2017

REPORT

Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2016

RAVN
Resistensovervåking av virus i Norge
Resistance against Antivirals in Norway

Norwegian Institute of Public Health

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folkehelseinstituttet

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Introduction

It is a pleasure to present the fourth report from the surveillance system Resistance against Antivirals in Norway (RAVN). Continuous surveillance is conducted in order to detect any emergence or change in antiviral drug resistance. Such information is essential to develop optimal treatment regimens and for implementation of preventive measures.

This report presents new data for 2016 on resistance against agents for the treatment of influenza, HIV-1 infection, hepatitis B virus (HBV) infection and human herpes virus infections. The surveys have been conducted by the Norwegian Institute of Public Health and the Oslo University Hospital. In addition to surveillance data, we have focused on some relevant topics in the field: Prophylactic treatment of HIV-infection (PrEP and PEP), a historical summary of treatment of HIV-infection and next-generation sequencing of hepatitis C virus. In this report Dr. Nina Weis, Amager-Hvidovre Hospital, Denmark, is an invited author on hepatitis B and gives a review on available therapy, recommendations of resistance testing and novel therapy strategies.

It is our hope that the report contains valuable data for clinicians, microbiologists and those developing treatment guidelines and strategies to prevent transmission of viral infection.

RAVN would like to thank those who contributed with data and writing this report, for excellent work.

Oslo, september 2017

Contributors and participants

Editor:

Anita Kanestrøm	RAVN, Norwegian Institute of Public Health (NIPH)

Authors:

Anita Kanestrøm	Summary	NIPH
Irene Litleskare	Usage of antivirals	NIPH
Karoline Bragstad	Influenza virus	NIPH
Bente Bergersen	HIV	Oslo University Hospital, Ullevål
Anne Marte Bakken Kran	HIV	Oslo University Hospital, Ullevål
Birgitta Åsjö	HIV	Haukeland University Hospital, Bergen
Anita Kanestrøm	HIV	NIPH
Nina Weis	Hepatitis B virus	Amager-Hvidovre Hospital, Denmark
Kathrine Stene-Johansen	Hepatitis B virus	NIPH
John Pettersson	Hepatitis C virus	NIPH
Halvor Rollag	Human herpes viruses	Oslo University Hospital, Rikshospitalet

Contributors:

RAVN council

Institutions submitting surveillance data to RAVN:

NIPH, Department of Virology, Regine Barlinn / Karoline Bragstad / Olav Hungnes / Kathrine Stene- Johansen.
The Norwegian Prescription database (NorPD), NIPH, Irene Litleskare
Oslo University Hospital, Rikshospitalet, Halvor Rollag
Oslo University Hospital, Ullevål, Anne Marte Bakken Kran, Mona Holberg-Petersen

RAVN council 2015:

Anne Marte Bakken Kran (chairman)	Oslo University Hospital, Rikshospitalet, Oslo
Bente Bergersen	Oslo University Hospital, Ullevål
Kathrine Stene-Johansen	Norwegian Institute of Public Health, Oslo
Andreas Christensen	St. Olav University Hospital, Trondheim
Tore Jarl Gutteberg	University Hospital of North Norway, Tromsø
Heidi Syre	Stavanger University Hospital
Susanne Dudman	Norwegian Institute of Public Health, Oslo
Birgitta Åsjø	Haukeland University Hospital, Bergen

Content

Sammendrag

Bruk av antivirale midler

I løpet av siste to tiår har det kommet en rekke nye antiviralia på markedet. Salget av antivirale medikamenter har økt årlig målt i definerte døgndoser (DDDs). Introduksjon av nye antiviralia for behandling av HCV-infeksjon har bidratt sterkt til økte kostnader for antiviralia til systemisk bruk (ATC gruppe J) de senere årene. Prisreduksjon på noen av anti-HCV medikamentene i 2016 har resultert i nesten uendret kostnader for antiviralia i 2016 sammenlignet med 2015, selv om forbruket har økt.

Influensavirus

2016/17 sesongen ble dominert av influensa A H3N2 virus, mens influensa B virus overtok fra uke 13. Rundt 12% av alle H3N2 positive ved FHI, 44% av H1 virus og 7% av influensa B virus, ble undersøkt for antiviral resistens. Ingen av prøvene viste redusert følsomhet for verken oseltamivir eller zanamivir. Screening for antiviral resistens er viktig for å hindre nye utbrudd av resistent virus. Virus med antiviral resistens kan vise rask spredning. Utvikling av alternative antivirale medikamenter er viktig for god beredskap mot influensa.

Hiv-1

SDRMs (Surveillance drug-resistance mutations) ble påvist i 5.2% av prøvene fra pasienter med nydiagnostisert hiv-1 infeksjon i Norge i 2016. Prevalensen av overført resistens har vært stabil de siste årene med bare små variasjoner. Introduksjon av profylaktisk behandling (PrEP og PEP) kan føre til endring i resistensforekomst og er et av hovedtema i årets rapport. Overvåking av hiv-resistens over tid er viktig for å gi grunnlag for å iverksette forebyggende tiltak for å hindre spredning av resistent virus samt for å kunne gi gode nasjonale behandlingsanbefalinger. Hiv-behandling i et historisk perspektiv er beskrevet i eget kapittel.

Hepatitt B virus

Med bruk av antivirale medikamenter med høy barriere mot resistens som entecavir og tenofovir, er utvikling av resistens under behandling et relativt lite problem. Dette viser også overvåkingsdata for HBV i Norge over tid. Dagens behandling med antivirale medikamenter foregår over år eller livet ut. Resistens oppdages ved å monitorere HBV DNA og serum ALT. Etterlevelse av medisinering bør alltid vurderes i tilfeller med stigning i HBV DNA. Behandling med nukleosidanaloger induserer sjelden HBsAg serokonversjon eller vedvarende HBsAg eliminasjon, men det er håp om at nye medikamenter under utvikling vil kunne gi helbredelse av kronisk HBV-infeksjon.

Humane herpesvirus

Ganciclovir og aciclovir er førstevalg ved behandling av henholdsvis cytomegalovirus- og herpes simplex virus infeksjoner. Til tross for utstrakt bruk av begge medikamenter både til terapi og profylakse er forekomsten av resistent virus minimal. Åtte av 43 CMV-prøver innsendt for resistensbestemmelse ble funnet å være ganciclovir-resistente. En pasient var infisert med CMV resistent mot ganciclovir, cidofovir og foscavir. Av 17 prøver innsendt

for påvisning av aciclovir-resistent herpes simplex virus ble det funnet aciclovir resistensmutasjoner i 3 prøver, en herpes simplex virus 1 og to herpes simplex virus 2.

Hepatitt C virus

Resistensbestemmelse av hepatitt C virus er utfordrende på grunn av stor genetisk variasjon og utvikling av antivirale medikamenter rettet mot nye målområder. Neste generasjons sekvensering (NGS) er nå blitt tilgjengelig teknologi for partiell- eller fullgenomsekvensering av virus og vil kunne løse en del av utfordringene man har med amplifikasjonsbasert deteksjon av HCV-genomet. Denne teknologien gir mulighet for påvisning av resistensmutasjoner for alle (sub)genotyper. En såkalt NGS "target enrichment" metode er under utvikling ved Folkehelseinstituttet.

Summary

The usage of antivirals

During the last two decades, the development of new specific antivirals has been accelerated. The sale of antiviral drugs has been increasing every year measured in defined daily doses (DDDs). Introductions of new antivirals for treatment of HCV infections has highly influenced the increased costs in this group the last years, but price reduction for some of these drugs has resulted in virtually the same costs of the antivirals in 2016 compared to 2015 even if the sales measured in DDD and number of users have increased.

Influenza virus

The 2016/17 season was dominated by influenza A H3N2 viruses with influenza B taking over from week 13. Approximately 12 % of all H3N2 positives at the NIPH were analysed for antiviral resistance, 44% of the H1 viruses and 7% of influenza B viruses. None of the viruses had reduced susceptibility towards either oseltamivir or zanamivir. Timely screening for antiviral resistance is important in preventing new outbreaks of resistant viruses. Viruses with antiviral resistance might spread rapidly. Development of alternative antivirals is urgently needed and policymakers should consider their stockpiles of antiviral drugs.

HIV-1

Surveillance for drug-resistance mutations resulted in 5.2% positive samples from patients with newly diagnosed HIV-1 infection in Norway in 2016. The prevalence of transmitted drug resistance has been stable for the last years with only minor variation. The introduction of prophylactic treatment (PrEP and PEP) could challenge this situation. The key question is how PrEP will influence on the incidence of drug resistance. Surveillance of HIV resistance over time is important in order to make decisions on implementing preventive measures to control dissemination of resistant HIV strains.

Hepatitis B virus

With the use of nucleos(t)ide analogues (NA) therapy with high resistance barriers, like entecavir and tenofovir, development of resistance during HBV therapy is a minor problem. Resistance should be identified by HBV DNA and serum ALT monitoring over time in patients on antiviral treatment, and the patient´s compliance taken into consideration in case of a rise in HBV DNA. At present, NA therapy is rarely able to induce HBsAg seroconversion (complete cure) or sustained HBsAg clearance (functional cure), but hopefully, the combination of new drugs in the pipeline, like direct-acting antivirals and immune modulators, will lead to a 'definitive cure' for chronic HBV infection.

Human herpes viruses

Ganciclovir and aciclovir are the drugs of first choices in the treatment of cytomegalovirus- and herpes simplex virus-infections. Despite the extensive use of these drugs for both therapy and prophylaxis, the incidence of resistant viruses is minimal. Eight of 43 CMV samples submitted for resistance determination were found to be ganciclovir resistant.

One patient was infected with CMV resistant to ganciclovir, cidofovir and foscavir. Of 17 samples submitted for detection of aciclovir-resistant herpes simplex virus, aciclovir resistance mutations were found in 3 samples, one herpes simplex virus 1 and two herpes simplex virus 2.

Hepatitis C virus

In much of routine diagnostics and research, genotyping and sequencing commonly rely on amplification of a conserved region of interest in combination with Sanger sequencing. At the same time, Next-Generation Sequencing (NGS) has become an available technology to perform partial and/or whole genome sequencing of viral pathogens. This approach allows for simultaneous screening of resistance-associated substitutions at all relevant sites in the HCV genome for any (sub)genotype. An NGS target enrichment-approach is currently under development at the Norwegian Institute of Public Health.

The usage of antivirals in Norway

During the last two decades, the development of new specific antivirals has been accelerated especially due to research into HIV medicines and hepatitis C virus (HCV) medicines. The sales of antiviral drugs has been increasing every year measured in defined daily doses (DDDs). According to The Norwegian Drug Wholesales statistics database, the cost of antiinfectives for systemic use (ATC group J) has in 2016 stabilized after a few years of significant increase. Introductions of new antivirals for treatment of HCV infections has highly influenced the increased costs in this group over the last years. However, price reduction for some of these drugs has resulted in almost unchanged total costs for the antivirals in 2016 compared to 2015 even if the sales measured in DDD and number of users have increased. Figure 1 shows the sales of all direct acting antiviral drugs (DAA)(ATC group J05A), measured in DDDs during the past five years.

