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Phylogenetic inferences on HIV-1 transmission: implications for the design of prevention and treatment interventions

Bluma Brenner[a], **Mark A. Wainberg**[a], and **Michel Roger**[b]

[a]Lady Davis Research Institute and the McGill AIDS Centre, Montreal, Quebec, Canada, Montreal, Quebec, Canada

[b]Département de Microbiologie et d'Immunologie et Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada

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Introduction

In 30 years, the HIV-1/AIDS pandemic has evolved into an increasingly complex disease composed of multiple epidemics, each influenced by a complex array of biological, behavioural and cultural factors [1–4]. The concentrated subtype B epidemics in Western world settings have been largely restricted to MSM and IDU populations [3]. The generalized heterosexual (HET) epidemics in Africa and Asia have expanded and diversified to include nine major HIV-1 subtypes (A–D, F–H, Jand K) and mosaic circulating recombinant forms (e.g. CRF01_AE and CRF02_AG) [1,5,6]. Migration and globalization has contributed to the spread of non-B subtypes contributing to 20–60% of new infections in Europe, Asia and America [1,2,7].

Highly active antiretroviral therapy (HAART) remains the key to the management of epidemics, reducing mortality, opportunistic diseases and HIV-1 transmission [8–17]. Global health initiatives to scale up antiretroviral therapy (ART) in Africa and Asia have led to population-level reductions in HIV-1 transmission despite weak healthcare systems [8,10,18,19]. The HIV Prevention Trials Network (HPTN) 052, CAPRISA 004, Preexposure Prophylaxis Initiative (iPREX) and Botswana Preprophylaxis Trial (TDF2) randomized clinical trials have shown the potential benefit of early ART initiation, microbicides and prexposure prophylaxis (PrEP) in averting HIV-1 transmission [10,13–16,20,21]. This has led to revisions in treatment guidelines recommending early initiation of ART to all HIV-infected persons and PrEP for high-risk HIV-seronegative populations [15,16]. The failure of the Female Preprophylaxis Trial (FEM-PrEP) underscores the importance of ART adherence and retention [13,14,21,22]. The resurgence of MSM epidemics in the post-HAARTera emphasizes the importance of linking ART with other prevention control modalities [23,24].

The development, implementation and maintenance of effective prevention and treatment interventions will require a thorough understanding of the driving forces of individual epidemics [25,26]. Phylogenetics is a molecular epidemiological strategy that characterizes

Correspondence to Dr Bluma Brenner, Lady Davis Institute, 3755 Cote Ste. Catherine Road, Montreal, QC H3T 1E2, Canada. Tel: +514 340 8260; fax: +514 340 7537; bluma.brenner@mcgill.ca.

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epidemics on the basis of the genetic interrelatedness of HIV-1 viral sequences, capturing the underlying structure of transmission networks or 'clusters' within a given population [27–32]. Molecular phylogenetic analysis can be combined with epidemiological, demographical and behavioural data to describe the spatial, temporal and biological dynamics of individual epidemics [7,27–34]. Phylogenetics provides an important dimension in HIV-1 surveillance by delineating:

1. The introduction and dissemination of HIV-1 viral subtypes in different regional settings;
2. The range of transmission patterns fuelling HET, MSM and IDU epidemics;
3. The interrelationships of biological, demographical and social determinants on viral 'cluster' networks;
4. The role of disease stage in transmission dynamics; and
5. Underlying trends in regional epidemics, important in the selection of control interventions to limit HIV transmission [26,35,36].

Biological basis of phylogenetic 'clustering'

High genetic diversity within and across HIV-1 viruses and subtypes can be ascribed to rapid rates of viral replication and turnover with an error-prone reverse transcriptase having high recombination potential [37–41]. Viral evolution within and across HIV-1 subtypes occurs under host, immunological and antiretroviral drug selection pressures, contributing to up to 40, 20 and 10% variations in HIV-1 envelope (*env*), *gag and pol* domains, respectively [37,41,42]. Open access websites, such as the Los Alamos (http://www.hiv-lanl.gov), Stanford (http://hivdb.stan-ford.edu) and REGAdb (http://jose.med.kuleuven.be/subtypetool/html/) databases, have sequence datasets and computer tools for classification of viral subtypes, immune epitopes and antiretroviral drug resistance pathways [5,7,43].

