Influenza vaccination.
How can we improve its effectiveness?

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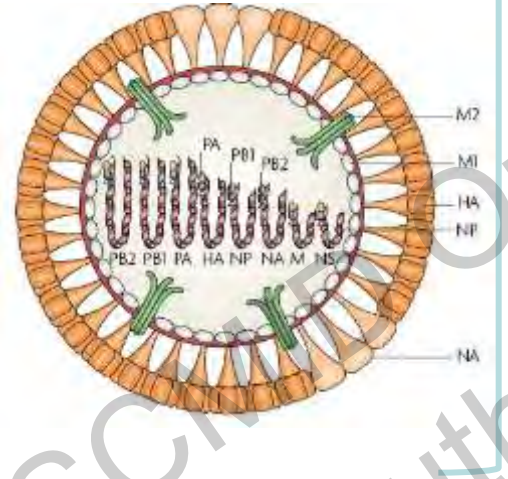
Influenza virus

- RNA Virus
- Orthomyxoviridae Family : A, B y C



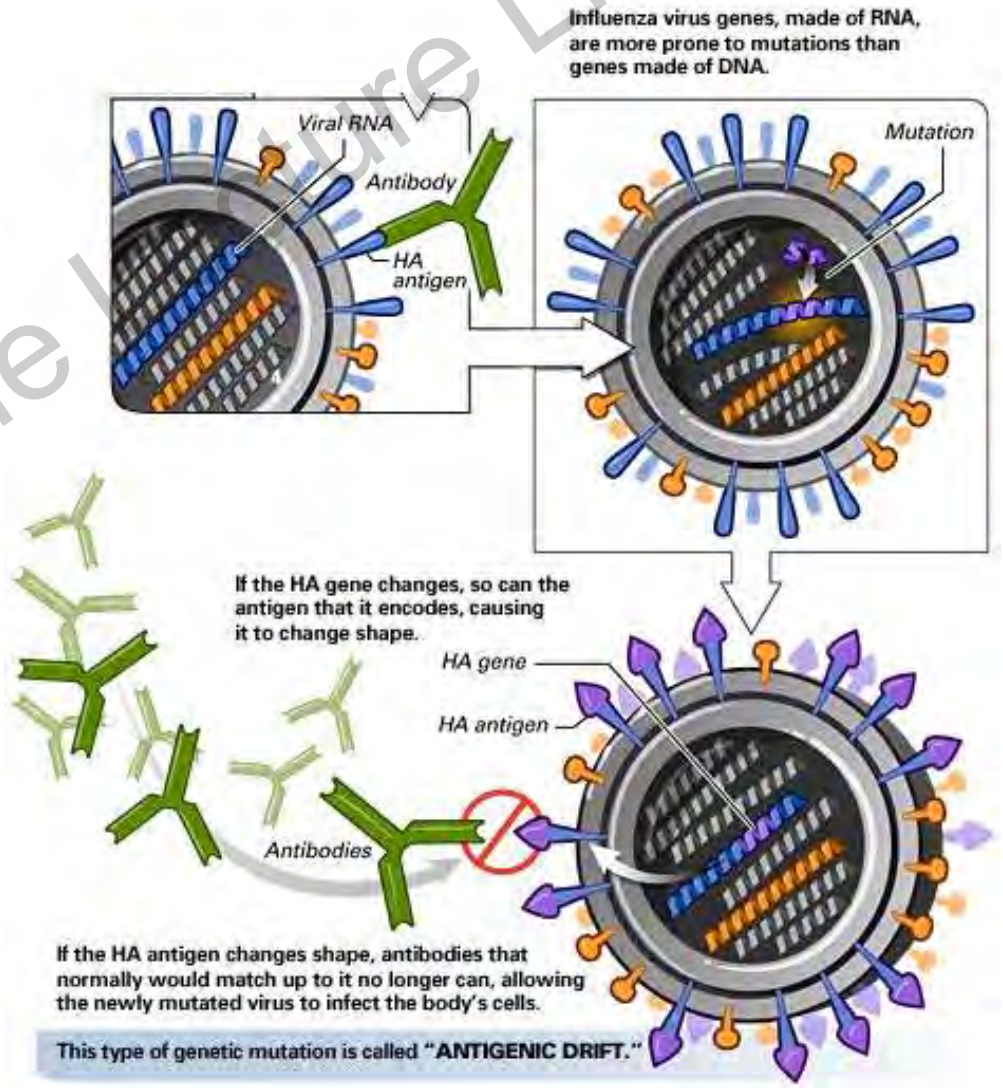
HA: hemaglutinin
NA: neuraminidase
M1 y M2: matrix protein
NP: nucleoprotein
PB1, PB2 y PA: polymerase
NS1 y NS2: non-structural

