MARY CROWLEY CANCER RESEARCH
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Personalized (aka Precision) Therapy-A Systems Analysis of Captured Molecular Data

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There are eight hallmark biological capabilities necessary for cancer development and persistence: (1) sustained proliferation, (2) evasion of growth suppressors, (3) death resistance, (4) replicate immortality, (5) angiogenesis, (6) invasion ± metastability, (7) reprogrammed energy metabolism, and (8) immune evasion. These capabilities support one or more of three core processes, i.e., cell survival, cell fate and genome maintenance. These core processes are sub served by one or more of 12 signaling pathways.

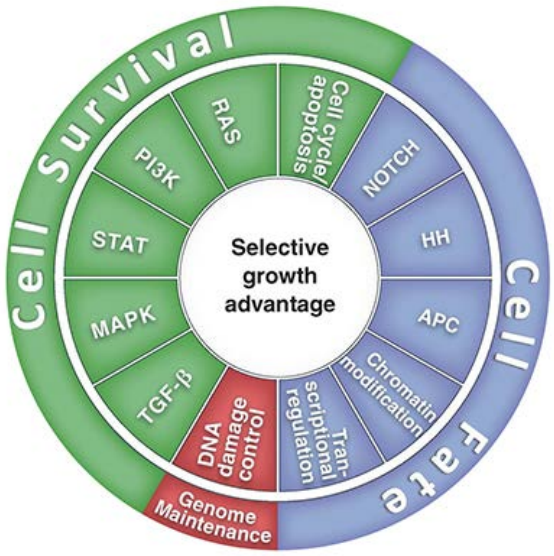

Figure 1. Twelve signaling pathways, which are responsible for 3 cancer core functions involving cell survival, fate, and genome maintenance.

Blocking the **cancer specific relevant pathway[s]** underlying the core processes will cripple cancer persistence and progression (Figure 1).

Fortunately, the multigenomic/proteomic components of each of the relevant pathways are dependent on a limited number of aberrant and rewired "hub" elements comprising "driver" genes (oncogenes and suppressor genes); rate-limiting genes/proteins, and high-information transfer genes/proteins. The targeting of these drive genes is feasible and can effectively block the cancer-specific relevant pathways. Examples of specific "target the target" benefits that have emerged from preclinical and clinical molecular targeting studies are B- vemurafenib/Raf^{V600E},

Tagrisso/EGFR^{T790M}, crizotinib/ALK, and Entrectinib/NTR1. The demonstration of dramatic responses, PFS (progression free survival) and OS (overall survival) benefits in numerous NDA applications has sufficed to justify FDA approval of an increasing number of targeting therapeutics.

As a case in point, the PAM pathway is a complex signaling network component with multiple negative feedback systems and a variety of mechanisms of development of adaptive resistance (see Figure 2).

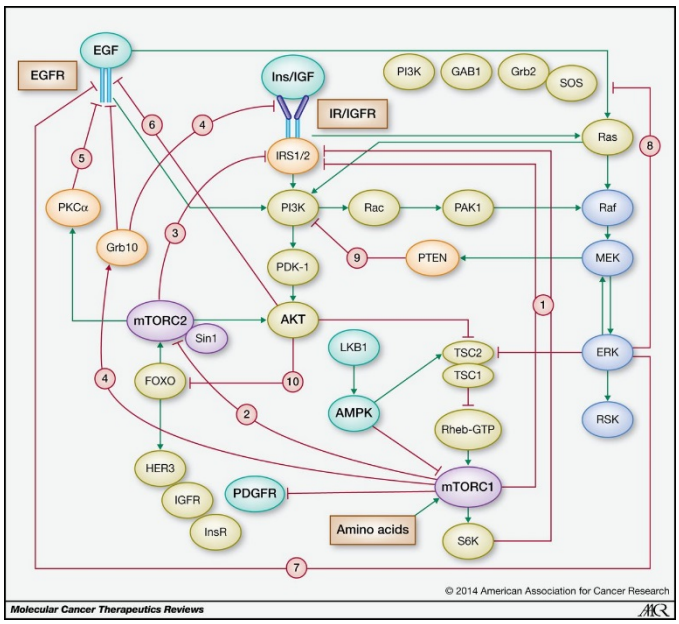

Figure 2. PAM pathway (stimulatory connections in green; negative feedback loops in red) (from Rozengurt E et al, 2014).

