

**Translational Medicine-Driven
Multi-Component Predictive Biomarkers
as an Emerging Enabler of
Personalized Medicine**

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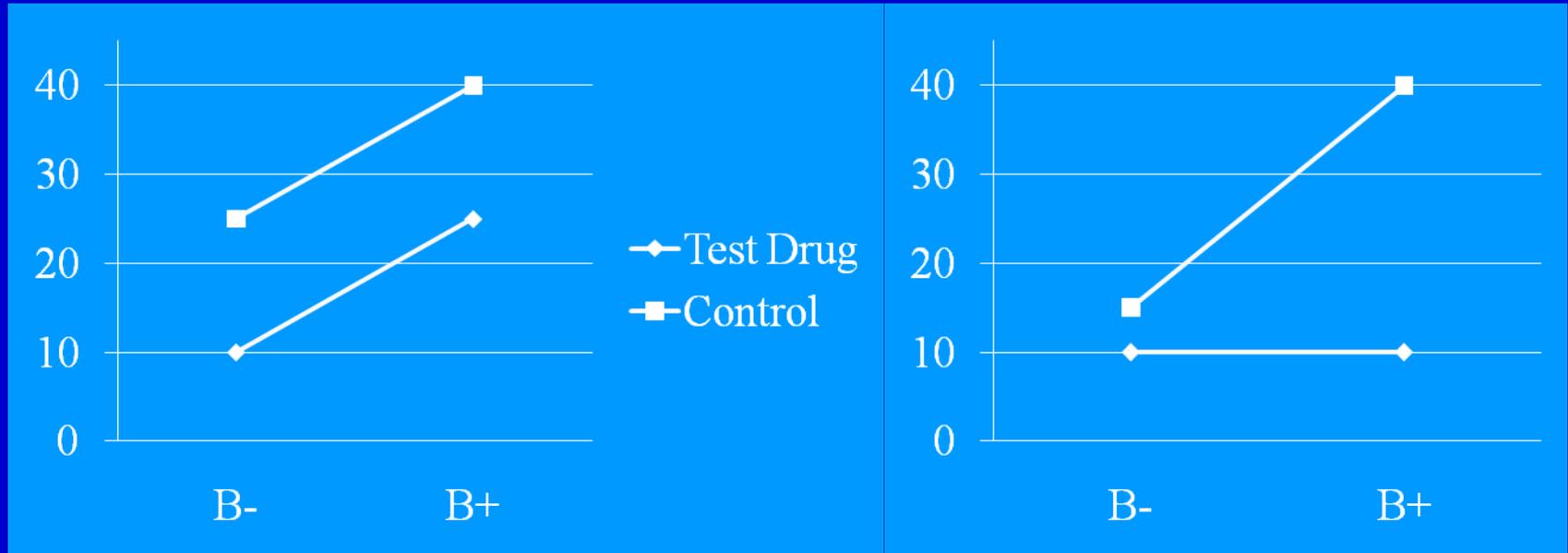
Outline of Presentation

- Introduction & Definitions
- Examples of Single Component Predictive Biomarkers
- Overview of this Suggested New Approach for Multi-Component Predictive Biomarkers
- Mechanisms of Primary & Secondary Resistance
 - Detailed Examples in CRC & NSCLC
- Further Methods to Identify Pts with Best Benefit-Risk
- Role of Phosphoproteomics in Predictive Biomarkers
- Brief Overview of Pre-Clinical & Early Clinical approaches to Identify most Critical Components out of the Set
- Summary & Conclusions

Historically Low Success Rates in Oncology Drug Development

- 5%-8% of Oncology Compounds Entering Man ultimately Obtain FDA Approval [Kola & Landis, 2004; Kaitin, 2008]
- Phase III failure rate for oncology reported at 66% [BIO & Biomed Tracker report, Feb. 2011]
- **80% of Phase III Oncology Failures are Due to Lack of Efficacy** [2006 *In Vivo* study]
- New Approaches to Cut these Failure Rates is area of great research
- Smith (2011) describes 27 different reasons for many of these failures
- Focus of current talk is on the development of certain kinds of multi-component predictive biomarkers to eliminate many of these reasons for failure.

Prognostic vs. Predictive Biomarkers



Prognostic Biomarker

Predictive Biomarker

Some Examples of Predictive Biomarkers within Oncology Drug Labels

| | | |
|--------------------------------------|-----------------------|---|
| Imatinib, Dasatinib, Nilotinib | CML | <i>BCR-ABL</i> translocation |
| Trastuzumab, Lapatinib | met. Breast Cancer | <i>HER2</i> gene copy # (originally considered <i>HER2</i> receptor expression for trastuzumab) |
| Cetuximab, Panitumumab | met. CRC | <i>KRAS</i> mutation absence (retrospectively derived) |

All of above are single component predictive biomarkers

Some Very Recent Successes in Oncology with Single-Component Predictive Biomarkers

Recently seen a few cases of drugs with outstanding efficacy:

- PLX-4032 in melanoma pts with B-RAF V600E mutation
 - Phase III: HR=0.37 for OS, HR=0.26 for PFS; Phase II: RR=53%
- Crizotinib in NSCLC pts that are *ALK*-positive
 - Phase I expanded cohort: RR=57%
- Olaparib in breast cancer and in ovarian cancer pts with *BRCA1* or *BRCA2* mutation
 - Phase II Br. canc. RR=41%; Ov. canc. RR=33% - pts on 400 mg *bid*

Development of Multi-Component Predictive Biomarkers

- Main part of presentation is:
 - Bottom-Up approach to Prospectively Rationalize Factors for (potential) inclusion in Multi-Component Predictive Biomarkers.
- Final part of presentation (covers very briefly):
 - Methods to Reduce this Set to the Most Critical Components, and how to convert this into a Validated Predictive Biomarker

Currently the factor(s) that could have been included in predictive biomarkers are generally Identified Too Late

Development of Multi-Component Predictive Biomarkers, cont'd

The Factors Included within Multi-Component Predictive Biomarkers by approach advocated here are derived from:

- Efficacy Signaling Pathways
- Safety Signaling Pathways (where this can be anticipated)
- Pharmacokinetic factors

Development of components related to efficacy is considered in greatest depth in this talk, making use of:

- Very broad view of the disease process
- Working Backwards from the Clinical to the Biological
- Working Forwards from refined Biological understanding to the Clinical

Impacts of Efficacy Target & of Patient Subpopulation

Two typical scenarios in early development, w.r.t. Efficacy Signaling (for drugs that do reach their target):

1. Drug Target is Inappropriate, but this is not yet recognized

Whatever subpopulation we use then the drug's impact on target is insufficient to meaningfully impact disease

In some cases target would be appropriate if drug directed at a 2nd particular target is given as add-on (before, after, or simultaneously)

Impacts of Efficacy Target & of Patient Subpopulation, cont'd

2. Drug Target is Appropriate, but drug's Degree of Efficacy Varies greatly with certain characteristics of the patient(s)
 - Historically Drug Developed in Full Population for a given stage of the particular cancer (allowing only for # of failed treatments)
 - Use of such Unselected Populations Contributes to High Drug Failure Rate in Oncology
 - Recent move towards Developing Drug in Subpopulation(s) where Drug Works Well
 - Requires the **Identification of Predictive Biomarker(s)**, which needs to be **started Early**, and carried out **Rigorously**
 - When done in this way it can help **Cut Oncology Failure Rate**, and Lead to **Faster Development**

Complexity of Cellular Signaling in Oncology is Much Greater than Often Assumed

Woodcock et al (2011, NEJM) article on "Development of Novel Combination Therapies" comments that:

"Cellular pathways operate more like webs than superhighways. There are multiple redundancies, or alternate routes, that may be activated in response to inhibition of a pathway. This redundancy promotes the emergence of resistant cells or organisms under the selective pressure of a targeted agent, resulting in drug resistance and clinical relapse. For this reason, combination therapies are often needed..."

See also "Guidance for industry co-development of two or more unmarketed investigational drugs for use in combination", December 2010

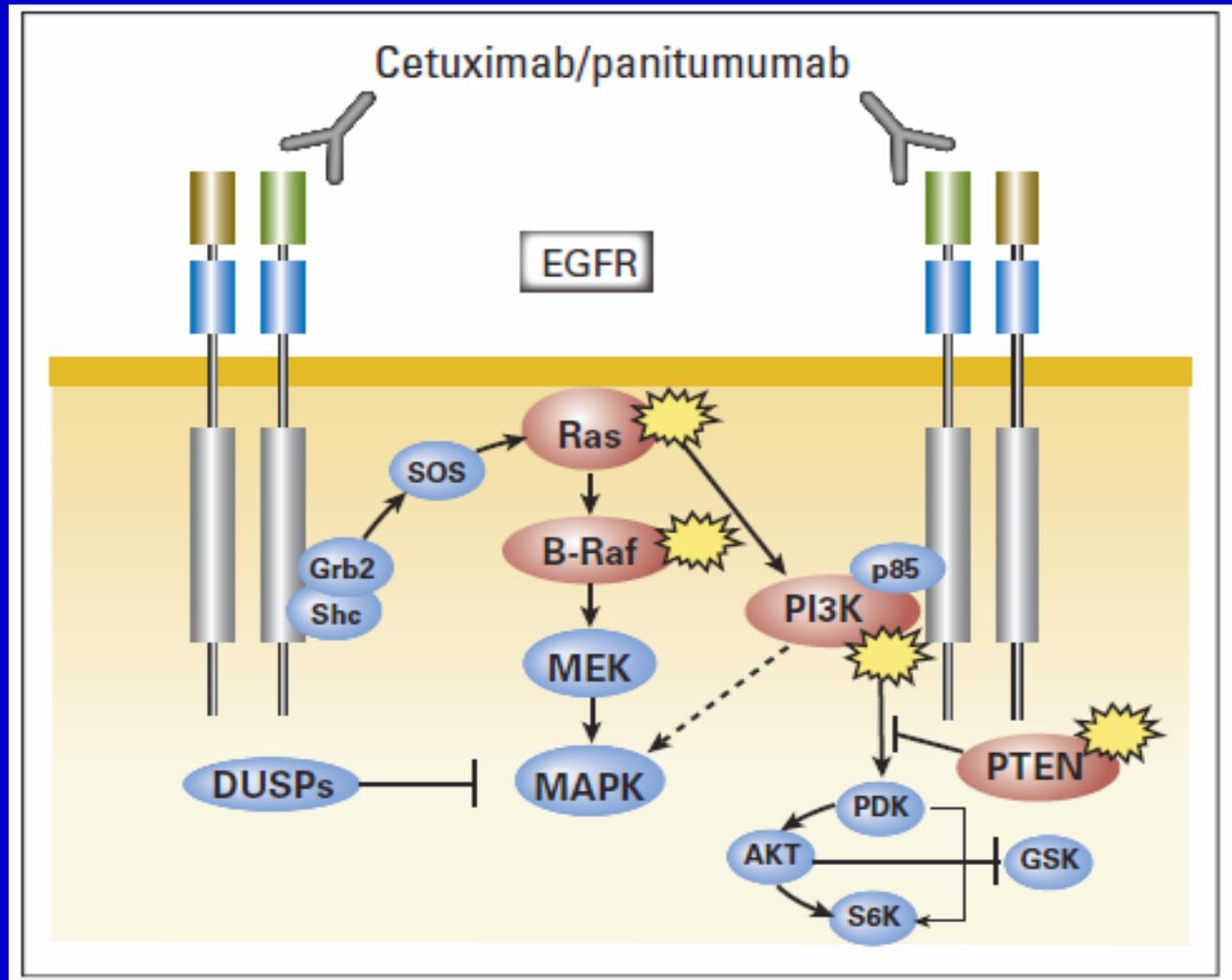
Two Approaches to Identify Potential Components of Predictive Biomarkers

