

Highly Sensitive Cytokine Profiling with Ella System *Reveals Hidden Vaccine Responses in Immunocompromised Subjects*

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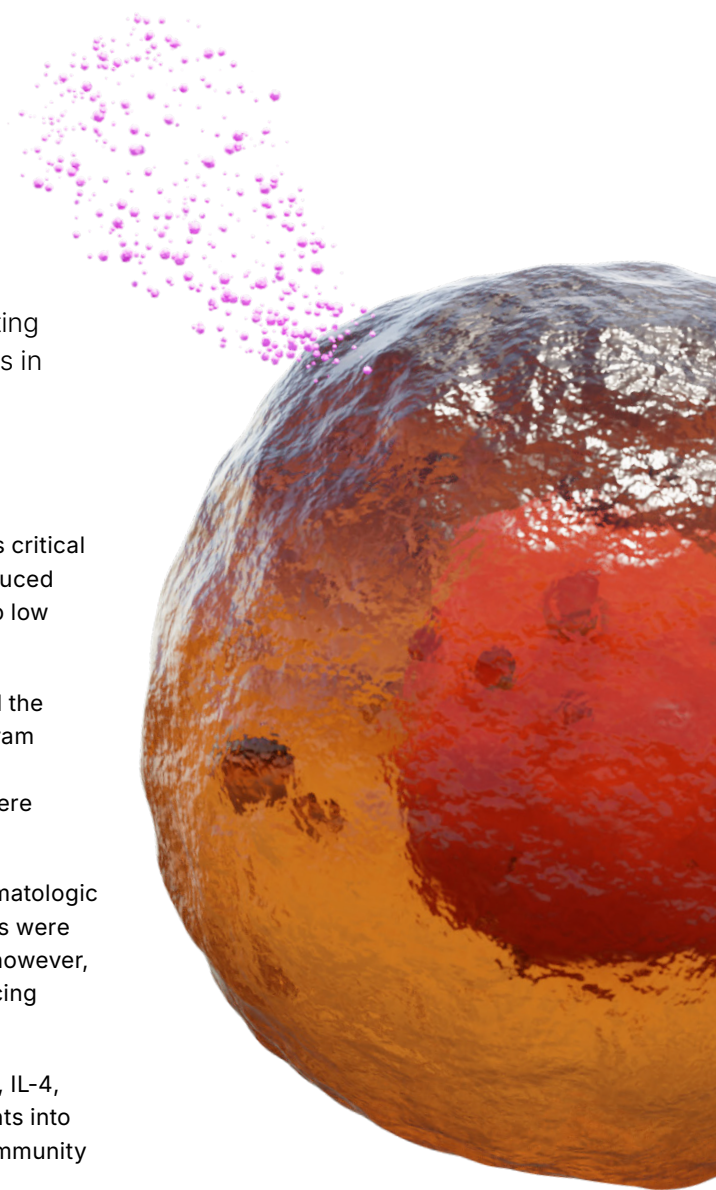
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Insights from a vaccine immunogenicity research study evaluating varicella-zoster virus (VZV) -specific cellular immune responses in immunocompromised subjects.

Study Highlights

- Varicella-zoster virus (VZV)-specific cell-mediated immunity (CMI) is critical for controlling herpes zoster reactivation, yet assessing vaccine-induced CMI in immunocompromised populations remains challenging due to low cytokine production.
- The Ella™* automated immunoassay system (R&D Systems™) enabled the low-abundance detection of cytokines concentrations at sub-picogram levels a critical capability for vaccine immune response in subjects with hematologic malignancies and primary immunodeficiencies where conventional assays fail.
- Using Ella and Simple Plex™ Assays* for IFN-γ detection, 32% of hematologic malignancy subjects and 24% of primary immunodeficiency subjects were classified as vaccine non-responders. Multiplex cytokine profiling, however, revealed additional immune responses missed by IFN-γ alone, reducing complete non-responder rates to just 11% and 7%, respectively.
- Simple Plex analysis measuring eight cytokines simultaneously (IL-2, IL-4, IL-5, IL-10, IL-17A, Granzyme B, IFN-γ, TNF-α) provided crucial insights into T helper cell polarization and identified IL-2 as a durable marker of immunity that increased even in IFN-γ non-responders.
- This Ella and Simple Plex-enabled approach offers a scalable, translational research tool that could help evaluate and guide future personalized vaccination strategies (including booster dose decisions and modified schedules) in vulnerable populations where standard antibody measurements are insufficient.



