

1 **Title page**

2 **Title:** Renal Impairment and Cardiovascular Disease in HIV-positive Individuals; The D:A:D Study

3 **Running title:** eGFR and Cardiovascular Disease in HIV

4 **This project was presented in part at CROI, February 23-26 2015, Seattle, abstract ID: 2099268**

5 **Word count abstract:** 200 words

6 **Word count text:** 3456 words

7  
8 **Authors**

9 Lene Ryom<sup>1</sup>, Jens D Lundgren<sup>1</sup>, Mike Ross<sup>2</sup>, Ole Kirk<sup>1</sup>, Matthew Law<sup>3</sup>, Philippe Morlat<sup>4</sup>, Colette Smit<sup>5</sup>,  
10 Eric Fontas<sup>6</sup>, Christoph A Fux<sup>7</sup>, Camilla I Hatleberg<sup>1</sup>, Stéphane de Wit<sup>8</sup>, Caroline A Sabin<sup>9</sup> and Amanda  
11 Mocroft<sup>9</sup> for the D:A:D Study Group

12  
13 <sup>1</sup>Department of Infectious Diseases, CHIP, Section 2100, Rigshospitalet, University of Copenhagen, Denmark

14 <sup>2</sup>Division of Nephrology, Mount Sinai School of Medicine, New York, USA

15 <sup>3</sup>The Kirby Institute, University of New South Wales, Sydney, Australia

16 <sup>4</sup>Université Bordeaux, INSERM U 897, CHU de Bordeaux, France

17 <sup>5</sup>Academic Medical Center, Div. of Infectious Diseases and Dept. of Global Health, University of Amsterdam  
18 and HIV Monitoring Foundation, Amsterdam, The Netherlands

19 <sup>6</sup>Nephrology department, Public Health department, CHU Nice, France

20 <sup>7</sup>Clinic for Infectious Diseases and Hospital Hygiene, Kantonsspital Aarau, Switzerland

21 <sup>8</sup>CHU Saint-Pierre, Department of Infectious Diseases, Brussels, Belgium

22 <sup>9</sup>Research Dept. of Infection and Population Health, UCL, London, United Kingdom

23 **Corresponding author**

24 Lene Ryom, M.D. PhD

25 Department of Infectious Diseases, CHIP, Section 2100, Rigshospitalet, Finsencentret, University of

26 Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen O

27 Tel: + 45 35 45 57 65/ Fax: +45 35 45 57 57/ email: [lene.ryom.nielsen@regionh.dk](mailto:lene.ryom.nielsen@regionh.dk)

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42 **Abstract** (200 words)

43 **Background** While the association between renal impairment and cardiovascular disease (CVD) is well  
44 established in the general population, the association remains poorly understood in HIV-positive  
45 individuals.

46 **Methods** Individuals with  $\geq 2$  estimated glomerular filtration rate (eGFRs) after 1/2/2004 were followed  
47 until CVD, death, last visit plus six months or 1/2/2015. CVD was defined as centrally validated myocardial  
48 infarction, stroke, invasive cardiovascular procedures or sudden cardiac death.

49 **Results** During 8.0 years median follow-up (Interquartile range 5.4-8.9) 1,357 of 35,357 developed CVD  
50 (incidence 5.2/1000 person-years [95%confidence interval, CI [5.0-5.5]). Confirmed baseline eGFR and CVD  
51 were closely related with 1.8% [95%CI 1.6-2.0%] estimated to develop CVD at five years at eGFR>90  
52 ml/min/1.73m<sup>2</sup>, increasing to 21.1% [95%CI 6.6-35.6%] at eGFR $\leq$ 30 ml/min/1.73m<sup>2</sup>. The strong univariate  
53 relationship between low current eGFR and CVD was primarily explained by increasing age in adjusted  
54 analyses, although all eGFRs $\leq$ 80 ml/min/1.73m<sup>2</sup> remained associated with 30-40% increased CVD rates and  
55 particular high rates at eGFR $\leq$ 30 ml/min/1.73m<sup>2</sup> (3.08 [95%CI 2.04-4.65]).

56 **Conclusions** Among HIV-positive individuals in a large contemporary cohort a strong relation between  
57 confirmed impaired eGFR and CVD was observed. This finding highlights the need for renal preventive  
58 measures and intensified monitoring for emerging CVD, in particular in older individuals with continuously  
59 low eGFR.

60  
61 **Keywords:** eGFR, renal impairment, kidney disease, cardiovascular disease, myocardial infarction, stroke,  
62 invasive cardiovascular procedures, sudden cardiac death, HIV

63

64

**Text (3456 words)**

65 **Introduction**

66 The association between impaired renal function and cardiovascular disease (CVD) is well established in the  
67 general population, in particular for severe levels of renal impairment [1-6]. As such more than 50% of all  
68 deaths in individuals with end-stage renal disease are related to a CVD event [7]. In contrast, most prior  
69 studies that have investigated the relation between renal impairment and CVD in HIV-positive individuals  
70 have been small, have used relatively broad definitions of CVD, or have focused on single measures of renal  
71 function which are subjected to random variation and the transient effects of acute illness [8-13]. The  
72 influence of a more sustained impairment of estimated glomerular filtration rate (eGFR) on well-defined  
73 CVD events in HIV-positive individuals is less clear.

74 Renal impairment is projected to become more prevalent among HIV-positive individuals in future years  
75 due to ageing and an accumulating burden of comorbidities and lifestyle related risk factors.

76 CVD is furthermore now one of the leading causes of non-AIDS death in HIV-positive individuals [14]. A  
77 better understanding of the rates of CVD among HIV-positives individuals with renal impairment is  
78 therefore warranted to assist identification of those at highest risk with a need for intensified monitoring  
79 and initiation of preventive measures [15]

80 The relationship between renal impairment and CVD is complex and may be mediated through a variety of  
81 different pathways [3, 6, 14]. These include accelerated coronary- and cerebrovascular atherosclerosis  
82 which may be mediated in part by increased inflammation and oxidative stress, atrial fibrillation and  
83 ventricular hypertrophy, which are common at severe levels of renal impairment and may, similar to  
84 electrolyte abnormalities, promote dysrhythmias resulting in stroke or sudden cardiac death [3, 15-20].

