A multiplexed Enzymatic Repair Assay stratifies head and neck cancers and patients treated by chemo-radiotherapy

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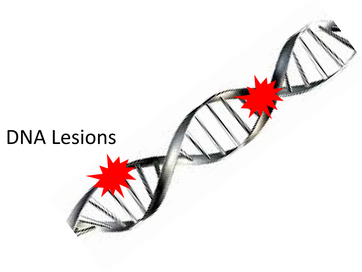
INTRODUCTION

Radiotherapy and chemotherapy induce a wide variety of lesions to the DNA.
Healthy tissues and tumor cells have the ability to sense this variety of lesions and elicit a coordinated response with an impact on treatment efficacy (resistant tumors) and treatment-induced toxicity. Both the abilities of treatments to induce several lesions into the DNA and of cells to repair these lesions influence tumors resistance and patients sensitivity.

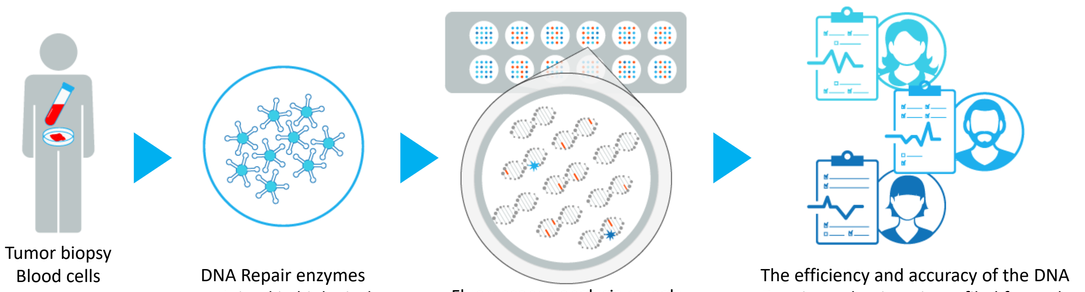


TOXICITY to anticancer treatments or **RESISTANCE** to anticancer treatments

Defining sub-group of patients according to their sensitivity/resistance is one of the main developments for increasing therapeutic effects.

LXREPAIR TECHNOLOGIES

Functional, Multiplexed and Quantitative Assays able to investigate the complexity of DNA repair for identification of markers able to predict the occurrence of adverse effects



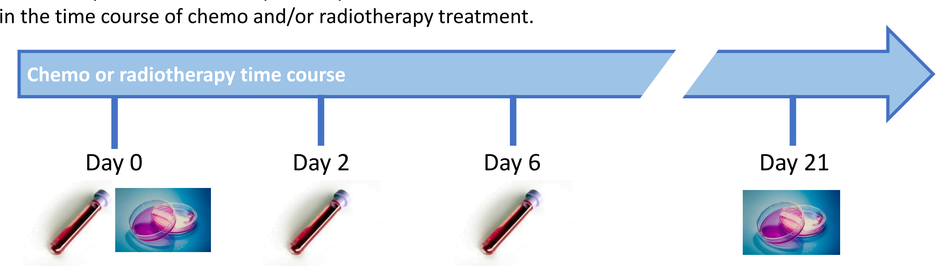
The DNA Damage Response profile characterizes the effective individuals DNA repair capacity → Patients stratification → Personalized Medicine

THE CHEMRAD CLINICAL TRIAL

- Identify predictive biomarkers of **Radio and Chemo Toxicity** or **Resistance**
  - Starting from **blood samples** of patients afflicted with Head and Neck Cancers
  - Starting from **tumor biopsies** of patients afflicted with Head and Neck Cancers

STEP 1: SAMPLES COLLECTION

Blood samples and tumor biopsies of patients afflicted with head and neck cancers were collected before and in the time course of chemo and/or radiotherapy treatment.

