TRUAGE BY TRUDIAGNOSTIC

Immune

This report explores the impacts of various types of immune cells and their concentrations on biological age by examining associated methylation patterns at various locations of your DNA.

Developed By TruDiagnostic's Bioinformatics & Research Department

UNDERSTANDING

Biological aging & the immune system.

Do you ever wonder why many older adults experience a harder time battling diseases like COVID-19 or the common flu, compared to younger people who typically have an **easier time recovering** from the same illnesses?

It all boils down to the capabilities of one's immune cells to effectively respond to internal and foreign health threats; capabilities which tend to decrease with age. **This age-related decline of the immune response in our blood is called immunosenescence**.

As we get older, and immunosenescence occurs, higher incidences of infections, cancer, and autoimmune disease emerge. As research indicates, the progression of one's immune system decline, to the point of immunosenescence, **occurs faster in men than in women**. It is also characterized by age-related changes in immune cells and inflammatory mediators.

Immunosenescence also **changes the number of immune cells** in our blood. The average adult has more than five liters of blood in their body that carries oxygen and nutrients to living cells and disposes of cellular waste. Blood also delivers various types of **immune cells, which are types of white blood cells** (WBC), to fight infections throughout the body. These WBCs come in many different shapes and sizes, and the concentration of each immune cell type has varying associations with age-related DNA methylation patterns.

THE REASON WHY

Immune cells are important to all epigenetic algorithms.

As we age, we have **overall fewer** Naïve T Cells, Natural Killer Cells, Macrophages, Dendritic Cells, and other immune cell types throughout our body. However, concentrations of immune cell types also change based on what kind of sample or tissue you are analyzing, such as blood or saliva, regardless of age.

Each immune cell type, and its respective concentration, can indicate vastly different aging implications from other types of immune cells when isolated; forcing algorithm developers to ask, *'Is this pattern actually caused by aging, or is this pattern caused by the type of cell and the type of tissue we are examining?'*

Further potential for data pollution rests in whether or not someone's immune system was actively or recently fighting an illness at the time of sample collection; which can cause temporary changes in immune cell concentrations.

Say we were to isolate Naïve CD8T immune cells from Memory CD8T immune cells (both of which are found in different concentrations in your blood sample) to determine your biological age based on those cells alone. **The Naïve CD8T cells might say you're 40 years old**, for example, **while the Memory CD8T cells would say you're 55 to 60 years old.**

Instead of addressing this challenge by completely excluding immune biomarkers, all of our algorithms were trained and developed with a weighted and controlled representation of each immune cell type and its concentration in blood tissue.

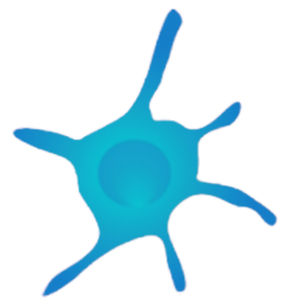
In doing so, we can ensure accurate results, free of potentially corrupting biodata. This includes our novel OMICm Age algorithm, as well as our other custom algorithms that have been developed to, as well as our other custom algorithms that have been developed to **ensure changes in immune cells don't give us false information about your biological age.**

THE IMPACTS OF

Immunosenescence on different immune cell types.

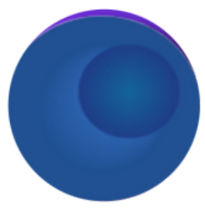
T Lymphocyte

- Reduced development and numbers of Naïve CD4T+/CB+ T cells
- Decline in CD4T+ function and in CD8T+ T cell totoxicity+ proliferation
- Reduced generation of Th subsets



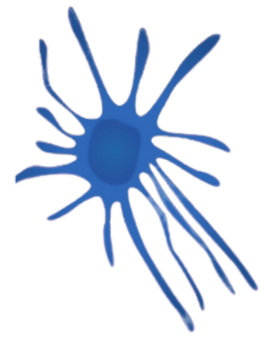
Dendritic Cell

- Reduced IFN production and expression CD25 and ICAM-1 in mature MODCs
- Reduction in lymphocyte cytotoxicity and greater of monocyte-macrophage derived APCs