Flu: acute, self-limited febrile illness

Subbarao et al. Nat Rev Immunol 2007

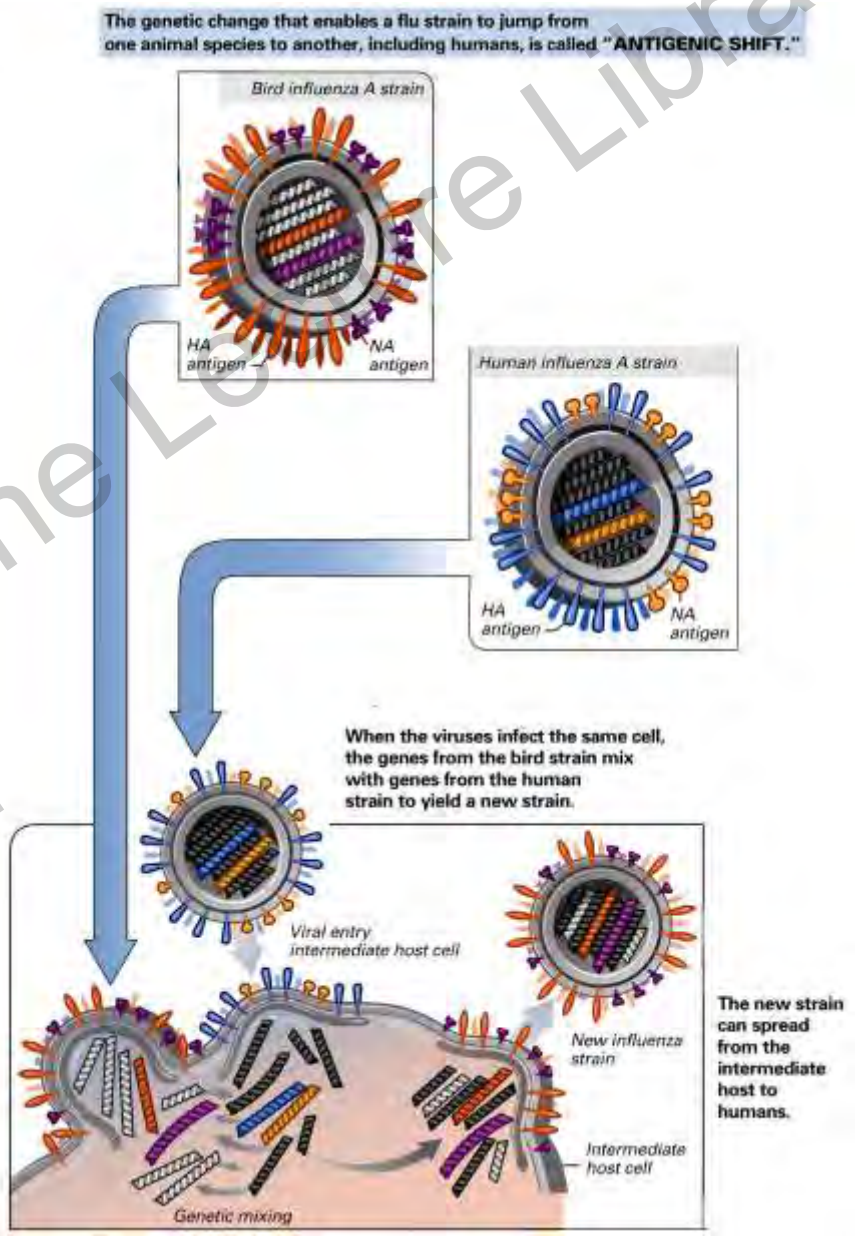
Antigenic drift of influenza virus

Enormous plasticity to select gene variations that affect the antigenicity of the HA and NA proteins



National Institute of Allergy and Infectious Diseases (NIAID)