Introduction

The Challenge of Vaccination in Immunocompromised Populations

Vaccines are essential tools in reducing the morbidity and mortality of vaccine-preventable infections in both the general population and individuals with compromised immune systems (1). The protection provided by vaccines results from a complex interplay between innate, humoral, and CMI. However, there is significant interindividual variability in both the magnitude and quality of immune responses to vaccination. This variability is influenced by intrinsic factors, such as genetics, sex, age, and comorbidities, as well as vaccine-related factors, including vaccine type, adjuvants, and dosing schedules (2).

Immunocompromised (IC) individuals may have weakened immune responses due to inherited immune dysfunctions or secondary causes, such as underlying diseases or immunosuppressive therapies (3). The number of individuals with compromised immunity is steadily increasing, largely due to advances in medical care that prolong life expectancy in aging populations and expand indications for immunosuppressive treatments (4). As a result, IC individuals are more susceptible to infections and their complications (5), making vaccination particularly important for this vulnerable population.

The Importance of Immune Correlates of Protection

A major focus of current research is identifying immune correlates of protection (CoP) to predict vaccine efficacy in preventing infection or severe disease. Historically, the measurement of vaccine-induced antibodies has served as the primary CoP for most vaccines. However, there has been a growing recognition of the importance of cellular immune responses, particularly since the SARS-CoV-2 pandemic (6). The development of practical, sensitive, and cost-effective assays to assess vaccine-induced CMI is now considered essential, especially in the context of immunocompromised populations.

Varicella-Zoster Virus: A Critical Target for Vaccination

Among vaccine-preventable infections, VZV is of particular concern. VZV causes chickenpox (primary infection), which can be severe in IC individuals, and may later reactivate to cause herpes zoster (HZ or shingles). Complications can include postherpetic neuralgia (PHN), myelitis, cranial nerve palsies, meningitis, stroke, and other serious conditions. HZ incidence ranges from 3 to 5 per 1,000 person-years across North America, Europe, and Asia, increasing with age, immunosuppression, and malignancy (7,8).

VZV is the only human herpesvirus for which effective vaccines exist for preventing both chickenpox (live attenuated) and reactivation (one live attenuated or LZV and one recombinant or RZV) (9). Although these vaccines have been available in the United States and Europe for several years (10,11), data on their immunogenicity in IC individuals remain limited. Importantly, VZV-specific CMI and not antibody titers, appears to be the key component in controlling viral reactivation. The strength of the CMI response is inversely correlated with HZ incidence, the severity of skin lesions and pain, and the risk of developing PHN (12).

Study Rationale and Objectives

The study investigated the VZV-specific CMI in subjects with hematologic malignancies (He) and individuals with primary immunodeficiencies (PrID) after RZV vaccination. This study aimed to develop an optimized interferon-gamma release assay (IGRA) workflow that can be translated to the clinical research setting by using the Ella automated immunoassay system (R&D Systems) for ultrasensitive IFN- γ detection in combination with multiplex cytokine profiling in RZV-vaccinated immunocompromised subjects. Traditional IGRAs often fail to detect low-magnitude responses in immunocompromised subjects due to sensitivity limitations. Furthermore, relying solely on IFN- γ may miss additional T-cell responses involving other cytokines. The development of highly sensitive, multiplex approaches is essential for comprehensive immune assessment in clinical research settings.

Materials and Methods

Study Design and Subject Selection

Subjects with hematologic malignancies (He, n=28) and primary immunodeficiencies (PrID, n=29) with a clinical recommendation for vaccination with recombinant vaccine for Herpes Zoster (RZV) attending the outsubject vaccination clinic at the Infectious Disease Unit of Tor Vergata University Hospital (Rome) were enrolled. All participants were adults (≥ 18 years old) who received a two-dose series (Figure 1A) of the recombinant subunit vaccine against herpes zoster (RZV) two months apart (T0 and T1). Heparinized whole blood was collected at T0 (before first dose) and T2 (one month after second dose). The study was approved by the local ethics committee (registration number 125.21).

Whole Blood Stimulation and Interferon-Gamma Release Assay

VZV-specific CMI was assessed using an in-house IGRA (Figure 1B) on fresh heparinized whole blood (HepWB) stimulated ex vivo with peptide libraries specific to VZV glycoprotein E (gE) and immediate early protein 63 (IE63) (PepMix™, JPT Peptide Technologies). A negative control (5 μ L DMSO only) and a positive control

