Annotation of Identified Variants

File Name: test_annotate_variants.tsv

Clinical Variant 1

Gene Name: EGFR

Protein Change: L858R

Coordinates: chr7:g.55259515T>G

Variant Annotation

EGFR L858R has long been recognized as a functionally significant mutation in cancer, and is one of the most prevalent single mutations in lung cancer. Best described in non-small cell lung cancer (NSCLC), the mutation seems to confer sensitivity to first and second generation TKI's like gefitinib and neratinib. NSCLC patients with this mutation treated with TKI's show increased overall and progression-free survival, as compared to chemotherapy alone. Third generation TKI's are currently in clinical trials that specifically focus on mutant forms of EGFR, a few of which have shown efficacy in treating patients that failed to respond to earlier generation TKI therapies.

Evidence Statements

Evidence statement 1

There is no statistical difference in progression free survival between lung cancer patients treated with gefitinib or erlotinib with EGFR L858R mutations (N=72/242; univariate: P=0.283; multivariate: P=0.250) compared to patients with Exon 19 mutations (N=170).

Evidence statement 2

Gefinitib has been shown to be effective in treating cell lines with L858R missense mutations.

Evidence statement 3

A phase III clinical trial (NCT00949650) found that median progression free survival among patients with exon 19 deletions or L858R EGFR mutations (n = 308) was 13.6 months for afatinib and 6.9 months for chemotherapy (HR, 0.47; 95% CI, 0.34 to 0.65; P = 0.001).

Evidence statement 4

In a phase 2 study of patients with lung adenocarcinoma (stage IIIb with pleural effusion or stage IV) and EGFR mutations, treated with afatinib were assessed by objective response. 129 patients were treated with afatinib. 66% of the 106 patients with two common activating EGFR mutations (deletion 19 or L858R) had an objective response compared to 39% of 23 patients with less common mutations.

Evidence statement 5

A randomized phase 3 trial (NCT00446225) involving 173 NSCLC patients with EGFR mutations (exon 19 deletion or L858R mutation in exon 21) with no history of chemotherapy for metastatic disease. Patients were randomly allocated (1:1) to receive either erlotinib or standard chemotherapy. The primary endpoint was progression-free survival (PFS). Median PFS was 9.7 months in the erlotinib group, compared with 5.2 months in the standard chemotherapy group.

Evidence statement 6

Cells harboring L858R were sensitive to afatinib. This study performed drug response assays using five human NSCLC cell lines with various combinations of EGFR mutations. In order to directly compare the sensitivity of multiple EGFR mutations to EGFR-TKIs the authors also generated multiple EGFR transduced Ba/F3 stable cell lines and evaluated sensitivity to EGFR-TKIs by MTS

assay.

Evidence statement 7

Afatinib is an irreversible covalent inhibitor of EGFR (second generation). This Phase III clinical trial (LUX-Lung 6; NCT01121393) was performed in Asian patients with EGFR mutant advanced NSCLC. 364 eligible patients with EGFR mutations were assigned to afatinib (n=242) or gemcitabine and cisplatin (n=122) treatment. The trial observed significantly longer median progression-free survival with afatinib vs. gemcitabine and cisplatin treatment (11.0 vs. 5.6 months). Afatinib/Chemotherapy group compositions: 51.2/50.8 % del 19; 38/37.7 % Leu858Arg; 10.8/11.5 % Uncommon.

Evidence statement 8

In NSCLC patients treated with EGFR tyrosine kinase inhibitors, the presence of L858R mutation is prognostic for better progression free survival.

Evidence statement 9

90 NSCLC patients with stage IIIB/IV chemotherapy-resistant tumors were treated with gefitinib, and the L858R EGFR mutation was associated with longer time to treatment failure than those with wild-type EGFR(median 9.1 versus 2.1). In multivariate analysis, L858R mutations plus adenocarcinoma was a significant predictive factor for TTF (HR = 0.1030; P = .0004).

Evidence statement 10

In a phase 3 clinical trial of non-small cell lung cancer (NSCLC) patients, a subset of patients with EGFR mutations (n=44) treated with gefitinib were associated with improved progression free survival (HR: 0.16, 95% CI: 0.05-0.49, P=0.001), and a higher objective response rate (ORR: 42.1% vs 21.1%, p=0.04) compared to patients treated with docetaxel. Of the 44 patients, 22 had an exon 19 deletion, 16 had an L858R mutation, one patient had an exon 20 T790M mutation, two had an exon G719A mutation and four had other mutations.

Evidence statement 11

In a phase 3 clinical trial of Japanese NSCLC patients with EGFR mutations (n=230), patients treated with gefitinib were associated with improved progression free survival (10.8 months vs 5.4 months, HR: 0.30, 95% CI: 0.22-0.41, P<0.001), compared to patients treated with carboplatin and paclitaxel combination therapy. The frequency of mutation of exon 19 deletion and L858R was 25.2% (58/230) and 21.3% (49/230), respectively.

Evidence statement 12

Mutational profiling was performed on EGFR exons 18-24 from a group of 10 retrospectively chosen patients who had shown partial response or clinical improvement in response to gefitinib. Of 7 tumors with EGFR mutations, 1 was L858R. The patient was a female former smoker who had lung adenocarcinoma histology, and demonstrated response for 5 months, with overall survival 8 months.