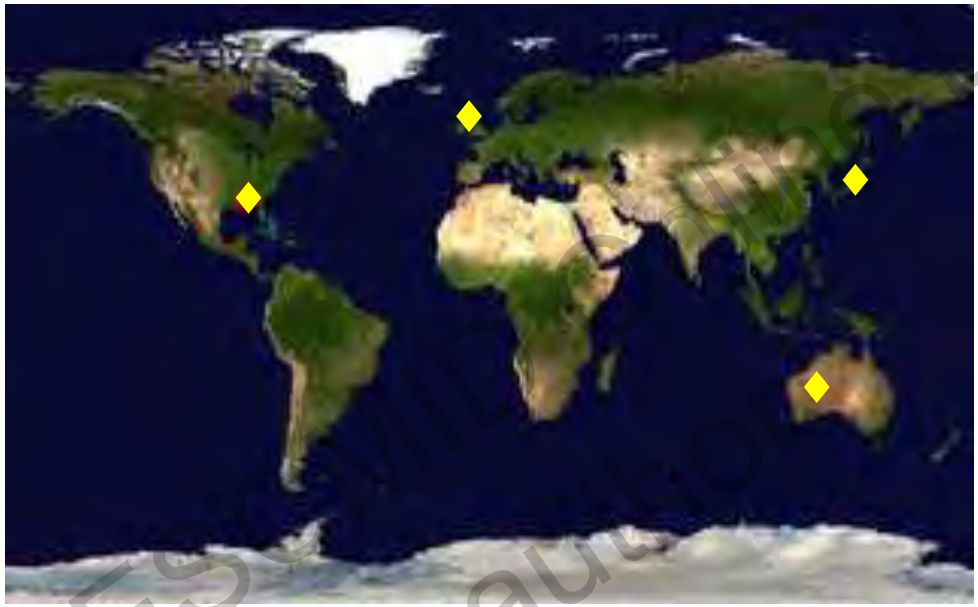
Antigenic shift of influenza virus



National Institute of Allergy and Infectious Diseases (NIAID)

Vaccine composition

WHO Global Epidemiological Surveillance Standards for Influenza



125 centers in 96 countries collect samples of the circulating virus

↓

Centralized in 4 reference centers

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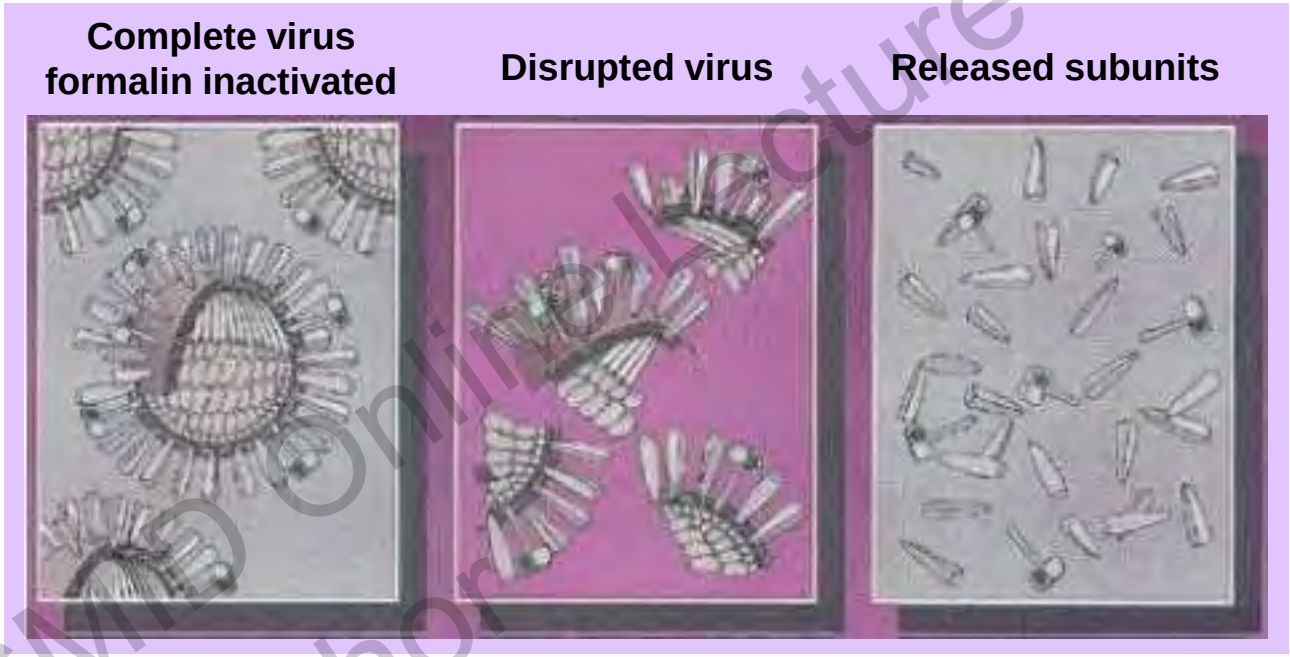
Annual composition proposal: including two influenza A subtypes (H3N2, H1N1) and one influenza B subtype

Type of vaccines

Trivalent inactivated vaccine (TIV)

