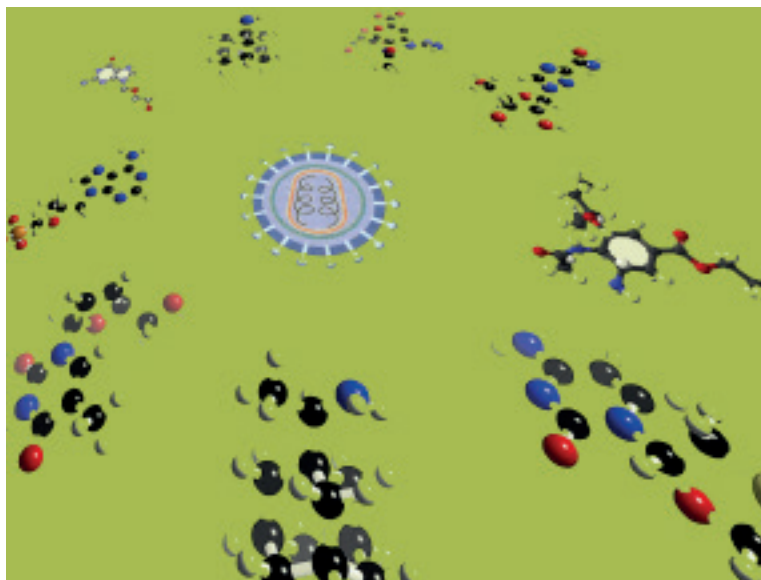2016

Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2015

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

Norwegian Institute of Public Health

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Introduction

The number of available antiviral drugs for treating viral infections is increasing. The emergence of drug-resistant viruses is well documented as a cause of treatment failure. Especially immunocompromised patients are regarded as an vulnerable group in this respect. Drug-resistant viruses may potentially be transmitted, as described for HIV-1 and influenza virus. Resistance to the new generation hepatitis C virus inhibitors is also likely to become a cause of concern. It is therefore important to conduct continious surveillance in order to detect any emergence or change in drug resistance and to develop optimal treatment regimen based on such information.

It is a pleasure to present the third report from the surveillance system Resistance against Antivirals in Norway (RAVN). This report presents new data for 2015 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: Clinical considerations for antiviral resistance in influenza infection, new aspects in treatment of HIV with particular focus on integrase inhibitors, and results of a small survey on clinical management of HBV in Norway. Dr. Johan Lennerstrand is invited by RAVN to give a review on new direct-acting antivirals and resistance testing for hepatitis C virus. It is our hope that the report contains valuable data for clinical doctors, microbiologists and those developing treatment regimens and strategies to prevent transmission of viral infection.

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

Oslo, September 2016

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Sammendrag

Bruk av antivirale midler

I løpet av siste to tiår har utviklingen av nye spesifikke antiviralia akselerert på grunn av forskning på nye hiv- og hepatitt C virus (HCV) medikamenter. Utgifter til antiinfeksiøse midler til systemisk bruk har økt med 23% i 2015. Introduksjon av nye antivirale midler for behandling av HCV infeksjon har sterkt bidratt til denne økningen.

Influensavirus

Alvorlig influensa krever spesifikk antiviral terapi. Behovet for resistensundersøkelse bør vurderes i noen situasjoner. Immunsupprimerte pasienter har høyere risiko for resistent virus på grunn av langvarig virusutskillelse og høy viruskonsentrasjon. Prøver tatt ved alvorlig eller fatal influensa bør resistensbestemmes, samt prøver fra pasienter som pådrar seg influensa under posteksponeringsprofylakse. Under utbrudd i institusjoner kan resistenstesting vurderes.

Under 2015/16 sesongen inneholdt 10 av 336 prøver virus resistente mot oseltamivir. Bare fire av disse tilfellene var hospitaliserte, og to av disse fire pasientene var behandlet med oseltamivir. De seks øvrige tilfellene var polikliniske pasienter som ikke fikk antiviral behandling. De oseltamivirresistente stammene var fortsatt følsomme for zanamivir. Selv om totalt antall H1N1 oseltamivirresistens var nesten tre ganger høyere i Norge (3 %) enn i Europa (~1%), er antallet fortsatt lavt.

HIV-1

De fleste hiv-positive pasienter i Norge blir i dag tilbudt tidlig oppstart av antiretroviral terapi, i samsvar med resultatene av START (HIV-1 Treatment Study). Integrase-inhibitorer i kombinasjon med revers transkriptasehemmere er foretrukket førstelinje-behandling, men eldre behandlingsregimer med proteaseinhibitorer kan fortsatt benyttes. God etterlevelse av behandlingen er viktig for å hindre resistensutvikling.

Det nasjonale overvåkingssystemet for hiv-1 i Norge monitorerer prevalens av overført antiviral resistens. Overvåkingen er basert på resistensbestemmelse av prøver tatt fra nylig diagnostiserte pasienter. I 2015 ble SDRM (Surveillance Drug Resistance Mutations) påvist i 8% av analyserte sekvenser. Nasjonal overvåking er viktig for å kunne implementere forebyggende tiltak for å hindre spredning av resistente hiv-stammer.

Hepatitt B virus

Ti pasienter med hepatitt B virus (HBV) infeksjon ble testet for antiviral resistens i 2015. Resistens ble påvist hos bare en virusstamme, fra pasient behandlet med telbuvidine. Blant pasienterprøver som ble HBV-genotypet (N=165) og som antas å være behandlings-naive, ble ingen resistensmutasjoner påvist, tilsvarende som for de tre foregående år.

En spørreundersøkelse om oppfølging av pasienter med kronisk hepatitt B og bruk av laboratorietjenesten ble utført i regi av RAVN, for å få data om grunnlaget for resistens-overvåkingen. Questback ble sendt via epost til medlemmer av Norsk forening for infeksjonsmedisin og Norsk gastroenterologisk forening. Resultatet fra undersøkelsen er presentert.

Humane herpesvirus

Det finnes antivirale midler mot følgende virus i herpesvirusgruppen: herpes simplex virus 1 og 2 (HSV-1, -2), varicella zoster virus (VZV) og cytomegalovirus (CMV). De mest brukte midler mot disse virus er guanosinanaloger slik som acyclovir (ACV) mot HSV-1, HSV-2 og VZV. Ganciclovir (GCV) er det mest brukte middel mot CMV-infeksjoner.

Til tross for stort forbruk av ACV både til behandling og profylakse mot HSV-infeksjoner, ser en sjelden HSV resistent mot ACV. Hvert år mottas 0 til 4 prøver for resistensundersøkelse. I tidsrommet 2009 til 2015 er det bare påvist tre ACV-resistente HSV-stammer.

Det er et økende forbruk av GCV både til behandling og profylakse mot CMV infeksjoner. I tidsrommet 2008 til 2015 er det årlig mottat fra 12 til 27 prøver, til sammen 160 prøver, for resistensundersøkelse. I 47 (29%) av disse prøvene ble det påvist GCV-resistent CMV. Antall påvisninger av GCV resistent CMV har variert mellom 4 og 8 per år.

Forekomsten av ACV-resistent HSV eller GCV-resistent CMV har ikke vist noen økning de siste 7-8 år.

Hepatitt C

Interferonfri behandling med direkte virksomme medisiner (direct acting drugs, DAA) har økt mulighetene å bli kvitt hepatitt C viruset (HCV). I Norge har utgiftene til antivral behandling steget med 23% for 2015. Økningen er forårsaket av DAA mot HCV.

Legemiddelinnkjøpssamarbeid (LIS) har bestemt rammer for forbruk av DAA og behandlingsrammer for pasienter med HCV. LIS vektlegger en at pasienten må ha påvisbar leversykdom for å få DAA.

Dr. Johan Lennerstrand er invitert av RAVN til å skrive en oversikt over DAA og resistensutfordringer. Tolkning av funn , valg av metoder og kvalitetssikring er utfordrende oppgaver for oss. HIV kan være en god læremester. RAVN dagen 17 november 2016 vil utdype disse utfordringene.

Det er lagt vekt på Y93H/N mutasjonen hos genotype 3a (den mest prevalente genotypen i Norge) og Q80K mutasjonen hos genotype 1a (den nest meste prevalente genotypen i Norge). Lennerstrand påpeker at på tross av gode kombinasjonsbehandlig, vil slik resistens kunne føre til mangel på antiviral respons.

Slik det fremkommer fra LIS er genotype 3a mest kostbar å behandle. Påvisbar leversykdom som inngangskriterium oppfattes av mange behandlende leger som utfordring.

Summary

The usage of antivirals

During the last two decades, the development of new specific antivirals has accelerated due to research into HIV and hepatitis C medicines. According to the Norwegian Drug Wholesales statistics database, cost of anti-infectives for systemic use increased by 23% in 2015. Introductions of new antivirals for treatment of hepatitis C virus infections highly influence the cost in this group.

Influenza virus

Severe influenza virus infection requires specific antiviral therapy. The need for resistance testing should be considered in some situations. Immunocompromised patients have a higher risk of developing antiviral resistance because of prolonged virus shedding and high viral load. Severe and fatal influenza cases can be selected for susceptibility testing as well as patients achieving influenza during post-exposure prophylaxis. During outbreaks in hospitals or nursing homes, resistance testing can be considered.

During the 2015/16 season 10 strains out of 336 tested were resistant towards oseltamivir. Only four of the patients infected with resistant strains were hospitalised and two of these four were treated with oseltamivir. The six other cases were out-patients not receiving antiviral therapy. The oseltamivir resistant viruses are still sensitive to zanamivir. Although the overall H1N1 oseltamivir resistance numbers from Norway were almost three fold higher in Norway (3 %) than in Europe (~1%), the numbers are still low.

HIV-1

Most HIV-1 positive persons in Norway are offered early treatment according to the results of the START study. Integrase inhibitors in combinations with reverse transcriptase inhibitors are preferred as initial therapy, but older regimen with protease inhibitors can still be used. Proper administration of medication is essential to avoid resistance development.

The national surveillance system for HIV-1 in Norway monitors the prevalence of transmitted drug resistance. The surveillance is based on resistance testing of samples taken from newly diagnosed patients. In 2015 SDRMs (Surveillance Drug Resistance Mutations) were detected in 8% of the analysed sequences. Surveillance of HIV resistance is important to make decisions on implementing preventive measures to control dissemination of resistant HIV.

Hepatitis B virus

Only ten patients were tested for HBV drug resistance in 2015. Resistance was only detected in one patient, currently on telbuvidine treatment. Among patients requested for HBV-genotyping (N=165) and presumed treatment naive, no drug resistance mutations were detected, the same as for the three previous years.

A small survey on HBV management and usage of laboratoty service was conducted by RAVN to gain more insight into HBV management in Norway and the basis for the drug resistance surveillance data. A questback was sent by email to members of the Norwegian

Society of Infectious Diseases and the Norwegian Society of Gastroenterology. The result is presentet in this report.

Human herpesviruses

There are anti-viral agents against the following viruses in the herpes virus group: herpes simplex virus 1 and 2 (HSV-1, -2), varicella zoster virus (VZV) and cytomegalovirus (CMV). The most commonly used agents for treatment of infections with these viruses are guanosine analogues such as acyclovir (ACV) against HSV-1, HSV-2 and VZV. Whereas ganciclovir (GCV) is the most widely used drug for treatment of CMV infections.

Despite the large consumption of ACV both for treatment and prophylaxis of HSV infections ACV resistant HSV is rarely seen. During the years 2009 to 2015 annually zero to four specimens were received for ACV resistance testing. Among the 14 specimens received during this period only three contained ACV-resistant HSV.

The consumption of GCV both for treatment and prophylaxis of CMV infections is increasing. During the years 2008 to 2015 we annually received from 12 to 27 samples totaling 160 samples for CMV- resistance testing. In 47 (29%) of these samples GCV resistant CMV was detected. The annual incidence of GCV resistant GCV strains ranged from 4 to 8.

