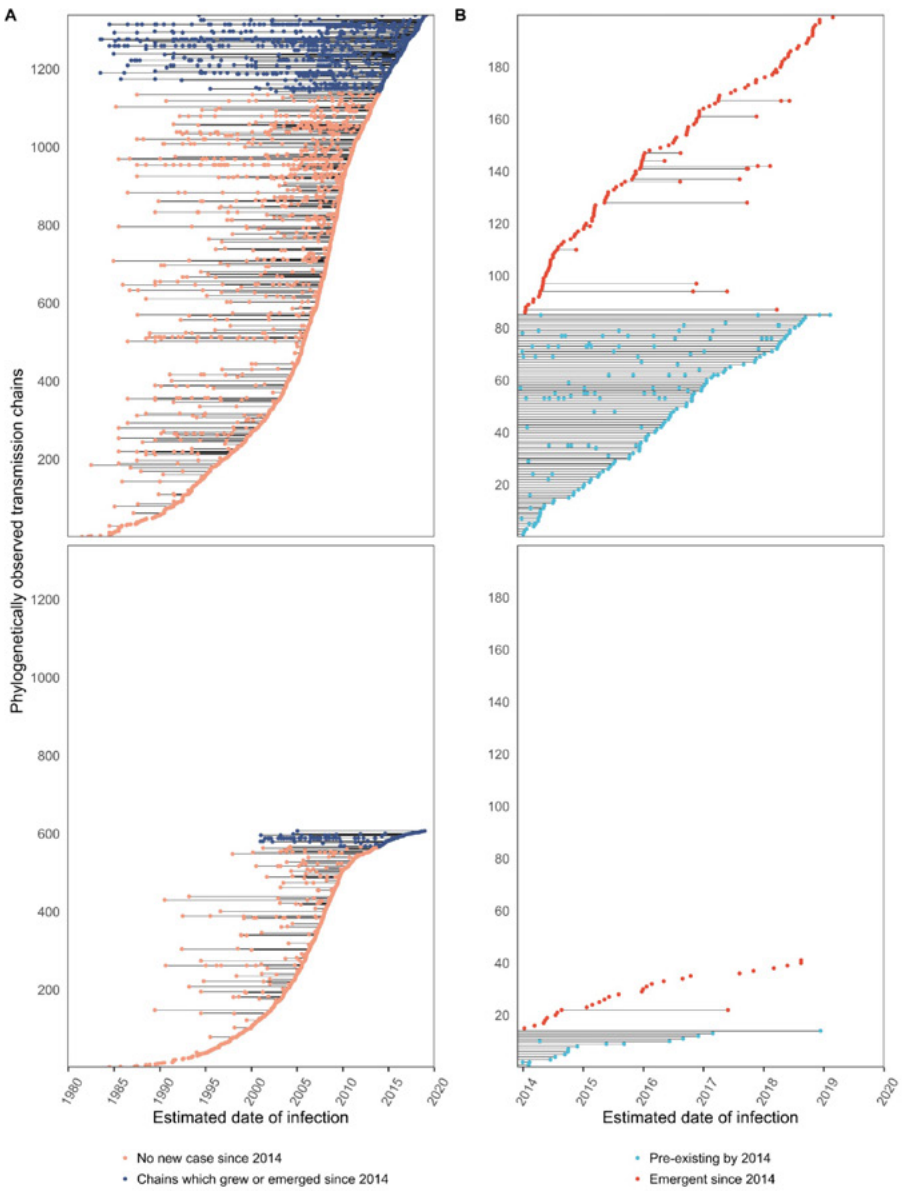


Figure 1: Phylogenetically reconstructed Amsterdam transmission chains *A) Each phylogenetic Amsterdam chain was identified in a background of Dutch and international HIV sequences, and is shown as a separate horizontal line. Chains in MSM and heterosexuals from Amsterdam are shown separately in the two rows. Phylogenetically observed chains are ordered by chains which had no new case since 2014, and those which continued to grow. B) Growth of transmission chains since 2014, by those which were pre-existing (blue), and those which emerged since 2014 (red).*

05 (Figure 2): Estimated proportion of locally acquired HIV infections amang Amsterdam residents in 2014-2019.

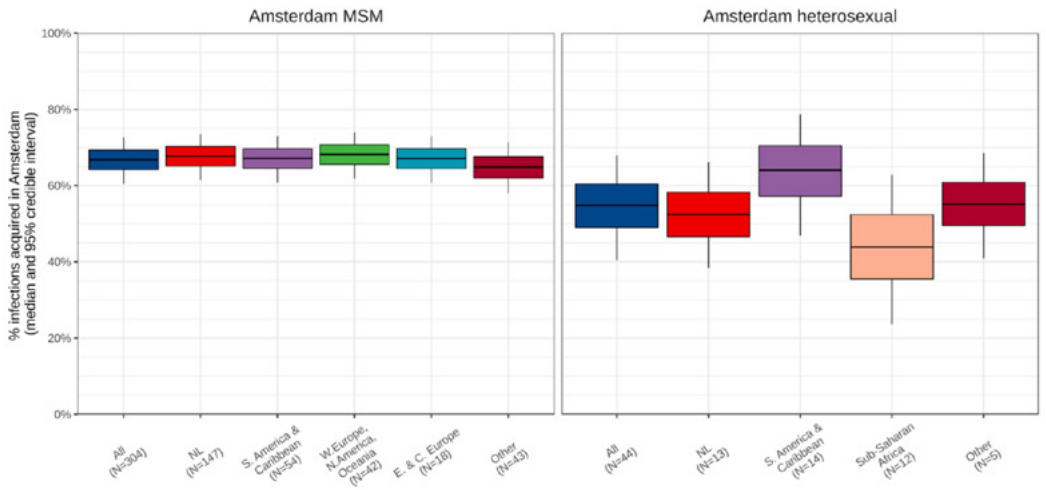


Figure 2: Estimated proportion of locally acquired HIV infections among Amsterdam residents in 2014-2019. *Estimates are based on phylogenetically reconstructed transmission chains, and independently estimated infection times, and account for undiagnosed and unsequenced individuals. Posterior median estimates (black horizontal bar) are shown with interquartile ranges (box) and 95% credible intervals (linerange) for Amsterdam MSM (left) and heterosexuals (right), stratified by place of birth. N=number of diagnosed infections in Amsterdam with an HIV sequence available.*

06

DEFINING HIV-1'S DOORWAY INTO THE INTESTINAL MUCOSA USING AN ADVANCED HUMAN PRIMARY GUT-EPITHELIAL-IMMUNE CELL CO-CULTURE ORGANOID MODEL

Anusca Rader, Alex Cloherty, Athanasios Koulis, Kharishma Patel, Dasja Pajkrt, Katja Wolthers, Adithya Sridhar, Renee Schreurs, Carla Ribeiro

Amsterdam UMC, Amsterdam, the Netherlands

Background
The gastrointestinal tract is an important entry site for HIV-1 during mother-to-child or sexual transmission. Additionally, the intestinal mucosa houses HIV-1 target cells, including dendritic cells (DCs) and CD4+ T cells, in which HIV-1 replicates and latently persists in reservoirs. Despite the key role of the intestine in HIV-1 pathogenesis, little is known about the mechanism of HIV-1 entry into the intestinal mucosa due to the lack of relevant *in vitro* human intestinal models. We developed a human primary gut-epithelial-DC co-culture organoid model, which recapitulates the cellular composition, polarization and barrier function of the human intestinal epithelium *in vivo*. Here, we utilized this co-culture model to elucidate the mucosal events and mechanisms required to establish intestinal HIV-1 infection, and define the impact of enteric HIV-1 infection on mucosal immunity.

Methods
Human primary intestinal organoids were used to generate gut epithelial monolayers on collagen-coated transwell inserts, which were subsequently co-cultured with human DCs. Barrier integrity was monitored by trans-epithelial electrical resistance and FITC-Dextran permeability assay. Intestinal inflammation was monitored by assessing cytokine production using ELISA assays. HIV-1 internalization route and cellular tropism were determined by confocal imaging with lineage and subcellular membrane markers. HIV-1 uptake and transmission rates were quantified by flow-cytometry.

Results
Exposure of gut epithelial organoid monolayers to different HIV-1 variants resulted in rapid internalization of HIV-1 viruses into a subset of human intestinal epithelial cells. Markedly, after viral internalization, we demonstrated HIV-1 sequestration into specialized intracellular vesicles. Subsequently, intestinal epithelial cells were able to transmit the sequestered replicative-competent HIV-1 virions across the gut barrier and into the mucosal compartment, without loss of tight-junction functioning. Furthermore, virus-induced intestinal inflammation was observed as indicated by the increased secretion of pro-inflammatory biomarkers including IL-1β. Gut epithelial monolayers were successfully co-cultured with human DCs. Imaging analyses confirmed DC attachment and formation of DC protrusions across the epithelial barrier. Notably, incorporation of lumen-sampling DCs, strongly increased HIV-1 transmission rate across the intestinal epithelial barrier.

Conclusion
Our findings identified a novel vesicle-mediated transcytosis pathway by human intestinal epithelial cells as a mechanism of enteric HIV-1 entry into the intestinal mucosa, and highlights the relevancy of lumen-sampling DCs in establishing intestinal HIV-1 infection.
Our co-culture model spearheads the application of *in vitro* organoid systems in human intestinal HIV-1 pathogenesis studies. Expanding the model with additional cell types (such as CD4+T cells) permits investigating HIV-1 reservoir formation at mucosal surfaces, and establishment of a platform for antiviral drug testing.

07

CONTINUOUS DECLINE OF INTACT PROVIRAL DNA AFTER TWO DECADES OF ANTIRETROVIRAL THERAPY

Marieke Nühn[1], Jori Symons[1], Kobus Bosman[1], Aridaman Pandit[2], Kiki Tesselaar[2], Joop Arends[3], José Borghans[2], Dorien De Jong[1], Theun De Kort[1], Sigrid Otto[2], Terry Huisman[4], Eduardo Lucio de Esesarte[4], Andy Hoepelman[3], Rob De Boer[4], Annemarie Wensing[1], Monique Nijhuis[1]

[1] *Translational Virology, Medical Microbiology, UMC Utrecht, Utrecht, the Netherlands*
[2] *Center for Translational Immunology, UMC Utrecht, Utrecht, the Netherlands*
[3] *Internal Medicine and Infectious Diseases, UMC Utrecht, Utrecht, the Netherlands*
[4] *Theoretical Biology, Utrecht University, Utrecht, the Netherlands*

Background
Implementation of antiretroviral therapy (ART) results in suppression of viral replication but does not eliminate HIV that persists as a proviral reservoir in $CD4^+$ T cells. The small population of replication-competent provirus forms the obstacle to HIV cure. However, the detection of this reservoir is challenging because of the predominance of defective proviruses. Therefore, limited data on the size and dynamics of this replication-competent reservoir, decades after ART initiation is available. Moreover, little is known about the contribution of the different T-cell subsets to this replication-competent reservoir after ART initiation. To elucidate this, we conducted a unique longitudinal study in 9 patients who initiated ART over two decades ago.

Methods
PBMCs were obtained before ART initiation, and 1, 10 and 20 years after start of treatment. DNA isolated from PBMCs and sorted T-cell subsets (naïve, central-memory, transitional-memory, effector-memory, and effector T cells) at 10 and 20 years after ART was analysed using the intact proviral DNA assay (IPDA). This allowed us to quantify both the defective and the intact proviral DNA. Samples in which the DNA was sheared for less than 50 percent and which showed at least 5 positive proviral DNA copies were included for analysis.

Results
The HIV DNA load in PBMCs declined significantly and continuously within both the defective and the intact reservoir during 20 years of ART. The intact reservoir declined significantly faster than the defective reservoir. At 10 and 20 years after ART initiation, we analysed T-cell subsets and observed that the memory T-cell subsets (transitional memory, central memory and effector memory) harboured the largest fraction intact proviral DNA compared to other T-cell subsets. Moreover, a trend of declining intact proviral DNA load was observed in the transitional memory cells during this decade.

Conclusion
We show that the size of both the defective and the intact proviral reservoir continuously declined during 20 years of ART. The decline of the intact reservoir is stronger than the decline of the defective reservoir and within the sorted T-cell subsets this difference is most pronounced in the transitional memory cells. The intact proviral DNA is most abundant in the memory T cells and could be a source of ongoing viral production. Consequently, the observed continuous decline within the intact proviral reservoir might be caused by virus-induced or immune-mediated cell killing. Altogether, this study provides more insights in the dynamics of the replication-competent reservoir during prolonged ART.

08

IDENTIFYING HOST GENETIC DETERMINANTS OF HIV-1 RESERVOIR MARKERS REVEALS PTDSS2, RNH1 AND IRF7 AS POTENTIAL MODIFYING FACTORS IN HIV-1 PATIENTS

Zhenhua Zhang[2], **Marc Blaauw[1]**, Wim Trypsteen[3], Jessica Dos Santos[1], Li Yang[2], Andre Van de Ven[1]

[1] *Radboudumc, Nijmegen, the Netherlands*
[2] *University of Groningen, Groningen, the Netherlands*
[3] *Ghent University, Ghent, Belgium*

Background
Combination antiretroviral treatment (cART) cannot eradicate HIV-1 from the body due to the establishment of persisting viral reservoirs which reinitiate new rounds of HIV-1 replication after treatment interruption. These HIV-1 reservoirs mainly comprise long-lived resting memory CD4+ T cells and show a high variability in size or activity among virally suppressed individuals. Therefore, the identification of host factors that contribute to this observed variation could open avenues for new HIV-1 treatment strategies.

Methods
In this study, we conducted a genome-wide quantity trait locus (QTL) analysis to probe functionally-relevant genetic variants linked to levels of cell-associated (CA)-HIV-1 DNA, CA-HIV-1 RNA and RNA:DNA ratio in CD4+T cells isolated from whole blood from a cohort of 207 (Caucasian) HIV-1 patients under long-term suppressive cART (median = 6.6 years).CA-HIV-1 DNA and CA-HIV-1 RNA levels were measured with corresponding droplet digital PCR assays and genotype information of 522,455 single nucleotide variants (SNV) was retrieved via the Infinium Global Screening array platform.

Results
The QTL mapping analysis involved an additive linear regression model with a correction for age, gender, CD4 nadir and HIV-1 duration and identified one significant genetic locus associated with CA-HIV-1 DNA (PTDSS2, p-value < 5 × 10-8), while four associations were found for RNA:DNA ratio (RNH1, IRF7, DEAF1 and RP11-1149M10.2, p-value < 5 × 10-7). Next, we validated that the IRF7 and RNH1 SNV are significantly correlated with higher expression (qPCR) of these genes in peripheral blood mononuclear cells (PBMC) from HIV-1 patients. IRF7 SNV influences the IFN-y production capacity of *ex-vivo* stimulated PBMCs with TLR7 agonist, supporting its functional role in HIV-1 infection.

Conclusions
The presented data suggests that the amount of CA-HIV-1 DNA and RNA:DNA ratio could be influenced through specific genetic variants of the PTDSS2, RNH1 and IRF7 loci. Especially, the IRF7 and RNH1 SNV are functionally linked to higher expression levels of its gene product and IRF7 SNV modifies IFN-y levels which contribute to the control of the relative HIV-1 transcriptional activity and associated immunologic burden. These observations provide novel knowledge on the molecular mechanisms involved in HIV-1 reservoir establishment and/or maintenance and could indicate targets for future therapeutic strategies to lower HIV-1 reservoir size or activity in patients.

09

GENETIC VARIATION IN AUTOPHAGY MACHINERY IS ASSOCIATED WITH ENHANCED ANTIVIRAL T-CELL IMMUNITY AND REDUCED T-CELL EXHAUSTION SIGNATURES IN HIV-1 INFECTED INDIVIDUALS

Renée Schreurs, Athanasios Koulis, Alexandra Cloherty, Neeltje Kootstra, Carla Ribeiro

Amsterdam UMC, Amsterdam, the Netherlands

Background

Antiretroviral therapy is effective, however treated HIV-1 patients suffer from severe comorbidities due to chronic inflammation and immune dysfunction. Autophagy functions as an antiviral defense mechanism and regulates adaptive T-cell-mediated immunity. We demonstrated that autophagy restricts mucosal HIV-1 infection by degrading HIV-1 through Tripartite motif-containing protein 5 (TRIM5α)-mediated autophagy pathway. Notably, we identified a genetic polymorphism within this pathway, which was associated with delayed disease progression and improved survival of HIV-1-infected individuals from the Amsterdam Cohort Studies (ACS), confirming the *in vivo* relevance of this genetic variant in chronic HIV-1 infection. Here, we present the functional impact of this autophagy polymorphism on T-cell proliferation and immunophenotype including signatures associated with T cell activation, differentiation and exhaustion.

Methods

Cryopreserved peripheral blood mononuclear cells homozygous for the major allele (wild-type) or homozygous for the minor allele (carrying autophagy polymorphism) from healthy donors or HIV-1-infected individuals were utilized. High-parameter flow-cytometry with cell-tracer and canonical autophagy marker (LC3) in combination with T-cell-associated markers including lymph-node homing (CCR7), antigenic stimulation (CD45RA), activation (CD25), regulation (FoxP3), co-stimulation (CD28), homeostatic proliferation (CD127), and co-inhibition/exhaustion (PD-1) were used to decipher T-cell proliferation and immunophenotype at baseline and upon T-cell receptor (TCR)-crosslinking or stimulation with viral peptide pools. T-cell transcriptional profiling was determined by RNA-sequencing.

Results

T cells from individuals homozygous for the minor allele displayed enhanced basal levels of autophagy. Notably, heightened autophagy activity was associated with increased proliferative capacity of both CD4+T and CD8+T cell compartments. At baseline, frequencies of CD4+T CCR7+CD45RA+ naïve, CD45RA-expressing CCR7- effector memory, and CD127-CD25+FoxP3+ regulatory cells were increased. Furthermore, TCR-crosslinking resulted in modulated expression levels of CCR7, CD28, CD127, CD25, and PD-1 in minor genotyped T cells, suggesting enriched regulation and suppression of T-cell exhaustion/senescence. In addition, gene set enrichment and pathway analyses identified transcriptome signatures unique to minor genotyped T cells with enrichment in cell-cycling, mitochondria metabolism and interferon-dependent networks.

Conclusion

Our findings on this newly described autophagy polymorphism underscore the protective role of autophagy in the clinical outcomes of HIV-1 infected individuals. Our data demonstrate the intrinsic impact of heightened autophagy on ensuing appropriate adaptive anti-HIV-1 T-cell immunity accompanied by skewing the T-cell compartment towards a regulatory and non-exhausted immunophenotype enriched in transcriptional networks associated with longevity. Thus, these findings underscore the therapeutical potential of autophagy-targeting strategies as innovative host-directed antivirals to potentiate the current antiretroviral therapy in restoring immune function and reducing immune activation in chronic HIV-1 infection.

