How to improve Outcome for ras mutant Tumors

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## Ras mutations in cancer

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mutation</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>95%</td>
<td>KRAS</td>
</tr>
<tr>
<td>Colorectal</td>
<td>45%</td>
<td>KRAS</td>
</tr>
<tr>
<td>Lung</td>
<td>35%</td>
<td>KRAS</td>
</tr>
<tr>
<td>AML</td>
<td>30%</td>
<td>NRAS</td>
</tr>
<tr>
<td>Melanoma</td>
<td>15%</td>
<td>NRAS</td>
</tr>
<tr>
<td>Bladder Cancer</td>
<td>5%</td>
<td>HRAS</td>
</tr>
<tr>
<td>Thyroid Cancer</td>
<td>5%</td>
<td>HRAS</td>
</tr>
</tbody>
</table>
Agenda

1. Mechanisms of Action of Ras Mutations
2. Preclinical Models and Early Drug Development
3. Clinical Trials
4. Future Research/Biomarker
5. How to treat mt ras mCRC
6. Conclusions
NIH Ras Initiative (2013): Major Goals

1. Discover/help discover small molecules that bind to RAS directly or disrupt RAS/effector interactions
   - In silico screening based on analysis of new structures
   - Biochemical assays
   - Cell-based screens
   - Covalent inhibitors

2. Molecular description of RAS/RAF signaling complexes in membranes
   - Imaging, biochemical and biophysical analysis
   - In silico modeling (collaboration with DOE)
RAF Activation Assay

- KRAS requires membrane interactions to activate RAF kinase
- Once activated, RAF phosphorylates MEK kinase
- MEK phosphorylation is measured by phospho-specific antibody based HTS assay
- The biochemistry of RAF activation is not fully understood
- Structural and biochemical work may further elucidate the membrane-RAS-RAF interaction and reveal druggable mechanisms
Potential mechanisms of KRAS$^{\text{mut}}$ inhibition

- Prevent GTP binding
- Accelerate GTP hydrolysis
  - GAP stimulated or intrinsic
- Prevent effector binding
- Inhibit effector pathways
- Disrupt membrane localization
- Promote KRAS degradation
- Synthetic lethal target
RAS Signaling Cascades = Drug Targets?
RAS Initiative: Structural Biology efforts

- Determine structures of wild-type and oncogenic mutants of KRAS in active (bound to GTP analogue) states
  
  Wild-type,  G12C,  G12D,  G12V,  G13D,  Q61L and  Q61R

- Determine structures of KRAS complexes with various effectors and regulatory proteins to aid structure-based drug design
  
  - **Effectors**:  RAF Kinase,  PI3-Kinase,  RALGDS,  RASSF1A
  - **GTPase Activating Proteins (GAPs)**:  NF1,  RASA1
  - **RAS-binding proteins**:  Calmodulin,  Argonaute-2
  - **Farnesyl-binding proteins**:  PDEδ,  smgGDS
RAF kinases

- One of the primary effectors of RAS (RAF-MEK-ERK pathway)
- Multiple family members (ARAF, BRAF, CRAF/RAF1, KSR1, KRS2)
- Complex structure, phosphorylation, accessory proteins/chaperones
- Disruption of RAS/RAF1 interaction is a high value target
- Structures of isolated domains are known, but little valuable information
- Production of larger fragments or full length RAF1 is challenging
Treatment Approaches

1. Preventing of ras to bind to plasma membrane: Farnelysation, Prenylation (post translational modification)
   a. Promising preclinical data on combination of both PDE and FTI.

2. G12C still between GTP and GDP: ARS-1620 inhibits GDP bound Kras G12C not others in preclinical models

3. Pan ras inhibitors: compound 3144 (kras G12D) and AZD4785 (in phase 1 trials)

4. TKI rigosertib binding to downstream RAF RalGDS and PI3K

5. Inhibitors of SOS in development

6. Inhibiting Dimerization critical for signaling such as NS1
HRAS but not KRAS is sensitive to Farnesyl Transferase Inhibitors
Future: Inhibitors of Phosphodiesterase
Testing Tipifarnib in the clinic

67% (4 of 6) confirmed PRs and 100% clinical benefit rate in HRAS mutant HNSCC patients treated with tipifarnib
Screening for RAS inhibitors