FIGURE 1

Study Protocol and IGRA Testing Workflow

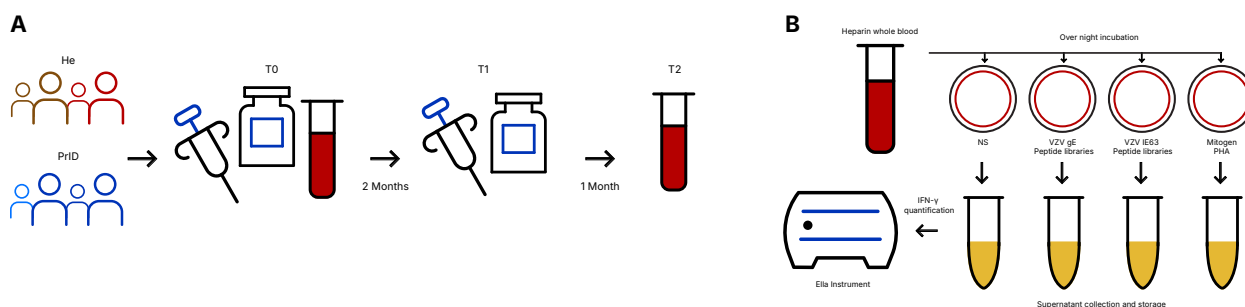


Figure 1. (A) Study timeline with T0, T1, and T2 timepoints. **(B)** IGRA workflow with whole blood stimulation using gE and IE63 peptides, followed by Ella platform analysis. **NS:** Whole blood stimulation and interferon-gamma release assay

IFN- γ Detection Using the Ella System

IFN- γ concentrations were measured using the Simple Plex Human IFN- γ (3rd Gen.) Assay (R&D Systems) on a 72-inlet cartridge with automatic triplicate analyses run on the Ella System. The Ella System enables sub-picogram quantification, offering markedly higher sensitivity than conventional ELISA methods. This increased sensitivity is particularly critical when assessing immune responses in immunocompromised populations where cytokine production may be substantially reduced.

Participants were defined as non-responders if post-vaccination IFN- γ levels (T2) did not increase at least 4-fold compared to pre-vaccination levels (T0) following gE peptide stimulation.

Multi-Cytokine Assessment

Supernatants from gE-stimulated HepWB were analyzed using a Simple Plex multiplex 8-plex cartridge (R&D Systems) optimized for T helper associated cytokines: IL-2, IL-4, IL-5, IL-10, IL-17A, Granzyme B, IFN- γ , and TNF- α . The 32-inlet sample cartridge automatically ran duplicate measurements for each cytokine. Lower and upper limit of quantitation and limit of detection are shown in Table 1.

TABLE 1

Limits of quantitation and detection for the 8 cytokines included in the Ella multiplex cartridge

Assay	LOD (pg/ml)	LLOQ (pg/ml)	ULOQ (pg/ml)
IL-2	0.18	0.54	2050
IL-4 (2 nd)	0.16	0.52	2020
IL-5	0.02	0.13	1248
IL-10 (2 nd)	0.170	0.38	1446
IL-17A	0.1	1.05	10000
GRANB	1.31	1.31	5000
IFN- γ (3 rd)	0.05	0.17	4000
TNF- α (2 nd)	0.03	0.30	1160

Table 1. LLOQ: Lower Limit of Quantitation; ULOQ: Upper Limit of Quantitation; LOD: Limit of Detection. IL: interleukin; GRANB: Granzyme B; IFN- γ Interferon- γ ; TNF- α : Tumor Necrosis Factor- α .

Statistical Analysis

Data are presented as median and interquartile range (IQR). To assess CMI the Wilcoxon signed-rank test was used for paired, non-parametric comparisons between T0 and T2. Between-group comparisons were analyzed using Mann-Whitney U test. Spearman's rank correlation was used for non-parametric correlation analyses. An alpha level of 0.05 was considered statistically significant.

Results

Subject Cohorts

A total of 57 participants (Table 2) were enrolled: 28 subjects (13 women) with hematological malignancies (He) and 29 subjects (15 women) with primary immunodeficiencies (PrID).

In the He group; median age was 56 years [IQR: 38–69]. The most common diagnosis was non-Hodgkin's lymphoma (22/28), followed by multiple myeloma (3/28), Hodgkin's lymphoma (1/28), angioimmunoblastic T-cell lymphoma (1/28), and chronic lymphocytic leukemia (1/28). Median peripheral blood counts: CD4⁺ cells 520 [356–728] cells/μL, CD8⁺ cells 630 [451–912] cells/μL, CD19⁺ cells 33 [1–127] cells/μL. Fourteen subjects were undergoing or had recently received chemotherapy; eighteen had received rituximab.

In the PrID group, median age was 55 years [IQR: 28–67]. The most frequent conditions were common variable immunodeficiency (8/29) and hypogammaglobulinemia (8/29). Median peripheral blood counts: CD4⁺ cells 765 [548–1122] cells/μL, CD8⁺ cells 523 [408–727] cells/μL, CD19⁺ cells 197 [96–269] cells/μL.

