DNA and mRNA Vaccines for Cancer: Rationale, Mechanisms and Progress

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Disclosures

- Member of the WHO drafting groups responsible for guidelines for:
  - DNA vaccines
    - https://www.who.int/publications/m/item/DNA-post-ECBS-1-sept-2020
  - mRNA vaccines
    - Approved by WHO Expert Committee on Biological Standards October 2021; to be posted soon
- Ipsen- Director
- ViroThera- SAB
- Blue Lake BioTechnology- SAB
- Jenner Institute - SAB

Organization of Talk

- Rationale for vaccines as immunotherapies
- Cancer from an immunotherapeutic perspective:
    - What types of immune responses may be effective
- Characteristics of DNA and mRNA vaccines that may make them useful for cancer
- Ongoing clinical trials as examples of types of cancer targeted

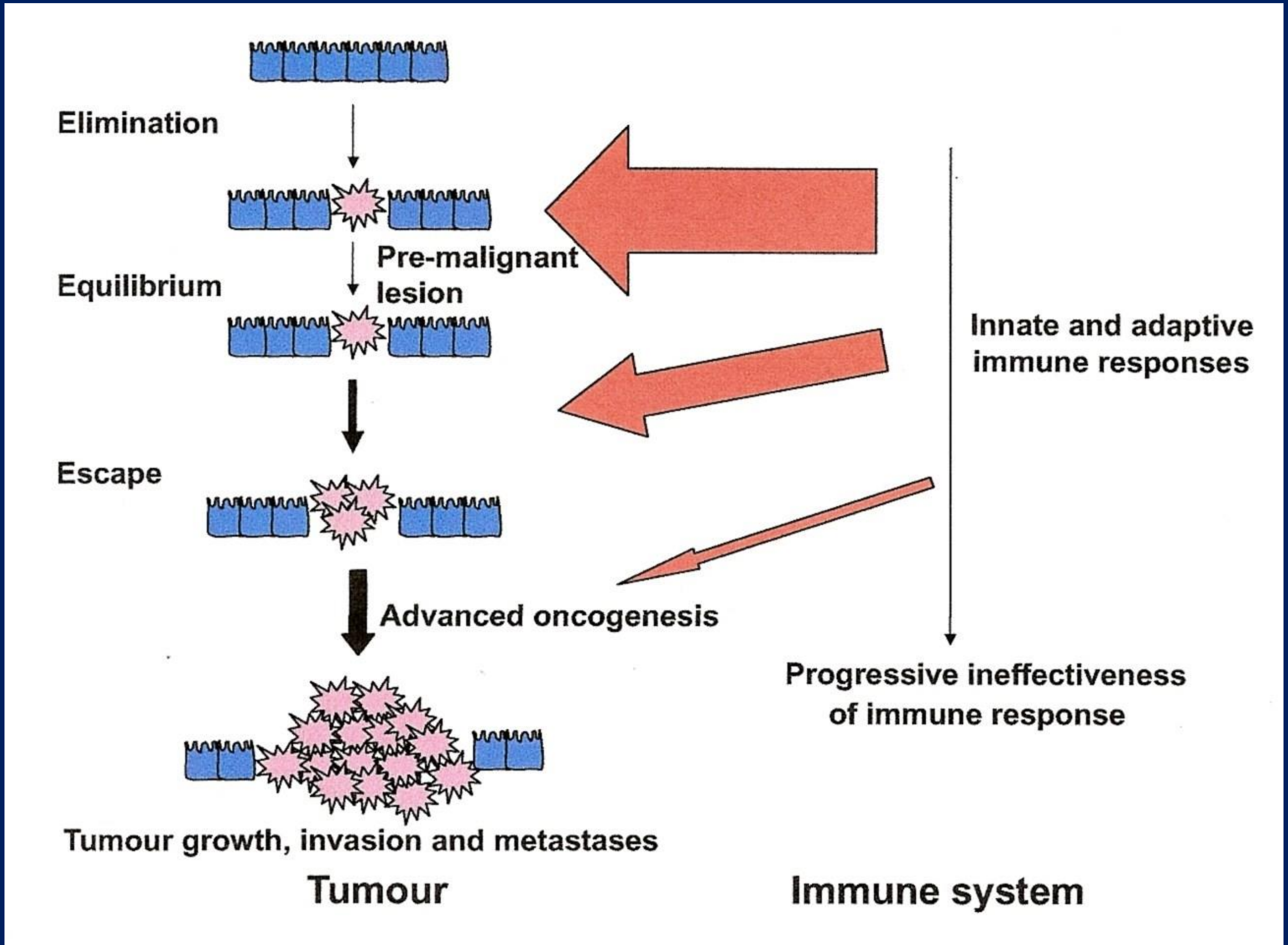
Rationale for Cancer Vaccines:

- Immunosurveillance Theory: Immune system recognizes and destroys neoplastic cells that arise whether from viral infection or de novo
  - Antigens targeted by the immune system:
    - Viral antigens on tumors that arise from viral infection (HPV E6 and E7)
    - Tumor antigens expressed or over-expressed on transformed cells (CEA)
  - Anecdotal cases of tumor regression
  - Disappearance of satellite lesions after biopsy or incomplete excision of “main” tumor
- Clinical cancer:
  - Failure of immune system
    - Senescence
    - Medical immunosuppression (such as for people with transplanted organs)
    - Decreased immune competence due to another disease such as HIV
  - Tumor escape

Rationale for Cancer Vaccines:
Success of Various Immune Interventions

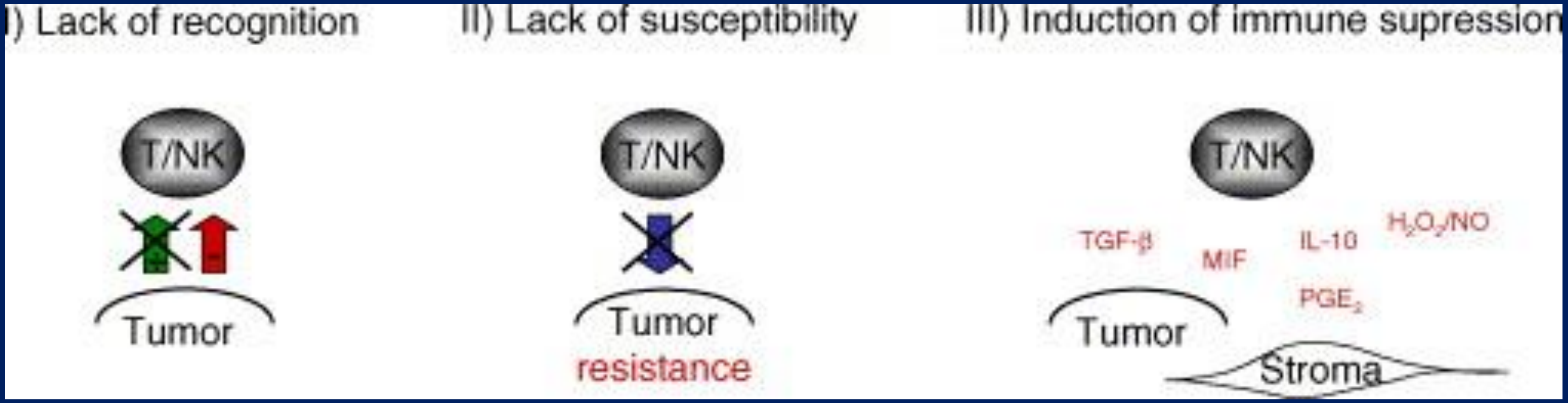
- Antibodies
  - Monoclonal antibodies (MAb)
  - Bispecific antibodies
  - Antibody drug conjugates (ADC)
- Immunostimulation
  - Cytokines (e.g., IL-2)
  - Non-specific (intravesicular BCG, Coley's toxin)
- T cell modalities
  - CAR-T cells
  - Check-point inhibitors

Sequential Escape from Immune Surveillance: What Happens to Immune Responses?



