PROJECT FINAL REPORT

REGENER-AR: Allogenic eASCs clinical trials phase I/IIb for treating rheumatoid arthritis

Acronym: REGENER-AR

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1. FINAL PUBLISHABLE SUMMARY REPORT

1.1 EXECUTIVE SUMMARY

Rheumatoid Arthritis (RA) is a major challenge for the health systems due to its vast prevalence and the special characteristics of the disease, particularly its long term sequelae. RA constitutes an inflammatory and chronic disease that affects over 21 million people worldwide (UN World Population Database, 2004). RA remains as an unmet clinical need because approximately 20-40% of RA patients do not have an adequate response to the current treatments. Cellular therapy with expanded adipose-derived mesenchymal stem cells (eASCs) can be an innovative therapeutic alternative for inflammatory diseases such as RA due to their capacity to modulate immune responses by reducing the activity of several types of immune cells.

The main goal of the REGENERAR Consortium was to investigate at preclinical and clinical levels, the safety and potential efficacy of allogeneic eASC treatment in RA, and therefore, a phase Ib/IIa, escalating dose, single-blind, clinical trial to assess the safety of the intravenous administration of eASCs to refractory RA patients was conducted. The trial was successfully finalized and the results obtained showed the safety of the treatment and provided a first indication (yet limited) of the potential efficacy of cell therapy with allogeneic eASCs in RA. The positive outcome of the trial, along with the knowledge generated (better understanding of mechanism of action, biodistribution and toxicology), encourages further clinical development of the use of allogeneic eASCs for the treatment of RA. TiGenix is currently working on the preparation of a new trial in early RA to further confirm the potential therapeutic effect of eASCs in this disease.

1.2 SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES

Introduction

Rheumatoid Arthritis (RA) is a major challenge for the health systems of the developed world and increasingly for developing countries due to its vast prevalence and the special characteristics of the disease, particularly its long term sequelae. RA constitutes an inflammatory and chronic disease that affects over 21 million people worldwide (UN World Population Database, 2004). It has a great impact on the patient's quality of life, and incurs important economic and social costs. The current treatment of RA involves early intervention with synthetic disease modifying anti-rheumatic drugs (so-called DMARDs). If inflammation cannot be adequately suppressed by these first line DMARDs, biologic DMARDs, being mainly antibodies against inflammatory molecules or cells, are employed. Despite all the available treatments, RA remains as an unmet clinical need because approximately 20-40% of RA patients do not have an adequate response to the current treatments. Moreover, several concerns about long term safety of current RA treatments have arisen.

Cellular therapy with mesenchymal stem cells (MSCs) is a promising new treatment approach addressing yet unmet medical needs in various inflammatory and autoimmune diseases such as RA. Human adipose-derived mesenchymal stem cells (ASC) are a source of adult stem cells that can be easily isolated from fat tissue and expanded by in vitro cell culture (eASCs). eASCs have been shown to modulate immune responses by reducing the

activity of several types of immune cells. These properties situate eASCs as a promising therapeutic approach for treating chronic inflammatory diseases.

Considering the immunoregulatory potential of ASCs, REGENERAR have made an effort to develop an innovative treatment for RA based on the use of expanded ASCs (eASCs) extracted from the adipose tissue of a healthy donor (allogeneic). We have clinically tested the capacity of these cells to modulate the inflammatory process that occurs in the RA with the aim to provide therapies that will be:

- Affordable, in terms of the production costs of the medicinal product;
- Readily and widely available, implying that the product may be easily stored and readied for application any time at a reasonable cost;
- Easy to apply and compatible with current clinical standard of care
- Produce a robust immunoregulatory response

Project objectives

To accomplish this goal, the following objectives were defined:

1. **Clinical objectives:** To conduct a multicenter clinical trial (EudraCT no.: 2010-021602-37), entitled "Phase Ib/IIa, escalating dose, single-blind, clinical trial to assess the safety of the intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells (eASCs) to refractory rheumatoid arthritis (RA) patients". This clinical trial aims to firstly determine the safety and secondly the efficacy of this novel RA treatment.

2. **Preclinical objectives:** To strengthen the scientific knowledge of treating RA with eASCs, by e.g. exploring the different immunological mechanisms of eASC or studying their distribution in the body. This should support further clinical development including future improved treatment regimens.

3. **Pharmaceutical development and manufacturing process objectives:** To provide a validated scaled-up eASCs manufacturing process, based on the use of cell bioreactors. These manufacturing developments are designed to meet the regulatory agencies quality requirements.

