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Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2021

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Introduction

It is a pleasure to present the ninth report from the surveillance system for Resistance against Antivirals in Norway (RAVN). In this report, we present data for 2021 on resistance against antivirals for treatment of influenza, HIV-1 infection, hepatitis B virus infection (HBV), hepatitis C virus infection (HCV), and human herpes virus infections, as well as data from The Norwegian Drug Wholesales Statistics Database showing the usage of antiviral drugs in Norway in 2021.

In addition to the surveillance data, we have invited clinicians with specific expertise and interest for antiviral treatment of selected viral infections to write this years' chapters on special topics. The clinicians have addressed the role of drug resistance testing in the clinical management of HIV, HBV, HCV, CMV, and genital herpes infections. They also present interesting case studies from their clinical practice to illustrate some of the practical challenges in handling drug resistance. The invited clinicians are leading experts in their fields, and the chapters provide valuable insight and learning points that hopefully will be appreciated by many of our readers.

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

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	Cytomegalovirus
CRF	Circulating recombinant form
DAA	Direct-acting antiviral
DDD	Defined daily dose
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus-1
HSV	Herpes simplex virus
INSTI	Integrase strand transfer inhibitors
MAb	Monoclonal antibody
MSIS	Norwegian Surveillance System for Communicable Diseases
MSM	Men who have sex with men
NA	Nucleoside/nucleotide analogue
NAI	Neuraminidase inhibitor
NIPH	Norwegian Institute of Public Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PEP	Post exposure prophylaxis
PI	Protease inhibitor
PrEP	Pre-exposure prophylaxis
RAS	Resistance-associated substitution
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SDRM	Surveillance drug-resistance mutation
SVR	Sustained virological response
TK	Thymidine kinase
TAF	Tenofovir alafenamide fumarate
TDF	Tenofovir disoproxil fumarate
WHO	World Health Organization

Sammendrag

Bruk av antivirale midler

Ifølge data fra Reseptregisteret, var det i 2021 en reduksjon i salget av antivirale medikamenter i Norge målt i definerte døgndoser (DDD) etter flere år med økning. Denne nedgangen skyldes først og fremst en liten nedgang i salg av midler mot hiv, som utgjør en stor andel av de antivirale midlene som selges i Norge. Det var ingen nedgang i antall personer som behandles med hiv-midler sammenliknet med tidligere, men når en stadig økende andel behandles med kombinasjonspreparater, reduseres antall medikamenter, og dette vises som en nedgang.

Dersom man ser på antall personer som behandles med antivirale midler, er det midler mot herpesvirus som brukes av flest personer, og det har vært en ytterligere økning i salget av midler mot herpesvirus i 2021. Denne økningen gjelder særlig salg av valaciclovir. Det var i 2021 en nedgang i salg av midler mot hepatitt C og influensa, mens midler mot hepatitt B var uendret fra tidligere.

Influensavirus

I likhet med forrige influensasesong, var også 2021/2022 en annerledes sesong som ble sterkt påvirket av smitteverntiltakene i forbindelse med pandemien. Det var svært lav forekomst av influensa i begynnelsen av sesongen, men betydelig smittespredning en kortvarig periode våren 2022. Det er ikke funnet noen influensavirus med resistensmutasjoner som gir nedsatt følsomhet for neuraminidasehemmere. I en enkelt prøve ble det funnet nedsatt følsomhet for det nye medikamentet baloksavirmarboksil, et medikament som knapt nok er tatt i bruk i Norge.

Humant immunsviktvirus-1

Trenden med nedgang i antall hiv-tilfeller de siste årene har fortsatt også i 2021. Totalt 64 prøver ble analysert som ledd i resistensovervåkningen i 2021, hvorav kun 13 prøver var fra pasienter smittet i Norge. For første gang har vi i år informasjon om hvor personen var bosatt ved smittetidspunktet. Blant nye infeksjoner meldt til MSIS hos personer bosatt i Norge ved smittetidspunkt, ble hele 88% av tilfellene også rapportert til RAVN. Dette tyder på gode nasjonale rutiner for oppfølging av nydiagnostiserte med tanke på antiviral resistens. Det ble funnet resistensmutasjoner i 11% av de undersøkte prøvene, noe som er på omtrent samme nivå som tidligere år. Det er i 2021 kun funnet resistens mot revers transkriptasehemmere, og ikke mot proteasehemmere.

Hepatitt B-virus

I 2021 ble totalt 134 prøver med hepatitt B virus (HBV) analysert med tanke på resistensmutasjoner. De fleste av disse prøvene (n=117) hadde blitt sendt inn til referanselaboratoriet for genotyping og var fra pasienter som ikke hadde fått antiviral behandling for sin HBV-infeksjon. Det er disse prøvene som utgjør den norske overvåkningen av primærresistens. De resterende 17 prøvene var fra pasienter med pågående antiviral behandling der det var spørsmål om resistens som årsak til behandlingssvikt. Relevante resistensmutasjoner ble funnet i fem av de 17 prøvene fra pasienter med behandlingssvikt. Det ble ikke funnet resistensmutasjoner i noen av overvåkningsprøvene.

Humane herpesvirus: Cytomegalovirus

I 2021 ble 19 prøver sendt inn til resistensundersøkelse ved referanselaboratoriet for cytomegalovirus (CMV). Relevante resistensmutasjoner ble påvist i fem prøver, hvorav fire hadde lav eller moderat resistans mot ganciclovir, mens det ble funnet moderat resistens mot det nye medikamentet maribavir i en av prøvene. Siden det ikke er noen systematisk resistensovervåkning av CMV, kan man ikke beregne den reelle forekomsten.

Humane herpesvirus: Herpes simplex virus

I 2021 var det kun fem prøver med herpes simplex virus (HSV) som ble undersøkt for resistens. Resistens mot aciklovir ble funnet i to av prøvene. 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 HSV en systematisk resistensovervåkning.

Hepatitt C-virus

Nasjonal resistensovervåkning av hepatitt C-virus (HCV) startet opp i Norge i mai 2022. Overvåkningen er basert på resistensbestemmelse av HCV fra alle pasienter med nyoppdaget HCV-infeksjon i Norge. I 2021 ble resistensbestemmelse utført på et begrenset antall prøver sendt inn for resistensbestemmelse. Resistensdata er sammenstilt med epidemiologiske data fra MSIS for å kunne sammenlikne ulike undergrupper. Det ble påvist mutasjoner som er assosiert med resistens i syv av de åtte analyserte prøvene. To av disse var fra pasienter som hadde mottatt behandling, en var fra en ubehandlet pasient og de resterende fire var fra pasienter med ukjent behandlingshistorikk.

SARS-CoV-2

I 2021 ble det ikke samlet inn noen overvåkningsdata for antiviral resistens hos SARS-CoV-2 til RAVN. Perorale medikamenter for behandling av covid-19 vil bli tilgjengelig i Norge i løpet av høsten 2022, og det planlegges et system for overvåkning av antiviral resistens som trolig vil bli implementert fra 2023. Denne overvåkningen vil baseres på de samme sekvensdataene som inngår i den nasjonale variantovervåkningen.

Summary

The usage of antivirals

According to The Norwegian Drug Wholesales Statistics Database, the sales of antiviral drugs measured in defined daily doses (DDDs) were reduced in 2021, after several years of increase. This reduction was primarily due to a small decrease in sales of antivirals against HIV, which make up a large proportion of the antiviral drugs sold in Norway. There was no decrease in the number of people being treated with HIV drugs compared to previous years, but as a growing proportion of patients is treated with single tablet regimens, the number of drugs sold is reduced.

When looking at the number of persons treated with antiviral drugs, the antiviral treatment received by the highest number of patients are drugs used against herpes viruses. In 2021, the sale of antivirals against herpes virus, especially valaciclovir, increased even further. There was a decrease in the sales of agents against hepatitis C and influenza, while treatments for hepatitis B were unchanged.

Influenza virus

Similar to the previous influenza season, the season of 2021/2022 was also unusual, mainly due to the infection control measures implemented in response to the pandemic. There was a very low incidence of influenza at the beginning of the season, followed by a short period with significant spread of infection during the spring of 2021. No drug resistance against neuraminidase inhibitors was detected, but a mutation conferring resistance to the new drug baloxavir marboxil was found in one sample.

Human immunodeficiency virus-1

The decreasing trend in the number of new HIV-infections has continued in 2021, which is also reflected in a reduction in the number of samples received for surveillance of primary drug resistance. A total of 64 samples were analysed as part of the surveillance in 2021, and only 13 of these were from patients infected in Norway.

For the first time, we have been able to classify cases according to site of residence at the time of infection. Among those living in Norway at the time of infection, as much as 88% of the cases reported to MSIS were also reported to RAVN. This indicates that national routines for follow-up of newly diagnosed patients with regard to antiviral resistance are good. Resistance mutations were detected in 11% of the examined samples, which is comparable to previous years. In 2021, only mutations affecting reverse transcriptase inhibitors and none affecting protease inhibitors were found.

Hepatitis B virus

In 2021, a total of 134 samples with hepatitis B virus (HBV) were analysed for resistance mutations. Most of these samples (n=117) had been submitted to the reference laboratory for genotyping prior to treatment. These samples constitute the Norwegian surveillance of primary resistance. The remaining 17 samples were from patients with ongoing antiviral treatment, and were submitted for investigation of resistance as a possible cause of treatment failure. Relevant resistance mutations were found in five of the 17 samples from patients with treatment failure. No resistance mutations were found in any of the surveillance samples.

Human herpes viruses: Cytomegalovirus

In 2021, 19 samples were submitted for resistance testing at the reference laboratory for cytomegalovirus (CMV). Relevant resistance mutations were detected in five of these samples. Low or moderate resistance to ganciclovir was found in four of these samples, while moderate resistance to the new drug maribavir was found in one sample. There is no systematic surveillance of resistance in CMV, and the true incidence of drug resistance cannot be determined.

Human herpes viruses: Herpes simplex virus

In 2021, only five samples with herpes simplex virus (HSV) were analysed for resistance. Two of the samples had mutations conferring resistance to aciclovir. Despite an increase in the use of aciclovir both as treatment and prophylaxis, samples are rarely submitted for resistance testing. Like CMV, there is no systematic surveillance of HSV drug resistance.

Hepatitis C virus

A systematic surveillance system for newly diagnosed HCV infections was launched in May 2022. In 2021, resistance testing was performed on a limited number of samples submitted for resistance testing. Drug resistance data is cross-referenced with epidemiological data from MSIS to enable comparisons of different subgroups. Resistance associated substitutions were detected in seven out of eight samples analysed for resistance, two of which were from treatment experienced patients, one sample was from a patient with no previous treatment exposure and the remaining four were from patients where treatment exposure was not known.

SARS-CoV-2

Surveillance of antiviral resistance in SARS-CoV-2 has not been collected to RAVN in 2021. Oral drugs for the treatment of COVID-19 will be available in Norway from the autumn of 2022, and a system for surveillance of antiviral resistance will probably be implemented from the beginning of 2023. This surveillance will be based on the same sequence data that is part of the national monitoring of variants.

1 Antivirals and development of drug resistance

Antiviral drugs act by inhibiting propagation and spread of virus, usually by interfering directly with one or more specific steps 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, including entry to host cells, replication of the genome, viral protein production, and particle assembly or release as shown in Figure 1.1 (1). 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 (2). Recently, therapeutic use of monoclonal antibodies (mAbs) directed against specific viral proteins has increased. Although traditionally thought of as passive immunization, mAbs can also be classified as antiviral agents, as they directly interfere with binding of the virus to the host cell, they are used in treatment of established viral infections, and they are subject to resistance.

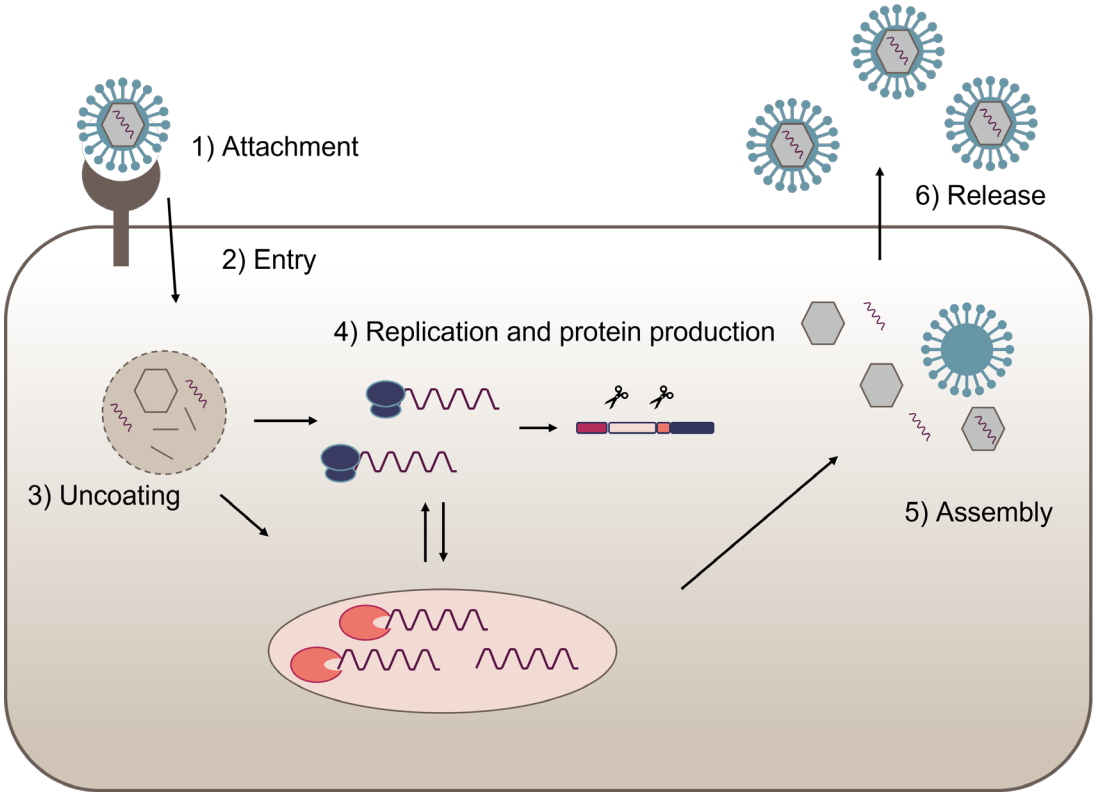

Figure 1.1. Generalized depiction of the viral replication cycle showing the major steps in replication. Antivirals inhibit the propagation of virus by inhibiting one or more of the steps in the replication cycle, such as 1) attachment of the virus to the host cell, 2) entry into host cell, 3) uncoating of the viral capsid and release of the viral genome and proteins, 4) replication of viral DNA/RNA and protein production including cleavage of viral polyproteins by proteases, 5) assembly of viral proteins and viral genome into new virions, and 6) release of viral particles. The replication cycle of different viruses may vary considerably, including variations in the sequential order of replication of the genome and translation of viral proteins.

Drug resistance against antivirals is caused by changes in the viral genome (mutations) leading to amino acid alterations (substitutions, insertions or deletions) in the protein targeted by the drug, thereby affecting the activity of the drug. Recombination or exchange of genetic material may also occur for certain viruses, which may introduce resistance into a new biological context. For example, antigenic shift in influenza transferred adamantane resistance from avian to human populations (3). 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 expansion of resistant 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, duration of both the infection and the treatment, and use of antiviral drugs in reservoir species. Immunocompromised patients are at particular risk. Furthermore, the genetic barrier for development of resistance, which roughly corresponds to the number of mutations needed is different for different drugs.

Antivirals against influenza

There are three classes of antiviral drugs targeting influenza entry, replication and release approved in Europe:

- Entry: M2-inhibitors block the M2 ion channel of influenza A virus, thereby inhibiting escape to the cytoplasm from endocytic vesicles. Influenza B is inherently resistant. Examples: amantadine and rimantadine.
- Release: Neuraminidase inhibitors (NAIs) 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. NAIs bind to neuraminidase on the surface of influenza virus A and B, preventing cleavage of sialic acid. NAIs thereby prevent release of the virus from the surface of the host cell and may possibly also affect viral entry by inhibiting viral penetration of mucus. Examples: oseltamivir and zanamivir (4;5).
- Replication: The polymerase inhibitor baloxavir marboxil was recently approved in Europe and is now available in Norway. The drug targets the endonuclease function of influenza RNA polymerase and inhibits transcription of viral mRNA by preventing the cap-snatching activity of the endonuclease.

Since 2016, oseltamivir has been the only antiviral drug against influenza on the market in Norway, until baloxavir marboxil was recently approved. Zanamivir is still registered but was withdrawn from the market in 2016 due to limited use. For many years, all circulating influenza viruses have been resistant to the two M2-inhibitors, and these drugs are not presently in use for treatment of influenza. Other NAIs have been developed, and are in use in the USA (peramivir) and Japan (peramivir, laninamivir).

