Molecular Predictors of Sensitivity to the Insulin-like Growth Factor 1 Receptor Inhibitor, Figitumab (CP-751,871)

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Abstract

Figitumumab (CP-751,871), a potent and fully human monoclonal anti-insulin-like growth factor 1 receptor (IGF1R) antibody, has been investigated in clinical trials of several solid tumors. To identify biomarkers of sensitivity and resistance to figitumumab, its in vitro antiproliferative activity was analyzed in a panel of 93 cancer cell lines by combining in vitro screens with extensive molecular profiling of genomic aberrations. Overall response was bimodal and the majority of cell lines were resistant to figitumumab. Nine of 15 sensitive cell lines were derived from colon cancers. Correlations between genomic characteristics of cancer cell lines with figitumumab antiproliferative activity revealed that components of the IGF pathway, including IRS2 (insulin receptor substrate 2) and IGFBP5 (insulin-like growth factor-binding protein 5), played a pivotal role in determining the sensitivity of tumors to single-agent figitumumab. Tissue-specific differences among the top predictive genes highlight the need for tumor-specific patient selection strategies. For the first time, we report that alteration or expression of the MYB oncogene is associated with sensitivity to IGF1R inhibitors. MYB is dysregulated in hematological and epithelial tumors, and IGF1R inhibition may represent a novel therapeutic opportunity. While growth inhibitory activity with single-agent figitumumab was relatively rare, 9 combinations comprising figitumumab plus chemotherapeutic agents or other targeted agents exhibited properties of synergy. Inhibitors of the ERBB family were frequently synergistic and potential biomarkers of drug synergy were identified. Several biomarkers of antiproliferative activity of figitumumab both alone and in combination with other therapies may inform the design of clinical trials evaluating IGF1R inhibitors.

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Introduction

The insulin-like growth factor (IGF) pathway plays an important role in normal growth and development. Binding of IGF to the receptor, IGF1R, induces receptor transphosphorylation and the recruitment of insulin receptor substrates (IRS) and Src homology adaptor proteins. Signal transduction is then mediated via two signaling pathways: phosphatidylinositol 3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/AKT) and RAS/RAF/mitogen-activated protein kinase (MAPK) (1–2).

Aberration of IGF signaling can occur at various levels involving overexpression of the ligands, the receptor, or ligand binding proteins. As IGF signaling is anti-apoptotic and pro-survival it is also key to the development and progression of cancer (1). High IGF1 serum concentrations have been associated with breast, prostate, and colorectal cancers (3), while increased production of IGF2 is associated with several tumor types including lung and colorectal cancer (4–5). Overexpression of IGF1R is frequently observed in tumor cell lines and human cancers including non-small cell lung cancer (NSCLC) and mediates both tumor growth and resistance to epidermal growth factor receptor (EGFR/ERBB) inhibitors (6). Compelling new data have shown that both the alpha and beta subunits of IGF1R can translocate to the nucleus and directly regulate transcription (7). Nuclear IGF1R has been detected in tumor cell lines and tumor biopsies, and early data suggest an association with poor prognosis (7). IGF1R has also been implicated in the ability of drug-resistant cancer cells to maintain viability (8).

IGF-binding proteins (IGFBPs) modulate the activity of IGFs, mainly by limiting IGF access to IGF1R. Growth inhibitors including vitamin D, anti-estrogens, retinoids, and transforming growth factor-β reduce IGF activity by increasing the secretion of IGFBPs. The wealth of evidence implicating the IGF system in tumorigenesis and cell survival has led to the development of targeted anticancer agents that inhibit IGF1R (2).

Figitumumab (CP-751,871) is a highly potent and fully human monoclonal antibody against IGF1R. Figitumumab can prevent IGF1 from binding to IGF1R (inhibitory concentration-50 [IC50] 1.8 nmol/L), and as a result inhibits IGF1-induced autophosphorylation of IGF1R (IC50 0.42 nmol/L) and indirectly inhibits AKT activation in
preclinical studies (9). Figitumumab also inhibited xenograft tumor growth via inhibition of IGF signaling, particularly when combined with chemotherapy or endocrine therapy (9).

In this paper, molecular analyses of multiple tumor cell lines were conducted to identify biomarkers potentially predictive of figitumumab antiproliferative activity, and thereby identify patients most likely to respond to treatment. In addition, the impact of figitumumab in combination with standard of care (SOC) and several other targeted agents on the growth of cancer cell lines was assessed.

Material and Methods

Cell lines, cell cultures and reagents

Figitumumab was tested in vitro in a panel of 93 cell lines derived from human small cell lung cancer (SCLC) and NSCLC, breast, and colorectal tumors. Tumor cells were obtained from ATCC (Manassas, VA), the National Cancer Institute cell line repository (Bethesda, MD), or from internal Pfizer cell banks. Cells lines acquired from ATCC were authenticated by Short Tandem Repeat analysis by the vendor and experiments were performed within 6 months after receipt, except for CACO2, Calu6, DLD1, HCC1143, HCC1395, HCC1419, HCC1954, HCC2935, HCC70, Ls174t, MX-1, NCI-H146, NCI-H1838, NCI-H187, NCI-H209, NCI-H23, NCI-H69, NCI-H82, NCI-N417, NCI-N592, RKO, SW48, and SW948 which were not re-authenticated. Cells were grown in appropriate culture media as recommended by the suppliers, including RPMI 1640, MEM, DMEM, and DMEM-F12 (all from Invitrogen, Carlsbad, CA). Supplements included HEPES buffer, sodium pyruvate, non-essential amino acids, Pen-Strep, ITS, glutamine (all from Invitrogen) and FBS, 5-20% (SAFC Biosciences, Lenexa, KS). Some of the lung cancer lines were grown in ACL-4 medium as described in ATCC’s online material (supplements from Sigma-Aldrich, St. Louis, MO).

