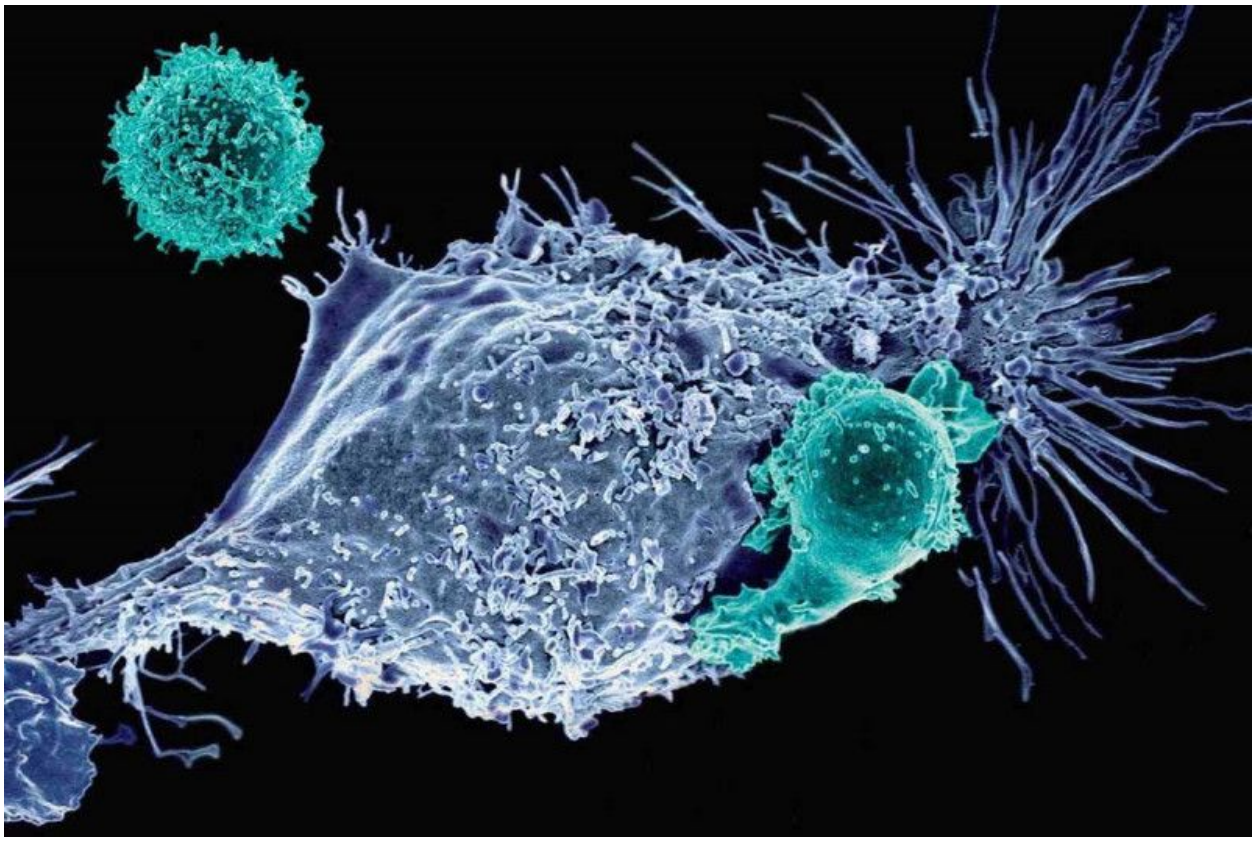

Credit: Prasad Adusumilli

Literature Review: Personalized Cancer Vaccines

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Background

The first documented successful immune-mediated treatment for cancer was the application of "Coley's Toxins" to sarcoma.

- The Treatment of Malignant Tumors by Repeated Inoculations of Erysipelas. With a Report of Ten Original Cases. Coley, W. B. *The American Journal of the Medical Sciences*, 105 (5):487-511. (1893).