During the last 8 years the incidence of ACV-resistant HSV or GCV-resistant CMV has not increased.

Hepatitis C

Interferonfree treatment with direct acting drugs (DAA) has increased the changes of getting rid of hepatitis C virus (HCV) . In Norway the expenses have increased by 23% for 2015. This is increase is due to DAA against HCV.

Legemiddelinnkjøpssamarbeid (LIS) has decided the frame for use of DAA for patients with HCV. LIS recommend DAA for patients with detectable liver disease .

Dr. Johan Lennerstrand is invited by RAVN to give a review on DAA and resistance. Interpretation of findings, choice of methods and quality assurance are challenging tasks for us. Our HIV experience can guide and teach us in our work. RAVN Day 17. November 2016 will eloborate these challenges.

Lennerstrand concentrates on Y93H/N mutations in genotype 3a (the most prevalent in Norway) and Q80K mutation in genotype 1a (the second most prevalent genotype in Norway). In spite of very good drug combinations, Lennerstrand points that antiviral resistance can lead to treatment failure.

The LIS circular shows that genotype 3a is the most costly to treat. Many physicians regard detectable liver disease as criterium for DAA treatment an ethical dilemma.

The usage of antivirals in Norway

During the last two decades, the development of new specific antivirals has been accelerated due to research into HIV medicines (1) and hepatitis C virus (HCV) medicines. The prescribed amount of antiviral drugs has been increasing every year. According to The Norwegian Drug Wholesales statistics database, antiinfectives for systemic use (ATC group J) cost increased by 23% in 2015 (3). Introductions of new antivirals for treatment of HCV infections highly influence the cost in this group. Figure 1 shows the sales of all direct acting antiviral drugs (DAA)(ATC group J05A), during the past five years.
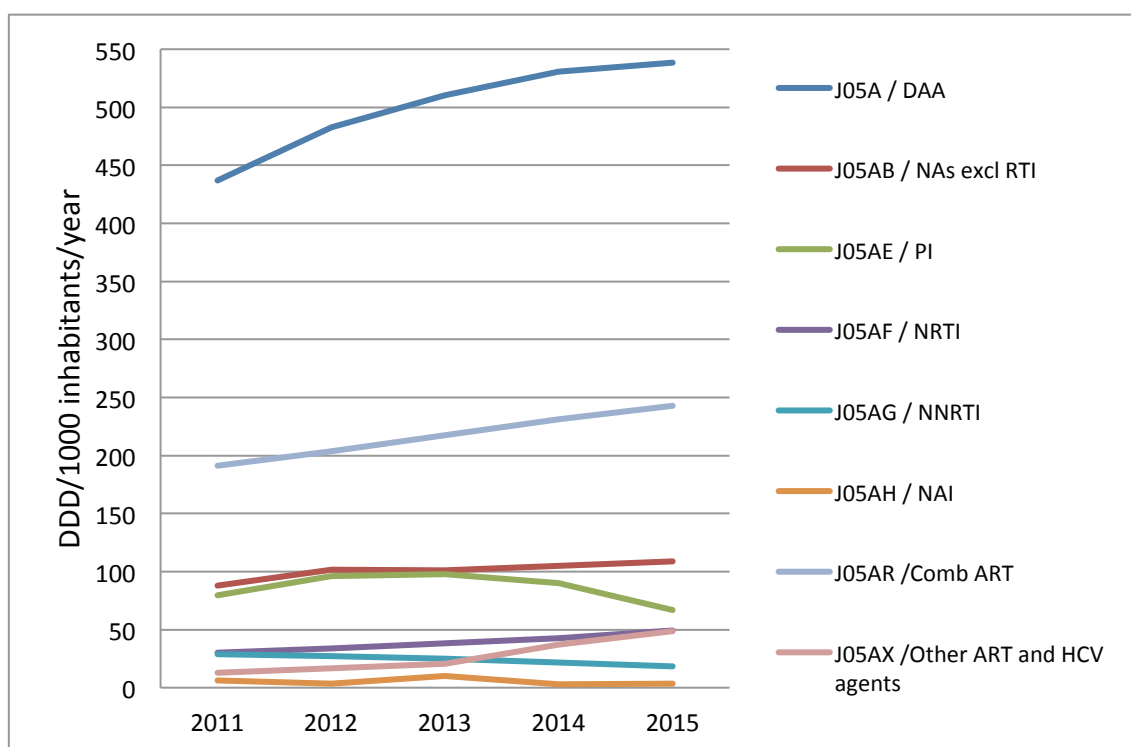
Figure 1. Sales of direct acting antiviral drugs (DAA), ATC group J05A for 2011–2015 given in DDD/1000 inhabitants/year. Source: The Norwegian Drug Wholesales statistics database.

Influenza virus

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

NI drug	Number of individuals with one or more prescription per annum				
	2011	**2012**	**2013**	**2014**	**2015**
Zanamivir	36	33	85	18	52
Oseltamivir	2612	1724	3911	1076	1473

Table 1. Number of individuals with at least one prescription of neuraminidase inhibitor (NI) drug according to year.
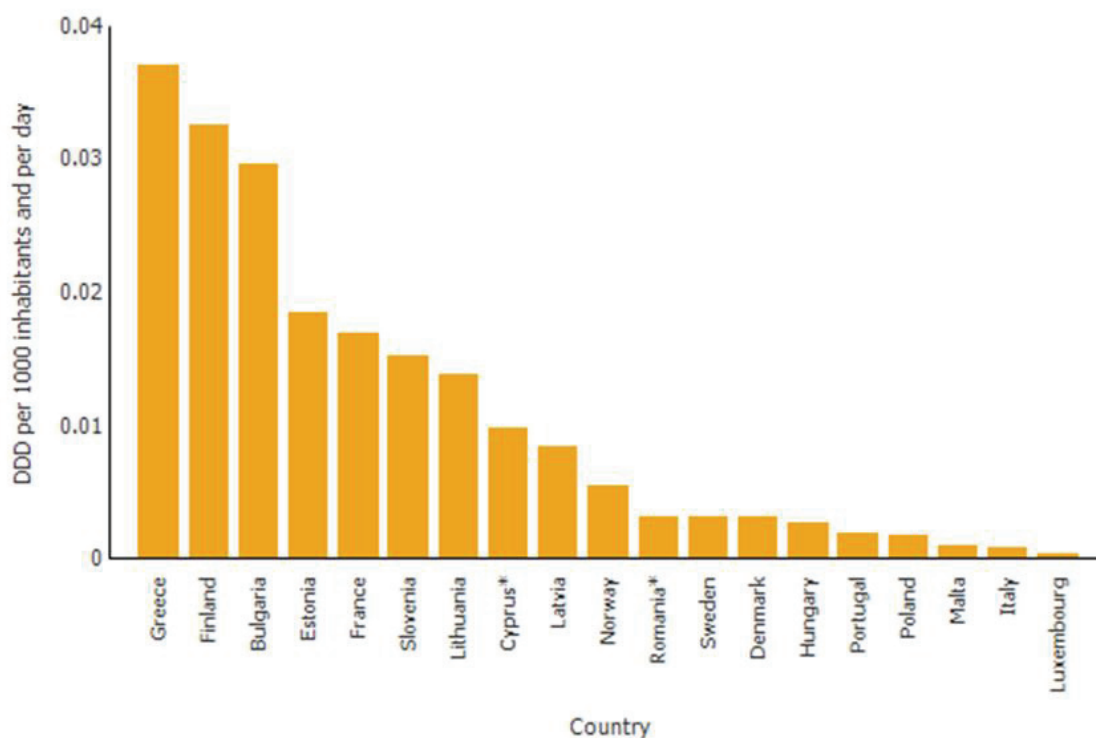
Figure 2: Consumption of neuraminidase inhibitors (ATC group J05AH) in the community and hospital sector in Europe, by country (reported 2014)

*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 in Norway. The number of patients given at least one prescription of these drugs has increased almost 50 % from 2011 to 2015. 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 2015 more than 3600 persons were treated with these fixed combinations in Norway. Single substance products could be given in addition to the fixed combination in 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 the latest years and it is expected that this trend will continue. Changes in usage may be due to these new combinations. The usage of the fixed combination of emtricitabine and tenofovir

disoproxil has been increasing the latest years but for the first time a small decrease is seen in 2015. The triple combination including emtricitabine, tenofovir disoproxil and rilpivirin has steadily increased since the introduction in 2012. Since 2012 two new fixed combinations of three active substances and one fixed combinations with four substances (emtricitabine, tenofovir disoproxil, elvitegravir and cobicistat) has been introduced. The combination of lamivudine, abacavir and dolutegravir (integrase inhibitor), introduced in 2014, are the most used of the combinations introduced after 2012.
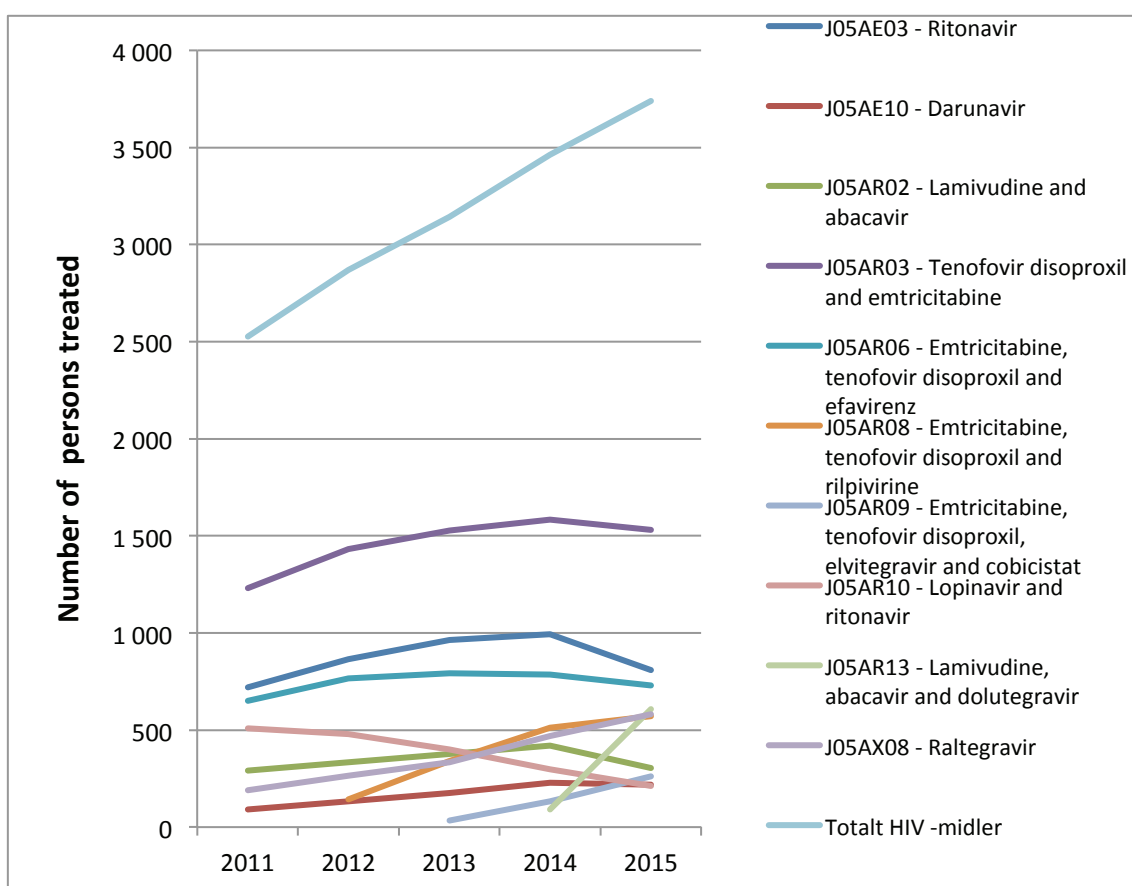
Figure 3: Trends in the use of antivirals for treatment of HIV for the period 2011–2015. The 10 most used agents. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Hepatitis B virus

There are currently 8 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 2011– 2015. 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 295–695 in 2015. 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 over 90% of the six NA treatments given in 2015.