- Identification of components to potentially include within a Predictive Biomarker will be Approached here from two directions. We consider in order:
 - Resistance Mechanisms that lead to **Drug NOT Working** in pts with particular characteristics
 - e.g., *KRAS* mutation in CRC pts when treated by EGFR MABs
 - Mechanisms that identify Pts in whom the **Drug is Expected to Work** well
 - e.g., *HER2* gene copy # in met. Br. C pts treated by trastuzumab

Mechanisms for Primary & Secondary Resistance in Oncology

- **Primary Resistance** - Certain patients who never get any (or only get limited) benefit from the drug
- **Secondary Resistance** - Patients who initially respond to drug but who ultimately develop resistance
 - Need to overcome to get Durable Long-Lasting Response
- The next few overheads cover examples of mechanisms of primary and secondary resistance
 - **The mechanisms of resistance will be different for each Drug** (in a given indication & given set of additional anti-cancer meds)
 - However, **Several Recurring Themes of Resistance** can be **Identified**

Some Key Pathways from the EGF Receptor in Metastatic Colorectal Cancer



Possible Mechanisms of Primary Resistance to EGFR MAbs in EGFR-expressing Metastatic CRC

- Patients with *KRAS* mutation (in codons 12 or 13) found from retrospective analysis to have no benefit from EGFR MAbs
 - Predictive biomarker based on having wild type (unmutated) *KRAS* now in labeling for both approved EGFR MAbs
 - Absence of effect in mutated *KRAS* likely due to constitutive activation (always on signaling) via K-Ras which by-passes blockade of EGFR

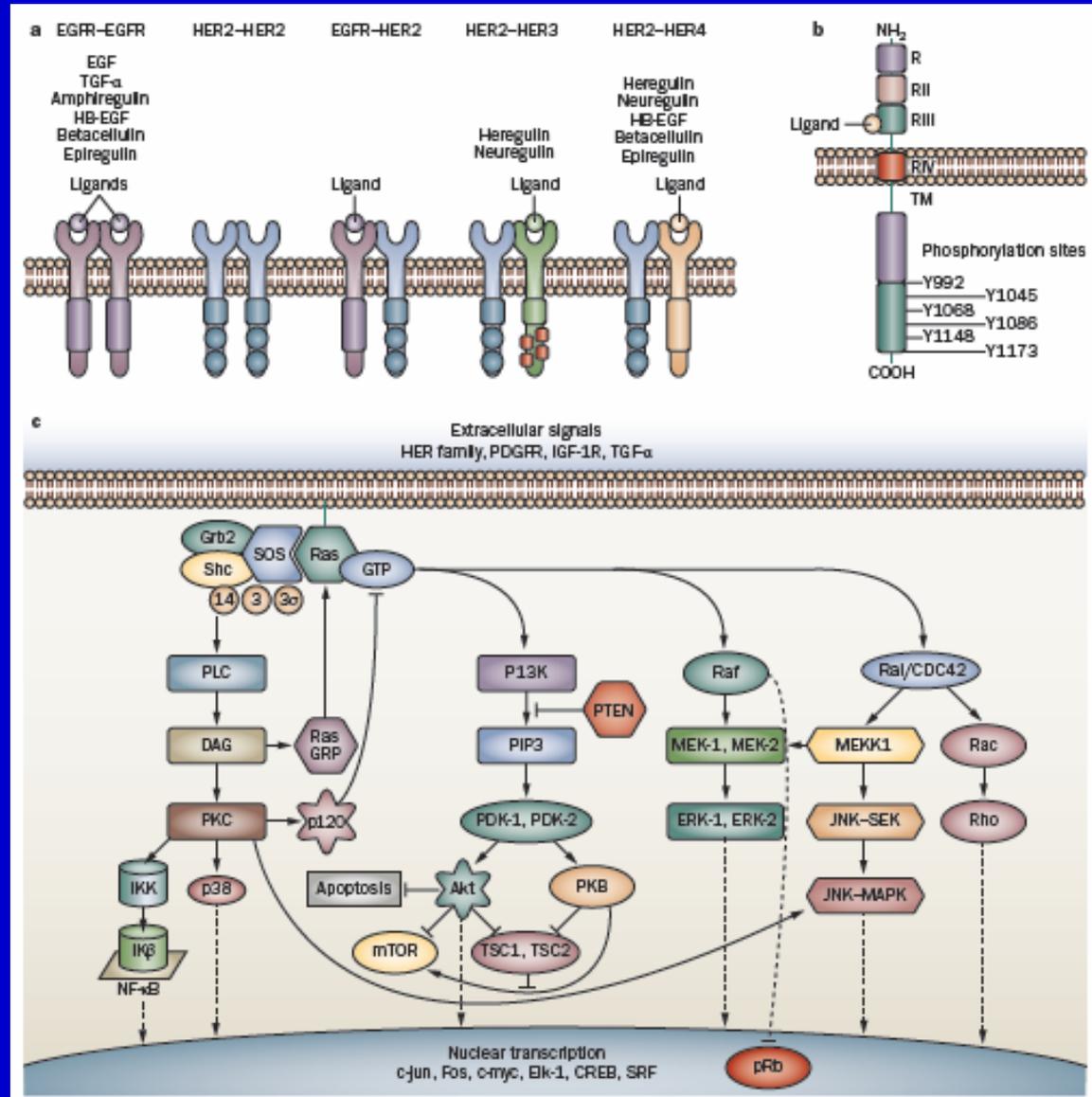
Possible Mechanisms of Primary Resistance to EGFR MAbs in EGFR-expressing Metastatic CRC, cont'd

- Many further primary resistance mechanisms found (with various degrees of clinical or pre-clinical evidence):
 - Absence of B-Raf V600E mutation possibly a weak predictive factor (but a very strong prognostic factor)
 - *PTEN* & *PIK3CA* mutations possibly predictive but evidence is contradictory
 - For *PIK3CA*, mutations at exon 20 & at exon 9 likely have different impact
 - Use of a quadruple negative predictive biomarker (-ve for mutations in *KRAS*, *BRAF*, *PIK3CA*, and *PTEN*) put forward by Bardelli & Siena (2010), but area needs further research

