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Title: A Pilot Clinical Study of Treatment Guided by Personalized Tumorgrafts in Patients with Advanced Cancer

Authors: Manuel Hidalgo¹⁻⁴, Elizabeth Bruckheimer⁵, N.V. Rajeshkumar¹, Ignacio Garrido-Laguna¹, Elizabeth De Oliveira¹, Belen Rubio-Viqueira²⁻³, Steven Strawn⁵, Michael J. Wick⁶, James Martell⁵, David Sidransky^{1, 7}

Affiliations: ¹Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD. ²Centro Integral Oncológico "Clara Campal", Madrid, Spain. ³Clinical Research Program, Spanish National Cancer Research Center (CNIO), Madrid, Spain. ⁴Universidad CEU-San Pablo, Madrid, Spain. ⁵Champions Biotechnology, Inc. Science and Technology Park at Johns Hopkins, Baltimore, MD. ⁶South Texas Accelerated Research Therapeutics, San Antonio, TX. ⁷Department of Otolaryngology - Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, MD.

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Corresponding Author: Manuel Hidalgo, MD., Ph.D, Clinical Research Program, Spanish National Cancer Research Center (CNIO), Melchor Fernandez Almagro 3, 28029, Madrid, Spain. Phone: + 34-91-224-6900, Fax: + 34-91-224-6980, E-mail: mhidalgo@cnio.es

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Abstract

Patients with many advanced solid cancers have very poor prognosis and improvements in life expectancy are measured only in months. We have recently reported the remarkable clinical outcome of a patient with advanced, gemcitabine-resistant, pancreatic cancer who was later treated with DNA damaging agents, on the basis of the observation of significant activity of this class of drugs against a personalized tumorgraft generated from the patient's surgically resected tumor. Here, we extend the approach to patients with other advanced cancers. Tumors resected from 14 patients with refractory advanced cancers were propagated in immunodeficient mice and were treated with 63 drugs in 232 treatment regimens. An effective treatment regimen in the xenograft model was identified for 12 patients. One patient died before receiving treatment and the remaining 11 patients received 17 prospectively guided treatments. Fifteen of these treatments resulted in durable partial remissions. In two subjects, no effective treatments were found. Overall, there was a remarkable correlation between drug activity in the model and clinical outcome, both in terms of resistance and sensitivity. The data supports the use of personalized tumorgraft model as a powerful investigational platform for therapeutic decision-making and to efficiently guide cancer treatment in the clinic.

Introduction

When it comes to anticancer drugs, a major obstacle is that one size does not always fit all (1). The individualization of cancer treatment may improve the outcome and patient compliance (2). While the rationale for this idea is strong and early clinical examples with targeted agents support this notion, the broad practical implementation of this concept remains difficult. In general, the field is mainly focused on finding the right patient for a given drug by implementing biomarkers predictive of drug action. For example, patients with lung cancer are now often assessed for mutations in the EGFR gene because such genetic alterations confer susceptible to inhibitors of the EGFR kinase (3, 4). Notwithstanding the importance of biomarker-driven approaches for cancer treatment, it has several challenges (5). First, it is a drug-centered, rather than patient-centered, where the main goal is to identify patients that may be good candidates for an agent. Second, often these biomarkers predict resistance rather than susceptibility (6). The frequency of most of these biomarkers is low within a given population and thus fails to provide a solution for most patients. Third, for multiple approved drugs, biomarkers are not known. Fourth, discoveries are in general restricted to diseases in which the drugs are approved thereby limiting the possibility of finding effective applications in other tumor types. Finally, the positive predictive values are not perfect and many patients despite having the appropriate biomarkers either do not respond or do it only transiently.

Personalized tumorgrafts developed in mice from patients' tumor tissues could potentially resolve some of these above-mentioned issues. These tumors recapitulate the biological characteristics of the disease of origin and suitable for the quick assessment of the chemosensitivity of patients' cancer (7). Here, we used the tumorgraft model to personalize the treatment course for patients with advanced cancers.