Figure 4. Patterns of prescriptions for HBV-treatment from 2011–2015 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). An additional fixed combination of ledipasvir (NS5A inhibitor) and sofosbuvir has also been marketed. With these 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 usage of antivirals is expected to increase further in the coming years in connection with the introduction of these new drugs.
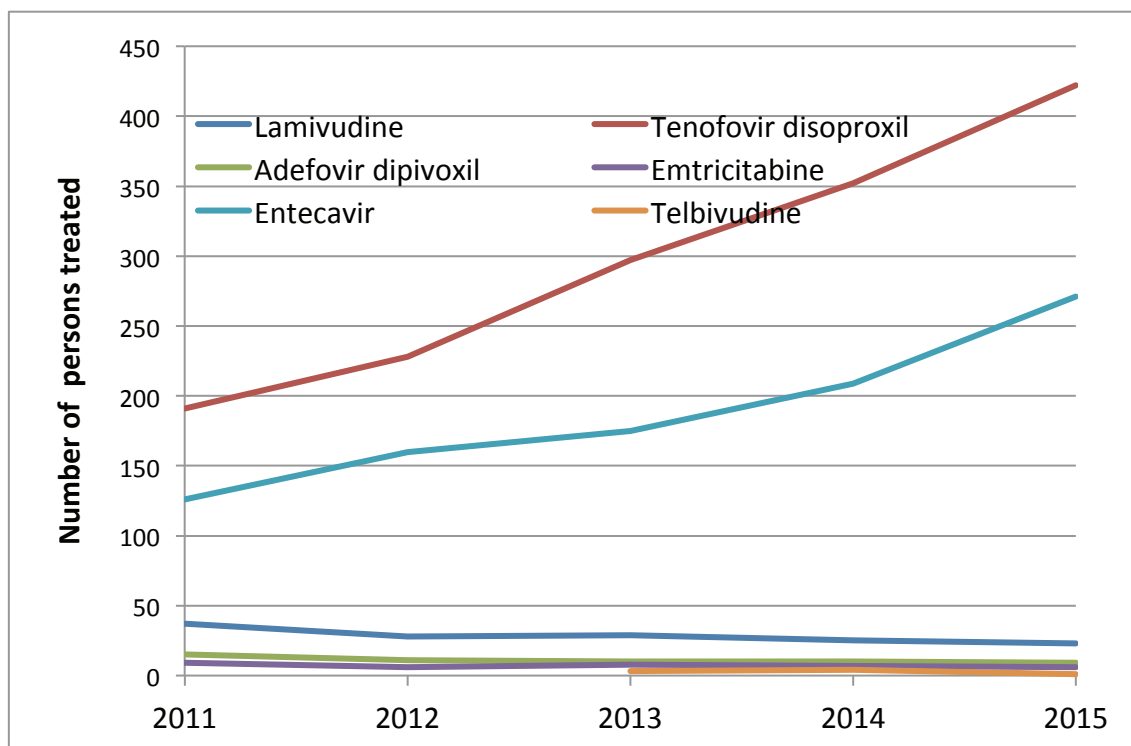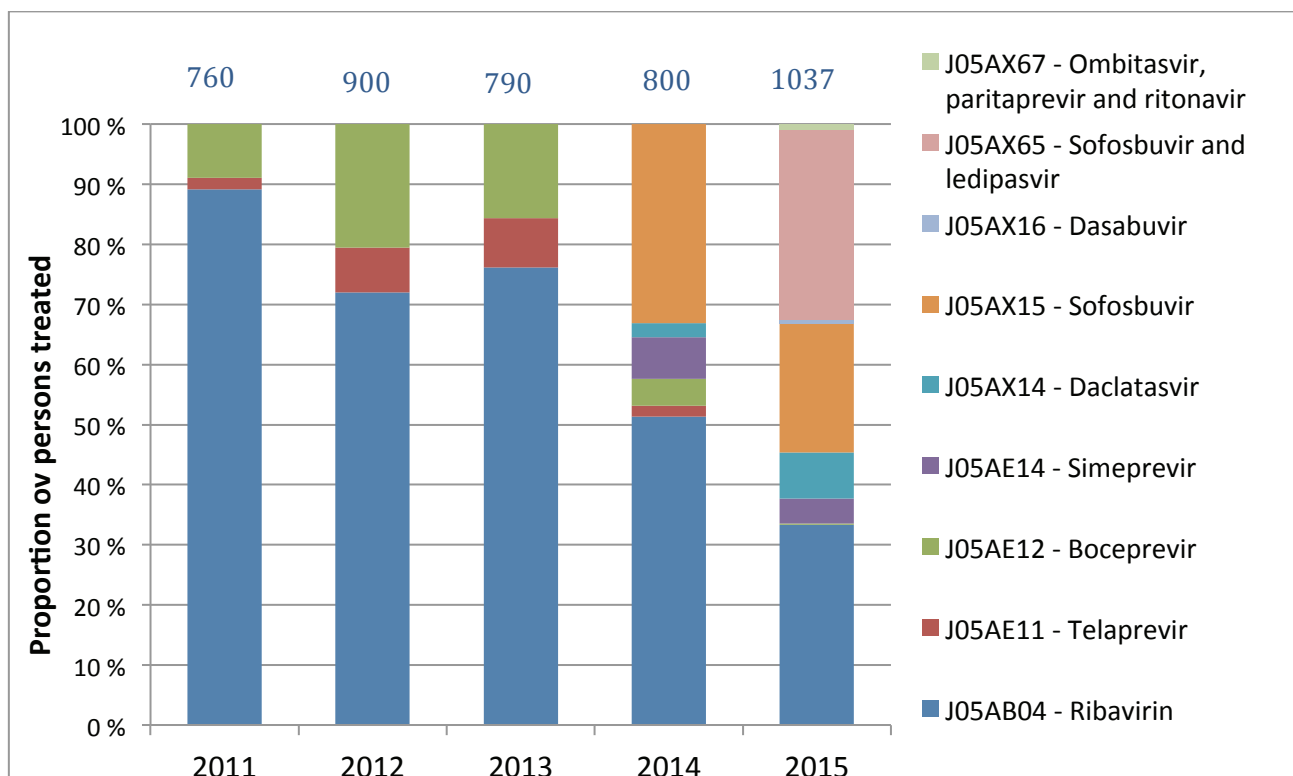
Figure 5. Patterns of prescriptions for HCV-treatment from 2011–2015 based on the number of patients given at least one prescription per year. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

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, valganciclovir is prescribed more often.
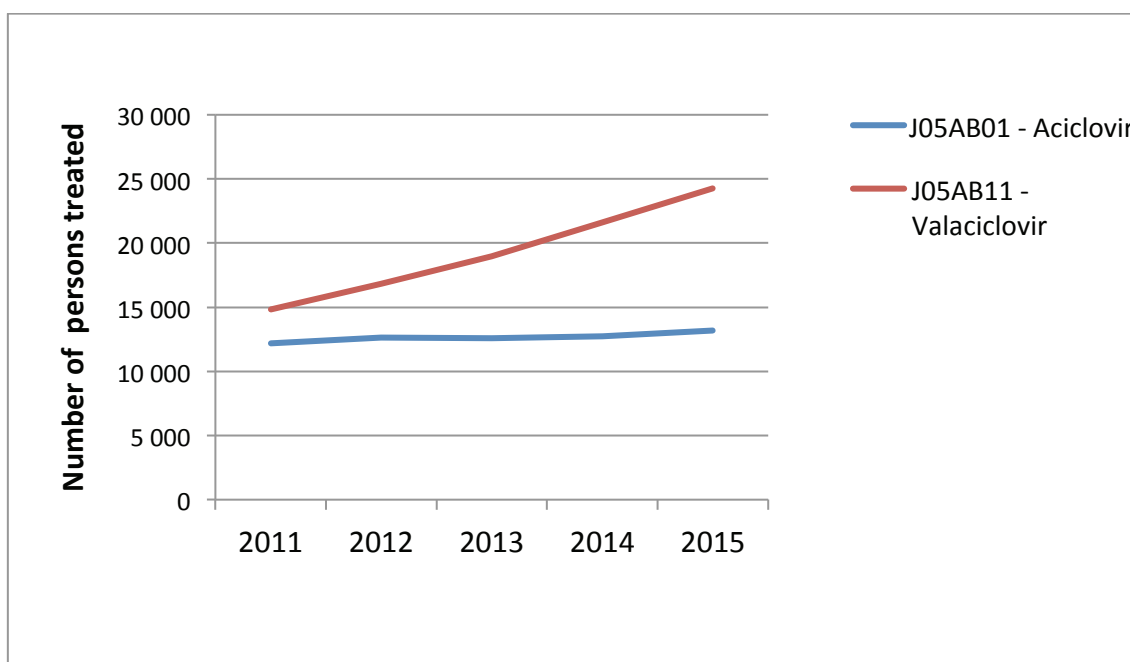
Figure 6. Number of individuals with at least one prescription of acyclovir and valaciclovir per year for the periode 2011–2015.

	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

Table 2. Number of patients given prescription for human herpes virus infections per year for the periode 2011-2015. Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health.

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1. Arts, E and Hazuda D. HIV-1 Antiretroviral Drug Therapy. Cold Spring Harb Perspect Med 2012;2:a007161.

2. Norwegian Prescription database (NorPD) www.norpd.no.

3. Sakshaug, S (ed), Drug Consumption in Norway 2011–2015 (Legemiddelforbruket i Norge 2015–2015), Norwegian Institute of Public Health, Oslo, legemiddelstatistikk 2016:1.

Influenza virus drug resistance

Fact box: Influenza virus drug resistance	
Resistance testing method:	Genetic by pyrosequencing, snp-real-time PCR and phenotypic by neuraminidase activity assay (MUNANA)
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.
Surveillence method:	Screening for resistance as part of the national influenza surveillance program. Samples from both untreated and treated patients. No active surveillance program for treatment induced resistance.
Indication for resistance testing:	Random screening of sentinel and confirmatory respiratory samples submitted through the influenza surveillance program. Antiviral treatment failure, long-time admitted patients with prolonged virus shedding.
Treatment options:	Oseltamivir, Tamiflu® Zanimivir, Relenza®

Antivirals against influenza

Two classes of antivirals have been developed for treatment or prophylaxis of influenza, M2-blockers and neuraminidase (NA) inhibitors. Oseltamivir (Tamiflu) a prodrug, Zanamivir (Relenza), Laninamivir (Inavir), and Peramivir belong to the neuraminidase class, but only Oseltamivir and Zanamivir are licensed in Norway. Most cases in need of antiviral treatment are given oseltamivir, as zanamivir is only available as an inhalation drug. When used profylactically, oseltamivir is proven to be effective in up to 89 % of healthy adults, and zanamivir similarly in up to 84 %. It has been shown that the drugs reduce the duration of symptoms, reduces the risk of lower respiratory tract complications and admittance to hospitalisation in patients with influenza-like illness (Dobson J Lancet 2015). Two other recently developed NA inhibitors, peramivir and laninamivir, have been approved for use in Japan. Peramivir (Rapivab) is also approved in USA. Other anti-influenza drugs that target different stages of viral replication such as favipiravir (T-705) and nitazoxanide (Alinia) are also in late-stage clinical trials.

