KU LEUVEN

EpiSense

KU Leuven

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Supervisor: Prof. Jeroen Lammertyn

Coaches: Lorenz Van Hileghem, Seppe Driesen, Sara Horta

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1. Summary for the SensUs website

EpiSense is a diverse team of motivated KU Leuven students that came together to create an innovative sensing device, designed to detect the blood plasma levels of free valproate (fVPA), a drug that is commonly used to prevent epileptic seizures.

Our benchtop device with disposable cartridges is a user-friendly, must-have tool for all hospitals. A drop of blood from the patient onto the inlet of the chip suffices for the neurologists to set the process in motion. The in-house developed passive microfluidic system ((i)SIMPLE) automatically pulls the sample into the albumin trapping zone where magnetic nanoparticles efficiently filter out albumin conjugated VPA, which interferes with the drug's function and final measurement. The sample is then pulled into the detection zone where catalytically active DNA molecules (DNAzymes), the core of the sensing process, generate a fluorescent signal upon cleavage of their substrates. A numerical value appears at the LED screen a few minutes later, indicating the patient's fVPA levels.

Despite COVID-19, the EpiSense device is already designed. We are confident that with our determination and the support of our scientists and sponsors, our idea will soon materialise and contribute to improving the quality of life of millions of epileptic patients.

2. Biosensor system and assay

2.1. Molecular recognition and assay reagents

Our sensing system is a competitive assay based on DNAzymes, which are catalytically active nucleic acids that have recently attracted the attention of the scientific community and whose use in biosensor applications is emerging (1). The basic component of the competitive assay (figure 1) is a conjugate of VPA and an amine-labelled DNAzyme. These are linked together using 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), a water-soluble molecule of zero length that is used for many conjugation techniques as a crosslinker (2). First, EDC links to the carboxyl group of VPA to create an amine-reactive O-acylisourea intermediate. This intermediate will link to the NH_2 group of the DNAzyme (appendix A), resulting in a VPA-DNAzyme conjugate (3).

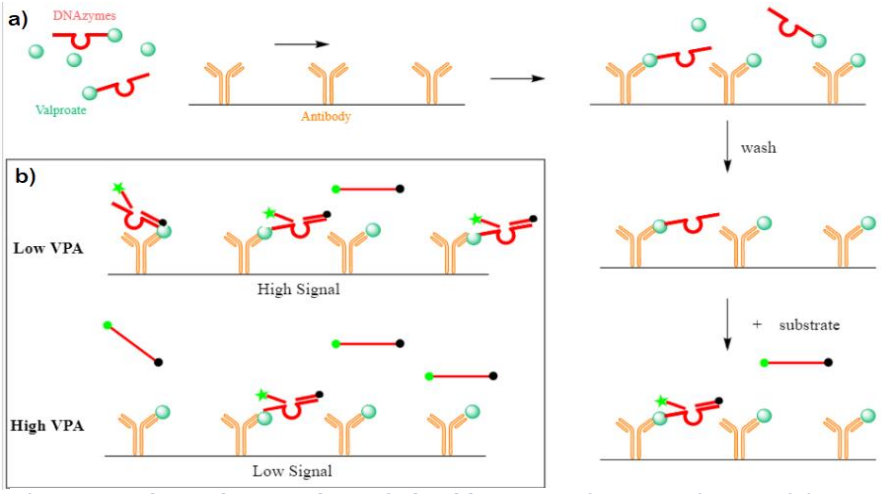


Figure 1 ***Schematic overview of the bioassay****. a) A sample containing an unknown amount of VPA (green) is mixed with a known amount of VPA-DNAzyme conjugates (green-red). At the detection zone, anti-VPA antibodies, will capture both VPA and the conjugates. After washing, the substrate is added, resulting in a fluorescent signal by DNAzyme cleavage. b) The competitive assay will results in a high signal for low amounts of fVPA and vice-versa*

This competitive assay is performed on-chip by flowing the assay components over a zone functionalized with specific monoclonal antibodies targeting VPA (detection zone) using a microfluidic system (see cartridge technology). Before performing the competitive assay, the sample containing an unknown concentration of VPA is mixed on-chip with a well-known amount of the VPA-DNAzyme conjugates. The immobilized antibodies in the detection zone will capture fVPA (if present in the sample) and DNAzyme-conjugated VPA when flowing over the plasma sample. Next, by flowing the pre-stored washing buffer over the detection zone all unbound components will be removed. Finally, the DNAzyme substrate is flown over the detection zone. This substrate is an oligonucleotide labelled with a fluorophore at one end and a quencher at the other end. This oligonucleotide consists of 11 DNA bases and 2 RNA bases (in the middle) to allow DNAzyme cleavage (appendix B). Upon cleavage, the fluorophore and quencher get separated, resulting in an increase in fluorescent signal(4).

2.2. Physical transduction

The detection of fVPA on the EpiSense chip is based on the catalytic action of DNAzymes on a fluorophore-quencher substrate that flows over the detection zone and will generate a fluorescent signal (optical transduction) upon DNAzyme cleavage. Due to the fact that it is a competitive assay, a high fluorescent signal indicates a low amount of VPA in the patient sample and vice-versa. (Figure 2)

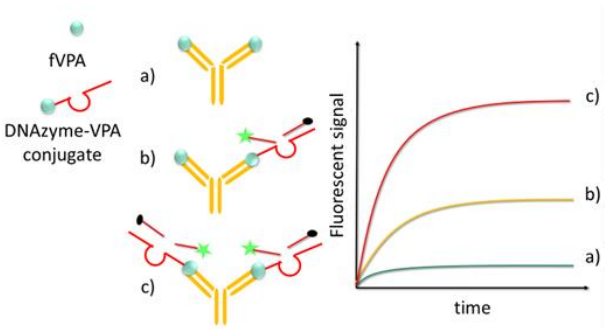


Figure 2 ***Intensity of the fluorescence signal in 3 situations****: a) High, b) intermediate and c) low VPA concentration in plasma*

2.3. Cartridge technology

The cartridge developed for the EpiSense reading instrument is a disposable, self-powered, easy-to-use and cost-effective microfluidic chip. It is based on the patented (infusion) Self-powered Imbibing Microfluidic Pump by Liquid Encapsulation ((i)SIMPLE) technology developed at the MeBios Biosensors group at KU Leuven (5, 6) With this concept, pressure gradients can be generated by the capillary action of a porous material (PM). To start the pump, the working liquid (WL) is brought into contact with the PM by pushing on the activation chamber. Then, the PM starts wicking in the WL and a negative pressure is created, which pulls the sample liquid first over a zone where albumin, and thus most of the bound VPA, is removed from the sample (see below). Then, the sample is pulled further toward the detection zone. After being flown over the detection zone, the sample reaches a second pump that pushes the washing buffer and the fluorescent substrate over the detection zone. The microfluidics to make this possible is depicted in Figure 3a.

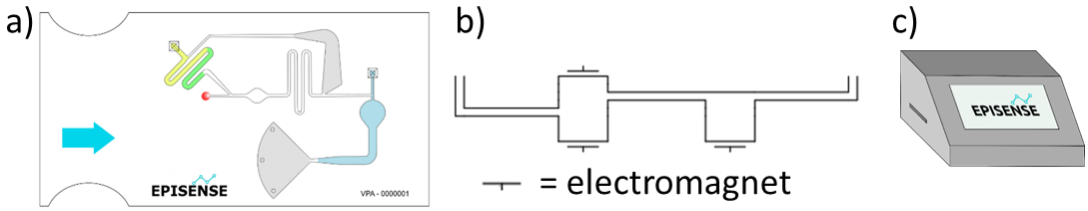


Figure 3 ***EpiSense technology:*** *a) Schematic representation of cartridge, b) side view of the microfluidic channels with albumin trap and c) design of the reader instrument.*

The sample interacts with magnetic nanoparticles that are coated with Cibacron Blue, a dye that has a high affinity for blood plasma proteins, and especially human albumin (7), to capture albumin in the two wells, acting as the albumin trap (Figure 3 b). These wells have a dual function, serving for both mixing and albumin depletion. When placed in the reader instrument, the first well is placed between two electromagnets (one above and one below), which apply complementary magnetic field pulses causing the particles to move, thus increasing sample and particles mixing and maximizing albumin intake. The second well is also placed on top of an electromagnet which will attract the nanoparticles that have escaped the first well by diffusion. In this way, the sample can be depleted of albumin (and hence, albumin-bound VPA), allowing the detection of fVPA in the detection zone.