Materials and Methods

Patients. Patients were enrolled in the Johns Hopkins University protocol J0507 (NCT00276744) or at the Hospital de Madrid protocol FHM.06.10. In these studies, patients with refractory solid tumors or early stage poor prognosis cancers have the opportunity to have their tumors implanted in nude mice. A fresh tumor specimen is

collected either at the time of surgical resection or by a tumor biopsy and implanted in immunodeficient mice and propagated (8, 9).

Preclinical studies. When tumorgrafts reached approximately 150 mm³, animals were randomized (5 mice with tumors on both flanks /group) and dosing initiated (Supplementary Table 1). Final tumor volumes were compared using a two tailed ANOVA adjusted for multiple comparisons. A rank list of effective treatments was provided to the treating physician who then selects the patient treatment.

Biological and pharmacological studies. Gene expression analysis was performed using Affymetrix U 133 Plus 2.0 gene arrays (10). Gene set analysis was performed using the GSEA software V2.0.2. Genes represented by more than one probe were collapsed using the Collapse Probes utility to the probe with the maximum value. We used unsupervised clustering analysis to classify responders and resistant tumors based on the expression of irinotecan pathway genes. The intratumor concentrations of irinotecan (CPT 11) and the metabolite SN38 were measured in tumors collected 6 hour after the last dose of CPT11 as previously described (11).

Results

A total of 14 tumorgrafts were obtained from 14 patients from either primary resected tumor (6 patients) or a resected metastasis (8 patients) and were treated with 63 different anticancer agents spanning 33 unique mechanism of action in 232 single agent or combination treatments. Supplementary Table 1 provides details regarding drugs, mechanism of action, combinations, dose and schedules, and activity noted in the mouse model. A regimen was considered active if it resulted in a TGI \geq 80 % and/or a PR rate \geq 50 %. In 2 tumors, JH082 (pancreatic cancer) and CBI-0701 (myoepithelioma of the salivary gland) no effective regimen was found in the 4 and 13 treatments tested, respectively.

Table 1 depicts the most relevant patient's characteristics. Three patients with standard of

care resistant metastatic cancers remain alive at 50+, 14+ and 50+ months. Patient CBI-0803 is a 54 year old male, who presented with stage IV gastroesophageal adenocarcinoma with liver and lung metastasis. The patient was initially treated with an epirubicin-cisplatin-capecitabine regimen with a partial response that lasted 8 months. Subsequently, developed disease progression with lung and liver metastasis and an elevation of the CEA tumor marker (Figure 1A). At that point, a tumorgraft generated from a resected liver metastasis had been treated with 17 different drugs in 35 combinations. As shown in Figure 1B, the tumorgraft responded to the combination of irinotecan, bevacizumab and cetuximab which was recommended for clinical use. With this treatment, the patient achieved a partial response in the liver metastasis (Figure 1C [pre treatment] and D) that lasted 14 months. At that point, his CEA started to rise again to 200 UI/mL. Data from his personalized tumorgraft indicated susceptibility to nab-paclitaxel (ABI-007) in combination with several angiogenesis inhibitors (Figure 1E). The patient received treatment with nab-paclitaxel in combination with bevacizumab with a normalization in CEA levels that has been maintained for 8 months (Figure 1A).

The second patient, CBI-0805, is a 44 year old female diagnosed with stage III colon cancer. The patient was treated with surgery and 5-fluorouracil-irinotecan chemotherapy. After 2 years, presented with liver metastasis and underwent tumor resection. Tumorgraft from this patient was extremely sensitive to irinotecan (Figure 2A). Six months after surgery, the patients progressed with a large pelvic mass (Figure 2 B [pretreatment] and C) that caused severe pain and hydronephrosis requiring nephrostomy tubes. She was treated with single agent irinotecan and achieved a partial response with resolution of her pain and restoration of urinary flow which is still maintained at 14+ months.

The third case is a 61 year-old male, who underwent a distal pancreatectomy for a pT3N1M0 ductal adenocarcinoma of the pancreas. The clinical outcome of this patient has been recently reported (12), and the patient remain disease free 50+ months after diagnoses.