Possible Mechanisms of Secondary Resistance to EGFR MAbs in EGFR-expressing Metastatic CRC

- Relatively little found on mechanisms of secondary resistance except:
 - ADAM-17 found *in vivo* to be increased in CRC by 5-FU, giving increased levels of TGF- α (plus other EGFR ligands), and then increased activity of EGFR, IGF-1R, and VEGFR2/3

Some Key Pathways from the EGF Receptor in NSCLC



Possible mechanisms of Primary Resistance to EGFR Tyrosine-Kinase Inhibitors in NSCLC

- Two EGFR tyrosine kinase inhibitors (TKIs) have been marketed in the US (erlotinib and gefitinib) for NSCLC
 - Erlotinib did demonstrate stat. sig. effect on OS in 2 settings, although proportion benefiting is clearly limited
 - Gefitinib originally gained AA on small RR effect, but later not show effect on OS
- ASCO Provisional Clinical Opinion recs. giving EGFR TKI as 1st-line treatment in NSCLC only in pts with *EGFR* mutations [Keedy, et al, 2011]
 - Largely based on IPASS study findings showing TKI benefit (HR=0.48) in pts with mutated *EGFR*, but worsening (HR=2.85) in pts without a mutation in *EGFR*

Possible mechanisms of Primary Resistance to EGFR Tyrosine-Kinase Inhibitors in NSCLC, cont'd

- Site of mutation in *EGFR* is critical
 - Deletions in exon 19, or L858R in exon 21 (which confer ligand-independent activation) Predict Benefit from TKIs
 - Point mutation T790M (in exon 20) leads to Primary Resistance
- Pts w' *KRAS* mutations also found to have primary resistance

Possible mechanisms of Secondary Resistance to EGFR Tyrosine-Kinase Inhibitors in NSCLC

- Point mutation T790M in EGFR found in approx. 50% of pts with acquired resistance
- *MET* gene amplification found in approx. 20% of pts with acquired resistance - possibly by HER3-dependent activation of PI-3 Kinase
- Some evidence that IGF-1R can cause acquired resistance to TKIs in NSCLC by forming heterodimers with EGFR
 - IGFBP3 found to combat this

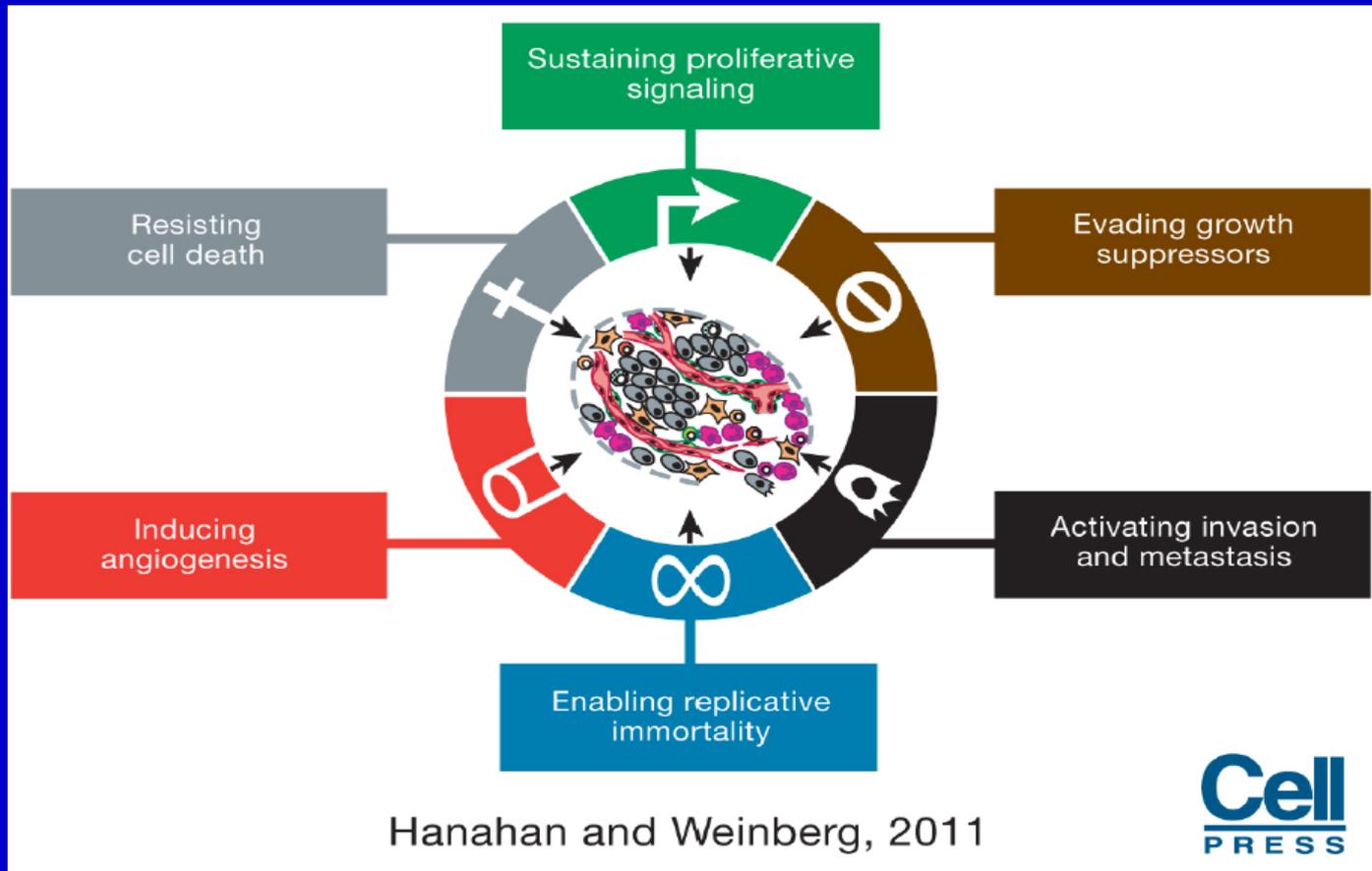
Some other Mechanisms of Primary or Secondary Resistance seen in Oncology