Proliferation assays and sensitivity tests

Cell viability assays were performed using cell lines treated with either single agent or pair-wise combinations of agents: trastuzumab; dacomitinib; PF4691502; cetuximab;
oxaliplatin; 5-FU; PD332991; erlotinib; gemcitabine. Three to five thousand cells per well were seeded into 96-well tissue culture plates (Costar 3997, Corning, Lowell, MA) and incubated at 37°C in 5% CO₂ humidified air. The following day, cells were treated with either single or double agents (equimolar ratio for combination) at nine serially diluted concentration points ranging from 10 μM to 152 pM (1 μM to 15.2 pM for figitumumab and trastuzumab). After 3 days of further incubation at 37°C, 0.1 mg/mL resazurin salt dye (Sigma Aldrich, St. Louis, MO) or 1/5 of manufacturer's recommended volume of Cell Titer Glo (CTG; Promega, Madison, WI) was added to indirectly measure cell viability/proliferation using an Envision multi-reader (Perkin-Elmer, Waltham, MA). Readings from the Envision multi-reader were obtained 6 hours after the addition of resazurin, or 30 minutes after CTG was added. Relative fluorescence (Resazurin) or luminescence (CTG) counts were first adjusted by subtracting the average counts taken from untreated cells assessed one day after cell seeding. The Cell Growth Inhibition (CGI) score for each compound concentration was calculated as:

\[
CGI = 1 - \frac{[\text{well_count} - \text{baseline}]}{[\text{platecontrol_mean} - \text{baseline}]} 
\]

The R dose-response curves package drc was used to fit the adjusted CGI values vs. the concentrations of the compounds (10). A four-parameter logistic model was utilized to fit the dose-response curves and generate estimations and inferences of the half maximal inhibitory concentration (IC₅₀), slope, upper and lower limits.

Synergy was determined by calculating the improvement of the area-under-the-curve (AUC) of the combination over the better of the single agents. Synergy is judged to have arisen if: 1) the better of the single-agent curves is not flat, synergy is defined when the percentage improvement of the AUC of the combination is >15% of the better of the single agents; or 2) both single-agent curves are flat (i.e. no response/resistant), synergy is defined when the intercept of the linear fit of the combination is statistically significantly different from the single agents and the linear slope of the combination is statistically significant.
Baseline molecular profile of cell lines

The mutation status of the cell lines was determined from the Catalogue of Somatic Mutations in Cancer (COSMIC) database (11) supplemented with custom OncoCarta (Sequenom, San Diego, CA) profiling. Baseline whole-genome copy-number variation in selected cell lines were obtained from public sources and complemented with internal profiling on Human Genome-U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA) and single nucleotide polymorphism 6.0 arrays (Affymetrix) according to the manufacturer's protocols. Publicly available reference profiles of cancer cell lines on the same platforms were obtained from the Welcome Trust Sanger Institute’s copy number analysis dataset (http://www.sanger.ac.uk/genetics/CGP/CopyNumberMapping/Affy_SNP6.shtml), GlaxoSmithKline (https://cabig.nci.nih.gov/caArray_GSKdata/), and Genentech (GSE10843). All new array data were deposited into the National Society for Biotechnology Information gene expression omnibus (GSE34211). For gene expression, we first excluded all arrays with a normalized unscaled standard error score > 1.05 and then normalized by GeneChip Robust Multiarray Averaging (GC-RMA) (12) using the GC-RMA Bioconductor package. When multiple arrays were available, we used the median of normalized values. Single-nucleotide polymorphism (SNP) 6.0 arrays were processed using the R based aroma.affymetrix methods (13) (http://www.aroma-project.org/). Relative copy number was inferred by computing the ratio of the arrays versus a baseline profile of the average of the 128 females from the International HapMap Project (http://www.affymetrix.com/estore/support/technical/sample_data/genomewide_snp6_data.affx).

Expression levels of MYB/c-Myb and IGF family mRNA were confirmed using TaqMan real-time polymerase chain reaction (RT-PCR; Applied Biosystems, Carlsbad, CA). Manufacturer’s assay codes are listed in Supplementary Table 1. The ABI Prism 7900 (Applied Biosystems, Carlsbad, CA) was used according to the manufacturer’s protocol. The mRNA expression levels were first normalized to GAPDH, and the relative gene expression was calculated by subtracting the average delta CT of triplicate reactions...
from the median of the average delta CT across each tumor type. Final values used in
the analysis are provided in Supplementary Table 2.

Data analysis and statistical methods

Any DNA variation leading to change at the amino acid level was classified as a
mutation; silent mutations were excluded from the analysis. Gene amplifications from
whole-genome SNP 6.0 arrays were defined as members of focally amplified
chromosomal segments ≤10 Mb containing at least five probes each with a mean log₂
≥1. There was no size constraint on the length of the chromosomal deletion: segments
were required to contain a minimum of five probes each with a mean log₂ less than –2.
As part of the TaqMan RT-PCR analysis, a given gene was defined as over-expressed
if its relative expression was more than one standard deviation above the median of all
lines.

In vitro growth inhibition was correlated with categorical predictors such as the presence
of genetic changes and tissue of origin using the nonparametric Kruskal–Wallis rank
test as well as Fisher’s exact binomial test as implemented in R. Spearman’s rank
correlation was used for continuous RNA expression profiles. The contributions of tissue
origin to the observed correlations were tested using analysis of covariance and
analysis of variance tests from the linear model function (lm) in R after factoring the
tissue effect.

