Signaling Pathway Biomarkers in Gastrointestinal Malignancies
Personalized Medicine May Help Guide Treatment Planning

- In medical practice, clinicians typically prescribe medications according to general information about what might be effective for patients based on product labeling; this trial-and-error approach is in contrast to the goal of personalized medicine.

- From the FDA’s perspective, “personalized medicine promises to increase benefits and reduce risks for patients by improving both the safety and efficacy of medical products.”

- For any individual patient, the actual safety and effectiveness of a treatment may vary as a result of genetic and environmental factors, as well as the interaction of these factors.

- Advances in diagnostic tools used to measure or evaluate an indicator of a normal biological process, pathogenic process, or response to a therapeutic intervention may permit clinicians to individualize or personalize treatment for patients by identifying those who may or may not benefit from a particular treatment plan.
The top panel illustrates an example in which patients get the same drug, regardless of genotype. The lower panel illustrates a personalized medicine approach in which treatment selection is based on specific patient genotype (e.g., presence of mutated gene).1,3
Biomarkers in Gastrointestinal Malignancies
That May Have Clinical Utility

- Aberrant signal transduction due to a variety of mechanisms drives carcinogenesis by promoting abnormal cell growth, proliferation, invasion, angiogenesis, and disrupting apoptosis\(^4\)

- Identification of particular molecules or biomarkers (proteins and genes) within specific signaling pathways may provide information that may aid in cancer detection, diagnosis, or may inform or guide treatment plans\(^5\)

- A biomarker is a characteristic that can be scientifically measured and evaluated to serve as an indicator of a normal or pathogenic biologic process and/or a response to a specific treatment plan\(^1\)

- Several biomarkers have been identified or are being investigated in various gastrointestinal malignancies\(^6\text{-}^9\)

- HER2, EGFR, VEGFR, mTOR, MET, PDGFR, and IGFR are putative biomarkers in esophagogastric adenocarcinoma; while FGF2, VEGF, EGFR, HER2, MET, PI3K, phospho-Akt, and RAS/RAF are potential biomarkers in gastric cancer

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HER2 = human epidermal growth factor receptor 2; EGFR = epidermal growth factor receptor; VEGFR = vascular endothelial growth factor receptor; mTOR = mammalian target of rapamycin; MET = mesenchymal-epithelial transition factor receptor; PDGFR = platelet-derived growth factor receptor; IGFR = insulin-like growth factor receptor; FGF2 = fibroblast growth factor-basic; CA 19-9 = carbohydrate antigen 19-9; MUC1 = mucin 1; SMAD4 = mothers against decapentaplegic homolog 4; HENT1 = human equilibrative nucleoside transporter 1
In pancreatic cancer, CA 19-9, a prognostic serum biomarker indicates disease recurrence after surgical resection; MUC1 and mesothelin expression are under investigation as prognostic biomarkers that predict survival, and SMAD4 levels have been reported to be associated with disease progression\(^{10}\).

The mutational status of \textit{BRAF}, \textit{KRAS}, \textit{NRAS}, and \textit{PTEN/PI3KCA} are under investigation in mCRC\(^{11}\).

The presence of aberrations such as gene amplification or protein overexpression in \textit{MET} is a negative prognostic indicator in gastric cancer\(^{8,9,12}\).

Other biomarkers, such as c-Kit, a growth factor receptor present in gastrointestinal stromal cell tumors (GIST), have been shown to be a predictive biomarker for response to specific treatment plans\(^{13}\).
**Biomarkers in Gastric Cancer**

- Multiple molecular abnormalities have been identified in gastric carcinomas, resulting in aberrant signaling that promote carcinogenesis.

- Aberrant activation of HER2 or MET stimulates downstream signaling through PI3K/AKT, key proteins in the regulation of genes that affect cell survival and apoptosis, and through the RAS/RAF pathway, which promotes cell growth and proliferation.

- HER2 is overexpressed in 12–22% of gastric cancers and in 20–30% of esophageal adenocarcinomas.

- While evidence shows that HER2 may act as both a prognostic and predictive biomarker for these malignancies, evidence indicates that HER2 is primarily a predictive biomarker in gastric cancer.

- MET, a receptor tyrosine kinase, whose ligand is hepatocyte growth factor (HGF), in some malignancies, abnormal stimulation of MET promotes transformation, tumor invasion, progression, metastases, and the epithelial-mesenchymal transition that accompanies some tumors.

- Aberrant signaling through MET can occur as a result of receptor overexpression, upregulated production of HGF, or amplification of the MET gene.