Tumor CBI-0805 showed remarkable sensitivity to irinotecan. To explore potential

mechanisms of sensitivity we compared this tumor gene expression profile with that of four additional colorectal cancer tumorgrafts with known response to irinotecan. Using a previously published irinotecan 24 gene expression signature, the three irinotecan sensitive tumors cluster together (Supplementary Figure 1) (11). A closer analysis of the expression of the candidate genes in this case shows that this patient tumor is characterized by high expression of the irinotecan membrane transporters ABCB1 and ABCG2 and low expression of the genes involved in the catabolism of SN38, the active metabolite of irinotecan (CYP3A4 and 5 and UGT1A1). Consistently with this gene expression profile, CBI-0805 had higher concentration of SN38 which represented 30% of the parental drug irinotecan (CPT11, Figure 2D). This suggests that this cancer's unique sensitivity to irinotecan is based on its ability to retain high concentrations of the active metabolite SN38.

Discussion

This report summarizes the results of a pilot study in patients with advanced cancer whose treatment were selected based on activity against a personalized tumorgraft developed from the patient's own cancer. The data shows a remarkable correlation between drug activity in the model and clinical outcome, both in terms of resistance and sensitivity. The treatments selected for each individual patient were not obvious and would have not been the first choice for a conventional second or third line treatment. This is perhaps best illustrated by the combination of cetuximab and bevacizumab in patient CBI-0803 as the combination of these two agents is not recommended in clinical practice (13). The objective response rate was 88 % for treatments deemed effective by the model and tested in the patients. Overall, 11 of 14 patients achieved a partial response. The expected response rate with phase I agents, the only available option for some of these subjects, is less than 10 % (14). This preclinical-clinical correlation supports the value of personalized tumorgraft models as predictive of clinical outcome. The abundant tumor materials obtained by propagating the cancer in mice allow biological and pharmacological studies in the validated models to understand the observed effects. This led, as recently reported, to the discovery of PALB2 mutations in a mitomycin C responsive patient (12, 15).

There are limitations to this approach that certainly challenge the broad clinical application of the process and that will need to be resolved before this can be first tested in a randomized clinical trial. The process requires large amounts of fresh tumor material and intense resources to generate the tumorgraft. Even in the best conditions 25-30 % of implants fail and those who engraft require 6-8 months of additional propagation to be useful for treatment.

In summary, this work shows that personalized tumorgrafts can be used to individualize patient treatment and to discover determinants of drug response. In those patients in whom an effective treatment is found, the clinical activity is remarkable. Nevertheless, this process has limitations with regards to efficiency, speed, and cost but given the promising response data, additional investigation to solve these issues is warranted.

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ID	Gender	Age	Tumor	Xenograft	Prior systemic therapy	Systemic therapy administered based on xenograft data	Response	Duration of Response
JH033	Male	63	Pancreatic ductal adenocarcinoma	Primary tumor	Gemcitabine	Mitomycin C/ Cisplatin	Partial Response	50+ months
JH082	Female	64	Pancreatic ductal adenocarcinoma	Primary tumor	None	Gemcitabine	Progressive Disease	
JH102	Female	66	Pancreatic ductal adenocarcinoma	Primary tumor	None	Gemcitabine	Stable Disease	
JH161	Female	70	Pancreatic ductal adenocarcinoma	Primary Tumor	None	Gemcitabine	Stable Disease	
CBI-0601	Female	55	Leiomyosarcoma	Pelvic metastasis	Adriamycin/Ifosfamide	Gemcitabine/Docetaxel/Bevacizumab	Partial Response	9 + months
					Gemcitabine/Doxetaxel	Capcitabine/Temozolomide	Partial Response	9 + months
CBI-0602	Male	45	Mesenchymalchondrosarcoma	Lung metastasis	VAC	Docetaxel/Irinotecan/Bevacizumab	Partial Response	9 months
					Sunitinib		Stable Disease	4 months
CBI-0603	Male	50	Non-small-cell lung carcinoma	Primary tumor	Carboplatin/Paclitaxel	Sorafenib/Irinotecan/Bevacizumab	Partial Response	9 + months
					Pemetrexed		No Response	
CBI-0701	Male	36	Myoepithelioma	Primary tumor	None	Doxorubicin/Cisplatin	Progressive Disease	
						Imatinib	Progressive Disease	
						Carboplatin-Bevacizumab	Progressive Disease	
CBI-0803	Male	54	Esophageal Adenocarcinoma	Liver metastasis	Epirubicin/5-Fluorouracil/Cisplatin	Irinotecan-Cetuximab-Bevacizumab	Partial Response	14+ months
						Abraxane-Avastin	Partial Response	9 months
						Ixabepilone-Avastin	Partial Response	9 months
						Ixabepilone-Navelbine	Partial Response	6 months
CBI-0805	Female	44	Colon Adenocarcinoma	Liver metastasis	FOLFOX	Irinotecan	Partial Response	50+ months
						Capecitabine/lapatinib	Partial Response	6 months
CBI-0810	Female	36	Breast cancer	Liver metastasis	5-Fluorouracil/Cyclophosphamide	None	No Response	
					Irinotecan/Bevacizumab		No Response	
CBI-0901	Male	49	Rhabdomyosarcoma	Lung metastasis	None	Docetaxel/Gemcitabine/Bevacizumab	Partial Response	8 months
CBI-0907	Female	62	Colon	Liver metastasis	FOLFOX	Cetuximab/Irinotecan	Partial Response	6 months
					Irinotecan		Partial Response	15 months
CBI-0914	Male	58	Melanoma	Lung metastasis	None	B-raf Inhibitor	Partial Response	6 months

