Towards personalized treatment for gastroesophageal adenocarcinoma: Strategies to address inter- and intra-patient tumor heterogeneity: PANGEA

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Disclosures

• Research Support
  – OSI Pharmaceuticals
  – Oncoplex Dx

• Honoraria/Consulting
  – Genentech/Roche
  – Amgen
  – AVEO
  – OSI/Astellas
### Management of Advanced Gastroesophageal Adenocarcinoma (GEC)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OS Months</th>
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<tbody>
<tr>
<td>BSC</td>
<td>1</td>
</tr>
<tr>
<td>FAMTX</td>
<td>2</td>
</tr>
<tr>
<td>SP</td>
<td>3</td>
</tr>
<tr>
<td>FP</td>
<td>4</td>
</tr>
<tr>
<td>IF</td>
<td>5</td>
</tr>
<tr>
<td>EOF</td>
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</tr>
<tr>
<td>DCF</td>
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</tr>
<tr>
<td>ECF</td>
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<tr>
<td>ECX</td>
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</tr>
<tr>
<td>XP</td>
<td>7</td>
</tr>
<tr>
<td>EOX</td>
<td>6</td>
</tr>
</tbody>
</table>

Chemotherapy Efficacy Plateau

- **BSC** = best supportive care;
- **MTX** = methotrexate; **S** = S-1; **A** = doxorubicin
- **F** = 5-FU; **C/P** = cisplatin; **I** = irinotecan; **E** = epirubicin; **O** = oxaliplatin; **D** = docetaxel

Putative Targets in Advanced Gastroesophageal Cancer

HER2
EGFR
VEGF
MET
FGFR2

RAS/RAF/MEK
PI3K/mTOR/AKT

Catenacci et al. Cancer Discov 2011
**Current therapies in advanced GC: ToGA**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Months</th>
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<tr>
<td>BSC^1</td>
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<td>FAMTX^2</td>
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<td>SP^3</td>
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<td>FP^4</td>
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<td>IF^5</td>
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<td>EOF^6</td>
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<td>DCF^4</td>
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<td>ECF^6</td>
<td>6</td>
</tr>
<tr>
<td>ECX^6</td>
<td>6</td>
</tr>
<tr>
<td>XP^7</td>
<td>7</td>
</tr>
<tr>
<td>EOF^6</td>
<td>6</td>
</tr>
</tbody>
</table>

**X/FP+/T^8**
- HER2 (+)  
- HER2 IHC3+/FISH+
- HER2 IHC3+/FISH+

^1 BSC = best supportive care; F = 5-FU; A = doxorubicin
^2 MTX = methotrexate; S = S-1; C/P = cisplatin; I = irinotecan
^3 E = epirubicin; O = oxaliplatin; D = docetaxel
^4 T = trastuzumab

<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>N</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; EP</th>
<th>Chemo</th>
<th>mOS (m)</th>
<th>ORR</th>
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</thead>
<tbody>
<tr>
<td>TOGA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>HER2</td>
<td>594</td>
<td>OS</td>
<td>CX CX + Tras</td>
<td>11.1</td>
<td>34.5%</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>13.8 (16.0)</td>
<td>47.3%</td>
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<tr>
<td>AVAGAST&lt;sup&gt;2&lt;/sup&gt;</td>
<td>VEGF</td>
<td>774</td>
<td>OS</td>
<td>CX CX + Bev</td>
<td>10.1</td>
<td>37%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.1</td>
<td>46%</td>
</tr>
<tr>
<td>REAL-3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>EGFR</td>
<td>553 (76%)</td>
<td>OS</td>
<td>EOC mEOC-Pmab</td>
<td>11.3</td>
<td>42%</td>
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<tr>
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<td></td>
<td>8.8</td>
<td>46%</td>
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<tr>
<td>EXPAND&lt;sup&gt;4&lt;/sup&gt;</td>
<td>EGFR</td>
<td>904</td>
<td>PFS</td>
<td>CX CX-Cetux</td>
<td>10.7</td>
<td>29%</td>
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<td></td>
<td></td>
<td>9.4</td>
<td>30%</td>
</tr>
<tr>
<td>AMG102&lt;sup&gt;5&lt;/sup&gt;</td>
<td>MET</td>
<td>118</td>
<td>PFS (phase II)</td>
<td>ECX ECX-Rilo</td>
<td>8.9</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>11.1</td>
<td></td>
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<tr>
<td>GRANITE-1&lt;sup&gt;6&lt;/sup&gt;</td>
<td>mTOR</td>
<td>656</td>
<td>PFS</td>
<td>Placebo Everolimus</td>
<td>4.34</td>
<td>2.1%</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>5.39</td>
<td>4.5%</td>
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<tr>
<td>REGARD&lt;sup&gt;7&lt;/sup&gt;</td>
<td>VEGFR-2</td>
<td>355</td>
<td>OS</td>
<td>Placebo Ramucirumab</td>
<td>3.8</td>
<td>2.6%</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>5.2</td>
<td>3.4%</td>
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<tr>
<td>TyTAN&lt;sup&gt;8&lt;/sup&gt;</td>
<td>HER2</td>
<td>430</td>
<td>OS</td>
<td>Paclitaxel +/- Lapatinib</td>
<td>11 (14)</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.9</td>
<td>9%</td>
</tr>
</tbody>
</table>