2.4. Reader instrument and user interaction

In the EpiSense reader device (15”x10”x5”), conceptually depicted in Figure 3 c, the generated fluorescence is focused through a lens and detected by photodiode, which comprises one half of the read-out system. The other half is composed of a touchscreen display that exposes a real-time fluorescence intensity curve. This system is coupled with an Arduino UNO motherboard which receives signals from the photodiode and then communicates it to the 3.5” touchscreen for the user to see. This measurement is internally compared with a calibration curve to determine the concentration of fVPA in the sample. The user is expected to apply a blood sample to the EpiSense cartridge and insert it in the EpiSense device (Figure 3 c). The chip will be locked inside the device, which will automatically begin processing the bioassay reactions. The user can see the device working as appropriate screens appear on the touchscreen. Additional options to see the previous usages of the device that log concentration of fVPA or to begin a new analysis will also be available.

3. Technological feasibility

Bioassay: The measurements to validate the assay were done with a Spectramax ID3 spectrophotometer in 384 well plates. The fluorescence was measured at an excitation wavelength of 485 nm and emission wavelength of 535 nm. The first test (Figure 4) confirmed that the DNAzymes can undergo the EDC-crosslinking process to make the DNAzyme-conjugated VPA and still be active. These conjugated DNAzymes were compared to normal DNAzymes to see what the effect is on the DNAzyme activity. All the solutions contained 0.25 µM DNAzyme. The average activity of DNAzyme conjugated with VPA is slightly lower than the DNAzymes that did not go through the EDC conjugation process but still acceptable in the same range

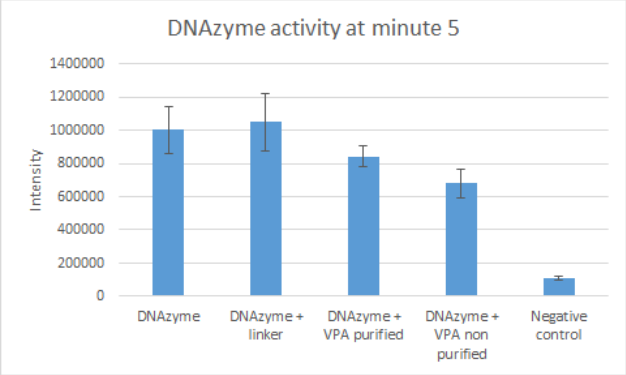


Figure 4 ***DNAzyme activity at minute 5 of different DNAzyme conformations****. DNAzyme concentration 0.25 µM. Error Bars represent standard deviation (n = 3).*

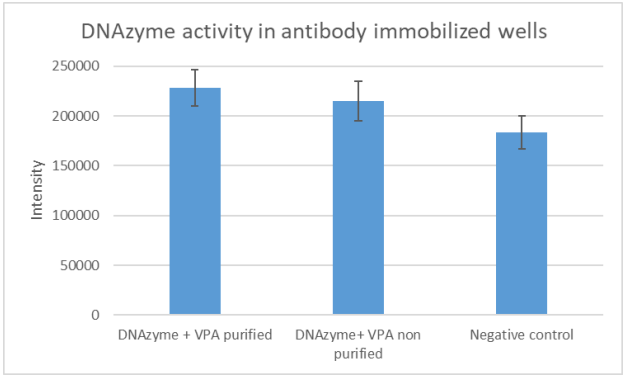


Figure 5 ***DNAzyme activity with immobilized antibodies in well****. DNAzyme concentration 1µM. Error Bars represent standard deviation (n= 4).*

After proving that the DNAzymes remained active after the conjugation process, it was necessary to show whether the conjugation between VPA and DNAzymes worked and to ascertain if these conjugates can still bind to the anti-VPA antibodies. Therefore, the wells were coated with antibodies (Figure 5). The concentration of VPA conjugated DNAzymes in these experiments was 1µM. VPA-DNAzyme conjugates can still bind to anti-VPA and the conjugation with the DNAzymes was successful. This experiment did show lower signals compared to the experiments in solution shown in Figure 4. With a subsequent buffer optimization (results not shown) we were able to increase the difference compared to the negative control by incubating in PBS at pH 5.

The measurement was then performed with different concentrations of DNAzyme-conjugated VPA, to determine if this difference is measurable (Figure 6). The highest concentration of DNAzyme (2 µM) did create a higher signal with a steeper slope compared to the lower concentrations. There is also a measurable difference between concentration 1 µM and 0.5 µM. This indicates the feasibility of using the DNAzyme-VPA conjugates in a competitive assay.

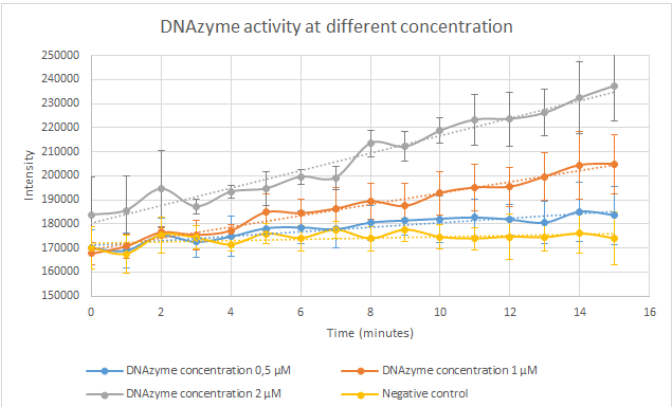


Figure 6 ***Performance of VPA conjugated DNAzymes at different DNAzyme concentrations (2 µM – 1 µM - 0.5 µM – 0 µM).*** *Measured in antibody-functionalized wells with PBS pH5 buffer. Error Bars represent standard deviation (n= 4).*

The next step would be adding fVPA to the assay to determine if the signal would change according to the concentration of VPA present in the sample. Furthermore, optimizing, upscaling and implementing the assay on the chip would be done in the following stages. However, the time in the lab was limited due to COVID-19 measure so we were not able to perform these tests.

Albumin trap: Preliminary experiments to test the feasibility of the concept of albumin depletion by functionalized particles was carried out by using a representative bovine serum albumin (BSA) model for the proposed on-chip albumin depletion. Magnetic microparticles were functionalized with antibodies against BSA through streptavidin - biotin chemistry. Then, they were mixed with fluorescently labelled BSA samples of known concentrations (0.01 mg/mL, 0.001 mg/mL). After incubation, these samples were tested for depletion activity using the Spectramax ID3 spectrophotometer. The depletion results obtained (Figure 7) using this model showed average 41% and 19% depletion for the 0.1 mg/mL and 0.001 mg/mL BSA samples.

