Massively parallel functional analysis of missense variants in the breast/ovarian cancer gene *RAD51C*

Montalban G[1], Milano L[1], Rodrigue A[1], Coulombe Y[1], Desjardins S[1], Dumont M[1], Joly-Beauparlant C[1], Soucy P[1], Masson J-Y[1]* and Simard J[1]*

[1]CHU de Québec-Université Laval, Québec City, QC, G1V 4G2, Canada
**Co-corresponding authors*

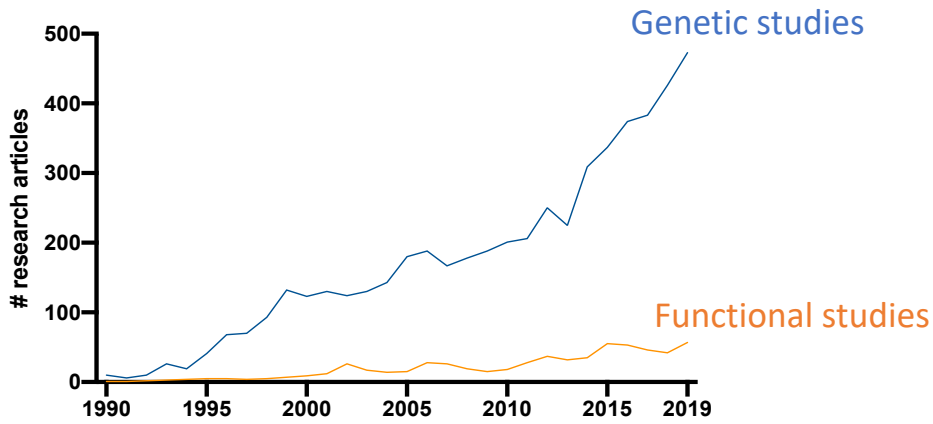
INTRODUCTION

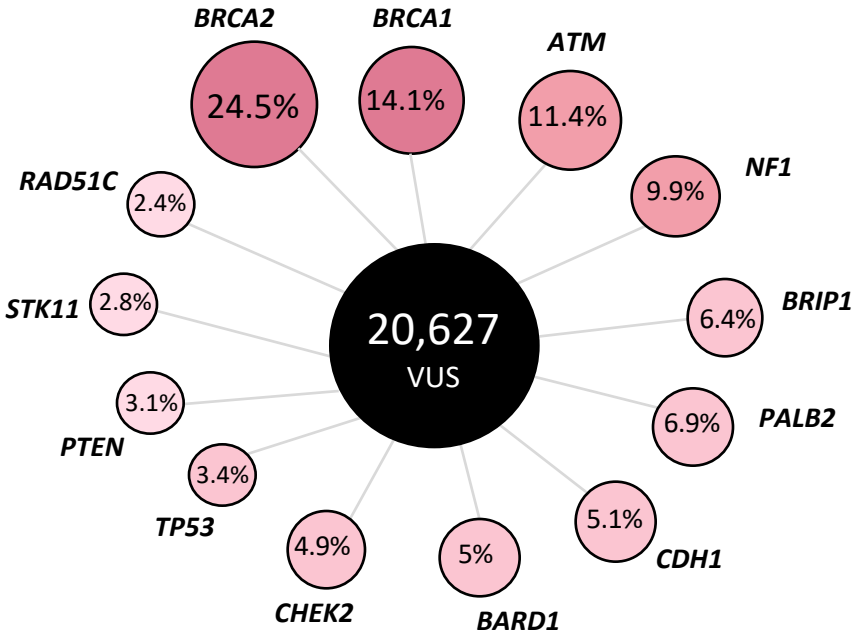
- A proportion of hereditary breast/ovarian cancer (BC/OC) cases are due to pathogenic mutations in genes involved in DNA repair (*BRCA1*, *BRCA2*, *PALB2*, *ATM, CHEK2*, *RAD51C* and *RAD51D*, among others).

- The application of next-generation sequencing has led to an increased detection of variants of unknown clinical significance (VUS).

