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Usage of Antivirals and the Occurrence of Antiviral Resistance in Norway 2020

RAVN

Resistensovervåking av virus i Norge

Resistance against Antivirals in Norway

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Resistance to antivirals in Norway

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Introduction

It is a pleasure to present the eighth report from the surveillance system for Resistance against Antivirals in Norway (RAVN). In this report, we present data for 2020 on resistance against antivirals for treatment of influenza, HIV-1 infection, hepatitis B virus infection, and human herpes virus infections. For the first time, we also present some data on drug resistance analyses of hepatitis C virus (HCV). In addition to the surveillance data, we have selected three relevant topics that are given special attention in the report.

The year 2021 has been strongly influenced by the pandemic, and the management of SARS-CoV-2 and covid-19 remains a main task for many of us working within the fields of virology, microbiology, and infectious diseases. The pandemic has also affected the surveillance in RAVN, as the preventive measures applied to contain covid-19 have reduced the incidence of other communicable diseases: Influenza was practically absent in 2020, and the number of new cases of HIV was also reduced, partly due to travel restrictions and social distancing. Furthermore, the massive sequencing of SARS-CoV-2 variants earlier this year has postponed the planned initiation of systematic surveillance of HCV drug resistance.

The pandemic situation is changing along with the massive roll-out of effective vaccines. Yet, the search for effective antiviral treatment of SARS-CoV-2 continues. Early efforts focused on exploring the repurposing of existing drugs with possible antiviral effects against SARS-CoV-2, and although some of these compounds have gained a lot of attention, no clinical efficacy has been demonstrated. Interestingly, the sales statistics from the Norwegian Prescription Database showed approximately 2.5 times higher sales of hydroxychloroquine in March 2020 compared to average monthly sales in 2019. Although no direct association to the pandemic is established, this remarkable increase suggests that prescription routines might have been briefly affected by speculations on the drug's effectiveness against SARS-CoV-2. There is a definite need for effective antiviral treatment of covid-19.

The pursuit of a cure has inspired two of the three special topics addressed in this report. New compounds with possible antiviral effects are currently being explored, including drugs not usually considered to be antiviral drugs, and treatments challenging the border between antiviral treatment and immunotherapy. This calls for clarifications and definitions and is discussed in the chapter called "What constitutes an antiviral drug?". The other covid-19-related topic presented, is the chapter "Possible antiviral treatment strategies for SARS-CoV-2". We also focus on HIV integrase inhibitors in the chapter "Perspectives on future surveillance of drug resistance against integrase inhibitors": Although an HIV integrase inhibitor is included in most first line treatment regimens, there is no surveillance of resistance against this drug class.

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.

The rise in antimicrobial resistance is considered one of the greatest threats to global health. Better knowledge and increased awareness are essential to be able to control emerging antiviral drug resistance, and surveillance will be a key tool for management.

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

Sammendrag

Bruk av antivirale midler

Ifølge data fra Reseptregisteret, har det i de senere år vært en økning i salg av antivirale legemidler målt i definerte døgndoser (DDD). I 2020 ser denne økningen imidlertid ut til å ha flatet ut. Det har vært en økning i forbruk av antiviralia mot hiv, hepatitt B og herpesvirus, og en nedgang i forbruk av midler mot hepatitt C. Til tross for lav forekomst av influensa denne sesongen, har salg av oseltamivir holdt seg relativt uendret sammenliknet med foregående år.

For medikamenter mot hiv har det vært en økning i salg målt i både DDD og antall behandlede pasienter. Økningen i antall behandlede personer de senere år kan i stor grad tilskrives økt bruk av kombinasjonen emtricitabin og tenofovir dispoproxil som er godkjent som pre-eksposisjonsprofylakse (PrEP), men økningen av dette kombinasjonspreparatet ser ut til å ha stagnert i 2020. I behandling av hiv-infeksjon brukes det stadig mer kombinasjonspreparater der en enkelt tablett utgjør komplett behandling. Behandlingsregimer basert på integrasehemmere er hyppigst brukt, og dette er i tråd med gjeldende retningslinjer.

Influensavirus

Det har vært svært lav forekomst av influensavirus i 2020/21-sesongen på grunn av omfattende smitteverntiltak for å begrense smitte med SARS-CoV-2. Det ble ikke påvist resistens mot oseltamivir eller zanamivir hos de få influensavirus som er undersøkt denne sesongen.

Humant immunsviktvirus-1

Både antall hiv-infeksjon meldt i Norge og antall prøver analysert som ledd i resistensovervåkningen var lavere i 2020 sammenliknet med foregående år. Blant de 75 undersøkte prøvene fra pasienter med nydiagnostisert hiv-1 infeksjon, ble resistensmutasjoner påvist i 13,3% av prøvene. Dette representerer en økning sammenliknet med tidligere år. Halvparten av pasientene som fikk påvist resistensmutasjoner var smittet i utlandet.

Kun en prøve hadde en mutasjon som medfører resistens mot tenofovir/emtricitabin som brukes forebyggende som PrEP. Det er derfor per i dag ingen tegn til økt resistens mot PrEP blant nydiagnostisert hiv-1 pasienter i Norge.

Hepatitt B-virus

I 2020 ble 146 prøver analysert med tanke på resistensmutasjoner hos hepatitt B virus (HBV). Av disse prøvene var det 14 prøver fra pasienter med pågående antiviral behandling der det var spørsmål om resistens som årsak til behandlingssvikt. De øvrige 132 prøvene var fra behandlingsnaive pasienter, og det er disse som utgjør den norske overvåkningen av primærresistens. Relevante resistensmutasjoner ble funnet i kun én av de 14 prøvene fra pasienter med behandlingssvikt, og ikke i noen av overvåkningsprøvene.

Humane herpesvirus

I 2020 ble 30 prøver sendt inn til resistensundersøkelse ved referanselaboratoriet for cytomelagovirus (CMV), og resistensmutasjoner ble påvist i fem prøver. Det har vært en økning i behandling av CMV-infeksjoner de senere år, men det er sjelden man påviser resistens. Det er imidlertid ingen systematisk resistensovervåkning av CMV, og den reelle forekomsten kan derfor ikke beregnes.

For herpes simplex-virus ble fire prøver analysert for resistens mot antivirale midler i 2020. I samtlige av de fire prøvene ble det påvist resistensmutasjoner og/eller delesjoner som gir resistens mot aciklovir. En av prøvene var i tillegg resistent mot cidofovir. Til tross for en økning i bruk av aciklovir, både i behandling og som profylakse, utføres det sjelden resistensundersøkelse. I likhet med CMV har man heller ikke for herpes simplex virus en systematisk resistensovervåkning. Påvisning av resistensmutasjoner i alle prøvene som ble undersøkt er imidlertid en indikasjon på at for få prøver blir sendt inn for resistensbestemmelse.

Hepatitt C-virus

For første gang presenteres data fra resistensundersøkelser av hepatitt C virus (HCV) i Norge. Det er undersøkt 21 prøver fra 2019 og 2020. Prøvene er ikke systematisk samlet inn, og er fra både ubehandlede pasienter og pasienter med behandlingssvikt. Resistensdata er sammenstilt med epidemiologiske data fra MSIS for å kunne sammenlikne ulike undergrupper.

Det ble påvist mutasjoner som er assosiert med resistens i 16 prøver, hvorav sju var fra ubehandlede pasienter. Et program for systematisk overvåkning av resistensmutasjoner hos nydiagnostiserte er under planlegging, og vil gi mer representative data om prevalens av resistensmutasjoner ved HCV-infeksjon i Norge.

Summary

The usage of antivirals

According to The Norwegian Drug Wholesales statistics database, there has been an increase over the last few years in the sales of antiviral drugs measured in defined daily doses (DDDs). However, in 2020, this increase seems to have stagnated. There has been an increase in the usage of antiviral drugs against HIV, hepatitis B, and herpesviruses, and a reduction in the usage of drugs for treatment of hepatitis C. In spite of very low prevalence of influenza in the season 2020/2021, the sales of oseltamivir in 2020 was comparable to last year.

The sales of HIV drugs increased in 2020, but to a lesser extent than in 2019. The previous rise in number of persons treated has been mainly due to increased use of the fixed combination of emtricitabine and tenofovir disoproxil as Pre-Exposure Prophylaxis (PrEP), but in 2020, this increase has stagnated. When looking at complete treatment regimens, the use of single-tablet regimens is increasing. Combinations containing integrase inhibitors are widely used, which is also in accordance with the Norwegian guidelines.

Influenza virus

There has been a very low incidence of influenza virus in the 2020/21 season due to extensive infection control measures for prevention of SARS-CoV-2. No resistance to oseltamivir or zanamivir was detected among the few influenza viruses tested this season.

Human immunodeficiency virus-1

The number of HIV infections in Norway reported in 2020 was lower than in 2019, and as expected, there was also a reduction in number of samples analysed as part of the resistance monitoring. Among the 75 samples from patients with newly diagnosed HIV-1 infection, resistance mutations were detected in 13.3% of the samples. This represents an increase compared to previous years. Among patients with detected resistance mutations, 50% were infected abroad.

Only one sample had a mutation that confers resistance to tenofovir or emtricitabine, the drugs used prophylactically as PrEP. Thus, there are currently no signals that indicate an increase in resistance to PrEP among newly diagnosed HIV-1 patients in Norway.

Hepatitis B virus

In 2020, 146 samples were analysed for hepatitis B virus (HBV) drug resistance mutations. Of these, 14 samples were from patients with treatment failure. The remaining 132 samples were from treatment naïve patients and can be considered surveillance of primary resistance. Relevant drug resistance mutations were detected in only one of the 14 samples from patients on treatment, while no resistance mutations were detected in samples tested for primary resistance.

Human herpes viruses

In 2020, 30 samples were submitted to the reference laboratory for cytomelagovirus (CMV) for resistance testing. Out of the 20 samples, resistance mutations were detected in five samples. Although there has been an increase in the treatment of CMV infections in recent years, resistance mutations are only rarely detected. There is, however, no systematic resistance

surveillance of CMV drug resistance, and the true prevalence of drug resistant CMV in Norway is therefore unknown.

Resistance mutations conferring resistance to aciclovir were detected in all of the four samples submitted for herpes simplex virus (HSV) drug resistance testing in 2020. One sample had an additional mutation which confer resistance to cidofovir. Despite increased usage of aciclovir, treatment failure is rare. As for CMV, there is no systematic surveillance of HSV drug resistance. Detection of resistance mutations in all the analysed isolates indicates that an insufficient number of samples are submitted for resistance testing.

Hepatitis C virus

For the first time, data from drug resistance analyses of hepatitis C virus (HCV) in Norway are presented. A total of 21 samples from 2019 and 2020 have been analysed. The samples were from both untreated patients and patients with treatment failure but have not been systematically collected. Resistance data have been cross-referenced with epidemiological data from MSIS to enable comparisons of different subgroups.

Resistance associated substitutions were detected in 16 samples, seven of which were from patients with no history of previous treatment. A program for systematic surveillance of HCV drug resistance in newly diagnosed patients is being planned and will provide more representative data on the prevalence of resistance mutations in HCV infection in Norway.

1 Antivirals and development of drug resistance

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

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

Antivirals against influenza

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

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

2) Neuraminidase inhibitors: Neuraminidase inhibitors are effective during the last stage of the replication cycle, inhibiting the release of newly formed virus particles. Normally, hemagglutinin on the surface of the virus binds to sialic acid on the cell surface. The virus is released after the viral enzyme neuraminidase cleaves residues on the sialic acid, thus destroying this binding. Neuraminidase inhibitors (NAI) bind to neuraminidase on the surface of influenza virus A and B, preventing cleavage of sialic acid. NAI 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) (2;3).

3) Polymerase inhibitors: 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. All currently circulating human influenza strains are resistant to the two M2-inhibitors, and these drugs are not presently in use for treatment of influenza.

New drugs are under development, some of which are already approved for treatment of influenza 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 it disappeared 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 (4). Furthermore, reassortment of the segmented genome may rapidly lead to major genetic changes that could involve domains of importance for drug resistance characteristics.

Antivirals against human immunodeficiency virus

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

1) Entry inhibitors: CCR5 blockers are drugs that block the binding between viral gp120 and the chemokine receptor CCR5 (example: maraviroc). Attachment inhibitors bind to and inhibit activity of gp120 (example: fostemsavir). The post-attachment inhibitor, ivalilzumab, is a monoclonal antibody directed against CD4. Fusion inhibitors preventing fusion between the viral gp41 and the cell membrane (example: enfuvirtide), are no longer registered.

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

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

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

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

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

a boosted PI or an NNRTI may replace the integrase inhibitor. Fixed-dose combination drugs are widely available.

Drug resistant HIV

HIV has a very high mutation rate and a considerable risk for development of resistant variants, mainly due to inaccuracy in viral replication and the lack of proofreading. There is vast genetic variation in the HIV-1 genome, and each patient harbors a mixture of coexisting genetic variants. This genetic variation increases over the course of the infection. Drug resistant viruses may evolve from wild-type viruses if viral replication persists during antiretroviral treatment. Because most drug resistance mutations impair viral fitness, wild type virus often rapidly reemerges when treatment is interrupted. Drug resistance rarely occurs without previous drug exposure, but individuals carrying virus with resistance mutations may transmit this virus to others. Drug resistance emerging during antiviral treatment is called acquired drug resistance. Drug resistance detected in previously untreated persons is usually transmitted from a person with acquired drug resistance and may subsequently spread to others. The term transmitted drug resistance is used when previously uninfected individuals are infected with virus that has drug resistance mutations (5).

