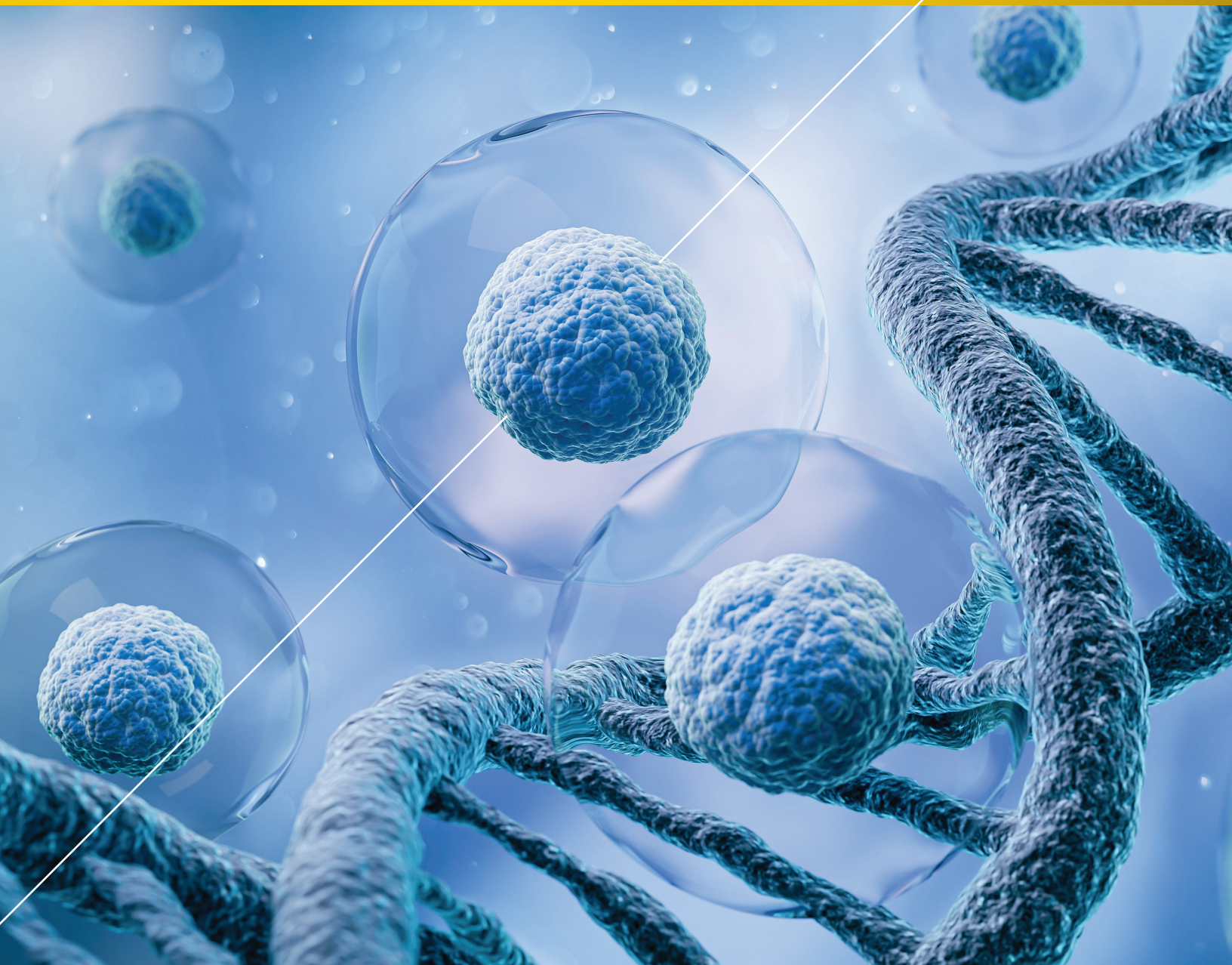
Epiontis ID®
Use Cases Across Diverse Therapeutic Areas

PRECISION for medicine®



Contents

Epiontis ID – Introduction

Epiontis ID is a robust, adaptable, cost-effective technology that uses specific epigenetic markers. It allows simplified trial logistics with consistent results and is ideal for immune monitoring in all stages of clinical trials. This document explores Epiontis ID's applications in various therapeutic areas, highlighting its role in precise immune monitoring for advanced research.

Easy Logistics, Reliable Results

Epiontis ID is versatile and compatible with diverse sample types, necessitating minimal processing. Samples can be transported ambient within a 3-day window or batch-shipped in a frozen state. All assays are uniformly performed in facilities accredited with ISO 17025 and compliant with GCP/ICH guidelines.

Choose from >35 fully validated assays ▶ **Collect samples at clinical site without processing** ▶ **Ship ambient or frozen in batches** ▶ **Samples arrive at central lab, measured with quick turnaround**

Epiontis ID offers more than 35 fully validated assays. Additional assays are continuously added following extensive validation, ensuring excellent accuracy and reproducibility.