Development of resistance

The rapid evolution of the influenza viruses also has an impact on the susceptibility against antiviral drugs. The high mutation rate in RNA viruses such as influenza provides an opportunity for selection of resistant viruses. Resistance can grow in the absence of antiviral agents as long as the mutation which confers resistance does not cause any significant evolutionary disadvantage for the virus. Resistant viruses vary in their ability to transmit from one person to another.

For reasons that remain unclear, the frequency of M2 blocker resistance in human influenza A (H3N2) viruses gradually rose from almost nil to 100 percent during the first decade of this century, and the pandemic influenza A (H1N1) virus that emerged in 2009 has been uniformly resistant to M2 blockers since the start. During the winter season

2007–2008 resistance to oseltamivir (substitution H275Y in the NA protein) was observed in an unexpectedly high proportion of influenza A (H1N1) viruses in Norway and the same development was reported from other countries conducting susceptibility surveillance. The emergence of oseltamivir resistance was not associated with prior use of anti-influenza agents.

Recent reports show that 98% of circulating influenza viruses are susceptible to neuraminidase inhibitors, however occasionally viruses with reduced inhibition against at least one neuraminidase inhibitor are found. There have been reported outbreaks of oseltamivir resistance in 2011 in Australia and in a large community cluster in Okaido, Japan in 2013-2014.

Zanamivir resistance has been detected sporadically in influenza B viruses. The structural similarity between the natural substrate and zanamivir, and a high concentration of the drug in the respiratory tract where virus replication occurs, help to reduce the risk of resistance development.

Clinical considerations for antiviral resistance in influenza infection

Severe influenza infection requires specific antiviral therapy. In cases with immunodeficiency, resistance could affect the course of the disease as these patients often have prolonged duration of infection and higher viral load, factors which in turn contribute to the development of resistance.

Normally in influenza infection, susceptibility testing will not be possible before the start of the treatment, as the treatment must be initiated within 48 hours to be efficient. Choice of empirical treatment is based on evidence from resistance surveillance and cross resistance. Active and timely sentinel surveillance for antiviral drug resistance is therefore important and evidence of community spread of resistant viruses should be reported rapidly. It is important for patient care that clinicians are aware of emerging resistance so that alternative drugs are considered in the event of a poor response to oseltamivir. Special care should be taken to minimize the risk of virus transmission from hospitalized patients undergoing oseltamivir treatment.

The need for susceptibility testing should be considered in some situations. Immunocompromised patients undergoing anti-influenza treatment have a higher risk of developing antiviral resistance because of the increased likelihood of prolonged virus shedding and high viral load. Severe and fatal influenza cases can be selected for susceptibility testing, both analysing the first and the last positive samples. Also patients becoming influenza-positive during or after finishing post-exposure prophylaxis, especially if insufficient drug dose has been used. During outbreaks in hospitals or nursing homes, susceptibility testing can also be considered.

In the 2015-16 influenza season, some severe cases treated with oseltamivir against influenza in intensive care wards have been specifically tested for the presence of drug resistant viruses. So far, all cases were susceptible to oseltamivir, except for one patient treated for a few days with oseltamivir.

Surveillance of influenza virus resistance

Surveillance of influenza virus resistance in Norway

The National Influenza Centre at The Norwegian Institute of public Health 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 are investigated phenotypically 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 2015/16 season was ~1% for H1N1pdm viruses and 0.6% for H3N2 viruses in Europe (ECDC). ECDC report concerning antiviral treatment and prophylaxis of influenza:
Expert Opinion on neuraminidase inhibitors for prevention and treatment of influenza
Review of recent systematic reviews and meta-analyses:
http://ecdc.europa.eu/en/publications/Publications/neuraminidase-inhibitors-flu-consultation.pdf

Resistance surveillance findings in the 2015/16 influenza season

The 2014/15 influenza season in Norway was dominated by influenza A H3N2 viruses in the south and influenza B-Yamagata viruses in the north. Late in the season the predominance shifted to influenza B in the south and influenza A in the north. H1N1pdm09 dominated during the 2015/16 season until week 12 when influenza B-Victoria took over predominance. Since the introduction of the pandemic H1N1 in 2009 and until last season (2014/15) only one virus from a polyclinic patient has been found to be resistant towards neuraminidase inhibitors. This case was a child with no travel history or antiviral treatment infected by H1N1pdm09 virus late in the 2014/15 season, in May. The virus had highly reduced sensitivity towards oseltamivir and possessed the H275Y substitution. From December 2015 to March 2016, the proportion of samples containing viruses resistant towards oseltamivir (275Y mutation) had increased from 1% to 4% in Norway. During the 2015/16 season as many as 10 strains out of 336 (3%) H1N1-isolates have been resistant towards oseltamivir, possessing the H275Y substitution. Only four of the patients infected with resistant strains were hospitalised and two of these four were treated with oseltamivir. The six other cases were out-patients not receiving antiviral therapy or being hospitalised. Seven out of the 10 cases were from March 2016 and from different parts of Norway. However; no cases were detected in April (55 tested) indicating that the increase in resistance observed in March has not continued. The oseltamivir resistant viruses are still sensitive to zanamivir (Relenza®). Although the overall H1N1 oseltamivir resistance numbers from Norway were almost three fold higher in Norway (3%) than in Europe (~1%), the numbers are still low and it is unknown if the resistant virus will persist in the community or spread to other countries like the previous resistant seasonal H1N1 viruses in 2007/8. H1N1pdm09 viruses circulating or introduced during the off-season will be carefully monitored for resistance.

All circulating influenza viruses are resistant towards adamantanes. These are therefore not available for antiviral treatment and prophylaxis in Norway.

Table 1. Norwegian influenza viruses resistant to the NIs oseltamivir and zanamivir and M2 blockers (adamantanes), during the influenza seasons 2005/6 through 2014/15.

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)
2009pdmH1	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

Conclusion

The results from Norway stress the importance for timely screening for antiviral resistance and increase the awareness of resistant viruses circulating. Viruses with antiviral resistance might spread rapidly, as experienced with the previous seasonal resistant H1N1 viruses in 2007/08, first circulating in Norway, spreading worldwide. Therefore development of alternative antivirals is urgently needed and policymakers should consider their stockpiles of antiviral drugs.

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Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV)	
Distribution in Norway	In Norway, a total of 5843 persons had by 2015 been diagnosed with HIV. There were 221 new cases of HIV-1 reported in Norway in 2015, the lowest number in 10 years.
Treatment	There are five classes of antiretroviral drugs available, targeting different steps in the replication cycle (figure 1). A combination of drugs from at least two different classes is recommended in order to avoid development of drug resistance
Resistance testing method	Genotypic assays involve a RT-PCR based amplification of the parts of the genome encoding the enzymes targeted by the drugs, followed by nucleotide sequencing. The sequences are analyzed for amino acid mutations associated with drug resistance. A plasma viral load > 500 copies/mL is required for the analysis.
Target gene	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. Genotypic assays for assessing drug resistance against protease inhibitors and reverse transcriptase inhibitors are performed routinely. In addition, assays for detection of resistance against integrase inhibitors and to determine CCR5 co-reseptor tropism are also available.
Indication for resistance testing	Virological failure is the main reason for performing HIV-1 drug resistance testing in clinical practice. Such samples are collected while the patient is still receiving the failing regimen.
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.

Figure 1. HIV-1 life cycle and
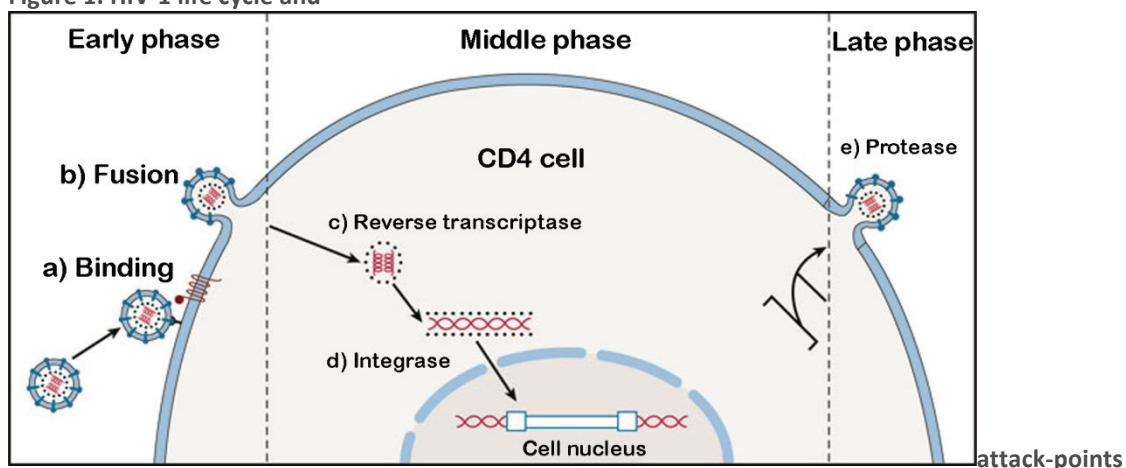
attack-points for the different classes of antiretroviral drugs. a. CCR5 antagonists blocking the binding to CCR5 co-receptor. b. Fusion inhibitors preventing cell entry. c. Nucleoside-and non-nucleoside inhibitors of reverse transcriptase targeting the transcription of viral RNA. d. Integrase inhibitors restraining the integration of viral gene transcripts into the host genome. e. Protease-inhibitors targeting the formation of new functional HIV proteins by inhibiting cleavage of polyproteins. (from Åsjø et al, TDNLF 2008)

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http://www.unaids.org/en/resources/campaigns/HowAIDSchangedeverything/factsheet

Integrase Strand transfer Inhibitors (INSTIs)

The first integrase inhibitor was licensed in Europe early 2008. At that time, the antiretroviral regimens used for treatment of HIV-infection were already very effective. Most patients obtained full suppression of viral load on regimens based on either non-nucleoside inhibitors (NNRTI) or boosted protease inhibitors (PI) combined with a backbone of various combinations of nucleoside reverse transcriptase inhibitors (NRTI). However, there were concerns about side effects and long term toxicity, and drug resistance seemed to be emerging. Therefore, the introduction of two different new classes of drugs was heartily welcomed: The first CCR5-co-receptor antagonist (maraviroc) was licensed in 2007, shortly followed by the first integrase inhibitor (raltegravir). Raltegravir was embraced by clinicians worldwide: A highly efficient drug, well tolerated, with very few side effects and almost no interactions with other drugs. There was only one catch; the low genetic barrier for development of drug resistance. With the arrival of the second generation of integrase inhibitors, it has become the drug class of choice for many different patient groups, and in the current European treatment guidelines, all the three drugs in the class are recommended as first line therapy.

The HIV enzyme integrase is responsible for the transfer of virally encoded DNA into the host genome. The integration process involves several steps, including formation and processing of a preintegration 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 viral enzyme integrase, and block the action of integrase by restraining the binding of the pre-integration complex with the host genome.

There are currently three different integrase inhibitors available: Raltegravir, Elvitegravir and Dolutegravir. The pharmacokinetics of the INSTI is favourable, and with boosting of elvitegravir with cobicistat, a standard dosage regimen of once daily is sufficient for both elvitegravir and dolutegravir. Both elvitegravir and dolutegravir are available as components of single tablet regimens in combination with other drugs.

Virological efficacy is excellent for all three integrase inhibitors, and they are all well tolerated with low levels of toxicity and little side effects. The most important difference is in the resistance profile and the genetic barrier for development of resistance to the individual drugs. In this respect the introduction of dolutegravir has been a true game changer. Studies have shown both an efficacy and a high genetic barrier that is at least comparable with the boosted protease inhibitors, but without the toxicity and with far less drug-interactions.

