Agenda
Management Committee Meeting

COST Action no. BM1406
Action Title: Ion Channels and Immune Response toward a global understanding of immune cell physiology and for new therapeutic approaches (IONCHAN-IMMUNRESPON)
IIMBC, Warsaw, Poland from _24 September_ to _25 September 2015____

1. Welcome to participants
   Quorum:
   a. 2/3 of country representatives
   b. 23 countries in BM1406
   c. 2/3 = 15 countries
   d. Today: 17/23 countries
   e. 32 participants (45,7%)

2. Adoption of agenda
   - The agenda has been approved by the Management Committee.

3. ~~Approval of minutes and matters arising of last meeting~~

4. Update from the Action Chair
   a. Status of Action, including participating countries
   New participating countries: Latvia and Turkey
   b. Action budget status
   There were fewer participants than expected (32 instead of 50 persons), so the unused money will permit more short-term scientific missions.
   On July 17th, 2015 by voting, the Management Committee accepted to decrease price for accommodation: 75 euros BB instead of 120 euros BB per night and per person.
   This rate is available only for this meeting in Warsaw.
   Charges on budget for meeting in Poland will be exact, once each participant will complete their own charges for this travel.

c. STSM status and new applications
Barbara M.; Purpose**:** *funding of short research exchange stays between laboratories, PhD students and postdocs* candidates STSM = 3 cf. doc in annexe 1. The duration of say to obtain the max amount is 91 days but shorter missions are possible. Remind that it's a contribution for a mission and not a global payment.
Applicants will send to Barbara a provisional budget and the core meeting will attribute corresponding amounts within the month.
- Decision to communicate a « red line » every 4 months

5. Promotion of gender balance and of Early Stage Researchers (ESR)
   - Full support to the COST directives
   - Gender balance at the proposal stage (% of women):
     - Initiators (27%)
     - Members (32,43%)
     - Proposed MC (26%)
   - Gender balance today (% of women):
     - Members (35,7%)
     - MC members (36,2%)
   - Early Stage Researchers (ECI)
   - Maria will give the number of ESR in the BM1406 Action, and details for DB

6. Update from the Grant Holder
   - Grant Agreement has been signed
   - 65 % of the total amount has been paid, the rest soon as there is need for cash

7. Update from the COST Association

   - New dissemination guidelines (August 2015)

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   - Website attack
     It's the reason why FVR wants to build the BM1406 website into the University's firewalls.

8. Follow-up of MoU objectives
   a. Progress report of working groups
      The scientific program of each WG is joined to the MCM report (annexe 2)
      Reports of each WG in meeting
9. Scientific planning
   a. Scientific strategy
   b. Action Budget Planning
   b. Long-term planning (including anticipated locations and dates of future activities)

Training School in Toulouse, February 2016
Lucette Pelletier needs to communicate more about budget and details. Date will be defined by FVR. A mail will be send with the program and dates to all participants for an expression of interest's call. Depending on the number of potential participants, the budget and organization will be planned.

   d. Dissemination planning (Publications and outreach activities)
A. Chemaly, new website, others dissemination Activities
   - Official Website: http://costbm1406.univ-tours.fr
   - Vote of the BM1406 logo; logo 2 was chosen (annexe 3)
   - Elesevier's proposal of a special issue on BM1406 thematic thanks to Pablo P.

10. Requests for new members
    - **LATVIA**:
      - Ms Zaiga **NORA-KRUKLE**
      - M. Martin **KALIS (present)**
    - **Turkey** :
      - Dr Ozlen **KONU (present)**
      - Prof Nuray **ERIN**

~~11. Non-COST applications to the Actions~~

12. AOB

13. Location and date of next meeting,
    - Lisbon
    - Place: **Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto 1649-003 Lisbon, Portugal**
    - **Accomodation**: SANA Metropolitan, http://www.metropolitan.sanahotels.com/en/)
    - Wednesday march 9th to friday march 11th, 2016
    - focused on WG3

Scientific program will be delivered by Friedrich KN, Pablo, Graca, FVR
Forecast: 30 participants,

14. Summary of MC decisions
    - The agenda proposed has been accepted
    - Training School in Toulouse:

    L. Pelletier needs to communicate about budget and details
    - Short Term Scientific Missions:

    Concerning the budget, B. Niemeyer will communicate a « red line » every 4 months. She will ask applicant to give a provisional budget for each short mission to allow the core committee to define the amounts of given funds. The three applicants will be funded by BM1406.