T Lymphocytes	Other Immune Cells	Exhaustion/Activation/ Migration Markers	Other Cell Types (Fibrocytes)
• CD3 T cells	• B cells	• PD1+ cells	• Col1A1+ cells
• CD4 T cells	• NK cells	• TIGIT+ cells	• PDGFRB+ cells
• CD8 T cells	• Neutrophils	• CTLA4+ cells	
• Regulatory T cells	• Eosinophils	• LAG3+ cells	
• Th17 cells	• Basophils	• CXCR3+ cells	
• TFH cells	• Monocytes	• Granulysin+ cells	
• Gamma delta (γδ) T cells	• NC monocytes	• CCR7+ cells	
• GATA3+ cells	• Monocytic MDSC	• IL6R+ cells	
• CD4 memory T cells	• Plasmacytoid DC	• CCR6+ cells	
• CD8-naive T cells	• Naive B cells	• CRTH2+ cells	
	• Memory B cells	• S1PR1+ cells	
	• IgM+ B cells	• S1PR5+ cells	
		• Integrin alpha 4+ cells	
		• CCR9+ cells	

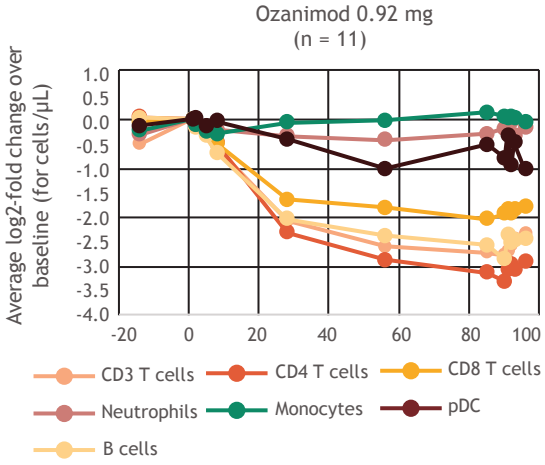
Autoimmune Diseases – Immune Cell Monitoring in Multiple Sclerosis

Monitoring of T and B cells is a key focus in autoimmune diseases; it aims to characterize the adaptive immune response that may overreact against self-antigens. Additional frequently monitored cell subpopulations include immune-suppressive regulatory T cells (Treg) and proinflammatory Th17 cells.

Advantage of Epiontis ID → The portfolio includes a wide range of fully validated assays for cell types of the adaptive and the innate immune system.

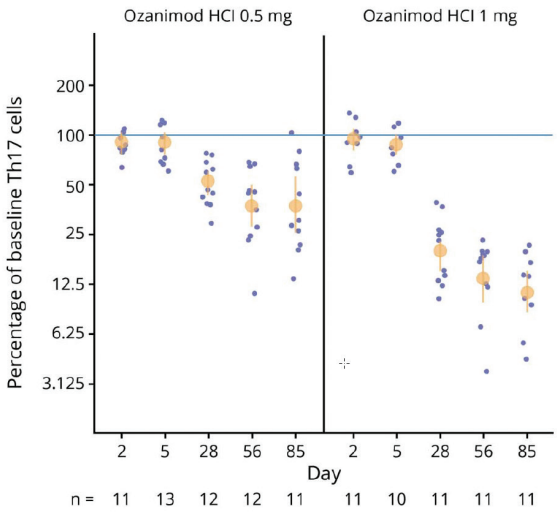
Ozanimod-induced reduction of T and B cells in a multiple sclerosis trial.

Results from this study show that ozanimod treatment significantly reduces adaptive immune system cells, including **CD3, CD4, and CD8 T cells**, as well as B cells, while largely preserving the cell types of the innate immune system, such as neutrophils, monocytes, and pDC cells.[1]



Ozanimod-induced reduction of proinflammatory Th17 cells in a multiple sclerosis trial.

In addition to the general reduction of T and B cells, ozanimod causes a very prominent reduction of proinflammatory Th17 cells.[2]



Phase I study by BMS investigating ozanomid in multiple sclerosis.

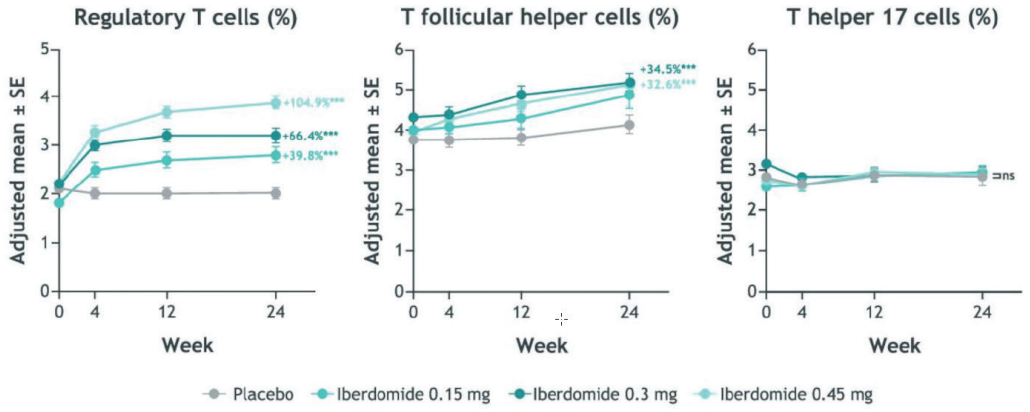
1. Harris S, Maddux R, Hoffmueller U, Raschke E. Effect of ozanomid on circulating leukocyte subtypes in patients with relapsing multiple sclerosis and comparison with healthy volunteers. Poster presented at: 37th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS); October 13-15, 2021; Vienna, Austria.
2. Harris S, Tran JQ, Southworth H, Spencer CM, Cree BAC, Zamvil SS. Effect of the sphingosine-1-phosphate receptor modulator ozanimod on leukocyte subtypes in relapsing MS. *Neurol Neuroimmunol Neuroinflamm*. 2020;7(5):e839.

