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REPORT

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Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2019

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Resistance to antivirals in Norway

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Introduction

It is a pleasure to present the seventh report from the surveillance system for Resistance against Antivirals in Norway (RAVN). The year 2020 has been different for all of us. The Covid-19 pandemic has vastly changed our world over the last eight months, and the workload has been immense for everyone working in the fields of virology, microbiology and infectious diseases. We are therefore particularly grateful to all the contributors to this years' report, for investing some of your limited time and resources in the making of this report.

Although the focus for many of us has been elsewhere, we must not forget that the rise in antimicrobial resistance is considered one of the greatest threats to global health. Antiviral treatment is a young, but rapidly growing field, and increased knowledge and awareness are essential to be able to control emerging antiviral drug resistance. Systematic surveillance will be a key tool for management.

In this report, we present data for 2019 on resistance against agents for treatment of influenza, HIV-1 infection, hepatitis B virus infection, and human herpes virus infections. The reference laboratories at the Norwegian Institute of Public Health and at the Oslo University Hospital have submitted the data. In addition to the surveillance data, we have selected three relevant topics that are given special attention in the report, presented by invited authors:

- *New antiviral drugs against cytomegalovirus:* An overview of new treatment options is presented, and their potential for drug resistance discussed.
- *Hepatitis C virus:* A national program for surveillance of antiviral drug resistance that will be launched in 2021 is presented.
- *SARS-CoV-2:* The potential risk of drug resistance to a future antiviral therapeutic treatment for SARS-CoV-2 is discussed

It is our hope that the report contains valuable data and interesting perspectives for clinicians, microbiologists, other colleagues with an interest in infectious diseases, and for those developing diagnostic- and treatment guidelines and strategies to prevent transmission of viral infections.

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

Enjoy!

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Abbreviations

ART	Antiretroviral therapy
CMV	Cytomelagovirus
DAA	Direct acting antivirals
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus-1
HSV	Herpes simplex virus
MSIS	Norwegian Surveillance System for Communicable Diseases
MSM	Men who have sex with men
NA	Nucleoside/nucleotide analogues
NIPH	Norwegian Institute of Public Health
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
PEP	Post exposure prophylaxis
PI	Protease Inhibitors
PrEP	Pre exposure prophylaxis
RAS	Resistance-associated substitution
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SDRM	Surveillance drug-resistance mutation
TAF	Tenofovir alafenamide
TDF	Tenofovir disoproxil
WHO	World Health Organization

Sammendrag

Bruk av antivirale midler

Ifølge data fra Reseptregisteret, fortsetter salget av antivirale medikamenter målt i definerte døgndoser (DDD) å øke også i 2019. For medikamenter mot hiv har det i 2019 vært en økning i salg målt i både DDD og antall behandlede pasienter. Det har særlig vært en økning for kombinasjonen emtricitabine og tenofovir dispoproxil, kombinasjons-preparatet som i 2016 ble godkjent som pre-eksposisjonsprofylakse (PrEP). Antall personer som ble forskrevet denne kombinasjonen har økt med 47% fra 2018 til 2019. Etter flere år med jevn økning av medikamenter mot hepatitt C virus (HCV), var det i 2019 en reduksjon i salget. For medikamenter mot hiv og HCV har det vært en dreining fra medikamenter med ett virkestoff til kombinasjonspreparater.

Influensavirus

Forekomst av resistens mot de antivirale midler som brukes i behandlingen av influensa er lav. Det ble ikke påvist influensavirus med resistens mot oseltamivir eller zanamivir i 2019/20-sesongen. Alle sirkulerende influensavirus er for tiden resistente overfor adamantaner, og adamantanresistens undersøkes derfor ikke rutinemessig ved FHI.

Humant immunsviktvirus-1

I 2019 ble data fra resistensovervåkningen for første gang sammenstilt med epidemiologiske data fra MSIS slik at man har kunnet analysere prevalensen av resistens-mutasjoner innen ulike undergrupper. Dette har også gitt bedre oversikt over dekningsgraden for resistensundersøkelser i de ulike pasientgruppene.

Resistensmutasjoner som overvåkes ble påvist i 10,3% av prøvene fra pasienter med nydiagnostisert hiv-1 infeksjon i Norge i 2019, noe som representerer en økning sammenliknet med tidligere år. Det var allikevel kun fire av de 11 prøvene med påviste mutasjoner som hadde et resistensmønster med klinisk betydning for valg av behandlingsregime, og alle disse fire var fra pasienter smittet utenfor Norge. Kun en av disse medførte resistens mot tenofovir/emtricitabin som brukes forebyggende som pre-eksposisjons-profylakse (PrEP). Det er derfor per i dag ingen tegn som tyder på økning i resistens mot PrEP blant nydiagnostisert hiv-1 pasienter i Norge. Det vil imidlertid være viktig å følge nøye med på resistens mot PrEP også fremover, sett i lys av den økte bruken av PrEP de senere år.

De aller fleste tilfellene av smitte med resistent virus i 2019 har skjedd i utlandet, noe som tyder på at spredning av resistent hiv i Norge fortsatt er lav.

Hepatitt B virus

I 2019 ble 217 prøver analysert med tanke på resistensmutasjoner hos hepatitt B virus (HBV). Av disse prøvene var det 14 prøver fra pasienter på antiviral behandling der det var spørsmål om resistens som årsak til behandlingssvikt. De øvrige 203 prøvene var fra behandlingsnaive pasienter, og det er disse som utgjør den norske overvåkningen av primærresistens. Relevante mutasjoner ble funnet i kun én av de 14 prøvene. Dette var i en prøve fra en pasient behandlet med entekavir, og mutasjonen var assosiert med resistens mot entekavir. Blant de 203 prøvene testet for primærresistens ble det ikke påvist noen resistensmutasjoner. Til tross for en økning i bruken av

førstelinjebehandlingene tenofovir og entekavir de siste 5 årene, ble det knapt funnet noen resistensmutasjoner. Dette tyder på at prevalensen av resistens mot antivirale midler brukt i behandling av HBV i Norge er lav.

Humane herpesvirus

I 2019 ble 21 prøver sendt inn til referanselaboratoriet for cytomelagovirus (CMV) for resistenstesting, og resistensmutasjoner ble påvist i seks prøver. Det har vært en økning i behandling av CMV-infeksjoner de senere år, men det er sjelden man påviser resistens. Det er imidlertid ingen systematisk resistensovervåkning av CMV, og dermed ingen sikker oversikt over den reelle forekomsten.

For herpes simplex-virus var det 12 prøver som ble analysert med tanke på resistens mot antivirale midler i 2019. Det ble påvist resistens mot aciklovir i to av prøvene, og mulig resistens i en tredje prøve. Til tross for en økning i bruk av aciklovir, både i behandling og som profylakse, utføres det sjelden resistensundersøkelse. I likhet med CMV har man heller ikke for herpes simplex virus en systematisk resistensovervåkning.

Hepatitt C virus

Verdens helseorganisasjon har fremmet et mål om å eliminere hepatitt C-relatert sykdom innen 2030, der behandling med antivirale midler mot hepatitt C virus (HCV) er en sentral del av strategien. Referanselaboratoriet for hepatitt ved FHI har nylig etablert en metode for helgenomsekvensering av HCV som vil gjøre det mulig å gjennomføre en systematisk nasjonal resistensovervåkning. Et slikt overvåkningsprogram vil være egnet til å monitorere hvordan økt bruk av antiviralia påvirker resistensforekomst, og vil dermed kunne bli et viktig bidrag til å nå målet om å eliminere hepatitt som et folke-helseproblem. Videre kan et overvåkningsprogram danne grunnlag for norske retnings-linjer for resistenstesting, og være et verktøy for å overvåke og justere norske behandlingsanbefalinger.

Sars-CoV-2

Det finnes foreløpig ikke noen antiviral behandling med dokumentert effekt mot sars-CoV-2. I temakapittelet om sars-CoV-2 i denne rapporten diskuteres potensiell risiko for resistensutvikling mot en fremtidig antiviral behandling mot sars-CoV-2. Hvis antiviral behandling blir en del av strategien for håndteringen av pandemien, vil en systematisk overvåkning av antiviral resistens kunne bli avgjørende.

Summary

The usage of antivirals

According to The Norwegian Drug Wholesales statistics database, the sales of antiviral drugs measured in defined daily doses (DDDs) continued to increase in 2019. For HIV drugs there was an increase in sales measured in DDDs and number of users. In particular, there was a significant increase in the fixed combination of emtricitabine and tenofovir disoproxil, the combination that was approved as Pre-Exposure Prophylaxis (PrEP) in 2016. The number of persons given at least one prescription of this combination increased almost 47 % from 2018 to 2019. The sales of antivirals against hepatitis C virus (HCV) was reduced in 2019, after several years of increase. Both for HIV- and HCV-drugs, there has been a significant change in the pattern of use with a transition from single ingredient drugs to fixed combinations.

Influenza virus

Resistance to antiviral drugs currently used to treat influenza virus remains low in Norway. No mutations conferring resistance to oseltamivir or zanamivir were detected in the 2019/20 influenza season. In recent years adamantanes have not been used in the treatment of influenza in Norway or most other countries due to universal resistance. Adamantane resistance testing is therefore not routinely performed at the Norwegian Institute of Public Health (NIPH).

Human immunodeficiency virus-1

In 2019, data from resistance testing were cross-referenced with MSIS data for the first time. This enabled the analysis of the prevalence of surveillance drug-resistance mutations (SDRMs) across different subgroups, as well as providing information on the coverage of primary resistance testing.

SDRMs were detected in 10.3% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2019. This is higher than what has been observed in previous years. However, of the 11 samples where SDRMs were detected, only four sequences harbored mutations associated with clinically relevant drug resistance, and all of these four patients were infected abroad. Furthermore, only one of these mutations was associated with reduced susceptibility to tenofovir/emtricitabine, the drugs used preventative as pre exposure prophylaxis (PrEP). At present there are no signs of an increase in drug resistance associated with PrEP among patients newly diagnosed with HIV in Norway. However, with the increased use of PrEP in recent years, continued surveillance of mutations associated with reduced susceptibility to these drugs is warranted.

As most of the cases harboring SDRMs were infected abroad, transmission of drug resistant HIV in Norway remains low.

Hepatitis B virus

In 2019, 217 samples were analysed for hepatitis B virus (HBV) drug resistance mutations. Of these, 14 samples were from patients with treatment failure. The remaining 203 samples were from treatment naïve patients and can be considered surveillance of primary resistance. Of the 14 samples from previously treated patients, only one sample had a drug resistance mutation. This sample was from a patient previously treated with

entecavir and the mutation was shown to confer resistance to entecavir. Among the 203 samples tested for primary resistance, no resistance mutations were detected. The use of tenofovir and entecavir, first-line drugs against HBV, has been increasing steadily the past five years. Nevertheless, relatively few drug resistance mutations have been detected, and the prevalence of HBV drug resistance in Norway remains low.

Human herpes viruses

Resistance mutations were detected in six out of the 21 samples submitted to the reference laboratory for cytomegalovirus (CMV) for resistance testing in 2019. Although there has been an increase in the treatment of CMV infections in recent years, resistance mutations are only rarely detected. There is, however, no systematic resistance surveillance of CMV drug resistance, and the true prevalence of drug resistant CMV in Norway is therefore unknown.

In 2019, 12 samples were submitted for herpes simplex virus (HSV) drug resistance testing. Mutations conferring resistance to aciclovir were detected in two samples, and one mutation that could possibly confer resistance was identified in a third sample. Despite increased usage of aciclovir, treatment failure is rare. However, as for CMV, there is no systematic surveillance of HSV drug resistance.

Hepatitis C virus

Treatment with antiviral drugs against hepatitis C virus (HCV) is a cornerstone in the World Health Organizations (WHO's) strategy towards eliminating HCV-related disease by 2030. A method for whole genome analysis of HCV has recently been established at the reference laboratory for HCV at NIPH. This new method enables the implementation of a surveillance program for baseline resistance. A national surveillance program for HCV drug-resistance will facilitate monitoring of the impact of escalating antiviral treatment on drug resistance, thereby contributing towards reaching WHO's target on combatting viral hepatitis. Furthermore, a surveillance program may in turn inform guidelines for resistance testing and provide a tool for monitoring and adjusting the Norwegian treatment recommendations.

SARS-CoV-2

At present there are no antiviral therapies with documented effect against SARS-CoV-2. In the chapter on SARS-CoV-2 in this report, the potential risk of drug resistance to a future antiviral therapeutic treatment for SARS-CoV-2 is discussed. If antiviral treatment also becomes part of the strategy for managing the current pandemic, systematic surveillance of drug resistance will be vital.

1 Antivirals and development of drug resistance

Antiviral drugs act by inhibiting viral replication, usually targeting a specific step in the virus' replication cycle. Most antiviral drugs are effective only against one particular virus or a group of viruses, and specific antiviral therapy is available only for a few viral infections. In principle, drugs may be designed to inhibit any step in the replication cycle of a virus. Most of the antivirals currently available work by inhibiting viral DNA- or RNA-synthesis, or by direct inhibition of other viral enzymes essential to the virus (1).

Drug resistance against antivirals is caused by genetic changes in the viral genome leading to amino acid alterations in the protein targeted by the drug, thereby affecting the activity of the drug. These genetic changes most commonly arise from random mutations. In addition, recombination or exchange of genetic material may also occur for certain viruses, for example antigenic shifts in influenza. Genetic alteration at a key site of the viral genome is usually a disadvantage for the virus, and most resistance mutations impair viral fitness. However, in the presence of antiviral drugs, resistant variants will have a fitness advantage over wild type virus. Resistant virus variants are therefore selected and may continue replication under these conditions. Compensatory mutations, restoring viral fitness of the resistant variants, may then be selected by similar mechanisms. This may ultimately lead to the persistence of these variants even in the absence of antiviral drugs.

The risk of developing drug resistance varies significantly between different viruses, depending on factors such as mutation frequency and replication accuracy of the virus, viral load, turnover, fitness of mutated virus, and duration of both the infection and the treatment. Immunocompromised patients are at particular risk. Furthermore, different drugs have different genetic barriers, meaning that the number of mutations needed for development of resistance is different for different drugs.

Antivirals against influenza

There are two classes of antiviral drugs for treatment of influenza that are approved in Europe:

1) M2-inhibitors: blocks the M2 ion channel of influenza A virus, thereby inhibiting the early stages of virus replication. No effect on influenza B (examples: amantadine and rimantadine).

2) Neuraminidase inhibitors: Neuraminidase inhibitors are effective during the last stage of the replication cycle, inhibiting the release of newly formed virus particles. Normally, hemagglutinin on the surface of the virus binds to sialic acid on the cell surface. The virus is released after the viral enzyme neuraminidase cleaves residues on the sialic acid, thus destroying this binding. Neuraminidase inhibitors bind to neuraminidase on the surface of influenzavirus A and B, preventing cleavage of sialic acid and thereby preventing release of the virus from the surface of the host cell (examples: oseltamivir and zanamivir) (2).

Oseltamivir is however the only antiviral drug against influenza currently on the market in Norway. All circulating influenza strains are currently resistant to the two M2-inhibitors, and these drugs are no longer used for treatment of influenza. Zanamivir is still registered but was withdrawn from the market in 2016 due to limited use.

New drugs are under development, and several are approved for treatment of influenza in the USA and Japan. The polymerase inhibitor baloxavir marboxil was recently approved in

the USA and is expected to be available in Europe in 2020. The drug targets the endonuclease function of influenza RNA polymerase and inhibits viral replication by preventing transcription of viral mRNA.

Drug resistant influenza

As mentioned earlier, drug resistant virus may propagate in the absence of antiviral agents as long as the mutation that confers resistance does not cause any significant evolutionary disadvantage for the virus. This is particularly evident for influenza virus. The largest outbreak of such a virus occurred in 2007, when an oseltamivir resistant H1N1 virus completely replaced the sensitive wildtype virus within one year after its first occurrence. Resistance may 'hitch-hike' on another advantageous feature that promotes one virus strain over others, such as fitness-enhancing mutations at other genomic sites (3). Furthermore, reassortment of the segmented genome may rapidly lead to major genetic changes that could involve domains of importance for drug resistance characteristics.