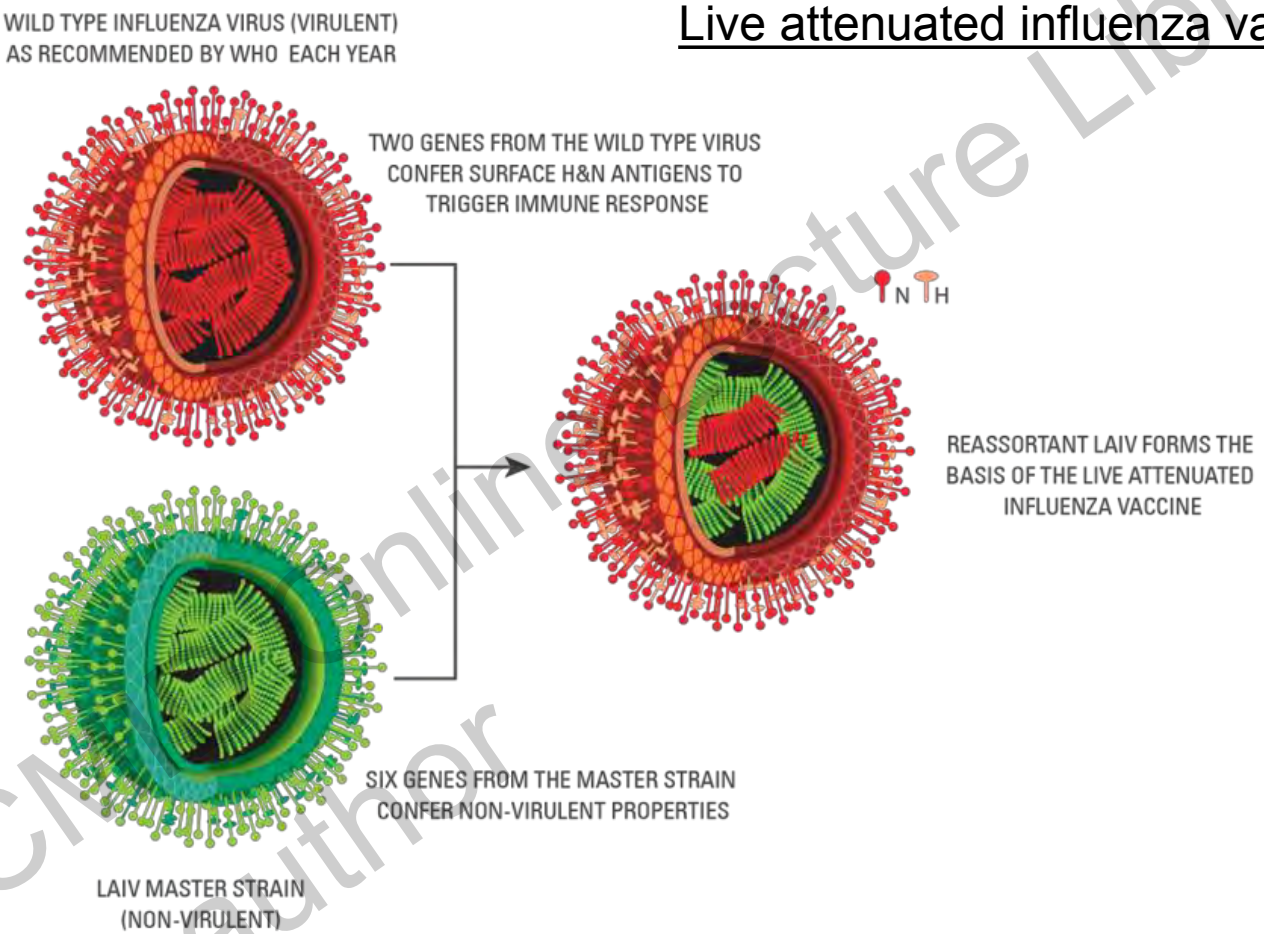


Administration:

- Intramuscular injection.
- Recommended for children > 6 months, pregnancy and chronic patients.
- No recommended for individuals allergic to egg proteins.

Type of vaccines

Live attenuated influenza vaccine (LAIV)



Administration:

- Nasal. Only available in few countries.
- Recommended for 5-49 year old healthy individuals.
- Not recommended for pregnant women, individuals allergic to egg proteins and transplant recipients.

Influenza vaccine recommendations

- Annual administration of seasonal trivalent inactivated influenza vaccine in pre- and posttransplantation.

- Live attenuated influenza vaccine is not recommended for transplant recipients.

- Not recommended for patients < than 3 months after transplantation or intensified immunosuppression for rejection.

- Close contacts of transplant patients should be immunized.

- Health care workers working with transplant recipients should be immunized.

