Vaccines
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Only clean water and antibiotics have had an impact on mortality and morbidity equal to that of vaccines.
In most instances vaccination is the most cost-effective method for disease prevention.

The eradication of smallpox demonstrated the power of large scale vaccination.

In 1966, 10-15 million cases of smallpox were reported and there were 2 million deaths in 31 endemic countries.
Variolation: the practice of deliberate induction of disease by transmission of material from smallpox pus or scabs.

Case fatality rate after variolation - 0.5 to 2%.

Case fatality rate after natural infection - 30%.

In the 17th and 18th centuries, 10% of all death in London were due to smallpox, thus making the benefit/risk ratio of variolation quite substantial.
The process of variolation not only saved lives but was commercially important.

Voltaire wrote in 1733, that in order to avoid the disruption of trade in young beautiful maidens to the Sultans of Turkey and the Persian Emperor, when epidemics of smallpox erupted, the body of children were inoculated with 'a pustule taken from the most favorable sort of smallpox that could be procured'.
• Worldwide eradication of smallpox was achieved in 10 years and with a budget (modest) of $300 million.

• This was translated into savings of U.S. $0.5 billion per year.

• Worldwide $2.5 billion per year which would otherwise have to be spent on smallpox control.
Lurking menace: The world signed up to eradicating polio in 1988. The Global Polio Eradication Initiative was launched, which is a partnership between governments and organizations such as Unicef, the WHO and Rotary International. The aim was to banish polio once and for all.
In 1988 there were 350,000 recorded cases. By 2012 cases had fallen to 223. But last year there was a rise in cases to 406 new infections.
The World Health Organization has declared its South East Asia region polio-free. The certification is being hailed a "historic milestone" in the global fight to eradicate the deadly virus.
It comes after India officially recorded three years without a new case of polio.
The announcement means 80% of the world is now officially free of polio, although the disease is still endemic in Afghanistan, Nigeria and Pakistan.

Rise and fall in endemic countries
Afghanistan: 2012, 37 cases 2013, 14 cases
Nigeria: 2012, 122 cases. 2013, 53 cases
Pakistan: 2012, 58 cases 2013, 93 cases
Vaccination began with Edward Jenners’ discovery in 1798 of the protective power of vaccination with cowpox to protect against smallpox.

Little more was done for another 80 years until Louis Pasteur discovered the attenuating effect of exposing pathogens to air or to chemicals.
Salmon and Theobald Smith (1886) demonstrated that heat killed cultures of chicken cholera bacilli could protect pigeons from disease.

This established that protection was not dependent on interaction of a live microbe with the host's cells. This established the use of killed vaccines.
• Roux and Yersin (1898) demonstrated that the filtrate of a culture of diphtheria bacilli (i.e. the toxin) would induce a protective response that involved the formation of antitoxins that neutralized the activity of the bacterial toxin.

• Glenny discovered the process of detoxifying bacterial toxins using formalin. This converted the toxin to a toxoid.
Glenny and colleagues also discovered the principle of primary and secondary responses to antigens. They noted that injection of a soluble antigen preparation gave a rather brief antibody response which correlated to the rapid disappearance of the antigen from the injection site.

They invented the use of alum-ppt. toxoid to slow down the absorption of antigen from the injection site thus reducing its rapid elimination.
Infectious diseases are the second leading cause of death worldwide, after heart disease, and are responsible for more deaths annually than cancer. Infectious diseases claim 16.2 percent of people who die each year. Children under the age of five are especially vulnerable, and infectious diseases account for a disproportionate number deaths in this group.

Of the top ten causes of death reported by the World Health Organization in 2008 (updated in 2011), four were due to infectious diseases. However, low-income countries are more severely affected by infectious diseases, and in these countries, five of the top killers were due to infectious agents, with lower respiratory infections being the number one cause of death.

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Cause</th>
<th>Estimated number of deaths (in millions)</th>
<th>Percent of all deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Lower respiratory infections</td>
<td>3.46</td>
<td>6.1</td>
</tr>
<tr>
<td>5</td>
<td>Diarrheal diseases</td>
<td>2.46</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>HIV/AIDS</td>
<td>1.78</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>Tuberculosis</td>
<td>1.34</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Prevention vs Therapy: a difficult sell

**Prevention**
- intervention in healthy people
  - compliance
  - willingness to pay
  - acceptance of side effects
- long term benefit risk/cost assessment
  - epidemiology/vaccination strategy

**Therapy**
- intervention in sick people
  - compliance
  - willingness to pay
  - acceptance of side effects
- short term benefit

Opposed interests of companies' shareholders and their customers

**SHAREHOLDERS**
want the best return on investment
- control/reduce
  - R&D costs
  - capital investments
  - operating costs

**CUSTOMERS**
want the best quality for the lowest price
- develop
  - price control mechanisms
  - competition
  - procurement mechanisms
- minimize expenditures

