Quanterix

The Science of Precision Health

Ultra-sensitive measurement of biomolecules and applications in precision health

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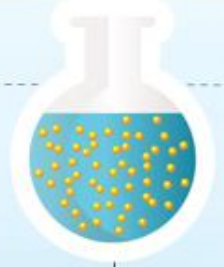
Disruption in detection and analytics

100 pg/ml

Disease and Aging

171 FDA Approved Protein Tests



"OLD ZERO" Old detection limit

4,000 Proteins[2]

ZERO

"NEW ZERO" SIMOA detection limit

Health

0.001 pg/ml

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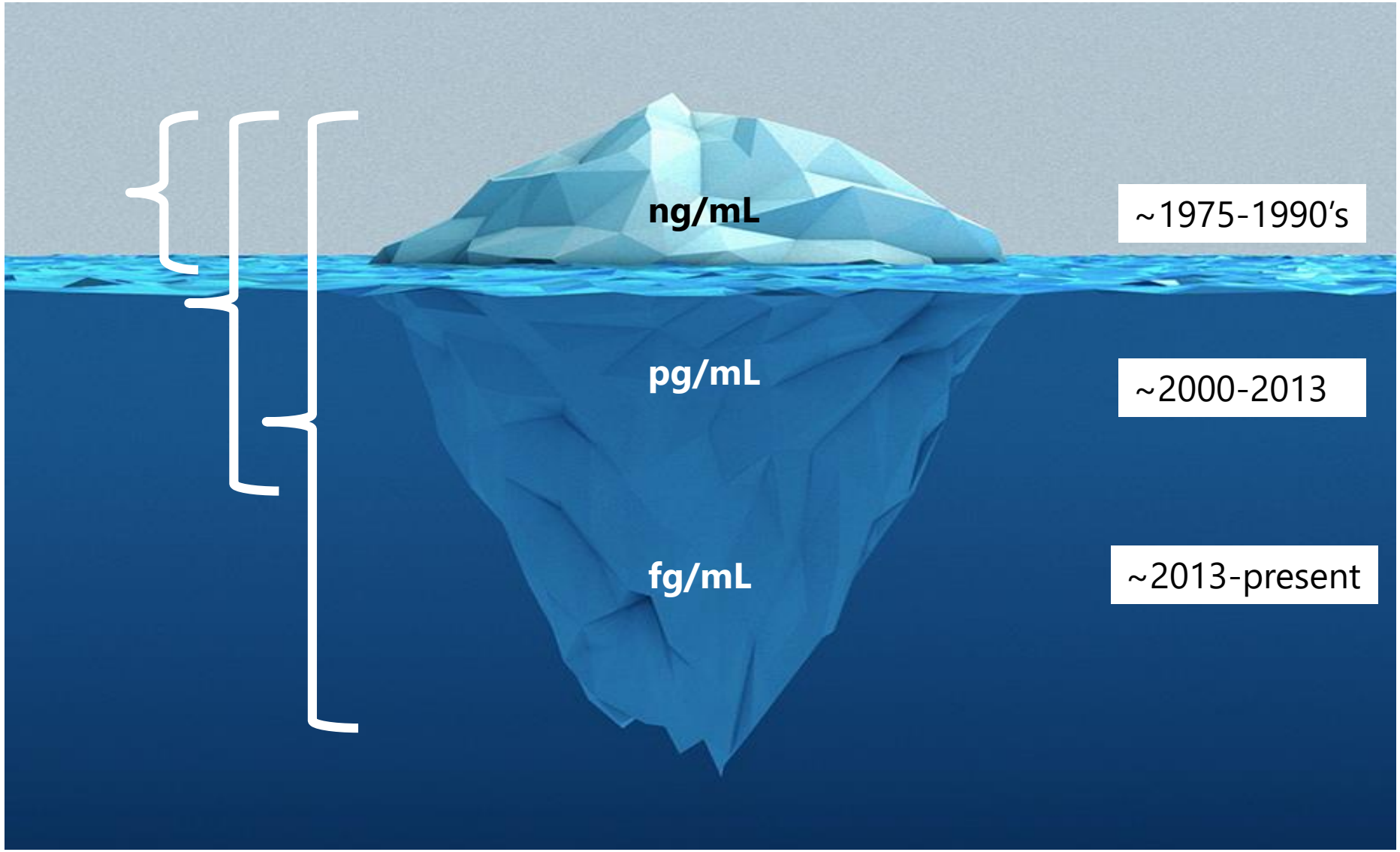
1) Peptide Atlas 2012; 2) Cui *et. al.* Bioinformatics (2008) 24 (20): 2370-2375; 3) Anderson, N.L. 2010. Clinical Chemistry February 2010 vol. 56 no. 2 177-185

Measuring What Could not be Seen Before

ELISA

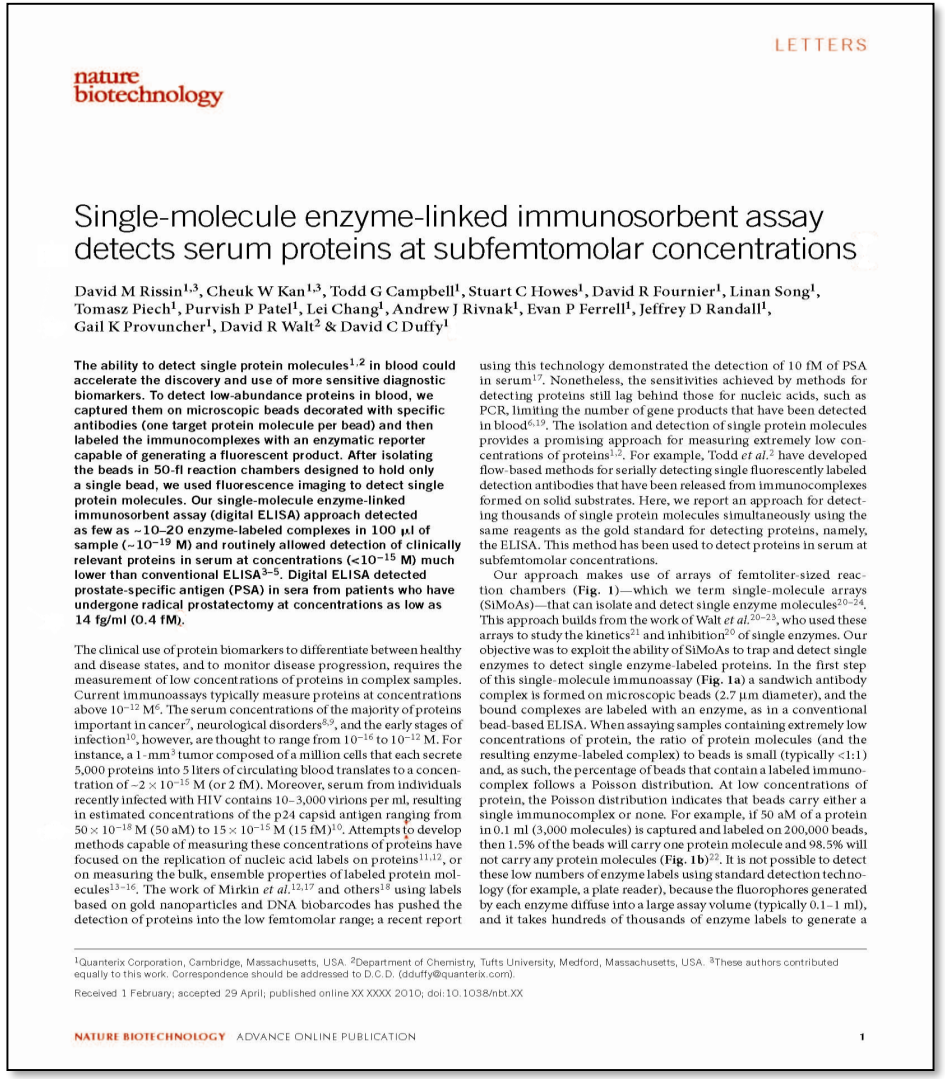
Enhanced ELISA

Simoa™



Historical Biobank samples have value!

Single Molecule Arrays (Simoa) described 2010



LETTERS

nature biotechnology

Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations

David M Rissin[1,3], Cheuk W Kan[1,3], Todd G Campbell[1], Stuart C Howes[1], David R Fournier[1], Linan Song[1], Tomasz Piech[1], Purvish P Patel[1], Lei Chang[1], Andrew J Rivnak[1], Evan P Ferrell[1], Jeffrey D Randall[1], Gail K Provuncher[1], David R Walt[2] & David C Duffy[1]

The ability to detect single protein molecules[1,2] in blood could accelerate the discovery and use of more sensitive diagnostic biomarkers. To detect low-abundance proteins in blood, we captured them on microscopic beads decorated with specific antibodies (one target protein molecule per bead) and then labeled the immunocomplexes with an enzymatic reporter capable of generating a fluorescent product. After isolating the beads in 50-fl reaction chambers designed to hold only a single bead, we used fluorescence imaging to detect single protein molecules. Our single-molecule enzyme-linked immunosorbent assay (digital ELISA) approach detected as few as ~10–20 enzyme-labeled complexes in 100 μl of sample (~10^{-19} M) and routinely allowed detection of clinically relevant proteins in serum at concentrations (<10^{-15} M) much lower than conventional ELISA[3–5]. Digital ELISA detected prostate-specific antigen (PSA) in sera from patients who have undergone radical prostatectomy at concentrations as low as 14 fg/ml (0.4 fM).