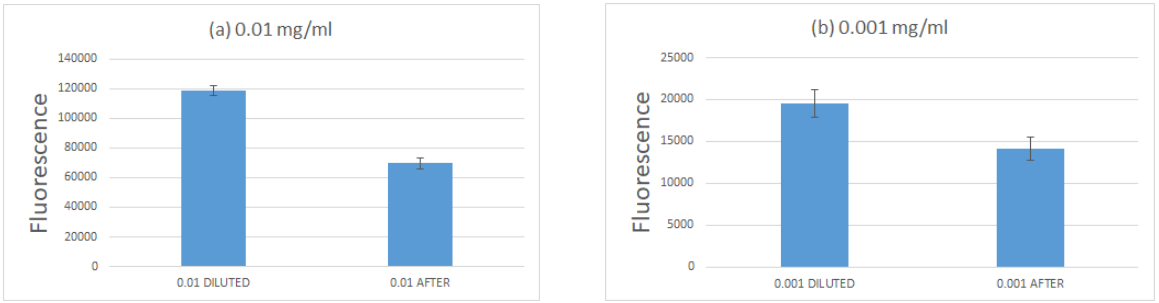


Figure 7 ***Albumin trap depletion using functionalized magnetic bead for BSA samples*** *with (a) 0.01 mg/mL (b) 0.001 mg/mL in PBS buffer, observed using fluorescent measurements*

These results on the model system can be improved significantly in the final design for two important reasons: (i) Cibacron Blue dye has a higher binding affinity for human serum albumin compared to the antibodies (anti-BSA) for bovine serum albumin; (ii) Functionalized magnetic nanoparticles can be used in lower concentrations on-chip by improved surface-to-volume ratio. The latter are also less prone to sedimentation compared to the micro-sized particles.

Microfluidics: A major technical issue of our concept was the need for flowing over the sample plug as first over the detection zone, and only afterward the other reagents (washing buffer + substrate). Therefore, an innovative microfluidic system, based on both SIMPLE and iSIMPLE, has been developed to allow the integration of the bio-assay on chip, as depicted in Figure 8 below.

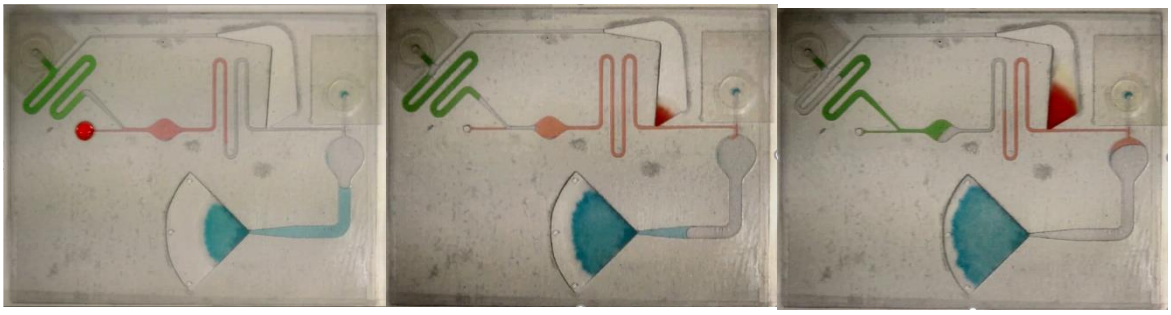


Figure 8 ***Two-plug system in action****: (a), (b) first (sample) plug flows over inlet of second pump and activates it, (c) second (pre-fluorescent) plug flows and allows detection of fVPA*

4. Originality

Team: The detection of fVPA by biosensors has already been proven by different researchers (8). However, the analyte's direct measurement from blood samples remains a challenge mostly because of (i) the small molecular size of VPA and (ii) the difficulties in purifying the fVPA due to its high affinity to blood plasma proteins, primarily albumin. The first problem was solved by Zabardasti et al. (9), which determined fVPA levels by a voltammetric assay. The second problem was tackled by Müge Andaç (10), which showed that the high affinity of Cibacron Blue F3GA (CB) to albumin could be used for albumin depletion in proteome studies. Besides, the purification of samples in a microchip can be achieved using modified magnetic nanobeads coupled with a direction-changing magnetic field (11). Furthermore, the combination of CB with magnetic nanobeads has been explored before (10). Finally, DNAzymes have been used in several studies, and are already used in biosensors for metal ions, glucose, and DNA detection, in which some of them use DNAzymes linked to another compound (12)(13). However, there is no report in the literature on the use of DNAzymes to detect VPA. With that in mind, our team decided to develop a biosensing concept based on DNAzymes with a purification step using magnetic nanobeads coated with CB. The novelty of the EpiSense biosensor lies in the use of DNAzymes to detect fVPA, and the mixing and purification of the sample by magnetic nanobeads in a single self-powered microfluidic chip (SIMPLE), with no need of intervention of the analyst after insertion of the sample. The idea was achieved by the collaboration of all team members guided by the coaches' supervision, and the originality of it was extensively checked. To the best of our knowledge, there is no biosensor or assay that combines magnetic nanobeads, DNAzymes, and the kind of competitive assay we use for the detection of fVPA.

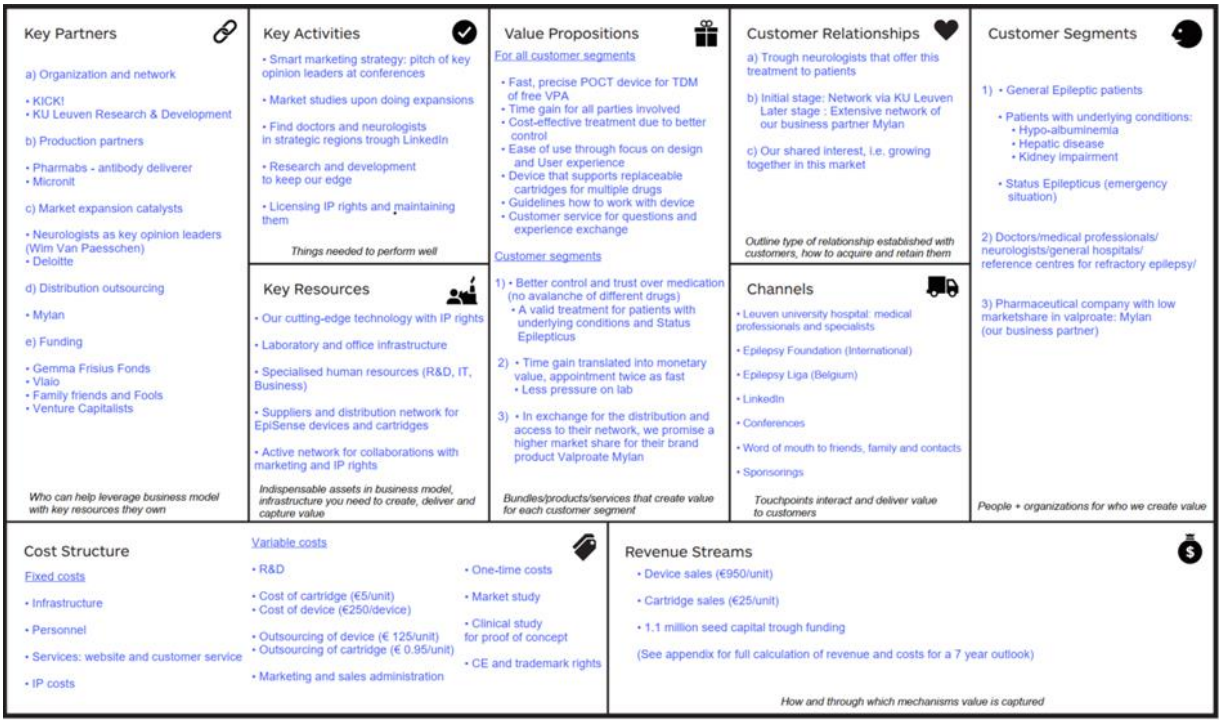
Team's Supervisor: The team received support from us, the supervisor and coaches, in every step of the development of this biosensing concept. More specifically, in the initial phase of the project, we discussed a selection of technologies and biosensing concepts with the team, inspired by the expertise and materials available within our group. Next, based on these concepts, an extensive literature search and their own interests and knowledge, the team came up with a number of ideas for the biosensor. Subsequently, we guided them in deciding on the final sensing principle, to end up with both a novel and feasible biosensing concept. Since the COVID-19 measures of KU Leuven severely limited the time that the students could spend in the lab (15 days in total from March till August), we offered additional support in efficiently organizing the lab work for collecting preliminary data. More specifically, we helped them in selecting the experimental tasks that would be feasible to carry out over this short period of time. In addition, we provided protocol drafts and training in the lab, to reduce the time required for experimental setup. Afterwards, the students planned, performed and interpreted the experiments independently. The business plan related to this biosensing concept was fully developed by the team. They looked independently for relevant information and partners to help them prepare a strong business plan, stressing the economic relevance of the proposed technology.