O10

TRENDS IN HIV STIGMA EXPERIENCED BY PEOPLE WITH HIV IN THE NETHERLANDS: A COMPARISON OF CROSS-SECTIONAL SURVEYS OVER TIME

Sarah Stutterheim[1], Kyran Kuijpers[1], Moon Waldén[1], Renee Finkenflügel[2], Pieter Brokx[2], Arjan Bos[3]

[1] *Maastricht University, Maastricht, the Netherlands*
[2] *Hiv Vereniging, Amsterdam, the Netherlands*
[3] *Open Universiteit, Heerlen, the Netherlands*

Background
Since its onset in the 1980s, HIV has been a highly stigmatized condition. It occurs across a variety of settings, and has detrimental consequences for the treatment cascade and quality of life. Developments in treatment and prevention such as U=U and PrEP have significant potential to reduce HIV stigma. As such, in this study, we investigated whether HIV stigma in the Netherlands has changed in recent years. We also set out to confirm established psychological, social, and physical correlates of HIV stigma in people with HIV in the Netherlands in 2020. Lastly, we assessed U=U and PrEP awareness and impact.

Methods
To ascertain trends in stigma over time, we compared data collected from people with HIV in the Netherlands in 2007 (*n*=667) and, specifically for the health care sector, 2009 (*n*=262), to data acquired in 2019 and 2020 (*n*=258). For each setting (e.g. family, friends, work) and manifestation (e.g. avoidance, blame, excessive preventive measures), we conducted Pearson's chi-square tests. Then, using the 2020 data, we confirmed associations between perceived HIV stigma and psychological distress (including depression and anxiety), self-esteem, social support, and both treatment and clinic adherence. Lastly, we established the extent to which people with HIV were aware of U=U and PrEP, and, among those aware, the perceived impact of U=U and PrEP on their experience of having HIV.

Results
Comparing the 2007 to the 2019/2020 data, we ascertained reductions in experienced stigma from friends, family, acquaintances, at work, in the financial services sector, and in media. However, stigmatizing messages in media remained highly prevalent (70.9%). Stigma in the LGBTQI+ community, with sexual partners, and while partying remained prevalent (56.0%-58.6%) and, disconcertingly, relatively unchanged. Stigma in health care increased (34.3% vs 26.2%) for a number of stigma manifestations, including physical distance, excessive preventive measures, awkward social interactions, avoidance, and unnecessary referrals. As expected, HIV stigma was positively related to psychological distress, and negatively related to social support and medication adherence. Further, most participants were familiar with U=U and PrEP, but 13.3% questioned the accuracy of U=U.

Conclusion
Stigma interventions are imperative, particularly given the negative psychological, social, and health impacts. Contexts currently most in need of stigma reduction interventions are the media, the LGBTQI+ community and dating, and the health care sector. U=U messaging is a key tool in this regard as it can significantly reduce fear of (occupational) infection and contribute to the normalization of HIV.

S1

AVAILABILITY AND ACCESSIBILITY OF HIV SELF-TESTS AND SELF-SAMPLE KITS AT COMMUNITY PHARMACIES IN THE NETHERLANDS

Chaima Kandil[1], Jacqueline Hugtenburg[2], Titia Heijman[3], Hanna Bos[4], Martina Teichert[5], Renee Finkenflügel[6], **Eline Op de Coul[1]**

[1] *RIVM, Bilthoven, the Netherlands*
[2] *Amsterdam UMC, location VU, Amsterdam, the Netherlands*
[3] *Public Health Service of Amsterdam, Amsterdam, the Netherlands*
[4] *Soa Aids Nederland, Amsterdam, the Netherlands*
[5] *Royal Dutch Pharmacists Association (KNMP), Den Haag, the Netherlands*
[6] *HIV vereniging, Amsterdam, the Netherlands*

Background
In 2016, the WHO declared HIV self-testing and self-sampling an effective and safe test option that can reduce testing barriers, and offers convenience and anonymity for end-users. Supporting the use of HIV self-tests and self-sampling kits (HIV-ST/SS) is not included in the Dutch national HIV policy, but HIV-ST/SS are available in the Netherlands for purchase online and at community pharmacies since 2019. We aimed to explore the availability and accessibility of HIV-ST/SS in community pharmacies.

Methods
A cross-sectional survey by email among all community pharmacies (n=1,987) in the Netherlands. Questionnaires were distributed in April-June 2021 through the Royal Dutch Pharmacists Association (KNMP). Pharmacy/pharmacist characteristics associated with the offering of HIV-ST/SS were explored by univariable and multivariable logistic regression analysis. Experiences of pharmacists with the HIV-ST/SS offer were descriptively analyzed.

Results
Of the 1,987 pharmacies, 465 (23.4%) participated in the survey. Availability of HIV-ST/SS in the responding pharmacies was low: 6.2% (29/465). Of these 29 pharmacies, the majority (78.6%) sold between 0 to 20 HIV-ST/SS per year (estimated total ± 170 self-tests and 190 self-sampling kits).

Community pharmacies offering HIV-ST/SS were less often located in low-to-medium SES neighborhoods (OR 0.36, 95%CI 0.16 - 0.80 versus medium-to-high SES), and in low-to-medium urban areas (OR 0.33, 95%CI 0.15 - 0.72 versus medium-to-high urbanity). Pharmacists of older age (45-69 years of age) were less likely to offer HIV-ST/SS at the pharmacy (OR 0.96, 95%CI 0.92 - 1.00 versus 24-44 years).

Most pharmacies (89.7%) kept the HIV-ST/SS behind the counter. Of pharmacists offering tests, 10.3% indicated that first-time buyers were their main customers, 13.8% reported repeat-buyers as main customers, 27.6% indicated both groups similar, and 48.3% didn't know. 79.3% of the pharmacists never recommend HIV-ST/SS to customers visiting the pharmacy.

Reasons for not offering the HIV-ST/SS were no/little demand (69.3%), not familiar with HIV-ST/SS (17.4%), other or unknown reasons (13.3%). Pharmacists saw options to improve the HIV-ST/SS test offer, such as placing it visible on the counter (51.7%), by advertisement (37.9%), or giving advice to customers who visit the pharmacy to purchase the HIV-ST/SS (72.4%).

Conclusion
HIV-ST/SS have a limited practical availability in community pharmacies in the Netherlands since its introduction in 2019, especially in low-to-medium SES and less urban areas. Pharmacists do see opportunities to improve the HIV test offer and could play a role in increasing access to HIV testing in the Netherlands.

S2

EFFECTIVENESS OF DIRECTLY ASSISTED AND UNASSISTED HIV SELF-TESTING AS AN ALTERNATIVE MODE OF HIV SCREENING FOR INDIVIDUALS FROM MIGRANT COMMUNITIES AT AHF CHECKPOINT AMSTERDAM DURING THE COVID-19 PANDEMIC

Michal Pawlega[1], **Anna Zakowicz[1]**, Balazs Bodnar[2]

[1] AHF Europe, Amsterdam, the Netherlands
[2] AHF Checkpoint, Amsterdam, the Netherlands

Background
By the end of 2019, an estimated 1,730 people in the Netherlands were unaware of their HIV status. Among people who are known to be HIV positive, 41,3% were born outside the Netherlands. This group faces more barriers reaching HIV services and is more likely to present late for HIV care. Due to the COVID-19 pandemic access to HIV testing services was reduced. HIV self-testing with a possibility of assisted service was introduced at AHF Checkpoint Amsterdam to provide an additional option for HIV screening.

Methods
The 'order online and receive via mail' model was used between 23 February and 15 July 2021. It was targeted at migrants, including LGBTI community and undocumented individuals. Targeted organic and paid posts on social media (FB, Instagram) and the AHF Checkpoint Amsterdam website were used to disseminate the information. Clients made initial contact through WhatsApp. After screening and regular pre-test counselling, they received a free test-kit by post. Depending on the client's preference, directly assisted (video or voice call) or unassisted HIVST was offered. Additional support by hotline was provided. All the clients were asked to report their results; for that purpose, SMS reminders were sent. Clients with reactive test results were offered linkage to care support.

Results
221 individuals were sent HIV self-testing kits; 215 of them (97,3%) opted for an unassisted option. 186 people (84,2%) reported their results back including one positive case (0,45% positivity rate). The person was successfully linked to HIV care. Main users of the service were men (72,3%) then women (25,8%) and transgender (1,9%). 112 individuals (50,7%) lived outside the Amsterdam metropolitan area, 178 individuals (80,5%) tested for HIV before. Most clients were of non-Dutch origin (65,6%); out of this 17,9% from Central and Eastern Europe, 15,9% from Sub-Saharan Africa and 14,5% from Middle East. The main reason to test was unprotected sex (168 individuals – 76%) and majority of the clients were MSM (120 individuals – 54,3%). Nine people who were initially interested in self-testing opted for an on-site testing.

Conclusion
Introduction of HIV self-test during the COVID-19 pandemic provided an additional option to test for non-Dutch community at AHF Checkpoint. The assisted option was rarely used by the clients, but appeared to be useful for those who needed support with the process. The intervention had a high percentage of results reported back. Some of the clients who came for self-testing switched to regular on-site testing.

S3

SPECIFIC VAGINAL MICROBIOTA ENHANCE HIV-1 TRANSMISSION

Nienke Van Teijlingen, **Leanne Helgers**,
Esther Zijlstra-Willems, John Hamme,
Carla Ribeiro, Karin Strijbis, Theo Geijtenbeek

Amsterdam UMC, location AMC, Amsterdam, the Netherlands

A dysbalance in the vaginal microbiome (vaginal dysbiosis) is a major health concern as vaginal dysbiosis is associated with increased HIV-1 susceptibility and attenuation of prophylactic antiretroviral therapy. However, the underlying cellular mechanisms causing this effect remain unclear. A specialized subset of dendritic cells, Langerhans cells (LCs), survey the vaginal mucosa and normally provide protection against HIV-1. Hence, we have investigated whether vaginal microbiota influence the antiviral function of vaginal LCs. Skin and vaginal LCs were treated *in vitro* and *ex vivo* with different vaginal bacteria associated with either a healthy vaginal microbiome or vaginal dysbiosis. Untreated LCs were able to clear the virus. Strikingly, in contrast to other bacteria, the gram-negative bacterium *Prevotella timonensis* strongly increased HIV-1 capture by exposed LCs and internalized virus remained infectious for several days, where after it was efficiently transmitted to target cells. HIV-1 capture was independent of HIV-1 (co-) receptors CD4/CCR5 or viral fusion. Both, HIV-1 capture as well as transmission by *P. timonensis*-exposed LCs were unaffected by prophylactic antiviral drug treatment. Notably, the observed *P. timonensis*-effect in transmission was even more profound when LCs were exposed to transmitted/founder (T/F) variants. To conclude, a *P. timonensis* containing vaginal microbiome affects the antiviral function of LCs and changes LCs from protective to HIV-1 reservoirs and disseminators. The inability to prevent HIV-1 infection by antiviral drugs underscores the importance of treating bacterial vaginosis and specifically the presence of *P. timonensis*.

S4

A NOVEL FLOW CYTOMETRY-FLUORESCENT IN SITU HYBRIDIZATION APPROACH TO DETECT TRANSCRIPTIONALLY LATENT AND TRANSCRIPTIONALLY ACTIVE HIV-1 IN CD4+ T CELLS FROM PEOPLE LIVING WITH HIV

Shirley Man, Stefanie Kroeze, Jade Jansen, Agnes M. Harskamp-Holwerda, Carla M.S. Ribeiro, Teunis B.H. Geijtenbeek, Neeltje A. Kootstra

Amsterdam UMC, location AMC, Amsterdam, the Netherlands

Background
Despite successful viral suppression by combination antiretroviral therapy (cART), a cure for HIV-1 has yet to be achieved. The main barrier to a cure is the persistence of the elusive HIV-1 reservoir. Novel cure strategies focus on the reactivation and subsequent elimination of this viral reservoir. Here we developed a novel flow cytometry-fluorescent in situ hybridization (flow-FISH) approach to detect transcriptionally latent and transcriptionally active HIV-1-infected cells without the need for ex vivo reactivation. Using this technique, we aim to detect and monitor the HIV-1 reservoir in peripheral blood mononuclear cells (PBMCs) from people living with HIV (PLWH).

Methods
We designed two RNA-binding probes targeting different HIV-1 mRNA transcripts to discriminate between transcriptionally latent (positive for probe 1) and transcriptionally active (positive for probe 1&2) HIV-1-infected cells by flow-FISH. The T lymphocytic ACH-2 cell line, which harbors a stable copy of the HIV-1 genome and has low levels of viral replication, was used for optimization and validation of the technique. *In vitro* infected primary cells from uninfected donors were used for further validation of the assay. Finally, flow-FISH was performed on unstimulated PBMCs from PLWH of the Amsterdam Cohort Studies (ACS).

Results
With our flow-FISH method we were able to detect a "transcriptionally latent" and a "transcriptionally active" population in the ACH-2 cells of around 3% and 10%, respectively. Overnight stimulation with TNF-α, which is known to activate viral transcription, led to a marked increase of the transcriptionally active population, whereas transcriptionally latent cells were hardly detected. Primary dendritic cells (DCs) which were infected with HIV-1 *in vitro* for five days were analyzed using the flow-FISH assay. Our probe combination was able to detect transcriptionally latent (approx. 1%) and transcriptionally active HIV-1 (approx. 15%) in primary DCs, which was confirmed by a p24-staining (approx. 17%). In PLWH (N = 5), transcriptionally latent (range 0.02-0.49%) and transcriptionally active HIV-1-infected $CD4^+$ T cells (range 0.04-0.35%) were detected by our flow-FISH method.

Conclusion
We have developed a novel flow-FISH approach that can detect and differentiate between transcriptionally latent and transcriptionally active HIV-1-infected cells. Our newly developed assay will be a useful tool in characterizing the viral reservoir and understanding the mechanisms underlying HIV-1 latency, ultimately leading to improved monitoring of HIV-1 and the development of cure strategies.

S5

USING THE CRISPR-CAS9 SYSTEM TO CURE CELLS FROM HIV AND AT THE SAME TIME PREVENT SUPER-INFECTION BY NEW HIV STRAINS

Mouraya Hussein, Elena Herrera-Carrillo, Ben Berkhout

Amsterdam UMC, Amsterdam, the Netherlands

Background
HIV continues to pose a major public health burden worldwide. Although combination antiretroviral therapy suppresses the infection, it has to be taken lifelong and it does not result in a cure. Therefore, the search for a HIV-cure remains an important area of research. Gene editing-based antiviral approaches offer an attractive alternative to target DNA or retroviruses. CRISPR-Cas-based gene editing enables sequence-specific gene-editing, providing a mean to inactivate viral genomes, including the integrated HIV proviral DNA.

In this study, we aimed at designing a CRISPR-Cas-based approach which leads to the HIV-inactivation, but at the same time prevents super-infection of these cured cells by new HIV strains. The approach is based on targeting of the Rev gene, which encodes the protein responsible for the switch from spliced HIV transcripts to partially spliced and unspliced transcripts. Rev-inactivation by means of CRISPR-induced mutations will restrict HIV gene expression to the early Tat and Nef proteins that are transcribed from the fully spliced viral transcript. Rev-inactivation will thus not only block HIV expression, but ongoing expression of the Nef protein will induce the “state of superinfection resistance” (SIR) by CD4 down-modulation at cell surface of infected cells.

Methods
Anti-Rev gRNAs were designed and their HIV-inhibition efficiency was measured in transfection with a molecular HIV clone and quantitated by CA-p24 ELISA assay. Lentiviral vectors were used to deliver Cas9 mRNA and the different Rev-targeting gRNAs in T cells which were subsequently infected with HIV. Viral replication was monitored to study virus inhibition and a possible cure.

Results
We describe a set of gRNAs targeting highly conserved Rev sequences. A strong reduction of virus production was measured for most gRNAs compared to the negative control. We now plan to perform a second viral challenge with a GFP-encoding HIV variant to study if the SIR mechanism was activated in some of these cured cells. Flow cytometry and Western Blot will subsequently be used to confirm the SIR mechanism.

Conclusion
Overall, our preliminary data shows potent targeting of HIV Rev sequences by CRISPR-Cas9 within the context of the designed gene-editing platform.

S6

A NANOBODY-BASED HIV-1 ENGAGER (NANO-HIVE) ANTIBODY FOR NK CELL-MEDIATED CLEARANCE OF HIV-1 INFECTED CELLS

Jeffrey Umotoy[1], Angela Schriek[1], Neeltje Kootsra[1], Steven de Taeye[1], Raimond Heukers[2], Marit van Gils[1]

[1] *Amsterdam Institute for Infection and Immunity, Amsterdam UMC, Amsterdam, the Netherlands*
[2] *QvQ, Utrecht, the Netherlands*

Four decades after the discovery of HIV-1 as the causative agent for AIDS, no vaccines or therapeutics for a total cure are available to date against HIV-1. Although the advent of combination antiretroviral therapy (cART) decreased the mortality and morbidity related to the disease, it is unable to fully eliminate HIV-1. Bispecific engager antibodies have been designed to redirect cytotoxic effector cells for targeting multiple tumors and virally-transformed antigens. We engineered a novel nanobody-based HIV-1 engager (Nano-HIVEs) targeting HIV-1 Env and redirecting NKG2D receptor positive effector cells such as NK cells and γδ T cells to HIV-1 infected cells. J3, a highly potent and broad CD4-binding site nanobody was conjugated with MHC class I-related protein A (MICA) extracellular domain via a 15-mer flexible linker. MICA is a stress-induced ligand expressed during tumor-transformation and viral infection and can activate NK cells and γδ T cells upon engagement to NKG2D receptor inducing activation and the subsequent lysis of target cells. The constructs were produced from HEK293 mammalian system, purified with Strep-Tactin® XT, and the monomeric structure confirmed via SDS PAGE. Nano-HIVEs displayed preserved affinity against their cognate antigens, HIV-1 Env and NKG2D, confirmed by ELISA and flow cytometry. The activity of MICA domain was confirmed by the increase of CD107a signal upon co-incubation with purified human NK cells and MICA-Fc. Nano-HIVEs will be further evaluated for targeted lysis of infected cells using HIV-1 transfected cell lines, reactivated HIV-1 latent cell lines, and ex vivo infected CD4+ T cells by flow cytometry and quantification of HIV-1 DNA. This nanobody-based HIVEs could potentially help eliminate HIV-1 infected cells and decrease the viral reservoir in HIV-1 infected individuals.

S**7**

PEOPLE LIVING WITH HIV USING CART HAVE HIGHER PERCENTAGES OF CIRCULATING CCR5+CD8+ T CELLS AND LOWER PERCENTAGES OF CCR5+ REGULATORY T CELLS COMPARED TO HEALTHY CONTROLS

Louise van Eekeren[1], Vicky Matzaraki[1], Zhen-Hua Zhang[2], Lisa Wijer[1], Marc Blaauw[1], Marien de Jonge[1], Linos Vandekerckhove[3], Wim Trypsteen[3], Leo Joosten[1], Mihai Netea[1], Quirijn Mast[1], Hans Koenen[1], Yang Li[2], André van der Ven[1]

[1] *Radboudumc, Nijmegen, the Netherlands*
[2] *UMC Groningen, Groningen, the Netherlands*
[3] *Ghent University Hospital, Ghent, Belgium*

Background
CCR5 is the main HIV co-receptor important for HIV pathogenesis. We aimed to (1) compare CCR5 expression on immune cells between people living with HIV (PLHIV) using combination antiretroviral therapy (cART) and HIV-uninfected controls, (2) relate CCR5 expression to viral reservoir size and (3) assess determinants of CCR5 expression.

