**Supplemental Table 1.** Patient characteristics of all six individuals with *MDM2/4* amplifications who received immunotherapy.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Case #** | **Cancer diagnosis** | **Age/Sex** | **Genomic alterations** | **PD-L1 status (IHC)** | **Immunotherapy** | **Time-to-treatment failure** | **Response by imaging** |
| 1 | Bladder cancer | 73/ Men | *MDM2* amplification  *AKT2* amplification  *BRIP1* truncation exon 19  *PIK3CA* H450\_V461 > GS  *RAF1* amplification ocal)  *MYC* amplification (equivocal)  *RNF43* S262\*  *ARID2* S889\*  *FRS2* amplification | Negative  (22C3 antibody) | Atezolizumab  (anti-PD-L1) | 1.9 months. | 258% increase from pre-immunotherapy imaging.  7.2-fold increase in progression pace compared to 2 months before therapy. |
| 2 | Breast cancer (triple negative) | 44/ Women | *MDM2* amplification  *PTCH1* T416S  *TP53* S127Y | Negative  (SP142 antibody) | Pembrolizumab  (anti-PD-1) | 1.5 months. | 55% increase from pre-immunotherapy imaging.  42.3-fold change in pace of progression compared to the 2 months before immunotherapy |
| 3 | Endometrial stromal sarcoma | 65/ Women | Profiling from initial surgical sample:  *CDKN2A* R58\* *p14ARF* P72L  *ZC3H7B-BCOR* Fusion  Profiling from liver mass biopsy 2 weeks after nivolumab:  *MDM2* amplification  *CDKN2A* R58\* *p14ARF* P72L  *FRS2* amplification  Profiling from new abdominal mass biopsy 2 months after nivolumab:  *MDM2* amplification  *CDKN2A* R58\* *p14ARF* P72L  *ZC3H7B-BCOR* fusion  *FRS2* amplification | Negative  (SP142 antibody) | Nivolumab  (anti-PD-1) | 1.5 months. | 242% increase from pre-immunotherapy imaging.  ~2.3 fold increase in rate of progression compared to the 2 months before immunotherapy |
| 4 | Adenocarcinoma of lung | 50/ Women | *MDM2* amplification  *KIF5B-RET* fusion | Not tested. | Pembrolizumab  (anti-PD-1) | 0.3 months. | 135% increase from pre-immunotherapy imaging.  7.1-fold increase in progression pace compared to 2 months before therapy |
| 5 | Adenocarcinoma of lung | 61/ Men | *MDM2* amplification  *CDK4* amplification | Not tested. | Pembrolizumab  (anti-PD-1) | 1.2 months. | Target lesions were stable. However, new brain metastases were found. |
| 6 | Squamous cell carcinoma of hypopharynx | 62/ Men | *MDM4* amplification  *EGFR* amplification  *FGFR1* amplification  *KRAS* amplification  *PIK3CA* amplification  *CDKN2A/B* loss  *IKBKE* amplification (equivocal)  *MYC* amplification  *NTRK1* M375I  *SOX2* amplification  *TP53* D259fs\*2, Y220C  *BCL2L2* amplification  *CUL3* splice site 379-1G>T  *NFKBIA* amplification  *NKX2-1* amplification  *PIK3C2B* amplification  *ZNF703* amplification | Not tested. | Investigational immunotherapeutic agent (OX40 agonist) | 1.4 months. | Stable disease on scan.  However had rapid clinical progression. |

