The Three Flavors of Danger

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- Disclosure: Founder, CEO of Zola Therapeutics

William B. Coley, M.D.

1862-1936

Zola

In 1891 Dr. Coley injected Zola's tumor with live bacteria[1]

"Zola's temperature … rose above 104 degrees, with nausea, vomiting and severe pain. The infection almost killed him, but within two weeks, the neck tumor was not observable… Zola was in excellent health when Coley saw him four years later."

- Dr. Coley reported >40% response rate in >1000 pts
- By the 1950's responses were rare; "Coley's toxins" mostly abandoned, except BCG for bladder cancer
- Why did the efficacy of "Coley's toxins" drop?
  - Patients' immune systems have changed.
  - "toxins" were not made fresh – active ingredient lost?
    - ***1984: BCG efficacy is mediated by bacterial DNA[2]***

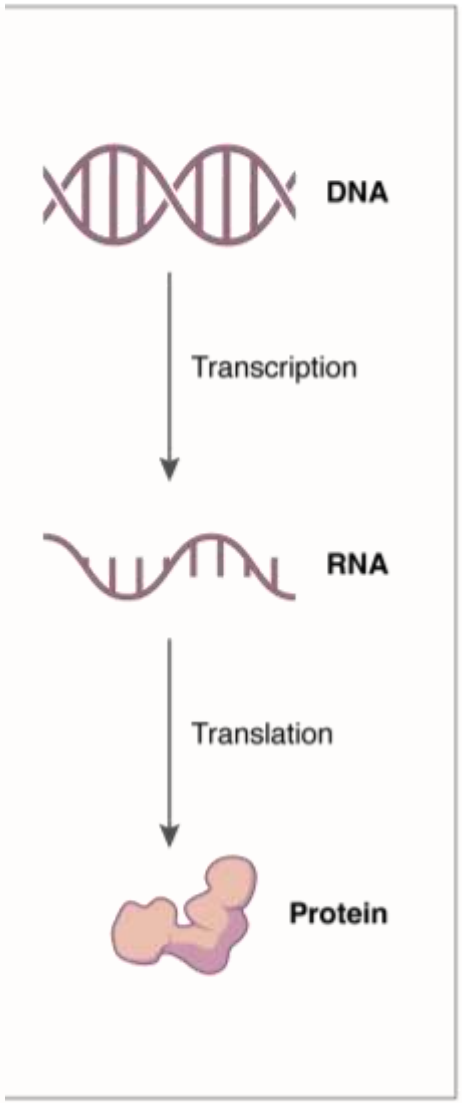
- Zola is considered to be the earliest example of successful "*in situ* vaccination" or "making cold tumors hot", thereby inducing anti-tumor **CD8+ T cells** that eradicate the cancer.
- Today, *in situ* vaccination for advanced tumors as monotherapy rarely succeeds in humans:
  - <10% response rate: cGAS-STING, RIG-I, NOD-like receptors, TLR7/8, CpG-B/C TLR9 agonists
  - >20% response rate: CpG-A DNA TLR9 agonist (vidutolimod)

Can we exceed Coley's 40% response rate? Can we do this with a safe IV formulation?

[1] https://www.americanscientist.org/article/healing-heat-harnessing-infection-to-fight-cancer; [2]Tokunaga et al., J Natl Cancer Inst., 1984; and Jpn J Infect. Dis., 1999

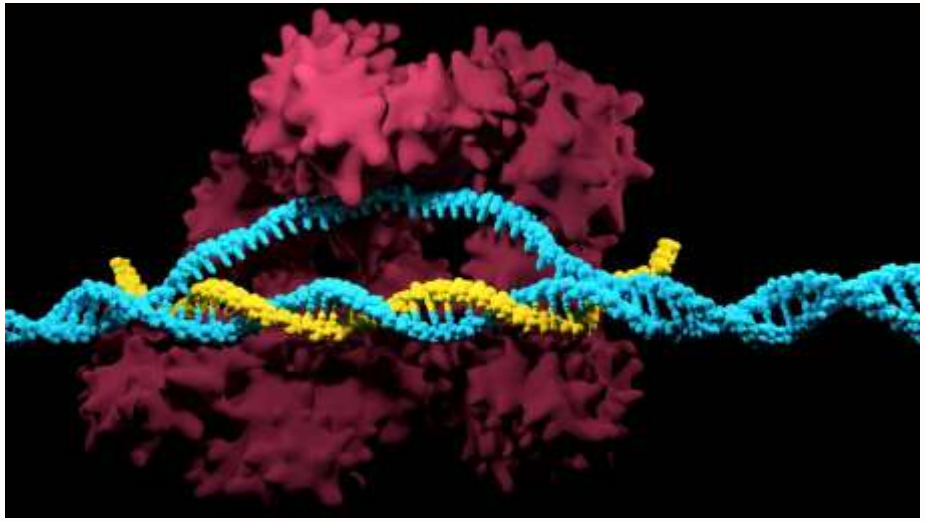
The Central Dogma

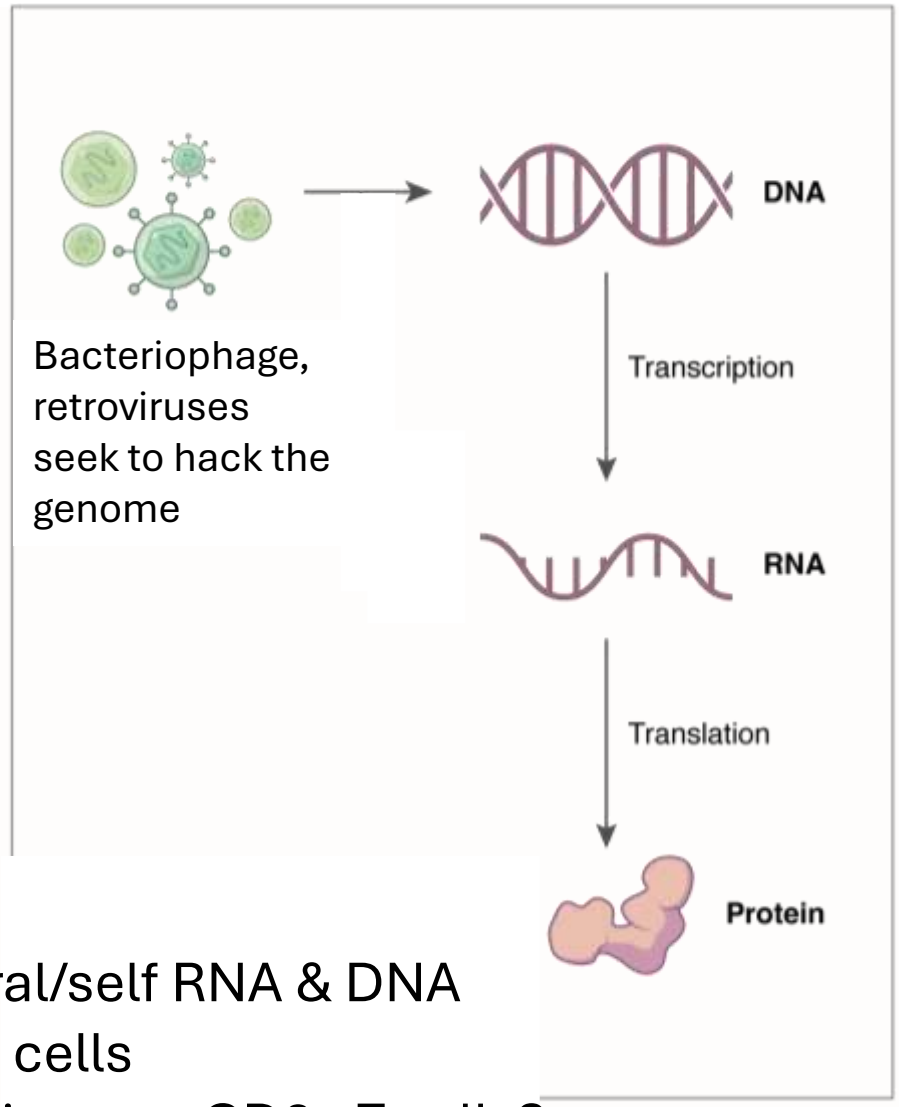
Since the first archaebacteria >3 billion years ago, all living organisms depend on the flow of information from the DNA genome through RNA, to make proteins.



The Challenge of Protecting Genome Integrity

All forms of life must protect their genome integrity against invaders to survive as a species. Archaebacteria evolved CRISPR-Cas systems to specifically cut foreign DNA.





Bacteriophage, retroviruses seek to hack the genome

Bacteria evolve new CRISPRs to cut each new phage

Higher organisms evolved receptors to distinguish viral/self RNA & DNA

- These induce CD8+ T cells to kill infected host cells
- Can we harness these receptors to induce anti-tumor CD8+ T cells?

Perception

Five primary senses perceive the external world:

1. Vision – photoreceptors in the retina
2. Smell – olfactory receptors in the nose
3. Taste – receptors in the tongue
4. Touch – pressure/texture/heat receptors in skin
5. Hearing – sound receptors in the ear

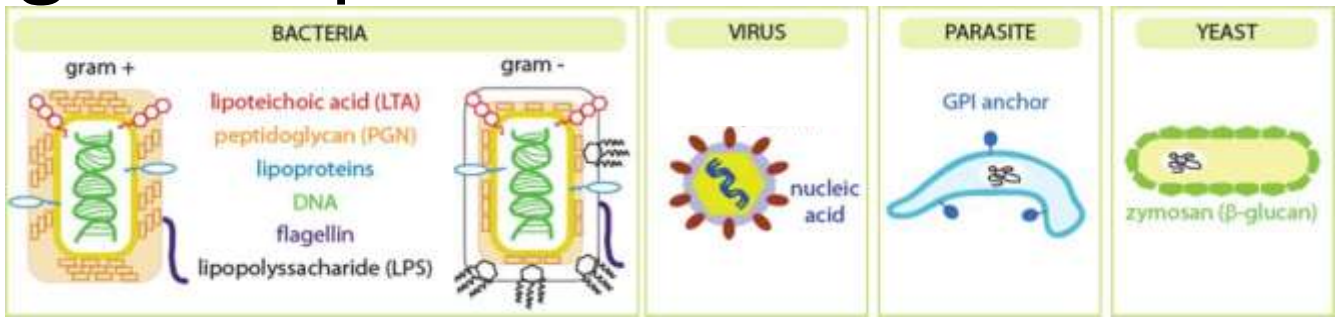
Interoception

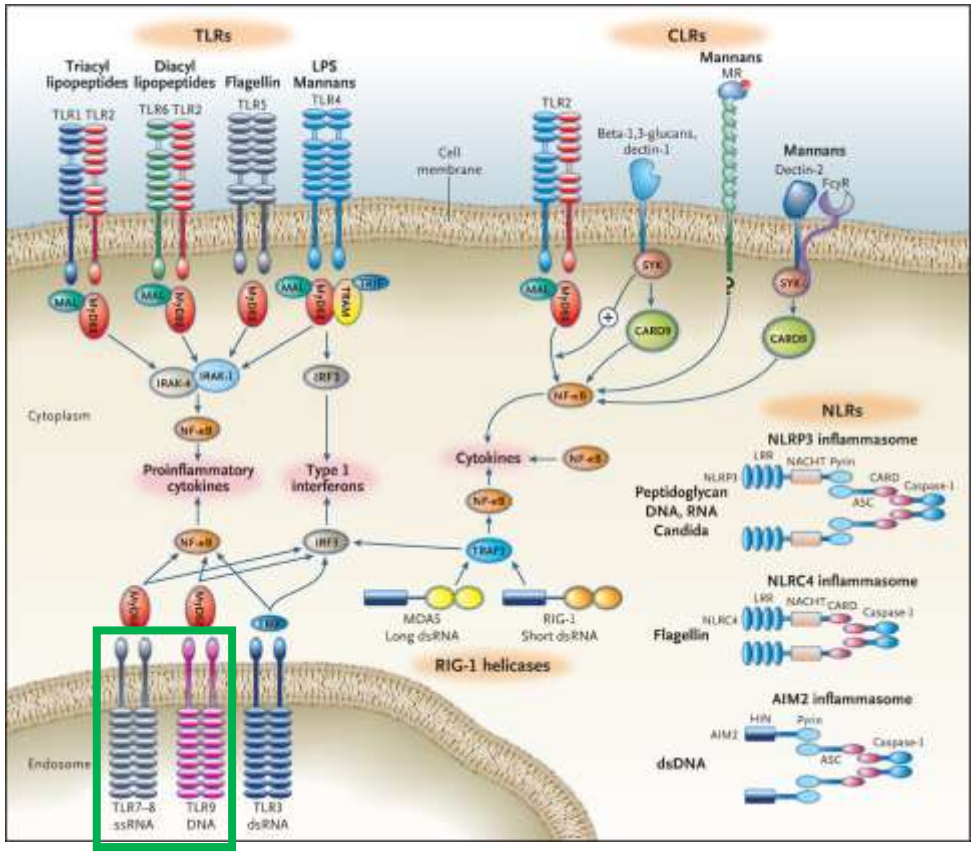
Sensing what's happening inside our bodies:

1. Semi-aware – receptors via vagus nerve, sympathetic
2. Unaware – immune system "**pattern recognition receptors**"
    a. Infection – *must tolerate normal microbiome*
        a. Intracellular (genome integrity) – *kill infected cell*
        b. Extracellular – *kill pathogen*
    b. Cell stress/hypoxia – *heal damaged tissue*

Image source: reframing autism

Pathogens Express Distinct Molecular Patterns

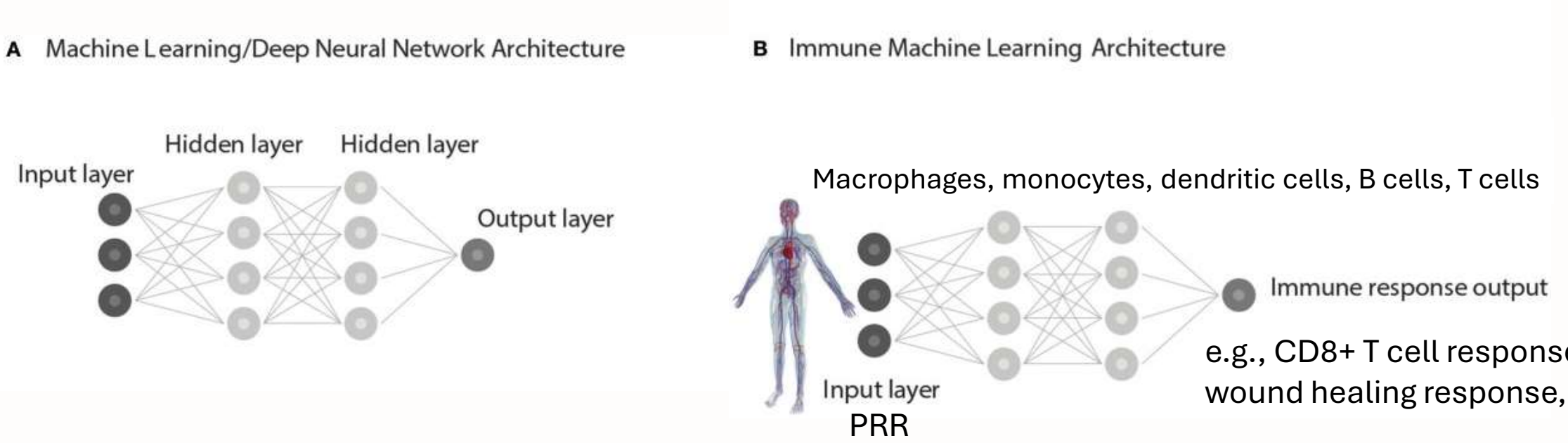




- *Dozens of Pattern Recognition Receptors (PRR) have evolved to detect pathogen patterns*
  - *TLR7/8 can detect retroviral RNA;*
  - *TLR9 can detect retroviral DNA*
- *PRR are differentially expressed in dozens of distinct immune cell subsets*
- *Each PRR can activate 1 or more defense pathways in 1 or more subset of immune cells*