Liège, 30 August 2000
Adolescents

- Booster for paediatric disease
- DTP
- Polio
- MMR

- Hepatitis A+B

Sexually transmitted diseases
- Herpes
- EBV
- CMV
- Chlamydia
- HPV

Travellers

- Hep A+B
- Hep E
- Salmonella
- Dengue
- Malaria
- STD's
- Travellers' diarrhoea
- ETEC
- Malaria
- Specific vaccinations
- Lyme

The Elderly

- Flu
- Pneumococcal
- RSV
- Zoster

Liège, 30 August 2000
Public health policy: the vaccination calendar of the future

<table>
<thead>
<tr>
<th>Paediatric</th>
<th>Adolescent</th>
<th>Adult</th>
<th>Travelers</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPV (eradication)</td>
<td>HBV-HAV</td>
<td>HBV-HAV</td>
<td>HBV-HAV</td>
<td>Improved flu</td>
</tr>
<tr>
<td>MMRV</td>
<td>+ Herpes</td>
<td>+ Herpes</td>
<td>+ Typhoid</td>
<td>+ RSV</td>
</tr>
<tr>
<td>DTP-HBV-Hib-IPV</td>
<td>+ Chlamydia</td>
<td>+ dtp booster</td>
<td>+ STDs</td>
<td>+ S. pneumo.</td>
</tr>
<tr>
<td>+ HAV</td>
<td>+ dtp booster</td>
<td>+ AIDS</td>
<td>+ ETEC</td>
<td>+ Zoster</td>
</tr>
<tr>
<td>+ Rotavirus</td>
<td>+ AIDS</td>
<td>+ Chlamydia</td>
<td>+ Shigella</td>
<td></td>
</tr>
<tr>
<td>+ RSV</td>
<td>+ CMV</td>
<td>+ Lyme</td>
<td>+ Malaria</td>
<td></td>
</tr>
<tr>
<td>+ PIV</td>
<td>+ Lyme</td>
<td>+ TB</td>
<td>+ Dengue</td>
<td></td>
</tr>
<tr>
<td>+ Otitis media</td>
<td>+ EBV</td>
<td>+ HPV</td>
<td>+ HEV</td>
<td></td>
</tr>
<tr>
<td>+ N. meningitidis</td>
<td></td>
<td></td>
<td>+ Lyme</td>
<td></td>
</tr>
<tr>
<td>+ Lyme</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>+ TB</td>
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</tbody>
</table>

Liège, 30 August 2000
A Gallery of Rogues

WEAPONS OF MASS DESTRUCTION

Old stand-bys

Anthrax—A staple of bioweapons arsenals for decades, the bacterium Bacillus anthracis might be engineered to resist antibiotics.

Plague—In an infamous episode in World War II, the Japanese Army unleashed fleas infected with the Yersinia pestis bacterium on Chinese forces in Manchuria. The attack backfired, inflicting heavy tolls on both sides.

Q fever—This disease is transmitted by the highly infectious and heat-resistant Coxiella burnetii rickettsiae. Not usually fatal, Q fever could be used primarily as an incapacitant.

Tularemia—one of the most infectious bacteria, Francisella tularensis would kill one out of five people in a hypothetical scenario in which 50 kilograms of the weaponized agent were released 2 kilometers upwind from a city.

Smallpox—Eradicated from the wild in 1980, variola major is known to exist in only two restricted laboratories in Russia and the United States. Experts don’t dismiss the unlikely scenario of theft or diversion of these stocks.

New possibilities

Aflatoxin—U.S. intelligence reports in 1998 suggested that Iraq was attempting to weaponize aflatoxin, a protein produced by a mold that grows on peanuts and other crops. Aflatoxin is highly toxic in people.

Ebola-influenza hybrid—A flu strain equipped with the hemorrhagic proteins of Ebola, presumably still a fantasy, would be a fearsome weapon.

TOOLS OF ASSASSINATION

Old stand-bys

Botulinum toxin—The most poisonous substance known, a single gram of this crystalline toxin from the bacterium Clostridium botulinum, evenly dispersed and inhaled, would kill more than 1 million people.

Ricin—In 1978, Soviet agents used ricin, a lethal toxin extracted from the castor bean, to murder Bulgarian defector Georgi Markov in London.

New possibilities

RNAi—Double-stranded “interference” RNA might be tailored to latch onto specific messenger RNA sequences, thus silencing virtually any gene.

Saxitoxin—Eating shellfish contaminated by this alkaloid neurotoxin, produced by dinoflagellates, can lead to paralysis and death.

Substance P—An aerosolized version of this neurotransmitter could be far more toxic than the potent chemical weapons sarin and VX.