4. **Regulatory objectives:** To ensure that all regulatory questions on the clinical trial are satisfactorily addressed in consultation with regulatory agencies. In addition, the technical development of the eASC process is also being steered in line with the regulatory expectations. This integrated regulatory strategy is aimed to ultimately meet the requirements needed for a Marketing Authorization of the product.

1.3 A DESCRIPTION OF THE MAIN S&T RESULTS / FOREGROUNDS

Clinical results.

The multicenter, randomized, placebo-controlled Phase Ib/IIa trial enrolled 53 patients with active refractory rheumatoid arthritis and was successfully finished. This is a challenging RA patient group as the patients failed to respond before to at least two biologics. The study design was based on a three cohort dose-escalating protocol, meaning that the three patient groups received different doses of eASCs, starting first with the group with the lowest dose. For both the low and medium dose regimens (1 or 2 million cells per kg body weight) 20 patients received active treatment (i.e. intravenous administration of eASCs) versus 3 patients who received a placebo treatment; for the high dose regimen (4 million cells per kg body weight) 6 patients received active treatment versus 1 placebo. eASCs were administered at day 1, 8, and 15, and patients were followed up monthly over a six-month period. The aim of the study was to evaluate the safety, tolerability and optimal dosing over the full 6 months of the trial, as well as exploring therapeutic activity. Only one patient suffered serious adverse events that led to discontinuation of the treatment. All other side effects were mild and transient. To gain a first insight into the therapeutic activity of the eASCs, several standard RA clinical parameters were evaluated every month for six months. The cumulated eASC results in percentage of the EULAR score are shown in the graph here below (EULAR is a disease score established by the European League Against Rheumatism and is a good indicator of disease activity).

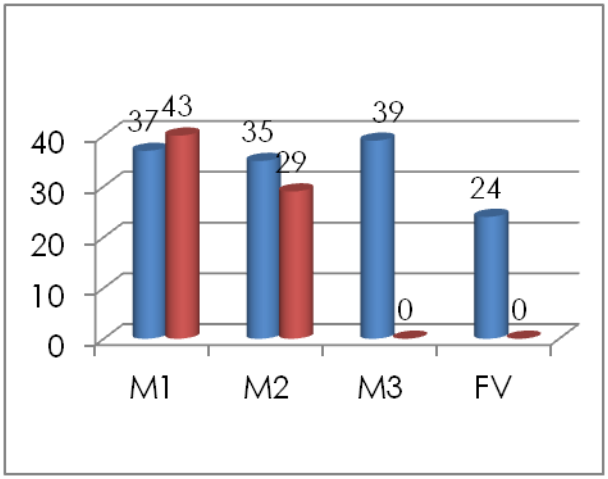


Figure 1: Clinical activity score (EULAR) in ASC or placebo-treated patients at different time points. EULAR disease activity was determined at months 1 (M1), 2 (M2), 3 (M3), and "final visit" (FV). Blue: ASC-treated patients (Cx611). Red: Placebo treated patients. N=46 for Cx611 and N=7 for placebo)

The combined three active treatment groups as well as the combined placebo groups at months 1 (M1), 2 (M2), 3 (M3), and "final visit at 6 months" (FV) are shown. Treatment with eASC resulted in a persisting therapeutic benefit compared to placebo treated patients as indicated by a sustained EULAR response at month 3 and final visit in the eASC-treated group (blue bars) which was not seen over time in the placebo group (red bars).

These results show the safety of the treatment and give a first indication of the potential efficacy of cell therapy with allogeneic eASC in RA. Due to these positive results we are currently preparing and designing a next clinical trial. For that purpose, advisory board meetings with key opinion leaders in the field of RA were organized. Based on the extensive scientific and clinical discussions a shift from refractory RA patients to early RA patients (non-responders to methotrexate) was recommended, as it was considered that this patient population is the one that

could benefit the most by ASC treatment. TiGenix is currently working on the clinical trial design of a new phase II trial in which the clinical relevance of eASCs and the applicability of the treatment to a significant proportion of RA patients could be substantiated. Based on the expert input, in-house research, recent stem cell research insights and literature analysis, TiGenix is planning to carry out a phase II double-blind, randomized, placebo controlled trial using intravenously administered eASCs in rheumatoid arthritis patients with active disease but refractory to methotrexate.

