**Proposal for the 2020 Imagine International PhD program**

**Laboratory**: Genome Dynamics in the Immune System (DGSI)
**Head of laboratory: Jean-Pierre de Villartay & Patrick Revy**
**Project and student supervisor: Jean-Pierre de Villartay (HDR Y)**
**Number of HDR in the lab: 4**
**Field of research: Immunology – Molecular Biology – Development – DNA repair**
**Number and names of PhD students currently in the lab in 2020: 1 in 1st y, 1 in 2nd y, 1 in 4th y**
**Number and names of PhD students under the Imagine program: none**
**Project Title: DNA damage/repair coupling as a safeguard against genetic instability: V(D)J recombination as a paradigm**

Project description (Max 2 pages including abstract and publications)

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Living organisms are constantly exposed to endogenous and exogenous genotoxic assaults. Highly conserved DNA repair mechanisms have been evolutionary selected to cope with these damages to maintain genome integrity. DNA double strand breaks (DSBs), which represent the most toxic lesions, are repaired by at least two DNA repair pathways (Homologous recombination and Non Homologous End Joining). Nevertheless, despite their "dangerousness", programmed DSBs (prDSBs) are part of essential physiological processes including meiotic recombination, V(D)J recombination, or genome rearrangements in ciliates [1].

**V(D)J recombination** is required for the assembly of genes encoding the variable domain of immunoglobulins and T cell receptors, thus ensuring the generation of diversified B and T lymphocyte repertoires [2]. It is essentially a mechanism related to "cut and paste" transposition, in which previously scattered variable (V), diversity (D), and joining (J) segments are physically associated on the DNA by somatic DNA rearrangement. It is initiated by a site specific prDSB introduced by the lymphoid specific, domesticated transposase **RAG1 and RAG2** on recombination signal sequences (RSS) that flank all the rearranging V, D, and J segments [3, 4]. **The repair of RAG1/2 induced DSBs strictly relies on NHEJ**. V(D)J recombination constitutes a critical checkpoint in the development of the adaptive immune system and its default results in **severe immune deficiency (SCID)** both in humans and animal models [5]. Moreover, V(D)J recombination is a potent pro-oncogenic process if not well controlled. Indeed, all animal models that combined NHEJ deficiency on the TP53-/- background result in the early development of **aggressive pro-B cell lymphomas** harboring RAG1/2 driven chromosomal translocations [6].

In previous work, we discovered an efficient two-tier mechanism that ensures the proper repair of RAG1/2 introduced DSBs, thus avoiding genome instability during V(D)J recombination [7]. This mechanism relies on the functional redundancy between the non-core **C-terminus region of RAG2** and the DNA repair factor **Xlf**. Very schematically, only when the two are missing is V(D)J recombination affected resulting in genome instability and the development of lymphomas when introduced on a TP53 KO background. We proposed that this RAG2/Xlf relationship might be at the basis of a **DNA damage/repair coupling "**strategy" that may be generalized to other instances of programmed DSBs [1]. We also demonstrated a functional redundancy between Xlf and PAXX a recently discovered NHEJ factor [8, 9].

In the present Thesis proposal, we wish to analyze the molecular basis of DNA damage/repair coupling and its function as a **potent tumor suppressor** by using the V(D)J recombination as a paradigm. V(D)J recombination can be easily analyzed in Abelson immortalized pro-B lymphocytes in culture. The project will follow several aims:

1. Analysis of several putative RAG2 binding partners that we identified through a large-scale proteomic study. The interactions will be validated by biochemical and *in cellulo* analyses (proximity ligation assay). The domains of interaction will be determined. The consequences of a loss of these partners on DNA repair will be addressed through i*n cellulo* CRISPR/Cas9 mutagenesis followed by functional complementation with wt. and mutant forms. *In vivo* murine model of gene inactivation (KO or KI) will be developed for the most interesting hits.
2. Functional analysis of Ku interacting DNA repair factors involved in the DNA repair phase of V(D)J recombination. Several NHEJ factors contain Ku binding motives (KBM). Genes coding for these factors will

be invalidated in Abl B cell lines through CRISPR/Cas9. Resulting mutants will be complemented with various combinations of wt. and mutated form to establish the functional interactome of these factors during the DNA repair phase of V(D)J recombination.

3. Transcriptomic analyses of Abl B cells undergoing V(D)J recombination will be conducted through RNAseq to identify the cellular machinery specifically involved in this process.
4. We'll take advantage of the powerful readout of V(D)J recombination in Abl B cells to setup a genome wide CRISPR/Cas9 screen to identify proteins critically involved in this process. Recovered candidates will be compared with the list of RAG2 partners as well as with the list of up regulated genes identified through RNAseq.
5. We will continue our survey of human patients presenting with immunodeficiency caused defect in V(D)J recombination. To identify these patients we have developed a biomarker based on the study of the T cell repertoire [10].

