Epigenetic Modifications After Ambient Air Pollution Exposure

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In collaboration with Fresno Asthma Children's Environmental Study

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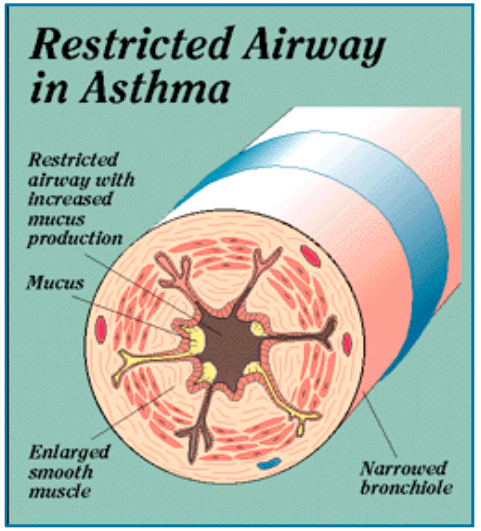
Asthma

- Chronic multifactorial disease characterized by :
  - Airway obstruction
  - Airway hyperresponsiveness (AHR)
  - Airway inflammation
  - Airway remodeling
- **Dramatic increase in the prevalence of asthma worldwide in industrialized countries**
- **34 million Americans with asthma**
- **$20 billion/year spent on asthma care in the U.S.**
- **<u>Possible Cause:</u>**
  - **Inappropriate immune response in a genetically susceptible individual driven by environmental exposures**
- **Asthma can be linked to allergies, viruses, pollution, and other events**



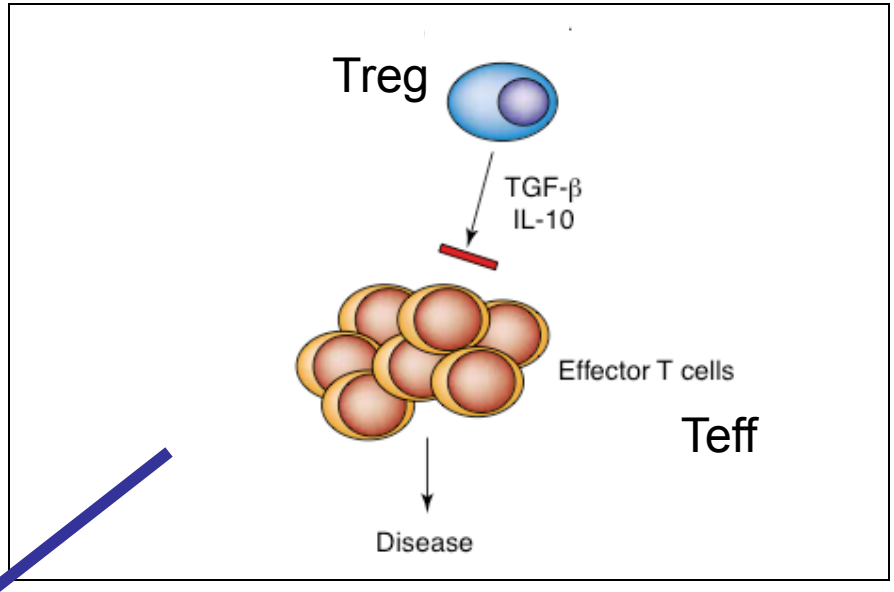
Regulatory T cells in Asthma

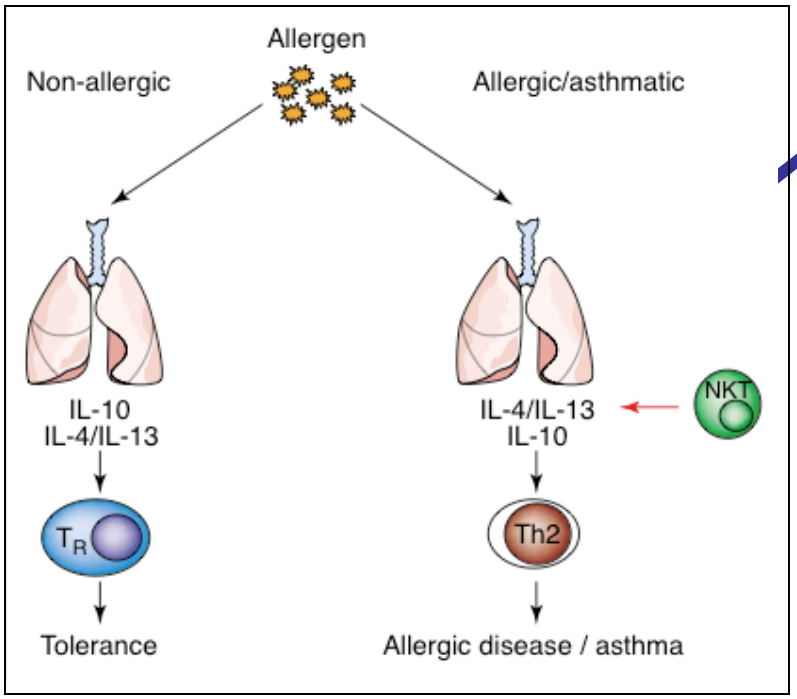
•Cells, which control or suppress the function of other cells

•**Foxp3** transcription factor associated with Treg

• Children lacking Foxp3 have severe allergies, asthma, GI disease, and diabetes type I.

•CD4+CD25^{hi}CD127lo cells can inhibit effector T cells





•Natural Treg(CD4+CD25^{hi}) suppress effector T cells

•What Environmental Factors worsen Treg function in asthma? Do they affect suppressive function? Is Foxp3 expression altered? If so, how?

GENERAL HYPOTHESIS and AIM

Overall: Treg are dysfunctional in subjects exposed to high levels of ambient air pollution exposure

Aim: The research aims to help elucidate the key role of air pollution in asthma, a link which is theoretically understood, circumstantially clear, but not yet proven.

Rationale: Understanding biological mechanisms is an important step towards developing target-driven treatments to reduce the burden of asthma in children who are exposed to high levels of air pollution.

Four cohorts chosen:

- Children's Environmental Study
  - 9 years of exposure data
  - Children 8-12 yrs with asthma (n=71, FA)
  - 315 families
  - Serial PFTs and clinical outcome score
  - Individual Estimates of Exposure to:
    - Elemental carbon, $PM_{2.5}$, PM_{10}, metals, ozone, polycyclicaromatic hydrocarbons, endotoxin, pollens, carbon monoxide
- Fresno control or FC: Age matched and sex matched children with no asthma and no allergies (n=40)
- Stanford Asthma or SA: Age matched and sex matched children with asthma (same clinical outcomes and PFTs used) (n=30)
- Stanford control or SA: Age matched and sex matched children with no asthma and no allergies (n=30)

Note: Stanford group must live 6 yrs or more at residence in Palo Alto, CA and must live 30 meters outside range of major highway

Nadeau, et al. JACI 2010.

PAHs about ~7x higher in Fresno Air

Volatile to semivolatile

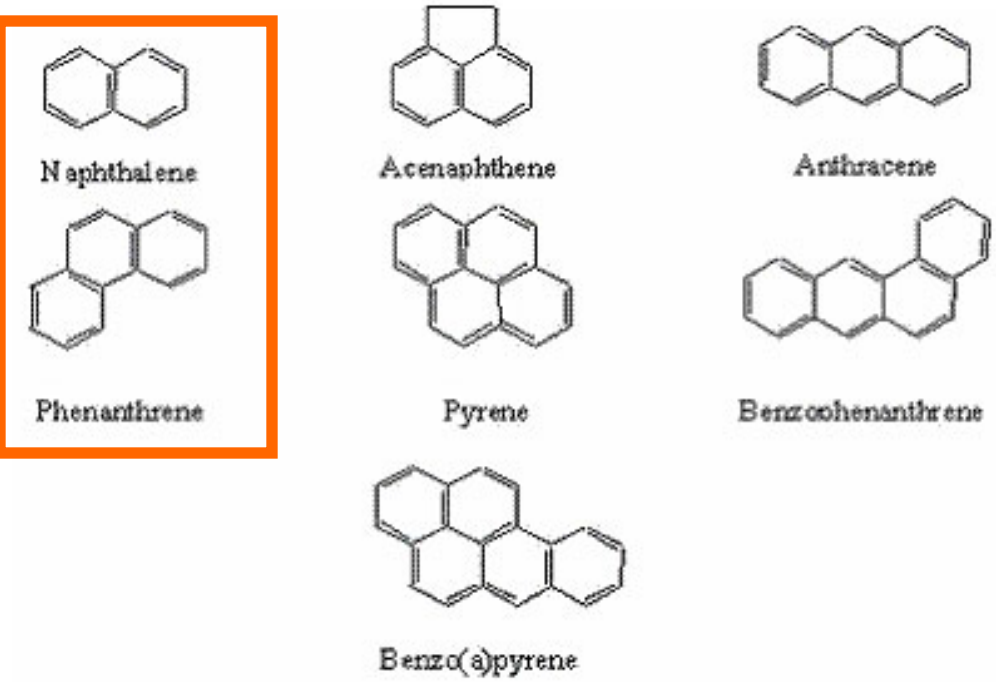


TABLE I. Comparison of area ambient pollutant concentrations between Fresno and Palo Alto, Calif, based on CARB compliance monitoring for 2008

Pollutant	Location of compliance monitor*	
	Redwood City	First Street, Fresno
PAHs, annual average (ng/m^3)	0.6	4.4
$PM_{2.5}$, annual average (μg/m^3)	10.5	21.2
$PM_{2.5}$, 24-h maximum (μg/m^3)	36.0	93.0
PM_{10}, 24-h high (μg/m^3)	41	78
O_3, highest 8-h average (ppb)	70	132
O_3, no. of days > state 1-h standard	0	44
O_3, no. of days > state 8-h standard	0	86

PM_{10}, Particulate matter with aerodynamic diameter ≤10 μm.
*Redwood City is the compliance monitor within 4.7 km of the Palo Alto residences of the Stanford cohort. All FA subjects live within a circle with the First Street monitor as its center and a radius of 20 km.