Sengupta, N., et al., Pathology-Research and Practice, 206:1, 2010

Mechanisms of Escape from Immunosurveillance



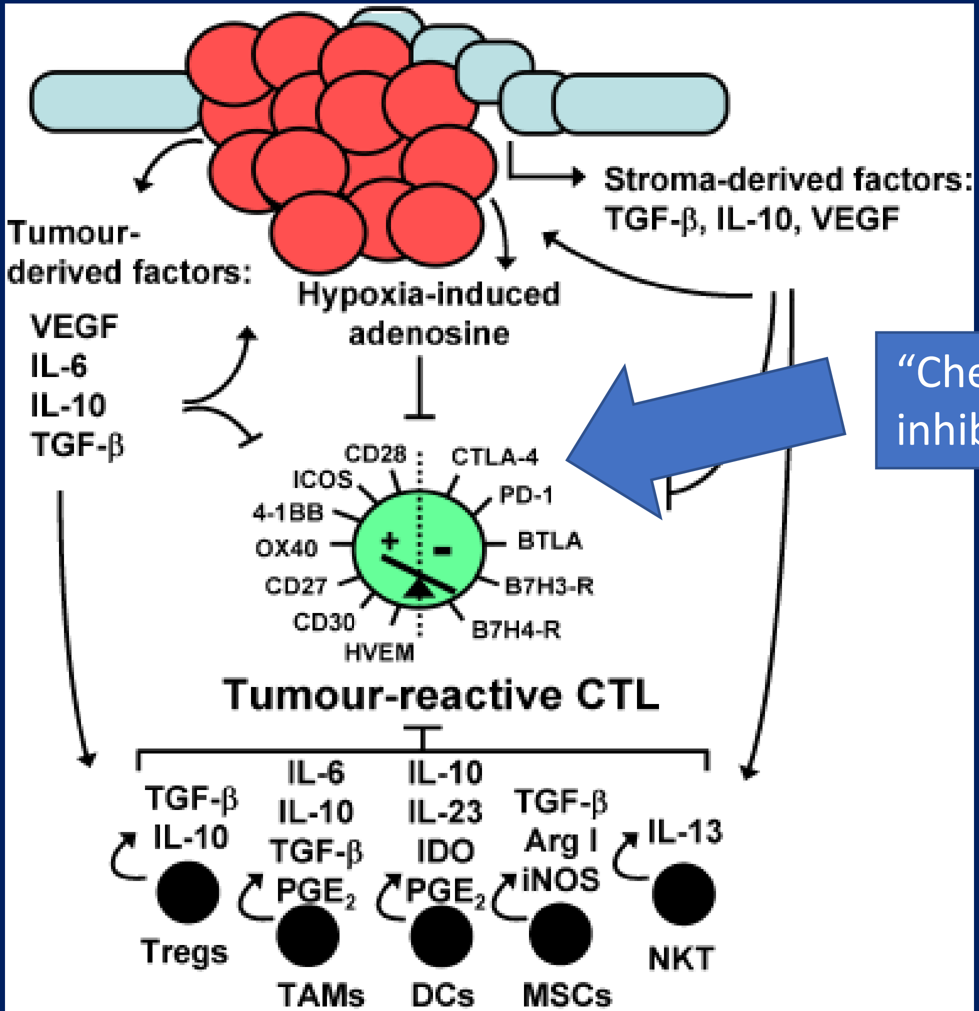
Immune system does not recognize the tumor as "foreign"

Escape from immune response due to genetic instability of the tumor and/or immune selection of cells not killed by the immune response

The tumor <u>and</u> stroma <u>suppress</u> the immune response

Modified from: Malmberg and Ljunggren, Sem. Cancer Biol. 16:16, 2006

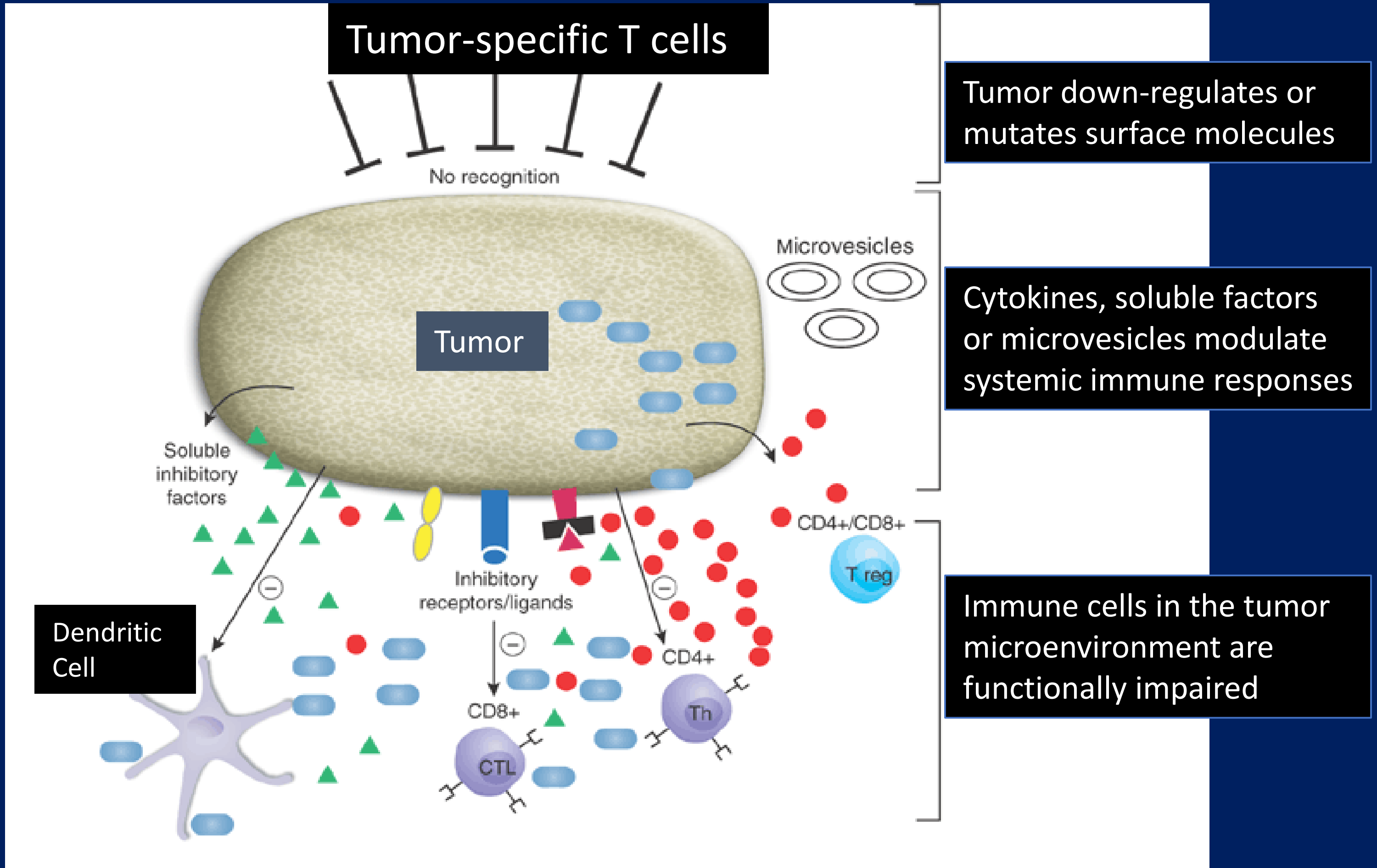
Tumor-Associated Immune Regulation



“Check point” inhibitors

Stagg, , J. et al. Imm. Rev. 220: 82, 2007

Because Tumor Cells Can Modulate Immune Responses: Simply Making a Vaccine May Not Effectively Kill a Tumor



