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 signaling 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:**
  - MAPK: vemurafenib (BRAF)
  - sunxitinib (RTKs)
  - pimarsertib (MEKI)
- **p53 reactivation:** ABT-199
- **Bcl-2 inhibition:** PRIMA 1
d- **Mnk1/2 inhibition:** SEL201 & ETP 45835
- **Short term proliferation assessment:** crystal violet assay
- **Long term proliferation assessment:** clonogenic assay
- **Apoptosis measurement:** FACS (annexin V)
- **Protein expression/localisation:** immunofluorescence
- **Metabonomic study:** U-H-RMN

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

Cell lines

- **Parental cells:** HBL (Suni (µM))
  - MM074 (Vemurafenib)
  - MM161 (PRIMA 15µM -16)
  - MMO74 (cKIT)

- **Resistant cells:**
  - HBL-R 0.1
  - MM161-R 0.5
  - HBL-R 1

Figure 1: Effect of p53 reactivation (PRIMA-1M) on proliferation (A&B) and apoptosis (C)
- (A) Effect of the combination of sunxitinib + PRIMA-1M on short term proliferation (72h) assessed by a crystal violet assay (n=3, results presented as M±SD)
- (B) Effect of the combination of sunxitinib + PRIMA-1M on long term proliferation (34 days) assessed by a clonogenic assay
- (C) Effect of the combination of sunxitinib + PRIMA-1M as apoptosis (ABI) assessed by FACS (annexin V)

Figure 2: Effect of Bcl-2 inhibition (PRIMA-1M) 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 (ABI) assessed by FACS (annexin V)

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

Cell lines

- **Materials:**
  - BRAF (10µM)
  - NRAS (15µM)
  - WT
  - MM162

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

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Figure 5: 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.