Nadeau, et al. JACI 2010.

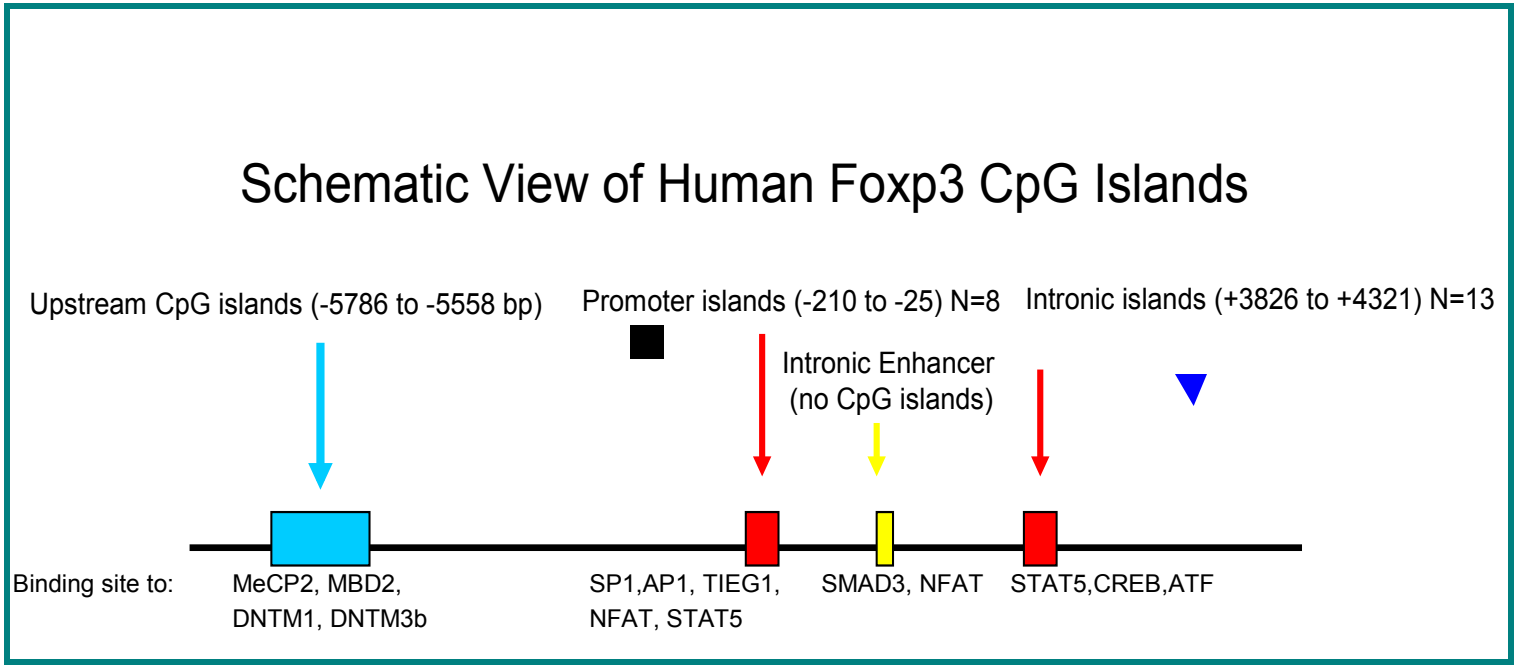
Data in children exposed to chronic, high levels of phenanthrene demonstrate a mean serum concentration of 2.0 μM, we chose a 10-fold higher concentration for our *in vitro* work performed over 7 days, similar to other publications.

Outcome Variables

For all 4 cohorts:

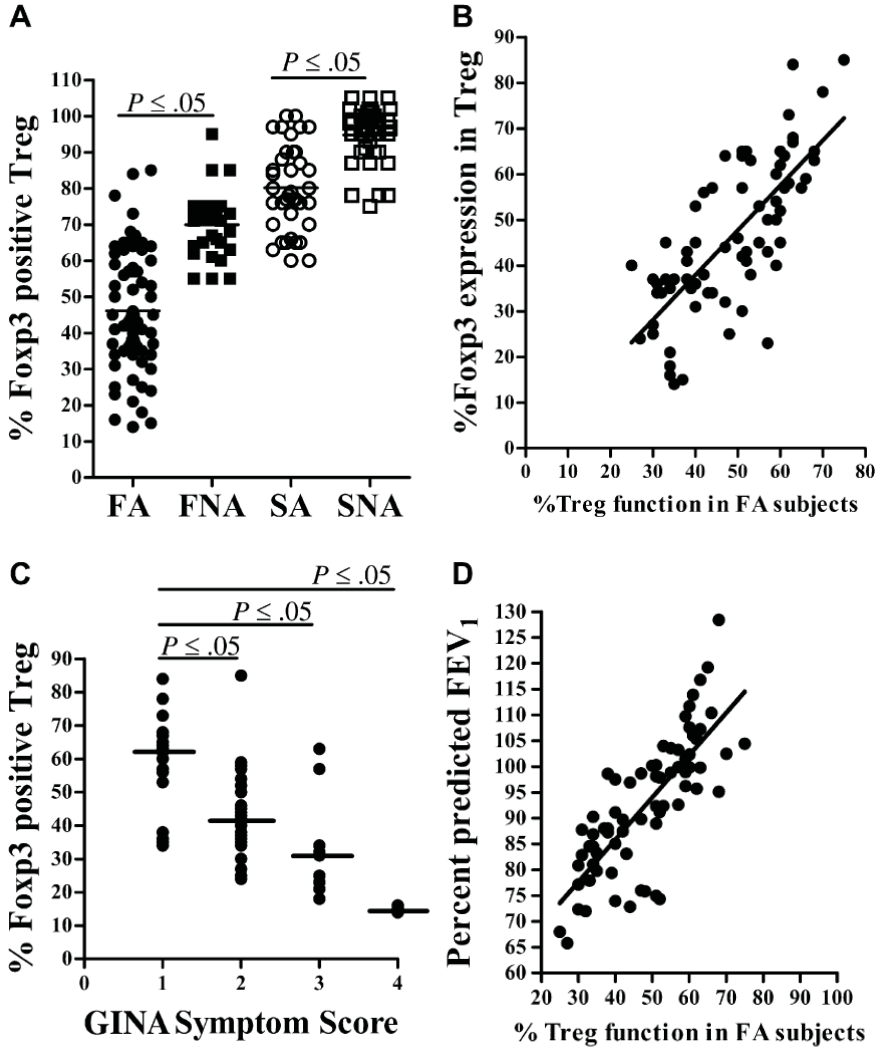
- Regulatory T cells ($CD4+CD127loCD25^{hi}$): molecular analysis and function
- Effector T cells or responder T cells ($CD4+CD127+CD25^{neg}$): molecular analysis (including epigenetics) and function
- Assays of interaction between the two above cell types: suppression assays, cell death assays
- Subject CBC with differential, physical findings and symptoms, pulmonary function and allergy tests.

Epigenetic Studies Foxp3 CpG regions are hypermethylated in FACES subjects



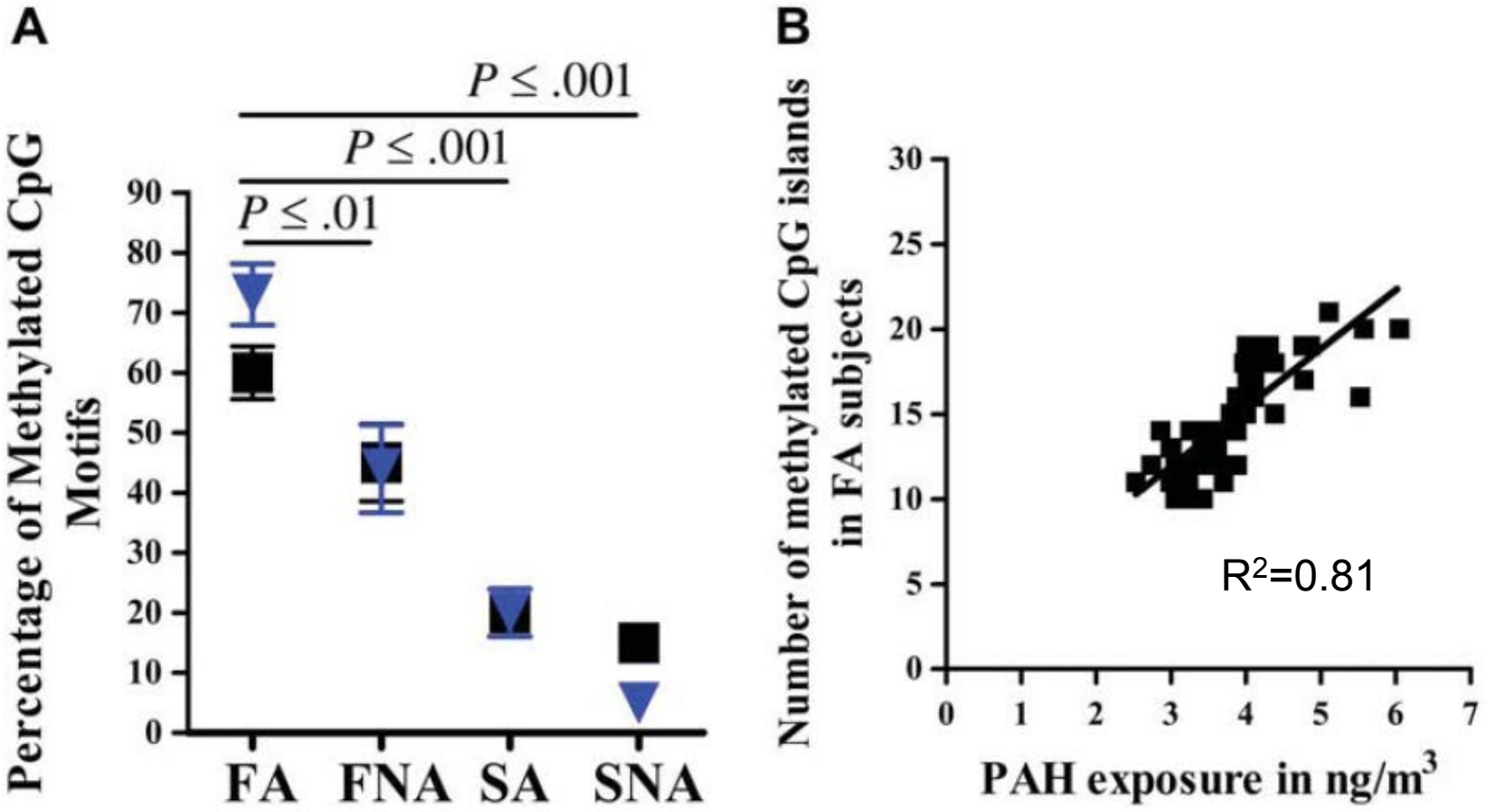
Nadeau, et al. JACI 2010.