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, before disappearing completely within the following two years. Resistance may 'hitch-hike' on another advantageous feature that promotes one virus strain over others, such as immune-escape mutations or fitness-enhancing mutations at other genomic sites (6). 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 HIV entry, replication and protein production:

- Attachment and entry: Attachment and entry inhibitors comprise four subclasses. CCR5 antagonists block the binding between viral gp120 and the chemokine receptor CCR5 (example: maraviroc). Attachment inhibitors bind to and inhibit the CD4-binding activity of gp120 (example: fostemsavir). The post-attachment inhibitor, ibalizumab, is a monoclonal antibody directed against CD4 which inhibits viral entry but not attachment. Fusion inhibitors, preventing gp41-mediated fusion of the viral envelope with the cell membrane (example: enfuvirtide), are no longer registered.
- Replication: Nucleoside reverse transcriptase inhibitors (NRTIs) are 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 reverse transcriptase. Examples: abacavir, lamivudine, emtricitabine, tenofovir, and zidovudine.
- Replication: Non-nucleoside reverse transcriptase inhibitors (NNRTIs) bind to the reverse transcriptase at a site distant to the nucleotide binding site inducing a conformational change, thereby inhibiting transcription of RNA into DNA. Examples: rilpivirine, etravirine, nevirapine, efavirenz, and doravirine.
- Replication: Integrase strand transfer inhibitors (INSTI) prevent integration of pro-viral DNA into the host cell DNA. Examples: dolutegravir, raltegravir, elvitegravir, and bictegravir.
- Protein production: Protease inhibitors (PIs) bind to the HIV protease and prevent 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 (7). 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 high turnover, resulting in a considerable risk for development of resistant variants, mainly due to inaccuracy in viral replication and the lack of proofreading. There is vast genetic variation in the HIV-1 genome, and each patient harbours 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 a virus that has drug resistance mutations (8).

Antivirals against hepatitis B virus

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

- Replication: Nucleoside/nucleotide analogues are 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. Nucleotide analogues may be directly incorporated into the DNA chain, whereas nucleoside analogues need to be phosphorylated prior to incorporation. 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 (9). 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 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 (10). Because of the rarity of resistant cases, the relevant mutation sites for tenofovir-resistance are not fully confirmed.

Antivirals against cytomegalovirus

There are two classes of antivirals used for treating cytomegalovirus (CMV) infection targeting CMV replication and assembly:

- Replication: 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). Examples: Ganciclovir, valganciclovir, cidofovir, foscarnet.
- Assembly: DNA terminase complex inhibitors 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 for prophylactic use after stem cell transplantation. Examples: letermovir.

Ganciclovir and its prodrug 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 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 ganciclovir resistance, CMV retinitis, or retinal necrosis.

Other anti-CMV-drugs are in development. Maribavir, a UL97-kinase inhibitor, has been used in clinical trials with mixed outcomes. The drug was approved by the FDA in 2021 for post-transplant CMV infection that does not respond to other CMV antivirals. Maribavir is currently under consideration by the European medicines agency (EMA). Unsurprisingly maribavir antagonises ganciclovir, since the UL97-kinase is required for activation of ganciclovir, and the two drugs should not be used in combination.

Drug resistant CMV

During ganciclovir anti-CMV therapy, resistance mutations usually develop after a cumulative exposure of six weeks or more. 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, targeting replication, is used for treating herpes simplex virus (HSV) infection:

- Replication: 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). Examples: guanosine analogues aciclovir, penciclovir and their prodrugs

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.

Helicase-primase inhibitors, also targeting viral genome replication, are in development for treatment of HSV (pritelivir).

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) (11).

Aciclovir resistance is frequently associated with cross-resistance to other HSV-TK dependent nucleoside analogues (12). Cidofovir and foscarnet are independent of HSV-TK and thus active against most of the strains that are resistant to aciclovir. Cross-resistance between foscarnet and aciclovir is rare (12). Although the prevalence of HSV resistance mutations is reported to be 0.1% -0.7% in immunocompetent patients and 3.5% to 10% in immunocompromised patients, treatment failures are relatively rare (11).

Antivirals against hepatitis C virus

There has been a rapid development of new and better drugs against hepatitis C virus (HCV) over the last years, replacing the early generations of direct-acting antivirals. 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) (13).

There are currently three groups of direct-acting antivirals (DAAs) targeting HCV genome replication/transcription, protein production or multiple stages simultaneously (14):

- Replication: NS5B inhibitors.
  - Nucleoside analogue polymerase inhibitors: Compete with nucleosides for the active site of the HCV RNA dependent RNA polymerase (NS5B). Example: sofosbuvir.
  - Non-nucleoside analogue polymerase inhibitors: Alter the shape of the polymerase and thus inhibit replication of HCV. Example: dasabuvir.
- Protein production: 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.
- Multiple stages: NS5A inhibitors target the multifunctional NS5A protein, 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 are now several options with documented effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targeting entry, genome replication/transcription and protein production.

- Replication: Analogues of naturally occurring deoxynucleotides which are incorporated by the RNA-dependent RNA polymerase (RdRp) into the growing RNA product and inhibits RNA synthesis. Example: remdesivir.
- Protein production: Protease inhibitors block the enzyme activity of the main protease (Mpro) involved in cleaving the viral polyproteins. Example: nirmatrelvir/ritonavir.
- Attachment and entry: Monoclonal antibodies specifically directed against the spike protein of SARS-CoV-2, thereby blocking the virus' attachment and entry into human cells. Examples: sotrovimab, casirivimab/imdevimab, cilgavimab/tiksagevimab.

In addition, the peroral ribonucleoside analogoue molnupiravir, a drug that inhibits SARS-CoV-2 replication by viral mutagenesis, showed promising results in early clinical trials. However, the results could not be reproduced, and the FDA emergency use authorization was withdrawn. A conditional recommendation on molnupiravir was included in WHOs guidelines on COVID-19 therapeutics in March 2022, in addition to their strong recommendation of the use of nirmatrelvir/ritonavir (15). Additional antivirals from new drug classes are under development, and some are in clinical trials.

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2 The usage of antivirals in Norway

Many new direct acting antivirals, especially against HIV and HCV, have been developed during the last decades, but in recent years new drugs introduced have mostly been fixed combinations of already established drugs. During the last two years, no new agents for treatment of HIV and HCV have been introduced in Norway. The only new DAA sold in Norway in 2021 was favipiravir, which was originally registered as an antiviral against influenza virus in Japan, but in 2021 has had some use in treatment of COVID-19 in several countries including Norway.

The sales for the different ATC subgroups of DAAs over time are shown in Figure 2.1. The sales of DAAs, measured in both defined daily doses (DDDs) and number of patients treated, increased yearly from 2017-2019. The sales measured in number of DDDs was slightly reduced in 2020 and 2021 while the number of users has continued to increase from 62 514 in 2019 to 66 071 in 2021 (Figure 2.1 and Figure 2.2, respectively) (1). Drugs used for treatment of HIV-infection make up a large proportion of the total of antiviral drugs sold in Norway, measured in DDDs. Because a growing proportion of patients is treated with single tablet regimens, the number of drugs sold is reduced despite an increase in number of users. 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. In 2021 the total cost of the DAAs had fallen by almost 55 % since 2017.

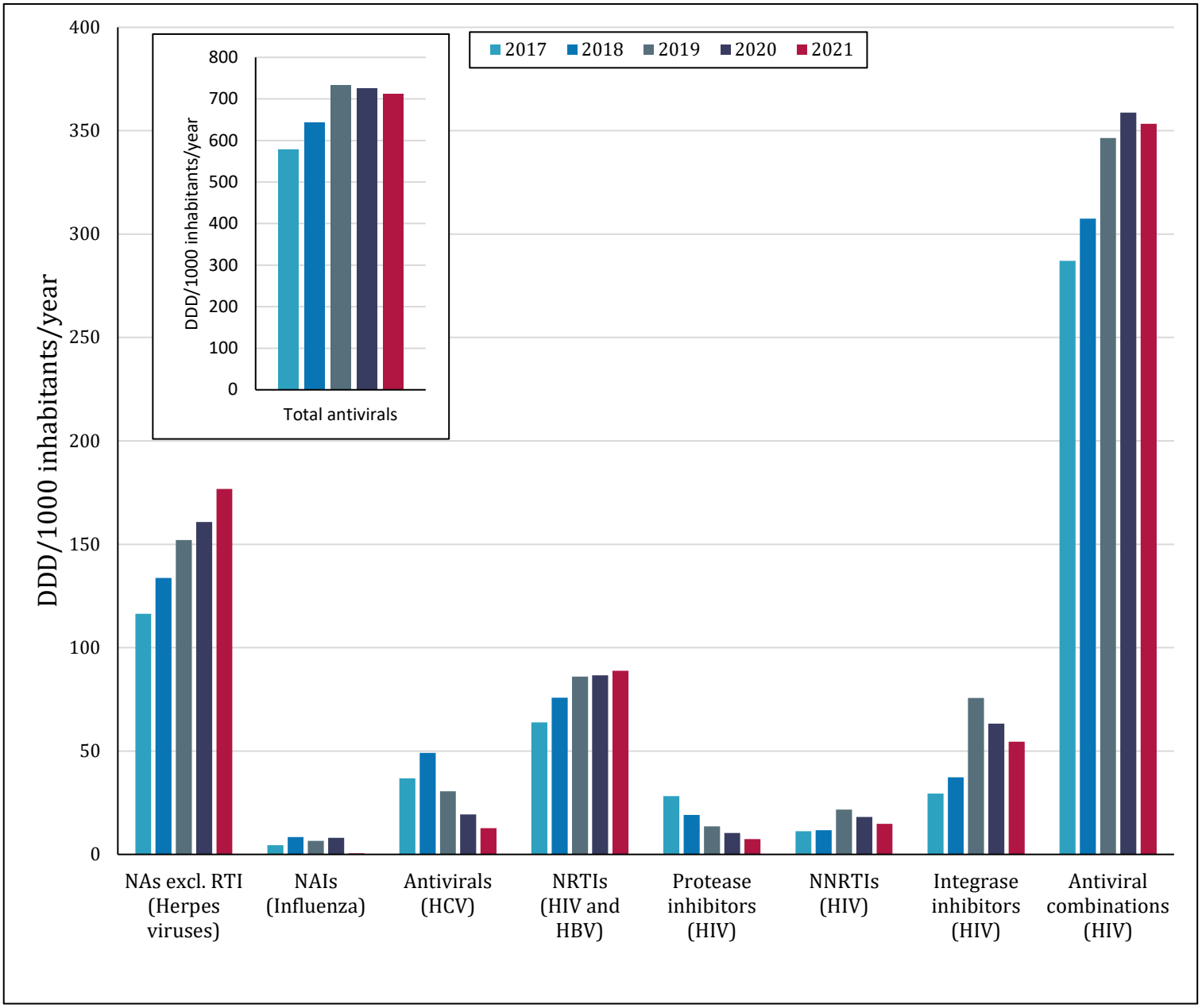

Figure 2.1: Sales of direct acting antiviral drugs for systemic use (ATC group J05A) for 2017-2021 (2). The figure shows the sales of direct acting antiviral groups over time. The numbers are given as defined daily doses (DDDs) per 1000 inhabitants per year. NAs excl. RTI: Nucleo(s/t)ide-analogues excluding reverse transcriptase inhibitors (J05AB); NAIs: Neuraminidase inhibitors (J05AH); Antivirals for treatment of HCV infections (J05AP); NRTIs: Nucleo(s/t)ide-analogue reverse transcriptase inhibitors (J05AF); Protease inhibitors (J05AE); NNRTIs: Non- nucleo(s/t)ide-analogue reverse transcriptase inhibitors (J05AG); Integrase inhibitors (J05AJ); Antiviral combinations, HIV: Antivirals for treatment of HIV infections, combinations (J05AR). The insert is a plot illustrating the total sales of antivirals in ATC group J05A in Norway. The total numbers also include phosphonic acid derivates (J05AD) used against herpesviruses and other antivirals (J05AX), due to low numbers these are not indicated in the main plot. In reports prior to 2021 integrase inhibitors were included in other antivirals (J05AX). In 2020 integrase inhibitors were reclassified in a new ATC group (J05AJ).

The number of individuals treated with DAAs for HIV, HBV and herpes has increased every year since 2017, while the number of individuals treated for influenza and HCV increased from 2017 to 2018 followed by a yearly reduction from 2019 to 2021(Figure 2.2).

The use of DAAs against influenza varies from year to year and is usually closely connected to the magnitude of the seasonal influenza outbreaks. 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 commonly used antivirals when measured in number of users (Figure 2.2). The high number of DDDs for HIV drugs reflects the long-term daily treatment, while antivirals against herpes infections are given in short courses. For DAAs against herpesvirus, the use of topical agents (creams and ointments) is not included in the measurement of DDD.

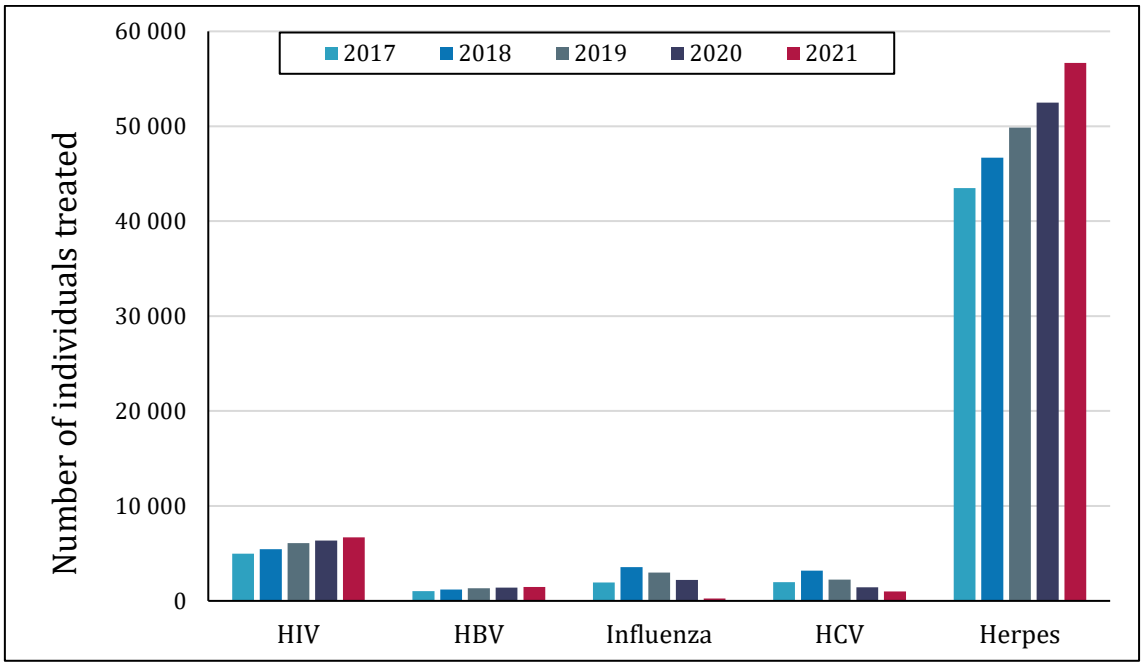

Figure 2.2: Trends in the use of direct acting antiviral drugs for systemic use (ATC group J05A) grouped by virus for 2017-2021 (2).

The figure shows the number of individuals 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 (lamivudine, Zeffix is excluded); HBV: All HBV pharmaceuticals (lamivudine, Epivir is excluded). Single component drugs approved for both HBV and HIV are included in the HBV numbers only; Influenza: Neuraminidase inhibitors; HCV antivirals; Herpes: aciclovir, ganciclovir, famciclovir, valaciclovir, cidofovir and foscarnet.

Influenza virus

The usage of the neuraminidase inhibitors, antivirals for the treatment of influenza (ATC group J05AH), is shown in Table 2.1. The variation 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. The low number of users of antivirals against influenza in 2021 coincides with the low number of reported influenza cases in the seasonal influenza epidemics in 2021. Due to limited use, zanamivir was withdrawn from the market in 2016; consequently, oseltamivir is now the only neuraminidase inhibitor available for treatment of influenza in Norway.

Table 2.1 Number of individuals with at least one prescription of neuraminidase inhibitor per year.

	2017	2018	2019	2020	2021
Oseltamivir	1 923	3 571	2 987	2214	248

Human immunodeficiency virus

There are currently 33 drugs or combination drugs in Norway that are used solely for treatment of HIV. The use of the different drugs has shifted in the last five-year period. Of the 33 HIV drugs or combination drugs used in 2021, seven of them have been introduced since 2017, while one older drug has been withdrawn in the same period. The number of patients retrieving at least one prescription of these drugs has increased by almost 35 % from 2017 to 2021, partly attributable to the concurrent increase in the number of persons receiving pre-exposure prophylaxis (PrEP).

During the whole period, nearly 99 % of persons treated, received combination drugs containing more than one active substance. Some of these combination drugs contain complete combination ART (single-pill regimens). Figure 2.3 shows the trends in use of antiviral drugs for treatment of HIV, measured in number of persons treated. The figure shows single tablet regimens; fixed dose combination drugs, which contain combinations of two substances, typically two NRTIs that are commonly combined; and single substance drugs that are given in addition to the fixed combinations in order to obtain complete ART.

Tenofovir disoproxil (TDF), 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 and tenofovir disoproxil (FTC/TDF) has been the most commonly used combination drug in recent years. It is usually used in combination ART together with either an integrase inhibitor, boosted protease inhibitor, or an NNRTI. For post exposure prophylaxis (PEP), the recommendation is to use FTC/TDF in combination with the integrase inhibitor raltegravir. In 2016, FTC/TDF was approved as PrEP to reduce the risk of sexually acquired HIV-1 infection in adults at high risk, with full reimbursement of the costs. PrEP is most likely the main reason for the observed yearly increase in the use of FTC/TDF since 2016. The number of patients receiving FTC/TDF in 2021 was 3218. The use of FTC/TDF increased almost 47 % from 2018 to 2019, while the increase was only one percent from 2019 to 2020 and two percent from 2020 to 2021. It is not unlikely that the extensive infection control measures applied in connection with the COVID-19 pandemic in 2020 and 2021 may have reduced the demand for PrEP, thereby contributing to this stagnation. However, from the drug statistics, it is not possible to separate the proportion of PrEP or PEP from the total use of these drugs, and the changes in the use of FTC/TDF seen the latest two years might also have other explanations.