Due to the variability of patient responses and primitive state of immunology, the role of the immune system in clearing tumors was never fully accepted. The advent of radiotherapy and later chemotherapy drew attention away from the possibility of immunotherapeutic treatments.

In the 1960s, there were more detailed studies of the immune system rejecting transplanted tumors and tumor cell lines, leading to a realization that the immune system does have some role to play in oncology.

- Immunology of experimental tumors. Old, L. J. & Boyse, E. A. *Annual Review of Medicine* 15:167–186. (1964).
- Tumor antigens. Klein, G. *Annual Review Microbiology* 20:223–252. (1966).
- Relationships of Immunology to Cancer: A Review. Chester M. Southam. *Cancer Research* 20 (3):271-291. (1960)

These initial studies eventually culminated in the concept of "immune surveillance", wherein the immune systems of healthy individuals continuously prevent the development of tumors by recognizing and killing cancerous cells.

- The Concept of Immunological Surveillance. Burnet, F. M. *Progress in Experimental Tumor Research* 13:1–27. (1970)

As a consequence of immune surveillance, tumors undergo a process called "immunoediting", where surviving cancer cells must evolve to escape the pressures of a hostile immune system. This "editing" can take the form either of accumulated defects in antigen processing, interferon response, or an evolutionary benefit conferred to sub-clones lacking immunogenic mutations. The concept of immunoediting was championed by Lloyd Old, Robert Schreiber, and Gavin Dunn:

- The Immunobiology of Cancer Immunosurveillance and Immunoediting. Dunn, Gavin P., Lloyd J. Old, and Robert D. Schreiber. *Immunity* 21 (2):137–48. (2004).

Therapeutic Cancer Vaccines

The first pre-clinical explorations of therapeutic vaccination against an established cancer happened in the late 1970s:

- Specific Immunotherapy of Established Visceral Micrometastases by BCG-Tumor Cell Vaccine Alone or as an Adjunct to Surgery. Hanna, M. G., and L. C. Peters. *Cancer* 42 (6):2613–25. (1978).

These vaccines consisted of irradiated tumor cells and Bacille Calmette Guerin (BCG) bacteria and were able to clear some tumors in guinea pigs.

Human research continued in this same direction through the 1980s, by focusing on therapeutic vaccines using tumor lysate.

Tumor-Associated Antigens

In 1991, the first tumor-associated antigen (MAGE-A1) was isolated:

- A Gene Encoding an Antigen Recognized by Cytolytic T Lymphocytes on a Human Melanoma. Bruggen, P. van der, C. Traversari, P. Chomez, C. Lurquin, E. De Plaen, B. Van den Eynde, A. Knuth, and T. Boon. *Science* 254 (5038):1643–47. (1991)

This resulted in significant expansion of cancer vaccine research, as many groups rushed to identify tumor antigens for different cancer types. In addition to the discovery of myriad tumor-associated antigens, there was also significant innovation in vaccine delivery platforms and adjuvants.

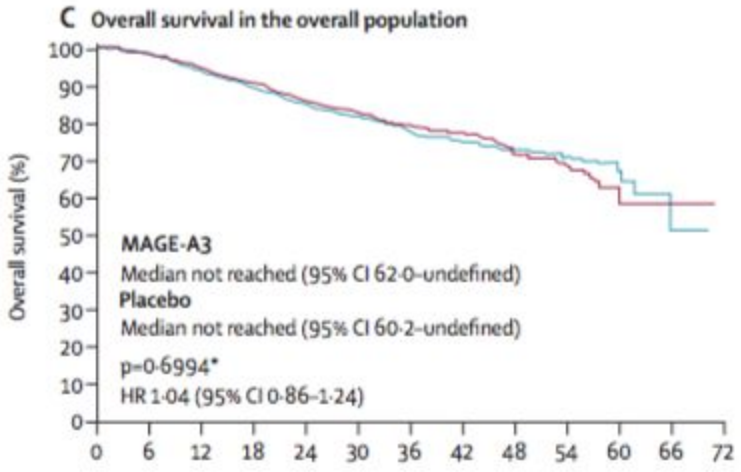
Despite these advances, therapeutic cancer vaccines only sporadically achieved clinical success. In 2004, Steve Rosenberg published a review of therapeutic cancer vaccines, highlighting their remarkably bad history of anti-tumor efficacy.