The presence of a predictive signature was tested using BRB-ArrayTools v4.1 (14). We
split the samples into two categories: sensitive, defined as IC₅₀ < 100nM, and resistant,
with IC₅₀ >1μM, and removed the cell lines with intermediate values of IC₅₀. We filtered
out control probesets, non-informative probe sets whose expression values deviated at
least 1.5-fold in either direction from the median value in fewer than 20% of the cell
lines, and those probesets with more than 50% of their data missing. Probe sets with a
univariate misclassification rate below 0.1 were used for class prediction using the
diagonal linear discriminant analysis (DLDA) model (Supplementary material: DLDA
expression signature). The leave-one-out cross-validation method was used to estimate the misclassification rate.

To satisfy the requirements of independence in statistical tests, we kept only one cell line derived from a particular tumor and excluded duplicates using genotyping information from the Sanger Institute (http://www.sanger.ac.uk/genetics/CGP/Genotyping/synlinestable.shtml). HCT-15 was removed and DLD1 kept, SW620 removed and SW480 kept. NICH69 was kept in place of NCIH249 and NCIH592.

**Classification of 300 cell lines with an IGF pathway response signature**

To develop the IGF pathway signature, we started with probesets from the Affymetrix HG-U133 Plus 2.0 arrays that correspond to 14 IGF pathway genes (without MYB). We used these probesets to develop an IGF pathway classifier in our panel of 93 lines using DLDA. This new classifier contained 14 probesets from 7 distinct IGF pathway genes (full model available in the supplementary material: DLDA-classifier_IGF-pathway_score). The performance of this IGF pathway-restricted (70% correct) classifier was lower compared with the whole-genome signature (85% correct). This new pathway signature was used to score IGF-pathway activity in 300 cell lines previously profiled by GlaxoSmithKline (https://cabig.nci.nih.gov/caArray_GSKdata/). This pathway score was compared with the relative copy number of all human genes using the Pearson’s linear correlation coefficient. The copy number data in the 300 cell lines were obtained from genome-wide human arrays processed as described above.

**Results**

*Molecular predictors of sensitivity to inhibition with single-agent figitumumab in vitro*

A panel of 93 cell lines was utilized to assess the pattern of response to figitumumab and the relationship of response to selected molecular biomarkers. The sensitivity profile of figitumumab in the panel of cell lines was bimodal with 15 cell lines highly
sensitive to figitumumab at IC$_{50}$ values $\leq$ 100 nM (Figure 1A). The remaining cell lines were insensitive to this agent and the IC$_{50}$ values exceeded 1 $\mu$M. The distribution of cell lines reflecting different tumor types of origin was non-random among sensitive lines ($p = 0.0017$; Kruskal–Wallis rank test); colon cancer cell lines were more sensitive than others (Figure 1B).

We evaluated the relationship of the figitumumab sensitivity profile with 19 established cancer driver mutations and found that APC and CTNNB1/b-catenin mutations were associated with cell-line sensitivity across different cancer types (Figure 1C). However, no such correlation was found within colon cancer cell lines, indicating that the observed pattern is related to the prevalence of these mutations in colon cancer models.

When the activity profile of figitumumab was correlated with whole-genome copy number alterations (Figure 1D; see Material and Methods), a total of 52 genes from six distinct genomic loci were found to be associated with sensitivity at a false discovery rate (FDR) of $< 0.25$ (Table 1; Supplementary Table 3). The most significantly amplified locus at 13q33.3 contains MYO16 and the IGF1R-associated adaptor protein, IRS2. The 17q22-24 amplicon harbors 42 significant genes including two loci encoding the human growth hormones, GH1 and GH2, which are known regulators of the IGF pathway (15). Four other significantly amplified loci 17q11.2, 11q24.2, 6p23.3, and 12q12 did not contain any well-established IGF pathway genes. Interestingly, the AHI1 gene on 6p23.3 is adjacent to a mitochondrial RNA gene, HSA-MIR-548A-2 and a well known oncogene, MYB/c-Myb.

Correlation between figitumumab activity and gene expression was investigated using two approaches: one biased toward known IGF pathway genes, and one unbiased that incorporated whole-genome data. The hypothesis-driven biased approach concentrated on mRNA expression of key IGF pathway members measured by TaqMan RT-PCR. Figure 1E shows the heat map of expression values sorted by figitumumab sensitivity.

In the combined panel, high insulin receptor (INSR; Spearman’s rank $R = -0.25$, $p = 0.03$) and low IGFBP5 (Spearman’s rank $R = 0.37$, $p = 0.001$) mRNA levels were associated with response. There was a strong association between high MYB mRNA, as determined by RT-PCR, and figitumumab sensitivity (Spearman’s rank $R = 0.45$, $p = 0.003$).
R = –0.44, p = 0.0001). An attempt was made to determine an unbiased figitumumab response signature from whole genome Affymetrix HG-U133 Plus 2.0 arrays. Twenty-five probesets with univariate misclassification rate below 0.1 were used for class prediction using the DLDA. However, this approach resulted in rather poor performance in cross-validation: sensitive models were identified with sensitivity equal to 0.533 and specificity to 0.91. The complete model, including the probesets and weights is available in the supplementary material (DLDA-classifier_IGF-pathway_score).

Table 2 shows correlations between IGF pathway genes and figitumumab activity in breast and colon cancer cell lines. High MYB and IGF2 expression predicted sensitivity in breast cancer cell lines, whereas high IGF1R, IGFBP4 and low IGF2BP3, and IGFBP6 expression was significant in colon cancer cell lines. The lack of overlap between the predictors suggests potential tissue-specific predictors of response to figitumumab. Only IGF1R itself and MYB mRNA expression were either significant or close to significant in both panels. Normalized expression values are listed in supplementary Table 2. No significant correlations were found in the SCLC and NSCLC panels, likely due to the small number of sensitive models.

