BRAF Mutant: What to do?
May need MSI

Heinz-Josef Lenz
Professor of Medicine and Preventive Medicine
Associate Director, Clinical Research
Kathryn Balakrishnan Chair for Cancer Research
Co-Director, USC Center for Molecular Pathways and Drug Discovery
USC/Norris Comprehensive Cancer Center
Los Angeles, California
Genetic changes in CRC

WNT signalling

- **DKK1-4**
  - **49%**
  - **33%**
- **FZD10**
- **LRP5**
- **AXIN2**
- **APC**
- **CTNNB1**
- **TCF7L2**
- **SOX9**

TGF-β signalling

- **TGFB1**
  - **3%**
  - **17%**
- **TGFB2**
- **ACVR2A**
- **ACVR1B**
- **SMAD2**
- **SMAD3**
- **SMAD4**

PI3K signalling

- **PTEN**
  - **4%**
  - **20%**
- **NRAS**
- **PIK3R1**
- **BRAF**

RTK-RAS signalling

- **ERBB2**
  - **6%**
  - **13%**
- **ERBB3**
- **KRAS**

P53 signalling

- **ATM**
  - **7%**
  - **87%**
- **TP53**

Pathway alteration pattern

- **WNT**
- **TGF-β**
- **RTK/RAS**
- **PI3K**
- **TP53**

BRAF Background

- Overall, approximately 8% of all tumors have a BRAF mutation; in CRC it ranges from 5-10%.

- The predominant mutation, similar to melanoma, is a single-base substitution of valine by glutamic acid at position 600 (V600E) within the activation segment.

- Signals through MEK/ERK activation pathway.

- BRAF mutation is an early event in CRC and there is a high concordance between primary and metastatic tissue.

- Associated with:
  - R-sided tumors; high grade
  - Older age, female
  - MSI-high (due to epigenetic mechanisms, not HNPCC)
  - Serrated (as opposed to tubular) adenoma pathway

Eckhardt, ASCO GI 2013
Questions we need to answer?

1. Why is braf mt associated with poor prognosis but not ras mt in metastatic disease?
2. Why is ras mt associated with efficacy of EGFR inhibitor but not braf mt?
2. How can MSI status reverse prognosis of braf?
3. Any immunophenotype in braf mutant tumors?
Distinct Biology of R v. L CRC

Analysis of PETACC-3 samples (n=2849)

High mutation Frequency

Poor Prognosis

Sensitive to Cetuximab

Good Prognosis

Exome analysis of 2849 samples from PETACC-3.

Right: BRAF mut, MSI, KRAS, PIK3CA, Mucinous differentiation

Left: EREG expression, 18q loss, 20q Gain, EGFR gain, HER2 gain

Missiaglia, ASCO 2013
**BRAF mut vs WT 2:** Differentially expressed probesets with fold change > 2 and <1% FDR (53 probesets)

Adjusted for MSI status and BRAF/MSI interactions

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*Popovici JCO 2012*
The BRAF - signature identifies:

1) Tumors that carry the BRAF V600E gene mutation with 96% sensitivity and 86% specificity