Raltegravir has a low genetic barrier, meaning that one or two mutations may be sufficient to cause a high grade of resistance leading to treatment failure. However, there is extensive but incomplete cross-resistance among the INSTI drugs. There are three main pathways for the development of resistance to raltegravir; Q148, N155 and Y143 (1). These are primary mutations that reduce virus susceptibility for raltegravir. These mutations do not themselves cause resistance to elvitegravir or dolutegravir. However, their presence is often followed by additional accessory mutations that further decrease virus susceptibility or compensate for the reduced viral fitness, especially if treatment with raltegravir is continued (2). Thus, continuing a failing regimen with raltegravir, often

promote selection of variants that are also resistant to elvitegravir, especially in patients with poor adherence. Most cases of resistance against INSTI are found in patients with current or previous exposure to the drug class. So far, there is very little transmitted drug resistance even to the first generation of integrase inhibitors. However, the documentation is scarce, as baseline surveillance of resistance to integrase inhibitors is not routinely performed either in Norway or in other European countries. It is not easy to predict the development of transmitted drug resistance for INSTI. For dolutegravir, baseline resistance does not seem to be a problem, as only very few isolated cases of transmitted drug resistance mutations have been described worldwide

In Norway, testing for genotypic resistance against integrase inhibitors has been available at the national reference laboratory for HIV at OUS Ullevål since 2009. The test is performed only in patients previously exposed to INSTI. So far, a total of 131 samples have been analyzed. Of the 35 samples analyzed in 2015, mutations associated with resistance against raltegravir or elvitegravir were detected in 5 samples (14 %). No resistance against dolutegravir was detected. Currently, there does not seem to be a rationale for recommending baseline surveillance of transmitted drug resistance against INSTI, and resistance testing for INSTI before initiating treatment is not necessary for patients that have not previously received integrase inhibitors. However, we need to maintain the awareness in order to avoid emerging resistant viruses with sufficient viral fitness to spread within a high risk group. All patients showing signs of virological failure on INSTI should be monitored closely with drug resistance testing. We need to be continuously alert, by following the development of drug resistance closely, and stay prepared to initiate baseline surveillance of INSTI drug resistance if and when it is needed.

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New aspects in treatment of HIV

Today, most people who are diagnosed with HIV in Norway are offered treatment within few weeks. In May 2015 the START Study (1) was terminated as the interim analysis showed that the early treatment group (CD4 >500) had less serious events than the deferred group (CD4< 350). At Oslo University Hospital 94% of the HIV-cohort are on treatment (2). Since the INSTIs became available as one-tablet-once-daily combinations a couple of years ago, INSTI has become the main choice for most treatment naïve patients. INSTI has generally few side-effects and few interactions with other drugs.

Cobicistat is a new booster and was introduced as INSTI-booster of elvitegravir in the "QUAD" elvitegravir/ cobicistat/ /emtricitabine and tenofovir study. Cobicistat is a CYP3A4 inhibitor without effect on the HIV virus but works as a booster for both INSTI and PIs by inhibiting their degradation. Two cobicistat/PI-combination tablets have been introduced, and cobicistat will probably replace ritonavir within few years.

The nucleotide analog tenofovir disoproxil fumarate (TDF) has been a cornerstone of many HIV regimens for more than a decade, but as the cohort of HIV-positive above 50 years is increasing, the negative long-term effects of TDF on kidneys and bone mass have been a growing concern. The introduction of tenofovir alafenamide (TAF) seems to reduce these negative effects and TAF has already replaced TDF in the "QUAD" study mentioned above.

A new principle of tailoring HIV drugs is introduced as TAF 10 mg is recommended for boosted combinations and TAF 25 mg for non-boosted. To avoid that patients with non-boosted regimens by mistake get TAF 10 mg only, some countries have decided to use TAF 25 mg to both boosted and non-boosted regimens of two-or-more-pills-regimens.

TDF will still be widely used, also as Post-Exposure Prophylaxis (PEP) and Pre-Exposure Prophylaxis (PreP) where price is crucial and long term toxicities are less important. The recommended PEP regimen today is raltegravir + TDF + emtricitabine within 48 hours after exposure and for 4 weeks (3). The recommended PreP regimen is TDF + emtricitabine – either once daily or "on demand" (3). TAF is still not approved as PreP or as hepatitis B treatment. In conclusion: The use of Tenofovir - both as TDF and TAF - has to be closely watched considering the risk for increasing resistance in coming years.

Even though INSTI is preferred as initial therapy today, patients without side effects or other problems with their older regimens may keep it unchanged. This may represent a cheaper option, as the patent of many HIV drugs have expired and cheaper generic are available. All modern HIV drug combinations are highly effective when they are taken as prescribed. "One pill once daily" instead of "three pills twice daily" has many benefits, especially for older patients with memory loss or illegal drug users with an irregular lifestyle. Coordinated administration and directly observed therapy of other medication and HIV drugs is easier with once daily regimens with few side effects. Still some of the drugs have to be taken with food to be absorbed properly. Others react with vitamin supplements when taken simultaneously. To avoid resistance, these details have to be emphasized – not only to the patient but to healthcare workers and others who support HIV-patients with administration of drugs.

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Surveillance of 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. 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. Acquired or secondary drug resistance develop in a person who has received antiretroviral therapy. This resistance results from selection of drug-resistant variants from a genetically heterogenous virus population during therapy.

Surveillance of transmitted HIV-1 drug resistance

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) are based on the following criteria: 1) Mutations should be commonly recognized as causing or contributing to resistance. 2) Mutations should be nonpolymorphic in untreated persons. A nonpolymorphic mutation is one that does not occur in the absence of therapy. The mutations should not accur at highly polymorphic positions. 3) The mutation list should be applicable to the 8 most common HIV-1 subtypes. 4) The list should be short and unambiguous. The WHO list of SDRMs was updated in 2009. There might be additional drug resistance mutations not included in the WHO list, that 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.

Surveillance findings in Norway in 2015

The national surveillance system for HIV-1 in Norway monitors the prevalence of transmitted drug resistance against protease inhibitors and reverse transcriptase inhibitors. 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 reference laboratory is taking measures to improve the reporting of MSIS report number. 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.
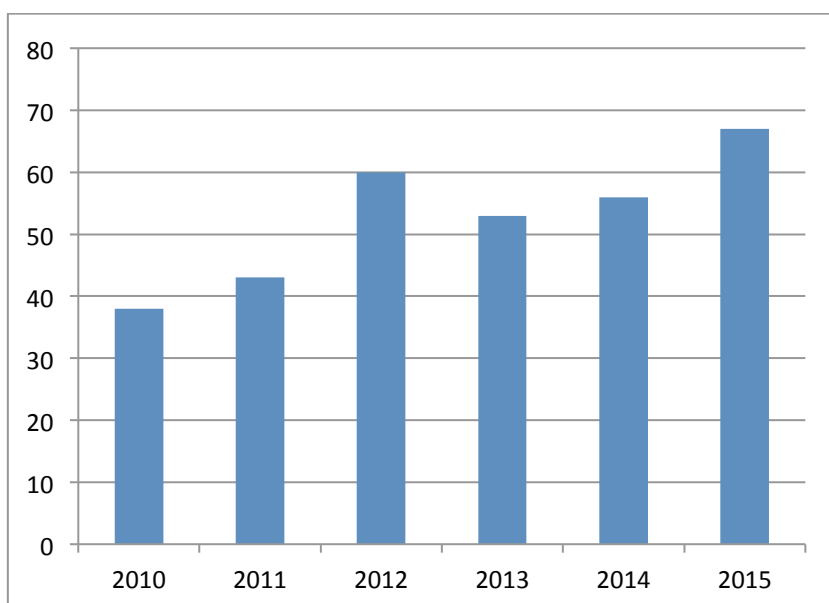
Figure 1. Percentage of newly diagnosed cases of HIV-1 infection where sequences were sent for resistance testing (2010–2015).

SDRMs detected in monitoring of primary HIV-1 resistance is presented in figure 2 as percentage of the sequences with detected SDRM in total. There may be several SDRM per sequence.
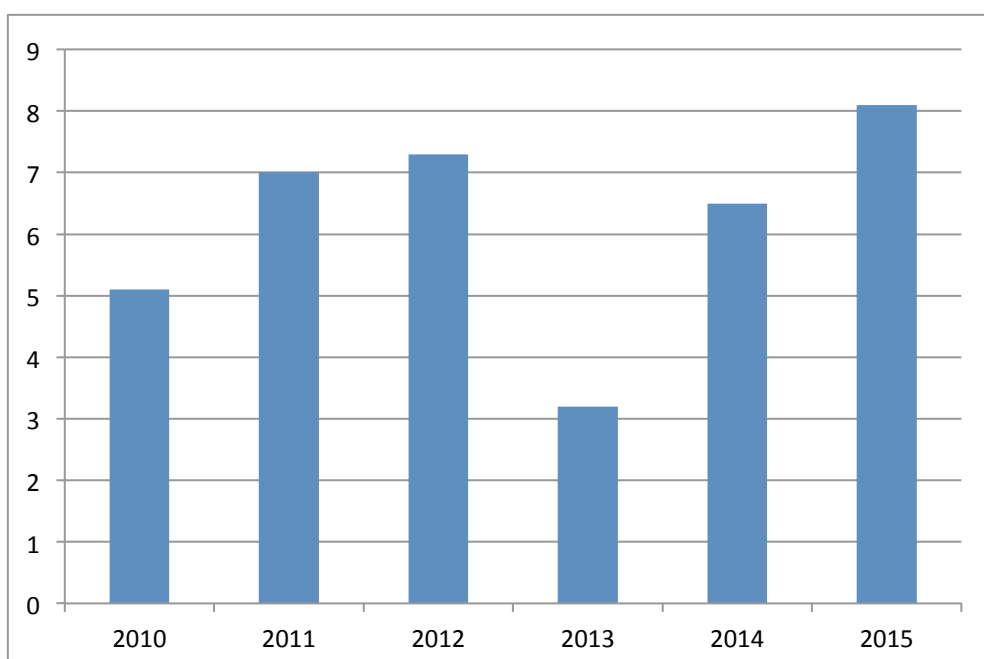
Figure 2. Percentage of analysed sequences containing one or more SDRMs (Surveillance Drug Resistance Mutations) (2010–2015).

In 2015, SDRMs from the WHO list were detected in 8% of the analysed sequences. The different mutations found is specified in table 1. Of the analysed sequences, 3% had SDRMs associated with PI, 5% with NNRTI and 2% with NRTI. One of the four samples with SDRMs associated with resistance to PI, had a single mutation of no clinical significance (I85V). Six samples had SDRMs resulting in resistance to efavirenz and/or

nevirapin (NNRTI), two samples would be interpreted as both NRTI and NNRTI resistant, while one sample was considered resistant to all groups of antivirals (NRTI, NNRTI and PI).

Table 1. Total sequences (n=149) with SDRMs in 2015 (% sequences with SDRMs).

SequenceID	NRTI SDRMs (2%)	NNRTI SDRMs (5%)	PI SDRMs (3%)
1	None	None	**L90M**
2	None	None	**I85V**
3	**M184V**	**K103N**	None
4	None	**K103N**	None
5	**T215D**	**L100I, K103N**	**V32I, I47V, F53L**
6	None	**K103N**	None
7	None	None	**L90M**
8	**M41L, M184V, T215Y**	None*	None
9	None	**Y181C**	None
10	None	**K103N**	None
11	None	**V106A, Y188C**	None
12	None	**K103N**	None

*Also detected K238T in one sample which is not a SDRM according to the WHO list, but is associated with reduced susceptibility to NNRTI.

Conclusions

The surveillance data are from patients who had their HIV-1 infection confirmed in Norway and anonymously reported to MSIS during the respective year. A majority 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 numbers above showing the frequency of resistance mutations in samples from all patients with newly diagnosed HIV-1 infection in Norway (8%), do 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, a legal authority with permission to connect epidemiological data from MSIS is urgently needed to get separate data on persons being infected in Norway.