Evidence statement 13

In an in vitro study using PC9 cells (EGFR exon 19 deletion), inhibition of cell viability was used as an assay to determine sensitivity to EGFR tyrosine kinase inhibitors. PC9 cells demonstrated an increased sensitivity to gefitinib (IC50: 23.0 nmol/L vs 200.0 nmol/L) compared to NCI-H2073 cells (EGFR wild type).

Evidence statement 14

In an in vitro study using NCI-H1666 cells (wildtype EGFR) and NCI-H3255 cells (EGFR-L858R), inhibition of cell growth was used as an assay to determine sensitivity to reversible tyrosine kinase inhibitor drugs. Cells with an EGFR L858R mutation demonstrated an improved response to lapatinib (IC50: 63nM vs. 534nM) compared to wildtype EGFR cells.

Evidence statement 15

In an in vitro study using NCI-H3255 cells (EGFR L858R), inhibition of cell growth was used as an assay to determine sensitivity to tyrosine kinase inhibitor (TKI) drugs. Cells with an EGFR L858R mutation demonstrated sensitivity to neratinib (IC50: 0.0049 μmol/L) compared to EGFRwt A549 cells (IC50 > 1 μmol/L).

Evidence statement 16

In an in vitro study using NCI-H1666 cells (wildtype EGFR) and NCI-H3255 cells (EGFR-L858R), inhibition of cell growth was used as an assay to determine sensitivity to irreversible tyrosine kinase inhibitor (TKI) drugs. Cells with an EGFR L858R mutation demonstrated an improved response to afatinib (IC50: 0.7nM vs. 60nM) compared to wildtype EGFR cells.

Evidence statement 17

In a phase 2b/3 study of 585 stage IIIB-IV lung adenocarcinoma patients who had a previous round of EGFR-TKI treatment, archival material was available for 141 patients. EGFR mutational analysis revealed that 96 tumors had an EGFR mutation, of which 76 (79%) were positive for either L858R or a deletion in exon 19. These mutations were associated with improved progression-free survival (median 3.3 months vs 1.0 month ; HR:0.51, 95% CI:0.31-0.85, P<0.009) compared to placebo control.

Evidence statement 18

In an in vitro study using NCI-H1666 cells (wildtype EGFR) and NCI-H3255 cells (EGFR-L858R), inhibition of cell growth was used as an assay to determine sensitivity to irreversible tyrosine kinase inhibitor (TKI) drugs. Cells with an EGFR L858R mutation demonstrated an improved response to canertinib (IC50: 1nM vs. 198nM) compared to wildtype EGFR cells.

Evidence statement 19

EGFR L858R mutation has been associated with increased sensitivity to first generation EGFR tyrosine kinase inhibitors, including erlotinib and gefitinib.In an in vitro study using PC9 cells (EGFR L858R mutation), inhibition of EGFR phosphorylation was used as an assay to determine sensitivity to EGFR tyrosine kinase inhibitors. PC9 cells demonstrated an increased sensitivity to gefitinib (IC50: 11.0-12.0 nmol/L vs 61.0 nmol/L) compared to NCI-H2073 cells (EGFR wild type).

Evidence statement 20

In a phase 3 clinical trial of Korean, never-smoker, lung adenocarcinoma patients, a subset of patients with EGFR mutations (n=42) treated with gefitinib were associated with improved overall response rate compared to gemcitabine plus cisplatin treatment (84.6%, 22/26, vs. 37.5%, 6/16, P=0.002). By contrast, in EGFR mutation negative cases, the response rate was much lower (25.9%, 7/27). The frequency of mutation in exon 19 deletion and L858R was 64.3% (27/42) and 36.4% (16/42), respectively. Exon 19 and L858R mutations were mutually exclusive in this cohort.

Evidence statement 21

MCF-7 cells were transduced with YFP tagged EGFR with E746_A750delELREA mutation, or YFP EGFR wildtype, and stained for ectopic YFP EGFR and phospho-Akt as a readout for EGFR pathway activity. Increased p-Akt stain was seen with ectopic mutant EGFR over wildtype cells. Parallel erlotinib incubations of cells with wildtype EGFR induced recompartmentalization of ectopic EGFR protien only at high concentration (10uM). Addition of erlotinib to mutant EGFR cell incubations reduced p-Akt signal and induced recompartmentalization of ectopic EGFR protien at considerably lower conecntration (100 nM) erlotinib. These results indicate the EGFR L858R variant as a growth pathway driver targetable by erlotinib.

Evidence statement 22

In an in vitro study using NCI-H1666 cells (wildtype EGFR) and NCI-H3255 cells (EGFR-L858R), inhibition of cell growth was used as an assay to determine sensitivity to reversible tyrosine kinase inhibitor drugs. Cells with an EGFR L858R mutation demonstrated an improved response to

erlotinib (IC50: 40nM vs. 110nM) compared to EGFR wild type cells.