Goldman, B., DeFrancesco, L., Nature Biotech 27:129, 2009
Reprinted from Whiteside, T.L., Semin.Cancer Biol. 16: 3-15, 2006

Milestones in the History of Cancer Immunotherapy

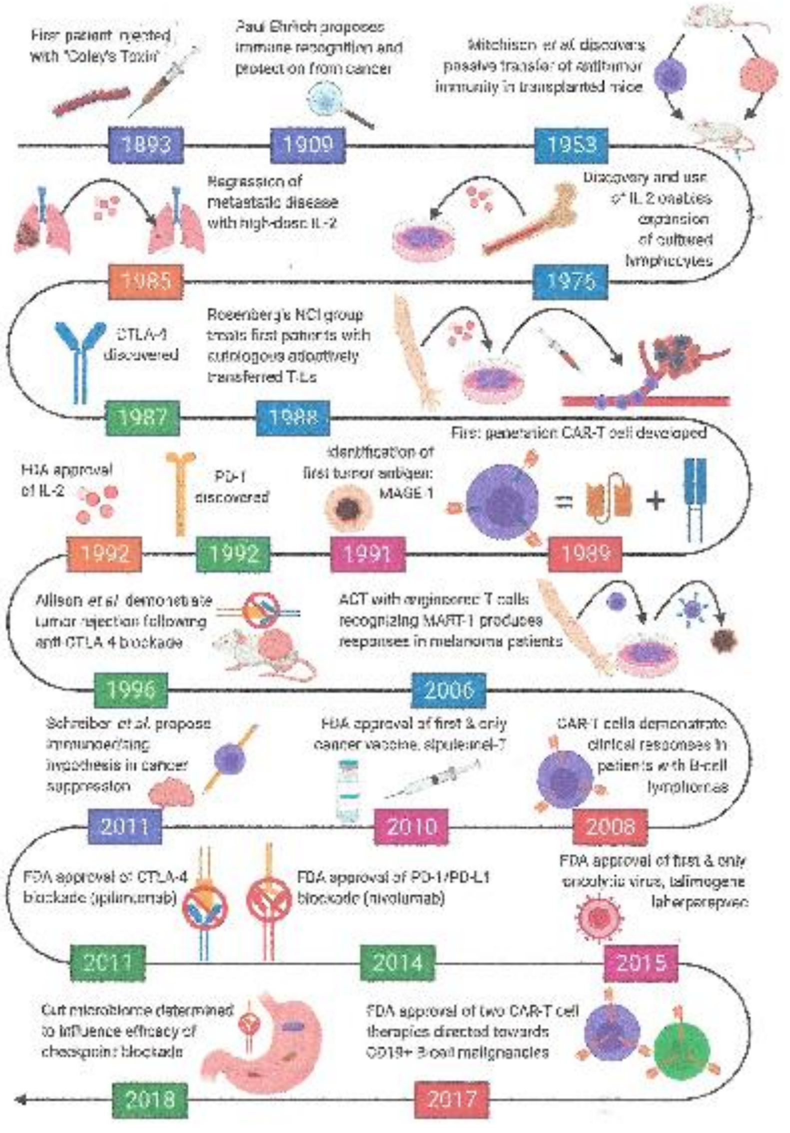


Figure 1. Milestones in the History of Cancer Immunotherapy

Carlson, R.D., et al., Toxins **2020**, 12, 241; doi:10.3390/toxins12040241

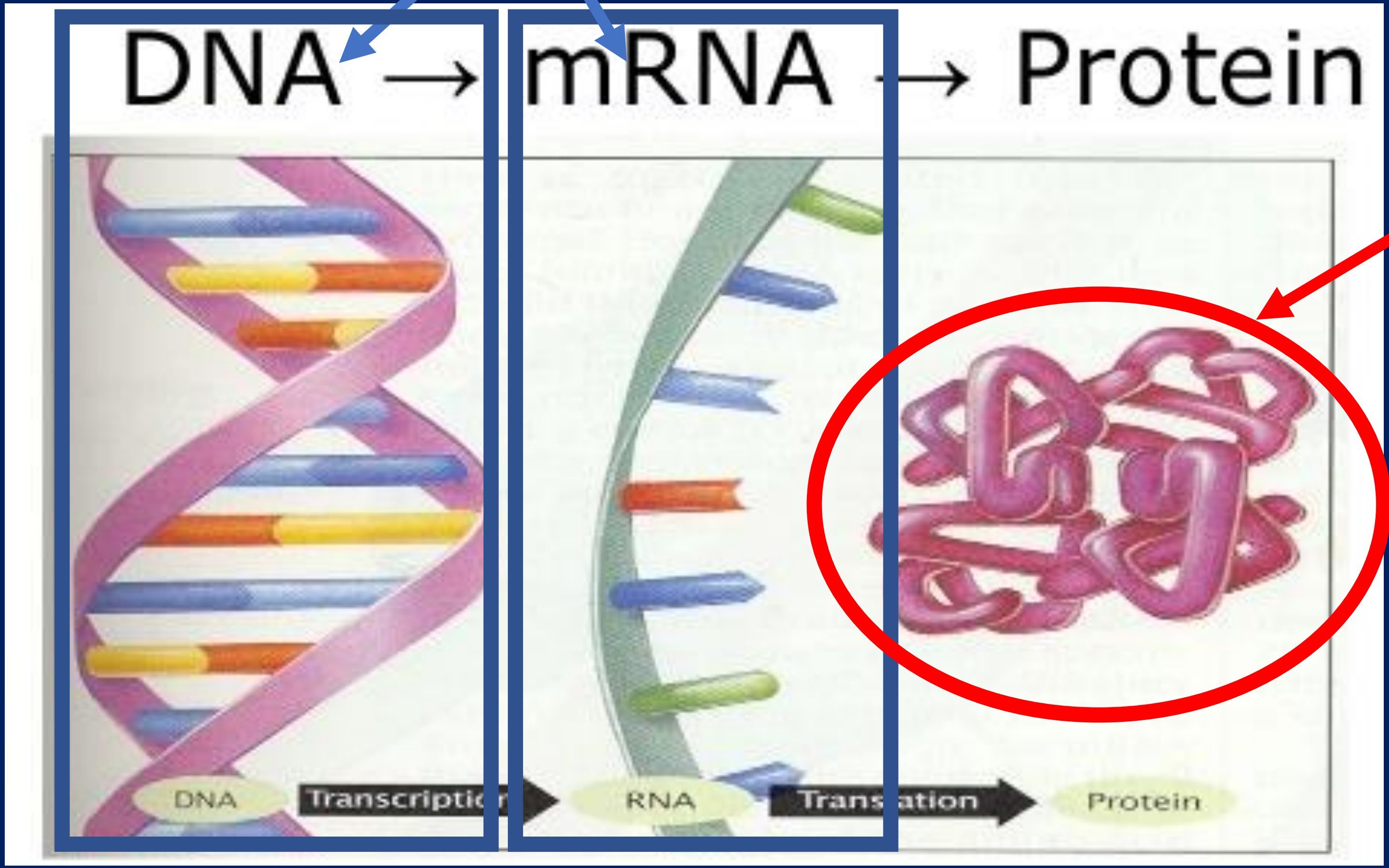
What Vaccines Will Be Effective for Treating Cancer?