Chronic inhibition of the PAM downstream node mTORC1 relieves redundant negative feedback loops on PI3K/AKT via IRS-1, thereby overriding pathway interference (see Figure 3) that, however, can be subverted by upstream element inhibition, e.g., PI3k/AKT. That p70S6K, a substrate of mTORC1, is amplified in 8.8% of primary breast cancer and overexpressed at the mRNA level in 38% of breast cancers may allow for mTORC1 independent expression, further

supporting the rationale for the use of a dual AKT/p70S6K moiety. In terms of mechanism, p70S6 kinase plays a critical role integrating the HER-family and PI3k pathways. Its activation is associated with resistance to both trastuzumab and lapatinib. In addition, overexpression of RSK3 (ribosomal S6 kinase, $p90^{RSK3}$) has also been shown to mediate resistance to PI3k/mTOR inhibition and is associated with resistance to lapatinib. A Phase I dual AKT/p70S6K targeting therapeutic has been shown to have *in vitro* potency against RSK3 although to a lesser degree than to S6k and AKT 1,3 (private communication).

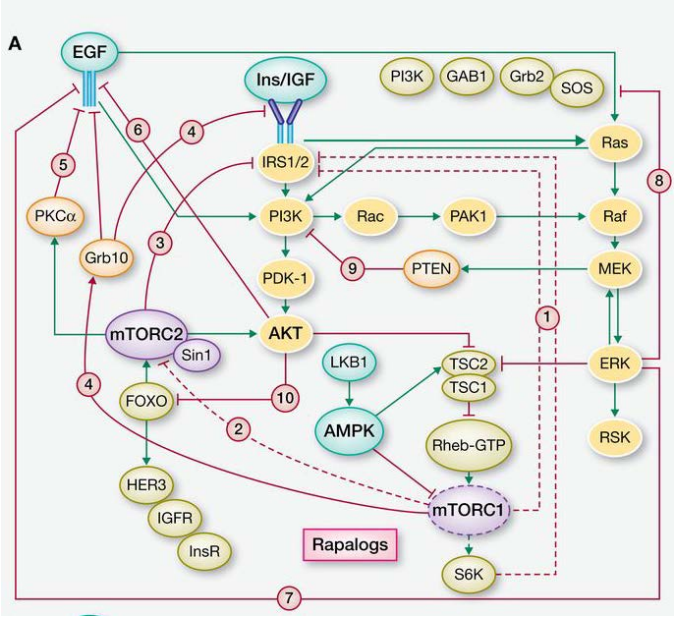

Figure 3. mTOR inhibition of the PAM pathway (stimulatory connections in green, negative feedback loops in red, pathways activated by suppression of negative feedback loops highlighted in yellow) (from Rozengurt et al, 2014).

However, even with a dual PI3k/mTORC1, 3 inhibitor, there are remaining avenues for resistance to emerge via suppression of multiple negative feedback loops resulting in over-activation of the MEK/ERK pathway via a PI3k-independent loop, as well as FOXO-mediated up-regulation of

tyrosine kinase receptors (e.g., HER3, IGFR, and InsR) (see Figure 4). Knowledge of these compensatory pathways informing the likely modes of development of adaptive resistance offers the opportunity for rapid assessment of tumor-specific genomic/proteomic mechanisms of resistance at time of progression. With such, this allows for the institution of mechanism-specific therapeutic combinations to circumvent resistance, e.g., a dual PI3k/mTORC1,3 inhibitor + MEK inhibitor, a dual PI3k/mTORC1,3 inhibitor + AZ8931 (EGFR/HER2/HER3 inhibitor, or a dual PI3k/mTORC1,3 inhibitor + IGFR/InsR inhibitor). Further, considering the continuing expansion of CTC (circulating tumor cell) detection and isolation techniques (e.g., microfluidic), both proteomic and genomic (next-generation sequencing (NGS), digital droplet PCR) analyses have the potential for detection of *emerging* pockets of resistance prior to clinical progression, increased frequency of assessment (especially in difficult to biopsy areas), and a broader view of heterogeneic tumor cell biology. Likewise, although limited to DNA analysis, cfDNA (circulating free DNA) would allow for genomic interrogation to identify resistance mediators, such as KRAS mutations and, possibly, some activating mutations of AKT.