Evidence statement 23

In an in vitro study, a MCF-7 cell line expressing EGFR L858R mutation demonstrated sensitivity to erlotinib treatment, compared to MCF-7 cells expressing EGFR wild-type. Sensitivity was determined by assessing YFP signal-EGFR relocation.

Evidence statement 24

In an in vitro study, Ba/F3 and NCI-H3255 cell lines expressing EGFR L858R demonstrated increased sensitivity to erlotinib treatment (IC50=0.006■M). Sensitivity was determined by assessing cell proliferation, EGFR, AKT and ERK phosphorylation, levels of BIM (a marker of EGFR TKI-induced cell killing) and cell viability.

Evidence statement 25

In an in vitro study, CHO and NCI-H3255 cells expressing EGFR L858R mutation were associated with sensitivity to erlotinib treatment. Sensitivity was determined by assessing cell density.

Evidence statement 26

In a non-small cell lung cancer patient with an EGFR L858R mutation, EGFR L858R was reported to be refractory to 61-day crizotinib treatment.

Evidence statement 27

In a prospective study of 46 Caucasian, advanced lung adenocarcinoma patients harboring EGFR mutations, first-line erlotinib treatment was assessed. Average PFS and OS for these 46 patients was 11 months (95% CI: 9.7-12.3 months) and 23 months (95% CI: 21.3-28.6+ months), respectively. A PFS rate of 81% at three months met the primary endpoint of presumed superiority over chemotherapy. Clinical benefit (CR+PR+SD) rate was 81%. Fifteen patients harbored EGFR L858R mutations, which was the only mutation found in exon 21. The authors note similar response profiles for exon 19 (27/46) and exon 21 mutations (15/46) to the overall population.

Evidence statement 28

In an in vitro study, Ba/F3 cell line expressing EGFR L858R was associated with sensitivity to erlotinib treatment (IC50 26.9 ± 12.4). Sensitivity was determined by assessing cell viability, AKT and ERK phosphorylation, and EGFR auto-phosphorylation.

Evidence statement 29

In an in vitro study, Ba/F3 cell line expressing EGFR L858R did not show sensitivity to cetuximab treatment, while cells expressing EGFR-RAD51 fusion were sensitive. Sensitivity was determined by assessing cell viability, AKT and ERK phosphorylation and EGFR auto-phosphorylation.

Evidence statement 30

In an in vitro study using NCI-H3255 cells (EGFR L858R mutation), inhibition of EGFR phosphorylation was used as an assay to determine sensitivity to EGFR tyrosine kinase inhibitors. NCI-H3255 cells demonstrated an sensitivity to erlotinib (IC50: 8.0-11.0 nmol/L vs 108.0 nmol/L) compared to NCI-H2073 cells (EGFR wild type).

Evidence statement 31

EGFR L858R mutation has been associated with increased sensitivity to first generation EGFR tyrosine kinase inhibitors, including erlotinib and gefitinib. In an in vitro study using NCI-H3255 cells (EGFR L858R mutation), inhibition of EGFR phosphorylation was used as an assay to determine sensitivity to EGFR tyrosine kinase inhibitors. NCI-H3255 cells demonstrated increased sensitivity to erlotinib (IC50: 8.0-11.0 nmol/L vs 108.0 nmol/L) compared to NCI-H2073 cells (EGFR wild type).

Evidence statement 32

In an in vitro study, a Ba/F3 cell line expressing EGFR L858R demonstrated increased sensitivity to erlotinib treatment, compared to Ba/F3 cells expressing EGFR wild-type. Variant function was assessed by EGFR auto-phosphorylation. Sensitivity was assessed by cell viability assay using stable transfection of each variant and increasing concentrations of erlotinib (0–10 uM).

Evidence statement 33

On May 14, 2013, the U.S. Food and Drug Administration approved erlotinib (Tarceva) for the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations.

Evidence statement 34

Afatinib, an irreversible inhibitor of the ErbB family of tyrosine kinases has been approved in the US for the first-line treatment of patients with metastatic non-small-cell lung cancer (NSCLC) who have tumours with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations as detected by a US FDA-approved test

Evidence statement 35

The authors pooled patients with exon 19 deletion and L858R EGFR (Exon 21) mutations from both studies (The ARCHER 1009 (NCT01360554) and A7471028 (NCT00769067)) to compare the efficacy of dacomitinib to erlotinib. 121 patients with any EGFR mutation were enrolled, 101 had activating mutations in exon 19 or 21. For those (exon19/21), the median PFS was 14.6 months with dacomitinib and 9.6 months with erlotinib The median survival was 26.6 months with dacomitinib versus 23.2 months with erlotinib.

Evidence statement 36

In a phase II trial for bronchioloalveolar carcinoma (BAC, or in situ pulmonary adenocarcinoma), EGFR exons 18-24 were analyzed in 7 patients who had shown a partial response to erlotinib. Two patients had the del L858R mutation. One patient was a male former smoker who demonstrated response for 3 months with overall survival of 3.5 months, and the other patient was a female never smoker who had response for 6 months and overall survival of 17+ months as the patient was alive at study end.