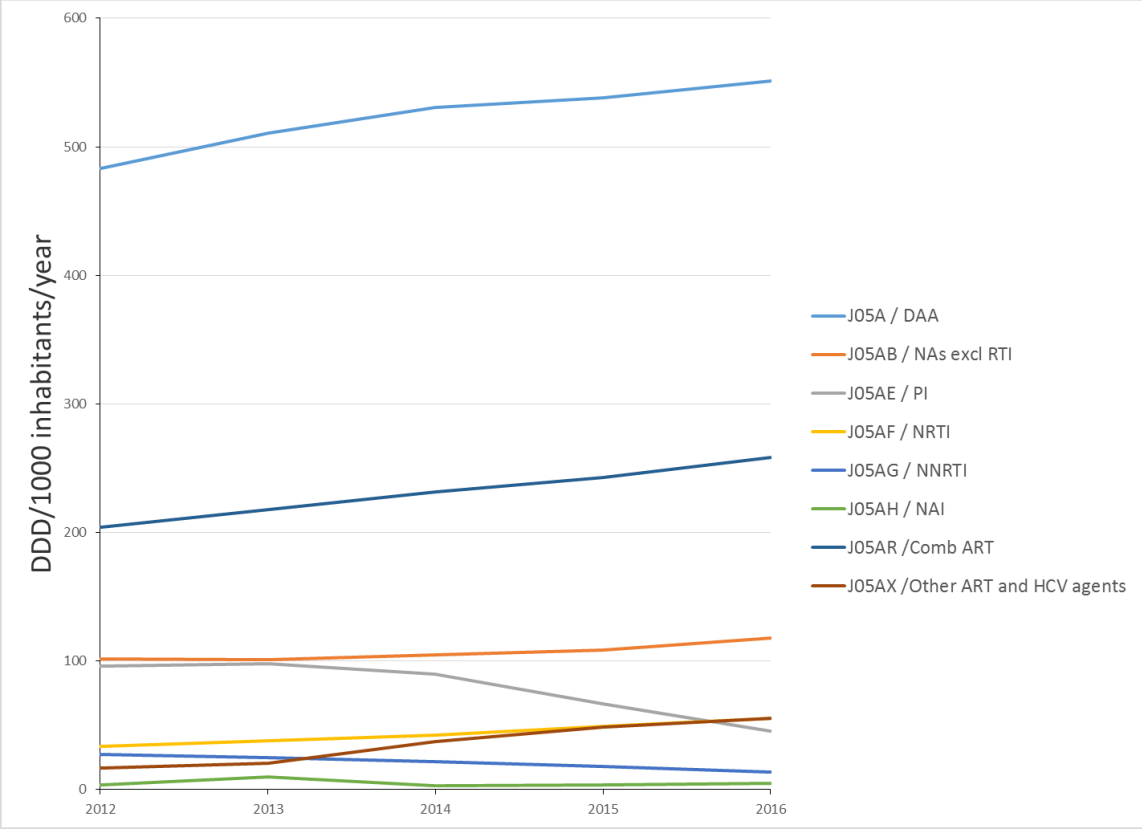


Figure 1. Sales of direct acting antiviral drugs (DAA), ATC group J05A for 2012-2016 given in DDD/1000/inhabitants/year. Source: The Norwegian Drug Wholesales statistics database.

Influenza virus

The usage of antivirals for the treatment of influenza (ATC group J05AH) in Norway is shown in Table 1. The variation between the years are probably linked to the size of the yearly influenza epidemic.

Table 1. Number of individuals with at least one prescription of a neuraminidase inhibitor (NI) per year, ATC group J05AH, in the period 2012-2016.

NI drug	Number of individuals with one or more prescription per annum				
	2012	2013	2014	2015	2016
Zanamivir	34	85	18	52	25
Oseltamivir	1776	3911	1081	1476	2 129

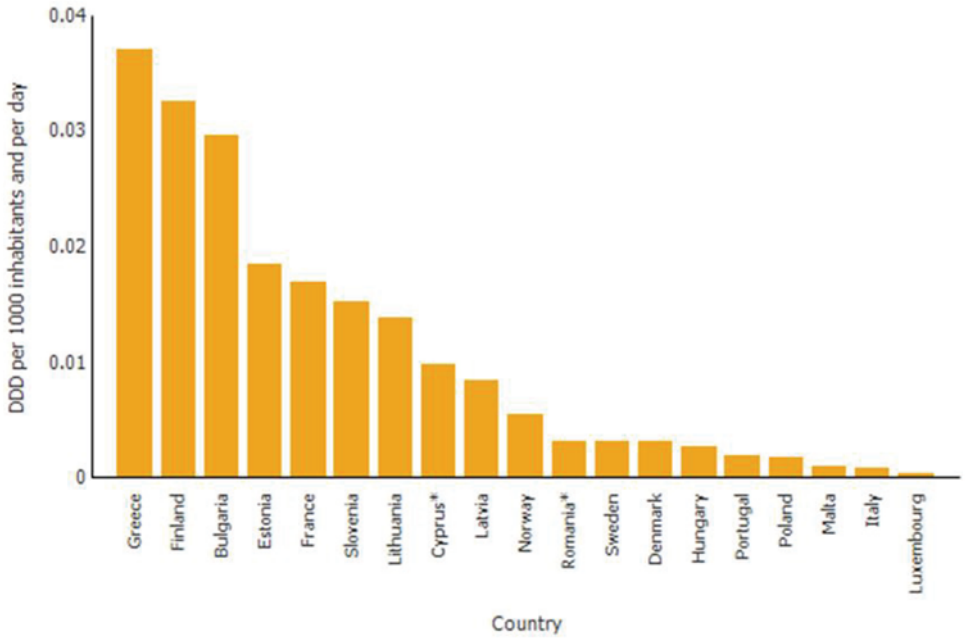


Figure 2: Consumption of neuraminidase inhibitors (ATC group JOSAH) in the community and hospital sector in Europe, by country (reported 2O14)

*Country provided only total care data.

Hungary and Luxenbourg reported data to ECDC only for consumption in the community sector. Source: Expert Opinion on neuraminidase inhibitors for prevention and treatment of influenza, ECDC.

HIV

There are currently 31 antivirals used in treatment of HIV infection in Norway. The number of patients given at least one prescription of these drugs has increased more than 50 % from 2012 to 2016. Figure 3 shows the number of patients given at least one prescription of the 10 most prescribed HIV agents per year. There is an increase in the number of persons receiving a combination product including more than one active entity. In 2016 more than 4000 persons were treated with these fixed combinations in Norway. Single substance products could be given in addition to the fixed combination for some patients. One example is ritonavir which is exclusively used as a protease-inhibitor enhancer (PK enhancer) and is always used in combination with other HIV drugs, decreasing pill burden and frequency of dosing.

Lamivudine, tenofovir disoproxil, adefovir dipivoxil and emtrivitabine are approved for treatment of HIV and hepatitis B virus (HBV) infection. These substances are not included in the number of users of HIV treatment in figure 3. The sum of the patients using the different products is higher than the total number of patients treated with HIV agents in figure 3. This is because some patients receive more than one product during a year.

Several new fixed combination products have been introduced over the last years and it is expected that this trend will continue. Changes in usage may be due to these new combinations.

The fixed combination of emtricitabine and tenofovir disoproxil (TDF) has been the most used combination product the last years but for the first time a small decrease was seen in 2015. This trend continued in 2016. A new prodrug of tenofovir, tenofovir alafenamide (TAF), was introduced in three different fixed combinations in 2016. One 2 component combination (emtricitabine+TAF), one 3 component combination (emtricitabine+TAF+ rilpivirine) and one combination with 4 substances (emtricitabine +TAF + elvitegravir and cobicistat). TAF is given in lower doses and has a greater bioavailability in relevant body tissues than TDF. Reduction in use of TDF containing combinations has partly been compensated for by the increased use of the new TAF containing combinations in 2016. The triple combination including emtricitabine, TDF and rilpivirin has steadily increased since the introduction in 2012 and is the only TDF containing combination that is still increasing in 2016. Since 2012 three new fixed combinations of 3 active substances and two fixed combinations of 4 substances (emtricitabine/TDF/ elvitegravir/ cobicistat) and (emtricitabine/TAF/ elvitegravir/ cobicistat) has been introduced. The combination of lamivudine, abacavir and dolutegravir (integrase inhibitor), introduced in 2014, is the mostly used of the combinations introduced after 2012.

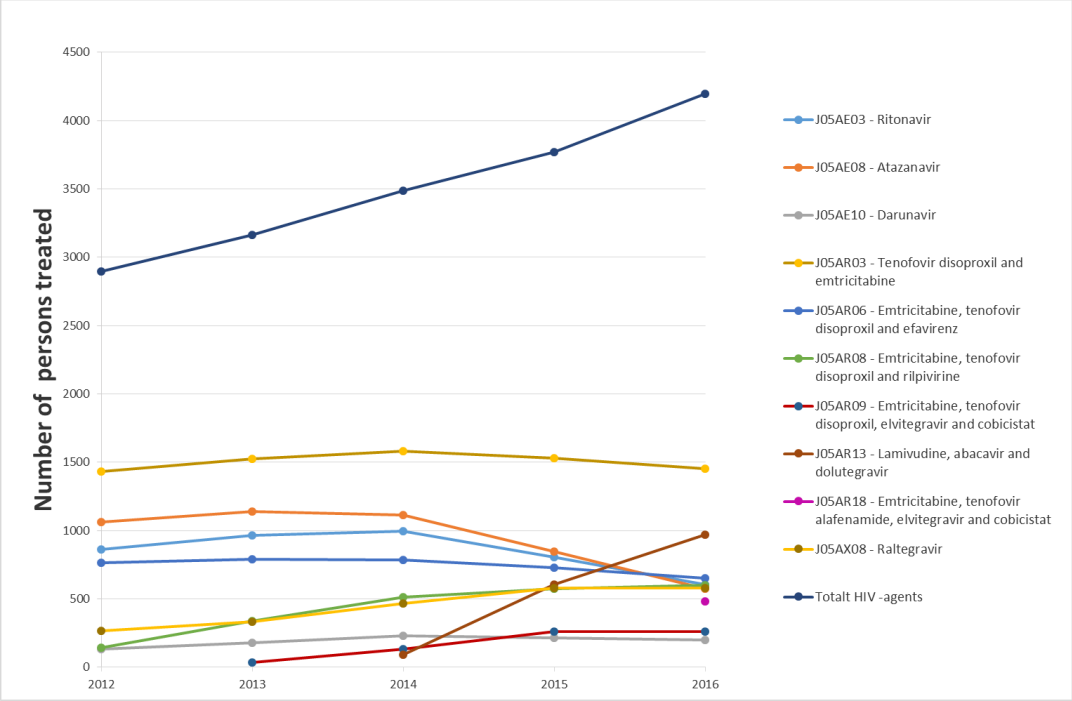


Figure 3: Trends in the use of antivirals for treatment of HIV for the period 2012-2016. The 10 most used agents based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health.

Hepatitis B virus

There are currently eight approved therapies for HBV infection including three interferon based and six nucleoside/nucleotide analogues (NA) (lamivudine, adefovir dipivoxil, emtricitabine, entecavir, telbivudine and tenofovir disoproxil). Treatment of HBV with antivirals is generally given as monotherapy. The use of these NA-drugs is shown in figure 4.

The data is based on the annual number of patients given at least one prescription per year for the period 2012- 2016. Lamivudine, adefovir dipivoxil, tenofovir disoproxil and emtricitabine are drugs that are approved for both HBV and HIV, while entecavir and telbuvidine are approved for HBV only. An estimate of the number of patients treated for HBV with antivirals in Norway will therefore be in the range of 359-866 in 2016. The lowest number is based on the number of patients using drugs approved for HBV only. The highest number is the total number of patients treated with the six NA-drugs (excluding lamivudine containing products approved for HIV only). First-line therapy (entecavir and tenofovir disoproxil) has been increasingly used for several years and account for more than 96% of the six NA treatments given in 2016. A new product containing tenofovir alafenamide (TAF) has been approved as monotherapy for treatment of HBV in January 2017. This may influence the pattern of use of anti-HBV drugs the next years.