Antivirals against human immunodeficiency virus

There are five different classes of antiretroviral drugs used in the treatment of human immunodeficiency virus (HIV) infection, targeting different phases of HIV's lifecycle:

1) Entry inhibitors: CCR5 blockers that block the binding between viral gp120 and the chemokine receptor CCR5 (example: maraviroc). Fusion inhibitors preventing fusion between the viral gp41 and the cell membrane (example: enfuvirtide), are no longer registered.

2) Nucleoside reverse transcriptase inhibitors (NRTI): Analogues of naturally occurring deoxynucleotides that are incorporated into the viral DNA chain in competition with the natural substrate. When incorporated, the drug stops further elongation of the viral DNA chain (chain termination), thereby inhibiting transcription of RNA into DNA (examples: abacavir, lamivudine, emtricitabine, tenofovir, and zidovudine).

3) Non-nucleoside reverse transcriptase inhibitors (NNRTI): Bind to the reverse transcriptase, thereby inhibiting transcription of RNA into DNA (examples: rilpivirine, etravirine, nevirapine, efavirenz, and doravirine).

4) Integrase inhibitors: Prevent integration of pro-viral DNA into the host cell DNA (examples: dolutegravir, raltegravir, elvitegravir, and bictegravir).

5) Protease inhibitors (PI): Bind to the protease, thereby preventing the cleavage of polyproteins in the maturing virus particle (examples: darunavir, atazanavir, and lopinavir). The effect is improved by addition of a pharmacokinetic enhancer (ritonavir or cobicistat).

In antiretroviral therapy (ART) for HIV-1, combinations of at least two drugs from different classes are used in order to reduce the risk of drug resistance. Currently recommended first line regimens consist of an integrase inhibitor in combination with two NRTIs. Alternatively, a boosted PI or an NNRTI may replace the integrase inhibitor. Fixed-dose combination drugs are widely available.

Drug resistant HIV

HIV has a very high mutation rate and a considerable risk for development of resistant variants, mainly due to inaccuracy in viral replication and the lack of proofreading of the viral enzyme reverse transcriptase. There is vast genetic variation in the HIV-1 genome, and

each patient harbors a mixture of coexisting genetic variants. This genetic variation increases over the course of the infection. Drug resistant viruses may evolve from wild-type viruses if viral replication persists during antiretroviral treatment. Because most drug resistance mutations impair viral fitness, wild type virus often rapidly reemerges when treatment is interrupted. Drug resistance rarely occurs without previous drug exposure, but individuals carrying virus with resistance mutations may transmit this virus to others. Drug resistance emerging during antiviral treatment is called acquired drug resistance. Drug resistance detected in previously untreated persons is usually transmitted from a person with acquired drug resistance and may subsequently spread to others. The term transmitted drug resistance is used when previously uninfected individuals are infected with virus that has drug resistance mutations (4).

Antivirals against hepatitis B virus

Only one class of antivirals is used for treating chronic hepatitis B virus (HBV) infection:

1) Nucleoside/nucleotide analogues: Analogues of naturally occurring deoxynucleotides that are incorporated into the viral DNA chain in competition with the natural substrate. When incorporated, the drug stops further elongation of the viral DNA chain (chain termination), thereby inhibiting transcription of RNA into DNA by the HBV polymerase. (examples: entecavir, tenofovir disoproxil, and tenofovir alafenamide)

The activity of the HBV polymerase is similar to that of HIV reverse transcriptase, and several of the nucleoside/nucleotide analogues have activity against both viruses. Currently, monotherapy with entecavir or tenofovir is recommended as first-line treatment, given their antiviral potency and favorable resistance profile (5). Another treatment option is interferon therapy, which works by enhancing the host immune response. Although interferon-based treatment strategies offer an opportunity for seroconversion, current use in treatment is limited, mainly due to considerable side effects.

Drug resistant HBV

The mutations associated with HBV drug-resistance are located in the reverse transcriptase domain of the HBV polymerase, and lead to reduced inhibitory effect of the drug on the viral polymerase. Aside from reducing the sensitivity of the virus to the drug, primary mutations often simultaneously reduce viral fitness. Compensatory resistance mutations restoring replication capacity, and secondary resistance mutations increasing drug resistance, may arise after the emergence of primary resistance mutations. Drug resistant HBV may develop under antiviral treatment but is rarely (if ever) transmitted. Reported resistance in HBV is mainly towards the less potent drugs lamivudine and adefovir, which have a low genetic barrier to resistance compared to tenofovir and entecavir. For entecavir, several mutations are required to confer drug resistance. Resistance to entecavir may still occur, but it is rare. For tenofovir, only a few cases of clinically significant drug resistance are described worldwide, all of them as part of multidrug resistance (6). Because of the rarity of resistant cases, the relevant mutation sites for tenofovir-resistance are not fully confirmed.

Antivirals against cytomegalovirus

Only one class of antivirals is used for treating cytomegalovirus (CMV) infection:

1) Nucleoside analogues: Analogues of naturally occurring deoxynucleotides that are incorporated into the growing strand of viral DNA by CMV polymerase (UL54), causing termination of the growing viral DNA strand (chain termination). Drugs of choice: Ganciclovir or its prodrug valganciclovir.

Ganciclovir and valganciclovir are the drugs of choice since they are quite effective in inhibiting virus replication and have few side effects. To become active, ganciclovir is monophosphorylated by the CMV UL97 kinase and then di- and tri-phosphorylated by cellular kinases. Cidofovir and foscarnet are also incorporated by the CMV-DNA polymerase but work independently of the CMV kinases. Because they do not require activation by viral enzymes, their action is not limited to infected cells. These drugs have more side-effects and are used only in special situations such as CMV retinitis or retinal necrosis.

Some new anti-CMV-drugs are in clinical trials. Letermovir binds to and inhibits the CMV-DNA terminase complex which is involved in cleaving and packaging of CMV-DNA genome into the capsid. The drug is approved by both the FDA and the European Medicines Agency for prophylactic use after stem cell transplantation and is already available in Norway. Maribavir, a UL97-kinase inhibitor, has been used in clinical trials with favorable outcomes but is not yet approved by the FDA.

Drug resistant CMV

During ganciclovir anti-CMV therapy, resistance mutations usually develop after a cumulative exposure of six weeks or more. Since ganciclovir has two points of interaction with CMV, two main types of resistance mutations arise. Resistance mutations are usually first seen in the UL97 kinase gene. The UL54 (DNA-polymerase) mutations tend to emerge later and add to the level of resistance conferred by preexisting UL97 mutations. UL54 resistance mutations in the absence of UL97 mutations are uncommon.

Antivirals against herpes simplex virus

Only one class of antivirals is used for treating herpes simplex virus (HSV) infection:

1) Nucleoside analogues: Analogues of naturally occurring guanosine that are incorporated into the growing strand of viral DNA by HSV DNA polymerase (UL30), causing termination of the growing viral DNA strand (chain termination). Drugs of choice: aciclovir or its prodrug valaciclovir.

To be effective, aciclovir has to be triphosphorylated, first by a viral thymidine kinase (TK) and then by the cellular kinases to the active aciclovir-triphosphate. Aciclovir and valaciclovir are effective against both HSV-1 and HSV-2, as well as varicella zoster virus. Penciclovir is available as ointment for topical treatment of herpes labialis. Second line drugs include foscarnet and cidofovir.

Drug resistant HSV

Resistance to aciclovir develops by mutations of either the HSV-TK- or HSV DNA polymerase gene. Mutations in HSV-TK are by far the most common, and about 95% of the

resistance mutations are localized in the thymidine-kinase gene (UL23) whereas 5% are localized in the DNA-polymerase gene (UL30) (7).

Aciclovir resistance is frequently associated with cross-resistance to other HSV-TK dependent nucleoside analogues (7). Cidofovir and foscarnet are independent of HSV-TK and therefore active against most of the strains that are resistant to aciclovir. Cross-resistance of foscarnet to aciclovir is rare (7). Although the prevalence of HSV resistance mutations are reported to be 0.1% -0.7% in immunocompetent patients and 3.5% to 10% in immunocompromised patients, treatment failures are relatively rare (8).

Antivirals against hepatitis C virus

There has been a rapid development of new and better drugs against hepatitis C virus (HCV) over the last few years, replacing the early generations of direct acting antivirals (9). There are now several pangenotypic combination tablets available, with high genetic barriers to resistance and excellent treatment responses. The goal of HCV therapy is to cure the infection. Treatment is usually given over 8-12 weeks, and most patients obtain sustained virological response (defined as absence of viremia) 12 or 24 weeks after completion of treatment (10).

There are currently four groups of direct acting antivirals (DAA) against HCV (11):

1) NS5B inhibitors:
    a. Nucleoside analogue polymerase inhibitors: Compete with nucleosides for the active site of the HCV polymerase, NS5B (example: sofosbuvir).
    b. Non-nucleoside analogue polymerase inhibitors: Alter the shape of the polymerase and thus inhibit replication of HCV (example: dasabuvir).
2) NS3/4A protease inhibitors: Target the active site of the protease enzyme, NS3/4A, inhibiting proteolysis of the HCV polyprotein. Genotype specific. (example: voxilaprevir, grazoprevir).
3) NS5A inhibitors: Target the proteins encoded by the NS5A region of the virus genome, thereby affecting the replication, assembly and release of the virus (examples: velpatasvir, ledipasvir).

Drug resistant HCV

Similar to HIV, HCV exhibits considerable genetic variation. The HCV RNA polymerase is relatively inaccurate and lacks proofreading, leading to a high mutation rate. As a result, a single infected person may harbour a vast population of variants, or quasispecies, dominated by the variants with the best viral fitness. Some of these random mutations may lead to amino acid substitutions associated with reduced susceptibility to antiviral drugs, called resistance-associated substitution (RAS). The RASs can be present prior to treatment, or they may develop during treatment. Continued replication under antiviral pressure increases selection of viruses with RASs. The clinical significance of the different RASs is variable, and the presence of a RAS does not necessarily predict treatment failure. After interruption of treatment, most RASs are reversed. However, some RASs may persist also in the absence of antiviral drugs, affecting future treatment options.

Antivirals against severe acute respiratory syndrome coronavirus 2

There is currently no direct acting antiviral treatment with documented effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Studies so far have been focusing on repurposing existing drugs approved for other infections and evaluate their antiviral effect against SARS-CoV-2 (12). A few of these antiviral drugs have shown promising results *in vitro*, and different candidates are being tested in clinical studies. For the nucleotide analogue remdesivir, preliminary results have indicated improved time to recovery among hospitalized patients (13). Remdesivir was the first COVID-19 treatment recommended for EU authorisation, and the drug received conditional marketing authorisation in June 2020 (14). However, antiviral treatment is not yet implemented as part of standard clinical care, and in Norway, treatment with remdesivir is offered within the frame of clinical trials.

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2 The usage of antivirals in Norway

Over the last decades, the development of new specific antivirals has accelerated, especially due to development of new drugs against HIV and hepatitis C virus (HCV) (1). The sales of direct acting antiviral drugs (DAA), measured in both defined daily doses (DDDs) and number of patients treated have increased every year (Figure 2.1 and Figure 2.2, respectively), and the introduction of new antivirals for treatment of HCV infections has highly contributed to increased costs. However, in 2018, price reduction for some of the drugs used in treatment of HIV and HCV resulted in reduced costs despite continued increase in sales. This trend continued for the HIV drugs in 2019 while the sales of HCV drugs this year were reduced both in DDDs and costs. According to The Drug Consumption in Norway 2015-2019 (2), the cost of anti-infectives for systemic use (Anatomical Therapeutic Chemical Classification (ATC) group J) was, for the first time in the last ten years, reduced in 2018, and then further reduced in 2019. This is mainly due to reduced costs for the DAA (J05).

For HIV drugs, sales measured in number of DDDs have been relatively stable in recent years, but since 2018 it has increased. During the years there has also been a significant change in the pattern of use with a transition from single ingredient drugs to fixed combinations. The sales for the different ATC subgroups of DAA over time are shown in Figure 2.1.

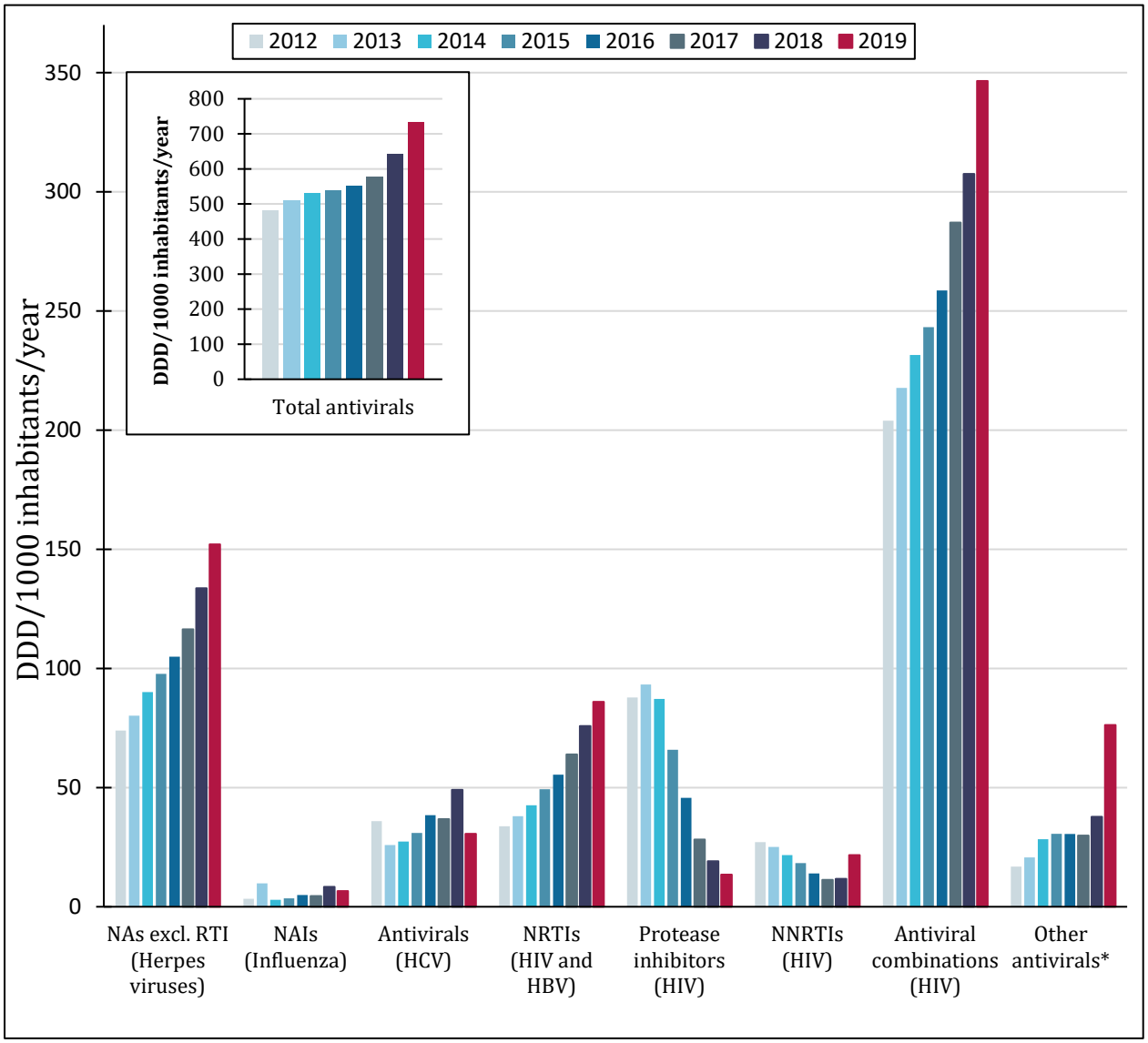

Figure 2.1 Sales of direct acting antiviral drugs for systemic use (ATC group J05A) for 2012-2019.