Autoimmune Diseases – Immune Cell Monitoring in Lupus and Psoriasis

Advantage of Epiontis ID → Offers assays for many cell types, including CD4+ T cells, Treg, and Th17 cells

Monitoring of Tfh, Treg, and Th17 cells in a lupus trial.

In this study, a concentration-dependent increase of Treg is observed in a lupus cohort, while proinflammatory Th17 cells do not show any change. This suggests a more suppressed immune state after treatment. In addition, Tfh cells were monitored, which showed only small changes.[3]

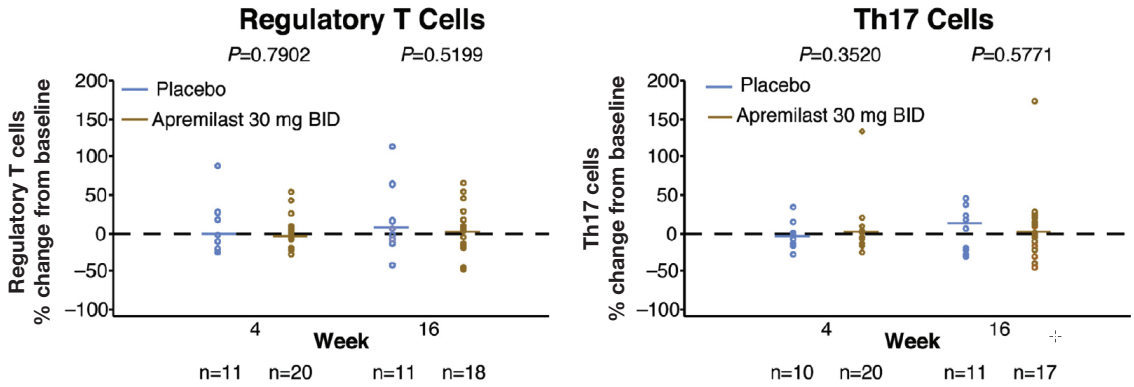


Phase II study by BMS investigating iberdomide in lupus.

3. Lipsky PE, van Vollenhoven R, Dörner T, et al. Biological impact of iberdomide in patients with active systemic lupus erythematosus. *Ann Rheum Dis*. 2022;81(8):1136-1142.

Monitoring of Treg and Th17 cells in a psoriasis trial.

This study observed no change in the peripheral blood levels of Treg and Th17 cells up to 16 weeks in a psoriasis trial with apremilast. This suggests that the observed cytokine change (not shown here) was due to increased activity rather than changes in cell numbers.[4]



Median Percentage Change From Baseline to Regulatory T Cell and Th17 Numbers at Week 4 and Week 16

Phase IV study by Celgene investigating apremilast in psoriasis.

4. Strober B, Alikhan A, Lockshin B, Shi R, Cirulli J, Schafer P. Apremilast mechanism of efficacy in systemic-naive patients with moderate plaque psoriasis: pharmacodynamic results from the UNVEIL study. *J Dermatol Sci*. 2019;96(3):126-133.

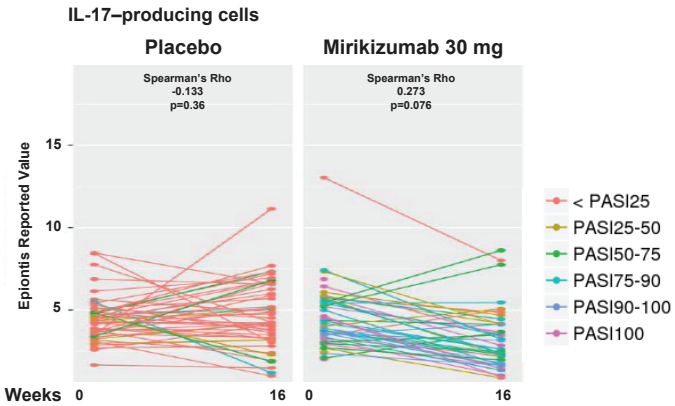
Autoimmune Diseases – Monitoring at the Site of Inflammation

Autoimmune diseases can affect various tissue types, such as the skin in atopic dermatitis or psoriasis, the joints in rheumatoid arthritis, or the kidneys in lupus nephritis. Monitoring immune cells both in the periphery and at the site of inflammation using the same technology enables seamless comparison of results.

Advantage of Epiontis ID → Use for immune infiltration in tissue, eg, skin or colon

Monitoring Th17 cells in skin tissue in psoriasis.

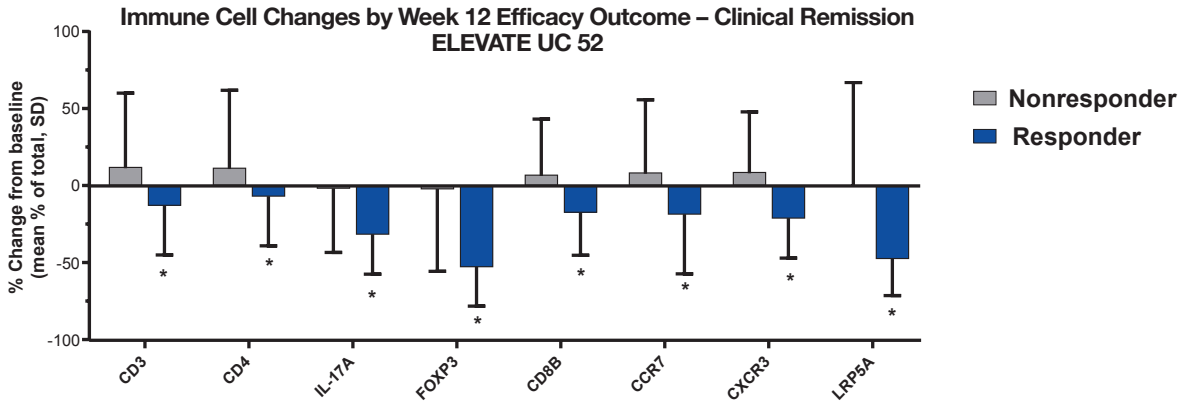
In this study, after 16 weeks of treatment with mirikizumab, a significant percentage of patients achieved clinical improvement (higher PASI scores), which was correlated with a substantial reduction in proinflammatory Th17 cells in the skin.[5]