- Is adaptive immunity (Antibodies and T cells) enough?
  - Is delivering an antigen (+/- adjuvant) adequate?
  - What antigens to target?
- Will additional immunostimulators help or be required?
  - Cytokines
  - Check-point inhibitors
- Is there a role for innate immunity?
- What do DNA and mRNA vaccines offer?

Nucleic Acid Vaccines:

Instead of using a tumor antigen as the vaccine-
Use the DNA or mRNA that codes for the protein

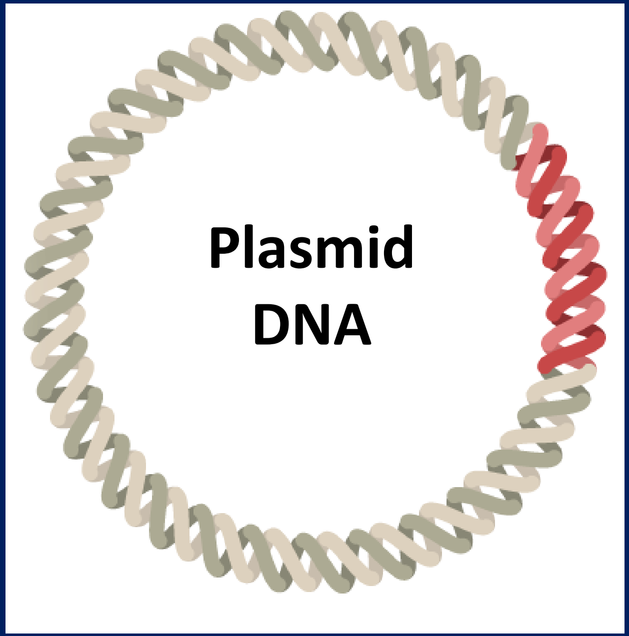
The “Drug Substance”



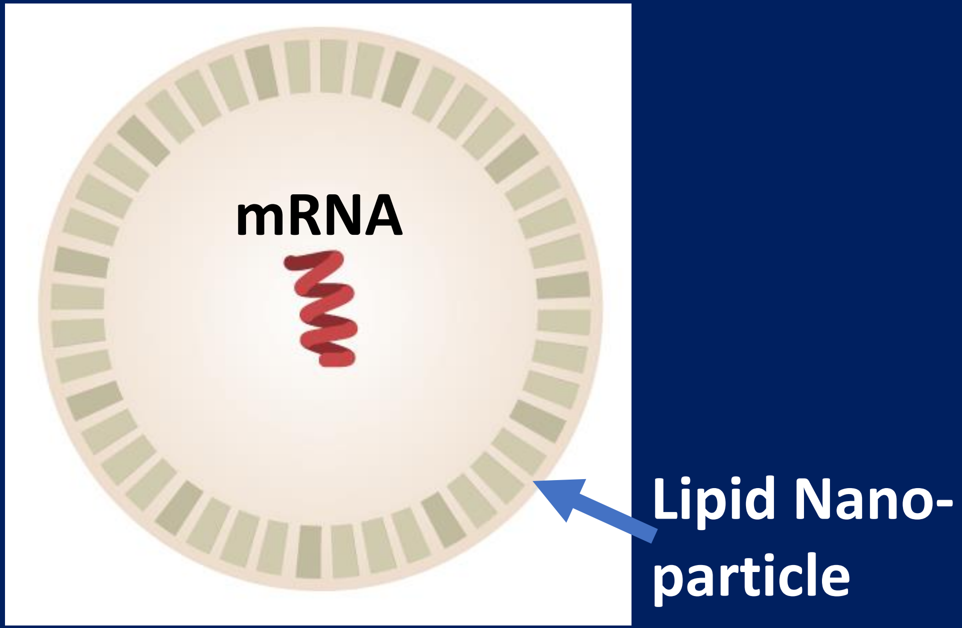
What Antibodies are directed against.
T cell responses are generated against peptide epitopes of the protein.

Slideshare.net

DNA and mRNA Vaccine Technologies

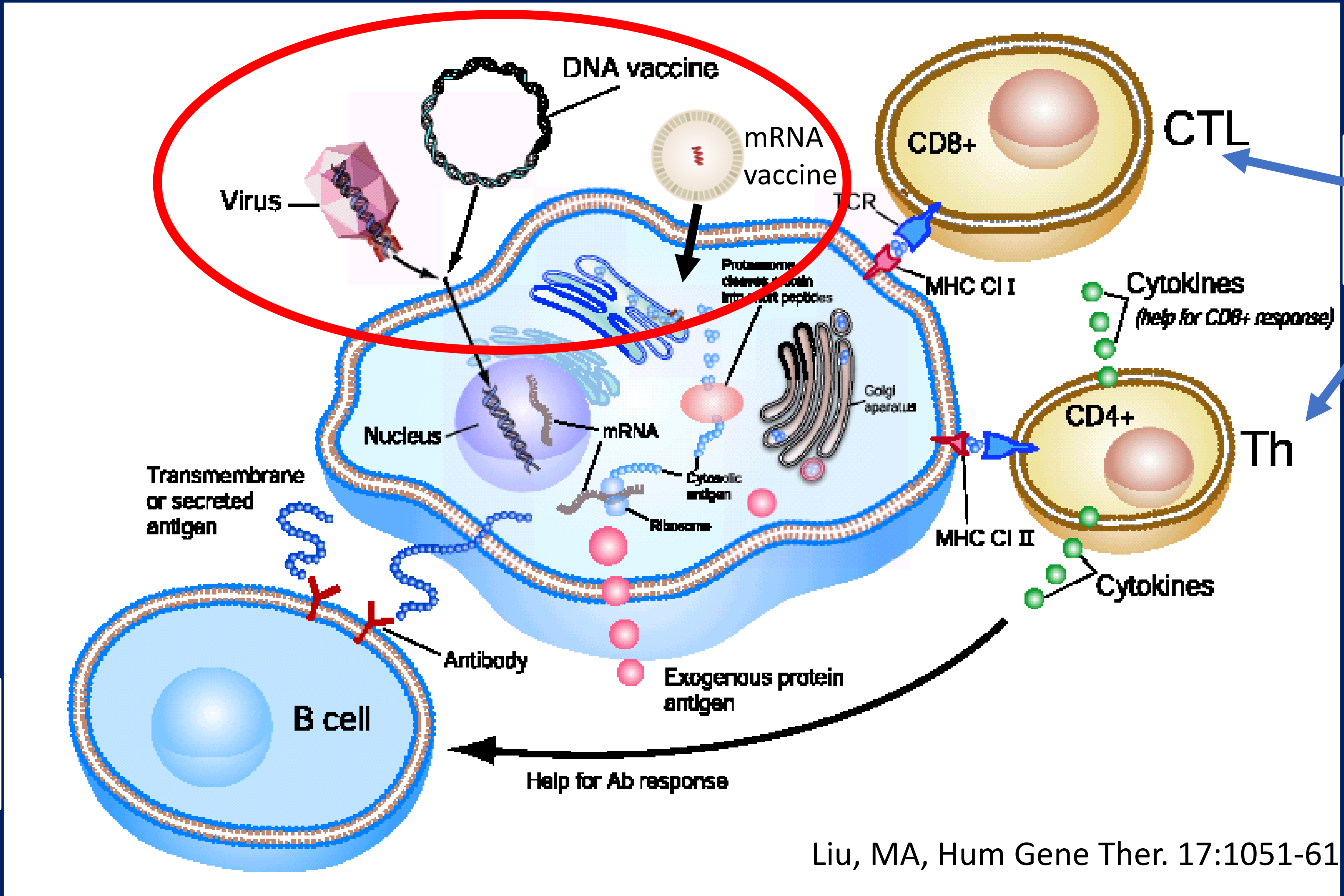


DNA Vaccine



mRNA Vaccine

Gene-based vaccines generate antibodies, T helper cells *and* CTL (Cytolytic T Lymphocytes)



