Influenza virus types

- **Influenza A**
  - Crosses species
  - Antigenic drift and shift
  - Seasonal epidemics and pandemics
  - Subtypes H1-H16, only H1,2,3 in human viruses

- **Influenza B**
  - Does not cross species
  - Antigenic drift, but not shift
  - Seasonal epidemics, not pandemics
  - No subtypes, but two distinct lineages

- **Influenza C**
  - Does not cross species
  - Mild respiratory illness
  - No antigenic drift or shift
  - No subtypes
  - May not be a true influenza virus
Comparative burden of vaccine-preventable diseases in Australia

AIHW 2019.

- “Some VPD cause more severe illness and have a higher burden of disease for each person with the disease”
- Others “(may not be as severe at the individual level but, due to the large number of cases, have a greater total burden”
- Despite contributing more than one-third of the total burden at the population level, influenza has a very low burden at the individual level, at 0.02 DALY per case.
Why is influenza important?

- Pandemics make lots of people ill and kill lots of people over 1-2 years
- Epidemic influenza makes lots of people ill and kills lots of people over many years
- It is the most serious of the respiratory virus infections due to
  - direct virus-related disease
  - secondary bacterial infections and
  - exacerbations of pre-existing chronic illness
- Immunity is transient, so reinfections are regular
- It is preventable and treatable
Genetic change in influenza viruses

- **Antigenic drift (Influenza A and B)**
  - **Point mutations**: minor change in HA producing low to moderate antigenic change but sufficient to reduce protection from previous infection or vaccination.

- **Antigenic shift (Influenza A only)**
  - **Major mutations or recombination**: Multiple mutations and/or recombination of sections of RNA within the segments. Produces a mixing of human, animal and avian gene sequences resulting in a human pathogenic strain with major antigenic change (1918).
Evolution of influenza A

- Antigenic drift
- Reassortment
- Recombination
- Multiple mutations

Humans → Pandemic strain → Migratory water birds

- Point mutations
- Antigenic Shift

Humans → Other species → Pigs → Domesticated birds → Humans
Influenza: circulating types/subtypes

- A/H1
- A/H3
- A/H2
- B

- A/H2N8
- A/H3N8
- A/H1N1
- A/H2N2
- A/H3N2
- A/H1N1
- A/H1N1(2009)
- B/Victoria
- B/Yamagata

- 1889
- 1918
- 1957
- 1968
- 1977
- 2009

Pandemic
Influenza A/H3 clades

C3.2a
Perth late 2018, early 2019 summer and winter

C3.3a
Perth 2018 winter and late 2019 winter

Vaccine 2018 and 2019
Antigenic shift: Leaping the species barrier

1918 H1N1
50 million extra deaths

1957 H2N2
2 million extra deaths

1968 H3N1
2 million extra deaths

2009 H1N1
0.3 million extra deaths

Hampson A. Mackenzie JS. MJA 2006; 185 (10 Suppl): S39-S43
Mortality due to pandemic influenza

- Death rates vary: 1918 - 50 million excess deaths, 1968 - 2 million excess deaths, 1968 - 1 million excess deaths
- 1918 virus produced more fulminant illness with a peak of deaths in young adults as well as the very young and the elderly.

Modelling

• 125,000-200,000 pandemic respiratory deaths due to influenza globally for the last 9 months of 2009, ~75% under 65 y of age.
• Compared with pre-pandemic seasonal influenza estimates 150,000–250,000 with only 19% in persons <65 y.
Influenza seasonality

- Estimated that over 250,000 people die each year from influenza, including 2-3000 in Australia – recognised to be an underestimate
- In temperate parts of the world influenza is a seasonal disease peaking in the winter months
- In tropical and subtropical areas it has more variable pattern – may be two seasons or continuous low level influenza
- Australia has two seasons - heavily dominated by the winter seasonal activity that occurs across the country – also a smaller summer season in tropical and subtropical northern Australia
Influenza Transmission and Incubation

- **Incubation period:** 1-4 days (typically 2 days)
  - Viral shedding can begin before symptom onset
  - Peak viral shedding on first day of symptoms
  - Adults typically shed viruses for 4-7 days
  - Children may shed viruses for longer periods