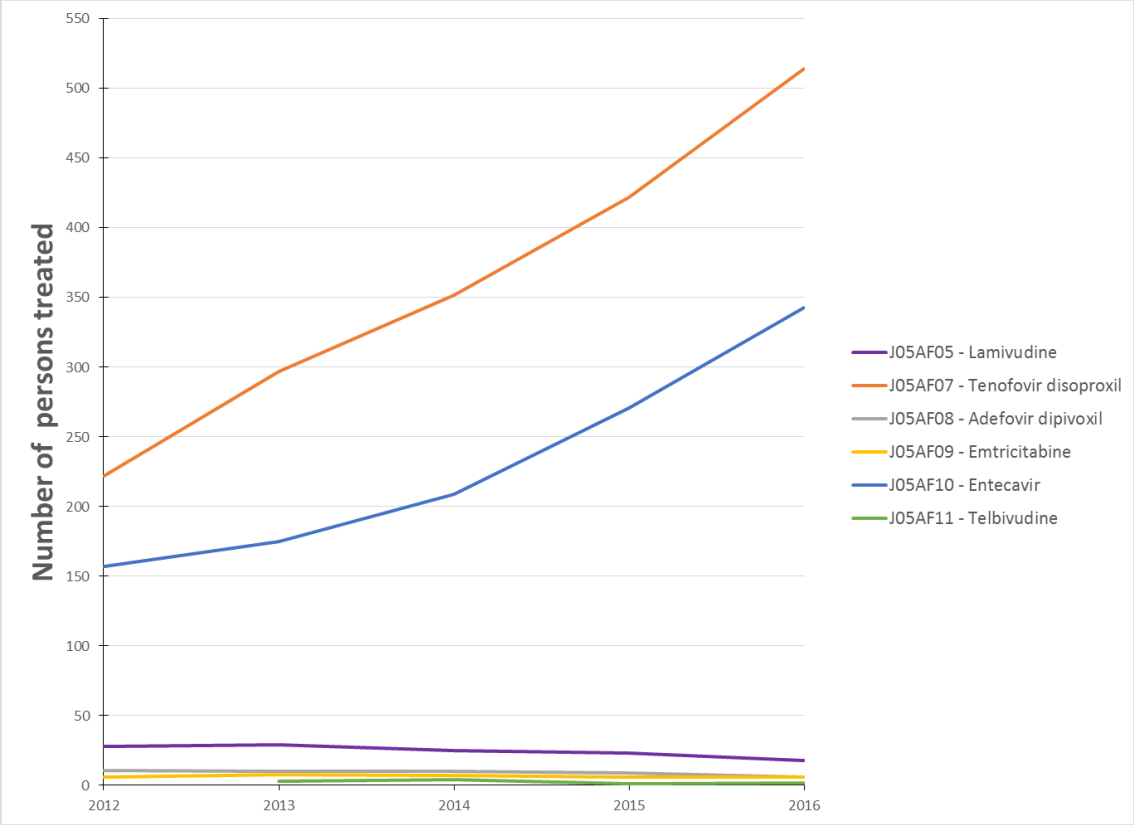
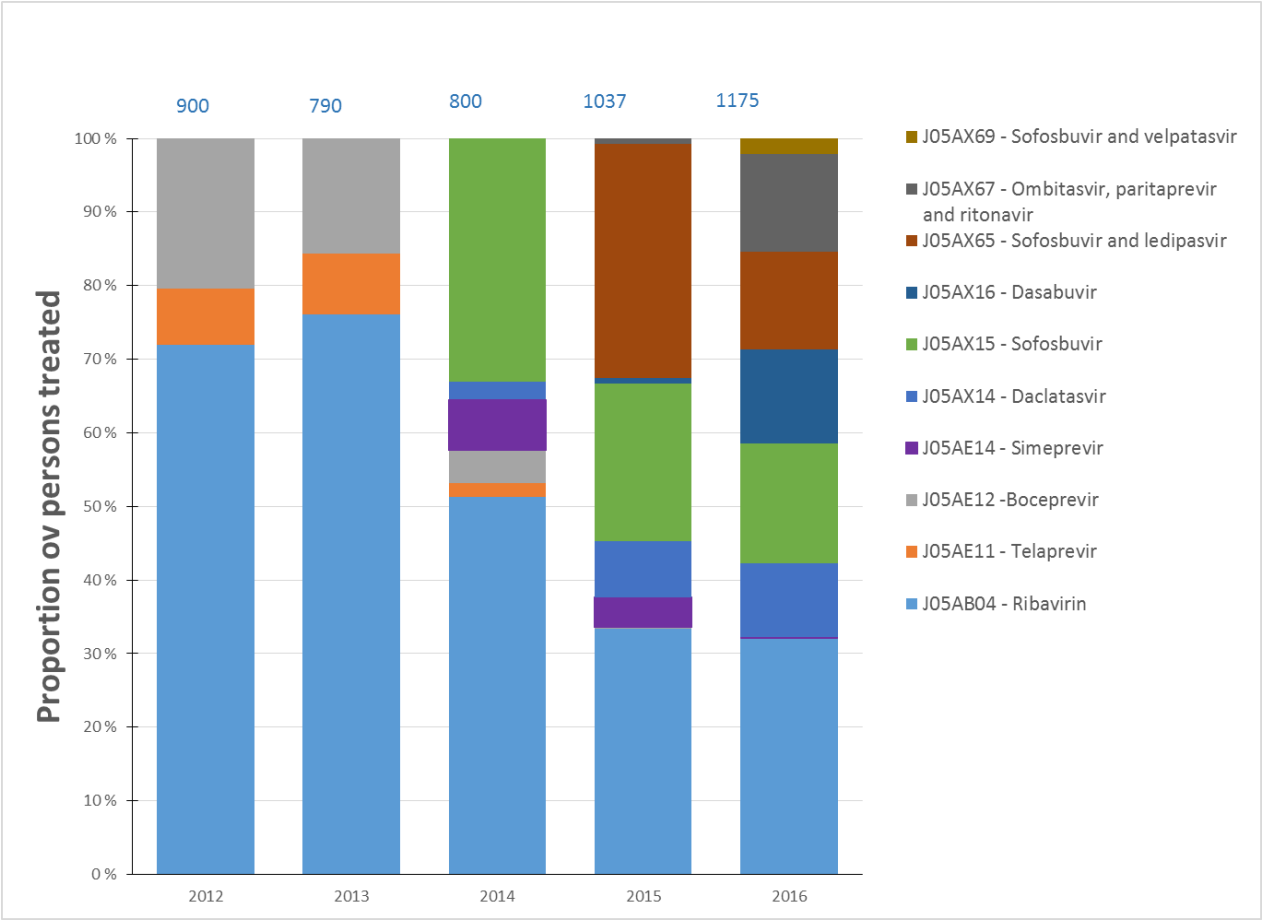


Figure 4. Patterns of prescriptions for HBV-treatment from 2012-2016 based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Hepatitis C virus

Until 2011 HCV-therapy was based on a combination of pegylated interferon and ribavirin for a given period depending on HCV-genotype. In 2011, two new protease inhibitors (PI), telaprevir and boceprevir, were licensed for combination therapy with ribavirin and interferon in HCV genotype 1 infections. In 2014, three new antiviral drugs targeting HCV entered the market: sofosbuvir; a pangenotypic polymerase inhibitor, simeprevir; a second-wave protease inhibitor and daclatasvir; a pangenotypic NS5A (non-structural protein 5A) inhibitor. The NS5B polymerase inhibitor dasabuvir entered the market in 2015. This substance is used in combination with a new fixed combination product including ombitasvir (NS5A inhibitor), paritaprevir (NS3 protease inhibitor) and ritonavir (pharmacokinetic (PK) enhancer). A fixed combination of ledipasvir (NS5A inhibitor) and sofosbuvir was also marketed. In addition, a new fixed combination of sofosbuvir and velpatasvir (NS5A inhibitor) was introduced in 2016. In 2016 the use of the three first PIs introduced to the market for treatment of HCV (telaprevir, boceprevir and simeprevir) was very limited. With the new direct-acting antivirals (DAA) the therapy for chronic HCV-infection has improved. One of the advantages with some of the new HCV products is the possibility to avoid the use of interferon. The overall number of patients on treatment has increased during the last five years with the new drugs on the market (figure 5). There is a number of new substances in the pipeline. The use of DAAs is expected to increase further in the coming years with introduction of these new drugs.

Figure 5. Patterns of prescriptions for HCV-treatment from 2012-2016 based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health



Human herpesviruses

Figure 6 shows the two most prescribed drugs for human herpes virus infections over the last five years. The use of the other drugs approved for treatment of human herpes virus is limited (table 2). While ganciclovir, famciclovir and foscarnet have been prescribed very rarely in this period, acyclovir is prescribed more often and valganciclovir is the substance most commonly prescribed and the use is steadily increasing.

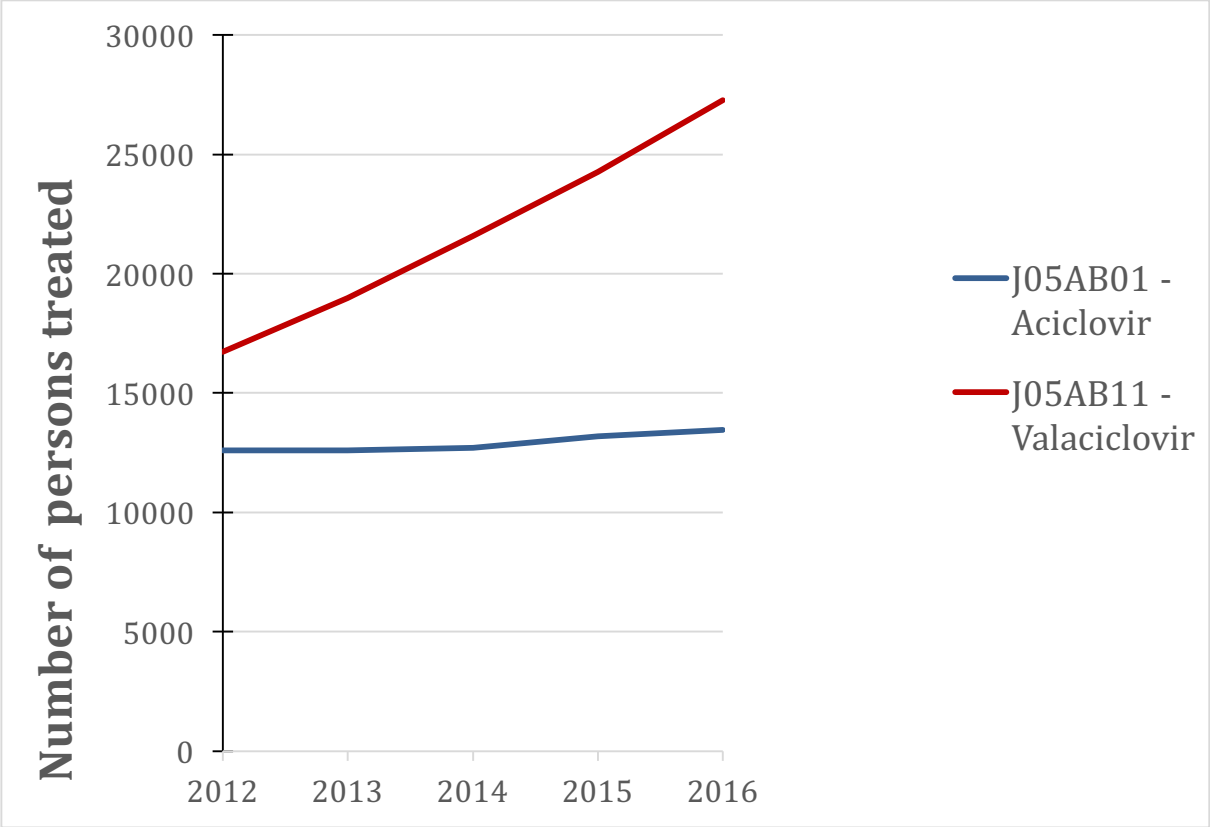


Figure 6. Number of individuals with at least one prescription of aciclovir and valaciclovir per year for the periode 2012-2016.

Table 2. Number of patients given prescription for human herpes virus infections per year for the periode 2011-2015. Data for 2016 not yet available. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health.

	2011	2012	2013	2014	2015
J05AB01 - Aciclovir	12 172	12 655	12 598	12 719	13 190
J05AB06 - Ganciclovir	1		1	2	2
J05AB09 - Famciclovir		1	2	4	3
J05AB11 - Valaciclovir	14 811	16 807	18 985	21 597	24 262
J05AB14 - Valganciclovir	319	347	365	378	371

Surveillance of influenza virus resistance

Fact box: Influenza virus drug resistance	
Treatment	Oseltamivir (Tamiflu®) As of 2016, zanamivir is no longer available on the Norwegian market
Resistance testing method	Genotypic by pyrosequencing snp-real-time PCR Phenotypic by neuraminidase susceptibility assay (MUNANA) In Norway, all influenza drug resistance tests are performed at the Norwegian Institute of Publich Health.
Target gene	Neuraminidase Currently all viruses are resistant towards adamantanes, which inhibits the function of the matrix protein. The matrix gene is therefore now not regularly screened for resistance.
Indication for resistance testing	Antiviral treatment failure Long-time hospitalised patients with prolonged virus shedding A selection of samples submitted through the influenza surveillance program
Surveillance	Screening for resistance as part of the national influenza surveillance program which involves samples from both untreated and treated patients No active surveillance program for treatment induced resistance.

Surveillance of influenza virus resistance in Norway

The National Influenza Centre at The Norwegian Institute of public Health (NIPH) performs antiviral drug susceptibility testing as part of the seasonal influenza surveillance program. Special attention is directed towards oseltamivir (Tamiflu®) resistance. Sensitivity towards the neuraminidase inhibitors like oseltamivir and zanamivir is investigated phenotypically by neuraminidase susceptibility assay and genetically by both pyrosequencing and by conventional sequencing (Table 1).

Surveillance of influenza virus resistance through WHO / European Influenza Surveillance Network

Influenza virus resistance data is reported to the European Centre for Disease Prevention and Control (ECDC) and WHO by the influenza network countries every week throughout the season. Influenza virus resistance during the 2016/17 season was ~1,9% for H1N1pdm viruses and 0.2% for H3N2 viruses in Europe (ECDC) (1).