    - Official website: http://costbm1406-univ-tours.fr and facebook site are activated.
    - The ideas about dissemination planning and others dissemination activities, should be discussed in next meeting
    - Logo number 2 has been voted
    - Next meeting in march 9th to 11th, 2016, in Lisbon, Portugal
    -

15. Closing
    - End of MCM at 10:35, 25th September 2015

Annexe 1
STSM applicants

Application for COST travel grant for ☑ **PhD student** ☐ **Postdoc**

Applicant / home institute / position

Name: **Alba Clara Sarti**
Department of Morphology, Surgery and Experimental Medicine
University of Ferrara
Status: Public Higher Education and Research Institutution
Address: Via Borsari 46
44121 Ferrara (Italy)

Date of birth: 16/06/1985
Ph.D. date: 01/2012

Proposed Dates of stay:
January 1st to March 31st 2016

Host institute

Name: Institute for Research in Biomedicine
Status: Higher Education Research Institution
Address Via Vincenzo Vela 6 - CH-6500 Bellinzona (Switzerland)

Host institute's collaborator:
Prof. Fabio Grassi group leader
T Cell Development

1. Current Research Topics *(max 11 lines)*

My current research interest focuses on the role of the ATP-gated ionotropic P2X7 receptor (P2X7R) in inflammation and regulation of cell energy metabolism. The P2X7 receptor is involved in several inflammatory processes including control of cytokine release (e.g. IL-1 β and IL-18) and cell responsiveness. Inflammation is tightly linked to cancer, and inflammatory cytokines, among which IL-1 β, contribute to tumor growth and metastatic spreading. The P2X7R, due to its pro-inflammatory and growth-promoting activity, has been shown to condition cancer pathophysiology (Adinolfi et al, 2015). The molecular bases of P2X7R-mediated effects on cell growth are poorly understood, but mitochondria likely play a crucial role and are also important organelles in tumor cell biology (Giorgi et al., 2015). I am currently investigating the role of P2X7R in the modulation of mitochondrial metabolism. Main experimental approaches I am using include the analysis of genetically-modified animals, the Seahorse technology as well as advanced cell fractionation and microscopy techniques.

2. Three most relevant publications of home institute related to proposed research

1 - Adinolfi E., Capece M., Franceschini A., Falzoni S., Giuliani A.L, Rotondo A., **Sarti A.C**, Bonora M., Syberg S., Corigliano D., Pinton P., Jorgensen N.R., Abelli L., Emionite L. ,Raffaghello L., Pistoia V., and **Di Virgilio F.***Accelerated Tumor Progression in Mice Lacking the ATP Receptor P2X7. Cancer Res* February 15, 2015 *75;* 635

2 - Franceschini A, Capece M, Chiozzi P, Falzoni S, Sanz JM**, Sarti A.C**, Bonora M, Pinton P, **Di Virgilio F**. The P2X7 receptor directly interacts with the NLRP3 inflammasome scaffold protein.FASEB J. 2015 Jun;29(6):2450-61. doi: 10.1096/fj.14-268714.

3 - Adinolfi E, Raffaghello L, Giuliani AL, Cavazzini L, Capece M, Chiozzi P, Bianchi G, Kroemer G, Pistoia V, **Di Virgilio F**. *Expression of P2X7 receptor increases in vivo tumor growth.* Cancer Res. 2012 Jun 15;72(12):2957-69. doi: 10.1158/0008-5472.CAN-11-194

