New combinations of targeted therapies in melanoma

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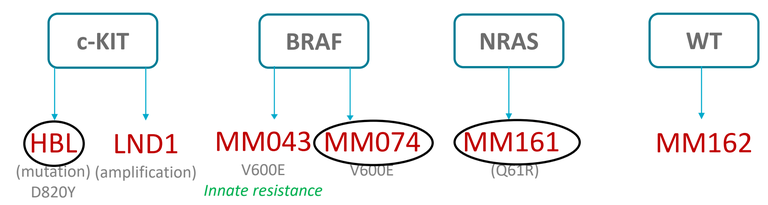
Introduction

Melanoma affects a large number of young adults and its incidence is constantly increasing. The diagnosis at metastatic stage is associated with a very poor prognosis in link to a very high mutational potential of such cancer. Advanced melanoma has also a high capacity to activate alternative signalling pathways for survival leading to the development of acquired resistance to targeted therapies. Combinations of targeted therapies are proposed as the most promising way to overcome these resistances. However, while many combinations have been evaluated in preclinical settings, only few ones have been approved for clinical use, mainly targeting the same MAPK pathway (BRAF and MEK) but with a limited remission rate or stabilisation of the disease.

Materials & Methods

- **Used inhibitors:**
  - ✓ MAPKi: vemurafenib (BRAFi), sunitinib (RTKi), pimasertib (MEKi)
  - ✓ p53 reactivation: PRIMA-1^{MET}
  - ✓ Bcl-2 inhibition: ABT-199
  - ✓ Mnk1/2 inhibition: **SEL201** & ETP 45835
- **Short term proliferation assesment:** crystal violet assay
- **Long term proliferation assesment:** clonogenic assay
- **Apoptosis measurement:** FACS (annexin V)
- **Protein expression/localisation:** immunofluorescence
- **Metabonomic study:** ^{1}H-RMN

Cell lines



Aim: focus on various potential targeting pathways for the development of new more effective combinatory treatments.

- Targeting apoptosis pathways : p53 and Bcl-2 (1)
- Studying resistance mechanisms (3)
- Targeting Mnk1/2 (2)
  - Validate the higher efficacy of SEL201 (2')

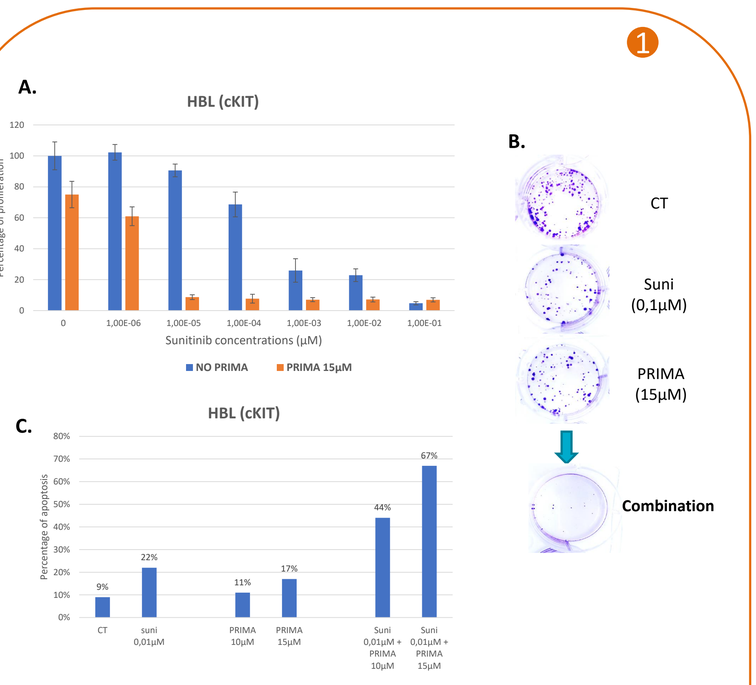


Figure 1: Effect of p53 reactivation (PRIMA-1^{MET}) on proliferation (A&B) and apoptosis (C)
(A) Effect of the combination of sunitinib + PRIMA-1^{MET} on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)
(B) Effect of the combination of sunitinib + PRIMA-1^{MET} on long term proliferation (14d) assessed by a clonogenic assay
(C) Effect of the combination of sunitinib + PRIMA-1^{MET} on apoptosis (48h) assessed by FACS (annexin V)