In summary, our analysis of the baseline molecular profile of figitumumab-sensitive cell lines uncovered several potential predictors of in vitro response to figitumumab at both the DNA and RNA levels. Figure 2 shows p-values comparing sensitive and resistant models plotted against odd ratios. From the various predictors, the DLDA multigene signature, colon cancer origin, MYB over-expression, and low expression of IGFBP5, represent the best biomarkers of response. However, one caveat of multigene signatures is the strong possibility of ‘over-fitting’ the data, and indeed our error estimation by cross-validation indicate that the discovery of a more robust signature would require profiling of additional samples.
**Whole-genome analysis of copy number changes associated with IGF pathway expression**

Given the importance of the IGF pathway, we looked for DNA aberrations associated with expression patterns typical for sensitive models. We used the signature comprising seven IGF pathway genes (14 probesets) that predict response to figitumumab in our panel of 93 cell lines described above to score IGF-pathway activity in over 300 additional cell lines, and then correlated IGF-pathway activity with copy number in these cell lines (see Methods). Figure 3 shows the correlation between copy number of all human genes (rank-ordered) and the predictive pathway signature. Circles represent rank-ordered human genes; red circles are genes significantly correlated with the IGF-pathway score at FDR < 0.25. In total, seven genomic regions were significantly correlated with IGF-pathway score in the 300 cell lines: 4q, 6q, 10q23, 13q, 15q, 17q and 18q. **BCL6** was the only gene on 4q that showed a significant correlation. Another gene with significant correlation, **FNDC1**, this time on 6q, is immediately adjacent to the insulin-dependent diabetes locus, **TAGAP**, and close to **IGF2R**. The 10q23 locus harbors many significant genes including the well known IGF-pathway regulator **PTEN** (16–17). The next significant region on 13q contains the key predictive gene **IRS2** as well as other IGF-pathway regulators including **FOXO1** (18). Another significant region on 15q contains many genes close to **IGF1R**. The 17q region contains several significant genes including the **BRCA1** tumor suppressor. The last significant region, 18q, contains several pathway regulators including **GRP**, **SALL3**, and **MBP**; the **SMAD4** locus is close but not significant.

**Combination screens**

Due to the low response rate observed for single-agent figitumumab in both nonclinical and clinical studies, figitumumab was tested in combination with several standard chemotherapeutic agents as well as other novel compounds in clinical development. Supplementary Figure S1 depicts combined activity of selected agents with figitumumab along with synergy calculations as outlined in Materials and Methods. Altogether, 14
combinations with 9 different compounds in NSCLC, colon and breast cancer cell lines are shown together with a brief summary of the results (Table 3). We noted frequent synergy between figitumumab and ERBB family inhibitors including synergy with trastuzumab in more than 50% of breast cancer cell lines, synergy with cetuximab in 67% of colon cancer cell lines, and with the pan-ERBB inhibitor, dacomitinib (PF299804), in 25% of NSCLC cell lines. Other frequent synergies were observed with the PI3K/MTOR inhibitor, PF4691502 (19), CDK4/CDK6 inhibitor, PD332991, and several chemotherapeutic agents, most notably with oxaliplatin in 78% of colon cancer cell lines.

We also attempted to identify molecular predictors of synergy between different drug combinations with figitumumab (Table 3). Given the limited number of models from individual cell line panels, we restricted the analysis to 19 key cancer drivers and IGF pathway genes to limit potential false discoveries from unbiased whole-genome analyses. Several potential predictors were found, most for combinations comprising figitumumab plus other targeted agents, and there were several common synergies across cell lines. Low NRAS mRNA was associated with synergy with trastuzumab in breast cancer cell lines and dacomitinib in colon cancer as well as NSCLC cell lines. High ERBB3 expression predicted synergies with trastuzumab in breast cancer cell lines and dacomitinib in both breast and colon cancer cell lines. Low IGF2BP3 mRNA was associated with synergies with trastuzumab in breast cancer cell lines, and also with gemcitabine in NSCLC cell lines. Intriguingly, breast cancer cell lines with wild-type and/or low TP53 mRNA levels were often synergistic for the figitumumab plus PF4691502 combination.

**Discussion**

There is growing interest in identifying predictors of response to anti-IGF1R therapy (20–22). The aim of this study was to identify biomarkers predictive of clinical efficacy with the IGF1R inhibitor, figitumumab, to help identify patient subgroups that may benefit from this type of targeted treatment. To do this, we combined in vitro screens
with extensive molecular profiling of genomic aberrations in a broad panel of 93 cell lines. The overall response was bimodal and the majority of cell lines were resistant to figitumumab. A small fraction of sensitive models was identified: 9 of 15 were from colon cancers.

To evaluate the correlation between results obtained in broad in vitro screening assays and in vivo tumor growth inhibition, the antitumor activity of figitumumab was assessed in 8 of the cell lines (4 sensitive and 4 resistant) implanted as subcutaneous xenografts in immunocompromised mice in vivo. The results from these experiments are captured in supplementary Table 4 and indicate that all 4 sensitive lines responded to figitumumab in vivo whereas figitumumab did not demonstrate significant tumor growth inhibition in all 4 resistant lines.

Figitumumab activity was correlated with copy number alteration in six distinct genomic loci, three of which contain known IGF pathway genes and regulators. The most significant amplified locus harbors IRS2. High IRS2 mRNA expression has been previously shown to correlate with response to an IGF1R monoclonal antibody in breast and colon cancer cell lines (21). The 17q22-24 amplification harbors 42 significant genes including those that encode the human growth hormones, GH1 and GH2. Growth hormones are recognized regulators of the IGF pathway (15). Interestingly, a recent study by Guevara-Aguirre et al. identified growth hormone receptor deficiency in cancer- and diabetes-free Ecuadorian families with severe short stature that, on the molecular level, was manifested by severe IGF1 deficiency, further corroborating the interplay between growth hormone signaling, the IGF pathway, and cancer (23).