The clinical use of protein biomarkers to differentiate between healthy and disease states, and to monitor disease progression, requires the measurement of low concentrations of proteins in complex samples. Current immunoassays typically measure proteins at concentrations above 10^{-12} M[6]. The serum concentrations of the majority of proteins important in cancer[7], neurological disorders[8,9], and the early stages of infection[10], however, are thought to range from 10^{-16} to 10^{-12} M. For instance, a 1-mm^3 tumor composed of a million cells that each secrete 5,000 proteins into 5 liters of circulating blood translates to a concentration of ~2×10^{-15} M (or 2 fM). Moreover, serum from individuals recently infected with HIV contains 10–3,000 virions per ml, resulting in estimated concentrations of the p24 capsid antigen ranging from 50×10^{-18} M (50 aM) to 15×10^{-15} M (15 fM)[10]. Attempts to develop methods capable of measuring these concentrations of proteins have focused on the replication of nucleic acid labels on proteins[11,12], or on measuring the bulk, ensemble properties of labeled protein molecules[13–16]. The work of Mirkin *et al.*[12,17] and others[18] using labels based on gold nanoparticles and DNA biobarcodes has pushed the detection of proteins into the low femtomolar range; a recent report using this technology demonstrated the detection of 10 fM of PSA in serum[17]. Nonetheless, the sensitivities achieved by methods for detecting proteins still lag behind those for nucleic acids, such as PCR, limiting the number of gene products that have been detected in blood[6,19]. The isolation and detection of single protein molecules provides a promising approach for measuring extremely low concentrations of proteins[1,2]. For example, Todd *et al.*[2] have developed flow-based methods for serially detecting single fluorescently labeled detection antibodies that have been released from immunocomplexes formed on solid substrates. Here, we report an approach for detecting thousands of single protein molecules simultaneously using the same reagents as the gold standard for detecting proteins, namely, the ELISA. This method has been used to detect proteins in serum at subfemtomolar concentrations.

Our approach makes use of arrays of femtoliter-sized reaction chambers (**Fig. 1**)—which we term single-molecule arrays (SiMoAs)—that can isolate and detect single enzyme molecules[20–24]. This approach builds from the work of Walt *et al.*[20–23], who used these arrays to study the kinetics[21] and inhibition[20] of single enzymes. Our objective was to exploit the ability of SiMoAs to trap and detect single enzymes to detect single enzyme-labeled proteins. In the first step of this single-molecule immunoassay (**Fig. 1a**) a sandwich antibody complex is formed on microscopic beads (2.7 μm diameter), and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA. When assaying samples containing extremely low concentrations of protein, the ratio of protein molecules (and the resulting enzyme-labeled complex) to beads is small (typically <1:1) and, as such, the percentage of beads that contain a labeled immunocomplex follows a Poisson distribution. At low concentrations of protein, the Poisson distribution indicates that beads carry either a single immunocomplex or none. For example, if 50 aM of a protein in 0.1 ml (3,000 molecules) is captured and labeled on 200,000 beads, then 1.5% of the beads will carry one protein molecule and 98.5% will not carry any protein molecules (**Fig. 1b**)[22]. It is not possible to detect these low numbers of enzyme labels using standard detection technology (for example, a plate reader), because the fluorophores generated by each enzyme diffuse into a large assay volume (typically 0.1–1 ml), and it takes hundreds of thousands of enzyme labels to generate a

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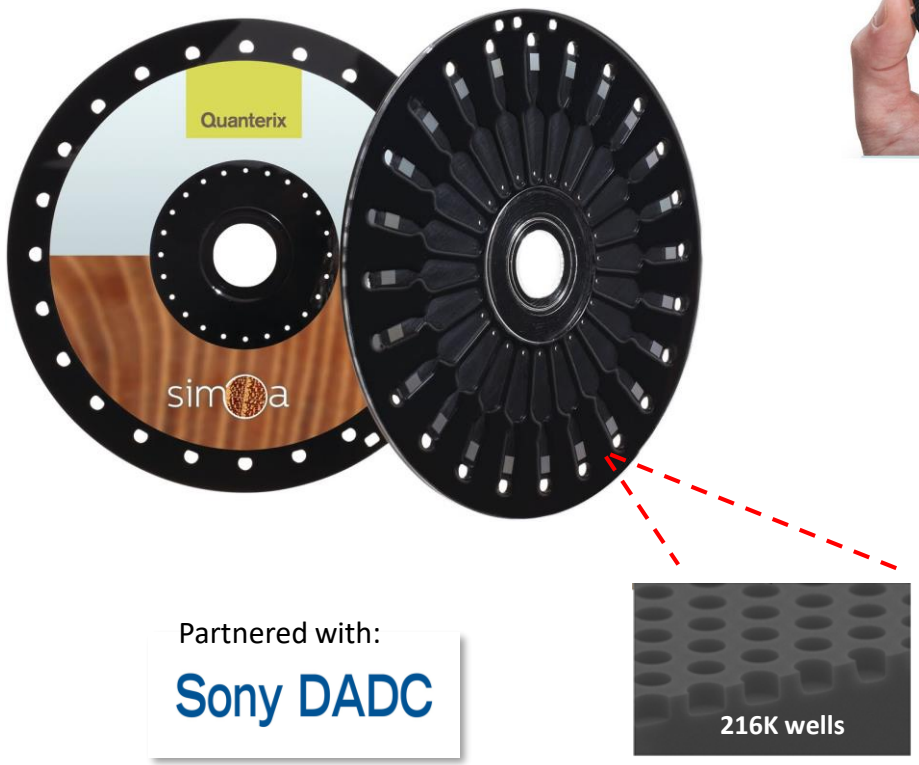
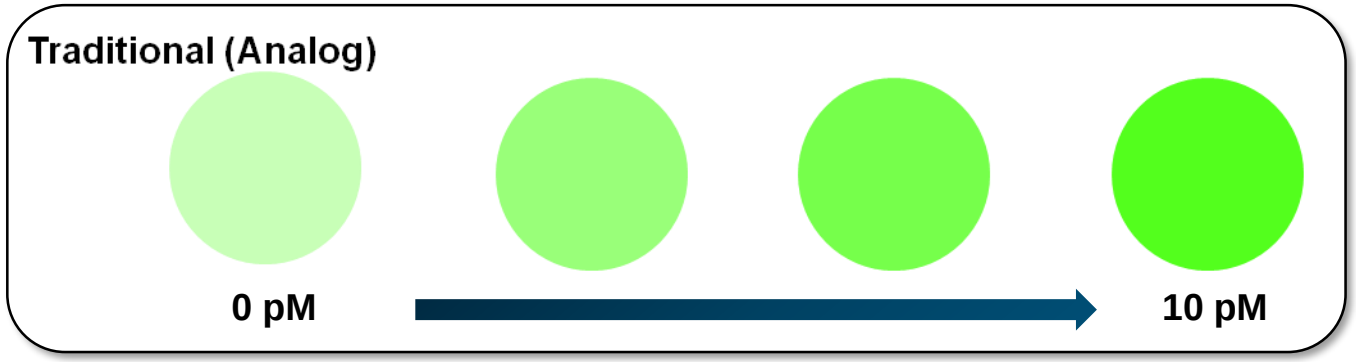
Simoa Disc enables technology

- Low-cost consumable
- 24 arrays/disc
- 240 results/disc (10-plex)
- 216,000 50-femtoliter wells per array
- Manufactured using state-of-the-art DVD technology by Sony DADC



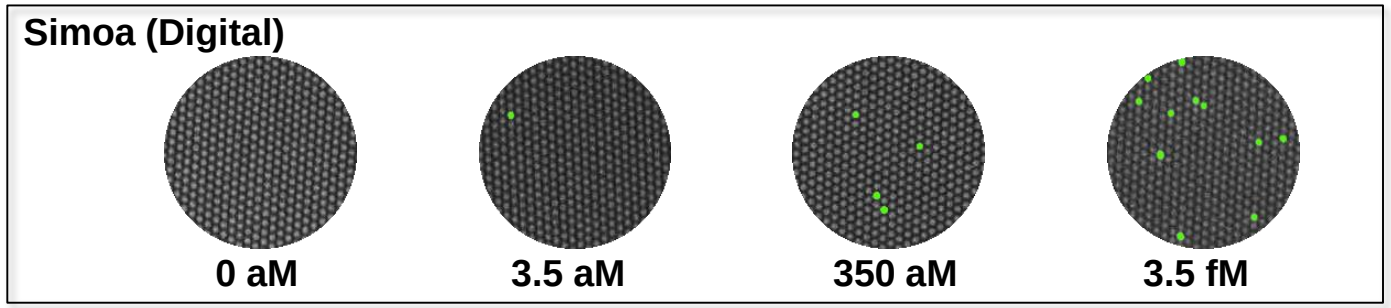
Partnered with:
Sony DADC

Simoa – single molecule sensitivity



Microliters (μL)

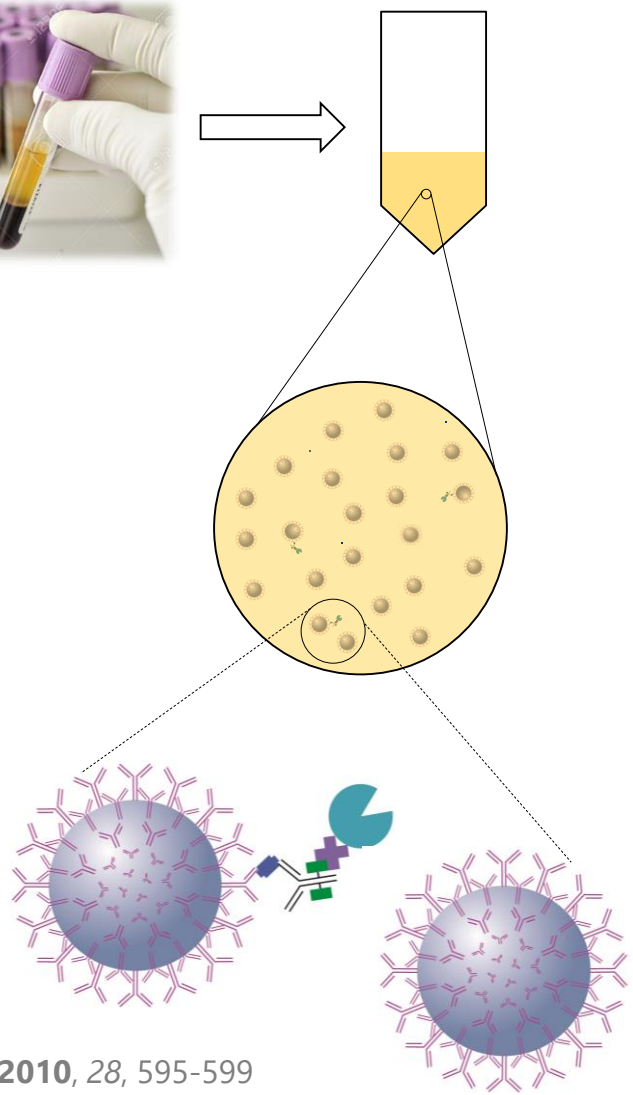
- Reaction volume = 100 $\times 10^{-6}$ L
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