**Abbreviations:** IHC = immunohistochemistry

**Supplemental Table 2.** Patient characteristics among patients who received anti-PD-1/PD-L1 (N = 102) **1**.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **All**  **(N=102)** | **TTF  <2 months (N=39)** | **TTF  ≥2 months (N=63)** | **Odds- ratio \***  **(95% CI)**  **(Univariate)** | **p-value \* (Univariate)** | **Odds- ratio #**  **(95% CI)**  **(Multivariate)** | **p-value # (Multivariate)** | **p-value § (Bootstrap)** |
| Age ≤ 65 years | 64 (62.7%) | 24 (61.5%) | 40 (63.5%) | 0.92 (0.42-2.10) | >0.9999 |  |  |  |
| Age > 65 years | 38 (37.3%) | 15 (38.5%) | 23 (36.5%) |  |  |  |  |  |
| **Cancer diagnosis** | | | | | | | | |
| Non-small cell lung cancer | 38 (37.3%) | 18 (46.2%) | 20 (31.7%) | 1.84 (0.83-4.13) | 0.21 |  |  |  |
| Squamous cell carcinoma of head and Neck | 9 (8.8%) | 2 (5.1%) | 7 (11.1%) | 0.43 (0.09-2.20) | 0.48 |  |  |  |
| **Cutaneous squamous cell carcinoma** | **9 (8.8%)** | **0 (0)** | **9 (14.3%)** | **<0.18 (0.02-1.31)** ¶ | **0.012** | 0.74 (0-6.31) | 0.83 | **0.001** |
| Melanoma | 6 (5.9%) | 1 (2.6%) | 5 (7.9%) | 0.31 (0.03-2.40) | 0.40 |  |  |  |
| Renal cell carcinoma | 5 (4.9%) | 2 (5.1%) | 3 (4.8%) | 1.08 (0.19-5.49) | >0.9999 |  |  |  |
| **Genomic alterations** | | | | | | | | |
| *TP53* | 50 (49.0%) | 20 (51.3%) | 30 (47.6%) | 1.16 (0.50-2.47) | 0.84 |  |  |  |
| *CDKN2A/B* | 25 (24.5%) | 9 (23.1%) | 16 (25.4%) | 0.88 (0.37-2.10) | >0.9999 |  |  |  |
| *TERT* | 17 (16.7%) | 5 (12.8%) | 12 (19.0%) | 0.63 (0.23-1.80) | 0.59 |  |  |  |
| *LRP1B* | 13 (12.7%) | 2 (5.1%) | 11 (17.5%) | 0.26 (0.06-1.20) | 0.12 |  |  |  |
| *KRAS* | 10 (9.8%) | 2 (5.1%) | 8 (12.7%) | 0.37 (0.08-1.70) | 0.31 |  |  |  |
| ***NOTCH1*** | **10 (9.8%)** | **0 (0)** | **10 (15.9%)** | **<0.16 (0.01-1.07)** ¶ | **0.012** | 0.47 (0-3.16) | 0.54 | **0.001** |
| *PIK3CA* | 10 (9.8%) | 4 (10.3%) | 6 (9.5%) | 1.09 (0.33-3.90) | >0.9999 |  |  |  |
| *MLL2* | 9 (8.8%) | 1 (2.6%) | 8 (12.7%) | 0.18 (0.02-1.30) | 0.15 |  |  |  |
| ***EGFR*** | **8 (7.8%)** | **7 (17.9%)** | **1 (1.6%)** | **13.6 (2.20-154.8)** | **0.005** | **11.8 (1.40-560.5)** | **0.02** | **0.014** |
| *MYC* | 8 (7.8%) | 4 (10.3%) | 4 (6.3%) | 1.69 (0.46-6.06) | 0.48 |  |  |  |
| *PTEN* | 8 (7.8%) | 2 (5.1%) | 6 (9.5%) | 0.51 (0.10-2.20) | 0.71 |  |  |  |
