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Corporate Headquarters:
Crown Bioscience Inc.
3375 Scott Blvd., Suite 108
Santa Clara, CA 95054 USA
Tel: 855.827.6968 | Fax: 888.882.4881
busdev@crownbio.com | www.crownbio.com

HuTrial Case Study
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AppNote

HuTrial™ Case Study Application Note

CrownBio's HuTrial: understanding who will benefit from your treatment before you enter the clinic

Cancer is a heterogeneous disease, in which one drug will not work for all patients. The foundation of clinical trials for new investigational agents is a population study, involving a cohort of patients. However, as preclinical research in oncology continues to focus on targeted therapies, and a move towards precision medicine, the way clinical trials are performed needs to be refined. Oncology failure rates historically stand at 95%[1], often due to a lack of efficacy rather than toxicity, with only small subsets of patients responding to a novel agent. As we gain further understanding about the molecular mechanisms that predict who will respond to a specific agent, correct patient selection or stratification within clinical trials is vital to increase response and reduce attrition rates.

Predictive biomarkers and gene signatures could greatly facilitate stratification in the clinic, and are driving forward precision medicine in oncology. Scientists are searching for methods which can accurately identify and validate biomarkers and gene signatures in a cost-effective manner, and have found such an approach in Preclinical Phase-II like mouse clinical trials, HuTrials, also known as human surrogate or "avatar" trials. HuTrials utilize patient-derived xenograft (PDX) models from our HuPrime® collection, with each PDX subject reflecting the pathology of its original patient (behaving as a patient avatar), and the cohort of patient avatars representing a diversity of the human patient population. Therefore, HuTrials support true population trials just as clinical trials do, leading to similar conclusions (predictive), which can involve single agents and combination treatments, and also have direct comparisons with standard of care (SoC) therapies.

Utilizing HuTrials with our proprietary state of the art genetic signature discovery and validation algorithm, HuSignature™ and our HuMark™ Translational Platform also allows the full elucidation of signatures and predictive biomarkers for clinical trial stratification.

This Application Note details three interesting and diverse uses for HuTrials:

- To identify a subset of small cell lung cancer (SCLC) PDX models which are sensitive to a poly(ADP-ribose) polymerase-1 (PARP) inhibitor[2]
- To evaluate oncogenic mutation alleles which better predict a response to cetuximab in colorectal cancer (CRC) than the current patient stratification of KRAS 12/13 mutations[3]
- To discover a HuMark predictive biomarker of EGFR gene amplification for cetuximab treatment of gastric cancer (GC)[4].

Case Study 1: Identifying a Subset of SCLC PDX Models Sensitive to the PARP Inhibitor Niraparib[2]

Small cell lung cancer is an aggressive form of disease that accounts for approximately 15% of all lung cancers[2]. SCLC is characterized by rapid growth and early development of metastases, and while patients are initially highly responsive to treatment, relapse commonly occurs within months. Historically, efforts at characterizing the molecular underpinning of SCLC have lagged behind those of non-small cell lung cancer (NSCLC), and the current treatment paradigm is dominated by platinum-based chemotherapy regimens[2]. Unlike NSCLC treatment, newer targeted therapies have shown little impact on SoC in SCLC or patient survival.

The first in class PARP inhibitor Lynparza™ was approved by the US Food and Drug Administration (FDA) in late 2014 for patients with deleterious germline BRCA mutated advanced ovarian cancer following previous treatment with chemotherapy[5]. Early 2016 has seen a Breakthrough Therapy designation follow for treatment of BRCA1/2 or ATM gene mutated metastatic castration resistant prostate cancer (mCRPC) following taxane-based chemotherapy and at least one newer hormonal agent[6]. Due to the role of PARP in DNA repair, PARP inhibitors were originally developed as chemo- and radio-potentiators, and *in vitro* data have shown that PARP inhibitors may be beneficial in tumors relying upon mechanisms of DNA repair for survival, including SCLC[7,8].