Surveillance of HIV resistance is important in order to make decisions on implementing preventive measures to control dissemination of resistant HIV strains.

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Hepatitis B virus

Fact box: HBV drug resistance	
Treatment	There are currently 8 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 infection with antivirals is generally given as monotherapy.
Resistance testing method:	Genotypic assays involve PCR based amplification and subsequent sequencing of the polymerase gene that overlap with the surface antigene (small S-gene). The sequences are analyzed for amino acid mutations associated with drug resistance. A plasma viral load > 800 IU/mL is required for the analysis.
Target gene:	small S-gene In Norway, all HBV drug resistance tests are performed at the Norwegian Institute fo Publich Health.
Indication for resistance testing:	Virological failure/breakthrough on antiviral treatment is the main reason for performing drug resistance testing in clinical practice.
Surveillance	Viral breakthrough on antiviral treatment and primary resistence surveillance of a selected population (treatment naive).

A small survey about treatment and virology analysis of patients with chronic hepatitis B.

There is limited data on hepatitis B virus (HBV) treatment practice and the effectiveness of HBV treatment in Norway. Further, HBV resistance testing is only requested in a limited number of patients. A small survey on HBV management and usage of laboratory service was therefore conducted by RAVN to gain more insight into HBV management in Norway and the basis for the drug resistance surveillance data. A questback was sent by email to members of the Norwegian Society of Infectious Diseases and the Norwegian Society of Gastroenterology. The results from the survey is presented.

A total of 56 members answered the Questback (table 1). The response rate for specialists in infectious diseases was 18,3%. A considerable number of specialists in gastroenterology are not involved in treatment of infectious hepatitis, and we presume that this is a major reason for low response rate.

Table 1.

On how many patients have you startet HBV treatment during the last 12 months?	Number
Yes, more than 10 patients.	1
Yes, 5 - 9 patients.	8
Yes, 1 – 4 patients.	34
None	13
Total number	56

HBV genotyping is important in clinical management and treatment with interferon. In 2015, 165 samples were genotyped for HBV. In most cases no clinical information are specified on these requests. We therefore asked the clinicians for the indication used for genotyping (tabell 2). The most frequent reason for genotyping according to this questback is virus breakthrough during treatment and genotyping before starting treatment. Eight clinicians never send samples for genotyping. Two out of these eight responders have not started HBV treatment on any patient during the last 12 months. A significant number requests HBV genotyping even if there is no or uncertain indication for treatment.

Table 2.

In what clinical situations do you request HBV genotyping?	Number*
Chronic HBV infection even if there is no indication for treatment	7
Chronic HBV infection before it is concluded regarding indication for treatment.	15
Before starting antiviral therapy	22
Viral breakthrough on antiviral treatment	23
Never	8
Other	3

*Nine responders specified two different answers, 7 responders specified three answers.

European guidelines recommend treatment of chronic HBV infection with entecavir or tenofovir. Lamivudin is associated with development of drug resistance and therapy failure (1). We wanted to know to what extent lamivudine is used in Norway today. According to this questback most clinicians do not use lamivudine (table 3). However, seven doctors specify that they prescribe lamivudine to patients that already are on this treatment as long as it is effective.

Table 3.

Entecavir and tenofovir are first choice in HBV treatment. Are there situations in which you use lamivudin?	Number
Patient is already on lamivudin treatment and has achieved virus suppression on this treatment.	7
Short / transient antiviral treatment during immunosuppressive treatment.	1
Short / transient antiviral treatment during pregnancy.	3
Never	41
Other*	4

*Among "other" reasons for treating with lamivudine was hiv coinfection and travelling to countries without access to modern medication.

Very few samples are analyzed for resistance mutations at the national reference laboratory for HBV. We wanted to find out if the clinicians request resistance testing when treatment fails, and hence the low number of requests is due to good treatment response. According to this questback 54% of the responders "always" or "almost always" send sample for resistance testing when patients experience viral breakthrough (table 4). 25% of the clinicians never or seldom request resistance testing in this clinical situation.

Table 4.

When patients have viral breakthrough on antiviral treatment, how often do you send bloodsample for HBV resistance testing?	Number
Always	21
Almost always	9
Frequently	7
Sometimes	4
Rare	9
Never	5

The reasons for not requesting resistance testing are indicated in table 5. Twenty-two responders find this question irrelevant. That could mean that they do not experience treatment failure, or that resistance mutations are not considered a likely cause when treatment fails. The second most common answer to this question is that there are other more likely reasons for treatment failure than resistance. Some clinicians change medication based on virus quantitation without looking for resistance mutations.

Table 5.

What is the reason if blood sample is not sent for resistence testing when viral breakthrough is detected?	Number
Resistance is an unsignificant problem when treating chronic HBV infection.	10
Changing treatment regime is sufficient.	4
Virus quantitation is sufficient to follow development of resistance.	4
There are other more likely reasons for virus breakthrough, like compliance and intercuirrent disease.	19
Other	3
Not relevant.	22

To provide relevant laboratory analysis, as well as interpretation of these data, clinical information is needed. We therefore asked for practice regarding clinical information on laboratory requests. Most of the responders indicate that they provide relevant information about therapy (table 6).

Table 6.

Do you inform about treatment and type of medicament when filling in forms for virus quantitation?	Number
Always inform about treatment.	44
Always inform about type of treatment.	31
Sometimes inform about treatment.	8
Sometimes inform about type of treatment.	17
Never inform about treatment.	3
Seldom inform about type of treatment.	6

Although antiviral treatment of chronic HBV infections has been regarded as lifelong, patients terminate therapy for several reasons. This group of patients should be followed thoroughly, also with respect to viral load and resistance mutations. Table 7 indicate that discontinuing of antiviral medication is an option also in Norway.

Table 7.

Have you discontinued treatment when chronic HBV patients on prolonged treatment have lasting virus suppresion?	Number
Yes, more than 10 patients.	1
Yes, between 5 and 10 patients.	1
Yes, less than 5 patients.	16
No	37

Table 8.

What clinical speciality do you have?	Number
Infectious diseases	42
Gastroenterology	12
Other	1
Total number	55

Conclusion

The survey is small, but still indicates some trends in management of patients with chronic HBV infection. The divergence in answers concerning HBV genotyping and resistance testing could indicate the need for guidelines for laboratory analysis in management and treatment of chronic HBV infection in Norway. The majority of clinicians request resistance testing frequently or always in case of viral breakthrough according to this survey. In relation to the low number of samples sent for resistance testing, this could indicate that the number of treatment failures is small. However, the criteria for requesting resistance testing is not clear when combining table 4 and 5. RAVN and the reference laboratory recommend that samples are sent for resistance testing in all cases of treatment failure, unless there are other obvious reasons for failure. Poor compliance increases the risk of resistance development and therefore do not eliminate the need for resistance analysis. Most of the responders give relevant clinical information about medication, but there are still potential for improvement.

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Surveillance of HBV drug resistance

Materials and Methods

The surveillance of HBV resistance is based on two populations; 1) patients that have been tested for drug resistance in relation to treatment and 2) patients that are genotyped for HBV as part of the elucidation of infections status before treatment and can therefore be regarded as surveillance of primary resistance. Sequencing of the polymerase gene that covers the mutations that give resistance to the NAs is frequently used for resistance determination (Table 1). It is the current method of choice at NIPH, although the proportion of resistant viruses must reach 20–30% before it is detectable by this method. The latter patient group (population 2) was selected because sequence information on antiviral resistance was available as part of a HBV-genotyping (S-gene) analysis previously requested. Drug resistance in patients are only counted the year it is first reported.

Table 1: Nucleos(t)ide analogue cross-resistance data for resistant HBV variants

Cross-resistance data for resistant HBV variants					
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

Ten patients were tested for drug resistance in 2015 (Table 2), whereof nine samples were successfully sequenced. Drug resistance (M204I) was only detected in one patient currently on telbuvidine treatment. The remaining patients showed no indication of genotypic resistance and were all on tenofovir treatment, except for one patient that had been on previous treatment, but current treatment status is unknown. Among patients tested for HBV-genotyping (N=165) no drug resistance mutations were detected, the same as in the three previous years.

Table 2. Surveillance of drug resistance among patients on HBV treatment in 2011–15.

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

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

Antiviral drug resistance of human herpes viruses

Antiviral drugs are available for treatment of herpes simplex virus 1 and 2 (HSV 1 and 2), varicella zoster virus (VZV) and cytomegalovirus (CMV) infections. The preferred drugs are nucleoside analogs, like acyclovir (ACV) and ganciclovir (GCV), which are inhibitors of virus DNA replication. To be effective these drugs have to be monophosphorylated by a viral enzyme and be accepted by the viral DNA-polymerase (1). Oral bioavailability is improved by prodrugs like valine esters of ACV and GCV. Other antivirals are foscarnet (FOS), an inhibitor of virus DNA-polymerase, and cidofovir (CDV), a cytosine nucleotide analog which is not dependent on monophosphorylation by a viral enzyme (1). FOS and CDV have serious side effects and are thus to be regarded as second line drugs.

Cytomegalovirus

Fact box CMV:	
Resistance testing method	Genotypic resistance testing
Target for resistance analysis	The CMV-genes UL97 and UL54
Surveillance method	Assessment of viral load in blood and other compartment
Indication for resistance testing	Persistent high viral load during antiviral treatment

GCV and valganciclovir (VGCV) are used for both treatment and prophylaxis of CMV-infections. GCV is initially converted to GCV-monophosphate by an enzyme encoded by CMV-*UL97* and then converted to GCV-triphosphate by cellular enzymes and is added to the growing CMV-DNA by its CMV-DNA-polymerase encoded by CMV-*UL54*. GCV acts as a DNA chain terminator. Genotypic GCV resistance testing is carried out by sequencing the CMV-*UL97* and *UL54* genes. In 2015 samples from 27 patients were received for GCV-resistance testing and resistance mutations were detected in samples from five patients. Resistance mutations were seen only in the *UL97* gene and were as follows: H520Q, A594V, L595S and C630Q. No GCV *UL54* resistance mutations were detected. The resistance mutations detected all confer intermediate or high levels of resistance requiring a switch to an alternative antiviral drug.

FOS and CDV resistance mutations are also located on *UL54* and thus detected when *UL54* is sequenced for GCV-resistance mutations. GCV *UL54* resistance mutations also confer CDV-resistance whereas FOS resistance show little or no GCV or CDV cross resistance. No FOS or CDV-resistance mutations were recorded in samples received for genotypic resistance testing in 2015.

In severely immunosuppressed, patients CMV-infection may become chronic despite GCV-treatment. The main indication for GCV-resistance testing in such patients is treatment failure lasting for at least two weeks. In the period 2009 to 2015, we have annually received samples from 12 to 27 patients and find resistance mutations in 29% (19 – 66%) of the cases (Table 1).

GCV genotypic resistance analysis is performed at Department of Microbiology, OUS-Rikshospitalet, Oslo

Table 1. Number of CMV-positive specimens analyzed for genotypic ganciclovir resistance during the years 2008-2015

Year	Number of specimens received	Number of specimens with ganciclovir resistant CMV
2008	14	5
2009	12	8
2010	22	5
2011	18	4
2012	23	5
2013	23	8
2014	21	7
2015	27	5

Herpes simplex virus

Fact box HSV:	
Resistance testing method	Genotypic resistance testing
Target for resistance analysis	HSV thymidine kinase gene
Surveillance method	Assessment of HSV-1-2 DNA in clinical specimens by PCR
Indication for resistance testing	Persistent HSV-infection during ongoing therapy

Folkhählsomyndigheten, Sweden (Dr. Lottie Schloss) performs the ACV genotypic resistance test. Resistance mutations are looked for by sequencing of the HSV thymidine kinase gene.

