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Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2011-2012

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Introduction

Long experience with antibiotic use has shown us that antibacterial agents may lose their effect because of the bacteria's ability to change. Our experience with antiviral agents is much shorter, limiting the knowledge about risks and challenges connected to antiviral treatment. However, one lesson has been learnt: – the importance of conducting surveillance in order to detect any developments in drug resistance and adjust the treatment accordingly.

Therefore it is with great pride that the Norwegian Institute of Public Health now presents the first report from the system for Resistance against AntiVirals in Norway (RAVN). The report presents data on resistance against agents for the treatment of influenza, HIV infection, hepatitis B infection and CMV infections from the years 2011 and 2012. The surveys have been conducted by the Norwegian Institute of Public Health and Oslo University Hospital. Our goal is that the surveillance will continue and expand to include other viral infections, such as hepatitis C. Annual reports will ensure the awareness of developing trends and thereby provide the opportunity to ascertain that patients will receive the most effective treatment.

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Sammendrag

Det finnes i dag nesten 50 tilgjengelige antivirale medikamenter i Norge og antallet er raskt stigende. Med den økende bruken av slike medikamenter har man sett en markant økning i virus' resistens mot medikamentene, slik man opplevde for bakterier etter inntoget av antibiotika på 40- og 50-tallet. Overvåking av denne utviklingen vil gi oss helt nødvendig kunnskap om utbredelse og forekomst av resistens for å kunne etablere forebyggende tiltak og dessuten gi grunnlag for behandlingsstrategier skreddersydd den enkelte pasient helt. Det langsiktige målet er å få utviklet medikamenter som effektivt behandler og utrydder kroniske virusinfeksjoner. Hepatitt C- infeksjon kan kureres ved behandling, men ikke alle pasienter kvitter seg helt med viruset. Nye medikamenter for behandling av hepatitt C- virus (HCV) vil gi nye muligheter, men krever også tett overvåkning av pasienter under behandling og bruk av mer presis diagnostikk og resistensbestemmelse. Dette er oppgaver som må løses de aller nærmeste årene.

Overvåking av virusresistens hos influensa og HIV-1 har foregått systematisk i Norge siden 2005–2006. I 2011 startet implementering av disse dataene i registeret RAVN (Resistensovervåking av virus i Norge). Samtidig i denne perioden har resistensdata for heptatitt B-virus (HBV) og cytomegalovirus (CMV) blitt innsamlet fra de respektive referanselaboratoriene og registrert i RAVN.

Influensa

- Influensavirus resistens overvåkning utføres ved Nasjonalt Folkehelseinstitutt (FHI) og er viktig for fortløpende å kunne gi kunnskapsbaserte råd om empirisk antiviral behandling ved årlig influensasesong, samt ved pandemi. Overvåkingen har avslørt nye resistenstrender som senere har vært påvist også i andre land.

HIV-1

- Overvåkingen har vist at resistens finnes hos nylig diagnostiserte HIV-1 tilfeller som ikke står på antiviral behandling, og at forekomsten er jevnt økende. Dette må følges nøye videre for å kunne oppdage en eventuell økende trend. Dette vil kunne ha betydning for legers valg av medikamenter ved oppstart av behandling.
- Siden starten av HIV-1 resistens overvåkingen har i underkant av halvparten av nydiagnostiserte tilfeller blitt sendt til resistensundersøkelse, men i løpet av det siste året har det vært et større antall undersøkte. Det er viktig å øke denne andelen ytterligere, samt tilstrebe at prøvene er representative for alle pasientgruppene. Insidensen av HIV-1 infeksjon i de senere år har økt i gruppen med menn som har sex med menn og det er derfor spesielt viktig at denne gruppen er godt representert i HIV-1 resistensovervåkingen.

HBV

- Virusresistens hos kroniske HBV-pasienter ser for tiden ut til å være et mindre problem i Norge. Pasientene gis nå i større grad effektiv førstelinjebehandling som undertrykker virusreplikasjonen slik at antiviral resistens motvirkes.
- Antall pasienter som behandles med disse midlene, opptil 485 i 2012, er lavere enn forventet ut i fra det estimerte tallet på 20 000 tilfeller av kronisk HBV-infeksjon i Norge.
- Det finnes ingen oversikt over totalt antall av pasienter som får behandling, informasjon om behandlingsregime, varighet av behandling og behandlingssvikt. I overvåknings sammenheng er det viktig å innhente og systematisere slik informasjon i tilknytning til tilgjengelige resistensdata. Dette arbeidet for bedret oversikt og systematisering av data med sikte på overvåkningen må derfor prioriteres.

CMV

- Alvorlig behandlingstrengende CMV-infeksjoner ser en først og fremst hos pasienter med nedsatt infeksjonsforsvar. Det er også i denne gruppen at de fleste tilfellene av behandlingssvikt forekommer.
- Ved behandlingssvikt vil omlag en fjerdedel av tilfellene skyldes at CMV utvikler resistens.

Anbefaling fra RAVNs fagråd

Resistensutvikling hos virus mot antivirale midler overvåkes, registreres og rapporteres nå via RAVN-organisasjonen. Virus som overvåkes er influensavirus, HIV-1, CMV og HBV.

Analyse av resistens hos influensavirus utføres ved Virusavdelingen, FHI. Overvåkningen er av høy kvalitet og standardisert i forhold til øvrige land i Europa og bør opprettholdes i nåværende form.

HIV-1 resistensovervåkning utføres ved Oslo universitetssykehus, Ullevål, og omfatter påvisning av primærresistens, dvs resistensmutasjoner i første prøve

fra pasient som er nydiagnostisert. Det nåværende system har tidligere bare fanget opp i underkant av 50 % av de nydiagnostiserte og tiltak bør iverksettes for å øke antall prøver fra nydiagnostiserte innsendt for resistenstesting.

Resistensovervåkning av CMV utføres ved Oslo universitetssykehus, Rikshospitalet. Analysen utføres ved terapisvikt under behandling med anti-CMV-midler. Undersøkelsen utføres etter en vurdering av behandlende lege og nåværende ordning fungerer greit så lenge behovet ikke øker betydelig. Det kan føre til kapasitetsproblemer.

Resistensbestemmelse av HBV utføres ved Virusavdelingen, FHI og har pågått siden 2004 i takt med de nye medikamentene som har kommet på markedet og blir innrapportert til RAVN fra 2011. Det har i de senere årene kommet nye og bedre midler for behandling av kronisk HBV-infeksjon, men det er viktig å følge langtidsbruken av disse medikamentene med tanke på resistens utvikling.

I 2011 kom to nye proteasehemmere for kombinasjonsbehandling av kronisk HCV-infeksjon (genotype 1), slik at behandling ble betydelig bedret for denne HCV- typen. Under behandling med proteasehemmere kan det oppstå resistensmutasjoner hos viruset. Resistensundersøkelse ved behandlingssvikt ved bruk av proteasehemmere er under etablering ved Virusavdelingen (FHI). Det anbefales at det etableres en protokoll for en mindre pilot studie mellom virusavdelingen og noen utvalgte klinikker for å avklare behov og kapasitet for resistensbestemmelse, samt høste erfaringer med tanke på en nasjonal overvåkning av HCV-resistens i RAVN.

Med den stadige utviklingen av nye antivirale medikamenter og nye behandlingsregimer bør situasjonen følges nøye med tanke på resistens utvikling og behovet for overvåking er økt. En god overvåkning er avhengig av systematisert og standardisert data innsamling.

RAVN fagråds anbefalinger oppsummert:
Influensavirusresistensovervåkning fortsetter som før, HIV-1 resistensovervåkning av primærresistens bør intensiveres, CMV-resistensovervåkning fortsetter som før og det bør lages en tilrådning for systematisk overvåkning av resistensutvikling ved HBV- og HCV-infeksjoner.

Summary

To date, there are almost 50 registered antiviral drugs available in Norway and this total is rapidly increasing. With the increasing usage of these antivirals, a marked rise of antiviral resistance against these drugs has been observed, as seen in the 1940s and 1950s with the flood of antibiotics used against bacteria. Surveillance of this development will give us the necessary knowledge on prevalence and spread of viral resistance to be able to establish preventative measures, thereby providing a solid basis for individual clinical treatment strategies. The long term goal is to develop drugs that effectively eradicate chronic virus infections. HCV infection is not effectively eliminated in many patients on treatment. New drugs for treatment of hepatitis C open up opportunities, but will also demand a closer follow up of patients and more precise diagnosis and resistance surveillance. These tasks must be solved in near future.

The surveillance of influenza and HIV antiviral resistance has been conducted continuously in Norway from 2005 and 2006 respectively, and the process of implementing this surveillance into the register RAVN (Resistance against Antivirals in Norway) started in 2011. At the same time, resistance data for HBV and CMV has been collected from the national reference laboratories for inclusion into RAVN.

Influenza

- Surveillance of influenza antiviral resistance is conducted at the NIPH and is vitally important to continuously be able to provide evidence-based advice on the empirical antiviral treatment during annual influenza season and pandemics.
- Monitoring has revealed new susceptibility trends that have subsequently been identified in other countries.

HIV-1

- The surveillance has shown that viruses with resistance mutations can be found among newly diagnosed HIV-1 patients, and this must be monitored closely to follow any increasing trend. This might give an impact on treatment regime at start of therapy.
- Resistance surveillance was carried out in less than half of the newly diagnosed HIV-1 cases during the first years of implementation, but in the last year there has been an increase in the percentage of samples tested. It is a necessity to improve the surveillance even further, ensuring a representative number of samples from all patient risk groups. The incidence of HIV-1 has increased among MSM in recent years and it is therefore important that the surveillance of this group is well covered.

HBV

- Antiviral drug resistance seems to be a minor health problem in Norway among chronic HBV (CHB) patients at the present time. Patients in Norway are increasingly given first-line therapy that effectively suppresses the virus replication and limits the development of drug resistance.
- The number of patients on nucleos(t)ide analogue (NA) therapy (upto 485 in 2012) appears to be lower than expected, given the estimated number of 20 000 cases of CHB infections in Norway.
- There is no overview of the total number on treatment for HBV-infection in Norway, including type and duration of treatment used and treatment failure. For surveillance it is important to obtain and systemise these data and link them with available data on drug resistance. A system should be put in place to get a better overview and systemise data for surveillance purposes.

CMV

- Serious CMV-infections that require antiviral treatment are mainly seen in severely immunosuppressed patients. Most treatment failures are seen in this group of patients.
- Upon treatment failure about one forth is caused by development of resistant CMV

Recommendation from RAVN council

The development of resistance to antiviral agents is monitored, recorded and reported through the RAVN organization. The viruses monitored are influenza virus, Human Immunodeficiency virus (HIV), Cytomegalovirus (CMV) and Hepatitis B virus (HBV).

Analysis of influenza virus resistance is carried out by the Department of Virology at the Norwegian Institute of Public Health (NIPH). The Influenza virus resistance surveillance has been carried out since 2006 and is necessary to provide evidence-based advice for empirical antiviral treatment during the annual influenza seasons and pandemics. This monitoring is

of highest quality and is standardized according to the network of influenza reference laboratories in Europe and should be maintained in its present form.

HIV resistance surveillance performed at the Oslo University Hospital, Ullevål, involves the detection of primary resistance, i.e. detection of resistance mutations in samples from newly diagnosed patients before the start of an antiviral treatment. The current system captures less than 50% of the newly diagnosed HIV patients. Measures should be taken to increase the number of samples from these patients for resistance testing.

Resistance monitoring of CMV infections is conducted at the Oslo University Hospital, Rikshospitalet. Resistance testing is performed in case of treatment failure during the therapy against CMV infections. Although the request for such testing is infrequent, the system is functioning satisfactory.

HBV resistance testing is performed at NIPH and this has been ongoing since 2004. New antiviral drugs that have come on to the market have been included in the assays and the results will be reported systematically to RAVN from 2011. In recent years new and improved drugs for the treatment of chronic HBV infection have been marketed, but the long-term use of these antivirals regarding resistance development should be followed closely.

In 2011, two new protease inhibitors for use in combination therapy of chronic HCV infection (genotype 1) were released. During treatment, resistance mutations may develop. Resistance testing is under establishment at NIPH. It is recommended to make a pilot study with the aim to build capacity to assess the needs and capacity for resistance monitoring, as well as experiences for a national monitoring of HCV resistance in RAVN.

With the constant development of new drugs and new treatment strategies, surveillance of antiviral resistance is of vital importance.To provide good surveillance data, monitoring of drug resistance needs to be systematic and standardised.

RAVN Council recommends:
Influenza virus resistance and CMV resistance monitoring continue as before, HIV resistance monitoring of primary infections is to be intensified, and a program for monitoring the development of HBV and HCV resistance is recommended.

Background

Virologists in Norway have been discussing the need for antiviral resistance surveillance for many years. In 2001 a working group appointed by The Ministry of Health and Care Services and led by the Norwegian Institute of Public Health (NIPH), made the first report on HIV resistance surveillance "Utredning om nasjonalt overvåkingssystem for HIV - resistens". The working group concluded that there should be a systematic surveillance of resistance in newly diagnosed HIV-1 positive patients, and this was implemented in 2006.

A survey of the susceptibility testing of other viruses in the country was conducted by NIPH in 2004. The conclusion was that the testing was very limited. Nevertheless, the development of new antivirals continued and in 2008 a plan was made "Nasjonal strategi for forebygging av infeksjoner i helsetjenesten og antibiotikaresistens (2008 – 2012)", advising further evolvement of the HIV susceptibility surveillance system and that surveillance of influenza should be established systematically together with a plan for a surveillance system for other viruses.

In the beginning of 2010, the NIPH published a report "Utredning om nasjonalt overvåkingssystem for virusresistens" resulting in the establishment of RAVN (Resistensovervåking av virus i Norge, Resistance against Antivirals in Norway) a national register recording antiviral susceptibility surveillance.

The newly launched RAVN Centre has been set up and is run by physicians and scientists at the Department of Virology at NIPH. Also, the RAVN Council has been appointed to make recommendations for the annual surveillance and will hold meetings twice a year. Recently the HIV resistance surveillance has been placed under the Department of Virology at NIPH. Representatives from both RAVN Council and RAVN Centre at the NIPH are participating in the European Society of Antiviral Resistance (ESAR). Some work remains on the rules and regulations of RAVN and the RAVN Centre has not yet been staffed according to the recommendations made in 2010.

In the following report all national data on viral resistance is presented. This biannual report for 2011–12 has a special focus on HIV-1 and influenza resistance as these are currently being systematically monitored. Later reports will contain more information on other viruses depending on the surveillance plan and recommendations from the RAVN council. The data has been collected and processed by the RAVN Centre and Council. We hope the report will show the benefits of the work accomplished to date, although there is still much more to be done. The plan is to expand and develop the surveillance system to all areas concerning medically important antivirals. The RAVN Centre should be staffed accordingly as planned, to ensure a national antiviral resistance surveillance thereby laying the foundations for participation within international networks.

