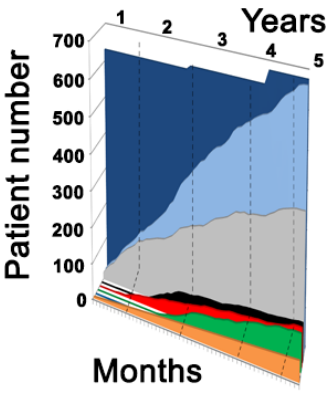
## 3.1 Publishable summary

### Summary of the EUTrigTreat project and objectives

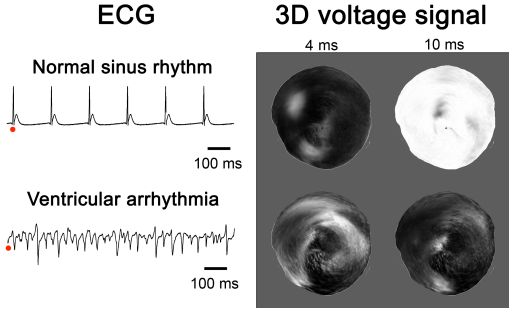
EUTrigTreat investigated significantly different, clinically highly relevant forms of electrical heart disease (arrhythmias). In particular, life-threatening arrhythmia phenotypes with a high risk for sudden cardiac death (SCD) and thrombembolic stroke were studied. This included rare genetic ion channelopathies, common environmental risk factors, and heart failure as the most prevalent and exponentially growing arrhythmogenic syndrome in aging. Furthermore, shared objectives were addressed through strategically interconnected clinical and experimental studies. In particular, molecular mechanisms of arrhythmia initiation, new experimental and clinical diagnostic readouts, better strategies to determine arrhythmia risk in individual patients, and novel therapeutic rationales were developed. Importantly, based on significantly improved mechanistic knowledge, new therapeutic concepts both for anti-arrhythmic drug compounds and implantable devices were developed (see below). In addition, new diagnostic strategies assessed the risk of arrhythmias both for rare genetic and highly prevalent forms of heart disease. Ultimately, **EUTrigTreat** investigators addressed a very critical area of health and disease by combining improved understanding of arrhythmia initiating **Trig**ger mechanisms with new **Treat**ment rationales to improve patient diagnosis and treatment through groundbreaking mechanistic insight and novel treatment strategies.

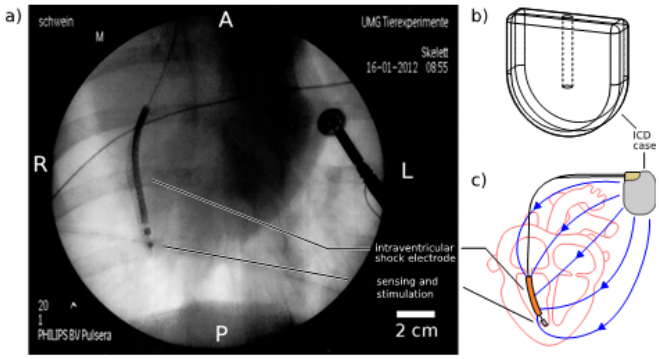
### Description of the work performed and main results



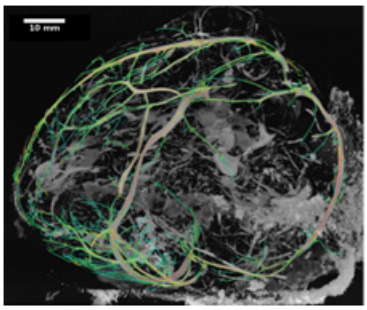

An unsolved dilemma in clinical medicine is how the individual patient's risk for arrhythmias and SCD can be accurately predicted to support rational therapeutic decisions. To address this, the EUTrigTreat Clinical Trial enroled and phenotyped patients with implanted cardioverter defibrillator devices (ICDs), the main guideline recommended therapy to prevent life-threatening arrhythmias in patients with a presumably increased SCD risk. Enrolment and in-depth diagnostic studies of 672 patients were successfully completed (**Figure**; see WP1 report). ICH/GCP guidelines were monitored at the study sites BRFAA, K.U.Leuven, UMCU, and UMG-GOE. UMG-GOE and BRFAA investigators identified potential arrhythmogenic gene variants (see WP1 and WP2 reports). Clinical database procedures, completeness, and inclusion criteria were confirmed. Finally, clinical and genetic data of 632 patients were analyzed as originally planned. In summary, novel diagnostic scoring and multivariate risk prediction strategies were established, and further implemented through therapeutic strategies.