Image sources: British Society for Immunology, NEJM

The Immune System Must Compute the State of the Body using PRR and "*Quorum Sensing*" or *"Crowd Wisdom"*[1]



We can use synthetic PRR agonists to change the input layer

Evolution has already determined the immune response output to certain inputs

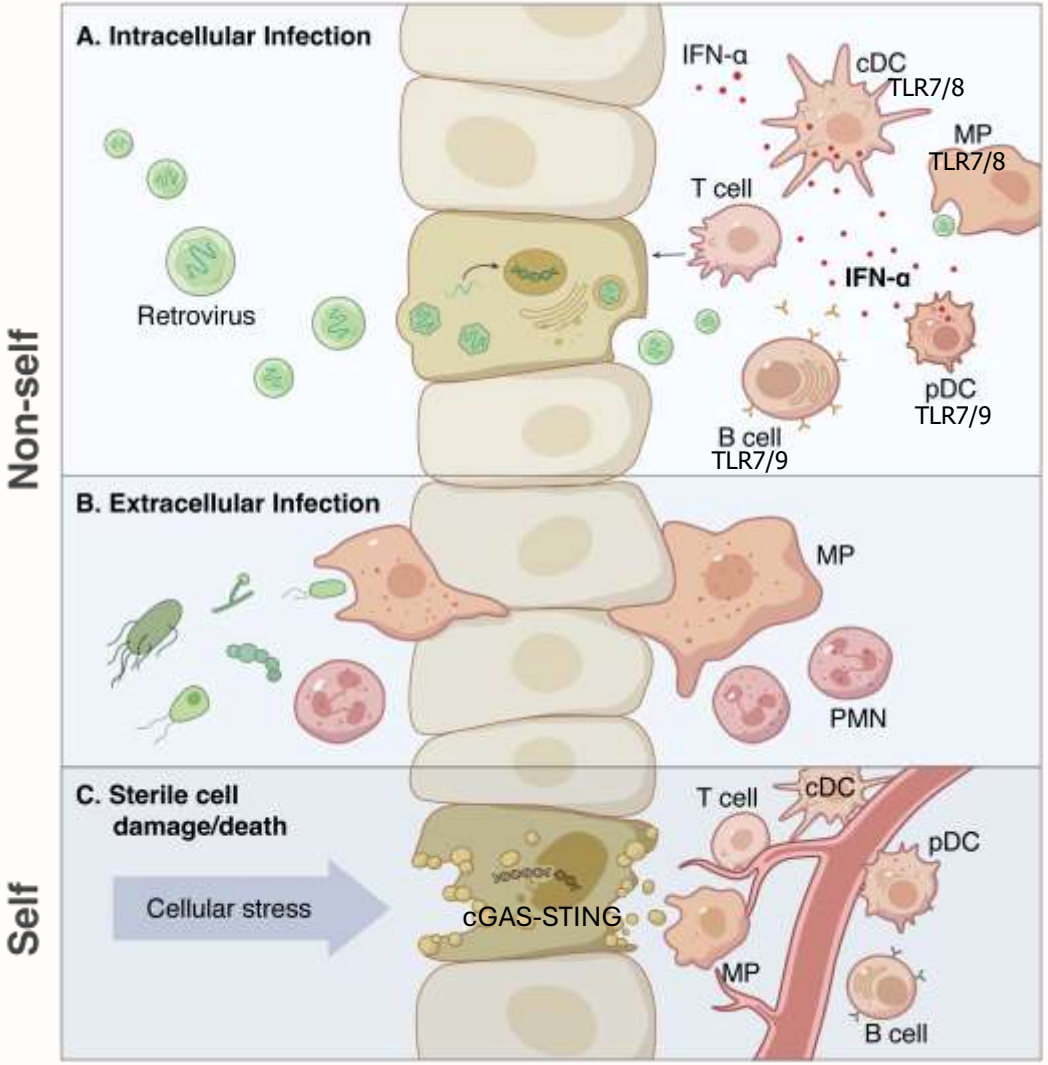
Retroviral infection MUST induce CD8+ T cells

Retroviral infection (RNA/DNA) is specified by activation of TLR7/8/9 alone

We can deduce that synthetic TLR7/8/9 agonists can be used to induce CD8+ T cells to treat cancer

[1]Antonioli et al., Nature Reviews Immunol. 2018; Cohen and Efroni, Front. Immunol. 2019; Medzhitov and Iwasaki, 2024

The 3 “flavors” of danger sensed by PRRs



cDC = conventional dendritic cell; pDC = plasmacytoid dendritic cell; MP = macrophage; PMN = neutrophil

All are recruited into any damaged tissue

Patterns: retroviral RNA (TLR7/8) / DNA (TLR9)

Detection: by macrophages (MP), dendritic cells (DC), etc.

Appropriate Response: DC make IFN-α, induce CD8+ T cells to kill infected cells, without killing uninfected cells

Inappropriate response: SLE, autoimmune diseases

Patterns: flagella, lipopeptides, peptidoglycans etc.

Detection: macrophage **(**MP), neutrophils (PMN), DC, etc.

Appropriate Response: MP, PMN kill pathogen

Inappropriate response: sepsis, “cytokine storm”

Patterns: damage molecules, blebs from dying cells

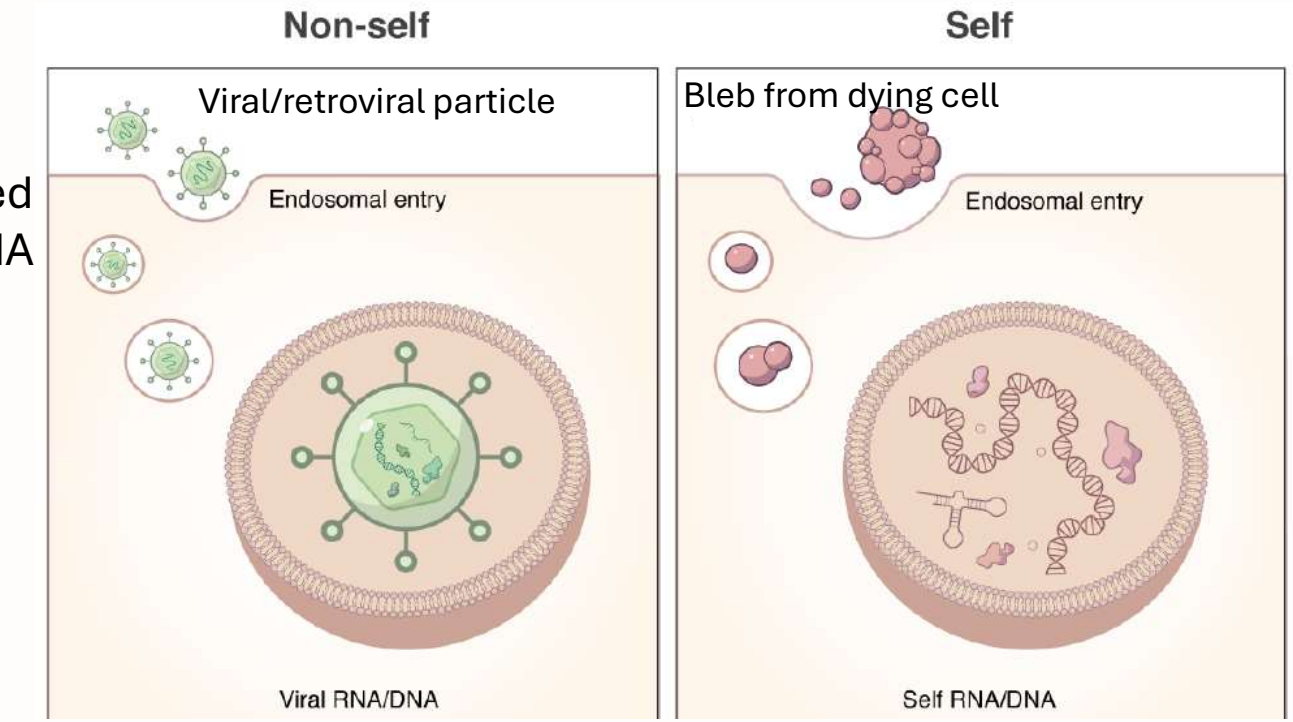
Detection: at least 13 subsets of MP, etc.

Appropriate Response: angiogenesis (new blood vessels), tissue healing, prevent immune attack

Inappropriate: suppression of anti-tumor immunity

TLR7/8/9 distinguish retroviral particles from blebs released by dying cells

GU RNA; unmethylated CpG DNA

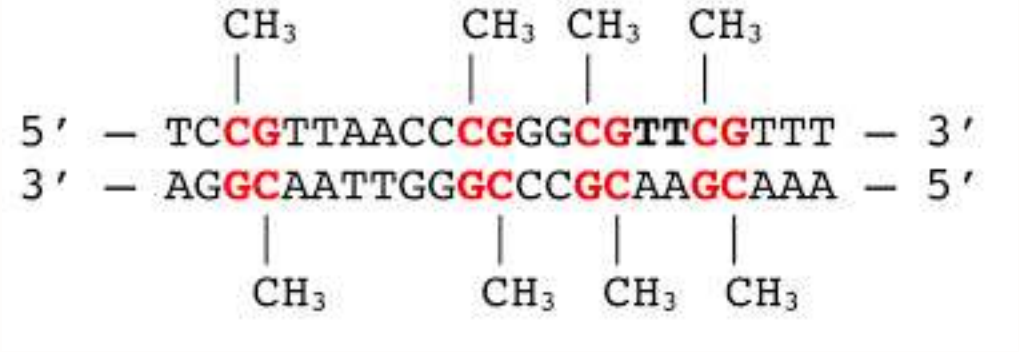


2’-OMe G RNA, pseudouridine; methylated CpG DNA

TLR7 and TLR8 distinguish self from retroviral RNA (in endosomes of DC, MP, B cells)
TLR9 distinguishes self from retroviral DNA (in endosomes of pDC, B cells)

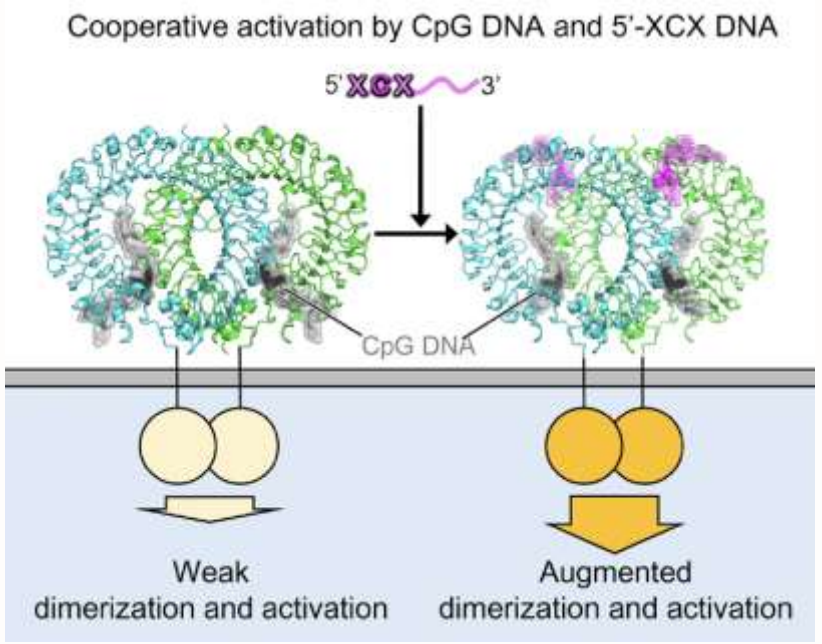
Immune cells detect CpG in viral DNA

In our DNA ~80% of CpG are methylated



In viral DNA CpG is unmethylated

Toll-like receptor 9 (TLR9) evolved to
i) detect unmethylated CpG DNA;
ii) activate DC that induce CD8+ T cells to kill retroviral infected cells



Could retroviruses evolve to avoid this defense?