Toxic brews. The United States and other countries are now drafting science briefs describing the state of the black art of bioweapons—including a few of the potential threats highlighted in this table—for the fifth BWC review conference in November. At the conference, signatories will discuss a declaration affirming the treaty that would account for threats that have emerged since the last review conference took place 5 years ago.
Rigorous criteria for assessment of a vaccine's efficacy and safety now exist.

eg. In the U.S. prior to human use the FDA requires extensive testing for toxicity, teratogenicity and efficacy in subhuman primates.

The primate species will vary according to its susceptibility to the organism.
Trials in humans occur in 3 stages.

Phase I trial: the putative vaccine is administered to human volunteers to test for safety and efficacy as measured by the induction of an appropriate immune response to an agreed level.

Phase 2 trial: these test the efficacy of the potential product to protect against disease after exposure to the live agent. The agent is given to volunteers or to members of a high risk group.

Planning for a phase 2 trial should include values for the level of efficacy and degree of confidence needed. This influences the number of participants needed.
Phase 3 trials are carried out in the field. One or more communities for which detailed epidemiological data are available are selected.

Already available is:

a) information on the risk of contracting the disease naturally.
b) the disease burden.
c) the infrastructure for delivery of the vaccine and assessment of efficacy following administration.

The ability of the vaccine to protect against disease and to ameliorate existing disease may both be tested. Very large numbers of people are vaccinated.
Any new or improved vaccine to be considered for inclusion in the WHO's EPI should ideally have the following attributes:

a) it must be inexpensive.

b) it must be safe.

c) it must be extremely effective (protection in 90-100% recipients).

d) it should provide lifetime immunity.

e) it should be heat stable (not need a cold chain).

f) it should require only one shot or be compatible with the schedule for other vaccines.
Ideal vaccine: single administration at birth will provide protection from multiple diseases.

Vaccines were developed as a *prophylactic* measure to *prevent* diseases provided their use caused only low levels of morbidity/mortality.

Seroconversion: measurement of antibody before and after vaccination.
The first cancer vaccines were composed of whole tumor cell lines that were previously irradiated or inactivated.

In mouse models this immunization strategy was successful, generating tumor specific immune responses and rejection of a tumor challenge.
Immunize mouse with irradiated tumor cells

Inject viable cells of the same tumor
Response to unique tumor rejection antigens eliminates tumor

Inject viable cells of a different tumor
Response to irradiated tumor will not eliminate unrelated tumors of a different cell type

Figure 14-10 Immunobiology, 6/e. (© Garland Science 2005)
The use of spontaneous tumors (that better mimicked human situations proved to be either non-immunogenic or weakly immunogenetic.

Immunologists had deciphered the exact requirements for antigen-specific T cell activation: T-cell receptors signaling and requirement for co-stimulatory signals.
The next generation of vaccines were therefore composed of gene-modified tumor cells that expressed various co-stimulatory molecules and/or cytokines.
Malignant tumor cells expressing TRA but no co-stimulatory molecules

Naive CD8 T cells specific for TRA cannot be activated by the tumor cells and may be rendered anergic

Tumor grows progressively

Transfect tumor cell with B7

Tumor cells expressing B7 can activate TRA-specific CD8 T cells

Activated CD8 T cells eliminate tumor

Transfect tumor cell with GM-CSF

GM-CSF recruits dendritic cells, which can present tumor antigens to T cells

Mouse can now reject parental B7-negative or GM-CSF-negative tumor

Figure 14-20 Immunobiology, 6/e. (© Garland Science 2005)
Manipulation of tumor cells: e.g. fusion with DCs, genetic engineering to express cytokines, chemokines, co-stimulatory molecules, or antisense for growth factors.
Unique Tumor Antigens

Products of random mutations or gene rearrangements often induced by physical or chemical carcinogens, and therefore expressed uniquely by individual tumors.

Vaccine comprised of whole tumor cells or whole tumor-cell lysates are complex mixtures which contain unique tumor antigens expressed only by individual tumor.
Shared Tumor Antigens

Molecules that are expressed by many tumors and not normal tissues, or expressed by normal tissue in a quantitatively or qualitatively different form.

Vaccines based on shared antigens elicit both an antigen specific response but can elicit responses to other antigens on the tumor by a process called “epitope spreading”
Epitope Spreading

A term originally applied to responses to autoantigens that tend to become more diverse as the response persists. This phenomenon is also known as determinant spreading. In the vaccine setting, it refers to responses that are generated to antigens other than those contained in the vaccine.
Major effort has been devoted by multiple groups to identifying tumor antigens.

Best known examples are HER2/NEU, MUCIN1, MAGE 3 (and other members of the MAGE gene family), CEA.