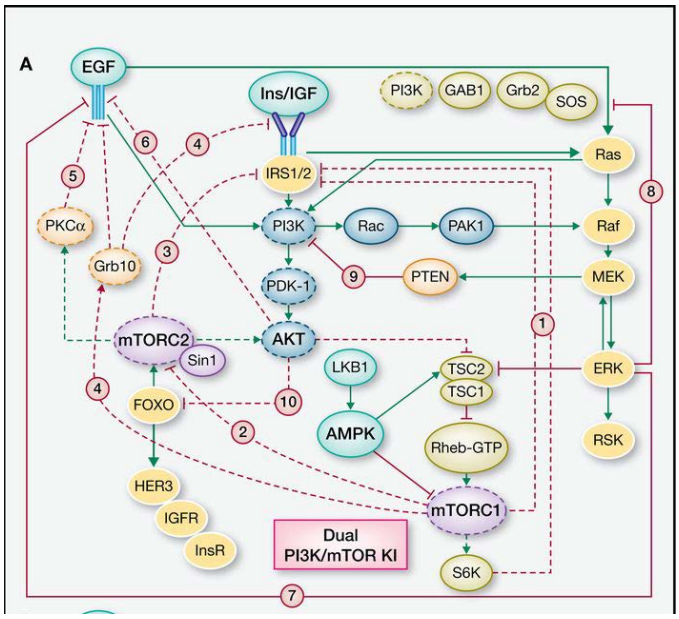

Figure 4. Dual PI3k/mTOR inhibition of the PAM pathway (stimulatory connections

in green, negative feedback loops in red, pathways activated by suppression of negative feedback loops highlighted in yellow (from Rozengurt E et al, 2014).

The following is a summary comparison of CTC and cfDNA.

Comparison	CTCs	cfDNA
Origin	Intact cells (not necessarily viable) [34]	Necrotic/apoptotic cells [33] and/or actively secreted from intact cells [35]
Definition	Tumor cells derived from primary/metastatic sites [6]	Fragmented DNA in circulation [6]
Capture & Analysis Techniques	Enrichment: size/density-, immunomagnetic-, or microfluidic-based [10] Detection: protein- or nucleic acid-based [10]	Enrichment: plasma collection [33] Detection: PCR-, or sequencing-based [33]
Advantages	Extensive downstream analysis (DNA, RNA, protein, functional assays) [22,36–38] Assessment of single cells [39] Clinically-validated technology available (CellSearch® system; metastatic breast, prostate, & colorectal cancers) [11–13] Captured viable cells can be used for in vitro culture or in vivo animal studies [38]	Easy to isolate/enrich from whole blood [33] Amenable to long-term storage for subsequent analysis [33] High-sensitivity read-out [28] Clinically validated test for EGFR mutations in non-small cell lung cancer [9,40]
Disadvantages	Low cell numbers in non-metastatic setting [41] Challenging to store long-term and subsequently analyze (Lowes, L.E., unpublished) Both detection and enrichment steps require highly sensitive and often expensive technology [6]	Limited (pre-)analytical/analytical SOPs, assay validation, & appropriate prognostic/predictive read-out (may be disease/mutation specific) [33] Limited downstream analysis (DNA only) Currently only feasible in high tumor burden setting [6] Need known target mutations to confirm cfDNA originated from tumor cells [6]

In conclusion, cancers evolve, as do all emergent processes. The traditional reductionist approach to therapeutic intervention will not suffice. At the Mary Crowley Cancer Center, in collaboration with each patient and their referring physician, interrogation of the molecular fingerprint of each patient's cancer followed by a system analysis of its integrated components has long been our approach to personalized (aka precision) therapy.