- Influenza vaccination for children should follow the standard age and dose recommendation.

- Not recommended for persons with known severe allergic reactions to chicken or egg proteins.

World Health Organization; Advisory Committee on Immunization Practices; American Society of Transplantation

Annual influenza vaccination

Annual administration of the influenza vaccine is the most effective measure for the control and prevention of influenza

70-90% Specific reduction of disease

60-80% Reduction of mortality and related complications

WHO position paper on Influenza Vaccines WHO 2005, Spanish Ministery of Health 2014;

Flu in Transplant Patients

Articles	N	Patients	Results
Lo et al. Pediatr Transplant 2013	166	TPH, Cancer, TOS	208 infections, 12% influenza virus
Camargo et al Transplantation 2012	22	TOS	41% Acute renal failure
Cordero et al. CMI 2012	51	TOS	29% pneumonia, 6% acute rejection, 8% mortality
Kumar et al. Lancet Infect Dis. 2010	237	TOS	32% pneumonia, 16% ICU, 4% mortality
Peck. et al. Blood 2007	3	TPH	Upper airway symptoms
Nichols et al. Am J Transplant 2004	62	TPH	25% Mortality
Martino et al. Clin Infect Dis 2003	130	Cancer	50% pneumonia, 32% ICU, 10% mortality
Vilchez et al. Am J Transplant 2002	15	TOS	62% Acute and chronic rejection
Whimbey et al. Bon Marr Tran 1994	68	TPH, Cancer	9% pneumonia, 17% mortality
Ljungman et al. Clin Infect Dis 1993	25	TPH, Cancer, TOS	Mild and autolimited disease

Associated with high morbidity and mortality

Vaccine recommendations in SOT

- After the 2009 pandemic, substantial morbidity and mortality has been described in solid organ transplant recipients due to influenza infection.

- Given the risk of severe disease post-transplantation recommendations for diagnosis, prevention, and therapy of influenza infection were given by several scientific societies.

- Annual influenza vaccination was recommended to prevent infection for transplant recipients.

Kumar et al. Am J Transpl 2009; Zhu et al. New England J Med 2009

Immunological response to influenza vaccination

Antibody response

Geometric mean titer (**GMT**): mean antibody titers in vaccinated individuals

Seroprotection: Antibody titers ≥1:40

Seroconversion: 4 fold increase in antibody titer from baseline

Geometric mean ratio (**GMR**): Postvaccination GMT fold increase from baseline

Effective vaccine: (European standard, 1997):

Seroprotection rate >70%

Seroconversion rate >40%

GMR: >2.5

Immunological response to influenza vaccination in SOT

Articles	N	Patients	Results
Cordero et al. Transplantat 2012	100	SOT	75% seroprotection
Cordero et al. Am J Transplant 2011	346	SOT	83% seroprotection
Manuel et al. Clin Infect Dis 2011	29	Kidney	52% seroconversion
Gaeta et al. Vaccine 2009	16	Liver	5–42% seroprotection
Candon et. al Am J Transplant 2009	66	Renal	9-22% seroprotection
Scharpe et al. Am J Transplant 2008	165	Renal	78-93% seroprotection
Birdwell et al. Am J Kidney Dis 2009	53	Renal	5-21% seroprotection
Lawal et al. Am J Transplant 2004	51	Liver	15% seroprotection
Duchini et al. Liver Transpl 2001	20	Liver	15% seroconversion
Burbach et al. Transplantation 1999	43	Liver	92-95% seroprotection
Admon et al. Vaccine 1997	26	Heart	8-36% seroprotection

High variability of results with low immune response

Immunological response to influenza vaccination in SOT

- Most studies show a significantly reduced humoral response in transplant recipients.

- Rate of seroprotection highly variable between 5% and 95%.

It is necessary to improve the effectiveness of flu vaccination in the transplant setting

Factors influencing immunological response to influenza vaccination

- Factors related with the vaccinated individual:
  - age: less response to vaccination in children and elderly
  - comorbidities
  - immune status

- Antigenic similarity between vaccine strains and the actual circulating virus (Ohmit NEJM 2006; Bridges JAMA 2000; Villari Vaccine 2004; Nichol NEJM 1995)
  - 70-90% efficacy in years with high antigenic similarity
  - 50-80% efficacy in years with low antigenic similarity

Route of administration

Randomized Controlled Trial of High-Dose Intradermal Versus Standard-Dose Intramuscular Influenza Vaccine in Organ Transplant Recipients

A. Baluch[a], A. Humar[a], D. Eurich[b], A. Egli[a], A. Liacini[c], K. Hoschler[d], P. Campbell[a], N. Berka[c], S. Urschel[a], L. Wilson[a] and D. Kumar[a,*]