85 Finally renal impairment and CVD are known to share a common underlying risk factor profile which include  
86 hypertension, diabetes, dyslipidemia, smoking, injecting drug use, obesity, on-going inflammation and black  
87 African origin [20, 21]. CVD, renal impairment, and many of the underlying shared individual risk factors,

88 are more prevalent among HIV-positive individuals than in the general population, hence the association  
89 between renal impairment and CVD may be stronger in HIV-positive individuals [22, 23]. The aim of this  
90 analysis is to investigate the nature and relationship of various levels of sustained eGFR impairment with  
91 centrally adjudicated CVD endpoints in a large heterogeneous and contemporary cohort of primarily  
92 Caucasian HIV-positive individuals.

## 93 **Methods**

### 94 *Study population*

95 The Data Collection on Adverse events of Anti-HIV Drugs (D:A:D) study is a large, prospective cohort  
96 collaboration established in 1999 following more than 49,000 HIV-1-positive persons from 11 cohorts in  
97 Europe, the United States and Australia; details have been published previously [17]. Data on centrally  
98 validated clinical events including myocardial infarction, sudden cardiac death, stroke, invasive  
99 cardiovascular procedures, end-stage renal disease and fatal cases is collected in real-time during routine  
100 clinical care. Information on socio-demographic factors, antiretroviral treatment, HIV viral load, CD4 counts,  
101 AIDS events, viral hepatitis, creatinine and other laboratory biomarkers and cardiovascular risk factors is  
102 collected electronically at enrolment and every six months.

### 103 *Endpoint definition*

104 CVD events are reported using designated event forms (more information at  
105 [www.chip.dk/Studies/DAD/Study-Documents](http://www.chip.dk/Studies/DAD/Study-Documents)) and are defined as centrally validated fatal and non-fatal  
106 myocardial infarction, stroke, coronary angioplasty, coronary bypass, carotid endarterectomy and sudden  
107 cardiac death. A fatal CVD event is defined as one of the above events leading to death within 28 days.  
108 Adjudication of CVD events is made in accordance with predefined algorithms, and only confirmed events  
109 are included in analysis. Sudden cardiac death is defined as a sudden death event in which the underlying  
110 cause of death could not be established as a myocardial infarction due to the lack of data on symptoms,

111 electrocardiogram findings and changes in cardiac biomarker, but with cardiovascular risks present at time of  
112 death according to the WHO MONICA Dundee score [24], and no evidence of other non-atherosclerotic or non-  
113 cardiovascular causes of death. All sudden cardiac deaths in the D:A:D study are reviewed by an external  
114 cardiologist.

#### 115 *Statistical methods*

116 D:A:D Study participants with  $\geq 2$  eGFR measurements after 1/2/2004 (baseline for initiation of systematic  
117 creatinine collection) were included and followed until the earliest of first CVD event, death, six months  
118 after last visit or 1/2/2015. Persons with less than three months follow-up from the first to last eGFR were  
119 excluded. The Cockcroft-Gault equation [25], standardized for body surface area [26], was used to estimate  
120 creatinine clearance, a surrogate for eGFR in this analysis [27, 28]. As several cohorts participating in D:A:D  
121 are prohibited from collecting ethnicity information, the Cockcroft-Gault equation was used rather than an  
122 equation including ethnicity. Where eGFR measurements were carried out more frequently than every 28  
123 days, the median value was used and assigned to the median date. Confirmed baseline and time-updated  
124 (current) eGFR levels were defined using two consecutive eGFR measurements, regardless of time between  
125 measurements (per definition minimum 28 days). The confirmed baseline and current eGFR values were  
126 subsequently allocated to the following eGFR strata:  $>90$ ,  $>60\text{-}\leq 90$ ,  $>30\text{-}\leq 60$  and  $\leq 30$  ml/min/1.73m<sup>2</sup>. Where  
127 two consecutive eGFR values ( $<15\%$  of all values) did not fall within the same eGFR strata, the mean of two  
128 eGFR values carried forward was used to assign an eGFR category.

129 Individuals with a prior CVD event were included, but only the first CVD event experienced during  
130 prospective follow-up after baseline was included as an event. Individuals could however experience two or  
131 more different types of CVD event on the same date.

132 Incidence rates were calculated per 1000 person years of follow-up (PYFU). Kaplan-Meier estimation was  
133 used to investigate time to CVD, stratified according to confirmed baseline eGFR levels (eGFR $>90$ ,  $\leq 90\text{-}>60$ ,  
134  $\leq 60\text{-}>30$ ,  $\leq 30$  ml/min/1.73m<sup>2</sup>).

135 Poisson regression models stratified according to the confirmed current eGFR level were used to model the  
136 CVD incidence rate ratios, overall and stratified by individual CVD events. Potential confounders included in  
137 multivariate models were age (per 10 years older), gender, ethnicity, D:A:D enrolment cohort, nadir CD4  
138 count, mode of HIV acquisition and family history of CVD. All remaining variables were adjusted for as  
139 time-updated, including HBV/HCV co-infection, HIV-RNA (per  $\log_{10}$ ), CD4 count, prior AIDS, hypertension  
140 ( $>150/>100$  or receipt of antihypertensive treatment), diabetes (confirmed diagnosis of DM or receipt of  
141 anti-diabetic treatment), confirmed eGFR strata, smoking status (current, previous, never), dyslipidemia  
142 (total cholesterol  $>6.2$  mmol/l, high-density lipoprotein cholesterol  $<0.9$  mmol/l, triglyceride  $>2.3$  mmol/l,  
143 or receipt of lipid-lowering treatment) and prior CVD (confirmed diagnosis). Antiretroviral drug use was  
144 fitted as time-updated cumulative use (per five years; zidovudine, didanosine, zalcitabine, stavudine,  
145 lamivudine, emtricitabine, tenofovir disoproxil fumerate, abacavir, efavirenz, nevirapine, indinavir,  
146 saquinavir, ritonavir, nelfinavir, (fos)amprenavir, atazanavir and darunavir) and current use (currently on and  
147 use with last six months; zidovudine, didanosine, zalcitabine, stavudine, lamivudine, emtricitabine,  
148 tenofovir disoproxil fumerate and abacavir).