They have all been demonstrated to be immunogenic and not cause autoimmunity.
## Potential tumor rejection antigens have a variety of origins

<table>
<thead>
<tr>
<th>Class of antigen</th>
<th>Antigen</th>
<th>Nature of antigen</th>
<th>Tumor type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor-specific mutated oncogene or tumor suppressor</td>
<td>Cyclin-dependent kinase 4</td>
<td>Cell-cycle regulator</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>β-Catenin</td>
<td>Relay in signal transduction pathway</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Caspase-8</td>
<td>Regulator of apoptosis</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Germ cell</td>
<td>MAGE-1</td>
<td>Normal testicular proteins</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>MAGE-3</td>
<td></td>
<td>Breast</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glioma</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Tyrosinase</td>
<td>Enzyme in pathway of melanin synthesis</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Surface Ig</td>
<td>Specific antibody after gene rearrangements in B-cell clone</td>
<td>Lymphoma</td>
</tr>
<tr>
<td>Abnormal gene expression</td>
<td>HER-2/neu</td>
<td>Receptor tyrosine kinase</td>
<td>Breast</td>
</tr>
<tr>
<td>Abnormal post-translational modification</td>
<td>MUC-1</td>
<td>Underglycosylated mucin</td>
<td>Breast</td>
</tr>
<tr>
<td>Oncoviral protein</td>
<td>HPV type 16, E6 and E7 proteins</td>
<td>Viral transforming gene products</td>
<td>Cervical carcinoma</td>
</tr>
</tbody>
</table>

Figure 14-11 Immunobiology, 6/e. (© Garland Science 2005)
• Paul Ehrlich in 1909 proposed that the immune system could protect the host from cancer.
• 50 years later Burnet and Thomas formally introduced the cancer immunosurveillance hypothesis. They predicted that lymphocytes were responsible for eliminating continuously arising cancer cells.
However nude mice neither developed increased incidence of spontaneous or carcinogen-induced tumors, nor showed shorter latency period.
Nude mice were an imperfect model

- Low but detectable nos. of functional $\alpha\beta$ T cells
- Existence of NK cells not well established
- Influence of innate immunity on adaptive immunity not recognized
In the 1990s two important studies resurrected interest in the theory of cancer immunosurveillance.

- Endogenously produced IFN-γ protected the host against the growth of transplanted tumors, chemically induced and spontaneous tumors.
- Mice lacking perforin were found to be more susceptible to MCA-induced and spontaneous tumor formation compared to their wild-type counterparts.
Definitive study demonstrating the existence of an IFN-γ and lymphocyte dependent cancer immunosurveillance came from experiment performed in RAG-2 K.O. mice and RAG-2⁻/⁻ x STAT⁻/⁻ mice (RkSk mice)
Does Cancer immunosurveillance occur in humans?

3 Lines of evidence suggest it does

- Immunosuppressed transplant recipients display higher incidence of non-viral cancers than age-matched immunocompetent controls.
- Cancer patients can develop spontaneous adaptive and innate immune responses to the tumors they bear.
- Presence of lymphocytes within the tumor can be a positive prognostic indicator of patient survival.
Immunologic sculpting

- Tumors are imprinted by the immunologic environment in which they form. By eliminating cells of high intrinsic immunogenicity, this process may select for variants with reduced immunogenicity. This favors the generation of tumors that are either poorly recognized by the immune system or have acquired mechanisms that suppress immune effector functions.
Cancer Immunoediting

• Term describes the dual role of immunity in not only preventing but also shaping neoplastic disease

The 3 Es of Cancer Immunoediting
Elimination, Equilibrium and Escape
The cancer immunoediting concept.

R D Schreiber et al. Science 2011;331:1565-1570

Published by AAAS
Model for evolution and fate of anti-tumor immune responses that develop coincidentally with tumor growth.

a. While the tumor is small it poses no danger and the immune system remains largely ignorant of it. DCs are not activated and B and T cells in draining lymph node remain in resting state.

b. When the tumor becomes larger, damage to normal tissue and products made by the tumor alert the immune system. Resident DCs get activated take up the products derived from tumor and damaged normal tissue and traffic to the LN. the DCs present the antigen to T and B cells.

c. Tumor specific antibodies, T cells and DCs reach the tumor site and attempt to destroy it. They are only partly successful owing to the large tumor size, and the many immune evasion mechanisms that allow the tumor to evade the immune effector cells.

d. The tumor that has evaded the immune responses continues to grow and disseminate and actively suppresses both local and activate immunity.
Manipulation of anti-tumor responses by therapeutic vaccination: Vaccine is administered after tumor is diagnosed and at a time when tumor and immune system have already interacted.

a. A vaccine based on autologous tumor or defined tumor antigen is administered along with adjuvant to activate Langerhan cells. The Langerhan cells take up the antigen and traffic to the draining LN where they present antigen. Expected outcome is clonal expansion of T cells and the production of tumor specific antibodies.

b. Tumor specific T cells migrate to sites of tumor metastases where they attempt to kill tumor cells that express antigens contained in the vaccine. Their function is compromised by the immunosuppressive tumor microenvironment which compromises their function and leads to their death. Additionally some tumor cells have lost the tumor antigen and others become resistant to immune attack. This allows many tumor cells to continue to grow.