| *BRAF* | 7 (6.9%) | 4 (10.3%) | 3 (4.8%) | 2.29 (0.58-9.41) | 0.42 |  |  |  |
| *PTCH1* | 7 (6.9%) | 1 (2.6%) | 6 (9.5%) | 0.25 (0.02-1.67) | 0.25 |  |  |  |
| **Variables** | **All**  **(N=102)** | **TTF  <2 months (N=39)** | **TTF  ≥2 months (N=63)** | **Odds- ratio \***  **(95% CI)**  **(Univariate)** | **p-value \* (Univariate)** | **Odds- ratio #**  **(95% CI)**  **(Multivariate)** | **p-value # (Multivariate)** | **p-value § (Bootstrap)** |
| ***ASXL1*** | **6 (5.9%)** | **0 (0)** | **6 (9.5%)** | **<0.3 (0.03-2.43)** ¶ | **0.08** | 0.47 (0-3.16) | 0.54 | 0.34 |
| *ATM* | 6 (5.9%) | 2 (5.1%) | 4 (6.3%) | 0.8 (0.15-3.57) | >0.9999 |  |  |  |
| *CTNNB1* | 6 (5.9%) | 2 (5.1%) | 4 (6.3%) | 0.8 (0.15-3.57) | >0.9999 |  |  |  |
| *GNAS* | 6 (5.9%) | 1 (2.6%) | 5 (7.9%) | 0.31 (0.03-2.43) | 0.4 |  |  |  |
| *NF1* | 6 (5.9%) | 1 (2.6%) | 5 (7.9%) | 0.31 (0.03-2.43) | 0.4 |  |  |  |
| *NOTCH2* | 6 (5.9%) | 2 (5.1%) | 4 (6.3%) | 0.8 (0.15-3.57) | >0.9999 |  |  |  |
| *RB1* | 6 (5.9%) | 3 (7.7%) | 3 (4.8%) | 1.67 (0.37-7.40) | 0.67 |  |  |  |
| *RET* | 6 (5.9%) | 3 (7.7%) | 3 (4.8%) | 1.67 (0.37-7.40) | 0.67 |  |  |  |
| *SETD2* | 6 (5.9%) | 2 (5.1%) | 4 (6.3%) | 0.8 (0.15-3.57) | >0.9999 |  |  |  |
| *SMAD4* | 6 (5.9%) | 2 (5.1%) | 4 (6.3%) | 0.8 (0.15-3.57) | >0.9999 |  |  |  |
| *APC* | 5 (4.9%) | 2 (5.1%) | 3 (4.8%) | 1.08 (0.19-5.49) | >0.9999 |  |  |  |
| *ARID1A* | 5 (4.9%) | 1 (2.6%) | 4 (6.3%) | 0.39 (0.03-2.52) | 0.65 |  |  |  |
| *ARID2* | 5 (4.9%) | 1 (2.6%) | 4 (6.3%) | 0.39 (0.03-2.52) | 0.65 |  |  |  |
| *BRCA2* | 5 (4.9%) | 2 (5.1%) | 3 (4.8%) | 1.08 (0.19-5.49) | >0.9999 |  |  |  |
| *CREBBP* | 5 (4.9%) | 0 (0) | 5 (7.9%) | <0.39 (0.03-2.52) ¶ | 0.15 |  |  |  |
| *FGFR1* | 5 (4.9%) | 2 (5.1%) | 3 (4.8%) | 1.08 (0.19-5.49) | >0.9999 |  |  |  |
| *MCL1* | 5 (4.9%) | 1 (2.6%) | 4 (6.3%) | 0.39 (0.03-2.52) | 0.65 |  |  |  |
| ***MDM2*** | **5 (4.9%)** | **5 (12.8%)** | **0 (0)** | **>7.09 (1.08-87.8)** ¶ | **0.007** | **11.1 (1.95-infinity)** | **0.02** | **0.001** |
| *STK11* | 5 (4.9%) | 1 (2.6%) | 4 (6.3%) | 0.39 (0.03-2.52) | 0.65 |  |  |  |
| *ZNF217* | 5 (4.9%) | 0 (0) | 5 (7.9%) | <0.39 (0.03-2.52) ¶ | 0.15 |  |  |  |
| *DNMT3A* | 4 (3.9%) | 3 (7.7%) | 1 (1.6%) | 5.17 (0.74-68.0) | 0.15 |  |  |  |

