Acquired resistance to BRAFi reverses senescence-like phenotype in mutant BRAF melanoma

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Gene mutation status of BRAF, p53, PTEN, and Cyclin D1 in the melanoma cell line panel

| Cell lines     | BRAF status | p53   | PTEN | Cyclin D1 |
|----------------|-------------|-------|------|-----------|
| Wild type BRAF cells |             |       |      |           |
| HBL            | WT          | WT    | WT   | WT        |
| LND1           | WT          | WT    | WT   | WT        |
| BRAFi sensitive cells |          |       |      |           |
| MM034          | V600E       | WT    | WT   | WT        |
| MM074          | V600E       | WT    | WT   | WT        |
| MM070          | V600E       | WT    | WT   | WT        |
| SK-MEL28       | V600E       | L145R | WT   | WT        |
| MM050          | V600E       | WT    | WT   | WT        |
| BRAFi resistant cells (intrinsic resistance) |           |       |      |           |
| MM164          | V600E       | A161V | R15G | WT        |
| MM032          | V600E       | WT    | WT   | WT        |
| MM043          | V600E       | WT    | WT   | WT        |
| MM133          | V600K       | WT    | WT   | WT        |
| MM029          | V600K       | WT    | WT   | WT        |
**Supplementary Table 3: Summary data recapitulating the reported findings in the study**

| Cell lines | BRAF status | Proliferation rate | Size (Volume) | Senescence like phenotype (SA-β-Gal activity) | IC50 (Vemu) | pRB | Cyclin D1 | pAKT | pERK |
|------------|-------------|--------------------|--------------|-----------------------------------------------|-------------|-----|---------|------|------|
| Wild type BRAF cells | HBL WT | +++ | + | – | ND | ND | ND | ND | ND |
| | LND1 WT | +++ | + | – | ND | ND | ND | ND | ND |
| BRAFi sensitive cells | MM034 V600E | ND | ND | + | 0.1 | +++ | – | – | + |
| | MM074 V600E | + | +++ | +++ | 0.1 | +++ | – | – | + |
| | MM070 V600E | + | +++ | +++ | 0.1 | +++ | – | – | +++ |
| | SK-MEL28 V600E | + | +++ | +++ | 0.3 | +++ | – | – | + |
| | MM050 V600E | + | +++ | +++ | 0.3 | – | +++ | +++ | – |
| BRAFi resistant cells (intrinsic resistance) | MM164 V600E | + | +++ | +++ | 2 | – | +++ | +++ | – |
| | MM032 V600E | + | +++ | +++ | 3 | – | +++ | +++ | – |
| | MM043 V600E | + | ND | +++ | 10 | +++ | – | – | +++ |
| | MM133 V600K | + | ND | +++ | 2 | +++ | – | – | +++ |
| | MM029 V600K | + | ND | + | 10 | – | – | – | + |

BRAF Status, Proliferation rate, size, SA-β-Gal activity, IC50 (Vemurafenib) and effect of BRAFi (vemurafenib) on SA-β-Gal activity and protein expression levels of pRB, cyclin D1, pAKT and pERK in wild type BRAF cell lines and mutant BRAF cell lines (BRAFi sensitive lines and lines with intrinsic resistance).

**Supplementary Table 2: Summary data recapitulating the reported findings in the study**

| Cell lines | BRAF status | Proliferation rate | Size (Volume) | Senescence like phenotype (SA-β-Gal activity) | IC50 (Vemu) | Senescence like phenotype SA-β-Gal activity (exacerbated) | Protein expression |
|------------|-------------|--------------------|--------------|-----------------------------------------------|-------------|-------------------------------------------------|------------------|
| Wild type BRAF cells | HBL WT | +++ | + | – | ND | ND | ND |
| | LND1 WT | +++ | + | – | ND | ND | ND |

BRAF Status, Proliferation rate, size, SA-β-Gal activity, IC50 (Vemurafenib) and effect of BRAFi (vemurafenib) on SA-β-Gal activity and protein expression levels of pRB, cyclin D1, pAKT and pERK in wild type BRAF cell lines and mutant BRAF cell lines (BRAFi sensitive lines and lines with intrinsic resistance).

**Supplementary Table 3: Summary data recapitulating the reported findings in the study**

| Cell lines | Drug inhibitor (Vemu) | Proliferation rate | Senescence like phenotype (SA-β-Gal activity) | Protein expression |
|------------|-----------------------|--------------------|-----------------------------------------------|------------------|
|野生型 BRAF细胞 | HBL WT | +++ | + | – | ND | ND | ND |

**药理学**

**高（+++），低（+）增殖率；大（+++），小（+）细胞体积；高（+++），低（+），负（-）SA-β-Gal活性；高（+++），低（+）负（-）蛋白质表达。**

**ND（Not determined），SA-β-Gal活性：与BRAFi治疗相关的β-D-葡萄糖苷酶活性。**

**IC50（Vemurafenib），Proliferation rate，size，SA-β-Gal activity and protein expression levels of pRB, cyclin D1, pAKT and pERK in lines with acquired resistance to BRAF inhibition in the presence of Vemurafenib and after 14 days of washing out vemurafenib (drug withdrawal).**
Supplementary Figure 1: Drug addiction/dependency phenomenon observed in a second cell line model with acquired resistance to vemurafenib. Western blots illustrating the evaluation of pERK, ERK, pAKT, AKT, PTEN, cyclin D1, pRB, p53, p21 and V600EBRAF (VE1) in a second line with acquired resistance (SKMEL-28-R) treated with 2 µM vemurafenib (vemu) or after 14 days of washing out vemurafenib (w/o). β-actin is used as loading control.
Supplementary Figure 2: The effect of mutant BRAF and BRAF inhibitor treatment on the secretory phenotype of melanoma cells. Fold changes of the relative mRNA expression of TGFβ and IL-8 in melanoma cells. In (A) data are presented as a fold change in BRAF mutant cell lines (MM074, SKMEL-28, MM164 and MM043) vs. wild BRAF cell lines (HBL). In (B) data are presented as a fold change in cells (MM074, SKMEL-28 and MM164) treated with vemurafenib (1 µM, 24 hours) vs. control. In (C) data are presented as a fold change in cells with acquired resistance (MM074-R, SKMEL-28-R) vs. parental sensitive lines (MM074, SKMEL-28). Data are normalized to 18S and representative of three separate reactions. *p < 0.05, **p < 0.01, ***p < 0.001 (Student’s t-test).