Table 1. Patient characteristics, and outcome of treatments based on xenograft predictions. When tumorgrafts reached approximately 150 mm³, animals were randomized (5 mice with tumors on both flanks /group) and dosing initiated. Details regarding drugs, dose and schedules were provided in Supplementary Table 1.

FIGURE LEGENDS

Figure 1. Clinical outcome of patient CBI-0803 and response to anti-cancer agents in patient's xenografts.

A) Time course of CEA levels; B) Tumor growth curve of patients' personalized tumorgraft treated with the indicated agents at the dose and schedules; C) and D) CT scan of the abdomens before and after treatment with irinotecan-cetuximab and bevacizumab showing a marked decrease in tumor volume; E) Tumor growth curve of patient's tumor treated with the indicated agents.

Figure 2. Clinical outcome of patient CBI-0805 and remarkable anti-tumor potential of irinotecan in patient's xenografts.

A) Response of patient's tumorgraft to single agent irinotecan illustrates a complete eradication of the tumor; B) CT scan of pelvis before treatment; C) CT scan of the pelvis showing a significant reduction in a metastatic pelvic mass; D) Intra-tumor concentration of irinotecan and SN38 in this patients xenograft and an irinotecan resistant CRC tumorgraft (CRC-005) showing heightened retention of SN38 in CBI-0805.