The biosensing concept is novel in several aspects of the proposed technology. Some of the major innovations are (i) the proposed microfluidic concept allowing the sequential flow of reagent plugs over a detection zone using (i)SIMPLE pumps, (ii) the use of magnetic particles to remove albumin-bound VPA from the plasma samples, (iii) the trapping of these particles on an (i)SIMPLE chip and (iv) the DNAzyme-based competitive assay for therapeutic drug monitoring, implemented on a microfluidic chip. It is, to the best of our knowledge, the very first time that these concepts are used in this way and joined to establish a novel biosensor for therapeutic drug monitoring.

Supervisor: Jeroen Lammertyn **Team captains:** Ine Mols & Laurens Goyvaerts

5. Translation potential

5.1. Business model canvas[1]



5.2. Stakeholder desirability

It is estimated that epilepsy affects between 5-6 million people in Europe, with a global prevalence between 0.6-1.2% of the population (14). For those treated with the drug VPA, there is a need for accurate and fast free VPA measurements, especially for epileptic patients suffering from underlying conditions such as hypo-albuminemia, hepatic disease, kidney impairment or for emergency situations like Status Epilepticus (17). Since responses to anti-epileptic drugs vary for each individual, limitations in determining drug levels in blood fast enough hampers proper treatment for the patients. Non-optimal dosing can negatively affect the patient's treatment success and health leading to severe side effects (15). Moreover, this cost-ineffective treatment leads to an increased annual cost for healthcare systems and implicit narrowing of the accessible market for pharmaceutical companies. A point-of-care (POC) test for therapeutic drug monitoring (TDM) may be applied at each doctor's visit to monitor therapy compliance, adjust therapeutic dose, and in emergency situations such as Status Epilepticus. Keeping the total addressable market in Europe in mind, an absolute minimum case of 5 million potential TDM tests per year for most commonly used anti-epileptic drugs (AEDs) could be achieved. Given that many patients are managed with several AEDs at the same time, and visit a specialist more than once per year, this number is potentially much larger.

Our stakeholder desirability comprises three parties: the epilepsy patients, the neurologists and our targeted pharmaceutical business partner Mylan. The choice for the latter is explained in the Business Feasibility paragraph. The proposed POC biosensor eliminates the need to send the samples to a lab. It brings down the time-to-result from several hours/days to less than ten minutes leading to a more efficient and cost-effective treatment. The main benefit is that the patients are immediately informed at the doctor's office and action can be taken by the doctor to adjust the patients drug intake. This also leads to a better informed patient and ultimately provides better control of their drug intake. This approach also results in the fact that they can stay longer at a specific prescribed dosage of drug, which eliminates the avalanche treatments that current epilepsy patients face. Moreover, reduced

[1] See appendix C for larger version.

time-to-result offers new possibilities for emergency cases and other patient's subgroups. Due to the time gain, the neurologist can meet by average two patients instead of one. An estimation has been made that the profit for this stakeholder, being €6 744 in the first year after paying the initial investment for the device. For Mylan, we aim to have a sustainable partnership to expand together in the valproate market for epilepsy patients. We have estimated that the gain in VPA usage could result in € 2 174 400 as a new income for Mylan per year, if VPA usage is increased by 3% in Europe and their market share is accounted at 10%. By proposing a cost-effective, fast biosensor with a precision equivalent to the gold standard lab tests of Roche Cobas, we aim to keep the trust of our three stakeholders. Our goal is to provide a stable drug intake to ensure a stable patient life.

5.3. Business feasibility

The foundation of the EpiSense start-up consists of a technical team assisted by an advisory board from MeBioS KU Leuven specialized in biosensor technology and microfluidics. The technical team is composed of a bioassay team, microfluidics engineer with support from an in-house innovation manager from KU Leuven. The expertise and network of KICK and the KU Leuven's R&D department will help in the organization of the start-up. They have a very high success rate in these venture types and have the most experience as an R&D incubator of its kind in Europe. For the production, EpiSense relies on Pharmabs, our key partner for exclusive antibodies. The manufacturing and scaling up of our products will be outsourced to the specialized manufacturing company Micronit. We have also contact with key opinion leaders at the university hospital that can be used as key partners for the proof of concept by carrying out a clinical study in the 4 centers for Epilepsy in Belgium. (UZ Leuven, UZ Gent, UCL Saint-Luc and ULB-Erasme). To acquire other key partners for our market expansion, EpiSense also trusts these key opinion leaders to give pitches about our start-up at conferences organized by the Epilepsy Foundation and the Epilepsy Liga. In return, they are given the opportunity to be the first ones to make use of our cutting-edge technology on a cost-free basis during the clinical studies. Deloitte is contacted to carry out market surveys upon expansion.
The commercialization strategy will consist of selling our devices and cartridges to a pharmaceutical company to be further sold to neurologists. To do so and to acquire other customers, EpiSense opts to close a deal with Mylan as our business partner for the distribution. Mylan is a smaller player in the valproate market, yet a big Pharma player, making it an interesting choice to grow together in this market. We plan to start this collaboration in 2023, after a proof-of-concept and clinical study. In the deal, Mylan will carry out the distribution with corresponding costs. In return, they receive a higher market share for their brand product Valproate Mylan by having an extra product over direct and very important competitors like Sanofi.
For a successful commercialization, the key activities are smart marketing to transmit adequately our value proposition, third party collaborations in particular with the pharmaceutical company, and continuous research for future opportunities to expand our customer base. The latter can be achieved by expanding into migraine or bipolar disorder or by developing a multiplexing chip to target other drugs at the same time. The multiplexing on chip has great promise as our technology has the technical feasibility to do that and there are many more anti-epileptic drugs in the market that have the same efficacy as valproate.

5.4. Financial viability

EpiSense's financial viability is supported by a business strategy, built upon surveys and interviews with patients, neurologists and investors. EpiSense has had meetings with Wim Van Paesschen, to understand the needs for doctors, Tim Buckinx CEO of epihunter whose son has epilepsy, to validate the needs and a day in the life of an epileptic patient, Filip Delport CEO of FOx Biosystems, to

understand the hurdles a start-up faces in the biosensor market, Marrit van den Heuvel from KICK, to get a view of the benefits of being a spin-off from the University, Francesco Dal Dosso an innovation manager from the KU Leuven, to validate our business case.
Our in-house developed benchtop EpiSense device and cartridges make up our revenue stream. The cartridge will be the key driver of EpiSense's financial growth. Both benchtop device and cartridge sales will be enabled through a strategic business partnership with Mylan and will be sold to them for €950/unit and €25/unit, respectively.
In Europe, the direct and indirect healthcare costs per patient were estimated to be more than €6 000/patient/year in France, Germany and the UK. This equates to total annual costs of almost €15 billion in these European countries alone in 2012 (based on total number of active prevalence cases) (18). Keeping the untapped market of TDM devices for Epilepsy in mind, it is clear that it holds a huge potential. Instead of having optimistic estimates, we did a worst case financial analysis. For the full calculation of our cost structure and revenue stream, we kindly invite you to have a look at the appendices. The summary of this analysis is visualized in figure 9 and table 1.
Keeping in mind the total addressable market of ± 66 000 epilepsy patients in Belgium, 184 000 in the Benelux and 5 million in Europe, from which ± 11% uses valproate(16). We ought to target 40% of these patients in the four refractory centres for epilepsy with the help of our key opinion leaders, resulting in 4.4% of the total market in Belgium in 2023. The following years, we assume to increase our market share by (i) expanding subsequently to the Benelux and Europe, (ii) reaching more than 40% of the VPA users each year, (iii) upscaling the percentage of VPA usage among epileptic patients due to our promising device. The accumulated market share over the next 7 years is presented in table 1.
In order to ensure a long-term sustainable business, critical initial and upscaling investments will need to be done in the first two start-up years while carrying out our study to have a proof of concept. After two R&D and upscaling years, we will start selling our devices and cartridges in 2023. The break-even point will be reached in 2026 years, 3 years after the initial product to market.