The prodrug of tenofovir, tenofovir alafenamide (TAF), is given in lower doses, and has a greater bioavailability in relevant body tissues than TDF. TAF is available in various combinations of emtricitabine and TAF (FTC/TAF), both as FTC/TAF alone, and in fixed dose combinations with substances from other drug classes as complete ART (3). FTC/TAF 25mg is approved as an alternative in continuous PrEP in persons with contraindications for FTC/TDF.

When looking at complete ART regimens, combinations containing integrase inhibitors are widely used, which is also in accordance with the Norwegian guidelines (3). This is illustrated in Figure 2.3, which shows that many combination drugs containing integrase inhibitors are among the most sold drugs the latest years, measured in number of users.

The recommendations from The Norwegian Hospital Procurement Trust (Sykehusinnkjøp HF) which negotiate prices and indicates the drugs of preference when it comes to reimbursement, have a great impact on the choice of drugs for treatment of HIV (4). No new combinations products were introduced in 2021, but the combination of cabotegravir and rilpivirin as injections was approved as complete dual therapy in December 2020 and available for use in 2021. The sales of these injections were very limited in 2021. An increase is observed in the number of individuals treated by the four one-tablet combinations introduced since 2019. These combinations are in fact the only combinations with increasing number of users in 2021 compared to the previous years. As shown in figure 2.3, the use of all the single component drugs except from doravirine which was introduced in 2020, has decreased in 2021.

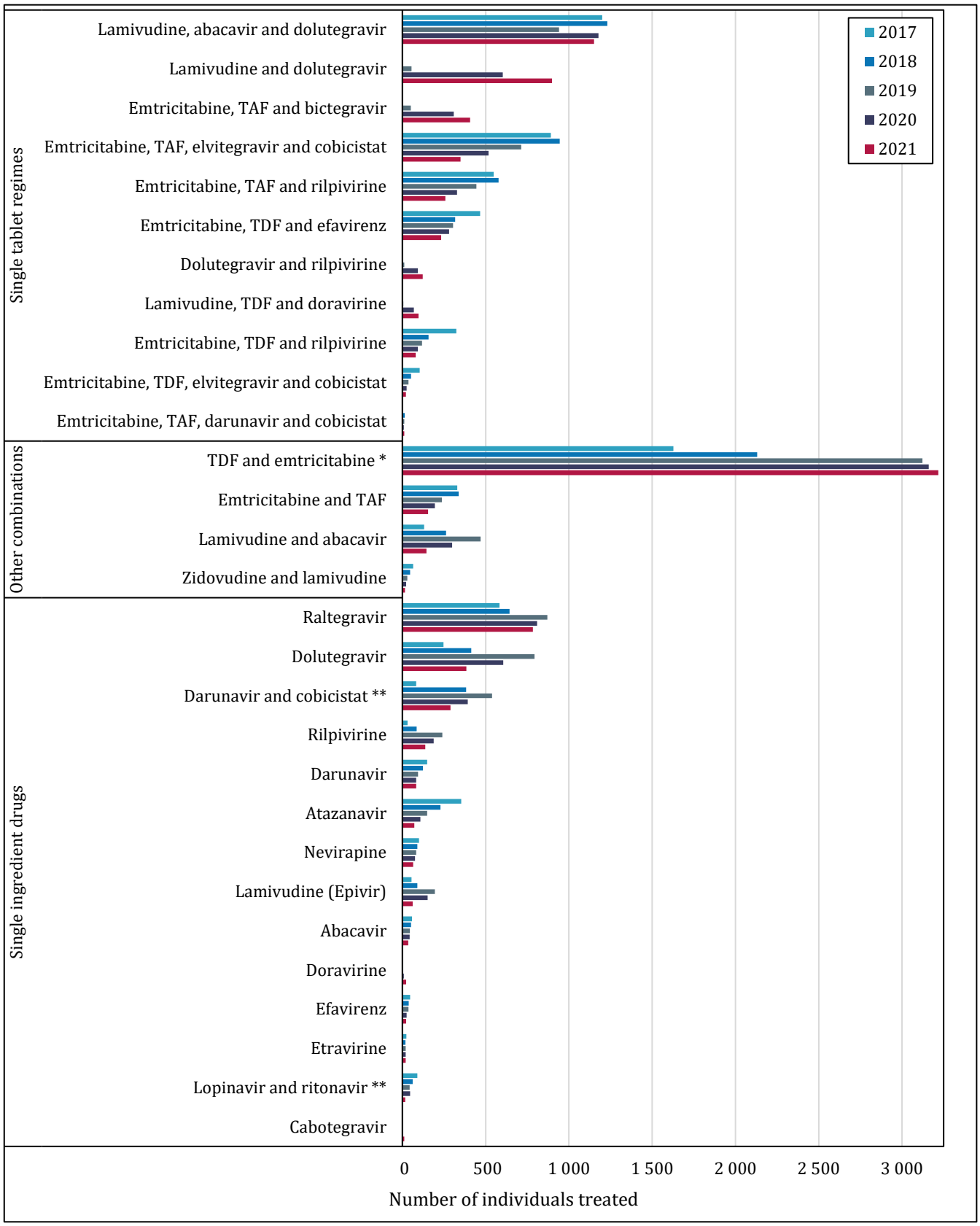

Figure 2.3: Trends in the use of antiviral drugs for treatment of HIV in the periods 2017-2021 (2). The figure shows the number of individuals given at least one prescription per year. Complete single tablet regimens, other fixed dose combination drugs, and single ingredient drugs are shown separately. Drugs prescribed to less than 5 individuals in 2021 are excluded (zidovudine; maraviroc; atazanavir and cobicistat; zidovudine). *Includes PrEP. **Boosted protease inhibitors are considered single ingredient drugs.

The use of the integrase inhibitors (INSTI) is increasing when measured in number of prescriptions per active ingredient. This is in line with the recommendations in the guidelines and the procurement recommendations.

The number of prescriptions per active ingredient over time is shown in Figure 2.4. For NRTIs, there are far more prescriptions for emtricitabine and tenofovir (TDF or TAF) than for lamivudine and abacavir, but the number of prescriptions for ART in comparison to PrEP is not known. Dolutegravir is the most used active ingredient that is not an NRTI. The use of the integrase inhibitors dolutegravir and bictegravir is increasing when measured in number of prescriptions per active ingredient while the use of raltegravir is slightly decreased.

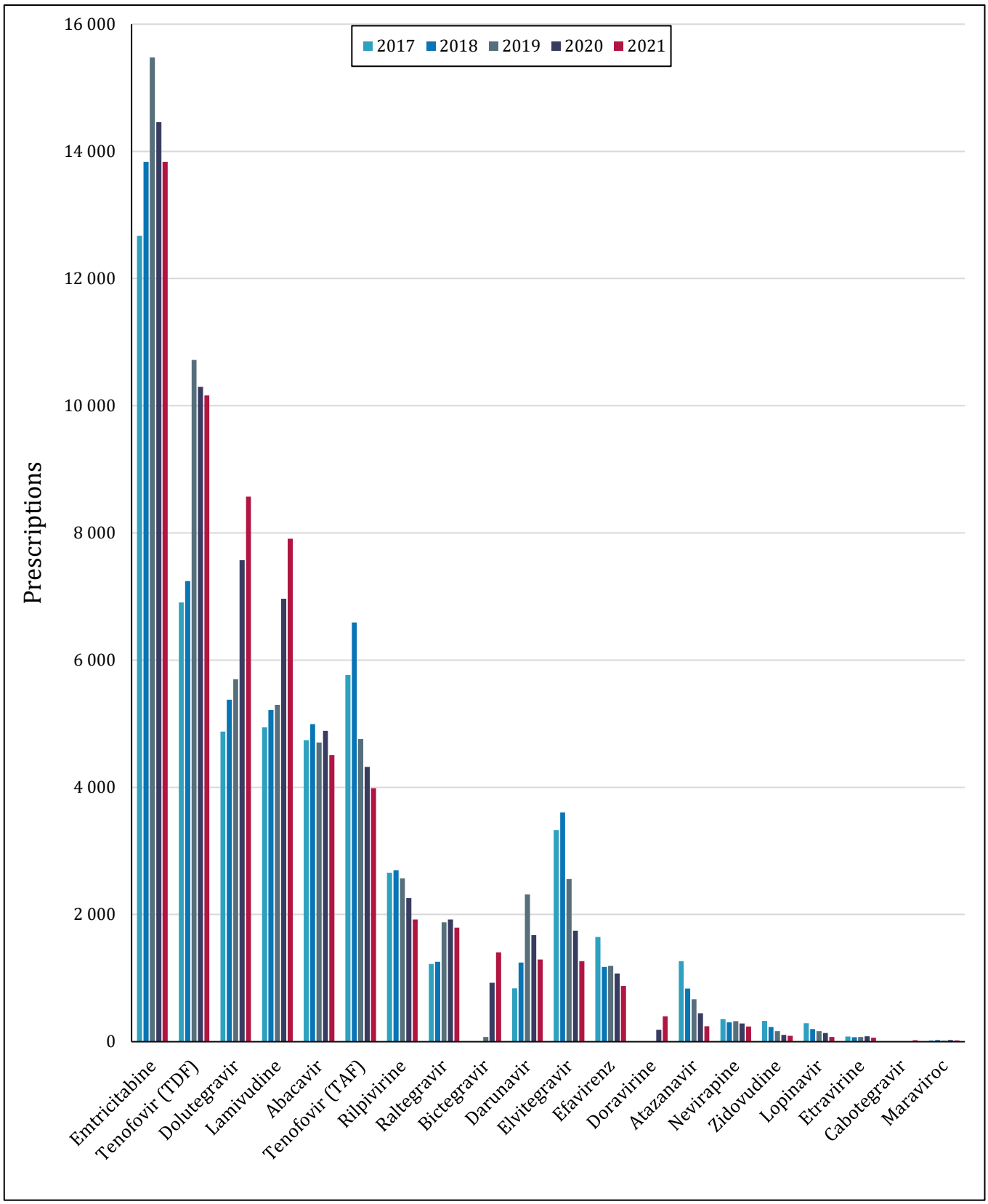

Figure 2.4: Number of prescriptions per active ingredient for HIV drugs (2).
This figure shows the number of prescriptions per active ingredient over time. Many prescriptions contain more than one active ingredient; these prescriptions are counted several times. TDF = tenofovir disoproxil, TAF = tenofovir alafenamide. Saquinavir was not prescribed in 2021 and is 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

No new antivirals have been introduced for treatment of HBV infection in 2021 and there are currently six nucleoside/nucleotide analogues (NAs) approved for this indication. Treatment of HBV with antivirals is generally given as monotherapy. The use of the NAs is shown in Figure 2.5.

The data is based on the annual number of patients retrieving at least one prescription per year for the period 2017-2021. Lamivudine, adefovir dipivoxil, tenofovir disoproxil (TDF), and emtricitabine are approved for both HBV and HIV, while entecavir and tenofovir alafenamide (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 588-1482 in 2021. The lowest number is based on the number of patients prescribed drugs approved for HBV only (entecavir and TAF). The highest number is the total number of patients prescribed one of the five 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 individuals receiving HBV treatments with NAs, more than 99% received one of these three drugs in 2021. The number of persons treated with TDF and TAF was stable from 2020 to 2021, while there was a 30 % increase in the number of users of entecavir. From April 2021, entecavir is recommended as the preferred drug according to recommendations from Sykehusinnkjøp HF, which might explain this observed increase (5).

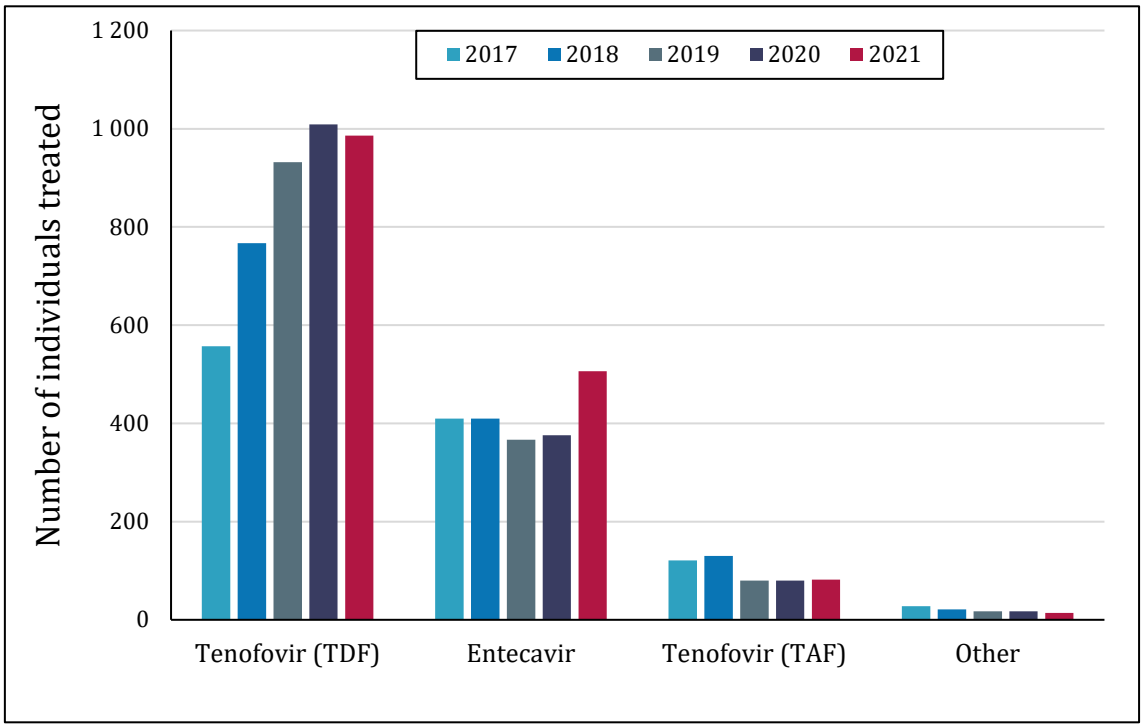

Figure 2.5 Trends in the use of antivirals for treatment of HBV for the period 2017-2021 (2).
This figure shows the trends in antiviral use for the treatment of HBV over time. The number of persons treated is defined as the number of patients given at least one prescription per year. Other: lamivudine (Zeffix), adefovir dipivoxil, and emtricitabine.

Human herpesviruses

Figure 2.6 shows the prescribed drugs for systemic use for human herpes virus infections over the last five years. Valaciclovir is the most commonly prescribed substance and there has been an increase of more than 30 % in the number of individuals treated with this antiviral since 2017. The use of aciclovir has been stable during the five-year period. Ganciclovir and famciclovir (Other) were rarely prescribed. In 2021 56 700 persons have been treated with systemic antivirals for herpes viral infections.

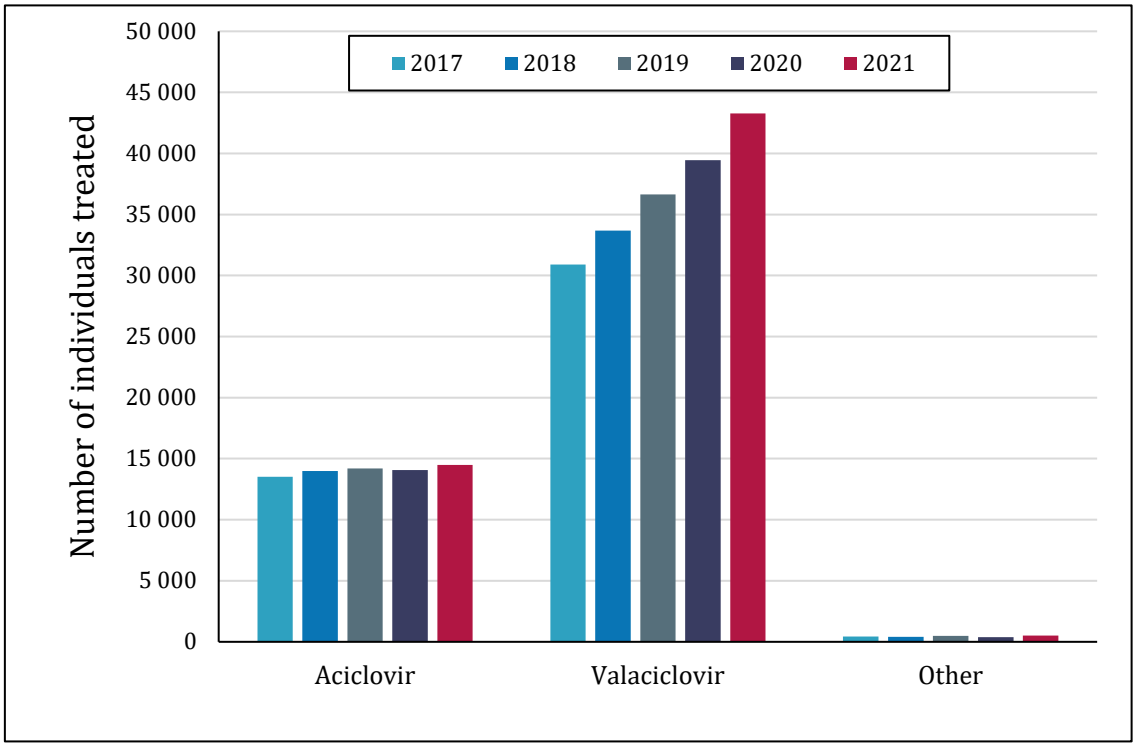

Figure 2.6: Trends in the use of antivirals for treatment of human herpes virus infection for the period 2017-2021 (2).
This figure shows the trends in direct acting antiviral use for treatment of human herpesviruses over time. Number of individuals treated is defined as the number of patients given at least one prescription per year. Other: ganciclovir, famciclovir and valganciclovir.