Methods
This cross-sectional study included 209 PLHIV on cART and 323 controls. The percentage of CCR5 positive cells (%) and CCR5 mean fluorescence intensity (MFI) were assessed by flow cytometry in monocytes and 12 lymphocyte subsets. CCR5 expression measurements were correlated to host factors, HIV-1 cell-associated (CA)-RNA and CA-DNA, plasma inflammation markers and plasma metabolites. Metabolic pathways were identified.

Results
PLHIV displayed higher percentages of CCR5+CD45+cells, monocytes and several CD8+T-cell subsets, but lower percentages of CCR5+ naive CD4+T-cells and naive and memory regulatory T-cells (Tregs). Age, sex and presence of anti-CMV antibodies correlated positively with percentages of CCR5+ cells in controls, unlike in PLHIV in whom CD4 nadir correlated negatively with percentages of CCR5+ cells. Quantities of HIV-1 CA-DNA and CA-RNA correlated positively with CCR5+ lymphocytes. There was no clear correlation observed between CCR5 expression and inflammation markers in PLHIV and controls. Metabolome analysis revealed three pathways involved in energy metabolism, i.e. glycolysis / gluconeogenesis, pyruvate, and propanoate metabolism, to be associated with percentage of CCR5+CD8+ cells in PLHIV.

Conclusions
CCR5 is differently expressed on various circulating immune cells in PLHIV. CCR5 dependent homing capacity of CD8+ T-cells is different from that of Tregs which may affect their interaction. The associations between different energy pathways and percentage of CCR5+ CD8+ cells in PLHIV, but not in controls, suggest higher energy demand of CCR5+CD8+T-cells in PLHIV.

Funding
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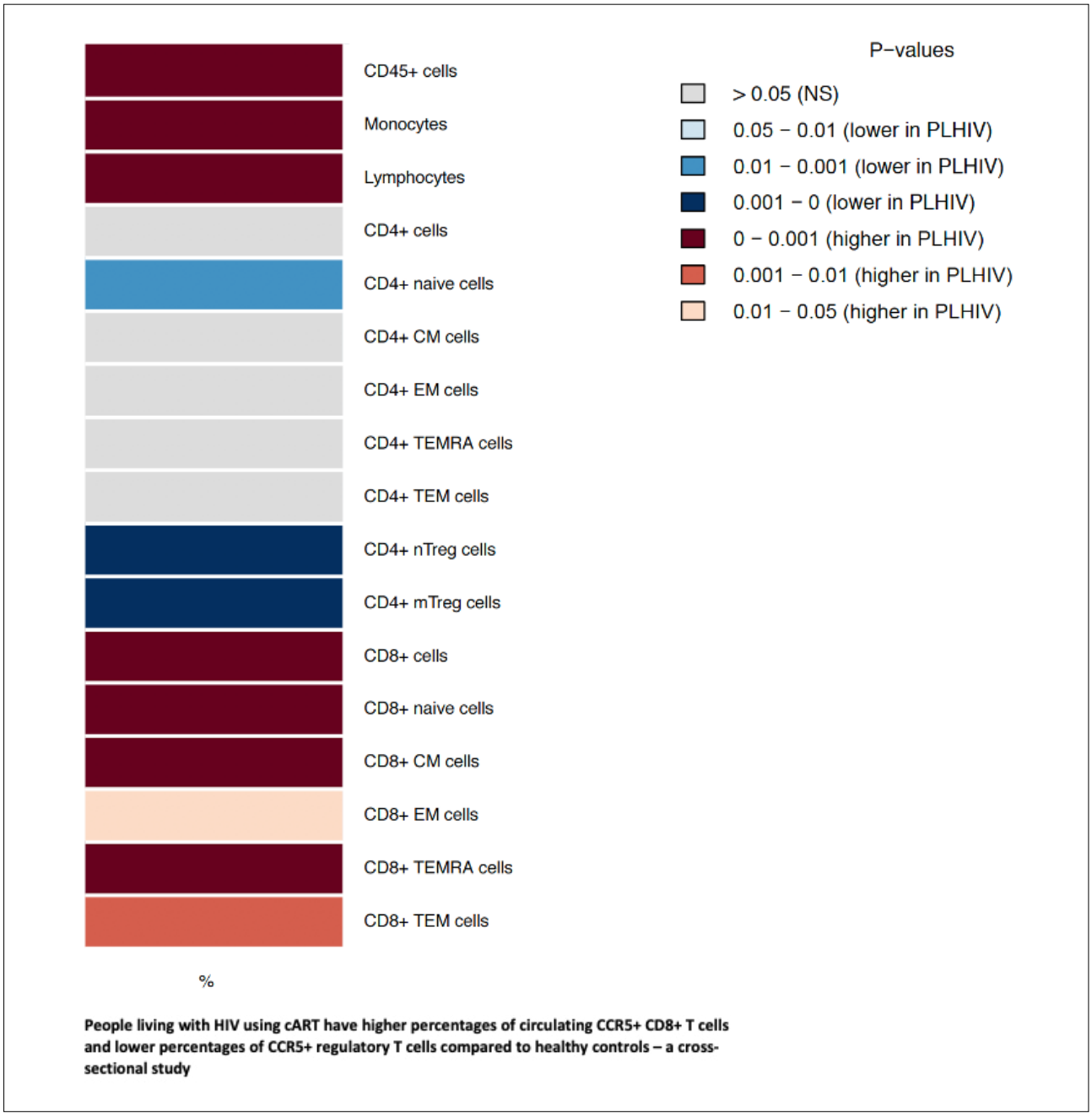
S7 (Figure 1)



People living with HIV using cART have higher percentages of circulating CCR5+ CD8+ T cells and lower percentages of CCR5+ regulatory T cells compared to healthy controls – a cross-sectional study

S8

INVESTIGATING HIV NEUROPATHOGENESIS USING HUMAN MONOCYTE-DERIVED MICROGLIA CULTURE MODEL

Lavina Gharu[1], Stephanie Gumbs[1], Pauline Schipper[2], Annemarie Wensing[2], Theo Geijtenbeek[3], Neeltje Kootstra[3], Lot De Witte[4], Monique Nijhuis[2]

[1] *Translational Virology, Dept. of Medical Microbiology, UMC Utrecht, Utrecht, the Netherlands*
[2] *Translational Virology, UMC Utrecht, Utrecht, the Netherlands*
[3] *Department of Experimental Immunology, Amsterdam UMC, Amsterdam, the Netherlands*
[4] *Department of Psychiatry, Icahn School of Medicine, New York, United States*

Background
Despite cART, HIV persists in the CNS and is associated with development of neurocognitive disorders. Microglia are the major HIV-1 reservoir within the CNS and thus it is extremely important to understand the pathobiology of these infected cells. However, current models using transformed cell lines are not representative of primary microglia and human brain tissue for isolation of primary microglia are difficult to obtain. Here, we describe the generation, characterization of human monocyte-derived microglia and further investigate it as an invitro model for HIV infection.

Methods
CD14+ cells isolated from healthy PBMC donors were cultured in presence of ACM, GM-CSF, MCSF, IFN-γ, TGF-β, IL34 for 10 days. These monocyte derived microglia (MDMi) were characterized for the morphology, microglial markers and HIV receptors by qPCR. MDMi were incubated +/- CCR5 inhibitor Maraviroc (MVC) for 1hr and subsequently infected with different CCR5-using replication competent lab adapted HIV strains with either luciferase or GFP reporter. To generate them, envelope coding region of CCR5-using lab adapted strains was amplified and cloned in either HxB2ΔENVluc or NL4.3ΔENVGFP backbone. Time course experiments were performed to monitor viral replication.

Results
MDMi acquired spindle shape with branches and expressed P2RY12, TREM2, CSF1R, IBA1, TMEM119, HLA-DR, CD4, CCR5 and CXCR4. HIV infection was detected by expression of GFP as early as 4 days post infection (pi) and subsequently increased up to 90-95% until day 16. Syncytia formation, the first cytopathic effect of HIV infection was detected on day 7 with Bal, AD8 and YU2 and further increased until day 13 in culture. Luciferase expression in supernatant was used to monitor viral replication and increased over time when the infected MDMi were maintained in culture. MVC completely inhibited viral replication, further demonstrating CCR5-mediated viral entry. BAL replicated to its highest titers; YU2 and AD8 had comparable but lower levels of viral replication. The released cell free virus was sufficient to infect TZM-bl cells, also indicating that the MDMi model supported HIV replication. JRCSF a T-tropic CCR5-using virus did not show any replication in MDMi.

Conclusions
We have developed and characterized a microglia culture model for HIV infection derived from peripheral blood monocytes using rapid, cost-effective and reproducible protocol. This model supports HIV replication kinetics with various lab adapted strains. This model can be used to investigate viral replication dynamics, latency and for screening anti-HIV agents. Same approach can be extended to generate HIV patient specific MDMi model for pharmacogenomics.

S9

IMPROVING FC-EFFECTOR FUNCTION OF ANTI-HIV-1 BNABS BY GLYCOENGINEERING

Steven de Taeye, Angela Schriek, Marloes Grobben, Rogier Sanders, Marit van Gils

Amsterdam UMC, Amsterdam, the Netherlands

Background

Broadly neutralizing antibodies (bNAbs) targeting the HIV-1 Env protein have shown great potential for the treatment of HIV-infected individuals. HIV-specific bNAbs are able to recognize a large percentage of circulating HIV-1 strains worldwide and have shown to delay viral rebound and reduce the latent reservoir in a subset of patients in recent studies. These results demonstrate the potential of antibodies, but also indicate that improvements are necessary before antibodies will lead to a functional cure. Recently, there is a growing interest in potentiating the effector functions of antibodies to improve the use of antibodies as therapeutics. The interaction of an antibody with Fc gamma receptors (FcγR) and other Fc engaging ligands is influenced by the composition of the *N*-linked glycan in the Fc domain of an antibody. Antibodies with an afucosylated *N*-linked glycan have a stronger interaction with FcγRs and are successfully used in the treatment of certain types of cancer.

Methods

Anti-HIV-1 antibodies were produced via transfection of HEK-293F cells with plasmids encoding for the heavy and light-chain of the antibody. Under standard procedures the degree of fucosylation of the *N*-linked glycan is >95%. We used a fucose decoy substrate (2-fluoro peracetylated fucose) during transfection to reduce the percentage of fucosylation to 10-20%. We analysed Env binding and neutralization capacity of the fucosylated and afucosylated variant of five different bNAbs (VRC01, 2G12, PGDM1400, PGT121 and PGT151). In addition, we determined FcγR binding in ELISA and NK-cell activation by measuring CD107 expression, a surrogate marker for degranulation.

Results

Afucosylated antibodies showed enhanced FcγRIIIa binding compared to WT IgG1 antibodies, while maintaining similar Env-binding and HIV-1 neutralization capacity. Furthermore, a stronger activation of NK-cells, as measured by CD107 expression and CD16 shedding, was observed with afucosylated antibodies. Currently, we are exploring the elimination of reactivated latently infected cells by afucosylated antibodies.

Conclusion

We show that afucosylation of anti-HIV-1 bNAbs enhances FcγRIIIa mediated NK-cell activation. In future experiments we will determine the potential of afucosylated antibodies in eliminating HIV-1 infected cells in vitro and in a therapeutic setting *in vivo*.

S10

UNEXPECTED HIGH INCIDENCE OF LOW DOLUTEGRAVIR TROUGH LEVELS IN PATIENTS TREATED WITH RIFAMPIN FOR TB/HIV CO-INFECTION, DESPITE DOLUTEGRAVIR DOSE ADJUSTMENT

David Burger, Ralf Stemkens, Martin Boeree, Rob Aarnoutse, Reinout van Crevel

Radboudumc, Nijmegen, the Netherlands

Introduction
It is well known that rifampin as a strong inducer of metabolic enzymes and drug transporters reduces plasma concentrations of many antiretroviral agents, including dolutegravir. A healthy volunteer study (Dooley et al. JAIDS 2013) demonstrated that a dose adjustment of dolutegravir from 50mg QD to 50mg BID compensated for the negative effect of this drug-drug interaction. As recommend by the 2020 KNCV guideline for treatment of TB/HIV co-infection *(https://richtlijnendatabase.nl/richtlijn/tuberculose_en_hiv/behandeling_van_gecombineerd_tuberculose_en_hiv.html)* we monitored dolutegravir trough concentrations in this patient population.

Methods
We reviewed all HIV-infected patients with TB co-infection treated since 2016 at Radboudumc with rifampin and dolutegravir (50mg BID), for which steady state dolutegravir trough levels were available. We collected demographic data such as gender and age, as well as drug doses and drug concentrations. For comparison, an extract from our national TDM database of dolutegravir plasma concentrations, excluding cases on rifampin, was composed. We evaluated two different targets for dolutegravir troughs: the in-vitro EC_{95} (0.064 mg/L) and the in vivo minimum effective concentration (MEC; 0.30 mg/L). Patients with documented evidence of nonadherence or malabsorption were excluded.

Results
We found 8 cases (7 males; 1 female) with a median (range) age of 51 (31-62) years. Five patients were treated with standard rifampin doses (450-600mg/day); the three other received increased rifampin doses (1350-1800mg/day). The median dolutegravir concentration was 0.25 mg/L, which is approximately 70% lower than the average dolutegravir trough concentration in the population of 0.83 mg/L. All dolutegravir trough levels were above the EC_{95}, 5/8 (62.5%) were between 0,064 – 0,30 mg/L (MEC), and only 3/8 (37.5%) were above the MEC. No apparent effect of optimized-dosed rifampin was visible. In one patient, we applied a dose increase of dolutegravir to 50mg TID, and this resulted in an increase in dolutegravir trough level from 0.21 to 0.27 mg/L.

For comparison, in our database of dolutegravir levels in 360 patients not on rifampin, 18 (5.0%) were below the lower limit of quantification (0.01 mg/L), and 18 (5.0%) were between 0.01 – 0.30 mg/L. The remaining 324 patients (90%) had dolutegravir levels > MEC.

Conclusion
We found an unexpected high incidence of low dolutegravir plasma concentrations in TB/HIV co-infected patients treated with rifampin, despite the dose adjustment for dolutegravir from 50mg QD to 50mg BID. No apparent effect of rifampin dose was observed. We recommend continued monitoring of dolutegravir plasma concentrations in this patient population as already mentioned in the KNCV Guideline.

S11

TENOFOVIR ALAFENAMIDE CONCENTRATIONS ARE REDUCED BY HALF IN PREGNANT WOMEN LIVING WITH HIV: DATA FROM THE PANNA NETWORK

Vera Bukkems[1], Coca Necsoi[2], Carmen Hidalgo-Tenorio[3], Coral Garcia[3], Irene Alba Alejandre[4], Fabian Weiss[4], John Lambert[5], Astrid Hulzen[6], Olivier Richel[7], David Burger[1], Deborah Konopnicki[2], **Angela Colbers[1]**

[1] *Department of Pharmacy, RIHS, Radboudumc, Nijmegen, the Netherlands*
[2] *Saint-Pierre University Hospital, Brussels, Belgium*
[3] *Hospital Universtario Virgen de las Nieves, Granada, Spain*
[4] *Department of Gynecology and Obstetrics, Ludwig-Maximilians-University, Munich, Germany*
[5] *Mater Misericordiae University Hospital, Dublin, Ireland*
[6] *Department of internal medicine, Isala Hospital, Zwolle, the Netherlands*
[7] *Department of internal medicine, Radboudumc, Nijmegen, the Netherlands*

Background
Tenofovir alafenamide (TAF) is included in the majority of the recommended first-line antiretroviral regimens for HIV, but only limited data are available to determine TAF's applicability for pregnant women living with HIV. Adequate therapy is of special importance for pregnant women to prevent mother-to-child-transmission of HIV, but physiological changes during pregnancy can alter the concentrations, and thus safety and efficacy, of antiretroviral agents. This study was a part of the PANNA study, which is an ongoing study established to prospectively collect pharmacokinetic profiles of newly developed antiretroviral drugs. The primary objective of the current analysis was to examine TAF and tenofovir (TFV) pharmacokinetic parameters during pregnancy.

Material and Methods
A non-randomized, open-label, multi-center, phase 4 study was performed. Pregnant women living with HIV treated with a TAF-containing regimen were included from HIV-treatment centers across Europe. Intensive pharmacokinetic sampling over 24 hours was performed in the third trimester (week 33) and postpartum (4-6 weeks after delivery). TAF and TFV pharmacokinetic parameters were subsequently determined with non-compartmental analysis. In addition, clinical efficacy and safety outcomes were collected.

Results
In total, 20 pregnant women living with HIV were included. At the third trimester, TAF geometric mean AUC_{last} (%CV) was 100.95 (43.5) ng*h/mL, and the geometric mean C_{max} (%CV) was 90.59 (56.9) ng/mL. This corresponded to a GMR (90%CI) third trimester/postpartum of 0.54 (0.43-0.68) and 0.48 (0.38-0.62) for AUC_{last} and C_{max}, respectively. For TFV GMR (90%CI) third trimester/postpartum for $AUC_{0\text{-}24h}$ was 0.67(0.62-0.74). One of the 20 women had a detectable viral load of 317 copies/mL at third trimester visit, which was attributed to prior non-adherence. All women had an undetectable viral load at delivery and postpartum visit. The median (range) gestational age at delivery was 39 (37-41) weeks. All children had a negative HIV DNA PCR test at birth, and no congenital abnormalities were observed. The birthweight of five infants (25%) was considered to be small for gestational age.

Conclusions
This European study observed that TAF plasma concentrations are reduced by about half in pregnant women living with HIV. Also, the plasma concentrations of TFV, the major plasma metabolite, are reduced by approximately 30% during pregnancy. Despite the observed exposure decrease high virologic efficacy was observed, and no mother-to-child transmission occurred in this study.

S12

DEXAMETHASONE IS A DOSE-DEPENDENT PERPETRATOR OF DRUG-DRUG INTERACTIONS: IMPLICATIONS FOR USE IN PEOPLE LIVING WITH HIV

Tom Jacobs[1], Catia Marzolini[2], David Back[2], David Burger[1]

[1] *Department of Pharmacy, Radboudumc, Nijmegen, the Netherlands*
[2] *Department of Molecular and Clinical Pharmacology, University of Liverpool*

Introduction
Global use of dexamethasone in COVID-19 patients has revealed a poor understanding of the drug-drug interaction (DDI) potential of dexamethasone, particularly with antiretroviral drugs (ARVs). Dexamethasone is both a substrate of cytochrome P450 3A4 (CYP3A4) enzyme as well as a dose-dependent inducer of CYP3A4. As many ARVs are substrates and/or inhibitors or inducers of CYP3A4, there is concern about drug-drug interactions (DDIs) with dexamethasone either as a perpetrator or a victim. Assessment of DDIs that involve dexamethasone is complex as dexamethasone is used at a range of daily doses (generally 0.5 up to 40 mg) and treatment course can be short, long or intermittent. This study aimed to establish recommendations for the management of DDIs with ARVs considering various dexamethasone dosages and treatment duration.