To test this hypothesis, TESARO, Inc. in partnership with CrownBio conducted a HuTrial study on a cohort of 31 SCLC PDX models, evaluating their orally active PARP inhibitor niraparib[2] (currently in Phase III trials as a maintenance agent in platinum sensitive ovarian and BRCA+ breast cancer patients). To mimic the maintenance therapy in the clinic, niraparib monotherapy followed a single cycle of cisplatin plus etoposide in the HuTrial. Initially an N of 1 design was used – with one animal per model receiving SoC treatment, and a partner animal receiving SoC followed by niraparib[2].

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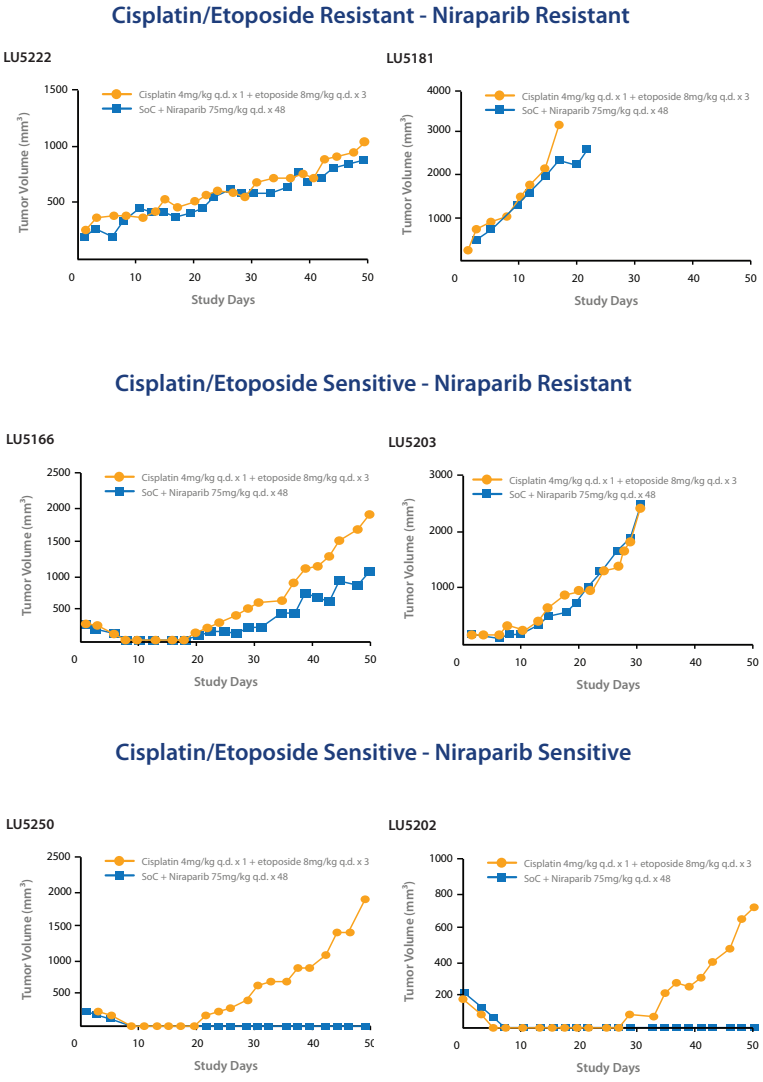
A range of responses were observed (**Figure 1**):

- models which were cisplatin/etoposide resistant and niraparib resistant
- models which were cisplatin/etoposide sensitive and niraparib resistant
- models which were cisplatin/etoposide sensitive and niraparib sensitive[2].

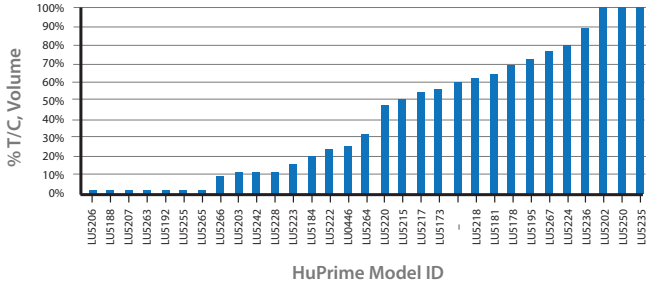
Figure 1: Example Range of Responses to SoC and SoC + Niraparib[2]



A summary of the N of 1 screening data is shown in **Figure 2**. From the cohort, 6/31 (19%) had a robust response (>75%) to niraparib following SoC, with all of these models also being sensitive to SoC. A moderate response (>50%) was observed in 13/31 (41%) of the models and a minimal response (<25%) to niraparib following SoC was observed in 15/31 (48%) of the models[2].