3. Proposed research plan at host institute *(max 1/2 page)*

The immune system has a key role in inflammation and cancer. T cell activation is central in the anti-tumor immune response as well as in the initiation and resolution of inflammatory processes. The ability of a T cell to shift from the naïve to effector and memory phenotype is closely linked to metabolic reprogramming (Pearce et al., 2013). Mounting evidence suggests that transcriptional regulation of P2X7R during T cell functional polarization crucially affects T cell responsiveness in distinct microenvironments (Proietti M. et al., 2014). I propose to investigate the role of the P2X7R in the modulation of mitochondrial functions and energy metabolism in different subsets of T cells from wild type (WT) and P2X7R knock-out (KO) mice. The role of P2X7 in regulating mitochondrial function will be addressed in several experimental models of T cell-mediated immunopathology, namely inflammatory bowel disease and type 1 diabetes, as well as in T cell anti-tumor response.
During my stay in Dr. Grassi's Laboratory I will first verify whether lack of P2X7R affects mitochondrial metabolism in T cells exposed to cognate antigen or inflammatory cytokines. We will measure mitochondrial oxygen consumption (OCR) as an index of mitochondrial respiration (Mito Stress Kit), and extracellular acidification (ECAR), an index of glycolysis (Glycolysis Stress Kit) using the XF24 analyzer from Seahorse Bioscience (Billerica, MA, USA). The sequential use of selective inhibitors of mitochondrial functions, such as ATP synthesis (oligomycin), proton selective permeability (FCCP), and electron flow (rotenone and antimycin A acting at site I and IIII of the respiratory chain, respectively), allows to generate a bioenergetic profile that directly correlates with cellular energy production based on oxidative phosphorylation and glycolysis. Bioenergetic data will be corroborated by the measure of mitochondrial membrane potential with the JC-1 probe. JC-1 is cationic dye that

(maximum length of proposal: 2 pages)

accumulates in energized mitochondria. At low concentrations (low mitochondrial membrane potential) is a monomer that yields green fluorescence. At high concentrations (high mitochondrial membrane potential) the dye aggregates yielding a red to orange fluorescence. Therefore a decrease in the aggregate fluorescent count is indicative of depolarization whereas an increase is indicative of hyperpolarization. In addition, we will analyse mitochondrial morphology and mass. Mitochondrial morphology and mass will be analysed with MitoTracker Orange and MitoTracker Green, respectively. Finally, we will measure mitochondrial ROS production with the MitoSOX assay. Mitochondrial activity is strongly calcium-dependent. Our laboratory has previously shown that P2X7R modulates both cytoplasmic and mitochondrial calcium levels. Therefore, we will measure cytoplasmic and mitochondrial calcium with Fluo-3AM and Rhod2/AM plus Mitotracker Green, respectively, in T-cells from WT, P2X7R-KO, OVA-specific TCR transgenic either WT or P2X7R-KO mice under different conditions of stimulation.

4. Reasons for choosing host institute/ Action'objectives *(max 1/2 page)*

The laboratory of Prof. Grassi has a long standing and widely recognized expertise in purinergic regulation of T cell functions. In particular the Grassi's lab has made fundamental contributions to the understanding of the role of extracellular ATP and P2X7R in T cell differentiation and activation. Recently, the lab has extended the research focus on the possible implication of P2X7R activity in immunopathological conditions such as type 1 diabetes and inflammatory bowel disease.An internship at the Institute for Research in Biomedicine in Bellinzona will give me the opportunity (i) to broaden my knowledge in T cell function and regulatory mechanisms; (ii) deepen my technical skills in T-cell isolation and characterization; (iii) extend my expertise in P2X7R pathophysiology. If my application will be successful, I will have the opportunity to merge in a single project state-of-the-art expertises on P2X7R (from Di Virgilio's laboratory), mitochondrial bioenergetics (from Pinton's laboratory) and T-cell function and regulation (from Grassi' s laboratory). In addition, the Institute for Research in Biomedicine offers a unique scientific environment that will help my growth both as a scientist and a person. I have already visited Dr Grassi' s laboratory in June, and I could directly experience the stimulating environment and the friendly atmosphere. Lab meetings and journal clubs to which all lab members participate are routinely held. This provides a wonderful situation in which data from a wide range of related projects are discussed in depth: the optimal setting to present and discuss my own data. I am sure that this opportunity will greatly accelerate my scientific growth, will increase my technical expertise and will improve my presentation skills.

5. Three most relevant publications of host institute related to proposed research

1- Proietti M., Cornacchione V., Rezzonico Jost T.,Romagnani A., Faliti C.E., Perruzza L., RigonI R., Radaelli E.,Caprioli F., Preziuso S., Brannetti B., Thelen M., McCoy K., Slack E., Traggiai E. and Grassi F. *ATP-Gated Ionotropic P2X7 Receptor Controls Follicular T Helper Cell Numbers in Peyer's Patches to Promote Host-Microbiota Mutualism* Immunity. 2014 Nov 20;41(5):789-801. doi: 10.1016/j.immuni.2014.10.010. Epub 2014 Nov 13.