TABLE 2

Clinical and immunological characteristics of subjects

	N	Age (years [IQR])	F/M Sex	CD4 (cells/μl) [IQR]	CD8 (cells/μl) [IQR]	CD19 (cells/μl) [IQR]
He Total	28	56 [38–69]	13/15	520 [356–728]	630 [451–912]	33 [1–127]
He R	19	70 [63–75]	7/12	477 [358–698]	654 [456–918]	53 [2–123]
He nR	9	56 [38–69]	6/3	670 [356–752]	606 [434–885]	18 [0–122]
PrID Total	29	55 [28–67]	15/14	765 [548–1122]	523 [408–727]	197 [96–269]
PrID R	22	43 [28–62]	10/12	956 [642–1194]	524 [437–726]	216 [188–296]
PrID nR	7	65 [56–71]	5/2	408 [395–512]	502 [373–714]	43 [21–74]

Table 2. N: number; IQR: Interquartile range; F: female; M: male; He: hematologic malignancies; R: responders; nR: non-responders; PrID: Primary immunodeficiencies.

Highly sensitive IGRA Reveals Vaccine Non-Responders

In both groups, stimulation with the VZV gE peptide library resulted in a significant increase in IFN-γ production after vaccination ($p < 0.0001$), as measured by IGRA using the Ella System. In contrast, no significant changes in IFN-γ levels were observed following stimulation with the IE63 peptide library (He: $p = 0.1578$; PrID: $p = 0.5917$), confirming assay specificity for the vaccine antigen (Figure 2).

An optimal response to vaccination was defined as a >4-fold increase in IFN-γ production from T0 to T2 following stimulation. Based on this criteria, 9/28 subjects (32%) in the He group and 7/29 (24%) in the PrID group were classified as non-responders.

Multiplex Cytokine Profiling Reduces Non-Responder Misclassification

While this study utilized whole-blood (heparin) samples, the Simple Plex Assays were verified in plasma and serum but not whole blood samples. Because whole blood samples are more prone to interference effects, we sought to assess the correlation between whole-blood IFN γ levels as measured across the single-analyte and the multi-analyte formats of the assay. A strong correlation was found between IFN- γ concentrations measured via IGRA and those detected in the multi-cytokine assay using Simple Plex multiplex cartridges (He: $\rho = 0.97$, $p < 0.0001$ at T2; PrID: $\rho = 0.99$, $p < 0.0001$ at T2), validating measurement consistency (Figure 3).

Multiplex analysis, however, revealed critical additional information. In the He group, gE stimulation induced significant increases in IFN- γ , IL-2, and IL-4 at T2. Among non-responders, IL-2 and IL-4 remained significantly increased post-vaccination (Figure 4), indicating residual functional T cell responses missed by IFN- γ assessment alone.

In the PrID group, post-vaccination gE stimulation led to a broader cytokine response with significant increases in IFN- γ , IL-2, IL-4, IL-5, IL-10, IL-17A, and TNF- α , suggesting activation of multiple T helper lineages. Among non-responders, IL-2 ($p = 0.0313$) and IL-17A ($p = 0.0469$) remained significantly elevated (Figure 4). Correlation analysis revealed broader inter-cytokine networks in the PrID group compared to He subjects (Figure 5), indicating more complex and coordinated immune responses in primary immunodeficiency subjects following vaccination.

Using the ≥ 4 -fold increase criterion for each cytokine, only 3/28 (11%) of He and 2/29 (7%) of PrID subjects were classified as complete non-responders, a substantial reduction from the 32% and 24% non-responder rates based on IFN- γ alone. Analysis of individual cytokine response breadth demonstrated that vaccine responders showed significantly more cytokines with ≥ 4 -fold increases (median 2-3 cytokines) compared to non-responders (median 1 cytokine, $p < 0.01$) (Figure 6), further supporting the value of multiplex assessment in capturing vaccine-induced immunity.

IL-2 expression has been associated with long-term T cell memory and has emerged as a potential hallmark of RZV-induced immunity. IL-2 production increased significantly post-vaccination in both subject cohorts, including those classified as non-responders by IGRA, highlighting its potential role as a durable marker of CMI.

Finally, multiplex cytokine profiling enabled the identification of responses in individuals who showed no significant IFN- γ increase (Figure 4), offering a more sensitive and comprehensive approach for evaluating vaccine-induced immunity. However, the clinical relevance of these non-IFN- γ cytokine responses (in terms of real-world protection from VZV reactivation) remains to be established.