Antivirals against hepatitis B virus

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

1) Nucleoside/nucleotide analogues: Analogues of naturally occurring deoxynucleotides that are incorporated into the viral DNA chain in competition with the natural substrate. When incorporated, the drug stops further elongation of the viral DNA chain (chain termination), thereby inhibiting transcription of RNA into DNA by the HBV polymerase. 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 (6). 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 (7). Because of the rarity of resistant cases, the relevant mutation sites for tenofovir-resistance are not fully confirmed.

Antivirals against cytomegalovirus

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

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

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

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

Drug resistant CMV

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

Antivirals against herpes simplex virus

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

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

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

Drug resistant HSV

Resistance to aciclovir develops by mutations of either the HSV-TK- or HSV DNA polymerase gene. Mutations in HSV-TK are by far the most common, and about 95% of the resistance mutations are localized in the thymidine-kinase gene (UL23) whereas 5% are localized in the DNA-polymerase gene (UL30) (8).

Aciclovir resistance is frequently associated with cross-resistance to other HSV-TK dependent nucleoside analogues (9). Cidofovir and foscarnet are independent of HSV-TK and therefore active against most of the strains that are resistant to aciclovir. Cross-resistance of foscarnet to aciclovir is rare (9). 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 (8).

Antivirals against hepatitis C virus

There has been a rapid development of new and better drugs against hepatitis C virus (HCV) over the last few years, replacing the early generations of direct-acting antivirals (10). 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) (11).

There are currently four groups of direct-acting antivirals (DAA) against HCV (12):

1) NS5B inhibitors:
    a. Nucleoside analogue polymerase inhibitors: Compete with nucleosides for the active site of the HCV polymerase, NS5B (example: sofosbuvir).
    b. Non-nucleoside analogue polymerase inhibitors: Alter the shape of the polymerase and thus inhibit replication of HCV (example: dasabuvir).
2) NS3/4A protease inhibitors: Target the active site of the protease enzyme, NS3/4A, inhibiting proteolysis of the HCV polyprotein. Genotype specific. (example: voxilaprevir, grazoprevir).
3) NS5A inhibitors: Target the 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 is currently no direct acting antiviral treatment with documented effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The nucleotide analogue remdesivir, has conditional marketing authorization in Norway (13). However, clinical efficacy data from randomized controlled trials are not consistent (14;15), and antiviral treatment is not implemented as part of standard clinical care in hospitals. Clinical trials with other antivirals are also ongoing, and the peroral ribonucleoside analogoue molnupiravir currently seems to be the most promising antiviral drug in pipeline (16;17). Furthermore, different treatments with monoclonal antibodies are in clinical trials, some of them with promising results (18-20).

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What constitutes an antiviral drug?

Margrethe Larsdatter Storm, Andreas Christensen

Since the first antiviral drug was approved in 1963 there has been a massive increase in development of new antiviral drugs to treat infectious viral diseases (1;2).

The development of drugs with novel mechanisms of action has received renewed interest with the SARS-CoV-2 pandemic and many new drugs are now in development. In this chapter, we aim to explore what defines an antiviral drug and give a short overview of typical mechanisms of action.

Antiviral agents are any agent or drug that is used in the treatment of an infectious disease caused by a virus, that inhibit the propagation and spread of virus by interfering directly with one or more of the steps in the virus' life cycle. This may happen by blocking entry to host cells, preventing replication of the genome or by inhibiting viral protein synthesis, assembly or release (3).

Vaccines, on the other hand, act indirectly by stimulating the host immune response. Vaccines are thus not included in this text, although they may be used therapeutically. Monoclonal antibodies (MAbs) and convalescenct plasma are somewhat in a grey area in this regard, as they contain complex molecules mimicking host immune responses. However, since they directly interfere with binding of the virus to the host cell, they are included here. Interferons are not included in this context as they are immunomodulators acting indirectly by eliciting complex cascades of immune responses. Drugs that interfere with host enzymes exploited by viruses also act indirectly, but since they are specifically designed for inhibiting defined steps in the virus life cycle, we have included them here as antiviral drugs. The definition of an antiviral drug can thus be considered to entail targeted/direct-acting drugs which exert a specific viral inhibiting effect.

Drugs that inhibit binding and entry

Most drugs in this category act by inhibiting viral receptor binding, either by blocking a viral surface protein or a host cell receptor. Synthetic drugs, MAbs or convalescenst plasma may all be used for this purpose (examples are pleconaril for enterovirus, maraviroc for HIV and MAbs such as bamlanivimab, bamlanivimab and etesevimab, or casirivimab and imdevimab for SARS-CoV-2 infections). A new concept under study today is the use of a soluble decoy receptor that binds the virus and prevents it from binding to the cell bound receptor, for example CTC-445.2d that mimics ACE2 and neutralizes SARS-CoV-2 infection of cells (4). Drugs interfering with other steps in the viral entry process include fusion inhibitors (palivizumab for RSV infections) and M2 inhibitors (amantadine for influenza infections).

Drugs that inhibit DNA- or RNA-synthesis

Another approach to disrupt the viral life cycle is to target proteins involved in viral genome replication, e.g. the DNA/RNA polymerase, reverse transcriptase or other parts of the replication machinery. Examples of such drugs include nucleoside and nucleotide analogues which are incorporated into the nascent chain during replication but block further elongation (e.g. aciclovir used against HSV, tenofovir against HIV, remdesivir

against SARS-CoV-2 and sofosbuvir against HCV). In contrast, non-nucleoside inhibitors act by binding to the polymerase and interfering with its active site (e.g. efavirenz against HIV). Proteins that act as supporting molecules for polymerases during replication may also be targeted. NS5A-inhibitors used against HCV (e.g. ledipasvir) and a newly registered endonuclease inhibitor active against influenza virus (baloxavir) are examples of this. Viral kinases involved in nucleotide production are other possible targets. The drug maribavir, active against CMV, inhibits the viral UL-97 kinase, and thus reduces substrate for viral replication. It is now in phase 3 trials.

Drugs that inhibit genome integration

For retroviruses, integration of the genome into the hosts' chromosomes is required for viral reproductivity. This is facilitated by the enzyme integrase, and inhibitors of this enzyme are now widely used in the treatment of HIV-infections (e.g. dolutegravir).

Drugs that degrade viral nucleic acids

This is a new category of antiviral drugs exploiting CRISPR/Cas-technology. Cas enzymes can be designed to cleave DNA or RNA at very specific sites, and a Cas13a enzyme specifically cleaving SARS-CoV-2 RNA has been developed.
It is now undergoing preclinical testing (5).

Drugs that inhibit proteolysis and assembly

Proteases play crucial roles in assembly and maturation of viral particles, and for many viruses they are necessary for protein production in general. Inhibitors of proteases are now well-established drugs in the treatment of HIV (e.g. lopinavir and ritonavir) and HCV-infections (e.g. glecaprevir). Protease inhibitors are also being investigated for the treatment of SARS-CoV-2 infections (e.g. the MPro inhibitor PF-07304814 and HCV protease inhibitors) (6;7). Drugs inhibiting other steps in the assembly process include the recently released letermovir, for treating CMV-infections. It inhibits the CMV terminase enzyme that is necessary for packing viral DNA into the capsid.

Drugs that inhibit particle release

Drugs in this category inhibit the budding or release of new virus particles from the cell. Examples are oseltamivir and zanamivir which inhibit the enzyme neuraminidase which is responsible for the cleavage of terminal sialic acid residues from carbohydrate moieties on the surfaces of host cells and influenza virus envelopes, thus promoting release of progeny viruses.

Drugs that inhibit host enzymes exploited by viruses

In addition to agents which target the virus itself, antivirals can also target cellular factors inherent to the host which are required for efficient viral infection or pathogenesis. For example, inhibitors of the cellular proteases TMPRSS2 and furin are currently being investigated for their potential antiviral activity against SARS-CoV-2 (8;9). Furthermore, the antivirals zotatifin and plitidepsin inhibits eEF1A and eIF4A, host proteins involved in translation of SARS-CoV-2 proteins necessary for viral replication (10). Other antiviral approaches include interfering with lipid biosynthesis and nucleic acid production, other cellular processes that are exploited by some viruses (11;12).

RNA-based therapeutics

RNA-based therapeutics have in the past 20 years received considerable attention as new promising therapeutic agents against a variety of diseases, including infectious viral diseases. RNA-based therapeutics comprise antisense oligonucleotides, microRNAs (miRNA), short interfering RNA (siRNA), as well as RNA aptamers and CRISPR/Cas, amongst others. RNA can act in multiple ways to modulate gene expression by for example translational repression or mRNA degradation. Several of these agents are being tried out against different viruses such as HIV, HCV and HBV with promising effects (13-15). However, most of these studies are limited to cell culture or animal models and few have entered clinical trials. The introduction of large nucleotide molecules into target cells has proven to be a major challenge *in vivo*.

Drugs that affect multiple steps of the viral life cycle

Drugs in this group have less specific targets and often have considerable side effects. Ribavirin is an example. It is a nucleoside analogue that inhibits viral RNA polymerase of several viruses. In addition, it reduces GTP-synthesis and stimulates cellular immunity. Furthermore, it has inhibitory effects on HIV reverse transcriptase. Due to its multiple side effects the use of this drug is rapidly declining as more targeted drugs are developed. A new antiviral drug against multidrug resistant HIV have shown promising effects and is currently in phase II/III trials.
The drug, lenacapavir, targets the HIV capsid protein which play a role in multiple steps of HIV's life cycle (16).

There has been a continuous development of new antivirals since the first antiviral was approved in 1963, which have been further accelerated the past year due to the SARS-CoV-2 pandemic. The majority of new antivirals being developed target one of the steps in the viral replication cycle, but several employ new or advanced technologies, such as small molecules acting as decoy receptors or CRISPR/Cas. Compared to conventional antiviral drugs, MAbs can be considered a distinct therapeutic class of antivirals as they enhance or mimic the host immune response. With the rapidly expanding literature on the use of MAbs in treatment of viral diseases it will be exciting to see what the future holds for treatment of infectious viral diseases with these agents. Furthermore, the advances in computational biology and artificial intelligence which enables digital high throughput screens, facilitate the identification of new targets and novel ways of affecting those targets.

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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. From 2019 to 2020 no new agents for treatment of HIV and HCV were introduced in Norway. The only new DAA introduced in Norway in 2020 was remdesivir, indicated in treatment of COVID-19. The sales of DAAs, measured in both defined daily doses (DDDs) and number of patients treated increased from 2016-2017 (Figure 2.1 and Figure 2.2, respectively) (1). The introduction of new antivirals for treatment of HCV infections contributed greatly to increased costs up to 2018. However, the total cost of the DAAs in 2020 had fallen by almost 50 percent since 2017. In 2018, price reduction for some of the drugs used in treatment of HIV and HCV resulted in reduced costs despite continued increase in sales. This trend has continued for the HIV drugs the two latest years while the sales of HCV drugs since 2018 have been substantially reduced both in DDDs and costs.

For HIV drugs, sales measured in number of DDDs have had a slight yearly increase for many years, but from 2018 to 2019 the increase was steeper than previous years. The sales further increased in 2020 but to a lesser extent than in 2019. The sales for the different ATC subgroups of DAAs over time are shown in Figure 2.1.

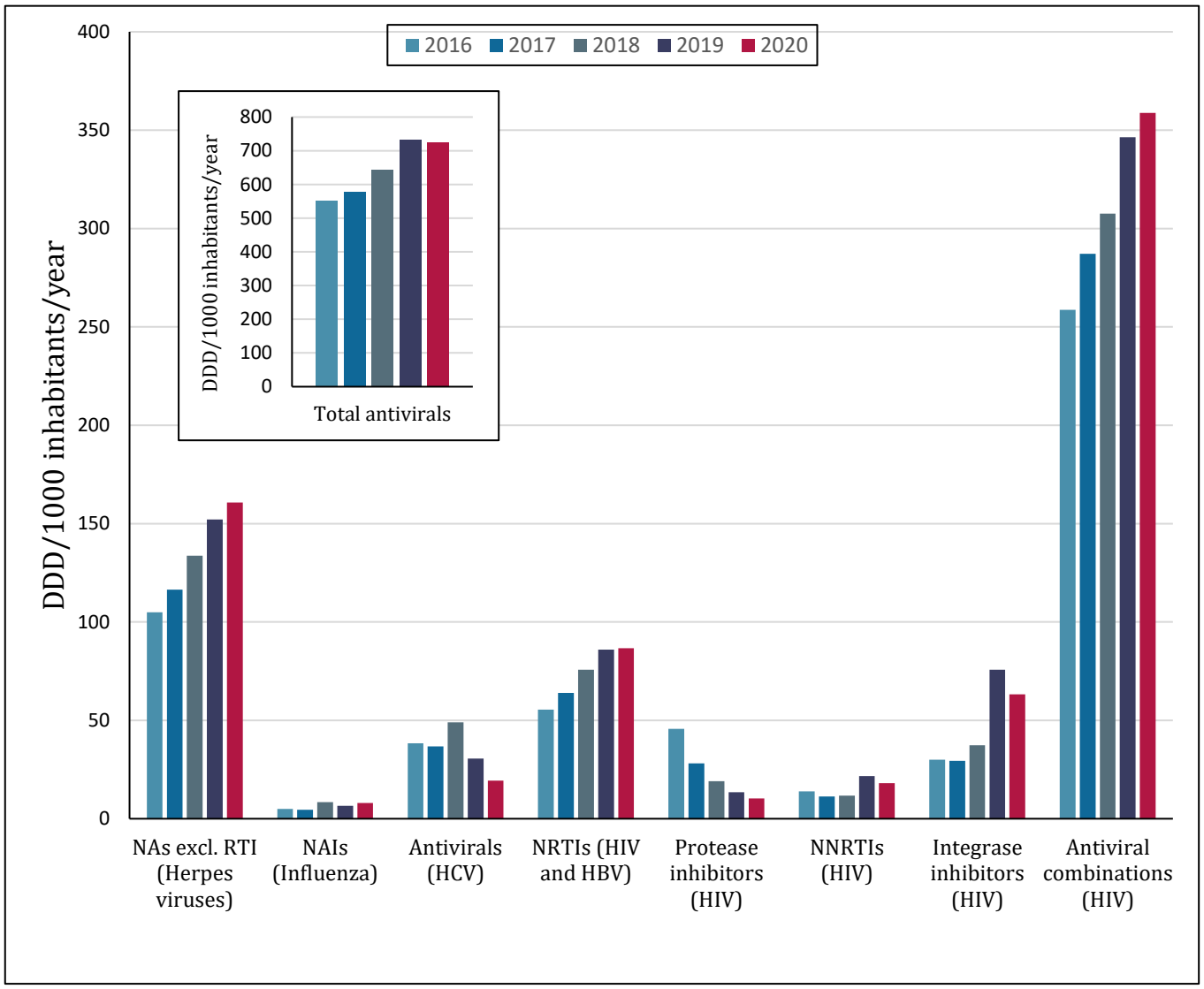

Figure 2.1 Sales of direct acting antiviral drugs for systemic use (ATC group J05A) for 2016-2020 (2).

