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Jury Meeting Minute
Reference of Fellowship IMM/BII/14-2024

Instituto de Medicina Molecular João Lobo Antunes (iMM) opens a call for one research fellowship under the project ***"Dependence of IL-7R-mediated signaling on sphingosine kinase activity in acute lymphoblastic leukemia cells as an exploitable therapeutic vulnerability"*** **(FPJ001584),** with the funding support from "Worldwide Cancer Research".

The ad was published at EURAXESS Portugal Portal on 5th of March 2024 and also disseminated in iMM website. The call was opened from 6th of March 2024 until 19th of March 2024 and during which the following applicant applied:

- ✓ Paulo Ricardo Viana Mendonça

The applicant Jaceline Gislaine Pires Sanches was excluded because she did not submit all documents required in the job ad.

On the 27th of March of 2024, the jury composed by João Taborda Barata (FMUL/IMM), Rita Cascão (IMM) and Rita Fragoso (IMM) met to analyze the application documents (motivation Letter, detailed CV, MSc certificate, recommendation letter(s) and candidates' declaration of honor indicating previous fellowships, if any, its typology and duration).

Work Plan and Goals:
Acute lymphoblastic leukemia (ALL), the most common childhood malignancy, is an aggressive cancer arising from B or T lymphoid progenitors. Although current therapies are highly effective, with 5-year survival rates reaching 80-90%, they associate with substantial short and long-term side effects, and a significant number of cases still relapse and have dismal prognosis. Importantly, therapeutic success in adults lags significantly behind, with only 30-40% of the cases surviving long-term. Thus, the current challenge is to develop novel, more efficient therapeutic strategies that specifically target the leukemic cells and minimize the detrimental side effects associated with conventional therapies. A majority of ALL cases express the IL-7 receptor (IL-7R) and ALL cells benefit from IL-7/IL-7R pro-survival and proliferative effects. Our preliminary data strongly indicate that there is a therapeutic window arising from the fact that IL-7R-mediated signaling depends on sphingosine kinase activity in leukemia cells but not in healthy lymphoid precursors. In this project we propose to validate this possibility, understand the molecular reasons for the differential dependence of IL-7R-mediated signaling on SK activity between leukemia and healthy lymphoid cells, and exploit them for therapeutic purposes. The selected candidate will be a highly organized, motivated and pro-active person that will participate in tasks that include:

- Single cell RNAseq analysis of B and T cell precursors collected from the BM and thymus, respectively, of 12-week old animals will be performed to evaluate transcriptomic alterations elicited by deletion of SKs in lymphoid cells in CD2-iCre.SK1fl/fl SK2-/- vs. SK1fl/fl SK2-/- control animals. This will be performed to evaluate transcriptomic alterations elicited by deletion of SKs in lymphoid cells;
- Single cell RNAseq analysis of CD2-iCre. IL7Rmutwt/fl.SK1fl/fl SK2-/- vs. CD2-iCre.IL7Rmutwt/fl.SK1wt/fl SK2wt/wt animals at 12 weeks, when most mice do not yet develop leukemia but are at a pre-leukemic stage with evidence of

increased self-renewal ability and partial developmental block, and also upon leukemia-related death (at which time full transformation has evidently occurred);

- Bulk RNAseq analysis of a) an IL-7-responsive T-ALL cell line (HPB-ALL) that has been deleted for SK1, SK2 or SK1 and SK2 using CrispR-Cas9 vs respective negative control, b) human IL7R+ T-ALL cells vs human thymocytes, and c) mouse heterozygous IL7R mutant B-ALL cells (which retain a wild type allele and thus are still responsive to IL-7) vs healthy mouse B-cell precursors, in response to IL-7 in the presence or absence of SK1 (PF-543), SK2 (compound 55) or pan-SK (compound 49 and SKi) inhibitors (or respective SK KO for HPB-ALL cells);
- Bulk RNAseq and whole exome sequencing of leukemias developed in Vav-iCre.IL7Rmutwt/fl, CD2-iCre.IL7Rmutwt/fl, Mb1-Cre.IL7Rmutwt/fl, CD19-Cre.IL7Rmutwt/fl, pLck-Cre.IL7Rmutwt/fl and CD4-Cre.IL7Rmutwt/fl mice.