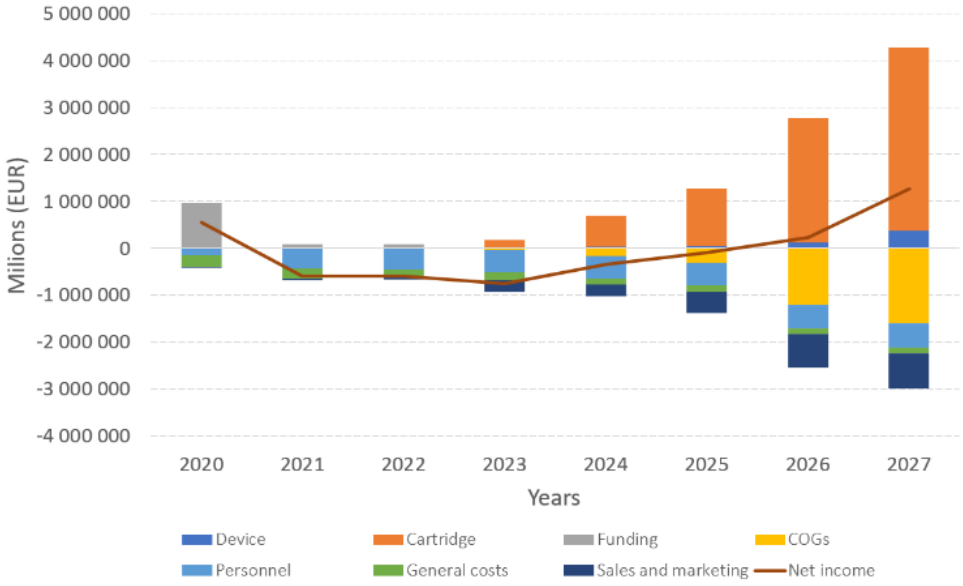


Figure 9 ***Estimated revenue, cost projection and net income for the first operating years***

Table 1 ***Estimate of our market share of total adressable market for the first operating years***

TOTAL ADDRESSABLE MARKET		
	Europe	**Benelux**
2023	2880/5 million= **0.06 %**	2880 / 66 000 = **4.4 %**
2024	11756 / 5 million = **0.24 %**	11756 / (66 000 + 124 000) = **6.2 %**
2025	21804/ 5 million = **0.44 %**	21804 / (66 000 + 124 000) = **11.5 %**
2026	47310 / 5 million = **0.9 %**	31800/ 190 000 = **16.7%**
2027	69020 / 5 million = **1.3 %**	38 000 / 190 000 = **20 %**

6. Team and support[2]

6.1. Contributions of the Team Members

- **Aditya Badola** is a member of the entrepreneurship and technology development team.
- **Yanjing Li** is part of the bioassay team and social media team.
- **Ine Mols** is the team captain of the bioassay team and part of the sponsoring team.
- **Torben Roy** is part of the technology, entrepreneurship and the sponsoring team.
- **Marina Ventouratou** is a member of the bioassay, the social media and sponsoring team.
- **Minerva Corrales** is part of the bioassay as well as the sponsoring team.
- **Gabriela Rath** is a member of the bioassay team and the social media team.
- **Chinmay Pendse** is a member of the technology and the social media team.
- **Laurens Goyvaerts** is the team leader of the technology and entrepreneurship team.
- **Roozbeh Yazdanpanah** is a member of the technology and social media team.
- **Sruja Dave** is part of the technology team.

6.2. People who have given support

- **Prof. Jeroen Lammertyn** is the head of the MeBioS-biosensors group of KU Leuven. He arranged the participation of the KU Leuven team in SensUs, allowed us to work in his lab and gave us the available equipment. Without this, our participation in SensUs would not be possible.
- **Dr. Dragana Spasic** is the research manager of the MeBioS-biosensors group. She offered her help when finalizing the bioassay concepts and was happy to give her opinion when we were not sure about which approach to choose.
- **Dr. Francesco Dal Dosso** is the IOF-manager of the Biosensors group. With his expertise, he was able to help us with the finishing touches of the business plan.
- PhD candidate **Sara Horta** is one of our coaches. Her expertise in (protein-based) bioassay development was of great value in the development of our biosensing concept.
- PhD candidate **Lorenz Van Hileghem** is one of our coaches, as an expert in (i)SIMPLE microfluidics, his knowledge was essential to make the technological part of the concept succeed.
- PhD candidate **Seppe Driesen** is one of our coaches, with his experience in DNA nanotechnology, he taught us to understand, develop and interpret DNAzyme-based bioassays.
- **Dr. Karen Ven** is one of our senior coaches. She offered her help in the DNAzyme-related work and was able to support the team in a practical way, thanks to her experience in coaching earlier SensUs teams.
- **Dr. Karen Leirs** is one of our senior coaches and helped both the bioassay and technology team with her experience in the field when asked. She was also able to offer practical help, thanks to her experience with SensUs in previous years.
- **Prof. Wim Van Paesschen** is the epilepsy expert at the neurology department at Gasthuisberg (the KU Leuven university hospital). He offered help to the business team by indicating the current needs for TDM in Epilepsy patients, based on his own experience.

6.3. Sponsors

- **Dr. Filip Delport** is the CEO of Fox Biosystems, a spin-off company of the Biosensors group. He offered help for the business plan by answering our questions based on his own experience of starting a company in the Biosensors and Diagnostics field.
- **Epihunter** is a resourceful and helpful company that readily shared their personal experience dealing with epileptic persons and how they generated feedback on the use of their technology. This input was particularly useful to develop our business canvas and predict market value in future.
- **KICK** is the entrepreneurship division of KU Leuven. Marrit van den Heuvel from KICK helped in creating the business plan, taking it to the next level with her insights and expertise.

[2] See appendix D for detailed version

7. Final Remarks

First of all, we want to start off with showing our gratitude to SensUs for organizing the event and to KU Leuven and Prof. Dr. Lammertyn for the opportunity to participate in the event. Being part of the EpiSense team allowed us to evolve as scientists and to learn new skills. We want to specifically thank our coaches for their support and the countless hours they put in the team. Additionally, we want to thank our sponsors for the shared knowledge and advice.

Our coaches and the team were challenged this year by the difficult situation we, and most of the other teams, faced due to the COVID-19 pandemic. Even though the coaches did everything they could to get us back in the lab, ongoing limitations and regulations made our time limited. This is the reason why we can only show preliminary data. We do believe that our biosensing concept is a realistic idea that could be implemented to help patients using VPA. The biosensor could also be modified to detect other drugs by changing the cartridge, by immobilizing different antibodies and by changing the DNAzyme conjugates. Despite all challenges we faced, we are grateful for the opportunity and would recommend participation to all.