TARGETED THERAPIES FOR TARGETED POPULATIONS

Next-Gen Sequencing

N = 45 patients

146/45 = 3.24 avg
## Inter-Patient Tumor Heterogeneity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Oncogenic</th>
<th>Tumor Suppressor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HER2 Amp+</td>
<td>TP53 mt, ARID1A mt, FANCA mt</td>
</tr>
<tr>
<td>2</td>
<td>HER2 Amp+</td>
<td>FBXW7 mt, EPHA3 mt</td>
</tr>
<tr>
<td>3</td>
<td>HER2 Amp+</td>
<td>TP53 mt</td>
</tr>
<tr>
<td>4</td>
<td>HER2 Amp+, KRAS Amp+, PIK3CA mt, CCND1 Amp+, CCNE1 Amp+, MCL1 Amp+, AKT1 Amp+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>MET Amp+, Notch mt</td>
<td>TP53 mt</td>
</tr>
<tr>
<td>6</td>
<td>KRAS Amp+</td>
<td>TP53 mt, CDKN2A mt, CDH1 Splice Site mt, ARID1A mt</td>
</tr>
<tr>
<td>7</td>
<td>KRAS mt</td>
<td>TP53 mt</td>
</tr>
<tr>
<td>8</td>
<td>KRAS mt, ERBB4 mt</td>
<td>BRC2A mt, ARID1A mt</td>
</tr>
<tr>
<td>9</td>
<td>KRAS mt, ERBB4 mt</td>
<td>BRC2A mt, ARID1A mt</td>
</tr>
<tr>
<td>10</td>
<td>KRAS mt, ERBB4 mt</td>
<td>BRC2A mt, ARID1A mt</td>
</tr>
<tr>
<td>11</td>
<td>KRAS mt, ERBB4 mt</td>
<td>BRC2A mt, ARID1A mt</td>
</tr>
<tr>
<td>12</td>
<td>KRAS Amp+, RICTOR Amp+, CCND1 Amp+, CDK6 Amp+, AURKA Amp+, FGFR1 Amp+, FGFR4 Amp+, ZNF217 Amp+, NFKB1A Amp+, NIK2-1 Amp+</td>
<td>TP53 mt, STK11 loss</td>
</tr>
<tr>
<td>13</td>
<td>PIK3CA mt, CTNNB1 mt</td>
<td>TP53 mt, PTCH1 mt, MLH1 mt, MSH6 mt</td>
</tr>
<tr>
<td>14</td>
<td>PIK3CA mt, CTNNB1 mt</td>
<td>TP53 mt, PTCH1 mt, MLH1 mt, MSH6 mt</td>
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<tr>
<td>15</td>
<td>AKT1 Amp+</td>
<td>TP53 mt, SMAD4 mt</td>
</tr>
<tr>
<td>16</td>
<td>AKT1A Amp+</td>
<td>TP53 mt, SMAD4 mt</td>
</tr>
<tr>
<td>17</td>
<td>MDM2 Amp+, AURKA Amp+, CTNNB1 mt, CSF1R mt</td>
<td>CDKN2A mt</td>
</tr>
<tr>
<td>18</td>
<td>CCND1 Amp+</td>
<td>TP53 mt</td>
</tr>
<tr>
<td>19</td>
<td>CCNE1 Amp+</td>
<td>TP53 mt</td>
</tr>
<tr>
<td>20</td>
<td>ZNF217 Amp+</td>
<td>TP53 mt, BRC2A mt, STK11 mt</td>
</tr>
<tr>
<td>21</td>
<td>IGFR1 Amp+</td>
<td>TP53 mt, SMAD4 mt, SMAD2 mt, MLL2 mt</td>
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<tr>
<td>22</td>
<td>IGF1R Amp+</td>
<td>TP53 mt, FANCA Loss</td>
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<tr>
<td>23</td>
<td>MDM2 Amp+</td>
<td>APC mt, CDKN2A/B loss, PTEN mt, DNMT3A</td>
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<td>24</td>
<td>MET Amp+</td>
<td>TP53 mt, SMAD4 mt</td>
</tr>
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<td>HER2 Amp+, SRC Amp+, TOP1 Amp+</td>
<td>TP53 mt, CDH1 mt</td>
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<td>26</td>
<td>FGF21 Amp+</td>
<td>TP53 mt, CDH1 mt</td>
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<td>27</td>
<td>SRC Amp+, AURKA Amp+, CCND1 Amp+, CDK6 Amp+, RICTOR Amp+, MDM2 Amp+, HER2 Amp+, PIK3CA mt, CDK6 Amp+</td>
<td>CDKN2A/B loss, ATM mt</td>
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<td>28</td>
<td>HER2 Amp+, PIK3CA mt, CDK6 Amp+</td>
<td>TP53 mt, PTEN mt</td>
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<td>29</td>
<td>MDM2 Amp+</td>
<td>TP53 mt, FANCA mt</td>
</tr>
<tr>
<td>30</td>
<td>MYC mt</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>PIK3CA mt</td>
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</tr>
<tr>
<td>32</td>
<td>HER2 Amp+, PIK3CA mt</td>
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</tr>
<tr>
<td>33</td>
<td>HER2 Amp+, PIK3CA mt</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>HER2 Amp+, PIK3CA mt</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>HER2 Amp+, PIK3CA mt</td>
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<tr>
<td>36</td>
<td>HER2 Amp+, PIK3CA mt</td>
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</tr>
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<td>37</td>
<td>HER2 Amp+, PIK3CA mt</td>
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</tr>
<tr>
<td>38</td>
<td>HER2 Amp+, PIK3CA mt</td>
<td></td>
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<td>40</td>
<td>HER2 Amp+, PIK3CA mt</td>
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</tr>
<tr>
<td>41</td>
<td>HER2 Amp+, PIK3CA mt</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>HER2 Amp+, PIK3CA mt</td>
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<tr>
<td>43</td>
<td>HER2 Amp+, PIK3CA mt</td>
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<td>44</td>
<td>HER2 Amp+, PIK3CA mt</td>
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<tr>
<td>45</td>
<td>HER2 Amp+, PIK3CA mt</td>
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</tr>
</tbody>
</table>
TARGETED THERAPIES FOR TARGETED POPULATIONS

