Last advances in HIV pharmacogenomics

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Disclosures

In the last five years I received:

• Research grants from BMS, Gilead and Viiv
• Consultancy fees from Gilead, MSD, Janssen-Cilag and Viiv
• Speaker’s honoraria from Abbvie, BMS, Gilead, MSD, Janssen-Cilag and Viiv
Outline

• A very short introduction
• PG (new drugs or hard endpoints)
  – drug disposition
  – tissues and cells
  – toxicity
  – Drug to drug interactions
• Concomitant conditions
• New targets
Why PG in Infectious Diseases?

Significant associations (PK→ efficacy or PK→ toxicity) may be observed when drugs (usually overdosed) are “pushed to their limits”

- Reduced dose or administration
- Extreme body composition (severe obesity or malnutrition)
- Extreme ADME conditions (ICU, ECMO, end-stage organ disease, etc.)
- Very high microbial burden (inoculum effect)
- Protected compartments or cells
On average each person is found to carry in his genome: 100 genuine loss of function genes and 20 genes completely inactivated

*McArthur Science 2012*
PG and clinical practice

Mechanistic studies
Define mechanisms and identify biologically plausible candidate genes.

Exploratory clinical studies
Candidate gene association studies to define whether an association exists.

Confirmatory clinical studies
Genotype – directed clinical management to define the clinical benefit.

Cost effectiveness studies
Risk – benefit analyses to assess whether the association is affordable.

Clinical validation

Clinical confirmation

Exploratory evidence for a monogenic association

Defined clinical utility

Implementable clinical test

Exploratory evidence for a weak association

Weak association

Exploratory clinical studies
Genome Wide association study (GWAS) to discover novel associations.

Mechanistic studies
Explore the mechanism for the association to ensure biological plausibility.

Confirmatory clinical studies
Genotype – directed clinical management to define the clinical benefit.

Cost effectiveness studies
Risk – benefit analyses to assess whether the association is affordable.
Clinical application - guidelines


1. UGT1A1 and atazanavir
2. HLAB and abacavir
3. IL28B and PegIFN/RBV
Figure 1. CYP450 allelic variants reported to be present in populations residing on the African continent.

1. DRUG DISPOSITION
Rilpivirine

- 150 patients
- Higher RPV Log_{10} C12 \textbf{CYP3A4*22} (7.8%), TDF/FTC (1.3%);
- Lower RPV Log_{10} C12 DRV/r (3.2%), ABC/3TC (6.5%), \textit{SLCO2B1} (2.26 less)

- PopPk model, 235 samples
- \textbf{No effect} of \textit{CYP3A4*22}, \textit{CYP3A5*3}, \textit{CYP2C19*2}, \textit{CYP2C19*17}, \textit{UGT1A1*28}, and \textit{UGT1A4*2}
- 29% \textbf{C\textsuperscript{\textsuperscript{th}}rough} <50 ng/mL

\textit{SLCO2B1} rs1789693

\begin{align*}
\text{Rilpivirine C\textsubscript{12}} & \quad \text{(ng/mL)} \\
\text{Genotype} & \\
\text{TT & TA} & \\
\text{AA} & \\
\end{align*}

\begin{align*}
\text{Rilpivirine concentrations (ng/mL)} & \\
\text{Time post-dose (h)} & \\
\end{align*}

Dolutegravir and PG

- **UGT1A1 poor metabolizer** status (*28/*28, *28/*37 and *37/*37) had a decrease in CL/F (~32%), increase in AUC (46%) and Cmax (32%);
- Higher **DTG Ctrough** if carrying *6 or *28 alleles in UGT1A1
- **ABCG2** (rs2231142) and **NR1I2** (rs2472677) variants were both independently associated with higher **DTG plasma peak concentrations** as were ethnicity and accompanying drugs.
  - Weight and height were inversely associated with Cmax.

Chen S, et al. Pharmacogenomics 2014; Yagura H, et al. BMC Infect Dis 2017; Elliot ER, CROI 2018 #467
Tenofovir and PG

- **ABCC4** (4131T>G) – higher TFV plasma concentrations in Thai patients (3463A>G) – 32% higher TFV plasma AUC, as well as 11% higher clearance in 342 Thai pts

- **ABCG2** (421C>A) – Lower TFV plasma and urinary concentration in 56 pts.

- **ABCC10** (1791 + 526 G>A) – Lower TFV urinary to plasma ratios

CNT2 (SLC28A2) and TDF/TAF?