The figure shows the sales of direct acting antiviral groups over time. Numbers are given as defined daily doses (DDD) per 1000 inhabitants per year. NA excl. RTI: Nucleoside-/nucleotide-analogues excluding reverse transcriptase inhibitors (J05AB); NAIs: Neuraminidase inhibitors (J05AH); Antivirals, HCV: Antivirals for treatment of HCV infections (J05AP); NRTIs: Nucleoside- and nucleotide-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 derivatives (J05AD) used against herpesviruses and other antivirals (J05AX), due to low numbers these are not included in the main plot. In previous reports integrase inhibitors were included in other antivirals (J05AX). In 2020, integrase inhibitors were reclassified in a new ATC group (J05AJ).

The number of people treated with different DAAs has increased for most treatable viral infections since 2016 (Figure 2.2). An exception is the reduction in people treated with HCV agents in 2020. In addition, the use of DAAs against influenza varies during the ten-year period, probably due to the strength 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 used antivirals when measured in number of users (Figure 2.2). The high number of DDDs for HIV drugs reflect the long-term daily treatment, while antivirals against herpes infections are given in shorter courses. For DAAs against herpesvirus, the use of topical agents (creams and ointments) is not included in the measurement of DDD.

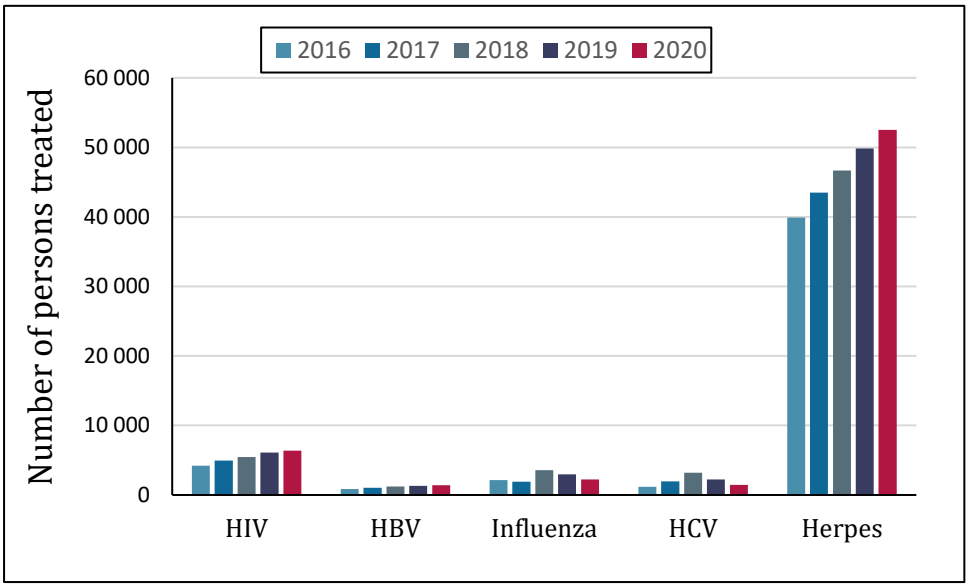

Figure 2.2 Trends in the use of direct acting antiviral drugs for systemic use grouped by virus for 2016-2020.

The figure shows the number of persons treated for different viruses with systemic direct acting antivirals over time. The number of persons treated is based on the number of patients given at least one prescription per year. HIV: All HIV pharmaceuticals (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, is shown in Table 2.1. The variations in the number of users of DAAs for treatment of influenza is probably related to the size and intensity of the seasonal influenza outbreak each year, the accuracy of the yearly influenza vaccine, and the vaccinated proportion of the population. 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 influenza season 2020/2021 was very mild, and by far the largest proportion of neuraminidase inhibitors were dispensed during the first three months of 2020 (influenza season 2019/2020). Zanamivir was withdrawn from the market in 2016 and as a result, oseltamivir has been the only neuraminidase inhibitor available for treatment of influenza in Norway in the period 2016-2020.

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

	2016	2017	2018	2019	2020
Zanamivir	25				
Oseltamivir	2 129	1 923	3 571	2 987	2214

Human immunodeficiency virus

There are currently 32 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 32 HIV drugs or combination drugs used in 2020, six of them have been introduced since 2016, while two older drugs have been withdrawn in the same period. The number of patients retrieving at least one prescription of these drugs has increased by more than 50 percent from 2016 to 2020, partly attributable to the concurrent increase in the number of persons receiving pre-exposure prophylaxis (PrEP).

Figure 2.3 shows the trends in use of single tablet regimens for treatment of HIV in 2020, measured in number of persons treated. During the whole period, nearly 99 percent of persons treated, received combination drugs containing more than one active substance. For some of these combination drugs, the drug contains complete combination ART (single-pill regimens). Others contain combinations of two substances, typically two NRTI 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 2.4. 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 combination drug most used in recent years. This combination has been commonly 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 2020 was 3160. The use of FTC/TDF increased almost 47 percent from 2018 to 2019, while the increase has only been one percent from 2019 to 2020. It is not unlikely that the extensive infection control measures applied in connection with the covid-19 pandemic in 2020 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 in 2020 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 is widely used, which is also in accordance with the Norwegian guidelines (3). This is illustrated in Figure 2.3, showing that many combination drugs containing integrase inhibitors are among the most sold drugs in 2020 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). Three new one-tablet combinations including an integrase inhibitor and doravirine/lamivudine/TDF have been introduced. All of them show increasing sales, indicating that a simple dosing regimen is preferred. As shown in figure 2.4, the use of all the single component drugs has decreased in 2020.

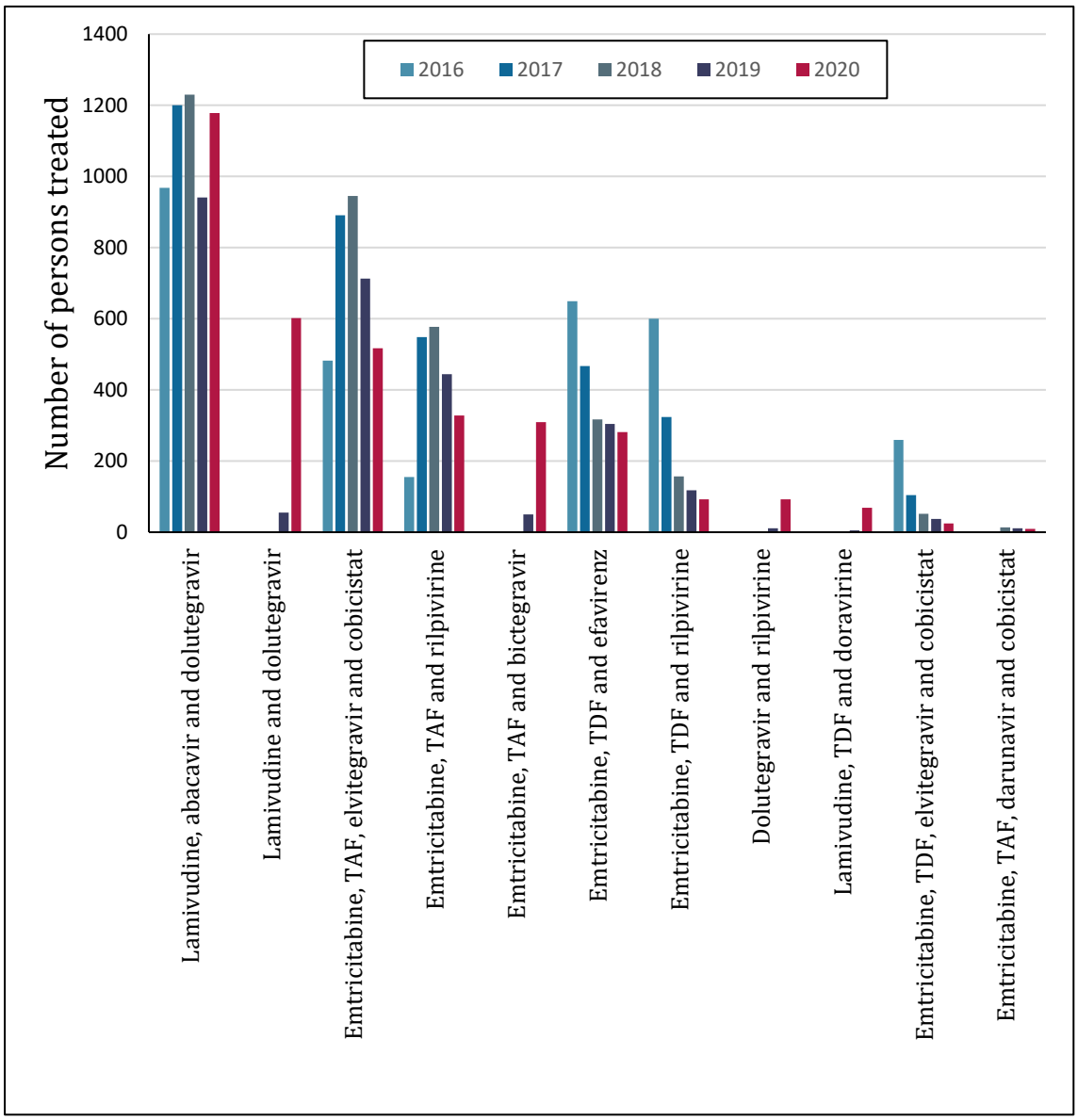

Figure 2.3: The use of single tablet regimens for treatment of HIV in the period 2016-2020, number of persons treated.

The figure shows the trends in the use of antiviral drugs for the treatment of HIV. The drugs comprising complete ART regimens are presented in the plot. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. The remaining antivirals used in treatment of HIV are shown in Figure 2.4. Number of persons treated is defined as the number of patients given at least one prescription per year.

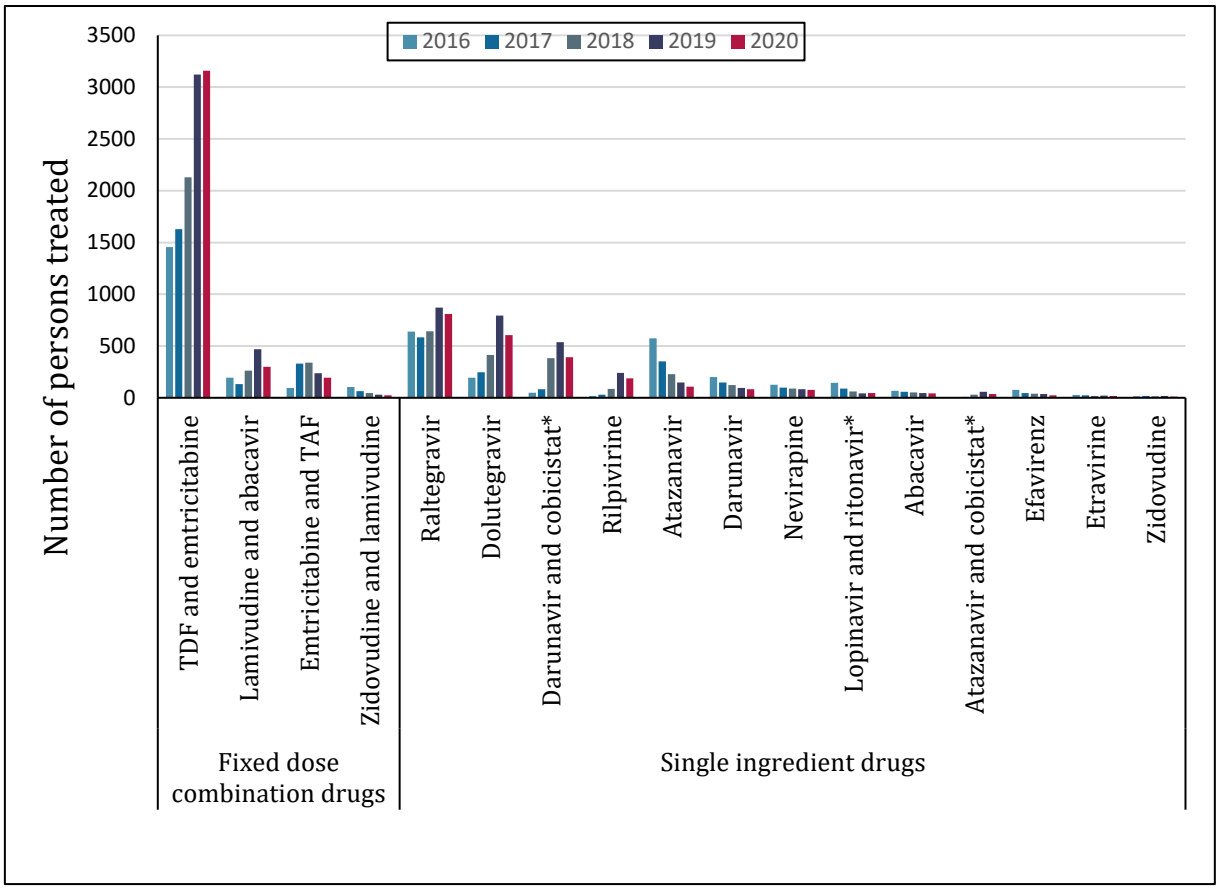

Figure 2.4 The use of antiviral drugs for treatment of HIV in the period 2016-2020, number of persons treated, other than single tablet regimens.