Antithetically, despite the amazing potential of HIV to diversify, surprisingly few viral variants and subtypes have contributed to individual infections and regional epidemics [27,44,45]. Severe bottlenecks in HIV transmission lead to a single transmitted/founder (T/F) virus seeding and dominating the virus population (quasispe-cies) [46–54]. Single-genome amplification and next-generation sequencing technologies have shown that 80% of MSM and HET infections may be attributed to a single virus [48,49,55–59]. An understanding of the genotypic and phenotypic signatures of these ancestral strains will be important in understanding critical events contributing to their preferential establishment of infection and evolution in the earliest stages of infection [50,52,56,60–62].

The critical determinant of HIV-1 transmission is viral burden in blood, genital fluid and semen [46,63,64]. Acute/primary stage infection has been postulated to disproportionately contribute to onward transmission related in part to high viraemia (>4.5 log copies/ml) and homogeneity of T/F variants [11,46,57,65,66]. ART limits transmission of chronic stage infection by reducing viral load at an individual and population level [11]. Geospatial bottlenecks add a further constraint, limiting the range of viral subtypes in different risk populations and demographic groups [67–71].

Taken together, high genetic diversity coupled with stringent transmission bottlenecks contribute a single ancestral species seeding infections. Phylogenetic 'clustering' elucidates transmission dynamics of individual epidemics and the geospatial expansion of viral subtypes in different regional settings.

Geospatial expansion of HIV-1 subtypes

Molecular phylogeny approaches were first used to classify HIV-1 subtypes and circulating recombinant forms (CRFs) [1,2,5,6,72]. In Western world settings, subtype B epidemics have predominated; in Africa, non-B subtypes have expanded and diversified. Phylogenetic analysis can follow the introduction and spread of specific subtypes and CRFs into local MSM, IDU and HET epidemics [2,71,73–80].

The subtype diversity of the global pandemic is reflected in the distribution of non-B subtypes in Quebec originating from francophone countries in West and Central Africa (Fig. 1) [1,2,6]. Subtype C infections account for half of infections worldwide, whereas subtypes A (12%), CRF01_AE (5%), CRF02_AG (8%) and G (5%) collectively contribute to 30% of the global pandemic [1,2,6]. The remainder of the non-B pandemic is composed of subtype D (Uganda), subtype F (Angola, Romania and South America) and CRFs [1,6]. Subtypes B MSM epidemics dominate in Europe and America, accounting for 9% of global infections. The intermixing of viral subtypes leads to the expansion of novel recombinant mosaics in different regions.

The fastest growing epidemics worldwide are the IDU epidemics in Eastern Europe where subtypes A1 and CRF03_AB are most prevalent [81]. In heavily populated regions, including India, China and Southeast Asia, epidemics have rapidly shifted from predominant IDU to HET epidemics with selective expansion of subtype C, CRF07_BC, CRF08_BC and CRF01_AE subtypes [82,83].

Non-B subtype epidemics comprise 20–60% of new infections in Europe, Asia and America [71,73–78,84]. Crossover of non-B subtype epidemics into domestic MSM, IDU and/or HETepidemics has been observed in the United Kingdom, France, Germany, Spain, Israel, Greece, the Balkans and Asia [74,84–90]. Domestic spread of non-B subtype infections was rarer in Holland, Switzerland and Quebec [91,92]. As illustrated in Fig. 2, secondary spread of non-B subtypes into Quebec has been limited to one founderCRF_AB(n=33)MSM clusterand one subtype D (n=9) HET cluster. This highlights the importance of providing healthcare to migrant populations.

Transmission dynamics of MSM epidemics

Phylogenetic analysis has provided a framework for an in-depth evaluation of transmission dynamics of MSM epidemics. Drug-resistance programmes, introduced in the 2000s, have provided large *pol* (RT/protease) sequence datasets for analysis of transmission trends of regional epidemics [27–29,31,90,93–96]. Molecular phylogeny approaches using neighbour-joining, maximum-likelihood and/or Bayesian (BEAST) methodologies have retraced transmission ‘clusters’ or networks that could not be otherwise identified [28,32].

Systematic surveillance has enhanced our understanding of MSM transmission dynamics by highlighting the role of primary/recent stage infection in onward spread of regional epidemics (Table 1) [27,29,90,93,94,97–101]. The temporal spread of clustered transmissions has been evaluated on the basis of chronological and stage of infection time scales.