### A. Tenofovir

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>0.1 M</th>
<th>1 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular uptake of tenofovir (pmol/mg protein)</td>
<td>MDCKII-CNT2</td>
<td>MDCKII</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### B. Tenofovir alafenamide

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>0.1 M</th>
<th>1 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular uptake of tenofovir alafenamide (pmol/mg protein)</td>
<td>MDCKII-CNT2</td>
<td>MDCKII</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
2. PHARMACOLOGY IN TISSUES AND CELLS
PgP and Brain parenchyma

- Cynomolgus monkeys
- Nelfinavir with zosuqidar
- **brain to plasma:**
  - huge effect 4.4% → 640%
- **CSF to plasma:**
  - no effect 0.6% → 0.7%

BCRP/Pgp and brain concentrations in macaques

Srinivas et al, IAS 2017, Abstract WEAB0105
PG and CSF concentrations

Intracellular concentrations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Intra PBMCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCB1</strong></td>
<td>↑ EFV</td>
</tr>
<tr>
<td></td>
<td>↑ ATV</td>
</tr>
<tr>
<td><strong>ABCC4</strong></td>
<td>↑ 3P-3TC</td>
</tr>
<tr>
<td></td>
<td>↑ 3P-AZT</td>
</tr>
<tr>
<td></td>
<td>↑ 2P-TFV</td>
</tr>
<tr>
<td><strong>SLCO1B1</strong></td>
<td>↑ RTV</td>
</tr>
<tr>
<td><strong>PXR</strong></td>
<td>↓ RTV</td>
</tr>
</tbody>
</table>

OCT1 and Lamivudine uptake

PG and pregnancy

- Few is known about transporters’ and CYPs’ expression during pregnancy (PXR mRNA ↑ towards term in mice)
- 20 HIV+ patients on ATV/r plus 2 NRTIs
- Plasma and IntraPBMCs’ PK, several SNPs

Lymphatic tissue

Higher [ARV] in SHIV+ Higher [ARV] in SHIV-

Higher [ARV] in males Higher [ARV] in females

Protein Concentration by QTAP (pmol/L protein)

** p<0.001

Female Male

BCRP ENT1 OCT3 Drug Transporter

Burgunder E, et al. CROI 2018 #476
3. TOXICITY
# PG and toxicity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Enzyme</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz</td>
<td>CYP2B6, CAR</td>
<td>CNS disturbances</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>P-glycoprotein, HLA:C04<em>01, HLA-DRB1</em>0102, HLA-B*5801</td>
<td>risk of hepatotoxicity</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>CYP2B6</td>
<td>risk of hypersensitivity syndrome</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>MRP2, MRP7</td>
<td>Tubular toxicity</td>
</tr>
<tr>
<td>Abacavir</td>
<td>HLA B57*01</td>
<td>HRS</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>UGT1A1</td>
<td>Hyperbilirubinemia</td>
</tr>
</tbody>
</table>
PG and Discontinuation

- **CYP2B6, 2A6, 3A4**
  - Score 1 (Ref.)
  - Score 2 ($P_a=0.648$)
  - Score 3 ($P_a=0.125$)
  - Score 4 ($P_a=0.234$)
  - Score 5 ($P_a=0.820$)
  - Score 6 ($P_a=0.185$)

- **SLCO1B1**
  - Score -2 (Ref.)
  - Score 0 ($P_a=0.499$)
  - Score +2 (risk, $P_a=0.473$)

- **UGT1A1**
  - *1/*1 (Ref.)
  - *1/*28, *37 ($P_a=0.158$)
  - *28/*28, *37 ($P_a<0.0001$)

- **ABCC2**
  - 0 (Ref.)
  - 1 ($P_a=0.371$)
  - 2 ($P_a=0.473$)

PG and tolerability

• 764 pts.
• Bonferroni
  - **SLCO2B1** → 2.37 higher risk of intolerance to NRTIs
  - **ABBC2** → 2.64 higher risk of intolerance to PIs
• Heterogenous definition of side effects

• 1181
• GWAS
  - **CYP2B6** → EFV PK
  - **APOE** → LDL Cholesterol
  - **APOA5** → triglyceride
• Other SNPs for CD4 recovery and HIV RNA