2- Schenk U., Frascoli M., Proietti M., Geffers R., Traggiai E., Buer J., Ricordi C., Westendorf A.M. and **Grassi F.** ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. *Science Signaling*, 4: ra12 (2011)

3- Schenk U., Westendorf A.M., Radaelli E., Casati A., Ferro M., Fumagalli M., Verderio C., Buer J., Scanziani E. and **Grassi F.** Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels. *Science Signaling,* 1 (39), ra6 (2008)

References

- Adinolfi E., Capece M., Franceschini A., Falzoni S., Giuliani A.L, Rotondo A., Sarti A.C, Bonora M., Syberg S., Corigliano D., Pinton P., Jorgensen N.R., Abelli L., Emionite L. ,Raffaghello L., Pistoia V., and Di Virgilio F. *Accelerated Tumor Progression in Mice Lacking the ATP Receptor P2X7*. *Cancer Res* February 15, 2015 *75;* 635

- Giorgi C., Bonora M., Sorrentino G., Missiroli S.,Poletti F., Suski J., Ramirez F.G., Rizzuto R., Di Virgilio F., Zito E., Pandolfi P.P., Wieckowski M.R., Mammano F., Del Sal G., and Pinton P. *p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner.* Proc Natl Acad Sci U S A. 2015 Feb 10; 112(6): 1779–1784. Published online 2015 Jan 26. doi: 10.1073/pnas.1410723112

- Erika L. Pearce, Maya C. Poffenberger, Chih-Hao Chang, Russell G. Jones *Fueling Immunity: Insights into Metabolism and Lymphocyte Function.* Science 11 October 2013: Vol. 342 no. 6155 DOI: 10.1126/science.1242454

- Proietti M, Cornacchione V, Rezzonico Jost T, Romagnani A, Faliti CE, Perruzza L, Rigoni R, Radaelli E, Caprioli F, Preziuso S, Brannetti B, Thelen M, McCoy KD, Slack E, Traggiai E, Grassi F. *ATP-gated ionotropic P2X7 receptor controls follicular T helper cell numbers in Peyer's patches to promote host-microbiota mutualism*. Immunity. 2014 Nov 20;41(5):789-801. doi: 10.1016/j.immuni.2014.10.010. Epub 2014 Nov 13.

Application for COST travel grant for ☐ PhD student ☒ Postdoc

Applicant / home institute / position

Name: Chiara Parisi
Status: Post doctoral scientist
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Date of birth: 10/04/1982
Ph.D. date: February 14th 2010

Host institute

Name: IMIB-Arrixaca
Address: 30120 El Palmar, Murcia, Spain

Host institute's collaborator:
Dr. Pablo Pelegrin

Proposed Dates of stay: from 01/03/2016 to 31/05/2016 (91 days)

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1. Current Research Topics *(max 11 lines)*

My research focuses on the biological effects of microRNA-mediated translational repression. Within this topic, during the last five years I approached the mechanisms of neuroinflammation in Amyotrophic Lateral Sclerosis (ALS), with particular emphasys on the role of microRNAs in microglia activated by the pro-inflammatory P2X7 receptor (P2X7r).
At the moment, I'm working on the project "MIR-125B AS MICROGLIA MODIFIER FOR ALS" granted by AriSLA foundation. In detail, having we recently demonstrated the up regulation of miR-125b in SOD1-G93A ALS primary microglia, and its further induction upon inflammatory stimulation by BzATP acting on P2X7r, the aim of the project is to verify if miR-125b and its consequent inhibition of the validated target A20 might promote the switch of SOD1-G93A microglia from a pro-inflammatory/toxic (M1) to an anti-inflammatory/protective (M2) phenotype.

2. Three most relevant publications of home institute related to proposed research

1: Volonté C, Apolloni S, Parisi C. MicroRNAs: newcomers into the ALS picture. CNS Neurol Disord Drug Targets. 2015;14(2):194-207
2: Parisi C, Arisi I, D'Ambrosi N, Storti AE, Brandi R, D'Onofrio M, Volonté C. Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. Cell Death Dis. 2013 Dec 12;4:e959.
3: Apolloni S, Parisi C, Pesaresi MG, Rossi S, Carrì MT, Cozzolino M, Volonté C, D'Ambrosi N. The NADPH oxidase pathway is dysregulated by the P2X7 receptor in the SOD1-G93A microglia model of amyotrophic lateral sclerosis. J Immunol. 2013 May 15;190(10):5187-95.