Ohto et al., Immunity, 2018

HIV Genome is highly CpG-suppressed

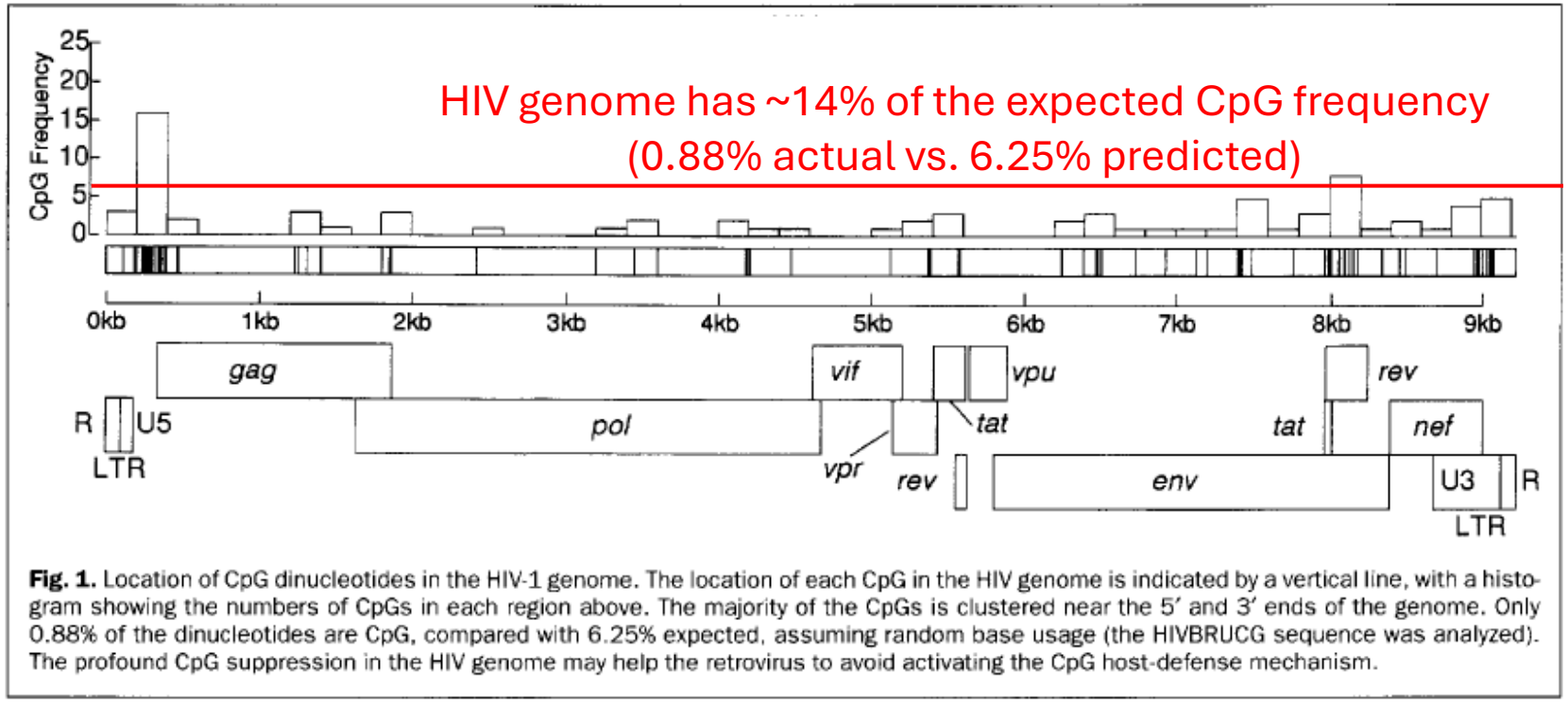


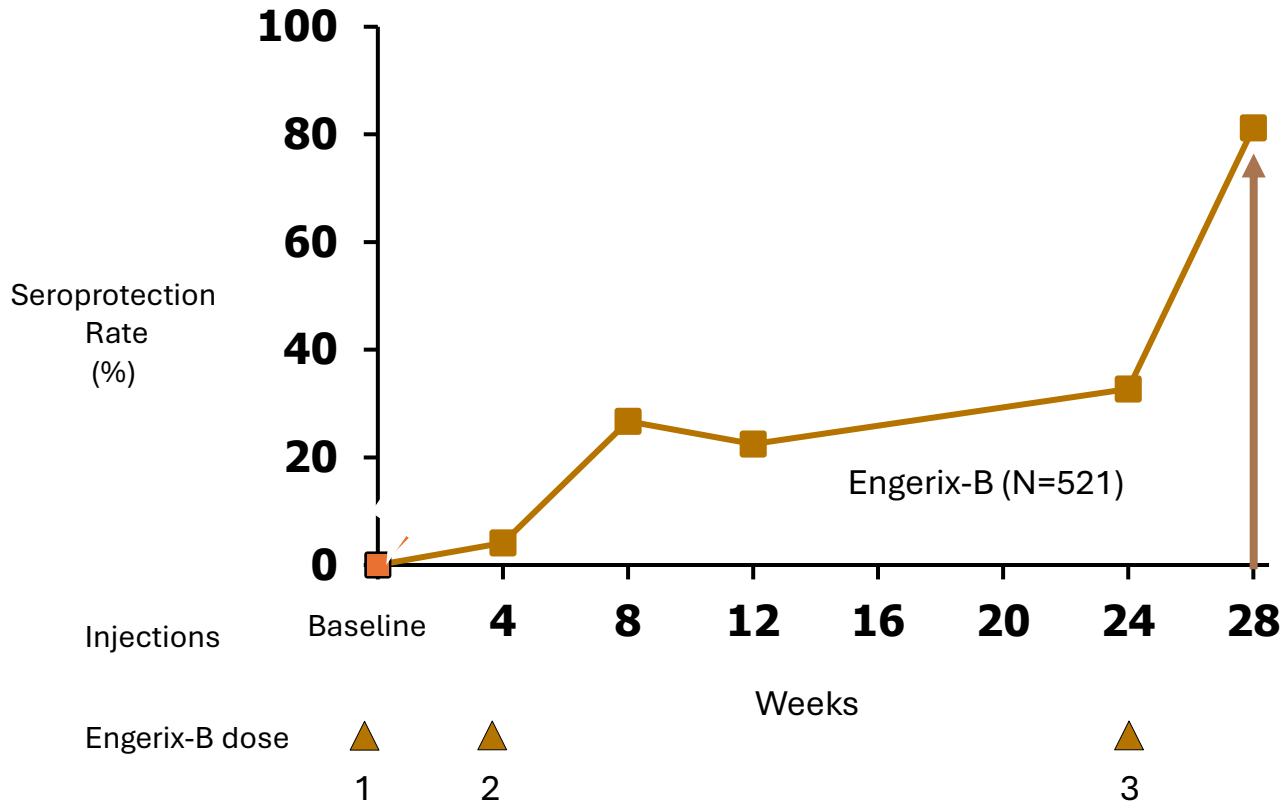
Fig. 1. Location of CpG dinucleotides in the HIV-1 genome. The location of each CpG in the HIV genome is indicated by a vertical line, with a histogram showing the numbers of CpGs in each region above. The majority of the CpGs is clustered near the 5′ and 3′ ends of the genome. Only 0.88% of the dinucleotides are CpG, compared with 6.25% expected, assuming random base usage (the HIVBRUCG sequence was analyzed). The profound CpG suppression in the HIV genome may help the retrovirus to avoid activating the CpG host-defense mechanism.

Adapted from Krieg, 1996, Trends In Microbiology 4:73.

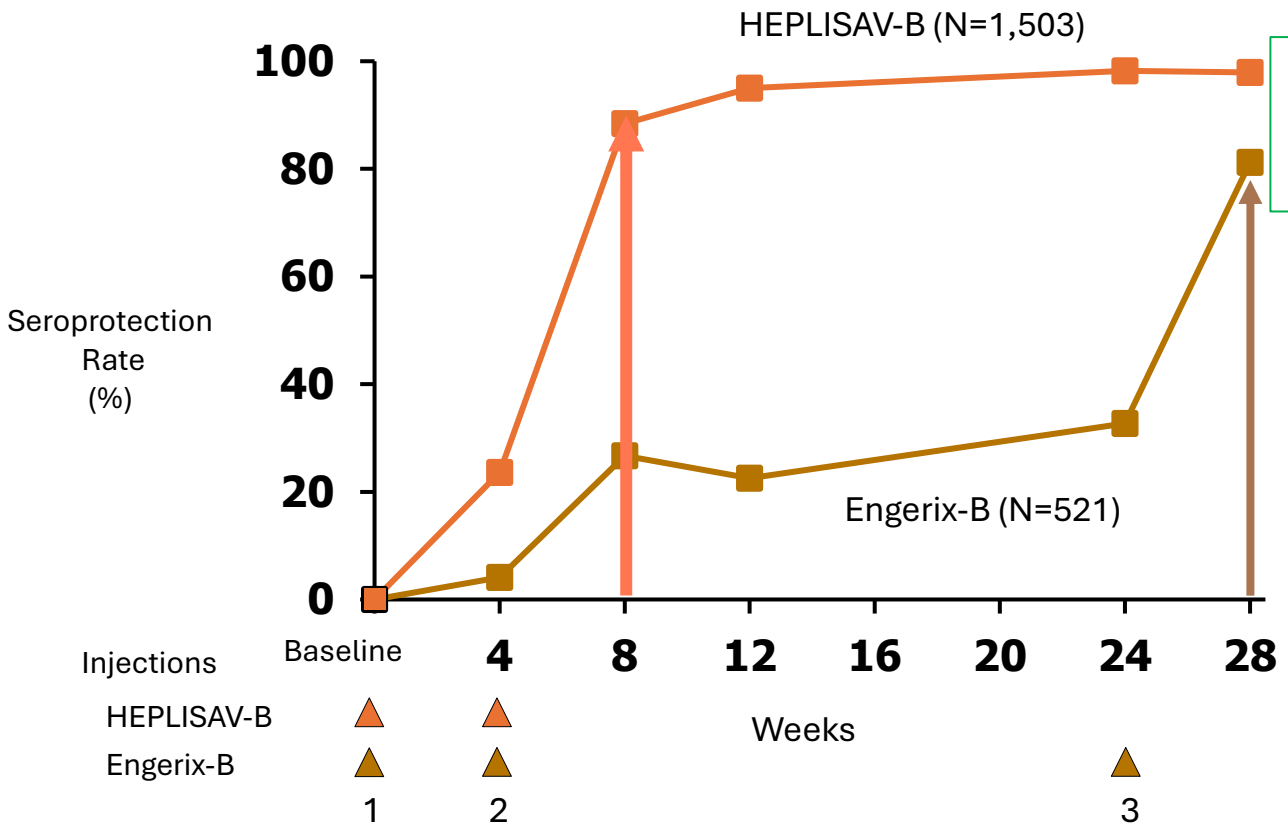
- All retroviral genomes are highly CpG suppressed!
- Conclusion: TLR9 detection of CpG DNA triggers effective anti-viral defenses

Could vaccines be enhanced by adding synthetic CpG DNA?

Standard hepatitis B vaccines require 3 doses to protect >80% of the population



HEPLISAV-B with CpG DNA: higher seroprotection by 8 weeks



2 dose Heplisav > 3 doses Engerix

- Safety shown in >13,000 humans
- Protects immune compromised pts

Could cancer be treated by injecting CpG DNA into a tumor?

Systemic Effects of Vidutolimod (CpG-A DNA, TLR9 agonist) Injection in Melanoma Groin Lesion

48 yo female with advanced melanoma following prior therapy:

IFN-α (1 month)

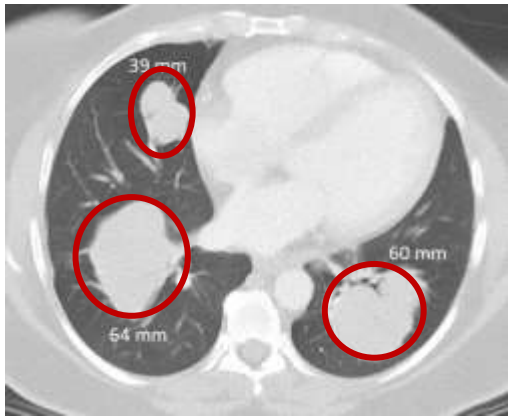
Pembrolizumab (3 months)

Aflibercept (6 months)

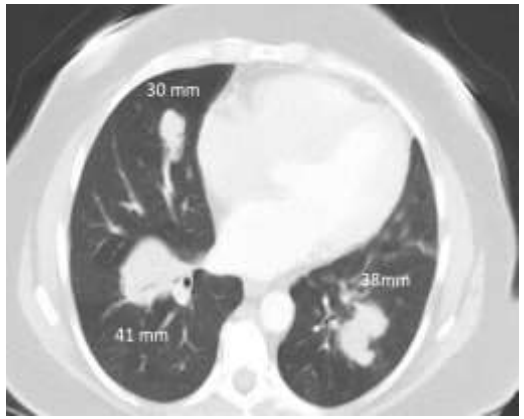
IL-2 (3 months)

Ipilimumab (14 months)

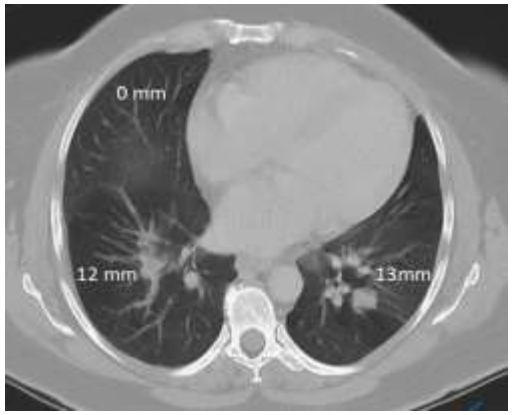
Baseline



Follow Up 1 (12 wks)



Follow Up 4 (48 wks)



*No recurrence after >4 years

Results of clinical trial of vidutolimod in patients with refractory melanoma:

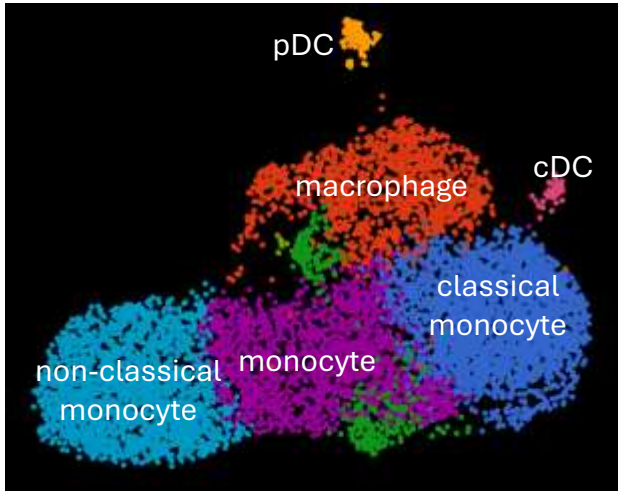
- 98 patients were treated with vidutolimod + checkpoint inhibitor: 27 responded (for average >2 years)
- 40 patients were treated with vidutolimod alone: 9 responded (for average <6 months)

Why didn't vidutolimod work in more patients?

Most immune cells in tumors can't detect CpG DNA, because they don't express TLR9!

The other immune cells in tumors express receptors for viral RNA structures, TLR7 and/or TLR8

Single cell RNA Sequencing is a tool to study gene expression in individual cells within a tumor or other tissue (many public data sets)



1. get a tumor biopsy, extract the immune cells
2. Separate each individual immune cell in a bubble (>100K cells)
3. Sequence all of the RNA in each bubble (containing 1 immune cell)
4. Cluster the cells into subsets using marker genes
   - Each dot in a cluster represents 1 cell that was in the biopsy

ALL of these innate immune cells are recruited by tumor "damage signals"

pDC = plasmacytoid dendritic cell, makes interferon-α (13 genes!)

cDC = conventional dendritic cell, present antigens and induce CD8+ T cells

Macrophage, monocytes = regulate/suppress DC, T cells, promote wound healing, angiogenesis

Which of these immune cells detects viral CpG DNA (via TLR9), and which detect viral RNA (via TLR7/8)?

Study link: https://pubmed.ncbi.nlm.nih.gov/33545035/

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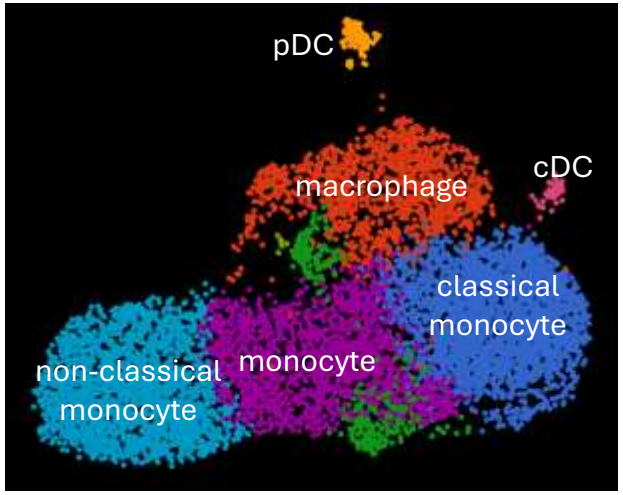

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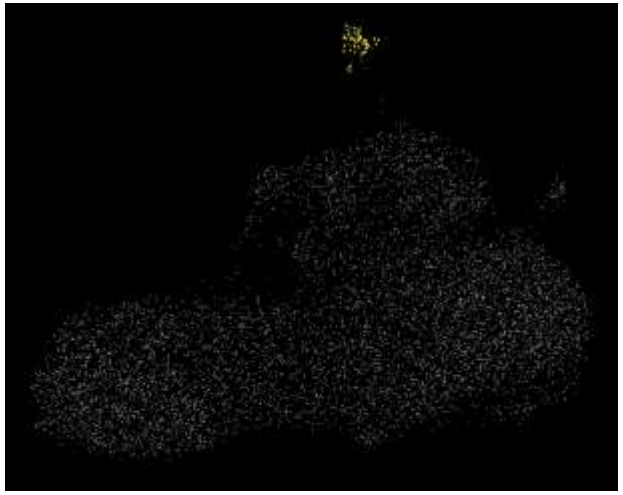
TLR9$^+$



Yellow cells detect CpG DNA (only pDC)

TLR7$^+$, TLR8$^+$, TLR7/8$^+$

Red, blue and yellow cells detect viral RNA (~all cells)

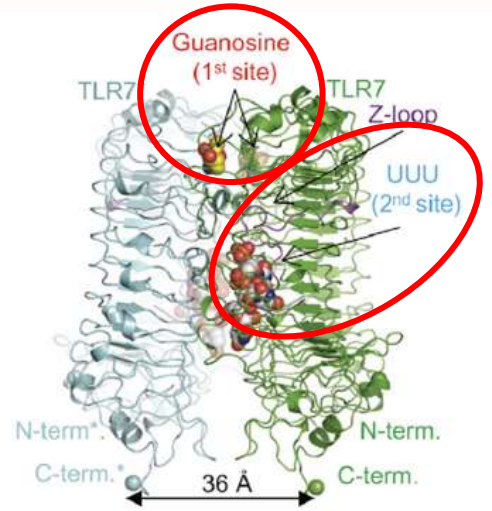
"Crowd wisdom": the TLR9$^+$ cells will be "out voted" by the macrophages/monocytes

