THE LANCET
Respiratory Medicine

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Kunisaki KM, Niewoehner DE, Collins G, et al, for the INSIGHT START Pulmonary Substudy Group. Pulmonary effects of immediate versus deferred antiretroviral therapy in HIV-positive individuals: a nested substudy within the multicentre, international, randomised, controlled Strategic Timing of Antiretroviral Treatment (START) trial. *Lancet Respir Med* 2016; published online Oct 20. http://dx.doi.org/10.1016/S2213-2600(16)30319-8.

ONLINE DATA SUPPLEMENT

Supplemental Data for Article:
Pulmonary effects of immediate versus deferred antiretroviral treatment in HIV-positive individuals: a nested substudy within the multicentre, international, randomised, controlled Strategic Timing of Antiretroviral Treatment (START) trial

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Contents of Online Data Supplement:

Supplemental Table S1a. FEV_1 slope comparisons in those randomised to immediate vs. deferred ART initiation, restricted to spirometry that met quality standards following central quality review.

	FEV_1 Slope	95% Confidence Interval	p-value
Baseline Smokers, Restricted to Good-Quality Spirometry			
Immediate ART (n=135)	-35·3 mL/year	-55·3 to -15·3 mL/year	
Deferred ART (n=153)	-33·8 mL/year	-52·7 to -14·9 mL/year	
Difference	**-1·5 mL/year**	**-29·0 to +26·0 mL/year**	**p=0·91**
Baseline Non-Smokers, Restricted to Good-Quality Spirometry			
Immediate ART (n=378)	-17·2 mL/year	-28·5 to -6·0 mL/year	
Deferred ART (n=352)	-26·6 mL/year	-38·5 to -14·6 mL/year	
Difference	**+9·3 mL/year**	**-7·1 to +25·8 mL/year**	**p=0·26**
Pooled Analysis, Adjusted for Baseline Smoking Status and Restricted to Good-Quality Spirometry			
Immediate ART (n=513)	-22·0 mL/year	-31·8 to -12·2 mL/year	
Deferred ART (n=505)	-28·8 mL/year	-38·8 to -18·7 mL/year	
Difference	**+6·8 mL/year**	**-7·3 to +20·8 mL/year**	**p=0·35**
Pooled Analysis, Adjusted for Smoking Status at Each Study Visit and Restricted to Good-Quality Spirometry			
Immediate ART (n=513)	-21·6 mL/year	-31·5 to -11·8 mL/year	
Deferred ART (n=505)	-28·0 mL/year	-38·2 to -17·9 mL/year	
Difference	**+6·4 mL/year**	**-7·8 to +20·5 mL/year**	**p=0·38**

ART: antiretroviral treatment; FEV_1: forced expiratory volume in 1 second

Supplemental Table S1b. FEV_1 slope comparisons in those randomised to immediate vs. deferred ART initiation, censoring spirometry data following ART initiation in deferred ART arm participants.

	FEV_1 Slope	95% Confidence Interval	p-value
Baseline Smokers, Censoring Deferred ART Arm Spirometry Data Following ART Initiation			
Immediate ART (n=135)	-33·9 mL/year	-61·2 to -6·6 mL/year	
Deferred ART (n=155)	-38·7 mL/year	-73·3 to -4·1 mL/year	
Difference	**+4·8 mL/year**	**-39·2 to +48·9 mL/year**	**p=0·83**
Baseline Non-Smokers, Censoring Deferred ART Arm Spirometry Data Following ART Initiation			
Immediate ART (n=383)	-27·9 mL/year	-45·0 to -10·8 mL/year	
Deferred ART (n=353)	-24·2 mL/year	-45·7 to -2·8 mL/year	
Difference	**-3·7 mL/year**	**-31·1 to +23·8 mL/year**	**p=0·79**
Pooled Analysis, Censoring Deferred ART Arm Spirometry Data Following ART Initiation, and Adjusted for Baseline Smoking Status			
Immediate ART (n=518)	-29·4 mL/year	-43·8 to -14·9 mL/year	
Deferred ART (n=508)	-28·0 mL/year	-46·2 to -9·9 mL/year	
Difference	**-1·3 mL/year**	**-24·5 to +21·9 mL/year**	**p=0·91**
Pooled Analysis, Censoring Deferred ART Arm Spirometry Data Following ART Initiation, and Adjusted for Smoking Status at Each Study Visit			
Immediate ART (n=518)	-28·7 mL/year	-43·2 to -14·2 mL/year	
Deferred ART (n=508)	-27·4 mL/year	-45·7 to -9·2 mL/year	
Difference	**-1·3 mL/year**	**-24·6 to +22·0 mL/year**	**p=0·91**

ART: antiretroviral treatment; FEV_1: forced expiratory volume in 1 second

Supplemental Table S1c. FEV_1 slope comparisons in those randomised to immediate vs. deferred ART initiation, restricted to spirometry that met quality standards following central quality review *and* censoring spirometry data following ART initiation in deferred ART arm participants.

	FEV_1 Slope	95% Confidence Interval	p-value
Baseline Smokers, Restricted to Good-Quality Spirometry, and Censoring Deferred ART Arm Spirometry Data Following ART Initiation			
Immediate ART (n=135)	-35·5 mL/year	-55·7 to -15·3 mL/year	
Deferred ART (n=150)	-38·0 mL/year	-63·7 to -12·3 mL/year	
Difference	**+2·5 mL/year**	**-30·2 to +35·2 mL/year**	**p=0·88**
Baseline Non-Smokers, Restricted to Good-Quality Spirometry, and Censoring Deferred ART Arm Spirometry Data Following ART Initiation			
Immediate ART (n=378)	-17·1 mL/year	-28·0 to -6·3 mL/year	
Deferred ART (n=350)	-30·3 mL/year	-45·0 to -15·7 mL/year	
Difference	**+13·2 mL/year**	**-5·0 to +31·5 mL/year**	**p=0·15**
Pooled Analysis, Restricted to Good-Quality Spirometry, Censoring Deferred ART Arm Spirometry Data Following ART Initiation, and Adjusted for Baseline Smoking Status			
Immediate ART (n=513)	-21·9 mL/year	-31·5 to -12·2 mL/year	
Deferred ART (n=500)	-32·2 mL/year	-45·0 to -19·4 mL/year	
Difference	**+10·3 mL/year**	**-5·7 to +26·4 mL/year**	**p=0·21**
Pooled Analysis, Restricted to Good-Quality Spirometry and Censoring Deferred ART Arm Spirometry Data Following ART Initiation, and Adjusted for Smoking Status at Each Study Visit			
Immediate ART (n=513)	-21·4 mL/year	-31·0 to -11·7 mL/year	
Deferred ART (n=500)	-31·7 mL/year	-44·6 to -18·9 mL/year	
Difference	**+10·4 mL/year**	**-5·7 to +26·5 mL/year**	**p=0·21**

ART: antiretroviral treatment; FEV_1: forced expiratory volume in 1 second

Impact of Shortened Follow-Up Time on Study Power

Due to early termination of the parent START trial, we had a relatively short median spirometry follow-up time of two years (mean = 2.2 years). The distribution of number of follow-up measures is shown in Figure 3 in the main paper.