- **Transmission:** person-to-person
  - Large droplets <2 meters: Coughing, talking, sneezing
  - Aerosols/droplet nuclei
  - Contact/Fomites
Clinical illness due to influenza

- Uncomplicated illness
  - Acute onset high fever, sore throat, muscle aches, headache, malaise, nausea, diarrhoea
- Complications
  - Pneumonia; primary viral and secondary bacterial
  - In children; Croup, tracheobronchitis, bronchiolitis, otitis media
  - Myocarditis and myositis
  - Febrile seizures, encephalopathy, Guillain-Barre, Reye’s syndrome
- Exacerbations of underlying disease
  - Chronic lung diseases, including asthma
  - Cardiovascular disease
  - Diabetes
  - Neurological disease
- Death
Annual illness impact of influenza USA

- Deaths: 10 per 100,000
- Hospitalisations: 12 per 10,000
- Medically attended: 12 per 100
- Acute respiratory disease: 26 per 100
- Infections: 31 per 100

Couch 1993
Age incidence of subtypes from fatal cases

- A/H3 higher incidence in elderly
- B higher incidence in older children
- A/H1 higher incidence in children and working age adults
Influenza mortality varies with patient age and influenza type/subtype

- Overall A/H3N2 causes the largest numbers of hospitalisations and deaths, mainly in the 60+ age group, then in young children
- A/H1N1 has a broader mortality range, especially relatively more in the 40-60 y.o. age group
- Influenza B causes few deaths, most of which are in the 60+ age group
Seasonal comparisons

Proportion of PathWest Tests Positive for Influenza
2015 to 2019

- 2019 – started earlier than usual, finished earlier than usual, and was a bit bigger than recent seasons
Australian Influenza Surveillance No. 12, 2019

It’s not the same everywhere
The number of laboratory-confirmed cases were very high, exceeded only by the pandemic year
The percentage positive rate was very high, but similar to a number of other years
This suggests that there was an effect due to increased testing rather than just increased activity.
Influenza in Western Australia 2017-2021

Data from WA Health Respiratory Pathogen Report
Influenza 2020

WHO Collaborating Centre Melbourne.

*Influenza Updates* Volume 9, Issue 3, December 2020

- “It has been an exceptionally quiet year for influenza around the world, due in part to restrictions on travel and social distancing measures that have been in place for the COVID-19 pandemic.”
- “It is difficult to predict when, where and how much influenza activity will return and which virus(es) will circulate.”
Influenza 2020

- Influenza activity dropped dramatically in April and did not reappear
- It was not due to any reduction in testing
- It coincided with travel restrictions related to the COVID-19 pandemic

Influenza diagnosis – antigen detection

• Antigen detection tests
  – Theses are designed to be used for testing symptomatic patients in the early stages of illness when there is a reasonable suspicion of infection
  – They should be used during periods or locations of influenza activity. Otherwise the risk of false positive result will be increased.
  – Where positive or negative tests are not consistent with the clinical and/or exposure history, they should be retested by PCR
  – Where tests are positive at least some should be referred for reference testing to allow subtyping of the influenza A and lineage typing of influenza B. This particularly applies to hospitalised patients, outbreaks, overseas travellers, first cases in an area, following exposure to animals, or other situations of higher clinical or public health concern
Influenza treatment and prevention

• Vaccination
• Antiviral agents
• Non-pharmacological interventions
  – Avoidance of exposure
  – Personal hygiene
  – Community mitigation strategies
Influenza vaccination

- Vaccination prevents milder illness in the community, as well as influenza-related hospitalisation and death.
- Severe illness is more likely in those aged 65y.o. or more, and people with pre-existing chronic illnesses.
- **Reduces the impact of influenza on individual health by**
  - Reducing the risk of infection
  - Reducing the severity of illness in those who are infected
- **Reducing the risk and impact of infection is achieved by**
  - Vaccinating the individual to protect them
  - Reducing the likelihood of them infecting others
    - Vaccinate those who have contact with people at high risk of serious illness – health care workers, close contacts of risk groups for severe disease
    - Universal vaccination of young children – protects them individually and reduces the spread within the community (herd immunity)
Influenza vaccine production