3. Proposed research plan at host institute *(max 1/2 page)*

As macrophages, also microglia can be polarized toward two different general states: M1/pro-inflammatory or M2/anti-inflammatory *(Franco R et al, Prog Neurobiol 2015)*. Neuroinflammation in most neurodegenerative diseases, among which ALS, is characterized by the exacerbation of microglia M1 phenotype with consequent release of pro-inflammatory mediators and toxicity towards neurons *(Philips T et al, Exp Neurol 2014)*.
In our lab, we investigate the role of P2X7r in ALS microglia and have highlighted a complex dual role for this receptor in modulating neuroinflammatory responses. In particular, we demonstrated that stimulation of P2X7r in vitro by BzATP exacerbated the M1 phenotype in ALS microglia and their toxicity towards motor neurons *(D'ambrosi N et al, J Immunol 2009;*

(maximum length of proposal: 2 pages)

Apolloni S. et al, J Immunol 2013), and pharmacological inhibition of the receptor in part ameliorated the disease pathology (Apolloni S *et al, Dis Mol Mech 2014)*. On the other hand, genetic ablation of P2X7r in ALS mice worsened the disease progression *(Apolloni S et al, HMG 2013)*, thus suggesting different roles for this receptor, perhaps depending on the microglia activation state. P2X7r-mediated microglia activation is controlled by miR-125b targeting the NF-kB inhibitor A20. In particular, we demonstrated that A20 is a target of miR-125b and that miR-125b inhibition is able to inhibit p65/NF-kB pathway in activated microglia by restoring A20 levels, with consequent decrease of M1 pro-inflammatory markers *(Parisi C et al., submitted).* M1 phenotype in both macrophages and microglia is characterized by NLRP3 inflammasome activity, that is strongly induced by ATP acting through P2X7r *(Mariathasan S et al, Nature 2006; Hanamsagar R et al, J Neurochem. 2011).* A recent work demonstrated that A20 restricts NLRP3 inflammasome activation by ATP in macrophages, through direct interaction with inflammasome proteins (*Duong BH et al, Immunity. 2015*). Surprisingly, in macrophages polarized towards the M2 phenotype, P2X7r is uncoupled from NLRP3-inflammasome activation and IL-1beta production *(Pelegrin P et al, EMBO J 2009)*.
In the context of testing if regulation of A20 by miR-125b is a mechanism to control microglia polarization, the general aim of the proposed research will now be to investigate if P2X7r-mediated activation of NLRP3 inflammasome proceedes in microglia through miR-125b/A20 interaction. In detail, will focus on:

1. the characterization of NLRP3-inflammasome in primary microglia over a dynamic polarity gradient *(Pelegrin P et al EMBO J 2009)*;
2. the expression of miR-125b and A20 in microglia during this polarity gradient;
3. the activity of NLRP3 inflammasome upon P2X7r activation after miR-125b and A20 overexpression/silencing in M1/M2 polarized microglia.

4. Reasons for choosing host institute/ Action'objectives (max 1/2 page)

The Molecular Inflammation Group directed by Dr. Pablo Pelegrin at Murcia BioHealth Research Institute-Hospital "Virgen de la Arrixaca" (IMIB-Arrixaca, Murcia, Spain) is a leading team in the scientific community involved since many years in studying the actions of P2X7r in macrophages during the switch from the pro-inflammatory to the anti-inflammatory phenotype.
Our reaserch also focuses on the analysis of those molecular mechanisms sustaining an M1/M2 progression in microglia, and in particular in the ALS context.
Thus, by complementing long lasting scientific interests and different areas of expertise, this proposed research strongly merges the common goal of understanding the role of P2X7r-mediated NLPR3-inflammasome activation during M1/M2 polarization, by translating and further developing in microglia the knowledge previously acquired in macrophages.
By this joint venture, we expect to obtain a comprehensive understanding of microglia/macrophages cell physiology and P2X7 ion channel role in immune responses, towards innovative therapeutic approaches.