Methods
First, (1) we identified and reviewed studies reporting *in vivo* or *in vitro* data on dexamethasone as a perpetrator of drug-drug interactions through CYP3A4. Then, (2) we developed a classification for assessment of dexamethasone's interaction potential based on its dose and treatment duration, followed by (3) extrapolation of dose recommendations for ARVs to overcome DDIs with other medications that have a comparable induction potential with dexamethasone.

Results
Only few clinical pharmacokinetic studies have determined the effect of concomitant dexamethasone use on CYP3A4 substrate compounds with a wide range of research methods being used in those studies. A dexamethasone dose of <1.5 mg is unlikely to cause a significant inducing effect. Daily dosages of >1.5 up to 16 mg are expected to cause a weak inducing effect (resulting in a ≥20% to <50% decrease of a CYP3A4 substrate AUC), whereas multiple daily doses >16 mg would cause moderate induction (≥50% to <80% decrease of CYP3A4 substrate AUC). In case of long the treatment courses, the risk of clinical significant DDIs increases. Hence, dexamethasone doses between 1.5-16 mg/day used for >10 days where considered to have a moderate risk of causing clinical significant interactions.

Rifabutin is a moderate inducer of CYP3A4 and DDIs between rifabutin and ARVs have been studied extensively. Therefore, recommendations for managing DDIs between rifabutin and ARVs were extrapolated to people receiving a high daily dose of dexamethasone or a moderate dose for a long treatment course.

Conclusion
High daily dose of dexamethasone could potentially cause reduced concentrations of many ARVs. The ARVs that are potentially affected by lower dexamethasone dosages, such as for treatment of COVID-19, include rilpivirine, doravirine, and maraviroc.

S**13**

IMPROVING PREVALENCE AND RISK ESTIMATES OF MSM LIVING WITH HIV IN THE NETHERLANDS: A PUBLIC HEALTH SERVICE LEVEL SMALL AREA ESTIMATION MODELLING ANALYSIS WITH A BAYESIAN APPROACH

Haoyi Wang[1], Chantal Den Daas[2], Eline Op de Coul[3], Kai Jonas[1]

[1] *Maastricht University, Maastricht, the Netherlands*
[2] *University of Aberdeen, Aberdeen, United Kingdom*
[3] *Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, the Netherlands*

Background
Epidemiological reports of MSM living with HIV (MSMLHIV) with annual updates have been successfully provided by SHM in the Netherlands, yet the true prevalence of MSMLHIV can be masked for areas with small population density or lack of data. Therefore, this study aimed to investigate the feasibility of small area estimation with a Bayesian approach to unravel a better estimate of the MSMLHIV epidemic while also providing an estimate on the Public Health Services (GGD) level, using data from two different surveys.

Methods
Data from European MSM Internet Survey 2017 (EMIS-2017, Dutch subsample, n=3,459) and Survey 'Men&Sexuality' 2018 (SMS-2018, n=5,653) were utilized in this study. We first calculated the observed prevalence of MSMLHIV and the standardized prevalence ratio to compare the observed risk of MSMLHIV per GGD region. We then used the Integrated Nested Laplace Approximation for the hierarchical Bayesian computation with appointing a weak-informative prior (PC prior). This hierarchical structure assumed that each GGD region can influence the risk of MSMLHIV by sharing the border or by proximity. We first modelled the relative risk (RR) of MSMLHIV by only considering the spatial influence and the random effects assuming the observed HIV cases in each GGD region to follow a Poisson distribution. We then conducted a spatial ecological regression modelling uni/multivariably to include the known areal determinants of HIV such as HIV testing history, age, number of sex partners, injecting drug use, condom use and prevalence of other STIs to explore how the other spatial information impact on the RR estimations.

Results
Results of the prevalence and risk estimations from EMIS-2017 and SMS-2018 (Figure1) revealed an overlapping estimation with minor differences. Both estimations confirmed that the risk of MSMLHIV is heterogenous in the Netherlands with some GGD regions, such as GGD Amsterdam [RR=1.21 (95% credible interval 1.05-1.38) by EMIS-2017, RR=1.39 (1.14-1.68) by SMS-2018], having a higher-than-average risk. Results from our ecological regression modelling showed some significant areal determinants which can impact the risk of MSMLHIV (Table1). However, results are slightly different between the two datasets, which may be due to collection biases.

Conclusion
Our Bayesian approach to assess the risk of HIV among MSM across the Netherlands at the GGD level was able to close data gaps and provide more robust prevalence and risk estimations over the use of crude proportions. We thus recommend using this methodology in addition to classic approaches in the future surveillance of HIV.

S13 (Figure 1)

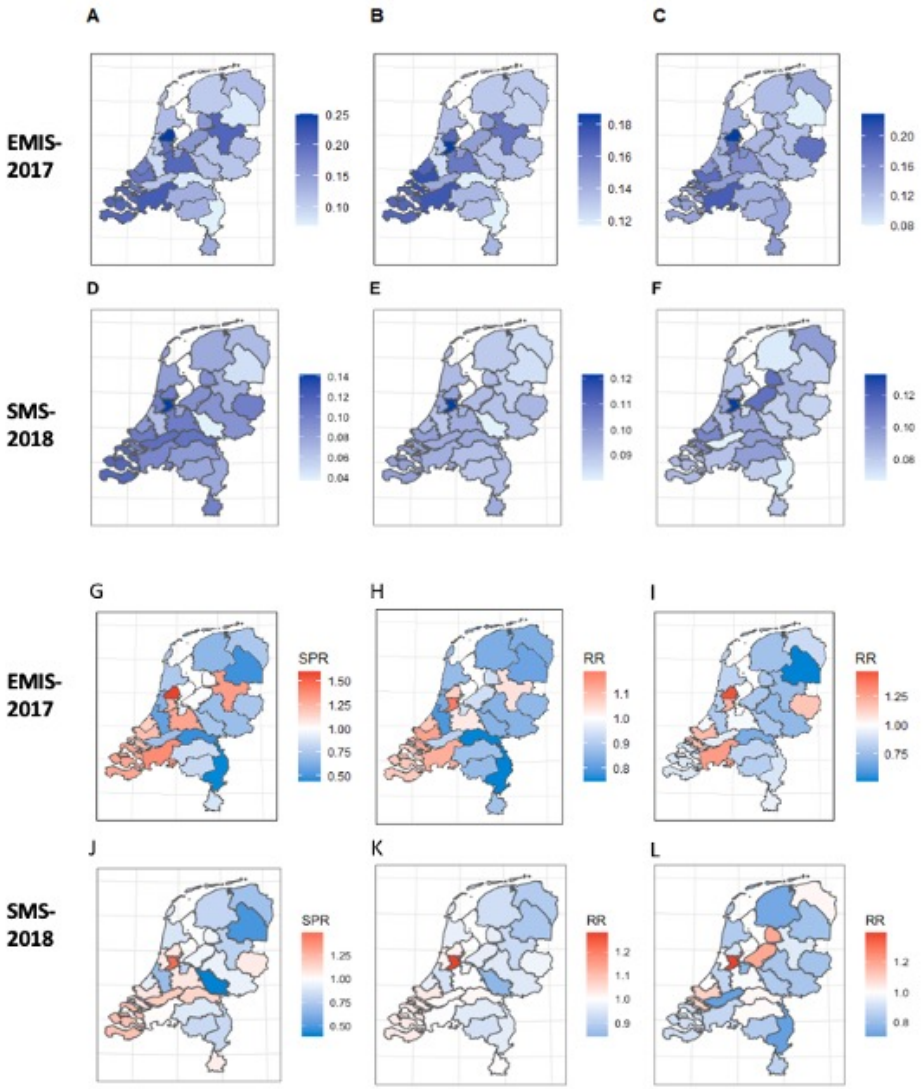


Figure 1. Choropleth map of the estimates of HIV prevalence by GGD regions in the Netherlands.
A: Observed HIV prevalence by EMIS-2017. B: Posterior mean of HIV prevalence estimated by Bayesian spatial modelling (null model) by EMIS-2017. C: Posterior mean of HIV prevalence estimated by Bayesian spatial ecological regression modelling (final model) by EMIS-2017. D: Observed HIV prevalence by SMS-2018. E: Posterior mean of HIV prevalence estimated by Bayesian spatial modelling (null model) by SMS-2018. F: Posterior mean of HIV prevalence estimated by Bayesian spatial ecological regression modelling (final model) by SMS-2018. G: Observed HIV standardised prevalence ratio by EMIS-2017. H Posterior mean of HIV relative risk estimated by the Bayesian spatial modelling (null model) by EMIS-2017. I: Posterior mean of HIV relative risk estimated by the Bayesian spatial ecological regression modelling (final model) by EMIS-2017. J: Observed HIV standardised prevalence ratio by SMS-2018. K: Posterior mean of HIV relative risk estimated by the Bayesian spatial modelling (null model) by SMS-2018. L: Posterior mean of HIV relative risk estimated by the Bayesian spatial ecological regression modelling (final model) by SMS-2018. For A to F: the darker a GGD region, the higher prevalence of HIV among MSM was estimated. For G to L: RR (or SPR) higher than 1 indicates a higher-than-average (average risk in the Netherlands) risk of HIV among MSM in that region (red); RR (or SPR) lower than 1 indicates a lower-than-average risk of HIV among MSM in that region (blue).

S13 (Table 1)

Table 1. Model comparison and selection for EMIS-2017 and SMS-2018

Models	HIV diagnosis									
	EMIS-2017					SMS-2018				
	Covariates	Coefficient	95%CrI	DIC	ICC	Covariates	Coefficient	95%CrI	DIC	ICC
Spatial Null model	Intercept	-0.108	(-0.257 - 0.025)	145.71	0.24	Intercept	-0.041	(-0.196 - 0.098)	123.99	0.27
Spatial Univariate models	Intercept	-2.359	(-4.052 - -0.597)	144.42	0.27	Intercept	-2.206	(-3.729 - -2.217)	118.97	0.31
	HIV test (%yes)*	2.724	(0.611 - 4.725)			**HIV test (%yes)***	2.674	(0.738 - 4.501)		
	Intercept	-0.591	(-1.376 - 0.172)	145.89	0.24	Intercept	-1.166	(-2.027 - -0.271)	119.84	0.28
	Age (%>= 35 y.o.)	1.089	(-0.613 - 2.786)			**Age (%>= 35 y.o.)***	2.454	(0.551 - 4.229)		
	Intercept	-0.063	(-0.232 - 0.083)	147.26	0.26	Intercept	0.073	(-0.498 - 0.645)	125.66	0.27
	Partner	-0.037	(-0.104 - 0.032)			Partner	-0.049	(-0.297 - 0.186)		
	Intercept	-5.305	(-10.373 - 0.715)	144.51	0.25	Intercept	-0.971	(-1.763 - -0.130)	121.04	0.29
	Condom (%never)	41.315	(-6.485 - 81.421)			**Condom (%never)***	3.121	(0.359 - 5.622)		
	Intercept	-0.398	(-0.752 - -0.056)	145.51	0.32	Intercept	0.005	(-0.235 - 0.227)	125.47	0.27
	IDU (%yes)	4.41	(-0.369 - 9.051)			SLAM (%yes)	-0.561	(-2.812 - 1.601)		
	Intercept	-0.87	(-1.384 - -0.352)	142.41	0.28	Intercept	-0.049	(-0.327 - 0.220)	125.62	0.27
	Syphilis (%yes)*	3.546	(1.263 - 5.703)			Syphilis (%yes)#	0.251	(-8.189 - 8.139)		
	Intercept	-0.662	(-1.281 - -0.029)	146.6	0.24	Intercept	-0.197	(-0.651 - 0.248)	125.42	0.29
	Chlamydia (%yes)	1.724	(-0.200 - 3.514)			Chlamydia (%yes)#	1.992	(-3.513 - 7.301)		
	Intercept	-0.856	(-1.552 - -0.132)	145.8	0.26	Intercept	-0.125	(-0.533 - 0.277)	125.64	0.27
	Gonorrhoea (%yes)*	2.255	(0.116 - 4.221)			Gonorrhoea (%yes)#	1.195	(-4.376 - 6.498)		
Spatial Multivariate final model	Intercept	-2.021	(-3.624 - -0.358)	141.74	0.28	Intercept	-2.225	(-3.701 - -0.739)	118.58	0.30
	HIV test (%yes)	1.607	(-0.6 - 3.737)			HIV test (%yes)	1.801	(-0.286 - 3.941)		
	Syphilis (%yes)	2.674	(0.192 - 5.099)			Age (%>= 35 y.o.)	1.515	(-0.549 - 3.571)		

Note: * = significant areal determinants of the univariate models. Partner = median number of partners. Condom = % never used condom with non-steady partners. # Indicates six-month prevalence instead of life-time prevalence. CrI = credible interval. DIC=Deviance Information Criterion, ICC= Intra-class correlation.

S14

TRAJECTORIES OF PREP USE AMONG MEN WHO HAVE SEX WITH MEN: A POOLED ANALYSIS OF TWO OBSERVATIONAL COHORT STUDIES

Vita Jongen[1], Thijs Reyniers[2], Maarten Schim van der Loeff[1], Elske Hoornenborg[1], Tom Smekens[2], Mark van den Elshout[1], Hanne Zimmermann[3], Liza Coyer[1], Chris Kenyon[2], Irith Baetselier[2], Udi Davidovich[1], Henry de Vries[1], Maria Prins[1], Marie Laga[2], Bea Vuylsteke[2], Anders Boyd[1]

[1] *GGD Amsterdam, Amsterdam, the Netherlands*
[2] *Institute of Tropical Medicine, Antwerp, Belgium*
[3] *Maastricht University, Maastricht, the Netherlands*

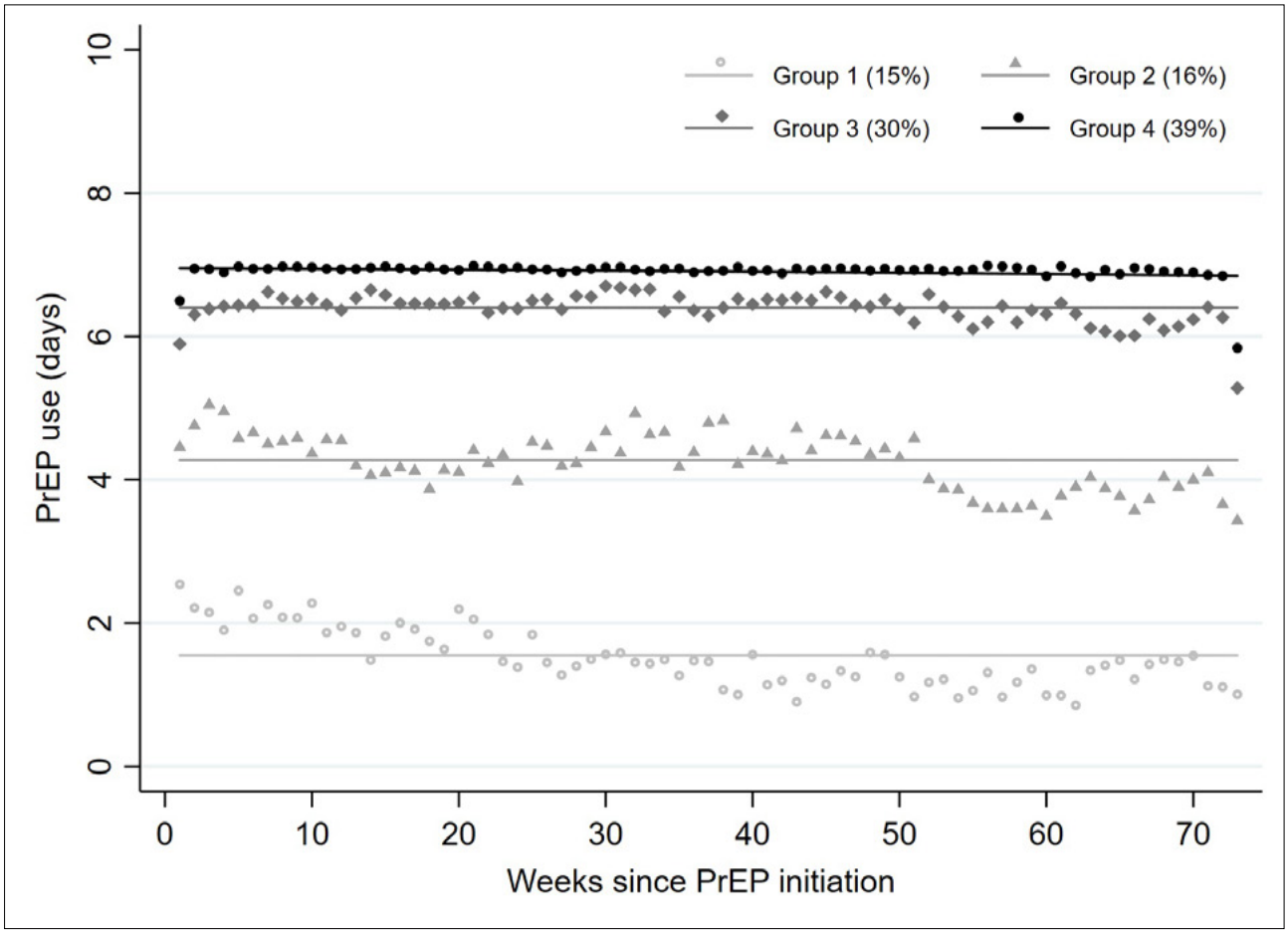
Background
Daily and event-driven pre-exposure prophylaxis (PrEP) are both efficacious in reducing the risk for HIV. In real-life, MSM may use PrEP for periods of elevated HIV risk and discontinue in-between. We aimed to assess PrEP use trajectories irrespective of chosen PrEP regimens, and determinants of these trajectories, among MSM and transgender persons participating in the AMPrEP and Be-PrEP-ared demonstration projects.

Methods
In AMPrEP and Be-PrEP-ared participants could choose and switch between daily and event-driven PrEP every 3-months. Data on PrEP use and sexual behaviour were collected daily and were pooled for this analysis. We included participants who contributed data on ≥10% of days during 73 weeks of follow-up. We used group-based trajectory modelling to identify groups of individuals who followed distinctive trajectories of PrEP use. Best model fit with respect to number of trajectories and polynomial order of each trajectory were determined using the Bayesian Information Criterion (BIC) and Entropy. This model was then used to examine determinants of belonging to specific trajectories.