- Influenza viruses are forwarded from National Influenza Centres and other laboratories to the WHO Collaborating Centre for Influenza Reference and Research in Melbourne.
- They undertake antigenic typing
- WHO has two meetings each year to select the best strains for inclusion in the upcoming season – meet in September for Southern hemisphere season and February for Northern hemisphere season
- Allows 6 months for vaccine formulation, testing and production
Influenza vaccines in Australia

• Prior to this year, all licensed vaccines available were made from virus grown in eggs, then inactivated and “split”.
• The vaccine variables
  – The number of different antigens –
    • Trivalent vaccines contain antigen from influenza A/H1, influenza A/H3, and one influenza B antigen (either the Yamagata lineage or the Victoria lineage)
    • Quadrivalent (also called tetravalent) vaccines contain antigen from influenza A/H1, influenza A/H3, influenza B Yamagata lineage and influenza B Victoria lineage
  – Vaccines with increased immunogenicity – these are aimed at getting better responses in the 65+ age group - two types
    • Those containing a higher amount of antigen
    • Those with an adjuvant that boosts immune responses
• Protective levels are best from about 2 weeks to 6 months after and vaccination.
• **Vaccine based on virus grown in cells has been registered for use in 2021**
• Live-attenuated vaccines are used overseas, but none yet licensed in Australia

Efficacy versus effectiveness and the recent experience with vaccine effectiveness in Australia

• Efficacy – how well does vaccine work in a controlled trial environment – compare outcome of vaccinated with that in unvaccinated individuals

• Effectiveness – how well does a vaccination program work in preventing particular

• You can measure efficacy and effectiveness against a range of markers, e.g. prevention of ILI, prevention of laboratory-confirmed influenza, prevention of hospitalization, prevention of death.

• For seasonal influenza we usually calculate vaccine effectiveness (VE) using a test-negative design, i.e. it compares the rate of vaccination in people who test positive or negative for influenza. This reduces bias due to possible difference in health-seeking behavior that can occur in cohort studies

• One of the major determinants of how effective the seasonal influenza vaccine is going to be is how well the strain in the vaccine matches to influenza strain that circulates that year.

Choosing the vaccine strains using antigenic cartography: Egg adaptation of vaccine viruses

- Vaccine viruses grown in cell-culture
- Vaccine viruses grown in eggs
- Recent influenza A/H3 viruses.

Sometimes the virus undergoes post-translational (i.e. non-genetic) change in glycosylation as an adaptation to the egg environment, which makes it antigenically different such that it may become less effective as a vaccine.

Egg adaptation has been a major contributor to low vaccine effectiveness in recent years, especially against influenza A/H3.

Antigenic cartography uses ferret antisera raised against circulating and vaccine strains of influenza virus. It then measures the strength of the haemagglutination inhibition.

Cartography figure from Barr I et al August 2012 Vaccine 30(45):6461-71
The benefits of increases in influenza vaccine effectiveness

- The benefits of an absolute 5 percentage point increase in VE varied by season
  - low-severity season (2011–12)
    - would have prevented an additional 228,000 (95% CrI 209,000–299,000) illnesses, 112,000 (95% CrI 102,000–147,000) medically attended illnesses, and 4,900 (95% CrI 4,000–7,700) hospitalizations
  - high-severity season
    - additional 1,050,000 (981,000–1,170,000) illnesses, 526,000 (95% CrI 486,000–589,000) medically attended illnesses, and 25,000 (95% CrI 22,000–30,000) hospitalizations would have been prevented.
    - the greatest numbers of additional illnesses prevented occurred in adults ages 50–64 years
How do we increase effectiveness?