- In silico, structure based design
- Biochemical screens
- RAS in Nanodiscs or membranes

FRET, BRET

Isogenic cell lines

Cancer-derived cell lines

3D models, organoids

PDX, GEMM, etc
KRAS MEF screen
Assay validation (Sanofi)

- 782 compounds: reference compounds in oncology + active in 3D phenotypic screen (Vitry, 2011)
- Good reproducibility (n1 vs n2 – same results for different batches on different plates)
- No difference between results obtained on adherent vs detached cells

Assay validated by reference compounds
No differential antiproliferative activity : MEK/ERK inhibitors
Differential antiproliferative activity : Pim-1, Hsp90 inhibitors & some hits from previous internal Sanofi screens
Oncogenic KRAS MEFs are more sensitive to HSP90 inhibitors. Currently investigating mechanism of action for HSP90 inhibitors in collaboration with Neal Rosen.
Levels of wt and oncogenic RAS.GTP

Patricelli MP et al, Cancer Discovery 6 p316 2016
Ras and effector dependencies

- **KRAS** subtype lines:
  - depend on the canonical RAS-RAF MAPK pathway
  - upregulate genes involved in the maintenance of the epithelial phenotype

- **RSK** subtype lines:
  - depend on the RSK-MTOR/PI3K axis to drive aerobic metabolism to supplement glycolysis
  - express mesenchymal markers ZEB1, TGFB, TWIST

Tina Yuan, Rachel Bagni, Cyril Benes, Arnaud Amzallag, Bob Stephens, Ming Yi, FNLCR
Cell Feb 2018
KRAS suptype: RAF/MEK/ERK dependencies

1. Inhibition of this signaling with MEK inhibitors such as trametinib, selumetinib alone or in combination

2. Inhibition of ERK with inhibitors such as MK-8353, BVD-523

3. Combination of MEK and ERK inhibition to overcome resistance

4. Pan raf inhibitors LY3009120

5. RAF/MEK inhibitor RO5126766

6. Combination of AKT/MEK PI3K/MEK inhibitors

7. Cdk4/6 and MEK inhibitors
Combination of MEK and CDK4/6 for mCRC

Regimen:
Palbociclib – 100 mpk QD
Trametinib – 0.25 mpk QD
Combo – 100/0.25 mpk QD
ACCRU GI-1618: Clinical Trial Framework

**Eligibility:**
1) RAS Mutant CRC
2) Prior Treatment with FOLFOX and FOLFIRI-based regimens

**Stratified:**
1) Prior regorafenib
2) KRAS 12/13 vs other

**Safety Lead-In:**
1) 9 patients at RP2D

**Arm A**
- TAS-102

**Arm B**
- Binimetinib + Palbociclib

Sample Size: 90 patients, HR = 0.66
1-sided alpha 5%, Power 85%

Primary endpoint: PFS
EGFR Resistance: A Phase II Enrichment Study of Panitumumab with and without Trametinib in Cetuximab-Refractory Stage IV CRC

Sym004 EGFR mAb RPhase 2: ctDNA selected to exclude of EGFR ECD mutations and RAS alleles frequency >20%

MEK + EGFR: Trametinib and Panitumumab

Circulating free DNA mutation analysis

Sensitive clones

Clones remain

Clones regressed

Cohort 1
EGFR S492R mutation without dominant KRAS/NRAS/BRAF mutation
N=12

Panitumumab 6 mg/kg IV q2wk

Optional Crossover
Panitumumab 4.8 mg/kg IV q2wk + Trametinib 2 mg po daily

Panitumumab 6 mg/kg IV q2wk + Trametinib 2 mg po daily

Cohort 2
KRAS/NRAS/BRAF mutation
N=36

Panitumumab 4.8 mg/kg IV q2wk + Trametinib 2 mg po daily

MDACC Study R01 Funded Amgen, Novartis

Cohort 3
No KRAS, NRAS, BRAF, or EGFR S492R mutation
N=36

Optional Crossover
Panitumumab 4.8 mg/kg IV q2wk + Trametinib 2 mg po daily

N=150 screened

Hazard ratios
Sym004 12 mg/kg vs IC=0.81 (0.49-1.33)
Sym004 9mg/kg vs IC=0.59 (0.35-0.96)
1. In a small series of 10 patients who all had mt ras in tissue and liquid biopsy treated with bev based chemotherapy. 5/10 changed to wt Ras under chemotherapy ) Gazzaniga et al Annals of Oncology (2017) 28 (suppl_5): v573-v594)