Resistance surveillance findings in the 2016/17 influenza season

In contrast to the previous H1N1 season, the 2016/17 season was dominated by influenza A H3N2 viruses and with influenza B taking over by week 13. The season was of medium intensity, but was severe for elderly as this was the main age group infected by the H3N2 viruses and they were most frequently admitted to hospital. An overall excess mortality was also observed for the elderly this season. Laboratories in Norway analysed more than 140 000 samples for influenza this season and 3000 samples were sent to the WHO National Influenza Centre at NIPH for further analysis. Approximately 12 % (n=174) of all H3N2 positives at the NIPH were analysed for antiviral resistance, 44% of the H1 viruses (n=10) and 7% of influenza B viruses (n=54). None of the viruses had reduced susceptibility to either oseltamivir or zanamivir, nor possessed known amino acid substitutions related to influenza antiviral resistance.

Table 1. Norwegian influenza viruses resistant to NIs (oseltamivir and zanamivir) and M2 blockers (adamantanes), during the influenza seasons 2005/6 through 2016/17.

Season	Oseltamivir resistance			Zanamivir resistance			Adamantane resistance	
	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)
2005/06	0% (n=6)	0% (n=13)	0% (n=21)	0% (n=6)	0% (n=13)	0% (n=21)	Nd	75% (n=4)
2006/07	0% (n=5)	0% (n=10)	nd	0% (n=5)	0% (n=10)	Nd	0% (n=6)	90% (n=10)
2007/08	67,8% (n=272)	0% (n=2)	0% (n=59)	0% (n=114)	0% (n=2)	0% (n=59)	0% (n=112)	100% (n=2)
2008/09	100% (n=33)	0% (n=13)	0% (n=1)	0% (n=5)	0% (n=12)	0% (n=1)	0% (n=5)	100% (n=65)
2009-pdmH1	0% (n=884)	Nd	0% (n=11)	0% (n=36)	nd	0% (n=9)	100% (n=258)	100% (n=2)
2010/11	0,82% (n=244)	0% (n=1)	0% (n=30)	0% (n=2)	0% (n=1)	0% (n=24)	100% (n=54)	100% (n=10)
2011/12	0% (n=27)	0% (n=72)	0% (n=5)	nd	0% (n=60)	0% (n=4)	100% (n=21)	100% (n=56)
2012/13	0% (n=256)	0% (n=22)	0% (n=24)	0% (n=20)	0% (n=22)	0% (n=19)	100% (n=11)	100% (n=5)
2013/14	0% (n=183)	0% (n=43)	0% (n=27)	0% (n=32)	0% (n=43)	0% (n=27)	100% (n=77)	100% (n=67)
2014/15	0,74% (n=136)	0% (n=169)	0% (n=92)	0% (n=136)	0% (n=166)	0% (n=92)	nd	100% (n=30)
2015/16	3,0 (n=339)	0% (n=32)	0% (n=50)	0% (n=106)	0% (n=31)	0% (n=48)	nd	nd
2016/17	0% (n=10)	0% (n=174)	0% (n=54)	0% (n=8)	0% (n=161)	0% (n=54)	nd	nd

Conclusion

Timely screening for antiviral resistance and increased awareness of community spreading of resistant viruses are important in preventing new outbreaks of resistant viruses. Viruses with antiviral resistance can spread rapidly, as experienced in the season 2007/08, when a resistant H1N1 circulating in Norway quickly spread worldwide. Therefore, development of alternative antivirals is urgently needed and policymakers should consider their stockpiles of antiviral drugs. To rely on one drug like oseltamivir, as is the current policy in Norway, is potentially hazardous.

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1. Expert Opinion on neuraminidase inhibitors for prevention and treatment of influenza. Review of recent systematic reviews and meta-analyses. https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/neuraminidase-inhibitors-flu-consultation.pdf

Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV) drug resistance	
Treatment	*Five classes:* Reverse transcriptase inhibitors (RTI's), which are subdivided in NRTI's (nucleoside RTI's) and NNRTI's (non-nucleoside RTI's) Integrase inhibitors Protease inhibitors CCR5 antagonists Fusion inhibitors
Resistance testing method	Genotypic assays based on amplification by RT-PCR and Sanger sequencing of the product. The sequences are analyzed for amino acid mutations associated with drug resistance. A plasma viral load > 500 copies/mL is required for the analysis. In Norway, all HIV-1 drug resistance tests are performed at the National Reference laboratory for HIV-1 at the Department of Microbiology at Oslo University Hospital, Ullevål.
Target genes	Reverse transcriptase Protease Integrase gp120 (envelope), V3 region (for CCR5 antagonist resistance testing)
Indication for resistance testing	Virological failure during antiviral treatment
Surveillance	It is recommended that samples from all patients with newly diagnosed HIV-1 infections are tested for resistance mutations in the protease and reverse transcripase genes.

PrEP and PEP in a clinical perspective.

PrEP (Pre-Exposure Prophylaxis) means that HIV-negative individuals take tablets before high risk exposure to reduce their risk of being infected with HIV. So far, TDF/FTC (tenofovir diproksil fumarat and emtricitabine) is the only medication approved for PrEP treatment.

PrEP with TDF/FTC has shown to be highly effective in high risk populations in two European studies; the PROUD Study (1) and the IPERGAY Study (2). The relative risk reduction was 86% in both studies and in this high risk population only 13-18 persons had to be treated to prevent one new HIV infection as the risk for acquiring HIV was very high: in 100 person years 7-9 were HIV-infected. The Norwegian PrEP guidelines reflect the importance of offering PrEP to HIV-negative persons with indicators of high risk (3). These indicators are:

- Recent sexually transmitted anal infection
- Recently used Post-Exposure Prophylaxis (PEP).
- Difficulties to always use a condom for anal sex
- Frequently sex without a condom with someone at high risk of HIV infection
- HIV-positive partner who is not receiving HIV treatment

PrEP can be taken continuously or intermittent, depending on risk behavior. Continuous treatment with daily TDF/FTC is suitable for HIV-negative persons with many sexual partners all the time or if it is unpredictable when they are going to have sex. Intermittent treatment regimen is suitable for people who rarely have sex, for example at weekends or when travelling abroad. Intermittent treatment with TDF/FTC means two tablets 2–24 hours before sexual contact, and then one tablet per day. The last tablets are taken 24 and 48 hours after last unprotected sex.

PrEP users have to come back for a check-up every three months to discuss problems, test for sexually transmitted diseases and renewal of the prescription.

Details can be read at https://oslo-universitetssykehus.no/avdelinger/klinikk-for-kirurgi-inflammasjonsmedisin-og-transplantasjon/avdeling-for-revmatologi-hud-og-infeksjonssykdommer/olafiaklinikken

Short term side-effects of TDF/FTC are few and mild. However, long term treatment with TDF may affect renal function and bone density and therefore, people on long term treatment with TDF/FTC should be checked yearly. An increase of condomless sex and other STI are found in some studies (4). In parallel with PrEP being made available in Norway, a study to evaluate the service is initiated at Olafiaklinikken, Oslo University Hospital.

PEP (Post-Exposure Prophylaxis) means that HIV-negative individuals take tablets after high risk exposure to reduce their risk of being infected with HIV. A case-control study from 1997, demonstrating an 81% reduction in the odds of HIV transmission among health care workers with percutaneous exposure to HIV who received zidovudine (ZDV) prophylaxis, was the first to describe the efficacy of PEP (5). Because of the ethical and operational challenges, no randomized controlled trials have been conducted to test the efficacy of PEP directly.

The Norwegian PEP guidelines recommend TDF/FTC and Isentress (raltegravir) within 48 hours and for 28 days. Side effects of this regimen are few and mild, but tracing and testing the index person is important to avoid unnecessary PEP. The main groups who are offered PEP are healthcare workers who are exposed to blood from persons known to have HIV infection or from persons from a group with a high prevalence of HIV infection (e.g., people who inject drugs and MSM), persons exposed to HIV through sexual assaults and MSM exposed through receptive anal intercourse.

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Comments on HIV drug resistance at the launch of pre-exposure prophylaxis (PrEP)

Prophylactic treatment with a fixed dose combination of tenofovir (TDF) and emtricitabine (FTC) has recently been implemented in Norway as a prevention strategy to reduce the incidence of new HIV-1 infections. Norway is one of two countries in Europe fully reimbursing PrEP to high risk groups. Studies have shown that if taken consistently, PrEP may substantially reduce the risk of HIV-infection in people who are at high risk, and is a cost-effective tool for prevention (1-3).

Regarding HIV drug resistance and PrEP, there are two main concerns: First, there is a risk that the use of PrEP may lead to an increased selection of mutations associated with resistance to TDF/FTC, and second, TDF/FTC offers limited protection against transmission of drug resistant strains. Both of these concerns would imply an increase in the spread of HIV drug resistance.

It is well known that HIV is a virus with a high mutation frequency, high genetic variability and ability to genetic adaptation, and thus, with a high risk for development of drug resistance. Lessons from the early 90's have shown that if even duotherapy with nucleotide reverse transcriptase inhibitors (NRTI) is continued during viral replication, drug resistance mutations are rapidly selected. In patients failing TDF/FTC-based ART regimens, especially mutations like M184V and K65R are selected, causing drug resistance and treatment failure. Concerns have been raised that a large-scale roll-out of PrEP may lead to an increase in the selection of mutations associated with resistance to TDF/FTC, thus enhancing the spread of drug resistance. A relevant question is whether using the same drugs for treatment and prevention may jeopardize the efficacy of one of the preferred regimens for first-line antiretroviral therapy (ART).

Over the last years, several clinical trials with TDF/FTC as PrEP in high-risk groups have been conducted. Thousands of people have received PrEP through these trials; very few have actually contracted HIV, and only a few cases of drug resistance have been observed. Among the 160 seroconverters in five early placebo-controlled PrEP trials with TDF/FTC, only 3% developed resistance to FTC, and even less (<1%) to TDF (4-8). Thus, if HIV-infection is not present at the start of PrEP, the risk for developing resistant strains seems to be very low, at least in the study trial setting. However, among persons having an unrecognized acute HIV-infection at the time of enrolment, the situation is quite different. In those five clinical trials, a total of 17 persons were retrospectively found to be in the seroconversion phase when starting PrEP. Among these patients, as many as 41% developed resistance to FTC, and 6% to TDF. These findings emphasize the importance of thoroughly excluding HIV-infection before starting PrEP.

The risk for selection of drug resistant mutations is the highest at high viral loads, and the major concern is that a person has an unrecognized acute HIV-infection at the initiation of PrEP. Otherwise, the risk of developing resistance to FTC during PrEP seems low, and the risk of development of TDF-resistance even lower. However, the available data are from clinical trial settings, and it is likely to expect that the compliance in such studies would be superior compared with the compliance in practical use in real life. Reduced compliance will most likely reduce the efficacy and increase the risk of PrEP failure.

Although the highest risk for drug resistance is associated with having an unrecognized acute infection at the start of PrEP, continuation of PrEP after contraction of HIV will also increase the risk for selection of resistance mutations. Therefore, we suggest three major

elements that need to be implemented in the protocol for initiation and follow-up of PrEP in order to minimize the risk for development of drug resistance: Most importantly, frequent and adequate testing for HIV-infection is crucial before PrEP is started and during the first few weeks. This would effectively identify possible acute infections as soon as possible. Second, consistent and systematic follow-up every 3 months during PrEP will minimize the risk for continuation of TDF/FTC after potential failure of PrEP. And finally, it is important to aim for as good compliance as possible in persons receiving PrEP in order to reduce the risk of failure.

In regard to the possible limited protection of PrEP against transmission of drug resistant strains, it is important to consider the prevalence of drug resistance mutations associated with resistance to TDF/FTC. Circulating TDF/FTC-resistant strains may reduce the efficacy of PrEP, as the drugs are not likely to protect against a variant transmitted from a partner on a failing TDF/FCT-based regimen, or another combination of NRTI selecting for M184V.

In Norway, transmitted drug resistance is monitored through RAVN, and the data are shown elsewhere in this report. The frequency of transmitted drug resistance is in general low, and in 2016 there were no resistance mutations affecting efficacy of TDF/FTC detected among newly diagnosed patients in Norway. However, in patients with virological failure, the frequency of especially M184V is much higher. As shown in figure 1, about 20% of analyzed sequences from patients with treatment failure in Norway contain mutations that are likely to reduce the efficacy of PrEP. This implies that as PrEP is now more widely used, it is not unlikely that there will be cases of PrEP failure even in adherent patients due to transmission of resistant strains of HIV.