Phase II study by Eli Lilly investigating mirikizumab in psoriasis.
5. Bissonnette R, Schmitz J, Patel D, et al. Effects on CD3, Treg, and TH17 cell numbers in skin biopsies after 16-week mirikizumab treatment, evaluated by an epigenetic assay. Poster presented at: 77th Annual Meeting of the Society for Investigative Dermatology; May 8-11, 2019; Chicago, IL.

Monitoring immune cell infiltration in colon tissue in an ulcerative colitis trial.

This study showed that clinical remission after 12 weeks of treatment correlated significantly with the loss of immune infiltration in colon biopsies.[6]



Phase III study by Arena/Pfizer investigating etrasimod in ulcerative colitis.
6. Siegmund B, Komori HK, Abreu MT, et al. Effect of etrasimod on immune cell subsets in colonic tissue of patients with ulcerative colitis: immunophenotyping analysis of colon biopsy samples from the phase 3 ELEVATE UC 52 and ELEVATE UC 12 trials. Poster presented at: 18th Congress of European Crohn's and Colitis Organisation; March 1-4, 2023; Copenhagen, Denmark.

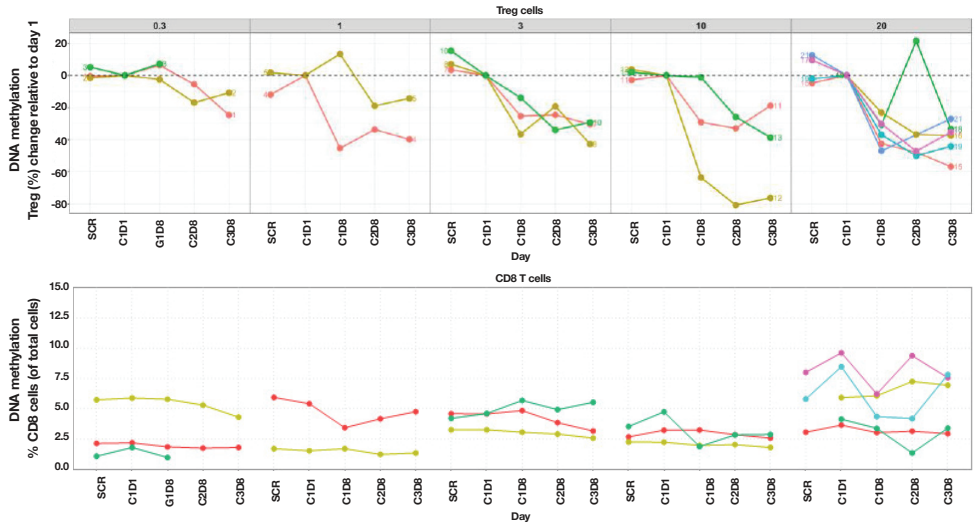
Oncology – Immune Monitoring in Peripheral Blood and Tissue

Characterizing the interaction between the immune system in the periphery and the tumor microenvironment (TME) is crucial for the successful development of cancer therapies. In this regard, qPCR immunophenotyping provides a unique solution, as it can be applied to both peripheral blood and the measurement of immune cell infiltration in tumor tissue.

Advantage of Epiontis ID → Use in blood and tissue samples

Treg monitoring in peripheral blood in a solid cancer trial.

In this study, treatment with etigilimab, an inhibitor of the immune checkpoint receptor TIGIT, resulted in a time- and concentration-dependent reduction of Treg cells, as observed through Epiontis ID and flow cytometry (not shown here). CD8 T cells, however, remained largely unchanged by etigilimab.[7]



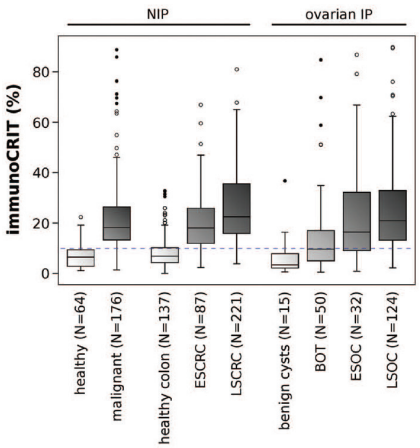
Treg cell results in 5 patient groups treated with increasing doses of etigilimab

CD8 T-cell results in 5 patient groups treated with increasing doses of etigilimab

Phase I study by OncoMed investigating etigilimab in solid cancer.
7. Huang Y, Brunner A, Cai S, et al. Interim biomarker analysis of etigilimab (OMP-313M32), an anti-TIGIT antibody, in advanced solid tumors supports TIGIT-associated mechanisms of action. Poster presented at: 33rd Annual Meeting of the Society for Immunotherapy of Cancer; November 7-11, 2018; Washington, DC.