c. Metastases that continue to grow are composed of tumor cells that lack antigen recognized by the vaccine activated T cells and antibodies and are resistant to immune destruction.
Manipulation of antitumor responses by prophylactic vaccination: Vaccine is administered before the occurrence of tumors to individuals at high risk or those diagnosed with premalignant changes.
Patients with immunodeficiencies are usually highly susceptible to viral infections and these individuals have an increased incidence of cancers caused by virus or uv light.
Malignant neoplasms with an increased incidence in immunodeficiency patients

<table>
<thead>
<tr>
<th>Type of immunodeficiency</th>
<th>Cancer</th>
<th>Carcinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary (inherited)</td>
<td>B-cell lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>HBV</td>
</tr>
<tr>
<td></td>
<td>Hematological malignancies</td>
<td>Germline</td>
</tr>
<tr>
<td>Secondary, drug induced</td>
<td>B-cell lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td>(patients with or without allograft)</td>
<td>Squamous cell carcinoma (skin)</td>
<td>HPV, UV</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>HBV</td>
</tr>
<tr>
<td></td>
<td>Cervical carcinoma</td>
<td>HPV</td>
</tr>
<tr>
<td>AIDS</td>
<td>B-cell lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td></td>
<td>Cloagenic or oral carcinoma</td>
<td>HPV</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>HBV</td>
</tr>
</tbody>
</table>
**Mechanisms by which tumors escape immune recognition**

<table>
<thead>
<tr>
<th>Low immunogenicity</th>
<th>Tumor treated as self antigen</th>
<th>Antigenic modulation</th>
<th>Tumor-induced immune suppression</th>
<th>Tumor-induced privileged site</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide: MHC ligand</td>
<td>Tumor antigens taken up and presented by APCs in absence of co-stimulation to tolerate T cells</td>
<td>Antibody against tumor cell-surface antigens can induce endocytosis and degradation of the antigen. Immune selection of antigen-loss variants</td>
<td>Factors (e.g., TGF-β) secreted by tumor cells inhibit T cells directly</td>
<td>Factors secreted by tumor cells create a physical barrier to the immune system</td>
</tr>
</tbody>
</table>

[Figure 14-14 Immunobiology, 6/e. (© Garland Science 2005)]
Mechanisms of tumor escape from immunologic destruction

A. Tumor-related

1. Failure of the tumor to provide a suitable target (defective immunosensitivity)
   - Lack of antigenic epitope
   - Lack of MHC class I molecule
   - Deficient antigen processing by tumor cell
   - Antigenic masking of the tumor
   - Antigenic modulation
   - Resistance of tumor cell to tumoricidal effector pathway
Tumor-related (continued)

2. Failure of the tumor to induce an effective immune response (defective immunogenicity)

- Lack of antigenic epitope (see above)
- Decreased MHC or antigen expression by the tumor (see above)
- Lack of costimulatory signal
- Production of inhibitory substances (e.g., cytokines) by the tumor
- Shedding of antigen and tolerance induction
- Induction of apoptosis in T cells by expression of Fas ligand by cancer cells
- Induction of T cell signaling defects by tumor burden
B. Host-related

1. Failure of the host to respond to an antigenic tumor
   - Immune suppression or deficiency of host including apoptosis and signaling defects of T cells due to carcinogen, infections, or age
   - Deficient presentation of tumor antigens by host antigen-presenting cells
   - Failure of host effectors to reach the tumor (e.g., Stromal barrier)

2. Failure of host to kill variant tumor cells because of immunodominant antigens on parental tumor cells
Infectious agents in use as vectors of nucleic acids from other sources

**Viruses** - animal
- Vaccinia, herpesvirus, varicella, adenovirus, papillomavirus, SV40, polio, retroviruses

**Viruses** - avipox
- Fowlpox, canarypox

**Viruses** - insect
- Baculovirus

**Bacteria**
- Salmonella, BCG, E. coli
Recombinant Live Vectors of Foreign DNA

Most work has been carried out with poxviruses, especially vaccinia.

Reasons: (Advantages)

(1) Ability to grow readily high titer virus stocks
(2) Thermostability of the virus
(3) Large capacity for insertion of foreign DNA
(4) High levels of gene expression
(5) Broad host range

Disadvantages:

♦ Vaccinia based constructs could only be used once in a given population because immunity to the vector would limit 'take' of a second administration.
Vaccinia and related viruses

- To circumvent the neutralizing antibody responses induced by vaccinia virus vaccination, attention has now focused on the use of attenuated v. v. and non-replicating poxviruses, such as avipoxviruses.

- Attenuated vaccinia strains: NYVAC, MVA Contain multiple gene deletions which prevent the virus from replicating in mammalian cells.