The organization of RAVN

RAVN stands for Resistance against Antivirals in Norway ("Resistensovervåking av virus i Norge"), and was established in accordance with the Ministry of Health's (MoH) "National Strategy for the prevention of infections and antibiotic resistance in health care (2008–2012)". RAVN consists of a Centre located at the Department of Virology at NIPH, and a Council who will work together to plan and manage the annual surveillance of viral resistance in cooperation with participating regional laboratories.

The RAVN Council was formally appointed for the first time in 2010, and holds meetings twice a year. The RAVN Centre has in 2010–11 been run by a team from the Department of Virology at NIPH. The team has worked closely with lawyers from The Ministry of Health and Care Services to prepare RAVN's rules and regulations. RAVN will be developed to include surveillance of viral resistance to influenza virus, human immunodeficiency virus type 1 (HIV-1), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV) and herpes simplex virus (HSV).

Aims of the Council:
The Council aims to promote high quality surveillance activities within RAVN. The Council collaborates closely with the RAVN Centre in order to organize the annual surveillance of viral resistance and evaluate the antiviral resistance data which are published annually. The Council will assess submitted applications for access to RAVN data. The Council will also consider applications for research grants and together with the RAVN Centre select the most relevant and appropriate applicants. The Council and the RAVN Centre combined will ensure the interests of the participating laboratories. The RAVN Centre's annual report and accounts will be presented to the Council.

The Council Meetings:
The head of the RAVN Centre is responsible to call the board meetings twice a year, and to keep the minutes of the meetings. The agenda is drawn up by the Chairman of the board and the RAVN Centre leader. All attendees are eligible to vote in all matters that require a vote and the chairman of the Council has a casting vote.

RAVN Council panel:
The Council should comprise of one medical microbiologist (virologist) from each of the four regional laboratories, and one from the NIPH to cover both geographic and institutional representation. In addition, there should be a molecular biologist and a specialist in infectious diseases. Also representation from the non-regional microbiology laboratories must be included. The Council should consist of no more than 8 people. The Council should be chaired by a medical microbiologist.

Council will therefore consist of the following professionals:
- Medical microbiologist from Oslo University Hospital
- Medical microbiologist from Haukeland University Hospital
- Medical microbiologist from St. Olav Hospital University Hospital of Trondheim
- Medical microbiologist from the University Hospital of North Norway
- Medical microbiologist from the National Public Health Institute
- Medical microbiologist from one of the non-regional microbiology laboratories
- Molecular Biologist
- Specialist in infectious diseases

Period of office for RAVN Council Members:
Members and the Chairman of the RAVN Council are elected for a 2 year term with the possibility for immediate re-election allowing for a 4 year consecutive period for each member. Members who have previously served on the Council can be elected again at a later date. The Council members are appointed by the Executive Director of the Division of Infectious Disease Control at the National Institute of Public Health. When appointing new members it will be taken into account that the continuity should be maintained, so only up to half of the Council's members will be replaced at any one time.

The usage of antivirals in Norway

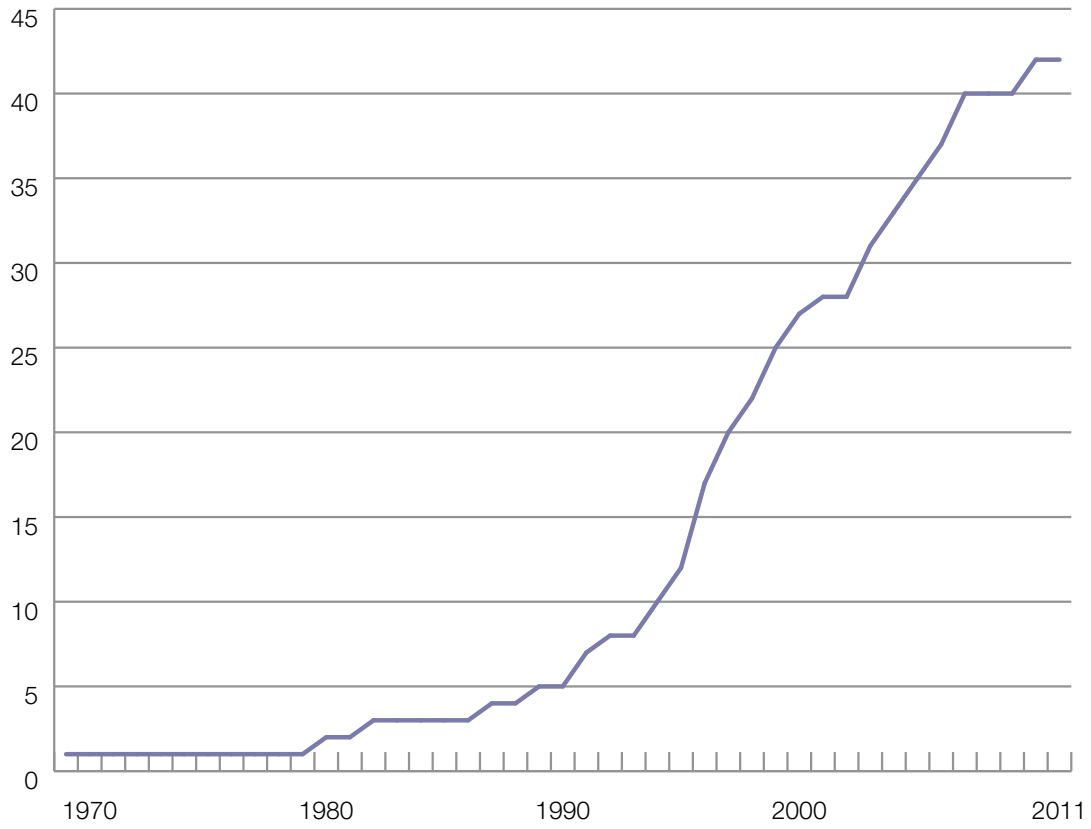


Figure 1. Cumulative number of direct acting antiviral drugs by year of approval (2).

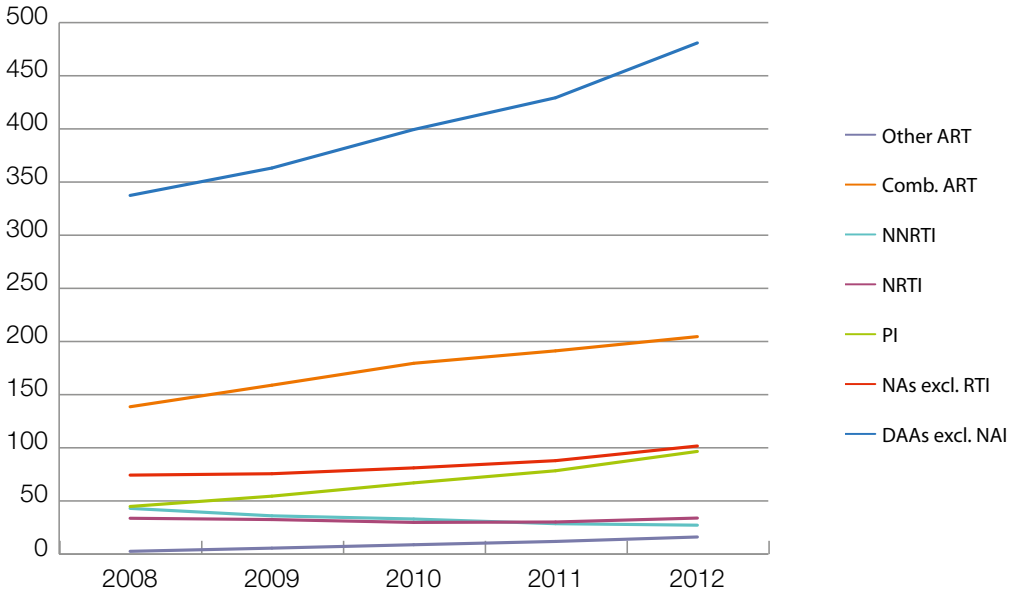
During the last 15 years, the development of new specific antivirals has been accelerated due to research into HIV medicines (1).

The prescribed amount of antiviral drugs has been increased every year as seen in figure 2. According to the Norwegian Prescription database (NorPD), anti-infectives for systemic use cost increased by 8% in 2012 (3). The increase is mainly due to increased sale of antivirals (4). Figure 2 shows the sales of direct acting antiviral drugs (DAA), excluding the neuraminidase inhibitors (NAI) during the past five years.

The usage of anitivirals for the treatment of influenza is shown in Table 1. Due to the pandemic in 2009, the annual number of individuals with neuraminidase inhibitor drug prescription was much higher during that year compared to previous or following years (3). The huge increase shown in 2009 includes stockpiling of neuraminidase inhibitors, and prescriptions given out that were unused.

There are currently 28 approved antivirals for HIV in Norway. The usage of these drugs is increasing and nearly doubled from 2007 to 2012, as indicated in figure 3 showing the number of patients given at least one prescription per year. In addition there is an increase in prescription with combination therapy indicating an even higher usage (figure 3). The number of patients on HIV therapy is difficult to estimate due to combination therapy of several drugs. The largest increase is seen for ritonavir and atazanavir (3). Ritonavir is exclusively used as a PI enhancer and is always used in combination with other HIV drugs, decreasing pill burden and frequency of dosing. Trends in usage may be due to new combinations drugs, as for example the usage of combination therapy emtricitabine and tenofovir disoproxil decrease, whereas triple combination with emtricitabine, tenofovir disoproxil and efavirenz is increasing.

There are currently 8 approved therapies for HBV infection including 3 interferon based and 5 nucleoside/nucleotide analogues (NA) (lamivudine, adefovir

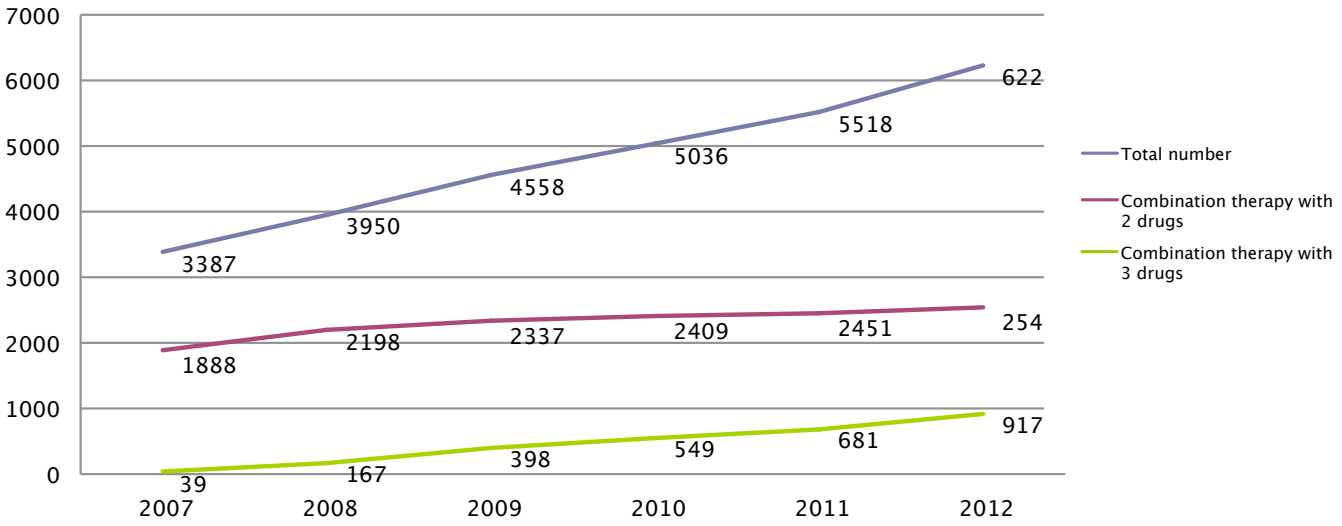


Source: The Norwegian Drug Wholesales statistics database.

Figure 2. Sales of direct acting antiviral drugs (DAA) excluding neuraminidase inhibitors for 2008–2012 given in DDD/1000 inhabitants/year.

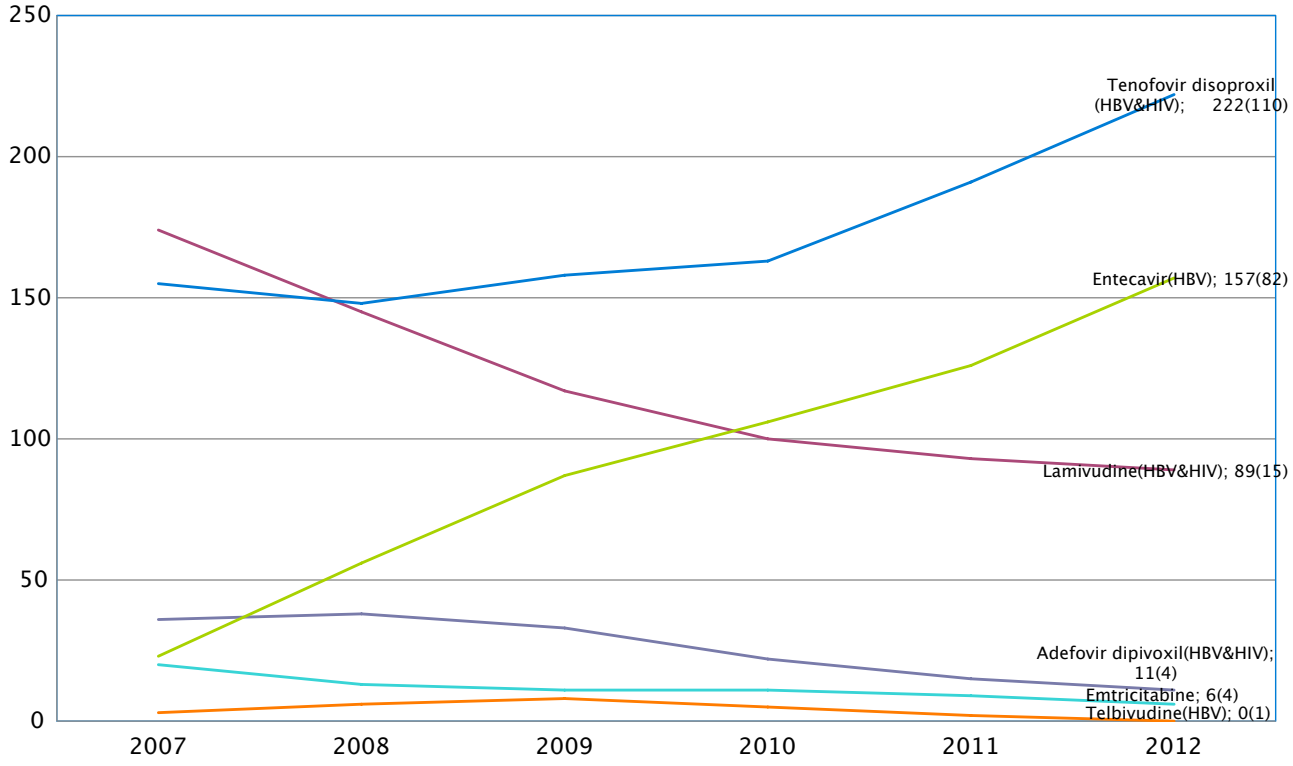
Table 1. Number of individuals with at least one prescription of neuraminidase inhibitor drug according to year.