- Examples of Other Mechanisms/Pathways Associated with Primary or Secondary Resistance (with various degrees of clinical or pre-clinical evidence), include:
 - Trastuzumab: Overexpression of p95^{HER2} (truncated HER2 receptor); Signaling via HER2/IGF-1R heterodimers; Loss of PTEN signaling and/or *PIK3CA* mutations; Hyperactivation of EGFR or of HER3; Pathways via MET receptor; SRC hyperactivation (particularly when phosphorylated at Tyr416)
 - Tamoxifen: Enhanced signaling via EGFR & HER2; Overexpression of ER α 36 (a splice variant of ER α 66); Expression level of ER β ; ER α phosphorylated at 2 specific residues
 - PLX-4032: Secondary resistance via: A-RAF or C-RAF pathways; other activators of MEK

Mechanisms often Leading to Lack of Efficacy (or Short Duration Efficacy) - Recurring Themes

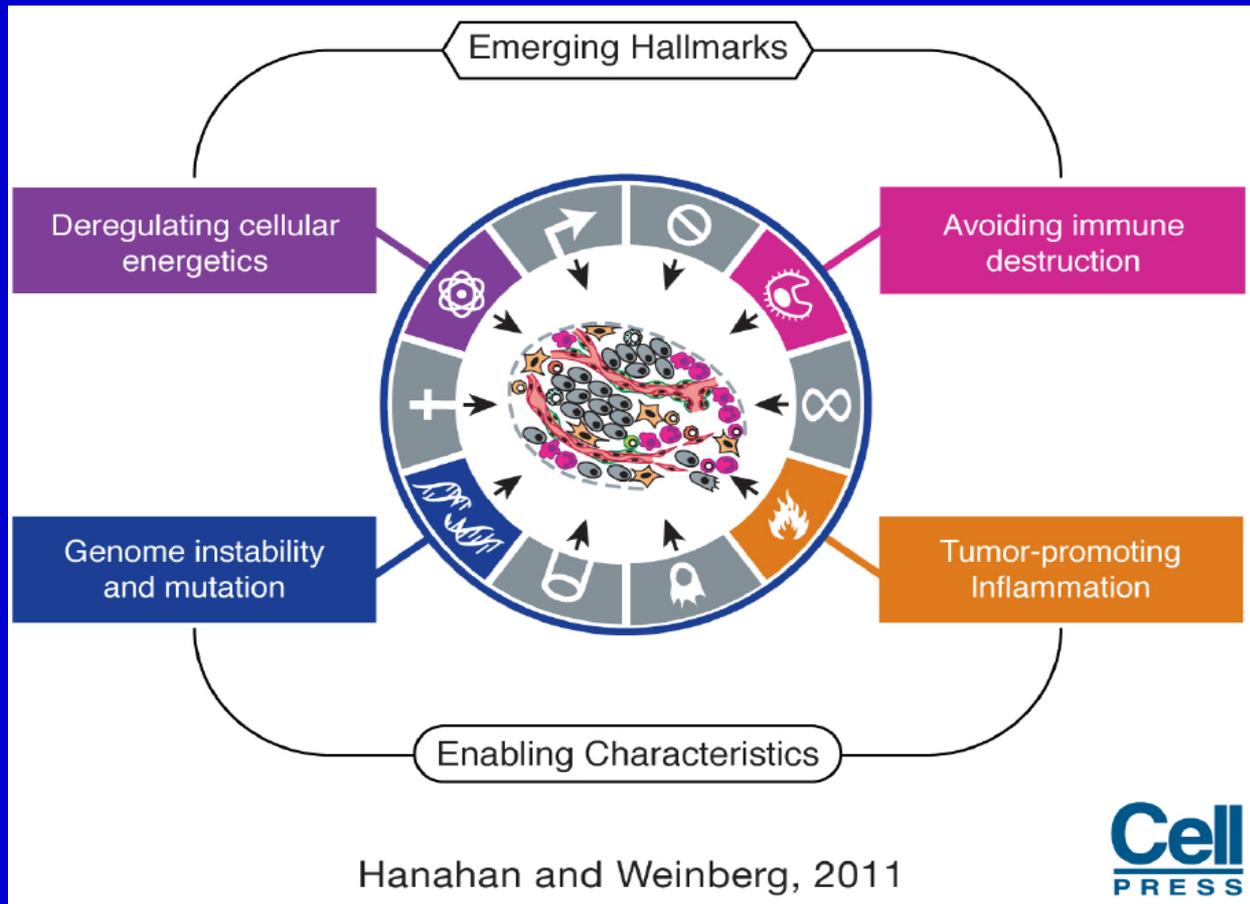
- Common Primary Resistance Mechanisms
 - Presence of key Downstream Effector(s) in "Always On" State(s)
 - Presence (or Absence) of Specific Mutations in Target itself (for many TKIs)
 - High Expression level of Alternative Forms of Target that drug does not impact, but which also transmit signal that drug seeks to block
- Common Secondary Resistance Mechanisms
 - Development of Additional Mutations in the Target (e.g., at drug's binding site on kinase for some TKIs)
 - Transactivation (via GPCR, SRC, ADAM-17 & related proteases, etc.) producing increased levels of ligand(s)
 - Hyperactivation of Closely Related Receptors, or upregulation of other signaling that leads to same effect (e.g., also leads to cell proliferation)

Hallmarks of Cancer - 2011



It is not just signaling pathways via growth-factor receptors leading to Cell Proliferation that are important. **Mechanisms leading to Invasion & Metastases are Responsible for Most Cancer Deaths** [Lazebnik, 2010]

Hallmarks of Cancer - 2011, cont'd



Inflammation: $IL1\beta$, $TNF\alpha$, $IL6$, and $RANKL$ all activate inflammation & **augment tumor cells' ability to metastasize**

- $NF-\kappa B$, $STAT3$, $STAT5$, and $IKK\beta$ each found in several cancer types to have "always on signaling" (constitutive activation)