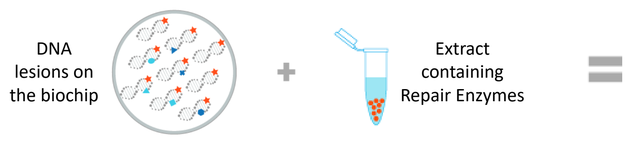


STEP 2: PROTEINS EXTRACTION

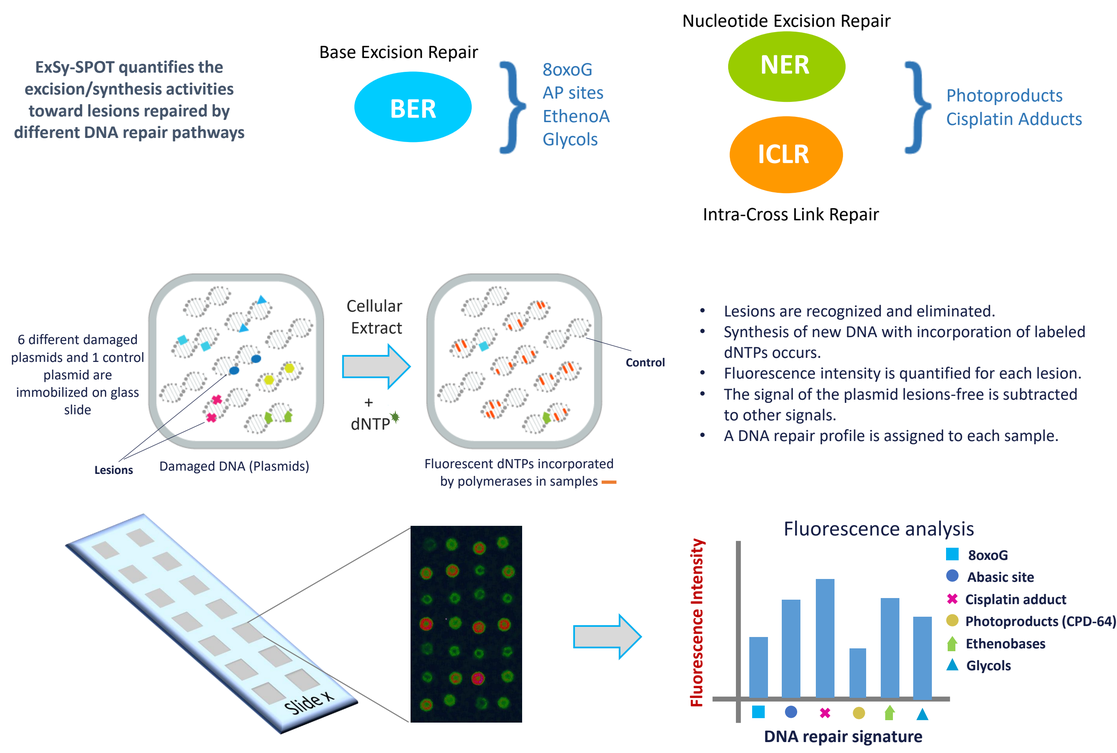
The nuclear or whole cell extracts were prepared from Peripheral Blood Mononuclear Cells (PBMCs) and tumor biopsies and contain all DNA repair enzymes.

STEP 3: FUNCTIONAL ANALYSIS

Multiplexed biochips are functionalized with a panel of specific DNA lesions which are repaired by the enzymes present in the cells extract.



LXRepair Assay ExSy-SPOT (Multiplexed Excision Synthesis Repair Assay)