6p23.3, perhaps the most interesting peak of association, is next to a well known oncogene, MYB/c-Myb. Analysis of MYB mRNA expression revealed a strong correlation between MYB levels and figitumumab IC50 values. While MYB expression has never been linked to response to IGF1R inhibitors, several reports indicate that MYB has a role in IGF pathway regulation. MYB has been shown to increase both IGF1 and IGF1R mRNA levels (24). MYB stimulates cell growth by regulation of IGF and IGFBP3 in K562 leukemia cells (25). The authors also found that MYB overexpression induced cell proliferation compared with control, and MYB-induced
cell growth was inhibited by anti-IGF1R antibodies, indicating the therapeutic potential of IGF1R inhibitors in MYB-driven malignancies.

MYB encodes a transcription factor essential for hematopoiesis and is frequently deregulated in human cancer (26). Recurrent MYB translocations were reported in adenoid cystic carcinomas (27). MYB translocations and amplification were also found in acute T-cell leukemia (28–29), pancreatic tumors (30), BRCA1-linked hereditary breast cancer (31), as well as other tumors. Notably, MYB over-expression has been reported in colorectal cancer (32) further supporting the link between figitumumab activity and MYB expression in colorectal cancer models. Finally, MYB is an essential regulator of hematopoietic stem cell proliferation and differentiation (33), suggesting that IGF1R inhibition could be considered for the treatment of general hematopoietic malignancies beyond acute T-cell leukemia.

Apart from MYB expression, IGF pathway members were among the strongest predictors identified in this study. In the whole panel of 93 lines, high INSR and low IGFBP5 were significantly associated with figitumumab activity. Our findings also reveal that tissue-specific predictors of figitumumab sensitivity exist. The molecular predictors of sensitivity to figitumumab depend on the tumor type: IGF2 and possibly also IGF1R mRNA expression predicted sensitivity to figitumumab in breast cancer cell lines, whereas IGF1R, IGF2BP3, IGFBP4, and IGFBP6 mRNA expression were significant predictors of activity in colon cancer cell lines.

Other studies have also demonstrated associations between components of the IGF pathway and administration of anti-IGF1R agents. High levels of total IGF1R were associated with moderate sensitivity to R1507, a fully humanized anti-IGF1R monoclonal antibody, in NSCLC models (20). Zha et al. suggested that colon and breast cancer models with low expression of IGF1R were unlikely to show dependence on the IGF1R pathway and were, therefore, unlikely to respond to an anti-IGF1R antibody (21). Furthermore, these investigators showed that other components of the pathway, such as the adaptor proteins, IRS1 and IRS2, and the ligand, IGF2, have predictive value in these tumor models. A similar study, which aimed to develop and characterize predictive biomarkers for the IGF1R tyrosine kinase inhibitor, OSI-906, in
colorectal cancer, also noted that the IGF1R/EGFR/insulin pathways were dominant in sensitive cells (22).

We also looked for genetic changes associated with IGF pathway activity with DNA aberrations in a broader panel of more than 300 cell lines. Altogether, seven genomic regions correlated with the predictive IGF pathway score; these genomic loci contain several known IGF or insulin pathway regulators such IRS2, PTEN, FOXO1, and TAGAP. Amplifications of BCL6 on 4q were also significant with the predictive IGF pathway score. BCL6 mRNA is strongly decreased after GH treatment (34). BCL6 downregulates expression of IGFBP4 and the IGFBP4 protein may play a role downstream of the BCL6 signaling pathway during B-lymphoid differentiation (35). We speculate that some of these genes may also affect response to IGF1R inhibitors due to their correlation with the predictive IGF pathway score.

The genetic complexity of most human malignancies indicates that ablation of a single target is unlikely to produce sustained growth inhibition and combination approaches are needed (36). In the tumor cell lines examined in this study, we demonstrated figitumumab activity in combination with several standard chemotherapeutic agents as well as novel compounds in clinical development. We found several synergistic combinations identified in NSCLC, colon and breast cancer cell lines. The most striking was figitumumab plus oxaliplatin that was synergistic in three-quarters of colon cancer cell lines. ERBB family inhibitors were also frequently synergistic with figitumumab. Synergy with trastuzumab was observed in more than 50% of breast cancer cell lines, with cetuximab in 67% of colon cancer cell lines, and with the pan-ERBB inhibitor, dacomitinib (PF299804), in 25% of NSCLC cell lines.

Other IGF1R inhibitors have also demonstrated synergy in combination with targeted agents. In a study similar to ours, Carboni et al. showed that BMS-754807, a small molecule inhibitor of IGF1R and the insulin receptor, was synergistic with cytotoxic, hormonal, and targeted agents in a variety of preclinical models including cetuximab (colon cancer), trastuzumab (breast cancer), lapatinib (lung cancer), bicalutamide (prostate cancer), and dasatinib (sarcoma and colon cancer) (37). Furthermore, dual
inhibition of EGFR (gefitinib) and IGF1R by the small molecule inhibitor, AZ12253801, was effective in reducing intestinal adenomas in the Apc^{min/+} mouse (38).

The synergistic associations observed in our study could be related to the cross-talk that exists between different signaling pathways that control tumor development. Tumor cells may counteract inhibition of a single signaling pathway by exploiting this cross-talk to invoke ‘escape’ mechanisms, hence the interest in administration of therapeutic regimens with multiple molecular targets. Sharma et al. demonstrated that the exposure of cancer models to several different inhibitors including anti-EGFR, -ERBB2, -RAF and -MET agents selected drug-tolerant subpopulations with activated IGF1 signaling (8). This chromatin-mediated, reversible state was efficiently ablated by treatment with IGF1R inhibitors. Also, acquired resistance to BRAF inhibitors via enhanced IGF1R/PI3K signaling was observed in BRAF^{V600E} melanoma cells, rendering them sensitive to concomitant MEK and IGF1R inhibition (39). Flanigan et al. reported synergies between PQIP, a dual IGF1R/insulin receptor tyrosine kinase inhibitor, and standard chemotherapies (5-fluorouracil, oxaliplatin, or SN38) in four colorectal cancer in vitro models (40).