This figure shows the antiviral drugs used in treatment of HIV which are not single-tablet ART regimens. Fixed dose combination drugs are shown to the left and single ingredient drugs to the right in the graph. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. Drugs prescribed to less than 10 individuals in 2020 have been excluded from the figure (zidovudine, lamivudine and abacavir; doravirine; maraviroc). Ritonavir which is used as booster to other drugs have been omitted from the figure. * Boosted protease inhibitors such as atazanavir/cobicistat, darunavir/cobicistat as well as lopinavir/ritonavir are classified as single ingredient drugs.

The use of the integrase inhibitors dolutegravir, raltegravir and bictegravir 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 2.5. For NRTI, 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, raltegravir and bictegravir 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.

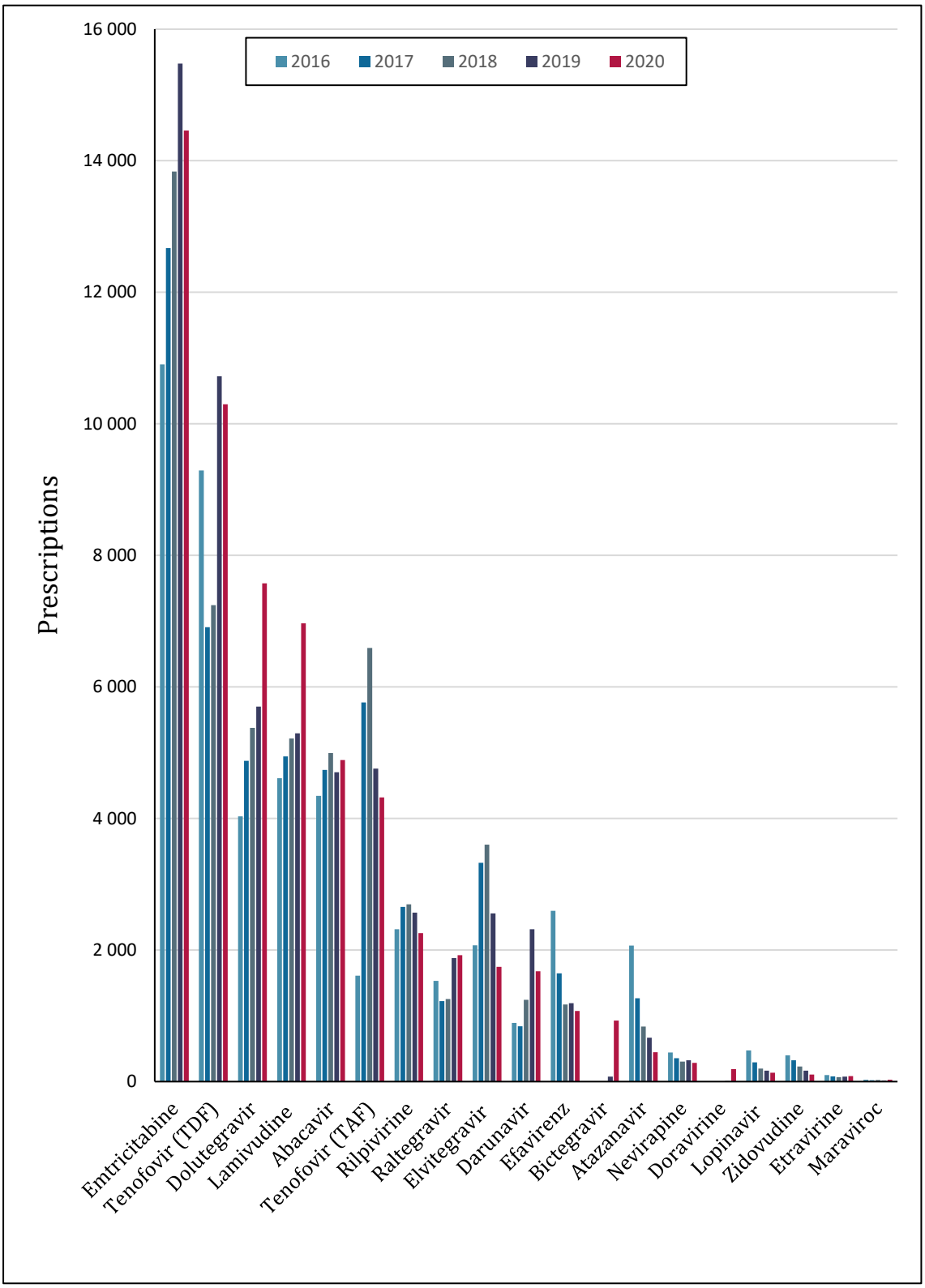

Figure 2.5: Number of prescriptions per active ingredient for HIV drugs

This figure shows number of prescriptions per active ingredient over time. Many prescriptions contain more than one active ingredient; these prescriptions are counted several times. TDF = Tenofovir disoproxil, TAF = Tenofovir alafenamide. Saquinavir and didanosine were not prescribed in 2020 and are excluded from the figure. Cobicistat and ritonavir which are used as boosters to other drugs have also been omitted from the figure.

Hepatitis B virus

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

The data is based on the annual number of patients retrieving at least one prescription per year for the period 2016-2020. Lamivudine, adefovir dipivoxil, tenofovir disoproxil (TDF), and emtricitabine are approved for both HBV and HIV, while entecavir, telbuvidine (withdrawn in 2016) 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 462-1406 in 2020. The lowest number is based on the number of patients prescribed drugs approved for HBV only (entecavir/TAF). The highest number is the total number of patients prescribed one of the six NAs (excluding combinations containing lamivudine that are approved for HIV only).

The number of persons treated for HBV has increased during the last five years. TAF, which was approved for monotherapy of HBV in January 2017, in addition to entecavir and TDF, are considered first line therapies for HBV. Of the patients receiving HBV treatments with NAs, almost 99% received one of these three drugs in 2020. The number of persons treated with entecavir and TAF was stable from 2019 to 2020, while there was an increase in the number of users of TDF.

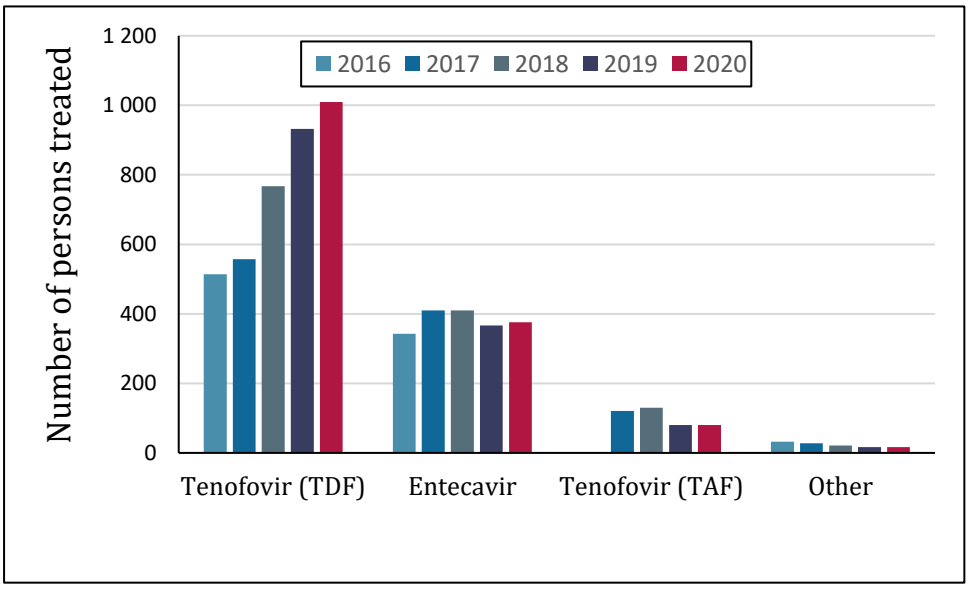

Figure 2.6 Trends in the use of antivirals for treatment of HBV for the period 2016-2020.

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

Human herpesviruses

Figure 2.7 shows the two most prescribed drugs for systemic use for human herpes virus infections over the last five years. The use of the other drugs approved for treatment of human herpes virus is limited. Valaciclovir is the most commonly prescribed substance and the increase of more than 30 percent in number of persons treated with systemic antivirals since 2016 is caused by the increased use of valaciclovir. The use of aciclovir has

been stable during the five-year period since 2016. Ganciclovir and famciclovir were on the other hand rarely prescribed in the period. 52 500 persons have been treated with systemic antivirals for herpes viral infections in 2020.

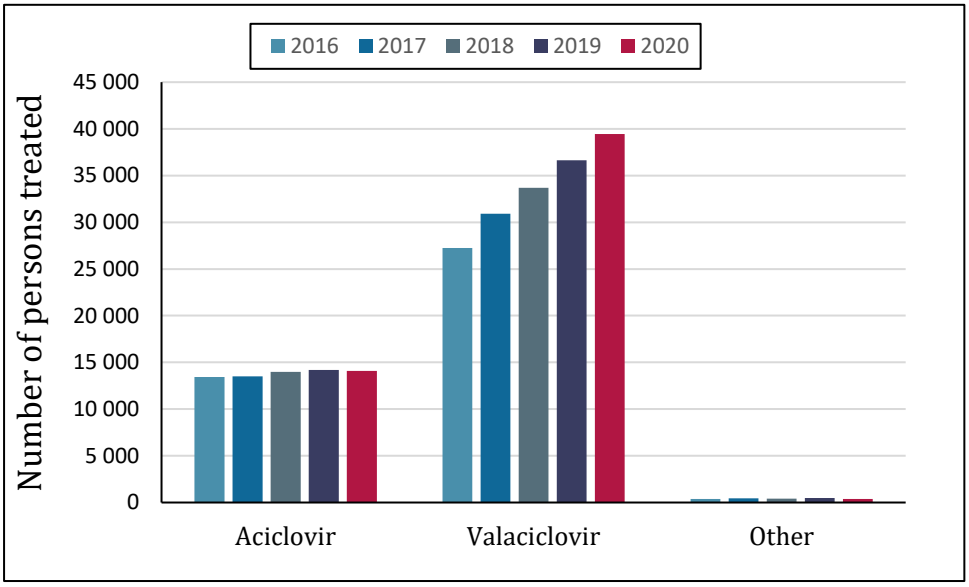

Figure 2.7 Trends in the use of antivirals for treatment of human herpes virus infection for the period 2016-2020.

This figure shows the trends in direct acting antiviral use for treatment of human herpesviruses over time. Number of persons treated is defined as the number of patients given at least one prescription per year. Other: vidarabine, 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. Since 2018 the use of a fixed combination of aciclovir and hydrocortisone has increased at the expense of topical aciclovir alone (Table 2.2).

Table 2.2 Sold packages of topical antivirals containing aciclovir, penciclovir and aciclovir and hydrocortisone in combination.

	2016	2017	2018	2019	2020
Aciclovir	206447	205818	212393	180880	169004
Penciclovir	30122	24062	18957	18664	17229
Aciclovir, combinations*			21794	40618	34727

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

The overall number of patients treated with DAAs against HCV was steadily increasing after the new HCV antivirals became available in 2015. The number of persons treated with HCV antivirals increased until 2018, but 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 1439 in 2020, a reduction by more than 50% from 2018. Fixed combinations of two or more active ingredients have almost completely replaced single component drugs as shown in Figure 2.8, and in 2020, ribavirin was the only single component drug still used to some extent.