Comparative analysis of MSM transmission dynamics has been confounded by the use of different selection criteria and methodology. Molecular phylogeny studies have been performed on different groups: acute/primary HIV infection (PHI), PHI (<6 months), recent infection (<12–18 months) and combined PHI and chronic cohorts. Statistical criteria have varied combining high bootstrap values (>95–98%) and low genetic distance (<0.015–0.045) for the designation of ‘clusters’ [28,29,93,101]. Correction for drug resistance is

necessary when using chronic treatment experienced populations. Robust criteria delineate 'transmission clusters' with internal controls for repeat sampling and include known source-index partnerships [28,29,93,101]. Clustering can be cross-validated by analysis of sequence congruency in natural polymorphisms and reproduction of *pol* gene clustering in *gag*, *env* and *integrase* domains [28,29,93,101].

The expansion of the MSM epidemic in Montreal evaluated clustering of primary infections (PHI < 6 months) over the last decade. Three patterns of onward spread of MSM epidemics were observed: unique transmissions, small clusters (2–4 PHI) and large clusters (5–60 PHI) (Fig. 2a, b) [27,29]. Large clusters were the driving force sustaining the MSM epidemic, increasing from 25% of primary infections in 2005 to 51% of infections by the end of 2011. The high rates of PHI-related clustering may be related to suppressed viraemia at a population level wherein 85% of diagnosed MSM are receiving HAART. Episodic expansion of large clusters (7–60, median 14 PHI) has arisen through the stepwise formation of new clusters unrelated to prior clusters, as well as secondary outbreaks of older clusters (Fig. 3) [27,29]. Whereas small clusters expanded over 4.75-month intervals (1–11.5 interquartiles), large clusters expanded over 11-month intervals (3.5–25.5 interquar-tiles).

Phylodynamic features of other regional MSM epidemics were consistent with that observed in Montreal (Table 1) [27,29,31,93,94,102–104]. Cluster membership (17– 70%) and size of clusters showed regional variation based, in part, on the depth of population sampling and incidence rates (Table 1) [27,31,93,101,102,105–110]. Transmission clustering was highest in concentrated urban settings and lowest in diffuse nationwide epidemics.

Time-resolved phylogenies on date-stamped sequences in the United Kingdom, Netherlands, France and Montreal cohorts indicated that 25–30% of clustering arose over 6-month time spans with median cluster intervals occurring over 14–17 month time spans (Table 1) [27,29,31,93,94,102]. Phylogenetic clustering in other studies was also related to acute/PHI/recent infection status and high CD4 cell count [29,101,103,111].

Temporal dynamics of MSM transmissions

Phylogenetic inferences project a role of primary infection (<6 months) and recent stage infection (<14–18 months) in sustaining MSM epidemics (Table 1, Fig. 4). This time span goes well beyond empirical and mathematical models that emphasize a disproportionate contribution role of the 3-week infectious period of 'acute' infection in onward transmission [46,112,113]. Newly infected persons in primary and recent stage infection may harbour homogeneous pools of ancestral founder viruses that may have a higher transmissible 'dose' of virus for an extended period of time. These early disease stages are distinct from chronically infected source partners that harbour a vast array of quasispecies, including variants having impaired replicative competence and/or fitness [54,114].

Accurate dating remains a serious caveat in time-resolved phylogenies and modelling of transmission dynamics. Acute infection is rarely detected in real time, as newly infected persons are often symptom-free and unaware of their status [115,116]. Those unaware of their status frequently engage in higher risk behaviour associated with a 3.5-fold increased risk for HIV transmission [117–120].

Control interventions to limit HIV transmission will be predicated on early diagnosis [46,113,121–126]. Serological incidence and p24 assays remain imprecise measures of recency of infection and are limited to those persons recruited into PHI cohorts [68,127–131]. Viral sequence-based assays may assist in estimating recency of infection by monitoring time-dependent evolution from a 'clonal' founder event [128,129,132,133]. The frequency of ambiguous calls in bulk sequencing can serve as a surrogate marker that

distinguishes recent infection (<0.44% ambiguity in first year) from chronic infection (predictive value 98.7%) [129,133]. Next generation sequencing and high-resolution melting assays may be applied in dating archived specimens [128,134,135].

There remain caveats in Bayesian analysis, time-resolved phylogenies and sequence diversity assays. Issues pertinent to phylogenetic metrics include sequence quality; interval between seroconversion and first genotyping, especially for chronic populations; and effects of ART on quasispecies dynamics. ART suppresses viral replication and quasispecies evolution; treatment failure leads to limited expansion of drug-resistant variants; treatment cessation leads to rebound of previously transmitted ancestral variants [114,136].