Selected Reaction Monitoring Mass Spectroscopy: ‘GEC-plex’
Tumor Heterogeneity: **Inter-patient!!**

How to characterize economically?

Stricker, Catenacci, Seiwert. *Semin Oncol* 2011
Biomarker Assessment & Treatment Algorithm

Newly Diagnosed/Recurrent Metastatic Gastric/EGJ adenocarcinoma

Required Testing:
- EGD/EUS, T,N Staging
- Biopsy of Primary Tumor
- Infused CT C/A/P, M Staging

Identify target metastatic lesion for biopsy (Prioritized: Liver, Lung, then Peritoneum*)
- Attempt 4 Core biopsies: each will have an H&E, and only used if ≥20% viable tumor

1. FFPE (3 cores aligned on single block) for Molecular Profiling & Treatment Assignment
   i) IHC: H&E, Her2, Met, FGFR2, KRAS, EGFR, PTEN (7 X 4uM = 28uM)
   ii) DNA/RNA extraction: NGS sequencing (236 genes, 20 translocations) (20uM)
   iii) SRM-Mass Spectroscopy: GEC-Plex for 10 oncoproteins (2X10uM LCM sides = 20uM)

- DNA recovered from extra lysate for confirmatory Sanger Sequencing of NGS
- Storage of remaining lysate and DNA for future currently unplanned studies

iv) FISH: HER2, MET, FGFR2, EGFR, KRAS (5 X 4uM = 20uM + 3 extra = 32uM total)

v) Storage of remaining tissue — section as needed for failed studies above, until exhausted.
   - Store remaining tissue for future currently unplanned studies

2. Fresh Frozen Core in OCT — (1 core) for Retrospective Analyses
   i) After confirmation of >40% tumor, process for PAMGene analysis
   ii) Storage of remaining tissue

* Can use malignant ascites fluid/block if ≥20% viable tumor

Biopsy of Selected Culprit Lesion(s) at Progression
Repeat assessment

Gene Copy Number (GCN) Assessment
- will use FISH for final determination:
  i) HER2, per standard HER assay
     - HER2/Cep17 ratio ≥2 and ≥6 HER2 copies/nucleus
  ii) MET, FGFR2, EGFR, KRAS will follow HER2 GCN scoring
     - NGS sequencing and SRM-MS and IHC protein expression will be used to assist in screening for GCN+

Gene Mutation Assessment
- NGS targeted exome sequencing (Foundation One) for screening for relevant mutations as previously reported (>10% alleles).
  Substratify within the 5 molecular groups based on ‘other’ mutants. Positive mutations confirmed with Sanger Sequencing.