- The association of protein truncating variants in ***RAD51C*** with **overall breast cancer risk** has been confirmed recently in a large case-control study (**Odds Ratio (95% CI) = 1.93 (1.20-3.11**) (*Breast Cancer Association Consortium, N Engl J Med 2021).*

- **OBJECTIVE**: To implement a massively parallel functional approach to measure the impact of all possible *RAD51C* missense substitutions.

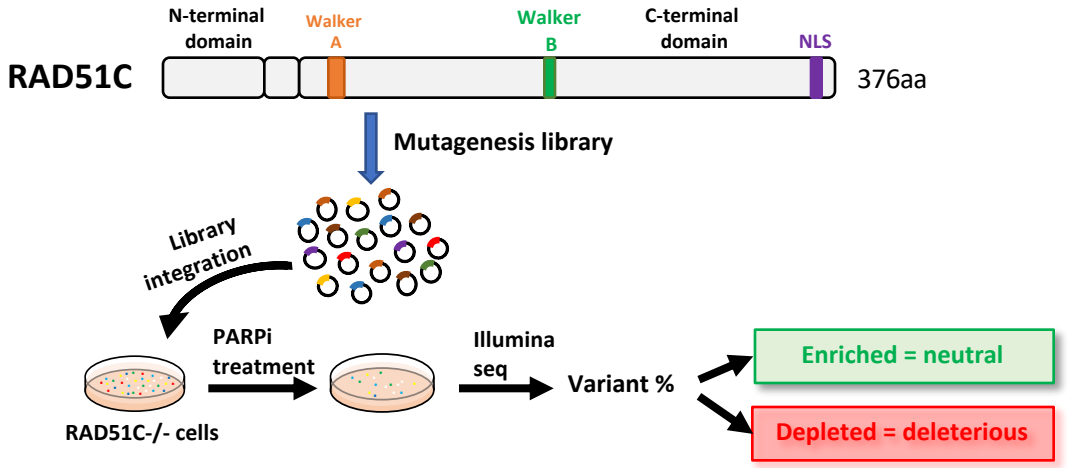




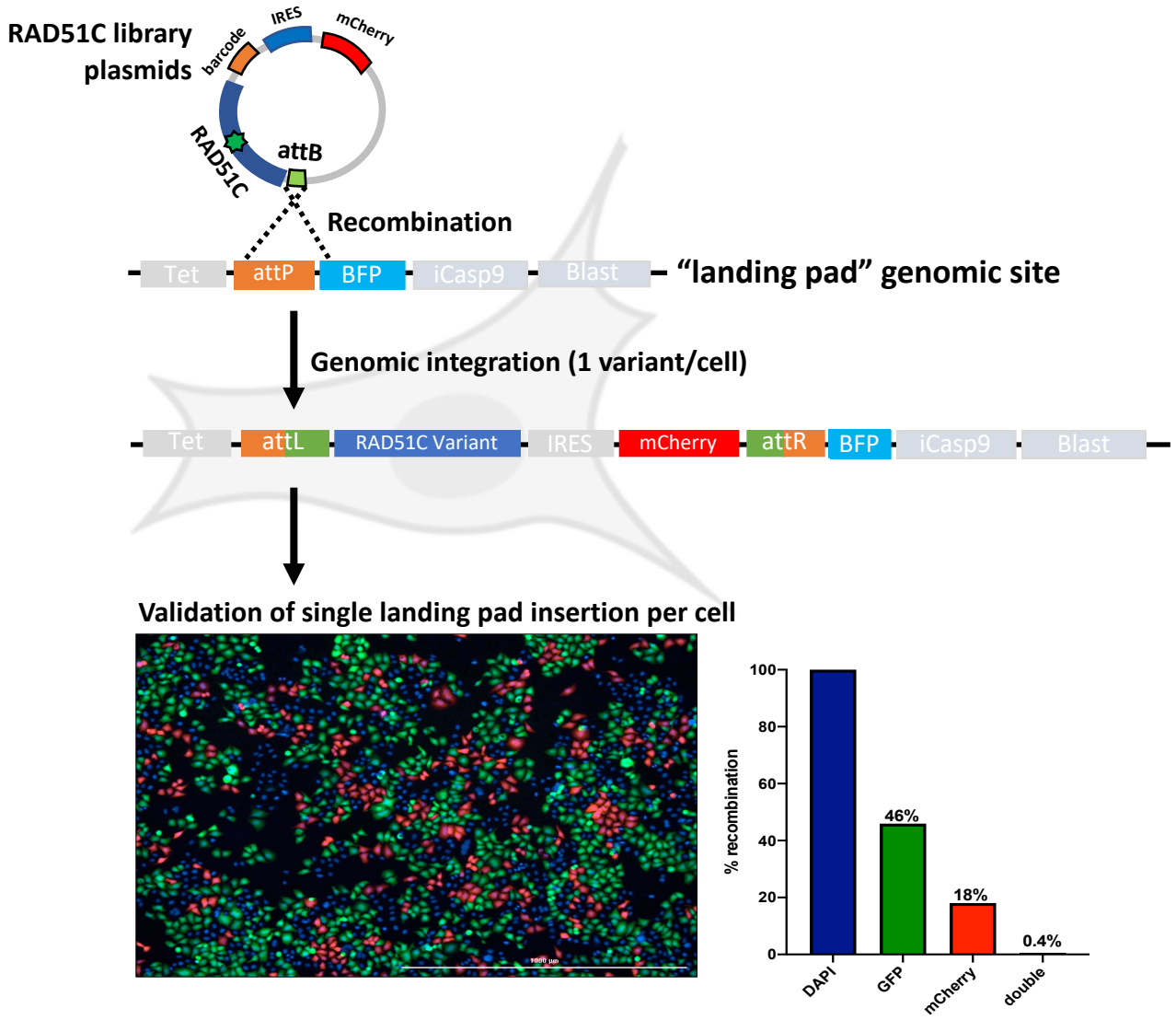
Data extracted from ClinVar and Pubmed databases

