Report of the PhD. Thesis

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Opponentus: Prof. Laurent Degos

The thesis on Preclinical model of acute promyelocytic leukemia: study of the anti-leukemic fect induced by ATRA and DNA vaccination, is proposed by Katerina Pokorna.

The aim of the study is to better characterize the efficacy of combined treatment and to determine molecular markers of clinical outcome.

Katerina Pokorna used a transplantable transgenic mouse model mimicking the human disease and established a minimal residual disease monitoring based on the detection of the transcript by PCR technology in “APL like” mice. By this technique she also demonstrated the possible malignant reservoir in extramedullary tissues. And then she investigated the immune response against malignant cells particularly in mice treated by ATRA and DNA vaccination.

The results are reported in three papers on (1) Frequent antibody production against RARA in both APL mice and patients (Blood), (2)DNA vaccination with ATRA treatment induces long term survival and elicits specific immune responses requiring CD4 and CD8 T cell activation in APL mouse model (Blood first author), (3) Tracking the extra-medullary PML-RARA positive cell reservoirs in a preclinical model: biomarkers of long term drug efficacy (submitted first author).

Katerina Pokorna through the broad scope of the study embraced several domains of the biology which are well presented in the introduction: the definition of the leukemia, the molecular origin of the disease, the cellular consequences, the mouse models, the vaccines and the immune response. She clearly reports theories, concepts and current managements of the treatments. This part of the thesis combines concision and extended references and is well done and helpful for the reader. Sometimes she refers to the last reviews and elsewhere she gives the reference of the seed papers. Both (first paper and review) would be useful.

Katerina Pokorna for the thesis settled a sensitive PCR technique in mice, considering the small amount of blood taken in mice (in comparison to human). She also used several techniques (mouse model of transplantable leukemia, immune testing, molecular and cellular techniques)

Considering first the three papers, the technical skills, the conceptual knowledge and the presentation by the excellent introduction we consider that Katerina Pokorna should be accepted as a PhD.

As opponent we have some comments and questions beyond the discussion presented by the candidate.

1- Comments

-The thesis reports and emphasizes positive results and does not discuss the artifices due to *the model*: What could be the role of the promoter hMPR8 in the results? Is the disease actually similar to human (papillomas..)? We do not know the exact status of the transplantable cell (caryotype, molecular defects, instability..) after so frequent transplantations since 15 years. Could these parameters be involved in the proliferation strength, in the extramedullary reservoirs)?

-The model uses suboptimal dose of ATRA as *reference treatment* in the study, while the ATRA dose used in human as well in mice in order to have a complete remission is much more higher. The suboptimal dose is useful for the proof of concept of the role of immune system but is not relevant for a clinical purpose. In human ATRA is combined with chemotherapy and what could be the (positive) role of a vaccine in patients with a depressed immune response after such a treatment?

- Details obtaining the DNA vaccination could be precised: plasmid vector, inserted gene, fragment C tetanus toxoid … and a figure of the construction (if it is not confidential) would be helpful for the reader.

2- Specific questions about the articles

-Antibody production against RARA in both APL mice and patients

What is the antibody production against PML the partner moiety of the oncogenic molecule?

Why 50% of patients have an antibody production in human and not in mice. Are those who possess antibodies at diagnosis are the patients with high white blood count or particular molecular feature (specific bcr? FLT3 mutation..), are those with a long delay between the diagnosis and the blood collection, those with an associated inflammatory symptoms(C-reactive protein?), are those with a strong DIC?.....? Could the chemotherapy be involved in the absence of increase of antibody production in some patients (additional strong high dose ARA-C is given in some patients).

- DNA vaccination: Since T cell infusion (CD4 + CD8) has no effect what is the result with the depletion of T cells (by an antiCD3).

Considering the previous article is it the antibody (positive correlation) or cellular immune response (T cell subpopulation depletion) who play the major role in the progression of the disease?

- Extramedullary reservoirs: Spleen is an hematopoietic tissue un mice and not in human. That could explain the enlarged spleen (as a standard relapse in mice).

If the mice “develop severe papillomatis of the skin before or at the onset of leukemia that may be due to the fact that the hMRP8 cassette allows the PML-RARA expression in the epidermal skin cells as well” why not also in the central nervous system cells? Both epidermal and central nervous cells came from the same origin (neuro-ectoderm tissue). Is it known which cell is involved in the brain “reservoir”, a hematopoietic cell (true reservoir) or a nervous cell (due to the cassette like the skin papillomas)? Could it be possible to investigate on the cell and its location involved in the brain taking half of the brain for PCR and the other (in positive mice) for a pathological study with specific probes and stainings?

Does such extramedullary reservoir occurs in mice with strong proliferation (as leukemic meningitis relapse in patients with high white blood cells count at diagnosis).

In conclusion, the thesis brings strong arguments and opens new areas in the scientific knowledge as well as a possible new management of the treatment of patients.

The results reported by Katarina Pokorna, well presented, published in high rank journals, and well discussed are the elements for the admittance as PhD.

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