- Cancer immunotherapy: moving beyond current vaccines. Rosenberg SA, Yang JC, Restifo NP. *Nature medicine.* 10 (9), 909-915. (2004).

Cancer type	Vaccine	Total patients	Patients responding
Melanoma	Tyrosinase + GMCSF	16	0
Melanoma	Peptides in IFA or on DC	26	3
Melanoma	MART-1 + IL-12	28	2
Prostate	Peptides	10	0
Melanoma	Peptides on PBMC + IL-12	20	2
Breast and prostate	Telomerase	7	0
Cervix	HPV16 E7	17	0
Colorectal	Peptides in IFA	10	0
Multiple	NY-ESO-1	12	0
Multiple	Ras in DETOX adjuvant	15	0
Multiple	Peptides in IFA	14	0
Prostate	Vaccinia-PSA	33	0
Prostate	Vaccinia-PSA	42	0
Colorectal	Vaccinia-CEA	20	0
Colorectal	Vaccinia-CEA and B7-1	18	0
Multiple	Avipox-CEA(IGMCSF)	60	0
Multiple	Avipox-CEA	15	0
Multiple	Vaccinia + avipox-CEA	18	0

Several years after Rosenberg's negative review, GlaxoSmithKline started MAGRIT, the largest cancer vaccine trial to date. MAGRIT vaccinated lung cancer patients against the tumor-associated antigen MAGE-A3. 13,849 patients were screened for MAGE-A3, of whom 1,515 were treated. No difference from placebo was observed.

- Efficacy of the MAGE-A3 Cancer Immunotherapeutic as Adjuvant Therapy in Patients with Resected MAGE-A3-Positive Non-Small-Cell Lung Cancer (MAGRIT): A Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial. Vansteenkiste, Johan F., Byoung Chul Cho, Tonu Vanakesa, Tommaso De Pas, Marcin Zielinski, Moon Soo Kim, Jacek Jassem, et al.. *The Lancet Oncology* 17 (6):822–35. (2016).



A more recent review of therapeutic cancer vaccines, published immediately before the advent of neoantigen vaccines, confirms a lackluster impression of limited clinical efficacy.

- Therapeutic Cancer Vaccines: Past, Present, and Future. Guo, Chunqing, Masoud H. Manjili, John R. Subjeck, Devanand Sarkar, Paul B. Fisher, and Xiang-Yang Wang. *Advances in Cancer Research* 119:421–75. (2013)

Categories of Tumor Antigens

Tumor antigens can be divided roughly into two categories (in increasing order of tumor-specificity):

Tumor Associated Antigens.

- Tumour Antigens Recognized by T Lymphocytes: At the Core of Cancer Immunotherapy. Coulie, Pierre G., Benoît J. Van den Eynde, Pierre van der Bruggen, and Thierry Boon. *Nature Reviews. Cancer* 14 (2):135–46. (2014).

These have been the antigens used in the long history of failed cancer vaccines:

1. Lineage differentiation (e.g. gp100)
2. Over-expressed relative to normal tissue (e.g. HER2)
3. Cancer/germline antigens (e.g. CTAG)
4. Onco-fetal (e.g. WT1)

Tumor Specific Antigens & Neoantigens

Tumor-specific antigens are those which are extremely unlikely to be encountered by the immune system anywhere other than cancer cells. This category includes onco-viral