2) 30% of KRAS mutant CC
   13% of double wild type CC
   which carry the same gene expression profile as BRAF V600E
BRAF mut vs WT 2: Differentially expressed genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>Pathway</th>
<th>Fold.change</th>
<th>P.Adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP5</td>
<td>Aquaporin 5</td>
<td>target of ESR1; promotes proliferation and inhibits apoptosis in chronic myelogenous leukemia; interacts with the MAPK and Rb pathway</td>
<td>5.5</td>
<td>9.7E-21</td>
</tr>
<tr>
<td>CTSE</td>
<td>cathepsin E</td>
<td>is found in highest concentration in the surface of epithelial mucus-producing cells of the stomach. Found in more than half of gastric cancers.</td>
<td>4.8</td>
<td>5.43E-11</td>
</tr>
<tr>
<td>SOX8</td>
<td>SRY (sex determining region Y)-box 8</td>
<td>Wnt/beta-catenin signaling</td>
<td>2.6</td>
<td>0.000217</td>
</tr>
<tr>
<td>REG4</td>
<td>regenerating islet-derived family, member 4</td>
<td>activator of the EGFR in CRC, involved in gastric and pancreatic cancers</td>
<td>3.7</td>
<td>1.68E-07</td>
</tr>
<tr>
<td>PIWIL1</td>
<td>piwi-like 1</td>
<td>intrinsic regulator of the self-renewal capacity of germline and hematopoietic stem cells.</td>
<td>2.5</td>
<td>9.43E-05</td>
</tr>
<tr>
<td>AXIN2</td>
<td>Axin 2</td>
<td>Wnt/beta-catenin signaling</td>
<td>-2.1</td>
<td>5.65E-06</td>
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<tr>
<td>CDX2</td>
<td>caudal type homeobox 2</td>
<td></td>
<td>-2.3</td>
<td>1.16E-10</td>
</tr>
<tr>
<td>HSF5</td>
<td>heat shock transcription factor</td>
<td></td>
<td>-3.1</td>
<td>5.79E-10</td>
</tr>
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</table>

Analysis of differentially expressed genes between BRAF-mutant-like and the rest of KRAS mutants identified genes responsible for colon crypt differentiation, Wnt pathway activation and, more intriguingly, 50 other genes, all located on chromosome 20q, that were significantly down-regulated in BRAF-mutant-like tumors. Among these genes, there are a number of important tumor suppressors, like PLAGL2, TP53RK and POFUT1.

Popovici JCO 2012 Adjusted for MSI status and BRAF/MSI interactions
TS Expression by BRAF Mutation Status in Right-Sided Colon

$P = 0.003$

- Mutant: N = 24
  - TS mRNA Expression Level: 6.4
  - 95% Confidence Interval: 3.2 - 9.6

- Wildtype: N = 130
  - TS mRNA Expression Level: 1.6
  - 95% Confidence Interval: 0.8 - 2.4
Mechanisms of Resistance to braf Inhibitors

a) Alterations that promote enhanced RAF dimerization such as NRAS mutation (NRAS Q61), expression of RAF splice variants (p61), CRAF overexpression, NF1 loss and RTK activation (latter two mechanisms not shown) cause resistance to RAF inhibitors. (b) Reactivation of ERK signaling and resistance to RAF inhibitors may also occur in a dimerization-independent fashion as a result of downstream mutations in MEK or RAF bypass resulting from activation of COT (an ERK kinase kinase).
Regulation of 14-3-3 target binding by protein phosphatases. Protein Trafficking of Braf


©2004 by The Company of Biologists Ltd
RANBP2 is selectively synthetic lethal with BRAF V600E in CC.

Vecchione WGI Barcelona 2014
Random effect model of Log hazard ratio (LogHR) with 95% confidence interval for studies comparing the effect of BRAF-V600E mutation on overall survival of colorectal cancer patients.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0047054
Kaplan–Meier survival plots for colorectal cancer according to combined MSI/BRAF subgroup.


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Kaplan–Meier plots of BRAF and MMR status

A

BRAFwt
BRAFmut

SAR time (yrs)
No. at risk
BRAFwt 510 230 93 43 8 1
BRAFmut 92 13 5 3 2

OS time (yrs)
No. at risk
MMR-p, BRAFwt 1,358 1,271 1,126 830 626 100
MMR-d, BRAFwt 130 123 114 88 67 15
MMR-p, BRAFmut 176 145 121 93 76 11
MMR-d, BRAFmut 71 61 57 45 35 3

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Forest plot of mutations and MMR status and their association with recurrence, OS, and SAR. HR > 1 indicates detriment.