The figure shows the sales of direct acting antiviral groups over time. Numbers are given as defined daily doses (DDD) per 1000 inhabitants per year. NA excl. RTI: Nucleosides and nucleotides excl. reverse transcriptase inhibitors (J05AB); Protease inhibitors (J05AE); NRTIs: Nucleoside and nucleotide reverse transcriptase inhibitors (J05AF); NNRTIs: Non-nucleoside reverse transcriptase inhibitors (J05AG); NAIs: Neuraminidase inhibitors (J05AH); Antivirals, HCV: Antivirals for treatment of HCV infections (J05AP); Antiviral combinations, HIV: Antivirals for treatment of HIV infections, combinations (J05AR) and Other antivirals (J05AX). A plot illustrating the total sales of antivirals in ATC group J05A in Norway is inserted in the main plot. The total numbers also include phosphonic acid derivatives (J05AD) used against herpesviruses, due to low numbers this is not included in the main plot. *mostly HIV integrase inhibitors.

Number of persons treated with different DAAs has increased for all the different virus infections since 2011 (Figure 2.2). Antivirals used for treatment of HIV dominate when sales are measured in number of DDDs (Fig. 2.1), while DAAs against herpesviruses are by far the most used antivirals when measured in number of users. The high number of DDDs for HIV drugs reflect the long-term daily treatment . For DAAs against herpesvirus, the use of topical agents (creams and ointments) are not included in the measurement of DDD.

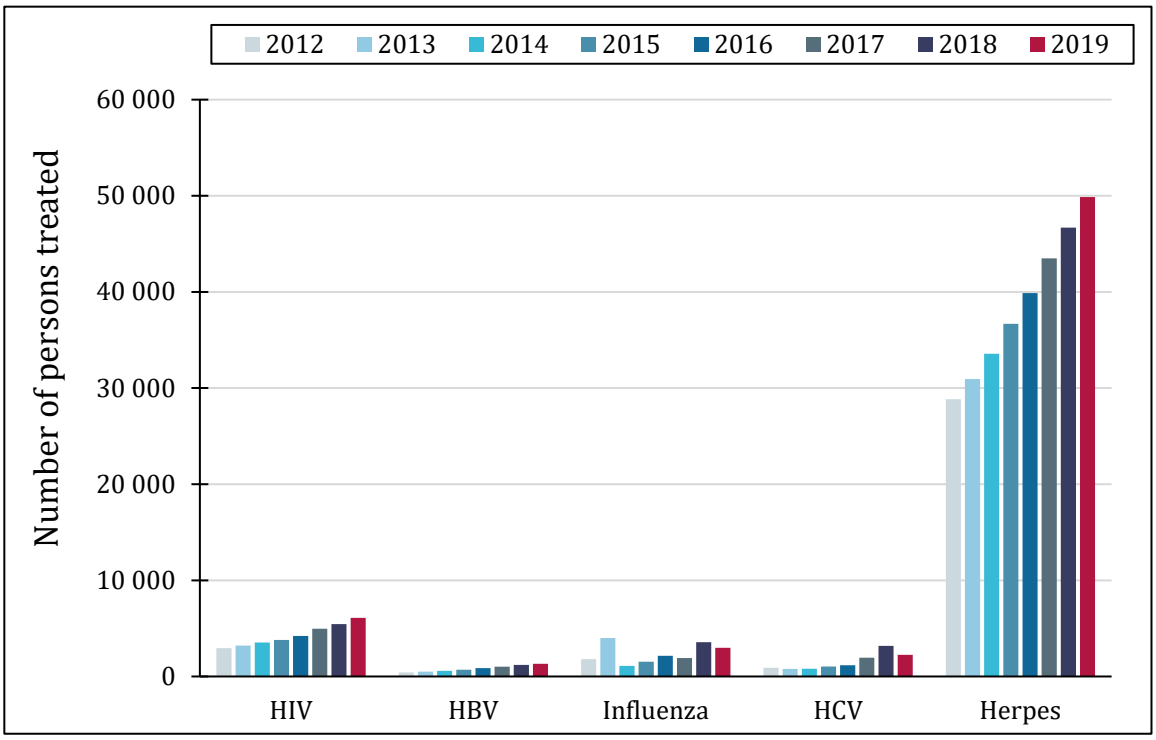

Figure 2.2 Trends in the use of direct acting antiviral drugs for systemic use (ATC group J05A) grouped by virus for 2012-2019.

The figure shows the number of persons treated for different viruses with systemic direct acting antivirals over time. The number of persons treated is based on the number of patients given at least one prescription per year. HIV: All HIV pharmaceuticals (ATC-group J05AF05: Lamivudine, Zeffix is excluded); HBV: All HBV pharmaceuticals (ATC-code J05AF05: Lamivudine, Epivir is excluded). Single component drugs approved for both HBV and HIV are included in the HBV numbers only; Influenza: ATC-group J05AH: Neuraminidase inhibitors; HCV antivirals: ATC-group J05AP; Herpes: aciclovir (J05AB01), ganciclovir (J05AB06), famciclovir (J05AB09), valaciclovir (J05AB11), cidofovir (J05AB12) and foscarnet (J05AD01).

Influenza virus

The usage of the neuraminidase inhibitors, antivirals for the treatment of influenza (ATC group J05AH), is shown in Table 2.1. The variations in the number of users of DAAs for treatment of influenza is probably related to the size and intensity of the seasonal influenza epidemic each year, the accuracy of the yearly influenza vaccine, and the proportion of the population vaccinated. The number of vaccines sold has increased substantially the last two years. It should be noted that the data on antiviral usage is collected per calendar year, which includes the end of one influenza season and the beginning of the next. Due to limited use, zanamivir was withdrawn from the market in 2016; consequently, oseltamivir is the only neuraminidase inhibitor available for treatment of influenza in Norway.

Table 2.1 Number of individuals with at least one prescription of a neuraminidase inhibitor (ATC group J05AH) per year.

	2015	2016	2017	2018	2019
Zanamivir	52	25			
Oseltamivir	1 477	2 129	1 923	3 571	2 987

Human immunodeficiency virus

There are currently 32 DAAs, defined by different ATC codes, used in treatment of HIV in Norway. The use of the different drugs has shifted in the last five-year period. Of the 32 HIV drugs or combination drugs used in 2019, nine of them have been introduced since 2015, while two older drugs have disappeared in the same period. The number of patients retrieving at least one prescription of these drugs has increased by more than 60% from 2015 to 2019. Figure 2.3 shows the trends in use of the 10 most frequently used drugs in 2019, measured in number of persons treated. During the whole period, more than 97% of persons treated, received combination drugs containing more than one active substance. In 2019, more than 6000 persons in Norway retrieved prescriptions for a fixed combination drug. Single substance drugs are given in addition to the fixed combinations for some patients.

Tenofovir disoproxil, adefovir dipivoxil and emtricitabine are approved for treatment of both HIV and HBV infections. However, since these single substance drugs are rarely used for HIV therapy, the users of these drugs are neither included in the total number of users of HIV treatment nor in the different groups in Figure 2.3. The sum of the patients using the different drugs is higher than the total number of patients treated with HIV drugs in Figure 2.2. This is because some patients receive more than one drug or may change treatment regimens during a year.

The fixed combination of emtricitabine (FTC) and tenofovir disoproxil (TDF) has been the combination drug most used in recent years. A small decrease was seen in 2015 and 2016 before the use again increased in 2017. In 2016, this combination was approved as Pre-Exposure Prophylaxis (PrEP) to reduce the risk of sexually acquired HIV-1 infection in adults at high risk and was given with full reimbursement of the costs. This may explain the increased number of patients retrieving at least one prescription of this fixed combination in 2017. The increased use of FTC/TDF has continued, and in 2019, 3122 persons were given at least one prescription of this combination. This corresponds to an increase of almost 47 % compared to 2018. For post exposure prophylaxis (PEP), the recommendation is to use FTC/TDF in combination with the integrase inhibitor raltegravir. The use of raltegravir is also increasing. From the drug statistics it is not possible to separate out the proportion of PrEP or PEP from the total use of these drugs.

A new prodrug of tenofovir, tenofovir alafenamide (TAF), was introduced in three different fixed combinations in 2016; emtricitabine /TAF, emtricitabine/TAF/rilpivirine and emtricitabine/ TAF / elvitegravir/ cobicistat. A second 4-component combination (emtricitabine/TAF/darunavir/cobicistat) has been available since 2018. Finally, bictegravir, a new integrase inhibitor, only available in a 3-component combination together with emtricitabine/TAF, entered the market in 2019. TAF is given in lower doses and has a greater bioavailability in relevant body tissues than TDF. The increased use of the new TAF-containing combinations started in 2017 and has continued in 2018, but the use was reduced in 2019. This is also the case for TDF combinations other than emtricitabine/TDF.

New treatment guidelines from the Norwegian Society of Infection Medicine in 2019 recommended the use of an integrase inhibitor in all antiretroviral therapy (ART) starting regimens (3). The guidelines also open for use of 2-component regimens for selected patient groups (e.g. low virus count, expected good compliance). The recommendations from the Procurement Services for Health Enterprises Ltd, which negotiate prices and indicates the drugs of preference when it comes to reimbursement, also have a great impact on the choice of drugs for treatment of HIV (4). The majority of patients use a

relatively limited number of drugs even if the selection of different drugs and possible combinations is extensive. The increased use of tenofovir disoproxil at the expense of tenofovir alafenamide in 2019 could partly be explained by the recommendations from the Procurement Services for Health Enterprises, but this shift may also be related to increased use of PrEP.

Of the five most sold drugs in 2019 measured in number of users, the fixed combination of emtricitabine/TDF is the only drug not containing an integrase inhibitor. The remaining four include an integrase inhibitor either as combinations; (lamivudine/abacavir/dolutegravir and emtricitabine/TAF/elvitegravir) or as single substances (raltegravir and dolutegravir). Lamivudine/abacavir was in 2019 one of the two recommended starting nucleoside reverse transcriptase inhibitor (NRTI) regimens together with emtricitabine/TDF. The use of lamivudine/abacavir in combination has increased, even if the use of the 3-component combination lamivudine/abacavir/dolutegravir is somewhat reduced.

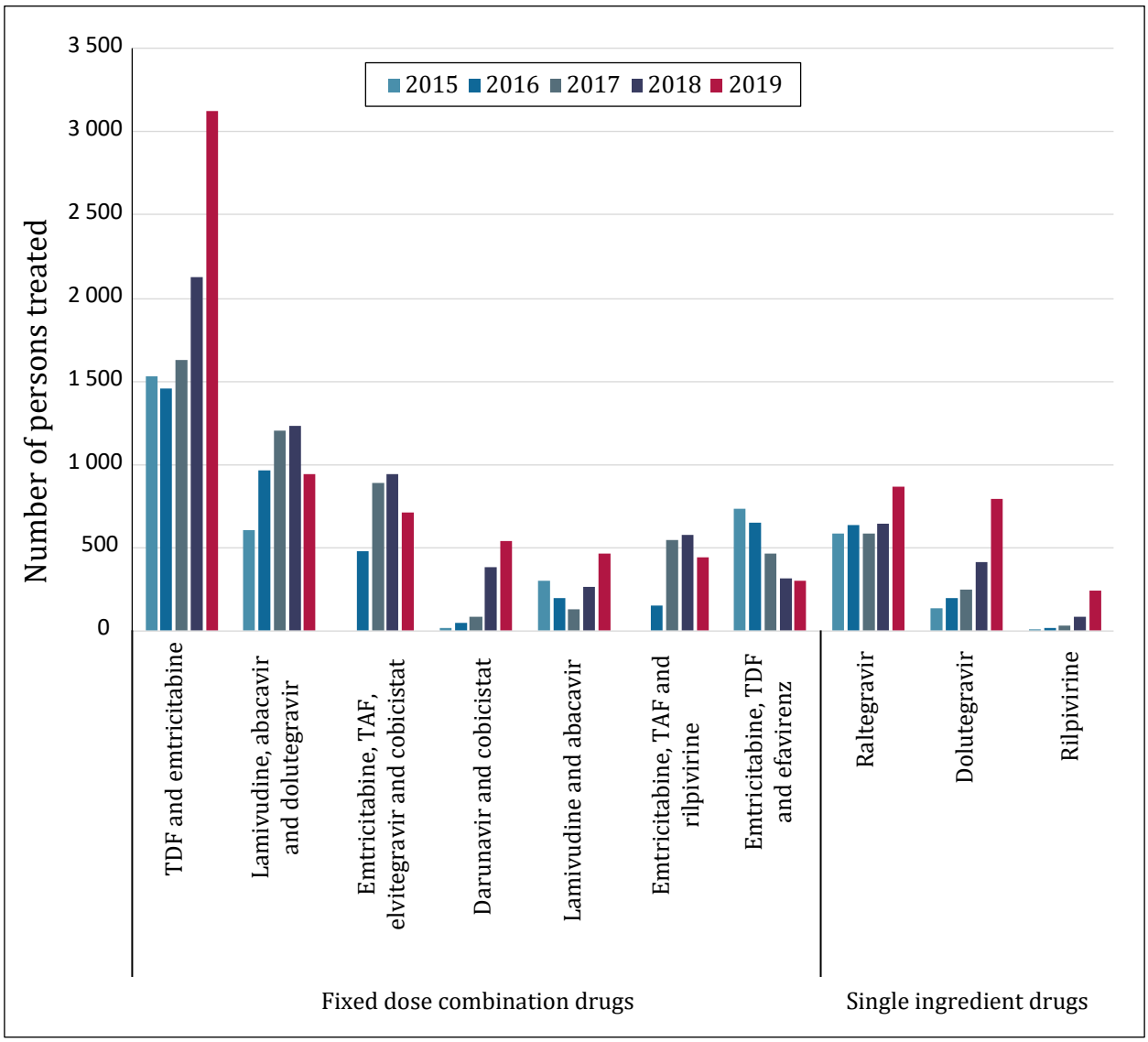

Figure 2.1: Trends in the use of antivirals for treatment of HIV in the period 2015-2019, number of persons treated (5).

The figure shows the trends in antiviral use for the treatment of HIV. The 10 most used drugs in 2019 are presented in the plot, separated by fixed dose combination drugs and single ingredient drugs. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. The remaining antivirals used in treatment of HIV are shown in Figure 2.4. Number of persons treated is defined as the number of patients given at least one prescription per year.

Only three of the 10 most used drugs in Figure 2.3 are single component drugs while there is a range of different single component drugs included in the "other group" (Figure 2.4). The non-nucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine and the integrase inhibitors dolutegravir and raltegravir are the only single component drugs that are increasingly used. The NRTI emtricitabine is included in four of the most used HIV drugs in Norway. It is also the most used active ingredient measured in number of prescriptions. The two different prodrugs of tenofovir, TDF and TAF are in second and third place, respectively. Together they are included in four of the 10 most sold drugs measured in numbers of users and in nine combinations in total. Also, the use of the integrase inhibitors dolutegravir and raltegravir is increasing, both in combinations and as single ingredient drugs when measured in number of prescriptions per active ingredient. This is

in line with the recommendations in the guidelines and the procurement recommendations.