Evidence statement 37

EGFR L858R was expressed in Ba/F3 cells which do not contain endogenous EGFR, and conferred growth factor independance to the cells. Cells were plated and treated with 1st generation EGFR inhibitors Gefitinib, Erlotinib, or the the tool compound AEE788, and IC50 values were measured. L858R EGFR cells had very low IC50 nmol/L values for all three inhibitors in comparison to other constructs (Gefitinib=12, Erlotinib=6, AEE788=6), indicating sensitivity to all three constructs.

Evidence statement 38

In an in vitro study performed after identification of the mutation in a malignant peritoneal mesothelioma patient, COS-7 cells expressing EGFR L858R demonstrated increased EGFR phosphotyrosine after EGF treatment over wildtype EGFR expressing cells. Cells expressing mutant EGFR also showed increased sensitivity to erlotinib treatment compared to cells expressing EGFR wild-type. Sensitivity was determined by assessing EGFR auto-phosphorylation.

Evidence statement 39

In a Japanese Phase III clinical trial, noninferiority of Gefitinib as compared to Erlotinib was assessed in 561 postoperative recurrent or stage IIIb/VI patients who had undergone prior chemotherapy treatment but no tyrosine kinase inhibitor therapy. In a subset of patients with sole EGFR L858R, there was an insignificant difference between the objective response and disease control rates for patients treated with erlotinib (N=67) and gefitinib (N=78). Progression free survival was also not significantly different and did not meet the noninferiority endpoint with Gefitinib and

Erlotinib arms at 8.1 and 8.5 months, respectively (HR, 0.938; 95% CI, 0.675 to 1.304; P = .704).

Evidence statement 40

In an in vitro study using NCI-H322 cells (wildtype EGFR) and NCI-H3255 cells (EGFR-L858R), inhibition of cell growth was used as an assay to determine sensitivity to irreversible tyrosine kinase inhibitor (TKI) drugs. Cells with an EGFR L858R mutation demonstrated an improved response to Dacomitinib (IC50: 0.007umol/L vs. >10umol/L) compared to wildtype EGFR cells or compared to reversible TKI drug gefitinib (IC50: 0.075umol/L vs. wild-type >10umol/L).

Evidence statement 41

A 62 year old patient with 4 pack-year smoking history with back and flank pain was found to have metastatic lung adenocarcinoma by CT and MRI. Targeted NGS sequencing discovered an EGFR L858R mutation and the patient started 80mg osimertinib treatment with excellent response of all disease sites, including brain metastases. The patient had continued response until 8.5 months after treatment initiation when a new liver lesion was found. NGS sequencing of a repeat biopsy detected the original EGFR L858R mutation as well as L718V and L718Q mutations. Afatinib treatment led to a partial response for 4.5 months, at which time disease progression occurred. Biopsy of a progressing liver lesion showed acquired EGFR T790M mutation as well as increase and decrease of L718V and L718Q mutation frequencies, respectively.

Evidence statement 42

Median survival of patients with EGFR L858R mutation is better than those with wild type EGFR.

Clinical Variant 2

Gene Name: KRAS

Protein Change: G13D

Coordinates: chr12:g.25398281C>T

Variant Annotation

While the KRAS G13 region is a widely studied recurrent region in cancer, its impact on clinical action is still debated. Often associated with tumors that are wild-type for other drivers (EGFR and ALK specifically), the prognosis for patients with this mutation seems to be worse than the KRAS wild-type cohort. This mutation, along with the mutations affecting the neighboring G12 position, may result in a less responsive tumor when treated with first-generation TKI's like gefitinib. However, results are conflicting with retrospective analyses suggesting a better response to EGFR-Inhibition. A recent prospective phase-II study (12 patients, Schirripa et. al. 2015) could not reproduce this finding and another prospective phase II trial is currently ongoing.

Evidence Statements

Evidence statement 1

Meta-analysis encompassing eight randomized controlled trials (n = 5967) for assessment of both overall survival (OS) and progression-free survival (PFS) of patients with KRAS mutant metastatic colorectal cancer. For other KRAS MT the hazard ratio for OS benefit with addition of anti-EGFR mAb therapy was 1.06 (95% confidence interval [CI]; 0.96, 1.17), compared to 1.08 (95% CI; 0.73, 1.60) for KRAS G13D [test for interaction p=0.99]. In contrast, the hazard ratio for KRAS wild-type (WT) tumours was 0.85 (95% CI; 0.76, 0.95). Regarding PFS benefit with anti-EGFR mAbs, the hazard ratio was 1.07 (95% CI; 0.92, 1.26) for other KRAS MT, 0.96 (95% CI; 0.73, 1.27) for KRAS G13D, and 0.68 (95% CI; 0.54, 0.85) for KRAS wild-type. Again, the test for interaction (p=0.46) demonstrated no significant difference in PFS benefit for anti-EGFR mAb therapy between KRAS G13D and other KRAS MT.

Evidence statement 2

Cells harboring KRAS G13D mutation were sensitive to cetuximab treatment in isogenic SW48 cells and in a mouse xenograft model.

Evidence statement 3

KRAS G13D mutation is associated with better response to Cetuximab with longer progression-free and overall survival in colorectal patients compared to other KRAS.