- Avipoxviruses (fowlpox and canarypox) ALVAC, are pathogenic in birds, unable to replicate in mammalian cells.
Recombinant Adenoviruses

Use of human adenoviruses as a vector for the other DNA molecules has several advantages.

(1) Virus can be readily grown in tissue culture.

(2) High copy number of viral DNA is present in the cell during replication.

(3) The virus has strong promoters.

(4) Up to 7 kilobase pairs of foreign DNA can be inserted.

(5) Can induce mucosal immunity to the agent whose DNA is inserted.
Oncolytic virotherapy mechanism of action: viral replication, cell killing, virus release and spread within cancer tissue but not normal tissues

Tumor selectivity of oncolytic vaccinia virus was achieved by deletion of thymidine kinase (TK) and vaccinia growth factor (VGF) genes:

TK- mutant requires thymidine triphosphate (TTP) for DNA synthesis from the nucleotides present in dividing cells. *This leads to the preferential replication in dividing cells and is the presumed explanation for the tumor specificity.*

VGF is a secreted protein produced early during infection that acts as a mitogen to prime surrounding cells for vaccinia infection. *Deletion of VGF gene causes decreases in viral replication in resting/nondividing cells.*
Principles for oncolytic virotherapy with vaccinia virus

**Favorable attributes**

- Rapid spread and broad-spectrum infectivity compared with other OVs
- Replication and gene expression in cytoplasm of the host cell
- Ability to travel systematically through the blood
- Capacity that permits cloning large fragments of DNA >25kbp
- High level of transgene expression
- Approved or experimental antiviral agents are available (cidofovir, ST-246, neutralizing Abs)
- Initiation of potent inflammatory responses by danger signals
- Human experience with vaccine strains (10⁸ humans vaccinated)

**Findings from clinical trials with oncolytic vaccinia virus**

Phase I-II clinical trials with JX-594 (Jennerex Biotherapeutics) have been completed in patients with advanced hepatocellular, colorectal, melanoma and lung cancer; *Park et al., Lancet Oncol. 2008*.

**Interpretation:** Intratumoral injection of JX-594 into primary or metastatic tumors was generally well-tolerated. Direct hyperbilirubinemia was the dose-limiting toxicity (MTD of 1 x 10⁹ PFU). Safety was acceptable in the context of JX-594 replication, GM-CSF expression, systemic dissemination, and JX-594 had anti-tumoral effects against several refractory carcinomas. Phase II trials are now underway.

According to Response Evaluation Criteria in Solid Tumors (RECIST), three patients had partial response, six had stable disease, and one had progressive disease (ten patients were radiographically evaluable for objective responses).
Limitations of oncolytic virotherapy?

- Insufficient single agent potency
- Inefficient systemic delivery to tumors
- Immune clearance may reduce oncolytic effects

How can we make it better?

- The efficacy of OV would benefit from combination therapies.
- The difficulty to translate the tumor-killing properties of OV from experimental model into effective “cures” in patients is in part related to the presence of tumor vasculature that serves as a major barrier for viral egress into tumors.
Delivery of protein antigens and DNA by virulence-attenuated bacteria.

- Bacterial carriers offer tremendous versatility of presenting antigens to the immune system.
- Some bacterial carriers grow extracellular on the surface of the host cells and tissues, whilst others can grow inside specialized vacuoles or in the host cell cytoplasm.
- Bacterial carriers can infect different organs of the mammalian host (intestine, respiratory tract, urogenital tract).
- Finally, the various specific bacterial protein secretion systems offer the unique possibility to secrete those antigens either into the surrounding environment or directly into the host cell cytoplasm.
Lipid-based delivery systems

- Liposomes are formed from phospholipid bilayers that enclose aqueous compartments.

- Concentric layers of phospholipids with aqueous phases in-between form multilamellar vesicles (MLV), average size of 1-50 μm.

- When phospholipids are mixed with water under low shear conditions, MLV usually form spontaneously.

- Liposomes can also be prepared with a single bilayer as either (a) large unilamellar vesicles (LUV) with a 100-500nm size or (b) small unilamellar vesicles (SUV) with a 25-100nm size range.
Quality Control Parameters

- Encapsulation efficiency
- Vesicle integrity
- Release properties *in vitro*

Route of Delivery of Liposomes

Liposome mediated delivery can be achieved by intramuscular, subcutaneous, oral, intranasal or topical application to intact skin.
New Delivery Systems

It is evident to most vaccinologists that for several practical reasons, one needs routes of immunization that do not depend on injections using syringe and needle.