\* Odds-ratio and p-value by Fisher's exact test.

# Odds-ratio and p-value by exact conditional logistic regression (multivariate) analysis. Included characteristics with p-value ≤ 0.1 from univariate analysis.

**§** Bootstrapping with multiple logistic regression analysis was conducted on characteristics with p-value ≤ 0.1 from univariate analysis. p-value based on 989 bootstrap samples.

¶ If a variable dichotomized as N versus zero, and the odds ratio is thus zero or infinity, we adjusted the events to be 1 (instead of zero) versus N-1. This produces a numerical odds ratio, which is less than the actual infinite odds ratio. For example, for *MDM2*, where there were N = 5 versus zero patients with TTF<2 versus ≥2 months, the actual odds ration is infinity. Using the adjustment above, the numeric odds ratio for N=4 versus one patient is 7.09 and we list it as “>7.09.” The p-value shown is the actual p-value for the unadjusted numbers.

**1** Included variables with N ≥ 5, except for DNMT3A (N=4).

**Abbreviations:** CI = confidence interval;TTF = time-to-treatment failure.

**Supplemental Table 3**. Patients with *EGFR* alterations and TTF less than 2 months on immunotherapy (N=8 of a total of 10 patients with *EGFR* alterations).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Case #** | **Cancer diagnosis** | **Age/Sex** | **Genomic alterations** | **PD-L1 status (IHC)** | **Immunotherapy** | **Time-to-treatment failure** | **Response by imaging** |
| 7 | Squamous cell carcinoma of unknown primary | 56/Men | *EGFR* amplification  *BRCA2* W1692fs\*3  *BRIP1* T733fs\*4  *MLH1* loss exons 12-19  *PTEN* R173C, S229\*, splice site 1027-  2A>G  *ARID1A* Q1334\_R1335insQ  *CDKN2A/B* loss  *MEN1* R521fs\*15  *MSH6* F1088fs\*5  *PIK3CB* E1051K  *BAP1* Q441\*  *CHD2* V175fs\*1  *CIC* P786fs\*138  *CTCF* E363fs\*5  *CTNNA1* A784T  *EP300* Q128\*  *HNF1A* G292fs\*25  *JAK1* K860fs\*16, P430fs\*2  *MLL2* P647fs\*283, R2105H  *MLL3* K2797fs\*26  *TP53* R282W | Negative  (SP142 antibody) | Pembrolizumab  (anti-PD-1) | 0.7 months. | 3% decrease from pre-immunotherapy imaging. |
| 8 | Glioblastoma | 62/Men | *EGFR* amplification, *EGFR* vIVa  *CDKN2A* loss *p16INK4a* and *p14ARF* exons 2-3  *PIK3R1* N453del  *QKI* E135fs\*5  *SETD2* splice site 5016-2\_5018delAGAAA  *TERT* promoter -124C>T | Negative  (22C3 antibody) | Nivolumab  (anti-PD-1) | 0.8 months. | 2.4 % increase from baseline imaging. |
| 9 | Squamous cell carcinoma of lung | 64/Men | *EGFR* amplification  *PIK3CA* amplification  *CCND1* amplification  *MCL1* amplification  *TP53* splice site 560-7\_561del9  *FGF19* amplification  *FGF3* amplification  *FGF4* amplification  *SOX2* amplification | Not tested. | Nivolumab  (anti-PD-1) | 0.8 months. | 30% increase from pre-immunotherapy imaging. |
| 10 | Squamous cell cancer of hypopharynx | 62/Men | *EGFR* amplification  *FGFR1* amplification  *KRAS* amplification  *PIK3CA* amplification  *CDKN2A/B* loss  *IKBKE* amplification (equivocal)  *MDM4* amplification  *MYC* amplification  *NTRK1* M375I  *SOX2* amplification  *TP53* D259fs\*2, Y220C  *BCL2L2* amplification  *CUL3* splice site 379-1G>T  *NFKBIA* amplification  *NKX2-1* amplification  *PIK3C2B* amplification  *ZNF703* amplification | Not tested. | Investigational immunotherapeutic agent (OX40 agonist) | 1.4 months. | Stable disease (0%) from pre-immunotherapy imaging. |
| 11 | Adenocarcinoma of lung | 54/Men | *EGFR* E746\_A750del, T790M  *AKT1* amplification  *CDKN2A/B* loss  *TP53* R248W  *NFKBIA* amplification  *NKX2-1* amplification | Not tested. | Nivolumab  (anti-PD-1) | 1.1 months. | 125% increase from pre-immunotherapy imaging.  (3% increase from baseline to pre-immunotherpay). 41.7 fold increase in progression pace. |
| 12 | Adenocarcinoma of lung | 44/Men | *EGFR* E746\_A750del  *MET* amplification  *CDK6* amplification  *MYC* amplification  *RICTOR* amplification  *TP53* R248W  *FGF10* amplification | Not tested. | Clinical trial with anti-PD-1 antibody | 1.6 months. | 18% increase from pre-immunotherapy imaging. |
| 13 | Adenocarcinoma of lung | 73/Man | *EGFR* amplification, L858R, T790M  *MITF* amplification  *TP53* C238\_N239>\*VGSDCTTIHYNYMC  *FOXP1* amplification  *NFKBIA* amplification  *NKX2-1* amplification  *NOTCH2* P6fs\*27 | Negative  (22C3 antibody) | Nivolumab  (anti-PD-1) | 1.7 months. | 53.6% increase from pre-immunotherapy imaging.  (Only 1.5% increase from 2 month pre-therapy baseline scan to pre-immunotherapy scan).  35.7-fold increase in the pace of progression. |
| 14 | Renal cell carcinoma | 44/Man | *EGFR* I1060fs\*18  *VHL* R120G  *PIK3CA* E545A  *ERRFI1* loss  *CDKN2A/B* loss  *BAP1* M231fs\*11  *TNFRSF14* loss | Not tested. | Nivolumab  (anti-PD-1) | 0.6 months. | Clinical progression with respiratory failure from known lung metastases. No imaging available to evaluate the response. |