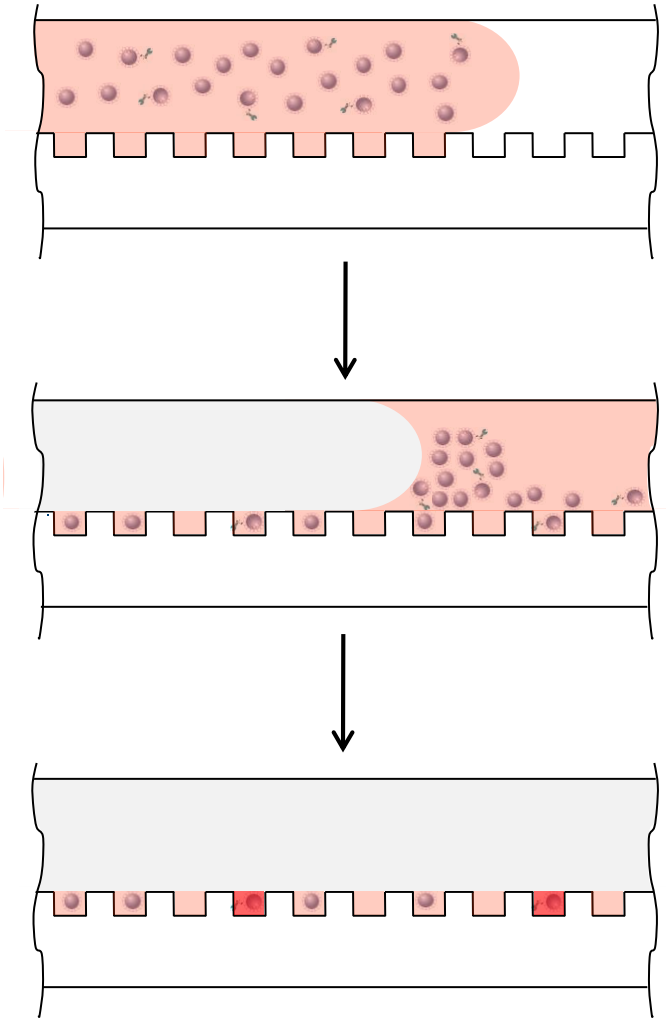


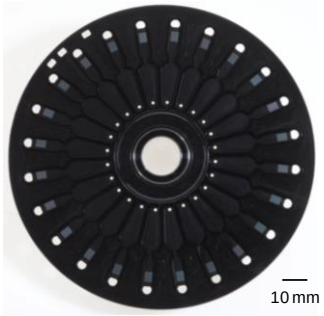
Femtoliters (fL)

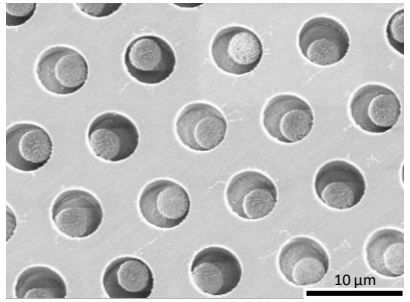
- Reaction volume = 50 $\times 10^{-15}$ L (2 billion times smaller)
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- One molecule needed to reach detection limit

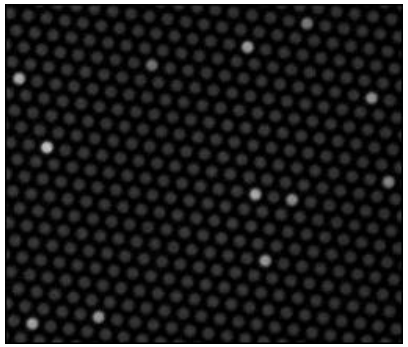
Counting Single Protein Molecules in blood using Simoa











Rissin *et al.*, *Nat. Biotechnol.* **2010**, *28*, 595-599
Kan *et al.*, *Lab Chip* **2012,** *12*, 977-985

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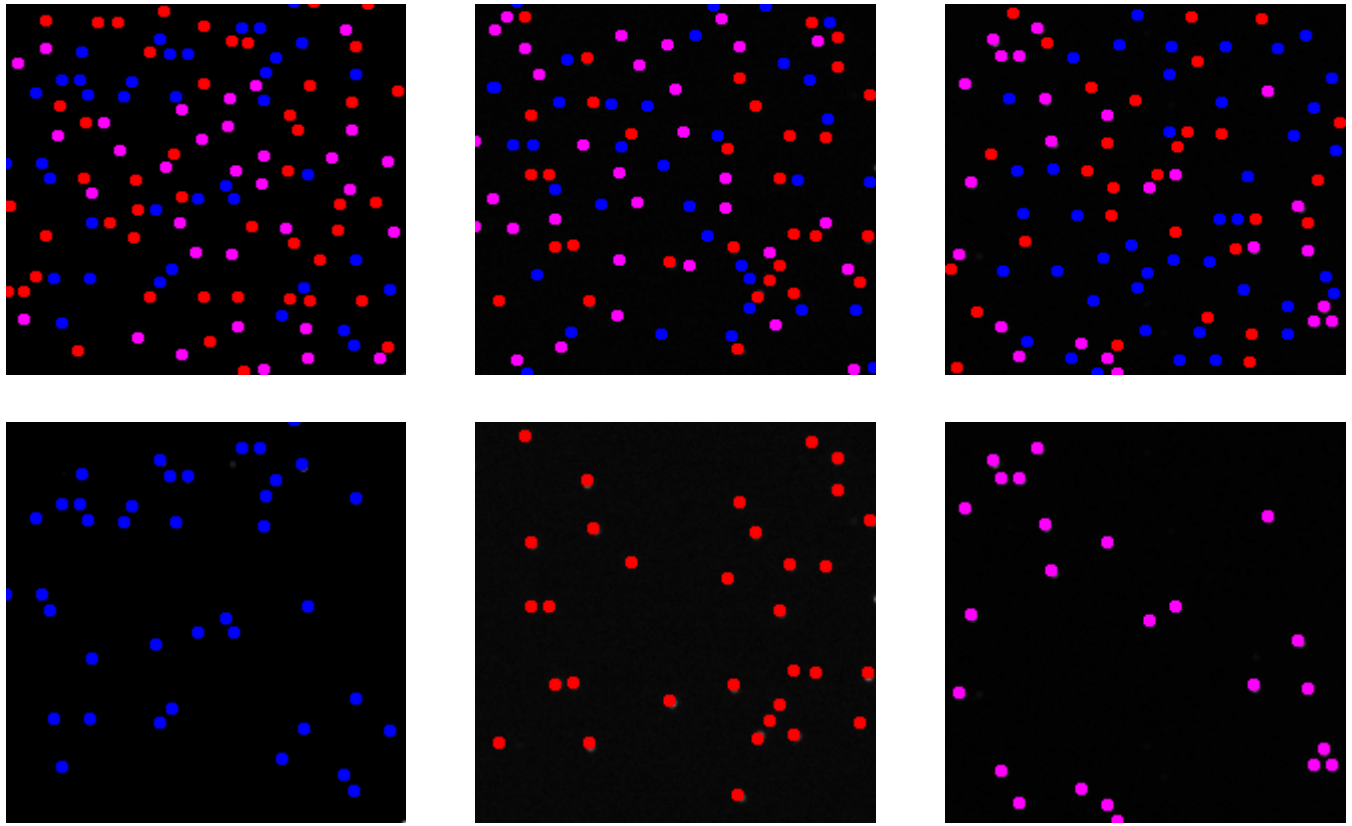
Simoa multiplexing

Bead Encoding Signal

Single Molecule Signal



[TNF-α] = 100 pg/mL
[IL-6] = 0 pg/mL
[GM-CSF] = 0 pg/mL

[TNF-α] = 0 pg/mL
[IL-6] = 100 pg/mL
[GM-CSF] = 0 pg/mL

[TNF-α] = 0 pg/mL
[IL-6] = 0 pg/mL
[GM-CSF] = 100 pg/mL

AF-488 encoded beads = TNF-α

AF-750 encoded beads = IL-6

AF-405 encoded beads = GM-CSF

Rissin *et al.* Multiplexed single molecule immunoassays. *Lab Chip* **2013**, 13, 2902.