Treg Foxp3 expression is associated with asthma severity



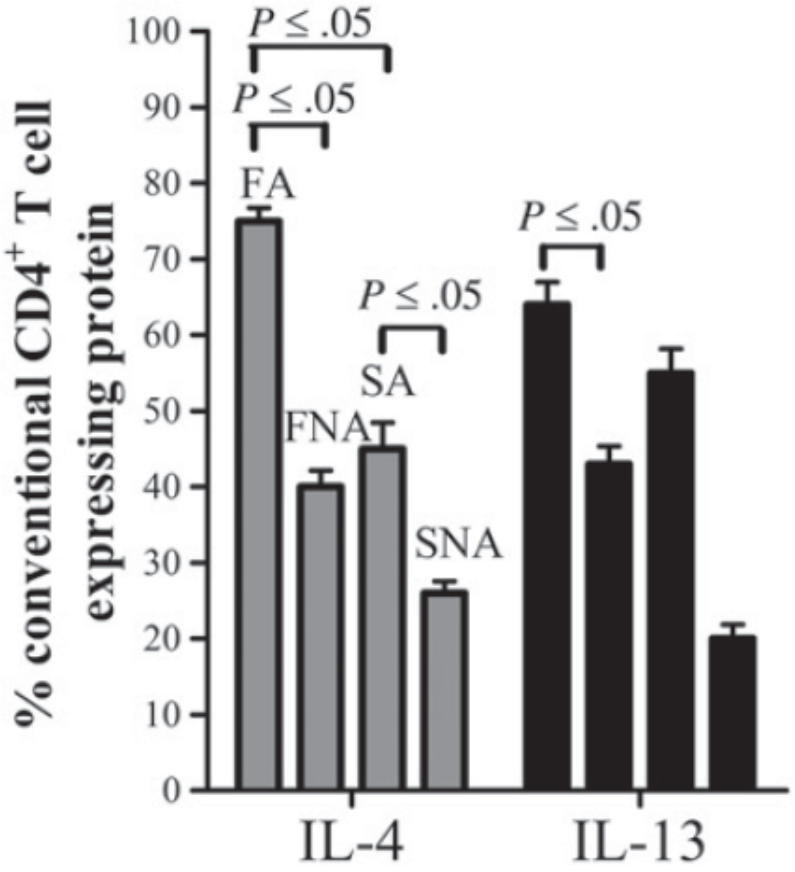
Nadeau, et al. 2010

Methylation of CpG sites on Foxp3 locus in Treg is associated with PAH exposure



Nadeau, et al. 2010

Th2 skewing occurred in FA subjects compared to other groups

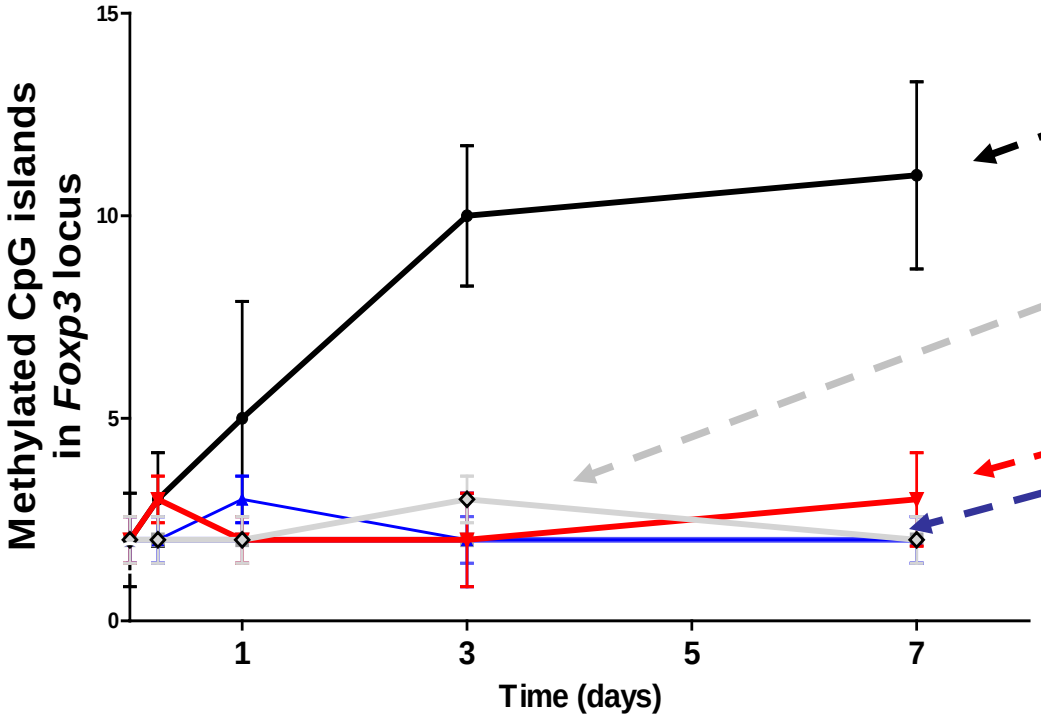


Nadeau, et al. 2010

Ex vivo studies using phenanthrene in culture with Treg

DNMT inhibitor or AhR inhibitor abrogate the effects of Phenanthrene

B



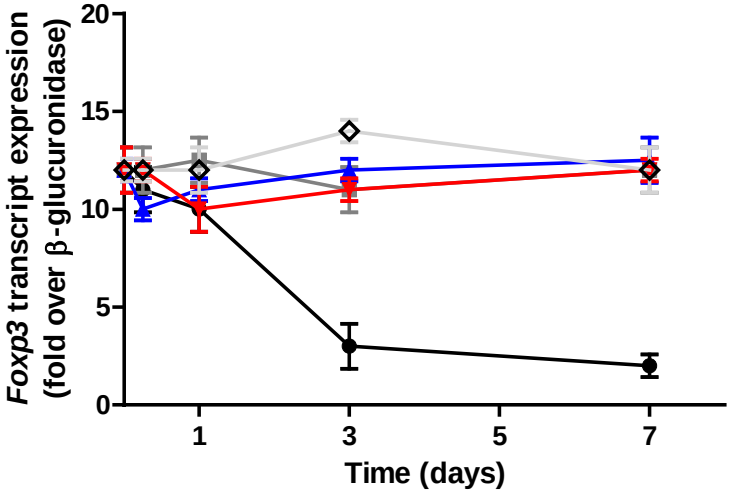
Phenanthrene

DNMT inhibitor

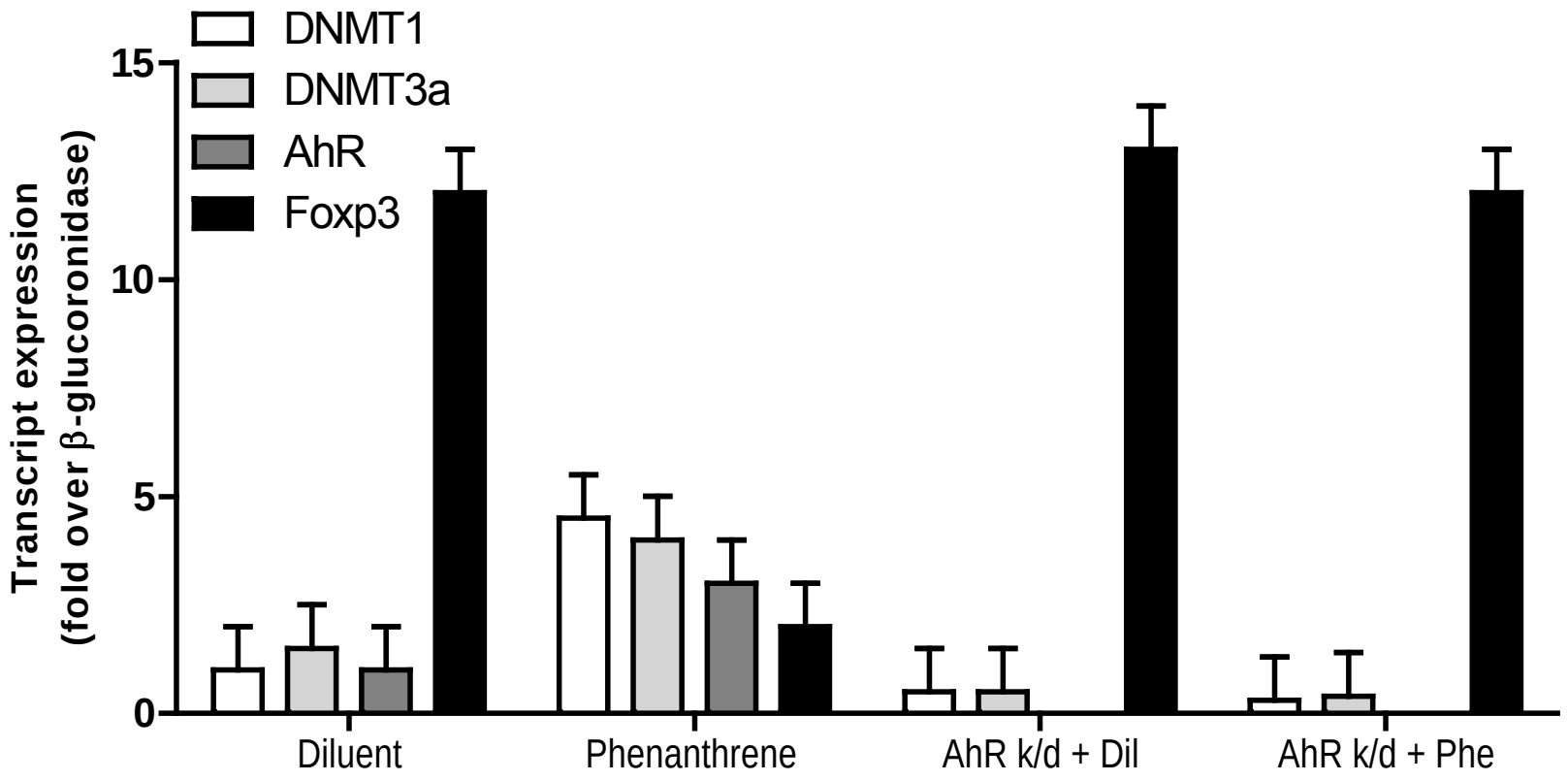
Media alone

AhR inhibitor

C



Gene Silencing Experiments demonstrate dependency of DNMT1 and 3a expression on presence of AhR

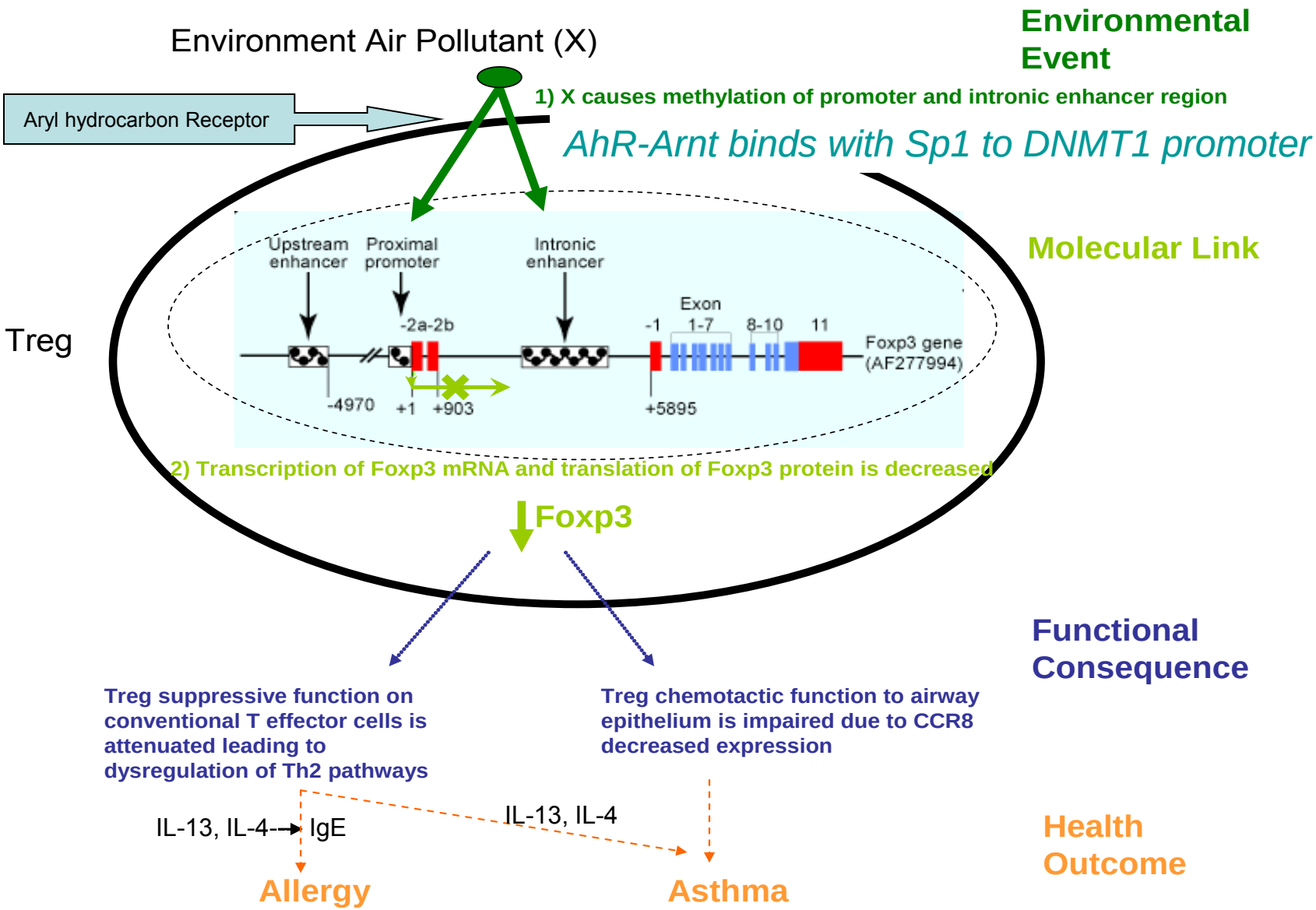


Molecular links between pollution, immune system changes and health outcomes

Summary and Next Steps

- Clinical Outcomes (ie. Severity of asthma) correlate inversely with presence of memory Treg with stable Foxp3 expression
- Memory Treg, not Naïve Treg, are most affected by air pollution effects
- Foxp3 is unstable in Treg after methylation of promoter and intronic regions