The truncated follow-up time likely decreased the precision of the slope measures (i.e. increased the variability of the slopes). Our original sample size assumptions were based on an anticipated standard deviation (SD) of FEV_1 slopes of ± 60 mL/year in both smokers and non-smokers. We powered our analysis to provide 85% power to detect a difference in FEV_1 slope (effect sizes) of 19·0 mL/year in smokers and 15·5 mL/year in non-smokers. At the time of unblinding, our actual SD was ± 94 mL/year in smokers and ± 107 mL/year in non-smokers. Therefore, study power for the effect sizes above was 41% in smokers and 50% in non-smokers. The table below provides actual study power for various effect sizes:

Effect Sizes	Power in Smokers (n=290)	Power in Non-Smokers (n=736)
15 mL/year	27·4%	47·8%
20 mL/year	44·1%	71·9%
25 mL/year	62·0%	88·8%
30 mL/year	77·6%	96·8%

In support of our suspicion that truncated follow-up time increased variability, when we restricted the sample to those with three or more follow-up visits (as originally planned), the SD was quite similar to our original assumptions, with an SD of ± 68 mL/year in smokers and ± 53 mL/year in non-smokers.

Statistical Methods Details

We used PROC MIXED in SAS 9·4 to model the primary outcome of FEV_1 slopes. Models were run for smokers and non-smokers separately. Rtreat is treatment group (coded as 1 for immediate and 2 for deferred), fev1 (millilitres) is the response variable, pid is the participant ID, and visit is the study visit year (coded 1 to 5). AR(1) was the covariance structure for the repeated measures on each participant.

The SAS code was as follows:

```
proc mixed ;
class  rtreat pid ;
model fev1 = visit rtreat visit*rtreat ;
random int visit / type=un subject=pid ;
repeated / type=ar(1) subject=pid ;
estimate 'Slope - immediate group' visit 1 visit*rtreat 1  0 / cl ;
estimate 'Slope - deferred group'    visit 1 visit*rtreat 0  1 / cl ;
estimate 'Difference in slopes'                visit*rtreat 1 -1 / cl ;
run ;
```

We also used alternate covariance structures and the results were robust, with no treatment group differences in FEV_1 slope when using a Huynh-Feldt covariance matrix (p=0·77 for smokers and p=0·96 for non-smokers) or compound symmetric covariance matrix (p=0·87 for smokers and p=0·63 for non-smokers).

Missing Data

The frequencies of missing spirometry data at each visit are provided below:

	Immediate ART			Deferred ART		
Study Visit	Expected number of visits	Missing visits	%	Expected number of visits	Missing visits	%
Year 1	517	61	11·8	505	77	15·2
Year 2	435	45	10·3	436	62	14·2
Year 3	217	31	14·3	223	40	17·9
Year 4	78	13	16·7	67	18	26·9
Year 5	1	0	0·0	5	0	0·0
Total*	1248	150	12·0	1236	197	15·9

'Expected' excludes participants who were known to have died or had withdrawn consent
**p=0·26 for comparison of missing data frequency between immediate and deferred ART arms.*

Although the primary statistical analysis mixed model incorporates data from those with no follow-up, we carried out a 'Complete Case' analysis that excluded all participants with no follow-up spirometry measures. Results were very similar to the Intention-to-Treat analyses, with no difference in slopes between the immediate and deferred ART arms in smokers (difference of -3·9 mL/year; 95%CI: -39·5 to +31·7 mL/year; p=0·83) or non-smokers (difference of -6·6 mL/year; 95%CI: -30·5 to +17·3 mL/year; p=0·59).

START Pulmonary Substudy Team Roster. Listing of team members, by coordinating centres and by sites in descending order of participant enrolment.

International Coordinating Centres
Copenhagen: B Aagaard, PO Jansson, MT Pearson.
London: AG Babiker, A Arenas-Pinto, NB Atako, E Dennis, S Forcat, F Hudson, B Jackson, D Maas, C Purvis, C Russell.
Sydney: S Emery, C Carey, M Clewett, S Jacoby.
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Site Coordinating Centres
Argentina: GR Loria, ML Doldan, A Moricz.
Germany: K Tillmann, V Müller.
Greece: G Touloumi, V Gioukari, O Anagnostou.
Spain: P Herrero, P Lopez.
Thailand: A Avihingsanon, P Rerksirikul.

Site Investigators by Country by Enrolment
(n=number of participants enrolled)

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Desmond Tutu HIV Foundation Clinical Trials Unit (n=104): R Wood, M Rattley.
Durban International Clinical Research Site (n=50): S Pillay, R Mngqibisa.
Durban International Clinical Research Site (WWH)(n=13): T Ndaba, P Madlala.

Uganda (n=137)
Joint Clinical Research Center (JCRC) (n=95): C Kityo, H Mugerwa.
MRC/UVRI Research Unit on AIDS (n=42): P Munderi, J Lutaakome.

Peru (n=96)
Asociación Civil Impacta Salud y Educación (n=51): J Valencia, M León.
Asociación Civil Impacta Salud y Educacion - Sede San Miguel (n=23): E Montalbán, J Alave.
Hospital Nacional Guillermo Almenara Irigoyen (n=14): R Salazar, J Vega.
Hospital Nacional Edgardo Rebagliati Martins (n=8): M del Portal, F Mendo.

United States (n=91)
Florida Department of Health in Orange County/Sunshine Care Center (n=12): N Desai, W Carter.
Hennepin County Medical Center (n=10): K Henry, R Givot.
Hillsborough County Health Department/University of South Florida (n=10): M Chow, B Holloway.
University of North Texas Health Science Center (n=7): S Weis, I Vecino.
University of Illinois at Chicago (n=6): R Novak, G Culbert.
Wake Forest University Health Sciences (n=6): A Wilkin, L Mosley.
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Virginia Commonwealth University (n=5): V Watson, C Clark.
Puerto Rico-AIDS Clinical Trials Unit (n=5): J Santana, I Boneta.
Henry Ford Health System (n=4): I Brar, L Makohon.
Newland Immunology Center of Excellence (n=4): R MacArthur, M Farrough.
AIDS Resource Center of Wisconsin (n=4) and Medical College of Wisconsin (n=2): M Frank, S Parker.
Temple University (n=3): E Tedaldi, M Santiago.
The Ohio State University Wexner Medical Center (n=2): S Koletar, H Harber.

Washington DC VA Medical Center (n=1): D Thomas.
Boston Medical Center (n=1): I Bica, B Adams.
Regional Center for Infectious Disease (n=1): C Van Dam.
University of Miami (n=1): M Kolber, K Moreno.
Infectious Diseases Associates NW FL, PA (n=1): A Brown, B Wade.