Figure 2: Summary of *In Vivo* HuPrime SCLC Model Response to Cisplatin and Etoposide Treatment followed by Niraparib Maintenance Therapy[2]

For each model: one animal dosed with chemotherapy to mimic front line SoC (Day 1: cisplatin 4mg/kg; Days 1 through 3: etoposide 8mg/kg); one animal dosed with the same representative SoC regimen followed by niraparib 75mg/kg maintenance regimen (dosing from Day 8, q.d. x 48)[2].

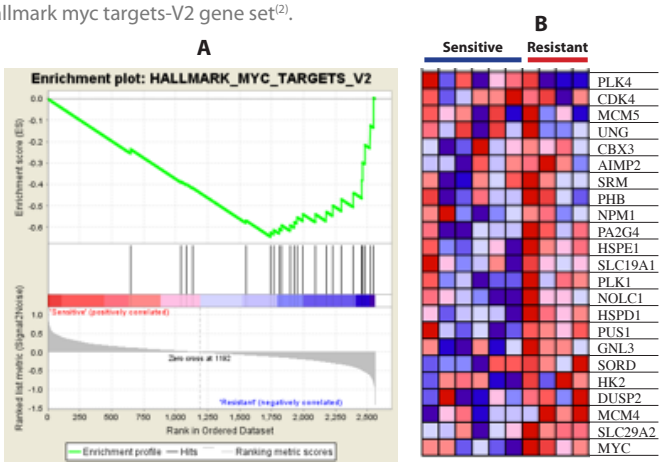


Six responsive models were chosen for confirmatory efficacy studies, utilizing n=5 animals per group. Five out of the six models reproduced the screening results, supporting the N of 1 design as an effective means of evaluating therapeutics in HuPrime SCLC models[2].

Whole exome sequencing was performed on untreated tumor samples from six highly sensitive and four resistant models to identify biomarkers predictive of response. Sequencing was performed via Illumina's Nextera Rapid Capture Exome Kit on Illumina HiSeq (140x mean coverage). Gene expression analysis was performed on 10 SCLC PDX models on the HTG EdgeSeq Oncology Biomarker Panel (HTG Molecular, Tuscon, AZ)[2]. Preliminary findings revealed genes with variants more prevalent in niraparib sensitive (i.e. NADK, TMEM14B) or resistant models (i.e. CBX4, MAP3K4). Expression values of approximately 2,500 genes were subjected to Gene Set Enrichment Analysis (GSEA). The top cancer hallmark gene set enriched in the niraparib-resistant group is the MYC target set (FDR q-value = 0.0001; **Figure 3**)[2].

Figure 3: Gene Expression Analysis[2]

A: Expression values of ~2,500 genes resulting from this assay were subjected to Gene Set Enrichment Analysis (GSEA)[2]. B: Heat map of core enrichment genes in hallmark myc targets-V2 gene set[2].



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Case Study 1 Conclusions

From a HuTrial with a cohort of 31 PDX SCLC models, approximately 40% showed benefit from niraparib maintenance after a single cisplatin and etoposide regimen. A robust response to niraparib maintenance was observed in 30% of the cisplatin and etoposide sensitive models. Preliminary biomarker analysis suggests that tumors characterized by overexpression of myc-related genes may be resistant to niraparib maintenance regimens. Together, these data provide support for the investigation of niraparib maintenance in SCLC patients after response to frontline therapy. The HuTrial also confirms that an N of 1 design is an effective means of evaluating therapeutics in HuPrime SCLC models.