- ChIP Seq demonstrates AhR-Sp1 binding site in promoter region of DNMT1
- Test in AhR knock out model in mice (Sunwoo lab)

Possible Overall Schematic



Thank you

- **Laboratory Team**
  - Nadeau lab: Marco Garcia, Olivier Humblet, Jennifer Jenks, Jing Liu, Takahiro Kanai, Shuchen Lyu, Jiming Rong, Scott Seki
- **Clinical Research Team**
  - Stanford:Tina Dominguez, Daniela Pineda, Margie Woch, Anne Keller, Grace Yu
  - Fresno: Leah Melendez, Cynthia Appel
- **Exposure/Epidemiology/Statistics Team**
  - Berkeley: Fred Lurmann, Katharine Hammond, Alan Hubbard
- **Current Collaborators**
  - Stanford: HIMC, CTRU, Boyd lab, Fire lab, Galli lab, Snyder lab, Sunwoo lab, Gary Shaw
  - Outside: Wesley Burks (Duke), Karagas lab (Dartmouth), Xiu-Min Liu (Mt Sinai), Miller lab (Columbia), Ira Tager (Berkeley), Umetsu lab (Harvard),

CHAPS SJV

Children's Health & Air Pollution Study
San Joaquin Valley

Berkeley
UNIVERSITY OF CALIFORNIA

STANFORD
UNIVERSITY

Funded By:

EPA
United States
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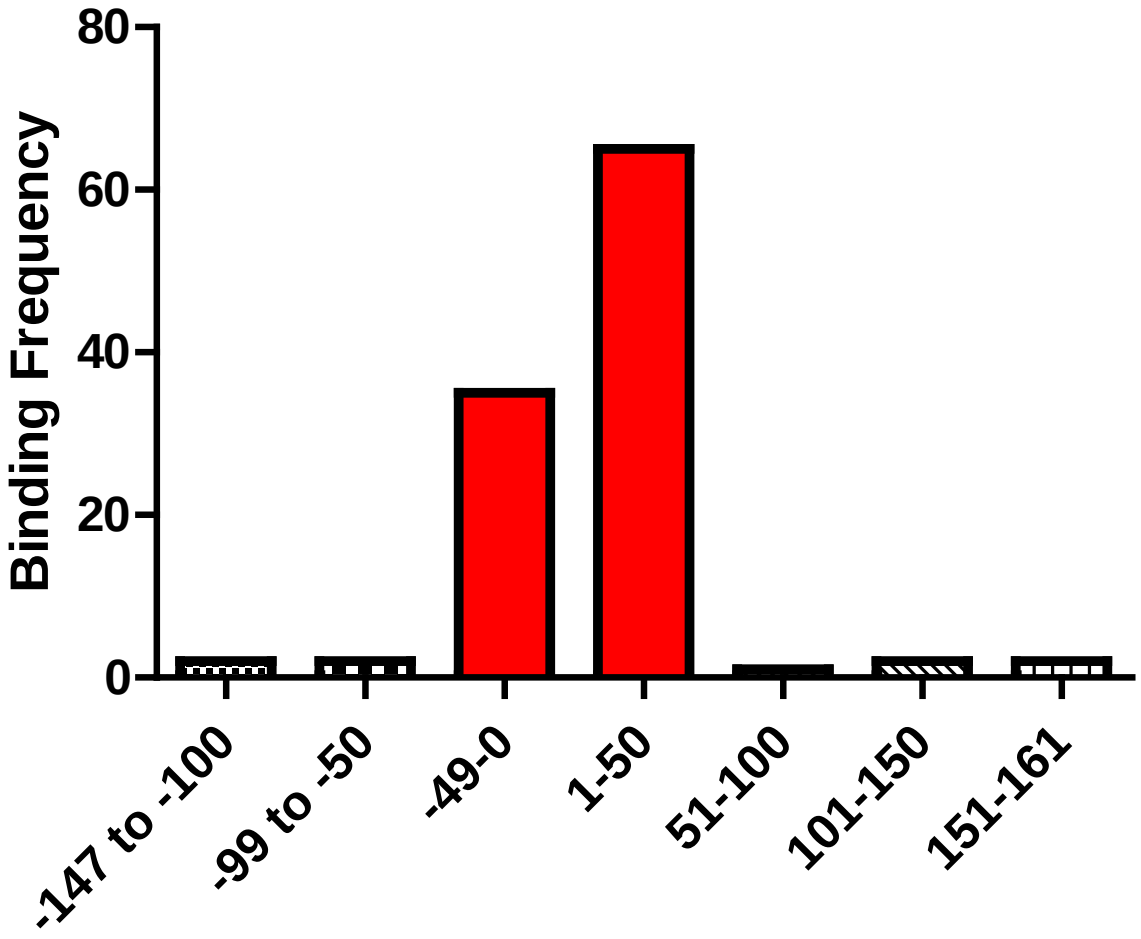
Back up slides