Protein Expression Assessment
- will use IHC for final determination for:
  i) HER2, per standard HER assay
  ii) MET, per standard DAKO Antibody assessment
     - other proteins (FGFR2, EGFR, KRAS, including HER2 and MET) will be assessed with SRM-MS (Oncoplex Dx) and IHC to assist in screening for GCN+

Treatment Assignment Algorithm

<table>
<thead>
<tr>
<th>Biomarker Result Priority</th>
<th>Treatment Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HER2 FISH+ (IHC 3/2+)</td>
<td>B1 - HER2</td>
</tr>
<tr>
<td>2. MET FISH+</td>
<td>B2 - MET</td>
</tr>
<tr>
<td>3. FGFR2 FISH+</td>
<td>B3 - FGFR2</td>
</tr>
<tr>
<td>4. EGFR FISH+</td>
<td>B4 - EGFR</td>
</tr>
<tr>
<td>5. MET IHC 3+/2+ - “MET Hi”</td>
<td>B1 - MET</td>
</tr>
<tr>
<td>6. KRAS – ‘Like’</td>
<td>B5 - KRAS-like</td>
</tr>
</tbody>
</table>
   - KRAS FISH +
   - KRAS mutant
   - BRAF mutant
   - PIK3CA mutant
   - MEK mutant
   - AKT mutant
   - PTEN mutant or IHC-
| 7. HER2 IHC 2+ and FISH-  | B1 - HER2     |
| 8. All negative          | B4 - EGFR     |

Proceed to subsequent line if previous is negative.
A Compromise Between Number of Potential Treatment Groups and Feasibility

PANGEA Classification: Genomics & Proteomics

- HER2
- MET
- FGFR2
- EGFR
- KRAS
The PANGEA-MBBP Trial

Personalized ANtibodies for Gastro-Esophageal Adenocarcinoma:
A Metastatic Trial of Biologics Beyond Progression

Historical Control → FOLFOX → FOLFIRI → FOLTA

i) Primary Endpoints:
- Feasibility: Time to treatment assignment
- Safety: toxicity
ii) mOS (HR 0.67)
- Historic Arm A v Arm B
  (Improve to 18 months from 12 months)
  HR: 0.67
  \{12 month survival rate \(\sim\) 63\%
  \(N = 68\) (80\% power)

Secondary Endpoints:
PFS\(_{1,2,3}\), PFS\(_{1-2,3}\)
2\(^{nd}/3^{rd}\) line treatment rates, RR
- Arm A\(_1\) v A\(_3\)
- Compared to Historic Controls
  - Tissue correlates
    - primary tumor to metastatic lesion
    - baseline v PFS\(_{1,2,3}\)

Diagnosis: metastatic cancer

Anticipated Incidence
- 15\% HER2 amplified
- 30\% MET amplified/Hi
- 10\% FGFR2 amplified
- 20\% EGFR/HER3 amplified/Hi
  KRA5 wild type
  NI HER2,FGFR2,RON,MET
- 20\% KRAS/BRAF/
  PIK3CA/AKT/PTEN del/mt/amplified
  NI HER2,FGFR2,RON,MET

ARM B:
Therapy based on molecular profile

Anticipated Incidence
- 15\% HER2 amplified
- 30\% MET amplified/Hi
- 10\% FGFR2 amplified
- 20\% EGFR/HER3 amplified/Hi
  KRA5 wild type
  NI HER2,FGFR2,RON,MET
- 20\% KRAS/BRAF/
  PIK3CA/AKT/PTEN del/mt/amplified
  NI HER2,FGFR2,RON,MET
The PANGEA -2MBBP Trial

Personalized ANtibodies for Gastro-Esophageal Adenocarcinoma: Phase II Metastatic Biologic Beyond Progression Trial (R 2:1)