Results
We included 516 MSM and 4 transgender women (n=323 AMPrEP; n=197 Be-PrEP-ared), of whom 24% chose event-driven PrEP at baseline. Participants reported data on 225,850 days during follow-up. Based on BIC and Entropy, a model with 4 trajectories and zero-order polynomials had the best fit (Figure): ≤2 tablets per week (Trajectory 1, 15% of total population), ±4 tablets per week (Trajectory 2, 16%), almost daily (Trajectory 3, 30%), and daily (Trajectory 4, 39%). There was more variation in weekly PrEP use for Trajectory 2 (mean variance per week over time 6.30, standard deviation (SD)=0.87) compared to Trajectory 1 (3.85, SD=1.08), Trajectory 3 (2.04, SD=0.75), and Trajectory 4 (0.20, SD=0.20).
Compared to Trajectory 1, participants of Trajectory 3 were younger (odds ratio (OR)=0.69, 95% confidence interval (CI)=0.53-0.90), participants of Trajectory 2 were more often highly educated (OR=2.39, 95%CI=1.03-5.50) and participants of Trajectory 4 were more often employed (OR=3.40, 95%CI=1.63-7.08). In addition, participants of Trajectory 1 reported less days on which anal sex had occurred compared to the other groups (p<0.001 for all).

Conclusion
While two different PrEP regimens were used in these studies, we found 4 distinct trajectories of PrEP use ranging from infrequent use (<=2 pills/week) to daily use. During 73 weeks of follow-up we did not see a clear trend towards more or less PrEP use within the trajectories. Future research should investigate how PrEP care could be adapted according to different usage patterns.

S14 (Figure 1)


Group 1 (15%)
Group 2 (16%)
Group 3 (30%)
Group 4 (39%)
PrEP use (days)
0
2
4
6
8
10
0
10
20
30
40
50
60
70
Weeks since PrEP initiation

S15

BIANNUAL VERSUS QUARTERLY BACTERIAL STI SCREENING OF PREP USERS IN AMSTERDAM, THE NETHERLANDS

Vita Jongen[1], **Hanne Zimmermann[2]**, Marit Goedhart[1], Johannes Bogaards[3], Udi Davidovich[1], Liza Coyer[1], Henry de Vries[1], Maria Prins[1], Elske Hoornenborg[1], Maarten Schim van der Loeff[1]

[1] *GGD Amsterdam, Amsterdam, the Netherlands*
[2] *Maastricht University, Maastricht, the Netherlands*
[3] *Amsterdam UMC, Vrije Universiteit, Amsterdam, the Netherlands*

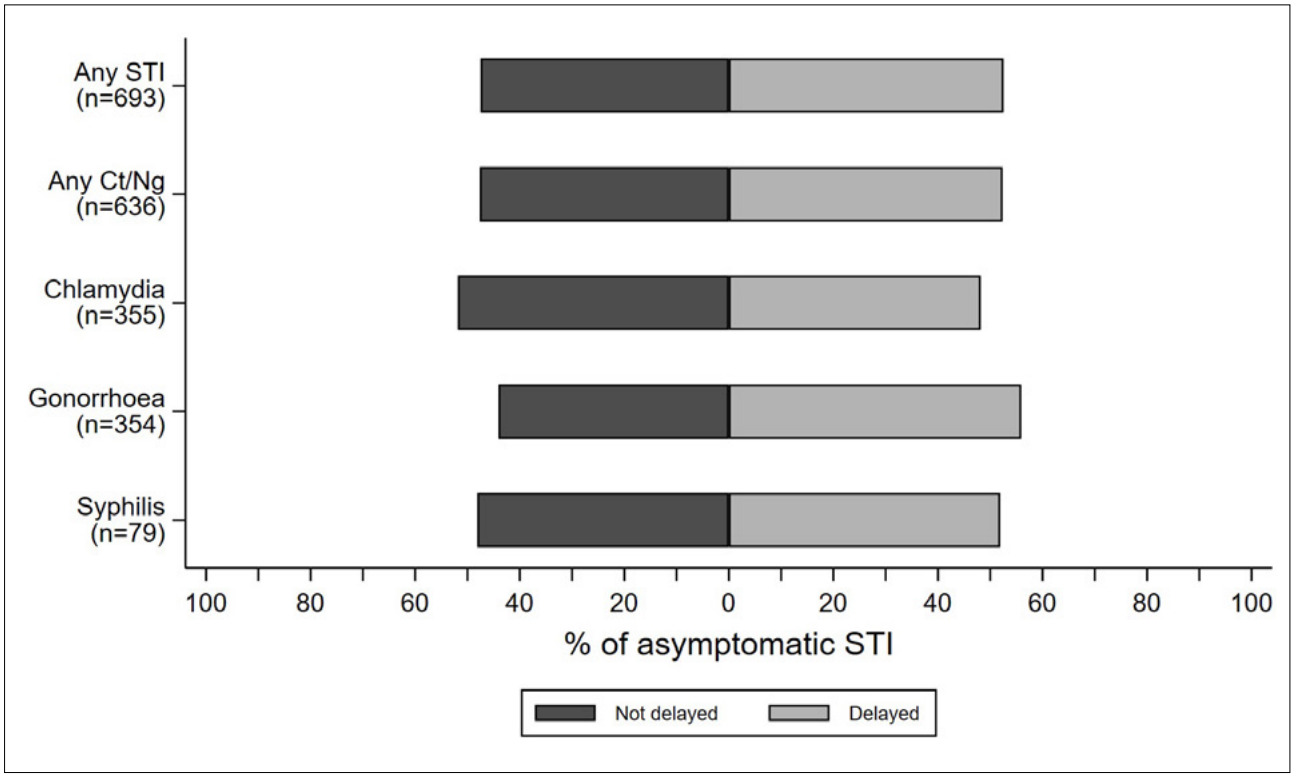
Background
In the Netherlands, pre-exposure prophylaxis (PrEP) users are screened quarterly for sexually transmitted infections (STIs) and, if tested positive, treated promptly. The optimal screening frequency of STIs is under debate as (1) screening for chlamydia and gonorrhoea is costly, (2) serious sequelae of gonorrhoea and chlamydia in men are rare; and (3) frequent antibiotic courses may lead to antimicrobial resistance (AMR). To optimize the STI screening frequency of PrEP programs, we aimed to determine the proportion of men with STIs for whom diagnosis would have been delayed if screening would have been conducted biannually instead of quarterly. Moreover, we assessed determinants of asymptomatic STIs to evaluate the possibility of targeted screening based on risk factors.

Methods
Using data from the Amsterdam PrEP (AMPrEP) study, we assessed the number and proportion of asymptomatic syphilis, chlamydia and gonorrhoea diagnoses which would have been delayed if STI testing was done biannually (i.e. at visit 6, 12, etc.) instead of quarterly. We also assessed opportunities for transmission at study visits where an STI diagnosis would have been delayed (i.e. at visit 3, 9, etc.), by assessing the number of visits where condomless anal sex (CAS) was reported in the 3 months prior. We assessed determinants of incident asymptomatic chlamydia, gonorrhoea or syphilis using Poisson regression.

Results
We included 367 participants, 365 (99%) MSM. Median follow-up time was 47 months [IQR 42-50], with a total of 4,974 study visits. 855 STIs were diagnosed at these study visits, of which 693 (81%) asymptomatic. 364 of 693 (53%) asymptomatic STIs were diagnosed at visits that would have been delayed if testing was done biannually (Figure). 126 (35%), 287 (79%), and 295 (81%) of these delayed STIs were diagnosed during visits at which the participant reported CAS with a steady, known casual or unknown casual partner, respectively. Younger PrEP users had a lower risk of asymptomatic STI (IRR=0.86 per 10 year increase, 95%CI=0.80-0.92). CAS with known (IRR=1.36, 95%CI=1.10-1.68) and unknown (IRR=1.85, 95%CI 1.47-2.32) casual partners and participating in chemsex (IRR= 1.51, 95%CI=1.28-1.78) increased the risk for asymptomatic STI.

Conclusion
Reducing the screening frequency of STIs to biannually among all PrEP users may result in many delayed diagnoses, with an opportunity of ongoing transmission to other sexual partners. Targeted STI screening may be possible among a group of PrEP users, thereby possibly improving allocation of limited recourses and reducing AMR risk.

S15 (Figure 1)


Any STI
(n=693)
Any Ct/Ng
(n=636)
Chlamydia
(n=355)
Gonorrhoea
(n=354)
Syphilis
(n=79)
100
80
60
40
20
0
20
40
60
80
100
% of asymptomatic STI
Not delayed
Delayed

S16

HUMAN MICROGLIAL CULTURE MODELS TO INVESTIGATE HIV NEUROPATHOGENESIS

Stephanie Gumbs[1], Raphael Kübler[2], Lavina Gharu[1], Pauline Schipper[1], Anne Borst[1], Gijsje Snijders[2], Paul Ormel[1], Amber Berdenis van Berlekom[1], Annemarie Wensing[1], Lotje Witte[2], Monique Nijhuis[1]

[1] *UMC Utrecht, Utrecht, the Netherlands*
[2] *Icahn School of Medicine, New York, United States*

Background
Despite years of antiretroviral therapy, HIV persistence in the central nervous system (CNS) may cause neurological disorders and poses a critical challenge for HIV cure. Understanding the pathobiology of HIV-infected microglia, the main viral CNS reservoir, is imperative. However, this is restricted due to the difficulty to obtain and study primary microglia. Here, we provide a comprehensive evaluation of different human microglial culture models as compared to primary microglia to advance the investigation of HIV infection in the CNS.

Methods
Uncultured primary microglia (pMG) were compared to cultured pMG, microglial cell-lines (SV40, HMC3), monocyte-derived microglia (MDMi), stem cell-derived microglia (iPSC-MG), and microglia grown in 3D cerebral organoids (oMG). All models were compared with respect to morphology, function (phagocytosis and LPS responsiveness) and microglia core transcriptomic signature. A head-to-head infection was performed using CCR5 M-tropic HIV-1 BaL reference strain equipped with a luciferase or GFP reporter.

Results
Functional characterization revealed phagocytic capabilities and LPS responsiveness across all culture models. Microglial transcriptome profiles of uncultured pMG showed the highest similarity to cultured pMG and oMG, followed by iPSC-MG then MDMi. Direct comparison of HIV infection showed a striking difference, with high levels of viral replication in cultured pMG and MDMi, relatively low levels in oMG resembling HIV infection observed in post-mortem biopsies, while the SV40 and HMC3 cell-line did not support HIV infection. Fluorescent staining of 3D organoids showed that all HIV (GFP)-positive cells express the microglial marker Iba1.

Conclusion
Altogether, based on transcriptional similarities to uncultured pMG and susceptibility to HIV infection, MDMi may serve as a first screening tool, whereas oMG, cultured pMG and iPSC-MG provide more representative microglial culture models for HIV research. This novel 3D organoid model serves as a tool to study the interaction of HIV with different CNS cell types in a CNS-like environment.

S17

FUSION OF A HUMAN IGG1 TO BROADLY NEUTRALIZING ANTI-HIV-1 NANOBODIES RESULTS IN IMPROVED NEUTRALIZATION POTENCY AND THE ABILITY TO MEDIATE FC EFFECTOR FUNCTIONS

Angela Schriek[1], Steven De Taeye[1], Gillian Dekkers[2], Raimond Heukers[2], Theo Verrips[2], Marlies van Haaren[1], Neeltje Kootstra[1], Marit van Gils[1]

[1] *Amsterdam UMC, Amsterdam, the Netherlands*
[2] *QVQ, Utrecht, the Netherlands*

Background
HIV-1 is still a major global problem due to its substantial mortality. Previous studies have shown that broadly neutralizing antibodies (bNAbs) targeting the HIV-1 envelope glycoprotein (Env) can mediate viral suppression and the elimination of virus-infected cells, showing their therapeutic potential. Another approach for HIV-1 treatment is the use of nanobodies. Nanobodies are more stable, easy to produce and their small size and relatively long CDR3 loop facilitates binding to epitopes on Env that might not be accessible to conventional bNAbs. The most potent anti-HIV-1 nanobody described so far is J3, targeting the CD4 binding site, that can neutralize more than 90% of all circulating strains.

Methods
We generated multivalent anti-HIV-1 nanobodies to improve neutralization capacity and used Fc-engineering to add Fc-dependent effector functions. Nanobodies were couples using 15-30 amino acid long glycine-serine (GS) linkers. To gain Fc effector functions, nanobodies were fused to an IgG1 Fc domain.

Results
Bivalent and trivalent J3 nanobodies showed enhanced neutralization potency compared to the monovalent variant, indicating that the avidity for HIV-1 Env binding is improved. The J3-Fc variant showed increased neutralization potency compared to the bivalent J3. This suggests that the orientation and flexibility of the nanobody domains determines Env recognition and neutralization capacity. In addition, the nanobodies fused to a human IgG1 domain were able to activate THP-1 cells hereby inducing antibody-dependent cellular -phagocytosis and –trogocytosis. Moreover, the antibodies were able to activate NK cells, measured by CD16 loss and CD107a upregulation.

Conclusion
Our nanobody-Fc constructs showed improved neutralization capacity and Fc-effector functions and these may prove to be valuable towards a functional HIV-1 cure.

S18

COMMUNITY ENGAGEMENT IN HIV CURE RESEARCH EFFORTS: EXPLORING PERSPECTIVES ON MEANINGFUL INVOLVEMENT, WHETHER A CURE WILL CHANGE STIGMA, AND THE IMPORTANCE OF LANGUAGE AMONGST PEOPLE WITH HIV

Tamika Marcos[1], Kai J. Jonas[1], Maaike A.J. Noorman[2], Chantal den Daas[3], John de Wit[2], Sarah E. Stutterheim[1]

[1] *Maastricht University, Maastricht, Netherlands*
[2] *Utrecht University, Utrecht, the Netherlands*
[3] *University of Aberdeen, Aberdeen, United Kingdom*

Background
An HIV cure is on the horizon and the implications for people with HIV are significant. Social engagement based on the meaningful involvement of people with HIV is crucial to ensure that HIV cure research and communication is in line with the needs of people with HIV. This study explored people with HIV's perspectives on how they can best be involved in HIV cure efforts, the possible implications of a cure for HIV stigma, and the importance of language when discussing HIV cure.

Methods
Between April and June 2021, we conducted online semi-structured interviews with 28 people with HIV. Participants were recruited through the Dutch HIV association, an HIV consultant and snowball sampling. Interviews were transcribed verbatim, and inductively coded and analyzed for themes.

Results
Preliminary results show participants emphasized the importance of involving people with HIV in cure research, and showed an interest in being involved. For most, meaningful involvement was defined as participation in clinical trials. Other forms of social engagement were not frequently mentioned. With regard to stigma, most participants conveyed that the stigmatization of people with HIV is likely to decrease if a cure becomes available. Some indicated that they believed a cure would mean that HIV will no longer be stigmatized at all. However, the extent to which stigma was expected to decrease was contingent upon the type of cure. A suppressing cure was perceived to have less impact on HIV stigma than an eradicating cure. Participants further conveyed that the language used to communicate about HIV should be clear, simple, and free of jargon. Additionally, they indicated that transparency in communication about HIV cure efforts is key, and that such transparency has potential for stigma reduction as HIV stigma was perceived to be driven by a lack of knowledge. Some participants proposed establishing a platform where current cure updates and calls for clinical trial involvement are communicated.

Conclusion
This study highlights the importance of social engagement in HIV cure research efforts, and provides important insights on how people with HIV perceive meaningful involvement in HIV cure efforts, the ways in which an HIV cure may impact HIV stigma, and the importance of communicating about HIV cure with clarity and transparency. To ensure HIV cure research and communication are in line with the needs of people with HIV, it is imperative these results are considered when establishing future cure efforts.

S19

A LONGITUDINAL ANALYSIS OF CEREBRAL BLOOD FLOW IN PERINATALLY HIV INFECTED ADOLESCENTS AS COMPARED TO MATCHED HEALTHY CONTROLS.

Jason van Genderen[1], Malon van den Hof[2], Anne ter Haar[1], Charlotte Blokhuis[1], Vera Keil[2], Dasja Pajkrt[1], Henk Mutsaerts[2]

[1] *Emma Children's Hospital, Amsterdam, the Netherlands*
[2] *Amsterdam UMC, location AMC, Amsterdam, the Netherlands*

Background
Despite effective combination anti-retroviral therapy (cART), perinatally HIV infected (PHIV) adolescents still experience cognitive complications, such as decreased executive function or lower IQ. We previously reported higher cerebral blood flow (CBF) in basal ganglia and white matter (WM) in PHIV children on cART compared to matched controls. CBF is associated with cognitive domains in healthy children.

Methods
To determine longitudinal changes in CBF and its impact on cognitive complications, we measured CBF – using the MRI modality arterial spin labeling – in 21 PHIV adolescents and 23 controls matched for age, sex, ethnic background and socio-economic status twice with a mean follow-up of 4.6 years (SD: 0.3). We determined CBF changes in gray matter (GM), WM and the following subcortical regions: caudate nucleus, putamen and thalamus. We explored associations between CBF changes and brain WM hyperintensities (WMH), WM microstructural markers, and cognitive domains using linear mixed models adjusted for age and sex.

Results
The median age (in years) at follow-up was comparable between PHIV adolescents 17.4 (IQR:15.3-20.7) and controls 16.2 (IQR:15.6-19.1). At baseline, PHIV adolescents had higher CBF in the caudate nucleus (p = 0.007) and putamen (p = 0.010). At follow-up, we found no differences in CBF between groups. CBF development was comparable in GM, WM and subcortical regions in both groups. In our cohort, we found that over time an increase of GM CBF was associated with an increase of visual motor function (coefficient = 9.18, p = 0.043) and executive function (coefficient = 1.36, p=0.045). Increase of CBF in the caudate nucleus, putamen and thalamus was associated with an increase processing speed (coefficient = 14.9, p = 0.033; coefficient = 16.1, p = 0.036; coefficient = 20, p = 0.003, respectively) and visual motor function (coefficient = 9.76, p=0.023; coefficient = 9.55, p = 0.045; coefficient = 12.5, p = 0.003 respectively). We found no association between CBF and WMH and WM microstructural markers.

Conclusion
CBF development is relatively normal in PHIV adolescents on cART. CBF decline is associated with cognitive impairment, irrespective of HIV status. Subtle changes in CBF might play a role in the occurrence of impaired cognitive function in PHIV adolescents.

S20

LONG TERM TRENDS OF COAGULATION PARAMETERS IN PEOPLE LIVING WITH HIV-1 TREATED WITH COMBINED ANTIRETROVIRAL THERAPY

Soerajja Bhoelan, Jaime Borjas Howard, Vladimir Tichelaar, Wouter Bierman, Karina Meijer

UMC Groningen, Groningen, the Netherlands

Background
Human immunodeficiency (HIV) infection causes a procoagulant state. However, haemostatic trends after long term treatment with combination antiretroviral therapy (cART) are unclear. Therefore, we aimed to longitudinally describe coagulation parameters in people living with HIV on cART.

Methods
We followed forty male HIV-1 infected subjects starting cART. Plasma levels of procoagulant parameters, factor VIII (FVIII), von Willebrand factor (vWF) and D-dimer, and anticoagulant parameter Protein S (PS), were measured before start of cART and three months, one and nine years later. We adjusted for cardiovascular risk factors (age, smoking and hypertension) at baseline as confounding variables.

