Dried blood spot (DBS) and segmental hair drug level analysis in 2 HIV seroconversions with genotypic resistance to TD/FTC in a PrEP programme

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Background

•A small number of seroconversions in individuals with full adherence to daily PrEP (Pre Exposure Prophylaxis) for HIV prevention have been reported internationally, including those where genotypic resistance has been detected.
• Seroconversions in trials and open-label extensions of on-demand PrEP have been similarly infrequent.
•Seroconversions on PrEP raise understandable concerns regarding the acquisition and potential onward transmission of drug-resistant virus.
•Scotland was the first UK nation to make PrEP available outside a clinical trial in July 2017 through existing routine clinical sexual health services.
•We describe two breakthrough cases in Men who have sex with men (MSM) who seroconverted after PrEP initiation during the first year of the programme in a single clinic.
•Investigation included DBS and segmental hair analysis, which has recently been employed to establish adherence over months prior to sampling.

Case 1:

A 25 year old white MSM eligible for PrEP and with a non-reactive HIV test was dispensed generic TD/FTC on 08/11/17. He delayed PrEP start due to perceived absence of risk and being unwell (significant flu-like illness from 12 to 16/11/2017). Daily dosing began with a single PrEP dose on 06/12/17. He then took daily PrEP until 22/12/2017 when he self-discontinued due to low perceived risk. He took two further short courses (2,1,1) of on-demand PrEP in January 2018. He reported no condomless anal sex without PrEP in the intervening period. On 31/01/18 (Day 0) at routine review he tested HIV antibody positive. Quantitative HIV viral load on Day 2 was 671 copies/ml, HIV subtype C. Antiretroviral therapy (ART) with TDF, Rilpivirine and Dolutegravir was commenced on Day 15. He self-enrolled in the SeroPrEP study; DBS and hair samples were taken on 19/03/18 (Day 47). Genotyping (Day 2) identified the NRTI mutation M184V, conferring resistance to lamivudine and emtricitabine. Retrospective testing of baseline sample from 08/11/17 was negative for HIV by PCR.

The TFV-D results from DBS testing suggested an average dosing of >/= 4 doses/wk over the ~ 8 weeks prior to draw (which included the period of 32 days on ART).
Segmental hair analysis showed that drug levels in the distal hair segment (corresponding to the time of suspected seroconversion) were consistent with 3-4 doses per week of PrEP.

Despite extensive investigation of sexual contacts by both patient and clinicians, no source of HIV infection was identified. No evidence of a risk exposure immediately prior to or just after baseline HIV testing explained a possible seroconversion illness in the month before starting PrEP.

Conclusions

•PrEP breakthroughs are very infrequent but demand immediate and intensive investigation at a time of significant user emotion and potential distress.
•Algorithms that include immediate sampling for evaluation of possible PrEP failure, including adherence metrics are required for PrEP programmes. Local or national arrangements for ethical approval, tissue sampling (hair) and data sharing are required unless testing is available locally.
•PrEP failure can result from inadequate adherence (which can select for NRTI resistance) or transmission of NRTI-resistant virus. It is not possible to definitively exclude either possibility unless other signature mutations (eg to NNRTIs) are present, or the index contact is identified and tested.
•Segmental hair analysis allows assessment of adherence over specific time periods. It can be extremely helpful in determining whether PrEP breakthrough is biological or due to user/system issues.
•Daily or on-demand PrEP appears to be extraordinarily 'forgiving' in terms of variations in dosing timing and frequency. Variations to established dosing regimens are commonplace. However limitations to effectiveness do exist.
•MSM users start, discontinue and restart PrEP according to perceived risk. There is no clear distinction between 'daily' and on-demand PrEP in reality and clear starting and stopping rules would be helpful for guidance.
•The incorporation of PrEP use into decision making and negotiated safety justifies further examination.
•PrEP provision in the UK was driven by and provided to MSM and expert clinicians with extremely high levels of motivation and PrEP literacy. PrEP delivery by less expert users to less expert patients requires additional support and clarity.

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Methods

PrEP was provided in integrated sexual health clinics following national Scottish eligibility criteria and protocols based on the BASHH/BHIVA Statement on PrEP and BASHH/BHIVA PrEP Guidelines 2018. 4th Generation (Architect) serological HIV antigen/antibody testing was done:

- •at baseline
- •at booked three-monthly appointments
- •additionally at 1 month in individuals reporting risk in the 4 weeks prior to PrEP commencement.