It is as yet unclear which pathway provides the optimal combination strategy with inhibition of IGF1R (41). A recent phase I combination study of figitumumab and the MTOR inhibitor, everolimus, in patients with advanced sarcomas and other solid tumors indicated that the combination was well tolerated with no unexpected toxicities. Stable disease was observed in the majority of patients and the combination also yielded a partial response in one patient with malignant solitary fibrous tumor (42). Furthermore, a phase I trial evaluating the humanized anti-IGF1R monoclonal antibody, dalotuzumab, combined with the MTOR inhibitor, ridaforolimus, demonstrated antitumor activity in patients with estrogen receptor-positive metastatic breast cancer (43). However, the anti-IGF1R monoclonal antibody, IMC-A12, combined with cetuximab in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer was inactive in all but one of 41 patients treated (44). It is clear that the identification of predictive biomarkers in order to select patients for combination treatment is required. In this work, we applied our predictive biomarker analysis to synergies and several potential
predictors of synergy were identified including high baseline ERBB3 expression that predicted synergies with trastuzumab in breast cancer cell lines and dacomitinib in both breast and colon cell lines. If validated in independent sets of models, these markers could potentially be used to stratify likely responders in future combination trials.

In this study, we analyzed the in vitro antitumor activity of figitumumab in a broad panel of cancer cell lines. While antitumor activity with single-agent figitumumab was relatively rare, numerous combinations comprising figitumumab plus SOC or other targeted agents were found to be synergistic. We also investigated correlations between genetic and genomic characteristics of the cancer cell lines with figitumumab antiproliferative activity in order to identify predictive biomarkers of response. We confirmed that components of the IGF pathway, including IGFBPs, play a pivotal role in determining the sensitivity of tumor cells to IGF1R inhibitors. For the first time, we report that amplifications of IRS2, growth factor genes, and the MYB oncogene greatly sensitized cancer models to IGF1R inhibitors. MYB is activated in both hematological and epithelial tumors and IGF1R inhibition may represent a novel therapeutic opportunity to treat these neoplasms. To conclude, we have identified several biomarkers of antiproliferative activity following administration of figitumumab both as a single agent and in combination with other agents. Although clinical development of figitumumab has now been discontinued, our findings add to the growing body of knowledge related to the identification of biomarkers of response and may assist in the improved design of clinical trials of IGF1R inhibitors.

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References


Table 1. Six copy-number loci associated with figitumumab sensitivity at a false discovery rate < 0.25

<table>
<thead>
<tr>
<th>Locus</th>
<th>Best hit</th>
<th>Nominal p-value (best hit)</th>
<th>FDR</th>
<th>Cancer driver (distance)</th>
<th>IGF pathway gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q33.3-34</td>
<td>IRS2, MYO16</td>
<td>0.0003</td>
<td>0.16</td>
<td>SUZ12 (6Mb apart), NF1 (2Mb)</td>
<td>IRS2</td>
</tr>
<tr>
<td>17q11.2</td>
<td>6 genes (DHRS13, FLOT2, PHF12, PIPOX, SEZ6, TIAF1)</td>
<td>0.0005</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11q24.2</td>
<td>PKNOX2</td>
<td>0.0006</td>
<td>0.17</td>
<td>FLI1 (4Mb)</td>
<td></td>
</tr>
<tr>
<td>6q23.3</td>
<td>AHI1</td>
<td>0.0010</td>
<td>0.23</td>
<td>MYB (170kb)</td>
<td></td>
</tr>
<tr>
<td>17q22-24.3</td>
<td>42 genes</td>
<td>0.0058</td>
<td>0.23</td>
<td>PRKAR1A</td>
<td>GH1, GH2</td>
</tr>
<tr>
<td>12q12</td>
<td>ARID2, LOC400027</td>
<td>0.0058</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The activity profile of figitumumab was correlated with whole-genome copy number alterations and six distinct genomic loci were found to be associated with sensitivity at a false discovery rate (FDR) of < 0.25.
Table 2. Correlation between figitumumab sensitivity and mRNA expression of key genes in breast and colorectal cancer lines