• Improve matching of vaccine with circulating strains
  – Universal vaccines?
• Increase antigen content or use adjuvants
• Reduce egg-adaptation changes
  – Cellular vaccines
  – Choose viruses that don’t undergo egg adaptation
  – Use egg-adapted viruses as the vaccine candidates
<table>
<thead>
<tr>
<th>EGGS-BASED PROCESS</th>
<th>ISOLATION OF CIRCULATING VIRUSES</th>
<th>CREATION OF SEED STRAINS</th>
<th>PROPAGATION OF VACCINE STRAINS</th>
<th>INACTIVATION &amp; PURIFICATION OF VIRUS</th>
<th>FORMULATION &amp; FILLING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonal reference strains selected¹ ²</td>
<td>Virus (seed) strains for manufacturing produced in eggs¹ ²</td>
<td>Live virus propagated in eggs then harvested¹ ²</td>
<td>Inactivation of virus and purification of Hemagglutinin¹ ²</td>
<td>Strains mixed and packaged¹ ²</td>
<td></td>
</tr>
<tr>
<td>CELL-BASED PROCESS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Virus (seed) strains for manufacturing produced in MAMMALIAN cells²</td>
<td>Live virus propagated in MAMMALIAN cells then harvested¹ ²</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Potential for egg-adaptation¹ ²**

**Does not undergo egg-adaptation¹ ²**

CVV = Candidate Vaccine Virus; WHO = World Health Organization.

In most studies in populations under 65 y.o. vaccine effectiveness for cell–based quadrivalent vaccine is increased by 5-15% over egg-based quadrivalent vaccines.

Similar studies in 65 y.o. and over populations showed effectiveness the same as unadjuvanted, standard antigen content vaccines.
Antivirals for influenza

**Matrix protein inhibitors**
- Only for influenza A
- High levels of resistance, including A/H1N1 2009
- No longer recommended as first-line treatment

**Neuraminidase Inhibitors**
- Active against all known strains of human and animal influenza
- Block release of virus from infected cells

**Polymerase inhibitors**
- Active against all known strains of human and animal influenza – varies with the specific antiviral
- Inhibit RNA polymerase
## Influenza antivirals in current clinical use

<table>
<thead>
<tr>
<th>Class</th>
<th>Active against</th>
<th>Target</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2 inhibitors</td>
<td>Influenza A</td>
<td>M2 ion channel. Inhibits uncoating</td>
<td>Amantadine# Rimantadine#</td>
</tr>
<tr>
<td>Neuraminidase inhibitors</td>
<td>All influenza viruses</td>
<td>Inhibits viral neuraminidase. Prevents release of progeny virions</td>
<td>Oseltamivir Zanamivir Peramivir Laninamivir</td>
</tr>
<tr>
<td>Polymerase inhibitors</td>
<td>Influenza A, B</td>
<td>Inhibits viral RNA polymerase</td>
<td>Baloxavir marboxil (Ribavirin)*</td>
</tr>
</tbody>
</table>

*Rarely used for influenza now. Toxic and limited effect, though may be useful in combinations  
# Rarely used due to high levels of resistance*
## Influenza antivirals in advanced evaluation

<table>
<thead>
<tr>
<th>Class</th>
<th>Active against</th>
<th>Target</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerase inhibitors</td>
<td>All influenza viruses (favipiravir), Influenza A only (pimodivir)</td>
<td>Inhibits viral RNA polymerase</td>
<td>Favipiravir (T-305) Pimodivir</td>
</tr>
<tr>
<td>HA receptor inhibitors</td>
<td>All influenza viruses</td>
<td>Removes sialic acid receptors form cell surface. Prevents virus binding.</td>
<td>DAS181</td>
</tr>
<tr>
<td>HA inhibition</td>
<td>All influenza virus</td>
<td>Blocks HA maturation. Also inhibits IF and IFN dependent immunomodulators</td>
<td>Nitazoxanide</td>
</tr>
</tbody>
</table>
NAIs for influenza


Oral oseltamivir and inhaled zanamivir have significant benefits in the treatment of influenza.
Influenza antiviral resistance in the Asia-Pacific region during 2011

*(Hurt A et al, Antivir Res 97;2013:206-210)*

<table>
<thead>
<tr>
<th></th>
<th>H1N1 (1162)</th>
<th>H3N2 (795)</th>
<th>B Vic (1073)</th>
<th>B Yam (52)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oseltamivir</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Reduced
susceptibility | 0           | 0          | 0.3%         | 0          |
| Highly reduced
susceptibility | 2.3% (0.8%)* | 0          | 0            | 0          |
| **Zanamivir**  |             |            |              |            |
| Reduced
susceptibility | 0.6%        | 0.1%       | 0.1%         | 0          |
| Highly reduced
susceptibility | 1.2%        | 0          | 0            | 0          |