Other Mechanisms that Often have a role in Drug Response or in Drug Resistance

- Protease Activity levels - particularly those which release ligands, cleave receptors & kinases, are involved in apoptosis, or are involved in breakdown of ECM leading to Invasion & Metastasis
- DNA methylation - silencing of certain genes, including those encoding for tumor suppressor proteins such as p53 and p16
- Micro RNAs - multiple types of small non-coding RNAs that regulate gene expression, inhibit protein translation, etc.
- Cancer Stem Cells - thought to have a role in cancer drug resistance

Further Methods for Identifying Patients Most Likely to have Good Benefit/Risk

- Efficacy - For the Target, as well as all proteins in Upstream, Downstream, & critical Interacting Pathways consider:
 - Mutations at particular site(s), SNPs (or other polymorphisms), increased Gene Copy Number, etc., and for each of these proteins:
 - Allow for Disparity Sometimes Seen (Stoecklein & Klein, 2010) between Mutations in Primary Tumor vs. Mutations at Particular Metastatic Sites
 - Levels of endogenous ligands, endogenous antagonists (e.g., IGFBP3)
- **Level of these Protein(s) in their Active Form** are generally **More Useful** than total protein level

Further Methods for Identifying Patients Most Likely to have Good Benefit/Risk, cont'd

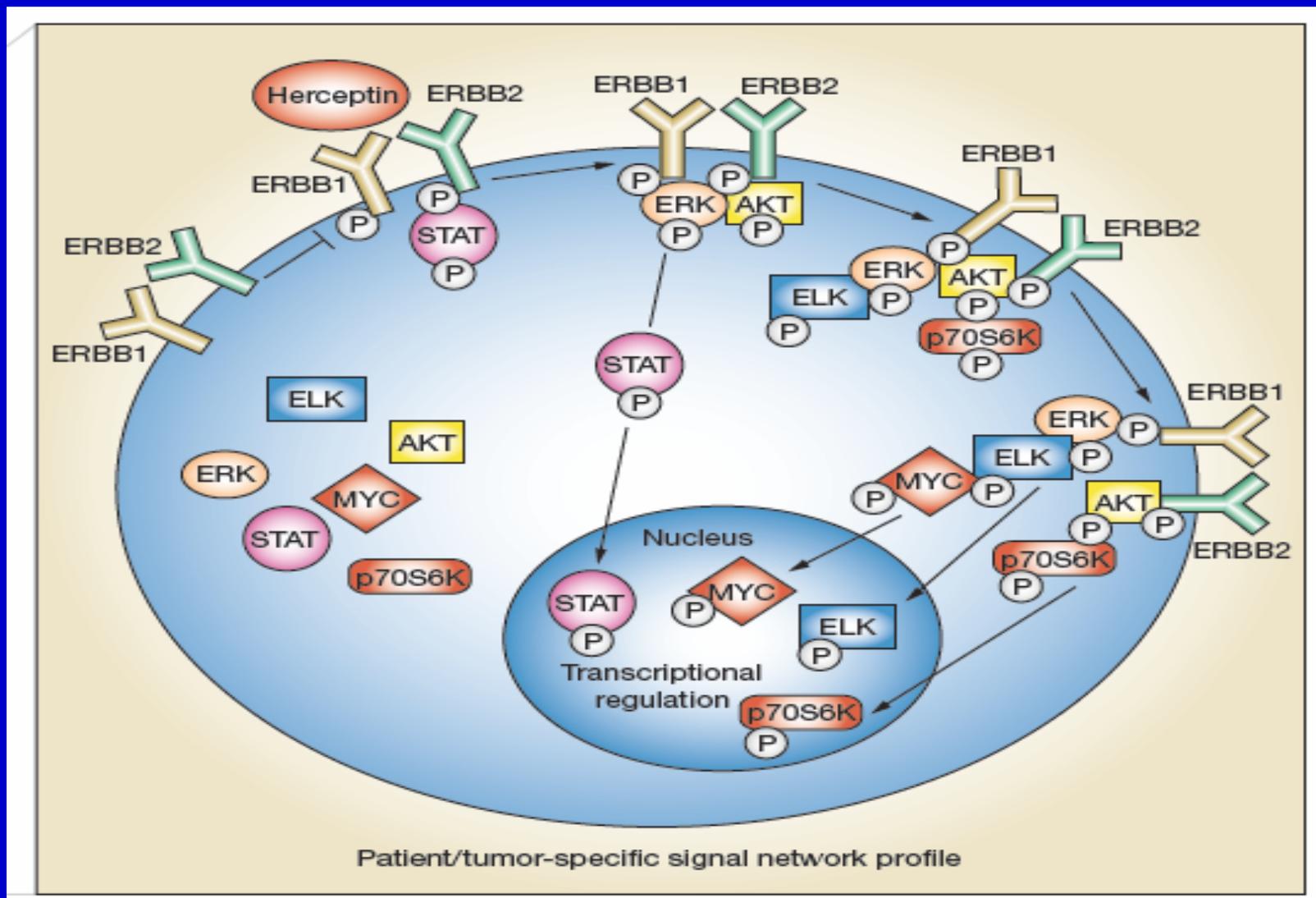
- Proteins in Safety Pathways (where this is able to be anticipated) can be assessed as for efficacy pathways
- Pharmacokinetics related to Drug Metabolizing Enzymes (& Drug Transporters)
 - Any SNPs (or other gene variants) corresponding to **Poor Metabolizers** leading to high drug levels are likely to cause **Safety Problems**, e.g. Irinocetan & homozygous for *UGT1A1**28
 - Any SNPs (or other gene variants) corresponding to **Ultra-Rapid Metabolizers** leading to low drug levels are likely to cause **Problems with Lack of Efficacy**

Approach advocated here is Bridging Silos as regards type of data, i.e., Pharmacogenomic, Proteomic, Phosphoproteomic, Metabolomic, etc.