Quanterix Product Offering

Instruments

HD-1/X

Ultra-sensitive Simoa bead assay technology

Floor-standing integrated system

Completely automated assay prep and detection (sample->answer)

600+ publications

SR-X

Ultra-sensitive Simoa bead assay technology

Benchtop form factor
Semi-automated assay prep using standardized benchtop devices

Equivalent assay performance to HD-1

SP-X

Ultra-sensitive Simoa planar assay technology

Benchtop form factor
Semi-automated assay prep using standardized benchtop devices

Unique multiplex capabilities

Assay kits

Plate based

Bead based

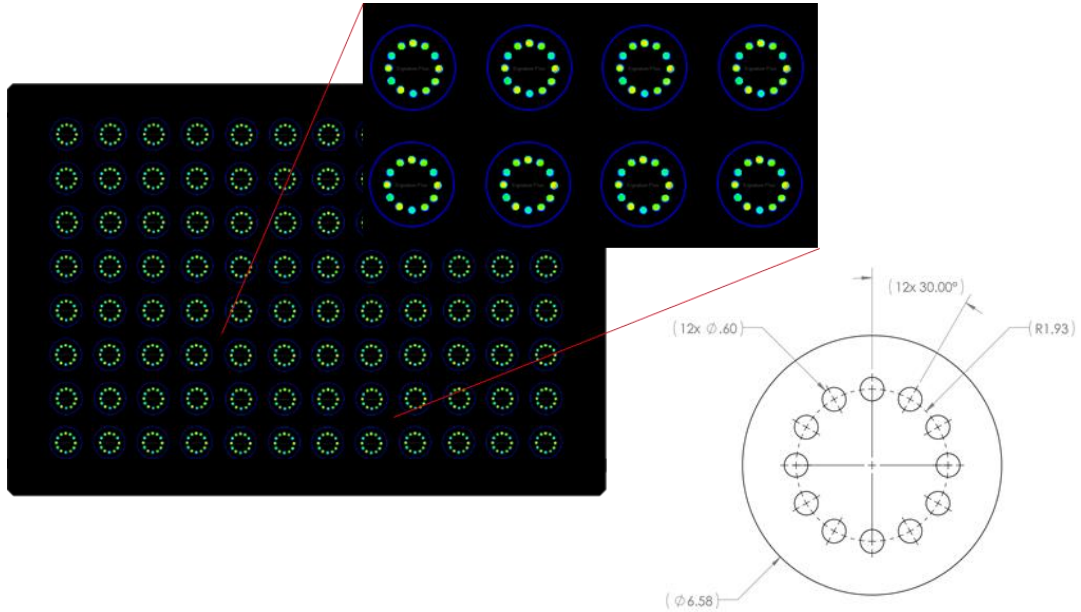
250+ assays developed for neurology, oncology, cardiology, infectious disease and inflammation research
Capable of assay customization with homebrew kits
Singleplex and multiplex formats

Services

- ✓ Contract research services through Simoa Accelerator Laboratory
- ✓ Sample testing services
- ✓ Custom assay development
- ✓ Custom reagent production and kitting
- ✓ **CLIA and LDT capabilities**

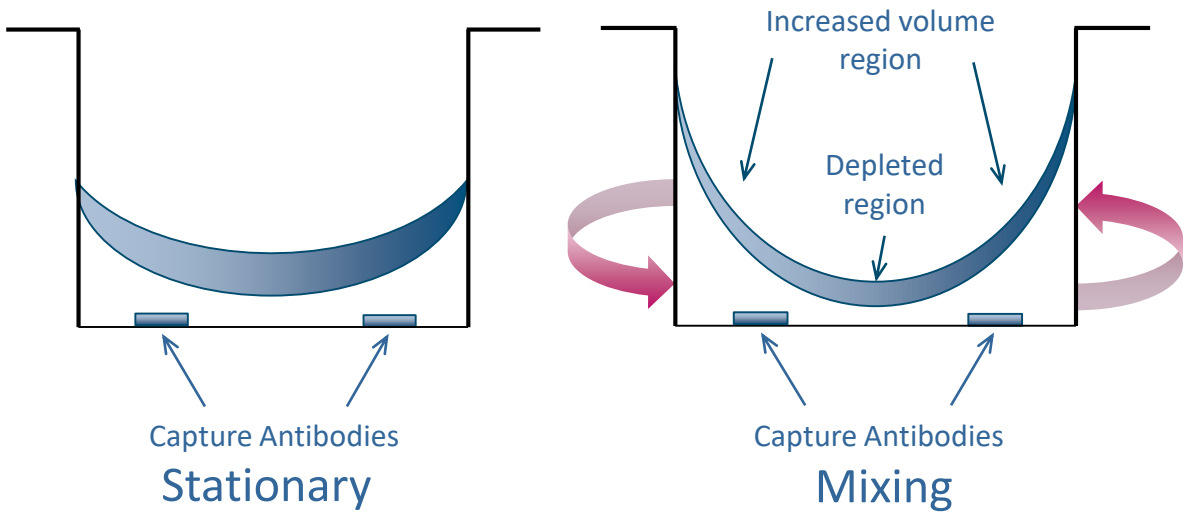
Simoa Planar Array Sensitivity: Capture Antibody Printing and Spot Geometry

Unique Antibody Deposition Tools

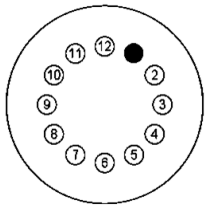


- High density capture Ab
- Surface chemistry optimization
- Precisely controlled spot size
- Highly reproducible positioning
- Uniform coating of blocking buffer

Unique Antibody Deposition Pattern

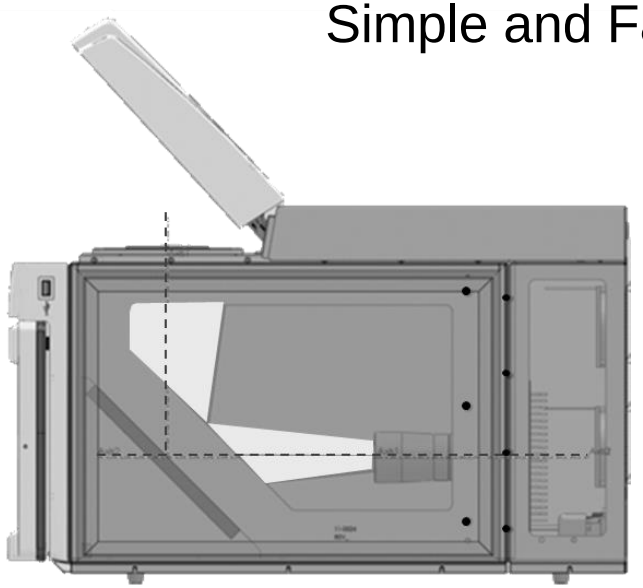


- Mixing drives binding reactions to equilibrium to maximize sensitivity (reduction in diffusion-limited kinetics)
- Circular pattern accounts for fluid dynamics to avoid depletion in center of plate well



Simoa Planar Array Sensitivity: Image Acquisition and Analysis

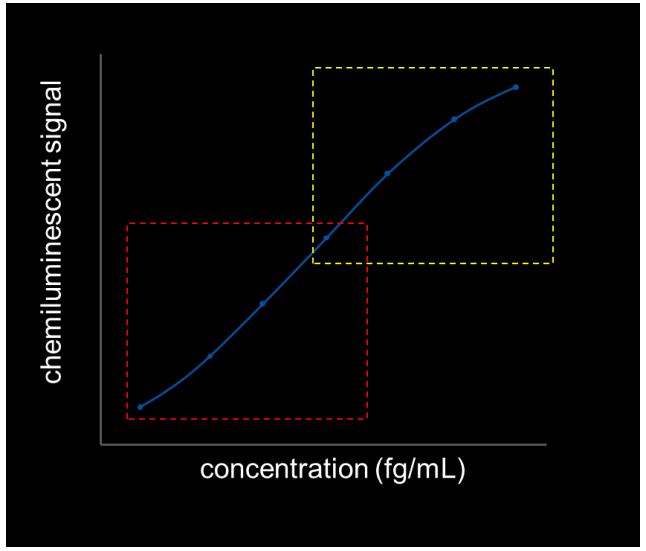
Simple and Fast Imaging



SP-X instrument features

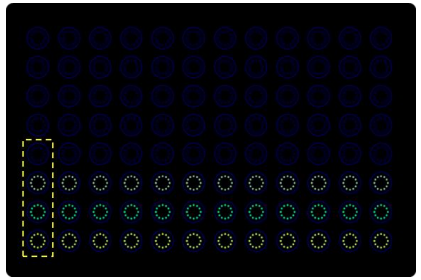
- Simultaneous whole plate imaging
- High-sensitivity scientific grade CCD camera
- No excitation light
- No moving parts
- ipad touch-screen interface
- Benchtop form factor