B Lymphocyte

- Reduced development and numbers of Naïve B cells
- Decreased diversity of B cell repertoires and B cells responses to new antigens



Macrophage

- Defective phagocytosis
- Decreased cytokine production, antigen presentation, and superoxide anion production

Neutrophils

- Decreased phagocytosis, chemotaxis, and apoptosis function

Natural Killer

- Reduced cytolytic potential and CD1 expression in NKT cells
- Decreased cytokine and chemokine production

Your Results.

IMMUNE CELL TYPE	95% CONFIDENCE INTERVAL RANGE	YOUR PERCENTAGE	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
Naïve CD4T	7.196%-7.35%	3.59%	7.273	0.0383	1.446	**HIGHER**
Memory CD4T	5.14%-5.284%	2.05%	5.212	0.0361	1.647	**HIGHER**
Memory CD8T	6.519%-6.691%	9.00%	6.605	0.0430	1.346	**HIGHER**
Naïve CD8T	1.09%-1.16%	0%	1.125	0.0175	1.545	**HIGHER**
Basophils	1.026%-1.056%	0.77%	1.041	0.0076	2.389	**HIGHER**
B Memory	1.689%-1.785%	1.03%	1.737	0.0241	-1.57	**LOWER**
Naïve B	2.207%-2.311%	0%	2.259	0.0260	2.987	**HIGHER**
Regulatory T	0.604%-6.408%	2.89%	3.506	1.4510	-2.316	**LOWER**
Eosinophils	0.376%-0.424%	0%	0.400	0.0121	3.205	**HIGHER**
Natural Killer	3.353%-3.459%	5.08%	3.406	0.0264	-1.365	**LOWER**
Neutrophils	62.899%-62.953%	74.86%	62.93	0.0136	-1.781	**LOWER**
Monocyte	4.453%-4.567%	0.73%	4.510	0.0285	-2.981	**LOWER**

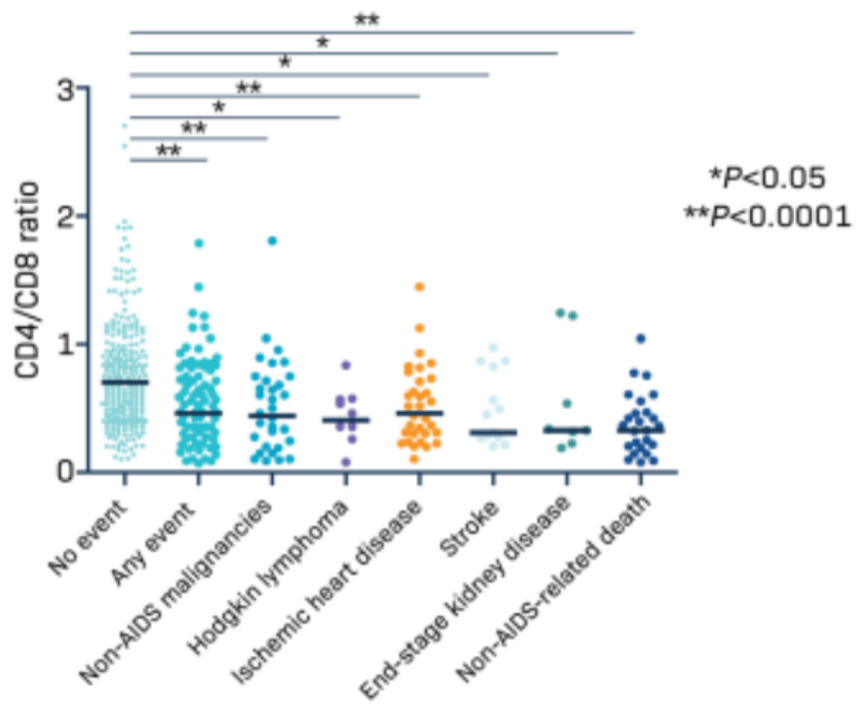
UNDERSTANDING

CD4T/CD8T Cell Ratio.