Treg increase in the TME with increasing tumor aggressiveness.

In this study, the immunocrit (Treg cells as a percentage of total T cells) significantly increased in tumor tissue compared to healthy tissue. Moreover, higher levels were observed with increasing tumor aggressiveness.[8]



BOT=benign ovarian tissue; ESOC/LSOC=early-/late-stage ovarian carcinoma; ESCRC/LSCRC=early-/late-stage colorectal carcinoma.

Study investigating the cellular ratio of immune tolerance. 8. Türbachova I, Schwachula T, Vasconcelos I, et al. The cellular ratio of immune tolerance (immunoCRIT) is a definite marker for aggressiveness of solid tumors and may explain tumor dissemination patterns. *Epigenetics.* 2013;8(11):1226-1235.

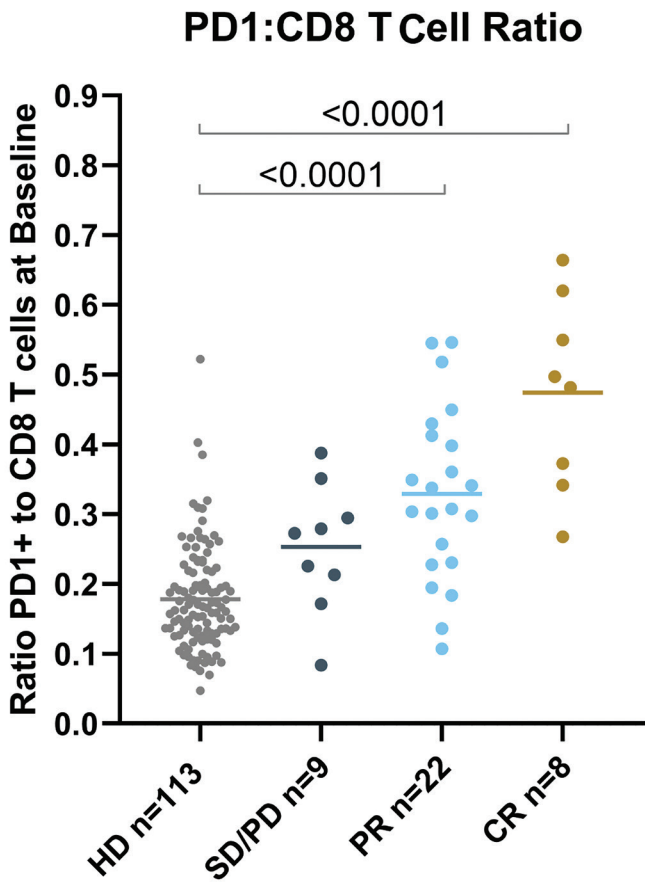
Oncology – Prognosis and Prediction of Clinical Response

Establishing the prognosis of a disease and predicting the clinical response to treatment require accurate and consistent measurement of biomarkers. Epiontis ID technology offers excellent interassay precision and reproducibility, even when samples are measured years apart. This makes it suitable for both establishing a disease prognosis and predicting clinical response.

Advantage of Epiontis ID → Precision and reproducibility allow for prognosis and clinical response prediction

Patients with high levels of immune exhaustion show better clinical response to a drug targeting immune exhaustion.

In a study on hepatocellular carcinoma, TACE (localized chemotherapy) was combined with nivolumab, an anti–PD-1 drug. Patients with stable or progressive disease (SD/PD) exhibited similar pretreatment ratios of PD-1+ and CD8 T cells compared to healthy donors (HD). However, patients with a partial or complete clinical response (PR/CR) showed significantly elevated pretreatment levels for this biomarker ratio.[9]



Phase II study of transarterial chemoembolization in combination with nivolumab performed for intermediate-stage HCC.

9. Saborowski A, Waldschmidt D, Hinrichs J, et al. IMMUTACE: a biomarker-orientated phase II, single-arm, open-label AIO study of transarterial chemoembolization (TACE) in combination with nivolumab performed for intermediate-stage hepatocellular carcinoma (HCC; AIO-HEP-0217) – updated efficacy results. Poster presented at: 2022 ASCO Annual Meeting I; June 3-7, 2022; Chicago, IL.

Respiratory and Infectious Diseases – Immune Cell Monitoring in Nasopharyngeal Swabs

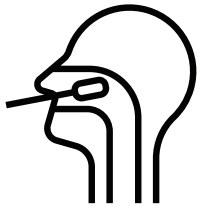
COVID-19 is linked to a significant imbalance in the immune system, characterized by relative lymphopenia, neutrophilia, and a decreased lymphocyte-to-neutrophil ratio, which correlates with a more severe disease progression.

Conducting self- and home testing for virus infection and relevant cell populations can aid in patient risk assessment. Epiontis ID facilitates the measurement of immune cells in sample types that do not necessitate venous blood collection, such as immune monitoring in capillary blood or at the site of infection, like nasopharyngeal swabs.

