ImmunoTools *FlowISiAM* Award 2024

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Identification of novel biomarkers for Arrhythmogenic Cardiomyopathy using the FlowISiAM technology.

Background: Arrhythmogenic cardiomyopathy (ACM) is a genetic cardiac disease characterized by cardiomyocyte death and subsequent fibro-fatty replacement of the myocardium [PMID:28052233]. ACM is classified as the second most common cause of sudden death among athletes and manifests itself with severe ventricular arrhythmias, syncope, and sudden death, which can be its first sign. The diagnosis of ACM is very complex, and it is based on the combination of major and minor criteria established by an international Task Force that take into account structural, electrical, and familial aspects of the disorder [PMID:20172912]. Affected patients are currently treated with palliative drugs, which do not represent a definitive solution for the disease. Considering that the risk of sudden death remains constant during all the progression of the disease and that up to now no efficient biomarker and therapy have been identified, there is urgent need to find novel diagnostic and therapeutic solutions to prevent the risk of sudden death. Studies performed on both ACM patients and murine models revealed the presence of inflammatory infiltrates, including macrophages, which may precede the fibro-fatty replacement [PMID:22944624, PMID:35845067, PMID:37936669]. These results suggest that the focus on immune cells, and in particular on macrophages and their cargo, may provide relevant information on the identification of noninvasive novel biomarkers for the disease.

Therefore, our **main objective** is to use the *FlowISiAM* technology to identify novel biomarkers for ACM in blood samples obtained from our TgQ mice [PMID:30304392]. These animals overexpress the p.Q558* pathogenic variant detected in the *DSG2* gene in ACM patients and show the typical signs of the disease, including fibrous replacement and myocardial structural changes. More in detail, the project is structured in **three aims**: **1**. Identification of ACM macrophage cargo signature in murine models **2**. Assessment of the potential of macrophage cargo as companion biomarkers in murine models treated with an antifibrotic drug. **3.** Definition of ACM macrophage cargo signature in affected patients.

Experimental plan:

Aim 1: Because ACM is progressive, first, we will perform the *FlowISiAM* assay to profile macrophages and their phagocytosed cargo in the blood in both 9-month-old TgQ mice, showing the disease, and in 5-month-old animals, which don't express any pathological feature yet, as well as in their wild type counterpart (24 mice/group, males and females in equal number).

Aim 2: The lack of effective biomarkers for ACM slows down the assessment of novel disease therapies. To overcome this issue, we will also apply the same approach described in Aim 1 in TgQ mice treated with an antifibrotic drug and in the untreated counterpart. Animals will be treated starting from 6 months of age for three months and potential circulating compamnion biomarkers will be detected by comparing the *FlowISiAM* output in treated vs untreated mice.

Aim 3: Finally, to detect potential circulating biomarkers in ACM patients, we will apply the *FlowISiAM* assay also to determine the blood profile of macrophages in 30 affected individuals and healthy subjects.

The availability in our lab of ACM models and patients and the collaboration with ImmunoTools and INVIGATE will make the study feasible.

Impact: Besides applying for the first time the *FlowISiAM* assay in the context of a cardiac disorder, this study can potentially allow an important step ahead in the diagnosis of ACM, the management of affected patients, and the evaluation of clinical trials for this complex disease.

Cooperation partner: Within this collaboration, ImmunoTools will provide technical support to conduct the *FlowISiAM* assay. INVIGATE will assist during the selection of specific antibodies (ie, $CD14^+$ and $CD16^+$ for activated monocytes; Desmoglein 2, cardiac Troponin-T, total and active Beta-catenin, Plakoglobin) and will share know-how for computer-aided scoring from *FlowISiAM* output. Dr. Calore and Dr. Sebastian Krause (INVIGATE) seek to explore possible strategies to identify biomarkers that could facilitate early detection of ACM and could support drug testing for this disorder.

ImmunoTools *FlowISiAM* AWARD for **Martina Calore** includes antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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