EVIDENT Summary

CONTEXT & OBJECTIVES

In response to the Ebola epidemic in West Africa, the Horizon 2020 EU research initiative 'EVIDENT' was launched in November 2014. Equipped with a budget of € 1.7 million, the two-year EVIDENT project provided a research platform which built upon the success of the European Mobile Laboratory consortium (EMLab) that was performing Ebola Virus Disease (EVD) diagnostics in affected West African countries since March 2014. EVIDENT's mandate was to scientifically exploit specimens from EVD patients collected as part of the outbreak response in Guinea, Liberia and Sierra Leone and to gather critical knowledge on B and T cell immunology, biomarkers, virus evolution, virulence determinants, and transmission of Ebola Virus.

The specific objectives of EVIDENT were:

- Providing key information needed to implement efficient convalescent plasma treatment and a toolkit to determine the suitability of plasma for treatment.
- Providing key information on immunity of survivors needed to estimate the efficacy of experimental vaccines.
- Improving supportive treatment of patients and reducing hospital case fatality rate by providing information on biomarkers and relevant co-infections.
- Providing information on pathophysiological changes and immunological determinants to infer new strategies for treatment of EVD.
- Monitoring development of mutations in EBOV genomes during the epidemic and enhancing our preparedness to determine the relevance of these changes in experimental systems.
- Protecting health care workers and communities by providing information on virus shedding and estimation of infectiousness in various stages of EVD.
- Support to operational research projects of other partners in the field, specifically vaccine and drug trails.
- Strengthening cooperation of biosafety level 4 (BSL-4) facilities and building a pan-European research area in the field of highly pathogenic viruses.

Main conclusions from the work of EVIDENT are:

- Convalescent plasma should be selected for treatment according to neutralising antibody titer.
- T cell and B cell response are likely correlates of protection in survivors; and vaccines should elicit both.
- EVD is characterised by serious disturbance of T cell homeostasis, a pathophysiological feature that might be amenable to therapeutic intervention.
- Ebola virus evolves during epidemics with a mutational rate comparable to other RNA viruses; emerging mutations may change the phenotype of the virus.
- Real-time molecular epidemiology based on sequencing in the field greatly enhances tracing of transmission chains and should be an integral part of outbreak response in the future.
- Ebola virus persists in various body fluids for long periods of time, remains infectious during persistence, and may be transmitted via breastfeeding or sexual contact.

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EVIDENT RESULTS

The two-year EVIDENT project has achieved the following main results:

1. Immune response of survivors: EVIDENT assessed the antibody titers, including neutralising antibodies, and T cell reactivity in survivors. The neutralising antibody titers remain stable after discharge for long periods of time (at least a year). Glycoprotein antibodies as measured in ELISA can serve as a surrogate for neutralising activity. As survivors are supposedly protected from re-infection, the observed level of antibody and T cell response should be considered protective.

2. Management of patients: To improve treatment of patients and support clinical trials EVIDENT set-up clinical chemistry measurement in the field. The analysis revealed frequent renal failure, electrolyte disturbance, and evidence for bacterial super infection. As main co-infection malaria was identified, while chronic virus infections were rare. The analysis of various pathogen and host factors showed that age, virus load, and co-infection with malaria in children 5-14 years of age are independent predictors of poor outcome.

3. Ebola virus evolution: Through its deep sequencing program EVIDENT revealed the evolutionary history and molecular clock of the virus during the epidemic with multiple spreading events of the virus across country borders. Furthermore, EVIDENT established nanopore sequencing technology in Guinea to follow the molecular epidemiology of the virus in real-time and support field epidemiology and contact tracing efforts. Replicon and reverse genetics systems were established to study the consequences of virus mutations that emerged during the epidemic.

4. Pathophysiology and immunology of EVD: EVIDENT measured a wide range of soluble cytokines, chemokines and growth factors, and studied the T, Vδ2T, and NK cell response during acute infection. Fatal and surviving EVD patients showed robust T cell activation consistent with biomarker analysis. However, in fatal patients markers of T cell 'exhaustion', in particular CTLA-4 and PD-1, were upregulated demonstrating that defects in the T cell homeostasis are associated with poor outcome of EVD.

5. Virus persistence and shedding: EVIDENT found that essentially all body fluids contain virus RNA until convalescence. Virus persists after convalescence in breast milk and seminal fluid. Time to clearance of EBOV RNA from seminal fluid is variable and may be >10 months.

6. Exploitation and dissemination: Results of EVIDENT have been presented to national authorities to facilitate outbreak response; to WHO to be considered in guidelines; to pharmaceutical companies to guide in the development of EVD vaccines; and to the wider scientific community at international conferences. The data have been published in high-ranking scientific journals, including "Nature".

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EVIDENT IMPACT

The antibody and T cell data from survivors provide key information needed to implement efficient convalescent plasma treatment and to estimate the efficacy of experimental vaccines, and facilitate the licensing of current and future EBOV vaccines.
The data on biomarkers, co-infections, and virus load will improve supportive clinical care, provide new prognostic information on the course of the disease, and predict complications, such as liver or renal failure. Some pathologic clinical chemistry parameters can be corrected (such as electrolytes, glucose) even in resource-poor settings. EVIDENT results have also implications for antimalaria and antibacterial therapy.

The EVIDENT sequencing data have shown to which extent and in which regions the virus changes during an epidemic; and EVIDENT explored the consequences of mutations of concern. The set-up of a sequencing facility in the field demonstrated the great value of combining field and molecular epidemiology in outbreak situations. This concept is a blueprint for future epidemics.

EVIDENT also has a long-term impact on research and innovation. Studies on pathogenesis of the acute infection, including the pathophysiology of the immune response and analysis of relevant pathways and mediators of disease will facilitate the design of therapeutic strategies interfering with this pathophysiological cascade.

The EVIDENT studies on virus shedding will improve the safety of health care workers and family members, who are in contact with patients in early phase and after discharge. We now know that a large variety of body fluids contains virus and patients are potentially infectious after discharge for a long time. EVIDENT also observed that sensitive PCR assays might not be able to detect the virus in a few patients at early stage.

In conclusion, main socio-economic impact of EVIDENT will result from application of the generated knowledge in the operational response to future epidemics as well as the development of medical countermeasures in the inter-epidemic time. Both will reduce the impact of future epidemics on the affected population and the economy of the affected countries.

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