149 A number of sensitivity analyses were performed to test the robustness of the results. One analysis  
150 investigated death as a potential competing risk of CVD. Another analysis excluded all those with a prior  
151 CVD event. Other analyses adjusted for the D:A:D CKD risk-score [29] and the predicted CVD risk based on  
152 the Framingham CVD prediction model [30] to estimate how much of the CVD risk is explained through  
153 common renal and CVD risk factors. The D:A:D CKD risk score is a nine-variable prediction score estimating  
154 the five year risk of developing CKD in HIV-positive individuals. Individuals in the low CKD risk group (score  
155  $<0$ ) have a 1:393 (0.3%) five year CKD risk, rising to 1:47 (2.1%) in the medium (score 0-4) and 1:6 (16.7%)  
156 high risk group (score  $\geq 5$ ) [29]. A final analysis investigated the association between current nadir eGFR  
157 and the percentage of follow-up time spent with  $eGFR \leq 60$  ml/min/1.73m<sup>2</sup> and CVD respectively.

158

159 **Results**

160 *Study population*

161 35,357 persons with follow-up after 2004 and at least two eGFR measurement were included in analysis,  
162 Supplementary Figure 1. Included individuals were predominantly Caucasian (48.1%) males (73.9%) with a  
163 median age of 41 (interquartile range, IQR, 35-48) years, Table 1. While 41.6% were smokers, 4.0% had  
164 diabetes, 8.9% had hypertension and 0.7% had experienced a prior CVD event. At baseline the median  
165 estimated five year risk of CKD was low overall (-1 (IQR -3 to4) corresponding to 0.3%) and medium (4 (IQR -  
166 1to 9) corresponding to 2.1%) in those developing a CVD event, Table 1. 558 persons were excluded from  
167 analysis due to missing CD4 counts or viral load at baseline, or because of insufficient follow-up. Excluded  
168 persons were more likely to be young, of Caucasian origin, cART-naïve, HCV-positive, have no family history  
169 of CVD and have experienced a prior AIDS event.

170 *Age and eGFR level*

171 Among individuals younger than 40 years 87.0% (n=13,660) had a normal (confirmed eGFR>90  
172 ml/min/1.73m<sup>2</sup>) baseline eGFR, and only 0.04% (n=7) had advanced renal impairment (confirmed baseline  
173 eGFR≤30 ml/min/1.73m<sup>2</sup>). In contrast, among individuals older than 60 years, only 15.8% (n=321) had  
174 confirmed baseline eGFR>90ml/min/1.73m<sup>2</sup> and 0.8% (n=17) confirmed baseline eGFR≤30 ml/min/1.73m<sup>2</sup>.

175 *CVD events*

176 Over a median follow-up time of 8.0 years (IQR, 5.4-8.9, total PYFU 258,480) 1,357 persons developed  
177 1,646 CVD events (incidence rate 5.2 per 1000 PYFU [95% confidence interval, CI, 5.0-5.5]). The CVD events  
178 included 586 myocardial infarctions (11.1% fatal), 430 strokes (8.6% fatal), 510 coronary angioplasties (1.6%  
179 fatal), 96 coronary bypasses (2.1% fatal), 19 carotid endarterectomies (0% fatal) and 5 sudden cardiac  
180 deaths respectively. A total of 284 persons (21.0%) experienced more than one CVD event on the same  
181 date, most commonly a myocardial infarction and coronary angioplasty (n=259).

182

183 *Median eGFR levels and incident CVD*

184 The median eGFR measured in individuals prior to their CVD event was significantly lower (85 (IQR 69-102)  
185 ml/min/1.73m<sup>2</sup>) than the median eGFR measured during follow-up in individuals not experiencing a CVD  
186 event (94 (IQR 79-110) ml/min/1.73m<sup>2</sup>, p<0.0001). Likewise, a greater proportion of individuals  
187 experiencing a CVD event had some level of confirmed reduced eGFR level, compared to individuals not  
188 experiencing an event, Figure 1. When comparing the individual types of CVD events, those experiencing a  
189 coronary bypass event had significantly lower confirmed eGFR levels compared to all other CVD event types  
190 (p=0.018). When excluding the coronary bypass events there was no statistically significant differences in  
191 confirmed eGFR levels prior to a CVD event (p=0.068). Likewise, when comparing those with an invasive  
192 cardiovascular procedures (coronary angioplasty, carotid endarterectomy or coronary bypass) to those with  
193 a myocardial infarction and/or stroke there was no statistical significant difference (p=0.55), Figure 1.

194 *Confirmed baseline eGFR levels and incident CVD*

195 We observed a clear inverse relationship between confirmed eGFR levels at baseline and incident CVD with  
196 1.8% [95% CI 1.6-2.0%] estimated to have progressed to CVD at five years among those with confirmed  
197 baseline eGFR>90 ml/min/1.73m<sup>2</sup>, increasing to 4.1% (95% CI 3.5-4.6) for eGFR 60-90 ml/min/1.73m<sup>2</sup>,  
198 10.8% (95% CI 8.7-12.9) for baseline eGFR 30-60 ml/min/1.73m<sup>2</sup> and 21.1% [95% CI 6.6-35.6%] among  
199 those with confirmed baseline eGFR≤30 ml/min/1.73m<sup>2</sup>, Figure 2.

200 Amongst individuals with moderately impaired baseline eGFR (confirmed eGFR≤60 ml/min/1.73m<sup>2</sup>) who  
201 developed a CVD event, we did not observe a statistically significant differences (p=0.63) in time to  
202 different CVD events with a median time to CVD event of 45 months (IQR 21-76).

203 *Confirmed current eGFR level and incident CKD*

204 There was a strong and inverse linear relationship between confirmed current eGFR and CVD in univariate  
205 analysis; incidence rate ratios (IRRs) increasing from 1.00 at eGFR>90 ml/min/1.73m<sup>2</sup> to 14.09 [95%CI 9.58-  
206 20.74] at eGFR≤30 ml/min/1.73m<sup>2</sup>, Figure 3. Adjusting for increasing age explained most of the relationship

207 between eGFR and CVD at eGFR levels  $>30$  ml/min/ $1.73\text{m}^2$ , although all eGFRs below  $80$  ml/min/ $1.73\text{m}^2$   
208 were associated with an increased incidence of CVD of approximately 30-40%. At a confirmed current  
209 eGFR $\leq 30$  ml/min/ $1.73\text{m}^2$  a significantly increased incidence of CVD remained independent of age (IRR 4.21  
210 [95%CI 2.81-6.30]), Figure 3. Further adjustment for other potential confounders including individual  
211 antiretroviral drugs had relatively limited impact on the overall association (IRR 3.08 [95%CI 2.04-4.65] at  
212 confirmed eGFR $\leq 30$  ml/min/ $1.73\text{m}^2$  compared to confirmed eGFR $\geq 90$  ml/min/ $1.73\text{m}^2$ , Figure 3. The  
213 exclusion of the 240 individuals with a CVD event prior to baseline led to entirely consistent results (data  
214 not shown).