<table>
<thead>
<tr>
<th>Time to recurrence</th>
<th>P</th>
<th>HR</th>
<th>95% Lower</th>
<th>95% Upper</th>
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<tbody>
<tr>
<td>BRAF</td>
<td>0.86</td>
<td>1.02</td>
<td>0.82</td>
<td>1.28</td>
</tr>
<tr>
<td>KRAS_G12V</td>
<td>0.16</td>
<td>1.22</td>
<td>0.93</td>
<td>1.60</td>
</tr>
<tr>
<td>KRAS</td>
<td>0.21</td>
<td>1.12</td>
<td>0.94</td>
<td>1.32</td>
</tr>
<tr>
<td>MET</td>
<td>0.64</td>
<td>1.11</td>
<td>0.73</td>
<td>1.68</td>
</tr>
<tr>
<td>NRAS</td>
<td>0.04</td>
<td>1.53</td>
<td>1.01</td>
<td>2.31</td>
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<tr>
<td>PIK3CA</td>
<td>0.25</td>
<td>0.88</td>
<td>0.71</td>
<td>1.09</td>
</tr>
<tr>
<td>MMR status</td>
<td>0.0001</td>
<td>0.48</td>
<td>0.33</td>
<td>0.70</td>
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</table>

<table>
<thead>
<tr>
<th>OS</th>
<th>P</th>
<th>HR</th>
<th>95% Lower</th>
<th>95% Upper</th>
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<tr>
<td>BRAF</td>
<td>0.0002</td>
<td>1.46</td>
<td>1.20</td>
<td>1.79</td>
</tr>
<tr>
<td>KRAS_G12V</td>
<td>0.49</td>
<td>1.11</td>
<td>0.83</td>
<td>1.47</td>
</tr>
<tr>
<td>KRAS</td>
<td>0.33</td>
<td>1.09</td>
<td>0.92</td>
<td>1.29</td>
</tr>
<tr>
<td>MET</td>
<td>0.40</td>
<td>1.19</td>
<td>0.79</td>
<td>1.80</td>
</tr>
<tr>
<td>NRAS</td>
<td>0.34</td>
<td>1.25</td>
<td>0.80</td>
<td>1.95</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>0.32</td>
<td>0.90</td>
<td>0.72</td>
<td>1.11</td>
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<tr>
<td>MMR status</td>
<td>0.0084</td>
<td>0.64</td>
<td>0.46</td>
<td>0.89</td>
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<table>
<thead>
<tr>
<th>SAR</th>
<th>P</th>
<th>HR</th>
<th>95% Lower</th>
<th>95% Upper</th>
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<tr>
<td>BRAF</td>
<td>&lt;0.0001</td>
<td>2.31</td>
<td>1.83</td>
<td>2.95</td>
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<tr>
<td>KRAS_G12V</td>
<td>0.55</td>
<td>0.91</td>
<td>0.66</td>
<td>1.25</td>
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<tr>
<td>KRAS</td>
<td>0.30</td>
<td>1.11</td>
<td>0.92</td>
<td>1.34</td>
</tr>
<tr>
<td>MET</td>
<td>0.27</td>
<td>1.31</td>
<td>0.81</td>
<td>2.12</td>
</tr>
<tr>
<td>NRAS</td>
<td>0.11</td>
<td>0.67</td>
<td>0.40</td>
<td>1.10</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>0.10</td>
<td>1.23</td>
<td>0.96</td>
<td>1.57</td>
</tr>
<tr>
<td>MMR status</td>
<td>0.02</td>
<td>1.60</td>
<td>1.07</td>
<td>2.41</td>
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</table>
The effect of bevacizumab (Bev) treatment on overall survival by mismatch repair (MMR) status for colon cancer: NSABP C-08.