5. Three most relevant publications of host institute related to proposed research

1: Compan V, Baroja-Mazo A, López-Castejón G, Gomez AI, Martínez CM, Angosto D, Montero MT, Herranz AS, Bazán E, Reimers D, Mulero V, Pelegrín P. Cell volume regulation modulates NLRP3 inflammasome activation. Immunity. 2012 Sep 21;37(3):487-500.
2: Lopez-Castejón G, Baroja-Mazo A, Pelegrín P. Novel macrophage polarization model: from gene expression to identification of new anti-inflammatory molecules. Cell Mol Life Sci. 2011 Sep;68(18):3095-107.
3: Pelegrin P, Surprenant A. Dynamics of macrophage polarization reveal new mechanism to inhibit IL-1beta release through pyrophosphates. EMBO J. 2009 Jul 22;28(14):2114-27.

(maximum length of proposal: 2 pages)

Application for COST travel grant for ☐ **Postdoc**

Applicant / home institute / position

Name: Iva Hafner Bratkovič
National Institute of Chemistry
Status: Postdoctoral fellow (research associate)

Address: Hajdrihova 19
1000 Ljubljana
Slovenia

Date of birth: 11 May 1978
Ph.D. date: 31 March 2008
(June 2008 – March 2009 not working in research due to maternity leave)

Proposed Dates of stay: 16th January 2016 – 16th April 2016

Host institute

Name: Pablo Pelegrin
Clinical University Hospital Virgen de la Arrixaca
Status: PI
Address: Carretera Buenavista s/n 30120 El Palmar, Murcia, Spain

Host institute's collaborator: Pablo Pelegrin

1. Current Research Topics *(max 11 lines)*

NLRP3 inflammasome is a multiprotein complex mediating inflammatory responses in a variety of diseases, such as CAPS, gout and Alzheimer's disease. Diverse triggers engaging ion channels such as P2X7 and TRP family inducing intracellular Ca^{2+} mobilization and K^{+} efflux were shown to be essential for NLRP3 oligomerization and inflammasome activation. The molecular mechanism of NLRP3 oligomerization remains largely unknown. We are particularly interested in how signaling downstream of ion channels induces NLRP3 oligomerization leading to inflammasome initiation, and in the role of NLRP3 domains in this process. The structure of NLRC4 suggested that LRR domain locks the protein in the inactive form. Our unpublished results define a minimal trigger-responsive NLRP3 molecule suggesting that LRR domain is redundant for activation. In this proposal we plan to further characterize the molecular determinants of NLRP3 inflammasome assembly, which is necessary to support the development of specific NLRP3 inflammasome inhibitors in the future.

2. Three most relevant publications of home institute related to proposed research

Hafner Bratkovič, Iva, Benčina, Mojca, Fitzgerald, Katherine A., Golenbock, Douglas, Jerala, Roman. NLRP3 inflammasome activation in macrophage cell lines by prion protein fibrils as the source of IL-1[beta] and neuronal toxicity. *Cellular and molecular life sciences*, 2012, 69(24): 4215-4228.

Manček Keber, Mateja, Frank, Mojca, Hafner Bratkovič, Iva, Smole, Anže, Zorko, Mateja, Pirher, Nina, Hayer, Silvia, Kralj-Iglič, Veronika, Rozman, Blaž, Ilc, Nejc, Horvat, Simon, Jerala, Roman. Toll-like receptor 4 senses oxidative stress mediated by the oxidation of phospholipids in extracellular vesicles. *Science signaling*, 2015, 8(381):ra60.

Hadži, San, Ondračka, Andrej, Jerala, Roman, Hafner Bratkovič, Iva. Pathological mutations H187R and E196K facilitate subdomain separation and prion protein conversion by destabilization of the native structure. *The FASEB journal*, 2015, 29(3):882-893.

(maximum length of proposal: 2 pages)

3. Proposed research plan at host institute *(max 1/2 page)*

Overview. The main aim of the proposed project is to define structural determinants of NLRP3 in response to various physiological processes triggering NLRP3 inflammasome assembly. Particularly we plan to follow inflammasome activation upon perturbed ion homeostasis mediated by P2X7 and other receptors. The candidate previously prepared 20 truncated NLRP3 mutants, introduced them into Nlrp3-deficient macrophages and followed their activation by particulate (alum, silica) and soluble activators (nigericin, imiquimod). In current proposal, an activation of representative NLRP3 mutants mediated by P2X7 will be followed to define NLRP3 domains involved in stimulation by ATP.