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 the use of these creams the next couple of years. Since then, the consumption has been quite stable. From 2018 the use of a fixed combination of aciclovir and hydrocortisone has increased at the expense of topical aciclovir and penciclovir alone (Table 2.2).

Table 2.2: Sold packages of topical antivirals containing aciclovir, pencixclovir and aciclovir in combination with hydrocortisone.

	2017	2018	2019	2020	2021
Aciclovir	205 818	212 393	180 880	169 004	176 013
Penciclovir	24 062	18 957	18 664	17 229	14 054
Aciclovir, combinations*		21 794	40 618	34 727	45 996

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. * In combination with hydrocortisone.

Hepatitis C virus

After the new HCV antivirals became available in 2015 the overall number of patients treated with DAAs against HCV increased steadily until 2018. However, in the following years the number of persons treated has again decreased. The number of persons who received at least one prescription for an HCV drug (except interferons) was 981 in 2021, a reduction by almost 70% from 2018 and 30% since 2020. Fixed combinations of two or more active ingredients have almost completely replaced single component drugs including ribavirin as shown in Figure 2.7, and in 2021.

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) were updated in 2019 (6). However, the recommendations from The Norwegian Hospital Procurement Trust (Sykehusinnkjøp HF) probably also have a great impact on the choice of drugs for treatment (7). These recommendations are similar but not identical to the NMA guidelines.

The treatment pattern for the use of the different combinations against HCV was the same from 2018 to 2020 with the single-tablet regimen of the NS5B inhibitor sofosbuvir (SOF) and the NS5A inhibitor velpatasvir (VEL) as the most used drug. This was one of the combination therapies recommended in the procurement for 2019 and was listed as the "recommended treatment" in genotype 3 HCV infections, one of the more common genotypes in Norway. In 2021 the 3-tablet regimen of the combination of glecaprevir and pibrentasvir (GLE/PIB), another pangenotypic fixed combination with high treatment response, almost reached the same level of use as SOF/VEL. GLE/PIB is from April 2021 the first-choice treatment for most cases of genotype 3 HCV infections according to the new procurement agreement.

Sofosbuvir and ledipasvir (NS5A inhibitor) (SOF/LDV) has since 2018 been the second most used combination but is now bypassed by both GLE/PIB and elbasvir and grazoprevir (EBR/GZR) which is now considered as the first-choice treatment by the 2021 procurement for treatment of most patients with HCV genotype 1, which is also commonly seen in Norway. The trends of use shown in Figure 2.7 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 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). Hopefully, the reduction in treated patients after 2018 indicates that the goal is achievable.

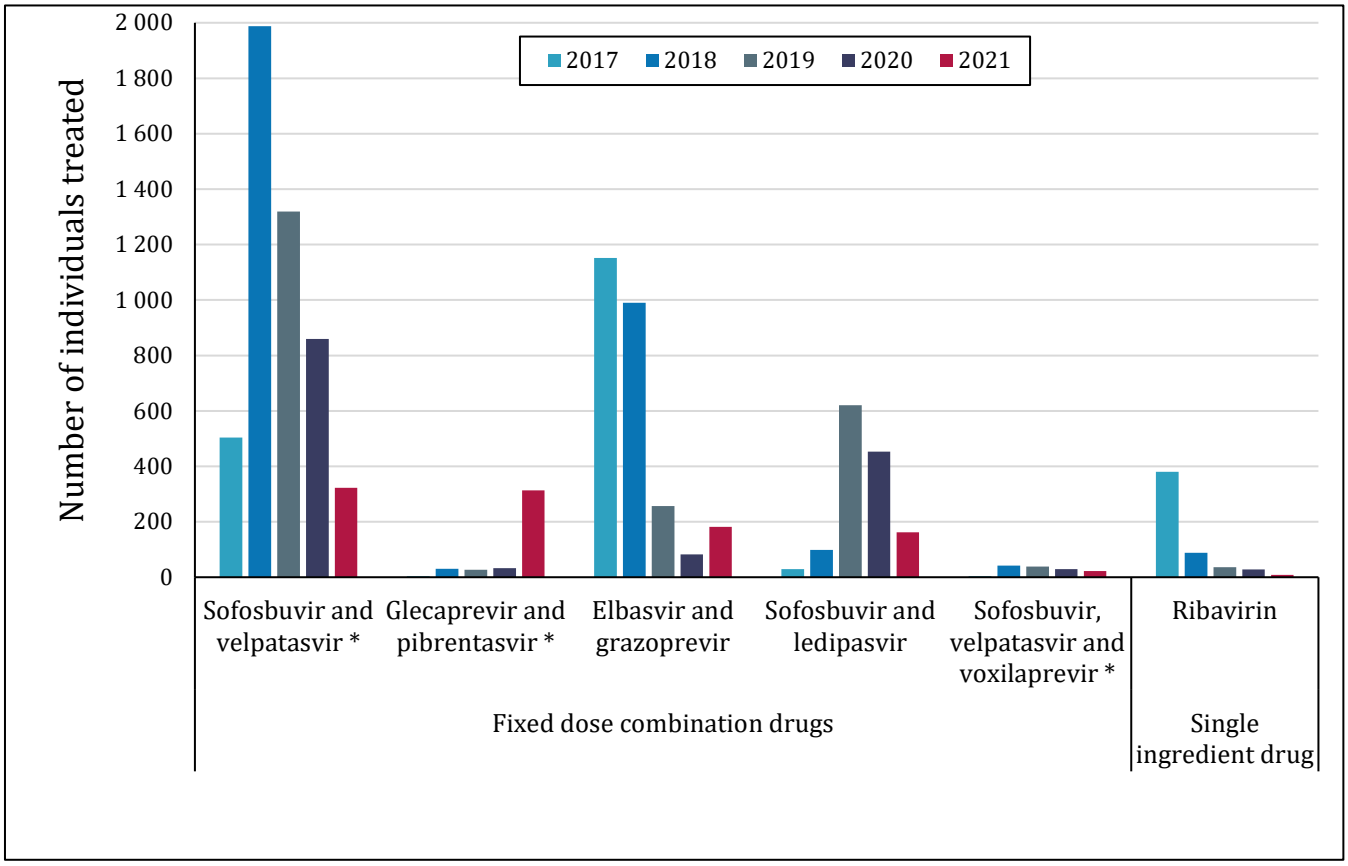

Figure 2.7: trends in the use of antivirals for treatment of HCV for the period 2017-2021 (2).
This figure shows the trends in the use of direct acting antivirals for treatment of HCV over time. The different drugs are sorted in fixed dose combination drugs and single ingredient drugs. The number of individuals treated is defined as the number of patients given at least one prescription per year. Drugs not sold in 2021 (ombitasvir, paritaprevir and ritonavir; simeprevir; daclatasvir; sofosbuvir; dasabuvir) are excluded from the figure.
* Pangenotypic drugs.

SARS-CoV-2

None of the oral antiviral drugs against SARS-CoV-2 were available in Norway in 2021, and the antiviral drugs used for treatment of COVID-19 in Norway were only for hospital use. Remdesivir has conditional marketing authorization in Norway and has since November 2020 been approved for use against SARS-CoV-2. There has been no sale of remdesivir registered in the Norwegian Drug Wholesales Statistics after 2020, but records of actual use are not available. However, as the recommendations was extended to also include treatment of severely ill patients, and not only early treatment of risk groups, the clinical use in hospitals most likely increased in 2021 compared to 2020. This indicates that some of the sales of remdesivir from wholesalers registered in 2020 was used in hospitals 2021.

The use of monoclonal antibodies for the treatment of SARS-CoV-2 has been limited in Norway. The clinical efficacy of the individual drugs against different variants is variable, and thus, recommendations for use depend on the variant currently circulating. In 2021, two new medicaments containing monoclonal antibodies (sotrovimab and casirivimab/ imdevimab) have been introduced for treatment of hospitalized patients with SARS-CoV-2 who are at increased risk of progressing to severe COVID-19. A total of 144 packages of sotrovimab and 100 packages of casirivimab/imdevimab were sold in the second half of 2021 according to data from the Norwegian Drug Wholesales Statistics. Records of actual use are not available.

Early efforts in the search for effective antiviral treatment of SARS-CoV-2 included repurposing of existing antiviral drugs used for other infections. Favipiravir is a nucleotide analogue approved in Japan for use against influenza virus, and its effect against SARS-CoV-2 is under investigation. In 2021, there were 1 641 prescriptions of favipiravir in Norway (mainly in December), prescribed to 1 473 individuals. Although favipiravir is registered as an antiviral against influenza virus, it is likely that in 2021, the drug was prescribed primarily against COVID-19 infection.

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3 Influenza virus

Fact box: Influenza virus drug resistance	
Treatment	Neuraminidase inhibitor: oseltamivir Polymerase inhibitor: Balaxovir marboxil (Licensed in Norway May 2021)
Resistance testing method	Whole genome sequencing Phenotypic by neuraminidase susceptibility assay (MUNANA) In Norway, all influenza drug resistance tests are performed at the WHO national reference laboratory for influenza, at the Norwegian Institute of Public Health (NIPH)
Target gene	Neuraminidase/ polymerase and matrix gene
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 provides samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit a subset of confirmed influenza-positive samples for analysis. 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 2021-22

Due to the COVID-19 pandemic, influenza has been absent in Norway and most parts of the world during 2020 and 2021. However, a late epidemic occurred in early spring of 2022 reaching a peak at week 14 with an influenza prevalence of 21%. Throughout the season more samples than ever have been analysed for influenza; 562 916 samples have been analysed, with 13 921 influenza positive cases detected. The season was dominated by influenza A (H3N2) (2 558 cases), with some influenza A (H1N1) (165 cases), and influenza B/Victoria (16 cases). Influenza B/Yamagata was not detected this season. 11 096 cases of influenza A and 98 influenza B were not subtyped.

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 and ECDC/WHO (1). During the 2021-22 season, a total of 674 samples were genetically analysed for antiviral neuraminidase resistance at the reference laboratory. No neuraminidase resistance was detected. However, in three of the H3N2 viruses, the I222V substitution was detected in neuraminidase. This substitution is not associated with resistance in H3N2 viruses, but together with substitutions in position 119, it could confer resistance. Such substitutions in position 119 were not found in any of the Norwegian viruses.

As baloxavir marboxil (hereafter baloxavir) was licensed in May 2021 in Norway, resistance towards baloxavir was also investigated, although the antiviral is not yet in use in Norway. Baloxavir appears to have a lower genetic barrier for development of resistance than neuraminidase inhibitors. Baloxavir has been the most widely used drug in Japan. In 2018 the resistance towards baloxavir in Japan was 1.5 % and in 2019 9.5 %, although mainly found in treated children. However, human to human transmission of resistant virus has also been described (1;2). Out of 442 influenza viruses investigated for baloxavir resistance in Norway, one single case (0.23 %) from week 19 was detected. The sample was from an >80 year old hospitalized, treatment-naïve patient who was infected with influenza A(H3N2) possessing the I38T substitution in the PA subunit of the polymerase complex, which is the most common substitution associated with reduced baloxavir susceptibility (2).

Phenotypic testing for neuraminidase susceptibility was not performed for any of the samples, as antiviral resistance testing of influenza virus has been deprioritized during the COVID-19 pandemic.

For many years, all circulating influenza viruses have been resistant to adamantanes, thus these antivirals are not used for treatment in Norway and most other countries. However, in recent years, a few susceptible cases have been detected. Therefore, the NIPH has resumed testing for adamantane resistance. All cases investigated for adamantane resistance possessed the S31N substitution in the M2 protein indicating high level resistance towards adamantanes.

Table 3.1: Norwegian influenza viruses resistant to the neuraminidase inhibitors oseltamivir and zanamivir, during the influenza seasons 2015/16 through 2021/22 (sequences with resistance/total number of analysed sequences. Percentages > 0 are shown in parentheses).

Season	Oseltamivir resistance			Zanamivir resistance			Baloxavir resistance			Adamantane resistance		
	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B	A(H1N1)	A(H3N2)	B
2015/16	10/339 (3.0%)	0/32	0/50	0/106	0/31	0/48	ND	ND	ND	ND	ND	ND
2016/17	0/10	0/174	0/54	0/8	0/161	0/54	ND	ND	ND	ND	ND	ND
2017/18	0/120	0/66	1/42 (2.4%)	0/28	0/54	0/30	ND	ND	ND	ND	ND	ND
2018/19	0/247	0/108	0/26	0/82	0/107	0/26	ND	ND	ND	ND	ND	ND
2019/20	0/103	0/63	0/42	0/32	0/60	0/42	ND	ND	ND	ND	ND	ND
2020/21	0/2	0/6	0/1	0/2	0/6	0/1	ND	ND	ND	ND	ND	ND
2021/22	0/31	0/634	0/9	0/31	0/634	0/9	0/0	1/442 (0,23 %)	0/0	19/19	476/476	0/0

ND: No data

Conclusions

Antiviral drug resistance in influenza remains low nationally as well as globally, based on the very few cases investigated during the COVID-19 pandemic. Global estimates made before COVID-19, indicated that approximately 0.5% of all viruses tested have reduced susceptibility towards neuraminidase inhibitors and this is expected to be similar for Europe (3). 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 (NRTIs) - Non-nucleoside reverse transcriptase inhibitors (NNRTIs) - Integrase strand transfer inhibitors (INSTI) - Protease inhibitors (PIs) - Entry inhibitors (CCR5 antagonists, fusion inhibitors, attachment inhibitors, post-attachment 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 (for CCR5/CXCR4 tropism)
Indication for resistance testing	Virological failure during antiviral treatment
Surveillance	The national surveillance program for HIV-1 monitors primarily drug resistance against protease inhibitors (PIs) and reverse transcriptase inhibitors (NNRTIs and NRTIs). 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 are 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 proxy for transmitted drug resistance. Since 2019, drug resistance data has been 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.

New HIV infections are reported to MSIS with full patient identification. Although resistance testing is recommended for all newly diagnosed patients, not all are included in the surveillance system. This could have different explanations: i) sample not submitted for resistance testing, ii) patient not identified as newly diagnosed on the referral form, or

iii) viral load was suppressed at the time of diagnosis, usually due to treatment initiated before arrival in Norway.

The World Health Organization (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). A standard list of SDRMs was published in 2009, but unfortunately, the list has not been updated since (2). The list is 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. The SDRM list is not designed for individual patient management as it excludes several clinically relevant drug resistance mutations and may include certain mutations with less clinical relevance for current regimens. 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 (CPR) tool at Stanford HIV Drug Resistance Database (3). All sequences are also analysed using the Stanford genotyping resistance interpretation algorithm in order to identify additional clinically relevant resistance mutations.

There has been an increase in the use of integrase strand transfer inhibitors (INSTI) 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 INSTI in Norway.

Surveillance data 2021

A total of 64 samples from newly diagnosed cases of HIV-1 in Norway were analysed for primary HIV-1 drug resistance in 2021, which equals 62% of the 102 cases reported to MSIS in 2021 (5). Of the 64 cases with samples submitted for resistance testing, 31% were female and 69% were male.

The distribution of the most common HIV subtypes detected in samples from newly diagnosed patients in Norway the last five years is shown in figure 4.1. Subtype B is the most commonly transmitted subtype in Norway, while the transmission of subtype C in Norway is very low. Distribution of subtypes in samples from patients infected abroad is more diverse, mostly reflecting variations in continents of transmission.

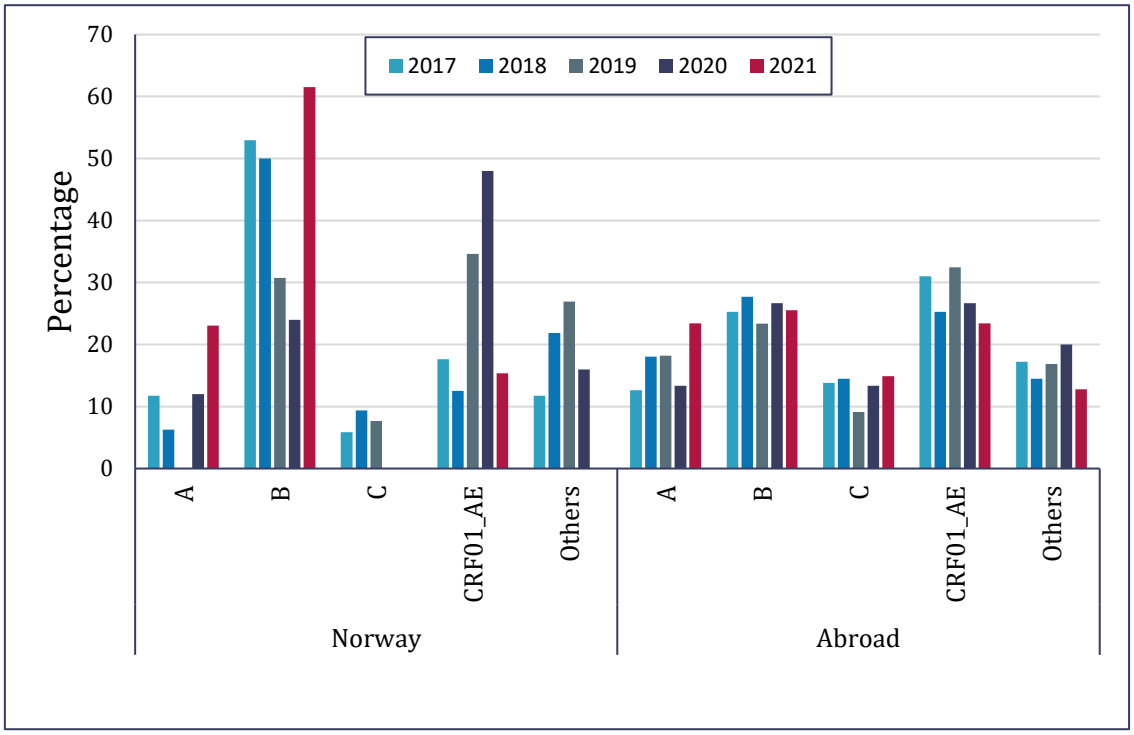

Figure 4.1: Percentage of subtypes among analysed sequences 2017-2021 by country of transmission. The five most frequent subtypes are shown. The group of others includes the subtypes CRF02_AG, CRF06_cpx, subtypes D, F, and G, as well as single cases of several other circulating recombinant forms (CRFs).