CD4T/CD8T cell ratio is incredibly informative on disease. A value between 1 and 4 is ideal. A value between 0 and 1 marks an “inverted ratio”. A low or inverted CD4T/CD8T ratio is an immune risk phenotype and is **associated with altered immune function, immune senescence, and chronic inflammation**.

The prevalence of an inverted CD4T/CD8T ratio increases with age. An inverted ratio is seen in 8% of 20-59 year olds and in 16% of 60-94 year olds. Women across all age groups are less likely to have an inverted ratio than their male counterparts.

Age and hormone-related atrophy of the thymus are theorized to explain the differences between populations. Hormonal influence on the ratio is supported by a correlation between low Plasma Estradiol levels, high circulating CD8T, and low CD4T/CD8T ratios in women with premature ovarian failure.

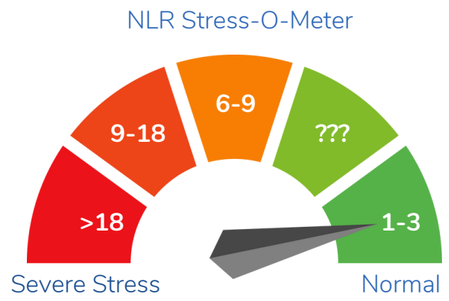


We have been able to refer patients for additional testing to diagnose HIV, Chronic Lymphocytic Leukemia, and even individuals taking their Rapamycin at too high of a dose. **If you see a low CD4T/CD8T ratio, it is not an immediate cause for concern but we might recommend testing via traditional labs just in case**. A value of 4+ marks hyperactivity or possible infection, autoimmunity, or additional immune risk phenotypes.

CELL TYPE	REFERENCE RANGE	YOUR RATIO	MEAN	SD	# OF STANDARD DEVIATIONS ABOVE OR BELOW MEAN	IS THIS HIGHER OR LOWER THAN ANTICIPATED?
CD4T/CD8T T Cell Ratio	1.00-4.00	1.61	2.59	0.074	1.469	**HIGHER**

RATIO	ABOUT THIS RATIO	YOUR VALUE
Regulatory T Cells to Total T Lymphocytes (RegT/all other T Cells)	There is evidence that Tregs exhibit atheroprotective properties by suppression of autoreactive T cell responses or by secretion of anti-inflammatory cytokines (Pastrana et al., 2012). Thus this might be a marker for cardiovascular disease. (www.sciencedirect.com)	0.147
Adaptive to Innate Immune (A/A Ratio)	The adaptive-to-innate immune ratio (A/I ratio) has been linked to response to several types of immunotherapy.	0.517

Other Ratios to Prioritize.

RATIO	ABOUT THIS RATIO	NORMATIVE RATIO	YOUR VALUE
Neutrophil to Lymphocyte	The NLR is simply the number of Neutrophils divided by the number of Lymphocytes. Under physiologic stress, the number of Neutrophils increases, while the number of Lymphocytes decreases. The NLR ***combines*** both of these changes, making it more sensitive than either alone: Effect of Physiologic Stress on the NLR: ↑↑ NLR= Neutrophils / Lymphocytes Endogenous cortisol and catecholamines may be major drivers of the NLR. Increased levels of cortisol are known to increase the neutrophil count while simultaneously decreasing the lymphocyte count. Thus, NLR is not solely an indication of infection or inflammation. Any cause of physiologic stress may increase the NLR (e.g. hypovolemic shock).	 Neutrophil-to-Lymphocyte ratio (NLR) reflects the amount of physiologic stress. The optimal cutoff value will vary depending on the specific patient population and disease state. The numbers provided above are. intended merely to provide a general concept of NLR interpretation.	1.724
Monocyte to Lymphocyte	MLR (Monocyte to Lymphocyte ratio) has been demonstrated to be a novel hematological and inflammatory parameter. MLR is associated with various diseases, such as community-acquired pneumonia, axial spondylarthritis, and coronary angiography, as well as the systemic inflammatory response, which reflects the abnormal immune status of diseases.	The mean Neutrophil-to-Lymphocyte ratio in the whole population was 1.70±0.70 (Range: 8.38, Min: 0.23, Max: 8.61), mean lymphocyte-to-monocyte ratio was 11.15±3.14 (Range:23.21, Min:3.46, Max:26.67), and mean platelet-to-lymphocyte ratio was 117.05±47.73 (Range:93.60, Min:19.11, Max:1598.77).	11.45