FIGURE 3

Correlation between whole blood IFN- γ concentrations measured via IGRA and the multi-cytokine assay

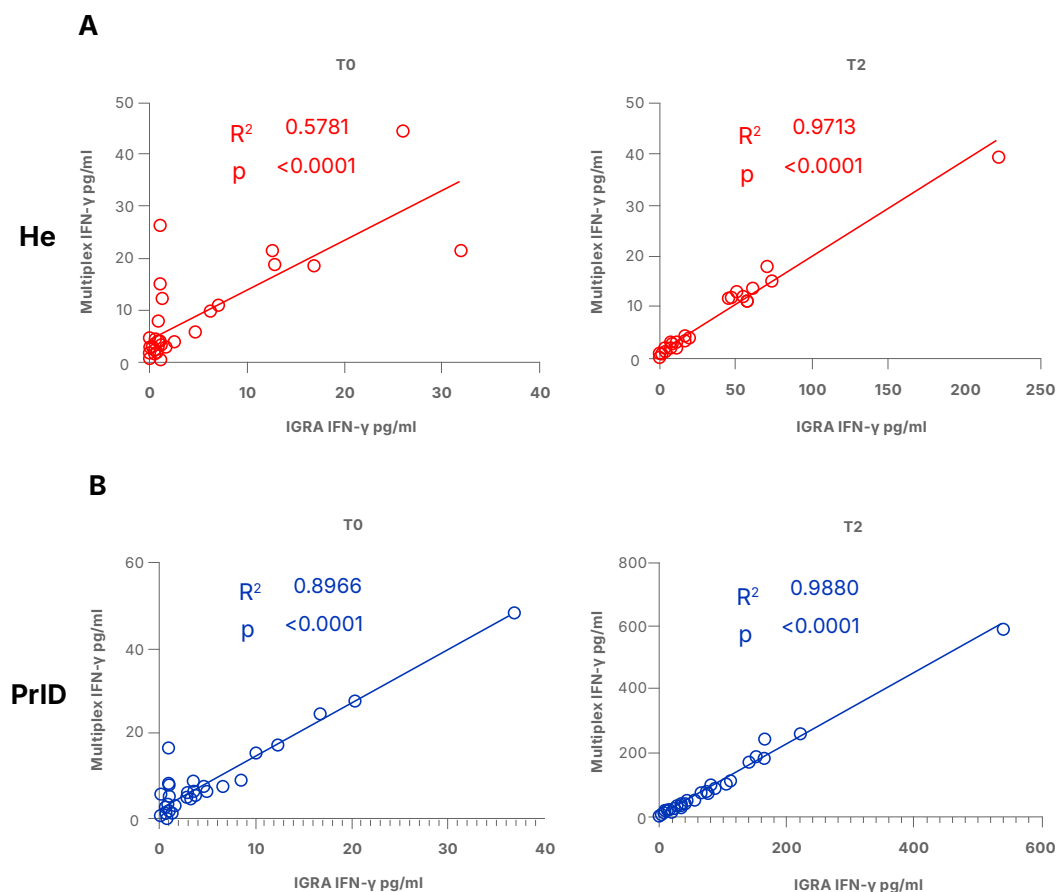


Figure 3. Correlation between IFN- γ measurements of whole-blood heparin samples using Simple Plex Human IFN- γ (3rd Gen.) cartridges, and IFN- γ measurements detected in the multi-cytokine cartridge format, at T0 and T2 in both He and PrID groups.

