Prioritizing Phase I Treatment Options Through Preclinical Testing in Personalized Tumorgraft

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A 29 years old young woman with a history of advanced adenoid cystic carcinoma (ACC) resistant to standard of care treatments presented to our phase I clinic seeking treatment with experimental therapeutics. The patient was diagnosed with ACC 11 years before and had been treated with surgery, radiation treatment, and several lines of conventional treatments including platinums, antracyclines, and imatinib mesylate. Eleven months before been seen in our clinic she had developed a brain metastasis that had been surgically resected. A personalized tumorgraft was successfully established from this lesion by implanting fragments of tumor materials in immunecompromised mice as described by our group.[1] At the time of enrollment in the clinical trial she had pulmonary and liver metastasis and was progressing with growth of a preexisting liver metastasis and development of a new liver lesion compared with a CT-scan performed six months before (Before Baseline CT-scan), as depicted in Figure 1. A brain MRI showed a stable 2 mm brain lesion. The patient was asymptomatic with ECOG 0 and normal liver, bone marrow and kidney functions.

In order to determine which phase I clinical studies could be more appropriate for the patient, we characterized her tumor for *KRAS* mutations and HER2 amplification and found that

it to be *KRAS* wild type and not HER2 amplified respectively. Given that she had a personalized tumorgraft model developed from her brain metastases we utilized the model to evaluate a battery of anticancer agents, both conventional and experimental. Briefly, a tumor specimen obtained at the time of removal of her brain tumor had been transplanted and propagated in nude mice. Once it was in exponential growth phase, cohorts of mice with tumor size of 150-300 mm^3 were randomized to several treatment groups. The results of these studies are listed in Figure 2. One of the most effective agents was a monoclonal antibody against the Insulin Growth Factor Receptor 1 (IGF1-R). As shown in Figure 2, intraperitoneal administration of this agent at a dose of 40mg/Kg every 3 weeks resulted in about a 76 % tumor growth inhibition compared to untreated control after 49 days of treatment. Although we did not observed tumor regression, treatment results in a significant cytostatic effect, and we considered the tumor sensible to treatment ($p<0.001$, t-test analysis).

At that time, we were conducting a phase I trial evaluating the combination of figitumumab, an IGF1R monoclonal antibody and PF00299804, a pan-HER inhibitor.[2] Based on the mouse data obtained with the IGF1R inhibitor, and on the fact that here tumor was KRAS wild-type, the patient was enrolled in this trial. Figitumumab was administered IV every three weeks and PF00299804 was given on a daily orally administered schedule. After four cycles of treatment with the combination of PF00299804 and fugitumumab patient developed a severe diarrhea, mostly related to the pan-HER toxicity and treatment with PF00299804 was discontinued, at that time patient tumor assessment was showing stable disease. Then patient continued treatment with figitumumab alone for four more cycles. Treatment with the IGF1R inhibitor showed a good safety profile and resulted in a minor response in the rapidly growing

liver lesion that lasted until cycle 8 of treatment.. At that time, the patient progressed with a new brain lesion and was taken off study. The tumor remained controlled outside the brain.

ACC are very rare variants of adenocarcinoma that most often arise from salivary glands. Roughly 500 new cases of ACC are diagnosed in USA each year[3]. The natural history of the disease can be characterized either by an indolent growth in some patients or by an aggressive and rapidly progressive disease. The overall 10-years survival for patients is about 50%, but when metastases occur the median duration of survival is about 3-years[4-6]. Because of the rarity of this disease there are few clinical trials investigating systemic therapy. Data, from a recently published meta-analysis, reported activity for platinum and/or doxorubicin-based regimen[7]. Imatinib and EGFR inhibitors have been recently evaluated in this disease with limited success.[8,9,10,11] There is no standard approach for patients who progress to conventional treatments. As such enrollment in clinical trials with novel agents is an accepted approach. Because of the paucity of molecular data in this cancer, however, selection of an appropriate trial is done, in general empirically. This situation is not limited to ACC and in fact is the norm for most solid tumors: patients are enrolled in early clinical trials mostly based on clinical and not molecular eligibility criteria. However, the failure rate in phase I clinical trials remains extremely high with most patients deriving no benefit. In fact, only in studies in which there is a clear connection between a genetic alteration and an inhibitor exist showed high response rate in phase I studies.[12-14]