Neuraminidase inhibitor drug	Number of individuals with one or more prescription per annum					
	2007	2008	2009	2010	2011	2012
Zanamivir	2	109	2 542	35	36	33
Oseltamivir	3 264	981	279 946	3 829	2 612	1 724



Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Figure 3. Trends in use of antivirals for treatment against HIV from 2007–2012



Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Figure 4. Patterns of prescriptions for HBV-treatment from 2007–2012 based on the number of patients given at least one prescription per year.

dipivoxil, entecavir, telbivudine and tenofovir disoproxil). Treatment of HBV with antivirals is generally given in mono-therapy. 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 subscription per year for the period 2007–2012 (3). Lamivudine, adefovir dipivoxil and tenofovir disoproxil are drugs that are approved for both HBV and HIV, while entecavir and telbuvidine are approved for HBV only. An estimated of patients treated for HBV with antivirals in Norway will therefore be in the range of 160–485 in 2012 based on the patients that used drugs approved for HBV only and the total number of patients treated with the 5 NA-drugs. Further, the number of patients on HBV therapy sending samples for analysis is to the department of virology is registered from 2012 and indicated in parenthesis (fig 4). These data together indicate around 400 patients in Norway given NA therapy for HBV in 2012. First-line therapy (entecavir and tenofovir disoproxil) has been increasingly used since 2007 and account for 78% of the five NA treatments given in 2012.

HCV-therapy is based on a combination of ribavirin and interferon for a given period depending on HCV-genotype. The effect of treatment is highly dependent on HCV genotype. In 2011 two new protease inhibitors (PI) were approved for combination therapy with ribavirin and interferon for patients infected with HCV genotype 1, improving the efficiency of therapy to this group of patients. Annually around 800 patients are given treatment for HCV (figure 5). In 2012 almost 40% of patients were given combination therapy with PI, and the overall number of patient on treatment seems to have increased slightly with the new drugs. In the pipeline, there are a number of new antiviral drugs targeting HCV that are ready for release from 2014–2016, so that the usage of antivirals is expected to increase further in the coming years. Figure 6 shows the two most prescribed drugs for Herpes-virus infections including CMV over the last six years. The following drugs Ganciclovir, Famciclovir, Cidofovir and Foscarnet have been prescribed very rarely in this period.

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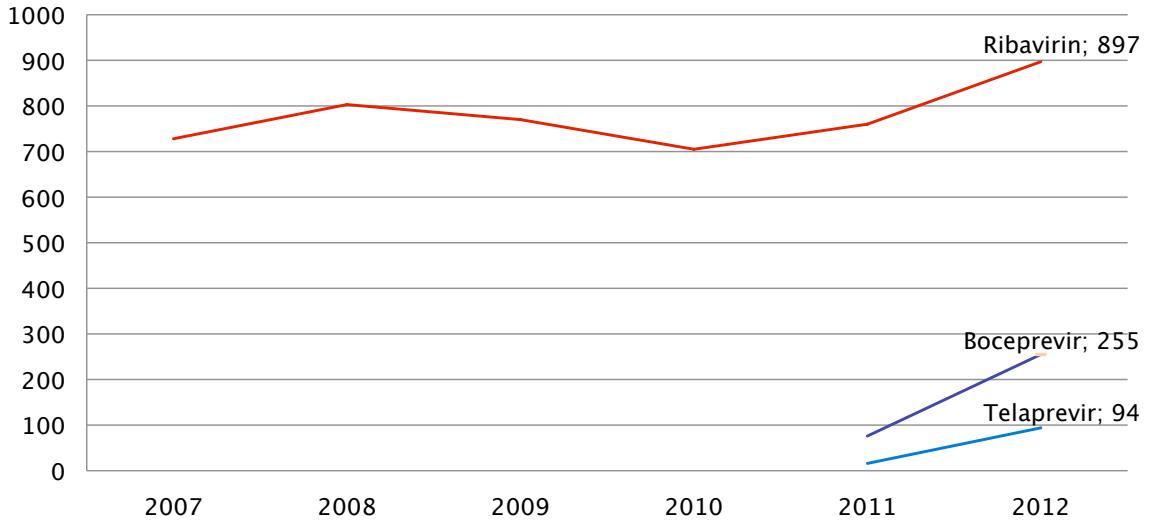
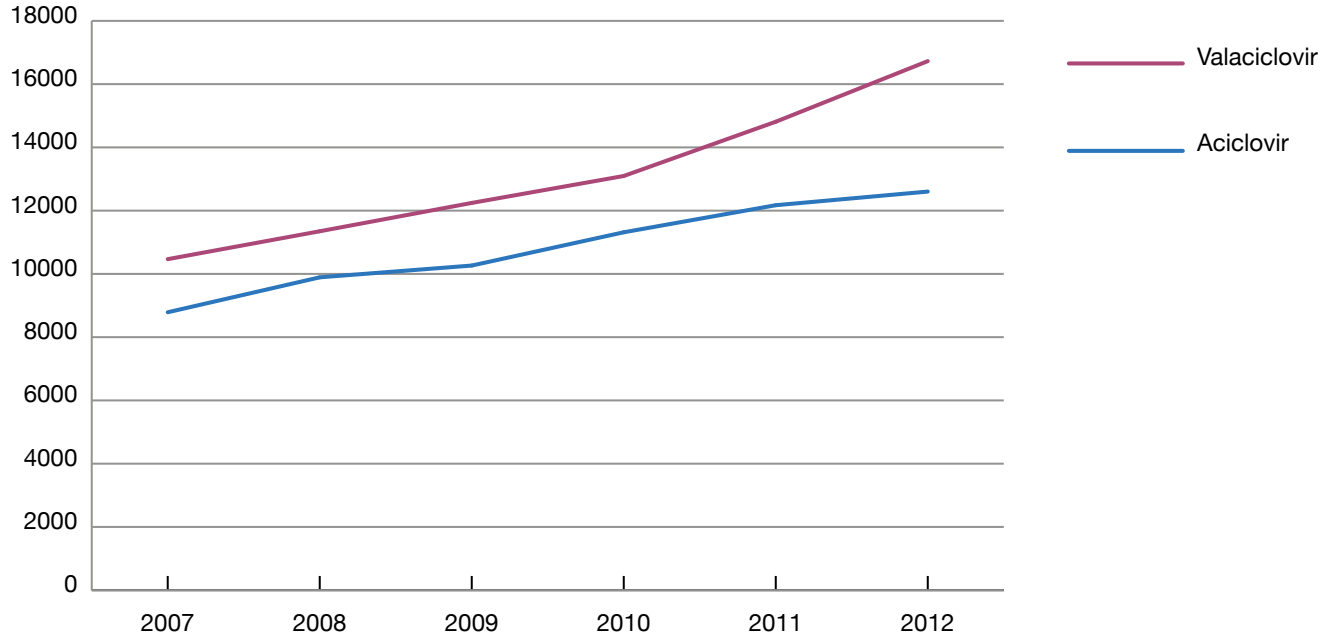


Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Figure 5. Patterns of prescriptions for HCV-treatment from 2007–2012 based on the number of patients given at least one prescription per year.



Source: The Norwegian Prescription Database (NorPD), Norwegian Institute of Public Health

Figure 6. Number of individuals with at least one prescription of acyclovir and valaciclovir per year for the periode 2007–2012.

Methods for detection of antiviral resistance

Resistance indicates the virus's ability to multiply in the presence of antiviral agents. Antiviral drugs are targeted against essential steps in the viral life cycle. It may be the viruses' own enzymes such as polymerase or protease, or viral mechanism to penetrate into or out of the host cell. Viruses can develop resistance to these drugs by the occurrence of one or more mutations in genes encoding for the antiviral target protein. The consequence is that the production of new virus particles is no longer inhibited by a drug at a concentration that would normally inhibit the virus.

There are two approaches for the detection of viral resistance, phenotypic testing of the infectious virus in the presence of an antiviral drug, or genotypic testing where mutations associated with antiviral resistance are detected using molecular biology techniques. The genotype describes the composition of nucleotides in the genome, while the phenotype is the functional expression of one or more genotypes in the virus population.

Phenotypic resistance testing is a direct measure of resistance where the virus's ability to replicate in the presence of various concentrations of antiviral drugs is analysed. The virus must first be isolated from the patient in question and then cultured in presence of serial dilutions of the drug. The resistance is analysed for one drug at a time, and the results must be compared with the results from a virus strain sensitive to the analysed drug. Phenotypic methods determine the drug concentration required to inhibit in vitro virus replication in the cell culture by 50%. The concentration is named "inhibitory concentration 50%" (IC50).

One problem with this method is to define the concentration that provides clinically relevant resistance (clinical cut-off level). The method is considered to be the gold standard, but is technically complex, labour- and time-consuming (depending on how fast the virus grows in cell culture), is costly and takes place in a cell culture laboratory (for HIV a biosafety level 3 laboratory is required). Therefore, this is usually not the preferred routine method.

Genotypic resistance testing is an indirect measure of the resistance by which nucleotide mutations, which correlate with resistance to one or more drugs, are detected. Genotypic methods require that the genetic cause of virus resistance has been identified. For interpretation of the genotype a map of known resistance mutations is used. For some medications, a complex interaction between several mutations is causing the resistance. It is common to use a sequencing-based method for the genotypic resistance testing, in which the gene involved in the antiviral activity is sequenced. The method is suitable for routine diagnostics. It requires sophisticated and expensive equipment and interpretation can be complicated, but viral culture is not necessary. It is less expensive in use and faster to perform than phenotypic resistance testing (takes 2–3 days).

Influenza virus drug resistance

Influenza viruses are divided into three types, A, B and C. Type C is the most rare and does not normally cause epidemic outbreaks, unlike influenza A and B that cause annual winter epidemics. Influenza A is subdivided into several subtypes based on the two surface antigens, hemagglutinin (H) and neuraminidase (N). Two subtypes, A(H1N1) and A(H3N2), have been circulating in the human population during the last few decades. These subtypes are so antigenically different that immunity acquired against one subtype will not protect against the other subtypes.

During the annual winter epidemics influenza illness has a large effect on society due to the large number of persons getting ill. For the affected individual, the illness is troublesome although most people recover without medication. What makes influenza a major public health problem are cases with serious complications, hospitalisations and death that occur during these epidemics. People with certain medical conditions are more likely to develop severe influenza infections and are highly recommended to be vaccinated against influenza every season (1).

Locally the viruses run their course and disappear completely after the outbreak is over. Other influenza viruses emerge the following winter, having been imported into the area from other parts of the world. Influenza virus is recognised by rapid evolution and annual global spread. Unlike many other viruses, for influenza virus the same virus variant is seen all over the world at the same time, but at a given place different influenza viruses occur from year to year. The rapid evolution of the influenza viruses also has an impact on the susceptibility against antiviral drugs.

Zoonotic and pandemic influenza

In addition to the seasonal epidemics in the human population, influenza A viruses exist in many animals – especially amongst ducks and other aquatic bird species, which are the most important hosts and probably the origin of influenza A found in most other species. The majority of these viruses has become very well adapted to their species and is not easily adapted in order to transmit to, and especially between, humans. These viruses represent an antigenic variability that is far wider than the human immune system will be prepared to combat. Zoonotic influenza infection can occur sporadically, normally without further transmission from person - to - person. Infection can result in a disease ranging from subclinical to severe course, e.g. the highly pathogenic A(H5N1) bird flu that has been circulating since the late 1990s.

Zoonotic influenza viruses are antigenically completely different from the viruses circulating in humans and therefore they have a huge epidemic potential if they develop the ability to efficiently transmit from human to human. Emergence of antigenically new influenza A virus has occurred earlier in history and resulted in exceptionally large and often severe epidemics, called influenza pandemics. Over the last hundred years, influenza A viruses of different subtypes emerged from animals and caused major pandemics among humans in the years 1918, 1957, 1968, 1976 and 2009.

Subsequent to a pandemic, the new virus is established and will remain a dominant influenza A subtype for the forthcoming seasons until possibly being replaced during the next pandemic. Previous influenza pandemics have emerged without warning, leaving little or no time to prepare efficient vaccines to combat the new virus during the pandemic on a large enough scale. Probably the same will happen during the next pandemic, especially in the beginning, and therefore many countries stockpile antiviral medicines as an immediate measure against the pandemic influenza (1).

Antivirals against influenza

There are two classes of antiviral drugs available against influenza, the M2-inhibitors amantadine and rimantadine, and the neuraminidase inhibitors, oseltamivir and zanamivir (3). The inhibiting effect of the M2-inhibitors amantadine and rimantadine has been well known since the 1960's. M2-inhibitors block viral replication in influenza A infection, but is ineffective against influenza B- or C-virus which do not possess M2 proton channels. Studies have shown that both drugs can prevent influenza A illness in 70–90 % of cases, and that they can reduce the duration and severity of influenza A if treatment is started within the first two days after the symptom debut (4). Usage has been limited due to side effects from the central nervous system, especially in the case of amantadine, but less so for rimantadine (4).

The Norwegian pandemic stockpile of anti-influenza medicines includes rimantadine meant for prophylactic use (2). The drugs are not registered in Norway for treatment of influenza, but amantadine has been used for Parkinson's disease.

In 1999, zanamivir (an inhalation drug), the first

neuraminidase inhibitor, was approved for clinical treatment against influenza A and B infection. In 2002 the first oral neuraminidase inhibitor, oseltamivir, was licensed in Norway. These medicines were amongst the first so-called designer drugs, where the molecular structure was designed by computers to fit the known viral protein. These two drugs are the only medicines licensed in Norway for chemoprophylaxis and treatment of influenza type A and B infections. Prior to the start of the pandemic in 2009, the use of these was quite limited in this country (www.reseptregisteret.no).

The mechanism of action of these drugs is shown in figure 1 – 3 and the clinical effect of these two medicines is almost identical. When oseltamivir is used profylactically, it is proven to prevent influenza in up to 89 % of healthy adults (5), zanamivir similarly in up to 84 % (6). It has been shown that the drugs reduce the duration of symptoms by two days and the degree of severity in healthy adults and children with laboratory confirmed influenza (5). The effect has not been studied to the same extent for infections with A(H5N1)-virus.