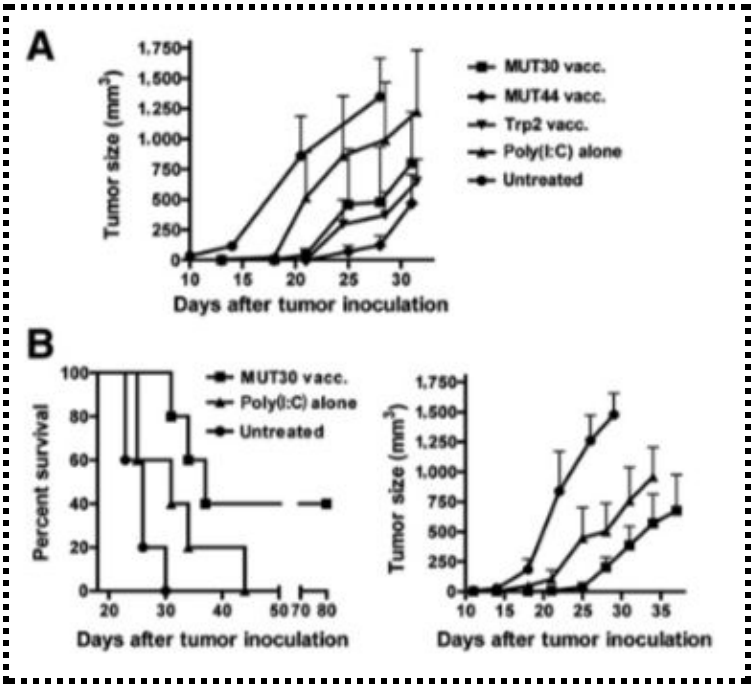
antigens, which can be screened for and potentially treated without personalization. For the vast majority of cancers, however, the most relevant category of tumor-specific antigens are “neoantigens” arising from mutations:

- Tumor Neoantigens: Building a Framework for Personalized Cancer Immunotherapy. Gubin, Matthew M., Maxim N. Artyomov, Elaine R. Mardis, and Robert D. Schreiber. *The Journal of Clinical Investigation* 125 (9). 3413–21. (2014)
- Neoantigens in Cancer Immunotherapy. Schumacher, Ton N., and Robert D. Schreiber. *Science* 348 (6230). 69–74. (2015).

Pre-Clinical Evidence for Neoantigen Vaccine Efficacy

The first pre-clinical demonstration of the anti-tumor efficacy of a therapeutic neoantigen vaccine was by Castle et al., who implanted the B16.F10 melanoma cell line in C567BL mice and then vaccinated them with 27mer peptides with a centered mutation and poly(I:C). Their paper demonstrated neoantigen-specific T-cell responses and slowed (but did not stop) the growth of B16 tumors. This paper also serves as an important landmark since their antigen selection pipeline (tumor+normal WES, tumor RNA-seq, MHC binding prediction with a neural network) is the basis of most subsequent neoantigen pipelines.

- Exploiting the Mutanome for Tumor Vaccination. Castle, John C., Sebastian Kreiter, Jan Diekmann, Martin Löwer, Niels van de Roemer, Jos de Graaf, Abderraouf Selmi, et al. *Cancer Research* 72 (5):1081–91. (2012).



Castle et al. (2012) - Figure 4

Studies Showing Murine Survival Benefit

Two studies in 2014 (Gubin et al., Yadav et al.) both showed that neoantigen vaccines can not only slow the growth of implanted tumors, but further demonstrated survival benefit in mice.

Gubin et al. transplanted the MCA-T3 sarcoma cell line into Taconic 129S6 mice. The mice were then vaccinated with two mutated peptides, along with Poly(I:C) as an adjuvant.

- Checkpoint Blockade Cancer Immunotherapy Targets Tumour-Specific Mutant Antigens. Gubin, Matthew M., Xiuli Zhang, Heiko Schuster, Etienne Caron, Jeffrey P. Ward, Takuro Noguchi, Yulia Ivanova, et al. *Nature* 515 (7528):577–81. (2014).