During the last years our group has been involved in the development of personalize mouse models from patients with cancer. These models are useful for drug screening and

biomarker development and thus can be used to prioritize and rank effective agents against an individual cancer. As illustrated in this case, we were able to screen a large set of agents, both experimental and conventional against the personalized tumorgraft model. The data from these studies suggest that this cancer may be sensitive to *nab*-paclitaxel, temozolomide, and inhibitors of IGF1R, mTOR and FGFR inhibitors providing several therapeutic opportunities. Because the IGF1R inhibitor trial was available we elected to enroll the patient in that study saving the other conventional opportunities for the future. In fact, she is now been treated with temozolamide based on the results from these experiments. Overall the data supports the use of personalized tumorgraft as a standard to test experimental agents prior to administration of patients. Furthermore, the personalized tumorgraft model, with a clinically validated susceptibility to IGF1R blockade is now an interesting preclinical tool to comparatively explore other IGF1R inhibitors, design combinations and investigate biomarkers. This is, for example, illustrated in our recent report showing that PALB2 mutations is a strong candidate biomarker of response to DNA damaging agents.[15]

There is however some limitations that will need to be addressed before this strategy can be broadly implemented in phase I clinical studies. Patients need to have a personalized tumorgraft model established. For this patient that was accomplished by implanting excess tissue from a clinically indicated surgical resection. However most phase I candidates do not need surgery thus collecting fresh tumor tissue for the generation of a personalized tumorgraft model can be a challenge. Additionally, the development, propagation of the personalized tumorgraft model and drug testing takes 6-8 months, which is often not available for most phase I candidates. Moreover, failure rate in tumorgraft establishment and the possibility of molecular

signature discordance between two lesions from different organs need to be considered. Thus, early selection of potential candidates is critical. Finally is the selection of agents to be tested. The number of drugs in development too large and there has to be a strategy to prioritize which drugs to be tested in the models. In this sense, integration of molecular testing to rank order candidates is be critical.

The next prevailing question is to test this strategy in a clinical trial to show is feasible and beneficial. A plausible design for example would be patients with advanced cancer with accessible primary tumor and metastatic lesions from whom a personalized tumorgraft model can be generated and available at the time of second line treatment. These lesions can be profiled with a battery of biomarkers to select a set of 5-10 agents to be tested in the model to select the ideal phase I study for the patient. Obviously, there are significant logistic and feasibility issues with such an approach but the reality is that the field is stagnant with little progress and new, albeit risky, approaches are needed.