All subjects provided informed consent. The study was approved by the medical ethics committee of University Medical Centre Groningen.

Results
At baseline, mean age was 44±12 years and median CD4/CD8-ratio 0.24(IQR 0.17-0.36). Procoagulant parameters were markedly elevated and PS was in the lower range of normal. At nine years CD4/CD8-ratio improved to median 0.87(IQR 0.74-1.31).

At three months, the procoagulant parameters were decreasing (FVIII -37.5%[95% CI -53.9, -21.2], vWF -52.7%[-77.9, -27.6], D-dimer -239.5 µg/L[-376.9, -102.2]).
This trend continued moderately until one year. Conversely, from one to nine years an increase was observed (FVIII 15.6%[4.5, 26.7], vWF 1.2%[-13.3, 15.7], D-dimer 102.4 µg/L[-33.3, 238.1]). After correction for age, smoking and hypertension, this increase was reversed (FVIII -6.5%[-23.5, 10.5], vWF -10.4%[-33.6, 12.7], D-dimer -9.1 µg/L[-254.3, 236.2]).

PS remained stable during the first year and slightly increased from one to nine years (total 12.2%[4.2, 20.2], free 13.2%[6.2, 20.2]).
In the first year, changes in mainly the procoagulant parameters were correlated to CD4/CD8 ratio improvement. After this period, this correlation was no longer present.

Conclusion
Immune recovery by cART restores the procoagulant state in HIV during the first year. On long term this restoration is reversed despite on-going immune recovery. This seems to be related to cardiovascular risk factors. Future research is needed to evaluate whether the observed increase in procoagulant parameters is similar to trends seen in the general population.

S21

POTENTIAL DRUG-DRUG INTERACTIONS IN MALE PATIENTS LIVING WITH HIV WHO USE DRUGS TO TREAT LOWER URINARY TRACT SYMPTOMS

David Burger[1], **Piter Oosterhof[2]**, Karin Grintjes[1], Manon Marneef-Pietersma[1], Frank Ancona[1], Xiaoye Zhu[1], Stephan Keijmel[1], Olivier Richel[1], Reinout van Crevel[1], David Jansen[1]

[1] *Radboudumc, Nijmegen, the Netherlands*
[2] *OLVG, Amsterdam, the Netherlands*

Introduction
Lower urinary tract symptoms (LUTS) in relation with benign prostatic hyperplasia is becoming more prevalent in the ageing population of male patients living with HIV (PLWHIV). Drugs to treat LUTS are known for their potential role as victims of drug-drug interactions (DDIs) as well as their side effects (e.g., hypotension, dizziness, abnormal ejaculation). We aimed to evaluate current use of drugs to treat LUTS in our cohort of adult male PLWHIV.

Methods
We reviewed pharmacy records of all male PLWHIV aged 18 years and older who visited the outpatient pharmacy of Radboudumc in the first 6 months of 2021. We recorded their combination antiretroviral therapy (cART) regimen as well as any use of drugs to treat LUTS (ATC codes G04CA/CB/CX and G04BD). DDIs were assessed using the Interaction Checker developed by the University of Liverpool (*https://www.hiv-druginteractions.org/checker*).

Results
A total of 411 male PLWHIV were included in this analysis. Median (+interquartile range, IQR) age was 53 (41-62) years. Nineteen (4.6%) patients used one or more drugs to treat LUTS; alpha-blockers were used by 13, anticholinergic agents by 5, and beta-3 agonists by 2 patients. As expected, older patients were at higher risk for receiving treatment for LUTS: Q1 (20-40 years): 0%; Q2 (41-52 years): 2%; Q3 (53-61 years): 7%; Q4 (62-79 years): 10%. Seven DDIs were noted in 6/19 (32%) patients. Tamsulosin (n=4), silodosin, mirabegron, and solifenacin (n=1 each) were the victims of a DDI. Boosted darunavir (n=5), boosted elvitegravir and nevirapine (n=1 for both) were the perpetrators of a DDI. In the remaining 13/19 (68%) patients non-interacting HIV drugs such as dolutegravir- and doravirine-based regimens were used in combination with treatment for LUTS. Following medication reviews of the 6 patients with DDIs between cART and LUTS treatment the following interventions were proposed: change in alpha-blocker (n=4), change in cART (n=2), and dose reduction of the anticholinergic agent (n=1).

Conclusion
Treatment for LUTS coincided with cART in 7-10% of patients aged above the median value in our cohort. Improvements in DDI management appeared to be possible in approximately 1/3 of the male PLWHIV with LUTS, which underlines the importance of regular and repeated medication reviews in this population.

S22

PRIMARY HIV-1-INFECTION WITH EMTRICITABINE/TENOFOVIR DISOPROXIL RESISTANT STRAIN IN A MAN WHO HAS SEX WITH MEN (MSM) ON PRE-EXPOSURE PROPHYLAXIS (PREP) DESPITE EXCELLENT SELF-REPORTED ADHERENCE FOR MULTIPLE YEARS: A CASE REPORT

Jeffrey Koole[1], Feline de la Court[1], Matthijs Welkers[2], Kenneth Yap[1], Eline Op de Coul[3], Maria Prins[1], Elske Hoornenborg[1]

[1] *Public Health Service Amsterdam, Amsterdam, the Netherlands*
[2] *Dept. of Medical Microbiology and Infection Prevention, Amsterdam UMC, Amsterdam, the Netherlands*
[3] *Centre for Infectious Disease Control, RIVM, Bilthoven, the Netherlands*

Background
Pre-exposure prophylaxis (PrEP), consisting of emtricitabine/tenofovir disoproxil fumarate, has been shown to be a safe and highly effective drug regimen protecting against HIV-1 infection. In 2015 and 2019, respectively, the Amsterdam PrEP (AMPrEP) demonstration project and the Dutch national PrEP program started, providing PrEP and related care for eligible individuals. A known reason for PrEP-failure is the acquisition of an HIV-1 strain resistant against one or both drugs used in the PrEP regimen. Between 2017-2019, baseline reverse transcriptase (RT) resistance determination at the AmsterdamUMC showed that in only 2/164 (1.2%) individuals recently diagnosed with HIV a 184V mutation was identified, indicating a low level of potential resistance to PrEP in Amsterdam.

Methods/Results
In August 2021, a primary HIV-infection was diagnosed in a 39-year-old male patient who has sex with men (MSM) who had used event-driven pre-exposure prophylaxis (PrEP) for two years with self-reported excellent adherence, defined as 2 tablets 2-24 hours prior to the sex act, followed by 1 tablet both 24 and 48 hours after the first intake. Three months prior to diagnosis, HIV test results (p24-antigen, HIV-antibody and HIV-RNA) and sexually transmitted infection (STI) test results were negative. He requested testing in-between the scheduled quarterly PrEP visits because of weight loss (6% of body weight), diarrhoea, oropharyngeal mucosal defects and myalgia. He had a positive p24-antigen and HIV-antibody test, HIV RNA load of 460,000 copies/mL, and indeterminate HIV western blot (gp120 and gp41 weak positive, p31-antigen negative), corresponding to Fiebig stage IV. Genome sequencing identified K65R and M184V reverse transcriptase mutations, indicating resistance to both emtricitabine and tenofovir disoproxil and potential cross-resistance to abacavir and lamivudine. In addition, V108I and E138A mutations were identified, associated with resistance to nevirapine and rilpivirine. No mutations associated with resistance to integrase nor protease inhibitors were identified. Unfortunately, due to the event-driven PrEP regimen, assessing adherence via dried blood spots was not possible. In the two months prior to diagnosis, receptive condomless anal intercourse with four male partners (no group sex) both in the Netherlands and in Belgium and sexualized drug use (GHB, poppers, XTC and kamagra) were reported. He did not inject drugs. He started antiretroviral therapy, consisting of dolutegraviur and darunavir/cobicistat.

Conclusion
Acquisition of a resistant HIV-1 resistant strain can occur among adherent PrEP-users. This case report emphasizes the importance of frequent HIV testing among PrEP-users, regardless of reported adherence, and genomic surveillance of new HIV infections.

S23

CHANGING BLOOD DONOR SELECTION POLICIES REGARDING MEN-HAVING-SEX-WITH-MEN (MSM): A POTENTIAL FOR HIV STIGMA REDUCTION?

Kennedy D'Abreu de Paulo[1], Ed Slot[2], Eva-Maria Merz[2], Kai Jesko Jonas[1]

[1] *Maastricht University, Maastricht, the Netherlands*
[2] *Sanquin, Amsterdam, the Netherlands*

Background
As of September 2021, the Dutch blood donor selection policy was changed, allowing sexually active men-who-have-sex-with-men (MSM) to donate blood under a monogamous relationship (>12months) criterion. This policy change aims to decrease discrimination of MSM while de-stigmatizing MSM sexual activity as high-risk behaviour for bloodborne infections. Given the close link between MSM and HIV, the effects of the policy change regarding blood donor selection has on HIV stigma for MSM remain unclear. There is both the potential of normalizing HIV and reducing HIV stigma due to the bloodborne infection transmission risk assessment, and for increased stigma since transfusion recipients now may receive labile blood products from MSM.

Methods
Alongside qualitative data collection from a survey among 214 MSM on attitudes towards blood donation (Mean age was 56, SD=12.75, range 24-89), social media statements were monitored and analysed after the introduction of the monogamous relationship criterion. Data collection for the social media comments took place on September 1st 2021, five hours succeeding the media attention. In total 889 comments were analysed after which 216 comments were eligible for coding. Both datasets were coded using Atlas.ti 9.1.2.

Results
MSM primarily perceived both the previous and current blood donation policies as discriminatory but did not see the link with HIV stigma (reduction). Normalization is seen in relation to heterosexual donors but not for HIV stigma reduction. In the general population sample the same link with HIV stigma was present in two directions: Statements addressed blood safety and the risk of an HIV infection in both a negative (increasing HIV stigma: receiving potentially HIV positive blood) as well as a positive way (equal rights, trust in blood safety).
The new policy bears potential to contribute to HIV stigma elimination. Yet, the MSM sample did not see a connection to HIV stigma reduction and was mainly focused on ingroup discrimination. That connection between blood donation and HIV stigma reduction was prevalent in the social media sample of the general population. Due to the absence of information on age, gender and sexual orientation in the social media sample further research is necessary to overcome this limitation.

Conclusion
The access for sexually active MSM to blood donations bears the potential to reduce HIV stigma even further, while additionally revealing that outdated stigmatizing attitudes still prevail. Communication strategies should aim at enhancing positive aspects of blood donor selection policy changes for MSM and alleviate HIV stigma further.

S24

USING PBPK MODELLING TO ESTIMATE LAMIVUDINE EXPOSURE WHEN APPLYING DOSE REDUCTIONS FOR CHILDREN WITH ABNORMAL KIDNEY FUNCTION

Tom Jacobs[1], **Thomas Nieuwenstein[2]**,
Joyce van der Heijden[3], Saskia de Wildt[3], David Burger[1],
Jolien Freriksen[3], Angela Colbers[1]

[1] *Department of Pharmacy, Radboudumc, Nijmegen, the Netherlands*
[2] *Department of Pharmacy, Radboudumc, Radboud Institute for Health Sciences, Nijmegen, the Netherlands*
[3] *Department of Pharmacology and Toxicology, Radboudumc, Nijmegen, the Netherlands*

Background
Lamivudine (3TC) is primarily excreted renally through glomerular filtration and active tubular secretion. In adults, dose reductions of 3TC are needed for patients with an estimated glomerular filtration rate (eGFR) <50ml/min/1.73m2 to prevent overdosing. Various studies suggest that 5-15% of children living with HIV have a decreased GFR. The recommended doses in the 3TC package insert for children with decreased eGFR are scaled from dose reductions in adults. Physiologically-based pharmacokinetic (PBPK) modelling could help inform dosing in this population in the absence of clinical data. The aim of this study was to support recommended 3TC dosing in children with moderate and severe renal impairment by simulating pharmacokinetics (PK) using PBPK modelling.

Methods
An existing 3TC Simcyp® model (v20), validated for adults with renal impairment, was used in this study. First, 3TC PK was simulated in virtual adults with various degrees of renal impairment and in virtual a healthy paediatric population. Model performance was evaluated by comparing predicted 3TC PK to observed clinical data (0.5 to 2-fold acceptance range). A model for children with abnormal kidney function was created by adjusting model input parameters to reflect renal impairment. Adjustments were extrapolated from the virtual renally impaired adult population. This paediatric abnormal kidney function model was validated using (val)ganciclovir as a surrogate compound, because it also undergoes glomerular filtration and tubular secretion.
Subsequently, simulations of 3TC PK were conducted in a virtual paediatric population <25kg with a normal renal function (eGFR >90ml/min/1.73m^2) receiving 10mg/kg 3TC once daily (QD), with moderately decreased GFR (eGFR: 30-49ml/min/1.73m^2) receiving 5mg/kg QD, and severely decreased GFR (eGFR: 15-29ml/min/1.73m^2) receiving a 5mg/kg loading dose followed by a 3.25mg/kg QD maintenance dose.

Results
The 3TC model was successfully validated (based on C_{max}, AUC, and CL/F) in adults and in children >2 years old (eGFR >90ml/min/1.73m^2). The abnormal kidney function model was successfully validated (based on AUC) using (val)ganciclovir for children with eGFR >90, 60-89, or 30-59ml/min/1.73m^2. Simulations of 3TC PK in children <25kg resulted in geometric mean AUC values of 19.7, 23.6 and 22.8mg*h/mL for normal renal function, and moderately and severely decreased GFR, respectively.

Conclusions
A PBPK model was developed for children GFR >15ml/min/1.73m^2 and validated for children with GFR >30ml/min/1.73m^2. The suggested 3TC dose reductions for children with abnormal kidney function resulted in similar simulated 3TC exposure compared to children without renal abnormalities. Clinical studies in children are needed to confirm these simulated findings.

S25

SUBGROUPS OF SEXUAL BEHAVIOUR IN MEN WHO HAVE SEX WITH MEN USING PRE-EXPOSURE PROPHYLAXIS IN THE NETHERLANDS: TRANSITIONS OVER TIME, DETERMINANTS OF TRANSITIONS, AND STI POSITIVITY RATES

Daphne van Wees[1], Liza Coyer[2], Mark van den Elshout[3], Eline Op de Coul[1], Fleur van Aar[1]

[1] *National Institute For Public Health And The Environment (RIVM), Bilthoven, the Netherlands*
[2] *Public Health Service of Amsterdam, Amsterdam, the Netherlands*
[3] *Municipal Health Service for the Utrecht region, Utrecht, the Netherlands*

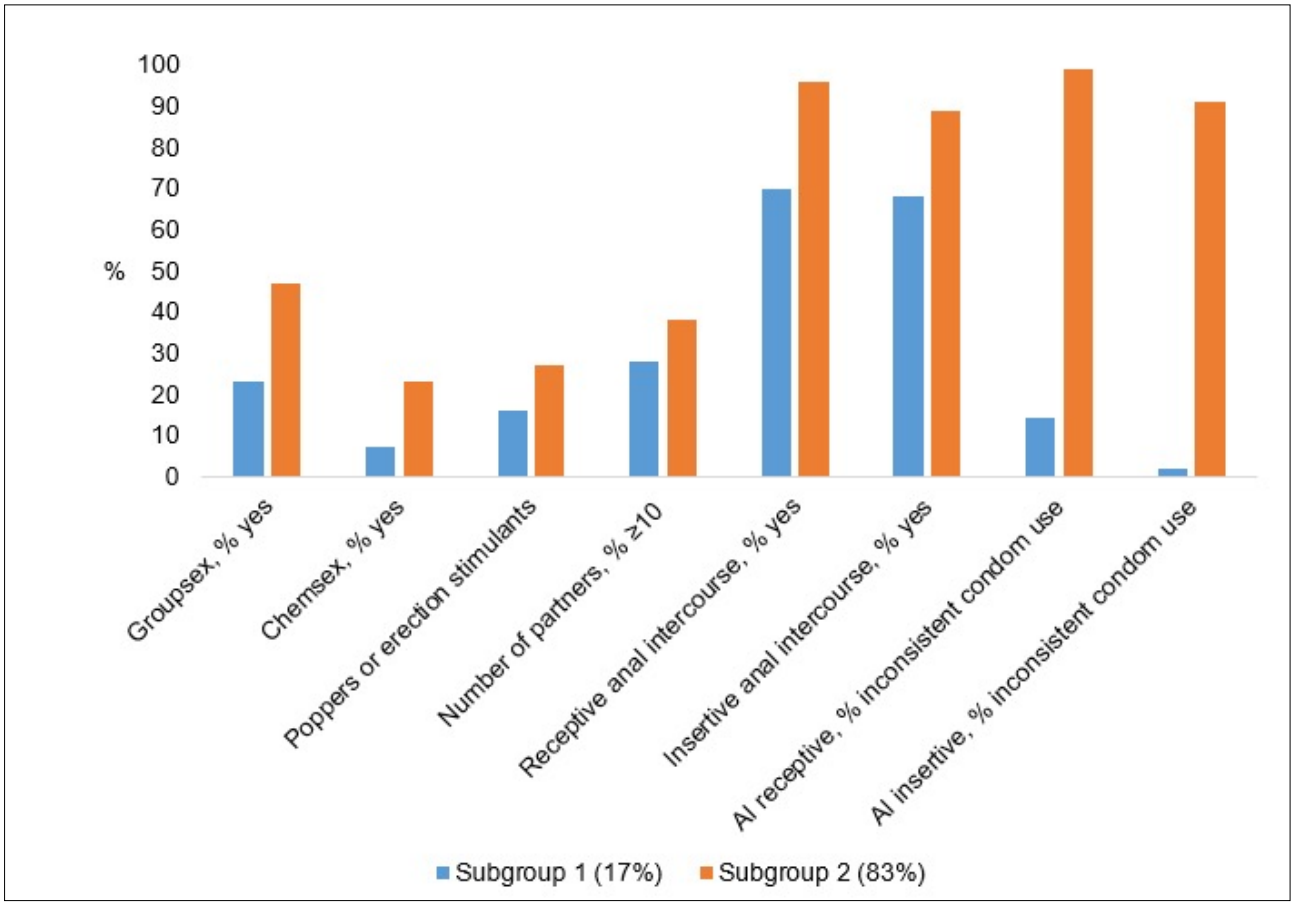
Background
Pre-exposure prophylaxis (PrEP) use significantly reduces HIV transmission, but may also lead to behaviour change and increased sexually transmitted infection (STI) incidence. We aimed to identify different sexual behaviour subgroups in men who have sex with men (MSM) using PrEP in the Netherlands, and to explore transitions between these subgroups before and after PrEP initiation.