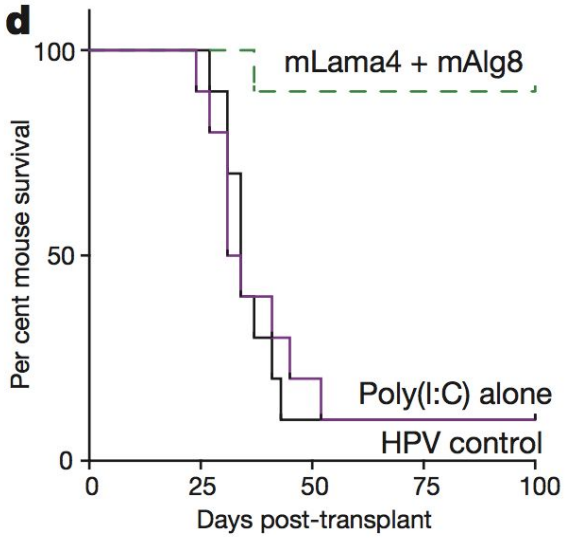


Figure 2d from Gubin et al. 2014

Yadav et al. also demonstrated survival benefit for (C56BL6) mice, which were vaccinated against the MC-38 colon cancer cell line. In addition to the "standard" pipeline of identifying somatic variants, filtering by expression, and further filtering by predicted MHC binding, they also used mass spectrometry of eluted MHC-bound peptides to narrow their set of candidate epitopes. The mice were immunized with minimal epitope peptides, poly(I:C), and an anti-CD40 antibody.

- Predicting Immunogenic Tumour Mutations by Combining Mass Spectrometry and Exome Sequencing. Yadav, Mahesh, Suchit Jhunjhunwala, Qui T. Phung, Patrick Lupardus, Joshua Tanguay, Stephanie Bumbaca, Christian Franci, et al. 2014. *Nature* 515 (7528):572–76. (2014).

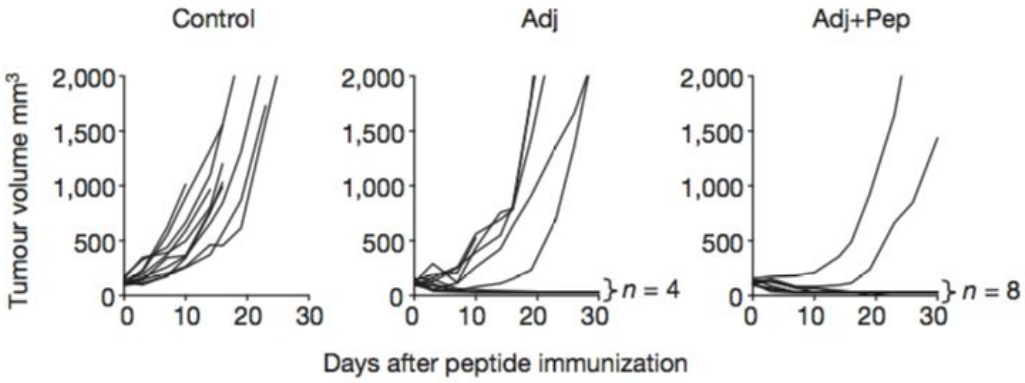


Figure 4j from Yadav et al. 2014

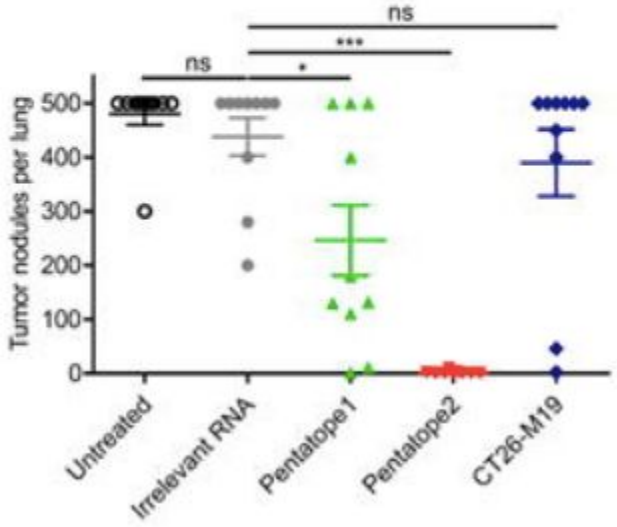
Epitope Synergy and Class II Predictions

In 2015, Ugur Sahin's group published a paper with two noteworthy findings:

1. epitopes predicted to bind to Class II can be useful for tumor clearance
2. while a group of 5 epitopes can be effective together, no component works alone

Tumoring bearing BALC/c mice were immunized against either the 4T1 or CT26 cell lines (depending on the tumor they had). The vaccine consisted of mRNA.