Universal genotyping at presentation will become increasingly more important, particularly with the introduction of test–treat paradigms. Genotyping is relatively inexpensive (~$125, homebrew assays), the frequency of transmitted drug resistance is high (10–20%) and subtype diversity is common.

Transmission patterns of IDU and heterosexual epidemics

Despite the overall decline in IDU epidemics associated with harm reduction and needle-exchange programmes and antiviral therapy ART, IDU epidemics continue to emerge in North America, Eastern Europe and Asia [85,110,137–141]. Transmission clustering has been implicated in the spread of IDU epidemics, including the spread of drug-resistant subepidemics (Table 1) [110,142–144]. IDU epidemics may bridge the crossover of HET and MSM epidemics, as well as contribute to the introduction and spread of non-B subtype infections and HIV/hepatitis C coinfections [110,137,145–148].

Phylogenetic clustering has been frequently observed in IDU epidemics [103,110,146,149,150] (Fig. 2c). Single-genome analysis has shown that 40–60% of IDU transmissions are associated with a single T/F variant, with remaining infections harbouring three to 16 closely related monophyletic strains [58]. Higher multiplicity of infection among IDU populations may be associated with an absence of the mucosal barriers, prison settings and poor general or mental health [58,149,151].

There is a paucity of data on the phylodynamics of HET non-B subtype epidemics, particularly in endemic Third World settings (Table 1) [57,152,153]. Single T/F viruses were observed in 68% of subtype C primary infections from Botswana, similar to that observed for MSM cohorts [153]. The overall membership size of transmission clusters (two to four infections/cluster) among HET cohorts has been far lower than that reported for MSM populations (Table 1) [42,154]. The role of early stage infection may also be less pronounced in HET partnerships (Table 1) [155,156]. Domestic spread of subtype A and C HET infections (cluster size >10) in the United Kingdom occurred over cluster intervals of 27 months, with only 2% of transmissions occurring over 6-month periods [90].

Individuals with extended high viraemia have been observed to disproportionately contribute to expansion of the subtype C epidemic in Botswana [9,154]. A substantial fraction (34%) of newly infected persons maintain extended high viraemia (>100 000 copies/ml) for median periods of 318 (282–459) days [57]. This suggests that treatment as prevention (TasP) initiatives may be selectively targeted to those persons showing extended viraemia [9,154].

Phylogenetics were important in randomized control prevention trials, showing that 10–30% of HIV-1 transmissions among serodiscordant partnerships were unlinked and likely involved third partners [157–160]. Analysis of virus transmitted in serodiscordent

partnerships in the Rakai cohort showed that there was a preferential transmission of the ancestral founder strain from the source partner's quasispecies [114].

Transmitted drug resistance

Genotypic drug resistance testing programmes have been instrumental in identifying mutations conferring resistance to nucleoside and nonnucleoside reverse transcriptase (NRTIs and NNRTIs), protease inhibitors and integrase inhibitors (INIs) [5]. Transmitted drug resistance (TDR) remains a serious public health concern, representing 10–20% of new infections among treatment-naive populations in different regional settings [161–164].

TDR is particularly noteworthy for NNRTIs wherein single point mutations (K103N, G190A, Y181C and E138K) confer high level resistance with a little impact on viral replicative fitness [5,162,163,165]. There is innate resistance to NNRTIs in subtype O and HIV-2, as well as a signature V106M pathway for subtype C [5,166,167]. The use of nevirapine to prevent vertical transmission may lead to NNRTI resistance in infants [168,169]. Another concern is the facilitated development of K65R in subtype C, in association with stavudine (d4T), didanosine (ddI) and/or nevirapine (NVP)-based regimens [170,171]. TDR may emerge more rapidly in Third World settings due to drugs having poor pharmacodynamic properties (d4T and ddI) [5].

High rates of TDR among drug-naive MSM and IDU populations have been related to clustering [5,162,163,172,173]. TDR to NNRTIs (17%) in Montreal can be ascribed to six MSM clusters harbouring K103N, V108I or G190A (n=9–60) (Figs. 1a and 2) [162]. Prospective monitoring of the expansion of drug-resistant subepidemics may help guide local treatment policies. Other frequently transmitted species include the L90M protease mutation and thymidine analogue mutations (e.g. M41L, D67N, T215 revertants and K219Q) [109,162,163,174–177].