Sp1 binds as a transcription factor to DNMT1

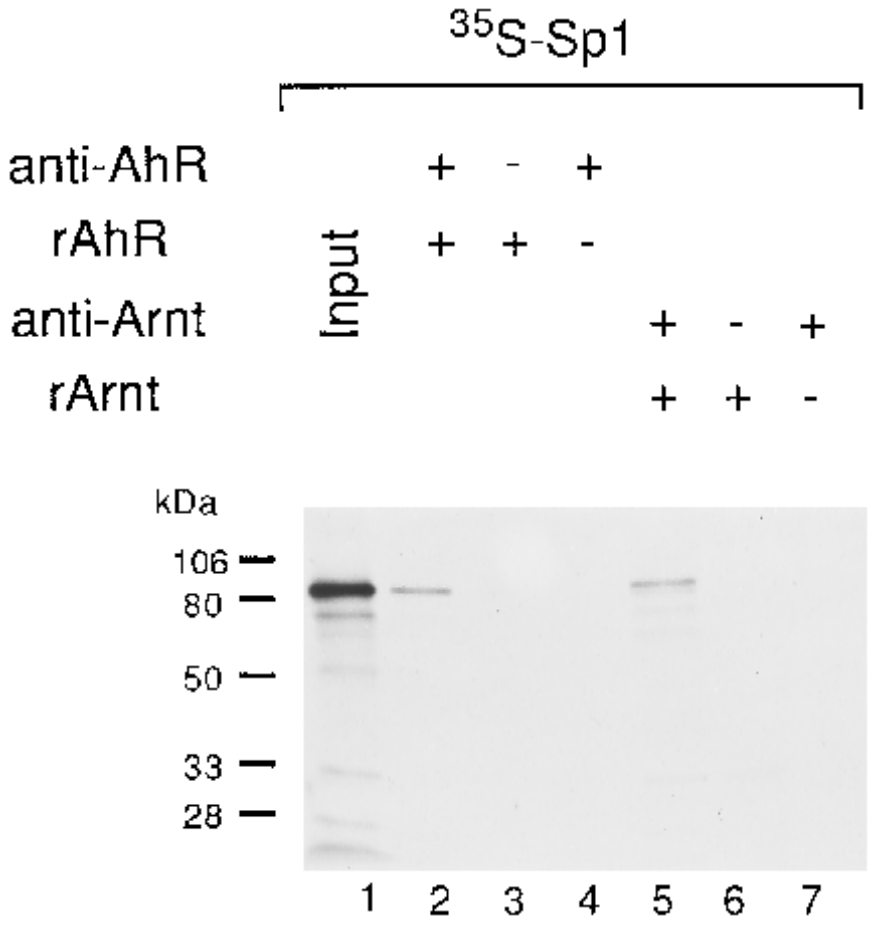


Binding Site of Sp1 in DNMT1 promoter

-147 TCCCCATCACACCTGAAAGAATGAATGAATGAATGCCTCGGGCACCG
-100 TGCCCACCTCCCAGCAAACCGTGGAGCTTGGACGAGCCCACTGCTCCGCG
-50 TGGGGGGGGTGTGTGCCCGCCTTGCGCATGCGTGTTCCCTGGGCATGGCC
1 GGCTCCGTTCCATCCTTCTGCACAGGGTATCGCCTCTCTCCGTTTGGTAC
51 ATCCCCTCCTCCCCCACGCCCGGACTGGGGTGGTAGACGCCGCCTCCGCT
101 CATCGCCCCTCCCCATCGGTTTCCGCGCGAAAAGCCGGGGCGCCTGCGCT
151 GCCGCCGCCGC

Liu, et al. 2008. Neoplasia

DNA ase I footprinting demonstrates Sp1 binds AhR-Arnt complex

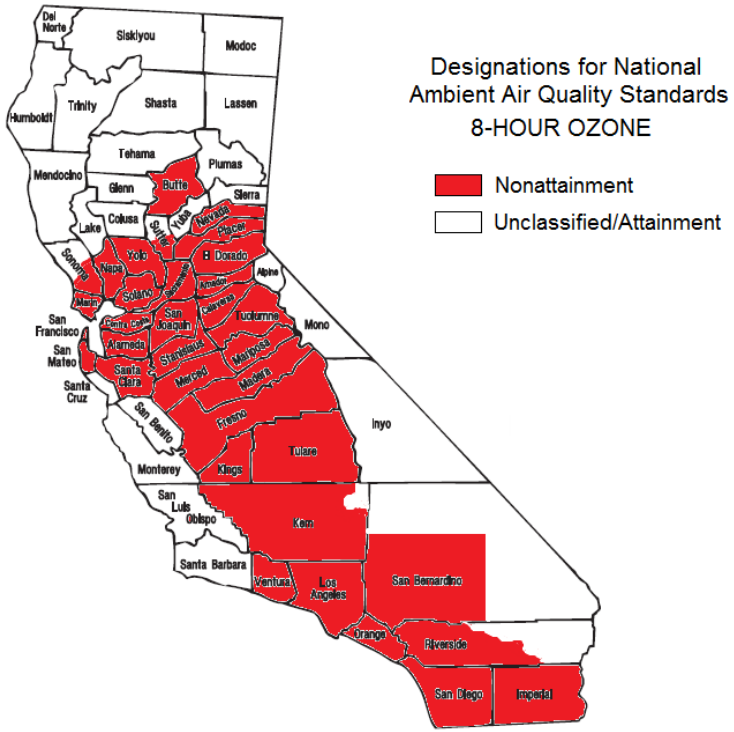


Kobayashi, et al. 1995. JBC

Why focus on Fresno?

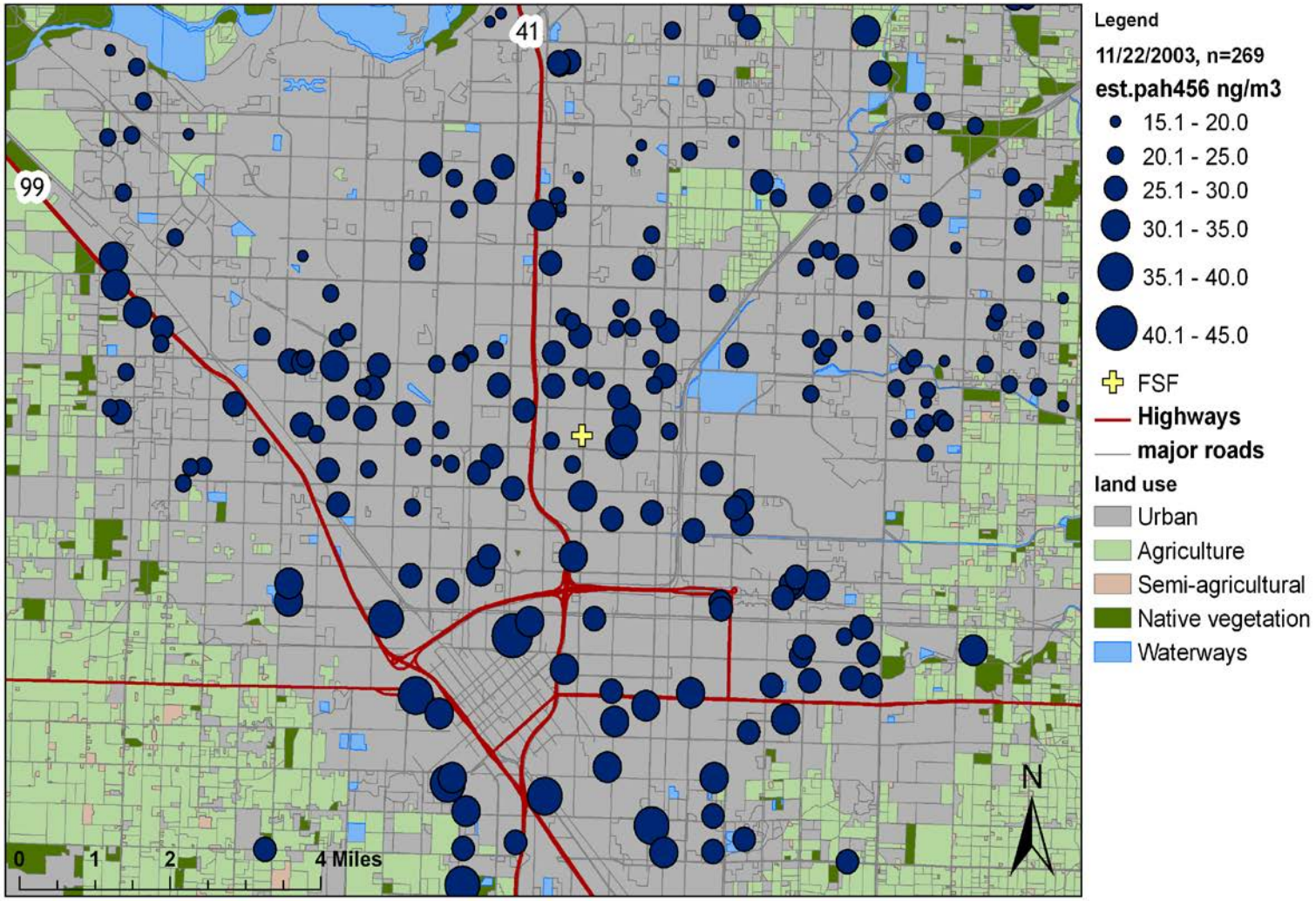
State of the Air 2011

Most Polluted Cities in America for Short-Term Particulate Pollution	
Metropolitan Area	**US Rank 2011**
Bakersfield	1
Fresno	2
Los Angeles	4
Visalia	7
Hanford	9
Sacramento	9
Modesto	12
Merced	13
San Diego	15
Stockton	16
San Jose – San Francisco – Oakland	**24**