United Kingdom (n=83)
Brighton and Sussex University Hospitals NHS Trust (n=13): A Clarke, A Bexley.
Coventry and Warwickshire NHS Partnership Trust (n=10): S Das, A Sahota.
Belfast Health and Social Care Trust (RVH)(n=10): C Emerson, S McKernan.
Royal Free London NHS Foundation Trust (n=8): MA Johnson, M Youle.
University Hospital Birmingham NHS Foundation Trust (n=8): J Ross, J Harding.
Lewisham and Greenwich NHS Trust (n=8): S Kegg, T Moussaoui.
Royal Berkshire Hospital (n=8): F Chen, S Lynch.
Gloucestershire Royal Hospital (n=7): A de Burgh-Thomas, I Karunaratne.
Sheffield Teaching Hospital NHS Foundation Trust (n=4): D Dockrell, C Bowman.
Imperial College Healthcare NHS Trust (n=4): A Winston, B Mora-Peris.
The James Cook University Hospital (n=3): DR Chadwick, P Lambert.

Germany (n=82)
Gemeinschaftspraxis Jessen-Jessen-Stein (n=35): H Jessen, A Jessen.
Universitätsklinikum Würzburg, Medizinische Klinik und Poliklinik II, Schwerpunkt Infektiologie CRS (n=11): S Wiebecke, H Klinker.
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EPIMED-Gesellschaft für epidemiologische und klinische Forschung in der Medizin GmbH (n=9): I Knaevelsrud, M Rittweger.
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Universitätsklinikum Erlangen (n=4): T Harrer, E Harrer.

Thailand (n=82)
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Bamrasnaradura Infections Diseases Institute (n=5): W Prasithsirikul, S Thongyen.
Khon Kaen University, Srinagarind Hospital (n=3): P Chetchotisakd, S Anunnatsiri.
Siriraj Hospital (n=1): W Ratanasuwan, P Werarak.

Argentina (n=55)
Enrolment for sites below equals less than 55 due to exclusion of a site de-registered during follow-up and not listed here.
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Hospital Universitari Mutua Terrassa (n=6): D Dalmau, C Badia.
Hospital Universitari Germans Trias i Pujol (n=5): B Clotet, JM Llibre.

Mexico (n=27)
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) (n= 27): J Sierra Madero, C Madrigal.

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Poland (n=23)
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YRGCARE Medical Centre VHS, Chennai CRS (n= 5): N Kumarasamy, F Beulah.

Nigeria (n=21)
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Pulmonary Substudy

A Substudy of
Strategic Timing of AntiRetroviral Treatment
(START)

A Multicenter Study of the
International Network for Strategic Initiatives in Global HIV Trials

Sponsored by:
The University of Minnesota
Minneapolis, Minnesota, USA

In collaboration with four International Coordinating Center (ICCs) of the INSIGHT Network:

Copenhagen HIV Programme (CHIP) -- Copenhagen, Denmark
Medical Research Council (MRC) Clinical Trials Unit -- London, United Kingdom
National Centre in HIV Epidemiology and Clinical Research (NCHECR), University of New South Wales -- Sydney, Australia
The Institute for Clinical Research at the Veterans Affairs Medical Center -- Washington D.C., USA

Funded by:
The National Heart, Lung & Blood Institute (NHLBI)
National Institutes of Health (NIH)

Substudy Co-Chairs: Ken Kunisaki, Dennis Niewoehner, John Connett

The START protocol is being managed and conducted by the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) with primary support by the U.S. National Institutes of Health through grants from the Division of AIDS, NIAID, NHLBI, and other NIH institutes to the University of Minnesota.

The University of Minnesota will serve as the sponsor for the study and will subcontract with four ICCs that will be responsible for implementation of Good Clinical Practice (GCP) and for oversight of the conduct of the trial at clinical research sites. The University of Minnesota is a constitutional entity under the laws of the State of Minnesota and assumes liability only to the extent provided under the Minnesota Tort Claims Act, Minnesota Statutes, Section 3.736.

The legal representative for the START trial in Europe is the Copenhagen HIV Programme (CHIP).

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1 BACKGROUND AND RATIONALE

1.1 HIV-Infection and COPD are Major Global Health Problems

The global HIV epidemic afflicts an estimated 33.2 million persons[1] and chronic obstructive pulmonary disease (COPD) afflicts over 600 million people worldwide[2]. While mortality rates for HIV-infected individuals have improved following the development of antiretroviral therapy (ART), COPD mortality is rising both in the United States and worldwide[3, 4]. By the year 2020, COPD is projected to become the 3rd leading cause of worldwide death (currently 4th) and the 5th leading cause of worldwide disability (currently 12th)[4].

1.2 COPD: Definition and Lung Physiology

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines COPD as a lung disease "characterized by airflow limitation that is not fully reversible"[5]. This definition highlights the airflow limitation that is a hallmark of COPD. COPD is associated with chronic inflammation of the small airways[6]. This inflammation causes the airways to narrow, which can be detected and quantified by spirometry. In COPD, spirometry demonstrates a reduction in the ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC). As COPD progresses, FEV_1 declines. Thus, FEV_1 is used to assess the severity of COPD and to follow patients for disease progression[5]. COPD is also associated with the destruction of parenchymal lung tissue (emphysema). This results in loss of alveolar attachments to small airways, thereby decreasing lung elastic recoil. Such structural changes result in early collapse of airways during expiration, and this expiratory airflow limitation can be quantified by spirometry.

1.3 HIV Infection is Associated with COPD

Early in the HIV epidemic, the pulmonary complications of HIV infection were mostly related to opportunistic infections. The first report of HIV-associated emphysema came from the radiology literature in 1989 [7]. Diaz and colleagues subsequently reported CT evidence of emphysema in 15% of 114 HIV-infected individuals, compared to 2% of 44 HIV-uninfected controls (p=0.025) [8]. Because cigarette smoking is the strongest risk factor for COPD, Diaz and colleagues matched the HIV-uninfected controls to the HIV-infected individuals on smoking history (along with age and gender). *Pneumocystis jiroveci* infection is also known to occasionally result in pulmonary cyst formation[9]; patients with a history of *Pneumocystis* were therefore also excluded. The potential role of other opportunistic infections, other bacterial pneumonias, or viral respiratory infections could not be ascertained, which may have been of significance, as this study was performed between 1994 and 1997, prior to the wide availability of highly active antiretroviral therapy (HAART).

When HAART became widely available, it was unclear how such therapy would affect the risk of developing COPD. To investigate this topic, Crothers and colleagues analyzed data from the HAART era (2001-2002), using a U.S. Veterans Affairs observational cohort of 1,031 HIV-infected patients and 740 HIV-uninfected controls[10]. After adjusting for age, ethnicity, smoking history, injection drug use history, and alcohol abuse, HIV infection remained an independent risk factor for COPD, as assessed by ICD-9 codes (odds ratio = 1.47, 95% confidence interval [CI] = 1.01 – 2.13) and self-reported chronic lung disease (odds ratio = 1.58, 95% CI = 1.14-2.19). These data were limited by a lack of pulmonary function testing.