T cell Responses

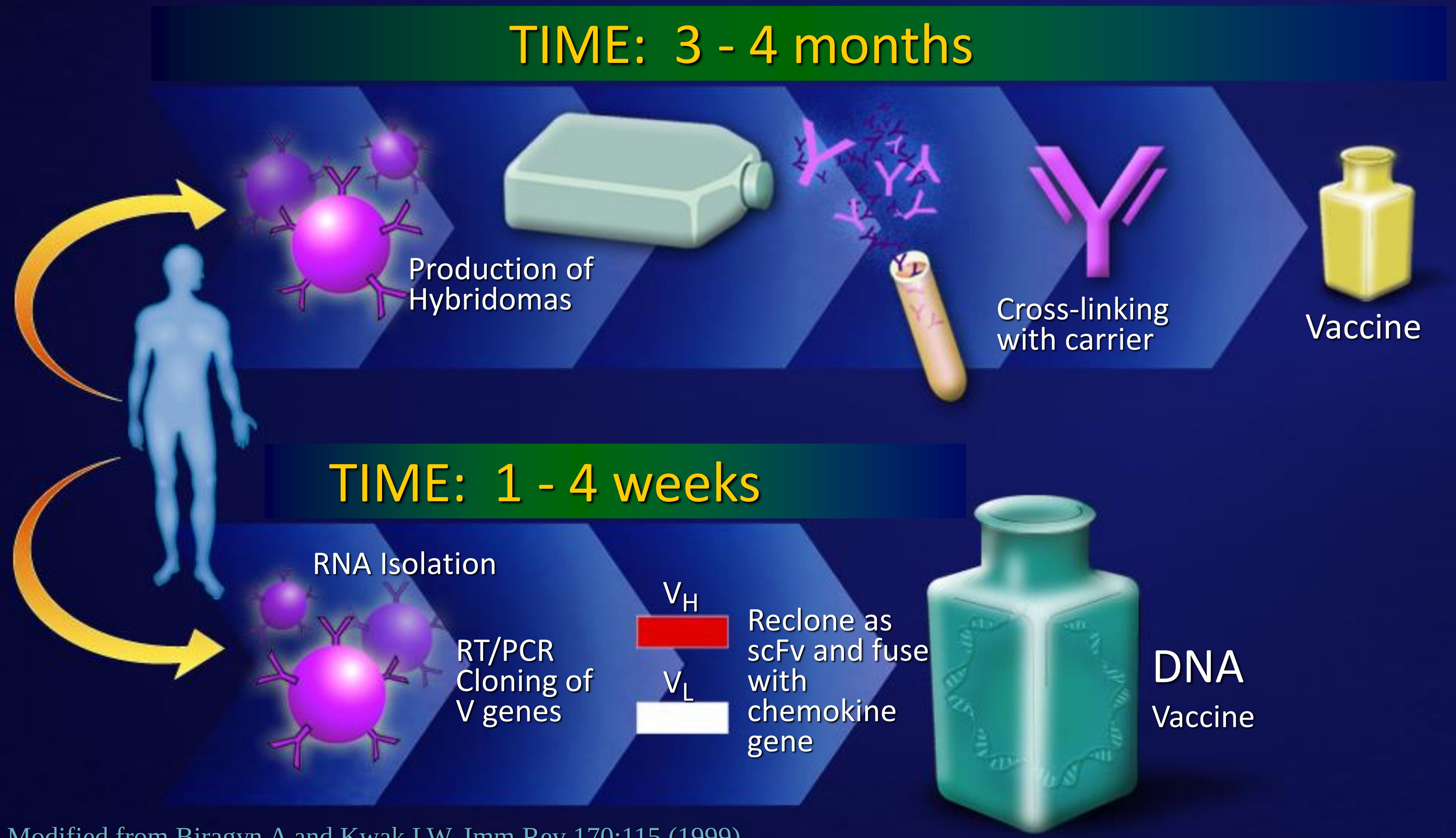
Antibody Responses

Liu, MA, Hum Gene Ther. 17:1051-61

Potential Advantages of DNA and mRNA Vaccines for Cancer

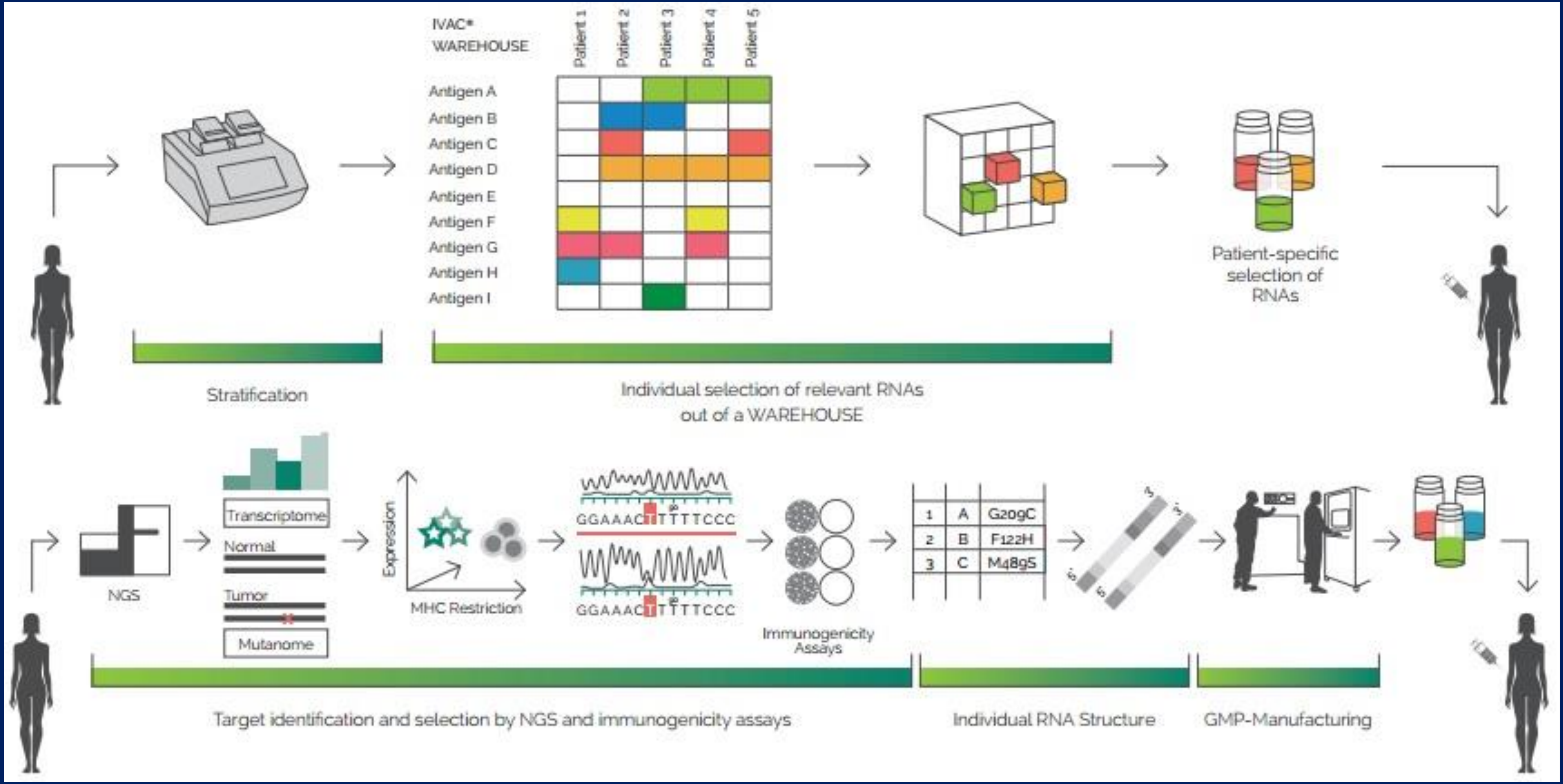
- Ability to generate all arms of adaptive immunity
  - CTL, TH cells, Antibodies
- Rapidity of construction and manufacture
- Ability to make desired form of antigen
  - Mammalian post-translational modifications
  - Transmembrane proteins
- Ease of construction and delivery of additional antigens
- Ability to co-deliver cytokines
- Innate immune responses due to DNA or mRNA