Information on the route of transmission for patients tested for drug resistance, was obtained by cross-referencing resistance data to epidemiological data from MSIS. The data is shown in Table 4.1. Only 13 (20%) samples were from patients infected in Norway, while 47 (73%) were infected abroad. Coverage of resistance testing among patients infected in Norway was 87%. Among those infected abroad in 2021, the coverage was significantly lower (55%). However, this group includes both travellers that are infected abroad but residing in Norway at the time of infection, and immigrants that may have been infected before arrival to Norway. Particularly among the latter, many may already be receiving treatment at the time of notification to MSIS and will therefore be ineligible for resistance testing due to a suppressed viral load. Among persons infected abroad while residing in Norway, surveillance resistance testing was performed in approximately 89% of the cases. Data is shown in Table 4.2.

Table 4.1: Route of transmission in samples from newly diagnosed HIV patients tested for antiretroviral drug resistance in 2021 compared to new cases reported to MSIS in 2021.

Route of transmission	Samples tested for resistance, 2021	Cases reported to MSIS, 2021
Heterosexual	32	58
- infected in Norway	0	*0*
- infected abroad	32	**57*
- unknown	0	*1*
MSM	23	36
- infected in Norway	12	14
- infected abroad	11	**22*
- unknown	0	0
IDU	3	4
Blood	0	0
MTC	2	4
Unknown	4	0
Total	64	102

MSM: men who have sex with men; IDU: injection drug users; MTC: mother to child.
*Includes cases on treatment and with suppressed viral load upon arrival in Norway.

Table 4.2: Coverage of resistance testing by country of transmission, compared to new cases reported to MSIS in 2021.

Country of transmission	Samples testes for resistance, 2021	Cases reported to MSIS, 2021	Coverage
Infected in Norway	13	15	87 %
Infected abroad	47	86	55 %
- before arrival in Norway	*30*	*67*	*45 %*
- residing in Norway	*17*	*19*	*89 %*
Unknown	4	1	
Total	**64**	**102**	**63 %**

In 2021, SDRMs from the WHO list were detected in seven samples, which equals 10.9% of the analysed sequences. In total, SDRMs were detected in 4 males and 3 females, corresponding to about 9% and 15 % of the analysed samples from 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, 7.8% had SDRMs associated with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), and 3.1% with nucleoside reverse transcriptase inhibitors (NRTIs). None of the sequences had SDRMs associated with resistance to protease inhibitors (PIs), as shown in Figure 4.3.

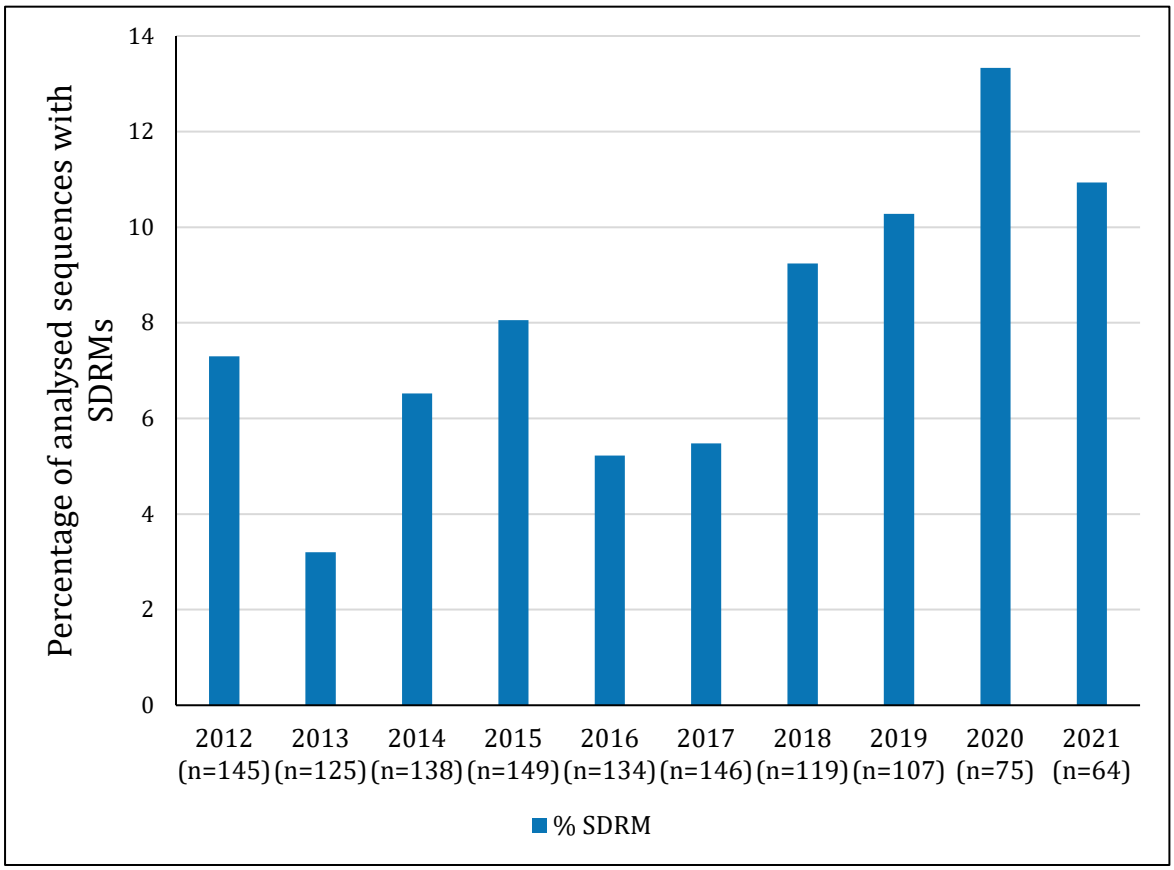

Figure 4.2: Percentage of analysed sequences with detected surveillance drug resistance mutations (SDRMs).
Percentages of the analysed sequences containing one or more SDRMs through the years 2012-2021 are shown as blue columns. There may be several SDRMs per sequence. n = number of sequences analysed for pre-treatment resistance.

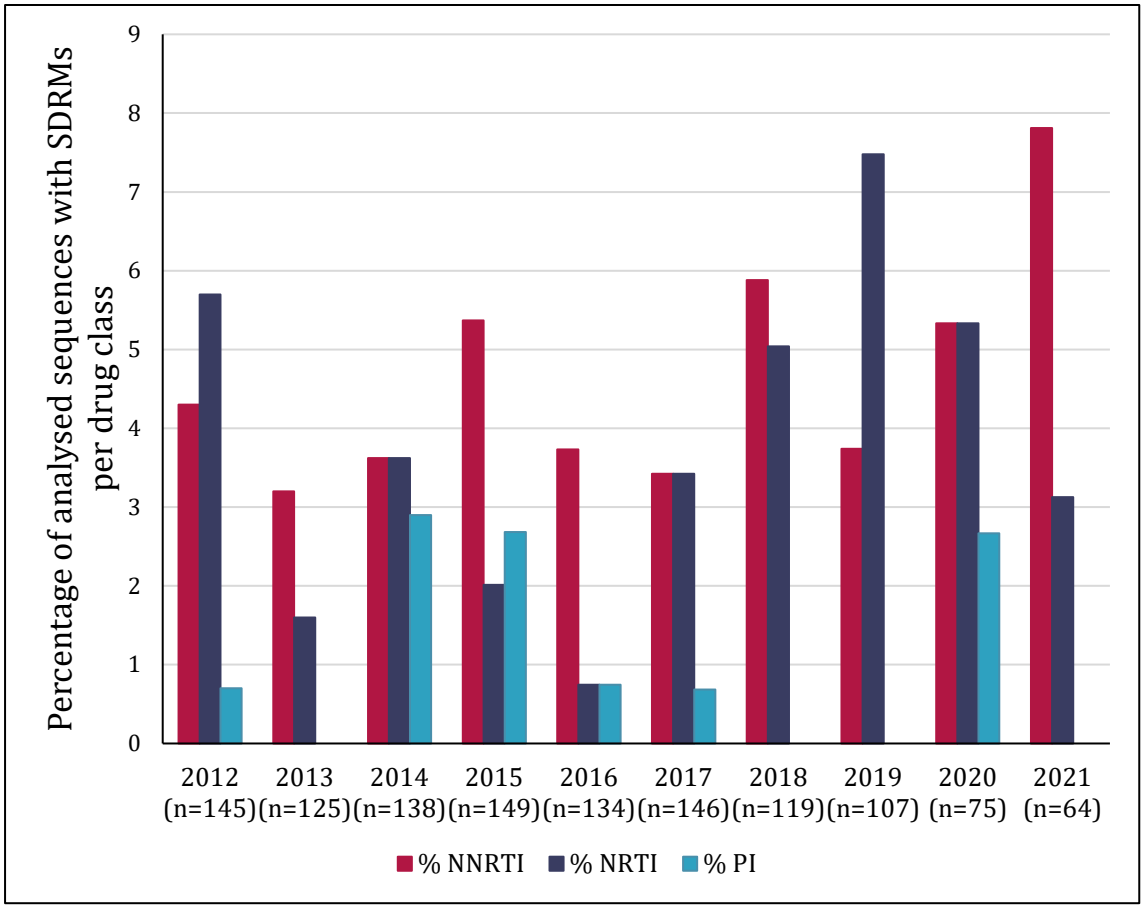

Figure 4.3: Percentage of analysed sequences with detected SDRMs per drug class.
Percentage of mutations affecting the individual drug classes are shown as colored bars; non-nucleoside reverse transcriptase inhibitors (NNRTIs) in red, nucleoside reverse transcriptase inhibitors (NRTIs) in dark blue, and protease inhibitors (PIs) in light blue. n = number of sequences analysed for pre-treatment resistance.

The individual mutations are specified in Table 4.3 for the seven patients with detected SDRM, along with country of transmission. Two patients (29%) were infected in Norway, and five (71%) were infected abroad. Both patients infected in Norway were men who have sex with men (MSM), while the patients infected abroad were infected through heterosexual- or mother-to-child transmission. Each of the five NNRTI-mutations is considered to be of clinical significance, whereas neither of the two NRTI-mutations detected confers reduced susceptibility to any of the drugs currently in use.

Table 4.3: Specification of the surveillance drug resistance mutations (SDRMs) detected in 2021.

Sekvens	NRTI	NNRTI	PI	Country of transmission
1	None	K101E	None	Norway
2	None	K101E	None	Norway
3	None	K103N	None	Abroad
4	T69D	None	None	Abroad
5	M41L	None	None	Abroad
6	None	Y188L	None	Abroad
7	None	Y188L	None	Abroad

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

Discussion

The surveillance is based on resistance data from patients who have 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. The data reported for 2021 have been cross-referenced to epidemiological data from MSIS, enabling 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.

In 2021, resistance data was available for 63% of the newly diagnosed patients reported to MSIS during that year. The coverage of resistance testing among newly diagnosed HIV patients was high among patients living in Norway at the time of infection, both among persons infected in Norway (87%) and persons infected abroad (89%). These numbers indicate that the local routines for submitting samples for resistance testing in newly infected patients in Norway are adequate. The coverage among persons infected abroad before arrival in Norway was only 45%, but it is important to remember that the data from MSIS includes patients that will never be included in the resistance surveillance, such as patients already receiving treatment or persons only temporarily residing in Norway.

The total number of new HIV-infections in Norway has been lower in 2020 and 2021 compared to previous years, probably influenced by travel restrictions and other COVID-19 containment measures during the pandemic. Consequently, the number of samples analysed for drug resistance surveillance, was also lower in 2021 compared to the years before 2020.

Overall, SDRMs were detected in 10.9% of the sequences analysed, thereby breaking the increasing trend observed over the last three years. Similar to previous years, the detected mutations with the most clinical impact such as K103N or Y188L, were found in samples from patients infected abroad, but two cases were infected in Norway with strains containing K101E, underscoring the importance of continued surveillance.

Since pre-exposure prophylaxis (PrEP) with tenofovir and emtricitabine was implemented with full reimbursement in Norway in 2017, there has been an enhanced surveillance of the mutations associated with reduced susceptibility for the two drugs used for PrEP. In

2021, none of the detected mutations are associated with reduced susceptibility for emtricitabine or tenofovir. This means that PrEP can be expected to be effective in preventing new cases. So far there are no signs of an increase in drug resistance associated with PrEP among patients newly diagnosed with HIV in Norway. Continued monitoring of possible PrEP-related resistance will be of importance.

For the first time, we present an overview of the subtypes identified in the analysed sequences. As expected, subtype B was the most common subtype detected among patients infected in Norway in 2021, but interestingly, CRF01_AE was more prevalent than subtype B in 2019 and 2020. Yearly surveillance of subtype distribution among patients infected in Norway and comparison with previous years, may be a useful tool for monitoring the dynamics of the epidemic in Norway.

Conclusions

There does not seem to be any increase in transmission of PrEP-associated resistance mutations, even after four years with widespread use of PrEP. Although the prevalence of transmitted drug resistance remains low in Norway, continued surveillance of HIV-1 drug resistance over time is important. High coverage of resistance testing in patients newly diagnosed with HIV-infection in Norway indicate that local routines for resistance testing in Norway are adequate.

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Drug resistance testing in the clinical management of HIV-infection

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The use of HIV drug resistance testing has become an essential tool in the clinical management of patients with HIV-infection. According to the Norwegian guidelines (1) , the two main indications for resistance testing are baseline testing in newly diagnosed individuals, and resistance testing at the time of virological failure. In addition, it is also recommended to perform resistance testing in pregnant women without full viral suppression, and in the source patient of needle stick injuries in order to guide post-exposure prophylaxis.

Resistance testing in newly diagnosed

Baseline HIV drug-resistance testing shortly after diagnosis serves two purposes: 1) basis for selection of the initial antiretroviral therapy (ART) regimen, and 2) epidemiological surveillance of transmitted HIV drug resistance. However, there is no need to wait for the resistance testing results before ART is initiated; the prevalence of transmitted drug resistance against the recommended starting regimens is very low, and the regimen may also be modified once the results are reported. Furthermore, most initial regimens are based on integrase strand transfer inhibitors (INSTI), which are not included in routine resistance testing due to the extremely low level of transmitted INSTI resistance (2). Over the last two decades, there has been a decreasing trend in the prevalence of transmitted drug resistance in Europe (3;4). Although the clinical value of baseline testing is limited, it is important for surveillance purposes, and for identification of the few individuals with virus harboring resistance mutations of clinical relevance.

Resistance testing in viral failure

HIV drug-resistance testing is recommended in patients with virological failure to guide the selection of active drugs when changing ART regimens. For patients currently or previously receiving an INSTI-based regimen, resistance testing for INSTI should be included in the analysis.

In the setting of virological failure, it is important that drug-resistance testing is performed while the patient is still taking antiretroviral drugs. Certain mutations may not be detected when there is no selective drug pressure, because the fitness advantage of resistant virus will disappear, and wild type virus with superior viral fitness will dominate the viral population. Previous mutations may however be archived in silent reservoirs and be reactivated due to changes in the selection pressure such as switch of therapy. Therefore, results of historical resistance testing are useful for clinicians when considering switching ART. According to the European guidelines (5), it is recommended to include at least two and preferably three active drugs in the new regimen based on resistance mutations present in current and earlier genotypic analyses. Two active drugs might be sufficient, but that implies that *the new regimen must be based on a fully active either boosted protease inhibitor or an integrase inhibitor with high genetic barrier*. The importance of this is illustrated by the case study below.