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9. Appendix

A. Sequence DNAzyme with linker

/5AmMC6/TTTTTTTTTTTTTTT *TGGTGA* GGCTAGCTACAACGA *GGTTGT*

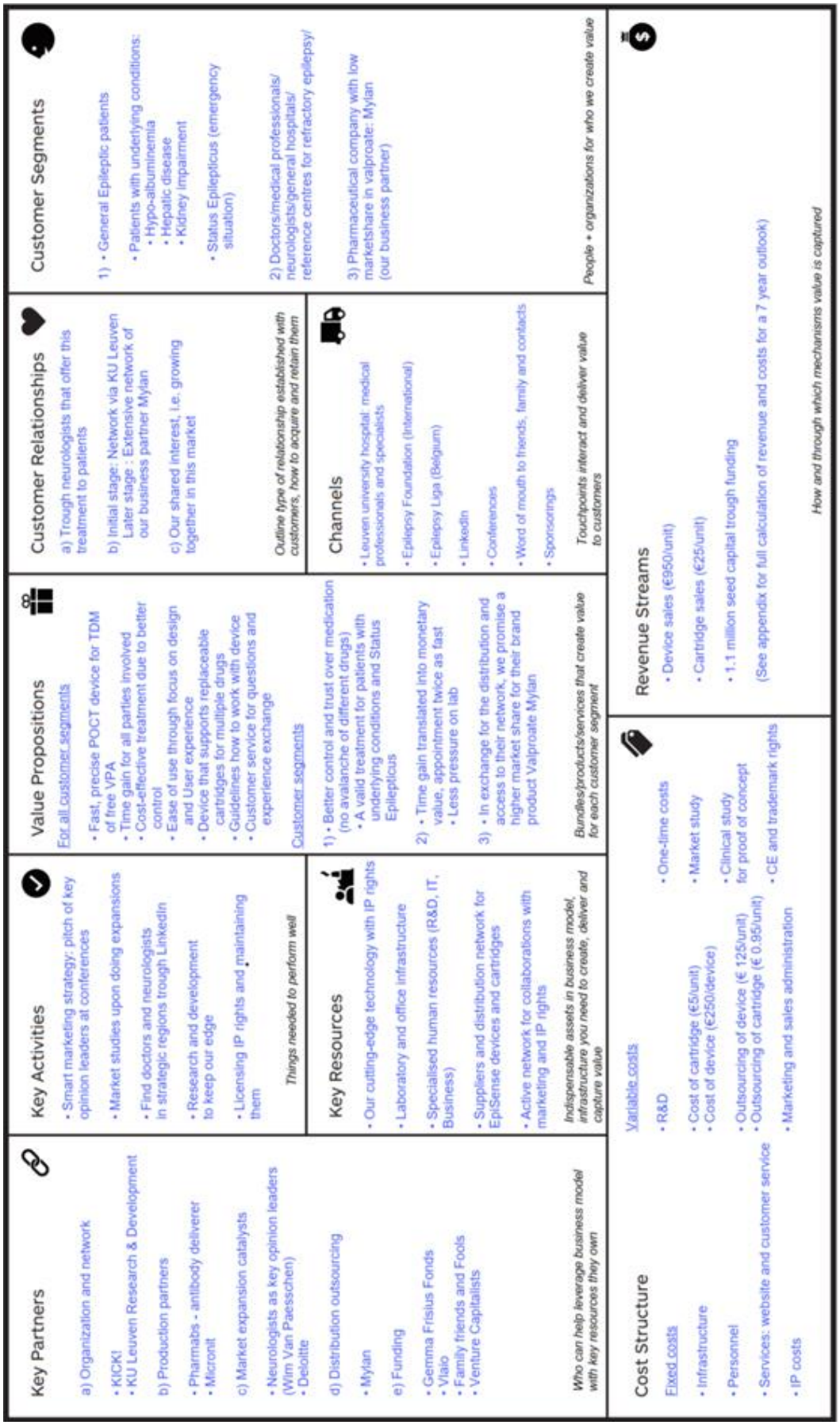
- /5AmMC6/ = Amino group
- PolyT spacer: 15 T nucleotides to ensure that substrate can bind to DNAzyme after conjugation to VPA
- Regular short DNAzyme

B. Sequence DNAzyme substrate

FAM / *ACAACC* gu *CACCA* / IowaBlackFQ

- FAM = fluorophore
- IowaBlackFQ = quencher
- gu = RNA bases, cleavage happens here
- *Complementary to substrate binding arms*

C. Business model



D. Team contribution in detail

Aditya Badola is a member of the entrepreneurship and technology development team. He contributed in the brainstorming of business-plan ideas, designing the biosensor device and creating the website for the EpiSense platform (https://episense.net/)

Yanjing Li is part of the bioassay team and communication team who mainly worked in the bioassay team, participating in idea raising and feasibility discussion. Besides, he was also responsible for posting on social media.

Ine Mols is as a team captain in charge of organizing team meetings, communication with SensUs and other outside partners/organisations and she makes sure the team meets the deadlines. Besides that, she is part of the bioassay team in which she contributed in the literature studies, team discussions and performed the experiments in the lab.

Torben Roy is part of the technology team, entrepreneurship team and the sponsoring team. He is one of the main members in the lab and helped brainstorming and setting up the technology plan. He also contributed to finding sponsors, interviewing, setting up the business plan and creating the logo.

Marina Ventouratou is a member of the bioassay, the communication as well as the sponsoring team. She is involved in the brainstorming, the feasibility discussion and the literature research. She also contributed to the dissemination of our work through our team's social media accounts and to finding sponsors.

Minerva Corrales is part of the bioassay as well as the sponsoring team. In the beginning she was actively searching for literature information and creating the brochure that we used to look for sponsors. She is also one of the members working in the lab testing and optimizing the theoretical idea.

Gabriela Rath is an active member of the Bioassay team and the communication team. She contributed to brainstorming sessions, literature studies, and feasibility discussions. Gabriela was also responsible for the Facebook page and helped in the organization and frequency of posts on social media, as well as the Instagram Take Over and Vlog Competition.

Chinmay Pendse is a member of the technology and the communication teams. He helped generate and execute new ideas for social media posts and to prepare the team mission statement. He also participated in team discussions and worked in the lab to test the technology feasibility.

Laurens Goyvaerts is the team leader of the technology and entrepreneurship teams. He contributed in establishing a proof of concept of the magnetic beads and albumin trapping specifically next to the user-interface. Moreover, he helped in making the business plan, doing the interviews and surveys.

Roozbeh Yazdanpanah is a member of the technology and communication team. He has mostly contributed to the feasibility discussions and literature reviews for the magnetic nanoparticles concept and brainstorming sessions with other team members.

Sruja Dave is part of the technology team. She contributed in the literature survey and feasibility discussions.

		2020 (Sep-Dec)		2021		2022		2023		2024		2025		2026		2027	
EpiSense												**P&L statement in EUR**					
Date	11th August 2020							Start product to market									
		2020		**2021**		**2022**		**2023**		**2024**		**2025**		**2026**		**2027**	
Revenue streams (EUR)			0		0		0		171500		691675		1272075		2775200		4262375
EpiSense cartridge	Unit Price (EUR)	25		25		25		25		25		25		25		25	
	#Units	0	0	0	0	0	0	6480	162000	26451	661275	49059	1226475	106448	2661200	155295	3882375
EpiSense biosensor device	Unit Price (EUR)	950		950		950		950	648	950	629,7857	950	545,1	950	506,8952381	950	493
	#Units	0	0	0	0	0	0	10	9500	32	30400	48	45600	120	114000	400	380000
Total devices								10		42		90		210		315	
Cost of Goods Sold (EUR)			0		4975		5950		46590		169383,5		309901,05		1195865,6		1599005,3
Material for production of Products/Services																	
EpiSense cartridge (Ab, magn NP, DNAzymes, ho	Unit Price (EUR)	5		5		5		5		5		5		5		5	
	#Units	0	0	500	2500	1000	5000	7200	36000	26451	132255	49059	245295	106448	532240	155295	776475
EpiSense biosensor device	Unit Price (EUR)	250		250		250		250		250		250		250		250	
	#Units	0	0	8	2000	0	0	10	2500	32	8000	48	12000	1500	375000	1800	450000
Outsourcing for production																	
EpiSense cartridge	Unit Price (EUR)	0,95		0,95		0,95		0,95		0,95		0,95		0,95		0,95	
	#Units	0	0	500	475	1000	950	7200	6840	26451	25128,45	49059	46606,05	106448	101125,6	155295	147530,25
EpiSense biosensor device	Unit Price (EUR)	0		0		0		125		125		125		125		125	
	#Units	0	0	0	0	0	0	10	1250	32	4000	48	6000	1500	187500	1800	225000
GROSS MARGIN (EUR)		0		-4975		-5950		124910		522291,6		962173,95		1579334,4		2663369,8	
(Revenue-Cost of Goods)																	
Funding (EUR)		960000		80.000		80.000		0		0		0		0		0	
IWT innovation mandate (VLAIO)		80000		80.000		80.000											
KUL C3 project		30.000															
Gemma Frisius		500.000															
Billateral collaborations		300.000															
Founders + family, friends and fools)		50.000															