1.4 Pulmonary Function Testing in HIV-Infected Patients

Diaz and colleagues reported a mean FEV_1 of 91.1% of predicted in 114 HIV-infected individuals, compared to 96.7% in 44 HIV-uninfected individuals[8]. This difference did not reach statistical significance (p=0.09), potentially due to the small sample size and lack of adequate statistical power. In a related study, the same group of investigators reported thoracic CT scan evidence of focal air trapping in 30 of 48 HIV-infected individuals (63%), compared to only 3 of 36 healthy controls (8%)[11]. Thus, it appears that air trapping may be a more frequent CT finding than emphysema, which was found in only 19% of the HIV-infected study participants. When spirometry data in the HIV-infected patients were analyzed, the FEV_1 was significantly lower in those with air trapping than in those without air trapping (FEV_1 = 89% of predicted vs. 101% of predicted, respectively; p=0.001).

Obaji and colleagues reported findings from a retrospective cohort identified from a single-clinic database of HIV-infected individuals who received nebulized pentamidine (for prophylaxis against *Pneumocystis jiroveci*) for ≥ 5 years and with serial spirometry data available[12]. Among the 79 patients who met these criteria, the rate of FEV_1 decline was -30 mL/year in non-smoking patients and -60 mL/year in the smoking patients—nearly identical to the rates of FEV_1 decline observed in other HIV-uninfected cohorts of non-smokers and smokers[13]. While this might suggest that HIV infection does not increase the rate of FEV_1 decline (and therefore does not increase the risk of developing COPD), the study was quite small and reflected only a small percentage of the 1,850 patients actually seen at this clinic over the study period.

A much more comprehensive study was published by The Pulmonary Complications of HIV Infection Study Group, jointly sponsored by the U.S. National Heart, Lung and Blood Institute and the National Institute of Allergy and Infectious Diseases. This study enrolled 1,183 HIV-infected persons with an average CD4+ cell count of 432 cells/mm^3 and 170 HIV-uninfected controls in the pre-HAART era between 1988 and 1990. Participants were prospectively followed until death or March 1994. The FEV_1 rate of decline was more than that accounted for by normal aging and smoking history[14]. The magnitude of this excess rate of FEV_1 decline was estimated to be an additional -27 mL/year.

To summarize, the spirometry data from cross-sectional and observational cohort studies, mostly from the pre-HAART era, support the notion that HIV infection is associated with a worse FEV_1 and faster decline of FEV_1. A rapid decline in FEV_1 has

important long-term consequences for patients with HIV infection. Their risk of COPD may increase, particularly as they experience significantly longer expected survival times. In addition, FEV_1 declines may have other clinically relevant effects. Population cohort studies have suggested that reductions in FEV_1 are an independent risk factor for all-cause death [15-18], cardiovascular mortality[16, 19], and lung cancer mortality[20].

The mechanisms of this more rapid decline in FEV_1 in patients with HIV infection remain unknown. Some data suggest that HIV viremia itself causes a pulmonary inflammatory response (a CD8+ lymphocytic alveolitis) that may lead to airflow obstruction and eventually, COPD[21]. Other data from the pre-HAART era suggest that *Pneumocystis jiroveci* pneumonia and bacterial pneumonia events may lead to permanent declines in FEV_1[14]. Bacterial pneumonia was the most frequently reported clinical event in the Strategic Management of Antiretroviral Therapy (SMART) trial[22]. Continuous ART was associated with a 36% lower rate of pneumonia as compared to episodic ART. Moreover, among patients with latest CD4+ counts >500 cells/mm^3 (as will be the case in the START trial), continuous ART was associated with a 50% lower risk of pneumonia. In SMART, smoking nearly doubled the risk of pneumonia[22]. ART has also been shown to eliminate the CD8+ lymphocytic alveolitis[23]. While these data suggest that ART might reduce the rate of FEV_1 decline (whether through elimination of the pulmonary inflammation or reduction in bacterial pneumonia risk), a cross-sectional study of 234 patients with HIV infection determined that ART use was independently associated with worse lung function[24], suggesting the possibility that ART might actually adversely affect lung function.

The effects of ART on rate of decline of FEV_1 in patients with HIV infection have not been prospectively studied in randomized trials, so the actual effects remain unknown.

1.5 Pulmonary Symptoms in COPD and HIV

Current standards define COPD using spirometry, but clinical manifestations of COPD also include radiographic emphysema and symptoms of chronic bronchitis—defined as cough or sputum production on most days for 3 or more months in the past year. As previously discussed, emphysema is found more frequently in patients with HIV infection than in HIV-uninfected individuals. Respiratory symptoms are also found more frequently in HIV-infected patients. In a pre-HAART era cross-sectional study of 327 HIV-infected individuals (with a mean CD4+ count of 370 cells/mm^3 and without a history of pulmonary HIV complications) and 52 HIV-uninfected controls (with similar age and smoking history to those with HIV infection), respiratory symptoms of dyspnea, cough, and sputum production were reported in a much higher proportion of those with HIV infection than in uninfected controls (40%-42% versus 8%-25%, respectively)[25]. These differences were present despite relatively similar FEV_1 in the two groups (92% of predicted in the HIV-infected group and 95% of predicted in the controls). Thus, spirometry alone could not sufficiently explain differences in proportions of participants reporting respiratory symptoms. Among the participants with HIV infection, there was also a reduced risk of dyspnea among those treated with the nucleoside reverse transcriptase inhibitor lamivudine (odds ratio = 0.31, p = 0.018). As a subgroup analysis, this must be interpreted with caution, but leads to the hypothesis that ART

might prevent the development of respiratory symptoms. Another observational study also found a reduced likelihood of respiratory symptoms with lower HIV RNA levels[24]. However, in an observational study of 127 HIV-infected children, those treated with ART used more asthma medications compared to those untreated, suggesting that ART might actually lead to an increase in respiratory symptoms[26].

No prospective or randomized studies have been conducted with longitudinal collection of respiratory symptom information.

1.6 Study Objectives and Hypotheses

Primary Objective: To determine if immediate initiation of ART alters the rate of lung function decline compared to deferral of ART until the CD4+ declines below 350 cells/mm^3 in HIV-1 infected persons who are antiretroviral naïve with a CD4+ count above 500 cells/mm^3.
Hypothesis: Early initiation of ART results in a slower rate of lung function decline compared to deferred ART.