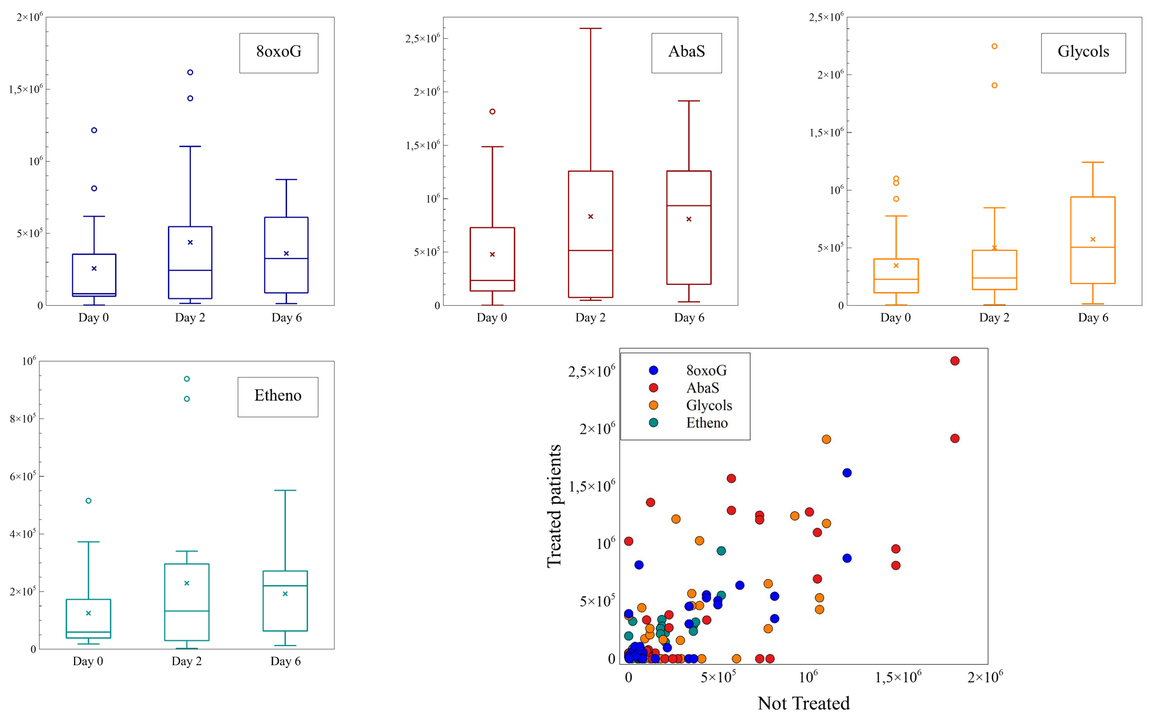
- Lesions are recognized and eliminated.
- Synthesis of new DNA with incorporation of labeled dNTPs occurs.
- Fluorescence intensity is quantified for each lesion.
- The signal of the plasmid lesions-free is subtracted to other signals.
- A DNA repair profile is assigned to each sample.

Each sample is characterized by a highly specific DNA Repair Signature

RESULTS

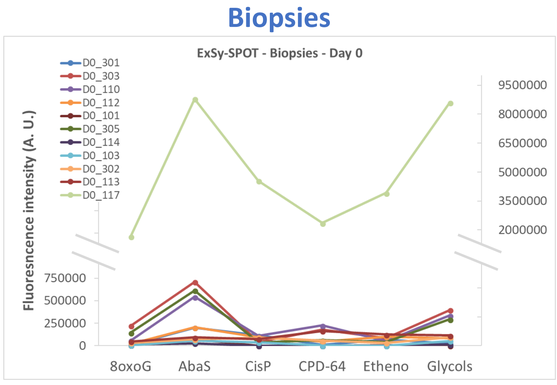
The preliminary results of this approach show an inter-individual variability in DNA repair capacities at basal level and treatment-induced in both PBMCs and tumor cells.

PBMCs

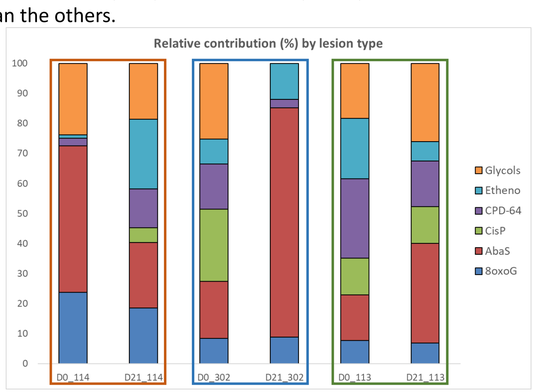


A high inter-individual variability in repair mechanisms of 4 specific lesions is shown before the treatment starts (Day 0). After two or five days of treatment, an induction of oxidized bases and abasic sites repair mechanisms was observed.
The scatter graph represents the correlation between different repair pathways induced before and after treatment in 22 patients. The induction of repair mechanisms is heterogenous highlighting some significant outliers that could suggest the apparition of treatment-induced adverse effects.

Biopsies



Analysis of Excision/Synthesis basal activities (before treatment) reveals 3 groups of tumors on the basis of their DNA repair profile. One sample in particular shows a repair activity 10 times higher than the others.



The relative contribution of Excision/Synthesis activities of three patients changes between the starting day and the 21st day of treatment revealing a progression of these mechanisms during the time course of treatment.

PERSPECTIVES

We want to correlate these experimental data with the clinical data at the end of the study in order to

- Identify patients at risk of adverse effects
- Classify tumours based on their real DNA repair capacities
- Refine mutation-based classification to define personalized treatments