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

The treatment pattern for the use of the different combinations against HCV has been the same since 2018 with the combination 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 is listed as the “recommended treatment” in genotype 3 HCV infections, one of the more common genotypes in Norway. SOF/VEL is one of the three pangenotypic fixed combinations with high treatment response. The second most used combination since 2018 has been the fixed combination of sofosbuvir and ledipasvir (NS5A inhibitor) (SOF/LDV). This was one of the combinations recommended by the 2019 procurement for treatment of most patients with HCV genotype 1, which is also commonly seen in Norway. The trends of use shown in Figure 2.8 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 (7). The reduction in treated patients after 2018 hopefully indicates that the goal is achievable.

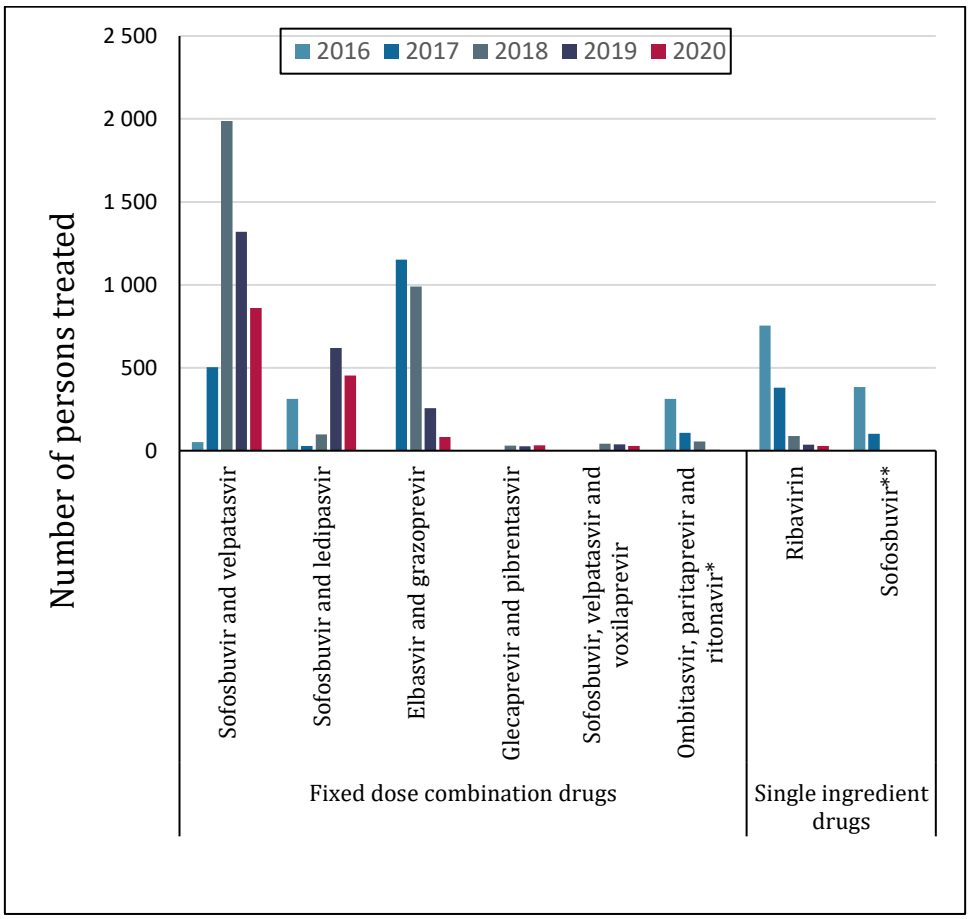

Figure 2.8 Trends in the use of antivirals for treatment of HCV for the period 2016-2020.

This figure shows the trends in the use of direct acting antivirals for treatment of HCV over time. The different drugs are separated by fixed dose combination drugs and single ingredient drugs. Number of persons treated is defined as the number of patients given at least one prescription per year. *Not prescribed in 2020. **Prescribed to five or less persons in 2018, 2019 and 2020. Dasabuvir and daclatasvir were not prescribed in 2020 and are excluded from the figure.

SARS-CoV-2

Remdesivir was approved for use against SARS-CoV-2 in November 2020, but the use in Norway has been very limited. Measured as defined daily doses (DDD), a total of 1210 and 920 DDD were sold to the pharmacies in November and December 2020, respectively, but the use in hospitals was negligible.

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

Surveillance methods

The WHO national reference laboratory for influenza in Norway is located at the NIPH and monitors the occurrence of influenza viruses in Norway. A volunteer network of sentinel physicians in all parts of the country provide samples taken from patients with influenza-like illness, and the medical microbiology laboratories submit a subset of confirmed influenza-positive samples. 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 2020-21

Due to the COVID-19 pandemic, influenza has been absent in Norway and most parts of the world. Throughout the season (week 40/2020 to week 35/2021), only 20 cases have been detected in Norway. Three B/Victoria, seven A/H3N2, and two A/H1N1, as well as eight

influenza B (weak samples, not subtyped). Nine samples were genetically analysed for antiviral resistance at the reference laboratory. Only one sample (B/Victoria) had neuraminidase susceptibility testing performed (by WHO CC), indicating normal inhibition. Antiviral resistance testing of influenza virus has been deprioritized during the COVID-19 pandemic.

No resistance to neuraminidase inhibitors or polymerase inhibitors was detected this season. However, one of the H3N2 samples possessed a V149I mutation in the NA gene that has been proposed to reduce susceptibility to zanamivir slightly. All circulating influenza viruses are currently resistant to adamantanes, which are not used for treatment in Norway and most other countries. Therefore, NIPH has stopped testing routinely for adamantane resistance. Virus resistance to antiviral agents in Norway is reported by the WHO national reference laboratory for influenza, NIPH via the Global Influenza Surveillance and Response system (1) and ECDC / WHO.

Table 3.1: Norwegian influenza viruses resistant to the neuraminidase inhibitors oseltamivir and zanamivir, during the influenza seasons 2015/16 through 2020/21 (sequences with resistance/total number of analysed sequences. Percentages > 0 are shown in parentheses).

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

Conclusion

Antiviral drug resistance towards influenza remains low nationally as well as globally, based on the very few cases detected 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 (2). Continued monitoring is important, both in samples from the community and in patients treated with antivirals.

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4 Human immunodeficiency virus

Fact box: Human immunodeficiency virus (HIV) drug resistance	
Treatment	Antiretroviral treatment (ART) of HIV-infection is always given as a combination of drugs from at least two of the five different classes: - Nucleoside reverse transcriptase inhibitors (NRTI) - Non-nucleoside reverse transcriptase inhibitors (NNRTI) - Integrase strand transfer inhibitors (INSTI) - Protease inhibitors (PI) - Entry inhibitors (CCR5 antagonists, fusion inhibitors, 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 antagonist tropism testing)
Indication for resistance testing	Virological failure during antiviral treatment
Surveillance	The national surveillance program for HIV-1 monitors primarily drug resistance against protease inhibitors (PI) and reverse transcriptase inhibitors (NNRTI and NRTI). Samples from all patients with newly diagnosed HIV-1 infections are tested for resistance mutations located in the protease and reverse transcriptase genes.

Surveillance methods

The Norwegian surveillance data 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 marker of 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, either due to treatment initiated before arrival to Norway, or for some other reason.

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;2). A standard list of SDRMs was published in 2009, but unfortunately, the list has not been updated since. The list is based on a set of criteria to ensure that the mutations included are nonpolymorphic and applicable to the most common subtypes, and do in fact contribute to resistance (1;2). The SDRM list is not designed for individual patient

management as it excludes several clinically relevant drug resistance mutations 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 (1-3). Because the list of SDRM has not been updated since 2009, 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 (INSTIs) in first line regimens, but resistance mutations affecting these compounds are still rare in treatment naïve patients. Baseline testing of resistance to integrase inhibitors is therefore not yet recommended (4), and there is no surveillance of primary resistance to INSTIs in Norway.

Surveillance data 2020

A total of 75 samples from newly diagnosed cases of HIV-1 in Norway were analysed for primary HIV-1 drug resistance in 2020, which equals 55% of the 137 cases reported to MSIS in 2020 (5). Of the 75 cases with samples submitted for resistance testing, 28% were female and 72% were male. The percentage of samples from newly diagnosed patients tested for resistance has increased steadily until 2017, as shown in Figure 4.1, and thereafter the percentage has declined.

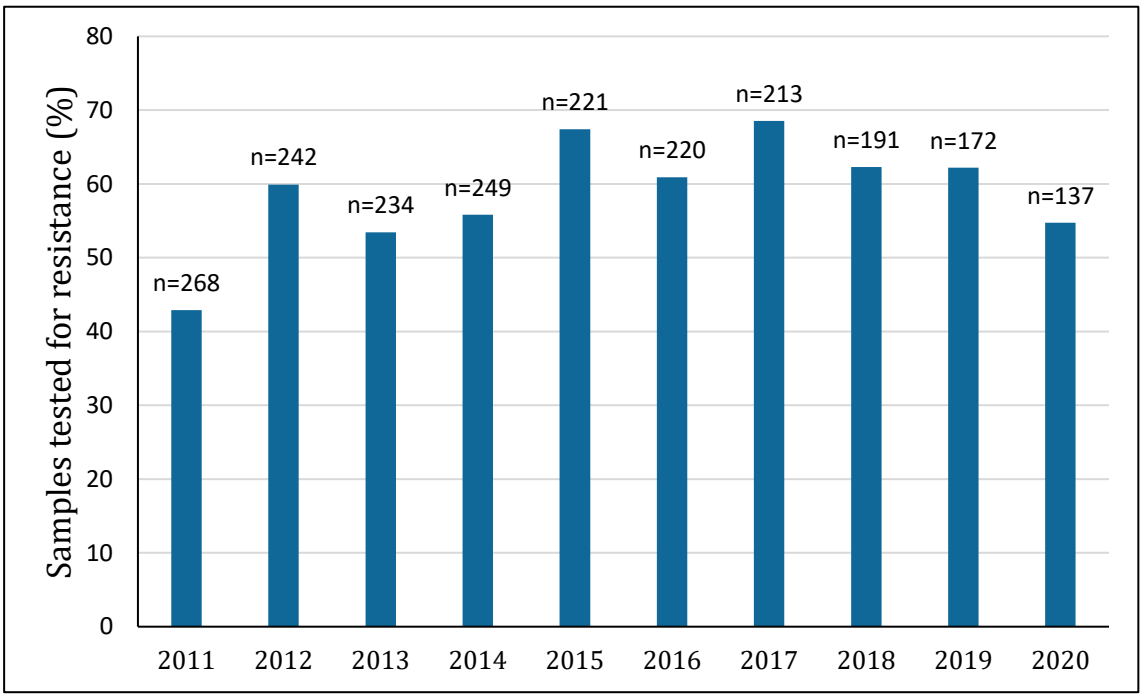

Figure 4.1: Samples tested for resistance (2011-2020).

Data shown as percentage of newly diagnosed cases of HIV-1 infection according to MSIS (6). n = total number of newly diagnosed cases reported to MSIS.

Information on the route of transmission for patients tested for drug resistance, was obtained by cross-referencing resistance data to epidemiological data from MSIS. Coverage of resistance testing among patients infected in Norway was 85% and was even higher among men who have sex with men (MSM) infected in Norway (95%). However, among those infected abroad in 2020, surveillance resistance testing was performed in only approximately 39% of the cases, which was even lower than the coverage of 50% observed in 2019. The latter group include persons residing in Norway that have been infected abroad, but also persons infected before arrival to Norway. In total, 78% of the cases reported to MSIS were infected abroad, while the corresponding number for cases reported to RAVN was 60%. Many of the patients infected before arrival may already be receiving treatment at the time of notification to MSIS, and thus, resistance testing cannot be performed due to suppressed viral load. Data is shown in Table 4.1.

Table 4.1: Route of transmission in samples from newly diagnosed HIV patients tested for resistance in 2019 compared to new cases reported to MSIS in 2020.

Route of transmission	Samples tested for resistance	Cases reported to MSIS
Heterosexual	30	**66**
- infected in Norway	4	***7***
- infected abroad	24	****57***
- unknown	2	***2***
MSM	34	**63**
- infected in Norway	19	**20**
- infected abroad	15	***43**
- unknown	0	
IDU	6	**8**
Blood		
MTC		
Unknown	5	
Total	75	**137**

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 to Norway

In 2020, SDRMs from the WHO list were detected in 13.3% of the analysed sequences. In total, SDRMs were detected in 7 males and 3 females, corresponding to about 13% and 14 % 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, 5.3 % had SDRMs associated with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI), 5.3 % with nucleoside reverse transcriptase inhibitors (NRTI), and 2.7 % had SDRMs associated with resistance to protease inhibitors (PI), as shown in Figure 4.3.