FIGURE 4

Multi-Cytokine Assessment in gE-stimulated whole blood at T0 and T2

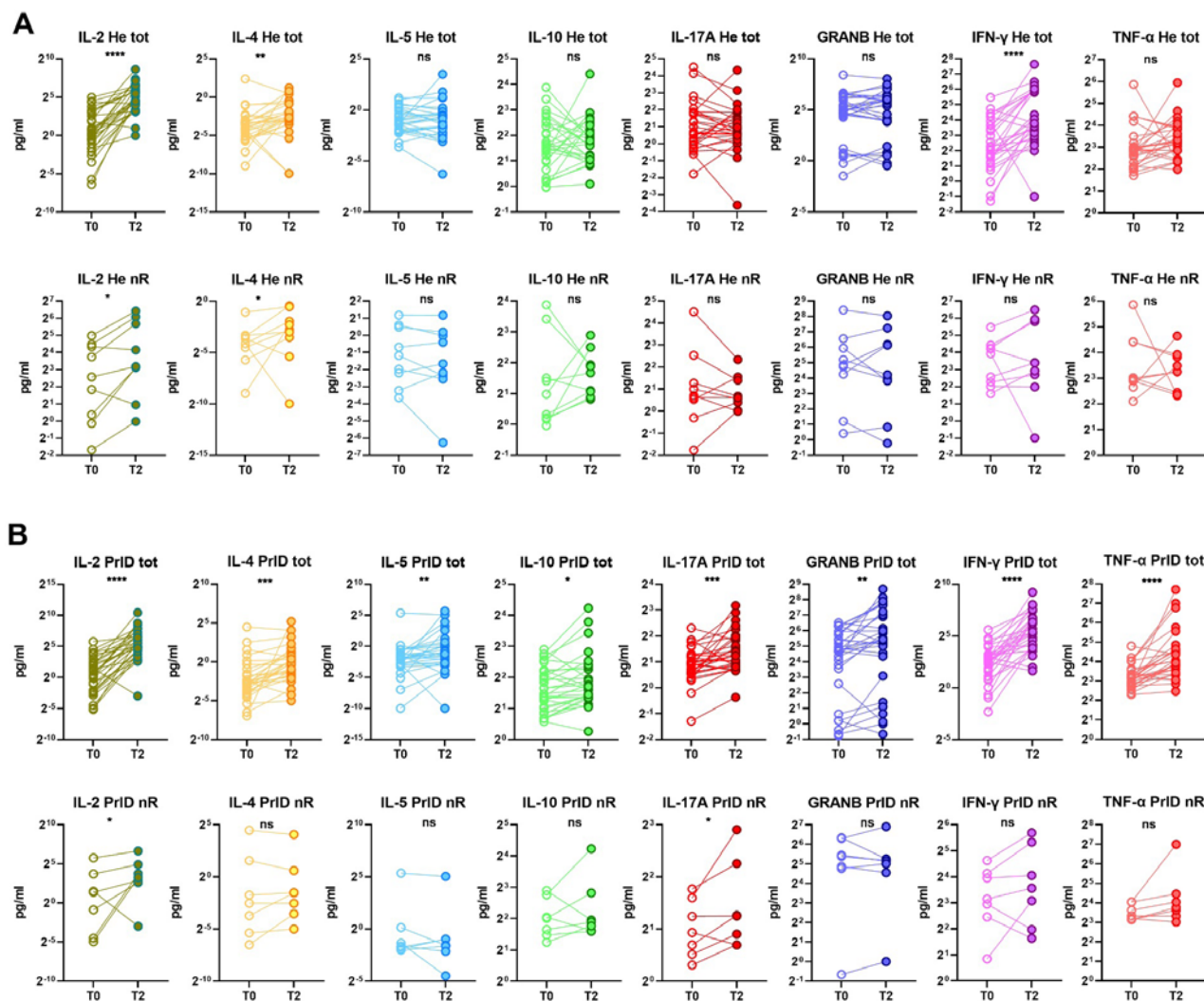


Figure 4. Cytokine production patterns for IL-2, IL-4, IL-5, IL-10, IL-17A, Granzyme B, IFN-γ, and TNF-α in He and PrID groups, comparing total and non-responder subgroups.