- Mutant MHC Class II Epitopes Drive Therapeutic Immune Responses to Cancer. Kreiter, Sebastian, Mathias Vormehr, Niels van de Roemer, Mustafa Diken, Martin Löwer, Jan Diekmann, Sebastian Boegel, et al. *Nature* 520 (7549):692–96. (2015).



Extended Data Figure 3b from Kreiter et al. 2015

Human Results

Identification of neoantigen specific T-cell responses in CLL patients after HSCT:

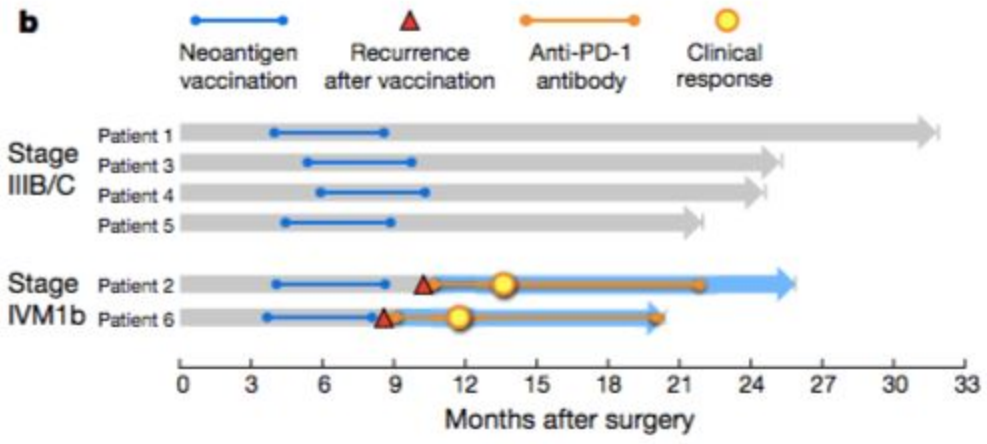
- Systematic Identification of Personal Tumor-Specific Neoantigens in Chronic Lymphocytic Leukemia. Rajasagi, Mohini, Sachet A. Shukla, Edward F. Fritsch, Derin B. Keskin, David DeLuca, Ellese Carmona, Wandi Zhang, et al. *Blood* 124 (3):453–62. (2014).

DC vaccine against melanoma neoantigens induces neoantigen specific T-cell responses:

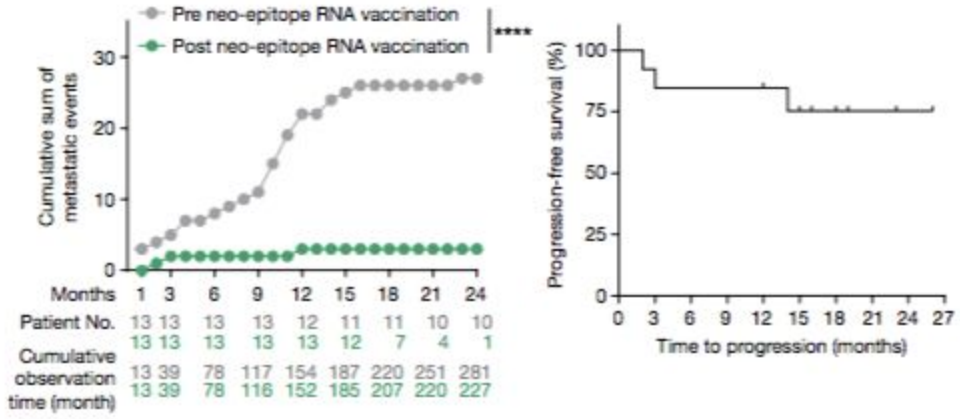
- Cancer Immunotherapy. A Dendritic Cell Vaccine Increases the Breadth and Diversity of Melanoma Neoantigen-Specific T Cells. Carreno, Beatriz M., Vincent Magrini, Michelle Becker-Hapak, Saghar Kaabinejadian, Jasreet Hundal, Allegra A. Petti, Amy Ly, et al. *Science* 348 (6236):803–8. (2015).