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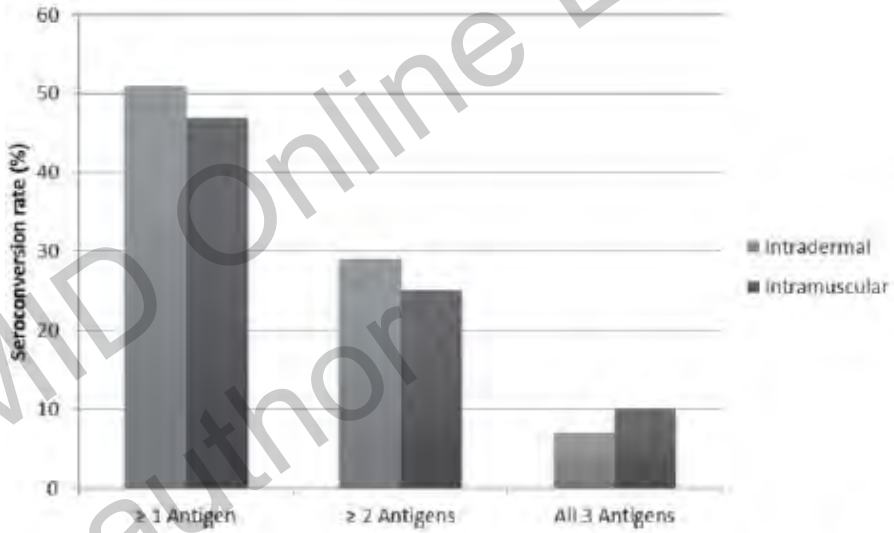


Figure 2: Seroconversion to at least one, two or all three antigens according to the vaccine type*. *No significant differences observed between the two groups.

Am J Transplant 2013

High-dose ID vaccination is an alternative to standard-dose IM vaccination

Type of vaccine

Trivalent inactivated vaccine *vs.* Live attenuated influenza vaccine

Articles	N	Patients	Results
Edwards JID 1994	5210	Immunocompetent subjects	Both safe and effective
Eick Vaccine 2009	70,325	Millitary personnell	Greater protection of LAIV vs. TIV
Jefferson et al. Cochrane Database Syst Rev 2008	18 cohorts	Children >2 years old	Efficacy: TIV: 59% - 65% LAIV: 72% - 82%

No clear advantage with one or the other and discordant results

Time after transplantation

Recommendations for timing of administration varies from 3 to 6 months after the transplant.

Patients diagnosed with influenza infection **within the first 3 months after the transplant have 5 times increased risk of acquiring severe disease** compared with those immunized later after transplantation.

A concern has been raised about the safety of administering the vaccine within the first six months after transplantation and the hypothetical possibility of triggering acute rejection.

Whether the strong immunosuppressive regimens given to patients, especially during the first months after transplantation may affect the response to the influenza vaccine is not resolved.

Vaccination within 6 months posttransplant

< 6 months

N= 150

> 6 months

N= 463

Variable	<6 months post-transplant	> 6 months post transplant	P-value	RR(95%CI)
Seroprotection rate (%)				
A/H1N1	115 (76.7)	373 (80.6)	**0.297**	0.79 (0.50,1.23)
A/H3N2	96 (73.8)	109 (77.3)	**0.885**	0.93 (0.53,1.65)
B	109 (86.5)	109 (77.3)	**0.052**	1.88 (0.98,3.58)

0% Acute rejection
0% Serious adverse events

Vaccination within 6 months posttransplant

Multivariate model

Time post transplant 1-6 months (yes *vs.* no)

Age

Male (yes *vs*. no)

Type of transplant

- Liver
- Kidney
- Heart
- Lung
- Others

Use of m-TOR (yes *vs.* no)

Diabetes (yes *vs.* no)

Hypogammaglobulinemia (yes *vs*. no)

Chronic kidney disease (yes *vs*. no)

Chronic liver disease (yes *vs.* no)

Previous season vaccine (yes *vs.* no)

GMT pre-vaccine

Factors influencing a lower immune response
mTOR inhibitor administration
Heart transplant recipients

Vaccinated patients that developed influenza infection 8 (1.3%)

< 6 months	> 6 months
1 (0.06%)	7 (1.5%)

Baseline antibody titers

• Having baseline antibody titers is significantly associated with the response to vaccination

Influenza strain	Seroprotection	*P*
Influenza A/2009H1N1: previous antibody titers (yes vs. no)	22 (100%) vs. 54 (73%)	0.006
Influenza A/H3N2: previous antibody titers (yes vs. no)	14 (100%) vs. 51 (62.2%)	0.005
Influenza B: previous antibody titers (yes vs. no.)	12 (100%) vs. 58 (69%)	0.02

Table 4. Seroprotection according to having GMTpre in patients with conserved long term response after 1 year.