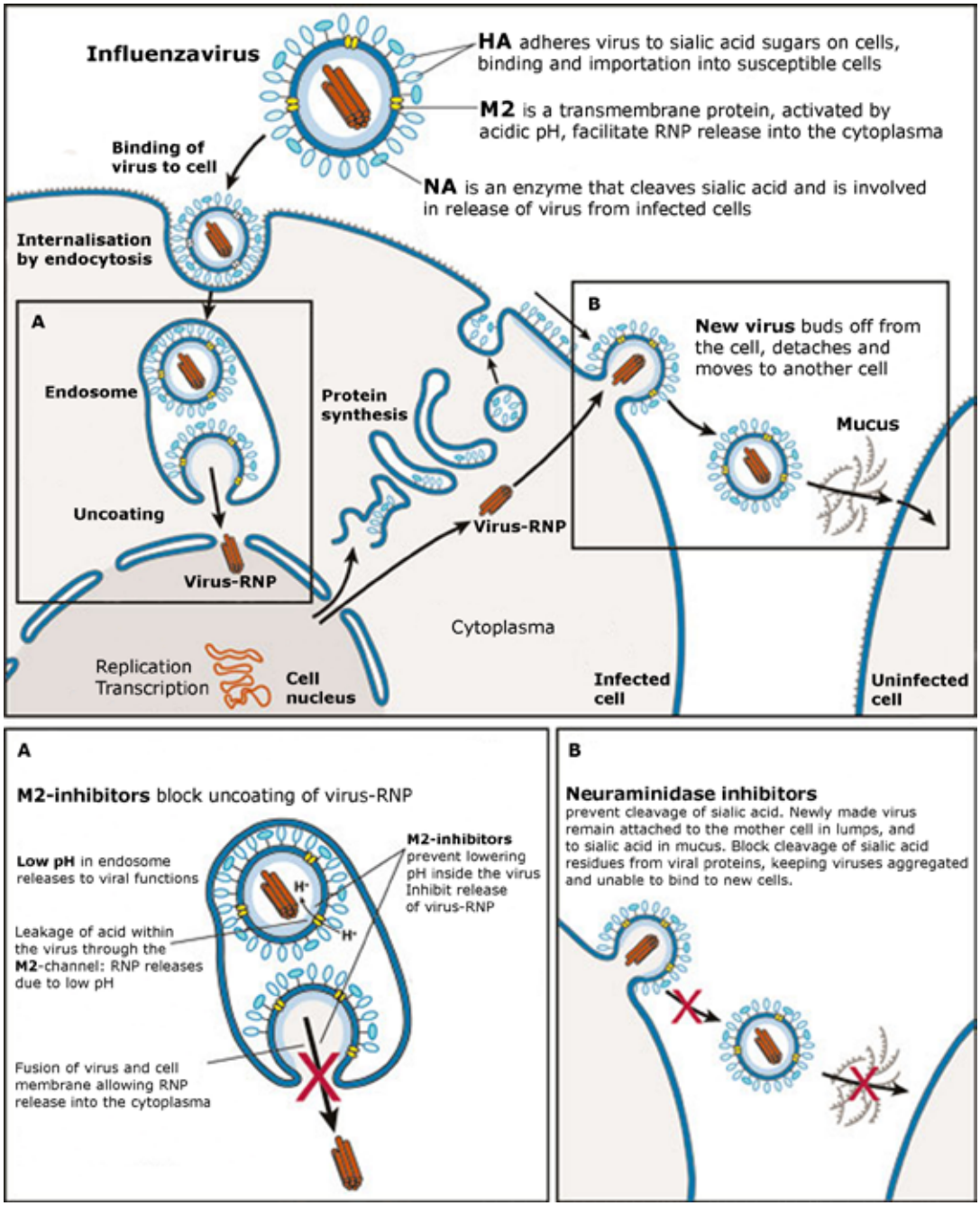


Figure 1. Targets for antivirals in the influenza virus replication cycle. M2 blockers work primarily early in the virus replication cycle - they prevent the virus's genetic material to be released from the particle when the virus enters a cell. Thus the viral genetic material will not be released into the nucleus where it can replicate and give rise to the synthesis of mRNA. Neuraminidase inhibitors prevent cleavage of sialic acid by the viral enzyme neuraminidase. Thereby, the release of virus from the infected cell and from respiratory secretions is prevented when the virus hemagglutinin remains bound to the sialic acid (19).

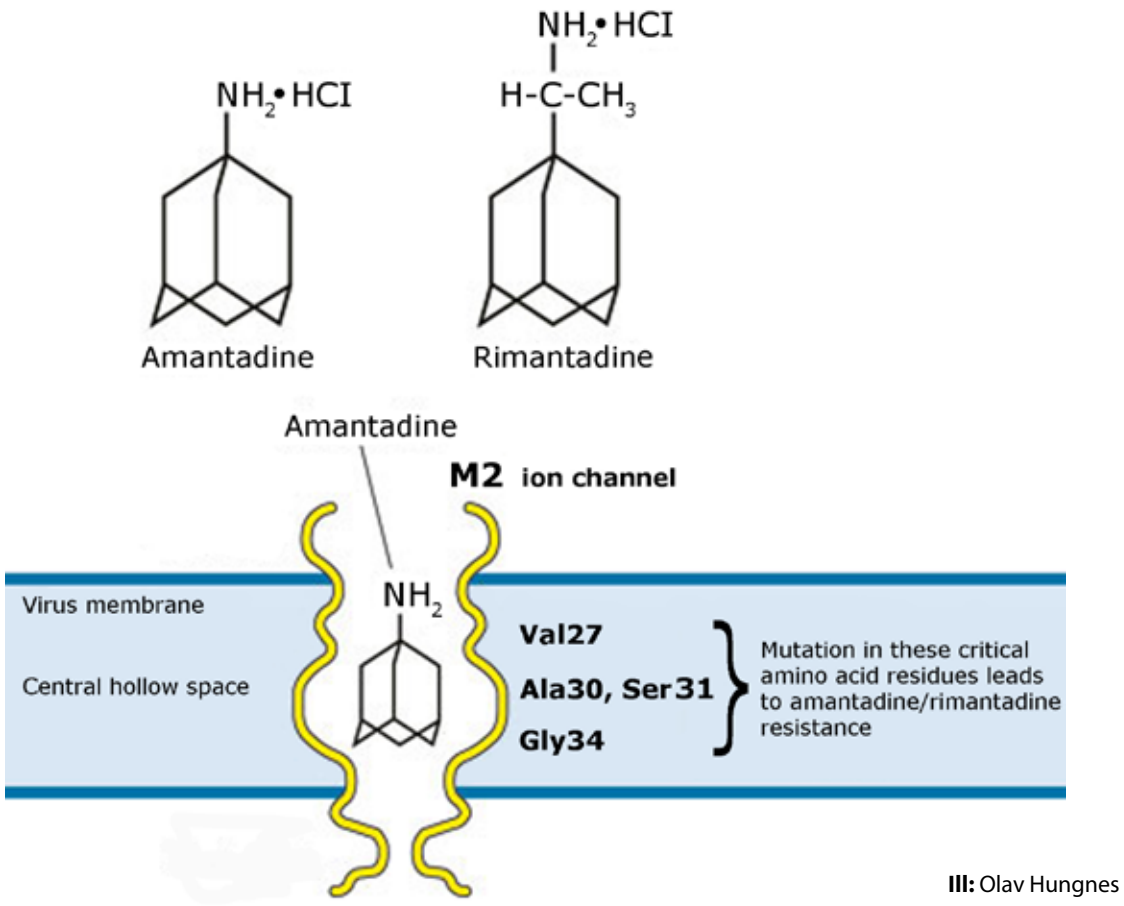


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Figure 2. Mutations that confer resistance to M2 blockers.
Relevant mutations in the M2 transmembrane region are plotted. This is a defined set of mutations in a small region of the polypeptide chain and is suitable for genotypic resistance testing (19).

Occurrence of resistance to anti-influenza agents

The high mutation rate in RNA viruses such as influenza provides an opportunity for selection of resistant viruses and can lead to a loss of clinical efficacy by the antiviral drugs. Shortly after the discovery of the antiviral effect against influenza A in the M2 blockers, amantadine and rimantadine, in the early 1960s, development of resistance against both these agents was observed in laboratory experiments (7). Studies in the 1980s showed that resistance to M2 blockers spread quickly during use in both children and adults (8). The resistant strains have both the ability to be transmitted between humans and cause illness (9). For reasons that remain unclear, the frequency of M2 blocker resistance in human A(H3N2) viruses gradually rose from almost nil to 100 per cent during the first decade of this century, and the pandemic A(H1N1) virus that emerged in 2009 has been uniformly resistant to M2 blockers since the start.

Resistance to oseltamivir has until recently been reported very rarely. But during winter 2007–08 resistance to oseltamivir was observed in an unexpectedly high proportion of influenza A (H1N1) viruses in Norway. Two-thirds of the Norwegian strains during the influenza season 2007–08 had a histidine to tyrosine mutation at position 274 in N1 neuraminidase (H274Y), leaving them highly resistant to oseltamivir and the related drug peramivir. The mutant viruses were still fully susceptible to zanamivir and the M2 blockers. Previously, this particular resistance generating mutation had been known for some years, but the virus viability was reduced and the mutation had not been observed in circulating A (H1N1) virus. The emergence of resistance occurred in almost complete absence of oseltamivir use in Norway. Co-occurring resistance to both M2-blocks and oseltamivir have earlier been detected in immunosuppressed individuals during subsequent treatment with these agents against influenza A infection (10).

Studies in which samples were taken before and after treatment with zanamivir, failed to demonstrate resistance, but zanamivir resistance has been reported in sporadic cases with influenza B (11). The structural similarity between the natural substrate and zanamivir, and high concentration of the drug in the respiratory tract where virus replication occurs, help to reduce the risk of resistance development. Different influenza viruses show varying sensitivity to neuraminidase (NA) inhibitors (10).

Oseltamivir has been the drug of choice, mainly because of its ease of administration in tablet form. The alternative, zanamivir, is inhaled and has not been

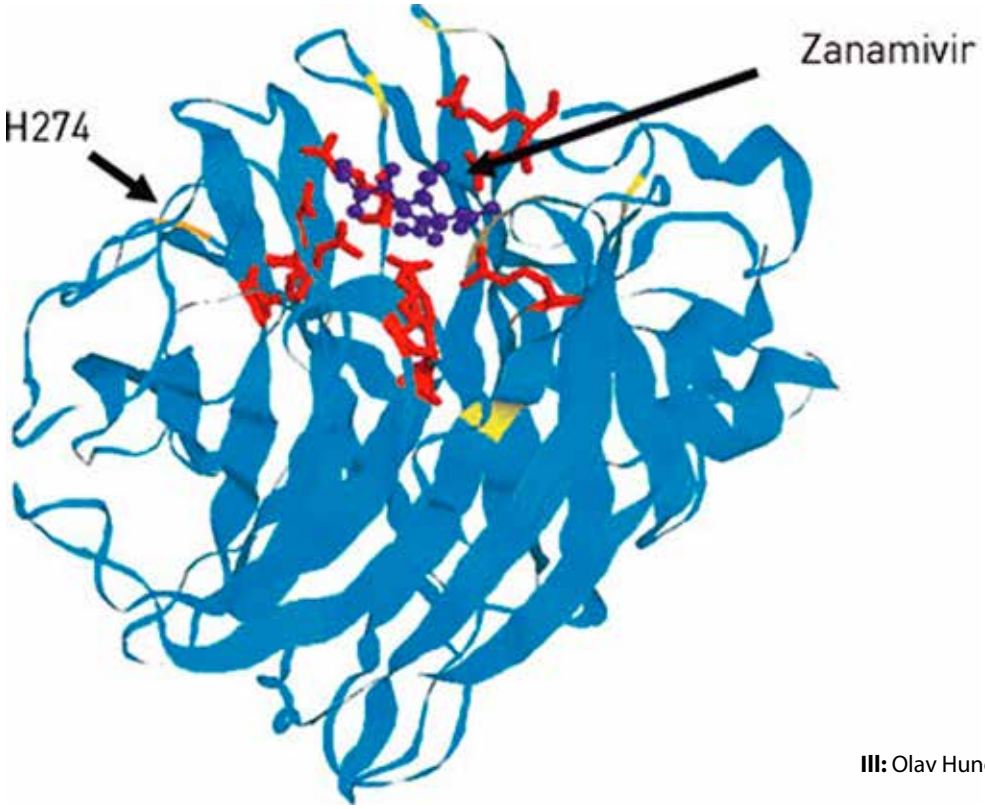

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Figure 3. Mutations that confer resistance to neuraminidase inhibitors.
The protein structure of the virus neuraminidase is shown with zanamivir (blue) bound in the active site. Mutations in the neuraminidase active site (red) affect the enzyme's function, but often also cause crossresistance. Mutations in important positions in the surrounding "protein framework," eg. H274Y (yellow color), can provide resistance to some agents and not against others. A large number of mutations may contribute to the resistance, and the relationships between genotype and resistance are incompletely known. Therefore, genotyping often do not provide definitive answers about the virus's susceptibility / resistance to neuraminidase inhibitors. (Molecular Structure visualized with RasMol (www.umass.edu/microbio/rasmol/) (19).

used nearly as extensively as oseltamivir, even though resistance has been detected rarely. Two other recently developed NA inhibitors, peramivir and laninamivir, are currently approved for use in Japan with additional clinical trials planned elsewhere (12). 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 (12).

Impact of neuraminidase inhibitor (NAI) treatment during the 2009–2010 Influenza A(H1N1) pandemic has been studied (13). Main conclusions were that early initiation of NAI treatment reduced the likelihood of severe outcomes compared with late or no treatment. Regarding mortality, significant reductions was observed for early treatment (≤48 hours after symptom onset) versus late and for early treatment versus none. Studies up to now have shown that the A(H1N1)pdm09 virus is sensitive to oseltamivir and zanamivir, but resistant to M2 blockers. Until now, only a few cases with oseltamivir resistant virus have been reported in the world and many of these have occurred in persons that have used the neuraminidase inhibitor as a prophylactic agent or immunocompromised patients being treated with oseltamivir during protracted infection.

Development of resistance

Resistance can develop in different ways. The resistant form may occur by de novo mutation, by exchange of genetic material between different influenza or it can be present initially as a rare variant. A minority resistant form can take over in a virus population by chance or as a result of selection. Selection can occur when an appropriate antiviral substance is present without virus replication prevented altogether, e.g. at suboptimal concentrations of the substance, or because the virus is not particularly sensitive. But it is also possible that resistance is located in a virus variant that has another advantage, and that the resistance is 'hitch-hiking' on another advantageous feature that promotes this variety over other viruses. Resistance can thus 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.