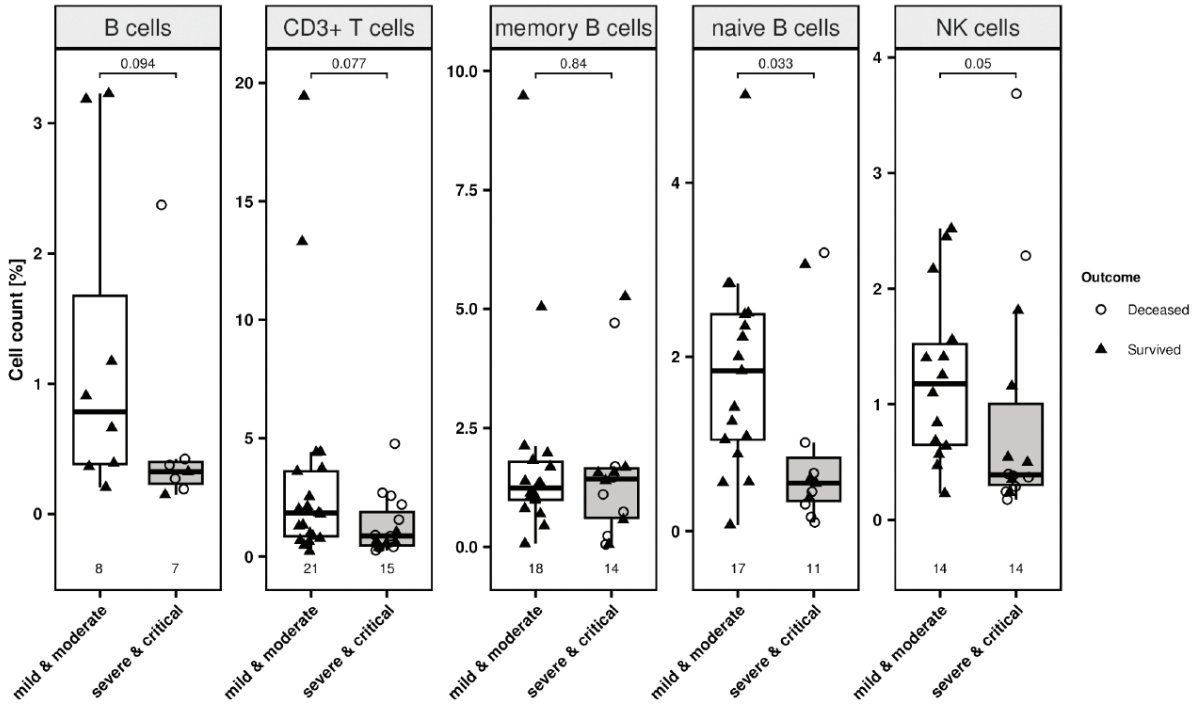
Advantage of Epiontis ID → Immune monitoring in finger-prick blood (dried blood spots [DBS]) or in nasopharyngeal swabs

Immune cell monitoring of T cells, B cells, and NK cells in nasopharyngeal swabs of patients with COVID-19.

In this study, patients with a severe disease course exhibited significantly reduced presence of immune cells from the adaptive immune system at the site of infection, namely the nasopharynx.[10]



Naso- and oropharyngeal swabs



Epigenetic immune monitoring for COVID-19 disease course prognosis.
10. Samans B, Chornet MR, Chornet AR, et al. Epigenetic immune monitoring for COVID-19 disease course prognosis. *Front Immunol.* 2023;14:1107900.

Sepsis Management – Immune Monitoring in Sepsis

The disease burden of sepsis is significant, both in terms of worldwide incidence and in associated mortality. Especially in low-resource settings and low- and middle-income countries, epigenetic immune monitoring offers a valuable tool due to the option of shipping blood samples at ambient temperatures or using DBS.

Advantage of Epiontis ID → Reliable immune monitoring with sample collection and shipment at ambient temperature

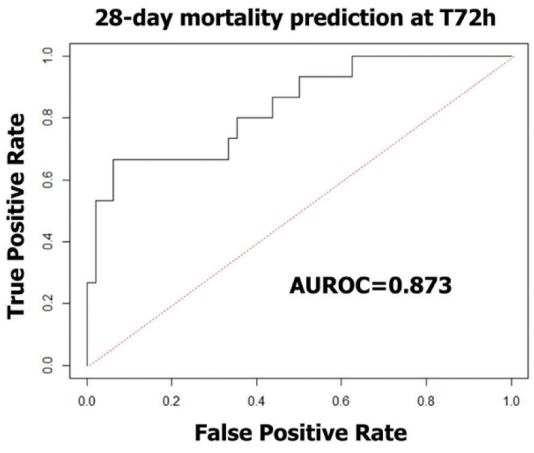
Observational study of sepsis in Ghana.

Fourteen epigenetic assays were used to analyze blood samples from 103 subjects upon admission with suspected infection. Results measured within 72 hours of admission showed predictive capability for sepsis survival of 28 days or more.[11]

Trends Over First 72h by Outcome

Epigenetic Assay	Non-Survivor <28d	Non-Survivor >28d	Survivor
T-Cells CD3+	n.s.	n.s.	**
T-helper CD4+	n.s.	*	**
Cytotoxic T CD8+	n.s.	n.s.	*
B cells	n.s.	n.s.	*
NK cells	n.s.	n.s.	n.s.
Th17	n.s.	n.s.	*
Neutrophils	n.s.	n.s.	n.s.
Basophils	n.s.	n.s.	*
Eosinophils	n.s.	*	**
Monocytes	n.s.	n.s.	n.s
PD1+ cells	n.s.	n.s.	**
CTLA4+ cells	n.s.	n.s.	**
LAG3+ cells	n.s.	n.s.	n.s.

Table showing changes in cell percentages among survivors and non-survivors (both <28d and >28d). Asterisk (*) represents $p<.05$; double asterisks (**) $p<.001$.