Case study

A male patient born in West-Africa in the 1960's moved to Norway at the age of 23 and was diagnosed with HIV shortly after arrival. The patient had several co-morbidities, including hypertension, hypercholesterolemia, and core-alone Hepatitis B. He received ART from 1998 to 2002, before he returned to his home country. When moving back to Norway in 2004, ART was resumed. The patient received several different ART achieving full viral suppression most of the time (Figure 4.4), but with a few exceptions:

- *In December 2007 when receiving tenofovir + emtricitabine + efavirenz, HIV RNA was found to be 970 copies/mL. The resistance mutations detected conferred reduced susceptibility to NRTI (V75L, V118IV, M184V) and NNRTI (K103N, V108I).*
- *In June 2014 on a rilpivirine-based regimen, HIV RNA was found to be 220 cp/mL.*

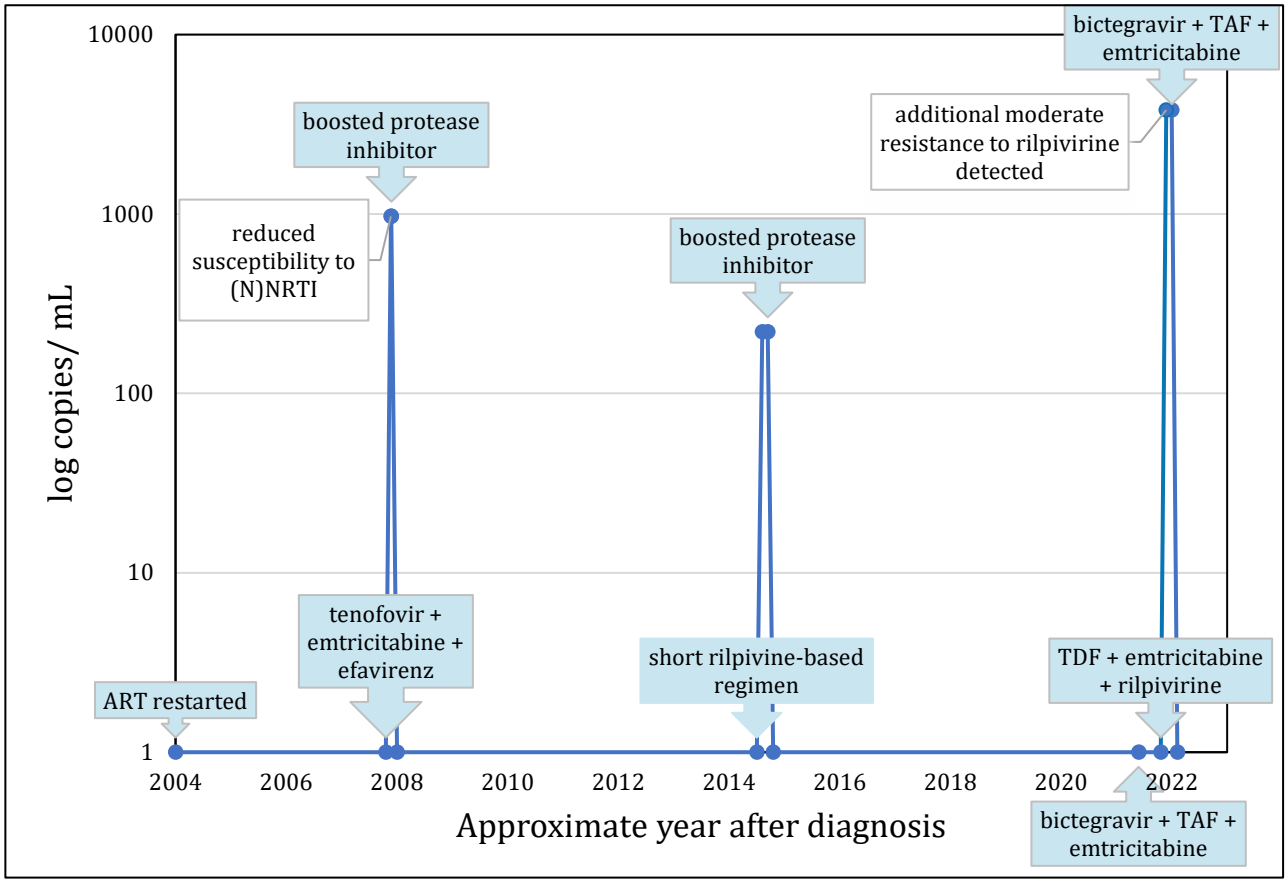

Figure 4.4: Timeline for HIV RNA copy number and antiretroviral therapy
The figure illustrates an approximate timeline for virus copy numbers relative to treatment after diagnosis for the case discussed in the text. Detected resistance to antivirals are indicated in the white boxes. ART= Antiretroviral therapy, TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide, (N)NRTI = (Non-) Nucleo(s/t)ide-analogue reverse transcriptase inhibitors.

After the virological failure in 2007 he was put on a boosted protease inhibitor, and full viral suppression was again achieved. The rilpivirine-based regimen in 2014 was for just a short period and was followed by a boosted protease inhibitor-based regimen from 2014 to June 2021, on which he also obtained full viral suppression. Due to patient-wish (One pill daily), the ART regimen was then changed to bictegravir + tenofoviralafenamide (TAF) + emtricitabine. In October 2021, the patient was concerned about weight gain after the switch to an integrase inhibitor-based regimen, and also the lipid profile was more unfavorable.

Thus, the treating physician thought that the TAF + bictegravir combination could be responsible for both the weight gain and the lipid changes. Based on patient-wish of one tablet daily, and the wish to avoid integrase inhibitors as well as TAF, a NNRTI-based, one-tablet-daily regimen was considered.

The mutations found in the genotypic resistance test performed during the virological failure in 2007 was updated in the Stanford HIV drug resistance database by a new search in October 2021, giving the following result:

High level resistance: Lamivudine, emtricitabine, efavirenz and nevirapine.
Low-level resistance: Abacavir and doravirine.
No resistance found for any other current drugs, including rilpivirine and tenofovir.

The regimen was switched to the one-pill combination of tenofovir disoproxil + emtricitabine + rilpivirine in October 2021. Four weeks after this switch he was found to have virological failure; HIV RNA was 3800 copies/ml. A new genotypic resistance test on this sample showed the same resistance pattern as the sample from 2007, but with one important addition, a moderate resistance to rilpivirine was detected.

Thus, a change in the resistance mutations had occurred, giving indeed significant rilpivirine-resistance in addition to the archived mutations found in 2007 that included high level resistance to emtricitabine. The patient now only had one fully active drug (tenofovir disoproxil), and therefore the virological failure was inevitable. The patient´s compliance with the drug regimen was considered to be excellent.

He then was switched back to bictegravir + tenofoviralafenamide + emtricitabine, and shortly thereafter, full viral suppression was again accomplished. According to the resistance interpretation of the last resistance analysis from 2021, the salvage regimen contained a fully active integrase inhibitor (bictegravir), a fully active NRTI (TAF), and an NRTI with high-level resistance. Thus, this regimen fulfilled the criterion of two active drugs, where one of those is an integrase inhibitor with high genetic barrier. The patient was once again in complete virological suppression.

Learning points:

In the case study described above, switching to a regimen with two fully active drugs based on a NNRTI with low genetic barrier like rilpivirine, led to development of virological failure after only 4 weeks. Even after the long period of virological suppression from 2007 to 2021, these archived mutations proved to be clinically relevant. On one occasion in 2014, he had a virological failure during a short period on a rilpivirine based regimen. Unfortunately, no resistance test was performed. Thus, it is possible that more NNRTI mutations were archived in addition to the ones found in 2007. Resistance testing performed during previous periods of virological failure is the key to reveal old mutations that might be of future clinical significance. Sequencing proviral DNA for the purpose of better detecting archived drug resistance mutations is technically possible, but the clinical relevance is still under investigation (6;7).

In conclusion, HIV drug resistance testing remains a cornerstone of ART, and resistance analyses and clinical interpretation of the results are crucial tools for both prevention and management of virological failure (8). Although drug resistance is rare, the consequences for individual patients and their future treatment options may be substantial. It is important that clinicians maintain focus on the risk of drug resistance in the clinical

management of patients with HIV-infection and stay updated on current guidelines and recommendations.

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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 Sanger sequencing of the RT domain of the HBV polymerase (P) gene. The sequences are analysed for amino acid substitutions 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 preferable 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 methods

The surveillance of HBV resistance in Norway aims to monitor two populations; i) samples submitted for drug resistance testing primarily in relation to treatment failure (acquired resistance) and ii) samples submitted for HBV genotyping in the course of diagnostic investigations, generally prior to treatment. Monitoring of the latter population can therefore be regarded as surveillance of primary resistance. Mutations altering amino acids in specific positions within the polymerase gene can give rise to resistance to the various antivirals for the treatment of HBV.

Surveillance data 2021

The resistance mutations detected in Norway between 2017 and 2021 are presented in Table 5.1

Table 5.1: Resistance mutations in samples submitted for HBV drug resistance testing in 2017 - 21.

HBV-variants resistant to antivirals	Drug resistance	2017	2018	2019	2020	2021
Total analysed		23	20	14	14	17
M204V + (I169T ᵛ S202G ᵛ T184A/S ᵛ M250V) ±L180M	**LAM (R), LDT (R), ETV (R)**	1	4	1	1	3
A181V/T + M204I/V ± L180M	**LAM (R), LDT (R), ETV (I), ADV (R)**					2
M204V + L180M	**LAM (R), LDT (R), ETV (I)**	1				
Unknown mutation in rated position			1[a]	1[a]		

LAM: lamivudine; LDT: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; R: resistant; I: intermediate; S: sensitive.
[a] A181S

In 2021, a total of 134 samples were analysed for HBV drug resistance mutations. Of these, at least 17 samples were submitted for resistance testing due to virological failure, mainly from patients on treatment and with suspected drug resistance. Drug resistance mutations were detected in five of the samples, and four of them were from patients on entecavir (ETV) treatment (Table 5.2). In addition, one sample (sample 4) from a patient with previous lamivudine (LAM) treatment, now on tenofovir (TDF), had multiple drug resistance to LAM, telbivudine (LDT), and ETV, but was sensitive to TDF and adefovir (ADV). No drug resistance mutations were detected in patient samples submitted for HBV genotyping only (N=117).

Table 5.2: Resistance mutations detected in samples from 2021 and the drug resistance they confer

Sample	Resistance mutations detected	Treatment*	Resistance				
			LAM	LDT	ETV	ADV	TDF/TAF
1	180M + 204V + 184S	ETV	R	R	R	S	S
2	180M + 204V + 202G	ETV	R	R	R	S	S
3	180M + 204V + 181V	ETV	R	R	I	R	S
4	184A + 204V	TDF	R	R	R	S	S
5	204I + 181T	ETV	R	R	I	R	S

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

Conclusions

The number of samples with drug resistance remains low in 2021. Drug resistance mutations were found only in samples from patients on antiviral treatment, and no resistance mutations were detected in the samples submitted for genotyping. All mutations detected give resistance to multiple drugs and were mainly detected in patients on entecavir treatment.

Tenofovir has been the primary drug of choice and was used for treatment of almost 1000 patients in 2021, whereas entecavir was prescribed for approximately 500 patients. However, entecavir is now the recommended first line therapy by The Norwegian Hospital Procurement Trust (Sykehusinnkjøp HF) (1). This calls for intensified awareness of drug resistance due to the lower genetic barrier of entecavir compared to tenofovir.

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Drug resistance testing in the clinical management of HBV-infection

Dag Henrik Reikvam, senior consultant, Department of Infectious Diseases, Oslo University Hospital and associate professor, University of Oslo, and Anne-Marte Bakken Kran

Case study

A man in his mid-30s was diagnosed with chronic HBeAg positive hepatitis B virus (HBV) infection with genotype D at a routine check-up when he immigrated from East-Africa in 2002. He had no additional risk factors for liver complications and was followed up without antiviral treatment until 2011, when transient elastography showed liver stiffness 9,3 kPa indicating significant fibrosis.

With HBV DNA 10 000 000 IU/ml he subsequently started emtricitabine and tenofovir disproxil fumarat (TDF). When one year later HBV DNA was below detection limit, the treatment was simplified to TDF as monotherapy (Figure 5.1). The following years the patient's adherence to the medication was inconsistent, reportedly due to various side effects to TDF, with relapsing viremia. An HBV resistance analysis in 2014 did not reveal any resistance mutations.

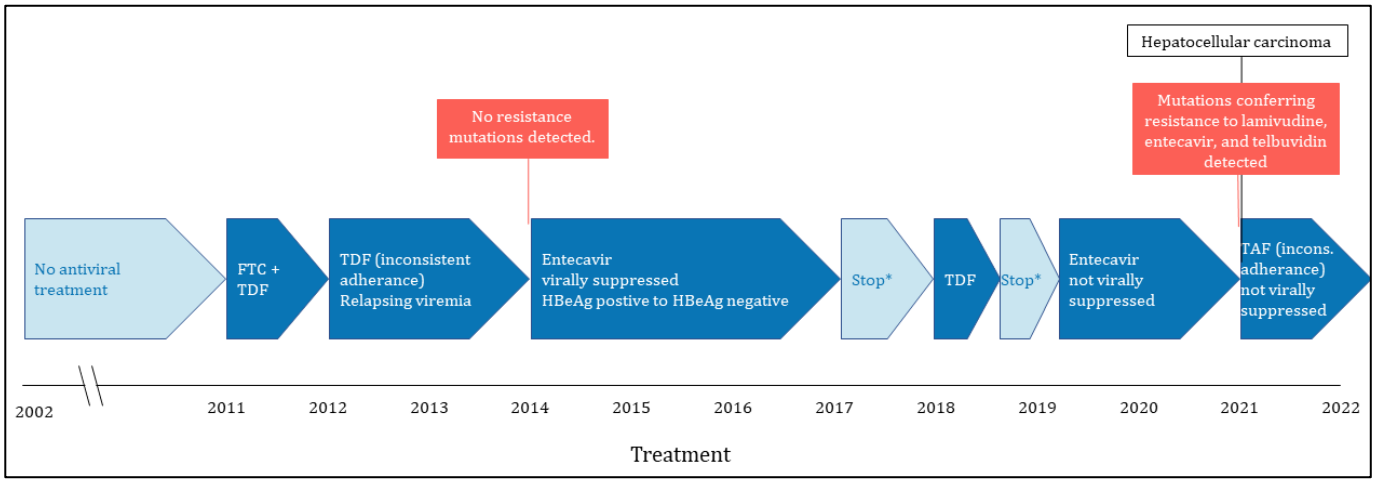

Figure 5.1: Timeline for antiviral treatment
The figure illustrates an approximate timeline for the antiviral treatment described in the case report. Occurrence of resistance mutations are indicated in the red boxes. FTC = Emtricitabine, TDF = tenofovir disproxil, TAF = tenofovir alanfenamide. * Patient's decision

Still, due to the side effects, TDF was stopped and entecavir started in 2014. The patient now consistently adhered to entecavir and was virally suppressed until 2017, when he for unknown reasons stopped taking the medicine. During the same time span, he converted from HBeAg positive to HBeAg negative status.

Antiviral medication was resumed in 2018, now again with TDF due to a more favourable cost of medicine. A year later the patient had once again stopped taking TDF due to side effects, and his HBV DNA load surged to 160 000 000 IU/ml in 2019. Once again, entecavir was re-started, but this time the patient was never fully virally suppressed with HBV DNA levels fluctuating between 220 and 2200 IU/ml. A new resistance analysis in 2021 detected mutations rtL180M, rtM204V, and rtT184S conferring resistance to lamivudine, entecavir, and telbuvidin. The same year he was diagnosed with and operated for hepatocellular carcinoma, underscoring the strong indication for antiviral HBV treatment.

The antiviral regimen was changed to tenofovir alanfenamide (TAF) in 2021, with HBV DNA at 8300 IU/ml. Still, the adherence was suboptimal and HBV DNA levels kept fluctuating between 21 and 11 000 IU/ml. A recent resistance analysis confirmed the presence of the three mutations detected in 2021 but discovered no new mutations, concluding that according to genotyping, the virus should be susceptible to tenofovir (TDF and TAF).

Learning points

This case raises several questions and concerns with regard to viral resistance in HBV. There are no certain answers to these questions, but they are still relevant to discuss.

Could the entecavir resistance have developed already during 2014-2017 when, according to routine HBD DNA quantification assays, he was virally suppressed?

Drug resistance develops under low-level viral replication in the presence of antiviral drugs, and the introduction and selection of drug resistance mutations in virologically suppressed patients is therefore unlikely. However, routine measures of viral load may not always reveal short periods of low-level viremia occurring between samplings, and early signs of entecavir-resistance in this period cannot be ruled out. Nevertheless, the development of clinically significant drug resistance will eventually lead to a detectable increase in viral load.

Should resistance analysis always be performed prior to initiating antiviral therapy?

Both tenofovir and entecavir have high genetic barriers for development of resistance and are very efficient inhibitors of viral replication. In HBV-infection, drug resistance in treatment-naïve patients is extremely rare. Although there are reports of pre-existing mutations (1), single mutations are not necessarily sufficient to cause drug resistance, and the clinical significance of their presence is still uncertain (2). In the case study presented above, even a resistance test after three years of treatment did not reveal any mutations. Thus, the clinical benefit of routine baseline resistance testing would probably be limited and not cost effective.

Entecavir is considered to have a high genetic resistance barrier, but nevertheless, the patient developed resistance. Can entecavir be considered equivalent to tenofovir, for which no resistance is yet reported?

Entecavir or tenofovir in monotherapy are both recommended as first line therapies. Tenofovir is the only oral antiviral with no evidence of genotypical resistance development during monotherapy treatment of HBV-infection (3), and one might therefore consider TDF superior to other drugs in regard to its resistance profile. However, for entecavir, drug resistance is also rare, and only a few cases are reported with ETV-resistance after complete viral suppression (4). As poor adherence increases the risk of developing drug resistance, TDF might be the preferred option when there is reason to suspect impaired adherence to treatment.

Can we be sure that the patient's current low-level viremia is due to inadequate therapy compliance and not reduced susceptibility caused by a yet unrecognized resistance mutation to tenofovir?

Genotypic resistance testing depends on detection of mutations known to be associated with reduced susceptibility for a particular drug. For the patient described, full viral suppression was not achieved in spite of complete susceptibility to tenofovir. There is always a risk that unrecognized additional mutations exist, that are capable of causing

drug resistance. It is therefore important that studies on drug resistance are performed regularly, and that viral strains from patients with treatment failure are investigated and further characterized.

In conclusion, although cases of drug resistance in chronic HBV-infection are rare, they often have substantial consequences for the patients affected. These cases are important reminders that patients should be cautiously monitored for drug resistance, especially if their adherence to medication is inconsistent.

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6 Human herpes viruses: Cytomegalovirus

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 methods

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. Therefore, as a standard, the UL97 gene is investigated first. For patients treated with ganciclovir alone, the UL54 gene is analysed only if resistance mutations are first detected in the UL97 gene. Foscarnet and cidofovir resistance is conferred by mutations in the UL54 gene, and both genes are always investigated in samples from patients treated with these drugs.