Methods for detection of resistance

Influenza resistance tests in Norway are currently performed at the National Influenza Centre at NIPH. The methods used in the laboratory to determine whether the virus isolates can be classified as sensitive or resistant to a drug, are either phenotypic or geno-

typic. By the phenotypic methods, one can determine the concentration of an antiviral agent that inhibits the virus.

Methods for resistance testing against neuraminidase inhibitors are commonly measuring decrease in neuraminidase enzyme activity with increasing concentration of the pharmaceutically active substance. One may thus determine the IC50, that is the drug concentration which gives 50% inhibition of the viral neuraminidase activity. NAI susceptibility is measured by enzyme inhibition assay. The MUNANA assay determines the sensitivity of influenza viruses to the NA inhibitor compounds, using the substrate MUNANA. MUNANA is a fluorescent substrate 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid (MUN or MUNANA). Cleavage of MUNANA by neuraminidase releases the methylumbelliferone which then fluoresces. The amount of fluorescence therefore directly correlates to the amount of enzyme activity. Any isolate suspected of showing reduced susceptibility in the NA inhibition assay is further characterised by sequencing the NA gene before resistance may be confirmed.

The genotypic methods detect mutations which already are known and have been shown to occur in resistant viruses by analysing gene sequences in specific target areas of the viral genome. Appendix 1 list substitutions in influenza neuraminidase associated with resistance or reduced susceptibility to neuraminidase inhibitors. Genotypic methods require that the genetic cause of virus resistance have been identified. The correlation between the finding of virus mutations and their impact on resistance should be evaluated in studies of the virus. Genotypic methods are used for susceptibility testing for both neuraminidase inhibitors and M2 blockers.

Pyrosequencing is a molecular technique that can be used for the detection and quantitation of neuraminidase inhibitor resistance mutations. This rapid technique can be used both on virus cultures and directly on clinical material which means that it can be used for individual management of severe cases. It has the advantage over alternative methods such as conventional Sanger sequencing which are more time consuming or lack the sensitivity to detect mutations in mixed virus populations.

Pyrosequencing is a real-time DNA sequencing technique which, via a cascade of enzymatic reactions, detects pyrophosphate (PPi) released during DNA synthesis as visible light. The light released is quantitative and enables the rapid generation of sequence information. This is a rapid technique suitable for high throughput surveillance or drug resistance screening, as demonstrated during the emergence of the oseltamivir resistant seasonal influenza A (H1N1) H274Y viruses in Europe in 2007–08 (14).

The clinical significance of resistant influenza virus

Cases with severe influenza infection require specific antiviral therapy. Viral resistance therefore will be of major clinical significance, especially for patients with high risk of complications. 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 (15). Clinical treatment failure can be due to other causes than viral resistance, and in such cases susceptibility testing will be invaluable.

Normally in influenza infection, susceptibility testing will not be possible before the start of the treatment, as the window of opportunity for efficient treatment is very narrow. Even laboratory confirmation of influenza infection can be too time-consuming, leaving empirical treatment as the only option. Choice of medicine should therefore be evidence based, by using knowledge 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.

Surveillance of influenza resistance

Surveillance of influenza resistance in Norway
The national reference laboratory for influenza in the NIPH monitors the occurrence of influenza viruses in Norway. A volunteer network of sentinel physicians in all parts of the country provide the reference laboratory with samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit confirmed influenza strains. These samples are analysed by virus cultivation and other methods. Resistance monitoring is performed using both the genotypic (part / full sequencing, pyrosequencing and PCR) and phenotypic susceptibility testing of virus isolates. The data is recorded in a format that is compatible with international database formats. Since 2007, the influenza reference laboratory has made annual reports of influenza resistance surveillance, and has published a number of research results in international journals. During the influenza season the results from resistance surveillance are published weekly on the NIPH's website www.fhi.no/influensa.

Surveillance of influenza resistance through WHO / European Influenza Surveillance Network
The WHO European Regional Office, in coordination with the European Centre for Disease Prevention and Control, conducts surveillance of seasonal influenza in the Region and publishes a weekly regional bulletin on seasonal influenza . The data are collected by clinicians' networks and laboratory networks, consisting primarily of WHO-recognized National Influenza Centres (NICs).

The regional surveillance network also participates in the WHO Global Influenza Surveillance and Response System (GISRS), mainly through the 50 NICs in 39 European countries. Data and viruses are submitted through the NICs and the surveillance focal points to one of the four global WHO collaborating centres for reference and research on influenza (for the European Region, the centre is located in the United Kingdom). This enables WHO to recommend the composition of the influenza vaccine for the following season, which it does twice a year, for the northern and southern hemispheres. In addition, the collaborating centres determine patterns of antiviral susceptibility of circulating strains and update reagents. GISRS also acts as a global alert mechanism for the appearance of influenza viruses with pandemic potential.

In the EU/EEA, the European Centre for Disease Prevention and Control (ECDC) coordinates the European Influenza Surveillance Network (EISN) which consists of contact points for influenza surveillance (epidemiological and virological) nominated by the Competent Bodies for surveillance of the Member States. Epidemiological and virological surveillance data on influenza are collected through The European Surveillance System (TESSy).

The National Influenza Laboratory at NIPH is part of the European monitoring system, EISN which collects both epidemiological and virological data from the participating European countries. The database, TESSy receives relevant clinical information for each case with influenza sequence data and susceptibility results from genotypic and phenotypic methods.

Monitoring of resistance is required for the correct choice of empirical treatment. Updated knowledge of resistance is crucial for the best possible pandemic preparedness. The 2009 pandemic caused by A (H1N1) virus originating from swine clearly demonstrated the need for continuous resistance monitoring.

The influenza season 2007/08 – a new era for resistance
The global emergence of resistance to oseltamivir during 2007/08 was discovered first through analysis of viruses from Norwegian influenza surveillance (14,16), and it took place with no association to previous recorded usage of drug (17). This particular resistance generating mutation had been known for some years, but in previous studies, it invariably affected the virus fitness (18) and had not been observed in circulating A(H1N1) virus.

The fact that the same mutation in the same virus the previous years led to sharply reduced viability, demonstrate that changes in the virus can facilitate increased fitness of viruses carrying this resistance mutations (19). Perhaps fortunately, the previous seasonal A(H1N1) viruses were completely displaced by the emerging pandemic virus of the same subtype in 2009, and now appear to be extinct.

Antiviral resistance in influenza viruses in Norway in the season 2011/12.
Historically, resistance has been known to develop quite easily against the M2 blockers. Over the last decade, the prevalence in A(H3N2) viruses of resistance due to the S31N substitution has increased and during the last few years almost all circulating H3N2 viruses

are resistant (20). Similarly, almost all A(H1N1)pdm09 viruses are resistant to the M2 blockers, also due to S31N.

The more recently developed NAIs initially seemed to be much less affected by resistance development and resistant mutants in general seemed less viable.

During the 2009 A(H1N1)pdm09 pandemic, a substantial peak in NAI (primarily oseltamivir) usage was recorded. This, however, did not lead to detectable emergence of resistant viruses. Also globally, very little oseltamivir resistance has been observed. Nonetheless, toward the end of the 2011 influenza season in Australia, local spread of oseltamivir resistant H1N1pdm09 viruses was observed (21). Apparently, these viruses did not spread beyond the initial area and ceased to circulate with the ending of the season there. No corresponding occurrence of resistant viruses has yet been reported from the 2011/12 Northern Hemisphere influenza season. Resistance to the other neuraminidase inhibitor available in Norway, zanamivir, appears to be extremely rare. Community spread of oseltamivir-resistant A(H1N1)pdm09 virus still remains a concern, given that data from animal studies suggest that the fitness of the H275Y variant is not significantly compromised and that there indeed was local spread of resistant virus in Australia in 2011 (19,21).

Surveillance findings in the 2011/12 influenza season

In Norway, seasonal H3N2 was the dominating strain in circulation during the 2011/12 season. There was limited circulation during the season of influenza type B, both Yamagata- and Victoria-lineage, representing about 3% of the identified viruses. The A(H1N1)pdm virus was encountered only sporadically that season.

Findings from the Norwegian influenza surveillance are summarised in table 1. The fewpandemic A(H1N1)pdm09 viruses analysed in 2011/2012 were 100% susceptible to the neuramidase inhibitors in the phenotypic assay (MUNANA), but 100% resistant to M2 blockers. The A(H3N2) viruses remained resistant to the M2 blocker adamantine, but susceptible to the neuramidase inhibitors oseltamivir and zanamivir. All influenza B viruses that were analysed were susceptible to both oseltamivir and zanamivir.

Conclusion

It is exceedingly important to have national antiviral susceptibility monitoring systems that can deliver timely data to inform public health and clinical recommendations for antiinfluenza drug use.

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Table 1. Norwegian influenza viruses resistant to M2 blockers (adamantanes) and the NIs oseltamivir and zanamivir, during the influenza seasons 2005/06 through 2011/12.

Season	Adamantane resistance			Oseltamivir resistance			Zanamivir resistance'	
	A(H1N1)	A(H3N2)	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B
2005/06	nd	75% (n=4)	0% (n=6)	0% (n=13)	0% (n=21)	0% (n=6)	0% (n=13)	0% (n=21)
2006/07	0% (n=6)	90% (n=10)	0% (n=5)	0% (n=10)	nd	0% (n=5)	0% (n=10)	nd
2007/08	0% (n=112)	100% (n=2)	67,8% (n=272)	0% (n=2)	0% (n=59)	0% (n=114)	0% (n=2)	0% (n=59)
2008/09	0% (n=5)	100% (n=65)	100% (n=33)	0% (n=13)	0% (n=1)	0% (n=5)	0% (n=12)	0% (n=1)
2009-pdmH1	100% (n=258)	100% (n=2)	0% (n=884)	nd	0% (n=11)	0% (n=36)	nd	0% (n=9)
2010/11*	100% (n=54)	100% (n=10)	1.6%** (n=244)	0% (n=1)	0% (n=30)	0% (n=2)	0% (n=1)	0% (n=24)
2011/12	100% (n=19)	100% (n=56)	0% (n=27)	0% (n=71)	0% (n=5)	nd	0% (n=59)	0% (n=4)

Two screening tools were used to determine oseltamivir/zanamivir resistance: sequence analysis of viral genes or a neuraminidase inhibition assay

* During influenza season 2010/11all A(H1N1) tested were pdmH1

** A(H1N1)pdm with the mutation 275Y in mixture commonly associated with oseltamivir resistance.

Nd=no data.

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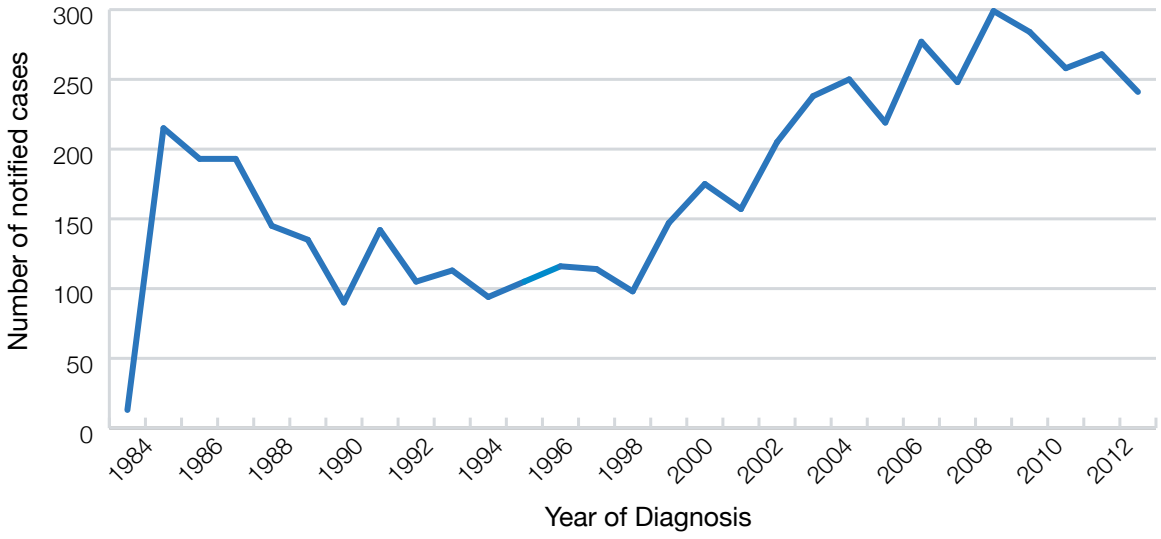
Human immunodeficiency virus drug resistance

HIV is a retrovirus that infects cells of the human immune system and destroys them or impairs their function. Infection results in progressive deterioration of the immune system leading to immune deficiency. Immunodeficient patients are more susceptible to a wide range of infections. Acquired immunodeficiency syndrome (AIDS) is a condition recognised by either the occurrence of specific diseases associated with HIV infection or a CD4+ T cell count below 200 cells per µL. HIV can be found in the bodily fluids of infected people (blood, semen, vaginal fluids and breast milk) and may be transmitted through unprotected sex, sharing of contaminated needles or other sharp instruments, from mother to child during pregnancy, childbirth or breast feeding, or through blood transfusion with contaminated blood.

There are two main types of HIV, HIV-1 and HIV-2, and since the 1980s HIV-1 has spread worldwide and accounts for the pandemic. In 2011 is was estimated that 34 million people live with HIV throughout the world and that approximately 1.7 million people died of AIDS related causes worldwide (1). Further, 8 million people living with HIV-1 had access to antiretroviral therapy in 2011.

By 2012 there were 5138 diagnosed cases of HIV-1 in Norway, 3 460 men and 1 678 women(2). Immigrants represent half of all newly diagnosed individuals every year (all transmission routes) and account for about 1400–1600 cases of HIV-1 in total. The situation during the recent years has been characterized by a continuous increase in the number of diagnosed HIV-1 cases (Fig. 1). The increase is among men having sex with men (MSM) and immigrants infected in their home countries before arrival in Norway (Table 1). The trend, with increasing HIV-1 prevalence among MSM, started around 2000 and is now spreading from MSM communities in Oslo to the larger cities and urban areas elsewhere in Norway(3). Similarly, the number of reported cases of syphilis and gonorrhoea in this group has also increased dramatically in recent years and underlines the extent of unsafe sex. The same trend is seen in most Western countries.



Source: MSIS, NIPH

Figure 1. HIV-1 infections in Norway 1984–2012, by year of diagnosis

Table 1. Transmissions routs of HIV-1 infections in Norway by year of diagnosis.