Moreover, we have studied blood samples (plasma cytokines, plasma microRNAs and peripheral blood cells) from patients participating in the clinical trial and compared in vitro a number of parameters before and after the treatment. This analysis aimed to identify potential prognostic biomarkers (that would allow selecting a patient population best suited for ASC treatment) and efficacy biomarkers (that would indicate the therapeutic effect caused by the eASCs). Interestingly, we observed some correlation between certain biomarkers and the response of patients to the treatment. These results, while promising, need to be further confirmed in next clinical trials with higher numbers of patients.

Preclinical results.

The preclinical work has been focused on the following activities:

- Understanding the mechanism of action of eASCs in vitro and in vivo. The mode-of-action of the immune-modulating capacities of mesenchymal stem cells (MSC), such as eASCs, has been investigated in vitro and in vivo by using MSC that are deficient in certain of their functions. Preliminary results show that some of those MSC indeed failed to reduce the severity of arthritis in model systems. This indicates that these factors are relevant for the therapeutic effect (mode-of-action) of MSC. In addition, the interaction of eASCs with different cells of the immune system has been studied. Notably, relevant information regarding the modulation by eASCs of immune cells with anti-inflammatory properties has been obtained.
- Understanding the interaction of eASCs with immunosuppressive drugs currently used in the treatment of RA. Contact of eASCs with DMARD immune-suppressor drugs routinely used in RA have been shown to not affect the biological activities of eASCs that are believed to play important role in their therapeutic capacity.
- Understanding the immune response against allogeneic (foreign) eASCs. The recognition of the eASCs by cells that are responsible for the recognition and possible elimination of foreign cells has been investigated. In addition, we have investigated the immune response of the patients against the allogeneic eASCs used. This has been done by measuring the generation of antibodies against the eASCs by the treated patients.
- Validation of alternative routes of administration. A new route of administration of eASC where the cells are injected directly in a lymph node (termed intralymphatic administration), has been optimized and successful results in controlling arthritis in a model system have been obtained.

- Understanding the capacity of eASCs to prevent arthritis before onset of the disease compared to their capacity to control the disease when already established. Different dose regimens have been explored in order to identify the best conditions of treatment.
- Understanding the differences between different donors of eASCs in order to be able to possibly identify the best ones (with higher therapeutic potential).
- Understanding the in vivo biodistribution of eASCs in healthy and arthritic conditions. Distribution of eASCs in the body has been studied, showing that the cell distribution varies depending on the route of administration.

The knowledge gained in these preclinical studies will help to better understand the biological activities and in vivo behaviour of the eASCs, and as such support the best therapeutic use of these cells.

Pharmaceutical and Manufacturing development results.

- The micro-RNA expression profile of eASC has been studied. This may allow identifying markers of eASCs identity as well as indicator molecules for comparing different eASC lots.
- Extensive work to find the optimal conditions for cryopreservation of eASCs has been done and new cryoformulations for eASCs have been developed. These new formulations improve the viability of the cells and might be incorporated in the next clinical trials.
- We have worked in the scaling-up process of manufacturing by using bioreactors. We encountered some unforeseen technical problems that did not allow us to progress as initially planned. Nevertheless, the efforts made will be of great help in the future progress of our manufacturing process.
- A new device for cell counting has been developed.
- The steps of the manufacturing process that can be automated were identified and a complete design of the automation process has been done. This may help to improve the efficiency of the manufacturing process of cell therapy products.

Regulatory results.

- Regulatory and clinical activities have been performed in close interaction to meet the regulatory requirements from the competent authorities and also to design the next steps in the clinical development of the use of allogeneic eASCs in the treatment of RA.
- The coordination of these both activities did ensure that the design of the clinical trial was meeting the regulatory requirements of the overviewing competent authorities, and did thereby allow a rapid start of the clinical trial.
- Other activities focused on the definition and implementation of a global regulatory strategy, and this in interaction with the regulatory authorities. This should support successful future clinical development.

1.4 EXPECTED FINAL RESULTS AND POTENTIAL IMPACT AND USE

Some of the above described results have already been presented in International Scientific Meetings across Europe and US, and ten scientific publications have been submitted or are currently in preparation for submission (at least eight publications). Based on the promising data, both in preclinical and clinical research, an important scientific impact of the research carried out in this project is expected both for the stem cell field as for progress in new RA treatment options.