American Lung Association

PAH accumulative exposure over 12 months



Objectives

- Provide an example of immune monitoring studies which led to improved understanding of toxicant mechanism
- Discuss a Systems Biology Approach and new applications in immune monitoring

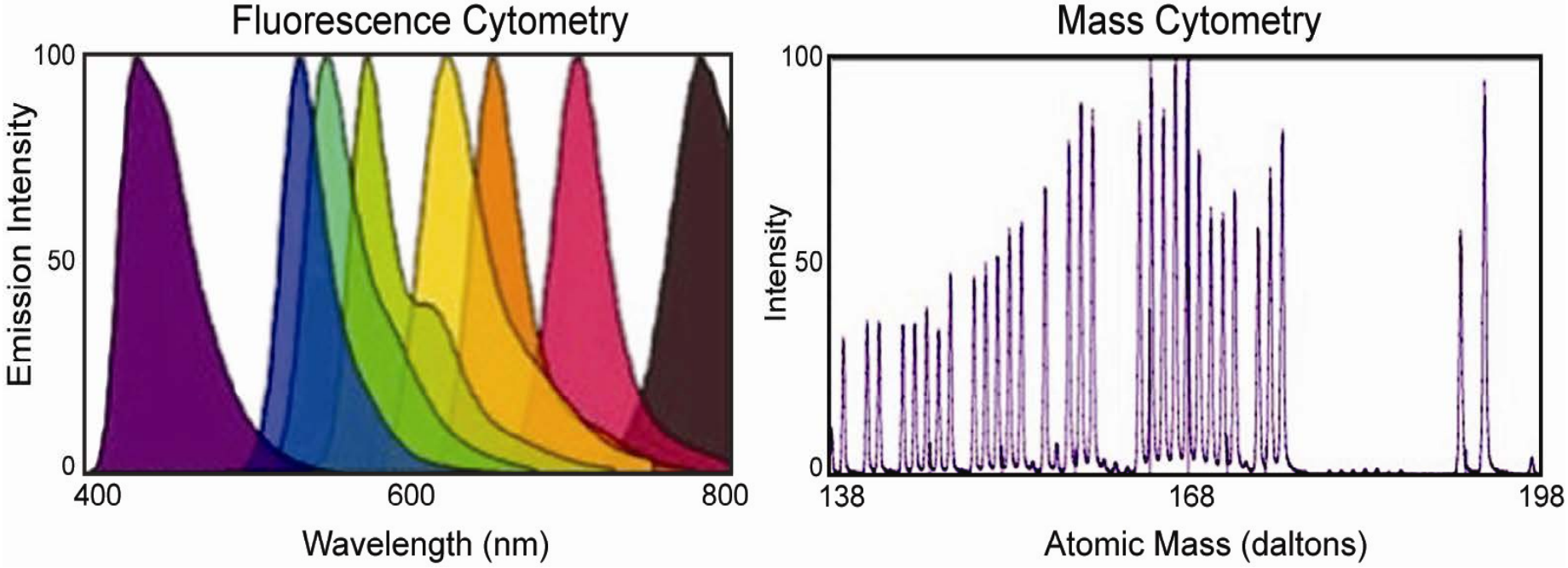
Technologies available for Immune Monitoring

- In addition to:
  - Proteomics (protein arrays using antibodies or HPLC)
  - Metabolomics (examining small molecules/products in human samples)
  - RNA-Seq (replacing microarrays)
  - Complete Genomic Sequencing
  - Methylomics of whole blood gDNA

CyTOF Technology

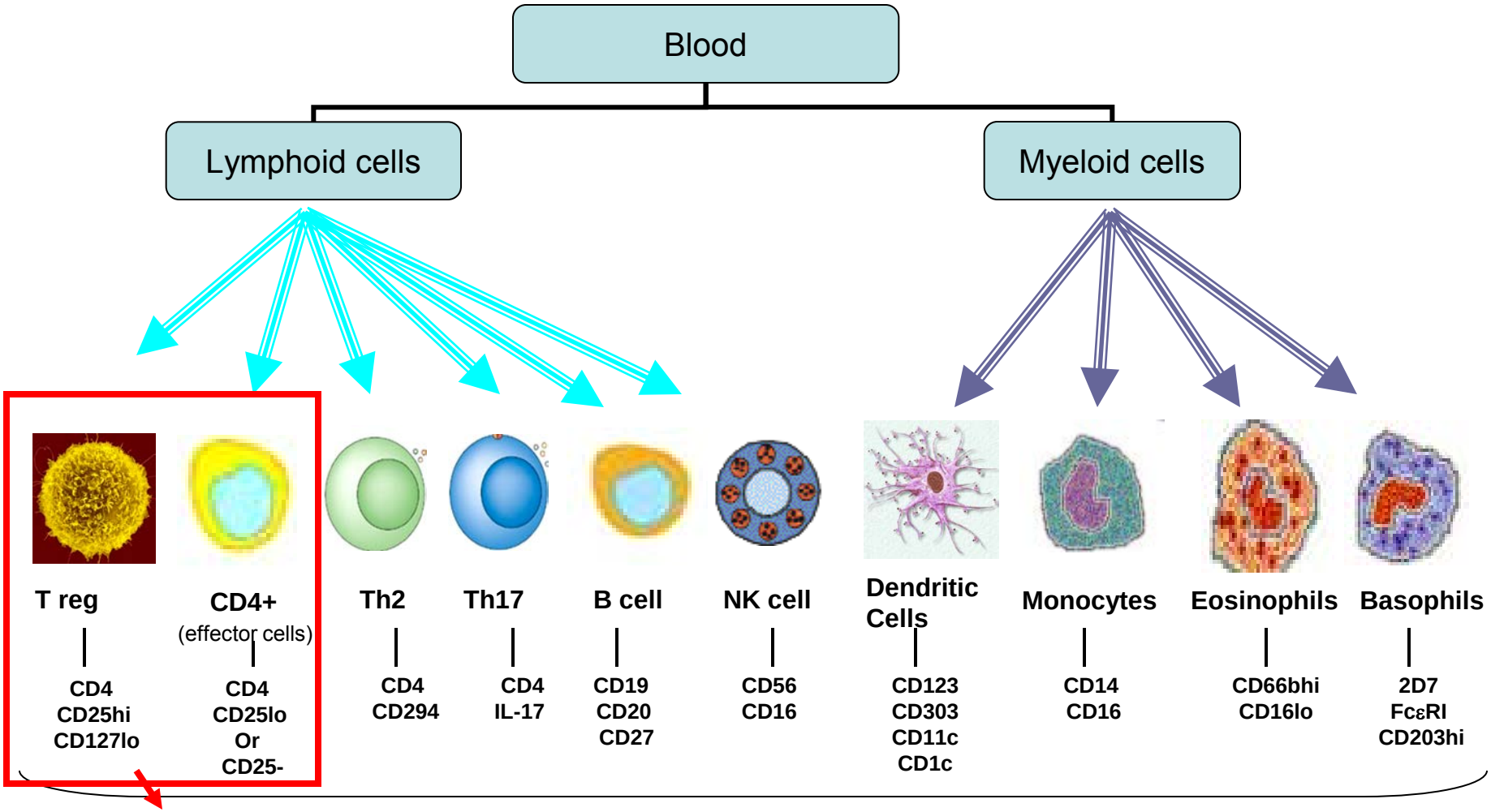
- Mass Spectrometry based cytometer
- Link 32 colors to be able to display many immune cells, intracellular products and activation pathways simultaneously
- Allows for functional studies to occur in small amounts of blood

Mass Cytometry (CyTOF) Accommodates More Antibody Specificities without Overlap, Greatly Improving Immunophenotyping of Cells involved in Allergy



The DVS Sciences (Toronto, CA) has produced inductively coupled plasma mass spectrometer (ICP-MS), known as CyTOF (for Cytometry by Time-of-Flight Mass Spectrometry). In the Stanford HIMC (Director, Holden Maecker, PhD) we have been able to detect up to 35 different parameters in a single sample.