Phylogenetic insights on future possibilities in HIV prevention

The HPTN 052 and PreP randomized control trials have advanced the vision that TasP paradigms may dramatically reduce HIV incidence and prevalence at a population level [10,13–16,20,21]. The development of prevention interventions will require metrics that characterize the drivers of individual epidemics [86,91]. Realistic models on how control interventions will impact on local networks will require a comprehensive understanding of transmission patterns of MSM, IDU and HETepidemics in different regional settings [27–29,31,90,93–96].

The cornerstone to HIV prevention is timely diagnosis of HIV in high-risk populations [15,16]. Acute infection is rarely detected in real-time, and half of newly diagnosed individuals are ‘late presenters’ (>1 year postseroconversion) [178–180]. Phylogenetic inferences project a median duration of 6 and 11–15 months of small and large clusters, respectively (Table 1, Fig. 4) [27,29,90,93,94,97–100]. This period substantiated a plausible benefit of early ART initiation to avert HIV clustering [46]. Seek–test–counsel paradigms are needed to address issues of poor testing habits and late presentation (Fig. 4).

The efficacy of ART requires adherence and retention, as viral rebound will occur on treatment cessation that may contribute to episodic bursts in transmission [136]. The observation of secondary outbreaks in the Montreal and Swiss epidemics has been linked to time frames when treatment interruption trials (e.g. SMART) were in place (Fig. 4) [136]. Individuals receiving PreP and early ART must be counselled on adherence and treatment continuity.

There have been few studies characterizing endemic non-B HETepidemics. In contrast to that observed for MSM and IDU epidemics, studies on HET populations show infrequent clustering and low cluster size. This would suggest that TasP would not have as dramatic an impact on reducing transmission. The Botswana study suggests a potential benefit of TasP for extended high viraemics [57].

It is important to recognize that phylogenetics define viral networks that are not necessarily synonymous to sex networks and individual-level risk. There is mixing of high and low-risk populations having high and low awareness of status [112,181]. Data from the Montreal SPOT rapid testing site show that although seropositivity was related to casual partnerships, cluster membership and size of cluster was related to poor testing habits. It has been estimated that 7–20% of MSM infections may be acquired abroad, contributing in part to unique transmissions [24,182].

Testing, treatment and other prevention interventions require major public health commitments. The integration of phylogenetic, epidemiological clinical and demographic data will be important in delineating the role of linkage to care, behaviour, socioeconomic factors and migration on transmission dynamics [96]. Universal genotyping of all newly infected persons could provide critical information needed to reconstruct underlying structures of local epidemics necessary for the design and evaluation of control interventions. Baseline resistance profiles could assess transmitted resistance and guide HIV-1 management. Molecular phylogeny strategies could help elucidate the role of disease stage and virus-specific determinants in transmission dynamics [128,129,132,133]. This analysis is particularly important for the design of cost-effective treatment and prevention combinations in resource-poor settings.

Whereas early stage infection may dominate in regional settings having universal access to healthcare and ART coverage, significant contributions of chronic stage infections may be related to socioeconomic factors, including lower awareness of status and poor linkage to care and treatment [115,116,183–186]. Phylodemo-graphics can be of importance in surveillance of the rise MSM epidemics among young adults (13–29 years) and racial/ethic minorities [109,184,187]. Phylogenetic inferences of local epidemics may assist in the design of targeted educational campaigns and prevention policies for distinct HIV-infected populations.

Future research may broaden our knowledge of underlying mechanisms leading to the preferential selection and expansion of transmitted ancestral strains. Phylodynamic inferences will be important in the design, implementation and assessment of candidate public health, therapeutic and behavioural interventions and the ultimate pursuit of HIV vaccines.