Candidate's Profile:
- Master's Degree in Bioinformatics or related areas, or in Biomedical Engineering or Biological Engineering, with clear evidence of expertise in bioinformatics (mandatory);
- Previous experience in cell culture (desirable);
- Previous experience in programming, e.g. Python, Matlab, Simulink, R+, Linux, GitHub, SQL (mandatory);
- Previous experience in bioinformatic techniques and tools (mandatory);
- High sense of responsibility, organization and method;
- Pro-active personality;
- Ability to work independently but also with team spirit;
- Excellent knowledge of English, spoken and written (mandatory).

Necessary Documents for Applications: *- Motivation Letter; - Detailed CV; - MSc certificate; - Recommendation letter(s); - Candidate's declaration of honor indicating previous fellowships, if any, its typology and duration. The non-compliance with these requirements determines the immediate rejection of the application.*
In case the applicant does not have yet the required degree certificate, a declaration of honor stating the conclusion of the necessary qualifications for the purposes of this process will be accepted and must be sent by the end date of the call.

Selection Methods: *Curriculum Analysis (50%), Motivation Letter (40%) and Recommendation letter(s) (10%). The Curriculum will be analyzed qualitatively, and in what concerns to its content and relevance for the tasks to be performed, with special emphasis on research experience and relevant knowledge in the area of the proposed work plan as described in the candidate profile (50%). The letter of motivation will be evaluated in what concerns the motivation and adequacy for the activities to be performed (25%), and command of the English language (15%). The recommendation letter(s) will be evaluated with regard to the work previously carried out by the candidate, reflecting autonomy, proactivity, responsibility, organization and method as well as the ability to work in a team (10%).*

Curriculum Analysis (50%)

The Curriculum Analysis took in consideration the following criteria:

- Master's Degree in Bioinformatics or related areas (20%)
- Research experience and relevant knowledge in the area of the proposed work plan (30%)

Motivation Letter (40%)

The Motivation Letter took in consideration the following criteria:

- Adequacy for the activities to be performed (25%);
- Command of the English language (15%).

Recommendation Letter (10%)

- Work previously carried out by the candidate, reflecting autonomy, proactivity, responsibility, organization and method as well as the ability to work in a team (10%)

The analysis and discrimination of the admitted candidate's classification in the sole phase of this process are presented in Annex I.

At this stage, the selected candidate is the one with the highest score.

Lisbon, 27th of March 2024

João Taborda Barata (FMUL/iMM)

Rita Cascão (iMM)

Rita Fragoso (iMM)

ANNEX I - EVALUATION: Fellowship Reference IMM/BII/14-2024

Applicant	Curriculum Analysis (50%)		Motivation Letter (40%)		Recomendation Letter (10%)	Total	Justification
	Master's Degree in Bioinformatics or related areas (20%)	Research experience and relevant knowledge in the area of the proposed work plan (30%)	Adequacy for the activities to be performed (25%)	Command of the English language (15%)	Work previously carried out by the candidate, reflecting autonomy, proactivity, responsibility, organization and method as well as the ability to work in a team (10%)		
Paulo Ricardo Viana Mendonça	17	30	25	15	10	**97**	The applicant holds a master's degree in Bioinformatics, with a final grade of 17. The candidate has previous experience in programming (in particular Python, Matlab, Simulink, R+, Linux, GitHub, SQL) as well as bioinformatic techniques and tools (mandatory). This experience is not only clearly stated in the curriculum but also supported by the recomentation letter. Moreover, the recomendation letter also reflects the adequacy of the applicant to integrate the research team. Lastly, the motivation letter showed that the candidate was fit for all of the activities to be performed, and has good command of the english language.

João Taborda Barata (FMUL/IMM)

Rita Cascão

Rita Cascão (IMM)

Ana Rita Fragoso

Rita Fragoso (IMM)

Instituto de Medicina Molecular João Lobo Antunes

Declaração de inexistência de conflito de interesses (CDI)

Os membros do júri do Concurso para Atribuição de 1 (uma) Bolsa de Investigação, Ref.ª IMM/BII/14-2024, aberto ao abrigo do projeto ***"Dependence of IL-7R-mediated signaling on sphingosine kinase activity in acute lymphoblastic leukemia cells as an exploitable therapeutic vulnerability"*** **(FPJ001584),** vêm por este meio declarar que não se encontram em situação de conflito de interesses que os impeça de participar no respetivo processo de seleção e atribuição de bolsa.

Declaram também manter a confidencialidade ao longo do processo de avaliação.

Lisboa, 27 de março de 2024

João Taborda Barata [FMUL/IMM]

Rita Cascão

Rita Cascão (IMM)

Ana Rita Fragoso

Rita Fragoso (IMM)