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Tumor Assessment

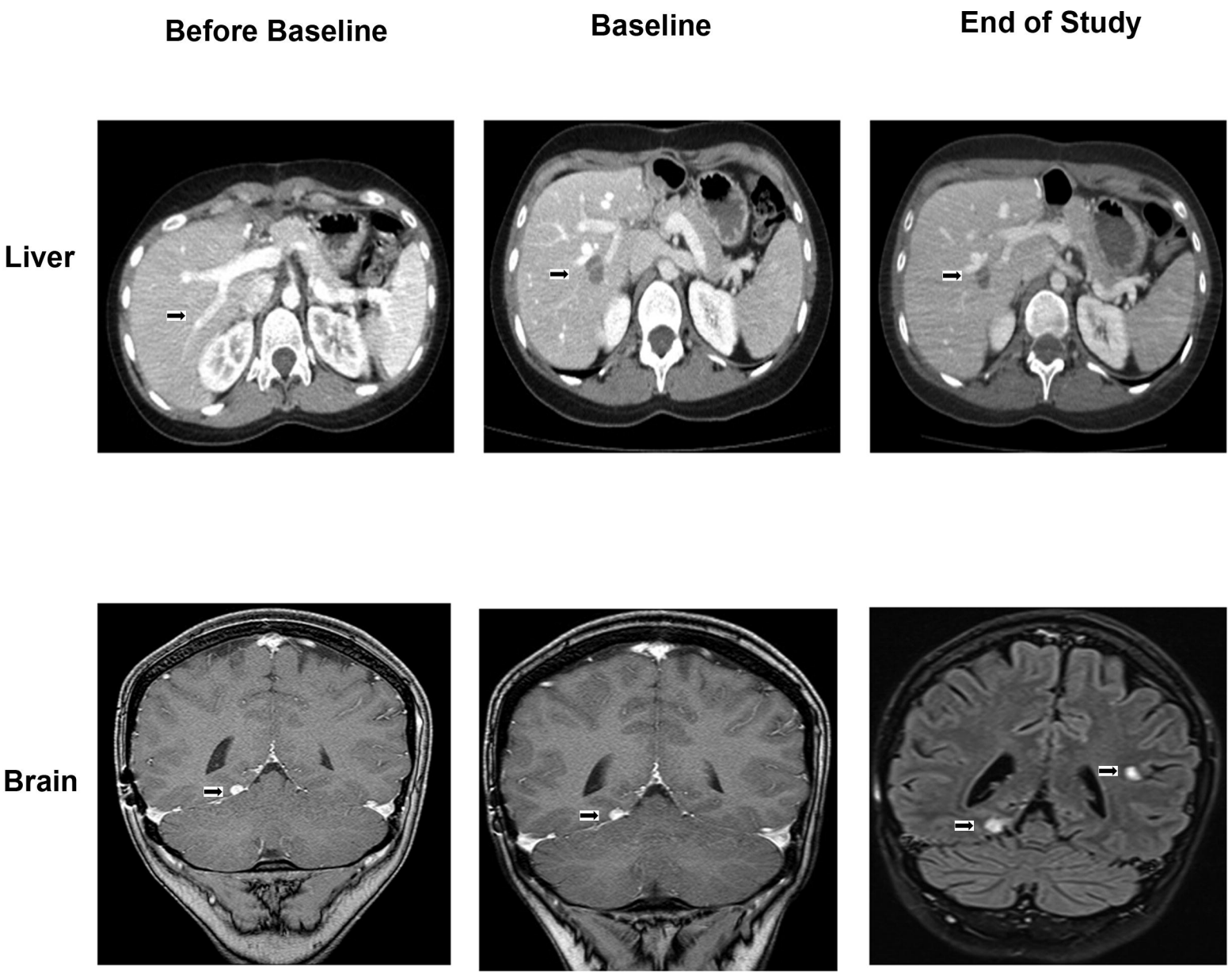
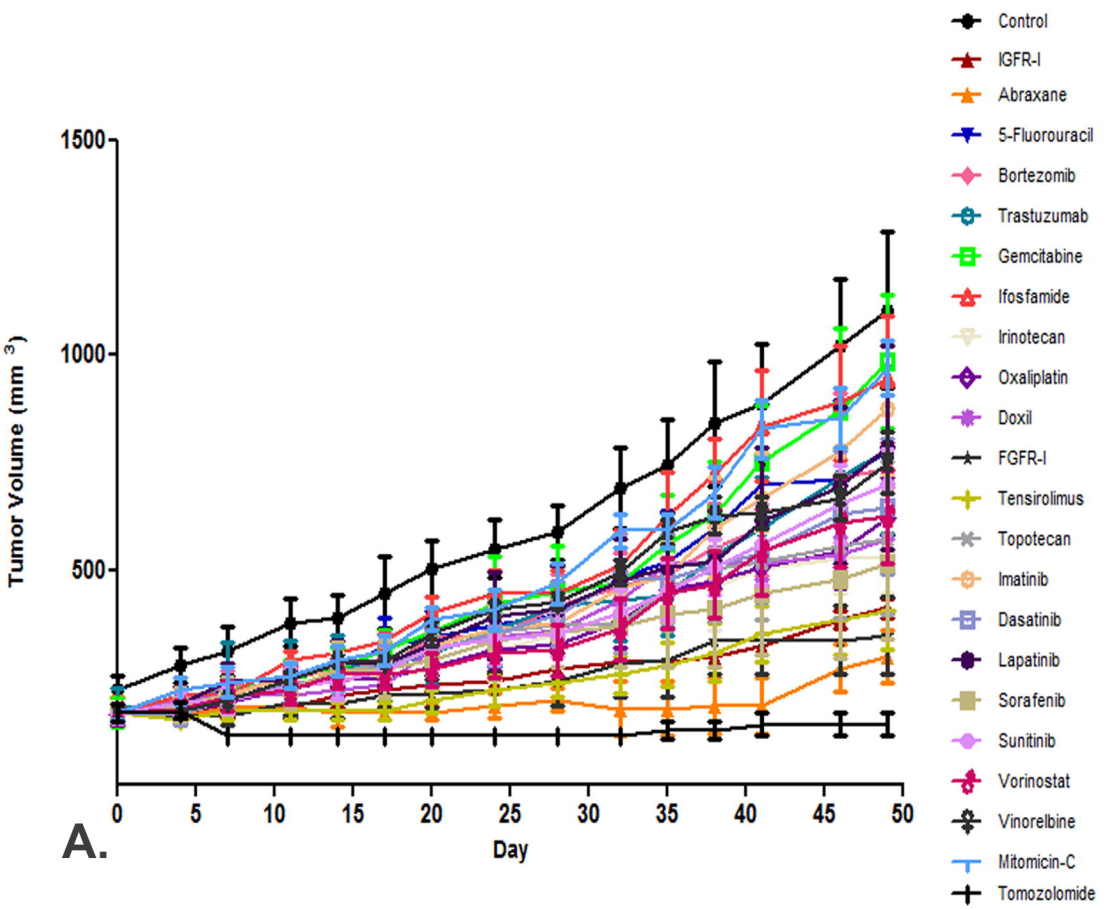