Impact of The Newly-Emergent Field of Phosphoproteomics

- Kinases exert their actions by Phosphorylation of particular proteins
 - High proportion of Drug Targets in oncology are Kinases, e.g., for TKIs
 - Most (if not all) Drugs have some Impact on Phosphorylation
- Phosphorylation (phos.) of particular Proteins modifies (usually increases) their Activity Level
 - Being phos. at different sites leads to different effects on the target protein
- Phosphoproteomics (proteomics of phos. proteins) can Assess Levels of key Signaling Proteins in their Phos. State(s)
 - Recently incorporated within a few Phase 0 & Phase 1 studies
 - Some phosphoproteomic measures can be prognostic & others can be predictive
 - Has also been used to obtain early efficacy assessments

Impact of The Newly-Emergent Field of Phosphoproteomics



Some Predictors of Response (or Resistance) Based on Phosphoproteomics

- Examples include (with various degrees of pre-clinical or clinical evidence):
 - High levels of co-expressed p-HER2 & p-HER3 highly predictive of response to Lapatinib in inflammatory Br. C
 - p-SRC at tyr416 (leading to hyperactivation) associated with resistance to trastuzumab in met. Br. C
 - p-AKT at ser473 associated with benefit of paclitaxel in node positive Br. C
 - p-ER α at 2 specific residues associated with resistance to tamoxifen in Br. C

Assessment of Net Effects of Signaling **by Use of Phosphoproteomics**

- Can use to **Measure Activity Level** of each of **Multiple Proteins** in the Efficacy Signaling Pathways of interest for a given drug.
- **Assesses Net Effect of All Signaling thro' that Protein** (allowing for Interacting Pathways, Feedback Mechanisms, etc.)
 - **Particularly useful for assessing Activity** (phos. level at a given residue) **of Downstream Proteins** such as ERK1/2, p70 S6 kinase, or p90 S6 kinase that **Integrate Many of the Signals for Cell Proliferation**, etc.
 - Similarly for downstream markers that integrate many of the signals for **Cell Migration, Apoptosis, Cell Cycle arrest**, etc.
 - This is Getting Much Closer to Clinical Measures

Pre-Clinical & Clinical Development of a Multi-Component Predictive Biomarker - Brief Overview

- Exploratory studies assessing each potential component of predictive biomarker need to begin Pre-Clinically, e.g., in mice with xenografts of human cancer cell lines (but better *in vivo* animal cancer models are under development)
 - This may give drug mechanism insights (via post-treatment biopsies of the mice linked to outcomes) leading to eliminating certain biomarker components
- In Phase I if subset of pts agree to post-treatment biopsy then it can more reliably inform re treatment impact on downstream effectors (in active state) and inform re mechanisms of drug resistance
- Enriched Phase I expansion cohorts have been considered, e.g., with PLX-4032

Note: Single arm trials on their own cannot separate prognostic from predictive biomarkers

Pre-Clinical & Clinical Development of a Multi-Component Predictive Biomarker - Brief Overview, cont'd

- Phase II options, depending on level of knowledge
 - If strong knowledge already that Biomarker +ve (B+) benefit but Biomarker -ve (B-) do not, then Enrichment Design in B+ pts could be used (subject to regulatory agreement)
 - If Biomarker fully developed but less evidence that drug does not work in B- then design stratifying by B-/B+ likely needed
 - If Biomarker needs further development (e.g., further identification of key components or determination of cut-point) then certain types of Adaptive Design may be useful, e.g., Adaptive signature design, Biomarker-adaptive threshold design
- **Prior to Phase III we need to have:**
 - **Fully defined predictive biomarker** (including any classifier)
 - **Completed Assay validation** for all retained components of biomarker

Multi-Component Predictive Biomarker: Simple Composite Variables or "Classifiers"

- Using a Multi-Component Predictive Biomarker **Does Not Mean** that you are **Left With a Very Small Subpopulation**
 - The quadruple negative biomarker (no mutation in any of *KRAS*, *BRAF*, *PIK3CA*, and *PTEN*) put forward by Bardelli & Siena (2010) still retains 40% of original met. CRC population
 - Olaparib Phase II studies considered mutation in either *BRCA1* or *BRCA2*
 - Phosphoproteomic Measures (of downstream proteins in their active state) may avoid eliminating as many patients because they **Reflect Integrated Signaling**
 - Classifiers (see Simon, 2008) will sometimes be needed (as with the OncoType Dx prognostic biomarker)
 - **Classifiers Combine Measurements statistically** (with different weights for each component) then **Use a Cut-Point to Separate Pts** into those:
 - **Most Likely to Benefit from the Drug**
 - **Least Likely to Benefit from the Drug**

Multi-Component Predictive Biomarker: **Defined Retrospectively After Failed Phase II or Failed Phase III**

- **If a Phase II or Phase III trial fails for lack of efficacy then it may be worth considering the retrospective development of a multi-component predictive biomarker**
 - The bottom-up signaling pathways based approach described here could be used to identify molecular characteristics of patients in whom drug worked best
- **Requires availability of archived tumor tissue from trial together with details on the patient's outcome (PFS, OS, or even just RR).**
- **Some further considerations:**
 - Need to be wary of spurious findings given that this would be retrospective
 - Bottom-up signaling pathways led approach would reduce this problem
 - Needs regulatory discussion before continuing development with an enriched (B+) randomized Phase III trial

Summary

1. Identification of potential Efficacy Components of Multi-Component Predictive Biomarkers is possible using the Bottom-Up Signaling Pathways-based method described here. This approach:

- Is relatively **Low Cost**
- Is complex, but **degree of complexity can be reduced** due to modular nature of signaling pathways (Fishman, NIBR, 2005)
- **Requires individuals with expertise and skills to:**
 - **Translate Clinical findings into the Biology**
 - **Translate the Refined Biological understanding back into the Clinical**
 - Step outside of their current paradigms and consider the particular disease process very broadly

Summary, cont'd

2. The set of Most Critical Components within such a Multi-Component Predictive Biomarker can then be Determined.
 - **Strategies that Enable Identification of the Most Critical Components** within Pre-Clinical & Early Stage Clinical Trials have been highlighted, while still Retaining a sufficiently Large Subpopulation
 - **Phosphoproteomic Measurements** (at baseline) of Activity Level for Downstream Proteins (**reflecting Integrated Signaling**) offer great **promise** as key Components of Predictive Biomarkers:
 - Example: **Phosphorylation levels for Protein(s) most closely related to Cell Proliferation**
 - Phosphoproteomics has the **potential to greatly reduce the # of components that are needed within the final Predictive Biomarker**