Case Study 2: Evaluation of Oncogenic Mutation Alleles to Better Predict Response to Cetuximab in CRC than KRAS 12/13 Mutations[3]

Colorectal cancer is the third most common cancer diagnosed in both men and women in the United States[9], and has a high metastatic frequency (mCRC) of approximately 50%. Common treatment options include combination chemotherapy regimens and targeted agents including bevacizumab, cetuximab, and panitumumab.

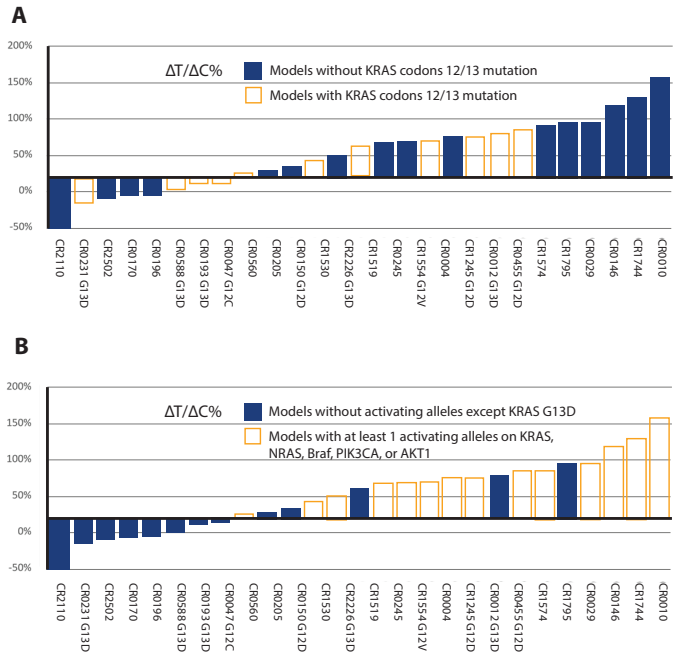
Cetuximab was initially approved by the FDA in 2004 in mCRC for the treatment of EGFR-expressing tumors refractory/intolerant to irinotecan-based chemotherapy without patient stratification[10]. Subsequently in 2009, patients with activating KRAS mutations at codons 12 and 13 were excluded from cetuximab treatment following retrospective subset analysis[11]. However, only 35% to 50% of patients with wildtype KRAS CRC actually benefit from cetuximab use[12,13], and a retrospective analysis in 2010 observed that patients with a KRAS mutation at codon 13 (G13D) could still benefit from the treatment[14]. Therefore, there is an apparent unmet medical need to refine cetuximab labeling to include previously excluded "responders" and to exclude the previously included "non-responders".

Reports have suggested that gene amplification and overexpression of EGFR or its ligands, epiregulin and amphiregulin, could potentially serve as positive predictors of cetuximab response. Other genetic alterations including activating mutations of EGFR and BRAF (e.g. V600E), and the activation of ERBB2 signaling, could serve as negative predictors in addition to KRAS mutations[3]. However, KRAS mutation is still the only biomarker used for patient stratification in the clinic.

CrownBio therefore investigated whether KRAS G13D mutation, or any other activating oncogene alleles, were predictive of cetuximab response in CRC. We conducted a HuTrial utilizing a randomly selected cohort of 27 EGFR+ PDX models from treatment-naïve Asian CRC patients. **Figure 4** shows a waterfall plot of ΔT/ΔC values for the CRC PDX models. The results demonstrated that 8/27 (approximately 30%) of the cohort were responders (ΔT/ΔC <20%), with the remaining 19/27 (70%) being non-/partial responders (ΔT/ΔC >20%). Among the PDX models, 15 contained KRAS mutations (5 codon 12 G12C/D/V; 6 codon 13 G13D, 2 at Q61H, and 2 at A146T). Studying these mutants and their response to cetuximab treatment we found that there were significantly no fewer KRAS 12/13 allele responders (4/8; 50%) than non-/partial responders (7/19; 37%). In particular, there were statistically no fewer G13D responders (4/8; 50%) than non-/partial responders (2/19; 10.5%). This suggests that KRAS 12/13 mutations are not predictive of poor response to cetuximab[3].