# EFV and Severe Neurological Diseases

<table>
<thead>
<tr>
<th>CNS Adverse Event</th>
<th>[Case 1]b</th>
<th>[Case 2]b</th>
<th>[Case 3]b</th>
<th>[Case 4]b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generalised tonic-clonic seizures</td>
<td>Acute progressive ataxia, with cerebellar signs</td>
<td>Generalised tonic-clonic seizures</td>
<td>Absence seizures</td>
<td></td>
</tr>
<tr>
<td>Aggressive behavior</td>
<td>Anti-social behavior</td>
<td>Progressively poor school performance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Event Described</th>
<th>Generalised tonic-clonic seizure</th>
<th>Cerebellar dysfunction</th>
<th>Generalised tonic-clonic seizure</th>
<th>Absence seizure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Starting ART Regimen</td>
<td>LPV/r, 3TC, d4T</td>
<td>LPV/r, 3TC, d4T</td>
<td>LPV/r, 3TC, d4T</td>
<td>RTV, 3TC, d4T</td>
</tr>
<tr>
<td>Age at ART start (months)</td>
<td>4.9</td>
<td>2.4</td>
<td>16.7</td>
<td>4</td>
</tr>
<tr>
<td>ART regimen at first event</td>
<td>EFV, 3TC, ABC</td>
<td>EFV, 3TC, ABC</td>
<td>EFV, 3TC, ABC</td>
<td>EFV, 3TC, d4T</td>
</tr>
<tr>
<td>Time on EFV at first event (months)</td>
<td>3.3</td>
<td>19.8</td>
<td>13.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Age at first event</td>
<td>4 years 6 months</td>
<td>4 years 10 months</td>
<td>7 years 6 months</td>
<td>4 years 7 months</td>
</tr>
<tr>
<td>EFV dose at first event (mg/kg/dose)</td>
<td>21 mg/kg/dose</td>
<td>21 mg/kg/dose</td>
<td>15 mg/kg/dose</td>
<td>21 mg/kg/dose</td>
</tr>
<tr>
<td>Age at second event</td>
<td>5 years</td>
<td>4 years 11 months</td>
<td>9 years 5 months</td>
<td>N/A</td>
</tr>
<tr>
<td>Time on EFV at second event (months)</td>
<td>9.6</td>
<td>21.3</td>
<td>37.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Time on EFV at time of drug level (months)</td>
<td>10.1</td>
<td>23.5</td>
<td>49.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| EFV level mg/L (reference range) | 20 mg/L (1-4 mg/L) | 60.54 mg/L (Ref > 1 mg/L) | 51.23 mg/L (1-4 mg/L) | 19.62 mg/L (1-4 mg/L) |
| Time since last dose prior to levels (hours) | 13 h post dose | 13 h post dose | 14 h post dose | 15 h post dose |

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Heterozygous CYP2B6 516G/T</th>
<th>Heterozygous CYP2B6 516G/T</th>
<th>Heterozygous CYP2B6 516G/T</th>
<th>Homozygous CYP2B6</th>
</tr>
</thead>
</table>
EFV and Suicidality

- 1833 pts. → 34 cases
- CYP2B6/CYP2A6
- Strong association in White, null in black

Mollan KR, et al. JID 2017
EFV/8-OH EFV and NC performances

8-OH EFV showed in vitro direct neurotoxicity

Table 4. Correlation Between Cerebrospinal Fluid 8-Hydroxy Efavirenz Exposure and Patient-Completed Questionnaires

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week</th>
<th>Spearman Correlation Coefficient</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASS-Depression</td>
<td>48</td>
<td>0.20</td>
<td>.31</td>
</tr>
<tr>
<td>DASS-Anxiety</td>
<td>48</td>
<td>0.11</td>
<td>.58</td>
</tr>
<tr>
<td>DASS-Stress</td>
<td>48</td>
<td>0.38</td>
<td>.04</td>
</tr>
<tr>
<td>ESQ</td>
<td>4</td>
<td>-0.43</td>
<td>.02</td>
</tr>
<tr>
<td>ESQ</td>
<td>48</td>
<td>0.13</td>
<td>.05</td>
</tr>
<tr>
<td>SF-12-Physical</td>
<td>48</td>
<td>0.13</td>
<td>.50</td>
</tr>
<tr>
<td>SF-12-Mental</td>
<td>48</td>
<td>-0.38</td>
<td>.05</td>
</tr>
</tbody>
</table>

Abbreviations: DASS, Depression Anxiety Stress Scales; ESQ, efavirenz symptom questionnaire; SF-12, 12-item short form.
Differential expression of CYP2B6 in tissues

- Rats administered EFV 9 mg/Kg or water for 10 and 36 days
- Increase in 8-OH-EFV over time
- Full auto-induction of CYP2Bs not until day 36
- Differential RNA expression in liver and brain
- Protein S-thiolation reduced in the hippocampus (drug-induced oxidative stress)
Atazanavir and UGT1A1 slow metabolizers