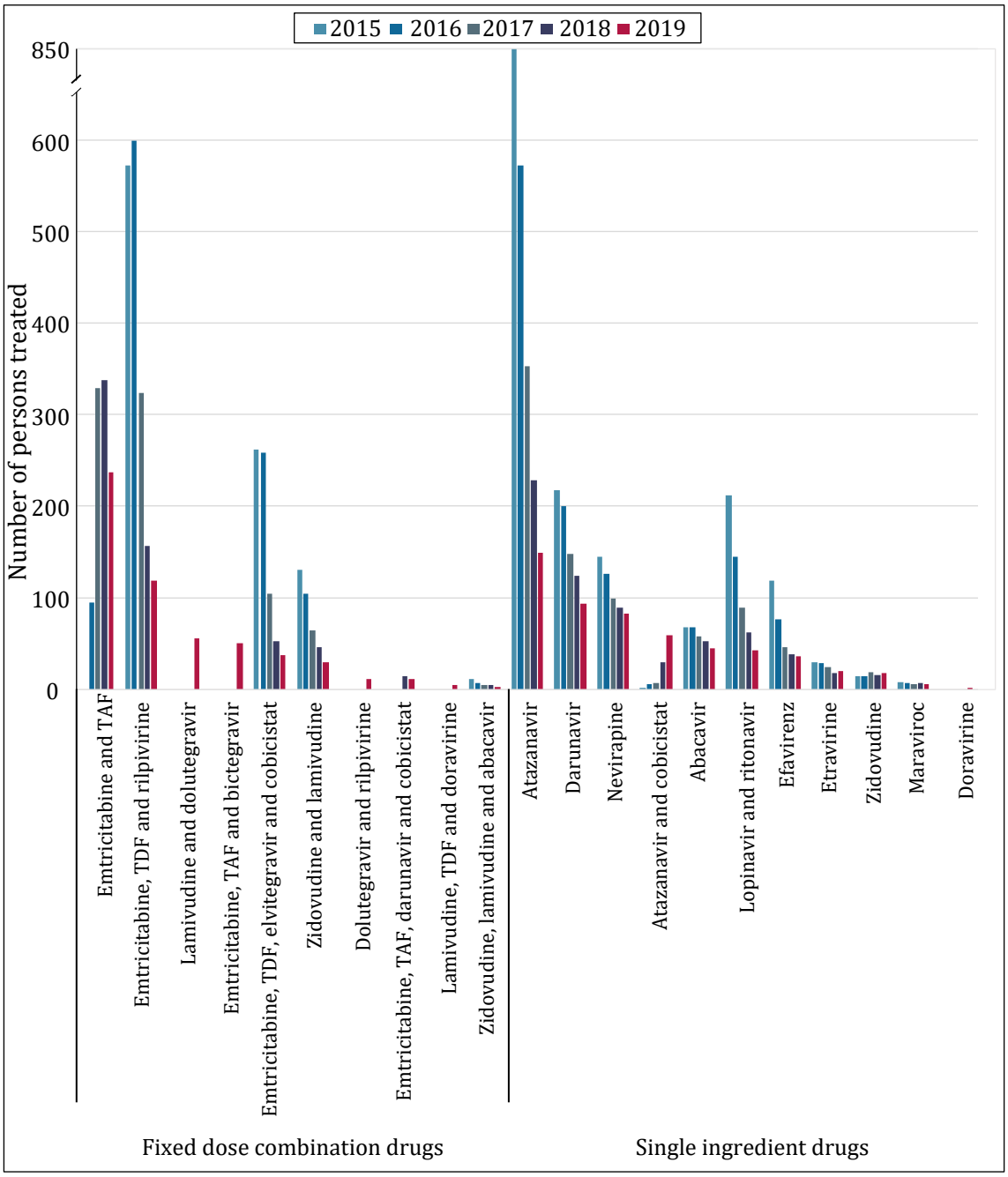

Figure 2.4 Trends in the use of antivirals for treatment of HIV in the period 2015-2019, number of persons treated, continued.

This figure shows the remaining antivirals used in treatment of HIV which are not among the top 10 most commonly used drugs. The different drugs are separated by fixed dose combination drugs and single ingredient drugs. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. Drugs prescribed to less than 10 individuals have been excluded from the figure (maraviroc, lamivudine, tenofovir disoproxil and doravirine, doravirine and zidovudine, lamivudine and abacavir). Ritonavir which is used as booster to other drugs have been omitted from the figure, and boosted protease inhibitors are classified as single ingredient drugs.

The number of prescriptions per active ingredient over time is shown in Figure 2.5.

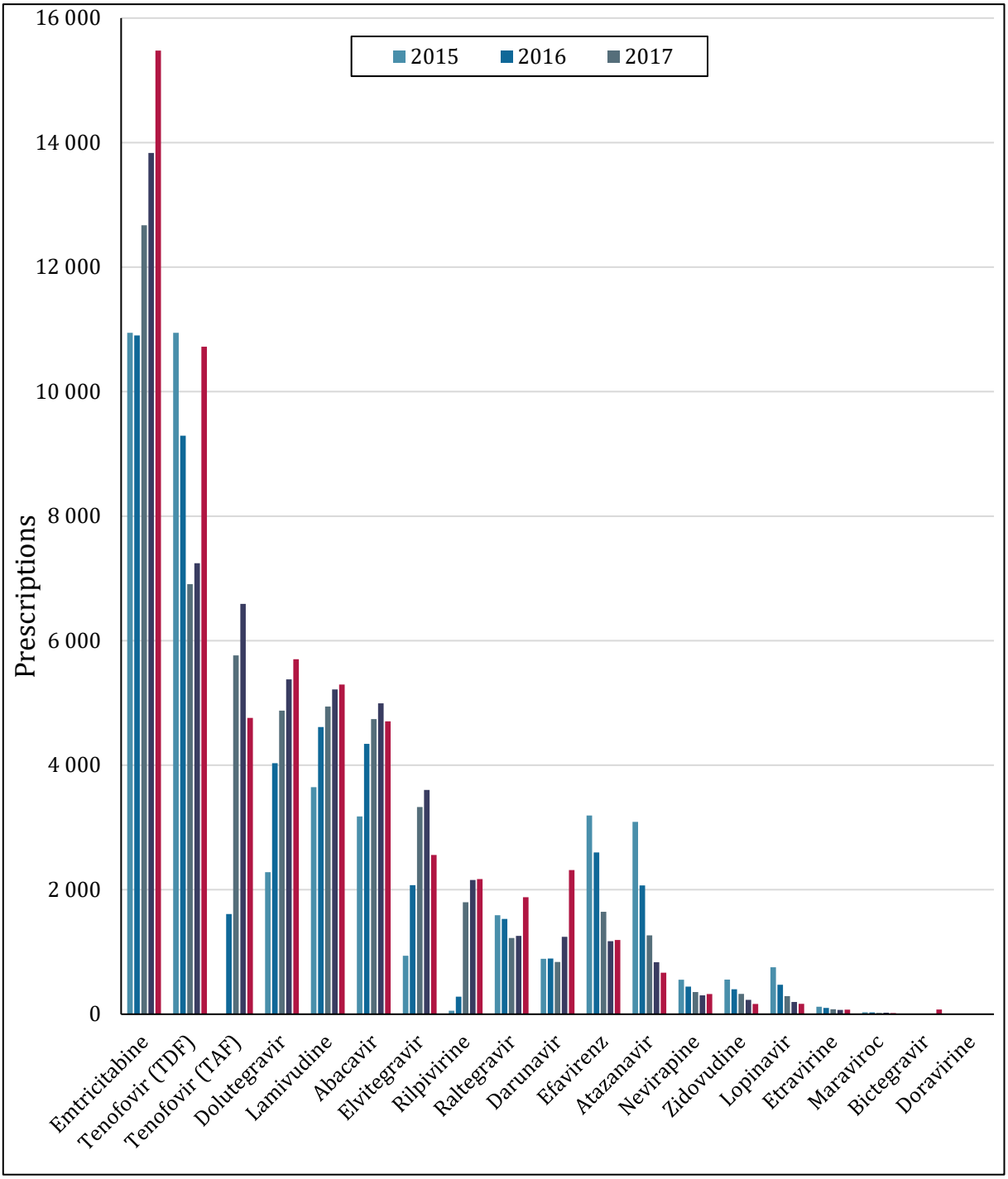

Figure 2.2: Number of prescriptions per active ingredient for HIV drugs

This figure shows number of prescriptions per active ingredient over time. Many prescriptions contain more than one active ingredient; these prescriptions are counted several times. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. Saquinavir and didanosine were not prescribed in 2019 and are excluded from the figure. Cobicistat and ritonavir which are used as boosters to other drugs have also been omitted from the figure.

Hepatitis B virus

There are currently six approved nucleoside/nucleotide analogues (NAs) approved for treatment of HBV infection. Treatment of HBV with antivirals is generally given as monotherapy. The use of the NAs is shown in Figure 2.6.

The data is based on the annual number of patients retrieving at least one prescription per year for the period 2015-2019. Lamivudine, adefovir dipivoxil, TDF, and emtricitabine are approved for both HBV and HIV, while entecavir, telbuvidine (withdrawn in 2016) and TAF as a single substance drug, are approved for HBV only. An estimate of the number of patients treated with antivirals against HBV in Norway will therefore be in the range of 375-1325 in 2019. The lowest number is based on the number of patients prescribed drugs approved for HBV only (entecavir/TAF). The highest number is the total number of patients prescribed one of the six NAs (excluding combinations containing lamivudine that are approved for HIV only).

The number of persons treated for HBV has increased during the last five years. TAF, which was approved for monotherapy of HBV in January 2017, in addition to entecavir and TDF, are considered first line therapies for HBV. Of the patients receiving HBV treatments with NAs, almost 99% received one of these three drugs in 2019. Following an increase in the use of entecavir and TAF in recent years, the number of persons treated with these drugs was slightly lower in 2019, while the use of TDF on the other hand, increased.

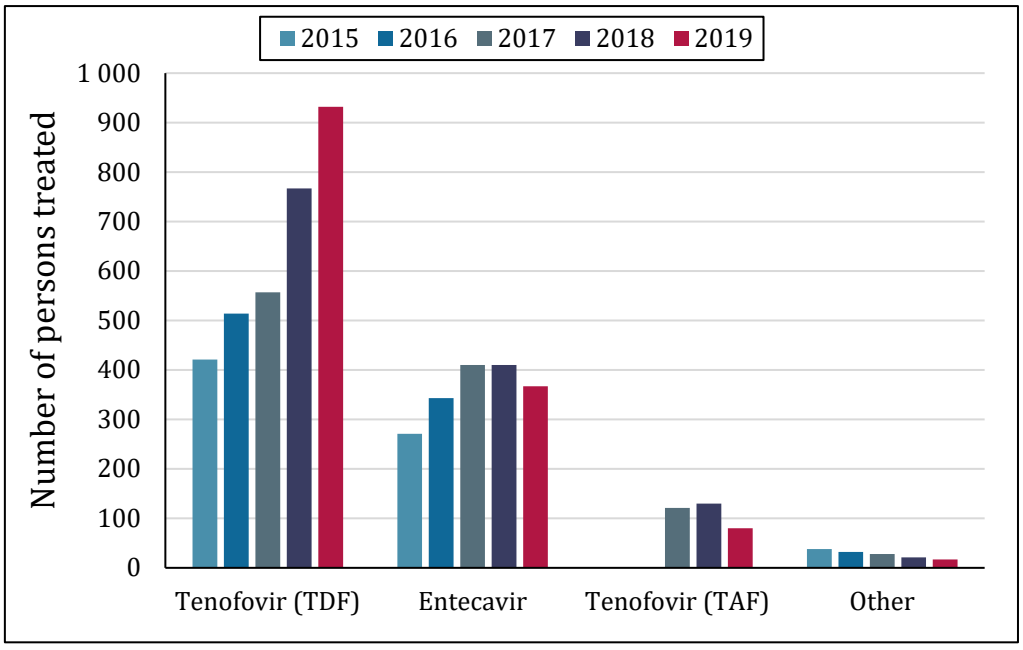

Figure 2.6 Trends in the use of antivirals for treatment of HBV for the period 2015-2019.

This figure shows the trends in antiviral use for the treatment of HBV over time. Number of persons treated is defined as the number of patients given at least one prescription per year. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. Other: lamivudine (J05AF05), adefovir dipivoxil (J05AF08), emtricitabine (J05AF09) and telbivudine (J05AF11).

Human herpesviruses

Figure 2.7 shows the two most prescribed drugs for systemic use for human herpes virus infections over the last five years. The use of the other drugs approved for treatment of human herpes virus is limited. Valaciclovir is the substance most commonly prescribed and the use of this drug is steadily increasing. The use of aciclovir has been stable during the five-year period. Ganciclovir and famciclovir are on the other hand rarely prescribed in the period. Almost 50 000 persons have been treated with systemic antivirals for herpes viral infections in 2019.

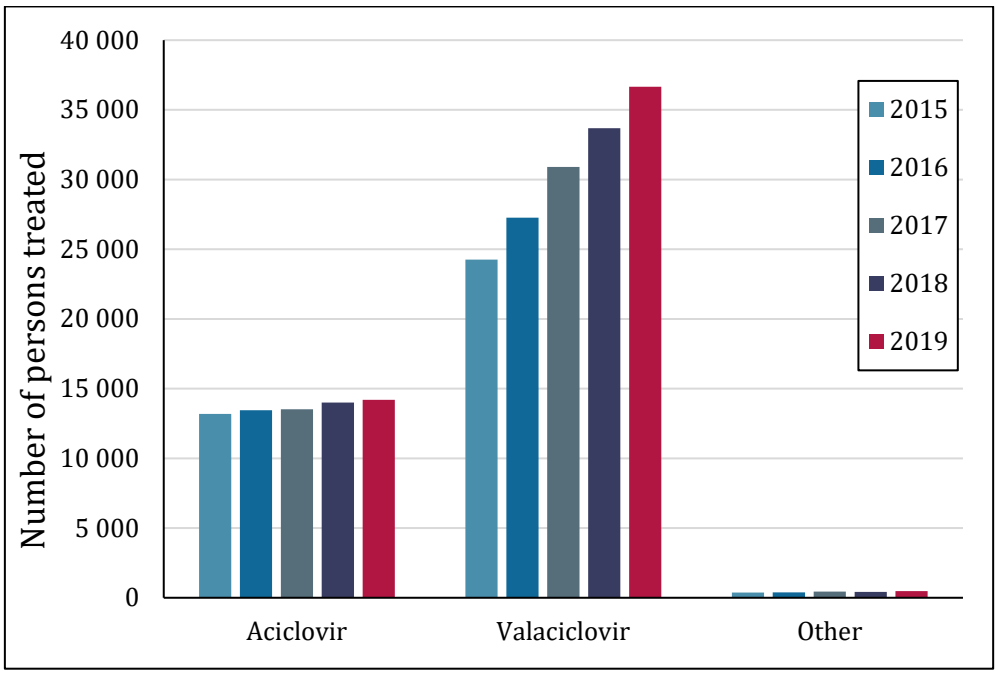

Figure 2.7 Trends in the use of antivirals for treatment of human herpes virus infection for the period 2015-2019.

This figure shows the trends in direct acting antiviral use for treatment of human herpesviruses over time. Number of persons treated is defined as the number of patients given at least one prescription per year. Other: vidarabine (J05AB03), ganciclovir (J05AB06), famciclovir (J05AB09) and valganciclovir (J05AB14).

Creams for topical treatment of herpes simplex virus infections of the lips and face (herpes labialis) are available in Norway. Aciclovir and penciclovir are the active ingredients in these creams. Small packages of aciclovir cream were made available for over the counter sales in 2006, and this resulted in a steep increase in use the next couple of years before the consumption stabilized. Since 2018, the use of a fixed combination of topical aciclovir and hydrocortisone has increased at the expense of aciclovir alone (Table 2.2).

Table 2.2 Sold packages of topical antivirals containing aciclovir (D06BB03), penciclovir (D06BB06) and aciclovir and hydrocortisone in combination ((D06BB53).

Active ingredient	2015	2016	2017	2018	2019
Aciclovir	201 829	206 447	205 818	212 393	180 880
Penciclovir	27 726	30 122	24 062	18 957	18 664
Aciclovir, combinations				21 794	40 618

Most packages contain 2 g of cream; the exception is a 5 g package with aciclovir as the active ingredient where prescription is needed. Approximately 90 % is nonprescribed medications.