Solution: add viral GU RNA mimic activating TLR7/8 to the CpG-A viral DNA mimic

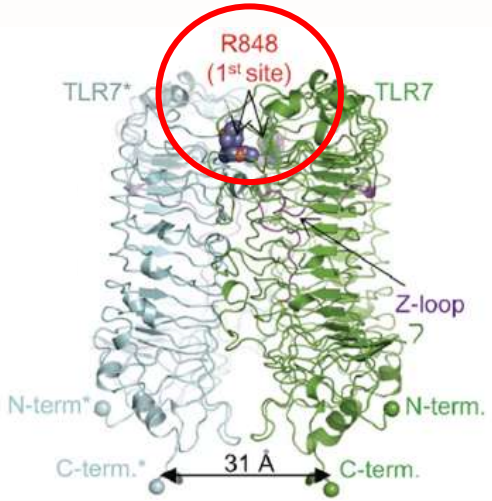
- Z-007 is the first TLR7/8/9 triple agonist, fully mimicking retroviral replicative complex
- Z-007 stimulates >50X more IFN-α **without inflammatory response** in:
  - human tumor-associated immune cells and normal blood cells
  - Monkey blood, liver, lymph nodes (IV dosing)
- Z-007 is expected to begin human clinical trials in mid 2026

RNA TLR7/8 agonist induces high IFN-α, low IL-6, TNF-α; small molecule TLR7/8 agonist* primarily induces inflammation (NFkB)

RNA binds TLR7 at 2 sites[1]

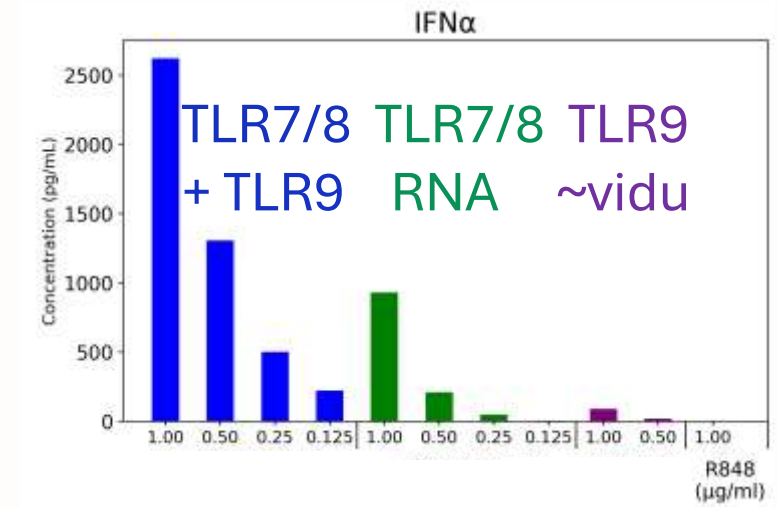


small molecules* only bind TLR7 at 1 site[1]



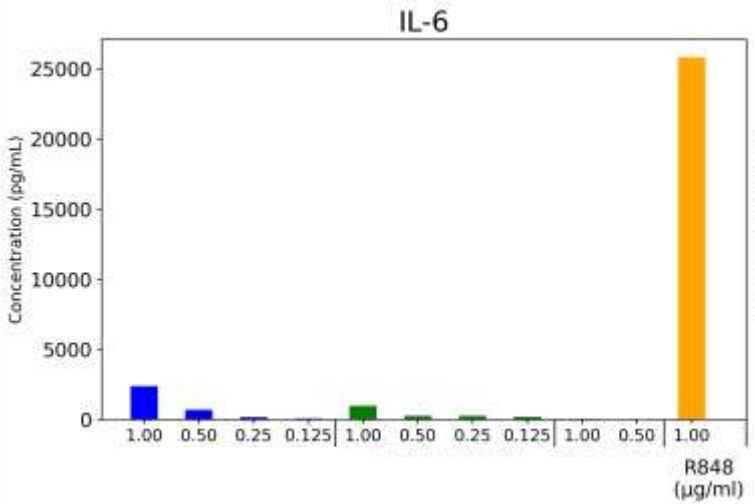
RNA binding to 2nd site induces allosteric shift, increasing 1st site affinity[2]

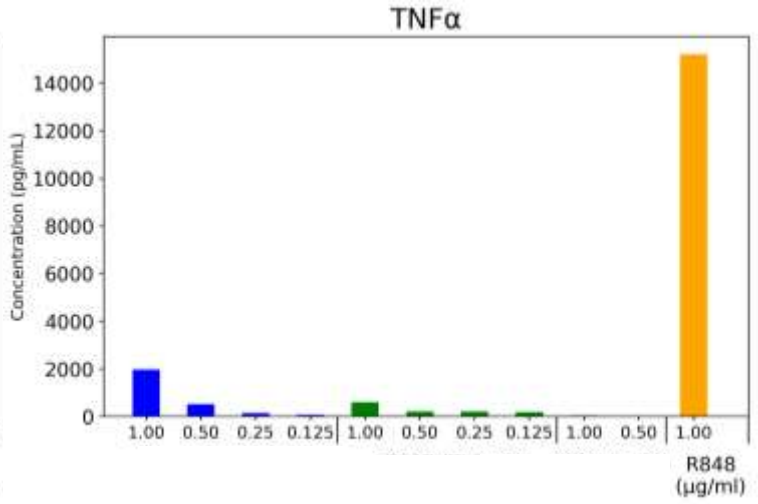
Human PBMC assay



Conclusions:

1. TLR7/8 RNA agonist is first to induce >IFN-α vs. CpG-A DNA[3-5]
2. TLR7/8/9 costimulation induces synergistic IFN-α with little inflammation.

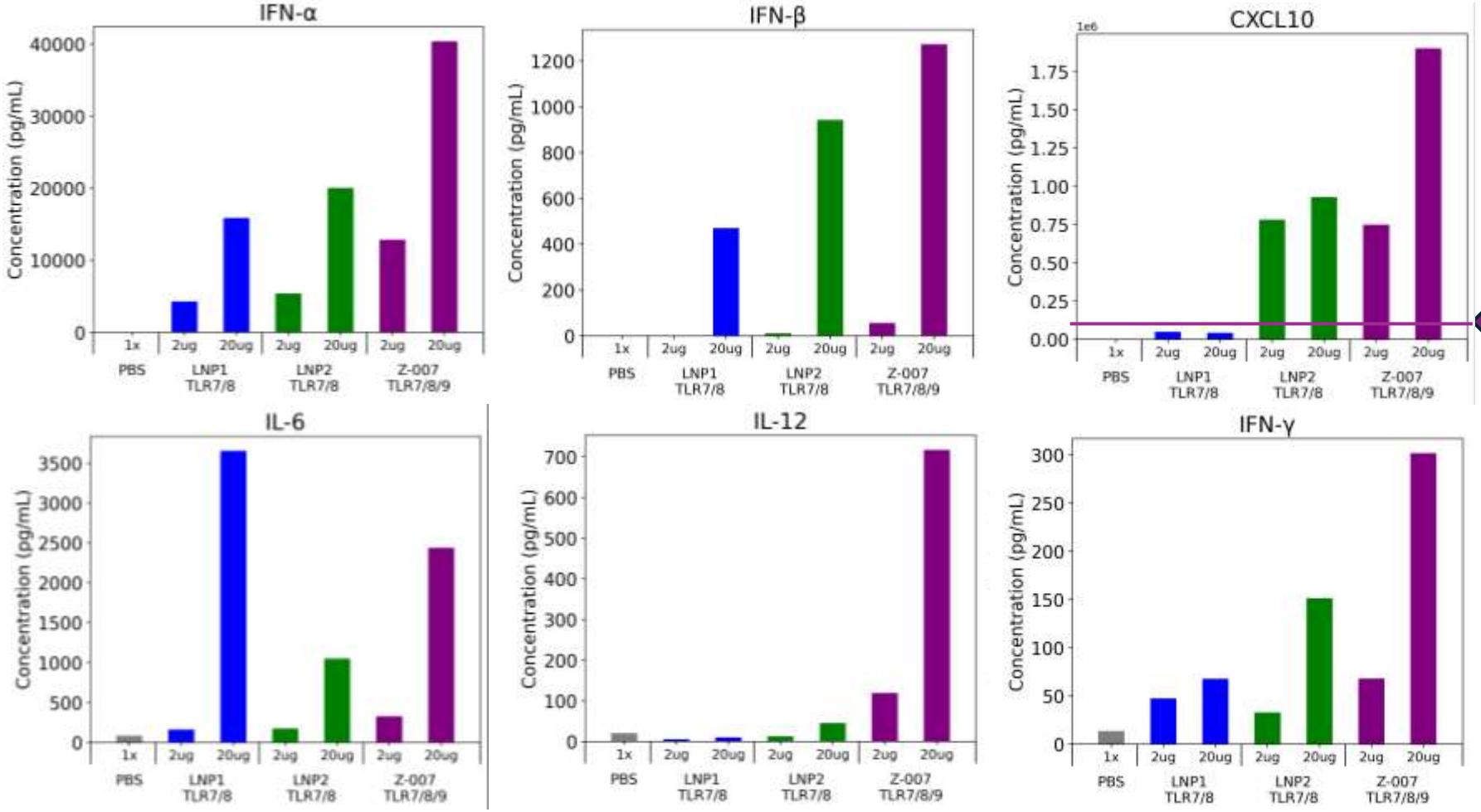




[1]Zhang et al., Immunity, 2016; [2]Ekimoto et al., Chem Pharm Bull, 2024; [3]Forsbach et al., J. Immunol., 2008; [4]Forsbach et al., Nucleic Acid Ther. 2011; [5]Tong et al., JEM 2024

*e.g, R848, Eikon, Bolt, Silverback, Primmune, Inimmune, Nektar, Medimmune, etc

Z-007 TLR7/8/9 co-stimulation in mice induces unprecedented IFN-α, CXCL10 response biomarkers



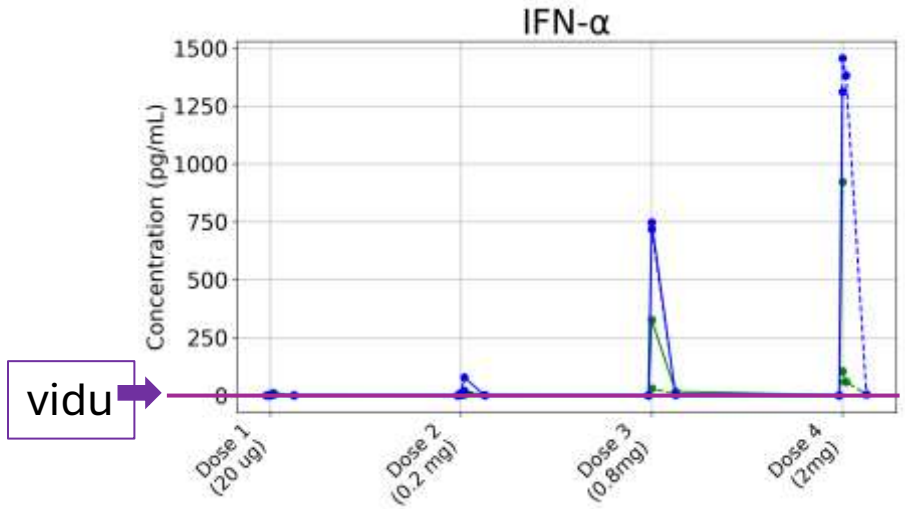
CXCL10 is primary pharmacodynamic marker; response to vidu. is <0.01 Z-007 induces 100X stronger response!

Conclusions

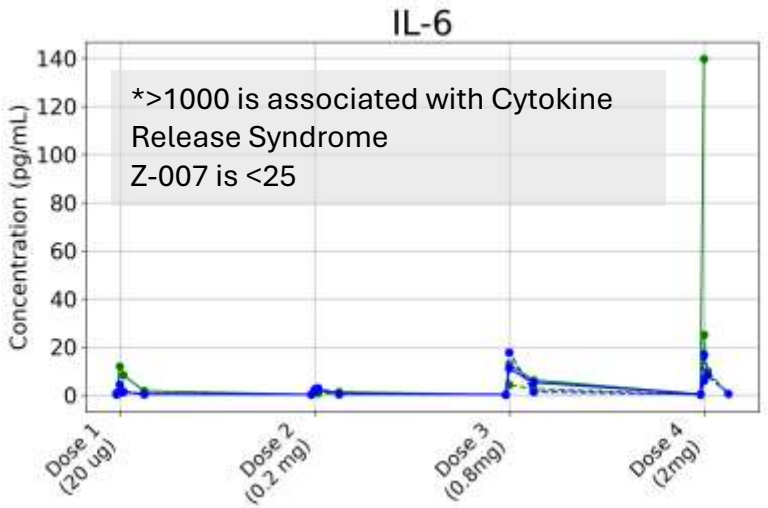
- **Unprecedented IFN-α/β ratio >30/1!**
- **Z-007 showed excellent safety at low and high dose**

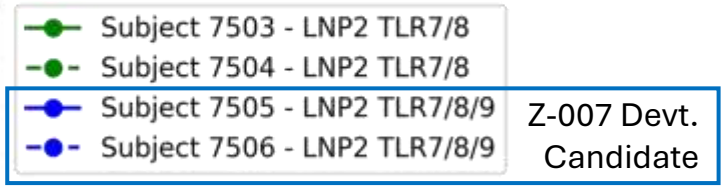
serum cytokines at 4 hr after the first IV injection, study performed at U. Montreal

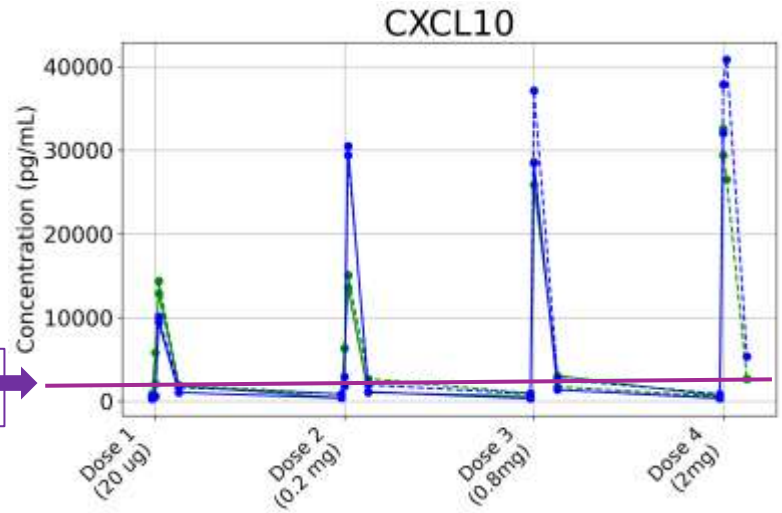
Z-007 induces unprecedented IFN-α and CXCL10 in NHP without inflammation: safe dose escalation of 100X



vidu







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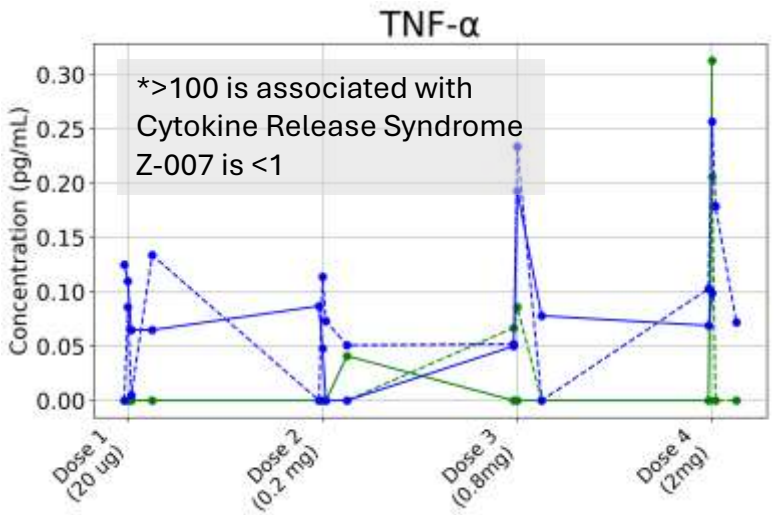