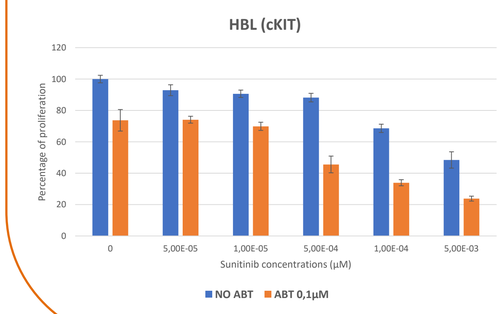


Figure 2: Effect of Bcl-2 inhibition (ABT-199) on proliferation
Effect of the combination of sunitinib + PRIMA-1^{MET} on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)

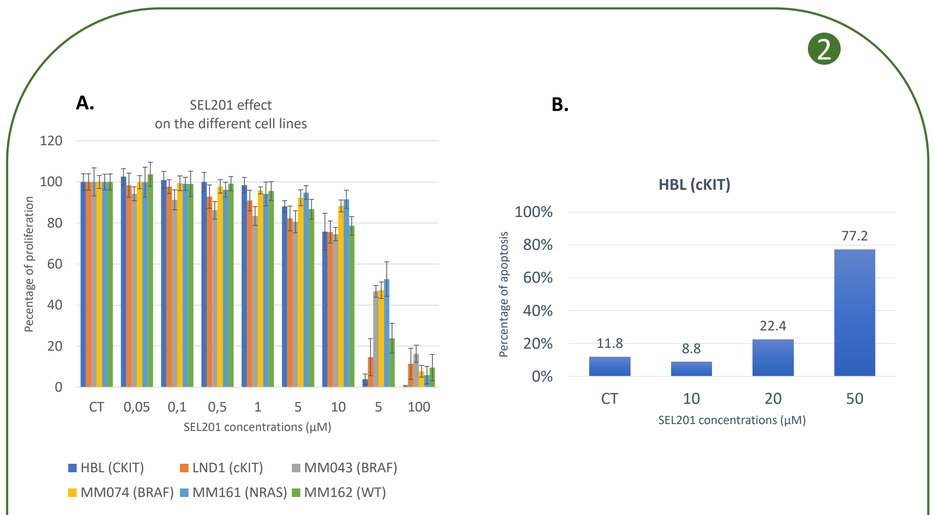


Figure 3: Effect of Mnk1/2 inhibition (SEL201) on proliferation (A) and apoptosis (B)
(A) Effect of SEL201 on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)
(B) Effect of SEL201 on apoptosis (48h) assessed by FACS (annexin V)

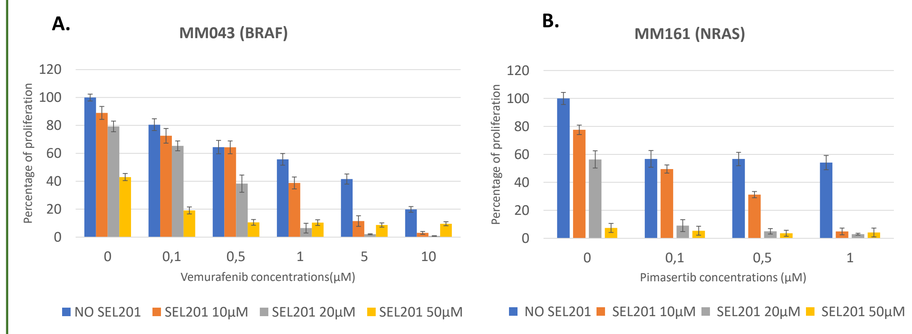


Figure 4: Effect of the combination MAPK inhibition / Mnk1/2 inhibition on proliferation
(A) Effect of the combination of vemurafenib (BRAFi) + SEL201 on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)
(B) Effect of the combination of pimasertib (MEKi) + SEL201 on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)