Intranasal, aerosol, transcutaneous, rectal and oral immunization routes are all being explored. They also offer the additional advantage that they generate not only systemic immunity but also local mucosal immunity.
Fig 1. (A) Nine volunteers in group one consumed placebo potatoes on days 0, 14 and 28 (open arrowheads). None of the volunteers had any significant changes in their anti-HBs specific titers during the study potatoes on days 0, 14 and 28 (open arrowheads). (B) Ten of 16 volunteers who ate three doses (group three) of HBsAg-containing transgenic potatoes on days 0, 14, and 28 (closed arrowheads) showed marked increases in anti-HBs titers. Each line represents changes in an individual volunteer’s antibody titer throughout the study period. (C) Nine of 17 volunteers (group two) who received transgenic potatoes on days 0 and 28 (closed arrowhead) and non-transgenic potatoes (open arrowhead) on day 14 showed marked increases in anti-HBs titers. Note the discontinuities in the ordinate axes in (B) and (C) and consequent breaks in the curves of higher-titer subjects.
Immuno-prevention

- Active immunization against the viral capsid proteins of oncogenic viruses can prevent infection and as a result prevent the development of cancer.

  eg. - HBV - hepatocellular carcinoma
  - HPV - cervical cancer

Other cancers, such as Karposi’s sarcoma associated with KSHV and human T cell leukemia associated with HTLV-1 offer additional opportunities.

- Immunization against the viral transforming protein (such as E6 and E7 of HPV) may also be effective in preventing development of the malignancy after infection has occurred.
<table>
<thead>
<tr>
<th>Vaccine platform</th>
<th>Example</th>
<th>Cancer type</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptides/proteins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptide</td>
<td>gp100 (modified), MUC-1 (Stimuvax), HER2/neu</td>
<td>Melanoma, lung</td>
<td>(1–9)</td>
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<tr>
<td>Protein</td>
<td>MAGE-A3, NY-ESO</td>
<td>Melanoma</td>
<td>(10)</td>
</tr>
<tr>
<td>Antibody</td>
<td>Anti-idiotype</td>
<td>Lymphoma</td>
<td>(11–14)</td>
</tr>
<tr>
<td>Glycoproteins</td>
<td>sTn-KLH</td>
<td>Melanoma</td>
<td>(15,16)</td>
</tr>
<tr>
<td><strong>Recombinant vectors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poxvirus</td>
<td>rV, rF-PSA-TRICOM (Prostvac)</td>
<td>Prostate</td>
<td>(17–29)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae (yeast)</td>
<td>yeast-ras</td>
<td>Pancreatic</td>
<td>(30,31)</td>
</tr>
<tr>
<td>Listeria</td>
<td>Listeria-mesothelin</td>
<td>Pancreatic</td>
<td>(32)</td>
</tr>
<tr>
<td>Alpha- and adenoviruses</td>
<td>adeno-CEA, alpha-CEA</td>
<td>Carcinoma</td>
<td>(33)</td>
</tr>
<tr>
<td><strong>Tumor Cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>adeno-CD40L, colon (BCG)</td>
<td>CLL, colon, melanoma</td>
<td>(34–36)</td>
</tr>
<tr>
<td>Dendritic cell/autologous tumor cell fusions</td>
<td>GVAX (+GM-CSF)</td>
<td>Myeloma</td>
<td>(37)</td>
</tr>
<tr>
<td>Allogeneic</td>
<td></td>
<td>Pancreatic</td>
<td>(38–40)</td>
</tr>
<tr>
<td><strong>Dendritic cells/APCs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC–protein</td>
<td>Sipuleucel-T (PAP-GM-CSF)</td>
<td>Prostate</td>
<td>(41,42)</td>
</tr>
<tr>
<td>Dendritic cell–peptide</td>
<td>Gliona peptides</td>
<td>Gliona, melanoma</td>
<td>(43–45)</td>
</tr>
<tr>
<td>Dendritic cells–vector infected</td>
<td>rV, rF-CEA-MUC1-TRICOM (Panvac-DC)</td>
<td>Colorectal</td>
<td>(46,47)</td>
</tr>
</tbody>
</table>