- Higher risk of **hyperbilirubinemia** (A5202 – 928 pts on ATV/r, + baseline Hb and Bil)

- Higher risk of **discontinuation** (321 pts, ATV/r) in White (HR 14.4) but not in Black (HR 0.8) individuals

- Higher risk of **kidney stones** in patients on ATV/r (31 vs. 47)

Table 4. Multivariate analysis to estimate the association of SNPs of the UGT1A-3’-UTR with atazanavir-induced nephrolithiasis

<table>
<thead>
<tr>
<th>UGT1A-3’-UTR</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype T/C versus C/C at position c.211</td>
<td>3.7</td>
<td>1.13–11.9</td>
<td>0.030</td>
</tr>
<tr>
<td>Genotype G/C versus C/C at position 339</td>
<td>5.8</td>
<td>1.56–21.3</td>
<td>0.009</td>
</tr>
<tr>
<td>Genotype G/G or G/C versus C/C at position 440</td>
<td>5.8</td>
<td>1.56–21.3</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Each SNP was tested in the model separately. Each variable was adjusted for sex, age and hepatitis C infection.
DTG and UGT1A1

34 patients (out of 107, 255 person-year follow up) reported NP-AEs (insomnia, dizziness, headache; mostly grade 1-2)
Tenofovir and tubular toxicity

Tenofovir and tubular toxicity

Single nucleotide polymorphisms

Higher plasma concentrations (Age, BMI, RTV, Cobi)

Tenofovir and proximal tubular toxicity

Frequency, %

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>KTD group</th>
<th>OR (95% CI)b</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCC10–ABCC2 haplotypea</td>
<td>8.4</td>
<td>22.1</td>
<td>3.0 (1.2–7.9)</td>
<td>.01</td>
</tr>
</tbody>
</table>

OR 4.67 (1.2–17.4)

p = 0.01

Tenofovir and proximal tubular toxicity (2)

- **Age**: Comparing different age groups, with a significant difference in cumulative incidence between age categories.
- **Male gender**: Comparing male to female groups, with a significant difference in cumulative incidence.
- **eCrCl**: Comparing different eCrCl categories, with a significant difference in cumulative incidence.
- **PI vs. NN vs. RAL**: Comparing different antiretroviral regimens, with a significant difference in CD4 cell count.
- **TFV C_{12} >70 ng/mL**: Comparing different TFV C_{12} levels, with a significant difference in cumulative incidence.

Calzagno A, et al. Submitted
4. DRUG TO DRUG INTERACTIONS
Voriconazole PG and DDIs

Figure 2. Metabolic pathways involved in the voriconazole-atazanavir-ritonavir interaction. Metabolic pathways involved in the interaction in CYP2C19 (A) normal metabolizers and (B) poor/intermediate metabolizers. Thick arrows indicate the main metabolic pathway.

VOR dose from 200 x 2 to 50 x 2

Calcagno A, et al. Pharmacogenomics 2014
Voriconazole PG and DDIs (2)
Voriconazole PG and DDIs (2)

“Poor and intermediate metabolizers”

VORICONAZOLE

HALOPERIDOL?

VORICONAZOLE

(CYP3A4 INHIBITION)

CYP2C19

CYP2C9

CYP3A4

DTG and INH/rifapentine

- 4 Healthy volunteers (3 on TT) with DTG + once weekly isoniazid and rifapentine
- 2/3 Flu-like syndrome and hepatic injury after the third dose → terminated

A Dolutegravir (DTG)

B Isoniazid (INH) on Day 19

NAT2 slow metabolizers

Brooks KM, et al. CID 2018
EFV/NVP and levonegestrel

- 47% lower LNG concentrations in EFV-treated HIV+ Ugandan women + high rate of unintended pregnancy

  - 20 women on EFV and 20 on NVP
    - MVA (EFV): Age + CYP2B6 SNPs
    - MVA (NVP): Age/weight/CD4 + albumin/SHBG + CYP2B6 SNP