Evidence statement 4

This is a retrospective analysis of 98 patients with metastatic colorectal cancer and KRAS mutations. 23 (23.5%) had KRAS p.G13D-mutated tumors. Of the 98 patients, 31 patients received cetuximab, of these, 9 (29.0%) had KRAS p.G13D mutations. Univariate analysis did not show any differences between these groups. Multivariate analysis showed a trend towards better PFS among patients with a G13D mutation (PFS: HR=0.29; 95% CI: 0.08-1.10; P=0.07; OS: HR=0.23; 95% CI: 0.04-1.54; P=0.13).

Evidence statement 5

12 patients with KRAS G13D mutant, metastatic colorectal cancer were treated with single agent cetuximab in a prospective phase II study. The primary endpoint of the trial was 4-month progression-free survival, with a benefit to treatment evaluated to be 50% of patients were progression free at this point. None of 12 patients achieved response by RECIST 1.1 criteria and three patients (25%) were progression-free at 4 months, with disease control rate at 6 months reaching 0%. Median PFS and OS were 1.9 (95% CI 1.7–3.8) and 7.2 months (95% CI 5.7–9.7).

Evidence statement 6

In a retrospective study of 89 metastatic irinotecan-refractory colorectal cancer patients, patients with a KRAS codon 12 or 13 mutation (n=24) treated with cetuximab plus chemotherapy were associated with reduced response rate (0% vs. 40.0%, P=0.001), shorter progression-free survival (10.1wk, 95% CI:8.0-16.0wk vs. 31.4wk, 95% CI:19.4-36.0wk, P=0.0001, multivariate analysis) and shorter overall survival (10.1mo, 95% CI:5.1-13.0mo vs. 14.3mo, 95% CI:9.4-20.0mo, P=0.026, multivariate analysis) as compared to patients without a KRAS codon 12 or 13 mutation (n=65).

Evidence statement 7

In a retrospective study of 59 metastatic, chemotherapy-refractory, colorectal cancer patients, patients with a KRAS exon 2 mutation (n=22) treated with cetuximab and standard chemotherapy were associated with higher disease progression rate (77.2% vs. 29.7%, P=0.0005, Fisher exact test) and decreased time to progression (3.0mo vs. 5.5mo, P=0.015, log-rank test) as compared to patients with wild-type KRAS (n=37).

Evidence statement 8

In a retrospective study of 113 metastatic irinotecan-refractory colorectal cancer patients, patients with a KRAS codon 12 or 13 mutation treated with cetuximab plus irinotecan (34/83) were associated with reduced progression-free survival (12.0wk, 95% CI:5.4-18.7wk vs. 34.0wk, 95% CI:28.5-40.0wk, P=0.016, log-rank test) and reduced overall survival (27.3wk, 95% CI:9.5-45.0wk vs. 44.7wk, 95% CI:28.4-61.0wk, P=0.003, log-rank test) as compared to patients with wild-type KRAS (49/83). However, in a cetuximab monotherapy cohort, patients with a KRAS codon 12 or 13 mutation (12/30) did not show significant differences in progression-free survival (12.0wk, 95% CI:7.0-17.0wk vs. 12.0wk, 95% CI:4.2-20.0wk, P=0.351, log-rank test) or overall survival (25.3wk, 95% CI:0.0-70.0wk vs. 27.0wk, 95% CI:8.9-45.1wk, P=0.33, log-rank test) as compared to patients with wild-type KRAS (18/30).

Evidence statement 9

In a retrospective study of 30 metastatic colorectal cancer patients, patients with a KRAS codon 12 or 13 mutation (n=13) treated with cetuximab and standard chemotherapy were associated with an absence of response (KRAS mutation in responder vs. non-responder patients: 0% , 95% CI:0-28.5% vs. 68.4% , 95% CI:43.5-87.5%, P=0.0003), while patients with a clinical response did

not have a KRAS mutation (11/17). Excluding the 3 patients treated with cetuximab and standard chemotherapy as first-line therapy, the patients harboring a KRAS codon 12 or 13 mutation had lower overall survival (6.9mo vs. 16.3mo, P=0.016) as compared to patients without a KRAS codon 12 or 13 mutation.

Evidence statement 10

In an in vitro study, a Lim1215 colorectal cancer cell line endogenously expressing KRAS G13D mutation demonstrated resistance to cetuximab treatment, compared to Lim1215 parental cells expressing wild-type KRAS. Resistance was determined by assessing cell viability.

Evidence statement 11

In a retrospective study of 23 EGFR-expressing metastatic colorectal cancer patients, patients with KRAS codon G12 or G13 mutations (n=6) treated with cetuximab were associated with decreased partial response (OR: 0.071; 95% CI:0.08?0.619, P=0.017) and decreased time to tumor progression (P=0.0443), as compared to patients with wild-type KRAS (n=17).

Evidence statement 12

In a retrospective study of 572 advanced colorectal cancer patients, patients treated with cetuximab and best supportive care with a KRAS exon 2 mutation (81/164) were associated with reduced response rate (1.2% vs. 12.8%), shorter progression-free survival (1.8mo vs. 3.7mo, P<0.001) and shorter overall survival (4.5mo vs. 9.5mo, P=0.01,), compared to patients with wild-type KRAS (117/230).