Methodology, tasks, feasibility. DNA constructs encoding NLRP3 variants, stable cell lines etc. will be prepared by the candidate in the home laboratory to enable the best use of stay in the host laboratory. ATP-mediated activation of macrophages expressing NLRP3 variants will be followed with real-time imaging, immunologic and biochemical techniques. Further, the ability of truncated NLRP3 variants to initiate inflammasome formation and intercellular spread will be evaluated as described in Baroja-Mazo et al, 2014. Furthermore, conformational change/interaction between NLRP3 molecules will be followed by a BRET assay (Compan et al., 2012). Encouraging preliminary results and techniques already established in the host lab suggest that the work (if supported by BM1406) will be successfully performed within proposed time frame.

Expected results. We expect that NLRP3 mutants will respond similarly to $P2X_7$ stimulation with ATP as to already tested NLRP3 triggers, although we cannot exclude that the minimal responsive NLRP3 variant will be different due to direct binding of ATP to NBD domain. The host lab has previously observed preformed inactive NLRP3 oligomers assembled through intermolecular interactions where LRR and PYD domains are in close proximity. These interactions should be lost in the case of mutants lacking LRR domain. It will be interesting to see how these interactions contribute to fine regulation of NLRP3 oligomers.

4. Reasons for choosing host institute/ Action'objectives *(max 1/2 page)*

While the applicant has knowledge in innate immunity she is lacking practical experience in cell physiology, particulary in measurement of ion fluxes in regard to specific ion channels. The PI at the host institute has documented experience in both NLRP3 inflammasome and ion channels. His group developed NLRP3 conformation BRET assay, which will be used in this research proposal. They also showed that upon NLRP3 initiation NLRP3-ASC aggregates leave the parent cell to seed ASC oligomerization in another cell, it will be interesting to see whether NLRP3 truncated mutants are able to do the same. The applicant on the other hand has experience in genetic manipulation of nontransfectable cell lines, which she will share with researchers at the host institution.

The project proposal fits well into Action's objectives. The main objective is to enhance the knowledge on ion channels in non-excitable cells. Several ion channels were previously linked to inflammasome activation. Although we will focuss on P2X7, we will not neglect Ca^{2+} signaling. The project is more specifically connected to plans of WG1 (ion channels on innate immune cells) and WG2 (the role of ion channels in inflammation). This proposal initiates collaboration between two groups, which despite common interests have not collaborated before.

5. Three most relevant publications of host institute related to proposed research

Baroja-Mazo A, Martín-Sánchez F, Gomez AI, Martínez CM, Amores-Iniesta J, Compan V, Barberà-Cremades M, Yagüe J, Ruiz-Ortiz E, Antón J, Buján S, Couillin I, Brough D, Arostegui JI, Pelegrín P. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat Immunol, 2014, 15(8):738-48.

Compan V, Baroja-Mazo A, López-Castejón G, Gomez AI, Martínez CM, Angosto D, Montero MT, Herranz AS, Bazán E, Reimers D, Mulero V, Pelegrín P. Cell volume regulation modulates NLRP3 inflammasome activation. Immunity, 2012, 37(3):487-500.

Compan V, Baroja-Mazo A, Bragg L, Verkhratsky A, Perroy J, Pelegrin P. A genetically encoded IL-1β bioluminescence resonance energy transfer sensor to monitor inflammasome activity. J Immunol, 2012, 189(5):2131-7.

(maximum length of proposal: 2 pages)

Annexe 2

BMBS COST Action BM1406 Ion Channels and Immune Response

PROGRAM
INTERNATIONAL Institute of Molecular and Cell Biology
4 Trojdena Street, Warsaw

SEPTEMBER 24th, 2015

08:30 – 08:45 ***WELCOME ADDRESS***

INTRODUCTION BY WORKING GROUP LEADER 1

08:45 - 10:25 WORKING GROUP 1

8:45 - 9:10
Activation and gating mechanism of STIM/Orai channels
Rainer Schindl University of Linz, Austria

9:10 - 9:35
Mechanisms regulating SOCE: Orai subunit composition and a novel inhibitory STIM2 splice variant
Barbara A. Niemeyer Saarland University, Homburg, Germany

9:35 - 10:00
Regulation of SOCE and Endoplasmic Reticulum Calcium levels by the Sigma1 receptor and its Ligands
Ruth Murrell-Lagnado University of Sussex, UK

10:00 -10:25
Regulation of neutrophil pro-inflammatory functions by Ca^{2+} signalling
Sabrina Bréchard University of Luxembourg

10:25 - 11:15 COFFEE BREAK

11:15 - 12:30 WORKING GROUP 1

11:15 -11:40
NLRP3 inflammasome modulation in macrophages by P2X7 receptor, hemichannels and swelling-sensitive channels
Pablo Pelegrín Instituto Murciano de Hospital Clínico Universitario Virgen de la Arrixaca (IMIB-Arrixaca), Murcia, Spain.