215 In a bivariate analysis, adjusting for the Framingham score (as a continuous variable) explained some of the  
216 association between confirmed current eGFR and CVD, but not to the same extent as age alone (data not  
217 shown). In another analysis adjusting for the estimated five-year D:A:D CKD risk score individuals with a  
218 medium CKD risk (score 0-4) had a 2.56-fold increased incidence of CVD (IRR 2.56 [95%CI 2.22 – 2.95]) and  
219 individuals with a high CKD risk (score  $\geq 5$ ) had almost a five-fold increased incidence of CVD (IRR 4.98 [95%  
220 CI 4.37 – 5.68]) compared to persons with a low estimated CKD risk (score  $<0$ ). After adjusting for other  
221 potential confounders (as shown in Figure 4) not included in the D:A:D CKD risk score (with the exception of  
222 age), those with a medium or high CKD risk score continued to have a significantly higher risk of CVD (IRR  
223 1.29 [95%CI 1.10-1.50] and 1.43 [95%CI 1.19-1.71] respectively).

224 There was no strong evidence suggesting that the observed association between confirmed current eGFR  
225 levels and CVD differed amongst the individual types of CVD events. When restricting the analysis to fatal  
226 CVD events only, all observed associations were further strengthened (data not shown). Our findings were  
227 furthermore consistent in different age groups (test for interaction,  $p=0.88$ ), and after accounting for death  
228 as a possible competing risk for CVD (data not shown). The association between CVD and confirmed eGFR  
229 seen in the primary analyses was largely unchanged by fitting renal function as current nadir eGFR and as

230 the percentage of follow-up spent with moderately impaired eGFR (eGFR $\leq$ 60 ml/min/1.73m $^2$ ) (data not  
231 shown).

#### 232 *Confirmed current eGFR levels and number of CVD events*

233 Individuals with higher confirmed current eGFR levels experienced two or more CVD events (at the same  
234 date) more frequently than those with lower eGFR levels (24.7% at eGFR $>$ 90 ml/min/1.73m $^2$  vs.4.2% at  
235 eGFR $\leq$ 30 ml/min/1.73m $^2$ , p=0.0034), most commonly a myocardial infarction and coronary angioplasty.  
236 Furthermore, the proportion of individuals experiencing a fatal CVD event (death within 28 days following  
237 the event) was strongly related to the confirmed current eGFR level, increasing from 4.4% in individuals  
238 with a confirmed current eGFR $>$ 90 ml/min/1.73m $^2$  to 25.0% in individuals with a confirmed current  
239 eGFR $\leq$ 30 ml/min/1.73m $^2$  (p $<$ 0.0001).

#### 240 **Discussion**

241 In this large heterogeneous cohort of HIV-positive individuals we found a strong association between  
242 centrally adjudicated CVD events and advanced levels of renal impairment (confirmed eGFR $\leq$ 30  
243 ml/min/1.73m $^2$ ).

244 Almost 60% of all individuals experiencing a CVD event had eGFR $\leq$ 90 ml/min/1.73m $^2$ , based on the latest  
245 median eGFR before the event, compared to less than 40% of those without an event. We further showed  
246 that development of a CVD event was considerably faster among those with a severely impaired eGFR at  
247 baseline. Among HIV-positive individuals with confirmed baseline eGFR $\leq$ 30 ml/min/1.73m $^2$  over 20% were  
248 estimated to have developed CVD after five years.

249 In previous studies from D:A:D we have investigated the inverse relation between CVD events and eGFR,  
250 focusing on CVD as a risk factor of various levels of chronic renal impairment [28, 29, 31]. Interestingly,  
251 these previous data also supported a strong association between CVD and renal function which significantly  
252 diminished after accounting for other risk factors suggesting an underlying biological mechanism at least

253 partly mediated by other factors. We have also previously showed an association between the use of  
254 certain antiretroviral drugs and CVD and renal impairment [28, 30, 32]. The results of this analysis are  
255 entirely consistent with these prior findings, and adjustment for the use of individual antiretroviral drugs  
256 did not have any major impact on the association between impaired eGFR and CVD. Data from this analysis  
257 points towards increasing age as the main underlying driver of the inverse relationship between eGFR and  
258 CVD, in particular at mild to moderately impaired eGFR levels [14]. At more advanced levels of renal  
259 impairment ( $\text{eGFR} \leq 30 \text{ ml/min/1.73m}^2$ ) there are additional pathways between renal impairment and CVD,  
260 not immediately related to any of the known common risk factors on the shared causal pathway such as  
261 diabetes, hypertension and immunosuppression. Regardless of the underlying pathology the high rates of  
262 CVD observed in older individuals with mild to moderate renal impairment highlight the need for  
263 intensified monitoring and search for effective prophylactic measures for impaired renal function and CVD  
264 in the ageing HIV-population.

265 In other studies of HIV-positive individuals, a smaller cross-sectional analysis in the FRAM study did not  
266 confirm an association between carotid intima-medial thickness and eGFR after accounting for older age,  
267 gender and ethnicity [13]. Likewise, a British study did not find an association between eGFR as a  
268 continuous variable and coronary heart disease, although those with  $\text{eGFR} < 75 \text{ mL/min}$  already had more  
269 than a 4-fold increased incidence [9]. In a recent EuroSIDA study both the follow-up time with a low eGFR  
270 and  $\text{eGFR} \leq 30 \text{ ml/min/1.73m}^2$  were predictive of non-AIDS events including CVD, but power was limited  
271 [12]. An older large cohort study among HIV-positive US veterans showed an almost 6-fold higher  
272 association between  $\text{eGFR} \leq 30 \text{ ml/min}$ , albuminuria and CVD, although this study also included peripheral  
273 artery disease and heart failure [10].