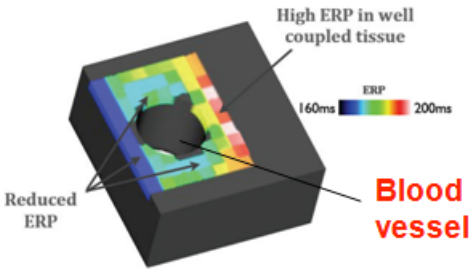
Participants UNIPD, UNIFI and SME Light4Tech investigated arrhythmogenic mechanisms of tissue, cell, and organelle $Ca^{2+}$ signaling and how these destabilize the membrane potential during arrhythmia initiation (see WP3). A new random access multi-photon (RAMP) microscope was developed for millisecond resolution intracellular $Ca^{2+}$ and voltage imaging in intact heart tissue. Participant UGLAS studied stress-induced repolarization abnormalities and developed new imaging strategies, to clarify clinically important re-entry arrhythmia initiating mechanisms (see WP4). Furthermore, participants MPG-a and UMG-GOE (see WP5, WP6) have developed new real-time panoramic voltage-imaging strategies and correlated electrocardiogram (ECG) with re-entry of action potential spread in transgenic hearts with patient mutations and arrhythmias (**Figure**). The work defined physiological behaviors versus pathological mechanisms of arrhythmia initiation relevant for the long-QT syndrome (LQTS) and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).





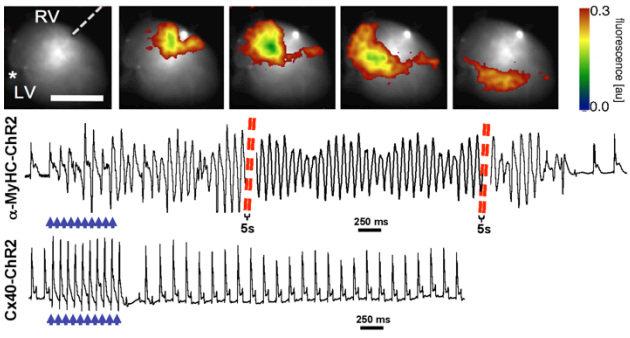

Proof-of-concept was established for low-energy anti-fibrillation pacing (LEAP), a new device strategy, which was further optimized for clinical translation by participants MPG-a, MPG-b, and UMG-GOE (see WP5, WP11). MPG-a has further improved the efficacy and robustness of LEAP for therapeutic arrhythmia control. These experiments indicate that pacing below the dominant arrhythmia frequency may be more efficient than faster pacing, resulting in a 80-90% energy reduction of the defibrillation threshold (DFT) energy. MPG-a and UMG-GOE have designed *in vivo* experimental protocols, which use clinically relevant electrode configurations as shown by real-time fluoroscopy of intracardiac ICD electrodes (**Figure a**), experimental design (**Figure b**), and pre-clinical experimental protocols (**Figure c**) that directly support further clinical translation.