Intelligent Analysis Algorithms

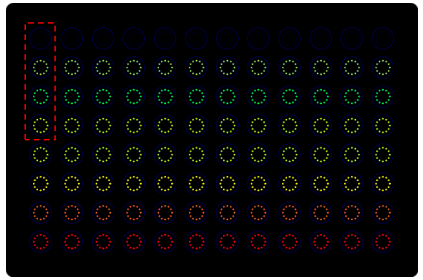


Step 1
- Short Exposure
- Evaluate signal from Cal H wells

Step 2
- Short Exposure for high analyte samples



Step 3
- Long Exposure for low analyte samples

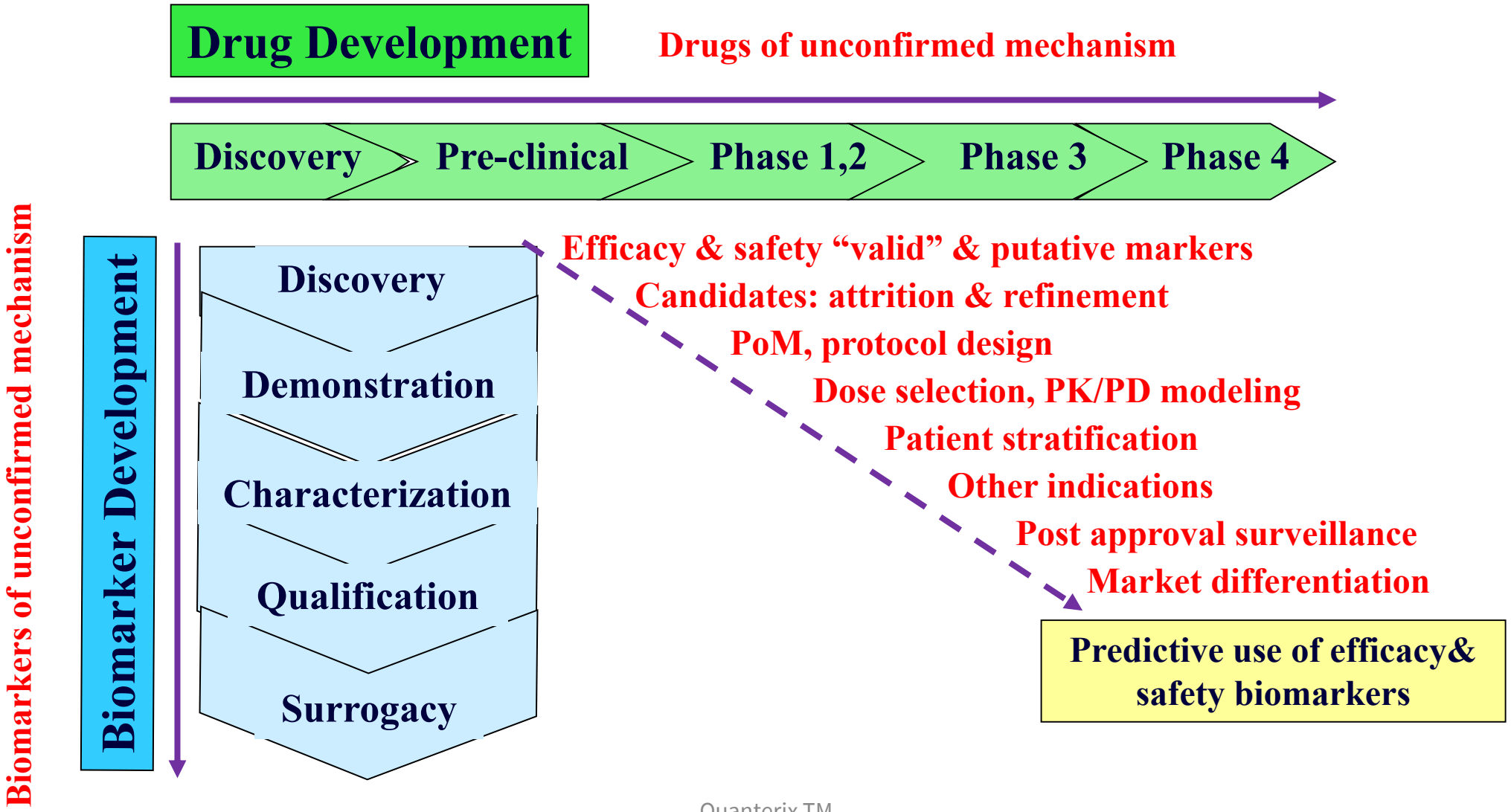


Step 4
- Generate composite virtual image
- Overlap and fit curve
- Determine concentrations

1 2 3 4

2.5 – 10 minutes Total Time

Biomarker Development & Drug Development are intertwined



FDA Food and Drug Administration

- **FDA News Release** May 23, 2017, **Second Drug approved in 2018**
- **FDA approves first cancer treatment for any solid tumor with a specific genetic feature**
- **CDER Biomarker Qualification Program**
- **List of Qualified Biomarkers**
- **Expect to see much more biomarker-driven drug development and approval in the Precision Health era**

A Blood Test That Can ID Alzheimer's Risk up to 16 Years Before Symptoms
Published: Nature Medicine, Vol 25, February 2019, 277-283

Blood test could detect Alzheimer's up to 16 years before symptoms begin, study says

By Nina Avramova, CNN

Updated 2:45 PM ET, Tue January 22, 2019

has opened

Simoa ~~is opening~~ a blood biomarker window to the brain

*Retrospective samples from longitudinal studies have **VALUE!***

Diabetes Type 1

FDA APPROVES THE SECOND PHASE OF DR. DENISE FAUSTMAN'S CLINICAL TESTING OF A TYPE 1 DIABETES VACCINE

Posted in Diabetes Research News

July 31, 2015

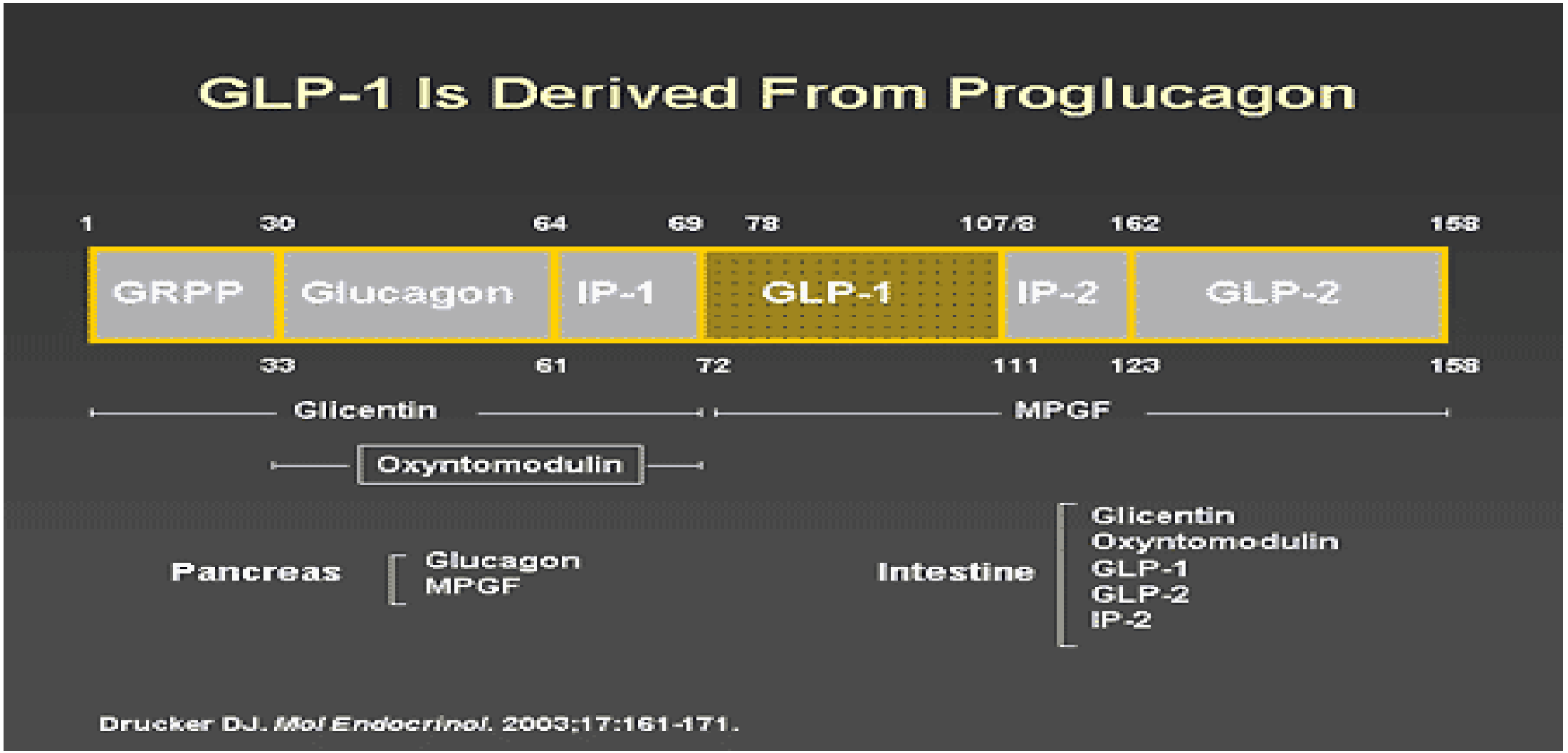
- Controversial at the moment, studies ongoing
- BCG vaccine (*Bacillus Calmette-Guerin otherwise known as (BCG) vaccine)*
- *Available since 1920's*
- *Increase TNF levels similar to healthy immune individuals*
- *Increase "good" T cells, reduce "bad/autoimmune" T cells*
- *Reduce attack on Beta Cells…??*

Health Stats USA (CDC)

- More than 30 million Americans have diabetes (1 in 10)
- 90-95% have T2D
- Predominantly over age 45, but shifting younger
- Approximately 80 million pre-diabetic!!!!

- EU and APAC on the increase as well!
- Perhaps biggest healthcare epidemic in USA? Globally?

Proglucagon peptides



Proglucagon peptides continued

- EBioMedicine. 2016 May; 7: 112–120.
- Published online 2016 Mar 31. doi: 10.1016/j.ebiom.2016.03.034
- PMCID: PMC4909640
- PMID: 27322465

- **Oxyntomodulin Identified as a Marker of Type 2 Diabetes and Gastric Bypass Surgery by Mass-spectrometry Based Profiling of Human Plasma**

- Nicolai J. Wewer Albrechtsen,[a,b,c,d] Daniel Hornburg,[c] Reidar Albrechtsen,[a,e] Berit Svendsen,[a,b] Signe Toräng,[a,b] Sara L. Jepsen,[a,b] Rune E. Kuhre,[a,b] Marie Hansen,[a,b] Charlotte Janus,[a,b] Andrea Floyd,[f] Asger Lund,[b,g] Tina Vilsbøll,[g] Filip K. Knop,[a,b,g] Henrik Vestergaard,[b] Carolyn F. Deacon,[a,b] Felix Meissner,[c] Matthias Mann,[c,d,1] Jens J. Holst,[a,b,∗,1] and Bolette Hartmann[a,b,1]

Proglucagon Peptides and Microbiome?

