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PhD Thesis by M.Sc. Oľga Babošová
Opponent: Meritxell Alberich-Jorda, Ph.D

Confidential report:

Myeloproliferative neoplasms (MPNs) are a diverse group of hematopoietic disorders characterized by clonal division of a myeloid progenitor cell and excessive production of mature blood cells. The Ph.D thesis by Oľga Babošová centers mainly on Philadelphia chromosome-negative MPNs, classified as polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF). These three disorders, despite their clinical differences, share a common mechanism of transformation, i.e. hyperactivation of JAK2 signaling. In the first part of her thesis, Oľga Babošová addresses how JAK2 germline mutations predispose patients to MPNs. To this aim, in collaboration with national and international laboratories, she explores the mutational landscape in MPN patients, and performs functional analysis of recurrent single or double JAK2 mutations identified in the screenings. Next, she investigates potential mechanisms that protect the progression of MPNs to AML. Given the high amount of DNA damage and the lack of efficient DNA repair mechanisms in cells from MPN models/patients, it would be expected a fast disease progression in MPN patients to AML. Nevertheless, MPNs are slow progressive disorders with a relatively low aggressiveness. In this context, Oľga Babošová investigates molecular mechanisms that grant a protective tool preventing the progression of MPNs towards AML. In particular, she proposes the protective role of KAP1 in the progression to acute leukemia, and generates several tools to address this question. The last part of her thesis drives away from MPNs, and focusses on mantle cell lymphoma (MCL). MCL is a lymphoid neoplasm characterized by uncontrolled proliferation of mature B lymphocytes. MCL cells overexpresses cyclin D1, due to the translocation t(11;14)(q13;q32), contributing to this B cell expansion. Iron chelation has been shown to downregulate cyclin D1 by inactivating EGLN2/PHD1 and stabilizing FOXO3A, which transcriptionally inhibits cyclin D1. In the last part of her thesis Oľga Babošová explores molecular mechanisms of cyclin D1 inhibition by iron chelators, and investigates the role of EGLN2/PHD I and FOXO3A in modulating cyclin D1 levels in MCL.

The results from this thesis have been compiled in three manuscripts. The first manuscript was published in 2016 in Blood (IF=15.132), and Oľga Babošová contributed as a second author. The second manuscript was published also in Blood in 2018, and Oľga Babošová was a first co-author together with C. Mambet and J.P. Defour. These two papers compiled her

work related to MPNs.The last manuscript derived from Oľga Babošová Ph.D. thesis compiles her work related to cyclin D1 regulation and has been prepared for submission. Oľga Babošová is the first author and I guess the paper is expected to be published in 2019. During her Ph.D studies Oľga Babošová also contributed to 2 other publications, investigating the role of Wnt signaling in colorectal cancer.

I would like to congratulate Oľga Babošová, on the present work. The thesis is nicely written and demonstrates your scientific maturity. The experimental work is solid, coherent, and consistent. I would also like to congratulate your Ph.D. supervisor, Lucie Lanikova, for her excellent work. Finally, I would like to ask Oľga Babošová few additional questions:

1. What additional experiments would you perform to further understand the functional cooperation of JAK2 V617F and JAK2 T108A or L393V? And would you consider overexpressing a JAK2 mutant form harboring the V617F and T108A mutation in cis?

2. Figure 5A, page 44: I'm not sure about how to interpret the data? For instance, the increase growth in V617F is the result of reduced apoptosis or increased proliferation? I understand that this graph is plotting cell numbers. Do you think it would help to complement this experiment with proliferation and apoptosis assays to make your statement stronger?

3. 7 patients with co-occurring JAK2 V617F and R1063H mutations were diagnosed with ET, 5 with PV, and 2 with PMF. What do you think is driving the distinct clinical features in these patients?

4. EPOR, TPOR and G-CSFR supported higher activation of signaling by JAK2 V617F/R1063H. You showed that the R1063H mutation increases the binding affinity of mutant JAK2 to G-CSFR. Was that experiment done in the presence of G-CSF? Should it be done in the presence of sub-optimal G-CSF doses? In addition, would it be possible that the R1063H mutation also affects the interaction with EPOR and TPOR?

5. Your data indicate that regulation of cyclin D1 in MCL is not controlled by EGLN2/PHD1-FOXO3A pathway. Is it possible that the reduction of cyclin D1 levels upon iron chelation treatment in MCL cells is a secondary and not direct effect of the treatment? For instance, merely reflecting metabolic changes?

To conclude, Oľga Babošová exhibits expectation for independent scientific work, and merits to be awarded the academic degree doctor (abbreviated as Ph.D. – after the name).

Sincerely,

Meritxell Alberich-Jorda