CXCL10 >1,000 is associated with clinical response to vidu

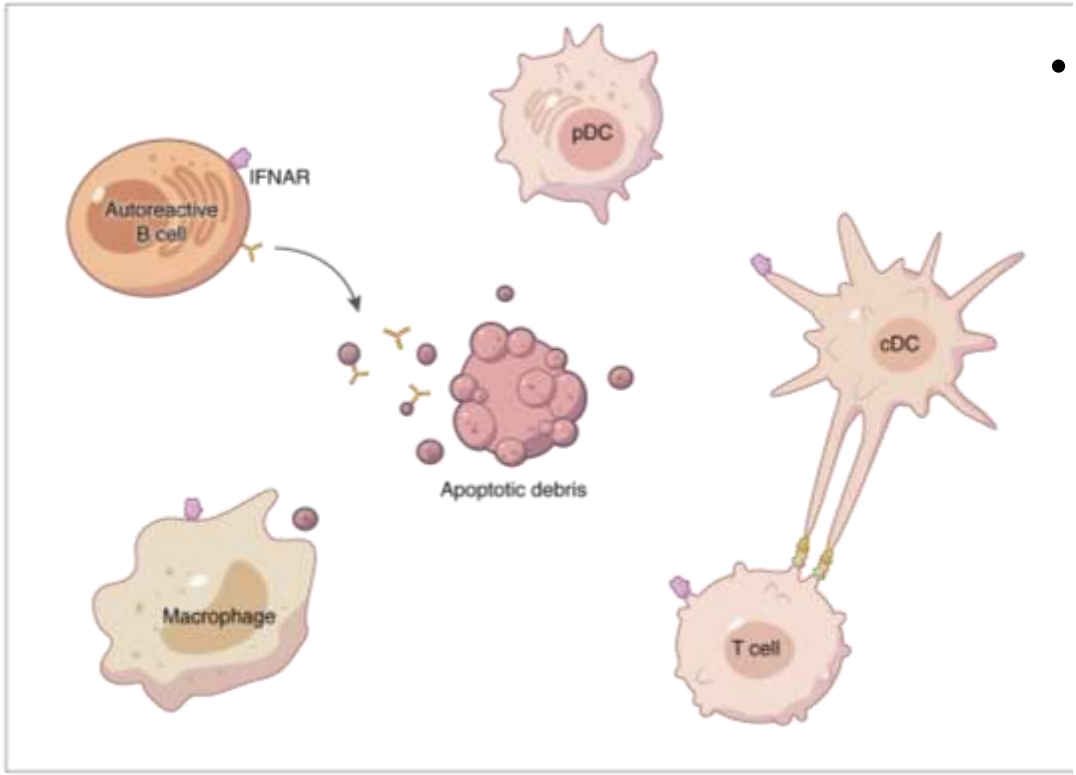
Lowest dose of Z-007 induces:

- **IFN-α >50X of vidutolimod**
- **>10X CXCL10 level associated with human clinical response**

- **The first innate immune activator that induces ~no inflammatory cytokines**

- **Monkeys showed no fever or other clinical signs or toxicity, all doses well tolerated**

Inappropriate TLR7/8/9 Activation Induces and Sustains Systemic Lupus Erythematosus (SLE)



- Autoimmunity to RNA- and DNA-associated antigens precedes SLE diagnosis by >3 years[1]

[1]Arbuckle et al., NEJM, 2003; [2]Sisirak et al., Cell 2016; [3]Leadbetter et al., Nature, 2002; [4]Vollmer et al., J Exp Med, 2005; [5]Lee et al., Lupus, 2016; [6]Garcia-Ortiz et al., Ann Rheum Dis., 2010; [7]Brown et al., Nature 2022; [8]Alharbi et al., bioRxiv, 2024

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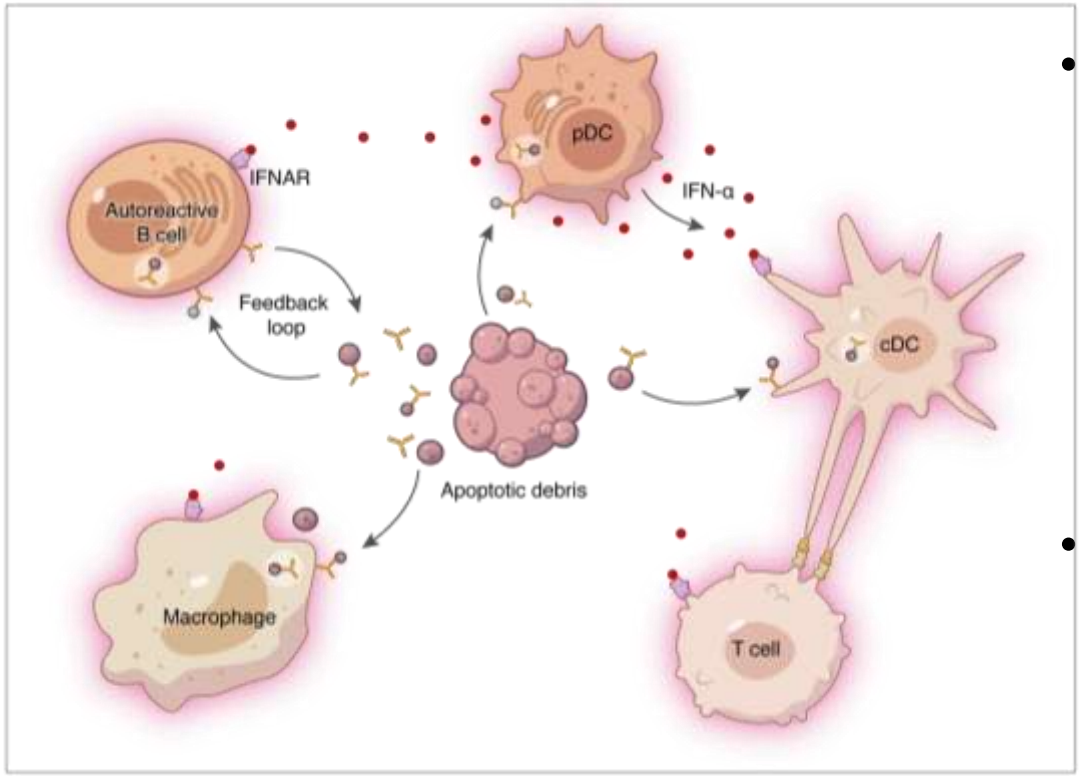
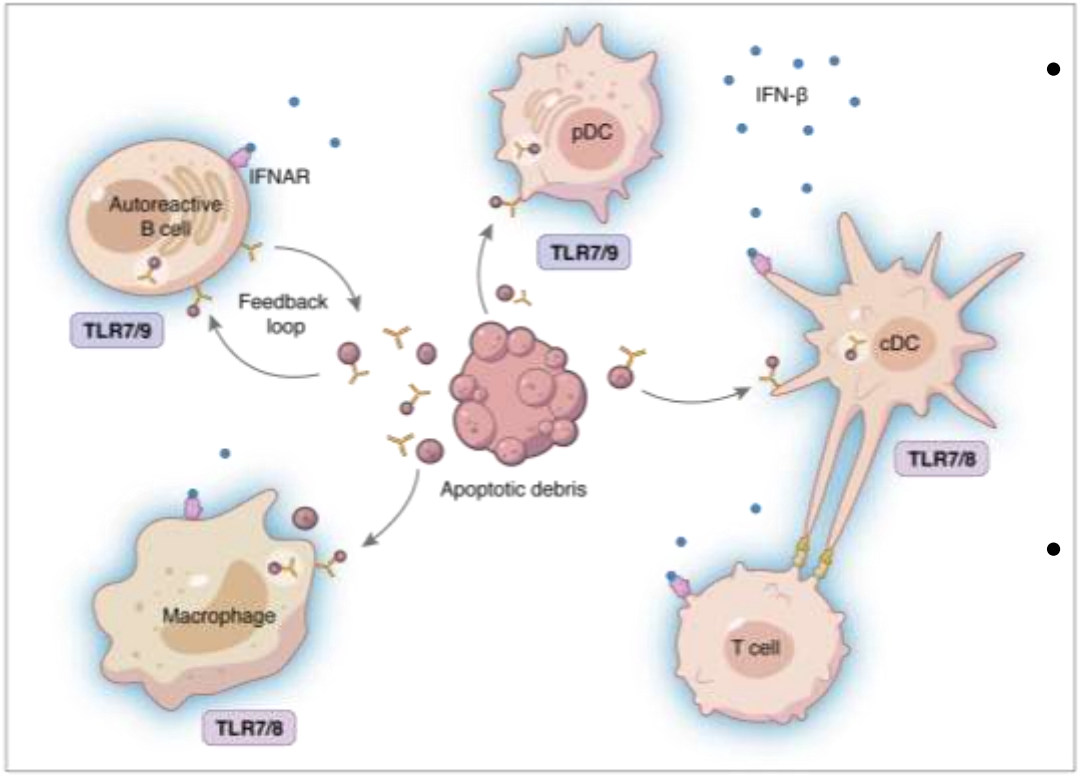


- Autoimmunity to RNA- and DNA-associated antigens precedes SLE diagnosis by >3 years[1]
  - Defective clearance of RNA/DNA in apoptotic cells precipitates SLE[2]
  - RNA/DNA immune complexes induce IFN-α secretion via TLR7, TLR9[3,4]
  - Dysregulated IFN-α secretion drives autoimmune disease
- Strong genetic linkage of TLR7/8/9 to autoimmune disease
  - TLR7/8 are on X chromosome: women have ~10X SLE
  - TLR7/8/9 mutations/polymorphisms associated with SLE[5-8]

[1]Arbuckle et al., NEJM, 2003; [2]Sisirak et al., Cell 2016; [3]Leadbetter et al., Nature, 2002; [4]Vollmer et al., J Exp Med, 2005; [5]Lee et al., Lupus, 2016; [6]Garcia-Ortiz et al., Ann Rheum Dis., 2010; [7]Brown et al., Nature 2022; [8]Alharbi et al., bioRxiv, 2024

Hydroxychloroquine suppresses inappropriate TLR7/8/9 activation in SLE



- Autoimmunity to RNA- and DNA-associated antigens precedes SLE diagnosis by >3 years[1]
  - Defective clearance of RNA/DNA in apoptotic cells precipitates SLE[2]
  - RNA/DNA immune complexes induce IFN-α secretion via TLR7, TLR9[3,4]
  - Dysregulated IFN-α secretion drives autoimmune disease
- Strong genetic linkage of TLR7/8/9 to autoimmune disease
  - TLR7/8 are on X chromosome: women have ~10X SLE
  - TLR7/8/9 mutations/polymorphisms associated with SLE[5-8]
- Standard in SLE since 1950's: hydroxychloroquine (HCQ)
  - At the concentrations present in SLE patients (~300 nM) the only detectable biologic effect of HCQ is TLR9 inhibition

[1]Arbuckle et al., NEJM, 2003; [2]Sisirak et al., Cell 2016; [3]Leadbetter et al., Nature, 2002; [4]Vollmer et al., J Exp Med, 2005; [5]Lee et al., Lupus, 2016; [6]Garcia-Ortiz et al., Ann Rheum Dis., 2010; [7]Brown et al., Nature 2022; [8]Alharbi et al., bioRxiv, 2024

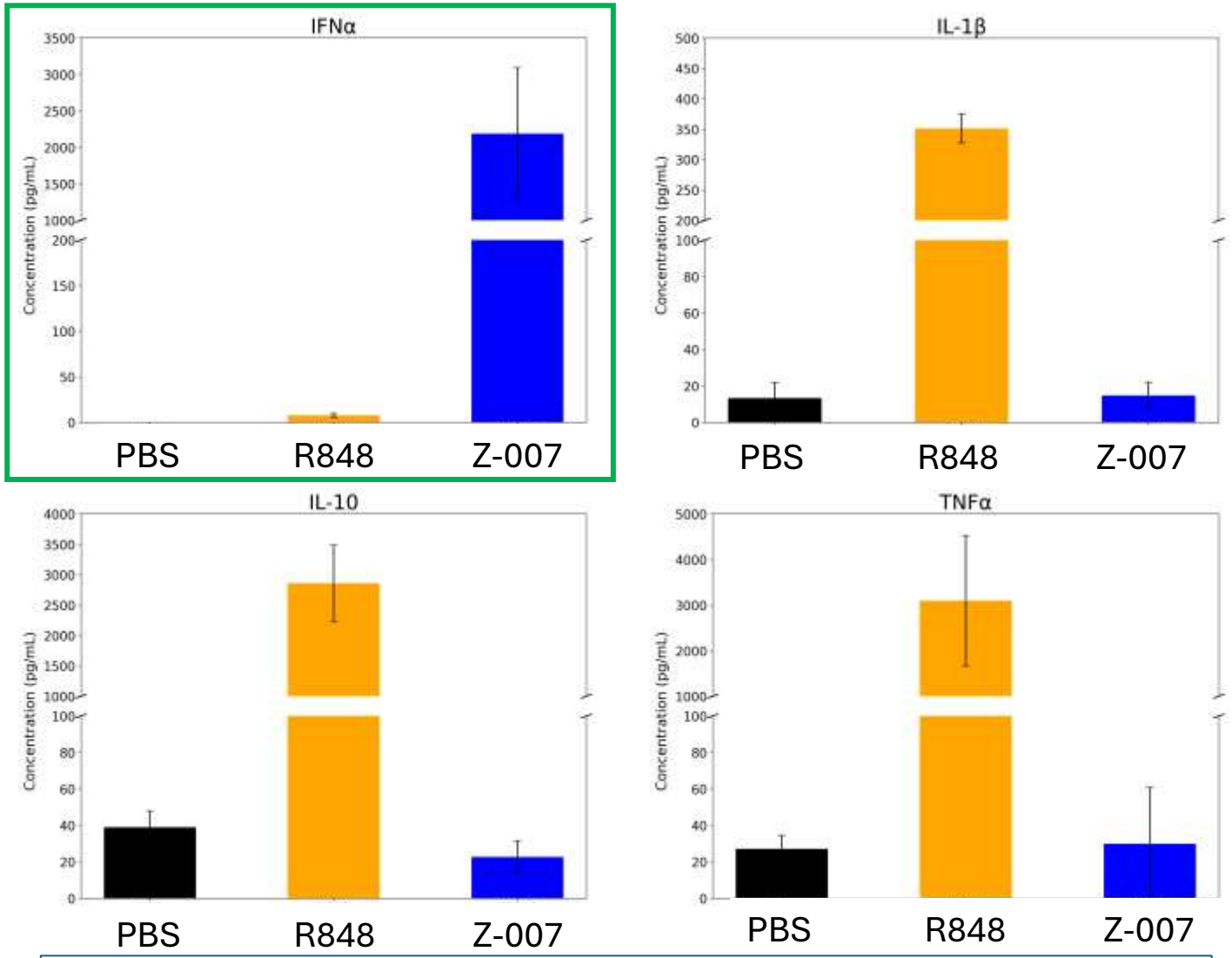
Conclusions

- Pattern Recognition Receptors evolved to distinguish at least 3 “flavors” of danger
  - Intracellular infection
  - Extracellular infection
  - Sterile tissue damage
- Understanding the “crowd wisdom” of the immune system may help in treating disease more than studying individual pathways in isolation
- TLR7/8/9 evolved to cooperatively distinguish retroviral particles from blebs of dying cells
  - They are not perfect: inappropriate sensing of self blebs as viral results in autoimmune disease (e.g., SLE)
- Synthetic TLR7/8/9 agonists (in LNP) contain the minimal signals for CD8+ T cell induction
  - These should be far more effective for cancer immunotherapy than any prior immune activator
- TLR7/8/9 antagonists may provide improved management of SLE and other autoimmune diseases driven by inappropriate responses to dying cells and their debris
  - Insights based on hydroxychloroquine are enabling design of improved antagonists

Questions?