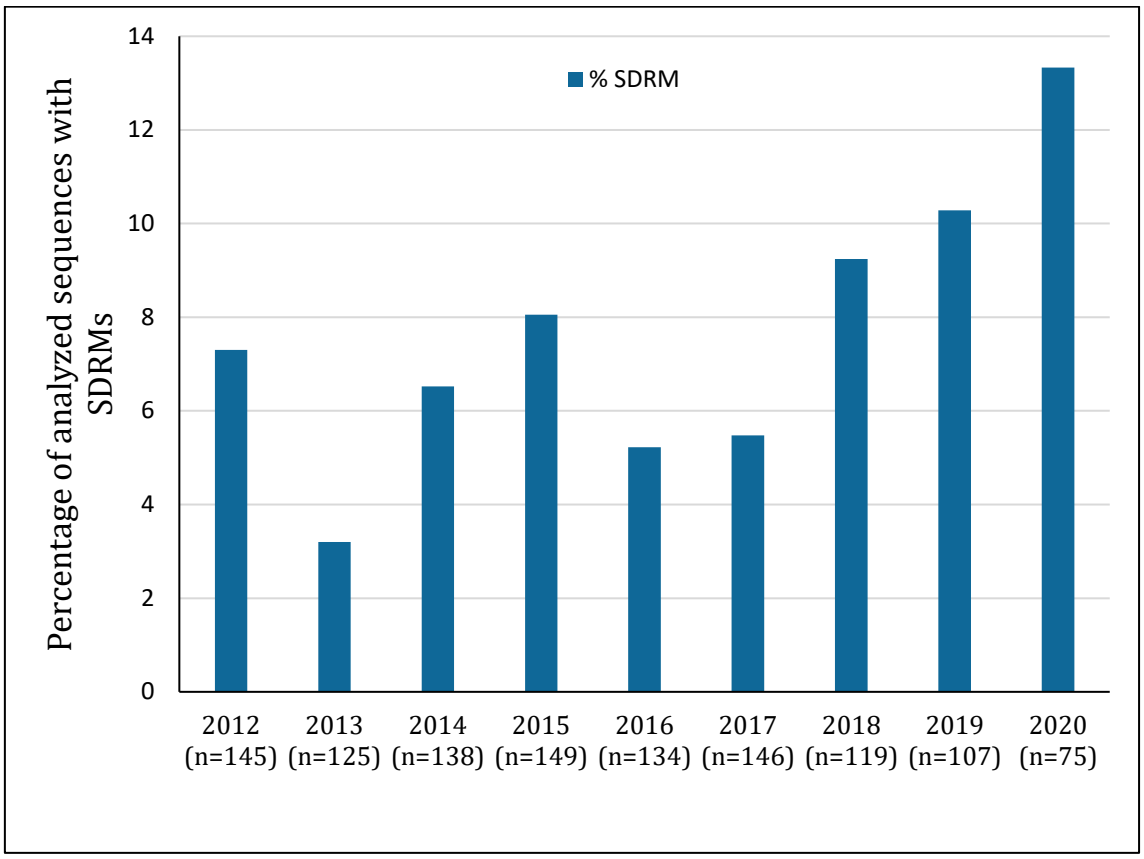

Figure 4.2: Percentage of analysed sequences with detected surveillance drug resistance mutations (SDRMs).

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

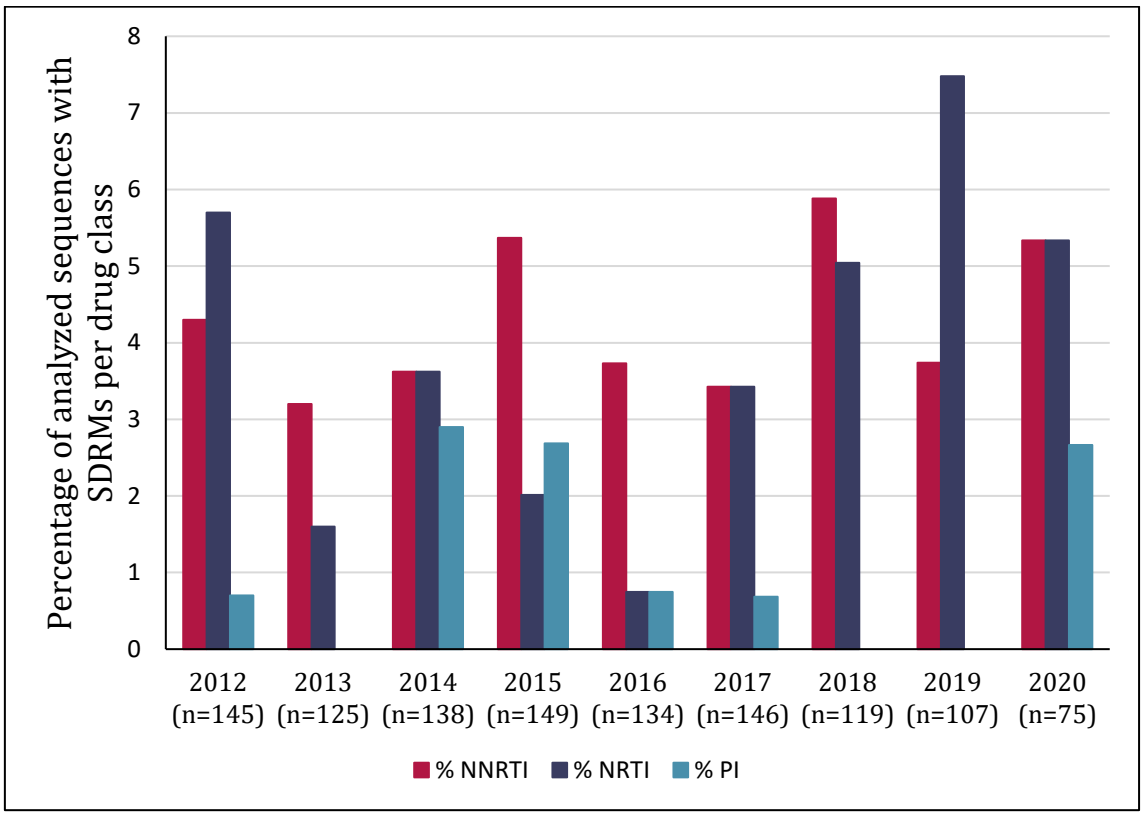

Figure 4.3: Percentage of analysed sequences with detected SDRMs per drug class.

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

The individual mutations are specified in Table 4.2, along with country of transmission and information on previous treatment exposure for the 10 patients with detected SDRM. The majority of the 10 patients with detected SDRM were treatment naïve. Five patients (50%) were infected in Norway, and five (50%) were infected abroad. Seven out of the 10 sequences had mutations that were of clinical significance (G190A, M184I, K103N, K70R, and M41L/T215D). Among these, four were infected in Norway, and three were infected abroad.

Table 4.2: Specification of the surveillance drug resistance mutations (SDRMs) detected in 2020.

Sequence ID	NRTI	NNRTI	PI	Country of transmission	Previous treatment
1	None	G190A	None	Norway	No
2	None	K103N	None	Abroad	No
3	None	K103N	None	Abroad	unknown
4	None	K103N	None	Abroad	No
5	K70R	None	None	Norway	No
6	T215E	None	None	Abroad	No
7	M184I	None	None	Norway	Yes
8	M41L, T215D	None	None	Norway	No
9	None	None	M46L	Abroad	No
10	None	None	I50V	Norway	No

SDRM: surveillance drug resistance mutations; 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 had their HIV-1 infection confirmed in Norway, and where a sample was sent to the National reference laboratory for HIV at Oslo University Hospital (OUH) for resistance testing. The data reported for 2020 have been cross-referenced to epidemiological data from MSIS. Cross-referencing was established in 2019, through a collaboration project between the NIPH and the National reference laboratory for HIV at OUH, and it enables detailed analysis of transmitted drug resistance in Norway by studying the prevalence of SDRMs in different subgroups, such as risk groups or country of infection. This also provides useful information on the coverage of primary resistance testing in the different subgroups.

Over the last few years, RAVN together with the National reference laboratory for HIV, have made efforts to increase the coverage of resistance testing among newly diagnosed HIV patients. In 2020, resistance data was available for 55% of the newly diagnosed patients reported to MSIS, which was slightly lower than the coverage of 62% for 2019. However, the MSIS-data includes patients that will never be included in the resistance data, such as patients already receiving treatment or persons only temporarily residing in Norway. This could explain why the coverage of surveillance resistance data in RAVN is low (39%) among patients infected abroad.

Coverage of resistance testing was high among patients infected in Norway, indicating adequate local routines for submitting samples for resistance testing in newly infected patients. Altogether, the combined data from RAVN and MSIS indicate that the majority of cases where surveillance resistance data is missing, are persons who were already diagnosed with HIV before arrival to Norway, and who were likely to have already started treatment.

Both the total number of new HIV-infections in Norway, and the number of samples analysed for drug resistance surveillance, were lower in 2020 compared to the previous years. SDRMs were detected in 13.3% of samples from patients with newly diagnosed HIV-1 infection in Norway in 2020, and thus, the increasing trend observed in 2018 and 2019 seems to continue also in 2020 (Fig. 4.2). Similar to previous years, mutations associated with clinically relevant drug resistance are rare, and the mutations with the most clinical impact such as

K103N, were mainly transmitted abroad. Most of the increase observed in 2019 was due to the presence of a single M41L mutation which does not confer clinical resistance to NRTI (7). However, this was not the case for 2020, where transmission of SDRMs was observed in Norway, including mutations of potential relevance for first line regimens and clinical management.

Since pre-exposure prophylaxis (PrEP) with tenofovir and emtricitabine was implemented with full reimbursment in Norway in 2017, an enhanced surveillance of the mutations associated with reduced susceptibility for the two drugs used for PrEP is warranted. In 2020, one patient had a mutation (M184I) that may have been selected in association with ongoing treatment with emtricitabine. There were no other mutations observed that are associated with reduced susceptibility for emtricitabine or tenofovir. This means that both tenofovir and emtricitabine would be effective against most HIV variants identified through the surveillance, and that the infections could potentially have been prevented by correct use of PrEP. So far there are no signs of an increase in drug resistance associated with PrEP among patients newly diagnosed with HIV in Norway, and PrEP can be expected to be effective in preventing most new cases. However, continued monitoring of possible PrEP-related resistance will be of importance.

Conclusions

There has been a small increase in transmission of the HIV drug resistance mutations that are monitored for surveillance, but altogether, the prevalence of transmitted drug resistance in Norway remains low. As in previous years, the mutations with the most clinical impact such as K103N, were mainly transmitted abroad. However, in 2020 there was also observed transmission within Norway of resistance mutations with potential relevance for first line regimens recommended for treatment of new HIV infections. Nevertheless, the clinical consequences of the increased prevalence are considered low. There does not seem to be any increase in transmission of PrEP-associated resistance mutations, even after three years with widespread use of PrEP. Continued surveillance of HIV-1 resistance over time is important in order to make informed decisions on implementation of preventive measures to control dissemination of resistant HIV-1 strains.

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Perspectives on future surveillance of drug resistance against integrase inhibitors

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There are currently five integrase strand transfer inhibitors (INSTIs) available for the treatment of HIV-1 infection. INSTIs work by blocking the action of the viral enzyme integrase. As a drug class, they are the most potent inhibitors of HIV replication. For the time being, they are used in the treatment of HIV infected individuals, as well as in Post-Exposure Prophylaxis (PEP). INSTIs have to be combined with one reverse transcriptase inhibitor (RTI) (for the INSTIs dolutegravir and cabotegravir) or two RTIs (all INSTIs). The use of the long-acting INSTI cabotegravir as Pre-Exposure Prophylaxis (PrEP) is currently under investigation, using intramuscular injections of cabotegravir in monotherapy every second month (1;2).

There are differences in the resistance profiles of the individual drugs. While the emergence of a single resistance mutation may be sufficient to confer high-level resistance to first-generation INSTI (raltegravir and elvitegravir), the second generation INSTI (dolutegravir, bictegravir, and cabotegravir) seem to have high genetic barriers. In combination therapy, resistance to dolutegravir is rarely seen when used as part of antiretroviral therapy regimens (3). Only rare cases of emerging resistance to dolutegravir have been reported among treatment-experienced patients (4;5). Furthermore, the efficacy of dolutegravir and the other second generation INSTIs seems to be well preserved also in the presence of resistance mutations selected by other INSTIs (6). However, studies on dolutegravir monotherapy have shown unacceptably high rates of INSTI resistance mutations in patients with virological failure. Therefore, monotherapy is not recommended (7).

Due to the favorable resistance profile, excellent viral efficacy and safety, regimens based on second-generation INSTIs have become the preferred first-line treatment, both in Norway and elsewhere (8;9). Nevertheless, in the national surveillance of HIV drug resistance in newly diagnosed patients, only resistance mutations associated with protease inhibitors and reverse transcriptase inhibitors are included. In this chapter we explain the reasons for not monitoring baseline resistance to integrase inhibitors. Further, we discuss the future need for surveillance of drug resistance against INSTIs, and the prospects of including analysis of the integrase-gene in the Norwegian programme for surveillance of baseline HIV drug resistance.

In Norway, testing for genotypic resistance against integrase inhibitors has been available at the national reference laboratory for HIV at Oslo University Hospital (OUH) Ullevål since 2009. The analysis is performed only for patients previously exposed to INSTI. This discernment is mainly due to the low prevalence of integrase resistance mutations reported among INSTI naïve patients. Population based studies have shown a very little baseline drug resistance against integrase inhibitors, and transmission of INSTI-resistance seems to be rare (10-14). In the rare instances where drug resistance is detected in INSTI naïve patients, it is mostly mutations associated with reduced susceptibility to raltegravir or elvitegravir (15). Case reports on dolutegravir-resistance in unexposed patients are very rare, mostly anecdotal (16;17).

There is no consensus on a standardized list of INSTI-resistance mutations for the purpose of monitoring transmitted drug resistance. However, there has been an initiative from a

WHO working group on HIV drug resistance, suggesting 24 mutations as candidates for inclusion on such a list (18)

There is also a question of capacity and resources. The current method used in Norway for resistance testing for INSTI is based on Sanger sequencing of a segment of the gene encoding the integrase enzyme. As this procedure is performed separately from the routine drug resistance analysis for protease- and reverse transcriptase-inhibitors, each additional sequencing of the integrase gene represents an increased laboratory workload. Therefore, the existing capacity in the laboratory is not sufficient to perform baseline INSTI resistance testing for all samples in Norway, and this is mainly limited by current availability of trained personnel.