<table>
<thead>
<tr>
<th>Gene</th>
<th>Breast cancer</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Colorectal cancer</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman R</td>
<td>P value</td>
<td>FDR</td>
<td>Spearman R</td>
<td>P value</td>
<td>FDR</td>
<td>Spearman R</td>
<td>P value</td>
</tr>
<tr>
<td>IGF1</td>
<td>−0.112</td>
<td>0.550</td>
<td>0.687</td>
<td>−0.067</td>
<td>0.784</td>
<td>0.840</td>
<td>−0.067</td>
<td>0.784</td>
</tr>
<tr>
<td>IGF1R</td>
<td>−0.334</td>
<td>0.066(^a)</td>
<td>0.331</td>
<td>−0.542</td>
<td>0.017(^c)</td>
<td>0.093(^b)</td>
<td>−0.542</td>
<td>0.017(^c)</td>
</tr>
<tr>
<td>IGF2</td>
<td>−0.369</td>
<td>0.041(^c)</td>
<td>0.307</td>
<td>0.228</td>
<td>0.349</td>
<td>0.638</td>
<td>0.228</td>
<td>0.349</td>
</tr>
<tr>
<td>IGF2R</td>
<td>0.036</td>
<td>0.848</td>
<td>0.848</td>
<td>−0.256</td>
<td>0.290</td>
<td>0.621</td>
<td>−0.256</td>
<td>0.290</td>
</tr>
<tr>
<td>IGF2BP3</td>
<td>0.182</td>
<td>0.337</td>
<td>0.569</td>
<td>0.487</td>
<td>0.758</td>
<td>0.840</td>
<td>0.487</td>
<td>0.758</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>0.167</td>
<td>0.386</td>
<td>0.569</td>
<td>−0.078</td>
<td>0.758</td>
<td>0.840</td>
<td>−0.078</td>
<td>0.758</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>−0.311</td>
<td>0.094(^a)</td>
<td>0.353</td>
<td>−0.070</td>
<td>0.775</td>
<td>0.840</td>
<td>−0.070</td>
<td>0.775</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>0.179</td>
<td>0.336</td>
<td>0.569</td>
<td>0.022</td>
<td>0.929</td>
<td>0.929</td>
<td>0.022</td>
<td>0.929</td>
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<tr>
<td>IGFBP4</td>
<td>−0.086</td>
<td>0.652</td>
<td>0.752</td>
<td>−0.545</td>
<td>0.016(^c)</td>
<td>0.093(^b)</td>
<td>−0.545</td>
<td>0.016(^c)</td>
</tr>
<tr>
<td>IGFBP5</td>
<td>−0.157</td>
<td>0.408</td>
<td>0.569</td>
<td>0.289</td>
<td>0.230</td>
<td>0.574</td>
<td>0.289</td>
<td>0.230</td>
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<tr>
<td>IGFBP6</td>
<td>0.226</td>
<td>0.221</td>
<td>0.553</td>
<td>0.534</td>
<td>0.019(^c)</td>
<td>0.093(^b)</td>
<td>0.534</td>
<td>0.019(^c)</td>
</tr>
<tr>
<td>INSR</td>
<td>−0.154</td>
<td>0.417</td>
<td>0.569</td>
<td>−0.110</td>
<td>0.654</td>
<td>0.840</td>
<td>−0.110</td>
<td>0.654</td>
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<tr>
<td>IRS1</td>
<td>−0.256</td>
<td>0.172</td>
<td>0.516</td>
<td>0.212</td>
<td>0.383</td>
<td>0.638</td>
<td>0.212</td>
<td>0.383</td>
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<tr>
<td>IRS2</td>
<td>0.055</td>
<td>0.777</td>
<td>0.833</td>
<td>−0.175</td>
<td>0.472</td>
<td>0.709</td>
<td>−0.175</td>
<td>0.472</td>
</tr>
<tr>
<td>MYB</td>
<td>−0.400</td>
<td>0.029(^c)</td>
<td>0.307</td>
<td>−0.444</td>
<td>0.057(^b)</td>
<td>0.171</td>
<td>−0.444</td>
<td>0.057(^b)</td>
</tr>
</tbody>
</table>

CRC, colorectal cancer; FDR, false discovery rate.

\(^a\)p < 0.1; \(^b\)significant at FDR < 0.1; \(^c\)p < 0.05
Table 3. Synergies between figitumumab and other anti-cancer agents

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Figitumumab combination</th>
<th>Mechanism of action</th>
<th>Synergy count</th>
<th>Biomarkers of synergy(^a)</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Trastuzumab</td>
<td>ERBB2 (HER2) inhibitor</td>
<td>13/25 (52%)</td>
<td>[RB1\ parent=\text{wt} ]</td>
<td>S1A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[ERBB3\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[ERBB4\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[NRAS\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGF2R\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IRS2\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGFBP6\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGF2BP3\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>Dacomitinib</td>
<td>pan-ERBB inhibitor</td>
<td>5/27 (19%)</td>
<td>[ERBB3\ parent=\text{mRNA high} ]</td>
<td>S1B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGFBP3\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>PF4691502</td>
<td>PI3K/MTOR inhibitor</td>
<td>4/27 (15%)</td>
<td>[TP53\ parent=\text{wt} ]</td>
<td>S1C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[TP53\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>[IGF2R\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[INSR\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IRS1\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGF2BP3\ parent=\text{mRNA high} ]</td>
<td></td>
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<tr>
<td>Colon</td>
<td>PF4691502</td>
<td>PI3K/MTOR inhibitor</td>
<td>7/19 (37%)</td>
<td>[BRAF\ parent=\text{mRNA low} ]</td>
<td>S1D</td>
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<tr>
<td></td>
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<td>[EGFR\ parent=\text{mRNA low} ]</td>
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<td></td>
<td>[PTEN\ parent=\text{mRNA low} ]</td>
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<td></td>
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<td>[SMAD4\ parent=\text{mRNA low} ]</td>
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<tr>
<td>Colon</td>
<td>Cetuximab</td>
<td>EGFR inhibitor</td>
<td>12/18 (67%)</td>
<td>[IGFBP3\ parent=\text{mRNA low} ]</td>
<td>S1E</td>
</tr>
<tr>
<td>Colon</td>
<td>Dacomitinib</td>
<td>pan-ERBB inhibitor</td>
<td>7/19 (37%)</td>
<td>[ERBB3\ parent=\text{mRNA high} ]</td>
<td>S1F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[NRAS\ parent=\text{mRNA low} ]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGFBP1\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>Oxaliplatin</td>
<td>Alkylating agent</td>
<td>14/18 (78%)</td>
<td>[EGFR\ parent=\text{mRNA high} ]</td>
<td>S1G</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[HRAS\ parent=\text{mRNA low} ]</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>5-FU</td>
<td>TYMS (thymidylate synthase) Inhibitor</td>
<td>4/19 (21%)</td>
<td>[IGF1R\ parent=\text{mRNA low} ]</td>
<td>S1H</td>
</tr>
<tr>
<td>Colon</td>
<td>PD332991</td>
<td>CDK4/CDK6 inhibitor</td>
<td>13/19 (68%)</td>
<td>[STK11\ parent=\text{mRNA high} ]</td>
<td>S1I</td>
</tr>
<tr>
<td>NSCLC</td>
<td>PD332991</td>
<td>CDK4/CDK6 inhibitor</td>
<td>6/24 (25%)</td>
<td>[SMAD4\ parent=\text{mRNA high} ]</td>
<td>S1J</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IGF1R\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[IRS2\ parent=\text{mRNA high} ]</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>Dacomitinib</td>
<td>pan-ERBB inhibitor</td>
<td>6/24 (25%)</td>
<td>[NRAS\ parent=\text{mRNA low} ]</td>
<td>S1K</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Erlotinib</td>
<td>EGFR inhibitor</td>
<td>4/24 (17%)</td>
<td>-</td>
<td>S1L</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Gemcitabine</td>
<td>Anti-metabolite</td>
<td>2/24 (8%)</td>
<td>[IGF2BP3\ parent=\text{mRNA low} ]</td>
<td>S1M</td>
</tr>
<tr>
<td>NSCLC</td>
<td>PF4691502</td>
<td>PI3K/MTOR inhibitor</td>
<td>4/24 (17%)</td>
<td>[KRAS\ parent=\text{mRNA low} ]</td>
<td>S1N</td>
</tr>
</tbody>
</table>