274 Our findings do not suggest that the association between declining renal function and CVD is stronger, or  
275 starts at higher eGFR levels in HIV-positive persons than in the general population, as was hypothesised  
276 based on the higher occurrence of common renal and CVD risk factors and increased immune activation [1,

277 4, 33, 34]. There is, however, ongoing ambiguity, in the general population, regarding the strength of the  
278 association between impaired renal function and CVD. Some studies report only on an association with  
279 CVD at advanced levels of renal impairment (eGFR $\leq$ 30 ml/min/1.73m<sup>2</sup>) while others report of associations  
280 already at higher eGFR levels [1, 4, 5, 9, 10, 14, 33, 34]. However, the definitions of CVD differ considerably  
281 in these studies ranging from subclinical imaging-verified diagnoses of atherosclerosis to various clinical  
282 events ascertained with different levels of certainty. The differences in the incidence of common risk  
283 factors and of CVD and renal impairment may also partly explain the conflicting results. Importantly, the  
284 D:A:D study focuses on 'hard' clinical CVD events exclusively and information on non-fatal heart failure or  
285 milder forms of ischemic CVD such as angina pectoris is not collected. This methodology may explain why  
286 more severe levels of renal impairment are necessary to establish an association with CVD. Interestingly,  
287 none of the widely accepted CVD risk prediction models currently include renal impairment in the  
288 estimates [30, 32], but the proportion of individuals with advanced renal impairment may be too limited  
289 to date.

290 We also found that fatal outcomes of a CVD event were more common at lower compared to higher eGFR  
291 levels, which may be related to a more severe clinical event or to the fact that those with advanced levels  
292 of renal impairment provide a more fragile phenotype with less ability to cope with CVD complications.  
293 Likewise, fewer multiple CVD events occurred on the same date among those with lower eGFR levels. This  
294 finding may be related to the increased fatality rate at lower eGFR levels or that those with lower eGFR  
295 levels are less likely to undergo invasive cardiovascular procedures as secondary prophylaxis, due to  
296 concerns about radiocontrast induced nephrotoxicity. Interestingly, there was no evidence of a relation  
297 between the eGFR level and type of CVD outcome i.e. a myocardial infarction did not seem to occur at  
298 different eGFR levels to other CVD events, with the exception of coronary bypass. Coronary bypass was  
299 more commonly carried out at lower eGFR levels, compared to the other CVD events, which may suggest  
300 more advanced atherosclerosis with multiple vessel disease in this population.

301 The potential limitations of the analysis should be acknowledged. We may have underestimated the  
302 proportion of individuals with an impaired eGFR level as those excluded from analysis were more likely to  
303 have common renal risk factors; hence the provided relation between eGFR and CVD is of a conservative  
304 nature. Proteinuria is a potential source of unmeasured confounding as it not collected systematically in  
305 the D:A:D study, and may further have moderating effects as it is a strong independent risk factor for both  
306 CVD and CKD [35].Furthermore, renal impairment may have developed secondary to a CVD event as part of  
307 a cardiorenal syndrome, with potentials of reverse causality. However, in this analysis eGFR impairment  
308 proceeded all prospectively investigated CVD events [36]. Finally, non-ischemic events such as cardiac  
309 arrhythmias and ventricular hypertrophy were not directly included in the CVD definition, but may have  
310 contributed more indirectly via stroke and sudden cardiac death events.

### 311 **Conclusion**

312 In a large, contemporary cohort of HIV-positive individuals we observed a strong relationship between  
313 confirmed impaired renal function and incident CVD. More than one in five of those with advanced levels of  
314 renal impairment were estimated to have developed CVD by five years, with an increasing 28-day CVD  
315 fatality rate as eGFR declined. Our findings highlight the need for an intensified monitoring for emerging  
316 CVD, in particular in older individuals with continuously low eGFR levels. Our findings also call for an  
317 increased focus on applying different renal and cardiovascular preventive measures in HIV-positive  
318 individuals.

319

320

321

322

323 **Funding**

324 The D:A:D study was supported by the Highly Active Antiretroviral Therapy Oversight Committee (HAART-  
325 OC), a collaborative committee with representation from academic institutions, the European Agency for  
326 the Evaluation of Medicinal Products, the United States Food and Drug Administration, the patient  
327 community, and pharmaceutical companies with licensed anti-HIV drugs in the European Union: AbbVie,  
328 Bristol-Myers Squibb, Gilead Sciences Inc., ViiV Healthcare, Merck & Co Inc. and Janssen Pharmaceuticals.  
329 Supported also by a grant [grant number DNRF126] from the Danish National Research Foundation (CHIP &  
330 PERSIMUNE); by a grant from the Dutch Ministry of Health, Welfare and Sport through the Center for  
331 Infectious Disease Control of the National Institute for Public Health and the Environment to Stichting HIV  
332 Monitoring (ATHENA); by a grant from the Agence nationale de recherches sur le sida et les hépatites  
333 virales [ANRS, Action Coordonnée no.7, Cohortes] to the Aquitaine Cohort; The Australian HIV  
334 Observational Database (AHOD) is funded as part of the Asia Pacific HIV Observational Database, a program  
335 of The Foundation for AIDS Research, amfAR, and is supported in part by a grant from the U.S. National  
336 Institutes of Health's National Institute of Allergy and Infectious Diseases (NIAID) [grant number U01-  
337 AI069907] and by unconditional grants from Merck Sharp & Dohme; Gilead Sciences; Bristol-Myers Squibb;  
338 Boehringer Ingelheim; Janssen-Cilag; ViiV Healthcare. The Kirby Institute is funded by The Australian  
339 Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, The  
340 University of New South Wales; by grants from the Fondo de Investigación Sanitaria [grant number FIS  
341 99/0887] and Fundación para la Investigación y la Prevención del SIDA en Españã [grant number FIPSE  
342 3171/00], to the Barcelona Antiretroviral Surveillance Study (BASS); by the National Institute of Allergy and  
343 Infectious Diseases, National Institutes of Health [grants number 5U01AI042170-10 , 5U01AI046362-03], to  
344 the Terry Beinr Community Programs for Clinical Research on AIDS (CPCRA); by primary funding provided  
345 by the European Union's Seventh Framework Programme for research, technological development and  
346 demonstration under EuroCoord grant agreement n° 260694 and unrestricted grants by Bristol-Myers  
347 Squibb, Janssen R&D, Merck and Co. Inc., Pfizer Inc., GlaxoSmithKline LLC, (the participation of centres from

348 Switzerland is supported by The Swiss National Science Foundation (Grant 108787)) to the EuroSIDA study;  
349 by unrestricted educational grants of AbbVie, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline,  
350 Pfizer, Janssen Pharmaceuticals to the Italian Cohort Naive to Antiretrovirals (The ICONA Foundation); and  
351 by a grant from the Swiss National Science Foundation (grant #148522) to the Swiss HIV Cohort Study  
352 (SHCS). The content of this publication is solely the responsibility of the authors and does not necessarily  
353 represent the official views of any of the institutions mentioned above.

