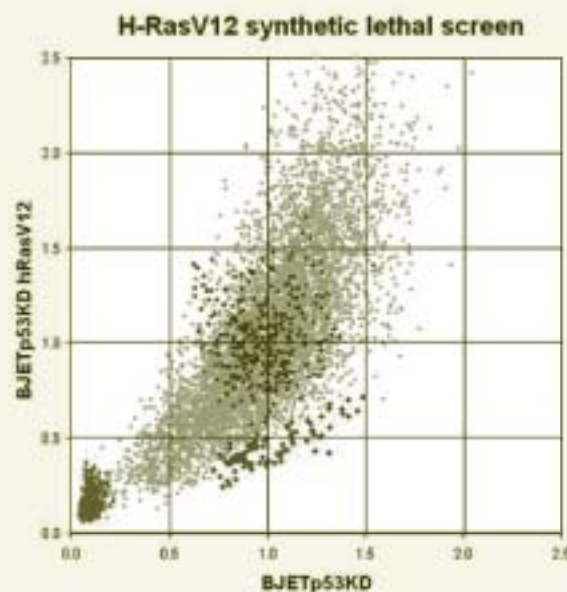


## THE RNAi STRATEGY IN TARGET DISCOVERY

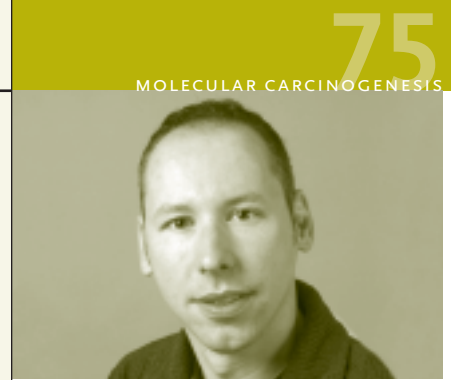
The research in my laboratory continues to evolve around the identification of novel drug targets in cancer, using large-scale cell-based screening technologies. We apply genome wide siRNA collections as well as large shRNA collections with the goal of identifying essential components in disease-related pathways that can be explored as drug targets in cancer therapy. Furthermore we use these RNAi technologies to search for synthetic lethal interactions with specific tumor-associated genetic alterations. We have begun to use RNAi technologies in combination with phosphoproteome profiling and cell-based assays to generate predictive models for therapy response to pathway targeted therapeutics in breast cancer.

**Synthetic lethal interactions** For the effective treatment of cancer, there is a great need for drugs that specifically target tumor cells without affecting normal cells. With the use of RNA interference, we explore synthetic lethal phenotypes in mammalian cells. Synthetic lethal phenotypes are defined as a combination of two mutations, which by themselves are non-lethal, but together result in a lethal phenotype. These interactions can lead to the identification of novel cancer drug targets that are only cytotoxic in the context of a tumor specific alteration and represent “genotype specific” drug targets. We have generated a panel of isogenic cell lines derived from primary human BJ fibroblasts that contain single or multiple defined genetic alterations that together are required for tumorigenic transformation of these primary cells. These genetic alterations include among others, loss-of-*TP53*, activation of *RAS* or activation of *PI3K*. These cell lines have been used in high throughput single well assays in combination with large siRNA collections targeting over 8000 genes to identify siRNAs that result in enhanced lethality only in the background of these tumor specific genetic alterations. We have completed these screens and have identified several siRNAs whose lethality is dependent the activation of *RAS*, *PI3K* or loss of *TP53* in our isogenic BJ model. For *RAS* synthetic lethal interactions, we have shown that the phenotypes caused by the siRNAs are on-target and cause a synthetic lethal phenotype also in the presence of *HRAS*, *NRAS* and *KRAS* oncogenic activation (see figure 2). These results indicate that these synthetic lethal phenotypes are associated with *RAS* pathway activation. We are extending the analysis of the *TP53*, *PIK3CA* and *RAS* dependent interactions in a panel of wild type and mutant cancer cell lines of different tissue origin to exclude context dependent effects. We are in the process of establishing the underlying biological mechanism for the synthetic lethal phenotypes.

Figure 2: Identifying synthetic lethal interactions with *RAS*  
Viability scores for 8000 siRNA smartpools in BJETp53KD and BJETp53KD/*RASV12* cell lines. Each dot represents an individual siRNA smartpool. Depicted in dark grey, in the middle under the cloud, are siRNA smartpools that display an enhanced reduction in viability of the *RASV12* expressing cells without effect on the control BJETp53KD cell line.



**Generation of computational models for predicting response to therapy in breast cancer** *PI3K* and *MAPK* signaling pathways are strongly implicated in triple negative breast cancer and invasive lobular carcinomas. At present, many drugs are



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### Publications

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Aguilar H, Solé X, Bonifaci N, Serra-Musach J, Islam A, López-Bigas N, Méndez-Pertuz M, Beijersbergen RL, Lázaro C, Urruticoechea A, Pujana MA. *Biological reprogramming in acquired resistance to endocrine therapy of breast cancer. Oncogene.* 2010;29:6071-83

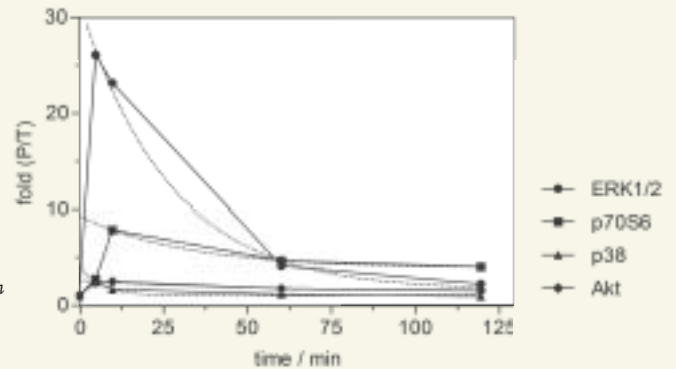
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in clinical development targeting specific components of these pathways although their successes are limited. Complicated interactions with other signaling pathways and unexpected feedback loops can limit the effectiveness of treatment and, in some instances, even accelerate the tumorigenic process. The understanding and modeling of these complex pathways using a systems approach can yield predictive models for therapy responses to pathway-targeted therapeutics in breast cancer. We are developing *in silico* models of therapy response in breast cancer by exploiting normal and tumor cell lines as *in vitro* model systems for the quantification of functional activation of pathway components and associated cellular phenotypes. We use RNAi for perturbation experiments in combination with a Luminex platform for quantification of the activation status of individual pathway components (figure 3).

Figure 3: Phosphoproteome analysis of the PI3K and MAPK pathways  
Quantification of levels of phosphorylation of pathway components involved in PI3K/MAPK signaling using the Luminex platform. Human mammary epithelial cells were starved for 24 hours after which they were re-stimulated with 10ng/ml human epidermal growth factor.



Phosphorylation of ERK, p70S6, p38 and AKT were measured at different time-points.

The data obtained from these experiments are used to create and validate a dynamic and quantitative computational model of pathway behavior and phenotypical outcome in relation to drug response. With this approach we anticipate to maximize the success rate of available targeted therapies against MAPK and PI3K pathway components.