Patient-Specific DNA and mRNA Vaccines are Faster to Generate: Example Immunotherapeutic Idiotype Vaccine for Lymphoma



Modified from Biragyn A and Kwak LW, Imm Rev 170:115 (1999)

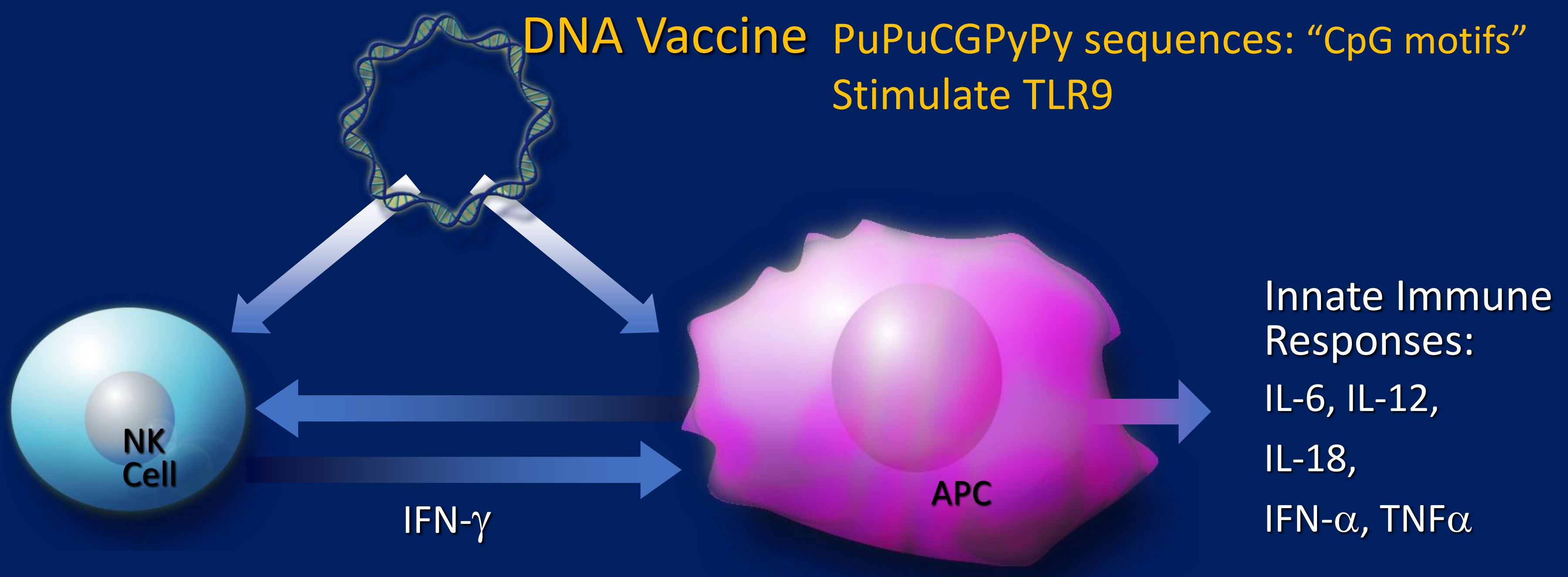
BioNTech "WAREHOUSE" vaccine concept



Harnessing/Optimizing Immune Responses: DNA Vaccines

Immune responses result from:

- Specific immunity against encoded antigen
- Non-specific immune effects of plasmid backbone

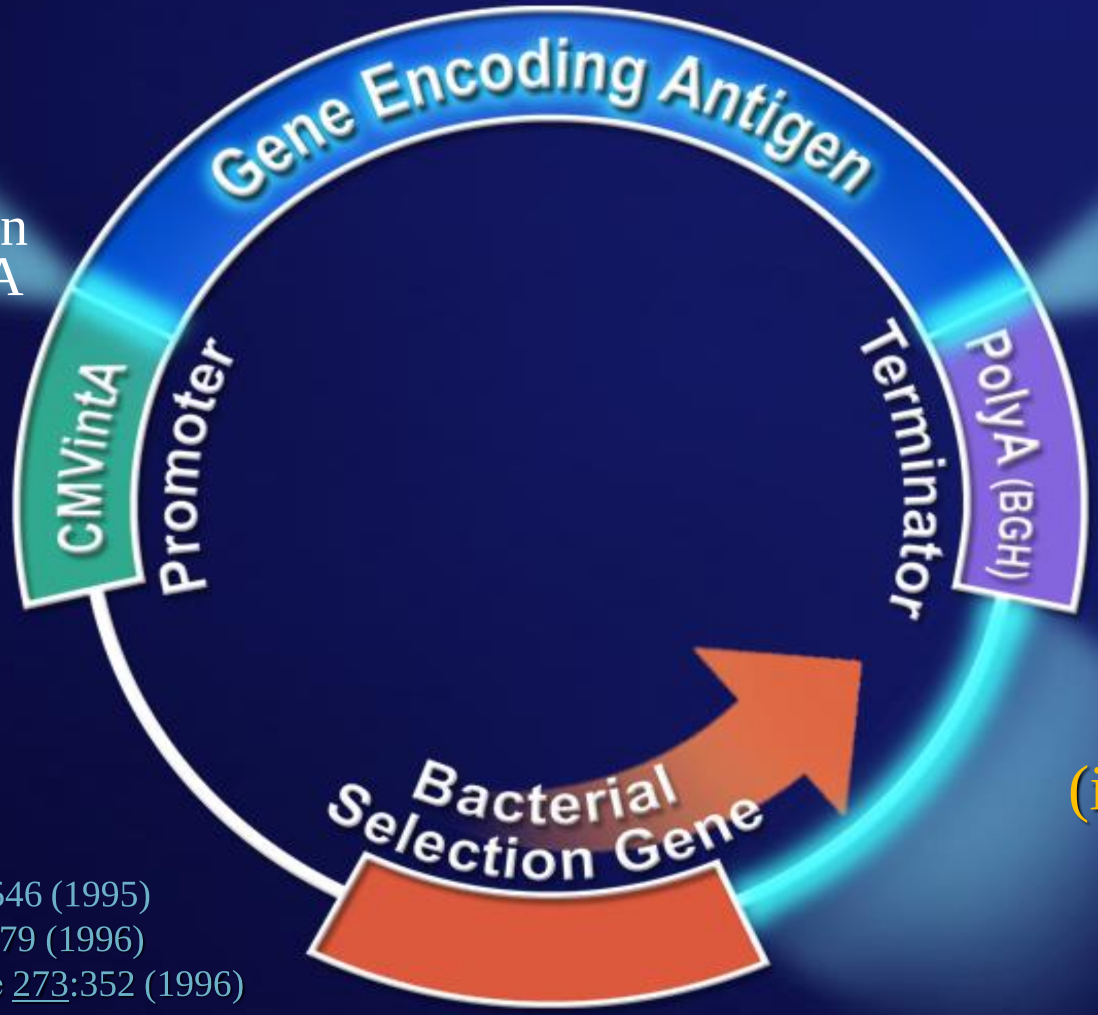


Modified from Krieg, AM, Current Op Imm 12: 35 (2000)