* APC = antigen-presenting cell; BCG = Bacillus Calmette–Guerin adjuvant; CD40L = CD40 ligand; CEA = carcinoembryonic antigen; CLL = chronic lymphocytic leukemia; gp100 = glycoprotein 100; GM-CSF = granulocyte macrophage colony-stimulating factor; MAGE-A3 = melanoma-associated antigen 3; MUC-1 = mucin 1; NY-ESO = New York esophageal carcinoma antigen 1; PAP = prostatic acid phosphatase; PSA = prostate-specific antigen; rF = recombinant fowlpox; rV = recombinant vaccinia; sTn-KLH = sialyl-Tn-keyhole limpet hemocyanin.
<table>
<thead>
<tr>
<th>Target type</th>
<th>Examples</th>
<th>Selected references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncoprotein</td>
<td>point mutated: ras, B-raf, frame shift mutations, undefined unique tumor mutations; HER2/neu, MUC-1 C-terminus, p53</td>
<td>(1,7,8,48,49)</td>
</tr>
<tr>
<td>Oncofetal antigen</td>
<td>CEA, MUC-1</td>
<td>(2–4, 19, 26)</td>
</tr>
<tr>
<td>Cancer-testes</td>
<td>MAGE-A3, BAGE, SEREX-defined, NY-ESO</td>
<td>(10, 50–52)</td>
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<tr>
<td>Tissue lineage</td>
<td>PAP, PSA, gp100, tyrosinase, glioma antigen</td>
<td>(5, 6, 24, 25, 27, 41, 44, 53)</td>
</tr>
<tr>
<td>Stem cell/EMT</td>
<td>Brachyury, SOX-2, OCT-4, TERT, CD44$^{high}$/CD24$^{lo}, CD133^+</td>
<td>(54–62)</td>
</tr>
<tr>
<td>Viral</td>
<td>HPV, HCV</td>
<td>(63, 64)</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>STn-KLH</td>
<td>(15, 16)</td>
</tr>
<tr>
<td>Antiangiogenic</td>
<td>VEGF-R</td>
<td>(65, 66, 67)</td>
</tr>
<tr>
<td>B-cell lymphoma</td>
<td>Anti-id</td>
<td>(11–14)</td>
</tr>
</tbody>
</table>

*BAGE = B melanoma antigen; CEA = carcinoembryonic antigen; EMT = epithelial–mesenchymal transition; gp100 = glycoprotein 100; HCV = hepatitis C virus; HPV = human papillomavirus; MAGE-A3 = melanoma-associated antigen-A3; MUC-1 = mucin 1; NY-ESO = New York esophageal carcinoma antigen 1; OCT-4 = octamer-binding transcription factor 4; PAP = prostatic acid phosphatase; PSA = prostate-specific antigen; SOX-2 = (sex determining region Y)-box-2; STn-KLH = sialyl-Tn-keyhole limpet hemocyanin; TERT = telomerase reverse transcriptase; VEGF-R = vascular endothelial growth factor receptor.*
**Stimuvax®** (also known as L-BLP25 or BLP25 Liposome Vaccine) is an investigational therapeutic cancer vaccine designed to induce an immune response to cancer cells that express MUC1, a glycoprotein antigen widely expressed on common cancers. MUC1 is over-expressed on many cancers such as lung cancer, breast cancer, prostate cancer and colorectal cancer. Stimuvax is thought to work by stimulating the body's immune system to identify and destroy cancer cells expressing MUC1.

**Lung cancer:** Phase IIb clinical trial of 171 patients with inoperable stage IIIb non-small-cell lung cancer (NSCLC), in which Stimuvax showed a trend towards extending median overall survival from 13.3 months for patients receiving best supportive care (BSC) to 30.6 months for patients receiving Stimuvax plus BSC. This result, from a subset of on stage 3b patients in the study, was not, however, statistically significant \( p = 0.16 \), *J. Thorac. Oncol.*, 2(8):S199-S201, August 2007). Reported side effects included mild-to-moderate flu-like symptoms, gastrointestinal disturbances, and mild injection-site reactions.

On December 19, 2012, Merck Serono and Oncothyreon announced that the pivotal Phase III clinical trial of L-BLP25 (formerly referred to as Stimuvax®) known as START did not meet its primary endpoint of an improvement in overall survival in patients with unresectable, locally advanced stage IIIA or stage IIIB non-small cell lung cancer (NSCLC). The trial was conducted by Merck Serono. Despite not meeting the primary endpoint, notable treatment effects were seen in certain subgroups. Further analyses are planned to explore the potential benefit-risk profile. This data will be discussed with external experts and regulatory authorities over the coming months. Latest announcement is that Merck has decided to push on with Stimuvax (now renamed tecemotide) in another ongoing late-phase trial in Asia and is now weighing the options of starting a further study.
Multicenter phase 3 IMPACT Trial: Results of the primary efficacy analysis of treatment with Sipuleucel-T (Patients APCs incubated with PAP-GM-CSF) as compared with placebo control. Sipuleucel-T did improve patients' OS 25.8 versus 21.7 months; P=.032. No tumor shrinkage seen. In April 2010, the FDA approved Sipuleucel-T for the treatment of minimal or asymptomatic metastatic castrate-resistant prostate cancer (mCRPC).

PAP=Prostatic acid phosphatase is present in 95% of prostate cancer cells.

Cost of vaccine: $93,000 for a course of treatment (3 injections every 2 weeks).

OS of a randomized, placebo-controlled 43 center trial of PROSTVAC vaccine consisting of recombinant vaccinia and fowlpox vectors containing transgenes for prostate-specific antigen, B7.1, ICAM1 and LFA-3 (PSA-TRICOM) in patients with metastatic castrate-resistant prostate cancer. The trial compared PROSTVAC against empty controls. There was an OS advantage of 8.5 months (25.1 months vs 16.6 months P=.006) and a 44% reduction in death in the vaccine arm.