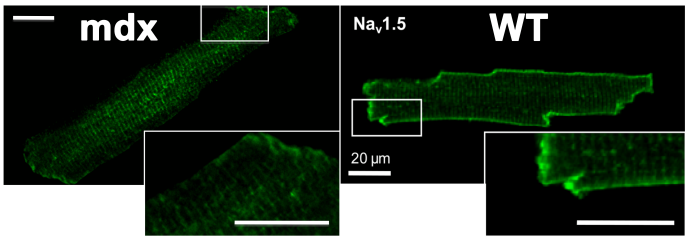

EUTrigTreat investigators developed and applied new *in silico* computer models of arrhythmia initiating mechanisms. This included all levels, from subcellular, intact cell, to whole organ and *in vivo*. Collaborative studies by participants USM, MPG-a, MPG-b, UMG-GOE and UGLAS revealed several important new insights (see WP5, WP6, WP11, WP12). For example, heterogeneous behaviors of action potential spread and the effective refractory period (ERP) were found to be modulated by cardiac blood vessels (**Figure**). Furthermore, participants MPG-a and MPG-b developed a mathematical theory that predicts quantitatively how external therapeutic electromagnetic fields interact with complex tissue heterogeneities to control arrhythmias. Generalizing on earlier theories about virtual electrodes, these studies identified tissue properties that require a particularly low external electric field strength for therapeutic LEAP pulses, a strategy requiring significantly less battery power and potentially significantly lower costs (economic potential).

A critical juncture for up/down translation of arrhythmia mechanisms through experimental and clinical arrhythmia models is to provide cell-specific information and for vastly different levels of investigation, from molecule to *in vivo*. For cell-specific arrhythmia trigger mechanisms *in vivo* and *ex vivo* we employed optogenetic light switches in transgenic models to test arrhythmia initiation mechanisms non-invasively by light stimulation. For example, collaborative work of participants MPG-a and UMG-GOE led to visualization of local action potential activation by light stimulation in intact hearts (**Figure** top); UNIPD used cell-specific expression of light switches in hearts with the same fiber-coupled light stimulation strategy to show that cardiac myocytes (α-MHC-ChR2) but not Purkinje fibers (Cx40-ChR2) trigger ventricular arrhythmias during myocardial infarction *in vivo* (**Figure** bottom). In addition, studies by participants UNIPD and GUF developed novel optogenetic surrogate models of arrhythmias to enable more rapid testing of pathogenic patient mutations or to screen novel drug compounds for efficacy versus

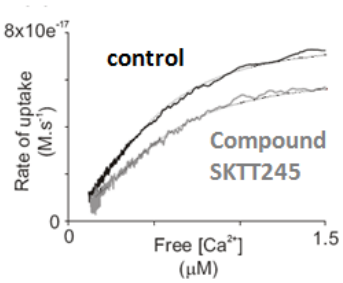


toxicity *in vivo* (see WP9, WP17). Additional transgenic models with patient mutations or molecular targeted mechanisms were generated by participants GUF and SME Polygene, and studied further by participants BRFAA, MPG-a, GUF, INSERM, UBERN and UMG-GOE.

Molecular mechanisms of arrhythmia initiation caused by the cardiac sodium channel Nav1.5 und interacting proteins were investigated by participants UBERN and INSERM (see WP7, WP10). INSERM studied novel patient mutations of Nav1.5 and mechanisms that lead to cardiac fibrosis as well as potential therapeutic approaches. UBERN discovered that loss of the dystrophin protein as occurs in Duchenne muscular dystrophy and other pathologies leads to loss of Nav1.5 protein and sodium current in the lateral membrane of cardiac myocytes (**Figure** mdx). In collaboration with participants UMG-GOE and MPG-a we showed that this dystrophin-dependent and cardiac myocyte specific mechanism causes significantly slower action potential propagation in intact hearts and ECG changes *in vivo*, indicating an important precursor mechanism of re-entry arrhythmias. UBERN further clarified important new genetic and targeting mechanisms of Nav1.5 channel dysfunction originally identified in patients.




Collaborative work between SME Endotherm and participants UGLAS, K.U.Leuven,UMCU and UMG-GOE led to development and evaluation of novel drug compounds. This includes identification of novel intracellular $Ca^{2+}$ leak inhibitors through own cellular high-throughput assays (**Figure**) and electrophysiological testing in ventricular myocytes. Cardiotoxic screening was tested in stem cells (iPS) by participant UGLAS, and SME Endotherm, K.U.Leuven, and UMCU developed novel NCX-targeted compounds with the potential to therapeutically modulate plasma membrane ion transport, a key pathophysiological mechanism.