FIGURE 5
Correlation analysis among the eight cytokines at T0 and T2

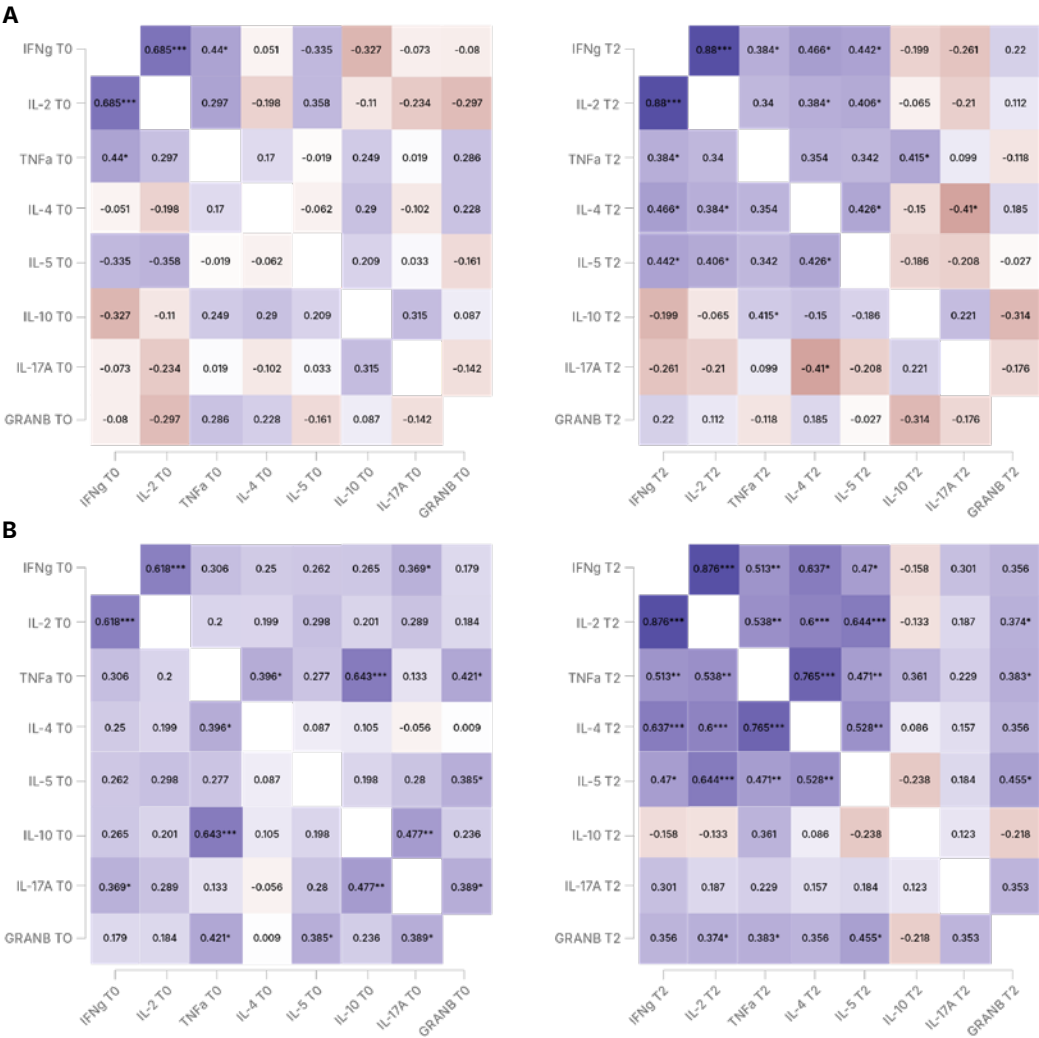


Figure 5. Heatmaps revealing broader networks of inter-cytokine associations in PrID compared to He groups.

FIGURE 6
Number of cytokines showing >4-fold increase per individual

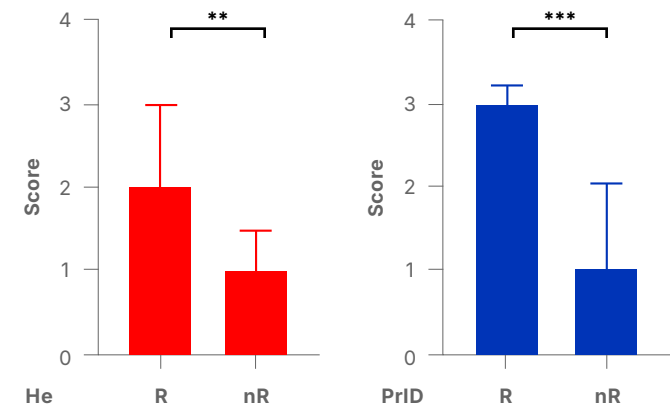


Figure 6. He responders had a median of 2 cytokines vs. 1 in non-responders (p = 0.0022). PrID responders had a median of 3 cytokines vs. 1 in non-responders (p = 0.0002).

Conclusion

In this study, we explored the utility of an in-house (IGRA) to assess VZV-specific CMI following RZV vaccination in two cohorts of immunocompromised subjects: individuals with hematological malignancies and those with primary immunodeficiencies.

Clinical efficacy of the adjuvanted recombinant subunit vaccine (RZV) has been shown to rely primarily on its ability to boost VZV-specific T cell responses (13), making CMI an ideal correlate of protection (CoP) for this vaccine. Thus, future development and translation of laboratory methods to monitor vaccine-induced CMI into clinical practice is essential for managing vaccine strategies in vulnerable populations.

The Ella automated immunoassay system provided critical capabilities for assessing vaccine responses in immunocompromised patients. The study shows that IGRA is a valuable, scalable, and translational tool to measure VZV-specific CMI following RZV vaccination in immunocompromised individuals. The integration of a highly sensitive IFN- γ detection system (Ella) with a multi-cytokine approach, improved assay sensitivity and gained insight into T helper polarization.

The approach shown here suggests a practical, scalable tool with the potential for clinical applications to guide personalized vaccine strategies, including booster timing and subject risk stratification in vulnerable populations where standard humoral correlates are insufficient to estimate protection.

Key Takeaways

The integration of highly sensitive IFN- γ detection with multiplex cytokine profiling on the Ella System enabled several key advances:

- ✓ Sub-picogram-level detection sensitivity in whole-blood samples, essential for identifying vaccine responses in immunocompromised subjects where conventional assays fail to detect meaningful signals.
- ✓ Identification of vaccine non-responders who may benefit from modified immunization strategies, including booster doses or alternative vaccination schedules.
- ✓ Discovery of additional immune responses through 8-cytokine multiplex analysis that were missed by single-analyte IFN- γ assessment, reducing complete non-responder misclassification by 65-71%.
- ✓ Comprehensive insights into T helper cell polarization and immune complexity that inform mechanistic understanding of vaccine responses.
- ✓ Identification of IL-2 as a potentially superior marker of durable immunity compared to IFN- γ alone.