Anxiety, Depression, and the Microbiome: A Role for Gut Peptides

Lach et al., Neurotherapeutics 2018 Jan., 15 (1) 36-59

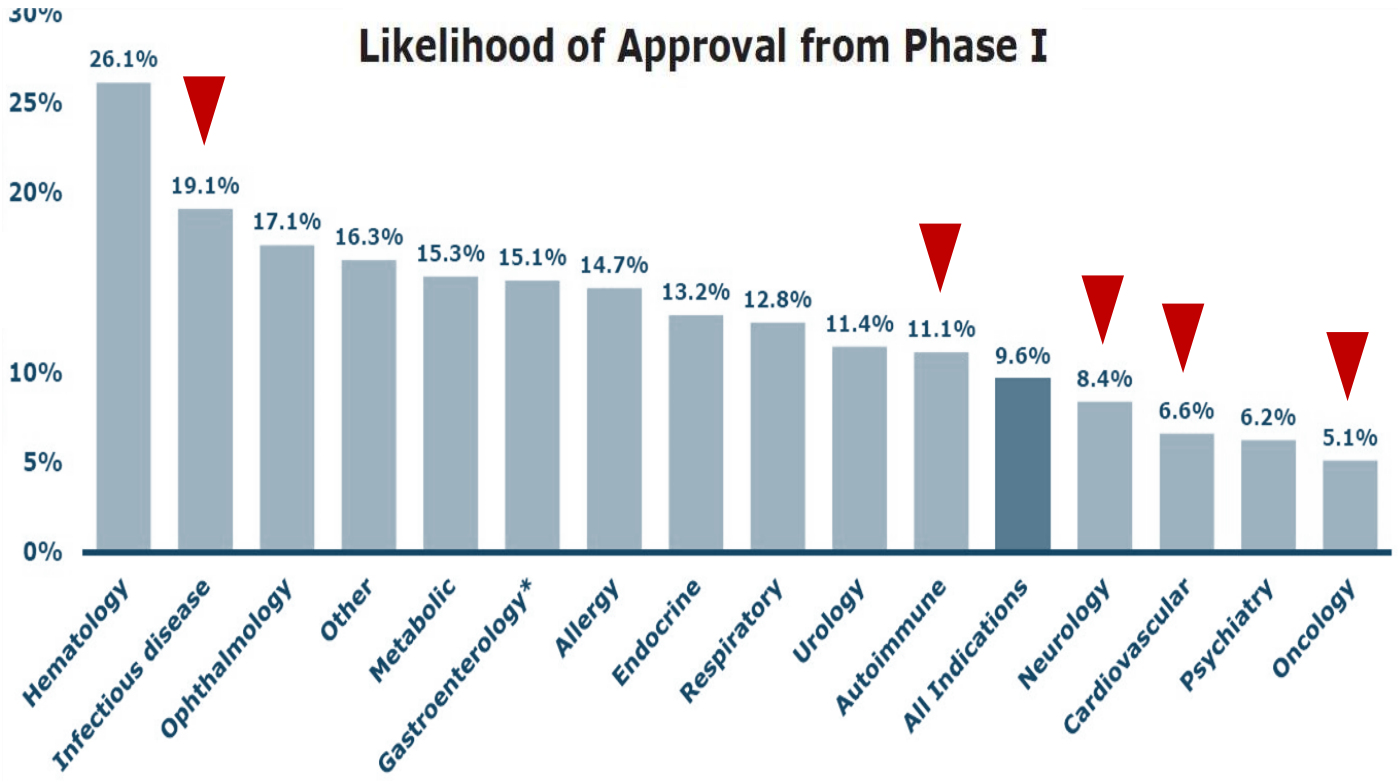
Gut to Brain linkages, complex.

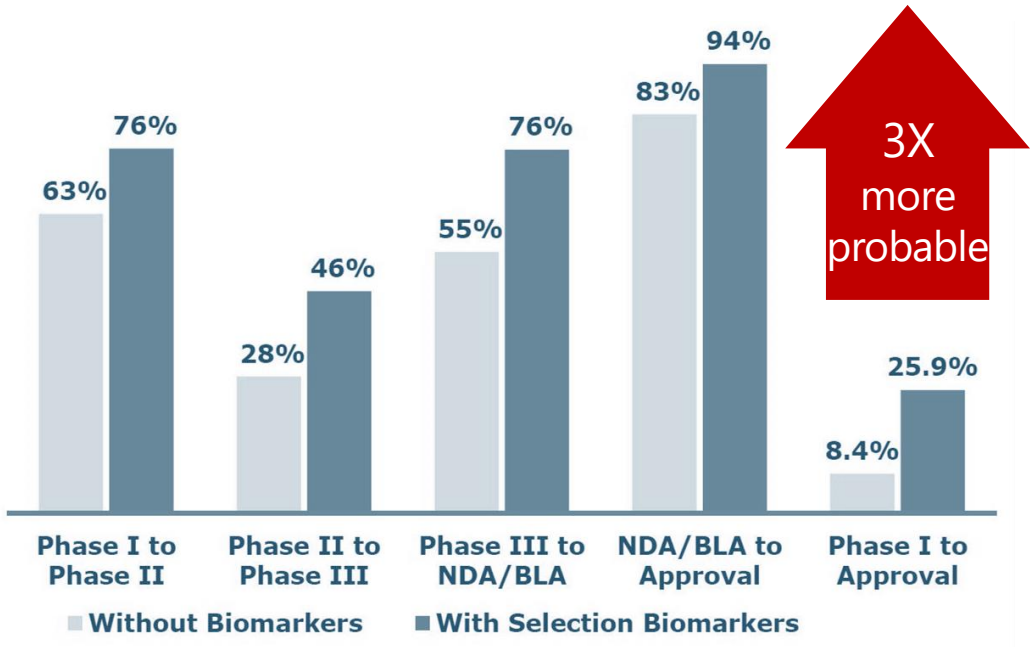
Measurement of peptides in CSF? Vs blood…

Very low concentrations. Ratio of constituents pre- and post-treatment?

Biomarkers Transforming Drug Development

Q excels where clinical trial success is historically poor





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Source:Clinical Development Success Rates 2006-2015, BIO Industry Analysis 2016

Crystal City VI: Points to Consider……

Many types of biomarkers,

Proteins, Biochemical markers, Cellular responses, Antibodies, Cytokines, DNA, RNA, SNPs, Radiographic images, Electrolytes, etc.

Many analytical platforms

LC/MS, LBAs, Cell based bioassays, Digital immunoassays Genomics

Used for many purposes

Candidate selection, Patient enrollment, Diagnosis, Treatment, Disease pathology, Regulatory approval, etc.

Biology

Acute, chronic, discrete, multiplexes, constitutive expression

Even approved diagnostic kits need to be validated for intended use

Brian Booth, Ph.D.
Office of Clinical Pharmacology
FDA/CDER/OTS/OCP**AAPS**
Crystal City VI: BMV on Biomarkers, 28-29 Sept., 2015

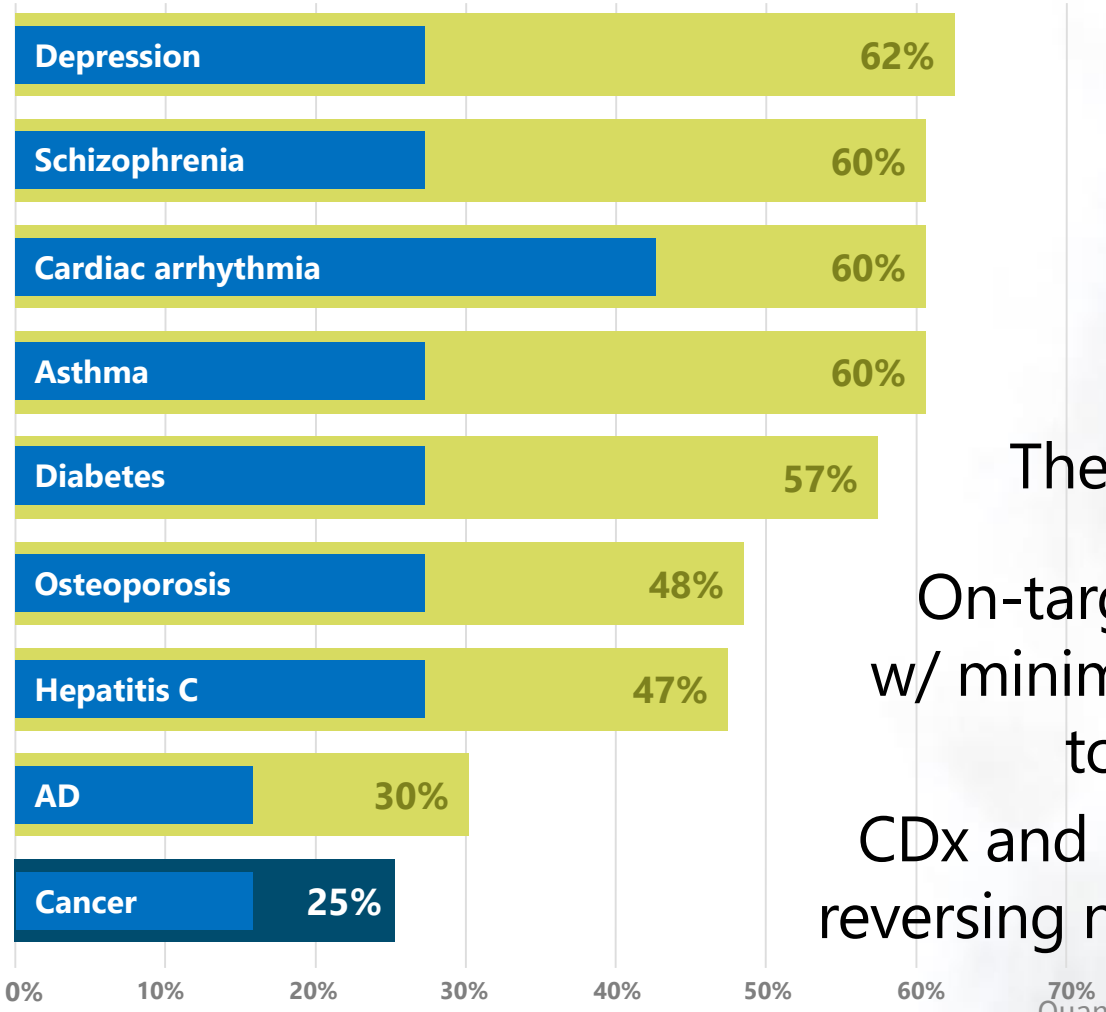
Drug Development and Clinical Practice present different challenges