The results obtained in the clinical trial confirm the safety of treatment with allogeneic eASCs and provide promising data regarding cell therapy of RA with eASCs. These clinical results, along with those obtained at the basic and preclinical level (mechanism of action, biodistribution, toxicology, etc), will support further clinical development not only in RA but potentially also in other inflammatory diseases. Due to the encouraging results obtained and knowledge generated in the clinical trial, TiGenix is currently working on the preparation of a new trial in early RA to further confirm the potential therapeutic effect of eASCs in this disease.

The progress in new cryoformulations and bioreactors, along with the potential automation of certain steps in the manufacturing process, should ensure a successful scale-up of the manufacturing.

Finally, successful regulatory strategies and approvals will pave the way for future clinical trials and also for other stem cell based therapies, leading eventually to broaden the therapeutic options in inflammatory diseases to also include cell therapies.

1.5 POTENTIAL IMPACT

The project has been successfully executed and the main objectives have been achieved. Among them, the finalisation of the first-in-human multicenter, randomized, placebo-controlled Phase Ib/IIa trial to test the safety of treatment with allogeneic eASCs of patients with active refractory RA is, with no doubt, the most important and relevant one. The fact that the safety profile of the intravenous treatment of RA patients with allogeneic eASCs was good, and that some indication of therapeutic benefit was observed, supports further research on the use of cell therapy for the treatment not only of RA, but also for other inflammatory diseases. Our results and experience could benefit others in the development of safe and efficacious treatments based on MSCs.

1.5.1 SOCIO-ECONOMIC IMPACT

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that primarily affects joints producing swelling, pain and deformation that can lead to loss of function. RA is a progressive disease, symptoms that usually appears in patients in their middle years of life and worsen over time creating a serious impact in the

quality of life of the patients. In fact, it has been estimated that 50% of the RA patients are not capable to hold a full time job 10 years after of being diagnosed[1].

Rheumatoid arthritis is considered the most common inflammatory disease of the joints affecting to 40 million people worldwide[2] and over 3 million people in Europe alone[3] representing a heavy burden to the healthcare economies. Total cost of RA has been estimated to be as high as €42M with an average expenditure per patient and year of €13,500. However this cost can rise significantly in the later stages of the disease when can reach the €40,000.

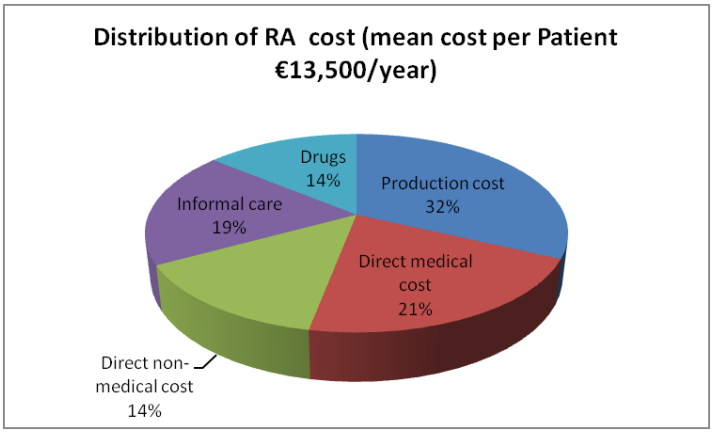


FIGURE 1. COST OF RA.

However, the majority of the costs generated by RA are not associated to medical expenses, 65% of costs linked to RA treatment occur outside the health care sector, being the cost associated to productivity losses the highest cost within the patient management. Production losses generate an enormous economic burden that is not limited to the society but has a significant impact on the economies of the patients.

As RA can severely restrict a person's ability to work even forcing them to leave the workforce which translates into income loss over the course of a lifetime as patients often live for 30 years or more with the disease. This data emphasizes the importance of aggressive treatment to prevent or delay the disability that can be caused by RA.

REGENERAR approach aimed to diminish the impact of the disease thanks to the immunomodulatory capacity of eASCs capable to balance the chronically imbalanced immune response of these patients. The outcomes obtained from the clinical trial have demonstrated encouraging results in a refractory population of RA patients (not responding to other therapies) where clinical and radiological improvement was identified in a subset of patients. These results motivate us to design a new clinical trial in early RA patients assisted by a group of leading clinical experts. The results of this new clinical trial will help us to demonstrate the efficacy of the proposed therapy and further continue with the development of an innovative treatment for RA.

1 Access to innovative treatments in Rheumathoid arthritis in Europe, 2009, A report of the European Federation of Pharmaceutical Industry associations.