2. Case report in JCO Precision Oncology from same group reported PR in one of this patient treated with cetuximab

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Pre-treatment</th>
<th>Post-Treatment 2 months</th>
<th>Post-treatment 4 months</th>
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<tr>
<td></td>
<td>KRAS exon 2</td>
<td>codon 12</td>
<td>KRAS exon 2</td>
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<td></td>
<td>KRAS exon 3</td>
<td>codon 13</td>
<td>KRAS exon 3</td>
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<td>codon 59</td>
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<td>KRAS exon 4</td>
<td>codon 61</td>
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<td></td>
<td>KRAS exon 146</td>
<td>codon 117</td>
<td>KRAS exon 3</td>
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<td></td>
<td>KRAS exon 146</td>
<td>codon 166</td>
<td>KRAS exon 3</td>
</tr>
<tr>
<td></td>
<td>NRAS</td>
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- Mutation detected
- No mutation detected
- Progression of disease
KRAS binds to and activates BRAF as part of the MAPK signaling pathway, a key regulator of cell growth and proliferation.

- **KRAS** (30-40%) activates **BRAF** (5-10%)
- **BRAF** activates **MEK**
- **MEK** activates **ERK**
- **ERK** activates **c-Myc**

**GLUT1** expression is upregulated in KRAS/BRAF mutations, leading to metabolic derangements:

- Increase expression of GLUT1
- Increase aerobic glycolysis → highly dependent on aerobic glycolysis
KRAS or BRAF mutant CRC cells rewire glucose metabolism by upregulating GLUT1 expression

Targeting KRAS or BRAF-mutant cancers by exploiting the selective high expression of GLUT1 and the high levels of reactive oxygen species (ROS) produced in these cells with vitamin C

Our preclinical studies have shown that ascorbate selectively kills KRAS and BRAF mutant CRC cells in culture and genetically engineered mouse models.
SVCT1 rs11242462 Outcome Data from KRAS mut mCRC patients in FIRE-3 FOLFIRI/bevacizumab arm

Berger et al ASCO 2017 oral presentation
SVCT1 rs11242462 Gene Expression Data from Normal Sigmoid Colon
Clinical and translational evaluation of high dose Ascorbate in KRAS/BRAF mutant tumors

- **Metastatic, refractory** KRAS or BRAF mutant solid tumor cancer
- **Vitamin C** 1.25 g/kg IV over 2 hours
  - 3-4 days / week
  - 3-4 weeks x 6 months or POD
- **Primary End point**
  - 3 month disease control rate (DCR)
- **Biostats**: 30% 3-month DCR vs. < 10%
  - 90% power, one-sided alpha 0.1 requires 25 evaluable patients
- **KRAS/NRAS/BRAF testing**

Patients who elect for tissue biopsy will be further analyzed.
T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer

Eric Tran, Ph.D., Paul F. Robbins, Ph.D., Yong-Chen Lu, Ph.D., Todd D. Prickett, Ph.D., Jared J. Gartner, M.Sc., Li Jia, M.Sc., Anna Pasetto, Ph.D., Zhili Zheng, Ph.D., Satyajit Ray, Ph.D., Eric M. Groh, M.D., Isaac R. Kriley, M.D., and Steven A. Rosenberg, M.D., Ph.D.
Ras mutant and Immune System

1. Ras signaling influence tumor microenvironment and immune function (reduced TIL, GM-CSF, increase of PDL1 on T cells)
2. TIL can recognize G12D mt ras: Adoptive T cell transfer? CART, engineered T cell receptor to recognize G12D (some preclinical pancreas model show efficacy)
3. G12D specific vaccine with GM CSF trial in pancreas cancer
4. Mek inhibition with PD(L)1 inhibitor in trials for mCRC (MHC class critical)
5. Braf mt increased CD 8 cells
This is a plot ranking SNPs from variable of most importance to least importance in predicting OS in KRAS mut mCRC patients from TRIBE and FIRE-3 FOLFIRI/bev arms.