METHODS

Deep mutational scanning pipeline



HeLa "landing pad" system

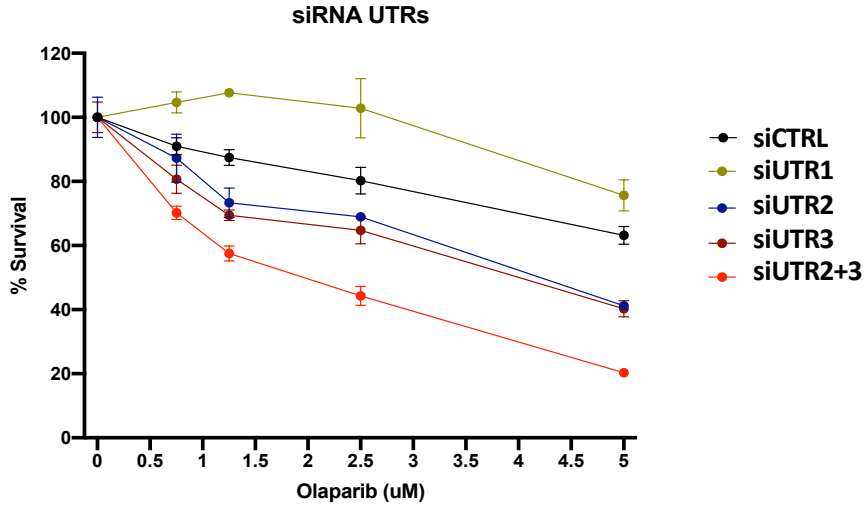


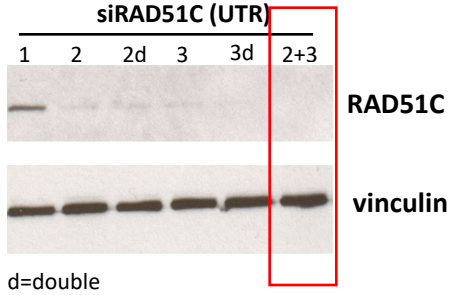
RAD51C LIBRARY - QC

Number of sites:	376 amino acid positions
Requested # of variants:	7516 (20 aa/position)
Sites passed:	369
Sites failed:	7
Variants passed QC:	98.04%