Binary logistic regression model using T-helper cell (CD4+), cytotoxic T cell (CD8+), PD1+ cell, and CTLA4+ cell data.

Epigenetic cellular immunophenotyping: a new approach for sepsis management in low-resource settings.
11. Chenoweth J, Brandsma J, Krishnan S, et al. Epigenetic assays identify leukocyte subpopulations that differentiate outcomes in a West African sepsis cohort. Poster presented at: 6th Annual Meeting of the European Sepsis Alliance (ESA 2023); March 21, 2023; Brussels, Belgium.

Pediatric Research – Immune Cell Monitoring in Pediatric Studies

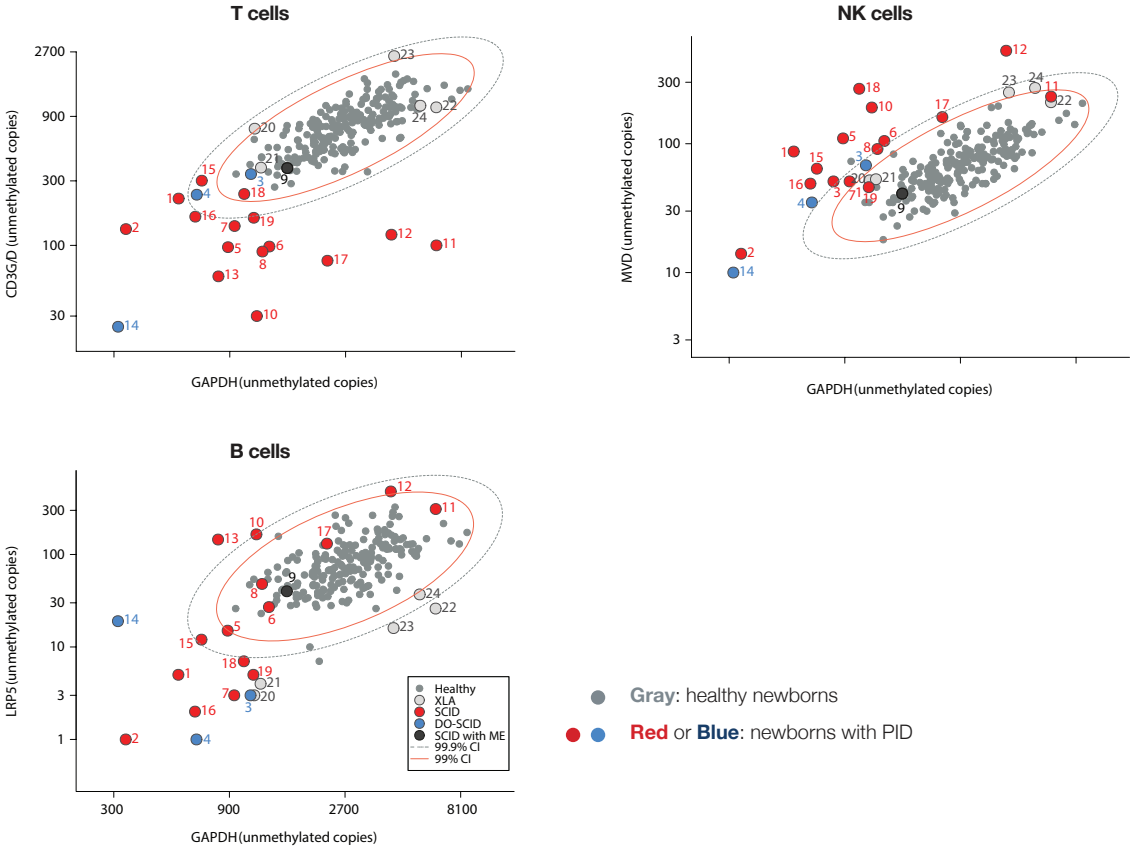
Monitoring immune cells with methods like flow cytometry necessitates significant blood draw volumes, which can be burdensome for pediatric patients. This hinders the detection of quantitative deficiencies of leukocyte subpopulations in newborns, critical for early detection and treatment of primary immunodeficiencies (PIDs).

Epigenetic monitoring with Epiontis ID requires minimal blood volumes below 100 microliters and can be performed on DBS, alleviating logistic and technical challenges for PID detection and pediatric immunophenotyping.

Advantage of Epiontis ID → Effective monitoring in pediatric patient populations with very small blood draw volumes

Monitoring of DBS from newborn heel blood for PIDs.

This study measured dried heel blood from 24 newborns with PID and 250 healthy newborns. Epigenetic assays were conducted for T cells, NK cells, and B cells as well as for total leukocytes. The sensitivity for PID detection was 0.958, with a specificity of 0.984.[12]



Investigating epigenetic qPCR for immune profiling in hematologic and immunologic diseases.

12. Baron U, Werner J, Schildknecht K, et al. Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med.* 2018;10(452):eaan3508.

