rational therapeutics®
The Art & Science of Personalized Cancer Care

EVA-PCD FUNCTIONAL PROFILE

Patient:	Sample NSCLC Patient	**Assay Date:**	
Dx:	Non-Small Cell Lung Cancer	**Assay Quality:**	High Yield/Mod Viability
Prior Rx:	Treated	**Report Date:**	
Physician:	Your Oncologist	**Specimen Number:**	

SINGLE DRUG DOSE EFFECT ANALYSIS

Drug	IC50	Units	Interpretation
Taxol	9.1	ug/ml	***Sensitive***
Cytoxan	1.4	ug/ml	Intermediate
Cisplatin	3.9	ug/ml	Resistant
Gemcitabine	238	ug/ml	Resistant
Irinotecan	36	ug/ml	Resistant

MULTIPLE DRUG DOSE EFFECT ANALYSIS

Drug	Ratio	IC50	Units	Interpretation	Synergy
Mitomycin-C & Irinotecan		12	ug/ml	Intermediate	N/A
Cisplatin & Gemcitabine		25	ug/ml	Resistant	***Synergy***
Cisplatin & Irinotecan		21	ug/ml	Resistant	***Synergy***
Cisplatin & Gemcitabine & Sunitinib		31	ug/ml	Resistant	Antagonism
Cisplatin & Taxol & Sunitinib		16	ug/ml	Resistant	Antagonism
Cisplatin & Taxol		8.9	ug/ml	Resistant	Antagonism
Taxol & Gemcitabine		68	ug/ml	Resistant	No Synergy
Geldanamycin & Taxol		6.3	ug/ml	Resistant	No Synergy
Cisplatin & Trimetrexate*		>28	ug/ml	Resistant	N/A
Cisplatin & Vinorelbine		2.3	ug/ml	Resistant	N/A

INTERPRETATION:

Laboratory results represent only one part of the overall determination of therapy for patients and do not guarantee outcomes nor indicate the specific drugs that should be used in a particular patient.

* The following compounds serve as in vitro surrogates for their respective drug classes, e.g., Nitrogen Mustard: = Cyclophosphamide, Ifosfamide, Melphalan, Chlorambucil and related mustard alkylators; Cisplatin: = Carboplatin; Doxorubicin: = Daunorubicin and Idarubicin; Trimetrexate: = Methotrexate; 5Fu + Interferon: = Xeloda.

Ex Vivo best regimen would be Taxol or Alkylating agent.

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EX VIVO TARGET Rx ANALYSIS

Patient:	Sample NSCLC Patient	**Assay Date:**	
Dx:	Non-Small Cell Lung Cancer	**Assay Quality:**	High Yield/Mod Viability
Prior Rx:	Treated	**Report Date:**	
Physician:	Your Oncologist	**Specimen Number:**	

SINGLE DRUG DOSE EFFECT ANALYSIS

Drug	IC50	Units	Interpretation
BIBW2992 ($HER_{1,2)}$	<3.1	ug/ml	***Active***
Phenformin (Mitochondrial)	18	ug/ml	***Active***
AUY922	15	ug/ml	Moderately Active
Gefitinib (EGF_R)	4.5	ug/ml	Moderately Active
AZD2281 (PARP)	54	ug/ml	Inactive
AZD6244 (MEK)	36	ug/ml	Inactive
BYL-719 (PI_3K)	4.2	ug/ml	Inactive
Crizotinib (ALK/MET/ROS)	6.8	ug/ml	Inactive
Geldanamycin (HSP_{90})	>11	ug/ml	Inactive
Pazopanib (VEGF/PDGFR/c-KIT)	>80	ug/ml	Inactive
Sunitinib (c-KIT/VEGF/FLT-3)	>13	ug/ml	Inactive
Vandetanib (VEGF)	5.1	ug/ml	Inactive

MULTIPLE DRUG DOSE EFFECT ANALYSIS

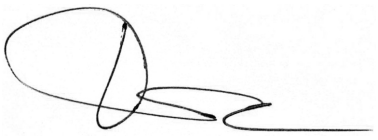
Drug	Ratio	IC50	Units	Interpretation	Synergy
BIBW2992 & Sunitinib		<1.9	ug/ml	***Active***	***Synergy***
AUY922 & Taxol		6.3	ug/ml	***Active***	Mixed Synergy
Sunitinib & Gefitinib		5.3	ug/ml	Inactive	Partial Synergy

INTERPRETATION:

Laboratory results represent only one part of the overall determination of therapy for patients and do not guarantee outcomes nor indicate the specific drugs that should be used in a particular patient.