		2020 (Sep-Dec)		2021		2022		2023		2024		2025		2026		2027	
EpiSense												**P&L statement in EUR**					
Date	11th August 2020							Start product to market									
		2020		**2021**		**2022**		**2023**		**2024**		**2025**		**2026**		**2027**	
Personnel (EUR) (bruto salary)			144666,7		434000		445580		457507,4		473792,6		486446,401		499479,7927		512904,19
CEO	Cost per year	50000		150000		154500		159135		163909,1		168826,3215		173891,1111		179107,8445	
	#FTEs	1	50000	1	150000	1	154500	1	159135	1	163909,1	1	168826,322	1	173891,1111	1	179107,84
Head of R&D	Cost per year	33333,33		100000		103000		106090		109272,7		112550,881		115.927		119405,2297	
	#FTEs	1	33333,33	1	100000	1	103000	1	106090	1	109272,7	1	112550,881	1	115927,4074	1	119405,23
Head of manufacturing	Cost per year	33333,33		100000		103000		106090		109272,7		112550,881		115927,4074		119405,2297	
	#FTEs	1	33333,33	1	100000	1	103000	1	106090	1	109272,7	1	112550,881	1	115927,4074	1	119405,23
Biofluidics specialist (PhD)	Cost per year	16000		48000		48000		48000		48000		52000		52000		52000	
	#FTEs	1	16000	1	48000	1	48000	1	48000	1	52000	1	52000	1	52000	1	52000
Administrator	Cost per year	12000		36000		37080		38192,4		39338,17		40518,31716		41733,86667		42985,88268	
	#FTEs	1	12000	1	36000	1	37080	1	38192,4	1	39338,17	1	40518,3172	1	41733,86667	1	42985,883
Sales manager	Cost per year	0		0		0		100000		103000		106090		109272,7		112550,881	
	#FTEs	0	0	0	0	0	0	1	100000	1	103000	1	106090	3	327818,1	5	562754,41
Other sales personel	Cost per year	0		0		0		36000		37080		38192,4		39338,172		40518,31716	
	#FTEs	0	0	0	0	0	0	1	36000	1	37080	2	76384,8	3	118014,516	4	162073,27
General costs (EUR)			258500		208.500		183.500		181.000		131000		131000		131000		131000
Infrastructure			36000		36000		36000		36000		36000		36000		36000		36000
IP (TM, simple + CE + own + legal costs)			22.500		22.500		27500		45.000		45000		45000		75.000		75.000
R&D costs			200.000		150.000		120.000		100.000		50.000		50.000		20.000		20.000
Clinical study cost (we pay patients pay-back by government)					8333		16667										
Sales and Marketing			7.000		32000		32000		245000		251080		445474,8		710832,616		749827,67
Marketing and sales costs			2.000		2000		2000		4000		6000		8000		10000		20000
Website			5.000		5000		5000		5000		5000		5000		5000		5000
Market Studies + networking			0		25000		25000		100.000		100.000		250.000		250.000		0

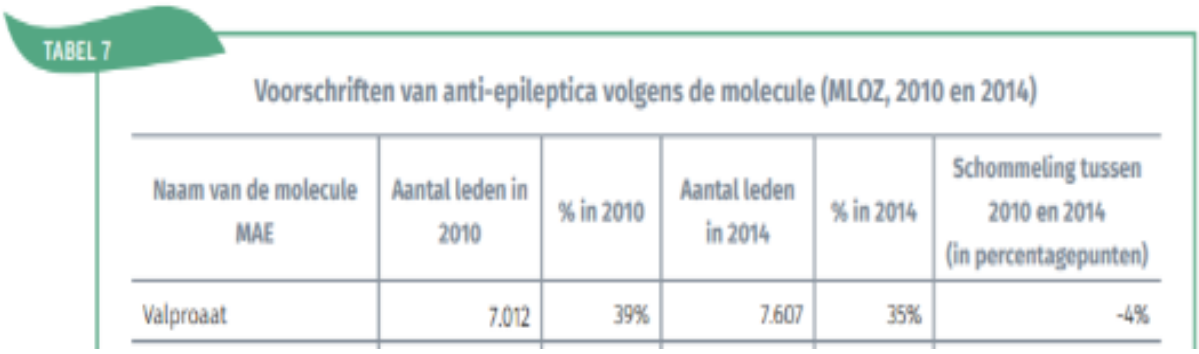
REMARK: Sales Team is in this total price

	Year	2020	2021	2022	2023	2024	2025	2026	2027
Revenue streams									
	Device	0	0	0	9500	30400	45600	114000	380000
	Cartridge	0	0	0	162000	661275	1226475	2661200	3882375
	Funding	960000	80000	80000	0	0	0	0	0
	Total revenue	960000	80000	80000	171500	691675	1272075	2775200	4262375
Costs									
	COGs	0	-4975	-5950	-46590	-169383	-309901	-1195866	-1599005
	Personnel	-144667	-434000	-445580	-457507	-473793	-486446	-499480	-512904
	General costs	-258500	-208500	-183500	-181000	-131000	-131000	-131000	-131000
	Sales and marketing	-7000	-32000	-32000	-245000	-251080	-445475	-710833	-749828
	Total costs	-410167	-679475	-667030	-930097	-1025256	-1372822	-2537178	-2992737
Net income		549833,3	-599475	-587030	-758597	-333581	-100747	238022	1269638

	2020	2021	2022	2023	2024	2025	2026	2027
Total revenue	960000	80000	80000	189499	793956,8	1698157	4008526	11805792
Total costs	410166,7	677475	666030	922897,4	1019058	1408128	2674931	4331047
Profit	549833,3	-597475	-586030	-733398	-225101	290028,7	1333595	7474745
Cumulative profit/debt	549833,3	-47641,7	-633672	-1367070	-1592171	-1302142	31452,85	7506198

Detailed calculation of cost structure and revenue streams to incorporate into excel file

8000 people in Belgium who use valproate (extrapolated) [1]



TABEL 7

Voorschriften van anti-epileptica volgens de molecule (MLOZ, 2010 en 2014)

Naam van de molecule MAE	Aantal leden in 2010	% in 2010	Aantal leden in 2014	% in 2014	Schommeling tussen 2010 en 2014 (in percentagepunten)
Valproaat	7.012	39%	7.607	35%	-4%

90% epilepsy = 7200 patients

Total persons in Europe 27 member states. As of 1 February 2020, the population of the EU is about 445 million people. [2]

+ Switzerland + Norway + Balkan = 45 million = 490 million

0.6-1.2 % active epilepsy out of 490 million [3]

490 million * 1% = 4.9 million so 5 million in Europe

2023

2880

4 Epilepsy centres = 0.4* 7200 = 2880 * 2.25 = 6480 cartridges

648 per device per year

2024

7200/ 66 000 = 11 %

11% scaling up to 16% estimate

16% of 66 000 = 10500

(10500 - 7200 = 3300 extra patients)

60 % instead of 40 %

0.6* 10500 = 6300 * 2.25 = 14175

14175/ 42 devices = 338

Nederland + Luxemburg

11 % * (120 000 + 4000) = 13640 [2]

13640 * 0.4 = 5456

Total patients

5456+ 6300 = 11756

Total cartridges

11756 * 2.25 = 26451

26451/ 42 = 630 per device per year

2025

20 % Belgium and 16 % the Netherlands and Luxembourg

0.2 * 66 000 = 13200

13200 * 0.75 = 9900

6 * 124 000 = 19840

19840 * 0.6 = 11904

Total patients

9900 + 11904 = 21804

Total cartridges

21804 * 2.25 = 49059

49059/90 = 545 cartridges per device per year

2026

We think our expansion in the first year is going to have troubles so we assume we could reach 3% of the total VPA usage in Europe.