Backup

Z-007 TLR7/8/9 agonist induces IFN-α from human tumor-associated immune cells; Small molecule TLR7/8 agonist induces inflammatory response



Unpublished Data from Weiner and Chan labs, University of Iowa Holden Comprehensive Cancer Center

Conclusions

- **These data support Zola's premise: we can convert tumor-associated immune cells to an antiviral state that safely induces IFN-α, CD8+ T cells**
- **No other immune activator has shown this profile of high IFN-α and low inflammation**
- **Prior clinical disappointments of small molecule TLR7/8 agonists are irrelevant for Z-007**

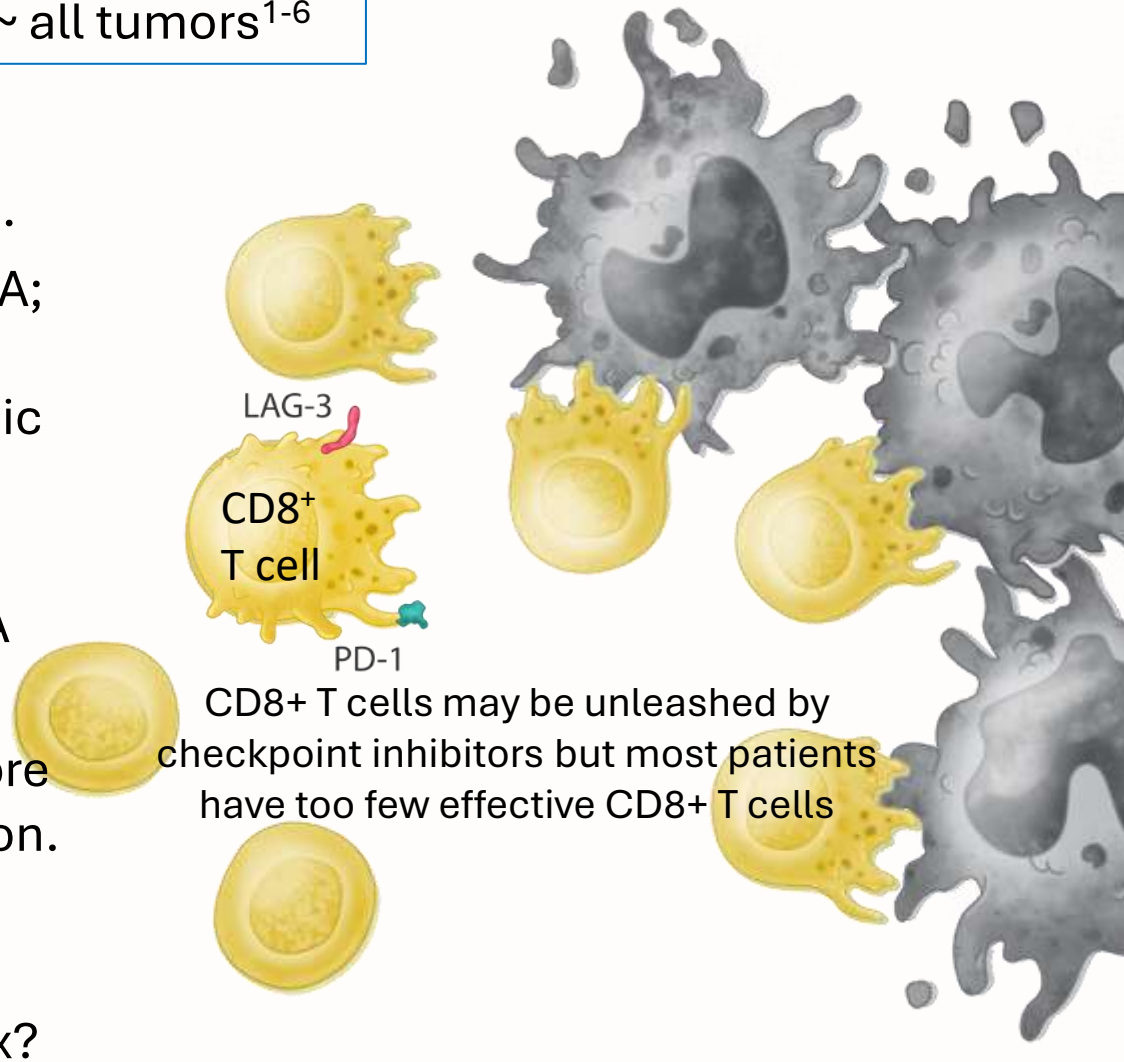
Methods: frozen ascites cells collected from patients with peritoneal tumors were cultured for 24 hr with various TLR agonists (0.5 μg/mL), and supernatants assayed by luminex

Zola's goal: *in situ* induction of anti-tumor CD8+ T cells, with systemic response as a monotherapy

Tumor CD8+ T cells associated with survival, prognosis in ~ all tumors[1-6]

Rationale for Z-007 as TLR7/8/9 agonist:

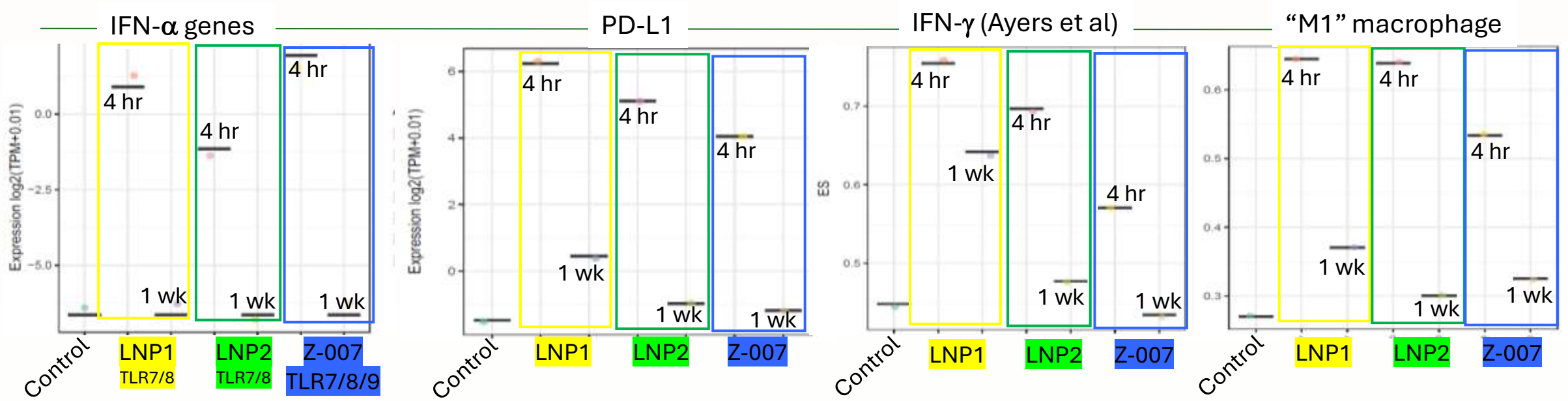
- CD8+ T cells evolved to kill viral/retroviral-infected cells.
- Immune cells use TLR7/8/9 to detect retroviral RNA/DNA; these signals are sufficient for CD8+ T cell induction.
- TLR9 agonist vidutolimod monotherapy induces systemic regression in >20% of PD-1-refractory melanoma.
- Past TLR7/8 agonists were small molecules, engage ½ agonist binding sites; Zola GU-rich phosphodiester RNA binds both sites, shifts TLR structure.
- Z-007 is the first TLR7/8/9 agonist, induces a never-before seen immune response: high IFN-α without inflammation.
- Delivery is the key question:
  - IT has been validated by vidutolimod
  - Will IV LNP deliver into liver, lymph node without tox?



CD8+ T cells may be unleashed by checkpoint inhibitors but most patients have too few effective CD8+ T cells

[1]Galon et al, Science, 2006; [2]Gooden et al., Br. J. Cancer, 2011; [3]Carstens et al., Nature Comm., 2017; [4]Yao et al., Scientific Reports, 2017; [5]Li et al., eClinical Medicine 2021; [6]Yang et al., Prostate, 2021

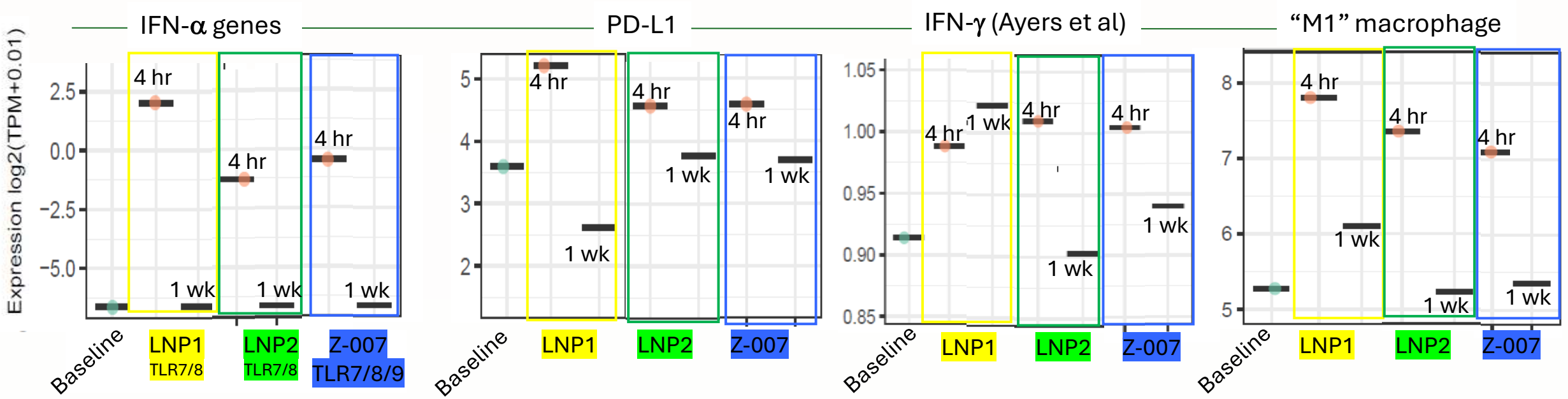
Zola's IV LNPs activate liver immune cells within 4 hrs (by RNA Seq)



Conclusions:

- Both the TLR7/8 formulations (LNP1, LNP2) and Z-007 strongly induce desired IFN-α, clinical response signatures in monkey livers by 4 hr
- These data support a systemic therapeutic effect in the liver after IV dosing of Zola LNPs
- Combined with published LNP efficient delivery of nucleic acid therapeutics into liver immune cells, these data support IV clinical development in patients with primary or metastatic liver cancer
    - Vidutolimod clinical experience with systemic responses predicts systemic response in extra-hepatic metastases to IV Z-007

Zola's IV LNPs activate systemic lymph nodes by 4 hrs (RNA Seq)

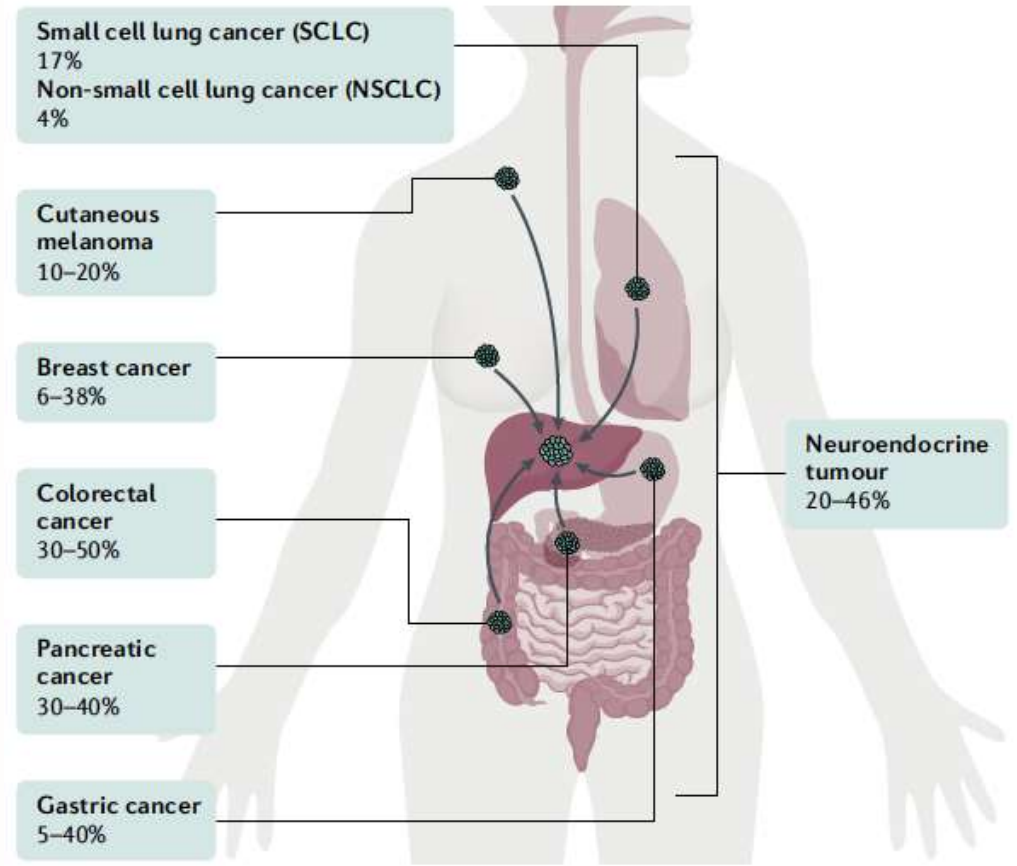


Conclusions:

- Both the TLR7/8 formulations (LNP1, LNP2) and Z-007 strongly induce desired IFN-α, clinical response signatures in monkey lymph nodes by 4 hr
- These data support a systemic therapeutic effect in lymph nodes after IV dosing of Zola LNPs
- Together with published lipid delivery of nucleic acid therapeutics into tumor-draining lymph nodes[1], these data support IV clinical development in patients with metastatic cancer

[1]Ganesh et al., Molecular Therapy, 2024

IV delivery of Z-007 LNP into liver can overcome immune suppression, promote systemic tumor regression in many tumors



% primary cancers that metastasize to the liver

MSS CRC with liver mets is common:

- Colorectal cancer (CRC) affects 150K people/year in the US, 80-85% considered microsatellite stable (MSS)[1]
- 30-50% of CRC patients develop liver mets[1]
- MSS CRC patients with liver mets: 36–64K/year in US

CRC liver mets respond to immune therapy:

1. 40-60% ORR to ICI in the MSI CRC subset
2. ~15% ORR to SBRT/ICI combos[3]
3. ICI resistance is mediated by tumor-associated macrophages[2] which express TLR7/8 (appendix)
4. High IFN signature associated with CRC response[4]
5. Z-007 *in vivo* NHP data confirm IFN, response signatures in the liver

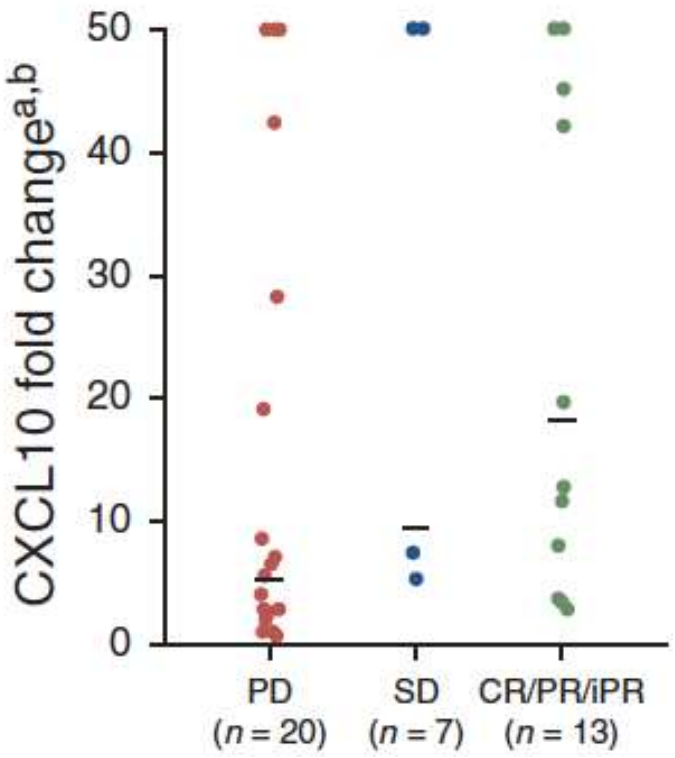
Image source: *Nat Rev Dis Primers* 2021;7(1):27.