Evidence statement 13

This was a retrospective study of 691 cetuximab treated patients with metastatic colorectal cancer. Of those, 50 patients harbored KRAS G13D, were BRAF, NRAS and PIK3CA wt, and had individual response data. Nine patients had partial response, 19 had stable disease, and 22 progressed. Treatments included cetuximab + irinotecan (n=38), cetuximab + FOLFIRI (n=5), cetuximab monotherapy (n=4), cetuximab + oxaliplatin + 5FU (n=2), and cetuximab + irinotecan + oxaliplatin + 5FU (n=1). Median PFS was 16 weeks (3-101 weeks), median OS was 33 weeks (3-161 weeks), and median number of previous chemotherapy lines was 2 (0-4). Median age was 65 years old (34-78), and there were 29 males and 20 females. Authors concluded that KRAS mutation was strongly associated with poor response to cetuximab and suggested that mutation status of KRAS is the most informative compared to BRAF, NRAS, and PIK3CA exon 20 for predicting cetuximab response.

Evidence statement 14

In a retrospective study of 148 treatment naive metastatic colorectal cancer patients, patients with RAS mutations (n=10), including KRAS A146T, KRAS G13D, NRAS G12D and NRAS Q179*, treated with FOLFOX4 plus cetuximab were associated with decreased overall survival (16.3mo vs. 28.5mo, HR:0.43, 95% CI:0.20-0.89, P=0.020) and decreased progression free survival (7.2mo vs. 9.7mo, HR:0.56, 95% CI:0.27-1.16, P=0.11), as compared to patients with wild-type KRAS and NRAS.

Evidence statement 15

In a retrospective study of 32 metastatic colorectal cancer patients, a patient with KRAS G13D mutation treated with cetuximab was reported to be a non-responder, whereas none of the 10 partial responders harbored KRAS mutation in the primary tumors or metastases.

Evidence statement 16

In a phase 3 clinical study of 533 metastatic colorectal cancer patients, patients harboring KRAS G13D mutation and treated with cetuximab plus standard chemotherapy (n=42) were associated with reduced overall survival (HR:1.61, 95% CI:1.13-2.29, P=0.0085) and reduced objective response (OR:0.50, 95% CI:0.26-0.97, P=0.040), as compared to patients harboring wild-type KRAS (n=398).

Evidence statement 17

In a phase 3 clinical study of 1,198 metastatic colorectal cancer patients, patients harboring KRAS codon 12 or 13 mutations and treated with cetuximab plus FOLFIRI were associated with reduced overall survival (16.2 vs. 23.5 months) and reduced progression-free survival (7.4 vs. 9.9 months), as compared to patients with wild-type KRAS.

Evidence statement 18

In a phase 2 clinical study of 344 metastatic colorectal cancer patients, patients harboring KRAS codon 12 or 13 mutations and treated with cetuximab plus FOLFOX-4 (n=52) were associated with reduced progression-free survival, as compared to patients with wild-type KRAS (n=61) (5.5 vs. 7.7 months; P=0.0009), and as compared to patients with KRAS codon 12 or 13 mutations treated with FOLFOX-4 alone (n=47) (5.5 vs. 8.5 months; P=0.0192).

Evidence statement 19

In a retrospective study of 95 metastatic colorectal cancer patients, patients harboring KRAS activating mutation (n=33) and treated with cetuximab plus standard chemotherapy were reportedly non-responders and were associated with decreased progression free survival and decreased overall survival, as compared to patients harboring non-activating or wild-type KRAS (n=62) (P<0.0001).

Evidence statement 20

In a phase 3 clinical study (NCT00154102) of 1198 metastatic colorectal cancer patients, patients harboring KRAS codon 12 or 13 mutations and treated with cetuximab plus FOLFIRI (n=105) were associated with reduced overall response (24.9 vs. 17.5 months) and reduced progression-free survival (7.6 vs. 9.9 months), as compared to patients with wild-type KRAS (n=172).

Evidence statement 21

In an in vitro study, a human colon adenocarcinoma SW48 cell line expressing KRAS G13D mutation demonstrated resistance to regorafenib treatment (IC50: 172.23 nM vs. 114.28 nM, P<0.01) compared to SW48 cells expressing KRAS wild-type. Resistance was determined by assessing cell viability. Authors concluded that KRAS G13D is more resistant to regorafenib than KRAS wt, but more sensitive than common KRAS G12 mutations.

Evidence statement 22

In an in vitro study, colorectal cancer SW48 cell line expressing KRAS G13D mutation demonstrated reduced sensitivity to sunitinib treatment (IC50: 3.4uM vs. 2.6uM, P<0.05) compared to SW48 cells expressing wild-type KRAS. Sensitivity was determined by assessing cell proliferation and viability.

Evidence statement 23

In an in vitro study, a Lovo cell line expressing KRAS G13D mutation was associated with sensitivity to palbociclib treatment as compared to cells treated with DMSO control. Sensitivity was determined by assessing cell growth, colony formation, Rb, ERK, and S6 phosphorylation. In in vivo experiments, KRAS G13D expressing Lovo xenografts in nude mice decreased in size when treated with palbociclib. Sensitivity was determined by assessing tumor volume.