11 % * 4.7 million = 517 000

0.03 * 517 000 = 15 510 extra in Europe due to problems with starting up

The rise in patients in Benelux

100 % total valproate users in Belgium = 13200 (everyone that uses this valproate can be reached, we assume that there is no further increase in VPA so we stay on 20%)

20 % * 124 000 = 24800 in Netherlands and Luxembourg

0.75 * 24 800 = 18600

Total patients in Europe

18600 + 13 200 + 15 510 = 47310

47310 * 2.25 = 106448

106448 / 210 = 507 cartridges per device per year.

2027

We assume that we will reach 6 % of the total VPA usage in Europe (raw estimate)

We assume that 11% is harder to scale up here to 16%, so we stay stuck on 517 000 VPA usage

0.06 * 517 000 = 31 020

Now also full saturation (100%) in NE and LUX (1 year of lagging on BE) so 24 800

We assume stagnation on total users in BE so we are stuck on 13 200

Total patients in Europe

13200+24800+31 020 = 69020

Total cartridges: 69020 * 2.25 = 155 295

155 295 /315 = 493 cartridges per device per year

Total addressable market		
	Europe	Benelux
2023	2880/5 million= **0.06 %**	2880 / 66 000 = **4.4 %**
2024	11756 / 5 million = **0.24 %**	11756 / (66 000 + 124 000) = **6.2 %**
2025	21804/ 5 million = **0.44 %**	21804 / (66 000 + 124 000) = **11.5 %**
2026	47310 / 5 million = **0.9 %**	31800/ 190 000 = **16.7%**
2027	69020 / 5 million = **1.3 %**	38 000 / 190 000 = **20 %**

Revenue stream pharma player:

11 % valproate initial

Scalable due to our device to 16 %

5% increase in usage for them, based on drug usage in Benelux the past 4 years

So distribution cost taken over by pharma player and in return VPA usage increases

7200 / 66000 = 11%

9 % (only Depakine in Europe) * 4.8 million = 432 000

Estimate that we can bring it to 12 percent in Europe = 576 000

Gain = 576 000 – 432 000 = 144 000

144 000 * unit cost of valproate

Market share of Mylan of these 144 000 is 10 % as an estimate

Thus resulting in 14 400

300 mg [4]

100 tablets per pack = € 10.06 [4]

Assumption (600-2000 mg) so 1200 mg per day, so 4 tablets (2 doses per day) [5]

25 days with 1 pack

365 / 25 = 15 packs per year needed

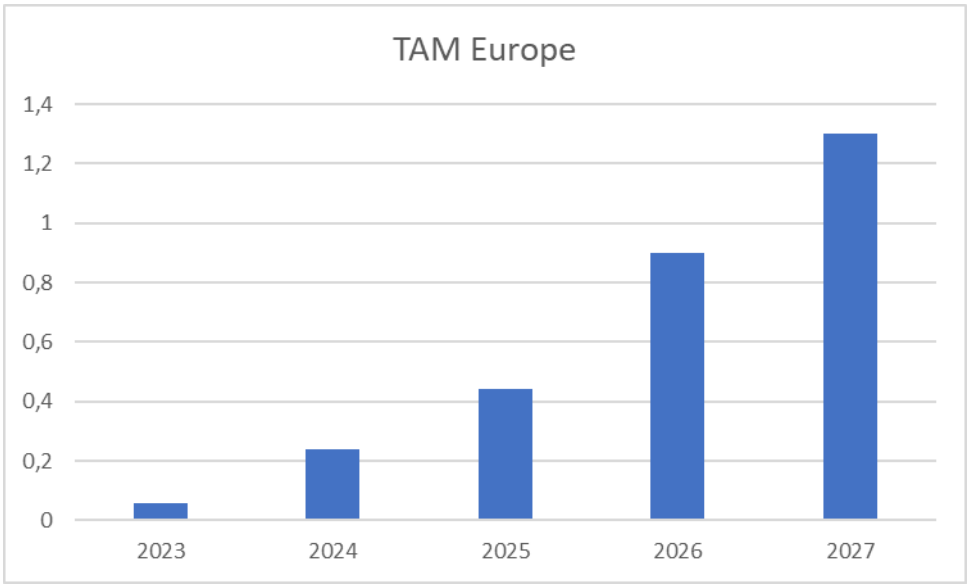
15*10.06 = €151

€151 * 14 400 new patients = € 2 174 400 is new income for pharma player per year

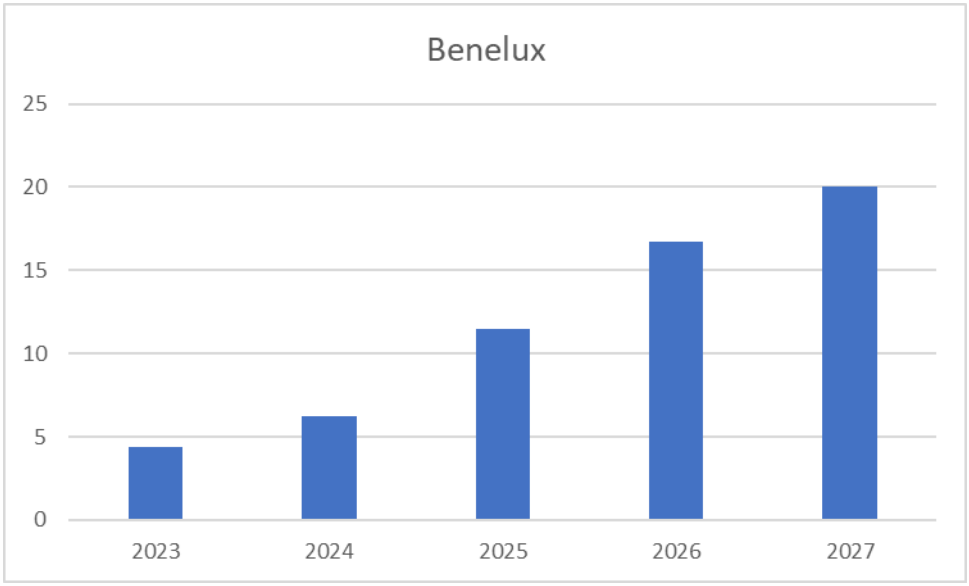
Final numbers for profit and loss statement

Year	Total patients	cartridges	cartridges per
2023	2880	6480	648
2024	11756	26451	630
2025	21804	49059	545
2026	47310	106448	507
2027	69020	155295	493

Total addressable market Europe



Total addressable market Benelux



Sources

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[4] https://www.bcfi.be/nl/chapters/11?frag=8730&trade_family=7229

[5] https://www.nhs.uk/medicines/sodium-valproate/