Phenotypical Studies



Molecules expressed on cell surface with CCR4, CCR8, CD45RO, CD45RA

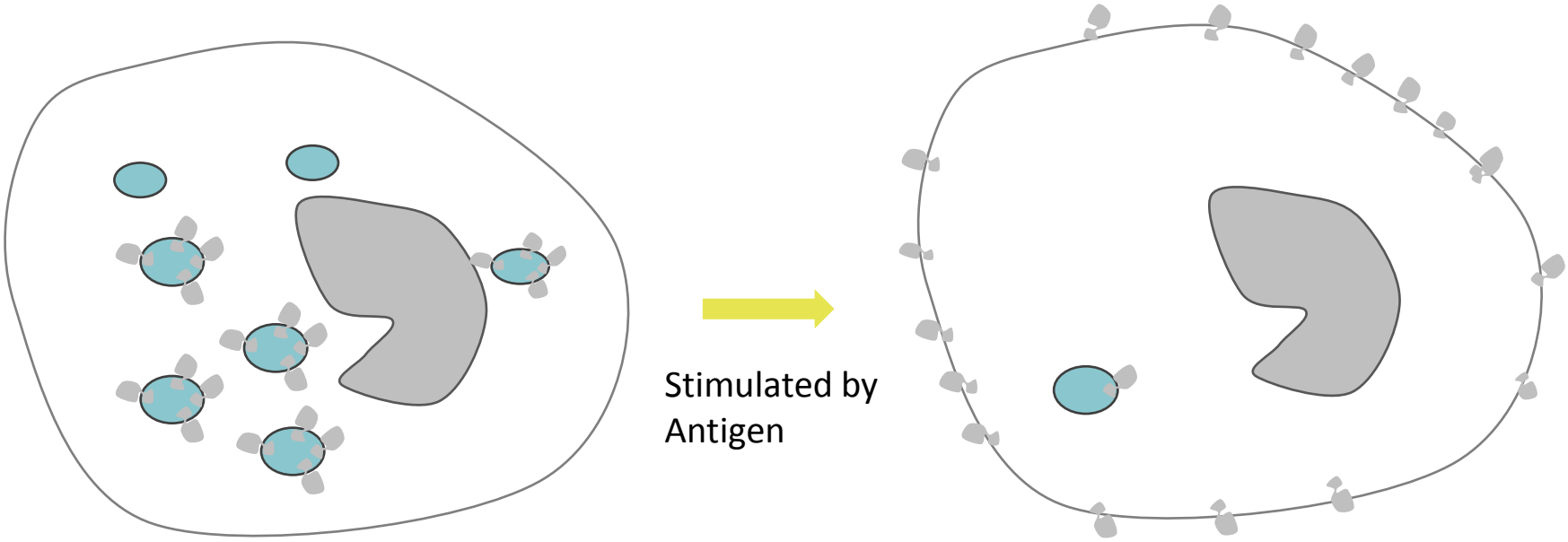
Intracellular markers to be stained: Foxp3, pSTAT5, CCL1, CCL22, CCL17, IL-4, IL-13, IL-10, TGF-β, IL-17, IFN-γ

Allergy Diagnosis

- Atopy risk vs Atopy
- IgE is not predictive
- Skin testing is not predictive
- Basophil activation test is being used currently as an improvement for allergy testing
- Gold Standard still remains the challenge test

New Diagnostic Allergy Test

based on rapid assessment of blood basophil activation

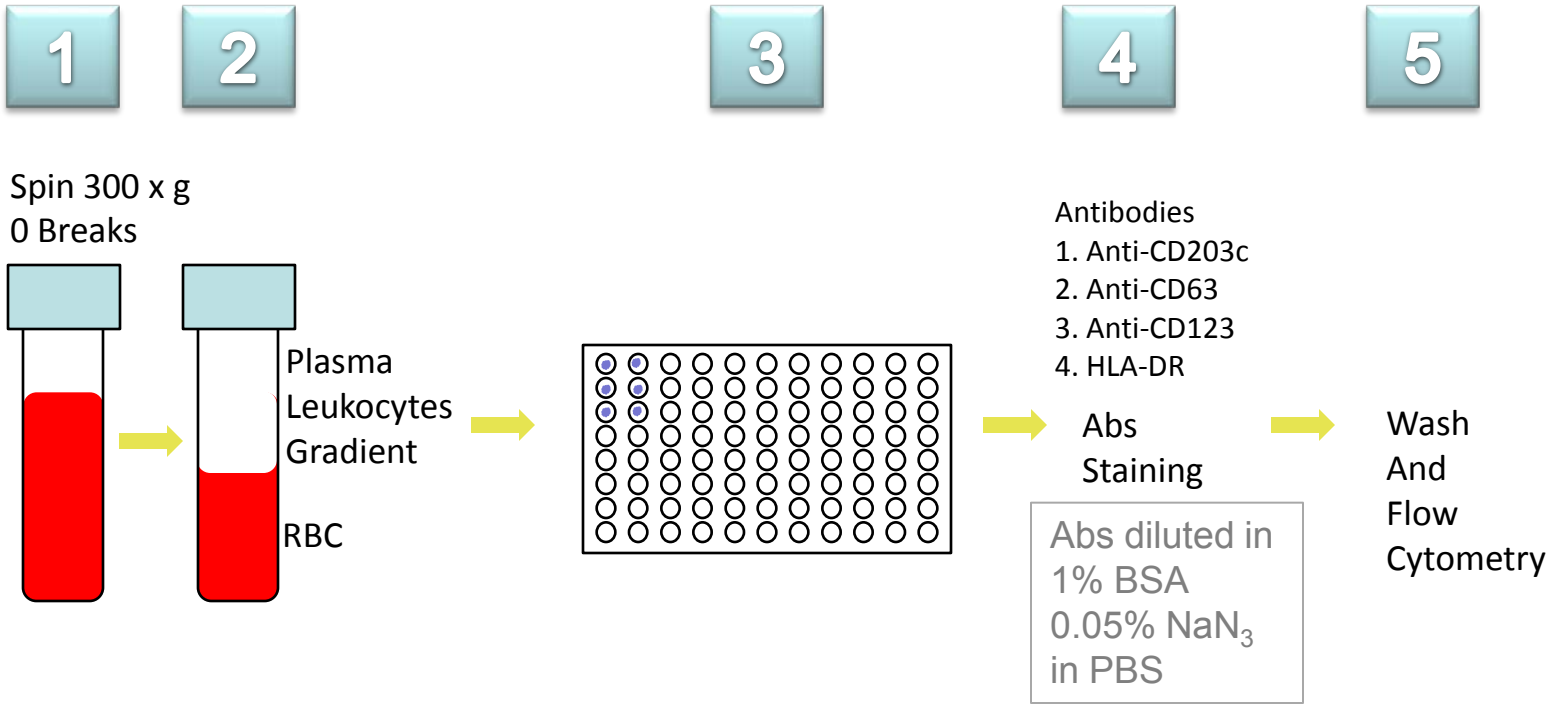


Unstimulated

Expression of molecules on the cell surface (expressed by cytoplasmic compartments) for example CD203c or CD63

New Diagnostic Allergy Test

Based on rapid assessment of blood basophil activation



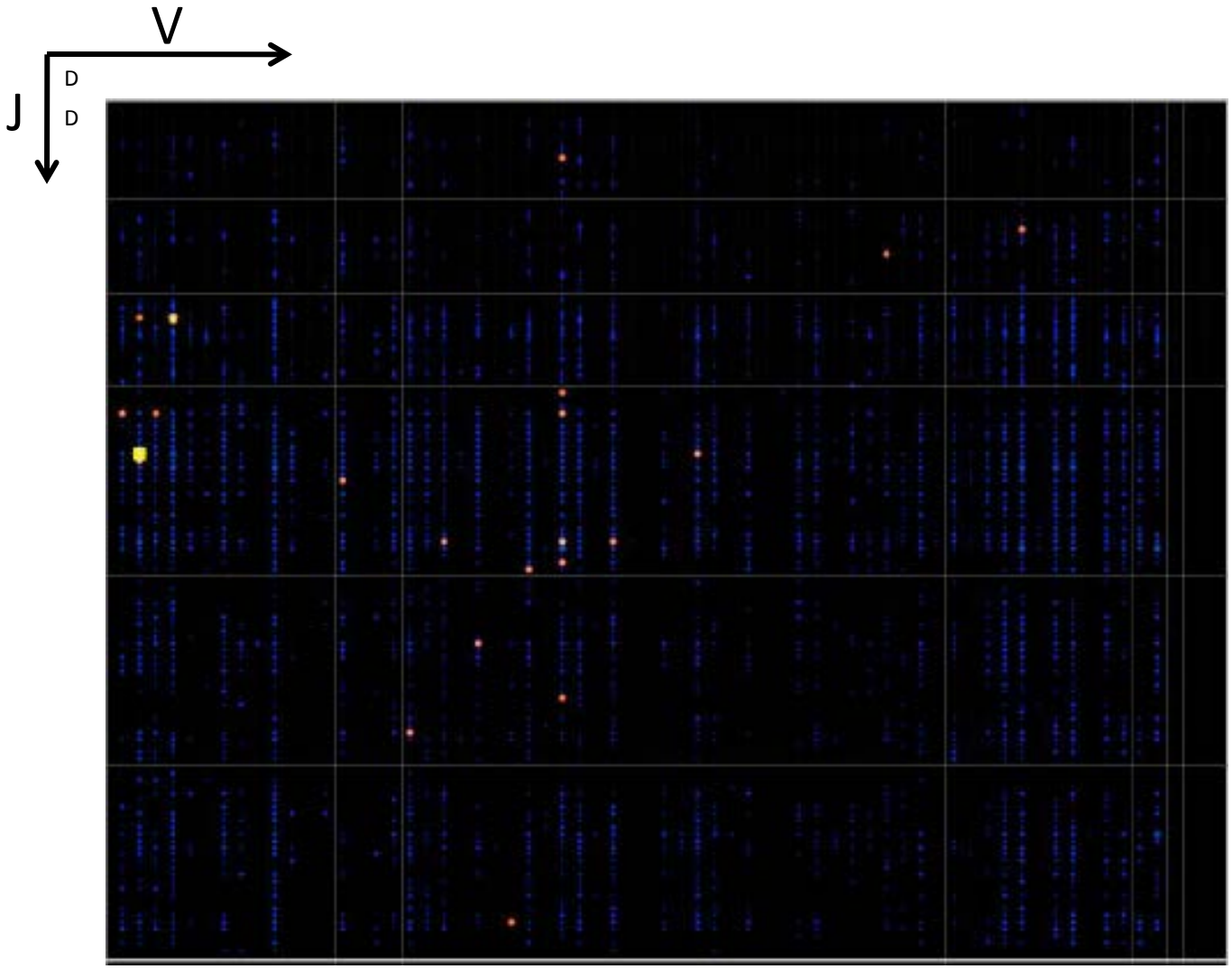
TIME To RESULT: ~ 1 HOUR

- **No RBC Lysis**
- **Activation stop during the staining with 1% BSA, 0.05% NaN_3**
- **Various Allergens/Patients can be tests at the same time**