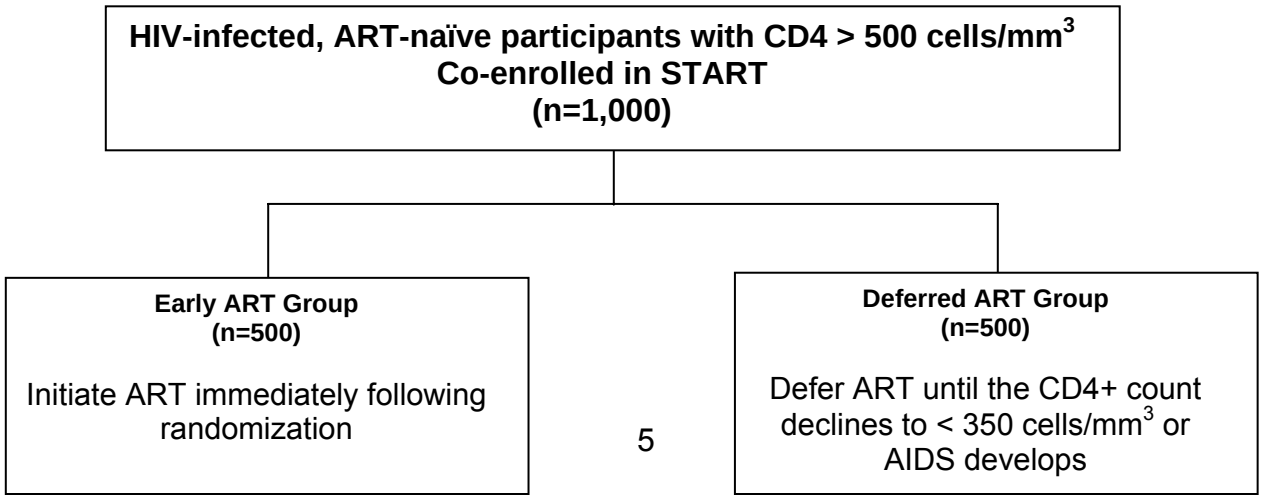
Secondary Objective: To determine if immediate ART alters respiratory health status compared to deferred ART.
Hypothesis: Early initiation of ART results in improved respiratory health status compared to deferred ART.

2 METHODOLOGY

2.1 Study Design

This is a substudy of the START study, which is an international clinical trial. The plan is to co-enroll 1000 participants over 3 years at selected clinical sites. Once the substudy is approved for a site, the goal is to co-enroll all eligible patients. Randomization (1:1 ratio) to the early or deferred treatment groups will be determined by the main START study. Participants will be followed to the common closing date of the START study. This is estimated to be 6 years after the beginning of enrollment (3 years of enrollment and a minimum of 3 years follow-up for each participant).

START Pulmonary Substudy Schematic



2.2 Participant Selection

Inclusion criteria

- Simultaneous co-enrollment in the START Study
- Signed informed consent to the Pulmonary Substudy
- Age ≥ 25 years

Exclusion criteria

- An episode of respiratory illness with 2 or more symptoms of cough, wheezing, breathlessness, or increase in sputum production within the 6 weeks before baseline spirometry.
- Use of asthma medications (bronchodilator, inhaled corticosteroid, leukotriene inhibitor, or theophylline) for 2 or more consecutive weeks within the 6 months before baseline spirometry.
- Relative contraindications to spirometry, such as chest or abdominal or eye surgery within the 3 months before baseline spirometry, known retinal detachment at the time of baseline spirometry.
- Known allergy to albuterol/salbutamol
- Relative contraindications to albuterol/salbutamol, such as resting heart rate of >110 beats per minute, or a known serious or recurrent or uncontrolled cardiac condition (such as unstable coronary artery disease, decompensated heart failure, or recurrent tachyarrhythmias).

2.3 Data Collection

To be collected at baseline and annually thereafter:

a) Post-bronchodilator spirometry
   Spirometry will be measured using a commercially available, handheld, portable spirometer that provides immediate test quality scores. Spirometry will be measured 10-15 minutes following administration of 200 micrograms (2 puffs) of inhaled albuterol/salbutamol.
b) St. George's Respiratory Questionnaire (SGRQ-C)
   The SGRQ-C will be self-administered and provided in the participant's native language.
c) Respiratory medication assessment (annually only)
   Participants will be asked to provide information on use of respiratory medications.
d) Smoking assessment
   Smoking status will be assessed in more detail than in the main START trial.
e) Respiratory illness assessment
   Participants will be asked to categorize the number of self-reported respiratory illnesses over the past 12 months and treatment of such illnesses with antibiotics and oral corticosteroids.

2.4 Study Outcome Measures

Post-Bronchodilator Spirometry

Spirometry is the gold standard by which obstructive lung diseases like COPD are diagnosed, quantified, and followed for progression of disease. Because the START participants will be relatively young and likely have a relatively short duration of HIV infection (because they will have a baseline CD4+ count >500 cells/mm^3), very few participants are expected to have significant COPD. However, data suggest that they will lose lung function faster than a general population (matched for other important confounders like age and smoking status), and therefore will be at higher lifetime risk of COPD, particularly in light of the increasing survival times for patients with HIV infection. Spirometry will be used to quantify this rate of longitudinal decline in lung function.

For all patients consenting to participation in this substudy, spirometry will be performed at baseline and again at each annual visit throughout study participation. Spirometry will be performed at least 6 weeks following any respiratory illnesses to allow accurate measurement of lung function. For patients who undergo chest or abdomen or eye surgery during the study, spirometry will be delayed for at least 3 months. For patients who develop retinal detachment, active pulmonary tuberculosis, or become pregnant during the study, spirometry will be delayed until complete resolution of such conditions. Post-bronchodilator spirometry will be performed, as has been performed in most studies assessing obstructive lung disease.

Inhaled albuterol (also called salbutamol, which is the same chemical compound) is a selective beta-2-agonist that relaxes bronchial smooth muscle and therefore optimizes lung function. Because data suggest that patients with HIV infection are more likely to have pulmonary inflammation, airflow obstruction, and bronchial hyper-responsiveness, their day-to-day lung function is likely to vary more than patients with no lung disease. Therefore, measuring spirometry following a dose of inhaled bronchodilator reduces the variability of the data, and thus provides a better estimate of true best lung function. Post-bronchodilator spirometry was used in the Lung Health Study[27], in most of the landmark COPD trials following longitudinal lung function decline[28-30], and in large population-based COPD prevalence studies[31, 32]. The main side effects are temporary tremulousness and tachycardia. The American Thoracic Society (ATS) and European Respiratory Society (ERS) have jointly published recommendations for the standardization of spirometry[33]. They recommend 400 mcg of albuterol/salbutamol for post-bronchodilator spirometry, but most studies cited above have used 200 mcg, which is the standard clinically prescribed dose. A dose of 200 mcg of albuterol/salbutamol will be used in this study. Because of the potential tachycardia side effects, spirometry will not be performed on patients with resting tachycardia and those with known, serious, uncontrolled, or recurrent cardiac conditions. Among 26,325 post-bronchodilator spirometry tests done in the Lung Health Study, three patients needed to be referred for medical care following spirometry (personal communication, Dr. John Connett, Lung Health Study data coordinating center principal investigator).