A maximum of 90 tablets of PrEP were supplied at each visit. Verbal advice was given on daily and on-demand (following a '2:1:1' IPERGAY regimen) dosing, supported by the patient guide 'PrEP in Scotland', derived from the i-base 'UK Guide to PrEP'. Genotypic resistance testing was performed locally by Sanger sequencing. DBS and hair samples were obtained for TDF/FTC drug level analysis following institutionally approved protocols for the SeroPrEP study. Liquid-chromatography–tandem-mass-spectrometric (LC–MS/MS) analysis in 1-cm scalp hair segments was performed.

Case 2.

A 38 year old MSM attended for advice and monitoring on 08/02/2017 after having purchased PrEP from an online supplier. HIV testing was non-reactive. He commenced 2 weeks of daily dosing reducing to 4 days weekly (Tues, Thursday, Saturday, Sunday: TTSS) for cost reasons. HIV serology was negative at two further reviews. On 15/11/2017 he commenced NHS funded PrEP. He wished to continue a TTSS regime because of an extended period of travel. HIV testing was again negative on 04/04/18; he reported taking 4-5 doses of PrEP weekly. At review on 27/06/18 HIV Ag/Ab testing was positive (Day 0). Quantitative HIV viral load on Day 2 was 10,421 copies/ml, HIV subtype B. On Day 4 he commenced treatment; he continued TD/FTC and commenced Dolutegravir, Darunavir and Ritonavir . SeroPrEP hair and DBS samples were obtained 26/07/18 (Day 29). Genotyping (Day 2) showed NRTI mutation K65R conferring resistance to Tenofovir.

DBS testing showed an average dosing of >/= 4 doses/wk over ~ 8 weeks prior to draw (which included the period of 25 days on ART). Segmental hair analysis showed relatively good adherence over the month prior to the hair cutting (normalized TFV concentration 0.0424 ng/mg in the proximal 1cm), but his adherence was lower prior to that. Between months 1 and 2 prior to hair cutting, adherence of about 6 doses a week. Between 5 and 2 months prior to the hair cutting, adherence was lower: 3-4 doses a week.

Approximate time prior to sampling	Sample distance from proximal	TFV concentration (ng/mg)	Normalized TFV concentration (ng/mg)	Approximate indicated TD/FTC dosing
1 month (July)	1cm	0.0406	0.0424	6 per week
2 months (June)	2cm	0.0381	0.0383	6 per week
3 months (May)	3cm	0.0300	0.0301	3-4 per week
4 months (April)	4cm	0.0303	0.0299	3-4 per week
5 months (March)	5cm	0.0329	0.0340	3-4 per week

Adherence was discussed and documented at appointments both prior to and following HIV diagnosis in detail and was confirmed as not lower than 4 doses per week. Contact history included anonymous contacts and the source of infection could not be ascertained. He had experienced no seroconversion symptoms.

Discussion

•Resistance is likely to have developed either as a result of acquisition of drug-resistant virus, or as a result of resistance evolution under selective drug pressure.
•'Daily' PrEP with lower adherence (4 doses a week) or intermittent dosing probably requires timing of PrEP doses with sexual risk exposure by incorporation into a complex existing network of heuristics and decisions about condomless anal sex.
•In the absence of a confirmed source of HIV infection in either case, it is highly likely, but not certain, that the viruses from both individuals evolved antiretroviral resistance due to continued PrEP dosing following unrecognised seroconversion.
•The K65R mutation seen in Case 2 is rarely seen at baseline and is not thought to be easily transmitted.
•In Case 1, M184V but not K65R was identified, although the latter appears to be more likely to develop in Subtype C infection.
•Drug levels added information, but DBS were obtained after ART initiation in both cases. It is not possible to conclude (in case 2) whether dosing was maintained at 4 doses per week, or dropped below at times of risk, but it provides a useful reminder that a regimen such as TTSS allows reduced forgiveness for further missed doses.
•Delays in sampling for drug level testing must be avoided in future to maximise the information to be gained from both DBS and hair samples.

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SeroPrEP study: www.how2offerprep.org/sero-prep