Hepatitis C virus

The overall number of patients treated with DAAs against HCV has increased steadily since the new HCV antivirals became available in 2015. The number of persons treated with HCV antivirals increased from 2016 to 2018, but in 2019 the number of persons treated was again reduced. The number of persons who received at least one prescription for an HCV drug (except interferons) was 2248 in 2019, a reduction by almost 30% from 2018. In 2018, new, improved DAAs became available, and there was a subsequent surge in patients treated. Thus, the reduction in 2019 does not necessarily represent a general reduction in HCV prevalence or in patients eligible for treatment, but may rather be a result of that patients had been waiting for better treatment options and were finally treated in 2018.

Fixed combinations of two or more active ingredients in 2019 almost completely replaced single component drugs as shown in Figure 2.8. In 2019, 98% of the patients treated for HCV used fixed combination drugs, and ribavirin was the only single component drug still used to some extent. The first fixed combinations were introduced in 2015.

Recommended treatment protocols for HCV-infection depend on both genotype and stage of liver disease. Norwegian treatment guidelines for HCV from the Norwegian Medical Association (NMA) was updated in 2019 (6). However, the recommendations from the Procurement services for Health Enterprises Ltd probably also have had a considerable impact on the choice of drugs for treatment of HCV (7). These recommendations are similar but not identical to the NMA guidelines.

The combination of the NS5B inhibitor sofosbuvir (SOF) and the NS5A inhibitor velpatasvir (VEL) is the most used drug in 2019. This was one of the combination therapies recommended by the Procurement services for Health Enterprises Ltd in 2019 and is listed as the “recommended treatment” in genotype 3 HCV infections, one of the most common genotypes in Norway. SOF/VEL is one of the three pangenotypic fixed combinations with high treatment response. The others are the combination glecaprevir (protease inhibitor)/ pibretasvir (NS5A inhibitor), and the triple combination SOF/VEL/voxilaprevir (protease inhibitor), both introduced in 2018. The second most used combination in 2019 was the fixed dose combination of sofosbuvir and ledipasvir (NS5A inhibitor) (SOF/ ledipasvir). This was one of the combinations recommended by the 2019 procurement for treatment of most patients with HCV genotype 1, which is also commonly seen in Norway. The trends of use shown in Figure 2.8 probably reflect the change in national recommendations for treatment of HCV in the five-year period, and the results of the procurement the last few years.

The number of prescriptions per active ingredient for HCV drugs are given in Figure 2.9.

“The National strategy against hepatitis 2018-2023" has two primary objectives: To reduce the prevalence of HCV by 90% by the end of 2023, and that no one in Norway should die or suffer serious illness caused by HCV (8). The usage of DAAs is expected to change further in the coming years because of possible changes in treatment guidelines and reimbursement rules, new HCV medicines introduced to the market, and changes in the prevalence of infection in the population.

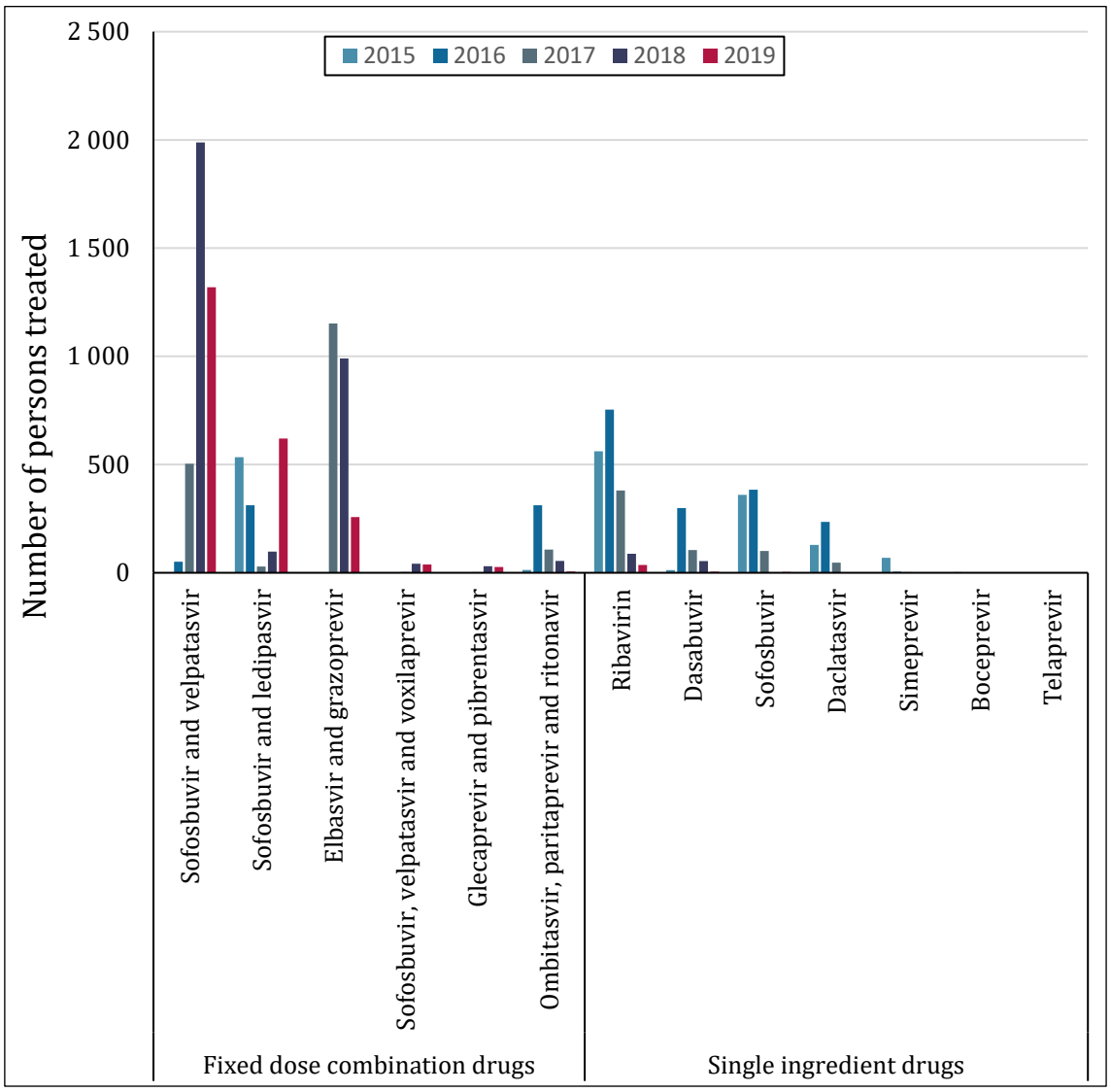

Figure 2.8 Trends in the use of antivirals for treatment of HCV for the period 2015-2019.

This figure shows the trends in the use of direct acting antivirals for treatment of HCV over time. The different drugs are separated by fixed dose combination drugs and single ingredient drugs. Number of persons treated is defined as the number of patients given at least one prescription per year.

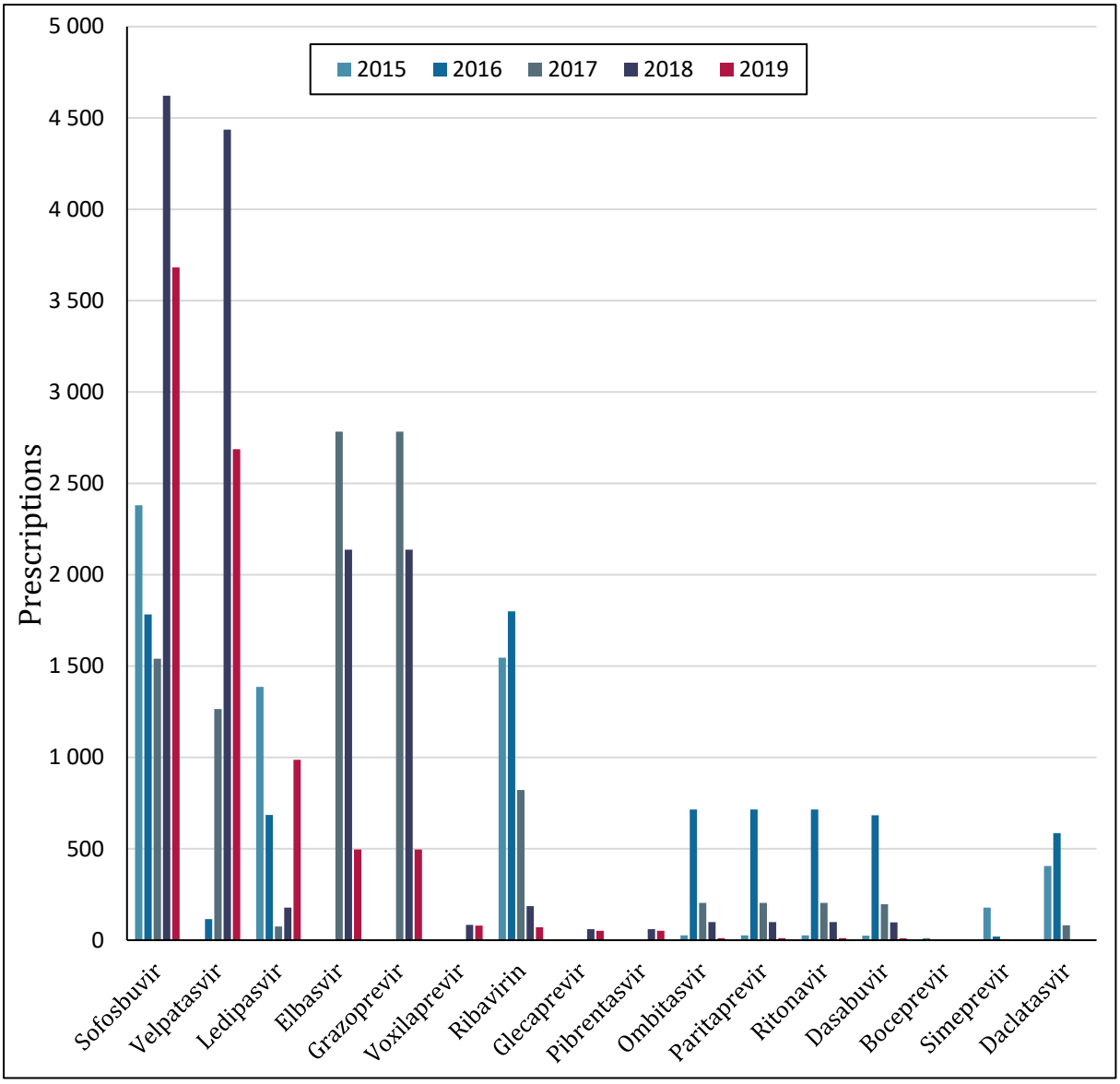

Figure 2.9 Number of prescriptions per active ingredient for HCV drugs

This figure shows number of prescriptions per active ingredient over time. Many prescriptions contain more than one active ingredient; these prescriptions are counted several times.

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3 Influenza virus

Fact box: Influenza virus drug resistance	
Treatment	Neuraminidase inhibitor: oseltamivir
Resistance testing method	Genotypic by pyrosequencing or Sanger sequencing Phenotypic by neuraminidase susceptibility assay (MUNANA) The WHO national reference laboratory for influenza, Norwegian Institute of Public Health (NIPH), performs influenza drug resistance testing in Norway
Target gene	Neuraminidase (Because adamantanes are not used in Norway, the matrix gene is currently not regularly screened for resistance.)
Indication for resistance testing	- Patients treated with antiviral drugs; with a particular focus on immunocompromised patients and young children as they often shed virus long-term, patients with severe or progressive illness who do not clinically improve, and patients with evidence of ongoing influenza virus replication through viral load monitoring. - Patients developing illness after or during antiviral chemoprophylaxis. - Patients infected after exposure to individuals receiving antiviral drugs. - Surveillance
Surveillance	Screening for resistance as part of the national influenza surveillance program, which involves samples from both untreated and treated patients. There is currently no active systematic surveillance for treatment-induced resistance.

Surveillance methods

The WHO national reference laboratory for influenza in Norway is located at the NIPH and monitors the occurrence of influenza viruses in Norway. A volunteer network of sentinel physicians in all parts of the country provide samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit a subset of confirmed influenza strains. Samples from both untreated and treated patients in the community are included. In order to facilitate detection of emergence and spread of viruses with resistance, there is a particular focus on samples from patients without known exposure to antiviral drugs.

Surveillance data influenza season 2019-20

Throughout the season (week 20 2019 to week 34 2020), 208 viruses have been analysed for resistance to the influenza antiviral drug oseltamivir and 134 for zanamivir. This represents in general 9% of all influenza positive specimens received by the influenza laboratory at NIPH for further analysis. No virus with resistance to neuraminidase inhibitors was detected this season. All circulating influenza virus groups are currently resistant to adamantanes, which are not used in treatment in Norway and most other countries. Therefore, NIPH has stopped testing routinely for adamantane resistance. Virus resistance to antiviral agents in Norway is reported by the WHO national reference

laboratory for influenza, NIPH via the Global Influenza Surveillance and Response system (1) and ECDC / WHO. The most recent observation of virus with reduced susceptibility to neuraminidase inhibitors in Norway was a double-deletion B/Victoria-lineage virus from August 2018, B/Norway/3241/2018, harboring the substitution D197N in the neuraminidase gene.

Table 3.1: Norwegian influenza viruses resistant to the neuraminidase inhibitors oseltamivir and zanamivir, during the influenza seasons 2005/06 through 2019/20 (sequences with resistance/total number of analysed sequences. Percentages > 0 are shown in parentheses).

Season	Oseltamivir resistance			Zanamivir resistance		
	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B
2005/06	0/6	0/13	0/21	0/6	0/13	0/21
2006/07	0/5	0/10	nd	0/5	0/10	nd
2007/08	184/272 (67.8%)	0/2	0/59	0/114	0/2	0/59
2008/09	33/33 (100%)	0/13	0/1	0/5	0/12	0/1
2009-pdmH1	0/884	nd	0/11	0/36	nd	0/9
2010/11	2/244 (0.82%)	0/1	0/30	0/2	0/1	0/24
2011/12	0/27	0/72	0/5	nd	0/60	0/4
2012/13	0/256	0/22	0/24	0/20	0/22	0/19
2013/14	0/183	0/43	0/27	0/32	0/43	0/27
2014/15	1/136 (0.74%)	0/169	0/92	0/136	0/166	0/92
2015/16	10/339 (3.0%)	0/32	0/50	0/106	0/31	0/48
2016/17	0/10	0/174	0/54	0/8	0/161	0/54
2017/18	0/120	0/66	1/42* (2.4%)	0/28	0/54	0/30
2018/19	0/247	0/108	0/26	0/82	0/107	0/26
2019/20	0/103	0/63	0/42	0/32	0/60	0/42

nd= not done

* Updated October 2019 due to post-season analysis. Differs from the data presented in the 2018 RAVN report.

Conclusion

Antiviral drug resistance towards influenza remains low in Norway. Global estimates indicate that approximately 0.5% of all viruses tested have reduced susceptibility towards neuraminidase inhibitors and this is expected to be similar for Europe (2). Continued monitoring is important both in samples from the community and in patients treated with antivirals.

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4 Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV) drug resistance	
Treatment	Antiretroviral treatment (ART) of HIV-infection is always given as a combination of drugs from at least two of the five different classes: - Nucleoside reverse transcriptase inhibitors (NRTI) - Non-nucleoside reverse transcriptase inhibitors (NNRTI) - Integrase strand transfer inhibitors (INSTI) - Protease inhibitors (PI) - Entry inhibitors (CCR5 antagonists and fusion inhibitors)
Resistance testing method	Genotypic assays based on Sanger sequencing of target genes, and identification of mutations associated with drug resistance. Plasma viral load > 500 copies/mL is usually required. In Norway, all HIV-1 drug resistance tests are performed at the National Reference laboratory for HIV at the Department of Microbiology at Oslo University Hospital, Ullevål.
Target genes	Reverse transcriptase Protease Integrase gp120 (envelope), V3 region (for CCR5 antagonist resistance testing)
Indication for resistance testing	Virological failure during antiviral treatment
Surveillance	The national surveillance program for HIV-1 monitor primary drug resistance against protease inhibitors (PI) and reverse transcriptase inhibitors (NNRTI and NRTI). Samples from all patients with newly diagnosed HIV-1 infections are tested for resistance mutations located in the protease and reverse transcriptase genes.