EUTrigTreat participants made many efforts to disseminate central scientific achievements and to include the public as much as possible. Dissemination activities included seminars, lectures, workshops, interviews, patient information events, and award competitions. The **Figure** highlights the exposition stand of participant MPG for the Max Planck "science tunnel", a global travel exhibition of important science megatrends. Their multimedia show demonstrated the complex spatiotemporal cardiac dynamics during arrhythmias and visualized how the novel LEAP device may work therapeutically. Furthermore, the BEYOND meeting organized in Berlin in June 2014 engaged the entire EUTrigTreat consortium through discussions with international arrhythmia experts and large organizations. Also, several students competed successfully for scientific awards at international meetings and over 100 publications about the collaborative work are already available and usually open access. Finally, EUTrigTreat developed novel SCD risk prediction scoring strategies and therapeutic mechanisms to improve public health in Europe, communicated as finalist for the European Health Award at the 2014 forum in Gastein.

**The above examples highlight important achievements of the EUTrigTreat large-scale collaborative project. Please note the above summary only presents a fraction of the overall consortium activities and results. For a comprehensive overview please refer to the detailed annual and the final EUTrigTreat work package reports.**

## Final results, potential impact and use

Both rare and common forms of arrhythmias occur due to exceedingly complex molecular and electrical mechanisms in the heart, which are modulated by genetic and environmental factors. EUTrigTreat applied a highly integrative translational research strategy to elucidate molecular, subcellular and tissue-specific arrhythmia mechanisms towards novel *in vivo* diagnostic and therapeutic strategies. Genotype-phenotype correlation studies were employed to address underlying molecular mechanisms, specific defects identified in patients, and targeted interventions developed. In Year-5, the consortium has completed research of key cellular, tissue, and in vivo arrhythmia mechanisms that converge in part through common mechanisms of arrhythmia initiation. This means despite the apparent multitude of genetic and environmental mechanisms, the cell and tissue specific organization of protein function in the heart may occur through tightly controlled interaction spaces e.g. membrane nanodomains, each combining hundreds or thousands of gene functions via subcellular elemental signals e.g. $Ca^{2+}$ sparks. Hence, electrical dysfunction for arrhythmias to occur needs to be modelled, detected, and targeted from subcellular domains to *in vivo*.

For this, complex scales from molecule to cell and intact tissue have been comprehensively addressed including entirely novel imaging strategies and microscopy prototypes, necessary to overcome resolution barriers in time and space. For the first time, complex electrical heterogeneities were visualized with nanometric and submillisecond detail in intact hearts and further correlated with physiological regional tissue properties, to explore novel diagnostic and therapeutic avenues. Furthermore, novel imaging strategies which overcome the fundamental resolution barrier of light microscopy in living heart cells addressed the critical subcellular compartments of excitable membrane systems. Novel arrhythmia models and computational modeling studies integrate and quantitate previously unknown early or precursor stages of electrical dysfunction leading to novel insights about disease mechanisms and surrogate biomarkers for new clinical diagnostic strategies. The unique combination of non-invasive imaging strategies to address different scales and cutting-edge mathematical modelling ultimately has the potential to transform and translate fundamental new insights about arrhythmia mechanisms for diagnosis and therapy.

EUTrigTreat has developed novel anti-arrhythmic device therapy (LEAP) concepts and high-throughput cell-specific screening strategies for novel drug compound development. This led to the identification of potential anti-arrhythmic lead compounds, some of which were tested in clinically relevant arrhythmia models. These therapeutic discoveries may ultimately improve patient treatment through new anti-arrhythmic device and drug options. Additionally, through the EUTrigTreat Clinical Trial we developed new diagnostic protocols and risk prediction scores for patients to better identify those patients with increased arrhythmia and SCD risk and to avoid unnecessary and costly device treatments in patients not at risk. In summary, this large-scale collaborative project has created impact through

- **Improved understanding of fundamental arrhythmia mechanisms**
- **Development of novel therapeutic strategies to prevent arrhythmias**
- **Novel strategies for patients to improve identification of individuals at increased risk for sudden cardiac death**
- **Risk driven strategies to support socio-economic analysis of patient treatment**
- **In summary, novel diagnostic and therapeutic strategies**

**The project website can be found at:** www.eutrigtreat.eu