- Mutations in the MET gene can lead to constitutive activation of the receptor and promote proliferation and anti-apoptotic signaling.

- Cross-talk between MET and other receptors, including EGFR, HER2, and HER3, can also lead to constitutive activation of the PI3K/AKT pathway.
• Overexpression of the MET protein and amplification of the MET gene has been observed in 26-74% and 2-23% of gastric cancers, respectively\textsuperscript{17-25}

• Investigators have examined the relationship between aberrant MET and different characteristics of gastric carcinomas such as depth of tumor invasion, lymph node metastasis, stage of disease, and poorer survival.

• When MET protein expression was assessed by immunohistochemistry (IHC), 14% of samples in one study showed no MET protein expression; immunoreactivity ranged from weak to strong in tumor cells of the remaining tumors; normal gastric mucosa weakly expressed MET protein\textsuperscript{26}

• Aberrant MET can be identified in a majority of esophageal adenocarcinomas, many colorectal adenocarcinomas, and high MET expression may be associated with development of metastases and poorer outcomes\textsuperscript{13}

• Other biomarkers under investigation in upper gastrointestinal malignancies include VEGF, SMO, and mTOR, which have been studied in patients with metastatic gastric cancer\textsuperscript{16}
Biomarkers in Pancreatic Cancer

- Pancreatic cancer is extremely aggressive, and it remains a leading cause of cancer-related deaths worldwide.\(^\text{27}\)
- CA 19-9 has long been thought to inform behavior of pancreatic carcinomas
- Tumor suppressor genes that have been implicated in the pathogenesis of pancreatic cancer include \(p53\), \(p21\), and \(SMAD4\), and activating mutations in oncogenes including \(KRAS\) and \(cyclin D\)—these mutations occur in up to 75%, 60%, 55%, 90%, and 82%, of pancreatic cancers respectively
- MUC1 and mesothelin expression, as measured by IHC, are prognostic biomarkers in pancreatic cancer that predict survival.\(^\text{9}\)
- Furthermore, the molecular markers HENT1, ERCC1, and RRM1 may predict response to a specific antimetabolite therapy
- Correlative analysis of blood based biomarkers from patients with advanced pancreatic cancer identified statistically significant prognostic markers for overall survival, including Ang2, CRP, ICAM-1, IGFBP-1, and TSP-2.\(^\text{28}\)
- MUC1 is expressed in >60% of pancreatic adenocarcinomas.\(^\text{29-35}\) Studies have shown that SMAD4 is inactivated in \(\geq 30\%\) of pancreatic ductal adenocarcinomas. KRAS has been shown to be mutated in \(\sim 80\%\) of pancreatic cancers.
Mutations in KRAS are common in pancreatic cancers; these gain-of-function mutations result in inappropriate stimulation through downstream signaling cascades that ultimately promote tumor initiation, progression, proliferation, and invasion. 

In addition, constitutively activated KRAS signaling contributes to up-regulation of other molecules that promote expansion of the desmoplastic stroma characteristic of pancreatic carcinoma.

Aberrant signaling through KRAS may also indirectly promote collagen production and further promotes tumor invasion and progression.

Reactivation of downstream mediators in pancreatic cancer bypass the EGFR pathway, allowing resistance to develop.

Such resistance mechanisms include the PI3K/AKT and Hedgehog pathways.
Biomarkers in Colorectal Cancer

- Chromosomal instability, which involves DNA copy number variation, may provide prognostic information in CRC\textsuperscript{10}
- Recent studies indicate that microsatellite instability, DNA methylation and DNA copy number may be helpful towards selection of an appropriate treatment plan
- Multiple signaling pathways have been implicated in the pathogenesis of mCRC\textsuperscript{38,39}
- Aberrant signaling through the RAS/RAF pathway occurs as a consequence of mutations in \textit{KRAS}, \textit{NRAS}, or \textit{BRAF} in mCRC—these aberrations in genes and proteins may impact treatment pans\textsuperscript{38,40}
- Oncogenic mutations in phosphoinositide-3 kinase (PI3K) are present in 12–30\% of colorectal cancers\textsuperscript{42,43}

**Genetic mutations in chromosomal instability–positive colorectal cancers\textsuperscript{41}**

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<thead>
<tr>
<th>Oncogenes</th>
<th>Tumor suppressor genes</th>
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<td>\textit{KRAS}: Cell proliferation, survival, and transformation</td>
<td>\textit{APC}: Inhibition of Wingless/Wnt signaling; cytoskeletal regulation</td>
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<tr>
<td>\textit{CTNNB1}: Regulation of Wnt pathway target genes that promote tumor growth and invasion</td>
<td>\textit{TP53}: Cell cycle arrest, apoptosis induction</td>
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<td>\textit{PIK3CA}: Cell proliferation and survival</td>
<td>\textit{SMAD4, SMAD2}: Intracellular mediators of the TGF-(\beta) pathway</td>
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<td>\textit{DCC}: cell surface receptor for netrin-1</td>
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Patients with mCRC having *BRAF* mutations have been shown to have decreased survival as compared with patients with unmutated *BRAF*.