The first hints of neoantigen vaccine efficacy in humans came from two papers published by DFCI and BioNTech in 2017. Both groups treated a small number of melanoma patients. Cathy Wu's group at DFCI used a peptide vaccine with poly-ICLC. BioNTech encodes antigens in mRNA, which is injected into lymph nodes using ultrasound guidance. In both trials, most patients achieved stable disease, and were recurrences reversable by checkpoint blockade.

- An Immunogenic Personal Neoantigen Vaccine for Patients with Melanoma. Ott, Patrick A., Zhuting Hu, Derin B. Keskin, Sachet A. Shukla, Jing Sun, David J. Bozym, Wandi Zhang, et al. *Nature* 547 (7662). Nature.com:217–21. (2017).



- Personalized RNA Mutanome Vaccines Mobilize Poly-Specific Therapeutic Immunity against Cancer. Sahin, Ugur, Evelyna Derhovanessian, Matthias Miller, Björn-Philipp Kloke, Petra Simon, Martin Löwer, Valesca Bukur, et al.*Nature* 547 (7662):222–26. (2017).



Vaccine Formulation

Antigen

Neoantigens can be encoded using any level of the "central dogma": DNA, mRNA, or peptides.

- Delivery Technologies for Human Vaccines. Moingeon, Philippe, Charles de Taisne, and Jeffrey Almond. *British Medical Bulletin* 62:29–44. (2002).
- Improvement of Different Vaccine Delivery Systems for Cancer Therapy. Bolhassani, Azam, Shima Safaiyan, and Sima Rafati. *Molecular Cancer* 10 (January):3. (2011).

DNA encoding of antigens can be injected as plasmids using electroporation, gene guns, or other techniques for getting DNA into cells:

- DNA Vaccines against Cancer Come of Age. Stevenson, Freda K., Christian H. Ottensmeier, and Jason Rice. *Current Opinion in Immunology* 22 (2):264–70. (2010).

Alternatively, DNA plasmids encoding cancer antigens can be introduced into live bacteria:

- Listeria and Salmonella Bacterial Vectors of Tumor-Associated Antigens for Cancer Immunotherapy. Paterson, Yvonne, Patrick D. Guirnalda, and Laurence M. Wood. *Seminars in Immunology* 22 (3):183–89. (2010).

A more logistically difficult approach to delivering the DNA encoding of cancer antigens is the use of viral vectors:

- Viral Vector-Based Therapeutic Cancer Vaccines. Larocca, Cecilia, and Jeffrey Schlom. *Cancer Journal* 17 (5):359–71. (2011).

mRNA vaccines can be injected directly into lymph nodes or delivered systemically when protected by a liposome:

- Tumor Vaccination Using Messenger RNA: Prospects of a Future Therapy. Kreiter, Sebastian, Mustafa Diken, Abderraouf Selmi, Özlem Türeci, and Ugur Sahin. 2011. *Current Opinion in Immunology* 23 (3):399–406.

Peptides are typically manufactured using solid phase synthesis:

- Solid Phase Synthesis. Merrifield, B. *Science* 232 (4748). Go.galegroup.com:341–47. (1986).

Peptides can be injected in combination with an immunostimulatory adjuvant or, alternatively, can be loaded onto dendritic cells:

- Dendritic-Cell-Based Therapeutic Cancer Vaccines. Palucka, Karolina, and Jacques Banchereau. *Immunity* 39 (1). Elsevier:38–48. (2013).

Adjuvants for Peptide Vaccines

Peptides typically lack intrinsic immunogenicity and thus must be accompanied by an adjuvant which stimulates an innate immune response and promotes uptake and presentation of peptides by antigen presenting cells.