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 developing drug resistance. Resistance mutations usually occur 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 2021

In 2021, only 19 samples were analysed for CMV antiviral drug resistance. Out of the 19 samples, relevant resistance mutations were detected in five samples as shown in table 6.1. The mutations detected are listed in Table 6.2.

Most of the resistance mutations detected that affect ganciclovir susceptibility, are located within the interaction region for ganciclovir in the kinase domain (1), and many of them clustering within a small region ranging from positions 590 to 607 in the UL97 (2) . The mutations detected all confer low or moderate resistance, but in immunosuppressed patients, even low-grade resistance to an antiviral drug may be of clinical importance.

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 - 2020.

CMV-variants resistant to antivirals	2017	2018	2019	2020	2021
Total samples analysed	32	21	21	30	19
Number of samples with CMV resistance mutations	7	4	6	5	5
Samples with UL97 mutations	7	2	6	4	5
Samples with UL54 mutations	1	2	2	1	1

UL97 encodes the viral kinase. UL54 encodes the viral DNA polymerase.

Table 6.2: CMV resistance mutations in samples tested in 2021

Patient	UL97 mutations	UL54 mutations	Resistance
1	T409M		Maribavir moderate
2	K599R		Ganciclovir low
3	C607Y		Ganciclovir moderate
4	A594V		Ganciclovir moderate; cidofovir low.
5	A594V, M460V	P522S	Ganciclovir moderate. Analysis failed for cidofovir and foscarnet.

UL97 encodes the viral kinase. UL54 encodes the viral DNA polymerase.

Conclusions

Despite an increase in the use of ganciclovir for therapeutic and prophylactic treatment of CMV-infections, drug resistance mutations are only rarely detected. However, in immunosuppressed patients with CMV-infection, early discovery of antiviral drug resistance can be of vital importance. Therefore, the reference laboratory encourages clinicians and laboratories to consider drug resistance testing in cases with suspected treatment failure, and to lower the threshold for submission of samples for drug resistance analysis from patients at risk.

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Drug resistance testing in the clinical management of CMV-infection

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In solid organ transplant (SOT) recipients, cytomegalovirus (CMV) infection or disease is associated with increased morbidity and mortality. Early post-engraftment, the most important risk factor for development of CMV disease in SOT recipients is mismatching CMV serology (positive donor-> negative recipient; CMV D+/R-). Other risk factors are related to the use of specific immunosuppressive drugs such as ATG/Rituximab. Active early post-transplant CMV surveillance is mandatory, and in kidney-transplant recipients, CMV PCR analysis is performed at least once a month during the first year following engraftment.

Treatment options and drug resistance

The anti-CMV agents currently available in Norway are effective but are often limited by their toxic effects, including myelosuppression (ganciclovir/valganciclovir), nephrotoxicity (foscarnet/cidofovir) and electrolyte imbalance (foscarnet). Ganciclovir, foscarnet and cidofovir are only available i.v. whereas valganciclovir is available in an oral formulation. Therefore, first-line treatment in SOT recipients for CMV re-activation or infection is oral valganciclovir, dosed according to creatinine clearance calculated by the Cockcroft and Gault formula (G&C).

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. Resistance towards foscarnet and cidofovir is conferred by mutations in the UL54 gene. Thus, if the patient only has received ganciclovir, the current procedure in the National Reference laboratory for CMV is to first analyse for UL97 resistance and subsequently analyse UL54 if resistance mutations are found in UL97.

In CMV negative kidney transplant recipients receiving a CMV positive organ, 6 months of valganciclovir prophylaxis is recommended. After completion of antiviral prophylaxis many transplant recipients develop late onset CMV disease. Some SOT recipients experience infection with drug-resistant CMV, which is often associated with prolonged hospitalization and poor outcome. Detection of drug resistance, optimized clinical management, and improved outcome require knowledge and awareness from the treating physician, and close collaboration with the laboratory performing the CMV analysis and the involved virologists. Some of the challenges one encounters is exemplified with the following case, which is currently ongoing.

Case study

A 65 year old male with an IgA-nephritis, in dialysis for 2 years, was transplanted with a kidney from a deceased donor in the autumn of 2021. There was a CMV donor positive to recipient negative constellation (CMV D+/R-), and according to national practice he therefore should receive oral valganciclovir prophylaxis dosed according to renal function, calculated by the Cockcroft & Gault formula (C&G) for 6 months. Maintenance immunosuppression consisted of prednisolone, tacrolimus (Prograf®, trough levels 4-7 µg/L), and mycophenolate mofetil (CellCept®, MMF). All kidney transplant recipients also receive 1 tablet/day of trimethoprim-sulfametoksazol for 6 months as Pneumocystis jirovecii prophylaxis.

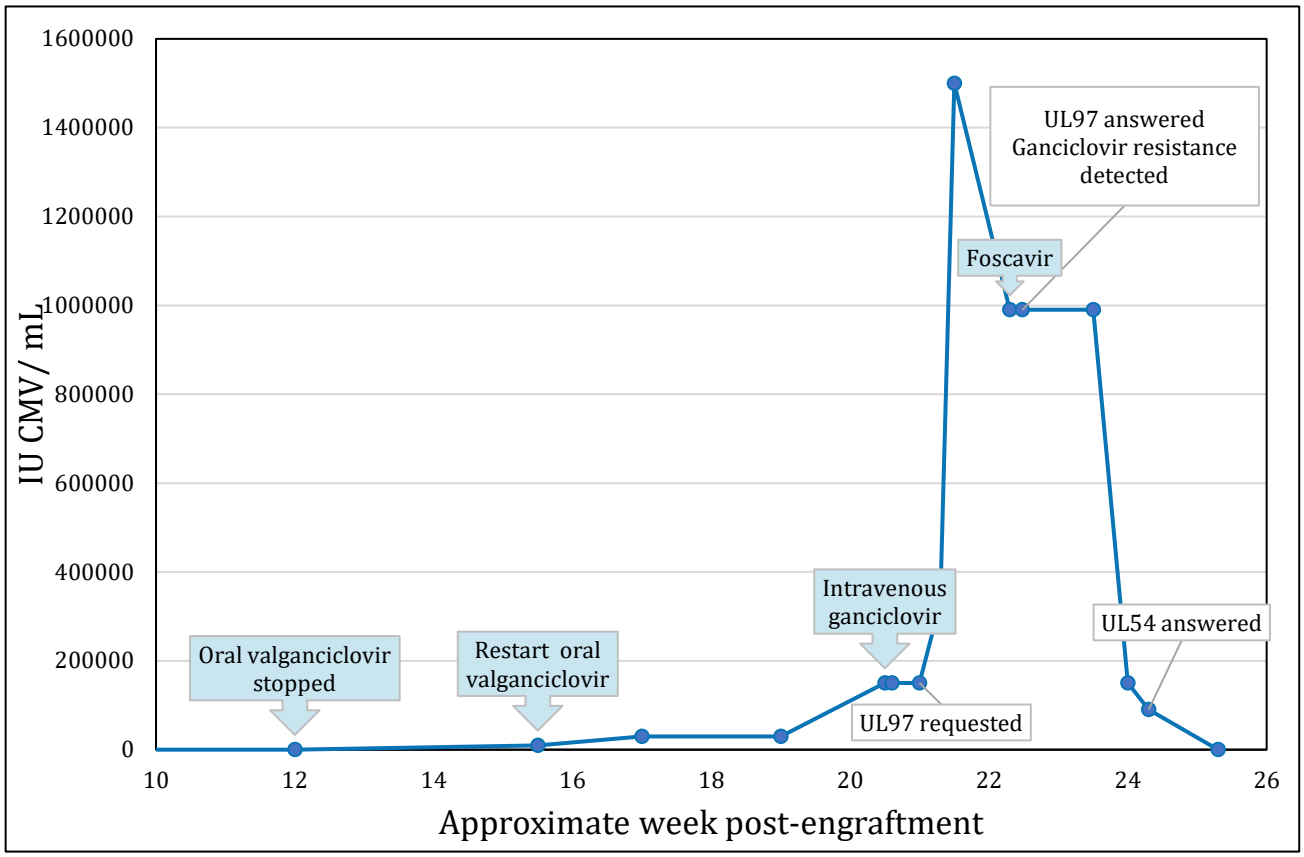

Figure 6.1: Timeline for CMV viral load and antiviral treatment
The figure illustrates a timeline for the CMV viral load and antiviral treatment post-engraftment for the current case study. Blue arrows indicate changes in medication, while resistance analyses are indicated in white boxes. UL97: CMV kinase; UL54: DNA polymerase. The timeline in the figure starts at 10 weeks post-engraftment as the weekly CMV PCR tests remained negative prior to this.

One month after engraftment he was hospitalized due to diarrhoea. General screening was performed including a colonoscopy with biopsies (CMV-colitis/MMF-colitis?). All tests came out negative or normal, and the diarrhoea disappeared. Initially there were circulatory challenges with the transplanted kidney, but eventually the graft function stabilized with creatinine of 250 µmol/mL. Weekly CMV PCR tests remained negative, as did a CMV-IGRA after 8 weeks. Three months post-engraftment he developed mild COVID-19 that was treated with sotrovimab and reduction of MMF (according to national guidelines). He also had leucopenia (total leucocytes of 1.6 x 10^9/L), and trimethoprim-sulfametoksazol and valganciclovir were therefore temporarily paused. Shortly after this he was again

hospitalized with an upper respiratory infection thought to be caused by bacteria and treated with ciprofloxacin i.v./oral.

After discharge from the hospital, his CMV PCR test turned positive (9900 IU/mL) and oral valganciclovir was reinitiated (Figure 6.1). The patient then developed diarrhoea, but did not want to be hospitalized. During ongoing valganciclovir-treatment, the plasma level of CMV DNA initially stabilized at around 30.000 IU/mL, and clinically the patient was improving. Four weeks after reinstitution of valganciclovir there was, however, an increase in CMV viral load (150.000 IU/mL) and treatment was switched form oral valganciclovir to intravenous ganciclovir to ensure that the antiviral therapy was sufficiently dosed. A ganciclovir resistance analysis was requested. Intravenous ganciclovir was continued (dosed according to C&G) and MMF was paused. While waiting for the resistance analysis, the CMV viral load continued to increase. Treatment was therefore switched to foscavir (complicated dosing due to reduced graft function/nephrotoxicity), anticipating development of resistance to ganciclovir. This was verified two weeks later by detection of a UL97/L595S mutation. Subsequently the UL54 gene was also analysed for resistance mutations. No resistance mutations were found in UL54.

Learning points

Temporary pausing (for any reason) of oral valganciclovir in CMV negative recipients receiving a CMV positive organ often leads to CMV-infection and disease.

In cases where the resistance mutations indicate that there is only a partial resistance, treatment with intensified dosing of valganciclovir is an option. The myelosuppressive properties of valganciclovir often lead to “under dosing”, and not all clinicians are aware of the fact that dosing should be done by calculating creatinine-clearance according to C&G (and not eGFR available from the lab). Measurements of plasma valganciclovir-concentrations were previously performed at OUH-Rikshospitalet and should be re-introduced as a clinical tool to help improve treatment of CMV-infection and disease. Sub-optimal plasma level of ganciclovir is a risk factor for selection of resistance mutations.

Ganciclovir-resistance is usually first suspected when there is an increase in viral load or a measurement of very elevated plasma CMV DNA during ongoing valganciclovir treatment. Sometimes resistance analyses may also be indicated when there is a persistent, long-lasting low-grade viremia under ongoing valganciclovir-treatment. It takes time from the analysis is requested until the CMV- resistance analysis report is available. Depending on the clinical situation of the patient, foscavir must often be started before the result from the lab is available.

Due to the delay, it is possible that the clinicians should order the analysis at an earlier time-point. However, such a strategy could result in an increase in unnecessary resistance analyses, as concomitant immunosuppression and not viral resistance often cause delayed antiviral response.

Increased awareness of resistance and optimization of sampling logistics and analytical-turnaround time for both CMV-DNA quantification and the resistance testing are essential and could reduce delay in the detection of antiviral drug resistance.

Human herpes viruses: Herpes simplex virus

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 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 methods

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 about the patients' immune status is not available for surveillance purposes. For routine testing, only genotypic tests are applicable.

Surveillance data 2021

In 2021, five samples from five patients in Norway were analysed for HSV drug resistance. In the five samples, one resistance mutation and one deletion were recorded as shown in Table 6.3. One sample had a mutation of unknown significance in the DNA polymerase. In two samples, no resistance mutations were detected.

Table 6.3. HSV resistance associated mutations

Sample	HSV-type	Sample material	TK mutations	DNA pol mutations	Aciclovir susceptibility
1	HSV-2	Genital secretion		K653T	Unknown significance
2	HSV-2	Secretion, unspecified	L98stop (del nt 280)		Resistant
3	HSV-1	Secretion, unspecified	R281stop		Resistant

Both the deletion and resistance mutation detected in the thymidine kinase gene lead to premature stop codons and were associated with aciclovir resistance (1). The L98stop codon is caused by a deletion that confers resistance to aciclovir, but has been shown to remain susceptible to foscarnet (1). The R281stop has been shown to confer resistance to aciclovir, brivudin and penciclovir, but is sensitive to cidofovir and foscarnet. The third

sample had a mutation (K653T) in the DNA polymerase gene. The clinical significance of this mutation is unknown, however, amino acid substitutions in the non-conserved region 628-698 have been shown to be relevant (1).

Conclusions

The consumption of aciclovir/valaciclovir for both therapeutic and prophylactic treatment has continued to increase the past five years. Treatment failure is rare and resistance to aciclovir appears to be uncommon. However, the data are scarce and there is no systematic surveillance of drug resistant herpes simplex virus.

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Drug resistance testing in the clinical management of genital HSV-infection

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Genital herpes infection is usually caused by herpes simplex-virus type 2 (HSV-2), but type 1 (HSV-1) may be the cause in about one-third of cases. Herpes virus infections are the most frequently treated viral infections in Norway in terms of number of individuals receiving a prescription. In 2016, the World Health Organization estimated that approximately half a billion people worldwide had genital infection with HSV (type 1 and/or type 2), and that the global prevalence of HSV-2 was 13.2 % in the population aged 15-49 years (5.3 % and 10.7 % in European males and females, respectively)(1). A study among Norwegian blood donors and pregnant women revealed a HSV-2 prevalence of 7% and 14 %, respectively (2) whereas a Finnish study detected HSV-2 seropositivity in 12.3 % of pregnant women and in 7.5 % of their spouses (3). Studies report seroprevalences of HSV-2 in people living with HIV to range between 60-95 % (4).

Clinical course and presentation including symptoms may vary: In general, HSV-2 infections have a greater tendency to be recurrent and to require antiviral therapy, and immunocompromised individuals will more often suffer from frequent reactivations and/or protracted courses. Therefore, a laboratory confirmed type specific diagnosis is recommended to guide clinicians in therapeutic strategy and advice to patients.

In Norway, first line medication in treatment of genital herpes is valaciclovir or aciclovir (5). Episodic treatment may be the first choice (6). In patients experiencing severe and frequent episodes (more than 6 recurrences a year), daily suppressive therapy is recommended. More recently, studies have shown that also patients with milder spectrums of disease may benefit from suppressive therapy with a reduced rate of recurrence.

Clinically refractory genital herpes in immunocompetent individuals is rare. However, in severely immunocompromised individuals it can be a major problem, including patients in late-stage HIV infection, patients with immune reconstitution inflammatory syndrome (IRIS) following combination antiretroviral therapy, and in organ transplant recipients. Today, seropositive patients undergoing allogenic stem-cell transplantation are given post-transplantation prophylaxis. Patients undergoing treatment for acute lymphocytic leukemia are routinely given valaciclovir for three months (6).

When should drug resistance be suspected?

A suboptimal response to therapy may raise the question about treatment failure, particularly in immunocompromised patients.

Drug-resistant infection should be suspected among patients with documented HSV-infection who have minimal improvement within a week (7-10 days) of appropriately dosed antiviral therapy, where lesions increase in size or do not heal.

Aciclovir resistance is most commonly (95 % of cases) due to one or more mutations located in the gene encoding HSV thymidine kinase (TK), which is involved in the phosphorylation of aciclovir to its active form, resulting in a nonfunctional TK or TK with reduced affinity for aciclovir. The remaining 5 % of resistant cases is due to mutations in the viral DNA polymerase gene (7;8). Several resistance mutations in both the TK and the DNA polymerase have been described which can facilitate interpretation of drug resistance testing (9).

Aciclovir resistance is found in less than 1 % of infections in immunocompetent persons. Studies among immunocompromised patients, especially those with/including HIV infected individuals indicate more than 5 times higher prevalence in these groups (10-12).

Case report

A 69- year old man with AIDS on hemodialysis for end-stage renal disease (ESRD), had a long history of recurrent genital and ocular HSV with poor adherence to prescribed aciclovir suppressive therapy, as well as ART. Initial resistance testing of genital HSV-ulcers revealed aciclovir-resistant and foscarnet-sensitive HSV, but due to his ESRD, foscarnet was contraindicated. However, the patient was successfully treated with intra-lesional cidofovir. Repeated testing showed a combination of aciclovir resistant and sensitive strains (13).

This case report highlights the clinical challenges as well as the high burden HSV infection can represent in immunocompromised patients. Whereas the combination of both aciclovir-sensitive and -resistant HSV strains further emphasizes the use of suppressive therapy, this requires good patient adherence to prevent drug resistant strains from developing.

When and why should drug resistance testing be performed?

According to UpToDate (7), drug resistance testing should only be performed in patients with clinical treatment failure in response to nucleoside analogue therapy (14;15).

The impression is that Norwegian clinicians almost never request this analysis, and there is no systematic surveillance. In 2020 and 2021, a total of nine samples from patients with HSV-infections in Norway were sent for drug resistance analysis.