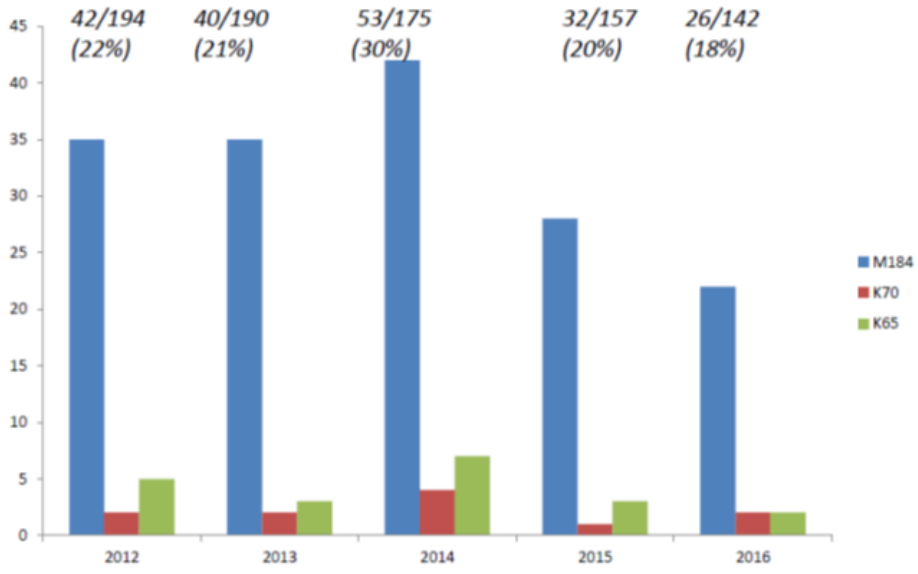

Figure 1. Frequency of drug resistance mutations with relevance for PrEP in patients with treatment failure in Norway.

The key question is how PrEP will influence on the incidence of drug resistance – will it increase, decrease or remain unchanged? There is an actual risk of increased resistance due to the factors discussed above, and an increased use of a drug might increase the selection pressure applied to the population. There is also a possibility that the incidence

of resistance against TDF/FTC will be unaffected: The transmitted drug resistance is low anyway, and the use of PrEP is not likely to affect the risk for treatment failure in patients receiving ART. Then there is the PrEP paradox, suggesting that although the actual number is unchanged or even reduced, the relative transmitted drug resistance will increase because new infections with sensitive strains will be prevented while the transmission of resistant strains remain unaffected.

Finally, there might actually be a true possibility that PrEP will reduce HIV drug resistance. Due to highly effective prevention of new infections, especially in groups with high transmission rates, the HIV incidence may decline. Fewer new infections mean fewer infections with transmitted drug resistance, and fewer infected people to drive the epidemic.

By taking the previously mentioned precautions to avoid starting PrEP in acute HIV-infection and to avoid continuation of PrEP in cases of failure, the risks may be reduced to a minimum. Nevertheless, roll-out of PrEP calls for intensified surveillance of drug resistance in order to get answers to these important questions.

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A brief history of antiretroviral therapy for HIV-1 infection

Background

The history of AIDS and HIV-1 started with the very well known 1981 report from Centers for Disease Control and Prevention (CDC) that a novel and distinct immunodeficiency syndrome ("acquired" rather than "primary") had been recognized in men who have sex with men (1). In the early 1980s, without any diagnostic tests available, only clinical diagnostics could be used to identify AIDS patients suffering from Kaposi sarcoma, *Pneumocystis (carinii) jiroveci* pneumonia or cryptococcal meningitis. The first major breakthrough was the isolation of HIV-1 (2). This opened up for the development of a diagnostic antibody test in 1985 (3) that could identify infected, but not yet sick persons and screen blood donors to prevent transmission of HIV-1 by blood and blood products.

The first attempts of antiretroviral treatment

The first drug against HIV-1 wasn't a new compound scientists had to develop from scratch. AZT, or azidothymidine, belongs to a group of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs interfere with retroviral replication by competitively inhibiting the enzyme, thus leading to chain termination of proviral DNA. AZT was originally developed in the 1960s as a cancer drug based on the knowledge that in the animal kingdom, retroviruses were the cause of several tumors. The compound was supposed to insert itself into the DNA of a cancer cell and mess with its ability to divide and produce more tumor cells. But it didn't work when it was tested in mice and was put aside. However, it didn't take long after the isolation of HIV-1 until the ability of AZT to block virus replication *in vitro* was tested with promising results (4). The first clinical trial was conducted in 1986 (5) and in 1987 the U.S. Food and Drug Administration recommended the approval of AZT for use as a "treatment of selected patients with acquired immunodeficiency syndrome, but only for those with advanced illness" (6).

Although AZT was the first drug to be approved for teatment of HIV-patients, the very first patients to be given an antiviral drug was in 1985 at Roslagstulls Hospital in Stockholm by dr. Susanne Bergdahl. Results from the 30 patients were published in 1988 (7). The drug was foscarnet, producd by the drug company Astra. Foscarnet is a pyrophosphate analogue and interferes with exchange of pyrophosphate from deoxynucleoside triphosphate during viral replication by binding to a site on the herpesvirus DNA polymerase or HIV reverse transcriptase (8). Foscarnet had to be given intravenously and it was associated with several, also severe, side effects. With access to AZT and newer NRTIs and other classes of antiretroviral drugs, there was no need for foscarnet so it is no longer in use.

Problems with resistance and need for new drugs and new combinations

Despite the initial positive effects of AZT and newer NRTIs on the patients' clinical conditions, the effect was not lasting due to the relatively rapid development of resistance to the drug when given as monotherapy. So, dual nucleoside combination was the next approach. When AZT was administered together with lamivudin (3TC) or zalcitabine (ddC) or didanosine (ddI) the CD4 cell numbers increased and the survival was better (9). Still, the results were not durable and the side effects and tolerability were problematic.

Because HIV is a retrovirus the initial efforts in drug development was to block the reverse transcriptase (RT) and, as mentioned above, NRTIs were the first to be approved for treatment.

During further development of NRTIs, a new type of drug was discovered, the non-nucleoside reverse transcriptase inhibitors (NNRTI). The NNRTIs are HIV-1 specific and have no activity against HIV-2 or other retroviruses (10). In addition, in contrast to the competitive inhibition of RT by NRTIs, the NNRTIs act by binding non-competitively to the RT. The binding causes a conformational change in the three-dimensional structure of the enzyme. This affects the catalytic activity of the enzyme and blocks the HIV-1 replication by inhibiting the polymerase active site. A single mutation in the NNRTI-binding pocket may result in high-level resistance to one or more of the NNRTIs. Resistance usually emerges rapidly when NNRTIs are administered as monotherapy or in the presence of incomplete virus suppression. However, when given as part of a three-drug regimen with two NRTIs, the combination is highly effective, as demonstrated in the INCA study, published 1998 (11). The first NNRTI, nevirapine, was approved by the FDA in 1996.

HAART and a new era of antiretroviral therapy

In many ways HIV has served as an important biomedical ”catalyst” by opening up for closer contacts and collaborations between scientists within basic cell biology, chemistry, diagnostics, pharmacology, clinicians and the Pharmaceutical industry. Therefore, it didn’t take long until the life cycle of HIV-1 was characterized, its DNA sequenced and the different proteins and enzymes identified in order to find new target molecules for treatment. As shown in Figure 1, the life cycle can be divided into Early phase with binding to the CD4 molecule and coreceptor CCR5 or CXCR4, followed by fusion with the cell membrane and entry into the cytoplasm. The Middle phase is represented by reverse transcription, synthesis of proviral DNA and integration of this viral DNA into the cellular DNA in the nucleus. Protein synthesis and assembly takes place in the cytoplasm. During Late phase immature HIV particles are budding from the cell membrane and the viral protease is activated to cleave the polyproteins into functional proteins and thereby produce infectious virions.

Protease inhibitors (PI), synthezised through advanced drug discovery processes (12), was a breakthrough and ritonavir and indinavir got FDA approval in 1996. The triple combination therapy also known as HAART (highly active antiviral therapy) included two NRTIs and one PI. HAART was quickly incorporated into clinical practise and rapidly showed impressive, almost ”miraculous” benefit with 60 – 80% decline in rates of progressison to AIDS, death and hospitalization (13).

The exitement and high expectations among patients and health-care workers about the use of HAART was tempered by the recognition of sometimes fatal metabolic complications like lactic acidosis, pancreatitis, severe diarrhea and problematic lipodystrophy and lipoatrophy (14). Another major obstacle to sustaining effective therapy, in the early days of HAART, was the difficulty for some patients to stay on complicated regimens with high pill burden, multiple daily administrations and interactions with food. Today the drugs have become more ”user friendly” with fixed dose combinations and once or twice daily dosing.

The search for new classes of antiretrovirals

The repertoire of available drugs to the RT and the protease are efficient and works well in different combinations. However, blocking HIV-1 from entering the cell would be an even better or an additional approach and became the new focus of attention for drug development.

Entry of HIV-1 is mediated by attachment of the envelope glycoprotein gp120 to the CD4 receptor, which leads to a conformational change that results in formation of the coreceptor (CCR5 or CXCR4) binding site on gp120. This in turn triggers glycoprotein 41 (gp41) mediated fusion of the viral envelope with the cell membrane. (See also Figue 1)

The first fusion inhibitor Enfuvirtide (T-20, Fuzeon) is a peptide based on the extracellular domain of the transmembrane segment of gp41 (15) and is the only FDA approved (in 2003) fusion inhibitor. It prevents membrane fusion by competitively binding to gp41 and blocking the formation of the post-fusion structure. T-20 is a very large molecule and can not be orally administrated. The dosing was subcutaneous injections twice daily and was only given to patients with resistance to most of the other available drugs. Because of side effects linked to the injections the drug is not anymore in use.

It has for a long time been known that HIV-1 isolates show differences in biological properties *in vitro* related to the stage of infection of the patient. The virus isolates have been named slow/low or rapid/high (16), M-tropic or T-tropic (17) or most commonly NSI (non-syncytia) or SI (syncytia inducing) viruses (18). In 1996 it was a stop to these nomenclatures because of a new major breakthrough, namely the identificatiton and characterization of the chemokine co-receptors used by the two major HIV-1 variants (19). It was demonstrated that CCR5 serves as co-receptor for the most commonly transmitted HIV-1 strains (20, 21), thus named R5 tropic virus. The other major co-receptor was fusin or CXCR4 and these virus strains were thus named X4 tropic (22). The vast majority of primary HIV infections occur with R5 virus and appears to represent a pure R5 population as determined by co-receptor tropism assays. Co-receptor usage, however, is a dynamic process and over time, R5 strains can eventually evolve into X4 strains as the disease progresses. Although the mechanisms behind this phenotypic switch are not clearly understood, the clinical consequences are well described. The appearance of X4 virus is associated with a faster rate of CD4+ T-cell loss, rapid disease progression, and an increased rate of development of AIDS and death (23).

Very soon focus was on the development of coreceptor antagonists as new antiviral drugs and the trigger for the synthesis of CCR5 antagonists was the sudden and unexpected identification of the CCR5-Δ32 mutation in the gene coding for the CCR5 receptor. This results in almost complete resistance against HIV-1 infection (24, 25). The first of these R5-antagonists to be FDA approved was maraviroc in 2007 and it is still the only one in clinical use.

This new class of drugs increases the repertoire for ART regimens. However, this option is coupled with the necessity to test the patient's virus for coreceptor tropism prior to initiating CCR5 antagonist-based therapy. Failure to screen for X4 usage increases the risk of using an ineffective drug and thereby increase the risk for developing antiretroviral resistance. There are a number of commercially and non-commercially genotypic and phenotypic tests available for tropism testing.

It is also particularly interesting that chemokine receptor antagonists are the first antiretroviral drugs in clinical practice to bind a cellular protein of the host and interact with a component of the human immune system rather than HIV itself . This could give rise to unique safety concerns. In fact, use of CCR5 antagonists may have negative consequences for diseases such as West Nile and Tick-borne encephalitis virus infections (26, 27).