#### 354 **Conflicts of Interests**

355 L. Ryom, J.D. Lundgren, M. Ross, E. Fontas, C. Smit, C.I. Hatleberg, and S. De Wit have reported no conflicts  
356 of interest. O. Kirk had prior/present board membership at ViiV Healthcare, Gilead Sciences and Merck,  
357 received payment for lectures and/or for development of educational presentations from Abbott, Gilead  
358 Sciences and Tibotec and had travel/accommodations/meeting expenses paid by Abbott, BMS, Gilead  
359 Sciences, Merck and ViiV Healthcare. P. Morlat has received honoraria, speaker fees, travel support or  
360 honoraria from AbbVie, Bristol-Myers Squibb, Gilead Sciences, ViiV Healthcare, Merck & Co Inc. and Janssen  
361 Pharmaceuticals. C.A. Fux is an advisory board member for Gilead Sciences and MSD, has pending grants  
362 from Gilead Sciences and Abbott and received payment for lectures by Gilead HIV and the body. M. Law has  
363 received research grants from Boehringer Ingelheim, Bristol Myer Squibb, Gilead Sciences, GlaxoSmithKline,  
364 Janssen Pharmaceuticals, Merck, Pfizer and Hoffman-LaRoche. C. Sabin received personal fees from Gilead  
365 Sciences, Bristol-Myers Squibb, Janssen Pharmaceuticals, Abbott Pharmaceuticals, and ViiV Healthcare. A.  
366 Mcroft has received consultancy fees/honoraria/speaker fees from Bristol-Myers Squibb, Pfizer, Merck,  
367 Boehringer Ingelheim, and Gilead Sciences.

368

369

370

371 **Acknowledgements**

372 **D:A:D participating cohorts:** AHOD (Australia), Aquitaine (France), Athena (The Netherlands), BASS (Spain),  
373 CPCRA (USA), EuroSIDA (multi-national), HivBivus (Sweden), ICONA (Italy), Nice (France), SHCS (Switzerland)  
374 and St. Pierre (Belgium)

375 **D:A:D Steering Committee:** Names marked with \*, Chair with #

376 **Cohort PIs:** W. El-Sadr\* (CPCRA), G. Calvo\* (BASS), F. Dabis\* (Aquitaine), O. Kirk\* (EuroSIDA), M. Law\*  
377 (AHOD), A. d'Arminio Monforte\* (ICONA), L. Morfeldt\* (HivBIVUS), C. Pradier\* (Nice), P. Reiss\* (ATHENA),  
378 R. Weber\* (SHCS), S. De Wit\* (Brussels)

379 **Members of the D:A:D SC from the Oversight Committee:** B. Powderly\*, N. Shortman\*, C. Moecklinghoff\*,  
380 G. Reilly\*, X. Franquet\*

381 **D:A:D Central Coordination:** C.I. Hatleberg, L. Ryom, C.A. Sabin\*, D. Kamara, C.J. Smith, A. Phillips\*, A.  
382 Mocroft\*, A. Bojesen, A.L. Grevsen, C. Matthews, D. Raben, J.D. Lundgren#

383 **D:A:D Cohort coordinators and data managers:** A. Lind-Thomsen (coordinator), R. Salbøl Brandt, M.  
384 Hillebrecht, S. Zaheri, F.W.N.M. Wit (ATHENA), F. Schöni-Affolter (SHCS) A. Travelli, I. Fanti (ICONA), O.  
385 Leleux (Aquitaine), E. Thulin, A. Sundström (HivBIVUS), G. Bartsch, G. Thompsen (CPCRA), M. Delforge  
386 (Brussels), E. Fontas, C. Caissotti, K. Dollet (Nice), S. Mateu, F. Torres, (BASS), R. Pühr (AHOD), D. Kristensen  
387 (EuroSIDA)

388 **Verification of Endpoints:** A. Sjøll (CVD), P. Meidahl (oncology), J. Helweg-Larsen (hematology), J. Schmidt  
389 Iversen (nephrology) **Kidney working group:** L. Ryom, A. Mocroft, O. Kirk\*, P. Reiss\*, C. Smit, M. Ross, C.A.  
390 Fux, P. Morlat, E. Fontas, D.A. Kamara, C.J. Smith, J.D. Lundgren#

391 **Mortality working group:** C.J. Smith, L. Ryom, C. I. Hatleberg, A. Phillips\*, R. Weber\*, P. Morlat, C. Pradier\*,  
392 P. Reiss\*, F.W.N.M. Wit, N. Friis-Møller, J. Kowalska, J.D. Lundgren#

393 **Cancer working group:** C. Sabin\*, M. Law\*, A. d'Arminio Monforte\*, F. Dabis\*, F. Bonnet\*, P. Reiss\*,

394 F.W.N.M. Wit, C.J. Smith, D.A. Kamara, J. Bohlius, M. Bower, G. Fätkenheuer, A. Grulich, L. Ryom,  
395 C.I.Hatleberg, J.D. Lundgren#

396 **For a complete list of acknowledgements for the members of the 11 Cohorts in the D:A:D Study, please**  
397 **see Supplementary Document 2**

