Development of a novel standardized and fully automated functional assay to assess and monitor global T cell immune function in 4 hours

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Abstract

Background

Primary immunodeficiency is associated with human cancer. It is also well recognized that Immunosuppressive therapy affecting the individual cellular immune response increase the risk of carcinogenesis. However, in routine practice, simple, rapid and standardized immune assays to functionally assess the individual level of immunosuppression are clearly missing. For instance, in solid-organ transplant patients who are more prone to cancer development, monitoring of immunosuppressive magnitude mainly relies on drug concentration measurements not known to be sufficiently accurate. Consequently, these approaches are often not precisely tailored to each individual patient's needs. Assessing immunity is therefore paramount to implement and monitor personalized treatment. This study aims to describe the potential and clinical relevance of a novel functional assay on fully automated VIDAS® instrument to answer the unmet needs in assessing cellular immunity for routine care.

Methods

Freshly blood was collected in lithium heparin tubes from different cohort of immunosuppressed patients and healthy volunteers. Cellular immune response was then assessed in 4 hours by our automated and standardized VIDAS® STIMM™-T, an assay in which cytokine released by T cells from whole blood was measured after stimulation. Peripheral blood count was also performed to assess T lymphocyte subsets (CD3, CD4, CD8) by flow cytometry.

Results

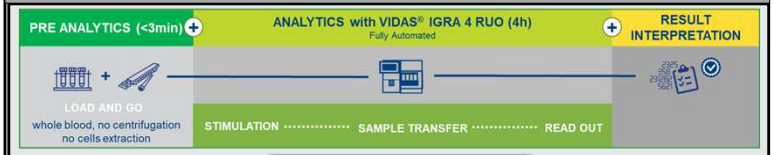
Cellular immune response was assessed by the VIDAS® STIMM™-T in 20 healthy volunteers, 20 patients after liver transplantation (sequential sampling after transplantation) and 22 septic shock patients (sequential samples after ICU admission). We observed that VIDAS® STIMM™-T allowed to stratify patients into low, medium and high T cell immune response subgroups, we assessed the correlation with the patients' clinical course, independently of the total T cell number. We demonstrated, that the transplant group under immunosuppressive treatment present homogeneous IFN-G secreted concentration at W3 and W4, while at W1, W2 and the septic shock patients at all time points were more heterogeneous.

Conclusion

VIDAS® STIMM™-T embodies the next generation of assays capable of assessing and monitoring global T cell functionality in a rapid, simple, fully automated and standardized way. It may help clinicians for routine care and clinical decision-making.

Methodology

Design of a standardized and fully automated IGRA to monitor antigen-independent T cell functionality



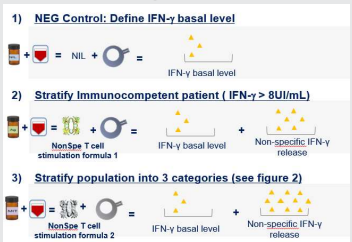


Fig.1- Principle of the VIDAS® STIMM ™T RUO. All the steps are fully automated (pipetting/ stimulation/ IFN-γ read-out) in VIDAS ® 3 system.

Definition of the VIDAS® STIMM™ T RUO cut-offs

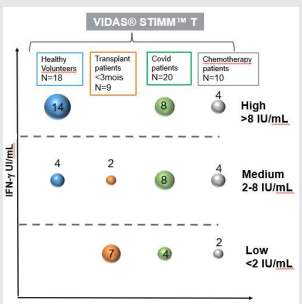


Fig.2- Preliminary data **of VIDAS® STIMM ™ T RUO with immunosuppressed populations** allow **to stratify patient's T cell functionality into low, moderate, and high** by placing 2 cut-offs of < 2 IU/ml and > 8 IU/ml

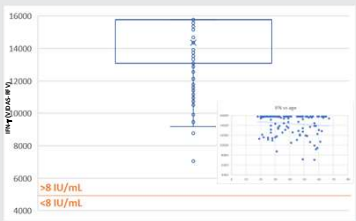


Fig.3- Distribution of VIDAS® STIMM™-T RUO value of 125 **healthy volunteers** (blood donors). All values are classified in **high** (> 8 IU/ml), suggesting immunocompetent patients. The signal ranging from 7,059 to 15,786 RFV may suggest a heterogeneity among patients on T cells functionality within this "normal" population. No correlation with age has been observed. A future investigation could determine if a signal below 25th percentile (13,000 RFV) could be link to **immune senescence**.

Results

Assessment and monitoring of T cell functionality in septic shock patients with VIDAS® STIMM™ T RUO

Study design and population

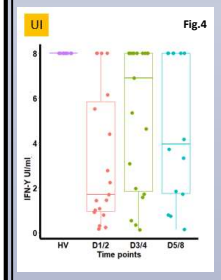
A total of 11 healthy volunteers (HV) and **22 septic shock (SS) patients** (Immunosepsis study) were enrolled. In terms of severity and mortality, patients were similar to those usually observed in this type of critically ill patients in term of SOFA score (from 6 to 16), Nosocomial infection (n=4, 18,2%) and 28 days mortality (n=4,18,2%). Blood was analyzed from SS patient at D1-2 (N=19), D3-4 (N=19), D5-8 (N=14) days of follow up. White blood cell counts and assessment of monocyte HLA- DR (mHLA-DR) were conduced by cytometry. T cell functionality was assessed by the VIDAS® STIMM™ T **(Fig.4 & Fig.5)** on VIDAS® 3 system.