Cell Therapy – Epiontis ID for Cell Therapy Characterization

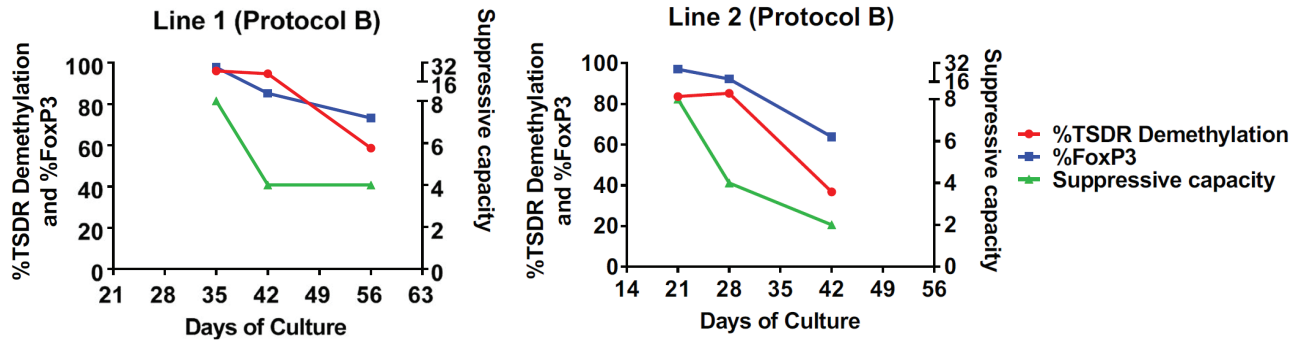
Cell therapy involves introducing in vitro expanded cells into a patient's immune system to treat various diseases, including cancer, autoimmune diseases like diabetes, graft-vs-host disease, and rare hereditary conditions.

An example is the monitoring of in vitro expanded Treg cells. The Epiontis ID Treg Assay tracks demethylation at the Foxp3 gene in human, murine, and cynomolgus samples. This assay offers a faster and more efficient alternative to functional testing or measuring Foxp3 protein levels, requiring less cell therapy product material.

Advantage of Epiontis ID → Determine purity of therapeutic cells and confirm that potentially contaminating cell populations are below a threshold or absent

Testing expanded Treg cells from Mauritian cynomolgus macaque.

In this study, the suppressive capacity, Foxp3 expression, and Foxp3 demethylation, as assessed by the Epiontis ID Cynomolgus Treg Assay, exhibited strong correlation in measuring the decline of cell therapy product quality during prolonged culture times.[13]



Optimizing Treg Expansion protocols in Mauritian cynomolgus macaques.

13. Alonso-Guallart P, Zitsman JS, Stern J, et al. Characterization, biology, and expansion of regulatory T cells in the Cynomolgus macaque for preclinical studies. *Am J Transplant*. 2019;19(8):2186-2198.

In conclusion, the versatility and adaptability of Epiontis ID underscore its value as an outstanding tool in diverse therapeutic areas. Its fusion of easy logistics, accurate and reproducible results, and customizability empowers scientists to precisely identify specific changes in the immune system.

We invite you to explore the potential of this technology in your research and development endeavors. Connect with us today to learn how Epiontis ID can align with your projects.

Epiontis ID Validated Panels Examples

Epiontis ID assays are fully validated and performed on an automated measurement platform under ISO 17025 accreditation. A continuously growing portfolio of assays includes monitoring options for T cells, B cells, NK cells, monocytes, and all types of granulocytes, each of which can be customized as needed.

Below are examples of the types of panels that can be constructed using Epiontis ID's validated markers.

Example Panels

Panel Description	Sample Matrix	Markers	Panels Can Be Combined and Customized Without Additional Validation											
T/B	Whole Blood, Paxgene, PBMC, Tissue	4	CD3	CD4	CD8	B								
T/B/NK/ Degranulation	Whole Blood, Paxgene, PBMC, Tissue	6	CD3	CD4	CD8	B	NK	GNLY						
T/B/NK/Monocyte/ Granulocyte	Whole Blood, Paxgene	9	CD3	CD4	CD8	B	NK	Monoc	Neutro	Eosino	Baso			
T/T-Memory	Whole Blood, Paxgene, PBMC	5	CD3	CD4	CD8	mem CD4	naive CD8							
T/B-Differentiation	Whole Blood, Paxgene, PBMC, Tissue	7	CD3	CD4	CD8	B	naive B	mem B	IgM B					
T/T-Cell Subsets	Whole Blood, Paxgene, PBMC, Tissue	6	CD3	CD4	CD8	Treg	Th17	Tfh	γδ-T cells					
T/T-Cell Subsets/NK/ Activation/Exhaustion	Whole Blood, Paxgene, PBMC, Tissue	12	CD3	CD4	CD8	Treg	Th17	Tfh	NK	CXCR3	LAG3	TIGIT	CTLA4	PD1
T/NK/MDSC/pDC	Whole Blood, Paxgene, PBMC	6	CD3	CD4	CD8	NK	MDSC	pDC						
Additional Markers	Marker Dependent	Add on	GATA3	CCR6	CCR7	CRTH2	S1PR1	S1PR5	Inta4	IL6R	PDGFRb	Col1A1		

For ease of navigation, we've color-coded markers in each panel to correspond to the cell type measured to quickly evaluate whether a validated panel contains a desired cell type marker.

- T Cell
- T Memory
- T Regulatory
- Monocyte/NK
- B-Cell Type
- Granulocyte
- Myeloid/DC
- Immune Checkpoint
- Degranulation
- Activation/Exhaustion

Additional Information

1. **qPCR immunophenotyping determines the number of all cell types independently in 2 readout formats:**

   a. Percent of total cells
   b. Cells per microliter blood

2. **To align with flow cytometry data, Epiontis ID results are calculated as ratios. For example, Treg cells within the parental CD4 T-cell gate:**

$$\frac{\text{Treg cells in sample}}{\text{CD4 T cells in sample}} = \%\ \text{Treg cells within the CD4 T-cell compartment}$$

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