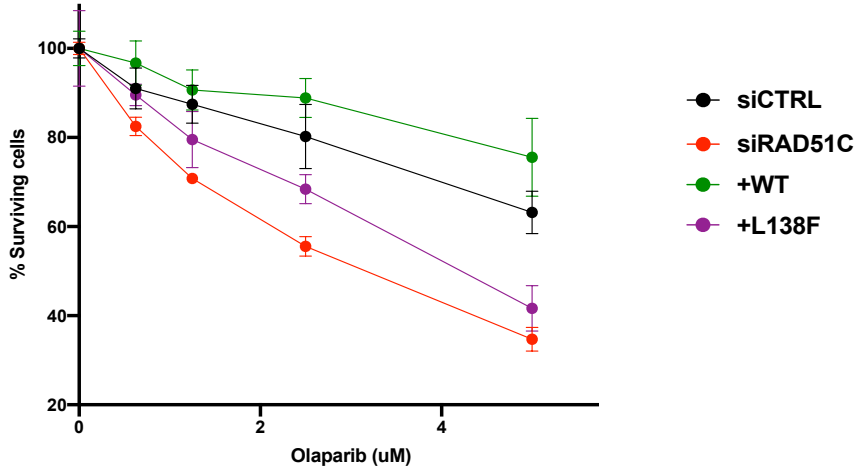
RESULTS I – Optimization assays

A.

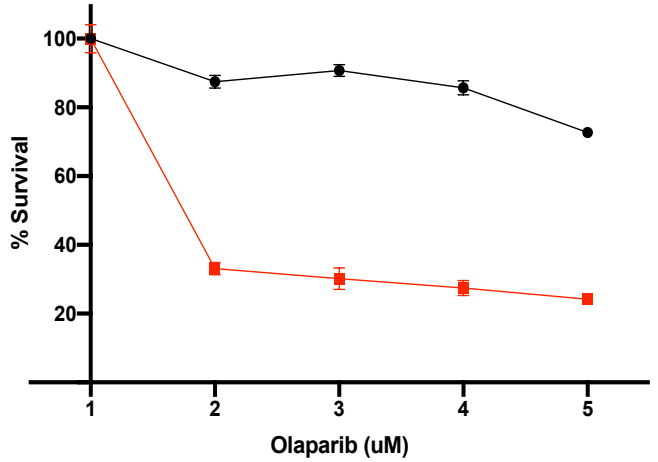


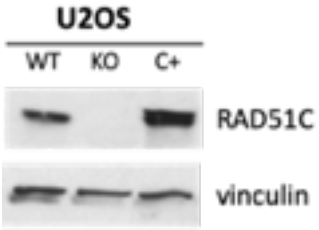


B.



C.