2 Symmonds et al. The global burden of rheumatoid arthritis in the year 2000. Available at: http://www.who.int/healthinfo/statistics/bod_rheumatoidarthritis.pdf (Accessed August 2014).

3 The Burden of Rheumatoid Arthritis (RA) and Patient Access to Treatment by prof. Bengt Jönsson, DR. Gisela Kobelt and PROF. Josef Smolen

1.5.2 DISSEMINATION ACTIVITIES

The scientific publication in peer reviewed journal are the most important dissemination activities carried out during the project, as well as the attendance to the most relevant congresses and meetings in the field of stem cells or RA (see section 2.1 of this report).

Regarding the articles published, it is still difficult to calculate their impact since they are very recent, however the number of citation is increasing quickly indicating the interest generated. The journals chosen by the REGENERAR researchers were appropriated to reach the intended audience. Moreover, most of the publications were done in open access journals to bring increased visibility, usage and impact for the Project outcomes.

The impact factor of the journals were the articles were publishes and the number of citations are detailed in the following table.

ARTICLE	JOURNAL IMAPCT FACTOR	NUMBER OF CITATIONS
Luz-Crawford, P., Noël, D., Fernandez, X., Khoury, M., Figueroa, F., Carrión, F., ... & Djouad, F. (2012). Mesenchymal stem cells repress Th17 molecular program through the PD-1 pathway. *PLoS One*, *7*(9), e45272.	3.73	32
Franquesa, M., Hoogduijn, M. J., Bestard, O., & Grinyó, J. M. (2012). Immunomodulatory effect of mesenchymal stem cells on B cells. *Frontiers in immunology*, *3*.	-	34
DelaRosa, O., Dalemans, W., & Lombardo, E. (2012). Toll-like receptors as modulators of mesenchymal stem cells. *Frontiers in immunology*, *3*.	-	18
Franquesa, M., Baan, C. C., Korevaar, S. S., Engela, A. U., Roemeling-van Rhijn, M., Weimar, W., ... & Hoogduijn, M. J. (2013). The effect of rabbit antithymocyte globulin on human mesenchymal stem cells. *Transplant International*, *26*(6), 651-658.	3.12	3
Mancheño-Corvo, P., Franquesa, M., de la Rosa, O., Ramírez, C., García-Benzaquén, L., Fernández, V., ... & Lombardo, E. (2013). Adipose mesenchymal stromal cell function is not affected by methotrexate and azathioprine. *BioResearch open access*, *2*(6), 431-439.	-	2
Roemeling-van Rhijn, M., Reinders, M. E., Franquesa, M., Engela, A. U., Korevaar, S. S., Roelofs, H., ... & Hoogduijn, M. J. (2013). Human allogeneic bone marrow and adipose tissue derived mesenchymal stromal cells induce CD8+ cytotoxic T cell reactivity. *Journal of stem cell research & therapy*, *3*(Suppl 6).	4,63	4
Franquesa, M., Mensah, F. K., Huizinga, R., Strini, T., Boon, L., Lombardo, E., ... & Hoogduijn, M. J. (2014). Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. *STEM CELLS*.	7.13	1
Barrio, L., Cuevas, V. D., Menta, R., Mancheño-Corvo, P., Dalemans, W., Lombardo, E., & Carrasco, Y. R. (2014). Human	3.1	0

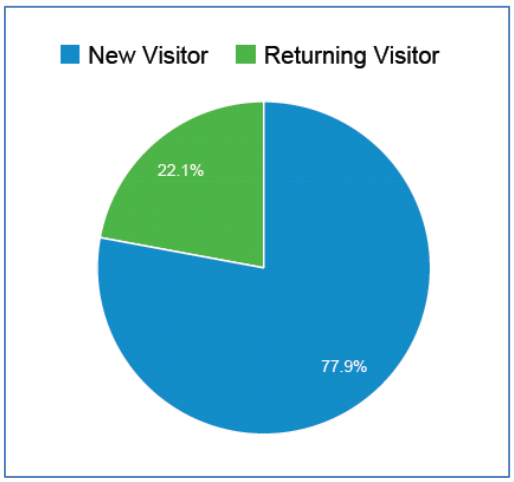
adipose tissue–derived mesenchymal stromal cells promote B-cell motility and chemoattraction. *Cytotherapy*, *16*(12), 1692-1699.		
Menta, R., Mancheño-Corvo, P., Del Río, B., Ramírez, C., DelaRosa, O., Dalemans, W., & Lombardo, E. (2014). Tryptophan concentration is the main mediator of the capacity of adipose mesenchymal stromal cells to inhibit T-lymphocyte proliferation in vitro. *Cytotherapy*, *16*(12), 1679-1691.	3.1	0
Toupet, K., Maumus, M., Luz-Crawford, P., Lombardo, E., Lopez-Belmonte, J., van Lent, P., ... & Noël, D. (2015). Survival and Biodistribution of Xenogenic Adipose Mesenchymal Stem Cells Is Not Affected by the Degree of Inflammation in Arthritis. *PloS one*, *10*(1), e0114962.	3.53	0