“Designer DNA Gene Vaccines”

Replicon:

- Amplify antigen mRNA



Genes Encoding:

- Cytokines
- Co-stimulatory molecules
- Targeting molecules

↑ CpG Content:

(immunostimulatory sequences)

Krieg AM…Klinman DM, Nature 374:546 (1995)
Klinman DM…Krieg AM, PNAS 93:2879 (1996)
Sato Y…Carson DA and Raz E, Science 273:352 (1996)

mRNA vaccines
Harnessing/Optimizing Immune Responses:

- Decrease inherent undesired types of immune stimulation
  - Modified nucleosides
  - Sequence modifications
- Potentially still maintain desired immune stimulation

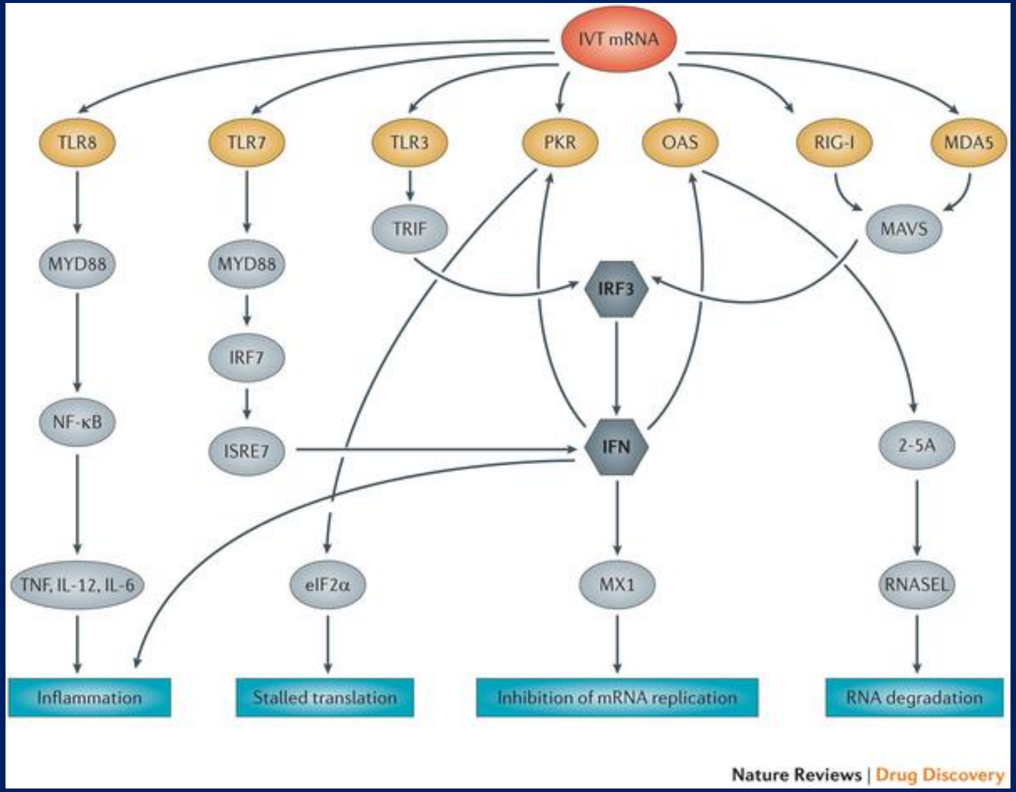
Stimulation of Innate Immunity mRNA Vaccines

- IVT (In Vitro Transcribed) mRNA immune activities
- LNPs can also have adjuvant activity
- Modified nucleosides decrease the immune reactivity of mRNA
- mRNA made with different modified nucleosides and/or mRNA sequences and different LNPs have different net profiles of activity
- RNA adjuvant activity has led to RNA itself being

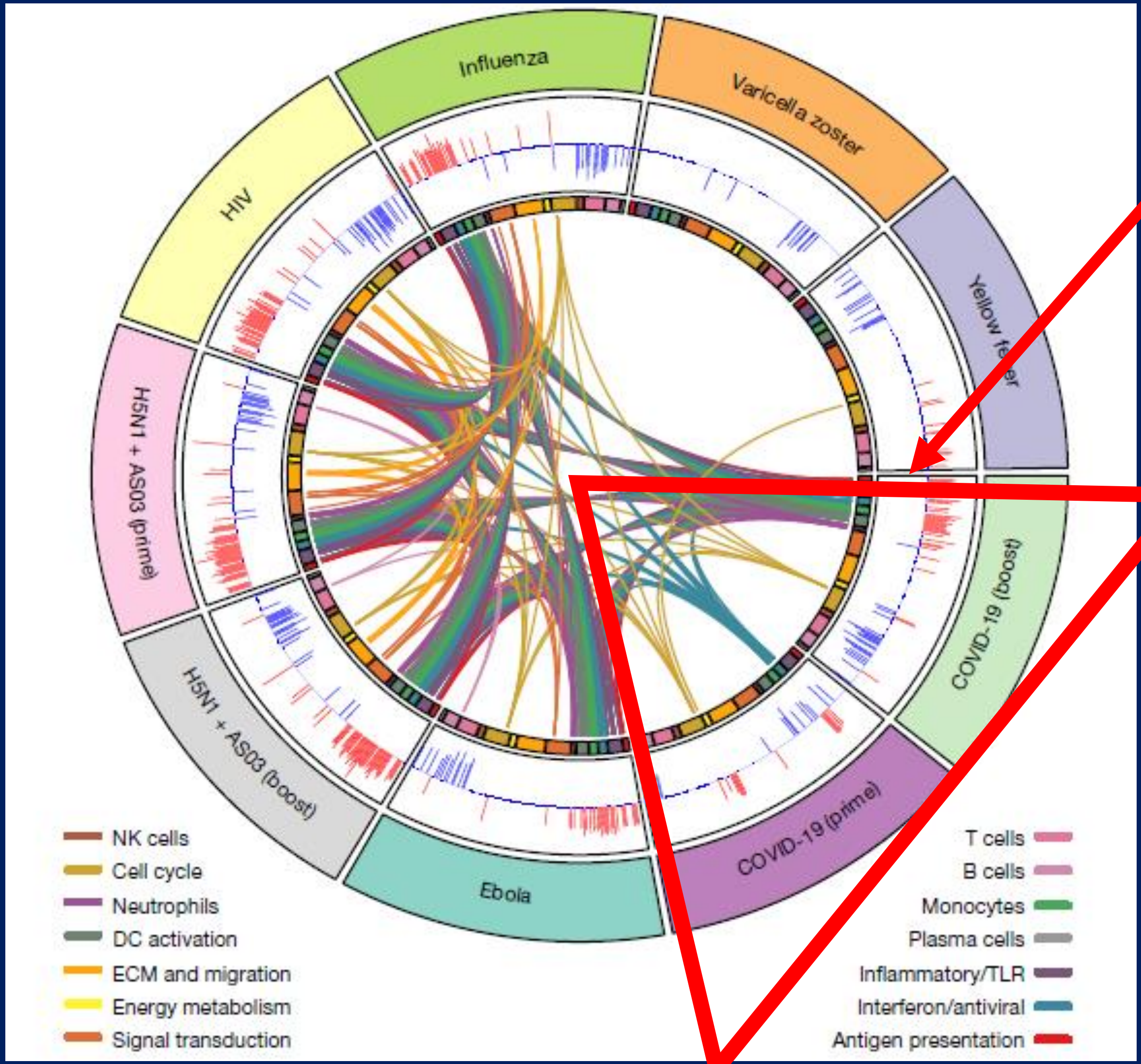
  used as an adjuvant

Biological Activities/Pathways of IVT mRNA

Source: mRNA-based therapeutics — developing a new class of drugs
Ugur Sahin, Katalin Kariko, Ozlem Tureci, *Nature Reviews Drug Discovery* **13**, 759–780 (2014)



Comparison of Transcriptomics Response for mRNA Vaccines Compared to Other Vaccines



mRNA Vaccine
Prime and Boost

Ab and T cell responses to Pfizer mRNA Vaccine depends on MDA5 signaling via Type 1 IFN and are independent of TLR signaling, inflammasome activation, and key cell death pathways.