**References**:

1 Betermier, M*., et al.* (2019) Coupling DNA damage/repair: an essential safeguard during programmed DNA double strand breaks? *Trends Cell Biol* in press

2 Jung, D*., et al.* (2006) Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annual review of immunology* 24, 541-570

3 Schatz, D.G. and Swanson, P.C. (2011) V(D)J recombination: mechanisms of initiation. *Annual review of genetics* 45, 167-202

4 Zhang, Y*., et al.* (2019) Transposon molecular domestication and the evolution of the RAG recombinase. *Nature* 569, 79-84

5 de Villartay, J.P*., et al.* (2003) The mechanisms of immune diversification and their disorders. *Nature reviews. Immunology* 3, 962-972

6 Ferguson, D.O. and Alt, F.W. (2001) DNA double strand break repair and chromosomal translocation: lessons from animal models. *Oncogene* 20, 5572-5579

7 Lescale, C*., et al.* (2016) RAG2 and XLF/Cernunnos interplay reveals a novel role for the RAG complex in DNA repair. *Nature communications* 7, 10529

8 Abramowski, V*., et al.* (2018) PAXX and Xlf interplay revealed by impaired CNS development and immunodeficiency of double KO mice. *Cell death and differentiation* 25, 444-452

9 Musilli, S*., et al.* (2020) An in vivo study of the impact of deficiency in the DNA repair proteins PAXX and XLF on the development and maturation of the hemo-lymphoid system. *The Journal of biological chemistry*

10 Berland, A*., et al.* (2019) PROMIDISalpha: A T-cell receptor alpha signature associated with immunodeficiencies caused by V(D)J recombination defects. *The Journal of allergy and clinical immunology* 143, 325-334 e322

***Lab members***

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***Major Publications***

1. Roch, B., V. Abramowski, J. Chaumeil and J. P. de Villartay (2019). Cernunnos/Xlf Deficiency Results in Suboptimal V(D)J Recombination and Impaired Lymphoid Development in Mice. *Front Immunol* **10**: 443.
2. Musili, S., V. Abramowski, B. Roch and J. P. De Villartay (2019). Redundancy of PAXX and Xlf for V(D)J but not for immunoglobulin class switch recombination: in vivo model *J Biol Chem* **in press**.
3. Betermier, M., V. Borde and J. P. Villartay (2019). Coupling DNA Damage and Repair: an Essential Safeguard during Programmed DNA Double-Strand Breaks? *Trends Cell Biol*. **In press**
4. Berland, A., J. Rosain, S. Kaltenbach, V. Allain, N. Mahlaoui, I. Melki, A. Fievet, C. Dubois d'Enghien, M. Ouachee-Chardin, L. Perrin, N. Auger, F. E. Cipe, A. Finocchi, F. Dogu, F. Suarez, D. Moshous, T. Leblanc, A. Belot, C. Fieschi, D. Boutboul, M. Malphettes, L. Galicier, E. Oksenhendler, S. Blanche, A. Fischer, P. Revy, D. Stoppa-Lyonnet, C. Picard and J. P. de Villartay (2019). PROMIDISalpha: A T-cell receptor alpha signature associated with immunodeficiencies caused by V(D)J recombination defects. *J Allergy Clin Immunol* **143**: 325-334 e322.
5. Lesport, E., A. Ferster, A. Biver, B. Roch, N. Vasquez, N. Jabado, F. L. Vives, P. Revy, J. Soulier and J. P. de Villartay (2018). Reduced recruitment of 53BP1 during interstrand crosslink repair is associated with genetically

inherited attenuation of mitomycin C sensitivity in a family with Fanconi anemia. *Oncotarget* **9**: 3779-3793.
6. Abramowski, V., O. Etienne, R. Elsaid, J. Yang, A. Berland, L. Kermasson, B. Roch, S. Musilli, J. P. Moussu, K. Lipson-Ruffert, P. Revy, A. Cumano, F. D. Boussin and J. P. de Villartay (2018). PAXX and Xlf interplay revealed by impaired CNS development and immunodeficiency of double KO mice. *Cell Death Differ* **25**: 444-452.
7. Zhang, S., C. Pondarre, G. Pennarun, H. Labussiere-Wallet, G. Vera, B. France, M. Chansel, I. Rouvet, P. Revy, B. Lopez, J. Soulier, P. Bertrand, I. Callebaut and J. P. de Villartay (2016). A nonsense mutation in the DNA repair factor Hebo causes mild bone marrow failure and microcephaly. *J Exp Med* **213**: 1011-1028.
8. Lescale, C., V. Abramowski, M. Bedora-Faure, V. Murigneux, G. Vera, D. B. Roth, P. Revy, J. P. de Villartay and L. Deriano (2016). RAG2 and XLF/Cernunnos interplay reveals a novel role for the RAG complex in DNA repair. *Nat Commun* **7**: 10529.
9. Rivera-Munoz, P., P. Soulas-Sprauel, G. Le Guyader, V. Abramowski, S. Bruneau, A. Fischer, F. Paques and J. P. de Villartay (2009). Reduced immunoglobulin class switch recombination in the absence of Artemis. *Blood* **114**: 3601-3609.
10. Soulas-Sprauel, P., G. Le Guyader, P. Rivera-Munoz, V. Abramowski, C. Olivier-Martin, C. Goujet-Zalc, P. Charneau and J. P. de Villartay (2007). Role for DNA repair factor XRCC4 in immunoglobulin class switch recombination. *J Exp Med* **204**: 1717-1727.
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12. Moshous, D., I. Callebaut, R. de Chasseval, B. Corneo, M. Cavazzana-Calvo, F. Le Deist, I. Tezcan, O. Sanal, Y. Bertrand, N. Philippe, A. Fischer and J. P. de Villartay (2001). ARTEMIS, a Novel DNA Double-Strand Break Repair / V(D)J Recombination Protein, is Mutated in Human Severe Combined Immune Deficiency. Cell 105: 177-186.