THE PERCENTAGE OF

Immune cells in our blood can be highly informative to health.

'Health outcomes' is a term used to encompass an interconnected set of attributes, that cumulatively describe the consequences of disease for an individual; aka, **how extensively does an illness impact your life and overall health**?

These attributes include impairments, symptoms, functioning capabilities, participation in activities and social roles, and overall health-related quality of life. Health outcomes also tell us how long, on average, people live within a given community, and how much physical and mental health they experience within their lifetime.

There are **many factors that impact health**, such as education, environment, lifestyle habits, access to healthcare, and socioeconomic stability.

There are also **many immune cell types** that are influenced by these factors, and that have direct associations with health risks and outcomes. Some of these include **Naïve CD4T** and **Naïve B** T-cells, which help protect the body from infection and cancer, **Natural Killer** immune cells that use enzymes to kill infected and cancerous cells, as well as **Memory CD4T** and **Memory B** T-cells which help the immune system coordinate and adapt its response.

Naïve CD4T

Decreased concentrations have been associated with an increased risk of COPD and Type 2 Diabetes, but decreased risk of all-cause mortality.

Naïve B

Decreased concentrations have been associated with a decreased risk of all-cause mortality.

Memory CD4T

Decreased concentrations have been associated with an increased risk of all-cause mortality.

Memory B

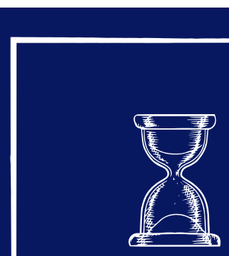
Increased concentrations have been associated with an increased risk of cancer, but decreased risk of Type 2 Diabetes.

Natural Killer

Decreased concentrations have been associated with a decreased risk of all-cause mortality.

Immune cells impact health outcomes regardless of age, sex, race, smoking habits, obesity, and alcohol consumption. However, **lifestyle habits and environmental factors can impact the quantity of, and health of, different immune cells**. For example, research shows a decrease in Natural Killer cell counts associated with smoking, obesity, and stress levels.

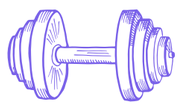
DNA methylation patterns show that certain lifestyle and environmental factors are **associated with increases and decreases in specific types of immune cells**. As the concentration of these cells changes, so does the risk for diseases such as stroke, Type 2 Diabetes, COPD, depression, cancer, and more.



AGE

BMI

ALCOHOL



EXERCISE

ASSOCIATED WITH **higher levels of**

Memory CD4T
B Memory
Natural Killer (NK)
Neutrophils
Monocyte

Memory CD4T

Memory CD4T
Basophils
Monocyte

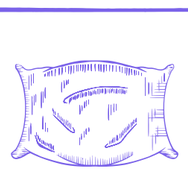
Natural Killer (NK)
Monocyte

ASSOCIATED WITH **lower levels of**

Naïve CD4T
Naïve CD8T
Regulatory

Naïve CD4T
Naïve CD8T
Regulatory
Natural Killer (NK)