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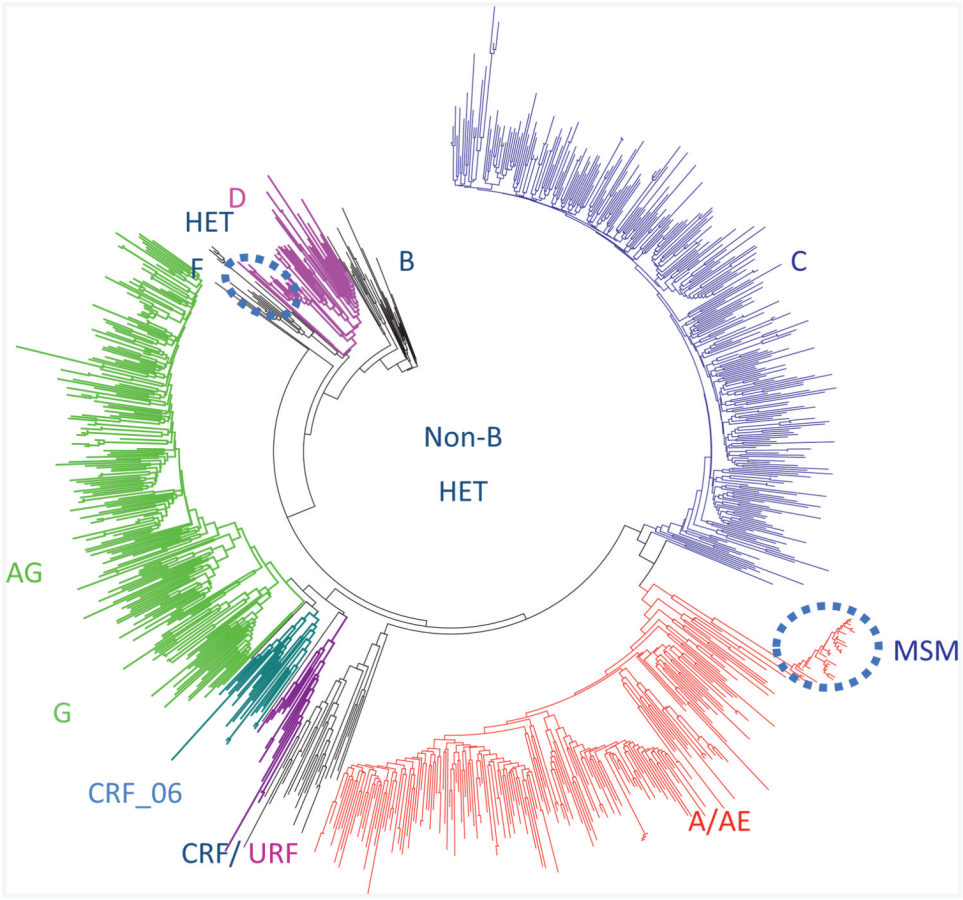

Fig. 1. Phylogenetic analysis of the Quebec non-B subtype epidemic (n=858) originating from West and Central Africa

Clustering (27%) was limited to conjugal families (*n*=2–4infections/cluster). Domestic spread of a subtype D (*n*=13) heterosexual cluster and a CRF_AB (n=30) MSM transmission clusters have been circled.