Evidence statement 24

In a retrospective study of 274 stage IIIB or IV non-small cell lung cancer patients, patients with a KRAS exon 2 mutation (25/55) treated with erlotinib plus chemotherapy were associated with decreased time to progression (3.4mo vs. 6.0mo, HR:1.9, 95% CI:1.1-3.6, P=0.03, log-rank test), decreased overall survival (4.4mo vs. 13.5mo, HR:2.1, 95% CI:1.1-3.8, P=0.019, log-rank test) and reduced response rate (8% vs. 23%) as compared to patients treated with chemotherapy (30/55).

Evidence statement 25

In a prospective study of 522 European non-small cell lung cancer patients, patients with a KRAS exon 2 mutation (42/307) treated with erlotinib as a second-, third-line or more treatment were associated with reduced progression-free survival (1.9mo vs. 2.3mo, HR:1.2, 95% CI:0.8-1.8, P=0.001, Wald test) and reduced overall survival (4.1mo vs. 5.3mo, HR:1.7, 95% CI:1.1-2.4, P=0.004, Wald test) as compared to patients with wild-type KRAS (n=221).

Evidence statement 26

In a phase 3 clinical study,■BRAF wild-type stage III colon cancer patients harboring KRAS G13D mutation (n=220) and treated with cetuximab plus chemotherapy were associated with decreased disease-free survival, as compared to patients harboring wild-type KRAS (n=1479; univariate HR:1.46, 95%CI:1.13-1.89, P=0.0035).

Evidence statement 27

In an in vitro study, a DLD-1 cell line expressing KRAS G13D mutation was associated with sensitivity to regorafenib treatment, as compared to Caco-2 and KM12SM cells expressing wild-type KRAS. Resistance was determined by assessing cell proliferation and migration.

Evidence statement 28

In this study, a large cohort of metastatic colorectal cancer patients were treated with anti-epidermal growth factor receptor monoclonal antibodies (cetuximab or panitumumab). KRAS, PIK3CA, and PTEN were tested for mutation; KRAS G13D was the only variant noted in the primary colon tumors of two patients who experienced partial response following treatment (Patients 37, 47; Supplemental Table 1). In the larger cohort, patients with tumors harboring any KRAS mutation had significantly decreased incidence of objective response than patients with wtKRAS tumors. Authors noted that patients with tumors harboring KRAS mutations tended to have decreased PFS and OS compared to those with wild-type tumors though the differences were not statistically significant.

Evidence statement 29

In this study, a large cohort of metastatic colorectal cancer patients were treated with anti-epidermal growth factor receptor monoclonal antibodies (cetuximab or panitumumab). KRAS G13D was the only variant noted in the primary rectal tumor of one patient who experienced disease progression following treatment (Patient 45; Supplemental Table 1). This is response is consistent with observations made of the larger cohort, where patients with tumors harboring any KRAS mutation had significantly decreased incidence of objective response than patients with wtKRAS tumors. Authors noted that patients with tumors harboring KRAS mutations tended to have decreased PFS and OS compared to those with wild-type tumors though the differences were not statistically significant. However, two other patients (37 and 47; Table S1) had G13D as their only noted variant, but experienced partial response.

Evidence statement 30

In a two armed Phase II study of 53 patients with molecularly selected G13D mutant, chemotherapy-refractory metastatic CRC, 25 patients were treated with cetuximab monotherapy, and 28 with cetuximab and ironotecan combination therapy. The six month progression free survival rate with monotherapy was 10% (95% CI, 2% to 26%), and 23% (95% CI, 9% to 40%) for cetuximab plus irinotecan combination with a hazard ratio of 0.74 (95% CI, 0.42 to 1.32). No responses seen with cetuximab monotherapy. The authors conclude that absence of response to monotherapy does not support sensitivity of these tumors to cetuximab.

Evidence statement 31

The results showed that cell lines with BRAF(V600E) or KRAS(G13D) mutation were resistant, whereas cell lines with wild-type of both KRAS and BRAF were particularly sensitive to BMS-754807 if they have either higher RNA expression levels of IR-A or lower levels of IGFBP6.

Evidence statement 32

In 28 out of 40 (70%) metastatic colorectal cancer tumors harboring KRAS, NRAS, PIK3CA or BRAF mutations and implanted into mice, the therapeutic combination of the MEK inhibitor AZD6244 and the PI3K/mTor inhibitor BEZ235 resulted in disease stabilization. Mean tumor growth was lower in the tumors treated with AZD6244+ BEZ235 (+77% vs. +267%, P=1E-6) compared to AZD6244 alone and (+77% vs. +222%, P=0.0014) BEZ235 alone. 32/40 mice harbored KRAS mutations including 5 G13D mutations where disease stabilization was achieved in 2/5 mice treated with AZD6244 or combination therapy and 0/5 mice treated with BEZ235 alone.