As drug resistance is low among INSTI-naïve patients, there does currently not seem to be a rationale for recommending neither baseline surveillance of transmitted drug resistance against INSTI, nor pre-treatment resistance testing in INSTI-naïve patients. However, the picture may change as INSTIs are now becoming more widely adopted globally, and there is a risk that more widespread use could lead to increased resistance against INSTIs (19). Of special interest is the question of to what extent cabotegravir will be used as PrEP. The study HPTN 083 reports superiority in favor of cabotegravir compared to tenofovir/emtricitabine in preventing HIV infections. However, INSTI resistance mutations are emerging in the cabotegravir-arm in patients failing PrEP and in patients with undetected HIV-infection at inclusion, reflecting the suboptimal effect of monotherapy. Altogether, these facts call for renewed evaluation of whether there is need for an intensified surveillance of emerging resistance against INSTIs, including close monitoring of the transmission of mutations with clinical impact on first line regimens.

There is also a risk that emerging INSTI resistance could go unnoticed if INSTIs are increasingly used in areas with limited drug resistance testing. In order to monitor possible consequences of the extensive roll-out of INSTIs as part of first line regimens, systematically collected surveillance data may contribute to detection of early signs of transmitted drug resistance.

The capacity in laboratories may be a limitation for implementing baseline surveillance of INSTI resistance. However, many countries are currently in the process of switching their routine method for resistance testing from Sanger-based sequencing to next generation sequencing (NGS). NGS allows the inclusion of integrase gene sequencing in the same assay as sequencing the protease and reverse transcriptase genes. Thus, resistance data for INSTIs may be obtained without major upscaling of laboratory resources assigned to resistance testing. However, the establishment and validation of methods, and the implementation of protocols will be laborious and costly. Furthermore, implementation of NGS-based resistance-testing will also require additional bioinformatic resources. In Norway, the possibility for a transition to NGS-based resistance analyses for HIV is currently being discussed.

In conclusion, the risk for emerging resistance to second generation integrase inhibitors such as dolutegravir or bictegravir is low, when INSTIs are given as part of a combination regimen. Transmitted drug resistance against INSTIs is very rare, and transmission of INSTI drug resistance that will affect the efficacy of the widely recommended first line regimens based on second generation INSTIs is currently unlikely.

Even though there is no immediate need for implementation of baseline INSTI resistance surveillance, monitoring of emerging drug resistance will be important as INSTIs are

becoming more widely recommended. Systematic surveillance may be the key to preserve INSTIs as first line treatment options, and we should therefore prepare to initiate baseline surveillance of INSTI drug resistance if and when it is needed. Transition to NGS-based resistance testing will open the opportunity for including sequencing of the integrase gene as part of standard resistance testing and may provide surveillance data on transmitted INSTI-resistance at a low cost.

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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 method

The surveillance of HBV resistance in Norway aims to monitor two populations; 1) patients that have been tested for drug resistance primarily in relation to treatment (acquired resistance) and 2) patients that are genotyped for HBV as part of diagnostic investigations, generally 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 2020

The resistance mutations detected in Norway between 2016 and 2020 are presented in Table 5.1.

Table 5.1: Resistance mutations in samples submitted for HBV drug resistance testing in 2016 - 20.

HBV-variants resistant to antivirals	Drug resistance	2016	2017	2018	2019	2020
Total analysed		23	23	20	14	14
M204I	**LAM (R), ETV (I), ADV (I)**	1				
L180M + M204I/V	**LAM (R), ETV (I), ADV (I)**	1	1			
L180M + M204V/I ± S202I/G/S ± T184G/A	**LAM (R), LDT (R), ETV (R)**	2	1	3	1	1
L180M + M204V ± I169T ±V173L ± M250V	**LAM (R), LDT (R), ETV (R)**	1				
I169T + L180M + T184A + M204V	**LAM (R), LDT (R), ETV (R)**			1		
Uncharacterized mutation*		1[a]		1[b]	1[b]	
Percentage of samples with drug resistance		22 %	9 %	20 %	7 %	7%

LAM: lamivudine; LDT: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate; TAF: tenofovir alafenamide; R: resistant; I: Intermediate, S: sensitive.

*Uncharacterized mutation: new mutation of undetermined significance in a position associated with major resistance. a N236D, b A181S.

In 2020, a total of 146 samples were analysed for HBV drug resistance mutations. Of these, 14 patient samples were submitted for HBV drug resistance testing, and 132 samples were submitted for HBV genotyping. Drug resistance was detected in only one sample (Table 5.2) from a patient on entecavir treatment, who switched to tenofovir alafenamide due to resistance.

No drug resistance mutations were detected in patient samples submitted for HBV genotyping (N=132) only. However, three of these samples had either an uncharacterized mutation in positions associated with resistance (I169L), or a compensatory single mutation (T184S), mutations that alone do not confer resistance.

Table 5.2: Resistance mutations detected in samples from 2020 and the drug resistance they confer

Sample	Resistance mutations detected	Treatment*	Resistance				
			LAM	LDT	ETV	ADV	TDF/TAF
1	180M + 204V + 202G	ETV	R	R	R	S	S

LAM: lamivudine; LDT: telbivudine; ETV: entecavir; ADV: adefovir; TDF: tenofovir disoproxil fumarate ; TAF: tenofovir alafenamide; R: resistant; S: sensitive.

*Treatment specified at the time of resistance testing.

Conclusion

Entecavir resistance mutations were detected in only one of the patient samples. In this case treatment was switched to tenofovir alafenamide. The few resistance mutations we have detected in recent years, are all directed against entecavir. Tenofovir is the primary drug of choice and was used for treatment of more than 1000 patients in 2020, whereas entecavir was prescribed for almost 400 patients. Based on our data, HBV drug resistance seems to be a minor problem in Norway.

6 Human herpes viruses

Surveillance of cytomegalovirus drug resistance

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

Surveillance method

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

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

Surveillance data 2020

In 2020, 30 samples were submitted for genotypic analysis of CMV drug resistance mutations. Out of the 30 samples, relevant resistance mutations were detected in five samples (Table 6.1). The mutations detected are listed in Table 6.2.

Table 6.1: Number of samples analysed for CMV antiviral drug resistance and number of samples with detected CMV drug resistance mutations for the years 2016 - 2020.

CMV-variants resistant to antivirals	2016	2017	2018	2019	2020
Total samples analysed	28	32	21	21	30
Number of samples with CMV resistance mutations	8	7	4	6	5
Samples with UL97 mutations	8	7	2	6	4
Samples with UL54 mutations	2	1	2	2	1

Table 6.2: CMV resistance mutations in samples tested in 2020

Patient	UL97 mutations	UL54 mutations
1	A594V[1]	
2	L959S[1]	
3	M460V/A594V[1]	
4	C603W[1]	
5		A505V/E756D2[2]

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

[1] Ganciclovir moderate resistance.

[2] Ganciclovir/foscarnet/cidofovir low resistance

Conclusion

Despite an increase in the use of ganciclovir for therapeutic and prophylactic treatment of CMV-infections, drug resistance mutations are only rarely detected. However, in patients under treatment for CMV-infection, discovering resistance can be of vital importance. Therefore, the reference laboratory encourages clinicians and laboratories to remember to consider drug resistance testing in cases with treatment failure.

Surveillance of herpes simplex virus drug resistance

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

Surveillance method

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

Surveillance data 2020

In 2020, four samples from four patients in Norway were analysed for HSV drug resistance. In these four samples, four resistance mutations and two deletions were recorded as shown in Table 6.3.

Table 6.3: HSV resistance associated mutations

Sample	HSV-type	Sample material	TK mutations	DNA pol mutations	Aciclovir susceptibility
1	HSV1	Vesicle	del C548-553	V573M	Resistant*
2	HSV1	Eye secretion	K62R		Possibly resistant
3	HSV2	Secretion	del G432-438		Resistant
4	HSV2	Secretion		N820NS, R1047L	Possibly resistant

*also cidofovir resistant

Both deletions found in the thymidine kinase gene were associated with aciclovir resistance (1;2). The significance of the substitution K62R has not been characterized, however other amino acid changes at this position (e.g. K62N) have been shown to confer aciclovir resistance (1). One sample had, in addition to a deletion in the thymidine kinase gene, also a V573M mutation in the DNA polymerase gene. Whereas the deletion C548-553 has been associated with aciclovir resistance, it has also been shown to remain susceptible

to cidofovir (1). In contrast, the substitution V573M has been shown to confer resistance to cidofovir, but not aciclovir or foscarnet (1;3). Thus, the combination of this deletion and mutation results in both resistance to aciclovir and the second-line treatment option cidofovir. The fourth sample had two mutations (N820NS, R1047L) within the DNA polymerase gene. The clinical significance of these mutations is unknown, however given that the N820NS mutation is within the conserved region of the genome, this might possibly confer resistance to aciclovir.

Conclusion

The consumption of aciclovir/valaciclovir for both therapeutic and prophylactic treatment has increased during the past five years. However, treatment failure is rare, and few samples are submitted for resistance testing. Thus, resistance to aciclovir appears to be uncommon, but the data are scarce and there is no systematic surveillance of drug resistant herpes simplex virus. However, as 100% of the analysed isolates exhibited resistance or possible resistance, this is a clear indication that too few samples were submitted for resistance testing.

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7 Hepatitis C virus

Fact box: Hepatitis C virus (HCV) drug resistance	
Treatment	Antiviral treatment of HCV infection consists of a combination of drugs from at least two of the four different classes: - Nucleoside analogue polymerase inhibitors (NS5B) - Non-nucleoside analogue polymerase inhibitors (NS5B) - Protease inhibitors (NS3/4A) - NS5A inhibitors Direct-acting antivirals may be supplemented with ribavirin. Treatment protocols depend on genotype and stage of liver disease.
Resistance testing method	Next generation sequencing of the complete HCV genome based on probe enrichment. This method can be used for genotyping, as well as detection of RAS. 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 Baseline testing of patients with HCV genotype 1a and high viral load (>800 000 IU/ml) considered for treatment with elbasvir + grazoprevir Baseline testing of cirrhotic genotype 3 patients
Surveillance	A systematic surveillance system for newly diagnosed HCV infections is under development and will be implemented in 2022.

Surveillance method

The plan for implementing a surveillance system for HCV drug resistance in Norway in 2021 has been postponed due to the SARS-CoV-2 pandemic and will be launched in 2022. The system will be based on resistance testing of samples collected from newly diagnosed patients in Norway, hence focusing on the surveillance of primary resistance. So far, resistance testing has only been performed on a limited number of samples submitted for either genotyping or resistance testing (baseline testing as well as after treatment failure).

As part of a drug resistance surveillance project approved by the regional ethics committee, data from national health registers are combined with HCV sequence data to better understand transmission patterns and spread of resistance associated substitutions (RAS). For 2019 and 2020, the drug resistance data has been cross-referenced to epidemiological data from MSIS, enabling an overview of RAS in different subgroups, such as route of transmission, country of infection, or previous treatment.

Surveillance data 2019-2020

In 2019-2020, a total of 21 samples from 20 patients were analysed for HCV drug resistance mutations. Descriptive characteristics of the cases included are shown in Table 7.1.

Table 7.1. Descriptive statistics of samples analysed for RAS in 2019-2020, n=21

	n (%)
Sex	
Female	5 (23.8)
Male	16 (76.2)
Genotype	
1A	10 (47.6)
2B	1 (4.8)
3A	8 (38.1)
3H	1 (4.8)
6A	1 (4.8)
Previous treatment*	
No	10 (47.6)
Yes	10 (47.6)
Unknown	1 (4.8)
Route of transmission	
Intravenous drug use	8 (38.1)
Injury/blood exposure	1 (4.8)
Other	1 (4.8)
Unknown	11 (52.4)
Country of transmission	
Norway	14 (66.7)
Abroad	-
Unknown	7 (33.3)

*Presumably, according to information on the submission form.

Surveillance data for 2019 and 2020 are scarce, and not systematically collected. About half of the samples analysed were from patients with treatment failure, where a high prevalence of RAS would be expected. For comparison, resistance data from samples that were submitted for genotyping are also presented.

Resistance associated substitutions (RAS were detected in 16 of the 21 analysed samples in 2019-2020 (Table 7.2). Eight of the samples with detected RAS were from treatment-experienced patients, and seven were from patients with presumably no previous treatment exposure. Seven of the samples had RAS associated with reduced susceptibility to more than one drug class, and these samples were from both treated and untreated patients. The impact on susceptibility to individual drugs of the detected RAS, is depicted in Figure 7.1.

Table 7.2. Mutation patterns in samples with detected HCV RAS from 2019-2020

Patient	NS3/4A	NS5A	NS5B	Genotype	Treatment*	Country of transmission
1	122G	31M		1A	Yes	Unknown
2	122G			1A	No	Norway
3	122G			1A	Yes	Unknown
4	122G	N/A	N/A	1A	Yes	Unknown
5	166S, 56Y, 168Q	93H, 30V	206E	3H	No	Unknown
6	55A			1A	No	Unknown
7	56Y, 168Q, 170I	93H, 62T		3A	Yes	Norway
8	56Y, 168Q, 170I		150V	3A	No	Norway
9	56Y, 168Q, 170I		150V	3A	Yes	Norway
10	56Y, 168Q, 170I		206E	3A	Unknown	Norway
11	56Y, 168Q, 170I			3A	Yes	Norway
12	80K, 132I, 156A, 168D	28V		1A	No	Norway
13		28L		2B	No	Unknown
14		93N		1A	Yes	Norway
15			150V, 206E	3A	Yes	Norway
16			206E	3A	No	Norway

*Presumably, according to information on the submission form. N/A – Insufficient coverage.