Transmission route	<03	03	04	05	06	07	08	09	10	11	12	Total	%
Heterosexual	1120	153	161	134	165	141	184	171	157	155	141	2682	52,2
- HIV-1 infected while living in Norway	409	34	42	33	42	41	46	44	57	46	46	840	-
-HIV-1 infected before imigrating to Norway	711	119	119	101	123	100	138	127	100	109	96	1843	-
Homosexual	836	57	71	56	90	77	93	88	85	97	76	1626	31,7
Intravenious drug abuse	473	13	15	20	7	13	12	11	11	10	11	596	11,6
Blod and blod products	46							1				47	0,9
From mother to child	29	5	1	5	6	9	4	4	1	4	7	75	1,5
Unknow/other	51	10	2	4	9	8	6	9	4	2	6	111	2,2
Total	**2555**	**238**	**250**	**219**	**277**	**248**	**299**	**284**	**258**	**268**	**242**	**5138**	**100,0**

Antiretroviral drugs and development of resistance

The introduction of an effective antiretroviral therapy has resulted in a significant reduction in HIV-1-related morbidity and mortality. There are currently five different drug classes targeting different phases of HIV's lifecycle; CCR5 blockers prevents binding between viral gp120 and the chemokine receptor CCR5, fusion inhibitors prevent fusion between the viral gp41 and the cell membrane, nucleoside and non-nucleoside reverse transcriptase inhibitors are directed against the reverse transcriptase and inhibits transcription of RNA into DNA, integrase inhibitors prevent integration of pro-viral DNA into the host cell DNA, and protease inhibitors prevent cutting of poly-proteins (Figure 2).

The antiretroviral therapy is based on the principle that during prolonged treatment of HIV-1 combinations of at least two drugs with different attack points must be used. Mono-therapy may favour the development of resistant viruses, while combination therapy targeting e.g. both reverse transcriptase and protease keep the replication so low that the risk of developing resistance decreases. Recommended treatment consists therefore of a combination of at least three different drugs from at least two different classes.

The treatment does not eliminate the virus, but can effectively reduce the production of new virus particles so that for most patients, HIV-1 RNA levels in plasma remain stable below the limit of detection. The effect of treatment is monitored by increase in CD4 counts and decrease in HIV-1 RNA copy numbers in plasma. Detectable HIV-1 RNA in plasma may indicate the development of resistant virus. There is a large genetic variation in the HIV-1 genome, not only from patient to patient, but also within the individual patient. This genetic variation is mainly due to the fact that the reverse transcriptase does not correct errors (mutations) that occur during DNA synthesis. Mutation rate is estimated to be approxi¬mately one substitution per viral genome per replication cycle. The variation is amplified by the fact that HIV-1 has a high replication rate, up to 1010 viral particles produced each day. Different variants will soon be able to be selected upon changes in the environment. At suboptimal treatment, resistant viruses are selected, resulting in therapy failure.

With today's antiretroviral treatment, effective control of viral replication and full suppression of plasma viral load are achieved for most patients with chronic HIV-1 infection. However, antiretroviral drug-resistant virus strains are emerging, and HIV-1 resistance testing has become an important component of the clinical management of patients with HIV-1 infection (5-8). There is some transmission of drug-resistant virus, but in most cases, resistance develops as a result of persistent viral replication during antiretroviral treatment, often due to suboptimal drug levels. Still, the dynamics of drug resistance development is not yet fully understood.

There are two main methods for determining the resistance of HIV-1, phenotypic and genotypic. Phenotypic susceptibility tests measure viral replication in cell culture in the presence of serial dilutions of the drugs in question, but these methods are slow and complicated, and are not used as routine tests. In Norway, as in most other countries, only genotypic assays are used, and all HIV-1 resistance testing is currently performed at the National reference laboratory for HIV at Oslo University Hospital Ullevål.

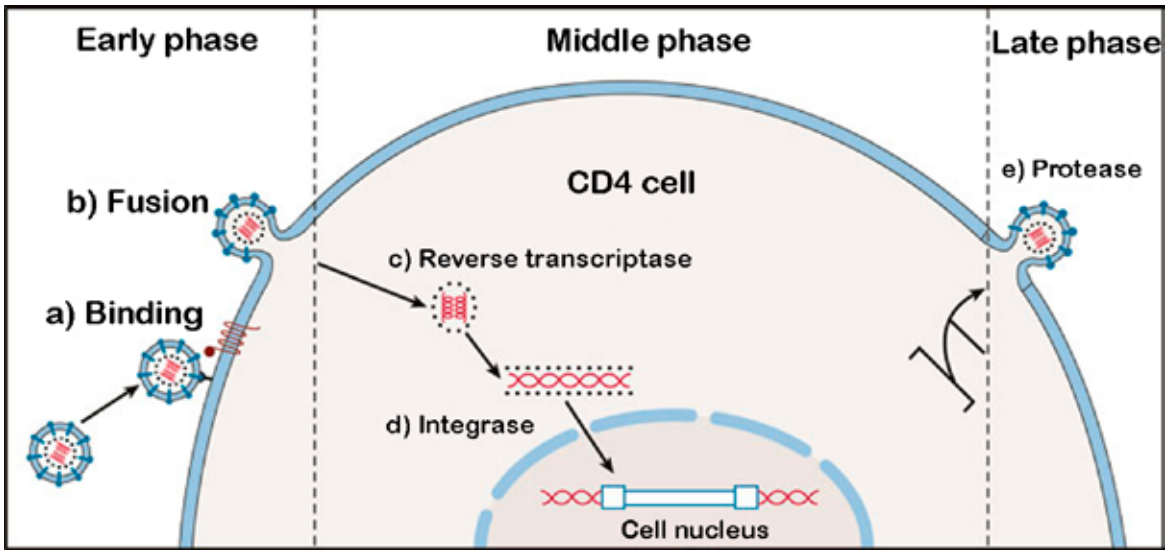
The genotypic assays involve amplification of the relevant part of the HIV-1 genome with RT-PCR,

followed by nucleotide sequencing of the PCR product. The routine assays include sequencing of the genes coding for the protease and reverse transcriptase, the viral enzymes targeted by the main classes of antiretroviral drugs. The integrase gene can also be investigated on request. The resulting amino acid sequence is subsequently interpreted through identification of amino acid alterations that have been found to be associated with reduced drug susceptibility. More than 200 amino acid sequence positions of relevance for resistance have been identified. There are numerous genotypic interpretation systems available that take accumulated clinical data into account and they are updated regularly. In addition, all samples showing genotypic resistance in Norway are individually interpreted by an experienced HIV clinician and microbiologist in collaboration, and the interpretation often includes treatment suggestions. In order to make such recommen¬dations, it is important that all information about previous and current antiretroviral treatment is communicated to the laboratory. A special referral form designed for this purpose is available at www.oslo-universitetssykehus.no (Avdeling for mikrobiologi, henvisningsrutiner).

A new class of antiretroviral drugs called CCR5 antagonists that work by blocking the binding of HIV-1 to CCR5 chemokine-receptors on the surface of the target cells. Most HIV strains depend on binding to CCR5 as a co- receptor for viral entry. However, some HIV strains use another chemokine receptor (CXCR4) as co-receptor, rendering CCR5 antagonists ineffective. Co-receptor usage is correlated with the amino acid sequence of the V3 loop of the HIV protein gp120. If viruses with CXCR4 tropism are detected by sequencing of the V3 loop, the patient should not be treated with CCR5 antagonists. Genotypic tropism testing can be performed on request at the HIV reference laboratory at Ullevål.

The most important rationale for performing resistance testing in clinical practice is virological failure. Resistance testing is also recommended in pregnancy, in case of a suspected "super infection" of a HIV-1 infected person with a different HIV-1 strain, or from the source after a needle stick injury. Furthermore, it is recommended that all patients with a newly diagnosed HIV-1 infection are tested for resistance mutations for surveillance purposes. It is not commonly recommended to perform resistance testing prior to initiation of treatment.

HIV-1 drug resistance testing requires plasma samples for analysis and in general a viral load of at least 500 copies/mL is required for genotypic resistance. However, samples with lower viral loads may sometimes be successfully sequenced, while some samples with higher viral loads may not, mainly due to variation in the quantification assay or individual sequence variations. Clinicians are encouraged to contact the laboratory if they have samples with low



Ill: Birgitta Åsjø

Figure 2. HIV-1 life cycle and attack points for antiviral drugs (4).

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.

viral loads where resistance testing is of particular importance.

One major limitation of genotypic resistance testing is its inability to detect variants of HIV-1 that represent only small fractions of the patients total virus population. For a mutation to be detected, it must account for 20–30% of the virus population in the sample. Therefore low-level mutations with possible clinical consequences cannot be ruled out. The presence of antiretroviral drugs acts as selection pressure, rendering HIV-1 variants containing resistance mutations a relative growth advantage in presence of the particular drug. This positive selection of resistant virus depends on the presence of the specific drug. When the medication is stopped or altered, the growth advantage of the mutant virus ceases, and wild type virus or other variants will usually reappear and dominate. Therefore, when testing for drug resistance mutations in a patient with virological failure, it is important that the sample is collected while the patient is still receiving the failing regimen. Otherwise, only wild type virus may be detected even though relevant mutations might be present at a very low level.

Surveillance of HIV-1 drug resistance

There are variations in the observed rate of transmission of drug resistant HIV-1 in countries where antiretroviral treatment is available. The variation in prevalence is due to several factors, among others, occupational testing bias, different treatment regimes at the population level, differences in risk behaviour and access to medicines among risk groups, different definitions of resistance, and different time periods between exposure and sampling. Different results from different countries illustrate the importance of national monitoring systems and standardises methods for surveillance monitoring. WHO recommend a set of Surveillance Drug Resistance Mutations (SDRM) that should be monitored in transmitted HIV-1 resistance surveillance. The list of SDRMs is updated regularly (Appendx 1), and used in the analysis tools provided by databases that can be used for genotypic interpretation of HIV-1 drug resistance, such as the Stanford HIV Drug Resistance Database http://hivdb.stanford.edu/hiv/ and the Los Alamos National Laboratory HIV Drug Resistance Database http://hiv -web.lanl.gov. The monitoring of primary HIV-1 resistance in Norway 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).

Surveillance of transmitted HIV-1 resistance in Norway

The monitoring of transmitted HIV-1 resistance commenced in Norway in January 2006 based on the recommendations in the action plan to combat antibiotic resistance 2000–2004 and the report from 2002 "Report on the national surveillance system for HIV-1 resistance". The purpose was to monitor the resistance at the population level and was not meant to guide therapy in the individual patients. It was important to initiate the surveillance, as it is needed for national treatment guidelines and for the assessment of infection control measures. In the period 2000–2008, genotypic HIV-1 drug resistance analysis was conducted at the hospitals; Ullevål, Rikshospitalet and Haukeland. From 2008 all primary drug resistance testing has been performed at Oslo University Hospital Ullevål, as the national reference laboratory for HIV-1 in Norway.

Surveillance of transmitted HIV-1 resistance in Europe

The SPREAD (Strategy to control spread of HIV drug resistance) database contains anonymised demographic data as well as CD4 count, HIV RNA level and gene sequence from a number of newly diagnosed patients from the participating countries included in the EU program. From 2003 till the end of 2009, Norway participated with 30 patients' data / year. All data is stored in Luxembourg. By application and permission from the respective countries' national coordinators, data can be retrieved in connection to specific projects. Available data are not suitable for general national surveillance due to the limited number of sequences submitted from each country.

InfCare HIV is a quality assured electronic system for the registration of many different data that may be aligned in relation to the resistance profile and disease progression. The system was developed in Stockholm at Huddinge Hospital. Karolinska Hospital and Sahlgrenska Hospital owns the rights to InfCare in Scandinavia and each participating clinic pays an initial fee and an annual fee to the owners. Each clinic decides how the data will be used and who will have access to the data. Each clinic owns its data and patients are anonymous to all but their own clinic. The system has been implemented in Denmark, Iceland and Greenland. The database is updated every night. It is possible for clinicians to access information generated in other clinics than their own. Research projects that will be based on the entire database have to be approved by the steering committee for the register after a written request. This database is a very important tool that provides the ability to monitor development of HIV-1 resistance and the influence of minor resistance mutations for development of resistance to various drug combinations. In Norway, a similar system, NorHIV is under development.

Surveillance findings in Norway in 2006–2012

Resistance surveillance was carried out in less than half of the newly diagnosed HIV-1 cases during the first years of implementation, but in the last year there has been an increase in the percentage of cases where samples are received for testing. The annual percentage of sequences analysed for primary HIV-1 drug resistance from newly diagnosed cases of HIV-1 in Norway since 2006 is shown in figure 3.

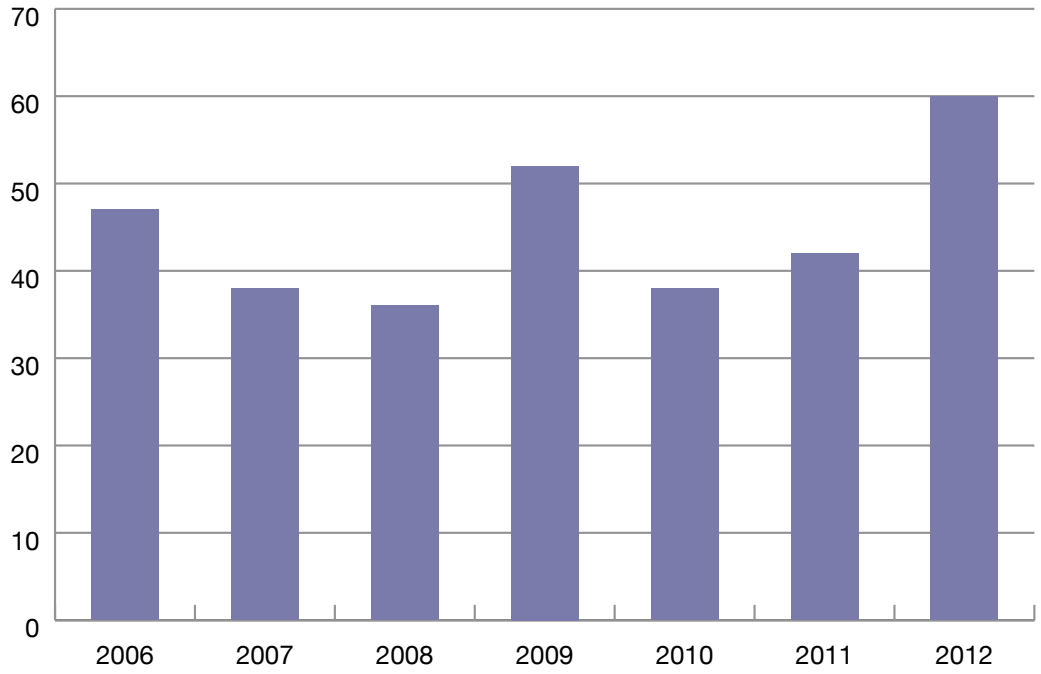


Figure 3. Percentage of newly diagnosed cases of HIV-1 infection where sequences where obtained (2006–2012).

