S2 Table. Melanoma ctDNA sequencing retrospective pilot study results. Blood samples were taken from melanoma patients who were known to have cancer (some patients were sampled pre-treatment, some were post-treatment). Tumour and plasma DNA were sequenced using Ampliseq Cancer Hotspot Panel (8-10 samples per 318 chip), and variant results were compared. For patients 9-14, ctDNA was also sequenced at higher coverage (using 1 ctDNA:gDNA paired sample per 318 chip).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient no.** | **Sample** | **Sample Date** | **Time dif (days btn tumour and blood sampling)** | **Variant** | **VAF (%)** | **Treatment notes** | **Pre or post treatment blood sampling** |
| 1 | Tumour biopsy | 01/10/2009 | 1206 | BRAF V600R | 35 | Started Vemurafenib (BRAF inhibitor) on 09/05/2013, then Ipilimumab (immune system activator), which finished 17/10/2013. | pre- treatment |
|  | ctDNA | 19/01/2013 |  | BRAF V600R | 7 |  |  |
| 2 | Tumour biopsy | 19/04/2013 | 62 | c-KIT V560D | 80 | Nilotinib (c-KIT inhibitor) started 03/07/2013. | pre- treatment |
|   | ctDNA | 20/06/2013 |   | c-KIT V560D | 19 |   |   |
| 3 | Tumour biopsy | 09/03/2012 | 594 | TP53 R213\* | 61 | Started chemotherapy on 14/11/2013. | pre- treatment |
|  | ctDNA | 24/10/2013 |  | TP53 R213\* | 4 |  |  |
| 4 | Tumour biopsy | 20/02/2013 | 36 | c-KIT N655T  | 36 | Started chemotherapy on 19/04/2013. | pre- treatment |
|   | ctDNA | 28/03/2013 |   | c-KIT N655T | 1.1 |   |   |
| 5 | Tumour biopsy | 15/05/2012 | 542 | BRAF V600E | 14 | Stage 3B melanoma excised in 2012. Started Vemurafenib 19/11/2013. | pre- treatment |
|  | ctDNA | 07/11/2013 |  | BRAF V600E | 1 |  |  |
| 6 | Tumour biopsy | 30/09/2013 | 31 | NRAS Q61R | 79 | Has brain metastases, started RADVAN study (whole brain radiotherapy and Vandetanib (kinase inhibitor)/placebo) on 14/11/2013. | pre- treatment |
|   | ctDNA | 31/10/2013 |   | NRAS Q61R | 18 |   |   |
| 7 | Tumour biopsy | 25/08/2011 | 588 | BRAF V600E | 25 | Started Vemurafenib 2/5/2013. | pre- treatment |
|  | ctDNA (low coverage) | 04/04/2013 |  | negative | - |  |  |
|  | ctDNA (high coverage) | 04/04/2013 |  | BRAF V600E | 0.8 |  |  |
| 8 | Tumour biopsy | 26/02/2013 | 93 | BRAF V600E | 49 | Started Vemurafenib on 30/5/2013. | pre- treatment |
|  | ctDNA (low coverage) | 30/05/2013 |  | negative | - |  |  |
|   | ctDNA (high coverage) | 30/05/2013 |   | BRAF V600E | 0.7 |   |   |
| 9 | Tumour biopsy | not available | na | BRAF V600E | not avail. | ctDNA seq detects mutation seen in tumour and one additional. Started Vemurafenib on 22/8/2013. | pre- treatment |
|  | ctDNA | 22/08/2013 |  | BRAF V600E | 14 |  |  |
|  |  |  |  | CTNNB1 T41A | 6 |  |  |
| 10 | Tumour biopsy | 18/07/2011 | 633 | BRAF V600E | 36 | Surgical treatment followed by Vemurafenib, 21/5/2013 start. | pre- treatment |