* Rate without the Newcastle cluster, which accounted for 32/42 resistant strains

Influenza A >99% amantadine resistant, influenza B 100% amantadine resistant
# The other neuraminidase inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Characteristics</th>
<th>Resistance and Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peramivir</td>
<td>Long acting intravenous. Single does, or daily dose for 5 days. Significantly improves outcomes in mild disease and in hospitalized patients. Mode of action like oseltamivir but tighter binding. Cross resistance.</td>
<td></td>
</tr>
<tr>
<td>Zanamivir</td>
<td>Short acting. Inhaled. Clinical benefits similar to oral oseltamivir. Binds to same NA site as oseltamivir, but no conformational change required. Resistance rare, and usually in N2</td>
<td></td>
</tr>
<tr>
<td>Laninamivir</td>
<td>Long-acting. Inhaled. High lung concentrations for 5 days. Single dose. Mode of action like zanamivir. Resistance rare Can be used in oseltamivir-resistant seasonal A/H1N1</td>
<td></td>
</tr>
</tbody>
</table>

Watanabe A. J Infect Chemother 2013;19:89
### Polymerase inhibitors in current use or advanced development

<table>
<thead>
<tr>
<th>Feature</th>
<th>Favipiravir (T-705)</th>
<th>Pimodivir (JNJ-63623872)</th>
<th>Baloxavir (S-033188)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza polymerase target</td>
<td>PB1</td>
<td>PB2</td>
<td>PA</td>
</tr>
<tr>
<td>Influenza virus-type spectrum</td>
<td>A, B, C</td>
<td>A</td>
<td>A, B</td>
</tr>
<tr>
<td>Inhibition of M2I and NAI-resistant viruses</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>In-vitro potency</td>
<td>μM</td>
<td>nM</td>
<td>nM</td>
</tr>
<tr>
<td>Synergy with NAIs for influenza A viruses</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Route of dosing</td>
<td>Oral (intravenous under development)</td>
<td>Oral (intravenous under development)</td>
<td>Oral</td>
</tr>
<tr>
<td>Antiviral efficacy in uncomplicated influenza</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical efficacy in uncomplicated influenza</td>
<td>Variable</td>
<td>Not formally tested</td>
<td>Yes</td>
</tr>
<tr>
<td>Emergence of variants with decreased in-vitro susceptibility during monotherapy</td>
<td>Not to date</td>
<td>Yes, common</td>
<td>Yes, common</td>
</tr>
</tbody>
</table>

Baloxavir marboxil

- Polycyclic pyridine derivative
  - Inhibits PA cap dependent endonuclease.
  - Prodrug. Active component (baloxavir) is released by enzymes in liver, blood and small intestine.
- Efficacy against
  - Influenza A,B; including NAI and adamantane resistant strains
- In vitro
  - synergistic with NAIs
  - Develop resistance due to PA/I38T substitution. Reduces endonuclease activity. Acquired resistance in up to 10% in trials. Isoleucine-38 variants of PA subunit (I38T, I38M, I38F)
- Route of administration
  - Oral
  - Trials show efficacy against for treatment of uncomplicated influenza and for prophylaxis

Baloxavir marboxil

• Treatment
  – CAPSTONE-2: Phase III study in high-risk influenza patients of baloxavir vs placebo or oseltamivir
  – Confirmed safety in this population
  – Baloxavir was superior to placebo in improving influenza symptoms by 29.1 hr
  – Baloxavir superior to oseltamivir by 7.7 hr
  – Baloxavir associated with significantly fewer influenza-related complications and reduced the requirement for antibiotics compared with placebo

• Prophylaxis for household contacts
  – Risk of influenza infection reduced by about 60%

International influenza activity
Feb 1-14, 2021.
Influenza vaccination in 2021

- Very important!
- Minimal influenza activity last year, potentially means a highly susceptible population.
- Difficult to estimate VE from 2020, and difficult to choose vaccine strains
- Coordinate with SARS-CoV-2 vaccines. No co-administration data as yet. Interaction with SARS-CoV-2 vaccines not expected, but recommend 2 week separation to allow accurate monitoring of reactions to the SARS-CoV-2 vaccine.²


Public health measure for prevention of influenza

- Maybe they do work!