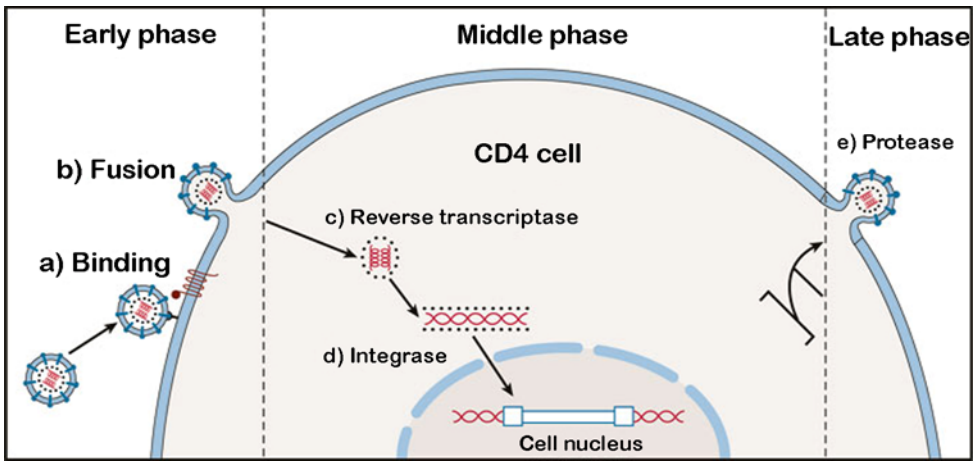

Figure 1. HIV-1 life cycle and attack points for antiviral drugs (29).

HIV-1 binds first to the CD4 molecule on the cell surface and thereafter to coreceptor CCR5 (or CXCR4). The fusion with the cell membrane is mediated by gp41. The viral RNA is transcribed to viral DNA by reverse transcriptase in the cytoplasm and is integrated into the cell nucleus by integrase. New virus particles bud off from the cell membrane and the protease cleaves the major poly-proteins to functional proteins. Early phase: a) blocking of CCR5, b) blocking of fusion with the cell membrane. Interphase: c) Nucleoside and non-nukleoside reverse transcriptase inhibitors, d) integrase inhibitor. Late phase: e) Protease inhibitors.

The last piece in the puzzle to disarm HIV with ART

Integrase is a key enzyme in retroviral replication as it catalyzes the insertion of the viral genome into the host chromosome. The unique properties of the enzyme makes it an ideal target for drug design. First, there is no functional equivalent in human cells and second, it is absolutely indispensable for viral replication. The integration process involves several steps, including formation and processing of a pre-integration viral DNA complex, followed by strand transfer of this complex into the host genome. The integrase strand transfer inhibitors (INSTI) work by binding to the active site of the enzyme and block the action of integrase by restraining the binding of the pre-integration complex with the host genome (28). Although integrase was seen as a rational target for drug design, it turned out to be more complex and complicated than anticipated and it took more than 10 years until the first drug, raltegravir, was FDA approved in 2007. There are currently three different integrase inhibitors available: raltegravir, elvitegravir and dolutegravir. Virological efficacy is excellent for all three inhibitors, and they are all well tolerated with low levels of toxicity and little side effects.

A presentation of integrase inhibitors was given in the RAVN report 2015.

Conlusions

AIDS started as a highly fatal infection about 40 years ago but has become a treatable, chronic infectious disease in the western world as a result of the development of extraordinary successful antireroviral drugs. In addition the drugs have become more ”user friendly” with few side effects and one or two pills once or twice daily, instead of 3-4 pills 4 times daily and severe side effects. This means that patients in our part of the world can get good quality of life and close to normal lives, which not many in the scientific and medical communities in the early days, would have expected.

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Surveillance of transmitted HIV-1 drug resistance

Primary or transmitted versus acquired or secondary drug resistance

Primary or transmitted drug resistance means resistance detected in previously untreated persons. Acquired or secondary drug resistance results from selection of drug-resistant variants from a genetically heterogenous virus population during antiretroviral therapy. Because drug resistance rarely occurs without drug exposure, primary drug resistance implies that a virus with resistance mutations was transmitted either directly, or through intermediates, from a person with acquired drug resistance.

Surveillance methods

To compare transmitted HIV drug resistance rates across geographic regions and time, the World Health Organization recommends the use of a consensus genotypic definition of transmitted HIV-1 drug resistance. The list of mutations for drug-resistance surveillance (SDRMs) is based on the following criteria: 1) Mutations should be commonly recognized as causing or contributing to resistance. 2) Mutations should be nonpolymorphic, which means that the mutation does not occur in the absence of therapy. The mutations should not occur at highly polymorphic positions. 3) The mutation list should be applicable to the eight most common HIV-1 subtypes. 4) The list should be short and unambiguous. There might be additional drug resistance mutations not included in this list, which are of clinical relevance. Furthermore, some of the mutations in the list may not be of clinical significance, but are included in the list as robust indicators of transmitted drug resistance.

The national surveillance system for HIV-1 in Norway monitors the prevalence of transmitted drug resistance against protease inhibitors (PI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTI). The monitoring is conducted according to WHOs SDRM-list of 2009 and analysed by using the Calibrated Population Resistance (CPR) tool at Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu).

The surveillance is based on resistance testing of samples taken from newly diagnosed patients. Results are included in the surveillance system when the MSIS report number is specified on the referral form. Without this report number, the patient can not be identified as newly diagnosed. Unfortunately, this report number is lacking in a number of cases, lowering the percentage of newly diagnosed cases of HIV-1 infection represented in the surveillance. The annual percentage of sequences analysed for primary HIV-1 drug resistance from newly diagnosed cases of HIV-1 in Norway since 2010 is shown in figure 1.

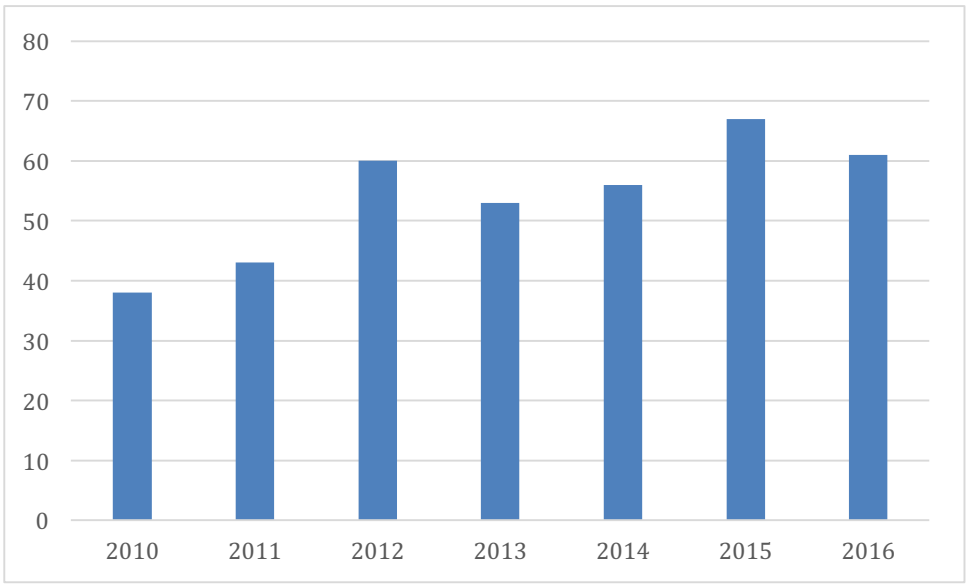


Figure 1. Percentage of newly diagnosed cases of HIV-1 infection with samples submitted to resistance testing (2010-2016).

Surveillance findings in Norway in 2016

SDRMs detected in monitoring of primary HIV-1 resistance are presented in figure 2. The percentage of the sequences with detected SDRMs in total is visualized with bar charts. There may be several SDRM per sequence. The lines show the percentage of sequences with SDRMs affecting NNRTI, NRTI and PI, respectively.

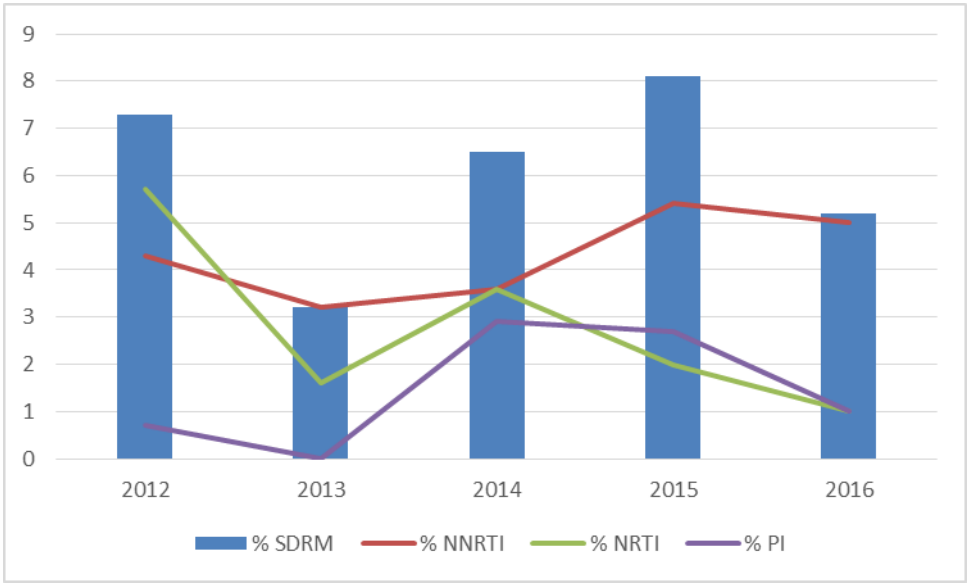


Figure 2. Percentage of analysed sequences containing one or more SDRMs (Surveillance Drug Resistance Mutations) and the distribution on NNRTI, NRTI and PI, respectively (2012-2016).

In 2016, SDRMs from the WHO list were detected in 5.2% of the analysed sequences. The different mutations found are specified in table 1. Of the analysed sequences, 1% had SDRMs associated with PI, 5% with NNRTI and 1% with NRTI. Five samples had SDRMs resulting in high-level resistance to efavirenz and/or nevirapin (NNRTI). Two samples (Sequence ID 3 and 4) had mutations that alone have no clinical significance, and confer resistance only in combination with other resistance mutations in the NRTI and PI region, respectively.

Table 1. The different mutations found in the sequences with SDRMs in 2016.

SequenceID	NRTI SDRMs (1%)	NNRTI SDRMs (5%)	PI SDRMs (1%)
1	None	G190S	None
2	None	K103N	None
3	M41L	None	None
4	None	None	M46L
5	None	K103N	None
6	None	K103N	None
7	None	K103N	None

Discussion

The surveillance data is based on patients who had their HIV-1 infection confirmed in Norway and anonymously reported to MSIS during the respective year. A number of these patients are immigrants who were infected before arrival to Norway. Some of these patients may have received treatment in their home countries. Thus, it should be noted that the data does not reflect the risk for being infected in Norway with a drug resistant strain of HIV. For patients infected in Norway, the corresponding numbers are even lower. Therefore, RAVN is working on a study which aims at linking epidemiological data from MSIS with resistance data which will enable separate data on persons infected in Norway. Another purpose of this study is to determine the incidence of transmitted drug resistant HIV within different patient groups and to identify risk factors involved.

Conclusions

Drug-resistance mutations were detected in 5.2% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2016. The prevalence of transmitted drug resistance has been stable for the last years with only minor variation. The introduction of prophylactic treatment (PrEP and PEP) could challenge this situation. Surveillance of HIV resistance over time is important in order to make decisions on implementing preventive measures to control dissemination of resistant HIV strains.