Green bars are SNPs which are most predictive of OS in KRAS mut patients.
MLH1 rs1799977 Outcome Data from KRAS mut mCRC patients in TRIBE FOLFIRI/bevacizumab arm

HR 3.14 (95% CI 1.37-7.18)

Median OS 25.8 vs 18.4 months
CCL2 rs4586 Outcome Data from KRAS mut mCRC patients in TRIBE FOLFIRI/bev Arm

HR 0.51 (95%CI 0.28-0.92)
Median PFS 25.8 vs 18.4 months
Current View of mCRC Treatment

- **RAS mutated**
  - FOLFOX + Bev
  - FOLFIRI + Bev
  - FOLFOX + cetux

- **BRAF mutated, MSS**
  - FOLFOXIRI + Bev
  - Vemurafenib/Cetuximab/Irinotecan or Clinical Trial

- **MSI-High**
  - FOLFOX + Bev
  - PD-1 inhibition

- **RAS/BRAF wild type**
  - **“Left Sided”**
    - FOLFOX + Cet/Pan (or Bev)
    - FOLFIRI + Bev (or Cet/Pan)
  - **“Right Sided”**
    - FOLFOX + Bev
    - FOLFIRI + Bev

Salvage Oral agents:
- Rego
- TAS-102

FOLFIRI + Bev
- Irinotecan + Cetuximab/Pantimumab
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  - FOLFIRI + Bev

- “Right Sided”
  - FOLFOX + Bev
  - FOLFIRI + Bev

Salvage Oral agents:
- Rego
- TAS-102

Irinotecan + Cetuximab/Pantimumab
### Overall survival

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td><strong>KRAS wt</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFL ± Bev</td>
<td>0.58</td>
<td>0.3-1.0</td>
<td>0.04</td>
</tr>
<tr>
<td>N=152</td>
<td></td>
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<tr>
<td>Pooled analysis</td>
<td>0.70</td>
<td>0.54-0.91</td>
<td>0.007</td>
</tr>
<tr>
<td>N=364</td>
<td></td>
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<tr>
<td><strong>KRAS mut</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFL ± Bev</td>
<td>0.69</td>
<td>0.4-1.3</td>
<td>0.26</td>
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<tr>
<td>N=78</td>
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<tr>
<td>Pooled analysis</td>
<td>0.85</td>
<td>0.60-1.22</td>
<td>0.38</td>
</tr>
<tr>
<td>N=166</td>
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</tbody>
</table>

*Hurwitz et al. The Oncologist 2009 and 2013*
**FIRE 3 results**
- No survival benefit for VEGF in RAS mutant disease –
- Higher RR with Bevacizumab ? -

<table>
<thead>
<tr>
<th></th>
<th>RAS wt (n=201)</th>
<th>RAS mutant (n=188)</th>
<th>HR/Odds ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FOLFIRI + Bevacizumab</td>
<td>FOLFIRI + Cetuximab</td>
<td>FOLFIRI + Bevacizumab</td>
<td>p</td>
</tr>
<tr>
<td><strong>ORR (95%-CI)</strong></td>
<td>56 % 48 – 64</td>
<td>38.1 % 28.5 – 48.6</td>
<td>50.5 % 39.9 – 61.2</td>
<td>0.60 0.34-1.08</td>
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<tr>
<td><strong>PFS (95% CI)</strong></td>
<td>10.2 mo 9.3 – 11.5</td>
<td>7.5 mo 5.7 – 8.5</td>
<td>9.6 8.5 – 10.9</td>
<td>1.25 0.93-1.68</td>
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<tr>
<td><strong>OS (95% CI)</strong></td>
<td>25.0 mo 23.0 – 28.1</td>
<td>20.2 mo 16.4 – 23.4</td>
<td>20.6 17.1 – 26.3</td>
<td>1.05 0.77 – 1.44</td>
</tr>
</tbody>
</table>

Similar observations in TML and CORRECT studies. Survival of all pts benefit driven by KRASwt.