**Abbreviations:** IHC = immunohistochemistry

**Supplemental Table 4**. Patients with *DNM3TA* alterations and TTF less than 2 months on immunotherapy (N=4 of a total of 5 patients with *DNM3TA* alterations).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Case #** | **Cancer diagnosis** | **Age/Sex** | **Genomic alterations** | **PD-L1 status (IHC)** | **Immunotherapy** | **Time-to-treatment failure** | **Response by imaging** |
| 15 | Cutaneous melanoma | 70/Man | *DNMT3A* P718L  *KDR* R1032Q  *NRAS* Q61H  *PDGFRA* E459K  *CDKN2A* R80\*  *MCL1* amplification  *TP53* S241F  *ARID1A* Y1377\*  *EPHA3* G766E  *FGFR2* W156\*  *RAD50* Q689\* | Not tested. | Ipilimumab (anti-CTLA-4) | 1.4 months. | 182% increase from 2 months prior to immunotherapy. No imaging just prior to the initiation of immunotherapy was available. |
| 16 | Cutaneous melanoma | 59/Man | *DNMT3A* R882H  *BRAF* V600K  *MYD88* L265P  *TERT* promoter -124C>T | Not tested. | Pembrolizumab  (anti-PD-1) | 7 days. | Clinical progression (Overall stable brain metastases per imaging). |
| 17 | Adenocarcinoma of lung | 57/Woman | *BRAF* V600E  *DNMT3A* R882H  *TP53* D281Y, Q331\* | Positive (Percent staining: 80%)  (22C3 antibody) | Nivolumab  (anti-PD-1) | 4 days. | No serial imaging available. Four days after nivolumab, patient was admitted for hydropneumothorax, and subsequently died 9 days after the therapy. |
| 18 | Adenocarcinoma of lung | 72/ Woman | *RET* CCDC6-RET fusion  *TP53* H193R  *DNMT3A* splice site 2478+1G>A  *LRP1B* D2600Y  *NFE2L2* R18Q | Not tested. | Nivolumab  (anti-PD-1) | 7 days. | No serial imaging available. Seven days after the initiation of nivolumab, patient was admitted for respiratory and kidney failure. Subsequently deceased 17 days after the therapy. |

**Supplemental Figure legend.**

**Supplemental Figure 1. Evaluation of tumor biopsy at the time of progression (Case #4).**

Two months after the initiation of nivolumab, tumor biopsy was obtained from the rapidly emerging abdominal mass which revealed high-grade endometrial stromal sarcoma (red arrow) with scattered apoptotic bodies (black arrow). However there was no finding suggestive of pseudoprogression including lymphocyte infiltration or tumor necrosis (Hematoxylin and eosin, 200x magnification).

