Supplementary Figure 1. CT scan at baseline and after 3 months since first introduction of vemurafenib.
**Supplementary Figure 2.** Copy number alteration (CNA) from whole exome sequencing data. Log ratio (logR) of coverage in tumor sample versus normal sample is indicated. Red lines indicate segmentation algorithm calls.
Supplementary Figure 3. B Allele Frequencies (BAF) of germline heterozygous SNPs located in CNA regions. Scatter plots compare the BAFs in Pre, PV1 and PV2 samples. Chromosome 7q is the only region showing duplication of different paternal/maternal chromosomes in PV1 and PV2.
Supplementary Figure 4. Sensitivity of a PV1-derived cell line to BRAF and MEK inhibitors. Cells were plated in 96-well plates and treated the following day with the BRAF inhibitor PLX4032 and MEK inhibitors AZD6244 and CI-1040 as described in suppl. Methods. Results, expressed as percent surviving cells relative to vehicle-treated cells, show the mean ± SD from a triplicate assay.
Supplementary Figure 5. Sanger sequencing of PI3KCA and NRAS amplified cDNA fragments from single cells clones isolated from the T1407A short-time culture (PV1 metastasis). Single nucleotide variations are indicated with an asterisk. Expression of both PIK3CA and NRAS is heterozygous. Clones also contained the BRAFV600E mutation (not shown).
Supplementary Figure 6. Sensitivity of PIK3CA mutated clones to combinations of MEK/PI3K and RAF/MEK inhibitors. A, clonal cultures carrying the PIK3CA H1047R or the indicated NRAS mutations were incubated, along with the original uncloned population, with AZD6244 (AZD, 0.1 μM, except for clone 11, 1 μM), GDC-0941 (GDC, 1 μM), or a combination of the drugs for 4 days. B, as in A, cells were treated with PLX4032 (PLX, 1 μM), AZD6244 (0.1 μM, except for clone 11, 1 μM) or a combination of the drugs. Results show average and standard deviation of triplicate measurements.
Supplementary Figure 7. Confirmation of the splice junction and presence of cT1799A/V600E mutation in the alternatively spliced BRAF sequence. Panel A, RT/PCR was performed with primers located in exon 3 and 18 so as to cover the splice junction and the mutation site. The alternatively spliced BRAF product from PV2 (red circle) was isolated for Sanger sequencing. Panel B, only a cT1799A sequence is detected in the alternatively spliced BRAF product. Panel C, splice junction showing in frame deletion of exons 4-10.
**Supplementary Figure 8.** Comparison of gene expression levels in PV1, PV2 and vemurafenib-naive metastasis. The number of RNA-seq reads mapped per gene were normalized and log2 transformed. The smoothed coloured density scatter plots compare the expression of all genes between PV1 and PV2 (A), PV1 and Vem naïve, a metastasis from a vemurafenib-naive patient (B), and PV2 and Vem naïve (C). In panel A, genes from the MAPK pathway are plotted in red, genes from the RTK pathway in green, genes from the PI3K/AKT pathway in magenta, genes from the mTOR pathway in orange, and GPCR and other genes in pink. Pathway genes among the top 10% most differentially expressed genes and with a minimum read count of 100 are named. In panel B and C, pathway genes from panel A with high expression in PV1 versus PV2 and PV2 versus PV1 respectively are indicated. Genes whose over-expression has been demonstrated to drive resistance to vemurafenib (HGF, MAP3K8 and PDGFRB) are also indicated. ARAF, BRAF, RAF1, NRAS are highlighted in yellow. The two external dashed lines indicate the 90-percentile of the fold-change distribution between the two samples being compared.