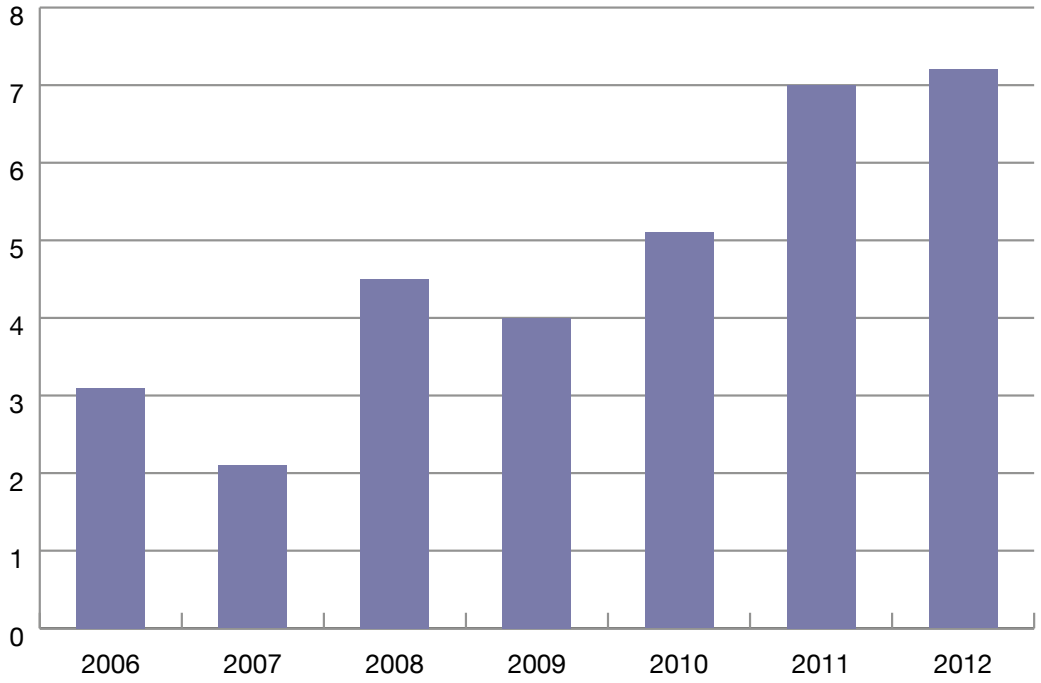


Figure 4. Percentage of analysed sequences with Surveillance Drug Resistance Mutations, SDRMs in 2006–2012.

SDRM detected in monitoring of primary HIV-1 resistance is presented in figure 4 as percentage of the sequences with detected SDRM in total. There may be several SDRM per sequence.

Findings of clinical significance in Norway in 2011–2012

In order to facilitate comparisons of the surveillance data, WHOs standard list of SDRM was used for the monitoring of primary HIV-1 resistance in Norway (appendix B1). The WHO list is designed for surveillance purposes, and does not give information on individual drugs, nor does it take into account the genetic barrier of a drug, and the presence of mutations from this list does not imply resistance of clinical significance. Therefore, the numbers above does not necessarily translate into the number of newly infected patients with clinical drug resistance. They represent surveillance data, and should not be used for recommendations and clinical practice.

In 2011 and 2012, SDRMs from the WHO list were detected in 7% of the analysed sequences (table 3 and 4). The SDRMs detected in the Norwegian material is shown in table 3, whereas the particular SDRMs and combinations of SDRMs detected in the Norwegian material is shown in appendix B2. However, only 3% (2011) and 4% (2012) of the samples had drug mutation patterns that would be interpreted as clinically relevant drug resistance. Most of these were cases of high level resistance to efavirenz and nevirapine, which are often used in first line regimens. Appendix B3 shows interpretation of clinically important single mutations detected in sequences from Norway in 2011 and 2012.

Table 2. Surveillance Drug Resistance Mutations, SDRMs present in sequences analysed in Norway 2006–2012.

NRTI		NNRTI		PI	
Pos	**Mut**	**Pos**	**Mut**	**Pos**	**Mut**
M41	L	K101	E, P	L23	I
D67	N, G, E	K103	N, S	I54	V, L, M, A, T, S
K65	R	V106	M, A	G73	S, T, C, A
K70	R, E	Y181	C, I, V	V82	A, T, F, S, C, M, L
M184	V, I	Y188	L, H, C	I85	V
L210	W	G190	A, S, E	L90	M
T215	Y, F, I, S, C, D, V, E				
K219	Q, E, N, R				

Results from sequence analysis for resistance mutations using the Calibrated population resistance, CPR tool at Stanford HIV DRUG RESISTANCE DATABASE, **http://hivdb.stanford.edu**

Table 3. Total sequences (n=108) with SDRMs in 2011

SequenceID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	None	None	I85V*
2	T215D	None	None
3	None	K103N	None
4	None	Y181C, G190A	None
5	None	K103N	None
6	None	K103N	None
7	T215E	None	None

* I85V is a nonpolymorphic PI-selected mutation, and does not give resistance.

Table 4. Total sequences (n=139) with SDRMs in 2012

SequenceID	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
1	M184V, T215Y	K103N	None
2	None	K103N	None
3	D67G, K70R, M184V, T215I, K219E	V106M, Y181C	None
4	T215I	None	None
5	K65R, M184I	V106A, Y181C, G190A	None
6	M41L	None	None
7	T215E	None	None
8	K70E, M184V	K103N	None
9	None	Y188L	None
10	M41L, T215D	None	M46L

Conclusions

In recent years, a large number of drugs have been developed to control HIV replication. This has dramatically improved both the patients' quality of life and their life expectancy. However, the treatment is very demanding, with a lifelong therapy and risk of serious side effects. Furthermore, if the drug regimen is not properly followed by the patients, there is a considerable risk of development of drug resistant viruses. The patient's health could deteriorate and there is a risk of spreading of resistant virus into the community. Resistance mutations was detected in between 2,1 – 7,3 % of the sequences from the newly diagnosed HIV patients in 2006–2012, and there was an increasing trend. Surveillance of HIV resistance is important to be able to make decisions on implementing preventive measures to control dissemination of resistant HIV strains.

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Hepatitis B virus drug resistance

Hepatitis B is a potentially life-threatening liver infection caused by the hepatitis B virus (HBV). It is a DNA virus (about 3 kb) within the Hepadnaviridae family that is converted to a highly stable mini-chromosome upon infection in liver cells. Despite the tiny size of its genome HBV is one of the most successful human pathogens. It is a major global health problem and the most serious type of viral hepatitis. It can cause chronic liver disease and puts people at high risk of death from cirrhosis of the liver and liver cancer.

Worldwide, an estimated two billion people have been infected with HBV and more than 240 million have chronic (long-term) liver infections (1). About 600 000 people die every year due to the acute or chronic consequences of hepatitis B. HBV is transmitted between people by direct blood-to-blood contact or contact with semen and vaginal fluid of an infected person. HBV can cause both acute and chronic disease. The likelihood that a HBV-infection becomes chronic depends upon the age at which a person becomes infected. Young children are the most likely to develop chronic infections. Ninety percent of infants infected during the first year of life and 30–50% of children infected between one to four years of age develop chronic infections. Twenty-five percent of adults who become chronically infected during childhood die from hepatitis B-related liver cancer or cirrhosis, whereas 90% of healthy HBV- infected adults will recover and get completely rid of the virus within six months.

Norway is generally a low prevalence country (0.5%) (2). The immigrant populations from highly endemic countries have an impact on overall prevalence, as the majority of cases infected with chronic HBV-infections (95%) are immigrants from middle- and high endemic regions infected before they entered Norway. However, further transmission of HBV from the immigrant population is quite limited. Although the mode of transmission is unknown in the majority of cases, it is assumed that almost all have been infected at birth or early in childhood. In recent years around 700 new cases of CHB are notified Norway (3), and the majority of these cases were among immigrants from Somalia, Afghanistan, Vietnam, Thailand and Eritrea. Among CHB with Norwegian ethnicity 50% are transmitted through sex or drug use, while in the remaining cases the transmission route is unknown.

Development of resistance

The ultimate goal of hepatitis B treatment is to prevent cirrhosis, hepatic failure, and hepatocellular carcinoma (HCC) (4). The nucleos(t)ide analogues (NAs) used in treatment for CHB suppress viral replication by inhibiting the viral polymerase, whereas interferon therapy works by enhancing the host immune response. The clinical benefit is dependent on the ability to maintain sustained suppression of HBV replication and to induce remission of liver disease. Despite recent advances in treatment of CHB using NAs, these approved treatments seldom eradicate the virus with the risk of viral resistance during long-term treatment. There are 8 primary mutations associated with drug resistance and cross-resistance occurs between several of these drugs (Table 1).

Currently, entecavir or tenofovir disoproxil are recommended as first-line monotherapy, given their antiviral potency and favorable resistance profile. The rates of resistance at 5 years in NA naive patients are <1.5% and 0% for entecavir and tenofovir disoproxil, respectively (4). Treatment response should be regularly monitored by quantification of the virus in blood. Resistance should be identified when there is a viral breakthrough (i.e. increase in viral load) as early as possible before biochemical breakthrough (increased ALT), and ideally identification of the pattern of resistance mutations should be used to adapt therapeutic strategies. Clinical and virological studies have demonstrated the benefit of an early treatment adaptation, as soon as viral load increases.

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

Materials & Methods

The NIPH is a national reference laboratory for hepatitis B receiving samples from microbiological laboratories in Norway for confirmation or characterization by alternative or supplementary analysis including antiviral resistance testing. Sequencing of the polymerase gene that covers the mutations that give resistance to the NAs is frequently used for resistance determination. It is the current method of choice at NIPH, although the resistance population must reach 20–30% before it is detectable by this method.

Surveillance of HBV resistance is based on a selection of chronic patients in 2011–12. These patients were selected because sequence information on antiviral resistance was available for these patients as part of a HBV-genotyping (S-gene) analysis previously requested as part of their clinical management. In addition available data on drug resistance among treated patients during this period is also presented.

Drug resistance surveillance data

Surveillance of chronic carriers (n=287) where no information had been given on antiviral treatment showed only one case harbouring drug resistance (Table 2). HBV-variants with resistance towards NAs were found in 4 of 17 treatment experienced patients.

Conclusion

Patients in Norway seem to be given first-line therapy (entecavir and tenofovir disoproxil) that effectively suppress virus replication and limit drug resistance (6). Since 2007 there is a clear increase in the use of

Table 2. Surveillance of drug resistance among patients on treatment and among patients where HBV-genotyping has been requested in 2011–12.

HBV–variants resistant to NAs	**Among treated patients**		**Among HBV-genotyped patients**	
	2011	*2012*	*2011*	*2012*
Total analysed	14	*3*	*131*	*156*
Wild type	11	*2*	*130*	*156*
M204I	1a	0	0	0
L180M + M204V	1b	1a	1c	0
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

a=entecavir, b=tenofovir, c=treatment unknown

first-line drugs. The use of entecavir has increased almost seven-fold during this time, whereas the less potent drugs (i.e. lamivudine, adefovir and telbivudine) commonly associated with drug resistance are decreasingly used.

Development of drug resistance during treatment of HBV infection seems to be a minor problem in Norway for the time being. However, very few samples are referred to antiviral susceptibility testing and the surveillance of resistance towards HBV antivirals drug treatment has not been done systematically on all patients on treatment in Norway. There is no national overview of the total number of patients on treatment for hepatitis B, only rough estimates. In 2012 NIPH received samples for diagnostic analysis from 225 CHB patients on therapy. The number of patients with subscription of NA for HBV and/or HIV was 483 in the same period (6), indicating the number of patients on treatment in the range of 225–483 as it not possible to discriminate HBV and HIV-patients given subscription on the same drugs. NIPH is the only laboratory in Norway offering HBV drug resistance testing, as well as one of a few that monitor viral load during treatment. Lack of information on referral forms regarding antivirals used is a general problem that makes surveillance of drug resistance among patients on treatment difficult. The need for a systematic surveillance system for HBV drug resistance will be reviewed and recommendations for future systems proposed by the RAVN council in 2013.

Given an estimate of 20 000 cases of CHB infections in Norway, the number of patients on NA therapy, upto 485 for HBV and/or HIV infections, is low. It is important that all chronic HBV patients are followed-up by a specialist with regard to further management and treatment.

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Cytomegalovirus

Cytomegalovirus (CMV) is a virus of the herpes virus group, and is highly prevalent in the population. The virus is secreted in saliva and infection often occurs when children lick on the same toy, or drink from the same cup. Later in life the virus is transmitted by sexual activity and also from children to parents. CMV remains latent in the body in hematopoietic stem cells and can be reactivated when these cells mature to macrophages or dendritic cells. CMV disease can be caused either by primary infection or reactivation of the virus. CMV disease requiring treatment is primarily seen in patients with impaired cell-mediated immunity as after allogeneic organ transplantation, stem cell transplantations and AIDS. At some hospitals newborn with congenital CMV infection receive antiviral treatment.

Antivirals used against CMV and sensitivity tests

Ganciclovir is an effective agent for intravenous treatment of CMV infections. The agent is also available as a "prodrug", valganciclovir, for oral administration. Ganciclovir is a nucleoside analogue that has to be activated i.e. trifosfosforylated. The initial phosphorylation is carried out by the viral protein UL97 which is a phosphotransferase. The virus has its own DNA polymerase (UL54) that incorporates ganciclovir in growing DNA chain. Development of resistance to ganciclovir occurs either by mutations in the UL97 gene or in the UL54 gene. By comparing the phenotypic resistance measured as virus replication in cell culture at various concentrations of ganciclovir and known genotypic resistance mutations, the various resistance mutations have been characterized with regard to degree of resistance (1). Some mutations make the virus fully resistant while others slightly decrease the sensitivity. In the daily routine genotypic analysis is performed.

Cidofovir and foscarnet are two other drugs that can be used in the treatment of CMV infections. When using these drugs serious side effects are often seen. These drugs are thus mainly used for treatment of ganciclovir resistant CMV infections. Cidofovir is a nucleotide analogue for intravenous treatment of CMV infections and infections by other viruses in the herpes group. Cidofovir is already monofosforylated and thus independent of UL97 activity. Resistance to cidofovir is caused by mutations in the viral DNA polymerase (UL54). Most cidofovir UL54 resistance mutations are also ganciclovir resistance mutations (cross-resistance).