Stintzing et al. ESMO 2014.
Mt Kras mCRC less benefit from anti VEGF therapies compared to wt ras

<table>
<thead>
<tr>
<th>Factor Subgroups</th>
<th>N</th>
<th>median survival</th>
<th>favor</th>
<th>HR</th>
<th>favor</th>
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<tr>
<td></td>
<td></td>
<td>placebo</td>
<td>treat</td>
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<tr>
<td>AVF2107 (bevacizumab 1st)</td>
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<tr>
<td>KRAS-WT</td>
<td>152</td>
<td>17.6</td>
<td>27.7</td>
<td>0.58</td>
<td>[0.30, 1.00]*</td>
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<tr>
<td>KRAS-MUT</td>
<td>78</td>
<td>13.6</td>
<td>19.9</td>
<td>0.69</td>
<td>[0.40, 1.30]</td>
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<tr>
<td>ML18147 (bevacizumab 2nd)</td>
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<tr>
<td>KRAS-WT</td>
<td>314</td>
<td>11.1</td>
<td>15.4</td>
<td>0.69</td>
<td>[0.53, 0.90]*</td>
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<tr>
<td>KRAS-MUT</td>
<td>297</td>
<td>10.0</td>
<td>10.4</td>
<td>0.92</td>
<td>[0.71, 1.15]</td>
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<tr>
<td>RAISE (ramucirumab 2nd)</td>
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<tr>
<td>KRAS-WT</td>
<td>542</td>
<td>11.9</td>
<td>14.4</td>
<td>0.82</td>
<td>[0.67, 1.00]*</td>
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<tr>
<td>KRAS-MUT</td>
<td>536</td>
<td>11.3</td>
<td>12.7</td>
<td>0.89</td>
<td>[0.73, 1.09]</td>
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<td>VELOUR (afiblercept 2nd)</td>
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<tr>
<td>KRAS-WT</td>
<td>281</td>
<td>11.6</td>
<td>14.9</td>
<td>0.74</td>
<td>[0.58, 0.95]*</td>
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<tr>
<td>KRAS-MUT</td>
<td>201</td>
<td>10.6</td>
<td>12.6</td>
<td>0.90</td>
<td>[0.65, 1.25]</td>
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</tbody>
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*Retrospective evaluation of a limited dataset can only be hypothesis generating*

What is the best treatment for RAS mutant disease?

**FOLFIRI/Bev +/- Oxaliplation**

<table>
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<tr>
<th>Group</th>
<th>Events</th>
<th>No.</th>
<th>OS (95% CI), months</th>
</tr>
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<tbody>
<tr>
<td>RAS/BRAF WT (Arm A)</td>
<td>51</td>
<td>79</td>
<td>25.2 (20.8-29.8)</td>
</tr>
<tr>
<td>RAS/BRAF WT (Arm B)</td>
<td>40</td>
<td>79</td>
<td>32.2 (26.1-46.1)</td>
</tr>
<tr>
<td>RAS MT (Arm A)</td>
<td>68</td>
<td>97</td>
<td>21.3 (19.6-23.0)</td>
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<td>RAS MT (Arm B)</td>
<td>65</td>
<td>97</td>
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<td>BRAF MT (Arm A)</td>
<td>11</td>
<td>12</td>
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<tr>
<td>BRAF MT (Arm B)</td>
<td>8</td>
<td>10</td>
<td>7.8 (4.7-13.5)</td>
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**FP/Bev +/- Irinotecan**

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<td>10</td>
<td>7.8 (4.7-13.5)</td>
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</table>

_Cremolini et al. Lancet Oncol 2015_

**Triplet is not better than doublet**

**Doublet is not better than FP+Bev**
Conclusions

1. Ras the most common mutant oncogene and one of the most important targets for drug development.
2. New understanding on functions of ras in the membrane, downstream signaling and metabolic dependencies have allowed novel insights and early promises of new therapeutic approaches.
3. Complexity, Redundancies and adaptive feedback or alternate signaling of ras pathway remain a significant challenge.
4. Combination therapies with novel inhibitors such as ERK, SOS, MEK, cdk4/6 and immunotherapy are ongoing.
5. Biomarker for ras mt are different than for wt ras may give us clues for novel targets.
6. We need better therapies for mt ras mCRC.