Evidence statement 33

Three patients participating in a large retrospective study of EGFR monoclonal antibodies in metastatic, treatment refractory colorectal cancer had tumors which harbored KRAS G13D, were wildtype for NRAS, BRAF and PIK3CA, and had individual response data. All three patients were males treated with panitumumab monotherapy. Their characteristics are as follows: 51 year old who experienced progressive disease (PFS: 8 weeks; OS: 24 weeks) and had three prior lines of chemotherapy; 54 year old who experienced progressive disease (PFS: 8 weeks; OS: 17 weeks) and had two prior lines of chemotherapy; 52 year old who experienced stable disease (PFS: 12 weeks; OS: 46 weeks) and had two prior lines of chemotherapy. Per the authors criteria, the clinical benefit rate is 1/3 and the response rate is 0/3.

Clinical Variant 3

Gene Name: MTHFR

Protein Change: A222V

Coordinates: chr1:g.11856378G>A

Variant Annotation

Variant annotation not found...

Evidence Statements

Evidence statement 1

The MTHFR C667T variant was associated with significantly lower relapse-free survival and overall survival in stomach cancer patients treated with 5-Fluorouracil-based therapies. 116 Chinese patients with histologically confirmed gastric cancer were used in this study, and all patients had radical surgery before treatment.

Evidence statement 2

Patients with the wild type (C/C) MTHFR gene are 2.91 times (95% CI: [1.23, 6.89]) more likely to have a positive response to neoadjuvant CRT and 3.25 times more likely not to experience relapse (95% CI: [1.37, 7.72]) than patients with the heterozygous MTHFR [rs1801133 (C>T)] mutation or the homzygous (T/T).

Evidence statement 3

Study of 1817 PCa cases and 2026 cancer free controls to clarify the association of (MTHFR)c.677C>T (and c.1298A>C) of pancreatic cancer risk in a population of Han Chinese in Shanghai. Results indicated a lower risk for the heterozygous CT genotype and homozygous TT genotype carriers of (MTHFR)c.677C>T which had a significantly lower risk of developing pancreatic cancer compared with the wild-type CC genotype.

Clinical Variant 4

Gene Name: FLT3

Protein Change: T227M

Coordinates: chr13:g.28624294G>A

Variant Annotation

FLT3 T227M (rs1933437) is a common polymorphism with a GMAF around .60 based on the Exome Aggregation Consortium (ExAC) data. Its role in cancer predisposition is still unknown, however it may be associated with the development of leukopenia in patients treated with sunitinib.

Evidence Statements

Evidence statements not found...

Clinical Variant 5

Gene Name: TP53

Protein Change: P72R

Coordinates: chr17:g.7579472G>C

Variant Annotation

This polymorphism is relatively widely studied across cancer types, but meta-analyses in breast, lung and cervical cancer cohorts have so far been inconclusive as to the significance of a patient's genotype at this locus as it relates to cancer susceptibility and prognosis.

Evidence Statements

Evidence statement 1

Pooled data from 13 studies of patients with lung cancer found little effect of codon 72 allele on the overall survival of patients with lung cancer. Proline homozygous patients were correlated with slightly poorer outcome, odds ratio 1.18 with a 95% confidence interval of 0.99-1.41. Heterozygotes had an OR of 1.02 (95% CI 0.86-1.20). Authors conclude that the effect of these alleles on prognosis in lung cancer is inconclusive at best.

Evidence statement 2

In a relatively small meta-analysis of 119 women with cervical cancer and 127 controls, associations with homozygosity for arginine, and heterozygosity at this locus were both associated with odds ratios above one (3.5 and 2.2, respectively) after adjusting for age and HPV infection. However, both confidence intervals had minimums below one (0.9 and 0.6, respectively). So the overall conclusion remains mixed on the significance of this polymorphism in the prognosis for cervical cancer.

Evidence statement 3

Large meta-analysis of 5,191 breast cancer patients and 3,834 controls did not find a conclusive link between codon 72 allele and breast cancer susceptibility. Odds ratios were 0.98 (95% CI, 0.91–1.05) for heterozygotes and 0.97 (95% CI, 0.86–1.11) in homozygotes (per-allele OR, 0.98; 95% CI, 0.91–1.04). Median age of onset was also not different between genotypes (GG: 49 years, GC: 50, CC: 50).

Evidence statement 4

In the evaluation of 275 oligodendroglial tumors, the specific genotype at amino acid 72 of TP53 (Arg/Arg, Arg/Pro, or Pro/Pro) was not associated grade of malignancy (low- vs. high-grade, P = 0.650) or overall survival (P = 0.857).

Clinical Variant 6

Gene Name: ERCC2

Protein Change: K751Q

Coordinates: chr19:g.45854919T>G

Variant Annotation

Variant annotation not found...

Evidence Statements

Evidence statement 1

The ERCC2 K751Q variant was significantly correlated with a lower response to cisplatin chemotherapy in osteosarcoma patients and shorter event-free survival. 91 osteosarcoma patients with a median age of 15 years were followed in this study.

Evidence statement 2

The ERCC2 K751Q variant is significantly correlated with increased response to paclitaxel and carboplatin therapies in non small cell lung cancer (NSCLC). The researchers utilized data from three previous clinical trials in Japan and the United States with a total of 526 NSCLC patients.

Additional information

Total Number of Variants Processed: 63

The Number of Clinical Annotations: 6