In addition, the Project website has been a useful tool in order to measure the impact of the project and the effectiveness of the project dissemination strategy being applied. The track of visits has been the main indicator. The dissemination strategy followed by the project dissemination team during the whole project seems to have been adequate and valid, as the visits to the website show a continuous increase since the website was launched until reach a constant number of visits per month (~1800-2000):

Informe: Gráfico de sesiones - regenerar.eu
Período de fechas: 05/01/2012 - 01/31/2014

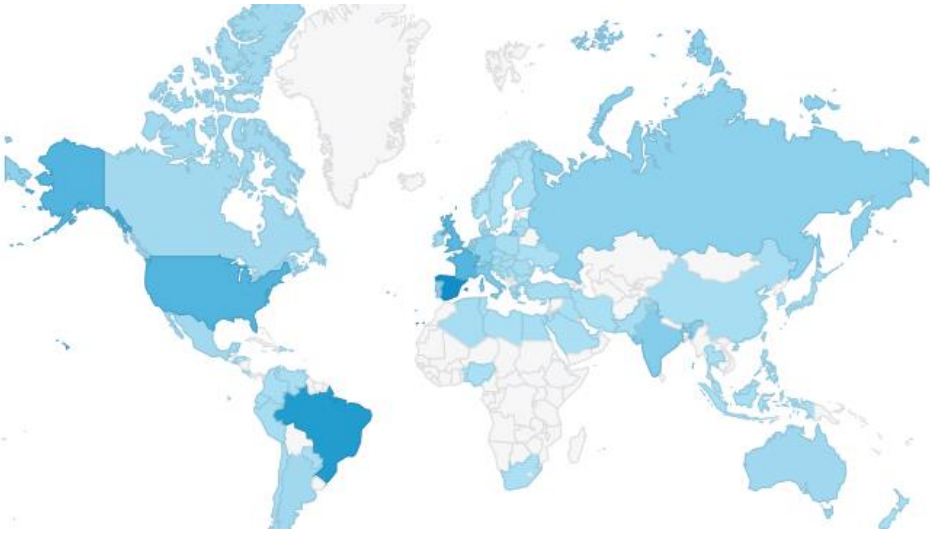
Gráfico de sesiones	3,000	Sesiones
5/2012		621
6/2012		924
7/2012		828
8/2012		778
9/2012		1.039
10/2012		1.292
11/2012		1.127
12/2012		1.336
1/2013		1.342
2/2013		1.188
3/2013		1.379
4/2013		1.537
5/2013		1.640
6/2013		1.515
7/2013		1.640
8/2013		1.637
9/2013		1.333
10/2013		1.798
11/2013		1.776
12/2013		1.790
1/2014		2.030
	Total:	28,550

Almost one quarter of the visitors were revisiting the site:



The origin of the visitors was monitored using the computers' IPs and the language of the visitor PC, those are the results:

	Language	% Sessions
1.	en-us	34.53%
2.	pt-br	13.36%
3.	es	9.49%
4.	fr	7.45%
5.	es-es	5.84%
6.	en-gb	4.36%
7.	it-it	3.23%
8.	ru	2.95%
9.	pt-pt	2.81%
10.	fr-fr	1.83%



1.5.3 EXPLOITATION OF RESULTS

The objective of Exploitation is to create revenues and/or provide social benefits.

In particular, the main target of the REGENERAR Exploitation activities is to find the best solution to take advantage of the REGENERAR project results and to reach an agreement among the project partners about the share of possible future incomes that may generate by the commercialization of the REGENERAR final products. To this end REGENERAR members will perform the required steps to protect the rights of the consortium trough patents, know-how or other adequate measures.

1.6 ADDRESS OF THE PROJECT WEBSITE

In February 2012 the REGENER-AR project website was launched: http://www.regenerar.eu/