Pogue-Geile K et al. JNCI J Natl Cancer Inst 2013;105:989-992

© The Author 2013. Published by Oxford University Press. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.
Due to confounding effect of MSI status in BRAF MT patients, Ogino proposed this strategy for classification. Must split, rather than lump, BRAF MT patients.
BRAF inhibition alone ineffective in BRAF-mutant CRC

BRAF mutant melanoma
~60-80% Response Rate

BRAF mutant CRC
~5% Response Rate

2. Kopetz et al., presented at ASCO 2010 (abstract 3534).
A murine study in a resistant BRAFmut CRC cell line combining venurafenib and an AKT inhibitor showed promising activity

Su et al CR 2012
EGFR and BRAF(V600E) inhibitors synergize to induce apoptosis of CRC cells and to suppress CRC tumour growth in a xenograft model.
New studies in the BRAF$^{V600E}$ mutant CRC population

• As examples of clinical trials evaluating the combination of BRAF$^{V600E}$ inhibitors plus anti-EGFR inhibitors in the BRAF mutant population in CRC:
  – NCT01524978: Vemurafenib + Cetuximab (BASKET) – Phase Ib
  – NCT01750918: BRAF/MEK Inhibitors (dabrafenib + trametinib) + Panitumumab – Phase Ib ➔ RP2
  – NCT01719380: LGX818 and Cetuximab or LGX818, BYL719, and Cetuximab – Phase Ib ➔ RP2
Pharmacodynamic and efficacy analysis of the BRAF inhibitor dabrafenib (GSK436) in combination with the MEK inhibitor trametinib (GSK212) in patients with BRAFV600 mutant colorectal cancer (CRC)

R. B. Corcoran¹, G. S. Falchook², J. R. Infante³, O. Hamid⁴, W. A. Messersmith⁵, A. Daud⁶, E. L. Kwak¹, D. P. Ryan¹, R. Kurzrock², C.E. Atreya⁶, J. Luan⁶, P. Sun⁷, M. Schaeffer⁷, M. Motwani⁷, M. Bleam⁷, C. Moy⁷, K. Patel⁷, K. Orford⁷, S. Kopetz⁸, A. P. Venook⁶

¹Massachusetts General Hospital Cancer Center, Boston, MA, ²Department of Investigational Cancer Therapeutics, The University of Texas, MD Anderson Cancer Center, Houston, TX, ³Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN, ⁴Department of Medical Oncology, The Angeles Clinic and Research Institute, Los Angeles, CA, ⁵Department of Medical Oncology, University of Colorado, Aurora, CO, ⁶University of California, San Francisco, San Francisco, CA, ⁷GlaxoSmithKline, Collegeville, PA, ⁸Department of Gastrointestinal Medical Oncology, The University of Texas, MD Anderson Cancer Center, Houston, TX

Presented at the 2013 ASCO Annual Meeting. Presented data is the property of the author.
Results: Efficacy

Duration on Study Treatment (40 patients)

Individual patients with CRC

Best Unconfirmed Response
- Complete response
- Partial response
- Stable disease
- Progressive disease
- Not evaluable

Treatment duration (months)
Updated model

Partial inhibition of MAPK pathway signaling with inhibition of BRAF and EGFR

Robust inhibition of MAPK pathway signaling with inhibition of BRAF, MEK, EGFR

Johanna Bendell triple inhibitor EGFR/MEK and BRAF ASCO 2014
Preliminary Efficacy: Time on Study

Plot represents duration on treatment by best unconfirmed response. The best response without confirmation is displayed at the end of the bar for each subject. * indicates that patient came off therapy for intercurrent illness. † indicates that the patient withdrew consent.
Potential Biomarkers

Mutational analysis by NGS

- NGS performed on archival tissues (n=17)
  - ION Torrent Ampliseq, 46 genes
  - PIK3CA mutations confirmed by Sanger Sequencing

- Results
  - Mutations were identified in a number of cancer-related genes
    - BRAF, APC, PIK3CA, TP53
  - No clear relationship between NGS results and clinical outcome
  - PIK3CA hotspot mutation present in tumor with CR
SWOG 1406 Braf driven Trial based on preclinical data (Kopetz/Lenz)

**Figure 1:** (A) Treatment with vemurafenib and cetuximab in a cell-line derived xenograft model reduces tumor volume relative to control (B) Waterfall plot of vemurafenib in patients with \(BRAF^{mut}\) mCRC (C) Waterfall plot of vemurafenib, cetuximab, and irinotecan in patients with \(BRAF^{mut}\) mCRC (D) Schema for SWOG S1406 phase II study
**SWOG 1406 Tumor Model Program with JAX**

**Figure 3:** Acquired mutations in a $\text{BRAF}^{\text{mut}}$ mCRC PDX after resistance to vemurafenib not seen in untreated/control mice from the same parent tumor.