Diagnosis: metastatic cancer

ARM A: Standard Chemotherapy + Placebo

ARM B: Therapy based on molecular profile

Biomarker Evaluation in all samples prior to randomization

15% HER2 amplified
30% MET amplified/Hi
10% FGFR2 amplified
20% EGFR/HER3 amplified/Hi
20% KRAS/BRAF/PIK3CA/PIK3CA/PIK3CA/PIK3CA/PIK3CA/PIK3CA/PIK3CA/PIK3CA/PIK3CA

Anticipated Incidence

Standard care: Control Arm

FOLFOX + placebo

Arm A1: HER2 amplified
FOLFOX-Trastuzumab
Arm A2: MET amplified/Hi
Arm A3: FGFR2 amplified
Arm A4: KRAS/PI3K wild type
Arm A5: KRAS/BRAF/PIK3CA mt/amp

Stratify:
1) Stage
2) PS
3) Biomarker
4) GEJ v distal stomach
5) Site of metastases

PD

FOLFOX-Trastuzumab
Arm B1
FOLFOX-METab
Arm B2
FOLFOX-FGFR2ab
Arm B3
FOLFOX-EGFRab
Arm B4
FOLFOX-VEGFR2ab
Arm B5

PD

FOLFOX + T
Arm A1 + T

PD

FOLFOX + M
Arm A2 + M

PD

FOLFOX + F
Arm A3 + F

PD

FOLFOX + E
Arm A4 + E

PD

FOLFOX + V
Arm A5 + V

PFS1
FOLFIRI + placebo

PFS2
DF + placebo

PFS3

Primary Endpoint: OS (HR 0.67)
1) Arm A v B (N=192, 128-B:64-A)
2) Arm A1 v B1
Secondary Endpoints:
PFS1,2,3, PFS1,2,3, 2nd/3rd line rates
RR, toxicity,
Arm A1 v A2, B1 v B2 etc
Tissue correlates

WIN 2013 - Paris, France - July 10-12, 2013
PANGEA STRATEGY

DIAGNOSTIC WORKUP

- Classic Histologic Diagnosis
  - i) Gastric Adenocarcinoma
  - ii) Esophagogastric Adenocarcinoma

- EGD/EUS and biopsy primary tumor
  - CT, C/A/P
  - PET
  - Biopsy Metastatic Lesion
    (Liver/Lung/Peritoneum/LN)

- Address Intra-Patient Heterogeneity Through Space

- Biomarker Assessment
- Medium Throughput
- Low Throughput

- Treatment Algorithm
- Prioritization Scheme

- Intra-patient heterogeneity
  - Space

- Inter-patient heterogeneity

- Treatment Resistance

BIOMARKER ASSESSMENT

- Adress Inter-Patient Heterogeneity
- Medium Throughput Targeted Profiling
  - Genes of Interest
  - Proteomics & Genomics
    "Economical" use of scarce tissue biopsy

- SRM Mass Spectroscopy (MS)
  - "GEC-Plex"

- Next-Gen-Sequencing (NGS)
  - "Foundation One"

- FGFR2 FISH
- CCND1 FISH
- KRAS FISH
- HER2 FISH
- MET FISH
- IGF1R FISH

- Select Low-Throughput Assay for Confirmation When Required
  (i.e. FISH for Equivocal MS Results for HER2 Expression)

PERSONALIZED TREATMENT ASSIGNMENT

- via Algorithm

- Assemble Molecular Profile Information
  - Run Through the Predefined PANGEA Treatment Algorithm
    (Prioritization System of Concurrent Aberrations)
  - Treatment Category and Treatment Assigned

ITERATIVE PROCESS

- i) Refine Medium-Throughput Assays
- ii) Refine Treatment Algorithm

- Assess Outcomes
  - Discovery of New Genes/Proteins and Available Treatment
  - Progressive Disease?

- ADDRESS RESISTANCE
  - Intra-Patient Heterogeneity
  - Time
  - Treatment Resistance

FUTURE GOALS Non-Invasive Approach for Biomarker Assessment:
- Circulating Tumor Cells
- Circulating Free DNA
- Imaging (e.g. Labelled HER2)

WIN 2013 - Paris, France - July 10-12, 2013
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OLOPADE LAB:
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Biostatistics
Theodore Karrison, PhD
James Dignam, PhD

Gi Medical Oncology
Richard Schilsky, MD
Hedy Kindler, MD
Blase Polite, MD
Manish Sharma, MD
Mark Kozloff, MD
Robert Marsh, MD
Victoria Villaflor, MD
Christine Racette, APN
Catherine Rogers RN
Kenisha Allen, RN
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