References

www.msis.no

https://hivdb.stanford.edu/

Hepatitis B virus

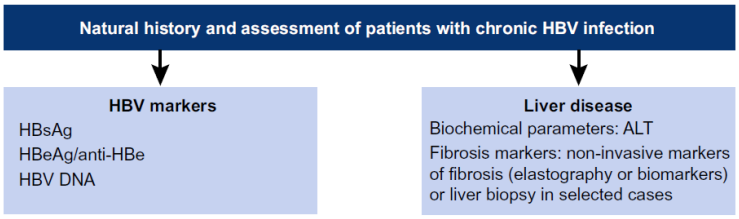
Fact box: HBV drug resistance	
Treatment	Nucleoside/nucleotide analogs (lamivudine, adefovir dipivoxil, emtricitabine, entecavir, telbivudine and tenofovir disoproxil) Interferon Treatment of HBV infection with antivirals is generally given as monotherapy.
Resistance testing method	Genotypic assays based on amplification by RT-PCR and Sanger sequencing of the product. The sequences are analyzed for amino acid mutations associated with drug resistance. A plasma viral load > 800 IU/mL is required for the analysis. In Norway, all HBV drug resistance tests are performed at the Norwegian Institute of Public Health.
Target gene	Polymerase gene (a segment overlapping with the small S-gene)
Indication for resistance testing	Virological failure/breakthrough on antiviral treatment
Surveillance	Population-level surveillance of a treatment naive population selected from samples submitted for genotyping

Hepatitis B virus: Available therapy, resistance mechanisms, recommendation of resistance testing and novel therapy strategies

Introduction

Hepatitis B virus (HBV) infection is estimated to chronically affect 240 million individuals worldwide (1), with a wide variation in national reported seroprevalence from e.g. 0.01% in Norway and the UK to >20% in South Sudan (2). Most chronic HBV infection (HBsAg positive in the blood > 6 months) is attributable to Mother-To-Child-Transmission (MTCT) in the absence of accessible infant vaccination programs (3). Other routes of transmission are sexual, household or horizontal contact, intravenous drug use or treatment with blood product or blood transfusion. The clinical outcome of transmission is determined by the immunological response, as the HBV itself is not directly hepatotoxic, despite high viral levels in liver and blood (3).

Recently, the nomenclature of the natural history and assessment of patients with chronic HBV infection has been changed, as shown in Figure 1 (adapted from EASL 2017 Clinical Practice Guidelines, (1)):



	HBeAg positive		HBeAg negative	
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis
HBsAg	High	High/intermediate	Low	Intermediate
HBeAg	Positive	Positive	Negative	Negative
HBV DNA	>10^7 IU/ml	10^4-10^7 IU/ml	<2,000 IU/ml°°	>2,000 IU/ml
ALT	Normal	Elevated	Normal	Elevated*
Liver disease	None/minimal	Moderate/severe	None	Moderate/severe
Old terminology	Immune tolerant	Immune reactive HBeAg positive	Inactive carrier	HBeAg negative chronic hepatitis

Fig. 1. Natural history and assessment of patients with chronic HBV infection based upon HBV and liver disease markers. *Persistently or intermittently. °°HBV DNA levels can be between 2,000 and 20,000 IU/ml in some patients without sings of chronic hepatitis.

In the following, available therapy for HBV infection, HBV resistance mechanisms and recommendations for resistance testing as well as novel therapy strategies will be presented.

Nucleos(t)ide analogue therapy for hepatitis B virus infection

The primary goal of chronic HBV therapy is to prevent disease progression to Hepatocellular Carcinoma (HCC) and/or mortality. Other goals are to prevent MTCT, HBV reactivation during immunosuppressive therapy and to prevent or treat HBV-related extrahepatic manifestations. Indications for treatment of patients with chronic HBV infection are: 1) compensated or decompensated cirrhosis, regardless of HBV DNA and serum alanine-amino-transferase (ALT), 2) HBV DNA >2,000 IU/ml, ALT>Upper Limit of Normal (ULN) and /or at least moderate necroinflammation or fibrosis, 3) HBV DNA > 20,000 and ALT > 2xULN, regardless of fibrosis, 4) HBeAg positivity, persistently normal ALT and high HBV DNA levels, if the patient is older than 30 years, regardless of the severity of liver histological lesions and 5) a family history of HCC (1).

Currently, there are two classes of drugs approved for the treatment of chronic HBV infection: direct antiviral agents as nucleos(t)ide analogues (NAs), that work by inhibiting the HBV DNA levels and inducing seroconversion from HBeAg to anti-HBe, and the immunomodulator pegylated interferon (PEG-IFN). In this review the focus will be on NAs.

The NAs approved are lamivudine (LAM), adefovir dipivoxil (ADV), entecavir (ETV), telbuvidine (TBV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF). Of these, the preferred regimens in Europe, including the Nordic countries, are ETV, TDF and recently TAF, with a high barrier to resistance, while, due to their low barrier to resistance, ADV, LAM and TBV are not recommended (1).

During treatment, HBV DNA and ALT should be monitored every 3 months for the first year, and every 6 months thereafter (1). The development of drug resistance begins with mutations in the polymerase gene, followed by an increase in HBV DNA, an increase in ALT levels several weeks to months later, and progression of liver disease (4).

Preventing emergence of resistance is based on the use of NAs with high or low resistance barrier. Failure to treatment can be defined as *primary non-response* (failure to achieve at least a 1.0 log 10 IU/ml decline in HBV DNA after 12 weeks of therapy), *partial virological response* (detectable HBV DNA using a real-time PCR assay during continuous therapy)

and *virological breakthrough* (an increase of at least 1.0 log 10IU/ml compared with the lowest value achieved during treatment (nadir), confirmed by a second test, in a treatment compliant patient) (4).

Hepatitis B virus resistance mutation mechanisms

Nucleos(t)ide analogues selectively target HBV DNA polymerase, resulting in premature chain termination of viral replication. Drug-resistant HBV strains have signature mutations in the reverse transcriptase domains of the viral polymerase gene (5). In case of resistance development, resistance mutations alter the interaction between the HBV polymerase and the drug, which interfere with the inhibitory effect of the drug on the viral polymerase. Compensatory resistance mutations, restoring replication capacity, and secondary resistance mutations, increasing drug resistance when they accumulate on the same viral genome, may arise after emergence of primary resistance mutations (5). Even after cessation of HBV therapy, once they have been selected, HBV resistance mutations will persist in the virus population (4).

As an example, resistance studies of patients treated for up to 5 years with entecavir (ETV) have shown that ETV-resistance (ETVr) is rare (1% over 5 Years) in NA treatment-naive patients, consistent with a high barrier to resistance (6). Resistance occurs more frequently in ETV-treated patients with LVDr HBV infection, due to the requirement for only one additional substitution at T184, S202 or M250 for high level ETVr. However, different ETVr substitutions result in various levels of phenotypic ETVr (7). In addition, ETVr substitutions in isolates with only the M204I substituted LVDr HBV and not the M204V+L180M LVDr virus, phenotypic resistance to ETV is diminished (8). Table 1, adapted from the EASL 2017 Hepatitis B Guideline (1) illustrates cross-resistance for most frequent resistant HBV variants (resistance mutations).

Table 1: Cross-resistance data for the most frequent resistant HBV variants.

HBV variant	LAM	LDT	ETV	ADV	TDF/TAF*
Wild-type	S	S	S	S	S
M204V	R	S	I	I	S
M204I	R	R	I	I	S
L180M + M204V	R	R	I	I	S
A181T/V	I	I	S	R	I
N236T	S	S	S	R	I
L180M + M204V/I ± I169T ± V173L ± M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

The amino acid substitution profiles are shown in the left column and the level of susceptibility is given for each drug: S (sensitive), I (intermediate/reduced susceptibility), R (resistant).
ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; LAM, lamivudine; ADV, adefovir.
* *In vitro* data for tenofovir, *in vivo* data for TDF, no clinical data for TAF.

Recommendation for hepatitis B virus resistance testing

With the introduction of the NAs entecavir and tenofovir, with high barriers to resistance development, antiviral resistance has become a manageable, although still very important, issue to consider. Resistance should be identified by HBV DNA monitoring, and might occur in patients with high HBV DNA baseline levels, previous suboptimal NAs therapy and slow decline in HBV DNA (1). Patient compliance is always important to consider; in a qualitative study of 29 HBV treated patients, only 59% of participants self-reported compliance, with fear of disease progression, patients´ understanding of treatment benefits and health outcomes, communication with hospital personnel and forgetfulness being factors of importance for compliance (9).

Resistance testing in Denmark

In Denmark, tenofovir and entecavir are the two NAs of choice. Resistance testing is done with Sanger Sequencing in the POL gene, in samples with a viral load of ≥ 1000 IU/ml, from amino acid 80 to amino acid 250. Baseline resistance testing is not done routinely. Since 2014, < 5 samples have been received annually for routine resistance testing in patients on NA therapy, and resistance mutations found in 66-100% of the samples tested (personal communication Henrik Krarup, Aalborg University Hospital).

Novel hepatitis B treatment strategies

Although NAs effectively suppress HBV replication, hereby reducing the risk of disease progression, they are, despite lifelong therapy, not able to clear the replication template of covalently closed circular DNA (10). This is why HBsAg seroconversion (complete cure) or sustained HBsAg clearance (functional cure), with undetectable viremia once therapy is stopped, only rarely occurs.

At present there are several potential therapies for hepatitis B under development, aiming to achieve a functional cure. Hepatitis B virus was discovered more than 40 years ago, but only very recently a milestone was reached with the discovery of the receptor for HBV entry (10). Interestingly, the receptor identified is human sodium taurocholate co-transporting polypeptide (hNTCP), a multiple transmembrane transporter required for liver bile acid transport. Myrcludex-B, which is an optimized synthetic lipopeptide, and a target of the hNTCP, is able to strongly inhibit HBV infection in both cell culture and in an in vivo mouse model (11). It is currently under investigation in phase II clinical trials. Other direct-acting antivirals are drugs aiming at cccDNA destruction or silencing, approaches targeting viral transcripts by siRNA or anti-sense oligonucleotides or approaches to decrease HBsAg release in serum (12).

Restoration of immune responses is a complementary approach. The restoration of innate immunity against HBV can be achieved, with Toll-like receptor (TLR) agonists or specific antiviral cytokine delivery. Restoration of adaptive immunity may be achieved with inhibitors of negative checkpoint regulators, therapeutic vaccines, or autologous transfer of engineered HBV-specific T cells. Novel targets and compounds will readily be evaluated using both relevant and novel in vitro and in vivo models of HBV infection (12).

Hopefully, the combination of new drugs that eliminate or functionally inactivate the genomic HBV reservoirs (cccDNA and integrated HBV-DNA) along with agents that enhance or activate immune responses against HBV will lead to a 'definitive cure' for chronic HBV infection (11).

Conclusion

With use of NA therapy with high resistance barriers, as entecavir and tenofovir, resistance development during HBV therapy has become a manageable issue. Resistance should be identified by HBV DNA and serum ALT monitoring, and the patient´s compliance always considered in case of a rise in HBV DNA. At present, NA therapy is rarely able to induce HBsAg seroconversion (complete cure) or sustained HBsAg clearance (functional cure), but hopefully, the combination of new drugs in the pipeline, direct-acting antivirals and immune modulators, will lead to a 'definitive cure' for chronic HBV infection.

Dr.Nina Weis, phd.
Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Denmark

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Surveillance of HBV drug resistance

Surveillance method: The surveillance of HBV resistance in Norway aim to monitor two populations; 1) patients that have been tested for drug resistance primarily in relation to treatment and 2) patients that are genotyped for HBV as part of the elucidation of infectious status before treatment (population-level surveillance).

Resistance determination of HBV is based on Sanger sequencing of the polymerase gene. There are currently eight amino acids within the polymerase gene that alone or in combination give rise to resistance to approved antivirals for the treatment of HBV (Table 1). The resistance patterns are indicated as sensitive, intermediate (partly sensitive) and resistant.

Table 1. Antiviral cross-resistance for HBV

Resistance mutations and their effect on antivirals for HBV					
HBV-variants (mutations)	Lamivudine	Telbivudine	Entecavir	Adefovir	Tenofovir
Wild type	S	S	S	S	S
M204I	R	R	I	S	S
L180M + M204V	R	R	I	R	I
A181T/V eller N236T	R	R	S	R	R
L180M + M204V/I ± I169T ± M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

S= sensitive, R= resistance, I = intermediate

Drug resistance surveillance data in Norway in 2016

Among the 23 patient samples tested primarily for drug resistance in 2016, five had drug resistance and one was inconclusive (Table 2). Drug resistance mutation M204V/I was detected in all five samples, whereas the combination L180M + M204V/I was detected in four of the samples. Among these four samples, more than 3 mutations were detected. In 12 of the 23 cases information on antiviral treatment was provided; 12 tenofovir, 2 entecavir and 1 lamivudine. No information was given about antiviral treatment for the cases with detected drug resistance, except for one patients receiving lamivudine. In population-level surveillance of patients primarily tested for HBV-genotype (N=163) no drug resistance mutations were detected.

Table 2. Surveillance of drug resistance among patients on HBV treatment in 2011–16.