Methods
We used longitudinal data from the national STI surveillance database (2018-2020) of HIV-negative MSM who first initiated PrEP in the Dutch PrEP pilot (start July 2019). All STI clinic visits of MSM who had ≥1 visit before and ≥1 visit after PrEP initiation were included. First, we identified subgroups based on sexual behaviour in the total time period using latent class analysis. Second, we modelled transitions of MSM between subgroups over time before (i.e., periods without PrEP use) and after PrEP initiation (i.e., periods using PrEP). Third, we identified predictors of transitioning using multi-state Markov models.

Results
Two subgroups were identified (n MSM=3,487, n visits=19,014), for which condom use was the most distinctive classification characteristic (Figure 1). Visits classified in subgroup 2 (83% of all visits) had a higher number of partners, and were more likely to report group sex, chemsex, use of poppers or erection stimulants, and condomless insertive and receptive anal sex, compared to visits in subgroup 1 (17%). Any STI positivity was 25% for subgroup 2 and 17% for subgroup 1. The probability of transitioning from subgroup 1 to 2 on the next visit was higher after (58%) compared to before PrEP initiation (44%; Table 1), as was the probability of staying in subgroup 2 (94% before versus 89% after). Predictors of transitioning were younger age (i.e., <35 years), and no anal STI or syphilis diagnosis. In 2020, STI positivity rates and percentage of transitions towards subgroup 2 were lower compared to 2018 and 2019.

Conclusion
MSM who started using PrEP in the national PrEP pilot were more likely to change their behaviour towards higher risk (e.g., group sex, condomless anal sex), and to stay at higher risk compared to periods without PrEP use, especially those who were older (i.e. ≥35 years) or diagnosed with an anal STI or syphilis. Behaviour change and reduction in STI positivity rates in 2020 may be explained by the impact of COVID-19 measures, such as physical distancing, on sexual behaviour. Behavioural and biomedical interventions are necessary to reduce STI risk among MSM using PrEP.

S25 (Figure 1)



S-25 (Table 1)

Before PrEP	Class	1	2
	1	0.42	0.58
	2	0.07	0.94
After PrEP	Class	1	2
	1	0.56	0.44
	2	0.11	0.89

S26

EFFECTS OF THREE PHASES OF COVID-19 RESTRICTIONS ON SEXUAL HEALTHCARE USE, PRE-EXPOSURE PROPHYLAXIS USE AND SEXUALLY TRANSMITTED INFECTION INCIDENCE AMONG MEN WHO HAVE SEX WITH MEN IN AMSTERDAM, THE NETHERLANDS

Feline de la Court[1], Anders Boyd[1,2], Liza Coyer[1], Mark van den Elshout[1], Henry J.C. de Vries[1,3], Amy Matser[1], Elske Hoornenborg[1], Maria Prins[1,4]

[1] *Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands*
[2] *Stichting HIV Monitoring, Amsterdam, the Netherlands*
[3] *Dept. of Dermatology, Amsterdam UMC, Amsterdam, the Netherlands*
[4] *Dept. of Inf. Diseases, AII, Amsterdam UMC, Amsterdam, the Netherlands*

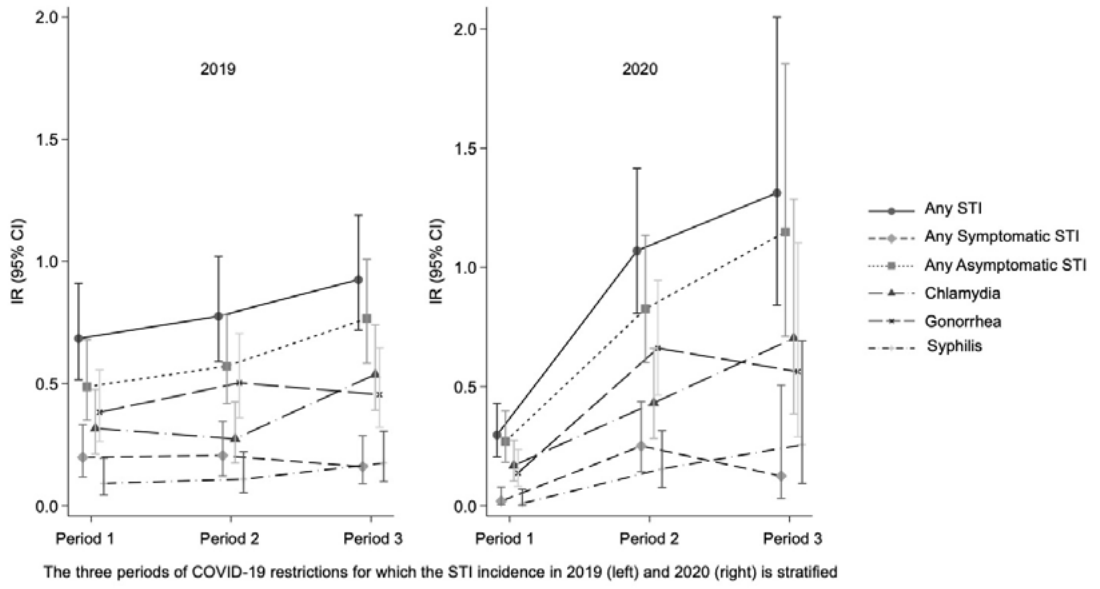
Background
COVID-19 restrictions have disrupted sexual healthcare provision, especially for pre-exposure prophylaxis (PrEP) using men who have sex with men (MSM). This study therefore explores the effects of COVID-19 restrictions on sexual healthcare use, PrEP use, and sexually transmitted infection (STI) incidence among MSM participating in a PrEP demonstration project in Amsterdam, the Netherlands (AMPrEP).

Methods
We retrieved data from 2019-2020 for AMPrEP participants with ≥1 study visit in 2019 (n=305), and two questionnaires on COVID-19 measures and sexual behaviour in 2020 and 2021 (n=203; n=160). Analyses were stratified for three periods of COVID-19 restrictions (first: 15/3/2020-15/6/2020 [lockdown]; second: 16/6/2020-15/9/2020 [social distancing]; third: 16/9/2020-31/12/2020 or 1/4/2021 for COVID-19 questionnaire data [partial lockdown and curfew]). Evaluated endpoints included proportion returning for sexual healthcare during COVID-19, change in PrEP use (increased/unchanged vs. deceased/stopped; relative to 2019), and incidence of any STI (chlamydia, gonorrhoea, or syphilis; diagnosed at clinic/study visit) or HIV. We modelled determinants of care and PrEP use via multivariable logistic regression, and STI incidence using piecewise Poisson regression; comparing 2020 periods to those in 2019.

Results
Of the 305 included in the analysis, 72.8% (n=222) of participants returned for care during the COVID-19 pandemic, among which 147, 190, and 148 visits took place during the first, second, and third period of COVID-19 restrictions, respectively. Daily (versus event-driven) PrEP use was significantly associated with returning for care across all periods (first: aOR=2.38 [95%CI=1.44-3.96]; second: aOR=2.67 [95%CI=1.62-4.39]; third: aOR=3.53 [95%CI=2.1-5.94]; all with p<0.001). Increased/unchanged PrEP use was reported by 55.2% (n=112), 58.1% (n=93), and 55.6% (n=89) during the first, second, and third periods, respectively. Increased/unchanged PrEP use was more likely among those reporting chemsex in the first (aOR=2.79; 95%CI=1.53-5.09; p=0.001) and third (aOR=2.19; 95%CI=1.13-4.24; p=0.020) periods, and those reporting increased/unchanged number of sex partners relative to 2019 during the second period (aOR=3.84; 95%CI=1.37-10.75; p=0.010). STI incidence was significantly lower in 2020 than 2019 during the first (IRR=0.43, 95%CI=0.28-0.68), yet seemed higher during the second (IRR=1.38, 95%CI=0.95-2.00) and third periods (IRR=1.42, 95%CI=0.86-2.33), albeit non-significantly (figure 1). No new HIV infections were diagnosed.

Conclusion
COVID-19 restrictions coincided with reduced care and PrEP use. The significantly lower STI incidence during the first period of COVID-19 restrictions and subsequent increase suggests a delayed diagnosis effect. Ways to stimulate PrEP re-uptake and restore regular STI testing are needed; particularly focusing on populations with missed care opportunities during COVID-19 to help ensure effective reinstatement of sexual healthcare.

S26 (Figure 1): STI incidence in 2019 and 2020 amang AMPrEP participants, both stratified for the three periods of COVID-19 restrictions imposed in 2020 in the Netherlands



The three periods of COVID-19 restrictions for which the STI incidence in 2019 (left) and 2020 (right) is stratified

Explanation of data: IR = incidence rate per person-year; 95% CI = 95% confidence interval. Explanation of periods of COVID-19 restriction in the Netherlands, applied to both 2019 and 2020 data: first=15/3/2020-15/6/2020 including a nationwide lockdown; second=16/6/2020-15/9/2020 including few public restrictions while social distancing remained; third=16/9/2020-31/12/2020 including partial lockdown with restricted access to public spaces and curfew. Any STI refers to any chlamydia, gonorrhoea, or syphilis diagnosis at a study or clinic visit. Any symptomatic STI refers to any chlamydia, gonorrhoea, or syphilis diagnosis at a study or clinic visit for which symptoms were reported by the patient. Any asymptomatic STI refers to any chlamydia, gonorrhoea, or syphilis diagnosis at a study or clinic visit for which no symptoms were reported by the patient.

S27

REDUCTION IN THE MAGNITUDE OF COVID-19-ASSOCIATED CHANGES IN SEXUAL ACTIVITY AND DISRUPTION IN HIV/STI TESTING OR PREP USE AMONG MSM RESPONDING TO THE 2ND COVID-19, SEX AND INTIMACY SURVEY

Philippe Adam[1,2], Wim Zuilhof[3], Chantal Den Daas[4], Eline Op de Coul[5], Paul Zantkuijl[3], Jean-Baptiste Paolucci[1], Itsada Ketsuwan[1], John De Wit[6]

[1] *Institute for Prevention and Social Research (IPSR Utrecht), Utrecht, the Netherlands*
[2] *UNSW Sydney, Sydney, Australia*
[3] *Soa Aids Nederland, Amsterdam, the Netherlands*
[4] *University of Aberdeen, Aberdeen, United Kingdom*
[5] *National Institute for Public Health and the Environment, Bilthoven, the Netherlands*
[6] *Utrecht University, Interdisciplinary Social Science, Utrecht, the Netherlands*

Background
Moderate reductions in sexual activity and substantial disruptions in HIV/STI testing and PrEP use were observed among MSM during the first Dutch lockdown, but what happened thereafter is less documented. We assessed COVID-19-related changes in sexual activity and partnerships, condomless anal intercourse (CAI), HIV testing, and PrEP use among Dutch MSM from January 2020 to mid-February 2021.

Methods
Respondents were recruited via social media in March-June 2021 to complete an online survey. 1520 adult MSM provided data for the analyses. MSM reported on their sexual and risk behaviour in six periods: pre-lockdown (T1, Jan–Feb 2020), 1st lockdown (T2, mid-Mar–mid-May 2020), relaxation of restrictions (T3, Jun–Jul 2020), normalisation (T4, Aug–Sept 2020), start of 2nd lockdown (T5, mid-Oct 2020–Mid-Dec 2021) and intensification of 2nd lockdown (T6, mid-Dec 2020–mid-Feb 2021). COVID-19 related disruptions in HIV/STI testing and PrEP use since the start of COVID-19 were also reported.

Results
MSM's behaviours are shown in Table 1. The proportion of sexually active MSM decreased by 17% from T1–T2, partially rebounded by T3 and was stable thereafter (75.5%-78.1%). Sex with casual partners was most affected by COVID-19 and decreased by 47% from T1–T2 and 17% from T4-T5/T6. CAI decreased by 13% from T1-T2 and proportions were similar to pre-lockdown at T3-T6. CAI with casual partners was less frequent at T2-T5 than pre-lockdown and returned to pre-lockdown level at T6. 34% of MSM had to postpone STI/HIV testing at some point during the studied periods because of COVID-19, and 48% of these had tested at the time of survey. 53% of the MSM who used PrEP in Jan-Feb 2020 stopped PrEP usage at some point; 74% had resumed PrEP at the time of survey.

Conclusion
COVID-19 changes in sexual activity were less pronounced during the 2nd lockdown than during the 1st lockdown, suggesting a possible effect of adaptation fatigue. Sexual risk practices with casual partners returned to pre-lockdown level at T6, when the 2nd lockdown became stricter. As sexual activity and risk rebound, it is concerning that disruptions in testing and PrEP use were only partially resolved.

S27 (Table 1): Behaviours per period (N=1520)

	T1	T2	T3	T4	T5	T6
Sexual activity	81.2%	67.6%***	76.0%***	78.1%**	75.5%***	75.7%***
Casual partners	31.4%	16.6%***	24.6%***	27.5%***	22.7%***	22.9%***
Any CAI	51.9%	45.2%***	50.2%	51.1%	51.9%	53.4%
CAI with casual parters	12.8%	7.2%***	11.0%**	11.4%*	10.1%***	12.0%

*p<0.05; **p<0.01; ***p<0.001; T1=reference.

S28

CHARACTERIZATION OF THE HIV-1 SUBTYPE C RESERVOIR DURING ART IN SOUTH-AFRICAN MEN AND WOMEN

Ninée Buchholtz[1], Lucas Hermans[2], Chijioke Umunnakwe[2], Dorien De Jong[1], Ayman Osman[2], Jori Symons[1], Hugo Tempelman[2], Annemarie Wensing[1], Monique Nijhuis[1]

[1] *UMC Utrecht, Utrecht, the Netherlands*
[2] *Ndlovu Medical Center, Elandsdoorn, South Africa*

Background
Subtype C is the predominant HIV subtype globally and in sub-Saharan Africa. Nonetheless, subtype B is most studied. The biggest obstacle to an HIV cure is the proviral reservoir, which can be categorized into an intact and a defective fraction. An essential part of cure studies is characterizing subtype C reservoir before and during antiretroviral therapy (ART). The aim of this study was to investigate the impact of late presentation (low CD4-count) and gender differences on size and activity of subtype C reservoirs before and during ART in rural South Africa, which remains under-studied in cure research.

Methods
78 participants were included, 65% of whom were female. Median CD4-count at ART initiation was 266.5 cells/mm3 (IQR: 290) and median viral load (VL) was 48,652 copies/mL (IQR: 138,158). All patients started protocolized first-line ART. At baseline and 48 weeks PBMCs were collected for analyses of intact and defective proviral DNA (IPDA) and msRNA as a measure of reservoir activity.

Results
At baseline a significantly higher CD4-count was observed for females compared to males (median 300 vs 203 resp. $p<0.05$). However, no significant difference in the intact or defective DNA reservoir, msRNA or VL was observed between genders. Significant correlations were observed between baseline VL and msRNA ($p<0.01$), VL and intact, and defective proviral DNA ($P<0.01$). Inverse significant correlations were found between VL and CD4 ($P<0.01$), and CD4 and msRNA ($P<0.05$). Interestingly, msRNA and CD4 were significant, positive and inverse correlated respectively, with defective proviral DNA ($P<0.05$) but not with intact proviral DNA at baseline. During treatment, a significant decrease of both the intact and defective reservoir, as well as msRNA was detected ($P<0.01$). The decrease of the intact reservoir was significantly more profound than the decrease of the defective proviral DNA ($P<0.05$).

Conclusion
Decrease of the HIV reservoir size during treatment is similar between genders, with a more pronounced decline of the intact reservoir. At baseline no impact of late presentation on the size of the (intact) reservoir was observed. Interestingly, levels of msRNA correlate with the size of the large defective viral reservoir rather than the smaller intact reservoir demonstrating that the defective reservoir actively transcribes msRNA. These results contribute to a better understanding of the subtype C reservoir. A combination of IPDA and msRNA could form the basis for the evaluation of HIV cure interventions and could serve as a predictor for treatment response.

S29

EXTINCTION OF ALL INFECTIOUS HIV IN CELL CULTURE BY THE CRISPR-CAS12B SYSTEM

Minghui Fan, Ben Berkhout, Elena Herrera-Carrillo

Amsterdam UMC, Amsterdam, the Netherlands

The CRISPR-Cas9 system has been used successfully for genome editing of various organisms. We previously reported inhibition of the human immunodeficiency virus (HIV) in cell culture infections and subsequent viral escape when a single guide RNA (gRNA) was used, but complete inactivation of all infectious HIV with certain combinations of two gRNAs. The recently described CRISPR-Cas12b system may provide an even more promising tool for genome engineering with increased activity and specificity. Full HIV inactivation in cell culture was achieved with only a single gRNA (called crRNA). We disclose that DNA cleavage by the Cas12b endonuclease and subsequent DNA repair causes mutations with a sequence profile that is distinct from that of Cas9. Both CRISPR systems can induce the typical small deletions around the site of DNA cleavage and subsequent repair, but Cas12b does not induce the pure DNA insertions that are routinely observed for Cas9. We propose that the different architecture of the Cas9 versus Cas12b endonuclease explains this effect. Then we tested the antiviral activity of dual crRNA combinations and analyzed the HIV proviral genomes at the target sites. We demonstrated that dual crRNA combinations can exhibit more robust antiviral activity than a single crRNA attack and, more importantly, that the dual-crRNA therapy can prevent virus escape in long-term cultures. We did not detect excision of HIV sequences located between the two Cas12b cleavage sites in dual strategy, but efficient HIV inactivation by ”hypermutation” at both sites as a result of DNA cleavage and subsequent error-prone DNA repair.

S30

SOCIAL ENGAGEMENT OF PEOPLE WITH HIV IN HIV CURE RESEARCH: AWARENESS, IMPORTANCE AND MEANING OF HIV CURE

Maaike Noorman[1], Chantal den Daas[2], Tamika Marcos[3], Sarah Stutterheim[3], Kai Jonas[3], John de Wit[1]

[1] *Utrecht University, Utrecht, the Netherlands*
[2] *University of Aberdeen, Aberdeen, United Kingdom*
[3] *Maastricht University, Maastricht, the Netherlands*

Background
Since the first cases of AIDS were reported, 40 years ago, much progress has been made in HIV treatment. The next frontier now is the quest for an HIV cure. As research into the development of an HIV cure advances, it is crucial to involve people with HIV in this research, to ensure alignment with their needs and lives. This research aims to assess awareness, importance and meaning of HIV cure among people with HIV in the Netherlands.

Methods
Online semi-structured interviews of 60-90 minutes were conducted. Twenty-eight participants from diverse backgrounds were recruited between April and June 2021 through the Dutch HIV Association, an HIV consultant and snowball sampling. Interviews were transcribed verbatim and thematic analysis was undertaken. Codes were developed to inductively identify main themes and subthemes.