HSV infections are almost exclusively treated with ACV. Despite extensive use both as therapy and prophylaxis ACV-resistant HSV-strains are rarely seen. In 2015, Folkhälsomyndigheten received specimens from two Norwegian patients with persistent HSV-2 infections. In one of the HSV-2 strains no ACV-resistance mutations were seen whereas the assay of the other strain showed an inconclusive result.

During the years 2009 to 2015, 14 HSV positive specimens were sent to Folkhälsomyndigheten for genotypic ACV resistance testing. ACV-resistant HSV-strains were detected in three specimens, two HSV-1 and one HSV-2. (Table 2).

Table 2. Number of HSV positive specimens analyzed for genotypic acyclovir resistance.

Year	Number of specimens	Number of resistant strains
2009-2010	0	0
2011	4	1 (HSV1)
2012	3	0 (two unsuccessful)
2013	2	1 (HSV1)
2014	3	1 (HSV2)
2015	2	0 (one inconclusive)

Conclusions

Treatment failure of CMV-infections is mainly seen in immunosuppressed patients. However, only a minority (29%) of these infections are caused by anti-viral drug resistant strains.

Persistent HSV infections caused by ACV-resistant strains seem to be a rare event since annually only 0 to 4 specimens are sent for genotypic resistance testing. During the last seven years, only three ACV resistant HSV- strains have been detected.

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Hepatitis C virus

Fact box: HCV drug resistance	
Resistance testing method:	Sequencing of the NS3-, NS4A-, NS5A/B – genes and/or complete HCV genome
Target gene(s):	NS3–NS4A (protease), NS5A (replication/assembly), NS5B (polymerase)
Surveillance method:	Patients on treatment with referral of drug resistance testing Primary resistance on selected genotype(s) and genomic region
Indication for resistance testing:	Treatment failure / Primary resistance surveillance
Treatment options:	Pegylated interferon and ribavirin Teleprevir (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)

Hepatitis C virus drug resistance

Hepatitis C virus (HCV) is widespread globally with more than 100 million individuals chronically infected. In Sweden and Norway about 0.5 % of the population is infected with HCV, approx 45 000 and 20 000 individuals, respectively. HCV is divided into 7 different genotypes (GTs), called GT 1-7, and several different subtypes, named a, b, c etc.

Treatment

Treatment of HCV infection is currently undergoing a paradigm shift with direct-acting agents (DAAs) which inhibits HCVs own proteins /enzymes such as protease (NS3), NS5A protein and polymerase (NS5B - either as nucleoside or non-nucleoside). To date (August 2016) the following DAAs are EMA (European medicines agency) approved: NS3 protease inhibitors: simeprevir, paritaprevir and grazoprevir; NS5A inhibitors: daclatasvir, ledipasvir, ombitasvir, elbasvir and velpatasvir; NS5B polymerase nucleoside inhibitor: sofosbuvir; and NS5B polymerase non-nucleoside: dasabuvir. Recently EMA has approved two new treatments (July 2016): Zepatier which is a combination of elbasvir (NS5A) with grazoprevir (NS3) for the treatment of GT 1 and 4, and Epclusa containing sofosbuvir (NS5B) and velpatasvir (NS5A) for pan-genotypic treatment of GT 1- 6, see Figure 1. All these DAA treatments are much more effective and have far fewer side effects than the previous interferon treatment. Most DAAs are effective against all GTs, for example, NS5A and NS5B inhibitors, nucleoside analogues. The current NS3 inhibitors are effective against all GTs except GT 3. Treatment with these DAAs combinations can lead to more than 90% cure. However, the cost of the new treatment is high for GT 1, 2 and 4, and even higher for GT 3 (which is the GT most difficult to treat). The cost depends on the drug combination and the duration of treatment. In Norway the most common GT is type 3a, followed by 1a. The time for starting therapy has depended on the fibrosis stage and inflammatory activity in the liver tissue. Due to the high cost of current DAA combinations, the treatment is primarily used for patients with fibrosis stage 2-4. It is expected that in coming years more DAAs will be approved (Figure 2), and with additional treatment options available the price for treatment would be reduced.

Resistance

Resistance mutations so called resistance associated variants or substitutions (RAV or RAS) can emerge in the HCV quasi species during the selection pressure of the DAA as the HCV viruses replicates rapidly and as it NS5B polymerase lack proof-reading activity. The mechanism of resistance mainly involves mutations (amino acid substitutions) at certain positions at HCV targets of the DAA. The mutation thereby reduces it´s affinity to the DAA.

Even though high cure rates/sustained viral response (SVR) are achieved with DAA-based interferon-free regimens, drug resistance is a problem when NS5A inhibitors, NS3-protease and NS5B non-nucleoside inhibitors are used in sub-optimal combinations/duration. With the exception of NS5B nucleoside (sofosbuvir), the current DAAs targeting NS3, NS5B and NS5A all have a low barrier of resistance. HCV drug resistance is associated with lower cures rates e.g. for certain patient that have other negative factors such as high fibrosis stage, genotype 3, previous treatment failures etc. Those approx 2–10% who fail treatment, mainly depending on fibrosis damage, almost always have emerging RAVs, where the NS3 and NS5A RAVs have a half-life of many years, obstructing retreatment with same drug class. Even treatment-naïve patients could have RAVs against DAAs, i.e. at baseline.

For a detailed report of RAVs, in NS3, NS5A and NS5B genes, see Lontok et al 2015 or Sarrazin 2016. Important RAVs level of fold-resistance to NS3 and NS5A inhibitors are summarized in Table 1 and 2 - notable is the broad cross resistance with "early generation" NS3 and NS5A inhibitors, respectively.

Baseline resistance

The probability that a DAA will select for and allow outgrowth of RAVs in viral populations depends on genetic barrier to resistance, the level of DAA exposure, and the viral fitness of the RAV (Lontok et al., 2015, Sarrazin 2016). The outgrowth of RAVs during treatment can therefore be enhanced by baseline RAVs in high levels in the viral population, which differ in their prevalence by GTs. Clinical studies have lately been performed to determine which baseline RAVs are the more important and at what level/frequency such RAVs appear in the viral population. However, as additional DAAs are approved, more studies are needed. Thus, pre-existing RAVs at baseline in treatment-naïve patients can together with other negative factors predict the SVR efficacy of DAA treatment (Sarrazin 2016).

One example of baseline RAV is the widespread NS3 Q80K in GT 1a, which specifically renders resistance toward simeprevir (Table 1). The prevalence of Q80K as a natural baseline RAV when using population-sequencing methods (cut-off 20%) varies geographically and is approx 10% in Nordic GT 1a population compared to 40% in USA. In 2013 FDA recommended screening for the Q80K polymorphism in the NS3 GT 1a before considering treatment with simeprevir and interferon/ribavirin. However, new interferon-free treatment in 2014 with simeprevir and the potent sofosbuvir drug resulted in high rates of SVR in GT 1a patients with Q80K. Thereby, baseline screening of Q80K was considered redundant. Nevertheless, interpretation of the OPTIMIST-2 study in 2015 revealed significant lower SVR for GT 1a patients with cirrhosis and baseline Q80K compared to those without Q80K; SVR rate of 74% compared to 92%. Hence, in Dec 2015, new guidelines (www.hcvguidelines.org) recommend monitoring Q80K for GT 1a patients with cirrhosis when considering treatment with simeprevir plus sofosbuvir.

Now when the NS3 inhibitor simeprevir is less used the focus has switched to study baseline NS5A RAVs in predicting the most effective DAA treatment and treatment duration, Evaluations from Sarrazin C, Review and www.hcvguidelines.org : the most important GTs to consider, in clinical practice, for NS5A baseline resistance analysis are 1a and 3a. In these GTs RAVs have very high fold resistance e.g. Y93H/N > 1000 fold with GT1a in vitro replicon assay (Table 2). It should be noted that there are many more NS5A RAVs at positions 28, 30 and 31 but the in vitro resistance might not be high enough to be of clinical importance for current approved NS5A inhibitors (with exception of elbasvir). Nevertheless the important Y93N/H are seldom found as baseline RAV in GT 1a patients, i.e. only in 1-2 % of DAA treatment-naïve patients when using population-sequencing method. However, EMA recently approved a new treatment for GT 1 and 4, called Zepatier, which consists of the NS5A inhibitor elbasvir, co-formulated with NS3 inhibitor grazoprevir. On the product information of Zepatier, EMA recommends NS5A baseline testing (of RAVs at position 28, 30, 31 and 93) for GT 1a prior to treatment. In this case the NS5A RAVs only need 5 fold-change towards elbasvir to be of clinical importance. If such RAVs are detected at baseline, the recommendation is to use prolonged Zepatier treatment from 12 to 16 weeks with the addition of ribavirin.

In contrast to the case in GT 1a, the Y93H is found quite frequently (9 %) at baseline for GT 3a patients with population sequencing. This RAVs possesses a high fold resistance to all

current approved NS5A inhibitors e.g. daclatasvir >1000 and velpatasvir >100 fold (Table 2.). Therefore, GT 3a baseline analysis is of importance for NS5A treatment combinations, since the ALLY-3 study showed that 33% without cirrhosis and 75% with cirrhosis of the GT 3 patients with baseline RAV Y93H failed a 12-week treatment with sofosbuvir and daclatasvir (Nelson et al. 2015). In other words, only 25% with both Y93H and cirrhosis reached SVR compared to 67% with cirrhosis alone, and in patients without cirrhosis 63% with Y93H reached SVR compared to 90% without Y93H.

Furthermore, the Epclusa product information revealed that velpatasvir is also hampered by baseline Y93H when treating GT 3 patients. In their ASTRAL-3 study the Y93H was detected in 9% of patients and the study demonstrated that 4 out of 10 non-SVR patients had Y93H at baseline, and that all these non-SVR patients then had Y93H at relapse. The study displayed a SVR rate of 84% for GT 3 patients with baseline Y93H compared to a 96% SVR for patients without baseline Y93H. However, in the product recommendation there is no clear endorsement to perform baseline analyses of Y93H for GT3 patients prior to treatment with Epclusa, but there are suggestions to prolong Epclusa treatment from 12 to 24 weeks with addition of ribavirin for patients (i.e. GT 1, 3 or 4) who have previously failed NS5A treatment.

Nevertheless, in the new guideline at www.hcvguidelines.org NS5A RAV testing is mentioned as a recommendation in patients with GT 3, who are considered for treatment with sofosbuvir/velpatasvir or for daclatasvir/sofosbuvir-based regimens. This guideline site is constantly being updated.

Hence, the Y93H, occurring at baseline for 9% of the GT 3 patients, should therefore be as strong factor as cirrhosis to predict treatment failure. Such patients would need longer treatment duration including addition of ribavirin, or treatment should be withheld until better DAAs against GT3 are approved, e.g. ABT 530 with different resistance profile, and ABT-493 a NS3 inhibitor potent against GT 3 (Table 1 and 2). On the other hand, 91% of the patients without baseline Y93H could be cured with shorter treatment duration at a lower expense and without ribavirin. Considering that the resistance analysis cost per individual is approx 200 fold lower than the cost of current DAA-treatment, selection of cost-effective treatment combinations/duration should be of greatest importance, both in a perspective of evidence based healthcare delivery, and in the case of the individual patient to avoid relapse with difficult retreatment options.

Methods

The general methods available for detecting RAVs are with population sequencing (Sanger) or with next generation sequencing (NGS). The population sequencing method renders a 20% cut-off level of RAVs in the viral population compared to 1% with NGS. However, the general consensus is to recommend a cut-off level of 10-20%, for detecting RAVs within the HCV quasispecies, in order to be of clinical relevance in predicting viral failures (Sarrazin 2016). Thus, the population sequencing method (or NGS set at a cut-off level of 10-20%) is the optimal method.