Surveillance methods

The Norwegian surveillance data is based on resistance testing of samples collected from newly diagnosed patients in Norway. Although some of these patients may be previously exposed to antiretroviral drugs, most are treatment naïve, and the data may serve as a marker of transmitted drug resistance. Starting from 2019, drug resistance data is being cross-referenced to epidemiological data from MSIS, enabling analysis of the prevalence of surveillance drug-resistance mutations (SDRMs) in different subgroups, such as risk groups or country of infection.

Although resistance testing is recommended for all newly diagnosed patients, not all are included in the surveillance system. This could be because the sample was not submitted or the patient was not identified as newly diagnosed on the referral form, or because the viral load was suppressed at the time of diagnosis, either due to treatment initiated before arrival to Norway, or for some other reason. Cases may also be lost if the MSIS report number is missing from the referral form. Since April 2019, new HIV infections are reported to MSIS with full patient identification, and inclusion in surveillance is based on information on the referral form.

The WHO recommends the use of a consensus genotypic definition of transmitted HIV-1 drug resistance to compare estimates of transmitted drug resistance rates across geographic regions, and over time (1;2). A standard list of SDRMs was published in 2009, based on a set of criteria to ensure that the mutations included are nonpolymorphic, are applicable to the most common subtypes, and do in fact contribute to resistance(1;2). The SDRM list is not designed for individual patient management as it excludes several clinically relevant drug resistance mutations, it may include certain mutations with less clinical relevance for current regimens, and the list has not been updated since 2009. The listed mutations are however robust markers of temporal trends in transmitted drug resistance. The monitoring in Norway is based on the WHO SDRM-list from 2009 and analysed using the Calibrated Population Resistance tool at Stanford HIV Drug Resistance Database (1-3).

There has been an increase in the use of integrase strand-transfer inhibitors (INSTIs) in first line regimens, but resistance mutations affecting these compounds are still rare in treatment naïve patients. Baseline testing of resistance to integrase inhibitors is therefore not yet recommended (4), and there is no surveillance of primary resistance to INSTIs in Norway.

Surveillance data 2019

A total of 107 samples from newly diagnosed cases of HIV-1 in Norway were analysed for primary HIV-1 drug resistance in 2019, which equals 62% of the 172 cases reported to MSIS in 2019 (5). Of the 107 cases with samples submitted for resistance testing, 28% were female and 72% were male. The percentage of samples from newly diagnosed patients tested for resistance has increased from 38% in 2010, to 62% in 2018 and 2019. The rates showing yearly coverage of resistance testing among newly diagnosed patients for the years 2010-2019 are shown in Figure 4.1.

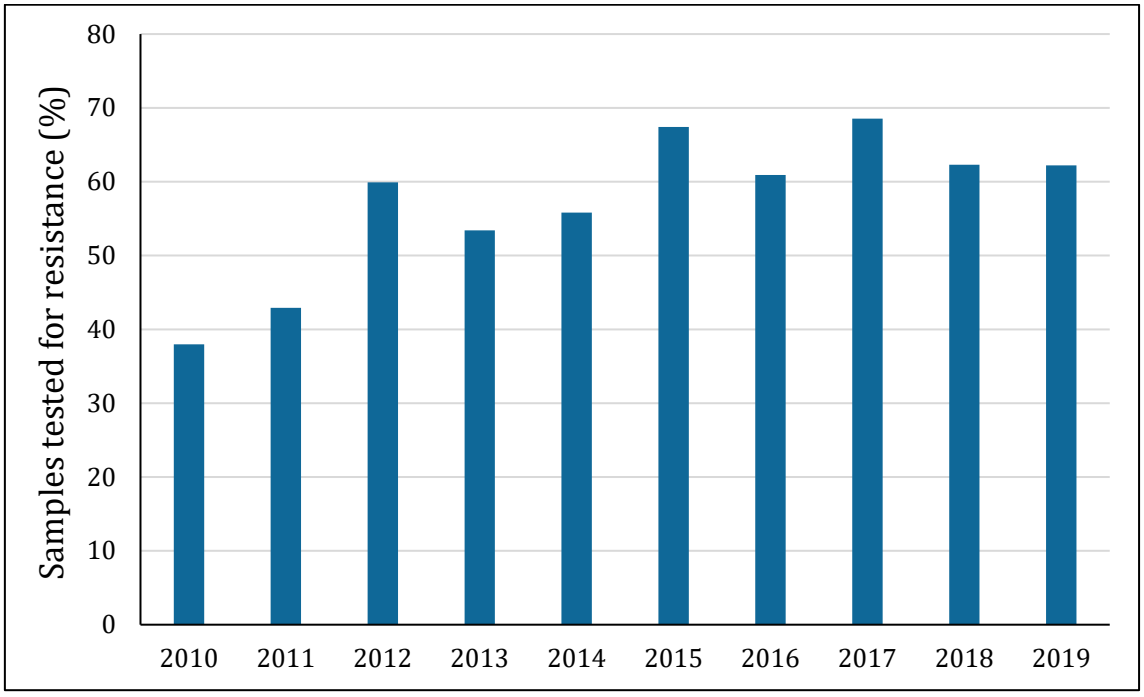


Figure 4.1: Samples tested for resistance (2010-2019).

Data shown as percentage of newly diagnosed cases of HIV-1 infection according to MSIS (6)

Information on the place and route of transmission for patients tested for drug resistance, was obtained by cross-referencing resistance data to epidemiological data from MSIS. Coverage of resistance testing among patients infected in Norway was around 90%, while it was only approximately 50% among those infected abroad. The latter group include persons residing in Norway that have been infected abroad, but also persons infected before arrival to Norway. Many of the patients infected before arrival may already be receiving treatment at the time of notification to MSIS, and thus, resistance testing cannot be performed due to suppressed viral load. Data is shown in table 4.1.

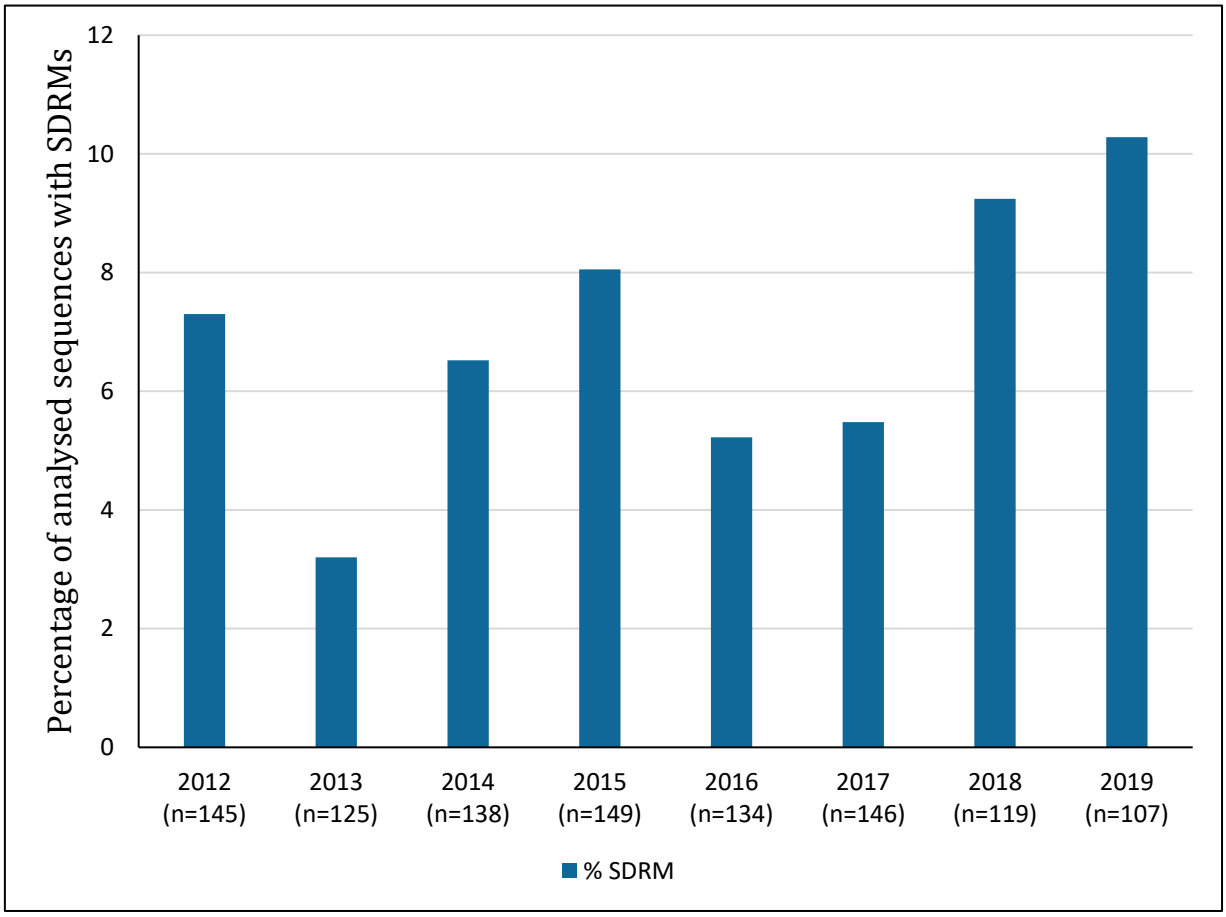
Table 4.2: Route of transmission in samples from newly diagnosed patients tested for resistance in 2019 compared to new cases reported to MSIS in 2019.

Route of transmission	Samples tested for resistance	Cases reported to MSIS
Heterosexual	**57**	**100**
infected in Norway	*7*	*7*
infected abroad	*49*	*92*
unknown	*1*	*1*
MSM	**36**	**61**
infected in Norway	*16*	*18*
infected abroad	*19*	*43*
unknown	*1*	
IDU	**5**	**8**
MTC		**2**
Unknown	**9**	**1**
Total	**107**	**172**

MSM: men who have sex with men; IDU: injection drug users; MTC: mother to child.

In 2019, SDRMs from the WHO list were detected in 10.3% of the analysed sequences. In total, SDRM were detected in 8 males and 3 females, corresponding to 10% of the analysed samples from both males and females, respectively. The frequencies of SDRMs are presented in Figure 4.2, showing the percentage of sequences with detected SDRMs during each year of surveillance. Of the analysed sequences, 3.7 % had SDRMs associated with non-nucleoside reverse transcriptase inhibitors (NNRTI), and 7.5 % with nucleoside reverse transcriptase inhibitors (NRTI), as shown in Figure 4.3. As in 2018, none of the sequences in 2019 had SDRMs associated with protease inhibitors (PI).

Figure 4.3: Percentage of analysed sequences with detected surveillance drug resistance mutations (SDRMs).



Percentages of the analysed sequences containing one or more SDRMs through the years 2012-2019 are shown as blue columns. There may be several SDRMs per sequence. n = number of sequences analysed for pre-treatment resistance.

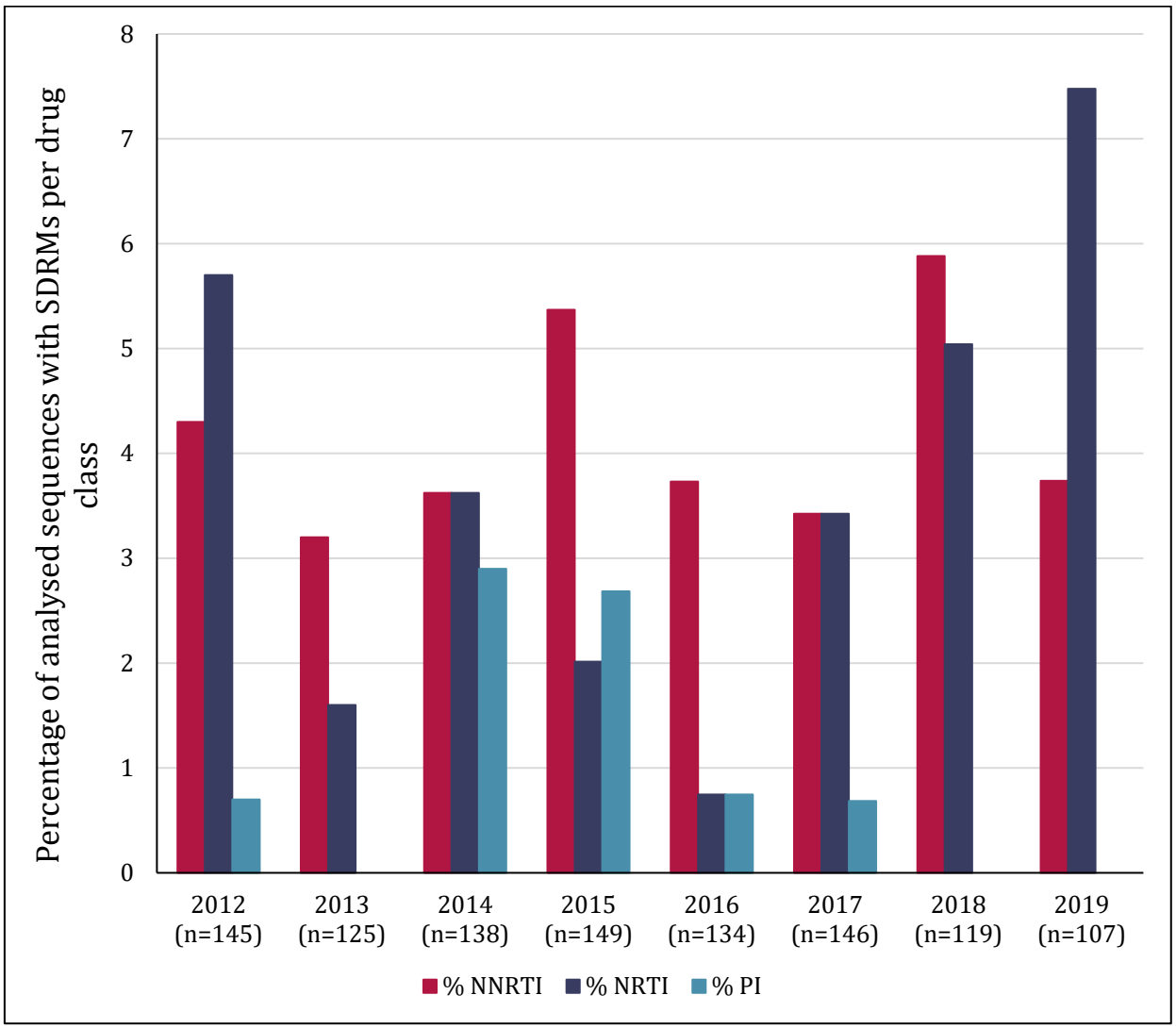


Figure 4.3: Percentage of analysed sequences with detected SDRMs per drug class.

Percentage of mutations affecting the individual drug classes are shown as colored bars; non-nucleoside reverse transcriptase inhibitors (NNRTI) in red, nucleoside reverse transcriptase inhibitors (NRTI) in dark blue, and protease inhibitors (PI) in light blue. n = number of sequences analysed for pre-treatment resistance.

The individual mutations are specified in Table 4.2, along with country of transmission and information on previous treatment exposure for the 11 patients with detected SDRM. All patients with detected SDRM were treatment naïve. Only two (20%) were infected in Norway, eight (73%) were infected abroad, and for one patient (9%), the country of transmission was unknown. Out of the 11 sequences, only four had mutations that were of clinical significance (G190A, K103N, M184V/Y181C). Among these four, one MSM was infected in Asia, and the remaining cases were infected in Africa through heterosexual transmission.