[1]Horn et al, Cancer Epidemiology, 2020; [2]Yu et al, Nature Med 2021; [3]Parikh et al., Nature Cancer, 2021; [4]Acha-Segrado et al., Cancer Cell, 2025

Serum CXCL10 response to vidutolimod is a biomarker for tumor regression

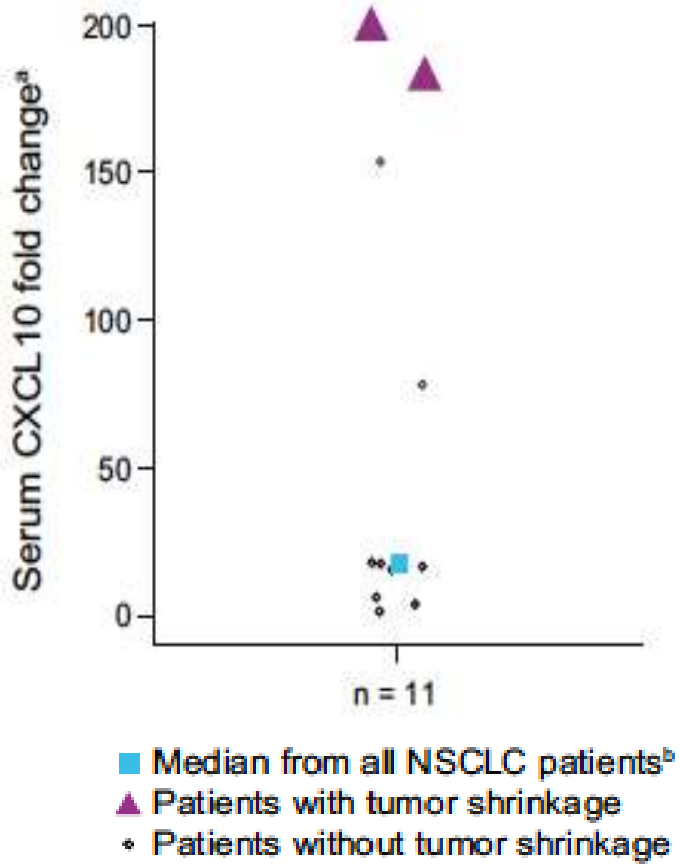
- Serum IFN-α is not detectable in most vidutolimod treated patients
- Serum CXCL10 is the best validated biomarker for IFN-α induction
- In PD-1 refractory melanoma, vidutolimod responders average 20X increase in serum CXCL10
- CXCL10 may be a useful surrogate marker for development of improved TLR9 agonists

PD-1 refractory melanoma



Ribas et al, Cancer Disc. 2021

PD-1 refractory NSCLC

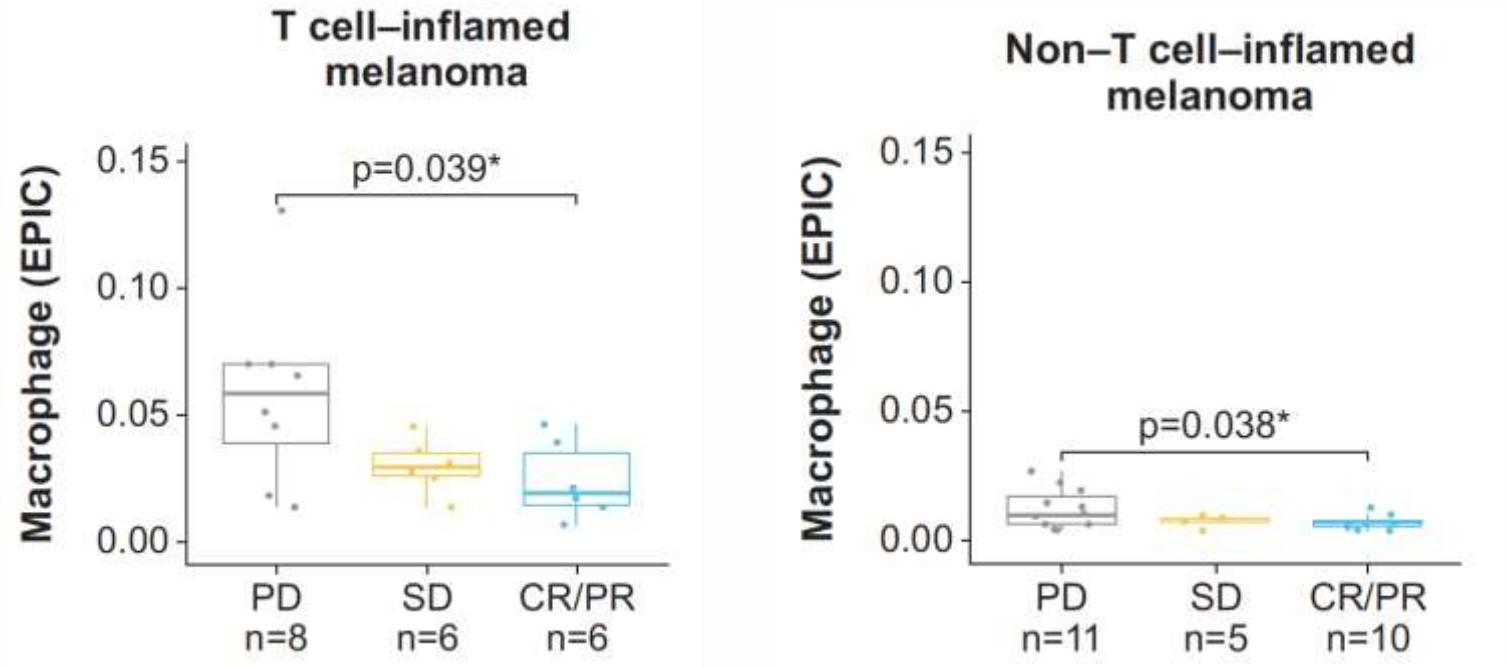


Negrao et al., JTO Clin Res Reports, 2022

High TLR7^{+}/8^{+} myeloid cells in baseline biopsy predict vidutolimod resistance in PD-1-refractory melanoma[1]

Novel Transcriptional Signatures Associated With Antitumor Activity in Vidutolimod-Treated Patients With Anti–PD-(L)1–Refractory Melanoma and Non–Small Cell Lung Cancer

Hong Liu,[1] Luping Zhao,[1] Ping Zheng,[1] Riyue Bao,[2] Jason J. Luke,[3] Marcelo V. Negrao,[3] Shakoora A. Sabree,[4] George J. Weiner,[4] Sujatha Kumar,[1] Dmitri Bobilev,[1] James E. Wooldridge,[1] Arthur M. Krieg[1]
[1]Checkmate Pharmaceuticals Inc., Cambridge, MA; [2]University of Pittsburgh Medical Center, Pittsburgh, PA; [3]The University of Texas MD Anderson Cancer Center, Houston, TX; [4]University of Iowa, Iowa City, IA

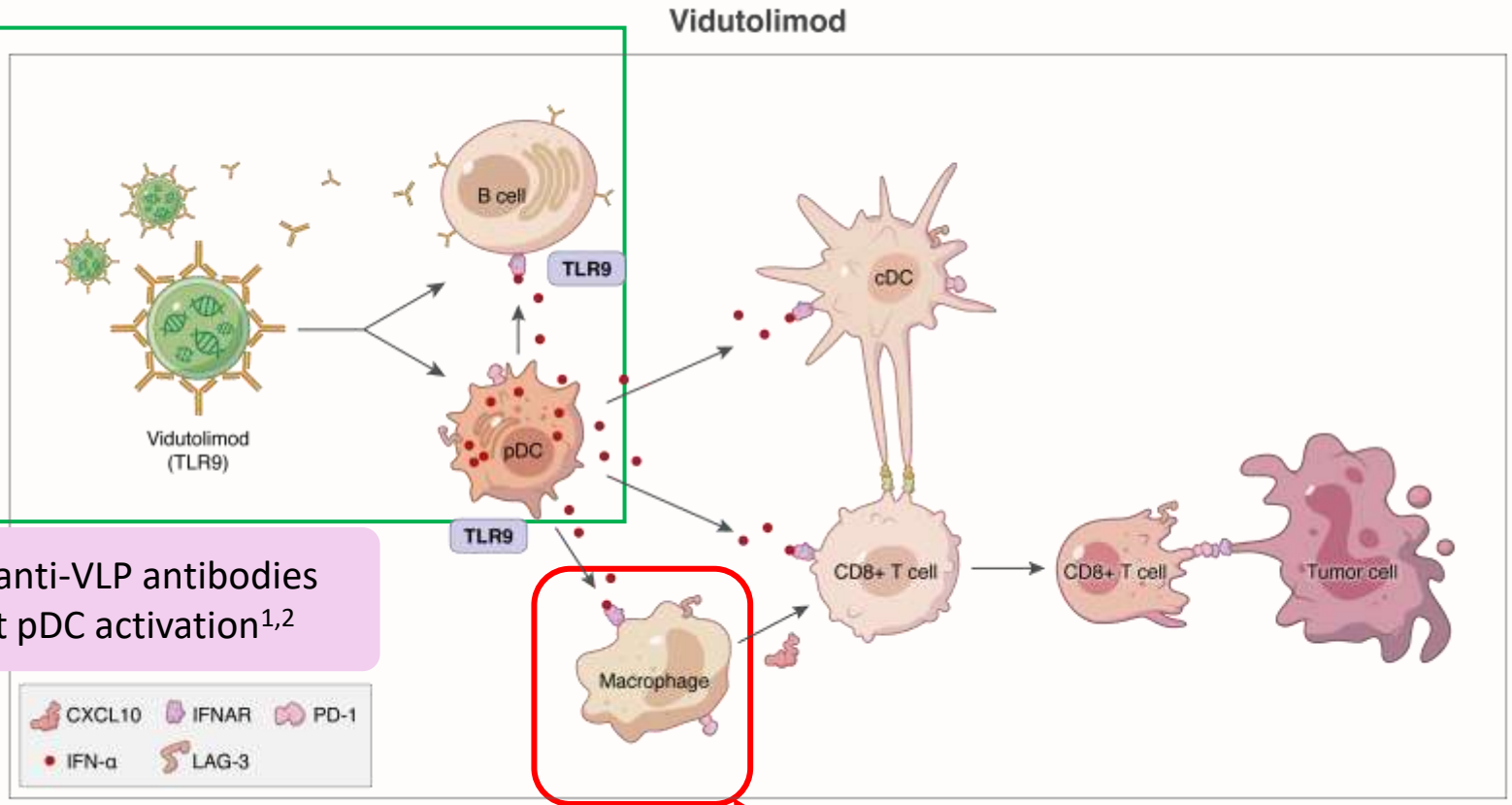


1. vidutolimod resistance is mediated by tumor-associated macrophages[2,3]
2. RNA-mediated activation of TLR7/8 in tumor-associated macrophages can overcome the known immunological resistance mechanism to TLR9 agonists, improving response rate

[1]Liu et al., AACR 2022
[2]https://doi.org/10.1111/j.1365-2567.2009.03134.x
[3]DOI: 10.1158/0008-5472.CAN-11-3468

Vidutolimod activates TLR9 in plasmacytoid dendritic cells (pDC) & B cells

Vidutolimod uptake is mediated by antibodies to the immunogenic virus-like particle (VLP)

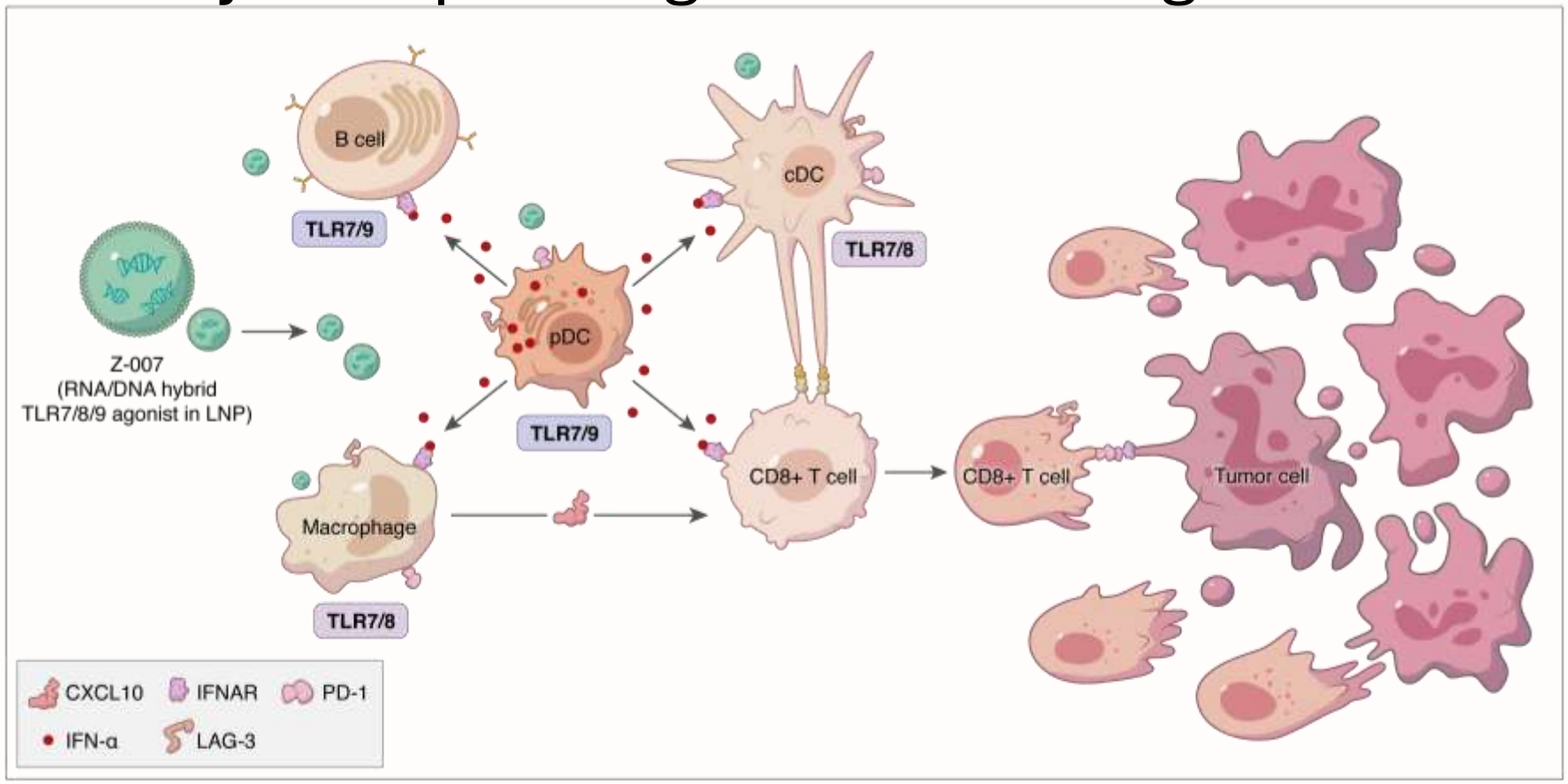


Excess anti-VLP antibodies inhibit pDC activation[1,2]