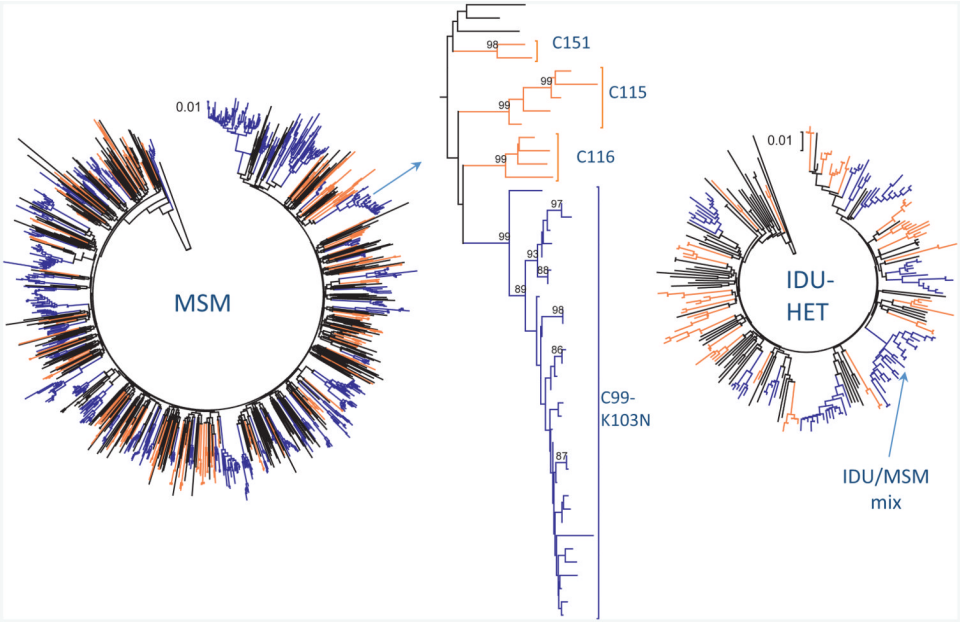

Fig. 2. Phylogenetic clustering of MSM and IDU epidemics in Montreal

(a) Phylogenetic tree of MSM infections (*n*=1359) with large clusters [6–30primary HIV infection (PHI)/cluster, *n*=553] and small clusters (2–4 PHI, *n*=272) denoted in blue and orange, respectively. (b) Section of MSM tree (arrowed) showing three unique transmissions, three small clusters and one large cluster harbouring the K103N resistance mutations. (c) Phylogenetic tree of IDU infections (*n*=244) with small (*n*=90) and large (*n*=77) clusters denoted in blue and orange, respectively. One mixed cluster (*n*=17) is arrowed.

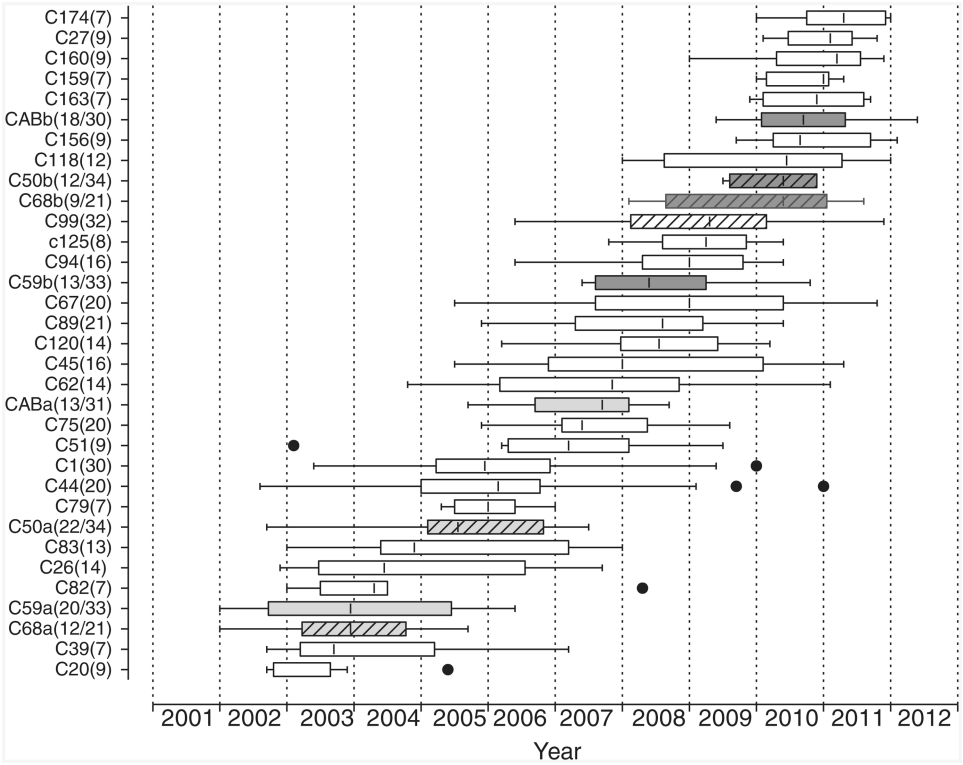

Fig. 3. Temporal expansion of 29 large MSM clusters (7+ primary infections) over the last decade

Box and whiskers plots represent the episodic transmission intervals of each cluster, with Tukey whiskers showing 1.5 time interquartile intervals and severe outliers (●). Four transmission clusters showed biphasic expansion with initial (light grey) and secondary outbreaks (dark grey). Clusters harbouring resistance to nonnucleoside reverse transcriptase inhibitors have been dashed. The sizes of each transmission cluster are denoted in parenthesis. Clusters 68, 50 and 99 harbouring transmitted resistance to NNRTIs are dashed.