Table 4.2: Specification of the surveillance drug resistance mutations (SDRMs) detected in 2019.

Sequence ID	NRTI	NNRTI	PI	Country of transmission	Previous treatment
1	None	G190A	None	Abroad	No
2	None	K103N	None	Abroad	No
3	None	K103N	None	Abroad	No
4	M184V	Y181C	None	Abroad	No
5	V75M	None	None	Abroad	No
6	M41L	None	None	Norway	No
7	M41L	None	None	Abroad	No
8	M41L	None	None	Abroad	No
9	M41L	None	None	Norway	No
10	M41L	None	None	Abroad	No
11	M41L	None	None	Unknown	No

SDRM: surveillance drug resistance mutations; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; PI: protease inhibitor.

Discussion

As for previous years, the surveillance is based on resistance data from patients who had their HIV-1 infection confirmed in Norway, and where a sample was sent to the National reference laboratory for HIV at Oslo University Hospital (OUH) for resistance testing. For the first time, the data reported for 2019 have been cross-referenced to epidemiological data from MSIS. This is achieved through a collaboration between the NIPH and the National reference laboratory for HIV at OUH, and it enables a detailed analysis of transmitted drug resistance in Norway by studying the prevalence of SDRMs in different subgroups, such as risk groups or country of infection. This also provides useful information on the coverage of primary resistance testing in the different subgroups.

Over the last few years, RAVN together with the National reference laboratory for HIV, have made efforts to increase the coverage of resistance testing among newly diagnosed HIV patients. In 2019, resistance data was available for 62% of the newly diagnosed patients reported to MSIS. However, the MSIS-data also includes patients that will never be included in the resistance data, such as patients already receiving treatment when they are diagnosed in Norway, or persons only temporarily residing in Norway. These are all patients that were most likely infected abroad before arrival to Norway. The epidemiological data from MSIS (5) showed that almost 60% of the cases reported to MSIS (102 out of 172) were immigrants infected before arrival to Norway. Corresponding numbers are not available for cases reported to RAVN, but the low coverage of primary resistance testing among patients infected abroad (50%), probably reflects that many of these patients were already receiving effective treatment. Coverage of resistance testing was high among patients infected in Norway (92%), indicating adequate local routines for submitting samples for resistance testing in newly infected patients. Data on patients infected abroad while residing in Norway is not available, but we may assume that local routines for submitting samples for drug resistance testing in this group do not differ significantly from those infected in Norway. Altogether, these findings suggest that we are able to include the majority of eligible cases through the current surveillance system for drug resistance.

Both the total number of new HIV-infections in Norway, and the number of samples analysed for primary drug resistance, were lower in 2019 compared to 2018, and considerably lower than for 2017. SDRMs were detected in 10.3% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2019, while the corresponding numbers for 2018 and 2017 were 9.2% and 5.6%, respectively. Thus, the increasing trend observed in 2018 seem to continue also in 2019. However, mutations associated with clinically relevant drug resistance were only detected in samples from four patients, all of them infected abroad. The increase observed is mainly due to the presence of a single M41L mutation, a mutation that is known to be commonly transmitted. As a single mutation, M41L does not confer clinical resistance to NRTI, and its presence does not seem to influence development of resistance (7). Given that only two of the cases with detected SDRM were infected in Norway, the data from 2019 indicates that transmission of drug resistance in Norway is low. However, data cannot be compared with previous years, as this information was not available until 2019.

Since pre-exposure prophylaxis (PrEP) with tenofovir and emtricitabine was implemented in Norway in 2017, an enhanced surveillance of the mutations associated with reduced susceptibility for the two drugs used for PrEP is warranted. In 2019, one patient had a mutation associated with reduced susceptibility for emtricitabine (M184V), and this patient was infected abroad. For the rest of the cases, both tenofovir and emtricitabine would be effective, and the infections could potentially have been prevented by correct use of PrEP. So far there are no signs of an increase in drug resistance associated with PrEP among patients newly diagnosed with HIV in Norway, and PrEP can be expected to be effective in preventing most new cases. However, continued monitoring of possible PrEP-related resistance will be of importance.

Conclusions

Most of newly diagnosed patients with detected surveillance drug-resistance in 2019 were infected abroad. Among patients infected in Norway, there was no transmitted resistance against any of the drugs currently used for treatment of HIV-1 infection. Furthermore, no transmission of PrEP-associated resistance mutations was detected, even after two years with widespread use of PrEP. Continued surveillance of HIV-1 resistance over time is important in order to make informed decisions on implementation of preventive measures to control dissemination of resistant HIV-1 strains.

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5 Hepatitis B virus

Fact box: Hepatitis B virus (HBV) drug resistance	
Treatment	Treatment of HBV infection with antivirals is generally given as monotherapy: - Nucleoside/nucleotide analogues, usually entecavir, tenofovir disoproxil, or tenofovir alafenamide
Resistance testing method	Genotypic assays based on amplification by PCR and Sanger sequencing of the product. The sequences are analysed for amino acid mutations associated with drug resistance using geno2pheno (version 2.0) resistance database (1) from Max Planck Institute of Informatics. A plasma viral load > 1000 IU/mL is required for the analysis. In Norway, all HBV drug resistance tests are performed at the Norwegian Institute of Public Health.
Target gene	Polymerase gene
Indication for resistance testing	Virological failure/breakthrough on antiviral treatment.
Surveillance	Surveillance of both treatment experienced and treatment naïve patients: 1) Monitoring of patients with virological failure (samples submitted for resistance testing) 2) Population-level surveillance in treatment naive patients (samples submitted for genotyping)

Surveillance method

The surveillance of HBV resistance in Norway aims to monitor two populations; 1) patients that have been tested for drug resistance primarily in relation to treatment (acquired resistance) and 2) patients that are genotyped for HBV as part of diagnostic investigations, generally before treatment. Monitoring of the latter population can therefore be regarded as surveillance of primary resistance. Mutations altering specific amino acid positions within the polymerase gene can give rise to resistance to the various antivirals for the treatment of HBV.

Surveillance data 2019

The resistance mutations detected in Norway between 2015 and 2019 are presented in Table 5.1.

Table 5.1: Resistance mutations in samples submitted for HBV drug resistance testing in 2012-19.

HBV-variants resistant to antivirals	Drug resistance	2015	2016	2017	2018	2019
Total analysed		10	23	23	20	14
M204I	**LAM (R), ETV (I), ADV (I)**	1	1			
L180M + M204I/V	**LAM (R), ETV (I), ADV (I)**		1	1		
L180M + M204V/I ± S202I/G/S ± T184G/A	**LAM (R), LDT (R), ETV (R)**		2	1	3	1
L180M + M204V ± I169T ±V173L ± M250V	**LAM (R), LDT (R), ETV (R)**		1			
I169T + L180M + T184A + M204V	**LAM (R), LDT (R), ETV (R)**				1	
Uncharacterized mutation*			1[a]		1[b]	1[b]
Percentage of samples with drug resistance		10 %	22 %	9 %	20 %	7 %

LAM: lamivudine; TBV: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate ; TAF: tenofovir alafenamide; R: resistant; I: Intermediate, S: sensitive.
*Uncharacterized mutation: new mutation of undetermined significance in a position associated with major resistance. [a] N236D, [b] A181S.

In 2019, a total of 217 samples were analysed for HBV drug resistance mutations. Of these, 14 patient samples were submitted for HBV drug resistance testing, and 203 samples were submitted for HBV genotyping. Drug resistance was detected in only one sample (Table 5.2) from a patient on entecavir treatment.

No drug resistance mutations were detected in patient samples submitted for HBV-genotyping (N=203) only.

Table 5.2: Resistance mutations detected in samples from 2019 and the drug resistance they confer

Sample	Resistance mutations detected	Treatment*	Resistance				
			LAM	LDT	ETV	ADV	TDF/TAF
1	L180M + M204V + S202G/S	ETV	R	R	R	S	S

LAM: lamivudine; LDT: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate ; TAF: tenofovir alafenamide; R: resistant; S: sensitive.
*Treatment specified at the time of resistance testing.

Conclusion

Entecavir resistance mutations were detected in only one patient of 14 samples tested. Tenofovir and entecavir are the first-line drugs for treatment of HBV, and their use has increased 2-3-fold from 2015 to 2019, although the use of entecavir declined slightly in 2019. Among the few resistance mutations we have detected in recent years, all are directed against entecavir rather than tenofovir even though tenofovir is the primary drug of choice with more than 900 prescriptions in 2019. Based on our data, HBV drug resistance seems to be a minor problem in Norway.

6 Human herpes viruses

Surveillance of cytomegalovirus drug resistance

Fact box: Human cytomegalovirus (CMV) drug resistance	
Treatment	Nucleoside/nucleotide analogues: ganciclovir/valganciclovir (first choice), cidofovir and foscarnet (second choice)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analysed for amino acid substitutions associated with drug resistance. In Norway, all CMV drug resistance tests are performed at the National Reference laboratory for CMV at the Department of Microbiology at the Oslo University Hospital, Rikshospitalet.
Target genes	CMV kinase (UL97) and DNA polymerase (UL54)
Indication for resistance testing	Persistent high viral load in blood or other compartments during antiviral treatment.
Surveillance	Population-level surveillance is currently not necessary.

Surveillance method

The antiviral drug resistance has been characterized by comparing phenotypic and genotypic test results. For routine testing only genotypic tests, looking for known resistance mutations, are applicable. Resistance to ganciclovir develops by mutations in the viral kinase CMV UL97-and/or the DNA polymerase CMV UL54 gene. Normally resistance mutations in the CMV UL97 gene precede mutations in the CMV UL54 gene, as ganciclovir is first choice of treatment, and the fitness cost of mutations in CMV UL54 is higher. Foscarnet and cidofovir resistance is conferred by mutations in the UL54 gene.

There is no population level surveillance of CMV drug resistance, and the surveillance is based on samples from patients with suspected resistance, usually due to persistent high viral load despite ongoing therapy. Immunocompromised patients are more prone to develop drug resistance. Resistance mutations usually develop after several weeks of treatment, and thus resistance testing is usually relevant in treatment failure only after at least 2-3 weeks of treatment or in patients that have previously received prophylaxis or treatment.

Surveillance data 2019

In 2019, 21 specimens from 18 patients were received for genotypic analysis of CMV resistance mutations. One specimen could not be analysed due to inhibitors in the sample material. Among the 20 other samples, CMV resistance mutations were recorded in six samples (Table 6.1) from six different patients. The mutations detected are listed in Table 6.2.

Table 6.1: Number of samples analysed for CMV antiviral drug resistance and number of samples with detected CMV drug resistance mutations for the years 2016 - 2019.

CMV-variants resistant to antivirals	2016	2017	2018	2019
Total samples analysed	28	32	21	21
Number of samples with CMV resistance mutations	8	7	4	6
Samples with UL97 mutations	8	7	2	6
Samples with UL54 mutations	2	1	2	2

Table 6.2: CMV resistance mutations in samples tested in 2019

Patient	UL97 mutations	UL54 mutations
1	**A594V**[1]	
2	**M460I + H520Q**[1]	
3, 4	**L959S**[1]	
5	**L959W**[1]	**K513N + D588N**[2]
6	**A594V**[1]	**L545S + P522S**[2]

UL97 encodes the viral kinase. UL54 encodes the viral DNA polymerase.
[1] Ganciclovir moderate resistance.
[2] Foscarnet/cidofovir moderate resistance

Conclusion

Despite an increase in the use of ganciclovir for therapeutic and prophylactic treatment of CMV-infections, drug resistance mutations are only rarely detected. However, there is no systematic resistance surveillance of CMV drug resistance, and the true prevalence of drug resistant CMV in Norway is therefore unknown. The reference laboratory encourages clinicians and laboratories to remember to consider drug resistance testing in cases with treatment failure.

Cytomegalovirus – new drugs and resistance against them

Grete Birkeland Kro

For many years the available treatment and prophylaxis against CMV has consisted of three drugs ganciclovir/valganciclovir, cidofovir, and foscavir. All of these drugs share a single target; the viral DNA polymerase (1). Therefore, many mutations that confer resistance against one drug also give some degree of resistance to the other drugs. Ganciclovir, foscavir, and cidofovir can only be given intravenously, while valganciclovir is an oral formulation. Foscavir and cidofovir are realtively toxic and cause a wide spectrum of side effects, including nephrotoxicity and neutropenia. They are therefore second-line agents. Ganciclovir and valganciclovir are usually better tolerated, but myelosuppression is frequently seen. All three drugs are associated with neutropenia and thus prophylactic use has not been recommended in stem cell recipients, leaving this group without effective CMV-prophylactic options (2).
Therefore, there is a need for medications with a different target and medications with less adverse effects, in particular less myelosuppression. Recently, a new antiviral drug with a different target became available; letermovir (3). Other alternatives in the pipeline include maribavir and brincidofovir.

Letermovir

Letermovir has a completely different target from the established drugs, as it targets the CMV terminase complex. The terminase complex is responsible for cleavage of freshly replicated concatameric viral DNA into individual genomic subunits and packaging them into the developing viral capsids. Inhibition of the terminase results in the formation of noninfectious viral particles. The genes UL56, UL89 and UL51 code for the three parts that comprise the terminase complex. The effect is specific for CMV, and thus letermovir has no known activity against the other herpesviruses (4).

Although letermovir has only recently been introduced, mechanisms of resistance and multiple resistance mutations have already been characterized (5;6). Resistance is mainly conferred by mutations in the UL56 gene, but mutations in UL89 and UL51 can confer low-grade resistance (5).

In general, viral mutations that confer resistance often reduce the fitness of the virus. An unfortunate property of letermovir resistance is that several different single mutations in UL56 codon 325 lead to absolute letermovir resistance (EC50>3000-fold), but with little loss of viral fitness. This suggests that sites critical for letermovir binding to the terminase complex are not important for biological activity (6).

Letermovir seems to have a lower genetic barrier to resistance than the traditional CMV drugs. In a study by Chou (6), mutations leading to resistance against letermovir were detected in cell culture at a median of three rounds of drug exposure, whereas mutations associated with foscavir resistance were detected at a median of 15 rounds. Furthermore, CMV exposed to letermovir showed a higher number of resistance mutations per experiment compared to the other drugs. Thus, the *in vitro* data indicate that resistance against letermovir may appear earlier than with the DNA polymerase inhibitors. In clinical studies of letermovir and in clinical use, the dominant mutations have been in the C325 position of UL56, conferring complete resistance. It has been suggested that this is because CMV with low grade mutations are suppressed by standard letermovir dosage and is thus not detected.

As letermovir has a different target than the other CMV antivirals, there is no cross resistance. The drug is available in both oral and intravenous formulations. Clinical studies have found that side effects are uncommon. The main side effects include gastritis, nausea, dyspnea and hepatitis (7). Importantly, letermovir has not been associated with myelosuppression.

Letermovir was approved for CMV prophylaxis in CMV seropositive stem cell recipients by the US Food and Drug Administration in November 2017. Data from the Letermovir trial (3) indicates that letermovir may also be effective for patients with active CMV viremia. However, there are no randomized controlled clinical trials designed to investigate treatment of viremia or CMV disease. Letermovir is currently not approved for any clinical indication in solid organ transplant recipients, but there is an ongoing trial on the use of letermovir for CMV prophylaxis in CMV seronegative kidney transplant recipients. The trial compares prophylaxis with letermovir and aciclovir to valganciclovir for 28 weeks and estimated study completion is September 2021.

Brincidofovir

Brincidofovir is an oral lipid formulation of cidofovir. It was designed to improve the bioavailability of cidofovir, but the new formulation also reduces the uptake in the kidney and thereby reduces the nephrotoxicity that is the main problem with cidofovir (8). Brincidofovir also shows activity *in vitro* against many double stranded DNA-viruses such as other herpesviruses, adenoviruses and polyomaviruses.