Li,C…Pulendra, B. Nature Immunol. 2022. 23(4): 543 doi:10.1038/s41590-022-01163-9

Figure from: Arunachalam, PS….Pulendran, B Nature, 2021 596:410

Cancer DNA Clinical Trials

- Many studies using DNA encoding HPV proteins
  - Cervical Intraepithelial Neoplasia (CIN)
  - Cervical Carcinoma
- Multiple other cancer targets: lymphoma, breast, prostate, head and neck, Merkel cell, bladder, melanoma, glioblastoma, neuroblastoma, lung

Published results (2017–22) from mRNA cancer vaccine trials by type of formulation

	Trial phase	Target antigen	Cancer type	Patients, n	Combination	Immune response	Clinical response
Non-formulated (naked)							
NCT02035956	1	An individualised tumour mutation signature with ten selected neoepitopes for each patient	Melanoma (stages III and IV)	13	None	T-cell responses against numerous vaccine neoepitopes	One (8%) patient had complete response and another patient (8%) had partial response10
NCT03394937	1	CD40L, CD70, caTLR4; tumour-associated antigens: tyrosinase, gp100, MAGE-A3, MAGE-C2, and PRAME	Resected melanoma (stages IIc, III, and IV)	20	None	Vaccine-induced immune responses in four (40%) of ten patients (low dose) and three (33%) of nine patients (high dose)	Not reported11

Lorenzten et al. Lancet Oncology 2022 Oct; 23(10): e450–e458.
Published online 2022 Sep 26. doi: 10.1016/S1470-2045(22)00372-2

Published results (2017–22) from mRNA cancer vaccine trials by type of formulation

	Trial phase	Target antigen	Cancer type	Patients, n	Combination	Immune response	Clinical response
Protamine formulation							
NCT01817738	1/2	PSA, PSMA, PSCA, STEAP1, PAP, and MUC1	Metastatic castration-resistant prostate cancer	197	None	Not reported	No significant differences in progression-free survival[12]
NCT00923312	1/2	MAGE-C1, MAGE-C2, NY-ESO-1, survivin, and 5T4	Non-small-cell lung cancer (stages IIIb and IV)	46	None	T-cell responses against at least one tumour-associated antigen in 19 (63%) patients	No objective responses; progression-free survival and overall survival not improved[13]
NCT01915524	1	MAGE-C1, MAGE-C2, NY-ESO-1, survivin, 5T4, and MUC-1	Non-small-cell lung cancer (stage IV)	26	With local irradiation (with or without pemetrexed and with or without EGFR tyrosine-kinase inhibitor)	Detectable antigen-specific immunity in 21 (84%) patients	One (4%) patient had partial response in combination with chemotherapy treatment, and 12 (46%) patients had stable disease[14]

Lorenzten et al. Lancet Oncology 2022 Oct; 23(10): e450–e458.
Published online 2022 Sep 26. doi: 10.1016/S1470-2045(22)00372-2

Published results (2017–22) from mRNA cancer vaccine trials by type of formulation

	Trial phase	Target antigen	Cancer type	Patients, n	Combination	Immune response	Clinical response
Lipoplex formulation							
NCT02410733	1	NY-ESO-1, tyrosinase, MAGE-A3, and TPTE	Melanoma	25 (monotherapy); 17 (combination)	With or without standard PD-1 therapy	Immune responses against a minimum of one tumour-associated antigen in 39 (75%) patients	mRNA vaccine with anti-PD-1 therapy: six (35%) patients had partial response and two (12%) had stable disease; mRNA vaccine monotherapy: three (12%) patients had partial response, and seven (28%) had stable disease[15]
NCT04503278	1/2	CLDN6 (CARVac)	Solid tumours (CLDN6 CAR T cells with CARVac)	7	With CLDN6 CAR T cells	Engraftment of CAR T cells in all patients	Four (57%) patients had partial response and one (14%) patient had stable disease at the 6-week evaluation[16, 17]

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Published results (2017–22) from mRNA cancer vaccine trials by type of formulation

	Trial phase	Target antigen	Cancer type	Patients, n	Combination	Immune response	Clinical response
Lipid nanoparticle formulation							
NCT03480152	1/2	Neoantigen-specific mRNA	Gastrointestinal cancer	4	None	Mutation-specific $CD4^+$ and $CD8^+$ T-cell responses against predicted neoepitopes in three (75%) of four patients	No objective clinical responses[18]
NCT03313778	1	Personalised cancer vaccine encoding several neoantigens	Solid tumours (resected)	13 (monotherapy); 19 (combination)	With pembrolizumab	Detectable neoantigen T-cell responses	Vaccine monotherapy: 12 patients were cancer-free on study treatment with a median follow-up of 8 months; combination treatment: one patient had complete response before vaccination, two patients had partial response, five patients had stable disease, five had disease progression, and two had unconfirmed disease progression[19]

Lorenzten et al. Lancet Oncology 2022 Oct; 23(10): e450–e458.
Published online 2022 Sep 26. doi: 10.1016/S1470-2045(22)00372-2

Additional Possible Mechanisms for Improving Anti-Cancer Immune Responses

- Non-specific Immune stimulation
  - BCG instillation to prevent progression or recurrent of bladder cancer
    - Mechanism unclear, multifactorial
      - Cytokines
      - Multicellular infiltration
      - Innate responders
  - Coley's toxin: mixture of killed bacterial toxins
    - Case reports of tumors shrinking
- Concept of "Immune Fitness"
  - Various vaccines (mainly live organisms) reported to make individuals less susceptible to other diseases
- Do nucleic acid vaccines have any non-specific beneficial immune effects?

ImmunoTherapeutic Vaccines of the Future

- Harness the synergistic characteristics of DNA and mRNA vaccines
  - Antibodies, CTL, and Th
  - Innate immune stimulation directly by DNA/mRNA
  - Ease of co-administration of cytokines as DNA/mRNA or directly
  - Likely need to reverse the immune impairment/tolerance of the tumor environment
    - Example: co-administration of check-point inhibitors
- Rapidity and manufacturing ease for making personalized tumor vaccines by sequencing the patient's tumor
  - Patient-specific constructs
  - Pre-made libraries encoding key antigen for combination
- Explore heterologous prime-boost immune responses to augment responses?
  - DNA/mRNA, DNA/protein, mRNA/protein, etc
- Explore any possibility of role for improving immune fitness

Thank you!

- More information provided in open access paper
- Link to paper: DNA and mRNA Vaccines for Chronic Viral Infections and Cancer: Rationale, Mechanisms, and Progress