Fig 1

Patient Xenograft vs Treatment

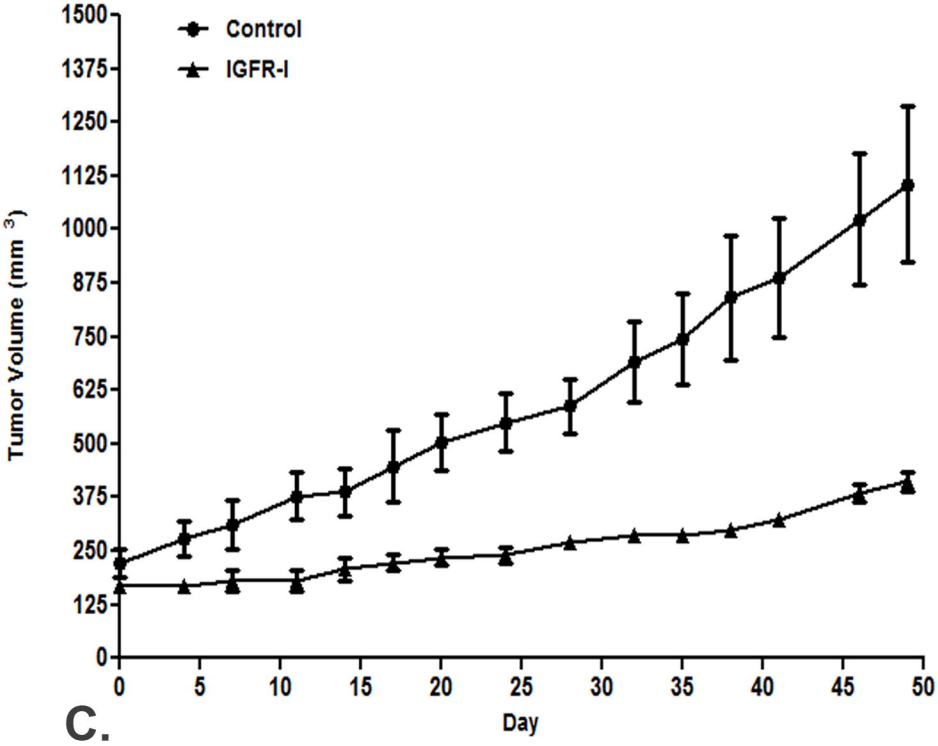


A.

Drug	Activity Rating (AR)%
IGFR-I	72%
Abraxane	73%
5-Fluorouracil	13%
Bortezomib	19%
Trastuzumab	12%
Gemcitabine	<0%
Ifosfamide	<0%
Irinotecan	48%
Oxaliplatin	34%
Doxil	41%
FGFR-I	74%
Temsirolimus	67%
Topotecan	41%
Tomozolomide	102%
Imatinib	<0%
Dasatinib	27%
Lapatinib	10%
Sorafenib	50%
Sunitinib	20%
Vorinostat	31%
Vinorelbine	14%
Mitomicin-C	<0%

B.

Patient Xenograft vs IGFR-Inhibitor



C.

Gene	Status
HER2/Neu	No amplified
KRAS	WT

D.

Fig 2