Figure 4: Waterfall Plot of Response for CRC HuTrial[3]

A: Per KRAS codons 12/13 mutation rule: wildtype vs mutations. B. Per the set of oncogenic alleles rule: wildtype/KRAS G13D vs at least one activating allele on KRAS G12C/D/V, -Q61X, -A146T, NRAS Q61X, AKT1 L52R, PIK3CA E545K/Q546L, and BRAF-V600E.



Antitumor activities were analyzed against common oncogenic mutation alleles frequently found in CRC, which had been identified within the PDX cohort by RNAseq (including KRAS, NRAS, AKT1, BRAF, and PIK3CA)[7]. Studying the non-responder population in detail, we found that 16/19 non-/partial responders had at least one of the activating alleles:

- KRAS G12C/D/V (5/19)
- -Q61X (2/19)
- -A146T (2/19)
- NRAS Q61X (1/19)
- AKT1 L52R (1/19)
- PIK3CA E545K/Q546L (5/19)
- BRAF V600E (2/19)

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These results are in contrast to 0/8 studied responders which were all wildtype for all of these alleles (Fisher's exact test $p=7.43x10^{-5}$). This suggests that a composite oncogenic allele profile could be more predictive for cetuximab response in CRC[3].

Case Study 2 Conclusions

Our data on an independent cohort of CRC PDX models support the recent clinical observation, but against the current practiced patient stratification, of using KRAS mutations at codon 12 and 13 to decide CRC treatment by cetuximab. Our data seem to suggest that a set of six oncogenic alleles may be of better predictive value than the current practiced stratification, justifying a new prospective clinical investigation on an independent cohort for confirmation[3].

Case Study 3: Discovery of EGFR Gene Amplification as a HuMark Predictive Biomarker for Cetuximab Treatment of GC[4]

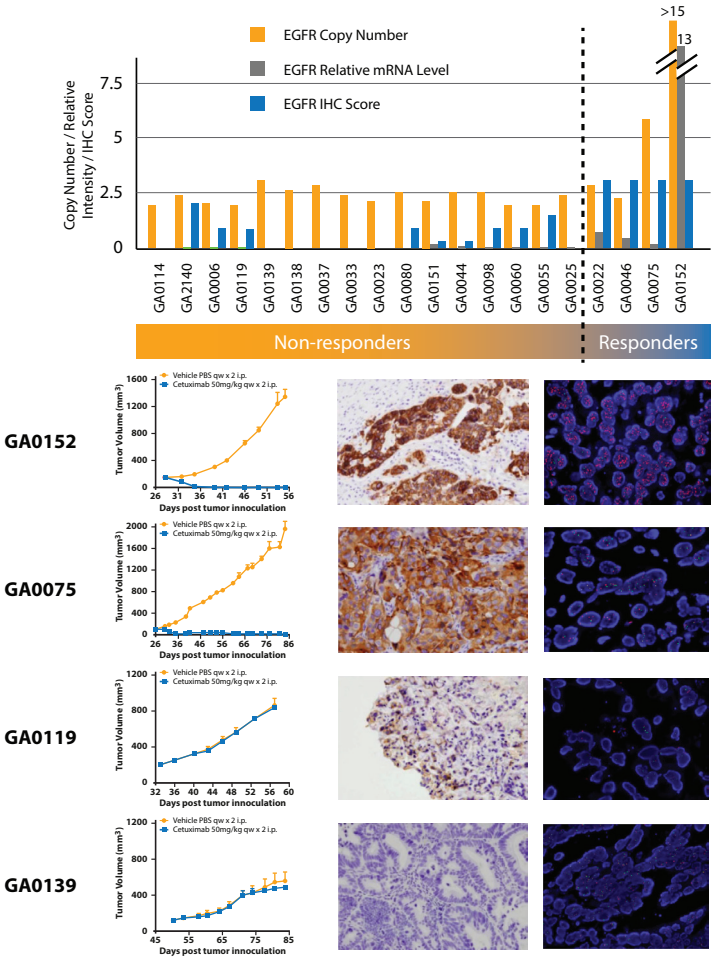
Gastric cancer, specifically gastric adenocarcinoma, is one of the leading causes of cancer mortality worldwide, with a poor treatment outcome for the majority of patients. Modest efficacy and the considerable toxicity associated with chemotherapy have prompted research into novel therapies targeting the genetic and molecular alterations that drive GC carcinogenesis[4].