The clinical utility and importance of a test result is however unclear. Firstly, because documentation of HSV-resistant strains does not necessarily predict failure of aciclovir (7). Secondly, it is shown that TK-deficient strains are less virulent (*in vitro*), they usually clear spontaneously without treatment, and there is no documentation of transmission between partners. These strains are also less likely to appear in latent stadium, and therefore subsequent clinical reactivations of genital herpes from drug-resistant strains are not seen (7;16).

Partially resistant strains may be successfully treated with high-dose intravenous aciclovir and other analogues, but fully aciclovir-resistant strains are also resistant to valaciclovir and ganciclovir. TK-deficient strains are susceptible to foscarnet and cidofovir which do not depend upon TK, but instead works by inhibition of viral DNA polymerase, and both are listed as alternatives in case of resistance (6;7).

Today, antiviral susceptibility testing for HSV is not easily accessible, as samples need to be sent abroad (Sweden). Therefore, the clinical response to antiviral therapy is often the preferred way to decide duration and dosage of first line therapy or switching to alternative treatment options. However, for selected groups of patients with defined risk factors, such as in immunocompromised individuals including HIV patients with a history of recurrent HSV infection, the clinical importance of resistance testing may be higher. In immunosuppressed patients having prior treatment history with aciclovir (particularly with suboptimal dosage) and a history of non-healing lesions, the clinicians should consider submitting a sample for resistance testing in subsequent episodes of recurrence.

Is there a risk of resistance emerging in the future and do we need systematic surveillance of drug resistance? There is reason to be cautious because prophylactic use of aciclovir in

the general population is increasing along with low doses in long term suppressive treatments. Also, the very short courses of episodic treatment taken more frequently on patient demands might be considered a risk.

Conclusion

Although risk of development and spread of drug resistant HSV in the general population is low, the individual risk in immunocompromised patients should not be neglected. Very few samples are currently being submitted for resistance testing. Increased awareness among clinicians may contribute to improved management of these patient groups. It is likely that improved availability of HSV drug resistance testing in Norway could lower the threshold for resistance testing. However, with only four or five samples submitted for analyses per year, it might not be worth the effort for a microbiology laboratory to establish the assay. We suggest starting in the other end – by increasing the frequency of resistance testing of infections in patients with risk factors for development of HSV drug resistance.

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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 three different classes: - Nucleotide 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	Next generation sequencing of the complete HCV genome based on probe enrichment. This method can be used for genotyping, as well as detection of RASs. The sequences are analysed using HCV-GLUE (1;2). In Norway, HCV drug resistance testing is only available 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. • New cases of HCV infection. • 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 considered for treatment with sofosbuvir + velpatasvir. • Patients with decompensated cirrhosis when liver transplantation is not an option.
Surveillance	A systematic surveillance system for newly diagnosed HCV infections was launched in May 2022, and data will be published in the 2022 report.

Surveillance methods

The plan for implementing a surveillance system for HCV drug resistance in Norway in 2021 was postponed due to the SARS-CoV-2 pandemic, but was launched in May 2022. The system is based on resistance testing of samples collected from newly diagnosed patients in Norway, hence focusing on the surveillance of primary resistance. In 2021, resistance testing was only performed on a limited number of samples submitted for resistance testing.

As part of a drug resistance surveillance project approved by the regional ethics committee, data from national health registers are combined with HCV sequence based data to better understand transmission patterns and spread of resistance associated substitutions (RASs). Several RAS may exist as natural polymorphisms in the HCV genome. The drug resistance data are cross-referenced to epidemiological data from MSIS, enabling an overview of RASs in different subgroups, such as route of transmission and country of infection.

Surveillance data 2021

In 2021, a total of 14 samples were submitted for HCV drug resistance analysis. Out of the 14 samples, six samples could not be analysed, in most cases due to low viral loads. Of the analysed samples, seven were from male patients and one was from a female patient. Four of the analysed samples were from patients infected through intravenous drug use, one sample was from a patient infected through blood transfusion and three samples were from patients that had an unknown route of transmission.

RASs were detected in seven of the eight analysed samples in 2021 (Table 7.1). Two of the samples with detected RASs were from treatment experienced patients, one was from a patient with no previous treatment exposure, and four were from patients where treatment exposure was unknown. Six of the samples had RASs associated with reduced susceptibility to more than one drug class, and these samples were from both treated and untreated patients. The sample with no RASs detected had limited coverage in the relevant areas of the genome.

Table 7.1 Mutation patterns in samples analysed for RASs from 2021

Sample	NS3/4A	NS5A	NS5B	Genotype	Treatment*	Country of transmission
1	122G		556G	1A	No	Norway
2	122T, 170I	37L, 54H		1B	Yes	Norway
3	56Y, 168Q, 170I		150V	3A	Unknown	Norway
4	56Y, 168Q, 170I		150V	3A	Yes	Norway
5	56Y, 168Q, 170I, 132I		188D	3A	Unknown	Abroad
6	80K, 132I, 156A, 168D	28V		1A	Unknown	Norway
7			150V	3A	Unknown	Norway
8	N/A	N/A	N/A	3A	Unknown	Abroad

* According to information on the submission form. N/A – Insufficient coverage.

The impact on susceptibility to individual drugs of the detected RASs, is depicted in Figure 7.1.

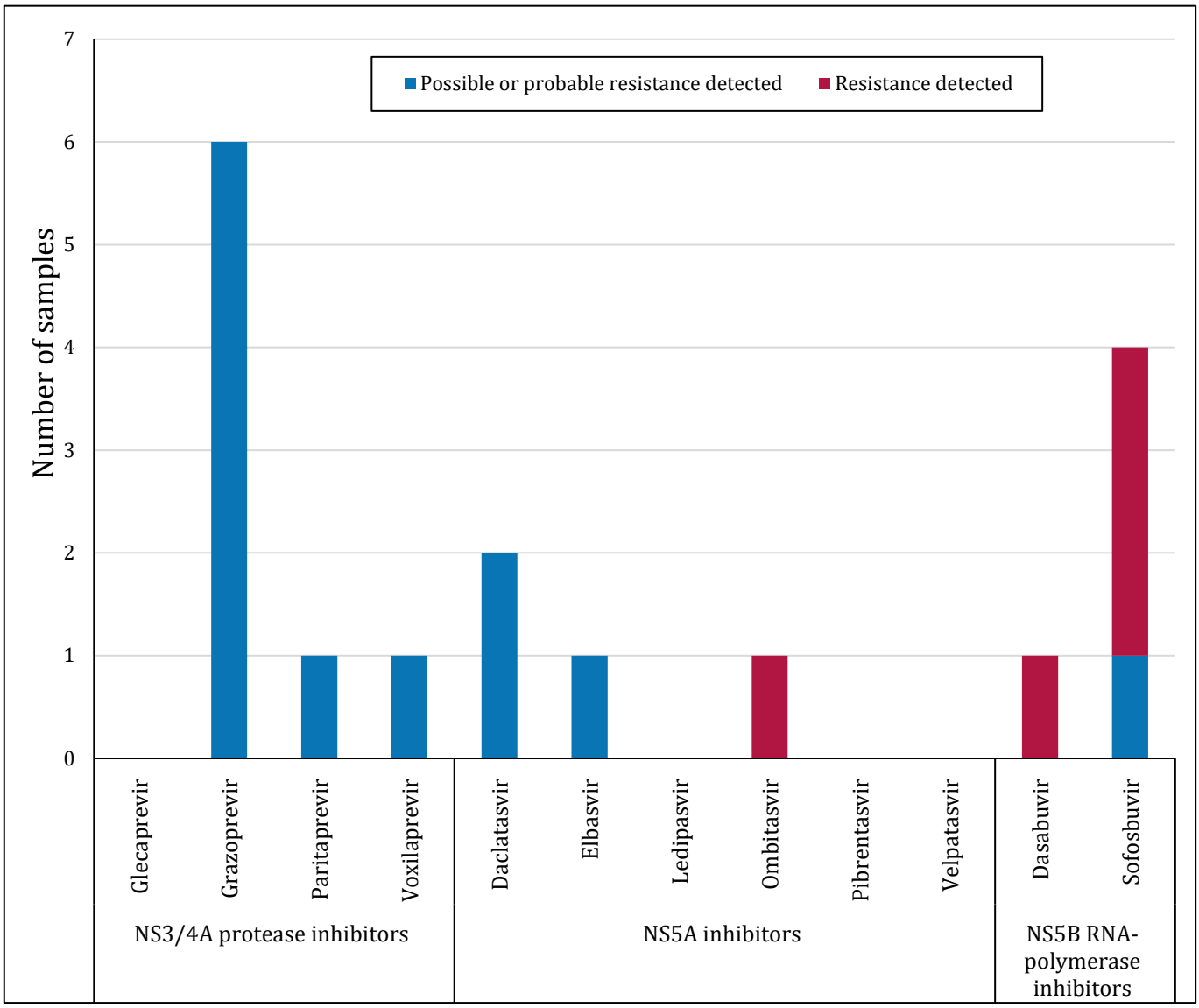

Figure 7.1. Number of samples with detected RASs for 2021, with corresponding resistance patterns against the individual HCV antivirals.
Number of samples with detected RASs (n=7) affecting the individual drugs and drug classes are shown (resistance in red and possible or probable resistance in blue). The sequences are analysed using HCV-GLUE which assigns resistance for a given drug to one of four categories: 1) Resistance detected: any category I polymorphisms, 2) Probable resistance detected: any category II polymorphisms, 3) Possible resistance detected: any category III polymorphisms, 4) No significant resistance detected: none of the above. Polymorphisms are assigned to one of three categories according to the strength of evidence for drug resistance (1;2).

Conclusions

RASs were detected in seven of the eight analysed samples in 2021 and were found in samples from both treatment-experienced and treatment-naïve patients. Most of the samples had resistance patterns associated with reduced susceptibility to more than one drug class.

The surveillance program for HCV resistance launched in May 2022 will aim at a continuous surveillance of the prevalence of RASs among newly diagnosed patients. This will provide information of the prevalence of RAS mutations in the population of HCV-infected in Norway and hence more insight into the HCV drug resistance.

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Drug resistance testing in the clinical management of HCV-infection

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Resistance-associated substitutions (RASs) exist as natural polymorphisms or mutations in the viral genome that appear during DAA treatment. Prevalence of RASs vary among genotypes. There are RASs among all HCV genotypes and for all classes of DAAs. Moreover, certain segments of the viral genome are more prone to harboring RASs than others.

The presence of RASs is one of several factors that increase the risk of treatment failure, and treatment-induced RASs may reduce future options for HCV re-treatment. Testing for HCV resistance by whole genome sequencing (WGS) is now offered by the Norwegian Institute of Public Health.

RAS testing prior to treatment initiation has so far not been routine practice in Norway for several reasons: HCV treatment failure is rare, there are excellent re-treatment options available, and there is no limit to how many times a patient can be prescribed treatment. Furthermore, as WGS is time consuming and may delay treatment-initiation, RAS testing may represent an inappropriate treatment barrier for marginalized individuals. However, there are several good indications for testing, as summarized in Table 7.2.

Table 7.2. Indications for HCV resistance testing.

Indications for HCV resistance testing
Treatment failure
New cases of HCV infection (where it is possible/feasible) as a part of national resistance surveillance
Patients with decompensated cirrhosis that are not candidates for liver transplantation
Patients with decompensated cirrhosis where achievement of SVR prior to liver transplantation is important
Cases with genotype 1a infection and compensated cirrhosis with high viral load (>800 000 IU/ml) where GZR/EBR is the treatment option of choice
Cases with genotype 3 infection and compensated cirrhosis where SOF/VEL is the treatment option of choice

SVR: Sustained virological response, GZR: Grazoprevir; EBR: Elbasvir; SOF: Sofobusvir; VEL: Velpatasvir.

Detectable HCV RNA after completed treatment can be a result of either treatment failure or reinfection. Treatment failure is caused by either virological failure (resistance or drug-drug interactions) or treatment discontinuation, but the exact cause can be difficult to determine.

Virological failure will in most cases present as a virological relapse with detectable HCV RNA after treatment completion, precluding sustained virological response (SVR). Virological breakthrough during treatment is very rare. Detectable viremia after documented SVR is most often due to reinfection.

Exposure to DAAs can result in selection of RASs in the viral genome, but clinical implications of RASs in patients receiving re-treatment (with regimens containing 3 DAAs) have not yet been demonstrated. Sofobusvir/Velpatasvir/Voxilaprevir is the treatment option of choice for most cases of re-treatment, but all regimens containing protease inhibitors are contraindicated in decompensated liver disease. Treatment options for cases of retreatment are summarized in Table 7.3.

Table 7.3. Treatment options retreatment

Degree of fibrosis		DAA	Duration	Dose
No cirrhosis or compensated cirrhosis	Alternative 1	SOF/VEL/VOX[1]	12 weeks	1 tbl daily
No cirrhosis or compensated cirrhosis	Alternative 2	GLE/PIB + SOF[2]	12 weeks	3 tbl once daily + 1 tbl daily
Decompensated cirrhosis		SOF/VEL + RBV	24 weeks	1 tbl daily + RBV dosage

[1] Monitor closely in patients with liver cirrhosis
[2] Can be considered in cases of multiple DAA treatment failures, advanced liver disease, or particular RAS profile
SOF: Sofobusvir; VEL: Velpatasvir; VOX: Voxilaprevir; GLE: Glecaprevir; PIB: Pibrentasvir; RBV: Ribavirin.

Important points regarding clinical practice

- There is rarely a clinical need for information on RASs prior to treatment initiation
- RAS testing of all newly diagnosed HCV infections is recommended for surveillance
- RAS testing should not delay rapid HCV treatment initiation in marginalized individuals
- RAS testing could affect choice of treatment in cases of virological failure or in patients with few treatment options

Patient case

The patient was a 56-year-old male from a country in central Europe. He had chronic HCV genotype 3 infection and no history of alcohol use in the previous year.

Liver disease assessment showed a liver stiffness measurement of 35 kPa and FIB-4 index of 4.2. Ultrasound showed a nodular liver surface and splenomegaly, but focal liver lesions. He had recently been admitted to hospital for treatment of ascites, spontaneous bacterial peritonitis, and hepatic encephalopathy. Child Pugh score was C 11 (decompensated).

Due to his religious beliefs, a liver transplantation was not possible.

Treatment for his HCV infection could be possible with either sofosbuvir/velpatasvir or sofosbuvir/velpatasvir + ribavirin.

Clinical assessment three months after the hospital admission confirmed that the patient still had decompensated liver disease (Child Pugh C). As liver transplantation was not possible, it was critical to reach SVR at the first attempt with a regimen without protease inhibitors. Therefore, RAS testing prior to treatment initiation was conducted.

RAS testing demonstrated that Y93H was not detected. Y93H confers some level of resistance to all NS5A-inhibitors (including velpatasvir) in patients with genotype 1 or 3 infection. The level of resistance depends on HCV genotype and drug (different levels of resistance for the various DAAs). As Y93H was not detected, treatment with sofosbuvir/velpatasvir for 24 weeks or sofosbuvir/velpatasvir + ribavirin for 12 weeks could be given. If Y93H had been detected, a different treatment approach would have been necessary.

Learning points:

- RAS testing prior to treatment initiation can have an impact on clinical decision-making in some cases
- Resistance to NS5A-inhibitors is most commonly detected, and detection of Y93H in genotype 1 and 3 infections can have important clinical implications
- Patients with decompensated liver disease should always be assessed by experienced specialists prior to treatment

8 Severe acute respiratory syndrome-coronavirus-2

Fact box: SARS-CoV-2 drug resistance	
Treatment	Nucleotide analogues (example: remdesivir) Protease inhibitors (example: nirmatrelvir/ritonavir) Monoclonal antibodies (examples: sotrovimab, casirivimab/imdevimab, cilgavimab/tiksagevimab)
Resistance testing method	Whole genome next generation sequencing.
Target genes	Protease RNA-dependent RNA polymerase (RdRP) Spike
Indication for resistance testing	Signs of virological failure in patients on treatment
Surveillance	A surveillance system for SARS-CoV-2 drug resistance is under development and will be implemented in 2023. Samples used for surveillance of variants will also be used for surveillance of resistance.

Launch of a national SARS-CoV-2 drug resistance surveillance program in Norway

A surveillance system for SARS-CoV-2 drug resistance is under development and will be implemented in 2023. The surveillance program is planned in cooperation with the national reference laboratory for SARS-CoV-2, which is situated at the Norwegian Institute of Public Health. The program aims to include surveillance of mutations associated with reduced susceptibility for the antiviral drugs used for treatment of COVID-19 in Norway, including specific therapeutic monoclonal antibodies.

To monitor the epidemiological situation in Norway and the emergence of new variants, the microbiology laboratories are encouraged to submit a proportion of positive SARS-CoV-2 samples to the reference laboratory for whole genome sequencing. In order to get a representative selection of samples, the drug resistance surveillance program will be based on the same samples used for national surveillance of SARS-CoV-2 variants. In addition, the reference laboratory receives samples from outbreak situations, from hospitalized patients, and samples from selected patients of particular clinical interest, but these samples will not be included in the surveillance of drug resistance.

After whole genome sequencing at the national reference laboratory, samples selected for surveillance will be subjected to Stanford SARS-CoV-2 Sequence analysis (1;2). The Stanford SARS-CoV-2 Sequence analysis output includes information on sequenced genes, amino acid mutations per gene, Pango lineage and neutralization susceptibility data (mAbs, convalescent plasma and vaccinee plasma). The Pango lineage and mutations identified in the target genes (Protease, RNA-dependent RNA polymerase and spike) will be included in RAVN.

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