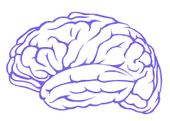
Naïve B



SLEEP

SMOKING

CAFFEINE



STRESS

MATERNAL SMOKING

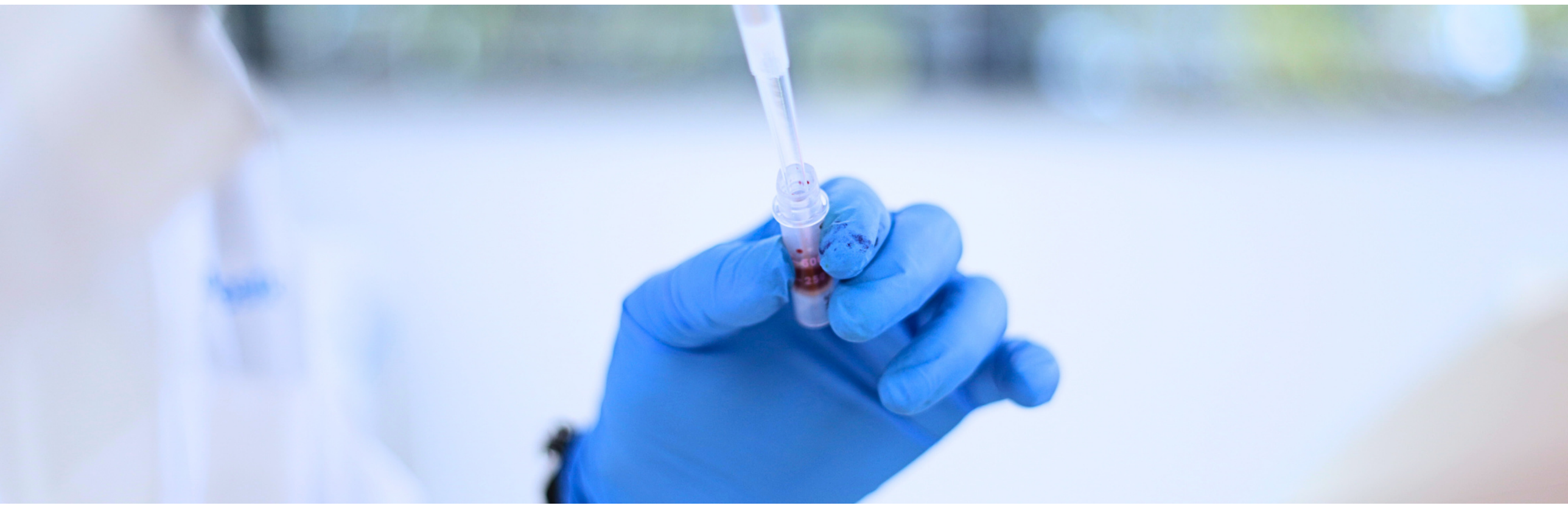
Natural Killer (NK) *Natural Killer (NK)* *Naïve CD4T* *Natural Killer (NK)* *Memory CD8T*

CELL TYPE	FACTOR WHICH ARE ASSOCIATED WITH HIGHER LEVELS	FACTORS WHICH ARE ASSOCIATED WITH LOWER LEVELS
Naïve CD4T	Alcohol, Caffeine	Age, BMI
Memory CD4T	Alcohol, Age, BMI	
Memory CD8T		Maternal Smoking
Naïve CD8T		Age, BMI
Basophils	Alcohol	
B Memory	Age	
Naïve B		Exercise
Regulatory		Age, BMI
Eosinophils		
Natural Killer	Sleep, Exercise, Age	Stress, BMI, Smoking
Neutrophils	Age	
Monocyte	Age, Alcohol, Exercise	

Notably, Naïve CD4T+ T-cell, Naïve B-cell, and Natural Killer cell fractions are all associated with a reduced risk of all-cause mortality, even after adjustment for all major disease risk factors. Interestingly, whilst the Naïve CD4T+ T-cell fraction also displayed negative associations with many health outcomes, notably with COPD and Type 2 Diabetes (T2D), the Memory CD4T+ T-cell fraction was only negatively associated with all-cause mortality. An increased Memory B-cell fraction was specifically associated with an increased risk of cancer but a reduced risk for T2D, whilst no associations were observed for the other outcomes.