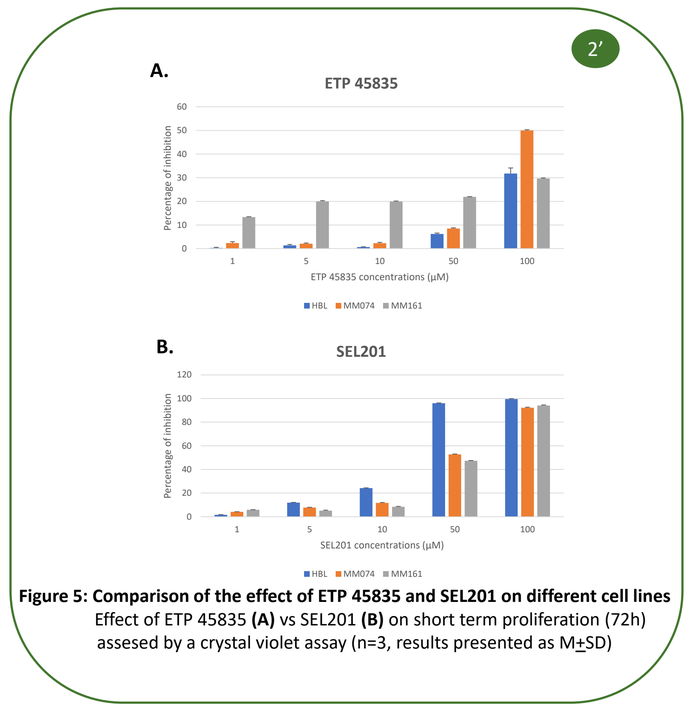


Figure 5: Comparison of the effect of ETP 45835 and SEL201 on different cell lines
Effect of ETP 45835 **(A)** vs SEL201 **(B)** on short term proliferation (72h) assesed by a crystal violet assay (n=3, results presented as M±SD)