* The following compounds serve as in vitro surrogates for their respective drug classes, e.g., Nitrogen Mustard: = Cyclophosphamide, Ifosfamide, Melphalan, Chlorambucil and related mustard alkylators; Cisplatin: = Carboplatin; Doxorubicin: = Daunorubicin and Idarubicin; Trimetrexate: = Methotrexate; 5Fu + Interferon: = Xeloda.

Ex Vivo best regimen would be Afatinib plus Avastin +/- Cetuximab.



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EVA-PCD FUNCTIONAL PROFILE
(Ex Vivo Analysis of Programmed Cell Death)

PATIENT:	**Sample NSCLC Patient**
DIAGNOSIS:	**Non-Small Cell Lung Cancer**
PRIOR THERAPY:	**Yes**
PHYSICIAN:	**Your Oncologist**
ASSAY DATE:	
REPORT DATE:	
ASSAY QUALITY:	**High Yield, Moderate Viability**
SPECIMEN NO.:	
SPECIMEN SOURCE:	**Fluid**

ASSAY TECHNIQUES

Ex Vivo Analysis of Programmed Cell Death (EVA-PCD) is a human tumor primary culture platform designed to address the complexity, redundancy, and promiscuity of human tumor signaling pathways at the systems level. "Functional Profiling" examines cancer cells behavior in *real-time*. Primary Culture microspheroids are isolated by mechanical and enzymatic disaggregation using fresh biopsy specimens. Drug induced cell death (apoptotic and non-apoptotic) is examined by morphology, cytochemistry, staining characteristics and cellular metabolism. LC50 values interpolated from dose response curves are compared with the Rational Therapeutics database to assess drug response probability and drug synergy.

Laboratory assays are not perfect predictors of drug activity and constitute only one portion of each patient's assessment. Drugs found active in vitro are approximately 7 times more likely to be clinically effective than drugs found inactive in vitro. The ultimate choice of therapy, however, must reflect the carefully considered compilation of all available data, both laboratory and clinical, toward the development of the most effective and least toxic drug regimen for each patient.

SYNERGY ANALYSES:

Drug combinations are evaluated for synergy by the median-Effect method of Chou and Talalay. (Biosoft, UK)

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SPECIMEN:
A 1000 cc fluid sample was submitted by overnight courier. Following density centrifugation, 4 x 10^7 cells were identified, of which 80% were consistent with adenocarcinoma with a viability of 80%. Sample was adequate for multiple analyses including both target analysis and cytotoxic drug analysis. Two endpoints were employed: The delayed loss of membrane integrity, as well as the ATP content by luciferase.

DISCUSSION:
Patient with previously treated non-small cell lung cancer reveals a pattern of moderate resistance to many forms of chemotherapy. The patient reveals some activity for Taxol as a single agent, as well as Cytoxan, an alkylating agent. There was modest activity observed for the combination of Mitomycin-C plus Irinotecan, with relative resistance to many other combinations. Based on these findings, the patient might consider Taxol or single agent Taxotere. Additional benefit might be obtained from related combinations such as Abraxane plus Avastin. Activity for Cytoxan and alkylating agents would suggest that there may be a role for Ifosfamide-based therapies, while Mitomycin-C plus Irinotecan have previously been reported in gastrointestinal and lung cancers.

Several other classes of treatments were evaluated in the target analysis. The most activity was observed for the multi-targeted HER1/2/4 inhibitor, BIBW2992, also known as Afatinib. Of interest, there was activity for Phenformin, the mitochondrial inhibitor. There was also good activity for the combination of BIBW2992 with the VEGF inhibitor and the HSP90 inhibitor AUY922 plus Taxol.

This patient with an EGF_R-mutated tumor reveals persistent activity for the more pluripotent BIBW2992 over Gefitinib. This would suggest that the EGF_R cascade can still be drugged using appropriate combinations. Among the most active combination is the combination of Cetuximab with oral daily Afatinib. One study reported for this combination provided an overall response rate of 32% (Janjigian, Cancer Discovery, 2014). This combination could also be combined with Avastin, as there was a favorable signal for Afatinib plus the VEGF inhibitors. Alternatively, Afatinib could be combined with Avastin, again based on the favorable finding. A final point would be that of the HSP90 inhibitors plus Taxotere. This clinical trial reported in 2013, known as the Galaxy-1, showed an improvement for the combination of Docetaxel plus Ganetespib, a member of the heat shock protein inhibitors. These findings are now part of the Galaxy-2 clinical trial in patients with advanced recurrent lung cancer.

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