A SCIENTIFIC DEEP DIVE

Methods & Applications



You may be wondering how it's possible that every cell in your body has the same DNA, but a heart cell behaves like a heart cell, and a hair cell behaves like a hair cell, etc. The answer is epigenetics! Epigenetics controls cell development and function by **switching certain genes on and off, which determines phenotype and how your cells behave**. It makes sense that the epigenetic regulation of each cell would depend on its cell type. You wouldn't want your heart to make the proteins found in your hair and vice versa. Thus, each cell has a different **epigenetic signature**.

This means that in order to create an accurate, predictive algorithm from DNA methylation data, one must know **what cell types** are being tested. Otherwise, the information from these **algorithms can give false information**.

For example, if you were to test your brain cells, you would see lower biological ages than if testing blood. We also see that breast tissue can age faster than other tissues across the rest of our body. The same is true with blood if someone is sick and they have increased B cells (cells that produce antibodies), that could alter results in a way that is not consistent with the actual health of an individual.

Therefore, the **rate of aging we calculate is dependent on what cell types we measure**. Using blood as the sample type, we determine what cells are we looking at, and more importantly, we control for different cell representations so our algorithm is accurate and predictive.

Immune Deconvolution.

As cells differentiate from pluripotent stem cells to the tissue type they become, they start to **form unique DNA methylation** patterns that can tell us which cell type they belong to.

By analyzing DNA methylation patterns in a tissue sample, we can infer the relative abundance of different cell types present in that sample. This is because different cell types have distinct DNA methylation profiles. With that information, we create algorithms that use DNA methylation to estimate the relative proportions of different cell types within a tissue sample.

Overall, this technique allows TruDiagnostic to gain a **better understanding of the cellular composition of a complex tissue sample**, which can be useful for understanding disease processes and monitoring the effects of interventions.

Accuracy of Results.

We've successfully demonstrated that our testing is comparable with the gold standard of Flow Cytometry with **less than 3% error**. We believe this is a needed algorithm to improve all methylation analysis algorithms in the future, and we have also developed a saliva deconvolution method for this very reason.

This immune deconvolution tool has been used in large biobank datasets to look at associations and trends. We believe that this might be a great tool to quantify the immune system and to find novel associations to disease conditions without having to use Flow Cytometry; which can be expensive, require high volumes of blood, and requires refrigerated sample processing.

Research & Partnerships.

The algorithms we've used to generate your results in this report are a product of academic processing and analysis from our private epigenetic database, along with research partnerships with Harvard University, Johns Hopkins University, and the Chinese Academy of Sciences.

Additionally, research into senescence has grown exponentially over the last few years. However, there are still very few tools to easily quantify this process. With Ohio State, we have created senescence predictors of t-cells through DNA methylation.

We are **now developing methods** to apply this to all tissues with additional datasets and believe this will be a valuable tool to quantify this hallmark of aging.

HARVARD

JOHNS HOPKINS UNIVERSITY

OHIO STATE

> **A meta-analysis of immune cell fractions at high resolution reveals novel associations with common phenotypes and health outcomes**
>
> Qi Luo, Varun B. Dwaraka, Qingwen Chen, Huige Tong, Tianyu Zhu, Kirsten Seale, Joseph M Raffaele, Shijie C. Zheng, Tavis L. Mendez, Yulu Chen, Sofina Begum, Kevin Mendez, Sarah Voisin, Nir Eynon, Jessica A. Lasky-Su, Ryan Smith, Andrew E. Teschendorff