|   | ctDNA | 11/04/2013 |   | negative | - |   |   |
| 11 | Tumour biopsy | 13/10/2010 | 981 | negative |   | Died Jul 2013. | pre- treatment |
|  | ctDNA | 20/06/2013 |  | KRAS G12R | 6 |  |  |
|  |  |  |  | TP53 R248Q | 3.4 |  |  |
| 12 | Tumour biopsy | 25/10/2011 | 604 | BRAF V600E | 45 | Treated with Dacarbazine, then Ipilimumab. Started Dabrafenib (BRAF inhibitor) in Sept 2012. | post- treatment |
|   | ctDNA | 20/06/2013 |   | BRAF V600E | 50 |   |   |
| 13 | Tumour biopsy | 26/04/2006 | 2559 | BRAF V600E | 27 | Treated with Vemurafenib Jan-Jun 2013, then Ipilimumab Jun-Aug 2013. Died Aug 2013 due to progressive disease. | post- treatment |
|  | ctDNA (low coverage) | 28/03/2013 |  | negative | - |  |  |
|   | ctDNA (high coverage) | 28/03/2013 |   | negative | - |   |   |
| 14 | Tumour biopsy | 23/03/2012 | 559 | BRAF V600E | 33 | Dabrafenib (BRAF inhib) and Trametinib (MEK inhib) started Jul 2012 (Combi-D study), stopped Apr 2013 due to toxicity. Died Nov 2013 due to progressive disease. | post- treatment |
|  |  |  |  | TP53 S241F | 53 |  |  |
|  | ctDNA (low coverage) | 03/10/2013 |  | negative | - |  |  |
|   | ctDNA (high coverage) |   |   | negative | - |   |   |
| 15 | Tumour biopsy | 22/08/2012 | 267 | BRAF V600E | 51 | Started Vemurafenib 14/3/2013.  | post- treatment |
|  | ctDNA (low coverage) | 16/05/2013 |  | negative | - |  |  |
|  | ctDNA (high coverage) |  |  | negative | - |  |  |
| 16 | Tumour biopsy | 06/07/2012 | 265 | NRAS Q22K | 44 | Started Dacarbazine chemotherapy 14/3/2013, stopped due to toxicity. Died May 2013 due to progressive disease. | post- treatment |
|  | ctDNA (low coverage) | 28/03/2013 |  | negative | - |  |  |
|   | ctDNA (high coverage) |   |   | negative | - |   |   |
| 17 | Tumour biopsy | 09/05/2012 | 253 | BRAF V600E | 49 | Started Vemurafenib June 2012. | post- treatment |
|  | ctDNA | 17/01/2013 |  | negative | - |  |  |
| 18 | Tumour biopsy | not available | na | BRAF V600E | not avail. | Treated with Vemurafenib, then IMCgp100 (immune-modulating agent), then Ipilimumab, completed Dec 2012. | post- treatment |
|   | ctDNA | 28/03/2013 |   | negative | - |   |   |
| 19 | Tumour biopsy | 14/01/2013 | 87 | TP53 Q60X | 63 | Melanoma removed surgically Nov 2012. | post- treatment |
|  | ctDNA | 11/04/2013 |  | negative | - |  |  |
| 20 | Tumour biopsy | 06/12/2012 | 126 | BRAF V600E | 30 | Vemurafenib started Feb 2013, stopped Jan 2015 (because of progressive disease). Died Mar 2015. | post- treatment |
|   | ctDNA | 11/04/2013 |   | negative | - |   |   |
| 21 | Tumour biopsy | 09/11/2011 | 624 | NRAS Q61H | 71 | Melanoma removed Jun 2011, Jan-Mar 2012 treated in DOC-MEK study (stopped due to progressive disease), treated with Ipilimumab Jun-Aug 2012. On follow-up. | post- treatment |
|  |  |  |  | PTEN L325F | 60 |  |  |
|   | ctDNA | 25/07/2013 |   | negative | - |   |   |