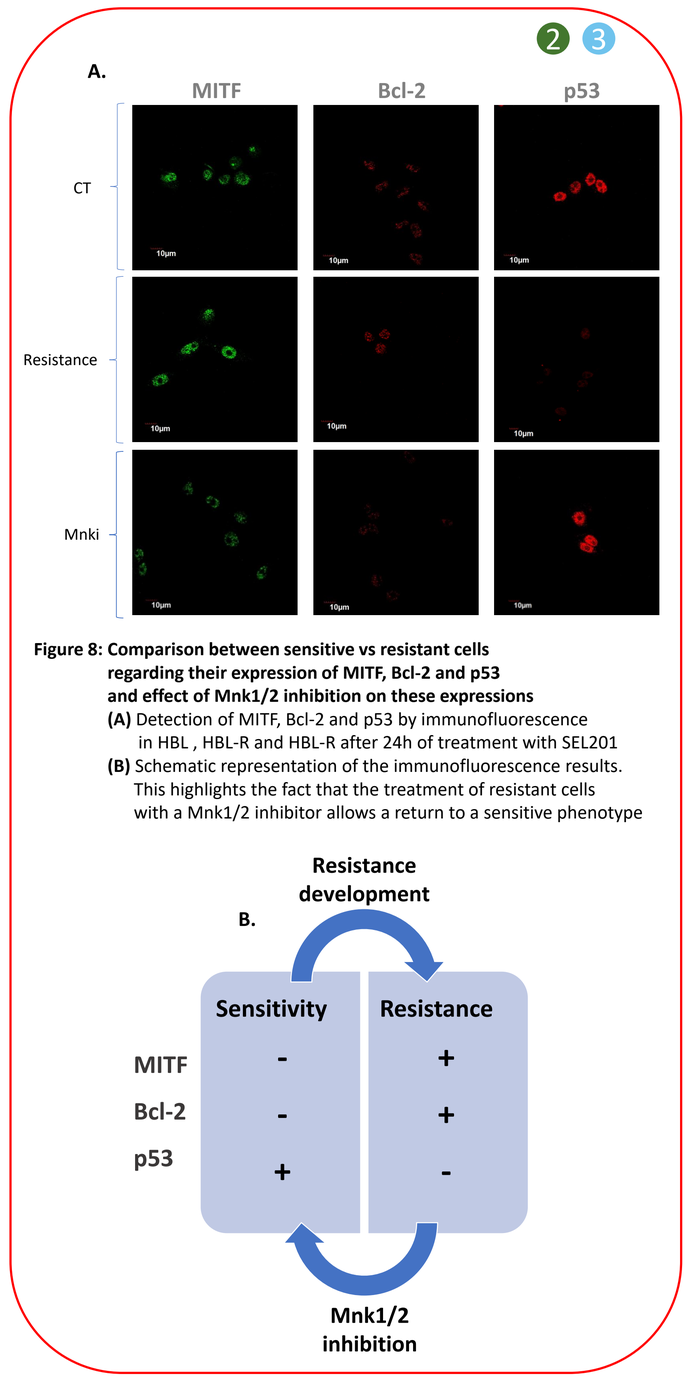


Figure 8: Comparison between sensitive vs resistant cells regarding their expression of MITF, Bcl-2 and p53 and effect of Mnk1/2 inhibition on these expressions
(A) Detection of MITF, Bcl-2 and p53 by immunofluorescence in HBL , HBL-R and HBL-R after 24h of treatment with SEL201
(B) Schematic representation of the immunofluorescence results. This highlights the fact that the treatment of resistant cells with a Mnk1/2 inhibitor allows a return to a sensitive phenotype

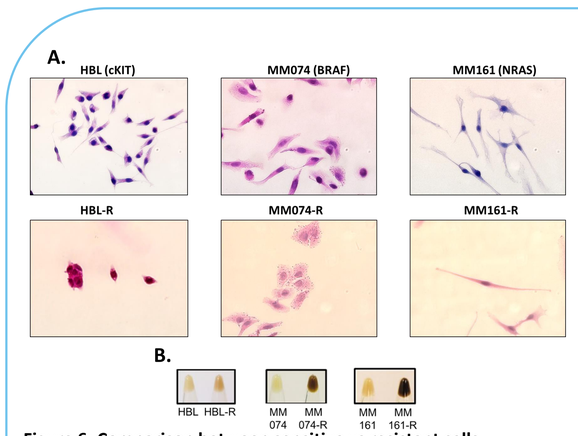


Figure 6: Comparison between sensitive vs resistant cells regarding their morphology (A) and pigmentation (B)
(A) H&E staining of 3 different cell lines in a sensitive or resistant state (optical microscope 400x)
(B) Sensitive and resistant cells after centrifugation and eluate elimination

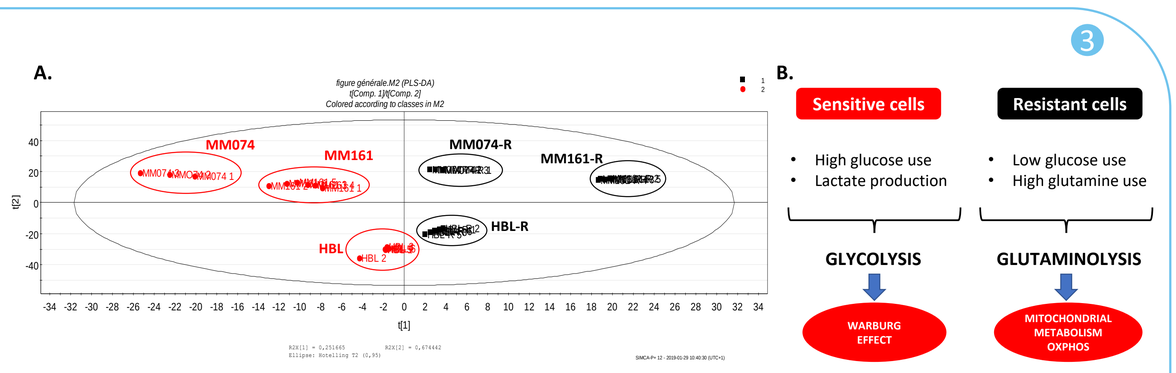


Figure 7: Comparison between sensitive vs resistant cells regarding their metabolite consumption and production
(A) Graph (score plot of PLS-DA) representing the statistical clustering of the different sensitive and resistant cells based on the identification of the metabolites in the intra and extracellular medium
(B) Schematic representation of the metabolism differences between sensitive and resistant cells. This highlights the major role of glycolysis and Warburg effect in cells sensitives to MAPKi. While resistant cells rely more on mitochondria for their metabolism with a major role of oxidative phosphorylation.

Conclusion:

Our data support the use of combinations of targeted therapies for the treatment of melanomas, breaking acquired resistance to the drugs. The inhibition of translation of specific proteins as well as the evaluation of resistance to targeted therapy at metabolite level, would be successful to propose novel therapeutic combinatory strategies against melanoma.

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