398

399

400

401

402

403

404

405

406

407

408

409

410

411

412

413 **References**

- 414 1. Lee M, Saver JL, Chang KH, Liao HW, Chang SC, Ovbiagele B. Low glomerular filtration rate and  
415 risk of stroke: meta-analysis. *BMJ* **2010**; 341:c4249.
- 416 2. Shara NM, Wang H, Mete M, et al. Estimated GFR and incident cardiovascular disease events in  
417 American Indians: the Strong Heart Study *Am J Kidney Dis.* **2012**; 60:795-803.
- 418 3. Wang AY, Lai KN. Use of cardiac biomarkers in end-stage renal disease. *J Am Soc Nephrol* **2008**;  
419 19:1643-52.
- 420 4. Di Angelantonio E, Danesh J, Eiriksdottir G, Gudnason V. Renal function and risk of coronary  
421 heart disease in general populations: new prospective study and systematic review. *PLoS Med*  
422 **2007**; 4:e270.
- 423 5. Arbel Y, Halkin A, Finkelstein A, et al. Impact of Estimated Glomerular Filtration Rate on Vascular  
424 Disease Extent and Adverse Cardiovascular Events in Patients Without Chronic Kidney Disease. *Can*  
425 *J Cardiol* **2013**.
- 426 6. de Bie MK, Buiten MS, Rabelink TJ, Jukema JW. How to reduce sudden cardiac death in patients  
427 with renal failure. *Heart* **2012**; 98:335-41.
- 428 7. Collins AJ, Roberts TL, St Peter WL, Chen SC, Ebben J, Constantini E. United States Renal Data  
429 System assessment of the impact of the National Kidney Foundation-Dialysis Outcomes Quality  
430 Initiative guidelines. *Am J Kidney Dis* **2002**; 39:784-95.
- 431 8. George E, Lucas GM, Nadkarni GN, Fine DM, Moore R, Atta MG. Kidney function and the risk of  
432 cardiovascular events in HIV-1-infected patients. *AIDS* **2010**; 24:387-94.
- 433 9. Campbell LJ, Desai M, Hegazi A, et al. Renal impairment is associated with coronary heart  
434 disease in HIV-positive men. *HIV clinical trials* **2012**; 13:343-9.

- 435 10. Choi AI, Li Y, Deeks SG, Grunfeld C, Volberding PA, Shlipak MG. Association between kidney  
436 function and albuminuria with cardiovascular events in HIV-infected persons. *Circulation* **2010**;  
437 121:651-8.
- 438 11. Serrano-Villar S, Estrada V, Gomez-Garre D, et al. Incipient renal impairment as a predictor of  
439 subclinical atherosclerosis in HIV-infected patients. *JAIDS* **2012**; 59:141-8.
- 440 12. Mocroft A, Ryom L, Begovac J, et al. Deteriorating renal function and clinical outcomes in HIV-  
441 positive persons. *AIDS* **2014**; 28:727-37.
- 442 13. Jotwani V, Scherzer R, Choi A, et al. Reduced kidney function and preclinical atherosclerosis in  
443 HIV-infected individuals: the study of fat redistribution and metabolic change in HIV infection  
444 (FRAM). *Am J Nephrol* **2011**; 33:453-60.
- 445 14. Natali A, Boldrini B, Baldi S, et al. Impact of mild to moderate reductions of glomerular  
446 filtration rate on coronary artery disease severity. *Nutr Metab Cardiovasc Dis* **2014**; 24:681-8.
- 447 15. Spoto B, Mattace-Raso F, Sijbrands E, et al. Association of IL-6 and a functional polymorphism  
448 in the IL-6 gene with cardiovascular events in patients with CKD. *Clin J Am Soc Nephrol* **2015**;  
449 10:232-40.
- 450 16. Klawitter J, Reed-Gitomer BY, McFann K, et al. Endothelial dysfunction and oxidative stress in  
451 polycystic kidney disease. *Am J Renal Physiol* **2014**; 307:F1198-206.
- 452 17. Matsushita K, Sang Y, Ballew SH, et al. Cardiac and kidney markers for cardiovascular  
453 prediction in individuals with chronic kidney disease: the Atherosclerosis Risk in Communities  
454 study. *Arterioscler Thromb Vasc Biol* **2014**; 34:1770-7.
- 455 18. Foley RN, Murray AM, Li S, et al. Chronic kidney disease and the risk for cardiovascular disease,  
456 renal replacement, and death in the United States Medicare population, 1998 to 1999. *J Am Soc*  
457 *Nephrol* **2005**; 16:489-95.

- 458 19. Baber U, Howard VJ, Halperin JL, et al. Association of chronic kidney disease with atrial  
459 fibrillation among adults in the United States: REasons for Geographic and Racial Differences in  
460 Stroke (REGARDS) Study. *Circ Arrhythm Electrophysiol* **2011**; 4:26-32.
- 461 20. Gupta SK, Kitch D, Tierney C, Melbourne K, Ha B, McComsey GA. Markers of renal disease and  
462 function are associated with systemic inflammation in HIV infection. *HIV Med* **2015**; 16:591-8.
- 463 21. Kong X, Ma X, Cui M, Xu D. Association of clustering of major cardiovascular risk factors with  
464 chronic kidney disease in the adult population. *Clin Nephrol* **2014**; 82:92-7.
- 465 22. Schouten J, group oboAHs. comorbidity and ageing with HIV. IAS. Washington DC, USA, **2012**.
- 466 23. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and  
467 cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin*  
468 *Endocrinol Metab* **2007**; 92:2506-12.
- 469 24. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial  
470 infarction and coronary deaths in the World Health Organization MONICA Project. Registration  
471 procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four  
472 continents. *Circulation* **1994**; 90:583-612.
- 473 25. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*  
474 **1976**; 16:31-41.
- 475 26. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med* **1987**; 317:1098.
- 476 27. Vrouwenraets SM, Fux CA, Wit FW, et al. A comparison of measured and estimated glomerular  
477 filtration rate in successfully treated HIV-patients with preserved renal function. *Clin Nephrol*  
478 **2012**; 77:311-20.