**Figure 4:** Proposal Schema. In **Specific Aim 1**, outcomes of patients will be compared to the matched PDXs given the same treatment to which the patient is randomized on the S1406 study. In **Specific Aim 2**, cfDNA from patients after progression will be compared to tumor DNA following tumor progression in PDXs treated with the same regimen as the matched patient.
Treatment option for mCRC if no clinical trials available

FOLFOXIRI plus Bevacizumab

Recommendations and Future Directions

• We recommend that routine screening for BRAF mutations and MSI should be considered early in ALL patients with metastatic CRC

• BRAFV600 mutant CRC patients have poor prognosis and typically do not respond well to standard therapy
• Early identification of BRAF mutations will enable CRC patients to consider several interesting and potentially effective therapies currently in clinical trials
• Consider clinical trials as an earlier line of therapy
• Ensure that patients do not miss window to consider clinical trial

• We hope that further studies will identify more effective treatments and will identify biomarkers to define the population of patients most likely to benefit
• FOLFOXIRI/BEV demonstrated promising PFS and OS data
Exposure to extracellular ligands results in the activation of RTKs and the recruitment of adaptor protein complexes, including GRB2-SOS, to the plasma membrane. SOS is a RAS-specific guanine nucleotide exchange factor and enhances the exchange of GDP for GTP by RAS, resulting in RAS activation. This, in turn, serves to promote RAF activation through a multistep process involving conformational changes, phosphorylation and dimerization. Active RAF phosphorylates and activates MEK1 and MEK2, which then phosphorylate and activate ERK1 and ERK2. Active ERK regulates a number of cytoplasmic and nuclear substrates, including several transcription factors that control genes responsible for cell cycle progression and proliferation. ERK also directly phosphorylates and inactivates upstream signaling intermediates (direct negative feedback, solid lines). Furthermore, ERK regulates the expression of genes encoding proteins such as SPRY and DUSPs; both of which have an indirect negative-feedback effect on pathway activity (dotted lines). SPRY proteins are thought to impede signaling by disrupting the GRB2-SOS interaction, and DUSPs are ERK-specific phosphatases.

In tumors harboring a BRAF V600E mutation, hyperactivated ERK signaling results in increased proliferation and evasion of apoptosis. In BRAF-mutant tumors, ERK-dependent negative feedback suppresses RTK-mediated signaling, resulting in low amounts of active GTP-bound RAS. In this state, BRAF V600E signals as a functional monomer.
Study Design

• Simon 2-stage adaptive design\(^1\)
  - Stage I was defined as follows: 7 patients with measurable disease in a corresponding cohort had a minimum of 8 weeks of treatment or withdrew early from the study, whichever occurred first
  - If a prespecified minimal response rate was not achieved in cohorts in the first stage of the study, the corresponding cohort would not enroll any additional patients unless a clear clinical benefit had been observed for these patients
  - The final decision about further recruitment into stage II was made by the sponsor in discussion with the study steering committee (SC)
  - Up to 19 patients were enrolled into stage II

- 7 Cohorts (Metastatic solid tumors and Multiple Myeloma)
- Up to 170 pts
- \(BRAF^{V600}\) positive (testing per local methods)
- **Vemurafenib** 960 mg BID orally

- Primary endpoint: response rate at Week 8
- Secondary endpoints: PFS, TTP, BOR, TTR, DOR, CBR, OS, safety

BOR, best overall response; CBR, clinical benefit rate; CLC, cholangiocarcinoma; CRC, colorectal cancer; DOR, duration of response; ECD, Erdheim-Chester disease; LCH, Langerhans cell histiocytosis; MM, multiple myeloma; NSCLC, non–small cell lung carcinoma; OS, overall survival; PFS, progression-free survival; TTP, time to progression; TTR, time to response.