5. CONCOMITANT CONDITIONS
<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>Alleles/SNP</th>
<th>Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>NAT2</td>
<td>*5-*12</td>
<td>Higher INH levels, higher variability, higher EBA</td>
<td>Parkin 1997, Kinzig 2005, Donald 2004</td>
</tr>
<tr>
<td>INH and RIF</td>
<td>CYP2E1</td>
<td>21, c2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RHZE</td>
<td>MnSOD</td>
<td>47C&gt;T</td>
<td>hepatotoxicity</td>
<td>Huang 2007, Gupta 2013</td>
</tr>
<tr>
<td>RHZE</td>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RHZE</td>
<td>TNF-alpha</td>
<td>308G&gt;A</td>
<td>hepatotoxicity</td>
<td>Kim 2011</td>
</tr>
<tr>
<td>RIF</td>
<td>SLCO1B1</td>
<td>463C&gt;A</td>
<td>Low RIF</td>
<td>Chiguitsa 2011, Weiner 2013, Kwara 2014</td>
</tr>
<tr>
<td>RIF</td>
<td>CES2</td>
<td>2263A&gt;G</td>
<td>Low RIF</td>
<td>Song 2013</td>
</tr>
</tbody>
</table>
Hepatotoxicity with antiTB/ARVs

- Significant variation in incidence (Ethiopia 16/30% vs. 6/10% Tanzania)
- Several candidate genes (CYp2B6, NAT2, ABCB1, HLAs)
- GWAS in Ethiopia
- **ERN1 gene** (MAP3K3) on chromosome 17 in ARV group (alters gene expression in ER-mediated stress signals)
- **rs4842407** (lincRNAs transcript variant located on chromosome 12) in ARV/antiTB group

Petros Z, et al. OMICS 2017
PG in the SOUTH Study

- 221 HIV+ patients starting antitB + TDF/3TC/EFV (58.8% male, age 34 yy, weight 50 Kg)
- 33 pts (14.9%) with SGOT/GPT elevation (x4) and 29 (13.1%) with peripheral neuropathy
PG in co-morbidities

Benefits of and Barriers to Pharmacogenomics-Guided Treatment for Major Depressive Disorder
Ahmed T. Ahmed¹, Richard Weinshilboum² and Mark A. Frye¹

Severe Cutaneous Adverse Reactions: The Pharmacogenomics from Research to Clinical Implementation
Shih-Chi Su ¹,², Shuen-Iu Hung ³, Wen-Lang Fan ¹, Ro-Lan Dao ³ and Wen-Hung Chung ¹,³,⁴,*

Pharmacogenomics of Rosuvastatin: A Glocal (Global+Local) African Perspective and Expert Review on a Statin Drug
Nyarai D. Soko¹, Collen Masimirembwa²,³ and Collet Dandara¹

A Landscape of Pharmacogenomic Interactions in Cancer
Francesco Iorio¹,²,⁴,²⁰ Theo A. Knijnenburg³,⁴,²⁰ Daniel J. Vis⁴,²⁰ Graham R. Bignell²,²⁰ Michael P. Menden¹,⁵,²⁰
Statins and PG

Pharmacogenomics of Rosuvastatin: A Glocal (Global+Local) African Perspective and Expert Review on a Statin Drug

Nyarai D. Soko¹, Colleen Masimirembwa²,³ and Collet Dandara¹

6. NEW TARGETS
Immune-driven selection of E138X

- NRTI RAM E138X (A/G/K) occurs naturally in a minority of HIV+ patients \( \leftarrow \) HLA-B*18

Gatanaga H, et al. AIDS 2017
Genetic susceptibility to infections

A

B

C

Evaluation of the association between the concentrations of key vaginal bacteria and the increased risk of HIV acquisition in African women from five cohorts: a nested case-control study


**Summary**

**Background** Disruptions of vaginal microbiota might increase women’s susceptibility to HIV infection. Advances in molecular microbiology have enabled detailed examination of associations between vaginal bacteria and HIV acquisition. Therefore, this study aimed to evaluate the association between the concentrations of specific vaginal bacteria and increased risk of HIV acquisition in African women.

![Graph showing the association between vaginal bacteria and HIV acquisition.](image-url)
Conclusions

• Several genetic variants have been associated with pharmacokinetics or tolerability of ARVs or companion drugs

• The effect size of these associations is often not clear and replication studies seldom performed

• Progress is being made in mining many associations but **prospective studies are needed** in order to implement decisions made on genetic tests into clinical practice

• The large scale up of ARVs’ use in LRCs warrant further efforts in characterizing drugs’ PK, DDIs, tolerability and pharmacogenomics
PUTTING GENOMES TO WORK IN AFRICA

Investment promises to bring precision medicine to Africans. But will it help?

BY LINDA NORDLING
Giovanni DiPerri
Stefano Bonora
Antonio D’Avolio
Jessica Cusato
Amedeo de Nicolò
Giovanna Fatiguso
Cristina Tettoni
Laura Trentini
Ilaria Motta

Stephen Okoboi
Mohammed Lamorde
Barbara Castelnuovo
Sylvia Nabukenya
Christine Sekaggya
Grace Turyasingura