**Supplemental Figure 2**.

**Supplemental Figure 2.A.**

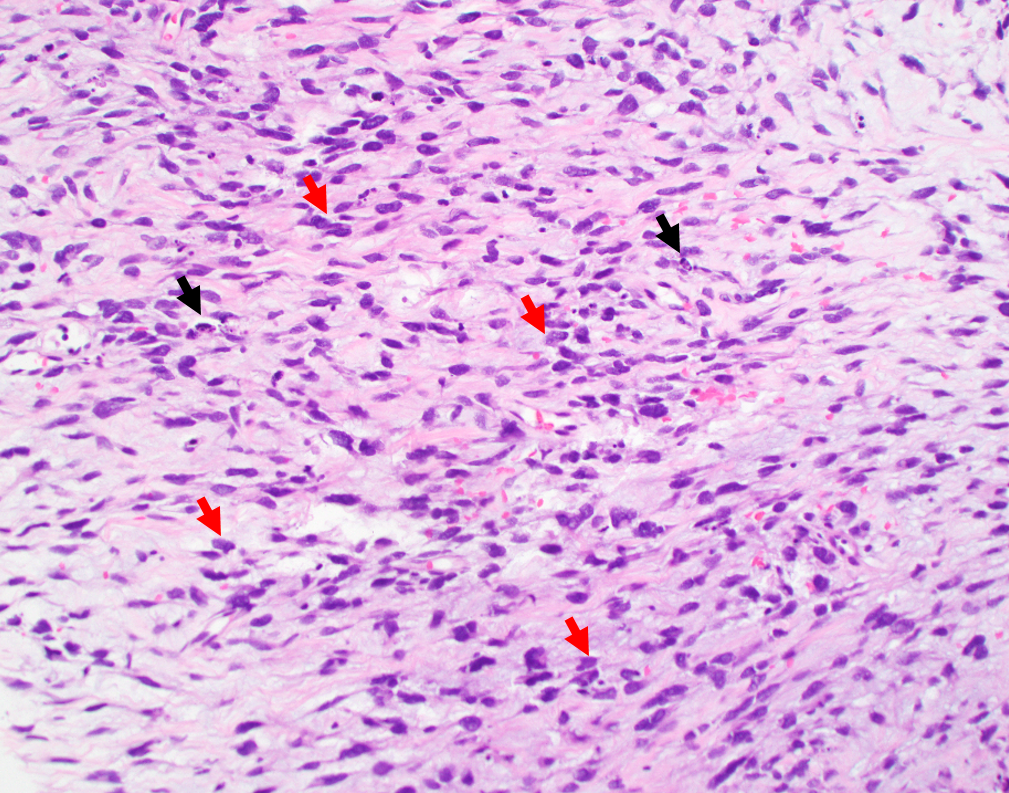
**Case #11**:

54 year old man with metastatic adenocarcinoma of lung. Genomic profiling revealed aberrations including *EGFR* E746\_A750del and T790M. After progressing on standard chemotherapy and anti-EGFR therapies (3% increase in last two months before immunotherapy), patient was started on nivolumab. However, 1.1 months after starting on nivolumab, patient was found to have worsening shortness of breath. Imaging revealed progression of thoracic lymphadenopathy as well as new liver metastases (125% increase; 41.7-fold increase in progression pace.) and taken off from therapy.