HBV–variants resistant to antivirals	Among treated patients					
Year	2011	2012	2013	2014	**2015**	**2016**
Total analysed	14	3	9	17	**10**	**23**
Wild type	11	2	8	15	**8**	**17**
M204I	1a	0	1a	1c	**1e**	**1**[d]
L180M + M204V	1b	1a		1c		**1**[d]
A181T/V eller N236T	1a	0	0	0	**0**	**0**
L180M + M204V/I ± I169T ± M250V	0	0	0	0	**0**	**1**[d]
L180M + M204V/I ± T184G ± S202I/G	0	0	0	0	**0**	**2**[c,d]
Sequencing inconclusive					**1**	**1**

, a=entecavir, b=tenofovir, c=lamivudine, d=treatment unknown, e= telbuvidin

Conclusion

The data presented on HBV resistance in Norway is based on samples from two patient populations; 1) samples where resistance testing has been requested and 2) samples that are analysed for genotype as part of the diagnosis (population-level surveillance).

We see a slight increase in the number of patients tested for resistance as compared to previous years. However, the numbers are low and we cannot draw any conclusions about trends. Information about antiviral treatment was available for only twelve of 23 received samples. First line treatment (tenofovir or entecavir) was given to eleven of these. Resistance mutations were detected in five of the patients. Unfortunately, information about antiviral treatment was available for only one of them. This patient received lamivudine. No resistance mutations were detected among the eleven patients known to receive first line treatment. These are drugs known to have a high barrier to resistance. Additionaly, among the 163 samples submitted primarily for genotyping, no resistance mutations were detected.

In conclusion, resistance against antiviral drugs for HBV-infections seem to be a minor problem in Norway, especially after the introduction of the drugs tenofovir and entecavir. However, our data are limited due to small numbers of collected samples, and to insufficient information given on sample requisitions.

Antiviral drug resistance of human herpes viruses

The guanosine analogues aciclovir (ACV) and ganciclovir (GCV) are the drugs of first choice for treatment of herpes simplex virus (HSV) and cytomegalovirus (CMV) infections respectively. Both drugs have a low bioavailability when administered orally. This can be improved substantially by prodrugs like valine esters of ACV and GCV. To be effective both drugs have to be triphosphorylated. In HSV infected cells ACV is monophosphorylated by a viral thymidine kinase (HSV-TK) whereas in CMV infected cells GCV is monophosphorylated by the phosphotransferase UL97. The drugs are then di- and tri-phosphorylated by cellular enzymes. ACV- and GCV-triphosphate are incorporated into the growing strand of viral DNA by HSV DNA polymerase or CMV DNA polymerase (UL54) and then causes termination of the growing viral DNA strand. Resistance to ACV develops by mutations of either the HSV-TK- or HSV DNA polymerase gene whereas resistance to GCV develops by mutations of either the CMV-UL97-or UL54 gene. The virus drug resistance has been characterized by comparing phenotypic and genotypic test results. For routine testing only genotypic tests, looking for known resistance mutations, are applicable.

ACV and GCV are effective antiviral drugs with few side effects and thus widely used. If resistance develops alternative drugs for anti-HSV and anti-CMV treatment are cidofovir (CDV) and foscarnet (FOS). CDV is a nucleotide analogue and act as a virus DNA chain terminator. It contains a phosphate group attached to the sugar analogue and is thus independent of HSV-TK or UL97 phosphorylation. Some mutations in the virus DNA polymerases may confer HSV and CMV resistance mutations. FOS is an inhibitor of HSV- and CMV DNA polymerase and some mutations coding for these viral DNA polymerases confer resistance to FOS.

Cytomegalovirus

Fact box: Human cytomegalovirus (CMV) drug resistance	
Treatment	Ganciclovir (nucleoside analog) Cidofovir (nucleotide analog) Foscarnet (polymerase inhibitor)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analyzed for amino acid mutations associated with drug resistance. In Norway, all CMV drug resistance tests are performed at the National Reference laboratory for CMV at the Department of Microbiology at Oslo University Hospital, Rikshospitalet.
Target genes	CMV-genes UL97 and UL54
Indication for resistance testing	Persistent high viral load in blood or other compartments during antiviral treatment
Surveillance	Population-level surveillance is currently not necessary

In the years 2008 to 2015 the laboratory annually received 10 to 27 specimens for CMV resistance tests. The number of specimens containing drug resistant CMV ranged from 4 to 8.

In 2016, 43 specimens were received for genotypic analysis of CMV resistance mutations. Fifteen specimens were not analyzed because the amount of CMV DNA was too low or resistance mutation had been detected in recent specimens from the same patients. In specimens from the remaining 28 patients, CMV resistance mutations were recorded in 8 patients whereas no resistance mutations were detected in specimens from 20 patients. With the exception of one colon biopsy the specimens were plasma samples. All samples were from patients on immunosuppressive therapy.

As can be seen from Table 1, patient 1 had a combination of UL97 and UL54 mutations that made the CMV infection resistant to all three available anti-CMV drugs (GCV, CDV, FOS). The CMV infection in patient 5 was resistant to ganciclovir and intermediately resistant to cidofovir.

Table 1. CMV-resistance mutations recorded in patients tested in 2016

Patient	UL97 mutations[1]	UL54 mutations
1	H520Q	A987R[2], A809V[3]
2	C592G	None
3	A594V,L595W	None
4	A594P	None
5	L595S	P522S[4]
6	L595S	Not analyzed
7	L595S	None
8	Del 597-599	None

[1]GCV resistance
[2]CDV resistance
[3] FOS and GCV resistance
[4]Intermediate CDV resistance

Herpes simplex virus

Fact box: Herpes simplex virus (HSV) drug resistance	
Treatment	Aciclovir (nucleoside analog) Valaciclovir (nucleoside analog)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analyzed for amino acid mutations associated with drug resistance. All HSV drug resistance tests for Norway are performed at The Public Health Agency of Sweden, Stockholm.
Target gene	HSV thymidine kinase gene
Indication for resistance testing	Persistent HSV-infection despite ongoing therapy
Surveillance	Population-level surveillance is currently not necessary

Indication for resistance testing is treatment failure when on ACV therapy for HSV-infection. In the years 2009 to 2015 zero to 4 specimens were sent and only 3 of these specimens contained ACV resistant HSV.

In 2016 seventeen samples from Norwegian patients on ACV treatment for HSV-1 or HSV-2-infections were analyzed. Seven patients were infected with HSV-1 and 10 with HSV-2. HSV-1 ACV resistance mutations were seen in one of the specimens whereas one of the specimens contained HSV-1 with no such mutations. The remaining 5 specimens contained insufficient amounts of HSV-1 DNA for PCR amplification and sequencing. In specimens from 10 HSV-2 infected patients 2 contained ACV resistance mutations, 7 had no resistance mutations whereas one contained insufficient amounts of HSV-2 DNA for amplification and sequencing

Table 2. HSV-1 and HSV-2 acyclovir resistance mutations detected in the thymidine kinase gene

	HSV-TK ACV resistance mutations
HSV-1	G206V[1]
HSV-2	280delG[2]
HSV-2	G201D

[1]Not previously recognized as acyclovir resistance mutation, but localized in a region of the gene where resistance mutations are clustered.
[2]No acyclovir, FOS or CDV resistance mutations in the HSV-2 DNA-polymerase gene

Conclusion

Despite increasing consumption of ACV and GCV for therapeutic and prophylactic use for HSV and CMV infections treatment failures due to resistance mutations seem to be a minor problem. One patient was infected with CMV resistant to all three anti-CMV drugs available Norway.

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Hepatitis C virus

Fact box: Hepatitis C virus (HCV) drug resistance	
Treatment options	Pegylated interferon and ribavirin Telaprevir (NS3/4A-inhibitor) Boceprevir (NS3/4A-inhibitor) Sofosbuvir (NS5B-inhibitor) Simeprevir (NS3/4A-inhibitor) Daclatasvir (NS5A-inhibitor) Dasabuvir (NS5B-inhibitor) *Fixed combinations:* Sofosbuvir + ledipasvir (NS5A-inhibitor) Paritaprevir/ritonavir (NS3/4A-inhibitor) + ombitasvir (NS5A-inhibitor)
Resistance testing method	Sequencing of relevant genes and/or the complete HCV genome Currently, a NGS-based method is under development at the Norwegian Institute of Public Health.
Target genes	NS3–NS4A (protease) NS5A (replication and assembly factor) NS5B (polymerase)
Indication for resistance testing	Virological failure during treatment
Surveillance	Primary resistance testing on selected genotypes and genomic regions from clinical routine samples

Next-Generation Sequencing of hepatitis C virus

In diagnostics and research, genotyping and sequencing commonly rely on commercial and/or in-house probe-assays and/or amplification of a conserved region of interest in combination with Sanger sequencing. In recent years, Next-Generation Sequencing (NGS), or high-throughput sequencing, has become a readily affordable and available technology to perform high-throughput partial and/or whole genome sequencing of viral pathogens. As such, with respect to detection of hepatitis C virus (HCV) resistance-associated mutations and/or partial or complete genomes for e.g. molecular epidemiological studies, there are in principle two main options; Sanger population/majority variant consensus sequencing or NGS.

In order to be suitable for clinically and epidemiologically relevant routine diagnostics, the method of choice should be able to detect and sequence (sub)genotypes that are commonly occurring in a certain country or, preferably, a larger geographic region as to accommodate potential influx of immigrants with uncommon (sub)genotypes. However, the main hurdle of designing a region-specific and pan-genotypic screening/sequencing approach for HCV is the high genetic variation between and within genotypes. With at least 7 genotypes and 67 (sub)genotypes, designing primers that capture the entire global or regional diversity of even a single (sub)genotype and genetic region becomes a significant challenge, with limited capability to detect minor variants and/or co-infections

of multiple (sub)genotypes that might be clinically relevant. Furthermore, a key difference between Sanger population sequencing and NGS is that while both Sanger population sequencing and NGS have the option to sequence amplicon products of primer-targeted specific regions, only NGS can produce truly template-independent relatively unbiased complete HCV genomes belonging to any genotype in a single sequence run.

In a recent review by Bartlett *et al*. (2017), eight good candidate methods have been identified, as well as limitations of current methods, which were suggested for further validation, standardisation and implementation. The review focused primarily on Sanger population sequencing, however the NGS method by Bonsall *et al*. (2015), which was not among the eight candidate methods, was said to be "*a candidate for determining the presence of all key [direct-acting antiviral] [resistance-associated substitutions] across all regions of interest in samples of any genotype*". The main difference with Bonsall *et al*. (2015) method compared to the other methods is that they used a target enrichment library prep approach followed by NGS. A 'problem' with RNA-sequencing by NGS is that all transcripts are amplified and sequenced regardless if they are host- or pathogen-associated transcripts (i.e. metagenomic sequencing). This gives you the option to identify other bacterial or viral pathogens present in a sample. At the same time, host-reads will take up the absolute majority of all sequence reads, which will be discarded in subsequent analyses and is thus cost-inefficient, and will commonly result in lower depth and only partial assemblies of virus genomes of interest, in particular for samples with low viral loads. The problem with low numbers of viral reads and coverage can to a large extent be minimised by sequencing on a platform with higher throughput, but for routine use this is not likely to be an option as the cost will be significant.

Target enrichment provides a good solution for several of the problems above. The basic principle of target enrichment is the utilisation of predesigned baits that are allowed to hybridise with RNA or DNA templates, e.g. covering genetic regions or genomes of interest, whereafter the baits and hybridised templates are 'pulled out' with streptavidin-coated magnetic beads. The result of this approach is that mainly RNA/DNA-templates of interest are enriched and subsequently sequenced, resulting in sequence data that has high number of template target reads and coverage. As such, target enrichment allows for sequencing on platforms with relatively moderate throughput. Several target enrichment approaches, directed against HCV, have been assessed in Thomson *et al.* (2016). Most of the methods tested had a lower limit of recovering complete or near-complete HCV genomes at somewhere between 1000–10000 IU/ml, thus being within range and relevance for routine sequencing of clinical samples. An NGS target enrichment-approach is also currently under development at the Norwegian Institute of Public Health. Initial test-runs have with optimisation proved very successful in recovering complete HCV genomes as well as detecting low-frequency minority variants and co-infections.

Importantly, this approach not only allows for simultaneous detection of resistance-associated substitutions at all relevant sites in the HCV genome for any (sub)genotype, but also allows for further molecular epidemiological analyses based on complete genomes. Complete genomic data are clearly superior to smaller targeted genetic regions for the study of pathogen transmission within and between populations and geographical regions etc. Therefore, target enrichment in combination with NGS is well suited for routine surveillance of resistance and transmission as well as guiding research efforts and public health intervention strategies.

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P. O. Box 4404 Nydalen
NO-0403 Oslo
Tel: +47-21 07 70 00
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