11:40 -12:05
Minimal responsive NLRP3 truncated variant
Iva Hafner Bratkovič National Institute of Chemistry, Ljubljana, Slovenia

12:05-12:30
Human dendritic cell migration requires calcium influx controlled by the calcium-activated K+ channelKCa3.1.
Florence Velge-Roussel University of Tours, France

12:30 - 13:45 **LUNCH**

13:45 - 14:00 ***INTRODUCTION BY WORKING GROUP LEADER (5 abstracts)***

14:00 - 15:30 **WORGING GROUP 2**

14:00 -14:15
The P2X7 receptor: an ion channel with a split personality in inflammation and cancer.
Francesco Di Virgilio University of Ferrara, Italy

14:15 -14:40
P2X7 receptor mediated control of adaptive immunity in the small intestine ensures host-microbiota mutualism and glucose homeostasis.
Fabio Grassi Institute for Research in Biomedicine, Bellinzona, Switzerland

14:40-15:05
The P2X7 receptor and other ion channels – roles and targets in pancreatic cancer.
Ivana Novak University of Copenhagen, Denmark

15:05-15:30
$P2X_7$ receptor mediates shedding of vesicles from microglia: implications of vesicle release in neurodegeneration and neuroinflammation
Claudia Verderio, *CNR Institute of Neuroscience, Milan*

15:30 - 16:00 **COFFEE BREAK**

16:00 - 17:00 **WORKING GROUP 2**

16:00-16:30
Evidence for non-redundant functions of Cav1.2 and Cav1.3 calcium channels in Th2 cell priming and allergic asthma development *in vivo*.
Magali Savignac Universitè Paul Sabatier, Toulouse, France

16:30-17:00
Restore Purkinje neuron calcium homeostasis to prevent Yo-Ab neuropathology.
Manya Schubert Haukeland University Hospital, Bergen, Norway

SEPTEMBER 25th, 2015

08:30 - 10:00 ***MC MEETING (MC Agenda)***

10:00 - 10:30 **COFFEE BREAK**

10:30 - 11:30 **Presentation H2020 by Anna Pytko**
Funds for research and innovation in Health, Demographic Change and Wellbeing - Horizon 2020

11:30 - 11:45 ***INTRODUCTION BY WORKING GROUP LEADER (5 abstracts)***

11:45 - 12:35 **WORKING GROUP 3**

11:45-12:10
Nanobodies that antagonize the ATP-gated P2X7 ion channel are promising anti-inflammatory drugs
Friedrich Koch-Nolte University Medical Center, Hamburg, Germany

12:10-12:35
Modulation of membrane potential by Proprietary Potassium Ionophores in
Breast Cancer Stem Cell Model
Katja Ester Ru□er Bošković Institute, Zagreb, Croatia

12:35 - 13 :35 LUNCH

13:35 - 14:25 WORKING GROUP 3

13:35-14:00
Hv1 proton channel, a new therapeutic target to treat cancer, inflammation and stroke
Antoine Chemaly University of Geneva, Switzerland

14:00-14:25
Native isolation of functional Ion channels: CALIXAR approach.
Vincent Corvest Calixar, Lyon, France

14:25 - 14:45 COFFEE BREAK

14:45 - 15:35 WORKING GROUP 3

14:45-15:10
Tailoring the selectivity of Anuroctoxin for Kv1.3 K^+ channels
Gyorgy Panyi University of Debrecen, Debrecen, Hungary

15:10-15h35
Action of clathrodin and oroidin, and their synthetic analogues on voltage-gated potassium channels
Nace Zidar University of Ljubljana, Slovenia

15:35- 15:45 CONCLUSION

annexe 3

9. Scientific planning

- d. Dissemination planning (Publications and outreach activities)
- 4 propositions to decide which logo for BM1406:

1

2

3

4

➢ **Logo number 2 has been voted**