Figure 1

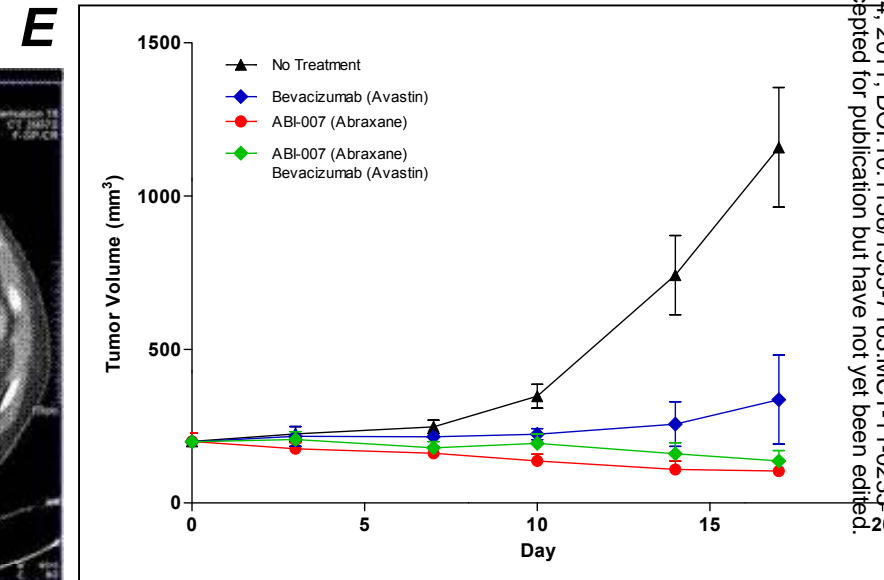
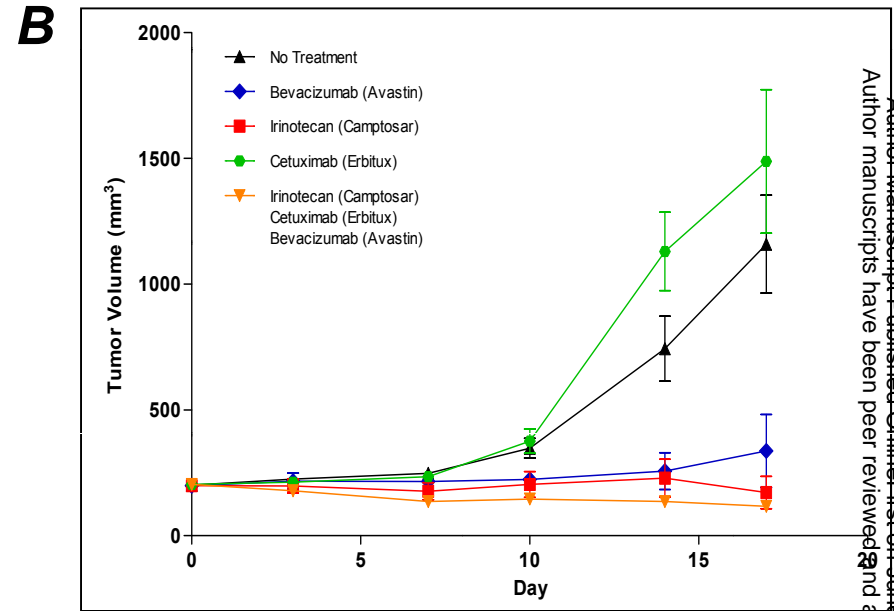
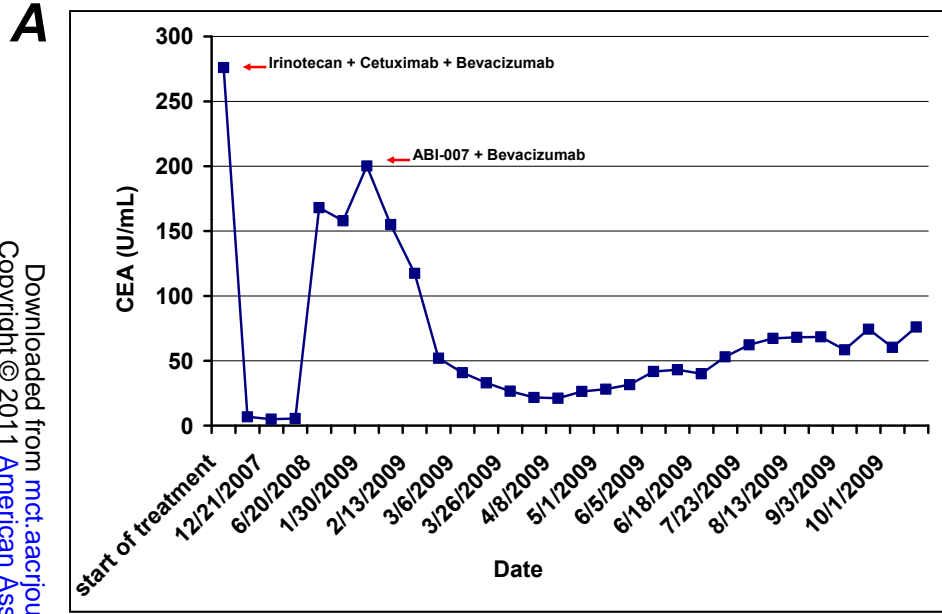


Figure 2