The ndd EasyOne spirometer (ndd Medical Technologies, Andover, MA, USA) will be used in this study. This is a handheld, battery-operated device and was chosen for its simplicity and proven feasibility of use in large, multi-center, international studies. This

device was used in the Burden of Obstructive Lung Disease (BOLD) Initiative, which successfully used this device to measure spirometry in 9,435 participants from multiple countries around the globe[32]. A similar COPD population prevalence study used this device to measure spirometry in 5,571 participants in Latin America[31].

St. George's Respiratory Questionnaire (SGRQ-C)

Patients in this substudy will also be asked to complete a St. George's Respiratory Questionnaire for COPD (SGRQ-C) at baseline and annually thereafter. The SGRQ-C is a standardized, validated, self-administered questionnaire that measures respiratory health status and includes domains of respiratory symptoms, activity limitations, and psychosocial impact. We do not expect many patients in this study to have COPD at baseline, but given their heightened risk of COPD and the data suggesting a high prevalence of respiratory symptoms, the SGRQ-C will allow reliable quantification of respiratory health status.

The SGRQ-C contains 40 items and takes approximately 10-15 minutes for participants to complete. It is scored on a scale of 0 to 100, with a score of 100 reflecting the most severe symptoms, limitations, and psychosocial impact of respiratory disease. The SGRQ-C has been translated into nearly 50 languages; participants will complete the SGRQ-C in their native language at baseline and annually.

Respiratory Medication Assessment

Study participants' use of inhaled medications, leukotriene inhibitors, and theophylline will be collected annually.

Smoking Assessment

Participants will be asked at baseline to provide a detailed smoking history related to current and previous use of cigarettes, cigars, cigarillos, and pipes. Questions will record intensity and duration of smoking. At each annual follow-up visit, participants will be asked if they are current smokers or not, allowing for documentation of changes in smoking behavior during the study.

Respiratory Illness Assessment

Respiratory illnesses might contribute to faster rates of lung function decline. Therefore, information on self-reported frequency of respiratory illnesses over the previous 12 months will be collected, along with questions about self-reported treatment with antibiotics or oral corticosteroids. These questions will be asked annually.

The Pulmonary Substudy Protocol Instructions Manual (PIM) includes detailed instructions on how to administer the bronchodilator, use the spirometer, create and store spirometry records, transmit records to the SDMC, administer the SGRQ-C, and perform the assessments of respiratory medications, smoking, and respiratory illnesses.

2.5 Sample Size Estimation

Sample size was estimated for the primary analysis of differences in rate of FEV_1 decline between immediate and deferred ART. Rates of FEV_1 decline for the HIV-infected START participants was estimated to be -87 mL/year in smokers and -57 mL/year in non-smokers[14]. ART is hypothesized to attenuate this additional rate of FEV_1 decline by effectively suppressing HIV viremia and reducing or eliminating pulmonary inflammation. The magnitude of this potential ART effect is unknown, but a change in rate of FEV_1 decline of 15-20 mL/year would be considered significant, both clinically and from a public health perspective.

The standard deviation (SD) of the rate of FEV_1 decline in patients with HIV infection has not been published. Using data extrapolated from the Lung Health Study (a study of patients with mild COPD) [13], and two other large general population cohort studies[18, 34], the rate of FEV_1 decline in our study sample is estimated to have a SD = 60 mL/year.

Sample size was estimated to ensure good power for comparing the immediate and deferred ART groups separately among smokers and non-smokers, as there may be a smoking x treatment interaction. For smokers, 166 participants per treatment group are needed based on the following assumptions: 1) a SD of rate of FEV_1 decline of 60 mL/year; a difference in rate of FEV_1 decline of 20 mL/year; a 2-tailed alpha of 0.05; and power of 0.85. For non-smokers, a smaller treatment difference of 15 mL/year was hypothesized. With the same assumptions for the SD, alpha and power, 290 patients per arm are needed. To account for the fact that some participants may not return for follow-up spirometry, sample size was increased by 10% in each arm, to 183 smokers per arm and 319 non-smokers per arm.

Based on these assumptions, 1,000 participants will be enrolled in this study. Based on smoking habits of participants in the SMART trial[35], this plan is expected to result in 400 smokers and 600 non-smokers. With 400 smokers, a difference between treatment groups of 19.0/mL per year can be detected with 0.85 power; with 600 non-smokers, a difference of 15.5 mL/year can be detected with 0.85 power. If the proportion of smokers and non-smokers differs from predicted, anticipated changes in the detectable treatment differences are described in the following table:

Total Numbers of Smokers / Non-Smokers	Detectable difference in smokers	Detectable difference in non-smokers
300 / 700	22.0 mL/year	14.3 mL/year
350 / 650	20.4 mL/year	14.9 mL/year
400 / 600 (anticipated)	**19.0 mL/year**	**15.5 mL/year**
450 / 550	17.9 mL/year	16.2 mL/year
500 / 500	17.0 mL/year	17.0 mL/year

The percentage of smokers enrolled in this substudy will be closely monitored to assure that the required number of smokers and non-smokers are achieved. Following the pilot phase of the main START trial, the sample size of the main trial and this Pulmonary Substudy will be re-calculated.

3 CLINICAL MANAGEMENT

3.1 Administration of Study Procedures

Research staff who perform study procedures will be trained on how to administer albuterol/salbutamol, perform spirometry, and collect reliable data on SGRQ-C scores, respiratory medication use, smoking assessments, and respiratory illness assessments. Research staff will participate in follow-up training to ensure correct spirometry procedures as necessary. Spirometry is simple to perform, and the total time per spirometry (including administration of albuterol/salbutamol 10-15 minutues prior to spirometry) will take about 20 minutes.

3.2 Study Withdrawal

Participants may withdraw from the study at any time at their request, as described in section 4.5 of the START protocol. A participant can withdraw from the Pulmonary Substudy and still be followed in the START study. If a participant withdraws from the main START study, the participant will be withdrawn from the Pulmonary Substudy.

Otherwise, participants should be followed according to protocol, even if a participant chooses not to adhere to the treatment assignment.

4 EVALUATION

4.1 Data Analysis

Primary Analysis

The primary analysis will compare the rate of FEV_1 decline between the two treatment groups, using strict intention to treat, separately for smokers and non-smokers. The primary analysis will employ a random effects model (with intercept and slope unique to each participant) and will make use of PROC MIXED in SAS or equivalent software. The rate of FEV_1 decline for each participant will be calculated using all available lung function measurements from baseline to the time of completion of the study. Linearity of rates of FEV1 decline will be assessed by studying the effect of adding time-quadratic terms to the model. Sensitivity analyses will also be carried out in which the propensity for missing data is considered using baseline covariates[36].

Changes in smoking behavior will be summarized. Rates of smoking cessation have typically been low in HIV-infected populations. However, due to the potential confounding effect of changes in smoking behavior, rates of FEV_1 decline between the immediate and deferred ART groups will be analyzed separately for continuous smokers, intermittent smokers, and sustained quitters, similar to the Lung Health Study analysis[27]. Analysis with smoking status as a time-dependent variable in linear mixed models will also be carried out.