TLR7⁺/8⁺ Tumor-Associated Macrophages can inhibit pDC, prevent T cell activation[3-10]

[1]Ribas et al., Cancer Discovery 2021; [2]Sabree et al., Vaccines, 2021; [3]Deb et al., J. Immunol 2020; [4]Sisirak et al., Int J Cancer 2013; [5]Bekeredjian-Ding et al., Immunology 2009 ; [6]Castellaneta et al., J. Immunol 2009; [7]Lotze et al., Immunol Rev. 2007; [8]Karki & Kanneganti Nat Rev Cancer 2019; [9]Tang et al., Nature Reviews Immunol., 2023; [10]Li et al., Nature, 2023;

Improving on Vidutolimod: i) ↑ Delivery Using LNP; ii) ↑ Efficacy Incorporating RNA TLR7/8 Agonist Domain



- LNP selected for efficient IV uptake into liver tumor-associated immune cells, avoids neutralizing Ab
- Tumor-associated immune cells generally have upregulated LNP uptake receptors (e.g., CD36[1,2])
- TLR7/8 activation: i) increases IFN-α production; ii) reverses macrophage-mediated immunosuppression

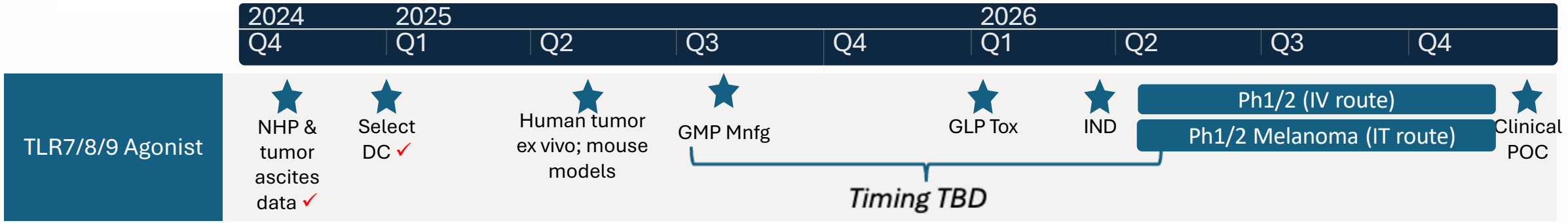
[1]Yang et al., Nature Comm. 2022; [2]Ganesh et al., Molecular Therapy, 2024

Z-007 Clinical Development Options

consider parallel IT and IV Z-007 clinical development

- IT monotherapy in PD-1-failed advanced melanoma
  - *Benchmark to vidutolimod >20%*
  - *Z-007 ORR expected to be substantially higher*
  - *Potential for accelerated approval TBD*
  - *Eventual expansion into neoadjuvant melanoma, prostate, breast, etc.*
- IV monotherapy in liver-metastatic cancers (potential basket trial)
  - *Supported by ex vivo human tumor data, RNA Seq in monkey liver*
    1. *POC is >20% ORR, potential for accelerated approval*

Potential Development Timeline To POC



2024 Seed: $2.8M

2025 Seed extension: $2.2M

Unfunded costs to IND filing: $7-9M

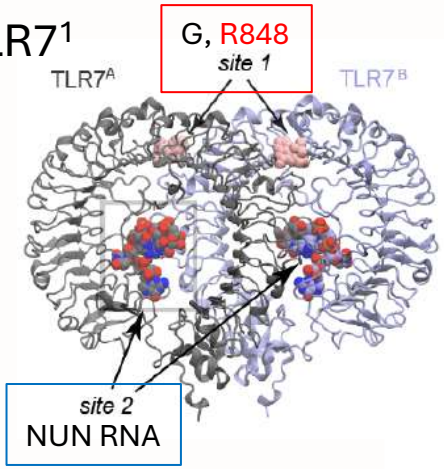
Zola has invested ~$5M in preclinical development of Z-007 development candidate; addl $7-9M needed
Clinical development options (pursuing both in parallel increases 2025 costs from $7M to ~$9M):

1. IV dosing for liver metastases initially based on efficient delivery into tumor-associated immune cells
   - Basket trial (many options being considered with KOLs)
2. IT monotherapy in PD-1-failed advanced melanoma, benchmark to vidutolimod
   - Longterm potential for neoadjuvant therapy in *many* tumor indications (prostate, breast, etc.)

Published TLR7/8/9 structures point to agonist and antagonist sites

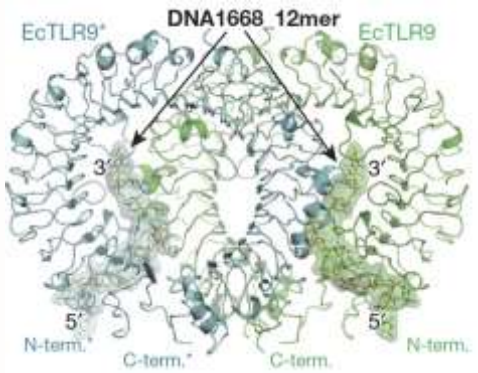
2 agonist binding sites for viral RNA/DNA

TLR7[1]



- RNase T2 processes viral RNA into G (1st site), NUN (2nd site)
- 2nd site binding increases 1st site affinity, causes allosteric shift[7]
- Binding both sites→ IRF7, IFN-α
- 1st site binding alone → NFkB

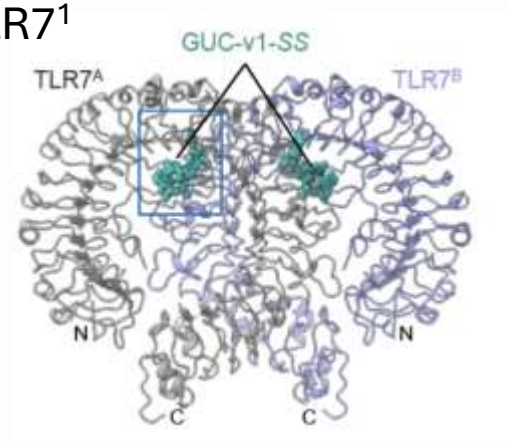
TLR9[3]



- DNase II processes viral DNA[6]
- Crystal structures with PS ssDNA
- Strongest published IFN-α inducer is CpG-A dsDNA (polyG, PO backbone) e.g., 2216, G10 in vidutolimod – no crystal structure

1 antagonist binding site for self RNA/DNA

TLR7[1]

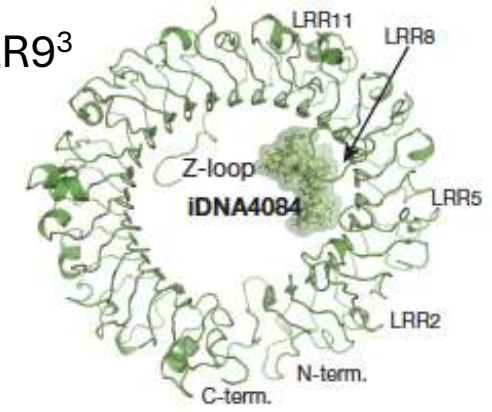


TLR7 antagonist pocket[2] binds:

- endogenous 2'-OMe G RNA degradation products;
- pharma small molecule TLR7/8 antagonists (4 in phase 2 for SLE etc.)

Mutations disrupting this pocket cause SLE[2]

TLR9[3]



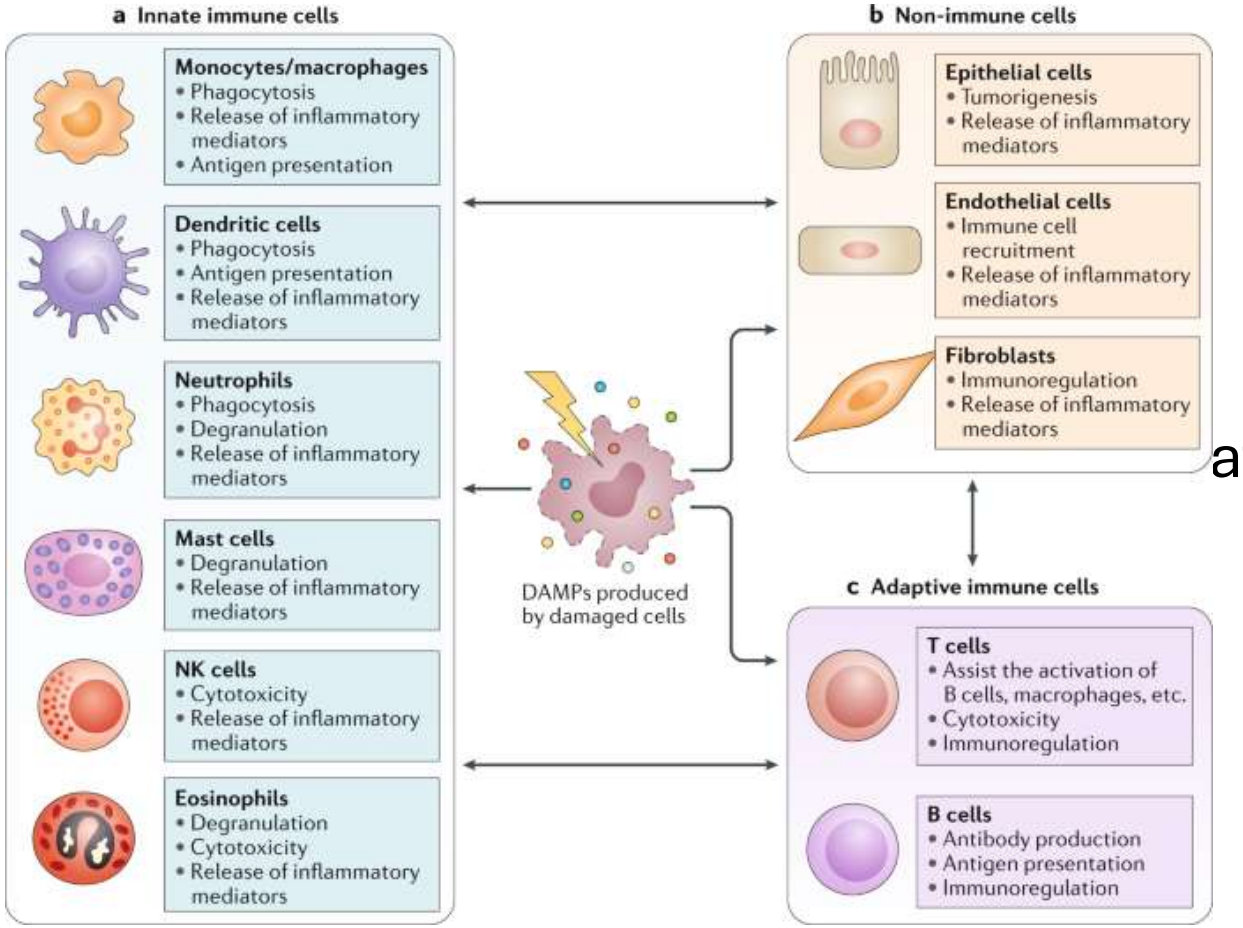
TLR9 antagonist pocket[3] binds:

- GGG[4] (endogenous source may be telomeres[5])

the mouse TLR9 antagonist pocket has been defined using PS DNA

[1]Zhang et al., Immunity, 2016; [2]Alharbi et al., bioXriv, 2024; [3]Ohto et al., Nature, 2015; [4]Lenert, Mediators of Inflamm., 2010; [5]Gursel et al., J Immunol, 2003; [6]Chan et al., Nature Comm., 2014; [7]Ekimoto et al., Chem Pharm Bull 2024

Damage-Associated Molecular Patterns



"DAMPs"

attern Recognition Receptors for PAMPs

Dozens of PRRs have been identified

PRRs are differentially expressed in dozens of distinct immune cell subsets

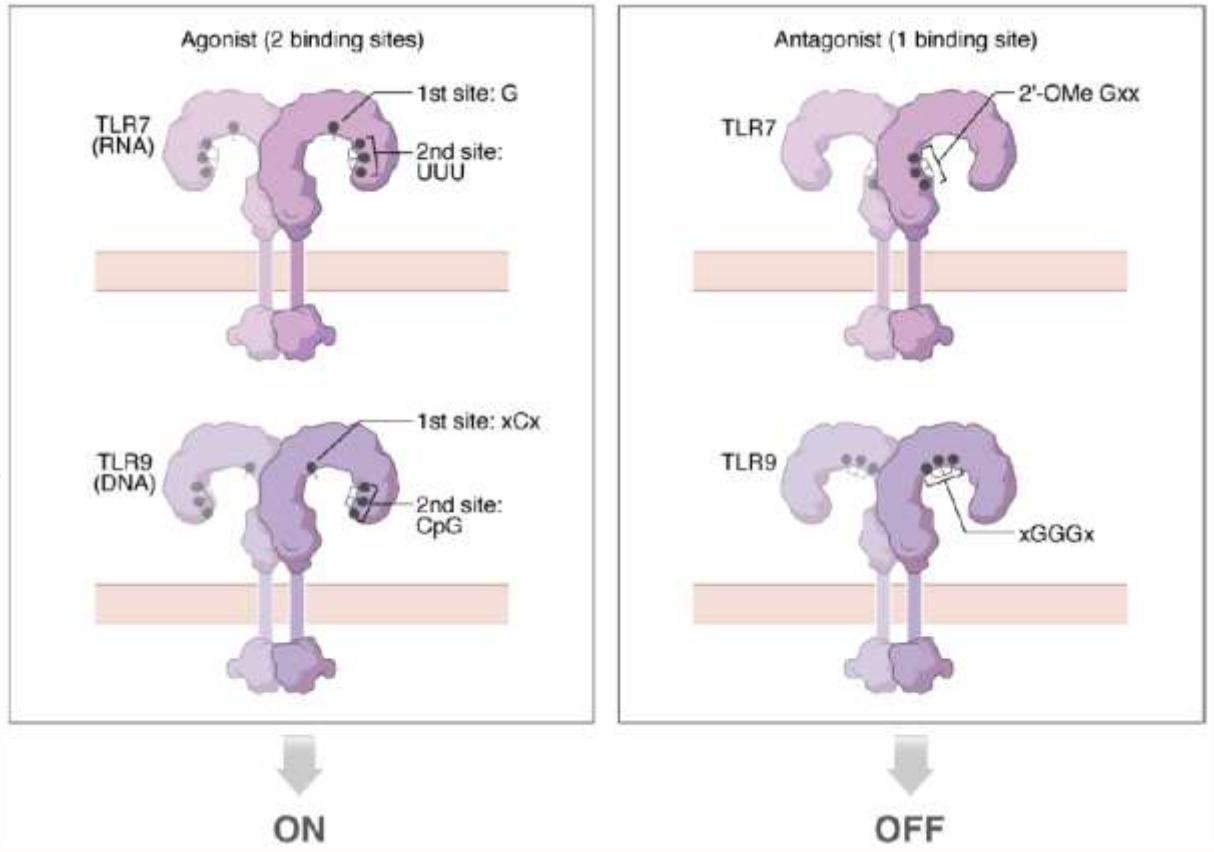
Immune cell subsets function via "crowd intelligence" AKA "quorum sensing" use PRRs in a combinatorial fashion to "decide" of the presence and type of infection

- *Different immune cells express different PRRs,*

TLR7/8/9 may be a rheostat for CD8+ T cell induction

viral GU RNA activates TLR7/8;

unmethylated CpG DNA activates TLR9



rRNA, tRNA fragments with 2’-O-Me ribose bind TLR7/8 antagonist sites;

telomere DNA degradation products bind TLR9 antagonist site

Proposal:

- Particles in endosomes are screened by DC/MP for their ratio of agonist/antagonist binding
- Hypothesis: the ratio of binding evolved as a surrogate measure of the “danger” in that particle
- Expression in separate DC and MP populations provides further levels of regulation