Adjuvants traditionally used to enhance the immunogenicity of prophylactic subunit vaccines, such as alum, have proven to be ineffective for therapeutic cancer vaccines. This is believed to be since they primarily elicit a Th2 rather than Th1 or CTL response:

- Adjuvants for Peptide-Based Cancer Vaccines. Khong, Hiep, and Willem W. Overwijk. *Journal for Immunotherapy of Cancer* 4 (September):56. (2016).

To skew the immune response toward activating Th1 and CTL cells, many adjuvants have been formulated to engage specific innate pattern recognition receptors, most commonly toll-like receptors (TLRs):

- Toll-like Receptor Agonists: Are They Good Adjuvants? Gnjatic, Sacha, Nikhil B. Sawhney, and Nina Bhardwaj. *Cancer Journal* 16 (4). (2010).

The synthetic double-stranded RNA Poly(I:C) has been used as a potent immunostimulatory agent and vaccine adjuvant since the 1970s:

- Protection Against Herpes Virus and Encephalomyocarditis Virus Encephalitis with a Double-Stranded RNA Inducer of Interferon. Catalano, Louis W., Jr., and Samuel

Baron. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine* 133 (2). SAGE Publications:684–87. (1970).

Though the underlying mechanism was not initially understood, Poly(I:C) was eventually discovered to be recognized by TLR3, which is also responsible for detecting RNA viruses.

Poly(I:C) has been an effective adjuvant in many murine peptide vaccine studies (including several of those linked above), but use was limited in humans due to both toxicity and rapid hydrolysis. Poly-ICLC, a modified form of Poly(I:C), stabilized with carboxymethyl cellulose and poly-L-lysine, is both safer and more effective in humans:

- A Modified Polyriboinosinic-Polyribocytidylic Acid Complex That Induces Interferon in Primates. Levy, H. B., G. Baer, S. Baron, C. E. Buckler, C. J. Gibbs, M. J. Iadarola, W. T. London, and J. Rice. *The Journal of Infectious Diseases* 132 (4):434–39. (1975).

Poly-ICLC was initially used in humans as a monotherapy against gliomas, where it induced anti-cancer T-cell responses:

- Induction of CD8+ T-Cell Responses against Novel Glioma--Associated Antigen Peptides and Clinical Activity by Vaccinations with α-Type 1 Polarized Dendritic Cells and Polyinosinic-Polycytidylic Acid Stabilized by Lysine and Carboxymethylcellulose in Patients with Recurrent Malignant Glioma. Okada, Hideho, Pawel Kalinski, Ryo Ueda, Aki Hoji, Gary Kohanbash, Teresa E. Donegan, Arlan H. Mintz, et al. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 29 (3). American Society of Clinical Oncology:330–36. (2010).

Poly-ICLC was eventually used as an adjuvant for a variety of cancer antigens, where it induced antigen-specific T-cell responses.

- Poly-ICLC as an Adjuvant for NY-ESO-1 Protein Vaccination with or without Montanide ISA-51 VG in Patients with Melanoma. Sabado, Rachel Lubong, Anna C. Pavlick, Sacha Gnjatic, Farah Hasan, Meredith Spadaccia, Bike Su Oner, Hanqing Dong, et al. *Journal of Clinical Orthodontics: JCO* 33 (15_suppl). American Society of Clinical Oncology:e14034–e14034. (2015).
- Phase I Trial of Overlapping Long Peptides from a Tumor Self-Antigen and Poly-ICLC Shows Rapid Induction of Integrated Immune Response in Ovarian Cancer Patients. Sabbatini, Paul, Takemasa Tsuji, Luis Ferran, Erika Ritter, Christine Sedrak, Kevin Tuballes, Achim A. Jungbluth, et al. *Clinical Cancer Research* 18 (23). AACR:6497–6508. (2012).

Poly-ICLC has now been repeatedly used in a variety of animal vaccines to clear established tumors but its role in human treatment remains experimental.