**Supplemental Figure 2.B.**

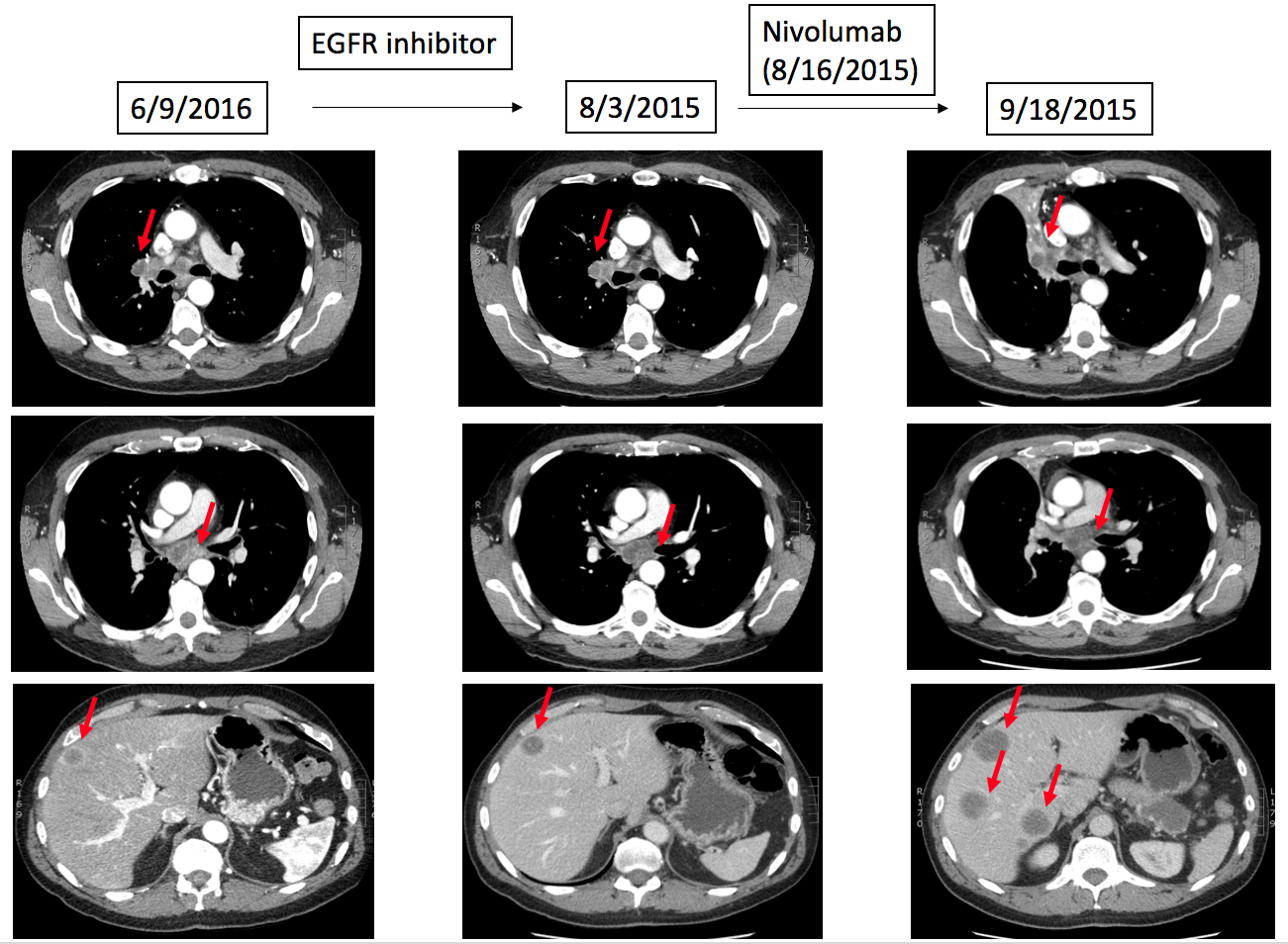
**Case #13**:

73 year old man with adenocarcinoma of lung. Molecular profiling showed *EGFR* amplification, L858R and T790M. After progressing on chemotherapy and anti-EGFR therapies, patient was started on nivolumab (1.5% increase in tumor size in the two months before immunotherapy). Restaging scan 1.7 months after the initiation of nivolumab showed disease stability of lung mass but progression of target liver mass as well as new liver metastases (53.6% increase; 35.7-fold increase in the pace of progression.). Nivolumab was therefore stopped.

**Supplemental Figure 1. Evaluation of tumor biopsy at the time of progression (Case #4).** 

**Supplemental Figure 2.**

**Supplemental Figure 2.A. Case #11**



**Supplemental Figure 2.B. Case #13**