The drug has been under clinical evaluation (9), and a phase 2 trial in stem cell recipients showed promising results. However, in the following phase 3 study the primary endpoints were not met. Brincidofovir did reduce viremia and the need for preemptive therapy, but showed increased gastrointestinal toxicity, including graft versus host disease, and there was a trend towards higher all-cause mortality (10). An intravenous formulation has been suggested to avoid the gastrointestinal accumulation.

There is limited data available on resistance against brincidofovir *in vivo*, but based on the clinical trials and *in vitro* experiments rapid emergence of resistant mutants is not expected (11). The pattern of resistance is expected to be the same for brincidofovir as for cidofovir. However, intracellular active drug concentrations are higher for brincidofovir and thus mutants with higher levels of drug resistance at the cost of reduced fitness could be positively selected.

Maribavir

Maribavir works through competitive binding to ATP-binding sites on UL97 kinase resulting in a specific inhibition of autophosphorylation of the UL97 kinase. This affects viral replication through mechanisms that are not fully understood, but defects in encapsidation, nuclear egress, or phosphorylation of replication related proteins have been suggested (12). However, the reduced UL97 kinase activity can be partly compensated by host cell kinases. Thus, the effect of maribavir is dependent on the host cell conditions, and in cell culture it has been shown that the inhibitory effect of maribavir can vary 100-fold for a single strain (13).

Mutations related to resistance have been found to appear earlier (median 5 passages) than with foscavir (median 15 passages) (6). Mutations in UL97 that map to ATP-binding sites have been observed in patients with maribavir treatment failure (i.e. T409M and

H411Y), while other mutations have only been observed in cell culture (5). The level of drug resistance ranges from < 2.2 to >100-fold increase in EC50. The most frequently observed clinical mutations confer 9-90-fold increase of EC50. With some exceptions, there is no cross-resistance between maribavir and ganciclovir, but maribavir strongly antagonize the action of ganciclovir by interfering with the initial phosphorylation step (5;14).

The drug is normally well tolerated; the main adverse effects are gastrointestinal, with distortion of the sense of taste (dysgeusia) being the most frequent. Maribavir has not been found to induce myelosuppression (15). Maribavir is in the late stage of therapeutic trials and has shown similar efficacy to ganciclovir in treatment of viremia in clinical studies (15;16).

Conclusion

Letermovir is approved for prophylaxis in stem cell recipients. It may potentially be used for other groups as well, and also for treatment of viremia. However, due to its low genetic barrier, awareness of development of resistance will be of importance. The use of brincidofovir seems to be limited by side-effects. Maribavir has a variable effect on cellular level and a lower genetic barrier, but does not induce myelosuppression, and thus has potential for use particularly in groups where ganciclovir is contraindicated. In conclusion, although the new antiviral drugs against CMV suffer from different limitations, they will be useful supplements covering some of the shortcomings of existing treatment.

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Surveillance of herpes simplex virus drug resistance

Fact box: Herpes simplex virus (HSV) drug resistance	
Treatment	- Nucleoside/nucleotide analogues: aciclovir/valaciclovir (first choice), cidofovir and foscarnet (second choice)
Resistance testing method	Genotypic assays based on Sanger sequencing. The sequences are analysed for amino acid mutations associated with drug resistance. All HSV drug resistance tests for Norway are performed at Sahlgrenska University Hospital, Gothenburg
Target gene	HSV thymidine kinase (UL23) and HSV DNA polymerase (UL30)
Indication for resistance testing	Persistent HSV-infection despite ongoing therapy
Surveillance	Population-level surveillance is currently not necessary

Surveillance method

The surveillance is based on samples from patients with persistent HSV-infection despite ongoing therapy. There is no population level surveillance of HSV resistance. Immunocompromised patients are more prone to development of drug resistance, but information on the patients' immune status is not available for surveillance purposes. For routine testing, only genotypic tests are applicable.

Surveillance data 2019

In 2019, 12 samples from 11 patients from Norway were submitted for HSV resistance analysis. Due to insufficient amounts of HSV DNA, two samples could not be analysed. In the 10 remaining specimens, two known resistance mutations were recorded in two samples, and a possible resistance mutation was detected in a third sample, see Table 6.3.

Table 6.3: HSV resistance associated mutations

Sample	HSV-type	Sample material	TK mutations	DNA pol mutations	Aciclovir susceptibility
1	HSV1	Secretion	R222H		Resistant
2	HSV1	Plasma		G841A	Possibly resistant/Unknown
3	HSV2	Secretion	G201D		Resistant

Of the two mutations found in the thymidine kinase gene, the G201D mutation is associated with resistance to aciclovir, whereas the R222H mutation was shown to confer resistance to both aciclovir and penciclovir. The third sample had a G841A mutation within the DNA polymerase gene. Previous studies have been unable to determine the significance of the substitution G841A on treatment resistance, however other amino acid changes at this position (e.g. G841S, G841C) have been shown to confer aciclovir and foscarnet resistance (1). Penciclovir resistance was not relevant in this case, as the mutation was detected in a plasma sample.

Conclusion

The consumption of aciclovir for both therapeutic and prophylactic treatment has increased during the past five years. However, treatment failure is rare, and few samples are submitted for resistance testing. Thus, resistance to aciclovir appears to be uncommon, but the data are scarce and there is no systematic surveillance of drug resistant herpes simplex virus.

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7 Hepatitis C virus

Fact box: Hepatitis C virus (HCV) drug resistance	
Treatment	Antiviral treatment of HCV-infection consists of a combination of drugs from at least two of the four different classes: - Nucleoside analogue polymerase inhibitors (NS5B) - Non-nucleoside analogue polymerase inhibitors (NS5B) - Protease inhibitors (NS3/4A) - NS5A inhibitors Direct acting antivirals may be supplemented with ribavirin. Treatment protocols depend on genotype and stage of liver disease.
Resistance testing method	Sequencing of relevant genes and/or the complete HCV genome Routine resistance testing is currently not available in Norway, but a next generation sequencing-based method is under development at the Norwegian Institute of Public Health.
Target genes	NS3–NS4A (protease) NS5A (replication and assembly factor) NS5B (polymerase)
Indication for resistance testing	Virological failure during treatment Baseline testing of patients with HCV genotype 1a and high viral load (>800 000 IU/ml) considered for treatment with elbasvir + grazoprevir Baseline testing of cirrhotic genotype 3 patients
Surveillance	Currently no surveillance. Will include resistance testing from clinical routine samples or surveillance projects.

Launch of a national HCV surveillance program in Norway

Kathrine Stene-Johansen, Rikard Rykkvin, Anne-Marte Bakken Kran

The WHO has declared a goal of eliminating viral hepatitis as a major public health threat by 2030. In response to this, the Norwegian government has published an ambitious national strategy against hepatitis (2018-2023). The strategy aims to reduce the prevalence of HCV in Norway by 90% by the end of 2023, and states that nobody should die or fall severely ill from HCV after 2023 (1). To achieve this goal, the Norwegian Directorate of Health organized a plan for implementation and preparation of national guidelines (2). The novel approach in the strategy is the attempt at using treatment as prevention (TasP), which means treating HCV-infected patients to reduce the risk of further transmission. Key recommendations in the guidelines include offering tests to all persons at risk, and to offer treatment to all patients with chronic HCV-infection. Thus, treatment with antiviral drugs against HCV is a cornerstone of the implementation of these guidelines. The WHO has in their guidelines for treatment of persons with HCV, emphasized that there is a need for surveillance of adverse events and drug resistance with the transitioning from clinical prioritization to a “Treat All” approach (3).

With current treatment, the majority of patients are cured, but not all patients obtain sustained virological response. This makes monitoring of treatment important. At present, treatment failures are only rarely caused by the presence of resistance-associated substitutions (RAS). However, an upscaling of antiviral treatment of HCV-infections could lead to changes in the prevalence of RAS that may have clinical consequences and therefore may affect recommendations for first-line treatment (4). Systematic national surveillance of HCV drug resistance is lacking in most European countries, and the majority of data stems from a few sets of studies restricted to specific regions or countries.

The reference laboratory for HCV at NIPH, has recently established a method for HCV whole genome analysis. The method is based on next generation sequencing with Illumina MiSeq technology, and can detect all recognized RAS in all target genes, as well as HCV genotypes and HCV co-infections with different genotypes, all in one analysis. For high quality deep sequencing of the complete genome including detection of all RAS, a viral load of > 50 - 100 000 IU/ml is required.

In the clinical management of individual patients with HCV-infection, resistance testing in patients failing treatment as well as analyses of pretreatment RAS that may affect the outcome of treatment, will be of importance. However, the implementation of nationwide surveillance of HCV drug resistance is needed in order to monitor whether an upscaling of antiviral treatment against HCV will have an effect on drug resistance and to tailor national treatment-recommendations. The new analysis at NIPH enables the implementation of a surveillance program for baseline resistance, because a high number of viral strains can be effectively analysed. The number of newly diagnosed patients in Norway was 769 cases in 2016 and was reduced to 532 cases in 2019 (5). The number is expected to decline further over the next years with continued implementation of the national strategy.

The surveillance program will be launched in January 2021, aiming at a continuous surveillance of the prevalence of RAS among newly diagnosed patients similar to the existing surveillance program for HIV drug resistance. This will require that when patients are diagnosed with HCV and notification is sent to MSIS, a sample should be sent to the HCV reference laboratory at the NIPH for sequencing and resistance analysis. The reference laboratory at NIPH will organize the program in collaboration with RAVN.

The sequence data will provide information on baseline resistance, genotype, and other genetic characteristics of circulating HCV on a national basis. In a surveillance project, data from national health registers will be combined with HCV sequence data to understand transmission patterns and spread of RAS, and patterns will be further characterized by molecular epidemiology analyses, providing new insight within this field. The project is approved by the regional ethics committee and will provide data on the prevalence of RAS using the state-of-the-art technology. Furthermore, a national laboratory database is currently being implemented, providing data on all newly diagnosed HCV infections, including genotype distribution. Combining data from this database with a systematic national surveillance of RAS, will provide an excellent overview of the HCV situation in Norway, and could be an important tool for monitoring the implementation of the ambitious Norwegian strategy to eliminate HCV-related disease.

A national surveillance program for HCV drug-resistance will contribute towards WHO's target for elimination of HCV-related disease and the sustainable development goal on combatting viral hepatitis by 2030. Furthermore, the surveillance program may in turn inform guidelines for resistance testing and provide a tool for monitoring and adjusting the Norwegian treatment recommendations.

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8 SARS-CoV-2

Future perspectives on drug resistance development in SARS-CoV-2

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At present, there is no antiviral treatment with documented effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This chapter will discuss how the existing knowledge of antiviral drug resistance can be employed to assess the risk of drug resistance against a future antiviral therapeutic treatment for SARS-CoV-2, and is partly based on a lecture held in June 2020 at The Norwegian Academy of Science and Letters (1).

The emergence and rapid spread of new viruses with the potential to cause serious illness in humans, elicits an urgent need for effective antiviral treatment. Characterizing a new virus, including its genome and replication cycle, and further identifying potential drug targets is complicated and time consuming. Moreover, drug development, from *in vitro* screening, to animal models and ultimately to clinical trials, is even more complicated and time consuming. A practical and swift approach is to re-purpose currently available drugs to investigate whether they can have an effect on the new virus (2). Several *in vitro* studies have been conducted with different available antivirals in the pursuit of finding an effective antiviral treatment against SARS-CoV-2 (3). The majority of the compounds tested are antivirals currently being used to treat infections caused by other RNA viruses, but therapeutic agents against other diseases, including cancer drugs and antimalarials have also been explored.

Not surprisingly, antivirals that inhibit RNA synthesis, such as nucleotide analogues, have been shown to be the most effective agents against SARS-CoV-2 *in vitro* and are currently being tested in clinical trials. Remdesivir, a nucleotide analogue which was originally developed for Ebola, seems to be the most promising drug so far (4). Remdesivir is the first antiviral drug to be approved for treatment of covid-19. Other nucleotide analogues such as favipiravir also seem to have some effect on SARS-CoV-2, whereas drugs targeting viral enzymes specific to a particular virus have not shown to have any effect, e.g. inhibitors of the influenza neuraminidase, or non-nucleoside inhibitors of the HIV reverse transcriptase.

It is likely that an effective antiviral treatment against SARS-CoV-2 will be found, either through the use of already existing drugs, or through development of new compounds. Subsequently, the potential risk of developing resistance towards this agent will become an issue. Development of drug resistance depends on a number of factors such as the mutation rate of the virus, the viral load, the treatment duration, and the selection pressure exerted by the drug in question. Increased risk of antiviral resistance is linked to high mutation- and recombination rates, prolonged treatment, and the presence of selective forces and factors that facilitate continued replication in the presence of the drug. For example, HIV has an extremely high mutation rate and requires life-long treatment. The high mutation rate helps HIV to adapt and compensate for loss of functions. The combination of extreme adaptability and long treatment duration elevates the risk of resistance. At the other end of the spectrum is herpes simplex virus, a DNA virus with a low mutation rate and, usually, a short treatment duration, resulting in a low risk of resistance. In the middle of the spectrum is influenza A virus. Influenza A has a high mutation rate, however the recommended treatment duration is only three days, and thus, the duration of selection pressure is short. The ability of a mutated virus to replicate

varies, and in certain seasons a resistant variant with a greater potential of infection and spread can emerge.

With regards to SARS-CoV-2's risk of resistance, there are many unknown factors. Studies so far have suggested a low mutation rate for SARS-CoV-2 (5;6), and major changes in the genome have not been reported. However, this is an RNA-virus where the potential for genetic changes is high. Unlike other RNA-virus, coronavirus have the ability of proofreading through a nonstructural protein exoribonuclease (7). On the one hand, this proofreading activity may reduce the risk of developing drug resistance because it helps stabilize the genome, but on the other hand, the exonuclease itself may hamper the effect of nucleotide analogues by removal of mismatched nucleotides during RNA synthesis (8).

So far, the main focus has been on characterizing mutations located in the Spike-region, as diversity of the Spike-protein is of particular importance in vaccine development. Variability in the Spike-region may also be of concern for potential drugs targeting viral entry, as mutations in this region could confer drug resistance. However, mutations seem to be evenly distributed across the genome, and mutations located in other regions of the genome may become of interest at later stages, depending on the target of the new antiviral drugs. The risk of genetic changes may increase as the virus is subjected to strong selective pressure through exposure to antiviral drugs.

The duration of the selective pressure is also of importance when evaluating the risk of development of resistance. A future antiviral treatment against SARS-CoV-2 will most likely be of short duration, which will reduce the risk of drug resistance.

What is harder to predict is whether a resistance mutation in SARS-CoV-2 will affect its ability to replicate, cause disease and spread infection, and if a massive roll-out of an antiviral treatment will affect the spread of antiviral drug resistance.

At present it is not known which steps in the replication cycle future treatments will target, however it is likely that there will be at least one drug inhibiting SARS-CoV-2's RNA synthesis. Combination treatment using drugs with different targets could be an option in order to reduce the risk for drug resistance, but this will depend on the development of new effective drugs with different points of action, and on the genetic barrier of each of these drugs.

To summarize, the risk of SARS-CoV-2 developing drug resistance of consequence for public health will probably be moderate. This level of risk provides an incentive for monitoring drug resistance once effective drugs are available. Independent of the potential targets of future drugs, the established high throughput assay for whole genome sequencing at NIPH will be suitable for resistance testing, and thus, we are already prepared. Test-and-treat-strategies and treatment as prevention (TasP) are well known approaches in combatting epidemics caused by viruses for which effective antiviral treatments are available, such as HIV and HCV. Antiviral treatment may also become part of the strategy for managing the current pandemic. We should therefore plan not only for resistance testing for clinical use but also for the implementation of a systematic surveillance of resistance that will be vital.

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