\(^a\) mRNA expression of IGF pathway genes and Myb were obtained from TaqMan RT-PCR, expression of other genes (main cancer drivers) is from Affymetrix whole-genome expression arrays. The statistical significance was calculated using the Fisher’s exact binomial test for mutation data, and by Kruskal-Wallis test for mRNA data.
b significant at $p < 0.05$; c significant at $p < 0.01$
Figure legends

**Figure 1.** Figitumumab activity in a cell panel correlated with the status of pathway genes and major cancer drivers

A. Ordered figitumumab *in vitro* antiproliferative activity measured as IC$_{50}$ in a panel of breast, colon, lung small cell and non-small cell cancer lines. B. Comparison of IC$_{50}$ values with the tissue of origin. C. Comparison with the genetic status of major cancer drivers. The red color shows protein-coding mutations as obtained from COSMIC and Oncocarta profiling, green indicates amplification (see Material and Methods), blue represents mutations or deletions in tumor suppressors, and yellow indicates likely wild-type status. On the right are the p-values from the Kruskal-Wallis test. D. Representative genes from six major copy-number loci in Table 1 associated with figitumumab sensitivity at false discovery rate < 0.25. E. Comparison with PCR-measured RNA expression of IGF pathway genes and *MYB*. Significant correlations are highlighted in colors; red mean positive correlation between high expression and IC$_{50}$ (and thus sensitivity), blue signifies correlation between high expression and resistance (Spearman’s rank correlation).

**Figure 2.** Molecular predictors of sensitivity to figitumumab

Odds ratio and p-value comparison for significant predictors of sensitivity. For each predictor, the genomic information was condensed into a binary absent–present status in each line. Then we divided cell lines into sensitive (IC$_{50}$ < 100nM) and resistant (IC$_{50}$ ≥3μM) and obtained a 2x2 table with frequencies. From the frequency tables, odds ratios and Fisher’s binomial test p-values were calculated. The dashed vertical line corresponds to odds ratio 1 (equal frequency in resistant and sensitive lines). Odds ratios > 1 indicate alterations positively correlated with response; ratios <1 characterize changes found in resistant lines. Changes found only in resistant or only in sensitive cell lines are plotted outside the odds ratio scale (grey vertical lines) on the left and right, respectively.

**Figure 3.** Genomic copy-number changes associated with changes in IGF pathway predictive signature in 300 cancer cell lines

The plot shows correlation between the predictive IGF pathway signature and gene copy number changes. The signature was developed as follows: we started with probesets from Affymetrix U133 Plus 2 arrays that correspond to 14 IGF pathway genes in Figure 1E (without *MYB*). We used these probesets to develop an IGF-pathway classifier in our panel of 93 lines using DLDA. This new classifier contains 14 probesets from 7 IGF pathway genes (full model is available in the supplementary information). The performance of this IGF-pathway-restricted (70% correct) classifier was lower compared with the whole-genome signature (85% correct). This new pathway signature
was used to score IGF-pathway activity in 300 cell lines and compared with copy number status of all human genes (see Methods). The plots show the correlation between copy number status of all human genes (rank ordered) correlated with the IGF-pathway signature. Circles represent rank ordered human genes, red circles are genes significantly correlated with the IGF-pathway score at a false discovery rate (FDR) <0.15. Key predictors and pathway genes are labeled, green circles correspond to genes significant at FDR < 0.15, blue are genes not significantly associated with IGF pathway modulation.
Figure 2

-log10(p-value) vs Odds ratio

1. 6q23.3/AHI1 amp
2. 6q23.3/MYB amp
3. 11q24.2 amp
4. 13q33.3.34/IRS2 amp
5. 17q22.24.3/GH1-2 amp
6. 17q11.2 amp
7. IGF1R over-expressed
8. INSR over-expressed
9. IRS2 over-expressed
10. IGFBP3 under-expressed
11. IGFBP5 under-expressed
12. MYB over-expressed
13. IGF pathway classifier (PCR)
14. DLDA classifier
15. Colon origin

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Molecular Cancer Therapeutics

Molecular Predictors of Sensitivity to the Insulin-like Growth Factor 1 Receptor Inhibitor, Figitumumab (CP-751,871)

Adam Pavlicek, Maruja E. Lira, Nathan V Lee, et al.

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