Currently NS3 and NS5A resistance analysis is generally only recommended by EMA at relapse when treatment failure occurs, with the exception for baseline analysis of NS3 Q80K GT 1a for simeprevir and NS5A baseline analysis for GT 1a prior to Zepatier treatment. However, once NS5A resistance analysis has become more accessible in Europe as a routine, the awareness of this analysis for determining the most cost efficient treatment choice should increase. In USA it is already done by large diagnostic companies,

e.g. LabCorp (by Monogram) and Quest. The website Geno2Pheno at Max Planck institute, where interpretation of HCV resistance is done, has increased the interest at European labs to set up their own in house resistance analysis.

The Clinical Microbiology Lab in Uppsala has developed assays with population sequencing for NS3 and NS5A resistance analysis (Palanisamy et al 2013; Lindstrom et al 2015). They are the first laboratory (since 2014) in the Nordic countries performing this analysis (including interpretation) in the routine. They have done more than 300 NS3 and 350 NS5A resistance analyses, mainly baseline analysis for GT1&3 patients in the Uppsala/Gävle region and the Tromsö region. In addition, since the summer of 2015, they have performed more than 80 resistance analyses of treatment relapse (i.e. non-SVR patients) from other Infectious Disease departments throughout Sweden.

In summary, as the future treatment will not be 100% effective, there will unavoidably be treatment failures with emerging resistance and in many cases it will be difficult to find re-treatment options. Hence, there should be a need for a surveillance system of HCV resistance in the Nordic countries that includes sample data collection for at least patients with relapses and possibly also baseline resistance data. This could be done in collaboration with the Clinical Microbiology Lab in Uppsala, InfCare Hepatitis and RAVN.

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Figure 1. Schematic overview of previous and current treatment recommendations in Sweden and Norway.
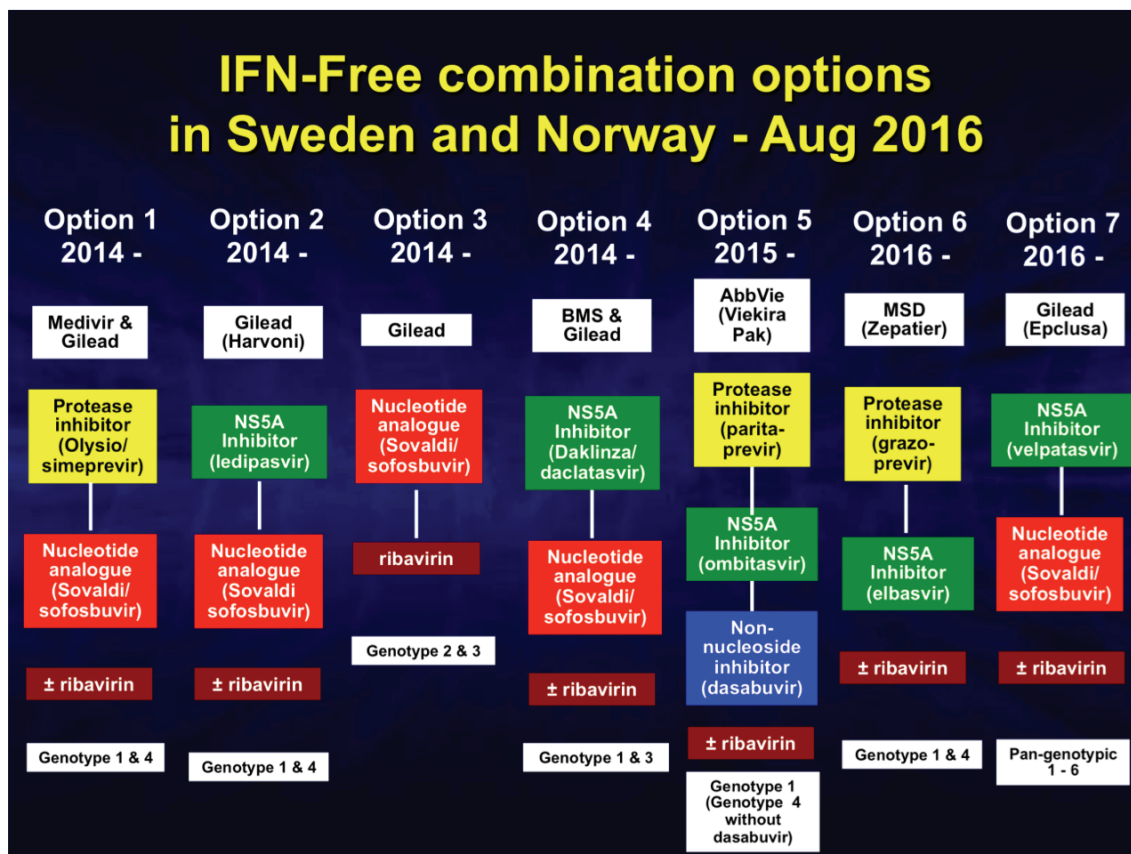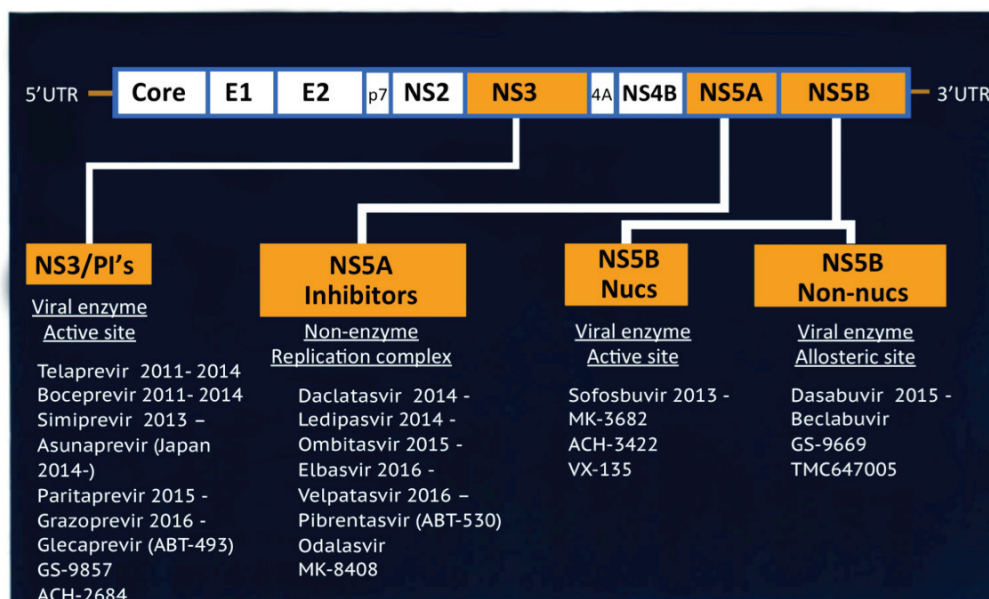

Figure 2.

Table 1 (NS3 RAVs). Fold change in resistance compared to wild type replicon of clinically-relevant NS 3 in genotype 1a and 1b, with addition of GT 3a to explain polymorphism Q168.

In vitro fold-resistance in genotype:		1a					1b			3a
Approval year	DAA	Q80K*	R155K	A156T	D168A/H	D168V/Y	R155K	R156T	D168A/H/V	Q168**
< 2016	Simeprevir	10x	>50x	>30x	>30x	>100	>30x	>30x	>50x	>500x
	Paritaprevir	<3x	>30x	20	>50x	>100x / NDA	>30x	<10x	>20x/>50x/>100x	NDA
2016	Grazoprevir	<3x	3x	NDA	>100x / NDA	NDA / >50	NDA	NDA	NDA	NDA
2017/18?	Glecaprevir (ABT-493)	<3x	<3x	>100x	<10x	<10x	<3x	>100x	<10x	NDA

NDA No data available
*Prevalence of 5 - 10% at baseline for DAA treatment naive patients when using Sanger sequencing method (20% cut-off).
** Natural NS3 polymorphism of 100% at baseline for genotype 3

Table 2 (NS5A RAVs). Fold change in resistance compared to wild type replicon of clinically-relevant NS5A RAVs in genotype 1a, 1b and 3a.

In vitro fold-resistance in genotype:		1a				1b		3a	
Approval year	DAA	M28T	Q30R/H	L31M/V	Y93H/N	L31V	Y93H*/N	A30K*	Y93H*
< 2016	Ledipasvir	20x	>100x	>100x/>100x	>1000x/>10000x	NDA	>100x	>1000x	>1000x
	Ombitasvir	>1000x	>100 / <3x	<3x / >100x	>10000x/>10000x	<10x	>70x/>100x	NDA	>1000x
	Daclatasvir	>100x	>100x	>100x/>1000x	>1000x/>10000x	<20x	20x/50x	50x	>1000x
2016	Elbasvir	20x	>100x	>10x / >100x	>1000x/>1000x	<10x	20x/50x	50x	>100x
	Velpatasvir	<10x	<3x	20x/50x	>100x/>1000x	NDA	<3x/–	50x	>100x
2017/18?	Pibrentasvir (ABT-530)	<3x	<3x	<3x	<10x/<10x	<3x	<3x/<3x	<3x	<3x
	MK-8408	<10x	<10x	<10x	<10x	<10x	<10x	NDA	NDA

NDA No data available
*Prevalence of 5 - 10% at baseline for DAA treatment naive patients when using Sanger sequencing method (20% cut-off).

Table 1 and 2 references: Lontok et al, Hepatology 2015 ; Sarrazin, J Hepatology 2016 ; Wyles D, AASLD presentation 2015 ; NG T et al, AASLD poster 2015 ; NG T et al, CROI poster 2014; Liu R et al, AAC 2015 ; Lahser FC et al, AAC 2016 ; Muir AJ EASL presentation 2016 ; Bergfors et al, AVR 2016.

Appendix A, HIV-1

Appendix A1. List of Surveillance Drug Resistance Mutations,SDRM, recommended by WHO.

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HIV-1 RT and Protease Mutations For Drug Resistance Surveillance

NRTI

Position	Mutation
M41	L
K65	R
D67	N, G, E
T69	D, Ins
K70	R, E
L74	V, I
V75	M, T, A, S
F77	L
Y115	F
F116	Y
Q151	M
M184	V, I
L210	W
T215	Y, F, I, S, C, D, V, E
K219	Q, E, N, R

NNRTI

Position	Mutation
L100	I
K101	E, P
K103	N,S
V106	M, A
V179	F
Y181	C, I, V
Y188	L, H, C
G190	A, S, E
P225	H
M230	L

PI

Position	Mutation
L23	I
L24	I
D30	N
V32	I
M46	I
LI47	V, A
G48	V, M
I50	V, L
F53	L, Y
I54	V, L, M, A, T, S
G73	S, T, C, A
L76	V
V82	A, T, F, S, C, M,L
N83	D
I84	V, A, C
I85	V
N88	D, S
L90	M

The following considerations were used to develop this list of drug resistance mutations*

the mutations should cause or contribute to drug resistance, defined as being present on three or more of five expert lists of drug resistance mutations **.
the mutations should not occur in untreated persons (i.e. they should be nonpolymorphic, and should not occur at highly polymorphic positions.),
the mutation list should be applicable to all group M subtypes, and
the mutation list should be simple, unambiguous, and parsimonious, excluding mutations resulting exceedingly rarely from drug pressure.

* HIV-1 pretease and reverse transcriptase mutations for drug resistance surveillance, AIDS 2007, 21:215-223 Shafer R et al.
Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update, PLoS One 2009;4:e4724. Bennett DE et al.

**ANRS drug resistance interpretation algorithm (2008.07),HIVdb drug resistance interpretation algorithm (4.3.7), IAS-USA Mutations Associated With Drug Resistance (March/April 2008), Los Alamos National Laboratories HIV Sequence database (2007), or Rega Institute Drug Resistance Interpretation Algorithm (7.1.1).

The prevalence of all protease and RT mutations according to subtype and treatment can be found at http://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi.

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www.fhi.no