The prevalence of different mutations that affect the RAS/RAF pathway in mCRC patients has been analyzed—overall, mutations in *KRAS* exon 2 have been observed in ~40% of mCRC.

Other distinct mutations in *KRAS*, as well as mutations in *NRAS*, account for another ~10% of patients with mCRC.

Taken together, this indicates that mutations in *NRAS* and *KRAS* may affect up to 50% of patients with mCRC.

Mutations in *BRAF* affect 8–10% of all mCRC patients, and up to 15% of mCRC patients with unmutated *KRAS*.
Evaluating Biomarkers With Companion Diagnostics May Help Inform Treatment Plan Decisions

- Increased understanding of the key molecular pathways in carcinogenesis has resulted in the identification of a variety of biomarkers\(^1,\,12\)

- Development of diagnostic tests based on biomarkers may help stratify patients into subpopulations that differ in their susceptibility to a different disease or their response to a specific treatment plan; which may include diet, therapy, and exercise\(^4,7\)

- A companion diagnostic (CDx) is an in vitro diagnostic (IVD) device or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product\(^2\)

- A CDx may identify the patients who are more likely to benefit or have increased risk of serious adverse events from treatment with a particular therapeutic product

- Biomarker-based CDx must demonstrate both analytic and clinical validity to achieve regulatory approval, and the robustness of the development process must be ensured and documented\(^1,\,2\)

- Additional investigation must demonstrate the relevance and usefulness of CDx

Identification of biomarkers and development of diagnostic tests may be used to determine whether certain patients may differ in their responses to specific treatment plans\(^1,\,2\)
• IVD, a reagent, instrument, or system intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae.\(^1\) Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body.

• Laboratory developed tests are designed, manufactured, and used by a single laboratory

• In contrast, IVD kits are generally developed by conventional manufacturers, submitted for FDA review, and if cleared or approved, are sold to labs, hospitals, and/or physicians’ offices, where these kits are used to perform the tests

• Deeper sequencing and more extensive mutational analysis (eg, next generation sequencing of multiple genes) is demonstrating the presence of biomarker mutations\(^48\)

• The FDA recognizes that regulatory oversight of CDx to ensure that tests “provide a reasonable assurance of safety and effectiveness, while also fostering innovation and progress in personalized medicine”\(^1\)
Amgen is Committed to Advancing the Science of Personalized Medicine

- New information is continuously emerging that refines our understanding of the molecular pathways and biomarkers that drive the carcinogenic process.
- Research to evaluate biomarkers in gastrointestinal malignancies is ongoing.
- CDx may play a role in predicting likelihood of response, patients unlikely to respond, or individuals at greater risk of adverse effects to a given treatment plan.
- Integrating biomarkers into routine clinical practice may aid in detection, diagnosis, and treatment planning.
- Amgen remains committed to the science of personalized medicine and biomarkers.
Definitions

A **biomarker** is a characteristic that can be scientifically measured and evaluated to serve as an indicator of a normal biologic process, a disease, or as a response to a therapeutic intervention. Biomarkers are generally measured using either a diagnostic test, IVD test, imaging diagnostic, or other objective measurement method.¹

**Prognostic markers** provide information regarding the natural history of a disease; they are useful in predicting probable clinical outcomes; prognostic enrichment strategies (those with a greater likelihood of having a disease-related endpoint event or a substantial worsening in condition) for example, could be selection of high-risk patients to include in a trial.¹³⁸

**Predictive biomarkers** provide information regarding the likelihood of response to a particular therapy or patients who may or may not benefit from certain treatments. Thus, a trial employing a predictive enrichment strategy would include patients more likely to respond to a particular intervention or treatment plan.³⁸

A **companion diagnostic (CDx)** is an IVD device or an imaging tool that provides information that is essential for the safe and effective use of a corresponding therapeutic product.¹²
References