About the ELLA System

The Ella automated immunoassay system from R&D Systems combines microfluidic technology with highly sensitive detection to deliver precise cytokine quantification from small sample volumes.

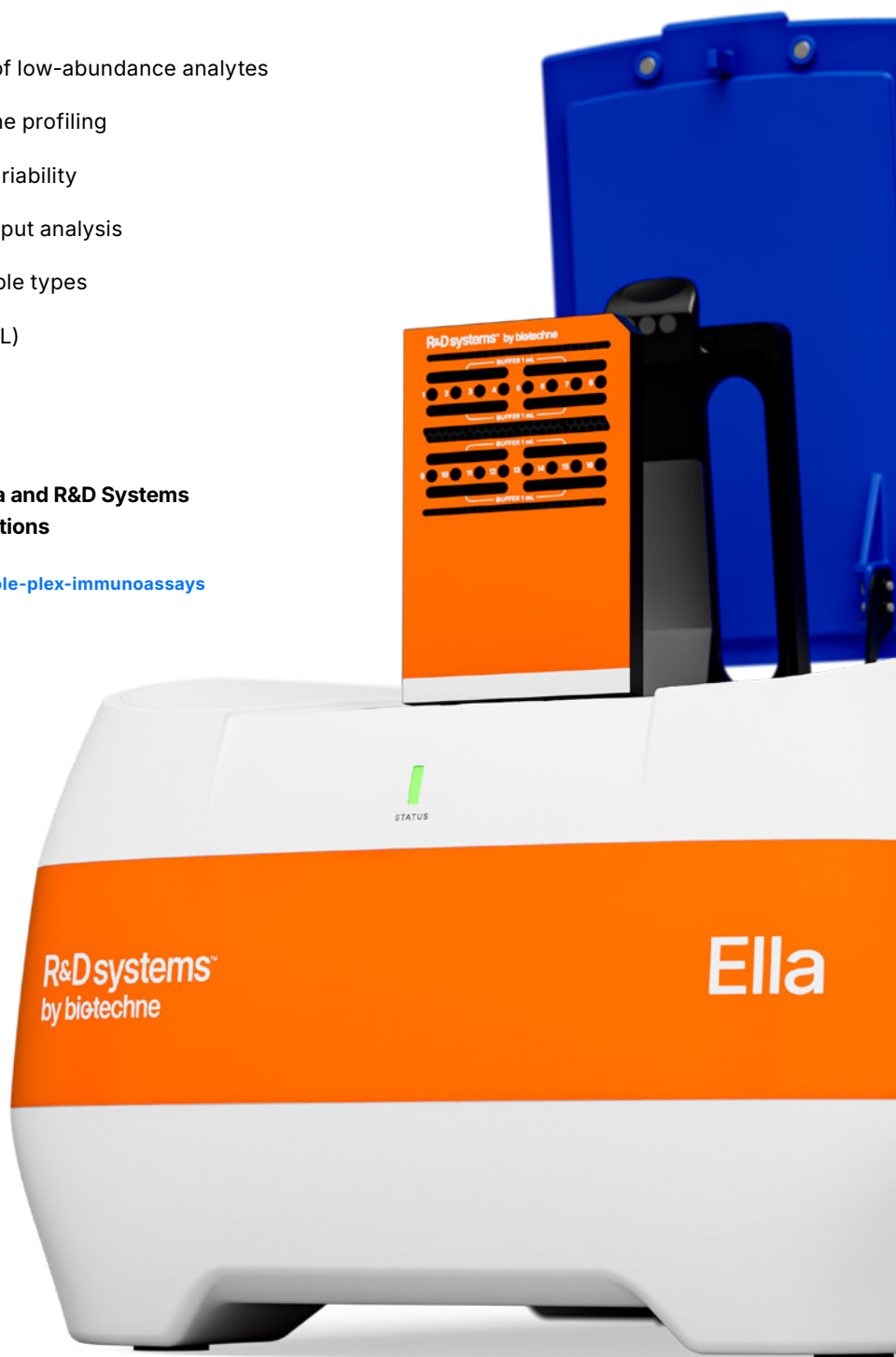
Key features include:

- Sub-picogram sensitivity enabling detection of low-abundance analytes
- Multiplex capability for comprehensive immune profiling
- Automated processing minimizing operator variability
- Simple Plex cartridges for rapid, high-throughput analysis
- Wide dynamic range suitable for diverse sample types
- Minimal sample requirements (as little as 25 µL)



**For more information about Ella and R&D Systems
SimplePlex Immunoassay Solutions**

Scan the QR code or visit
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