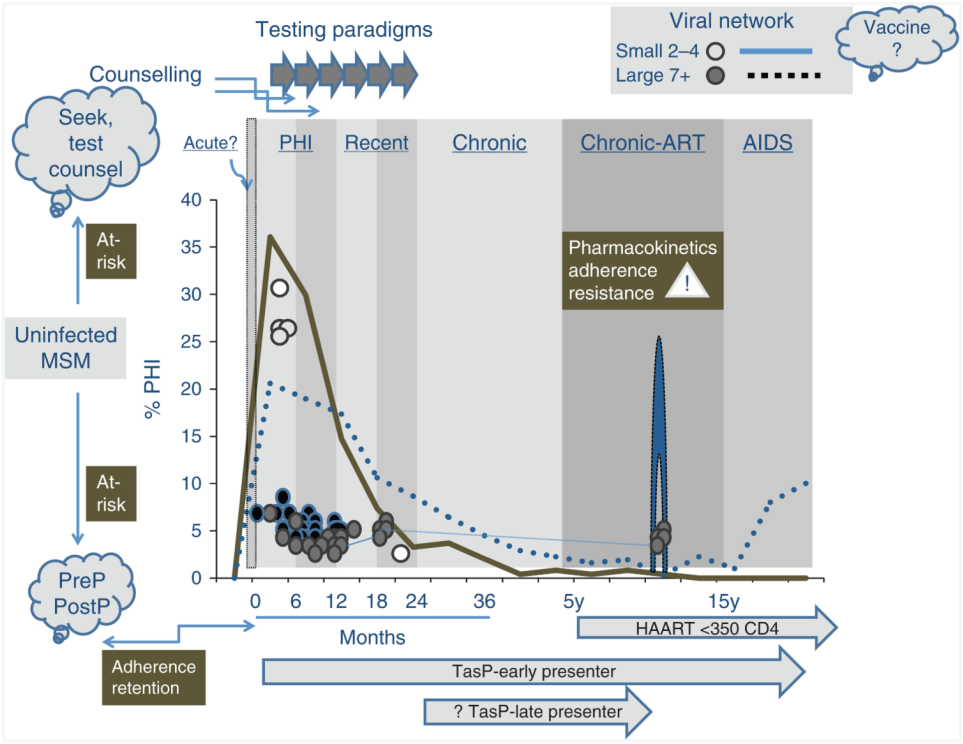

Fig. 4. Phylogenetic insights on future possibilities in HIV prevention

Temporal expansion of small (2–4 PHI) and large (5–60) clusters are denoted by solid and dashed lines, respectively. Testing must be frequent to capture individuals in a timely fashion for ‘Treatment for as Prevention’ (TasP) paradigms. Antiretroviral drug (ART) pharmacokinetics and patient adherence to ART is necessary to prevent viral bound and chronic stage transmissions.

Table 1

Comparison of the phylodynamic patterns of MSM, IDU and non-B heterosexual epidemics

			Frequency of infections in cluster groups (% cases)			Cluster interval	
Risk group	**Location**	**Inclusion criteria**	**Unique**	**Small 2–10**	**Large +10**	**Median (months)**	**<6 months (% cases)**
MSM	Montreal	Acute/PHI[a]	30	42	28	15	27
MSM	Amsterdam	Recent < 18 months	43	57	0	17	25
MSM	UK/London	PHI/Chronic	60	28	12	22/14	16/25
MSM	France	PHI (Cohort)	83	17	0	15	30
MSM	Denmark	PHI/Chronic	51	18	31		
MSM	Belgium	Recent <1 year	53	28	19		
MSM	Switzerland	Acute/PHI/Recent	64	0	36		
Mixed	North Carolina	PHI/Chronic	66	29	4		
IDU/HET	Switzerland	Acute/PHI/Recent	41	0	63		
IDU/HET	Montreal	Acute/PHI/Recent	30	45	25		
HET	Denmark	PHI/Chronic	85	9	6		
Non-B	Montreal	PHI/Chronic	74	24	2		
Non-B	UK	PHI/Chronic	75	20	5		
Non-B	Switzerland	Acute/PHI/Recent	78	22	0		
Non-B	Belgium	Recent (<1 year)	87	12	0		

Data were extracted from studies performed in Quebec [27,29], Netherlands [94,182], United Kingdom [31,90,93], France [105], Denmark [101], Belgium [111], Switzerland [86,103] and North Carolina [106] to elaborate the relative role of nonclustered (unique), small clustered (2–10) and large clustered (10+) networks in different settings. The median cluster intervals and the percentage of linked infections occurring within 6-month intervals substantiate the relative role of early infection. HET, heterosexual; PHI, primary HIV infection.

[a]The Montreal study included drug-naive and treated chronic populations to establish cluster size but excluded these samples from analysis of cluster intervals.