Results
Preliminary findings show that most participants had heard about major developments in cure research (e.g., Berlin patient) and several participants followed developments more closely (e.g. naming strategies such as CRISPR-Cas). All participants found HIV cure research important. Nevertheless, the majority indicated that being cured was not a priority. They would welcome an HIV cure as it increased quality of life (i.e., no longer having to take medication, more experienced freedom). However, it was not considered life changing for participants who experienced little impact of HIV in their daily life and for people living long-term with HIV who experienced permanent side effects that could not be remedied with an HIV cure. Instead, most participants contended that a cure would be more important for those who do not have equal access to treatment, are affected by stigma, are young or recently diagnosed. Furthermore, participants had high expectations of a cure. An ideal cure was described as non-invasive HIV eradication (i.e. sterilizing cure), that would improve their lives, without any side effects and was available to everyone.
HIV suppression (i.e. functional cure) was not viewed by all participants as a cure but rather as improved treatment. It was often compared to long-acting injectables. Regardless, most participants would welcome improvements in HIV suppression. Some participants also expressed concerns about HIV suppression as HIV remained in the body.

Conclusion
These findings underscore the importance of social engagement in HIV cure research as it provides important insights on how aware people with HIV are of HIV cure developments, how important they find an HIV cure and what an HIV cure would mean to them.

S31

HIGH RATES OF DIFFUSION IMPAIRMENT AFTER PNEUMOCYSTIS JIROVECII PNEUMONIA IN PEOPLE LIVING WITH HIV: A RETROSPECTIVE CROSS-SECTIONAL STUDY

Patrick Oomen, Berend Welzen, Andy Hoepelman, Tatiana Mudrikova

UMC Utrecht, Utrecht, the Netherlands

Background
The incidence of pulmonary disease is increased in people living with HIV (PLWH) and is attributed to a higher prevalence of traditional risk factors such as smoking and to HIV itself. Previous studies have mainly focussed on pulmonary restriction and obstruction and little research has been conducted on diffusion impairment. Pneumocystis jirovecii pneumonia (PJP), an opportunistic infection, is characterised by severe hypoxemia due to reduced alveolar diffusion capacity. It is therefore important to assess whether this abnormality recovers after treatment of PJP or whether residual pulmonary impairment persists.

Methods
We performed a retrospective cross-sectional analysis of pulmonary function parameters in PLWH with a history of PJP under follow-up in the UMC Utrecht. Pulmonary function tests (PFTs) were performed as part of standard care, were conducted in a standardised manner and included spirometry and CO diffusion measurement. All PFTs were reviewed by a pulmonologist for the presence of obstructive, restrictive or diffusive capacity impairment. Only PLWH with PFTs performed at least one year after the PJP episode were included. Pulmonary restriction was diagnosed with a total lung capacity Z-score <-1.64 and obstruction with a Tiffeneau-index <0.70. Impaired diffusion capacity was diagnosed with a transfer factor for carbon monoxide Z-score <-1.64, with a diffusion capacity of >60%, 40-60% and <40% defined as mild, moderate and severe, respectively. Continuous and categorical variables were compared using Mann-Whitney U or independent samples t-tests and Chi-square or Fisher's exact tests.

Results
Forty-nine participants met the inclusion criteria, of whom 42 were male (85.7%). Eight participants smoked (16.3%) and the median time since PJP and start of antiretroviral therapy was eight years (IQR 2.5-13.5). Median CD4 at PFT was 478 cells/μL (IQR 391.5-564.5). The occurrence of restrictive and obstructive pulmonary disease was limited (n=2, 4.1% and n=4, 8.1%, resp.). However, a considerable number of PLWH displayed diffusion impairment (n=19, 38.8%), mostly mild (n=15, 30.6%). Although there was a trend towards a difference between groups with and without diffusion impairment, no association with smoking was found (p=0.069).

Conclusion
In our cohort of PLWH with a history of PJP, a considerable proportion showed residual diffusion impairment years after infection. Further research is needed to determine whether and to which extent this is related to previous PJP-related damage and to what extent traditional risk factors such as smoking and HIV itself augment these abnormalities.

S31 (Table 1)

Table 1. Patient characteristics

Demographics	**n = 49**	**(IQR) / (%)**
Age (years)	54.00	9.00
Gender (male)	42	85.7
Clinical characteristics		
Time since HIV diagnosis (years)	8.00	10.00
Time since PJP diagnosis (years)	8.00	11.00
Time since start antiretroviral therapy (years)	8.00	11.00
Smoking		
- Current	8	16.3
- Former	13	26.5
- Never	28	57.1
Mode of transmission		
- MSM	25	51.0
- Heterosexual	8	16.3
- Other	16	32.7
Current cART regimen		
- INSTI-based	6	12.2
- PI-based	24	49.0
- NNRTI-based	13	26.5
- Other	6	12.2
History of pneumothorax	5	10.2
Admission to ICU during PJP	9	18.4
Adjunctive steroids during PJP	31	63.3
Biochemical characteristics		
Nadir CD4	29.00	50.00
CD4 at PFT*	478.00	173.00
Undetectable VL at PFT	46	93.9

*4 missing

S31 (Table 2)

Table 2. Pulmonary function test results

Restrictive pulmonary disease	**n = 49**	**(%)**
- No	47	95.9
- Yes	2	4.1
Obstructive pulmonary disease		
- No	45	91.8
- Mild	1	2.0
- Moderate	3	6.1
- Severe	0	0
Impaired diffusion pulmonary disease		
- No	30	61.2
- Mild	15	30.6
- Moderate	4	8.2
- Severe	0	0

S32

RETINAL STRUCTURE IN RELATION TO BRAIN FUNCTION IN PERINATALLY HIV-INFECTED ADOLESCENTS: A LONGITUDINAL AND CROSS-SECTIONAL ASSESSMENT

Jason Genderen[1], **Charissa Verkade[1]**, Malon Hof[2], Nazli Demirkaya[2], Anouk Schrantee[2], Frank Verbraak[2], Dasja Pajkrt[1]

[1] *Emma Children's Hospital, Amsterdam, the Netherlands*
[2] *Amsterdam UMC, location AMC, Amsterdam, the Netherlands*

Background
Subtle ocular abnormalities – including retinal changes – are reported in perinatally HIV-infected (PHIV) children despite effective combination antiretroviral therapy (cART). We previously found a thinner fovea in PHIV children compared to matched controls. Other studies found a thinner peripapillary retina nerve fiber layer (pRNFL) in PHIV children compared to controls. We also reported on associations between retina thickness (RT) and brain white matter (WM) microstructure. To assess the development of ocular abnormalities in PHIV children, we measured RT and its determinants in PHIV adolescents and controls longitudinally and cross-sectionally.

Methods
We measured RT using optical coherence tomography (OCT) on two occasions in 21 PHIV children or adolescents and 23 controls matched for age, sex, ethnic background and socio-economic status with a mean interval of 4.6 years (SD 0.3). We also included 22 participants (11 PHIV children and 11 controls, additionally matched for adoption status) together with the follow-up group for a cross-sectional assessment using a different OCT device. Magnetic resonance imaging (MRI) was performed to assess gray matter (GM) and white matter (WM) volume, WM hyperintensities and the white microstructure. We used linear (mixed) models to assess changes in RT and its determinants (over time), adjusting for age and sex.

Results
The median age (in years) in the longitudinal study at second assessment was 17.5 (IQR: 15.5-20.7) in PHIV adolescents and 16.4 (IQR: 15.8-19.5) in matched controls. The RT and its development were similar between the PHIV adolescents and controls. In our cohort, we found that changes in RT were associated with changes in GM volume (coefficient = 0.141, p = 0.014). Changes in the pRNFL was significantly associated with changes in WM microstructural makers: fractional anisotropy (coefficient = 0.030, p = 0.022) and radial diffusivity (coefficient = -0.568, p = 0.025). In our cross-sectional study the median age (in years) at assessment was 15.4 (IQR: 11.2-19.2) and 15.3 (10.9-17.6) for PHIV adolescents and controls, respectively. We found comparable RT between groups. A thinner pRNFL was associated with lower WM volume (coefficient = 0.117, p = 0.030).

Conclusion
PHIV children or adolescents appear to have a similar development of retinal structure. In our cohort, the associations between RT and MRI markers underscore the relation between retina and brain.

S33

PREFERENCE FOR NON-DAILY ART IS ASSOCIATED WITH ART-RELATED EMOTIONAL, PSYCHOSOCIAL, PHYSICAL AND ADHERENCE CHALLENGES AMONG PEOPLE LIVING WITH HIV IN THE NETHERLANDS

Reon van Dyk[1], Bart Lammertink[1], Ama Appiah[2], Hiskya Plieger[1], Patricia Rios[3], Chinyere Okoli[2]

[1] *ViiV Healthcare, Amersfoort, the Netherlands*
[2] *ViiV Healthcare, Brentford, United Kingdom*
[3] *ViiV Healthcare, Quebec, Canada*

Background
Daily antiretroviral treatment (ART) can be challenging for some people living with HIV (PLHIV). Long-acting regimens (LAR) allow for non-daily dosing. We explored unmet needs associated with daily dosing and LAR preference.

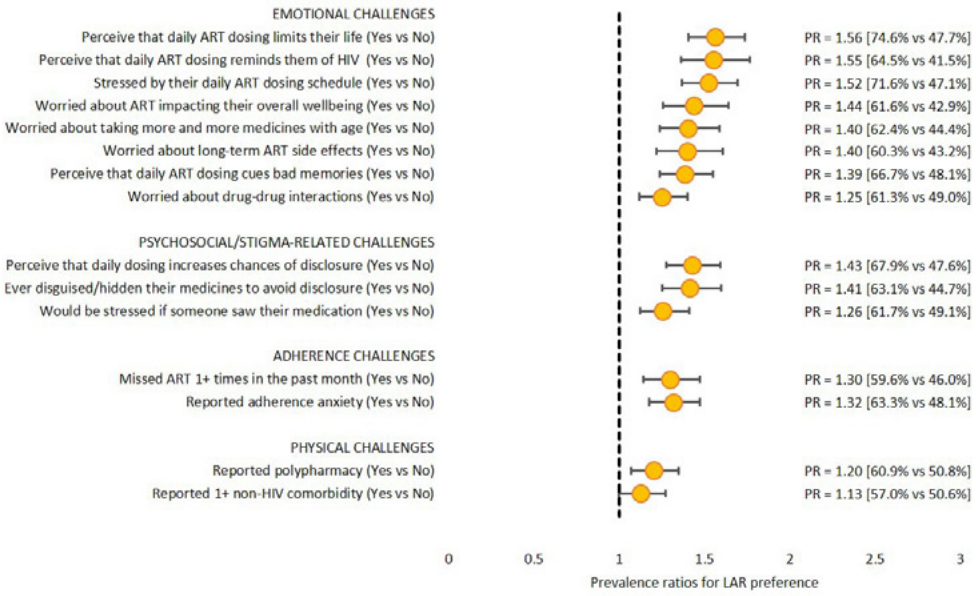
Methods
We analyzed the European Positive Perspectives PLHIV survey (Europe-wide, n=969; Netherlands, n=51]). Within four domains of ART-related challenges (emotional, psychosocial/stigma, physical, adherence), we tallied indicators of unmet need. Tertiles divided frequency distributions into three groups (low/moderate/high), each containing one-third of the pooled European population. Associations with LAR preference were explored using logistic regression.

Results
Of Dutch participants, 58.8%[30/51] indicated LAR preference with no significant socio-demographic differences; 32.2% ranked LAR as the single most important ART improvement. Regarding emotional challenges with daily dosing, 11.8%[6/51] felt it limited their life; 3.9%[2/51] felt stressed, and 35.3%[18/51] said daily dosing constantly reminded them of their HIV. Regarding psychosocial/stigma-related challenges, 23.5%[12/51] hid/disguised their medication and 25.7%[9/35] of those with sexual partners withheld their status from them. Regarding adherence challenges, 13.7%[7/51] reported adherence anxiety, and 37.2%[19/51] missed ART 1+ time during the past month. Physical challenges included difficulty swallowing (13.7%[7/51]) and comorbidities (84.3%[43/51]). Not only were these individual indicators associated with LAR preference in pooled analysis (Fig-1), within each domain, LAR preference increased with extent of challenges (Fig-2). LAR preference odds were 1.76 (95%CI=1.45-2.13) and 4.05 (95%CI=3.26-5.03) among those with moderate and high burden of ART-related emotional challenges, respectively, vs low; 1.50 (95%CI=1.11-2.04) and 2.33 (95%CI=1.68-3.21) among those with moderate and high anticipated stigma, respectively, vs low; and 1.53 (95%CI=1.14-2.04) and 2.06 (95%CI=1.45-2.91) among those with moderate and high levels of adherence barriers, respectively, vs low. LAR preference was 1.71 (95%CI=1.25-2.34) higher among PLHIV with 2+ non-HIV comorbidities vs HIV only, and 1.57 (95%CI=1.12-2.20) higher among those on 2+ comedications vs ART exclusively. Aggregated across domains and compared to those with unmet need on none of the domains, LAR preference odds were higher among those with unmet need on one (AOR=1.55, 95%CI=1.02-2.35), two (AOR=2.78, 95%CI=1.87-4.14), three (AOR=3.25, 95%CI=2.18-4.84), and all four domains (AOR=4.70, 95%CI=3.08-7.19). LAR preference was 67.6%[230/340] vs 47.2%[297/629] among those with vs without the perception that their ART needed improvement respectively; decomposition analysis revealed that 48.1% of this gap was explained by differences in ART-related emotional challenges while 20.4%, 16.5% and 7.3% attributable to differential anticipated stigma, adherence challenges, and physical challenges, respectively.

Conclusion
Many PLHIV have unmet needs with daily dosing and many expressed preferences for non-daily ART.

S33 (Figure 1)

Figure 1 Likelihood of indicating preference for non-daily (longer-acting) ART regimens [a] based on individual indicators of treatment challenges (present or absent) among people living with HIV in the Netherlands and 11 other European countries combined, 2019 (n = 969)

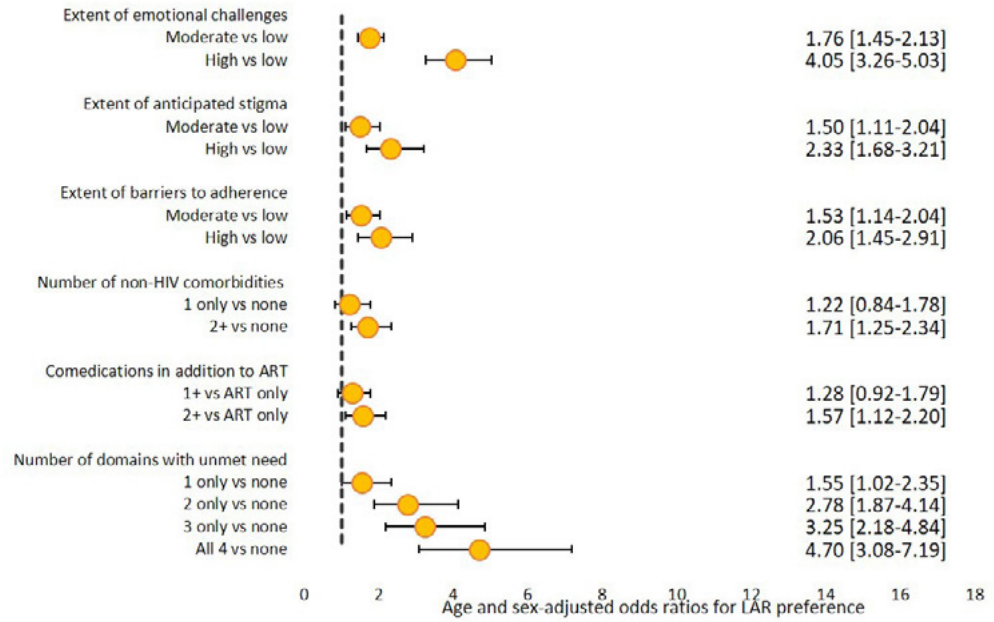


Note: ART = antiretroviral therapy; LAR = longer-acting regimens; PR = prevalence ratio, unadjusted (i.e., yes vs no). Other than the Netherlands, the remaining 11 European countries that participated in the Positive Perspectives, wave 2 were Austria, Belgium, France, Germany, Italy, Poland, Portugal, Republic of Ireland, Spain, Switzerland, and the UK.

[a] Answered "Agree" or "Strongly agree" to the statement "As long as my HIV stays suppressed, I would prefer not having to take HIV medication every day

S33 (Figure 2)

Figure 2 Adjusted odds of indicating preference for non-daily (longer-acting) ART regimens [a] by aggregated extent of treatment challenges (low, moderate, high) within [b] and across [c] domains of unmet need among people living with HIV in the Netherlands and 11 other European countries combined, 2019 (n = 969)



Note: ART = antiretroviral therapy; LAR = longer-acting regimens. Other than the Netherlands, the remaining 11 European countries that participated in the Positive Perspectives, wave 2 were Austria, Belgium, France, Germany, Italy, Poland, Portugal, Republic of Ireland, Spain, Switzerland, and the UK. The classification of extent of self-reported treatment challenges into three categories of low, moderate, and high was based on statistical distributions using tertiles. Tertiles are quantiles that split a frequency distribution into three groups comprising the bottom one-third, the middle one-third, and the highest one-third. Within the context of tallied/aggregated treatment challenges, we have referred to these three groups as those with low (bottom-third), moderate (middle-third), and high (top-third) extent of treatment challenges.

[a] Answered "Agree" or "Strongly agree" to the statement "As long as my HIV stays suppressed, I would prefer not having to take HIV medication every day"

[b] Emotional challenges assessed included report of having concerns about the risk of drug-drug interactions, the potential effect of ART on their overall wellbeing, side effects, perceived stress from their daily ART dosing schedule, the perception that their daily ART dosing schedule limited their life, that daily dosing cued bad memories, or that daily dosing served as a daily reminder of their HIV status. As a proxy for the number of stigma stressors, we tallied the different contexts in which participants refused to share their HIV status to avoid discrimination (range: 0 to 10). These reasons included anxiety over the prospect of any of the following happening simply because of their HIV status: "criminal prosecution", "being denied access to financial benefits/support", "my physical safety/potential violence", "being denied access to health care services", "it might affect my friendships", "I might lose my job", "it might affect my romantic or sexual relationships", "I might be excluded from activities", "they would see or treat me differently", and "they might then disclose my HIV status to others". As a proxy for the number of adherence barriers, we tallied the different reasons for which participants missed their HIV medication for at least once in the past month (range 0 to 15). These reasons were: depressed/overwhelmed; to forget about having HIV; bored of taking pills every day; to avoid side effects; work; busy; to reduce risk of long-term side effects; issues with dosing requirements such as meals; substance use; had no pills; trouble swallowing pills; privacy concerns; traveling/away; couldn't afford pills; or other. From a list of 21 health conditions, participants were asked to select which conditions they had ever been diagnosed with, and which ones they were currently taking medicines for at the time of the survey. From these data, we created a tally of non-HIV conditions participants had ever been diagnosed with, as well as conditions for which they were currently taking medicines at the time of the survey; these were both categorized as 0, 1 only, or 2+.

[c] Number of domains with unmet needs was assessed as a tally (range 0 to 4) of the following affirmative indicators on assessed domains (categorization as low, moderate, or high based on tertiles): moderate/high ART-related emotional challenges, moderate/high levels of anticipated stigma, moderate/high number of adherence barriers, or having been diagnosed with at least one non-HIV comorbidity.