Foscarnet, fosfonoformic acid, is a compound that inhibits viral DNA polymerase (UL54). The drug is given as an intravenous infusion. Development of resistance is due to mutations in the UL54 gene and there is little cross-resistance with the other two anti-CMV drugs.

Treatment failure is the main indication for doing genotypic analysis. Significantly reduced T-cell immunity against CMV is another common cause for treatment failure.

Genotypic sensitivity testing is performed at Department of Microbiology, OUS-Rikshospitalet. Table 1 shows the number of samples received for genotypic CMV ganciclovir sensitivity testing in the years 2008 to 2012. All samples were from organ transplant recipients.

Clinical trials

A retrospective study on ganciclovir resistant CMV-infections among renal transplant recipients during the years 2004 to 2009 was carried out at OUS-Rikshospitalet (2). This study included 1130 patients of whom 27 (2.2%) had ganciclovir resistant CMV. Ganciclovir resistant CMV was detected predominantly (26/27) among the 209 patients with primary CMV infection (R-). This tells us that 12.5% of those with CMV primary infection developed ganciclovir resistance while such resistance was seen in only 0.1% of those with reactivated infection or reinfection (D± / R +). The resistance mutations were mainly in the CMV-UL97 gene and not in the UL-54 gene. Furthermore, ganciclovir resistance appeared relatively late in the course of treatment, median 108 (41–205) days after start of valganciclovir/ganciclovir therapy. The high incidence of ganciclovir resistance in primary CMV infection is striking also in an international context. One reason may be that the valganciclovir dose given was too low which allowed the selection of resistant mutants. The first three months after transplantation the patients were monitored weekly with CMV PCR in plasma samples. Preemptive valganciclovir therapy was started immediately after the first positive test and lasted until the patient had two negative CMV PCR tests in plasma. It turned out that the dose chosen was only half of the dose eventually recommended. This shows that appropriate valganciclovir dosage is important to prevent development of resistance during anti-CMV-treatment.

In an international study, which emanated from the OUS-Rikshospitalet, plasma samples were collected from 275 organ transplant patients with verified CMV

disease who were treated with ganciclovir/valganciclovir (3). In 13 of these patients ganciclovir resistant CMV was detected. Five patients had ganciclovir resistance mutations already at start of the therapy. They had all previously received valganciclovir or ganciclovir prophylaxis or treatment. The other resistance mutations appeared between day 21 and day 49 after start of the treatment. The genotypic resistance tests showed that 9 patients had UL97 mutations, 3 had UL54 mutations whereas 1 patient had both UL97 and UL54 mutations.

Conclusion

Treatment failure during ganciclovir / valganciclovir treatment of CMV disease is primarily a problem in patients with impaired cell-mediated immunity. Development of resistance to ganciclovir causes 25–50% of cases of treatment failure. In organ transplant patients ganciclovir resistance is predominantly seen in patients with primary infection (D + / R-). Resistance mutations occur more frequently in the CMV UL97 gene than in the CMV UL54 gene. Adequate doses of ganciclovir/valganciclovir are important to prevent the development of resistance.

References

1. Chou S. Recombinant phenotyping of cytomegalovirus UL97 kinase sequence variants for ganciclovir resistance. J Infect Dis 54:2371,2010.
2. Myhre HA et al. Incidence and outcomes of ganciclovir-resistant cytomegalovirus infections in 1244 kidney transplant recipients. Transplantation 92:217,2011.
3. Boivin G et al. Cytomegalovirus resistance in solid organ transplant recipients treated with intravenous ganciclovir or oral valganciclovir. Antiviral Ther 14:697,2009.

Table 1 The number of samples received for genotypic CMV ganciclovir sensitivity testing in the years 2008 to 2012.

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

Abbreviations

ACV	Aciclovir
AIDS	acquired immunodeficiency syndrome, caused by HIV
CMV	Cytomegalovirus
CHB	chronic hepatitis B infection
ESAR	European Society of Antiviral Resistance
GCV	Ganciclovir
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSV	Herpes Simplex virus
MSIS	Meldingssystem for smittsomme sykdommer
NA	nucleoside/nucleotide analogues
NAI	neuraminidase inhibitor
NIPH	Norwegian Institute of Public Health
NNRTI	nonnucleoside RTinhibitor
NRTI	nucleoside RTinhibitor
PCR	polymerase chain reaction
PI	proteaseinhibitor
PFA	Foscavir
RT	reverse transcriptase
SDRM	Surveillance Drug Resistance Mutations
SPREAD	Strategy to Control Spread of HIV Drug Resistance
VZV	Varicella Zoster virus

Appendix A, Influenza

Appendix A. Substitutions in influenza neuraminidases associated with resistance or reduced susceptibility to NAIs*.

Substitution	N2 No [a]	Reduced Inhibitor Sensitivity [b]		
		Oseltamivir	Zanamivir	Peramivir
Substitutions in NA that are known to occur clinically and cause clinical resistance.				
NI NA				
H275Y	274	221-2597	1-3	66-1095
Substitutions in NA that are known to occur clinically and cause reduced sensitivity in vitro but the clinical impact is currently unknown.				
D199N [c]	198	3	2	unk
I223R [c]	222	28-45	10-12	unk
N295S	294	12-208	3-5	12
N2 NA				
E119V [d]	119	18-2057	1-3	1-3
R292K	292	>10000	3-20	14
N294S	294	300-1879	8	1
Influenza B				
R150K	152	38-252	5-1000	214-400
D197E	198	12-26	6-7	18
D197N	198	4-10	3-10	5
I221T	222	6-7	2-5	unk
N294S	294	17-23	1	unk
G407S	402	4	7	unk

a The corresponding position in N2 neuraminidase is indicated.
b Fold changes in IC, compared to wild-type (NAI sensitive) viruses is shown: unk currently unknown.
c These substitutions (and 223V) are known to synergise with H275Y.
d Occurrence with I222Vcariyield greater fold change in IC compared to wild-type (NAI sensitive) viruses.
* Substitutions table developed by the WHO GISRS antiviral susceptibility expert working group (AVWG). WER No. 39, 2012,87, p 372.

Appendix B, HIV-1

Appendix B1. List of Surveillance Drug Resistance Mutations,SDRM, recommended by WHO.

HIV-1 RT and Protease Mutations For Drug Resistance Surveillance

NRTI		NNRTI		PI	
Position	Mutation	Position	Mutation	Position	Mutation
M41	L	L100	I	L23	I
K65	R	K101	E, P	L24	I
D67	N, G, E	K103	N,S	D30	N
T69	D, Ins	V106	M, A	V32	I
K70	R, E	V179	F	M46	I
L74	V, I	Y181	C, I, V	LI47	V, A
V75	M, T, A, S	Y188	L, H, C	G48	V, M
F77	L	G190	A, S, E	I50	V, L
Y115	F	P225	H	F53	L, Y
F116	Y	M230	L	I54	V, L, M, A, T, S
Q151	M			G73	S, T, C, A
M184	V, I			L76	V
L210	W			V82	A, T, F, S, C, M,L
T215	Y, F, I, S, C, D, V, E			N83	D
K219	Q, E, N, R			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.

Appendix B2. Surveillance Drug Resistance Mutations, SDRMs present in sequences analysed in Norway 2006-2012.

		2006 n=129	2007 n=95	2008 n=108	2009 n=149	2010 n=95	2011 n=115	2012 n=139
NRTI	M184V	1						
	M41L	1		1			1	1
	V75A		1					
	T215D/E/I						2	2
	M41L+ T215D			1	1			
	M41L+T215D+ L210W					1		
NNRTI	K103N	1			3		3	1
	K101E			1		1		
	V106M			1				
	Y188C			1				
	Y188L							1
NRTI and NNRTI	K219N+ Y181C		1					
	Y181C+G190A						1	
	M184I+K219N, K103N+Y188H					1		
	M184V+K103N+ V106M					1		
	M184V+T125Y+ K103N							1
	D67G, K70R, M184V, T215I, K219E, V106M, Y181C							1
	K65R, M184I V106M, Y181C, G190A							1
	K70E, M184V K103N							1
PI	M461L	1						
	L23I				1			
	G73S					1		
	I85V*						1	
NRTI and PI	M41L, D67N, K70R, M184V, T215F, K219Q + I54V,V82A,L90M				1			
	M41L, T215D M46L							1

Appendix B3. Drug Resistance Interpretation of mutations present in sequences from 2011 and 2012.

NNRTI Resistance Mutations:	**Y181C and G190A**
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
etravirine (ETR)	Intermediate resistance
nevirapine (NVP)	High-level resistance
rilpivirine (RPV)	Intermediate resistance

NNRTI

Y181C causes high-level resistance to NVP, ~2-fold decreased susceptibility to EFV, and ~5-fold decreased susceptibility to ETR and RPV. Although Y181C reduces EFV susceptibility just 2-fold, older salvage therapy studies found that EFV was only transiently active in treating patients developing this mutation while receiving NVP.

G190A causes high level resistance to NVP and intermediate resistance to EFV. By itself, it does not decrease ETR susceptibility. However, it is synergistic with Y181C at reducing ETR susceptibility.

NNRTI Resistance Mutations:	**K103N**
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
nevirapine (NVP)	High-level resistance

NNRTI

K103N causes high-level resistance to NVP, and EFV. it has no effect on ETR or RPV susceptibility.

NRTI Resistance Mutations:	**T215D or T215E**
Nucleoside RTI	
abacavir (ABC)	Potential low-level resistance
zidovudine (AZT)	Low-level resistance
stavudine (D4T)	Low-level resistance
didanosine (DDI)	Potential low-level resistance

NRTI

T215Y/F cause AZT and D4T resistance and reduce susceptibility to ABC, ddI, and TDF. T215S/C/D/E/I/V/N/A/L do not decrease NRTI susceptibility but arise from viruses that once contained T215Y/F.

NNRTI Resistance Mutations:	**Y188L**
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
etravirine (ETR)	Potential low-level resistance
nevirapine (NVP)	High-level resistance
rilpivirine (RPV)	High-level resistance

NNRTI

Y188L causes high-level resistance to NVP, EFV, and RPV. It does not appear to reduce ETR susceptibility.

NNRTI Resistance Mutations: NRTI Resistance Mutations:	**K103N** **M184V, T215Y**
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
etravirine (ETR)	Susceptible
nevirapine (NVP)	High-level resistance
rilpivirine (RPV)	Susceptible
Nucleoside RTI	
lamivudine (3TC)	High-level resistance
abacavir (ABC)	Intermediate resistance
zidovudine (AZT)	Intermediate resistance
stavudine (D4T)	Intermediate resistance
didanosine (DDI)	Low-level resistance
emtricitabine (FTC)	High-level resistance
tenofovir (TDF)	Susceptible

NRTI

M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF, and d4T and are associated with clinically significant decreased HIV-1 replication.

T215Y causes AZT and D4T resistance and reduces susceptibility to ABC, ddI, and TDF particularly in combination with M41L and L210W.

NNRTI

K103N causes high-level resistance to NVP, and EFV. it has no effect on ETR or RPV susceptibility.

NNRTI Resistance Mutations: NRTI Resistance Mutations:	**V106M, Y181C** **D67G, K70R, M184V, T215I, K219E**
Nucleoside RTI	
lamivudine (3TC)	High-level resistance
abacavir (ABC)	Intermediate resistance
zidovudine (AZT)	High-level resistance
stavudine (D4T)	Intermediate resistance
didanosine (DDI)	Intermediate resistance
emtricitabine (FTC)	High-level resistance
tenofovir (TDF)	Low-level resistance
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
etravirine (ETR)	Intermediate resistance
nevirapine (NVP)	High-level resistance
rilpivirine (RPV)	Intermediate resistance

NRTI

D67N contributes resistance to AZT and d4T. D67E/G/S/T/Q generally occur in viruses with multiple NRTI-resistance mutations and their effects on drug susceptibility have not been well-characterized.

K70R causes intermediate resistance to AZT and low-level resistance to d4T and TDF.

M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF, and d4T and are associated with clinically significant decreased HIV-1 replication.

T215Y/F cause AZT and D4T resistance and reduce susceptibility to ABC, ddI, and TDF. T215S/C/D/E/I/V/N/A/L do not decrease NRTI susceptibility but arise from viruses that once contained T215Y/F.

K219Q/E decrease AZT and probably d4T susceptibility when present with K70R or T215Y/F but have little if any effect on the remaining NRTIs.

NNRTI

V106M causes high-level resistance to NVP and EFV.

Y181C causes high-level resistance to NVP, ~2-fold decreased susceptibility to EFV, and ~5-fold decreased susceptibility to ETR and RPV. Although Y181C reduces EFV susceptibility just 2-fold, older salvage therapy studies found that EFV was only transiently active in treating patients developing this mutation while receiving NVP.

NNRTI Resistance Mutations: NRTI Resistance Mutations:	**V106M, Y181C, G190A** **K65R, M184I**
Nucleoside RTI	
lamivudine (3TC)	High-level resistance
abacavir (ABC)	High-level resistance
zidovudine (AZT)	Susceptible
stavudine (D4T)	Low-level resistance
didanosine (DDI)	Intermediate resistance
emtricitabine (FTC)	High-level resistance
tenofovir (TDF)	Intermediate resistance
Non-Nucleoside RTI	
efavirenz (EFV)	High-level resistance
etravirine (ETR)	Intermediate resistance
nevirapine (NVP)	High-level resistance
rilpivirine (RPV)	Intermediate resistance

NRTI

K65R causes intermediate resistance to ddI, ABC, 3TC, FTC, and TDF; low-level resistance to d4T; and increased susceptibility to AZT.

M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF, and d4T and are associated with clinically significant decreased HIV-1 replication.

NNRTI

V106M causes high-level resistance to NVP and EFV.

Y181C causes high-level resistance to NVP, ~2-fold decreased susceptibility to EFV, and ~5-fold decreased susceptibility to ETR and RPV. Although Y181C reduces EFV susceptibility just 2-fold, older salvage therapy studies found that EFV was only transiently active in treating patients developing this mutation while receiving NVP.

G190A causes high level resistance to NVP and intermediate resistance to EFV. By itself, it does not decrease ETR susceptibility. However, it is synergistic with Y181C at reducing ETR susceptibility.

NRTI Resistance Mutations:	**K70E, M184V**
Nucleoside RTI	
lamivudine (3TC)	High-level resistance
abacavir (ABC)	Intermediate resistance
zidovudine (AZT)	Susceptible
stavudine (D4T)	Susceptible
didanosine (DDI)	Low-level resistance
emtricitabine (FTC)	High-level resistance
tenofovir (TDF)	Low-level resistance

NRTI

K70E/G reduce TDF, ABC, DDI, and to a lesser extent 3TC and FTC susceptibility.

M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF, and d4T and are associated