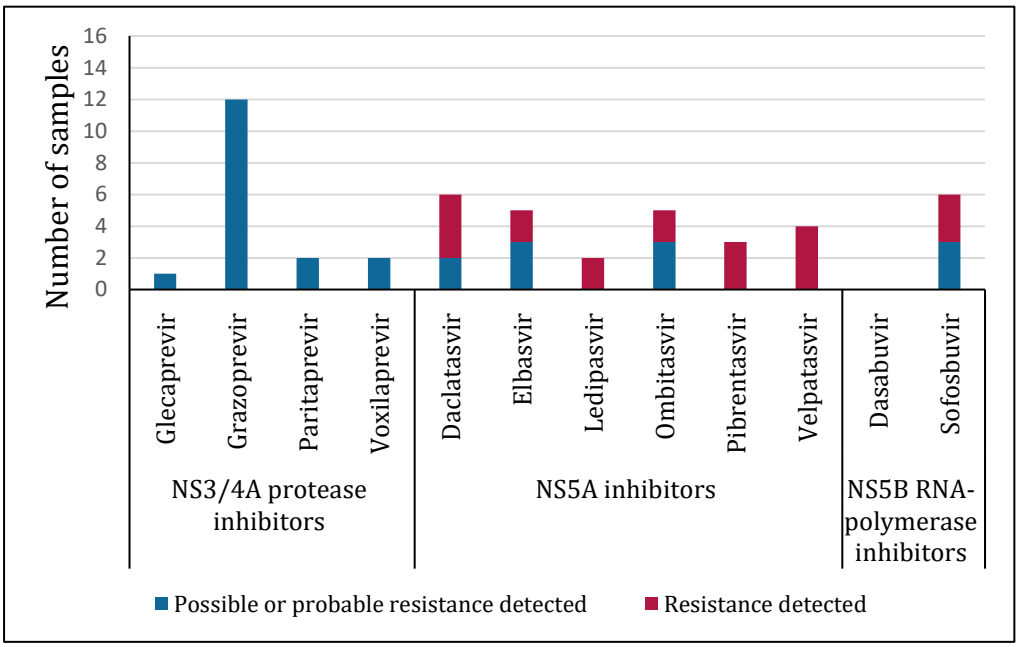

Figure 7.1. Number of samples with detected RAS for 2019-2020, with corresponding resistance patterns against the individual HCV antivirals.

Number of samples with detected RAS (n=16) affecting the individual drug classes are shown (resistance in red and possible or probable resistance in blue). For one sample the coverage of the genomic areas NS5A and NS5B was insufficient, and the resistance pattern could not be distinguished.

Conclusion

RAS were detected in 16 of the 21 analysed samples and were found in samples from both treatment-experienced and treatment-naïve patients. Many of the samples had resistance patterns associated with reduced susceptibility to more than one drug class.

The upcoming surveillance program will aim at a continuous surveillance of the prevalence of RAS among newly diagnosed patients. This will hopefully provide more representative numbers on the circulation and transmission in Norway of HCV strains containing RAS that may impact first line treatment regimens.

8 SARS-CoV-2

Possible antiviral treatment strategies for SARS-CoV-2

SARS-CoV-2 viral dynamics and disease progression. The importance of timing.

Garth Tylden

Relative to chronic viral infections, antiviral treatment of transient viral infections such as coronavirus-, influenza- and respiratory syncytial virus infections, has had limited success. One major reason for this is tied to infection kinetics. In transient viral infections the viral load often peaks before the onset of symptoms and the remaining course of the disease is determined by the damage already inflicted by the virus, and later by the ensuing immune response, and secondary infections. Antiviral treatment initiated after symptom debut has to exert its effects in a background of naturally declining viral replication. This simple kinetic problem undermines attempts to prove the clinical efficacy of treatments with antiviral effect in preclinical studies.

The Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic has provided the global medical community with the opportunity to observe the viral dynamics and disease progression of a transient viral infection reproduced in large numbers within a short period of time. It has become clear that COVID-19 has an initial viral phase followed by an inflammatory phase. This has consequences for treatment as well as design and interpretation of clinical trials, in that optimal patient management requires a transition between essentially opposite treatment principles at the correct stage of the disease (1). The phases of COVID-19 are now reflected in stage-dependent treatment recommendations (https://www.idsociety.org/COVID19guidelines, https://www.covid19treatmentguidelines.nih.gov/). If timing of treatment is not considered in trial design, moderately effective antiviral drugs are likely to produce indeterminant results. Interventions with directly acting antiviral mechanisms that have failed to show efficacy in clinical trials in hospitalized patients (2;3) have shown significant benefits when instituted early in the course of infection (4;5).

There is a longer window for antiviral treatment in immunosuppressed patients with persistent/chronic SARS-CoV-2 infection. These patients present an opportunity to observe direct antiviral effects of SARS-CoV-2 therapy on an otherwise stably high viral load and without immunological confounders (6).

Current status of SARS-CoV-2 antiviral treatment

Only three therapies with any demonstrable in vitro antiviral effects have also shown clinical efficacy so far. These are the nucleoside analog Remdesivir, neutralizing antibodies and the kinase inhibitor baricitinib.

Remdesivir is so far the only small-molecule-drug with direct antiviral activity that has also shown some degree of clinical efficacy against SARS-CoV-2. Remdesivir is an intravenously administered prodrug adenosine analog. The active metabolite is incorporated into the nascent RNA strand by the RNA-dependent RNA polymerase (RdRP). Remdesivir is a delayed terminator of RNA replication, causing the polymerase to stall after incorporating three more nucleotides (7). This displacement from the active site, further into the tunnel of the RdRP-complex, may protect remdesivir from excision by the

viral exonuclease to some extent (7), explaining why remdesivir inhibits the coronavirus RdRP more potently than other nucleoside analogues. Replication sometimes continues after incorporation of remdesivir, leading to inhibition of subsequent rounds of RNA replication through a template-dependent mechanism (8). Remdesivir was originally developed for Ebola virus (9), but was inferior to other treatments in a clinical trial during an Ebola outbreak (10). It has well documented antiviral effect in cell culture (11;12) and animal studies (13), but its clinical utility is debated (14-16). The preclinical data together with subgroup analyses of clinical trials (17) and case studies in immune compromised patients with persistent infection (6), indicate that remdesivir may have clinically relevant antiviral activity in the viral (presymptomatic and early symptomatic) phase of SARS-CoV-2 infection. Unfortunately, studies of preemptive treatment and post-exposure prophylaxis with remdesivir are lacking.

Neutralizing antibodies include the receptor binding domain (RBD)-specific monoclonal antibodies (MAbs) bamlanivimab, etesevimab, casirivimab, imdevimab and sotrovimab as well as neutralizing antibodies in convalescent- or vaccine-boosted plasma. These antibodies prevent the viral RBD from binding angiotensin converting enzyme-2 (ACE-2), thereby preventing cell entry. The clinical benefits of neutralizing antibodies are best documented for MAbs, which achieve optimal effect when administered early in the viral phase of infection (2-5). SARS-CoV-2 sensitivity to neutralizing antibodies is influenced by mutations in the spike protein that occur naturally during the course of antigenic drift, as has been clearly demonstrated for MAbs (18). Surveillance of circulating variants and possibly rapid individual variant analysis will therefore be necessary for rational use of MAbs.

Baricitinib, an orally bioavailable small-molecule-drug used in the treatment of rheumatoid arthritis, has been promoted as having antiviral activity against SARS-CoV-2 in addition to its previously well characterized immunomodulatory effects. Baricitinib reduces inflammatory cytokine production by inhibiting Janus kinases (JAK) and the JAK-signal transducers and activators of transcription (STAT) signaling pathway. The theoretical basis for the antiviral activity of baricitinib is inhibition of clathrin mediated endocytosis via inhibition of AP2-associated protein kinase 1 (AAK1) and G-associated kinase (GAK). However, the antiviral effect is poorly documented (19) and the immunomodulatory properties are probably much more important. A clinical trial including 1033 hospitalized adults found that baricitinib in combination with remdesivir reduced the time to recovery compared to remdesivir alone (20).

Other potential targets for inhibition of viral propagation

Molnupiravir and other nucleoside analogues: Coronaviruses are giants among RNA-viruses, with a genome size more than 3 times that of HCV and twice that of influenza. The coronavirus proof reading ability (or 3´-5´exonuuclease activity) is a key feature facilitating maintenance of the large single stranded RNA-genome. It is also the main reason why nucleoside antivirals do not work well on coronaviruses. Incorporated nucleoside analogues that cause the RdRP to stall are actively removed from the nascent genome by the exonuclease, allowing replication to continue and conferring natural resistance to this important class of antivirals. Molnupiravir is an orally available cytidine (C) analog originally developed to treat influenza that has also shown activity against SARS-CoV-2 (21). The SARS-CoV-2 RdRP incorporates molnupiravir opposite guanosine (G) and Adenosine (A) without stalling, producing a nascent RNA strand laced with molupiravir. Subsequently the incorporated molnupiravir base-pairs with either G og A

leading to lethal mutagenesis in the next round of replication. Preliminary clinical data has recently been released indicating a 50% reduction in the risk of hospitalization or death following treatment with molnupiravir (22).

Exonuclease inhibitors in combination with nucleoside inhibitors: Inhibitors of the HCV NS5A multifunctional protein have recently been shown to inhibit the SARS-CoV-2 exonuclease and increase the antiviral effect of nucleoside analogues (23). Exonuclease inhibition may open the door for a wider range of nucleoside analogues.

Protease inhibitors: The SARS-CoV-2 nonstructural genes are translated to give a large polyprotein, which is cleaved by the SARS-CoV-2 main protease (Mpro) and papain-like protease (PLpro) to give the functional nonstructural proteins. Protease inhibitors have proven to be very efficacious in the treatment of HCV and HIV-1 infection. Repurposed HIV-1 protease inhibitors ritonavir and lopinavir for treatment of SARS-CoV-2 infection have been widely used without success. Strangely the protease inhibitors of HCV, a virus that much more closely resembles SARS-CoV-2, have not yet been tested in controlled clinical trials. Recently, several HCV protease inhibitors have been shown to bind well to Mpro and PLpro, inhibiting viral replication in cell culture (24).

Host targets: Aside from the host kinases inhibited by baricitinib, many putative host targets for antiviral treatment have been identified. Gordon et al used SARS-CoV-2 proteins as bait to map host-virus protein-protein interactions. Many of the identified host proteins already have FDA-approved inhibitors that might be suited for repurposing (25). They simultaneously identified functions of uncharacterized SARS-CoV-2 proteins that may later become attractive drug-targets.

Spread of resistance in SARS-CoV-2

It has been predicted that SARS-CoV-2 will eventually assume a pattern similar to the currently circulating human coronavirus 229E, becoming a seasonal virus with antigenic drift and rapid global dissemination (26). In this scenario, changing population immunity will be the single most important positive selective force and the selective pressure exerted by antivirals will be very small in comparison. Similar to influenza, the spread of antiviral resistance will probably hitch hike with antigenic novelty. If resistance without major fitness costs should emerge in a "founder" virus, the spread of resistance is likely to be rapid, global and persistent through following seasons.

So far only one mutation associated with remdesivir treatment failure (D484Y in RdRP) has been identified (27). The distribution of this mutation has not been formally investigated, but it is present in sequences from diverse geographic localizations (personal communication from Olav Hungnes, NIPH). The current spread of resistance to neutralizing MAbs is more complex, in that it is linked to chaotic post-zoonotic antigenic adaption. Nonetheless, it provides a useful preview. The E484K and L452R mutations are examples of convergent evolution. Both mutations have arisen multiple times independently and both facilitate escape from convalescent humoral immunity. While both the Beta variant (carrying E484K) and the Delta variant (carrying L452R) are resistant to bamlanivimab, only the Beta variant is resistant to etesevimab (18). Despite this, the Delta variant has rapidly replaced the Beta variant and Delta is currently globally dominant (https://covariants.org/per-variant). Use of MAbs is far too restricted to have played a role in the selection of either variant. As such, the rise of the Delta variant illustrates how resistance determinants (in this case sensitivity to etesevimab) can rapidly spread globally, independent of antiviral treatment practices.

Conclusion

Over the next few years, while SARS-CoV-2 settles into a seasonal pattern and while global population immunity is established, we may continue to experience elevated numbers of serious viral respiratory infections. Given the short viral phase of COVID-19, the main focus of antiviral treatment, and clinical trials should be moved from the hospital to the community. Hospital based studies of antiviral treatment should focus on persistently infected immunocompromised patients. In the community, it is important to identify high risk individuals and make plans for rapid diagnosis and preemptive antiviral treatment. Currently, the benefits of MAbs, though SARS-CoV-2 variant-dependent, are most thoroughly documented. Isolation of treated individuals to contain resistant variants emerging under treatment is recommended, with particular attention to immunocompromised patients with persistent/chronic infection. There should be a low threshold for sequencing of isolates in the event of treatment failure in order to generate data on resistance associated variants.

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