Several Phase II and III trials have evaluated cetuximab in GC. However, as there are no established biomarkers to predict GC patient response to cetuximab, some trials have not shown an increase in survival following treatment[15]. CrownBio therefore investigated the activity of cetuximab in a HuTrial utilizing 20 GC PDX models from treatment-naïve Asian patients, to identify such a biomarker. The HuTrial was combined with our HuMark Translational Platform (fully detailed in our Translational Oncology Application Note) to elucidate a predictive biomarker for patient stratification in the clinic.

Following treatment with cetuximab, 4/20 (20%) models showed an almost complete response to therapy (ΔT/ΔC <0), whilst 16/20 (80%) of PDX models showed partial or complete resistance (ΔT/ΔC >30%). Analysis showed that the models that responded to cetuximab treatment corresponded to a GC subset with EGFR amplification and overexpression. EGFR gene copy number was shown to be significantly higher for all four responders (≥4) than most of the non-responders. The highest gene copy number observed was 15, which corresponded to the model which was the 'best responder' (GA0152). The four responders were also shown to express statistically significantly higher levels of EGFR mRNA, with the highest value of 10.5 observed in model GA0152, corresponding to the increased gene amplification. The four responder PDX models also had a significantly higher EGFR immunostaining score (IHC score 3+) than non-responders (IHC score 0 to 2, **Figure 5**).

Figure 5: Response to Cetuximab Treatment and Genetic Profile of GC PDX Models[4]

PDX GC models sorted by ΔT/ΔC following cetuximab treatment. Example responders and non-responders to cetuximab treatment. GA0152 and GA0075: both have EGFR IHC score 3+ and gene amplification of copy number >15 and = 5.8, respectively. GA0119 and GA0139: both have low IHC score and no gene amplification. IHC antibody: monoclonal antibody against human EGFR (Cell Signaling), score based on staining intensity of the membrane. FISH probes: dual EGFR Spectrum Orange/CEP7 Spectrum Green Probe (Vysis, Abbott Molecular). Average tumor size for 10 animals per model.



Mutation analysis of common oncogenes associated with the EGFR pathway (KRAS, BRAF, c-MET, EGFR, AKT, and PI3KCA) also did not reveal any aberrations that could easily explain the non-response of the majority of the GC PDX models to cetuximab[4].

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Case Study 3 Conclusions

Our HuMark analysis suggest that a GC subset with high EGFR mRNA expression and EGFR gene amplification (also IHC score 3+) may benefit from cetuximab treatment, both of which can be used as a gene signature/biomarker to predict cetuximab responders. These HuMark biomarkers can help to guide future potential success in clinical trials, and act as a basis for a patient stratification guide for clinical treatment.

Conclusions

Increasing the efficiency of the transition from successful preclinical to clinical research in oncology is essential to reduce drug attrition rates and to enable the development of precision medicine. CrownBio offers a full Translational Oncology Platform to improve molecule selection and identify patients who will benefit most from a treatment regimen.

CrownBio HuTrials on a cohort of intent-to-treat patient populations enables a "go or no-go" decision. It also allows the identification of signatures and biomarkers of responders and non-responders, by leveraging HuPrime the world's largest commercial collection of genomically characterized and diverse PDX models, in a fast and cost-effective approach. The identification of appropriate signatures and biomarkers which define responders and non-responders allows the selection of appropriate models to accelerate the understanding of likely success of your candidate in the clinic.

The clinical predictivity of PDX models is changing the way preclinical data is viewed, how drug discovery programs are progressed, and how clinical trials are designed. CrownBio is leading the way in making these models accessible, affordable, and translatable.

CrownBio can be contacted at **busdev@crownbio.com** for any further questions or information required on our Translational Oncology Platform, or for information on other CrownBio products and services.

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