• Annual vaccination to maintain long-term antibody titers.

• <u>Dificulties:</u>

Variations in circulating strains.
Long term response is deficient with only 30% of SOT recipients maintaining antibody titers one year after vaccination

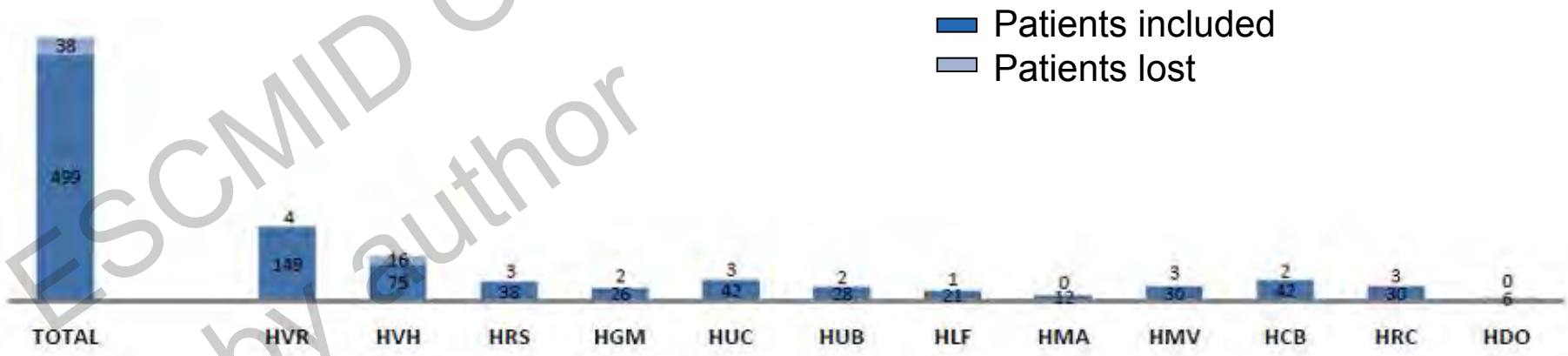
Cordero et al. Am J Transplant 2011, Cordero et al. Transplantation 2012.

Using a second vaccine dose to boost the immune response

Different studies have evaluated the use of a booster dose with different results may be explained by the different immunosuppression regimens employed, the different influenza strains and different rates of baseline seroprotection in the community. Further randomized clinical trials are warranted to generate strong evidences.

TraNsgripe1-2. Prospective ramdomized comparative clinical trial of the efficacy and safety of one versus two doses of the seasonal influenza vaccine to prevent flu in solid organ transplant recipients

Nº EudraCT: 2011-003243-21



CÓDIGOS DE CENTROS: **HVR**, Hospital Virgen del Rocío; **HVH**, Hospital Vall d'Hebron; **HRS**, Hospital Reina Sofía; **HGM**, Hospital Gregorio Marañon; **HUC**, Hospital Universitario de Cruces; **HUB**, Hospital Universitario de Bellvitge; **HLF**, Hospital La Fe; **HMA**, Hospital Virgen Macarena; **HMV**, Hospital Marqués de Valdecilla; **HCB**, Hospital Clinic de Barcelona; **HRC**, Hospital Ramón y Cajal; **HDO**, Hospital Doce de Octubre.

Possible alternatives currently under development

Approaches for new formulations of the vaccine:

- DNA vaccine
- Virus-like particles
- Using vectors

Table 2
Universal influenza vaccines based on the HA protein.[a]

Types	Carrier	Vaccination	Challeng subtypes	References
Fusion peptide	Peptide (synthetic)	Peptides (100 μg)/CFA, IFA/SC (mice)	H1N1 (A/PR/8/34)	Horvath et al. (1998)
Fusion peptide	Protein (conjugates)	Influenza B HA fusion Peptide-OMPC (1 μg peptides)/Q521, Aluminum adjuvants/IM (mice)	B/Ann Arbor/4/55 B/Hong Kong/330/2001 B/Yamanashi/166/1998	Bianchi et al. (2005)
Fusion peptide	Peptide–protein conjugates	Synthetic Peptides-KLH (mice)/broadly cross-reactive antibodies	H1–H13 subtype HA proteins	Li et al. (2010)
Head-less HA	Transfected cells	Transfected cells (1×10^6 cells) expression headless HA (A/Okuda/57 (H2N2))/IP	H1N1 (A/FM/I/47)	Sagawa et al. (1996)
Head-less HA	DNA/VLPs	Genetically engineered DNA/VLP vaccines/IM, Freund's adjuvants (mice)	H1N1 (A/PR/8/34)	Steel et al. (2010)
HA2 peptide	Peptide–protein conjugates	Synthetic Peptides-KLH (25 μg)/SC/(mice)	H3N2 (X31), H1N1 (A/PR/8/34)	Wang et al. (2010b)

New protein targets for vaccine development

M2 protein highly conserved between influenza strains.