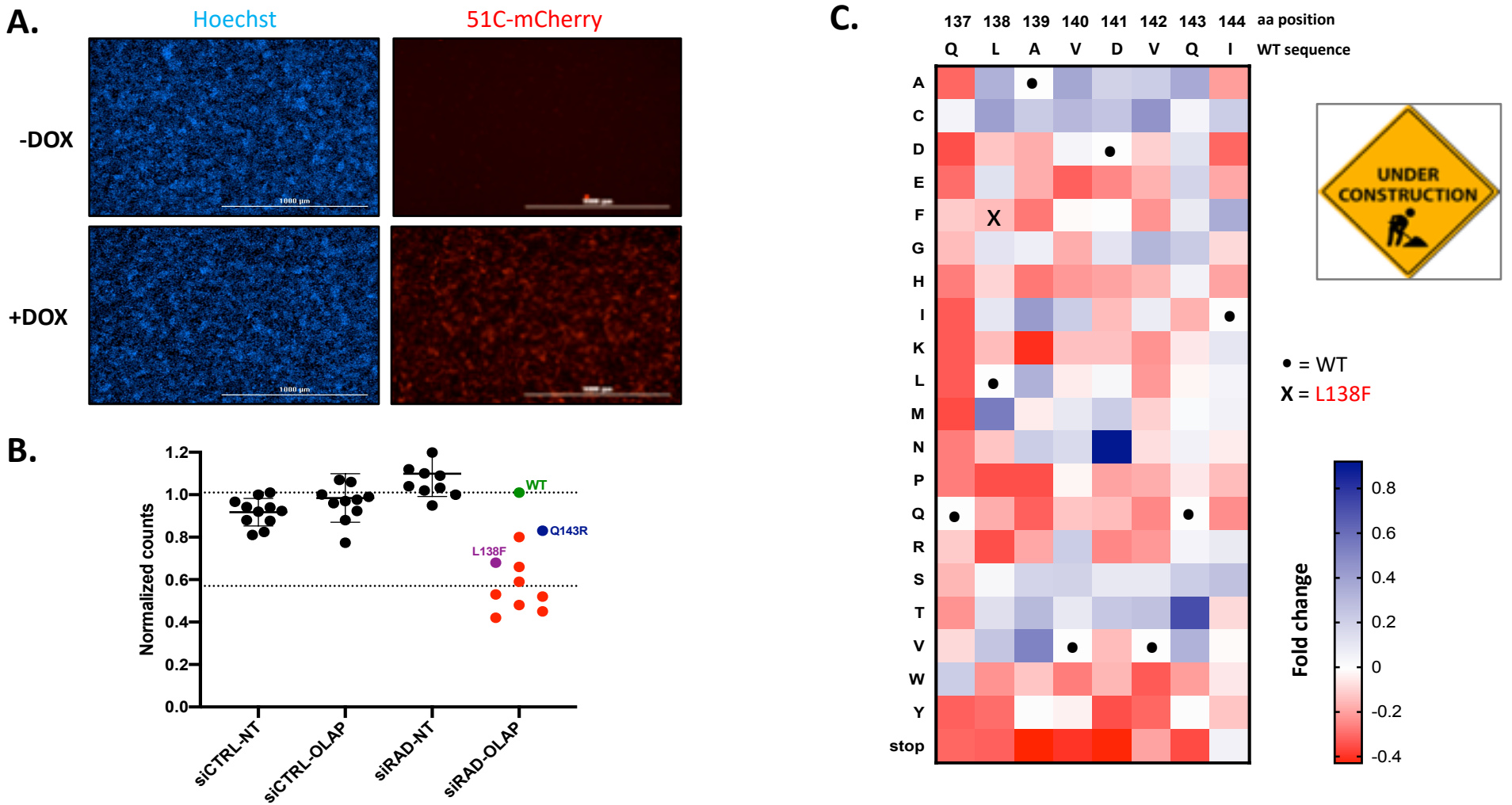


A. **Optimization of siRNA silencing** of endogenous *RAD51C.*

B. **Complementation assay** of HeLa landing pad cells with WT and L138F (pathogenic missense). WT complemented cells rescue drug sensitivity, whereas L138F expressing cells are sensitive to olaparib treatment.

C. **Complementation assay of U2OS RAD51C KO model** generated for validation of candidate missense variants.

RESULTS II – Pilot screen (integration of 160 missense variants – RAD51C residues 137-144)



A. **Microscopy images** confirming the expression of **RAD51C-mCherry** integrated in the HeLa landing pad cell system after 24h of doxycycline induction.

B. **Normalized variant read counts** obtained for **11 variants** that were used as controls (8 stop codons, the pathogenic missense L138F, the VUS Q143R and the WT) in siRNA control and siRNA RAD51C non-treated and olaparib treated conditions, respectively. A reduction of read counts is observed for the Olaparib treated cells when RAD51C is not functional.

C. **Functional scores** obtained for 160 missense variants. Negative scores (red) indicate potential loss of functionality.

CONCLUSIONS

- We have developed a large-scale functional approach to measure the impact of all missense variants in the *RAD51C* gene using PARP inhibitors sensitivity as a readout.
- Experimental replicates and calculation of loss-of-function scores (LOF) using other DNA damaging agents is ongoing.
- Future work will focus on validating our data with published works, clinical databases and complementary assays (RAD51 foci scoring).
- The final goal is to generate a functional atlas for the *RAD51C* gene in order to improve the interpretation of missense VUS and accelerate their clinical translation.

Thank you!

@gemma_montalban