Detailed guidelines exist for assay development and validation preMAA/BLA

- We need to adopt a conservative approach during development phases
- But assay results are pivotal to clinical management throughout a drug's life cycle
- **So how do we make sure our assays translate to the wider world?**

Adverse Events Are Also A Major Problem

Efficacy



Toxicity

Adverse drug reactions

Cost: $200B / yr

4th leading cause of death

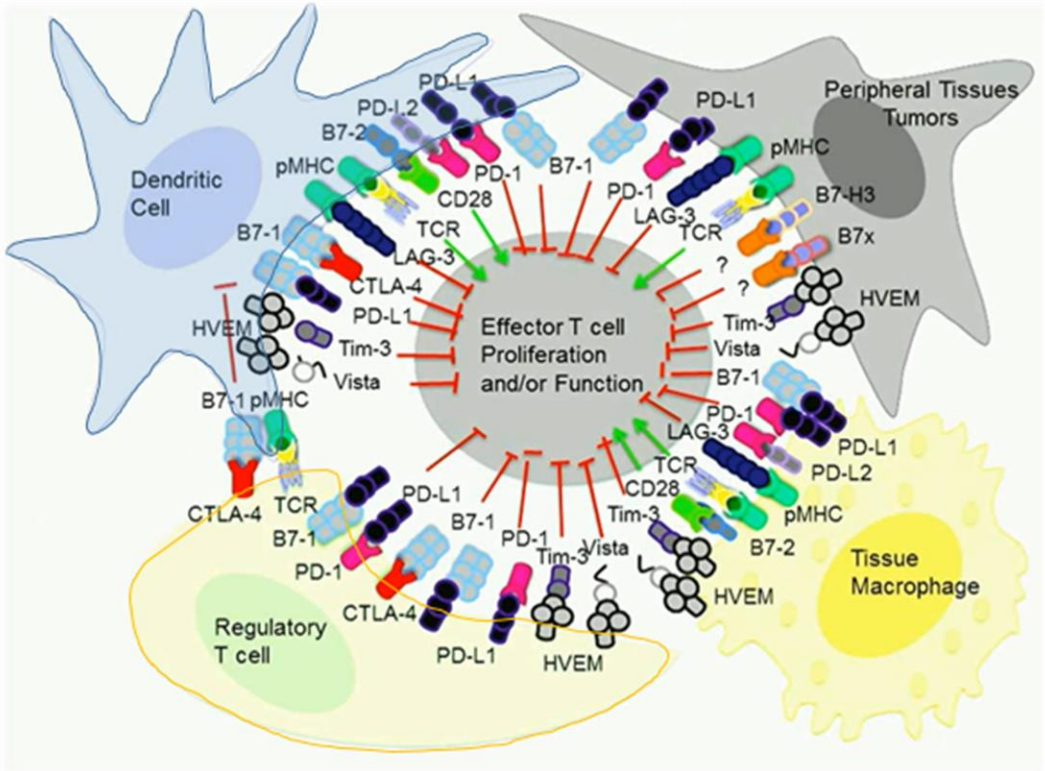
The Future:

On-target efficacy w/ minimal off-target toxicity

CDx and IO therapies reversing negative trend

Role of serum protein biomarkers in immuno-oncology

- Reversal of T cell exhaustion resulting from checkpoint inhibition is a direct way to determine effectiveness of therapeutic strategy
- T cell activation is characterized by an increase in expression of specific cytokines, e.g., interferon-gamma activation pathway
- Measurement of specific cytokines secreted by T cells into serum is an attractive biomarker approach
- Potential path to Dx and CDx (blood test)
- ***Technical challenge is availability of immunoassays with sufficient sensitivity to measure cytokines (femtogram per mL sensitivity)***



Jim Allison, ASCO 2015

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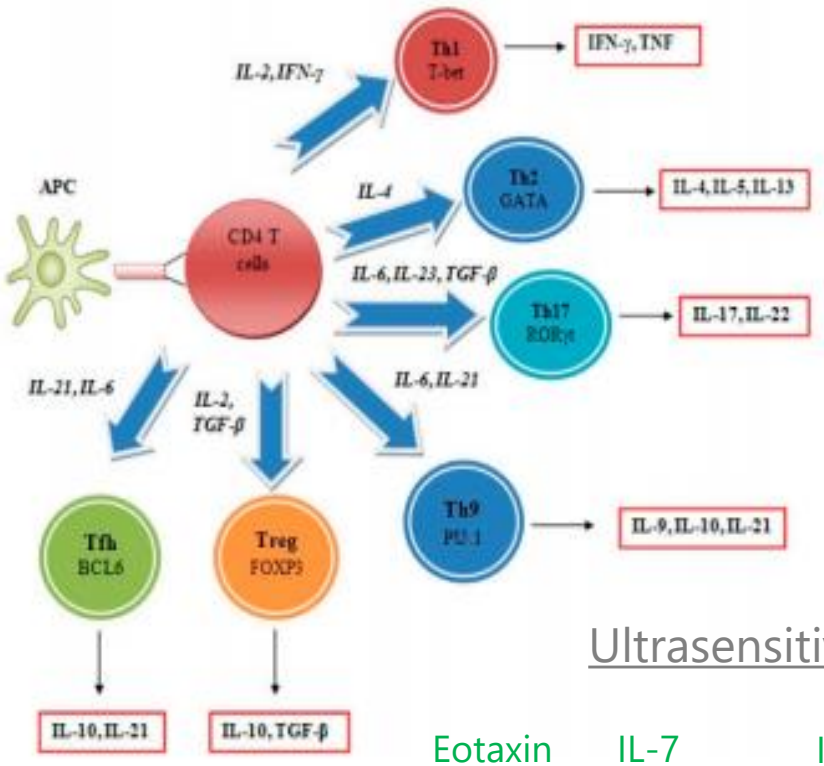
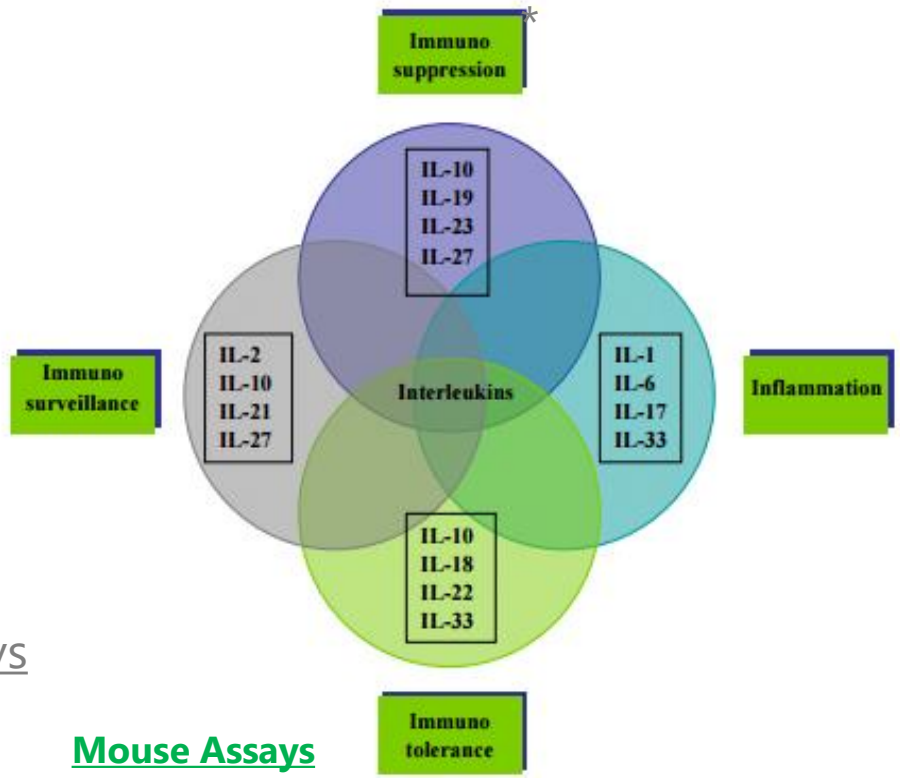
Biomarker monitoring in cancer immunotherapy approaches





Ultrasensitive (fg/mL) Simoa assays

Eotaxin	IL-7	IL-15	IFN-γ	IL-3
GM-CSF	IL-8	IL-17A	IP-10	Il-22
IL-1α	IL-10	IL-17F	MCP-1	Il-28A
IL-1β	IL-12p40	IL-18	TGF-α	IL-33
IL-2	IL-12p70	IL-23	TNF-α	IL-36β
IL-4	IL-13	IFN-α	TNF-β	TGF-β
IL-5	CP3A	IFN-β	TRAIL	PD-L1
IL-6	CP3B	VEGF	Homebrew	