Conclusions

- The **Approaches Covered Here Are Now Possible**, and can be applied by taking into account various degrees of complexity:
 - Considering just the **Drug Target** together with All (or even just a key subset of) **Proteins** that are **Upstream or Downstream** (together with the Drug Metabolizing Enzymes) is likely to **give large gains**.
 - Also **taking into account the Interacting Pathways** and other disease mechanisms, seems likely to **give rise to even greater gains**.
- The **Early** identification of **Appropriate Multi-Component Predictive Biomarkers** in this way should: **Decrease Cost, Decrease Time, and/or Increase Chance of Success**
 - Most Importantly it should **Greatly Reduce the Phase III Failure Rate in Oncology**

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Supplementary Overheads

Characteristics of Team Needed to Implement Approach Described here

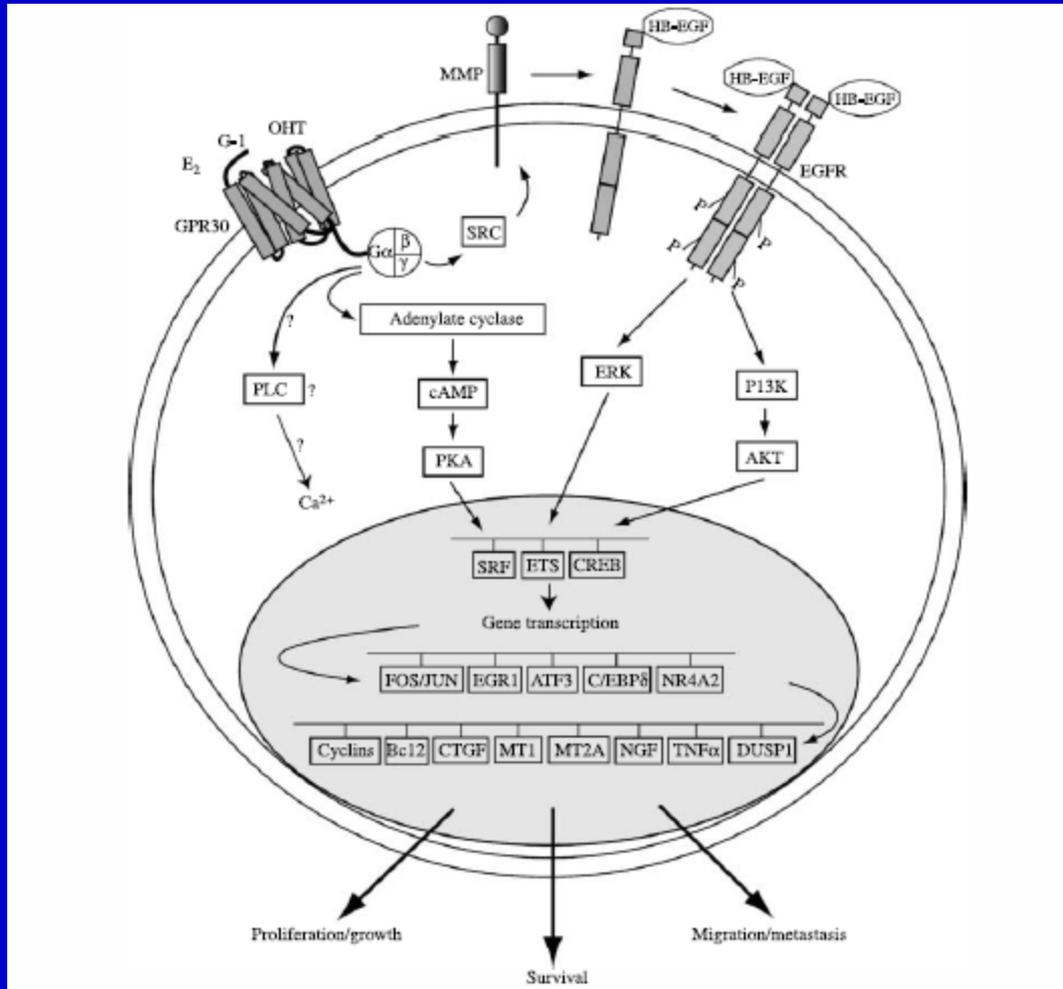
This Translational Medicine based approach needs sets of Individuals within the development team with at least the following skill sets:

- Understanding of the whole drug development process from discovery to approval
- Ability to translate clinical findings (on test drug, and on past trials for approved and failed drugs) back to the biology
- Ability to translate biological (particularly signal transduction) findings (from discovery/pre-clinical studies of test drug, from literature on newly recognized mechanisms in targeted disease, etc.) into clinical implications
- Ability to consider disease process very broadly, and how it differs from the non-disease state
- Ability to step outside of their paradigms, and not dismissive of other theories

Characteristics of Team Needed to Implement Approach Described here, cont'd

- Experience with assay development for each type of component of the predictive biomarkers, as well as experience/expertise with assay validation
- Experience/expertise with Predictive Biomarker validation
- Statistical Expertise, particularly relating to prognostic vs. predictive factors, sensitivity/specificity, validation, spurious findings, multiplicity, Adaptive Designs (e.g., Adaptive Signature design, biomarker adaptive threshold design), Enrichment Designs, methods to identify the most critical components within the multi-component predictive biomarker, & methods for combining these components into a classifier (where necessary)
- Regulatory experience/knowledge in drug/diagnostic co-development, and in the particular regulatory expectations when developing predictive biomarkers that are multi-component.

Contribution of Transactivation to Resistance by Increasing Levels of Endogenous Ligand(s)



Receptors such as EGFR, HER2/3/4 have been shown to be activated in certain cells by Transactivation. This has been found to occur via pathways from multiple different receptors which cause particular proteases to release extra amounts of the ligands for EGFR, HER2, HER3, and/or HER4.