- 479 28. Ryom L, Mocroft A, Kirk O, et al. Association Between Antiretroviral Exposure and Renal  
480 Impairment Among HIV-Positive Persons With Normal Baseline Renal Function: the D:A:D Study.  
481 *JID* **2013**; 207:1359-69.
- 482 29. Mocroft A, Lundgren JD, Ross M, et al. Development and validation of a risk score for chronic  
483 kidney disease in HIV infection using prospective cohort data from the D:A:D study. *PLoS Med*  
484 **2015**; 12:e1001809.
- 485 30. D'Agostino RB, Sr., Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in  
486 primary care: the Framingham Heart Study. *Circulation* **2008**; 117:743-53.
- 487 31. Ryom L, Mocroft A, Kirk O, et al. Predictors of advanced chronic kidney disease and end-stage  
488 renal disease in HIV-positive persons. *AIDS* **2014**; 28:187-99.
- 489 32. Friis-Moller N, Ryom L, Smith C, et al. An updated prediction model of the global risk of  
490 cardiovascular disease in HIV-positive persons: The Data-collection on Adverse Effects of Anti-HIV  
491 Drugs (D:A:D) study. *Eur J Prev Cardiol* **2015**.
- 492 33. Matsushita K, van der Velde M, Astor BC, et al. Association of estimated glomerular filtration  
493 rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a  
494 collaborative meta-analysis. *Lancet* **2010**; 375:2073-81.
- 495 34. Arbel Y, Halkin A, Finkelstein A, et al. Impact of estimated glomerular filtration rate on vascular  
496 disease extent and adverse cardiovascular events in patients without chronic kidney disease. *Can J*  
497 *Cardiol* **2013**; 29:1374-81.
- 498 35. Hemmelgarn BR, Manns BJ, Lloyd A, et al. Relation between kidney function, proteinuria, and  
499 adverse outcomes. *JAMA* **2010**; 303:423-9.
- 500 36. Lekawanvijit S, Krum H. Cardiorenal syndrome: acute kidney injury secondary to cardiovascular  
501 disease and role of protein-bound uraemic toxins. *J Physiol* **2014**; 592:3969-83.

**Table 1, Baseline Characteristics**

		<b>All</b>		<b>Persons developing CVD</b>	
		<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
<b>All</b>		35,357	100	1,357	3.8
<b>Gender</b>	Male	26,124	73.9	1,181	87.3
<b>Ethnicity</b>	Caucasian	17,016	48.1	697	51.4
	Black	2,450	6.9	40	3.0
	Other	716	2.0	12	0.9
	Unknown	15,175	42.9	608	44.8
<b>Mode of HIV acquisition</b>	MSM	16,234	45.9	728	53.7
	IDU	4,529	12.8	154	11.4
	Heterosexual	12,436	35.2	386	28.4
	Other	2,158	6.1	89	6.6
<b>HBV<sup>1</sup></b>	Positive	1,597	4.5	46	3.4
	Negative	31,169	88.2	1,213	89.4
	Unknown	2,591	7.3	98	7.2
<b>HCV<sup>2</sup></b>	Positive	6,479	18.3	236	17.4
	Negative	25,535	72.2	973	71.7
	Unknown	3,343	9.5	148	10.9
<b>cART</b>	On	26,425	74.7	1,197	88.2
<b>Prior AIDS event</b>	Yes	8,768	24.8	462	34.1
<b>VL&lt;400 (copies/mL)</b>	Yes	20,828	58.9	956	70.4
<b>Smoking</b>	Current	14,715	41.6	688	50.7

<b>BMI (Kg/m<sup>2</sup>)</b>	>30	1,830	5.2	78	5.7
<b>CVD Family History</b>	Yes	2,712	7.7	179	13.2
<b>Prior CVD<sup>3</sup></b>	Yes	240	0.7	72	5.3
<b>Hypertension<sup>4</sup></b>	Yes	3,133	8.9	264	19.5
<b>Diabetes<sup>5</sup></b>	Yes	1,425	4.0	163	12.0
<b>eGFR (ml/min/1.73m<sup>2</sup>)<sup>6</sup></b>	>90	24,937	70.5	656	48.3
	>60-<=90	9,378	26.5	559	41.2
	>30-<=60	999	2.8	13.5	10.0
	<=30	43	0.1	7	0.5
<b>Fragminham risk score</b>					
	Low (0-5%)	24,111	68.2	275	18.9
	Moderate (5-10%)	5,821	16.5	290	21.4
	High (>10%)	5,425	15.3	810	59.7
<b>D:A:D CKD risk<sup>7</sup></b>	Risk score	-1	-3 to 4	4	-1 to 9
<b>(median, IQR)</b>					
<b>Age (median, IQR)</b>	Years	41	35-48	50	44-59
<b>CD4 (median, IQR)</b>	cells/mm <sup>3</sup>	44	290-625	441	289-640

503 Baseline defined as 01/02/2004

504 1. HBV defined as positive: HBV surface antigen, HBV e antigen, or HBV DNA positive

505 2. HCV defined as anti-HCV positive and HCV-RNA positive/unknown

506 3. Prior CVD, as diagnosed on a D:A:D CVD event form

507 4. Hypertension defined as blood pressure >150/>100 or antihypertensive treatment

508 5. Diabetes as diagnosis on a D:A:D event form or by use of anti-diabetic treatment

509 6. eGFR calculated using Cockcroft-Gault

510 7. Score <0: low 5-year CKD risk (0.3%), Score 0-4: medium 5-year CKD risk (2.1%) and Score ≥5: high 5-year CKD risk

511 (16.7%)

512

**Figure 1, Confirmed Current eGFR Level Prior to CVD Event**

513

Confirmed current eGFR level for those with a CVD event is the last measured median eGFR level prior the event. For

514

those without a CVD event confirmed current eGFR level is the last measured median eGFR level during follow-up.

515

516

**Figure 2, Kaplan-Meier Progression to CVD By Confirmed Baseline eGFR Level**

517

518

**Figure 3, CVD Incidence Rate Ratios by Confirmed Current eGFR Level**

519

Multivariate analysis adjusted for age, gender, ethnicity, D:A:D enrolment cohort, nadir CD4 count, HIV mode of

520

acquisition and family history of CVD at baseline. Time-updated variables include HBV/HCV co-infection, HIV-RNA, CD4

521

count, prior AIDS, hypertension, diabetes, confirmed eGFR strata, smoking status, dyslipidemia, prior CVD, exposure

522

to antiretroviral drugs fitted as cumulative use (to zidovudine, didanosine, zalcitabine, stavudine, lamivudine,

523

emtricitabine, tenofovir disoproxil fumerate, abacavir, efavirenz, nevirapine, indinavir, saquinavir, ritonavir, nelfinavir,

524

(fos)ampreavir, atazanavir and darunavir) and current use (zidovudine, didanosine, zalcitabine, lamivudine, stavudine,

525

emtricitabine, tenofovir disoproxil fumerate and abacavir).

526

527

**Supplementary Document 1, Figure 1, Inclusion of Individuals in Analysis**

528

529

**Supplementary Document 2, Full cohort acknowledgements**

530