Secondary Analyses

Analyses of change in SGRQ-C scores between the two treatment groups will be by strict intention to treat and will be analyzed separately for smokers and non-smokers, analyzing change in SGRQ-C scores between baseline and month 12. It is unclear whether or not changes in SGRQ-C scores over time remain linear in patients with HIV infection. Intervention studies in patients with COPD have shown initially linear improvements in SGRQ scores followed by a progressive decline over time[37]. As such, rates of decline of SGRQ-C over the entire study are not expected to be analyzed in the same way as FEV_1 data. However, within-individual changes in SGRQ-C scores before and after ART initiation (in the deferred ART arms) will be analyzed.

Secondary analyses will analyze ART effects on respiratory illnesses and respiratory medication use.

4.2 Monitoring

The DSMB for START will also review data from this substudy. Summary data of treatment arm comparisons for the substudy will be blinded to all participating investigators and study participants throughout the START trial.

5 HUMAN SUBJECTS

This protocol must receive the approval of the participating site's IRB or IEC prior to implementation. All study participants must sign an informed consent form (see sample in Appendix A).

Appendix A: START Pulmonary Substudy Sample Consent

Pulmonary Substudy: A Substudy of Strategic Timing of AntiRetroviral Treatment (START) INSIGHT Protocol 001E, Version 1.0

A Multicenter Study of the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT)

University of Minnesota: SPONSOR
NHLBI: PRIMARY FUNDER
DAIDS Protocol 10839

Short Title of the Study: **START Pulmonary Substudy**

CONSENT FOR PARTICIPATION IN A SUBSTUDY OF AN NIH-FUNDED RESEARCH TRIAL

SITE LEADER: _____ PHONE: _____

ALL SITE INSTRUCTION THAT IS INCLUDED IN A TEXT BOX SHOULD BE REMOVED FROM THE SITE'S INFORMED CONSENT FOR SUBJECTS

INTRODUCTION
You are being asked to take part in this substudy because you are infected with HIV, the virus that causes AIDS, and because you have joined the START study. This substudy is being done to look at whether there is any difference in lung function depending on whether you start HIV medicines early or wait until the guidelines suggest doing so. Before you can decide whether or not to take part in this substudy, we would like to explain the purpose of the substudy, how it may help you, any risks to you, and what is expected of you.

YOUR PARTICIPATION IS VOLUNTARY
This consent form gives you information about this substudy that will be discussed with you. Once you understand the substudy, and if you agree to take part, you will be asked to sign this consent form. You will be given a copy to keep.

Before you learn about this substudy, it is important that you know:

- your participation is completely voluntary;
- you can refuse to take part in this substudy without losing any of the benefits of your usual medical care, and you can still be in the main START study;
- if you agree to this substudy and then decide to stop participating in the substudy, you may do so at any time for any reason. If you stop participating in this substudy, you can still be in the main START study, and you will not lose any of the benefits of your regular medical care.

If you agree to take part in this substudy, you will be asked to sign this consent form. The doctor in charge of this substudy at this location is **[insert the name of the substudy investigator]**. He or she will keep the original copy of this consent to place in your medical record. You will also receive a copy to keep.

This substudy is being funded by the National Heart, Lung and Blood Institute (NHLBI), in the U.S. National Institutes of Health (NIH), through a grant to the University of Minnesota. The University of Minnesota is the sponsor of the main START study and this substudy.

WHY IS THE PULMONARY SUBSTUDY BEING DONE?

People with HIV infection appear to be at higher risk for a common lung condition called chronic obstructive pulmonary disease (COPD). COPD is also known as emphysema or chronic bronchitis. In early stages of COPD, people may have very few symptoms, but as the disease progresses, COPD causes more and more shortness of breath. The most common risk factor for COPD is smoking cigarettes, but several studies have shown that HIV infection also increases the risk for COPD.

It is unknown how HIV infection might cause COPD. Several studies have shown that people with untreated HIV infection have abnormal inflammation in their lungs. It is possible that the way the body tries to fight off the HIV virus might injure the lungs and lead to COPD. It has also been shown that drugs that treat HIV infection can reduce this lung inflammation. Because of this, it is possible that treating HIV infection early may reduce the need for the body to fight HIV so hard, and prevent some of this damage to the lungs and lower the chances of COPD. On the other hand, some studies have suggested that drugs used to treat HIV might actually worsen lung function. There is very little information to help answer this question. This substudy will see if lung function is different between people in the early treatment group and people in the deferred treatment group.

HOW MANY PEOPLE WILL TAKE PART IN THE SUBSTUDY?

We expect that we will need about 1000 people to answer this question.

HOW LONG WILL YOU BE IN THE SUBSTUDY?

You will continue to be followed in the substudy until the main START study comes to an end. Right now we think it will take about 6 years of follow-up to complete the study.

WHAT DO YOU HAVE TO DO IF YOU ARE IN THE PULMONARY SUBSTUDY?

Screening visit

After you consent to the main START study and this substudy, your doctor or study nurse will do a test called spirometry. In order to improve the test's accuracy, we perform this test 10 to 15 minutes after having you take a dose of an inhaled medication called a bronchodilator. This inhaler medication (albuterol or salbutamol) is typically used for patients with asthma or COPD and allows us to measure your best lung function.

Spirometry measures the amount and speed of air movement out of your lungs. This test is the standard way to diagnose COPD. This test is performed while you are seated, with a nose clip over your nostrils. You will hold a small device with your hands and seal your lips around a mouthpiece. You will then inhale as large a breath as possible, and then quickly blow out all the air in your lungs as hard as possible until no more air is coming out of your lungs. You will then be allowed to rest until you are ready to repeat the test again. We will require at least 3 tests to make sure we are measuring your lung function accurately. You will be asked to perform no more than 8 tests. Most people will need to perform 4 to 6 tests, and the tests are usually completed within 3-5 minutes.

You will be asked to complete a questionnaire about lung symptoms like cough and shortness of breath. The questionnaire takes around 10 to 15 minutes to complete. You will be asked questions about your smoking habits and respiratory illnesses you may have had in the past 12 months.

Follow-up visits

Every 12 months, we will ask you to perform spirometry, complete the questionnaire about lung symptoms, and ask you about medications you may be using, your smoking habits, and respiratory illnesses you may have had in the past 12 months. If you are having a respiratory illness at any of these visits (or you recently had a respiratory illness), we will delay these assessments for at least 6 weeks after you have fully recovered.

WILL YOU GET THE RESULTS OF THE TESTS DONE IN THIS SUBSTUDY?

The results of the spirometry tests will be made available to you.

The results of the questionnaire will not be available to you because this questionnaire is only for research purposes and is not used to decide whether or not you require any treatment.

WHAT ARE THE RISKS AND/OR DISCOMFORTS OF BEING IN THIS SUBSTUDY?