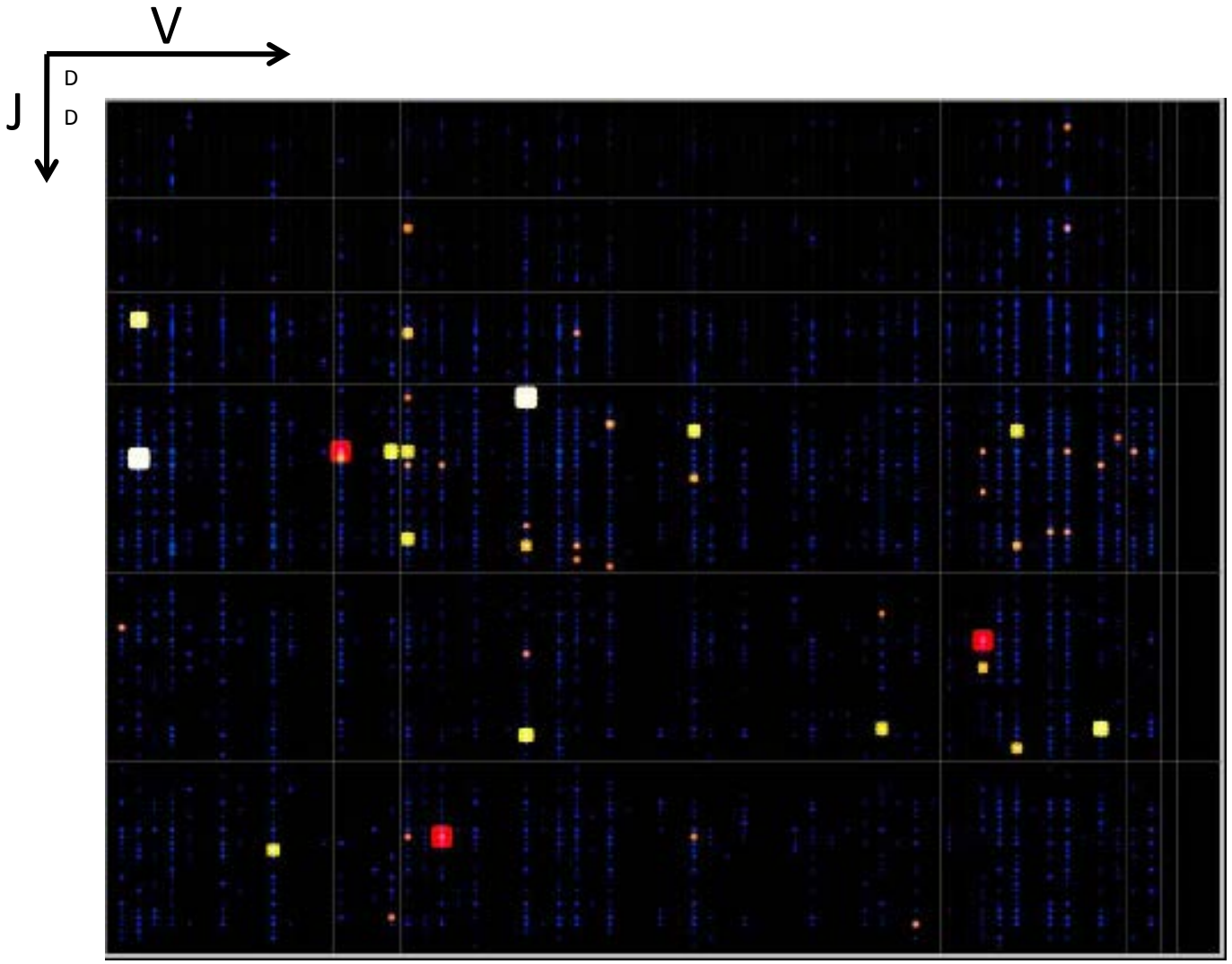
Immune Cell High Throughput Sequencing

- Hypothesis-based
- Certain VDJ recombinations are associated with allergies or neoplasia
- Can we detect these early before manifestation of disease?

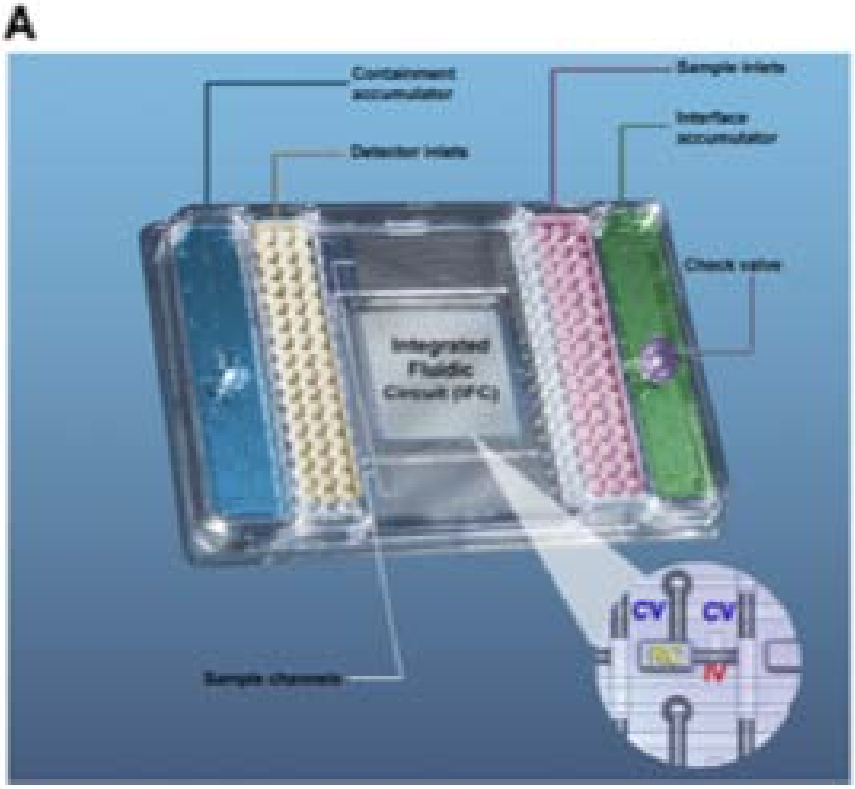
Detection of Clonal Sequences: pre

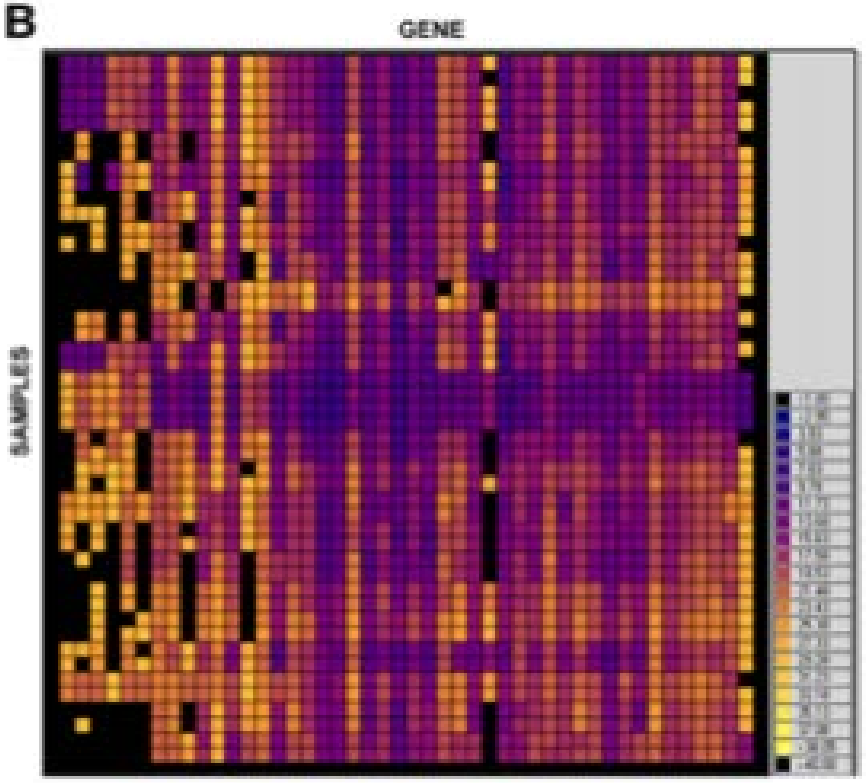


Detection of Clonal Sequences: post



Fluidigm Biomark Platform for Single Cell qPCR on Many Genes





Systems Biology Approach to Immune Monitoring in Human Samples

Summary and Next Steps

- High Throughput sequencing on small amts of blood
- Detailed phenotypical and functional studies on small amts of blood
- Single cell PCR will allow detection of critical changes in specific cell populations

Next steps

- Application of use to samples (thousands)
- Determine correlation with immune development and risk assessments in human development to toxican exposure