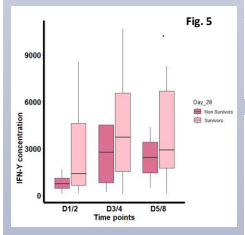
Assessment of SS patient T cell functionality with VIDAS® STIMM™ T RUO – Fig.4

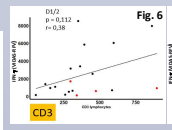
-T cell functionality of SS patients can be categorized as low (IFN-γ < 2IU/ml), moderate (2<IFN-γ >8 IU/ml) or high (IFN-γ > 8 IU/ml) compared to HV (IFN-γ > 8 IU/ml)

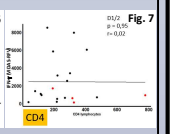
-**On the first day of ICU admission, T cell functionality of SS patients appears predominantly low (group IFN-γ < 2IU/ml in 53% of the patients)** compared to D3/4 and D5/8. Interestingly, there is no significant correlation between the level of IFNγ and the total lymphocyte counts (**Fig.6**) or T cell subset CD4+ (**Fig.7**). Only, an association between IFN-γ and CD8 T cells was observed (**Fig.8**) and has to be confirmed on larger study

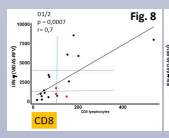
- On clinical standpoint, **low T cell functionality in SS patients seems to be associated with severity/ mortality at D1/2 .** 3 patients died amongst 4 categorized with low IFNγ secretion (< 2IU/ml) at D1/2 (**Fig.5**). The other non survivor had an IFN at 2,77IU/mL. Interestingly, there is no significant correlation between SOFA score and IFN-γ **(Fig.5)**

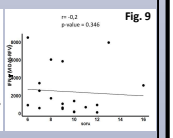












Assessment of T cell functionality in Liver transplantation (LT) patients with VIDAS® STIMM ™ T RUO

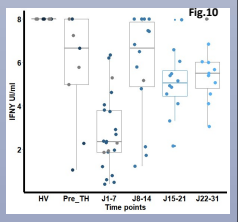
Study design

11 healthy volunteers (HV) and **20 LT patients** (EdMonHG study) were enrolled. T cell functionality from whole blood of LT patients was assessed before and at 1, 2, 3, 4 weeks following transplant by the VIDAS® STIMM ™ T

Assessment of LT patient T cell functionality with VIDAS® STIMM™T RUO - Fig.10.

- Starting before transplantation, VIDAS® STIMM™ T RUO values (IFN-γ levels after stimulation) were significantly lower in LT patients compared to HV during the month of follow-up (including pre-transplant). Results between patients are quite homogeneous at W3 and 4 despite the fact that some patients have high T cell functionality (IFN-γ> 8UI/mL)

- Correlation between T cell functional immune response assessed by VIDAS® STIMM™ T RUO and the occurrence of clinical events reflecting post LT immunodysfunction will be analyzed at the end of the EdMonHG study.



Perspectives in ImmunoMonitoring

CAR-T

Chemotherapy alters phenotype and functionality of T lymphocytes which will be used to produce CAR-T cells. In addition, the presence of T cells expressing exhaustion markers (PD-1, LAG3, TIM-3) at the time of apheresis is associated with a poorer prognosis in patients with Acute Lymphoblastic Leukemia [1]. In Chronic Lymphocytic Leukemia it has been shown that treatment with ibrutinib before apheresis increased T cell functional capacity/proliferation and therefore CAR-T cells generated from these T lymphocytes [2]. These data suggest that prior leucapheresis, **assessment of T lymphocyte functionality could be a good predictor of CAR-T cells treatment success**.

1) Finney OC, et al. CD19 CAR T cell product and disease attributes predict leukemia remission durability. J Clin Invest 2019;129(5):2123–32.
2) Fraietta JA, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. Blood 2016;127 (9):1117–27.

Immune Checkpoint inhibitor Therapy (ICIT)

Monitoring tools to assess immune response and guide decision-making as early as possible is lacking in routine practice. Today monitoring ICIT consist mainly to assess immune-related adverse events. Assessing **the restauration of T lymphocyte functionality** with VIDAS® STIMM™-T RUO could be an easy way and a new tool to predict success of treatment.

Conclusion

VIDAS® STIMM™ T RUO is a new and powerful tool for monitoring antigen-independent T cell functionality in only 4 hours and in a simple, standardized, fully automated way. This solution was well integrated in a routine laboratory without any failure in 5 months and may democratize the assessment of cellular immunity in the future.

Further investigations are now needed to specify the potential usefulness of this test in better assessing immune senescence and determine how the assay can be used to tailor and monitor treatment in patients with various immunosuppressive conditions: transplant, sepsis, cancer, immunotherapy…