Stratification of melanoma patients based on their DNA Repair Enzyme Signature: relationship with driver genes mutations and insights into DNA Repair defects

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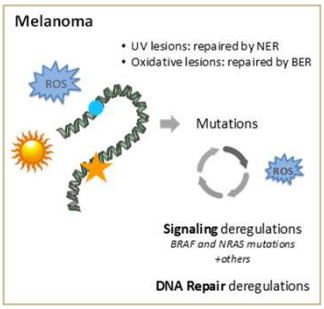
Introduction - Objectives

Melanoma is classified according to activating mutations in specific genes (BRAF, NRAS, …) which serve as a basis for **targeted therapies** prescription.
Exposure to UV radiation is a major risk factor for melanoma. This carcinogen promotes elevated mutation frequencies in tumors and affects **DNA Repair** pathways. Interestingly, DNA Repair mechanisms are regulated by the MAPK/PI3K/AKT signaling pathway. Functional links associate the different networks.
In addition, defective DNA Repair could be responsible for the observed elevated mutation frequencies in these tumors. Interestingly, mutation load predicts clinical benefit of **immunotherapy** in various cancers.

We believe a **unified strategy** is required **to stratify metastatic melanoma and identify relevant biomarkers** to choose the best therapeutic option. Because of the central role played by DNA Repair in this carcinogen-induced tumor, we propose a new classification of melanoma based on **functional DNA Repair analysis**.



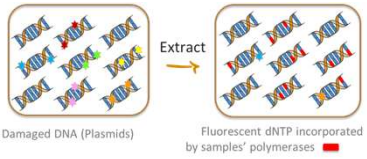
Materials and Methods

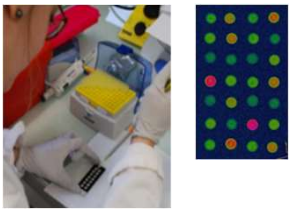
● Assay workflow

12 Patients with metastatic melanoma
(CHU Grenoble Alpes – CRB)
Tumor or Lymph nodes
↓
Cell extracts
↓
Functional DNA Repair assay on biochip
↓
DNA Repair Enzyme Signature

● ExSy-SPOT functional assay

The DNA repair reaction was performed in the presence of differently labeled dNTPS to:
- determine the DNA Repair capacities of different pathways
- Identify DNA Repair defects possibly leading to mutations





Repair pathway investigated :
Base Excision Repair (BER)
Nucleotide Excision Repair (NER)
- UV-induced lesions (NER) (CPD-64 and CPD)
- Oxidative damage (8oxoG, Glycols) (BER)
- Alkylated bases (BER)
- Abasic sites (BER)

● Data Analysis- Reporting

- **DNA Repair Enzyme Sig**nature: represents the DNA repair capacity for each repair pathway, expressed as **Fluorescence Intensity** (FI). It is used for the **classifications** and **box-plots.**
- **Contribution** of each pathway to total repair: allows a precise comparison between samples and DNA Repair regulation, independently of FI level.
- **Preferred incorporated nucleotide**: for each lesion, determines which base is preferentially inserted and gives information about the specific repair pathway involved while identifying **missing activities** and **polymerases defects**.

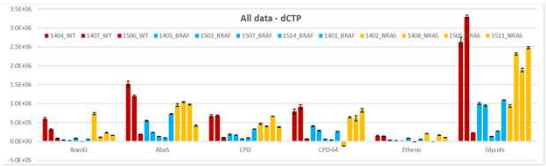
Results

•Samples

Sample	Gender	Age	PhotoType	Type	Mutation	Survival
1401	M	42	III	Node	BRAF	M9
1402	M	32	II	Node	NRAS	M3
1404	F	62	III	Node	WT	M7
1405	M	50	II	Metastase	BRAF	M3
1407	M	69	III	Metastase	WT	> M24
1408	M	60	III	Node	NRAS	>M24
1502	F	50	III	Node	BRAF	Lost M3
1505	F	83	II	Node	NRAS	Lost M3
1506	M	78	II	Node	WT	M3
1507	M	46	III	Node	BRAF	> M24
1511	M	64	III	Node	NRAS	> M24
1514	M	50	III	Node	BRAF	M18

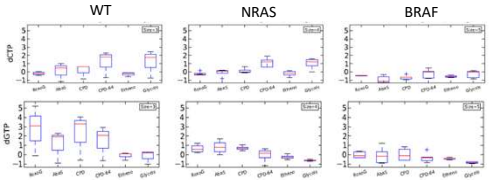
•DNA Repair Enzyme Signature – All samples

Fluorescence Intensity



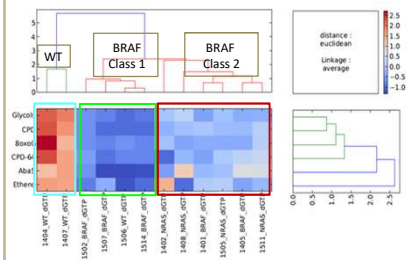
• Profiles by Mutation Group and dNTP – Box-Plots

Normalized data (mean:0; SD:1)

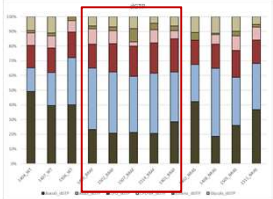


Impact of mutation in MAPK genes

• Classifications: Samples are clustered according to their profiles similarities



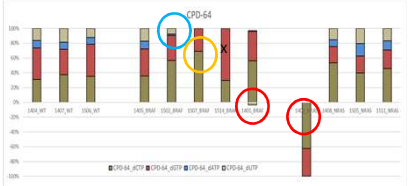
•Contribution



Functional link between MAPK pathway and DNA repair
There is a dominant pattern for each mutation group.
This pattern is revealed in different dNTPS conditions.
In certain experimental conditions, we identified 2 classes of BRAF mutated samples that could be associated with different response rates to targeted therapy (consistent with preclinical data).

•Preferred incorporated nucleotide

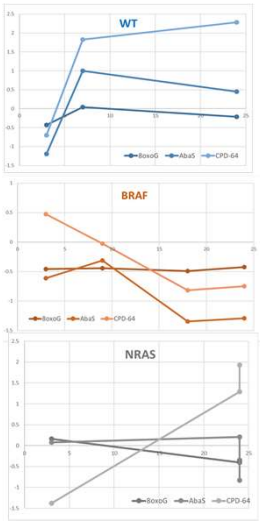
Missing activities
Unbalanced repair
Polymerase defect



This graph combines the data obtained with the 4 dNTPS: it visualizes the DNA Repair defects responsible for a mutator phenotype.

Repair of photoproducts is associated with patient survival
Repair capacity of Photoproducts correlates with survival for patients from **WT and NRAS Mutation** Groups and is **inversely correlated** for patients from **BRAF Mutation** Group.

•DNA Repair and survival



Conclusion

DNA Repair mechanisms and melanoma are intimately linked. Our powerful tool allows revisiting this link by covering different functional aspects of DNA repair and giving an exhaustive characterization of each sample's DNA Repair network. Interestingly this unified functional approach could be used to choose the best therapeutic option for each patient.
Consolidation of these biomarkers is required on a larger cohort of melanoma patients.

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