Mouse Assays

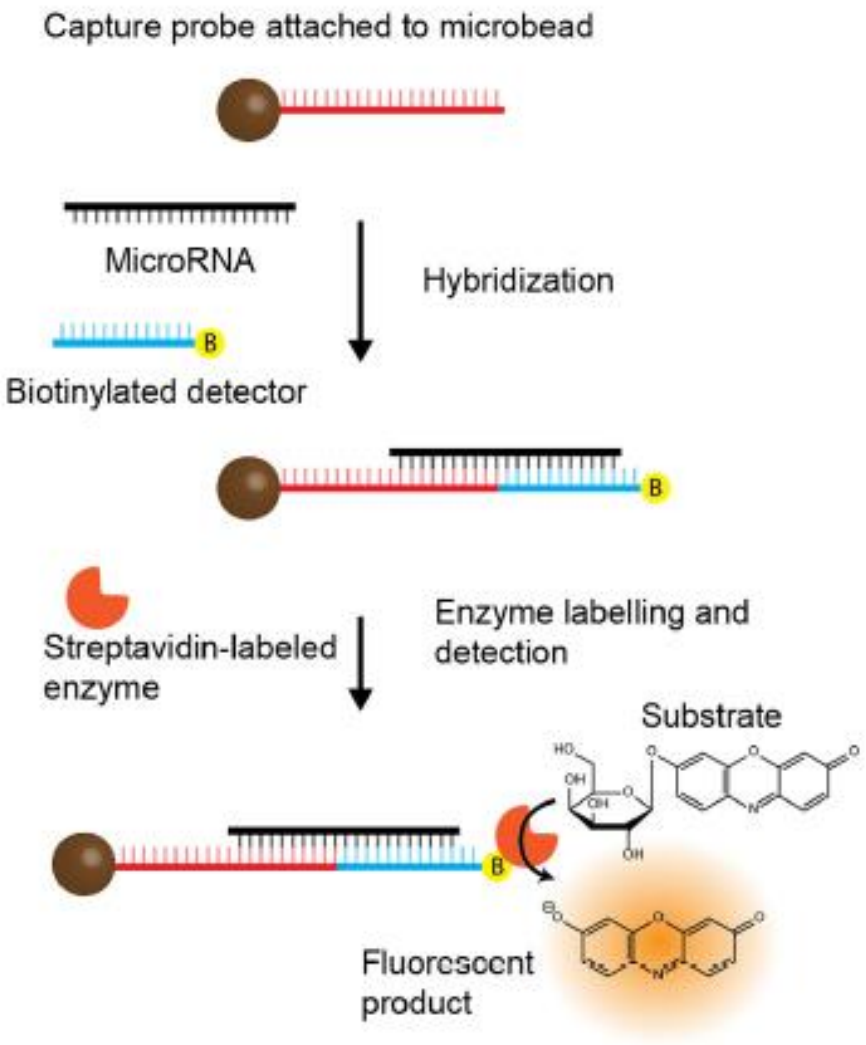
IL-6
IL-17A
IL-17A/F
IL-17F
IL-22
IL-23
TNFα

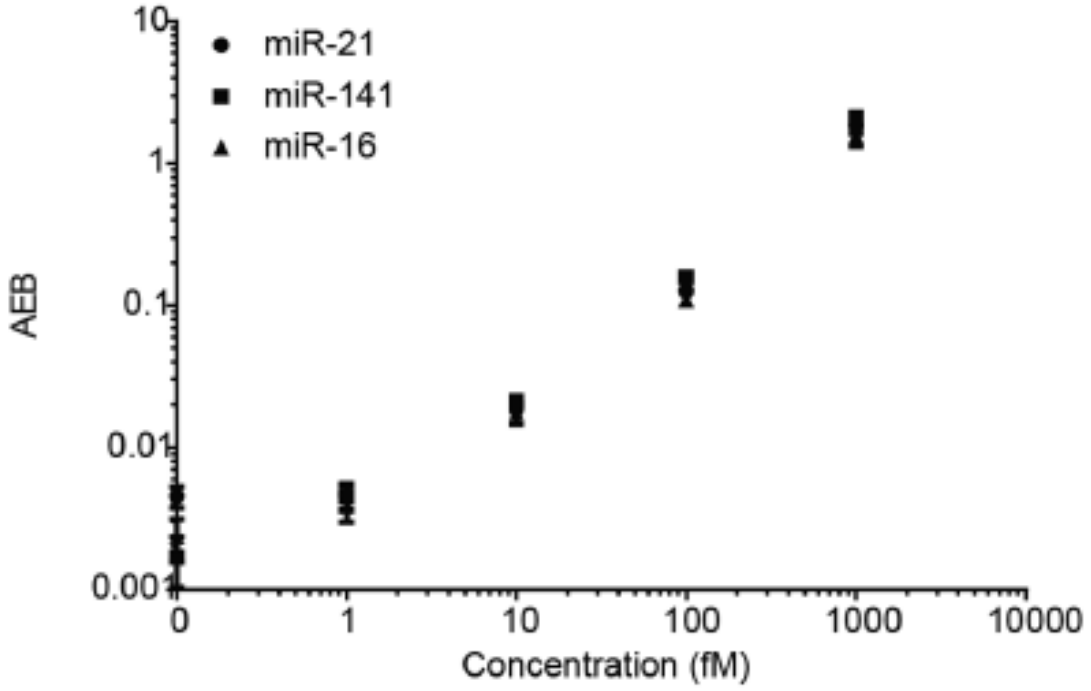
** Anestakis, et. al. , Mechanisms and Applications of Interleukins in Cancer Immunotherapy. Int. J. Mol. Sci. 2015, 16*

Example of Samples Tested on Simoa

- Serum
- Plasma
- Urine
- CSF
- Tissue extracts
- Interstitial fluid
- Brain extract
- Liver Extract
- Whole Blood
- Breath condensate
- Blood spots
- Tears
- Saliva
- Cell or IVF culture supernatant
- PBMC (lysate, culture supernate)
- Blood Fractionations (IP)
- Exosomes
- Ocular fluids

Detection of microRNA (miRNA)



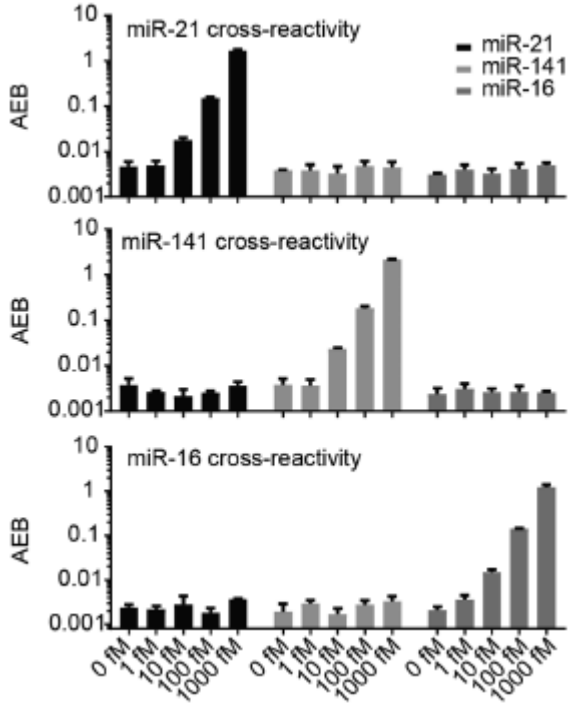


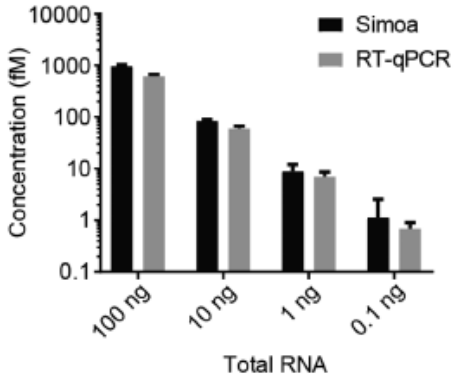
Cohen et al., *Nucleic Acids Res.* (In Press) 2017

Direct Detection of microRNA (miRNA) by Simoa technology

- Simoa 3-plex assay designed to measure and test sensitivity and cross-reactivity in serum spike recovery assays
- Highly sensitive and low cross-reactivity achieved; LOD close to 10 fM achieved
- Levels of miR-141 (and miR-21 and miR-16) from human total lung RNA similar for Simoa and RT-qPCR
- miR21: most frequently upregulated miRNAs in solid tumors
- miR141: is associated with numerous human malignancies, like breast cancer, gastric cancer
- miR16: is a key tumor-suppressive miRNA that can target numerous oncogenes in various human cancers.

- Simoa direct detection approach for miRNA is sensitive, accurate and does not require amplification techniques for detection





Cohen et al., *Nucleic Acids Res.* (In Press) 2017

Biomarkers Apply to All Drug Development

Biomarker Category	Comments
Target Engagement Markers	Quantify the drug & target (total target, drug-bound target, and free target molecule).
Safety Markers	Incidental or targeted safety markers (e.g. cardiac, liver, kidney, cytokines and inflammatory markers)
Efficacy Markers	Direct or indirect measurement of associated markers for efficacy, surrogate of efficacy..(oncology, diabetes)
Pharmacodynamic	Downstream effects of treatment (drug effect/MOA), surrogates of efficacy
Prognostic, Diagnostic, Surveillance…	Medical practice for routine assessments

Services: Quanterix Accelerator Lab (CLIA Certified)

- CRO for Ultrasensitive Immunoassays
- Maximize Adoption of Simoa: “Game Changing” Ultrasensitive Immunoassay Platform
- Integrate service with manufacturing for maximum flexibility

SIMOA will enable New Clinical Validation!

SIMOA

RESEARCH

Variable Quality

5000+ Analytes

Relative Quantitation

Diverse Species

CLINICAL DEVELOPMENT

5000+ Analytes
Diverse Genetics

Rigorous Quality

Limited Sample

Multi-Analyte

High Throughput

Absolute Quantitation

CLINICAL VALIDATION

Humans
100 Analytes?
Rigorous Quality
Single Marker
Absolute Quantitation

THANK YOU!

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