This spirometry test may pose risks to people who have had recent surgery in their chest, abdomen, or eyes, so if you have had any of these procedures in the 3 months before starting this study, you will not be allowed to be in this pulmonary substudy. If

you undergo any of these procedures during the study, we will delay spirometry for at least 3 months or until you have fully healed from your surgery. Other conditions which prevent safe testing include a detached retina (the light-sensing tissue of your eyes) and tuberculosis of the lungs. If you have any of these conditions at the start of the study, you will not be allowed in this study. If you develop any of these conditions during the study, we will delay spirometry until you have fully recovered from that condition.

You may become tired or lightheaded during the spirometry test. You may also experience coughing or chest discomfort during the test. If any of these happens, it is fine for you to rest for a while before continuing.

The main risks with the inhaled bronchodilator drug (albuterol or salbutamol) are a fast heart rate, shakiness of your muscles, headache, irritation of the mouth or throat, shortness of breath, and cough. Most of these side effects are temporary and not dangerous. However, a fast heart rate could pose more serious risks if you have a resting heart rate of over 110 beats per minute or if you have been diagnosed with serious, recurrent, or uncontrolled heart conditions. If you have any of these conditions at the start of the study, you will not be allowed in this pulmonary substudy. If you develop any of these conditions during the study, we will delay spirometry until you have fully recovered from that condition. The bronchodilator may also pose some risk to a pregnant woman and her fetus. If you become pregnant during the study, spirometry will be delayed until you are no longer pregnant.

In a previous study with 5887 patients, out of 26,325 of these spirometry with bronchodilator tests done, three patients had to be referred for medical care.

You might feel uncomfortable answering the questionnaire. You will be allowed to skip any questions which you do not want to answer.

WHAT ARE THE BENEFITS OF BEING IN THIS SUBSTUDY?

If you take part in this substudy, there may be a direct benefit to you from having your lung function tested regularly, but no guarantee can be made. It is also possible that you may receive no benefit from being in this study. Information learned from this study may help others who have HIV.

WHAT ABOUT PREGNANCY AND BREASTFEEDING?

The spirometry test will not be performed during pregnancy. There are no risks to breastfeeding.

WHAT IF THERE ARE NEW FINDINGS?

You will be told about any new information learned during this substudy that might cause you to change your mind about staying in it. At the end of the main START study, you will be told when substudy results may be available and how to learn about them.

WHAT IF YOU WANT TO WITHDRAW FROM THE SUBSTUDY?
If you enroll in this substudy, you may decide to stop participating at any time. Withdrawing from this study will not affect your regular medical care, and you can continue to be in the main START study.

CAN YOUR SUBSTUDY PARTICIPATION BE STOPPED WITHOUT YOUR CONSENT?
You may be taken off the pulmonary substudy without your consent if:

- your study doctor decides that continuing in the substudy would harm you;
- the substudy is cancelled by NHLBI, regulatory authorities in your country, or your site's Institutional Review Board (IRB)/Independent Ethics Committee(IEC);
- you are in jail or prison;
- other administrative reasons.

WHAT ARE THE ALTERNATIVES TO BEING IN THIS SUBSTUDY?
You can choose not to be in this substudy. Please talk to a study team member or your doctor about this and other choices available to you.

ARE THERE ANY COSTS TO YOU?
There is no cost to you to be in this substudy. The substudy will cover all costs for doing the tests described in this consent.

WHAT IF YOU ARE INJURED?

There is very little chance that you would be injured by participating in this substudy. If you are injured as a result of participating in this substudy, you will receive proper medical care. The cost for such medical care will be paid by you or by another party. There is no program to compensate you, either through this substudy or the US National Institutes of Health (NIH). You will not be giving up any of your legal rights by signing this consent form.

HOW IS YOUR CONFIDENTIALITY PROTECTED?

Researchers will take every reasonable step to protect the confidentiality of your health information and to prevent misuse of this information. For example, your research records will be identified by a code. You will not be identified by name or any other way in any publication about this substudy.

[The following paragraph is for U.S. sites only]

In addition to these efforts to keep your information confidential, the START study and its substudies are covered by a Certificate of Confidentiality from the U.S. Department of Health and Human Services. This certificate means that researchers cannot be forced to give information collected as part of this substudy to people who are not

involved with the substudy, such as the court system. However, this certificate has limited protection rights. You should know that it does not stop the doctor in charge of this substudy from taking appropriate steps to prevent serious harm to yourself or others.

[The following paragraph is for international sites only]

Efforts will be made to keep your personal information confidential, but we cannot guarantee complete confidentiality. Your personal information may be released if required by law. Any publication of this substudy will not use your name or identify you personally.

[The following paragraph is for all sites]
Your medical and research records may be reviewed by the *[insert the name of the site]* ethics committee (institutional review board, IRB), the US National Institutes of Health (NIH), the US Office for Human Research Protections (OHRP), and the research staff and monitors, and their designees. Also, the research staff at *[insert the name of the site]* is required to make sure that people not involved with this substudy do not have access to your research and medical records while collecting personal information about you. They will keep your files in a locked cabinet in a safe place and will handle your personal information very carefully. This will also help to protect your privacy.

WHOM DO YOU CALL IF YOU HAVE QUESTIONS OR PROBLEMS?

For questions about this substudy contact:

- *insert name of the investigator or other study staff*
- *insert telephone number of above*

For questions about your rights as a research participant, contact:

- *insert name or title of person on the ethics committee (Institutional Review Board, IRB) or other organization appropriate for the site*
- *insert telephone number of above*

SIGNATURE PAGE FOR THE START PULMONARY SUBSTUDY

You have already agreed to join the main START study, and you can still be in the main START study even if you do not want to join this substudy.

If you have read this informed consent (or if you have had it explained to you) and understand the information, and you voluntarily agree to join ***the START Pulmonary Substudy***, please sign your name below.

_____	_____	_____
Participant's name (typed or printed)	Participant's signature	Date

OR

_____	_____	_____
Participant's legal guardian or representative	Legal guardian's signature	Date

_____	_____	_____
Witness' name (typed or printed)	Witness' signature	Date

A witness to the volunteer's signature is strongly encouraged.

NOTE: This consent form with the original signatures MUST be retained on file by the principal investigator. A copy must be given to the volunteer. A copy should be placed in the volunteer's medical record, if applicable.

Appendix B: START Pulmonary Substudy Time & Events Schedule

Requirement	Baseline (≤60 days **before** participant randomized)	Follow-up visits **after** randomization
		Annual (e.g. 12, 24, 36, etc.)
Post-Bronchodilator Spirometry	X	X
St. George's Respiratory Questionnaire-C	X	X
Respiratory Medication Assessment		X
Smoking Assessment	X	X
Respiratory Illness Assessment	X	X

Appendix C: START Pulmonary Substudy Protocol Team

The INSIGHT START Pulmonary Substudy Protocol Team will oversee the implementation of this substudy. The Substudy Protocol Team includes representatives from the four International Coordinating Center (ICCs) of the INSIGHT Network.

Members are:

- Substudy protocol co-chairs: Ken Kunisaki, Dennis Niewoehner, John Connett
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