M2-based influenza vaccines.[a]

Types	Carrier	Vaccination	Challenge subtypes	References
M2	M2 protein (rBV), GST fusion (*E. coli*)	M2 (9 μg)/IFA/IP/3 times (mice) M2-GST fusion	H1N1 (A/Taiwan/1/86), H2N2 (A/Ann Arbor/6/60), H3N2 (A/Hong Kong/1/68)	Frace et al. (1999), Slepushkin et al. (1995)
M2	M2 DNA/rAd NP DNA/rAd vaccines	50 μg DNA/10^{10} particles rAd/IM (mice, ferrets)	H1N1 (A/PR/8/34, A/FM/1/47), H5N1 (A/Thailand/SP-83/04, A/Vietnam/1203/04)	Jimenez et al. (2007), Lalor et al. (2008), Tompkins et al. (2007)
M2e	Peptide (synthetic)	M2e peptides/IFA, Aluminum/CpG ODN (mice)	H1N1 (A/PR/8/34)	Wu et al. (2007, 2009b)
M2e	Protein (conjugates)	M2e-KLH (40 μg)/CFA (mice)	H1N1 (A/PR/8/34, A/FM/1/47)	Tompkins et al. (2007)
M2e	Protein (conjugates)	M2e-KLH, M2e-OMPC (20–100 μg)/CFA, QS1, Aluminum adjuvants (mice, ferrets, rhesus monkeys)	H1N1 (A/PR/8/34) H3N2 (X-31) H5N1 (A/Hong Kong/97)	Fan et al. (2004)
M2e	Fusion proteins (*E. coli*)	M2e-GST (mice, rabbit) M2e-CTA1-DD (5 μg)/IN/(mice) M2e-tGCN4 (10 μg)/MPL, Alhydrogel/CTA1-DD adjuvants/IP, IN M2e-ASP-1 (20 μg)/IM, 3 times	H1N1 (A/PR/8/34) H3N2 (A/Victoria/3/75, X47) H5N1 (A/Vietnam/1203/04, A/Shenzhen/406H/06)	Liu et al. (2004) Eliasson et al. (2008) De Filette et al. (2008) Zhao et al. (2010)
M2e	VLPs (*E. coli*)	M2e-HBVc/CTA1-DD, LT(R192G), QS1, ISA720 adjuvants IP, IN/twice, 3 times	H1N1 (A/PR/8/34) H3N2 (A/Victoria/3/75, X47)	De Filette et al. (2006a), De Filette et al. (2005), Fiers et al. (2004), Heinen et al. (2002), Neirynck et al. (1999)
M2e	VLPs (*E. coli*)	M2e-PaMV (100 μg)/Alum, M2 peptide/VLP adjuvants	H1N1 (A/WSN/33)	Denis et al. (2008)
M2e	HPV VLPs (Yeast) conjugates	M2e-HPV (45 μg)/Alum adjuvant/IM, twice	H1N1 (A/PR/8/34), H3N2 (X-31, reassortant A/Aichi/68)	Ionescu et al. (2006)
M2e	Liposome	M2e-liposomes (15–60 μg)/MPL adjuvant/2–3 times	H1N1 (A/PR/8/34), H5N1 (A/HK/483/97), H6N2 (X-88), H9N2 (A/HK/1073/99),	Ernst et al. (2006)
M2	M2 VLPs (rBV)	M2 VLPs (10 μg)/IN, 2 times	H1N1 (A/PR/8/34),H3N2 (A/Philippines/82) H5N1 (reassortant A/Vietnam/1203/04)	Song et al. (2011b)

Conclusions

1. Influenza infection contributes to increased comorbidities.

2. Vaccination remain the most effective strategy to protect and prevent influenza infection for the vulnerable population such as transplant population.

3. Due to incomplete vaccination efficacy, it is necessary to implement strategies for vaccination to improve vaccine response.

4. Intradermal administration, vaccine administration after 1 month posttransplantation, and promoting baseline antobody titers have shown improvement.

5. New alternative approaches would be desirable.