Ex Vivo 3D Drug Response Profiling of Primary Human Ovarian Cancer Differentiates Treatment Naïve and Relapsed Patients and Molecular Subtypes

Teresa M. DesRochers[1], Stephen Shuford[1], Christina Mattingly[1], Lillia Holmes[1], Matt Gevaert[1], Jeffrey Elder[2], David Orr[1], Larry Puls[2], Chris Corless[3], Howland Crosswell[1]

[1]KIYATEC Inc.; Greenville, South Carolina 29605 USA | [2]Greenville Health System; Greenville, South Carolina 29605 USA | [3]OHSU Knight Diagnostic Laboratories; Portland, Oregon 97239 USA

Background

Epithelial ovarian cancer (EOC) affects nearly 22,000 women annually and is the leading cause of death from gynecologic cancer in the United States. Five year cure rates are <40% and approximately 14,000 will die each year. Large-scale efforts are currently underway to use molecular profiling via next generation sequencing (NGS) technology to guide treatment in cancer patients with poor prognosis, but limited application of NGS in ovarian cancer has been reported. In this report, our previously described Ex Vivo 3D Drug Response Profiling (EV3D™ DRP) was used to identify response differences between newly diagnosed and relapsed ovarian cancer patients and correlated with NGS of primary ovarian tissue.

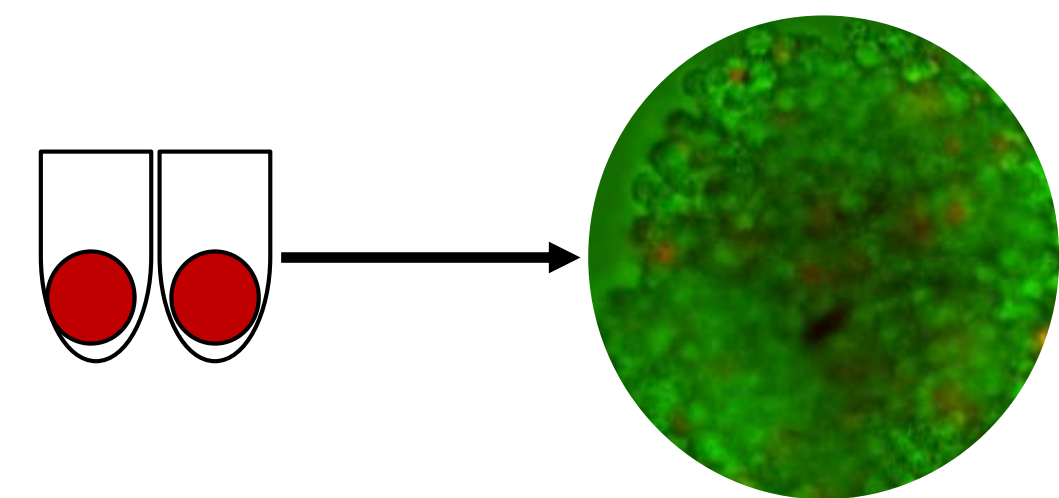## Methods

1. Tumor Acquisition and Processing

Patient Consented for IRB Approved Prospective Biology Study
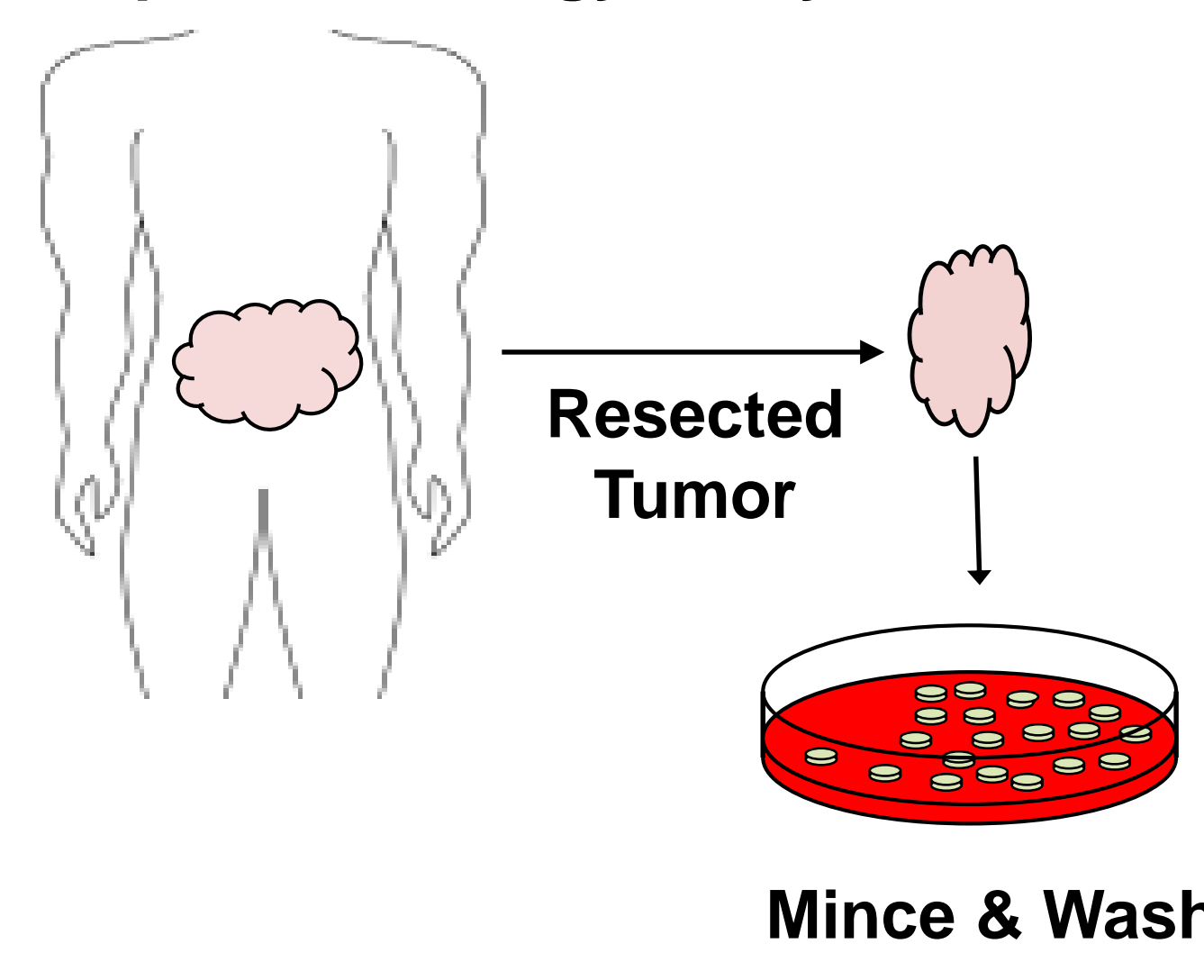
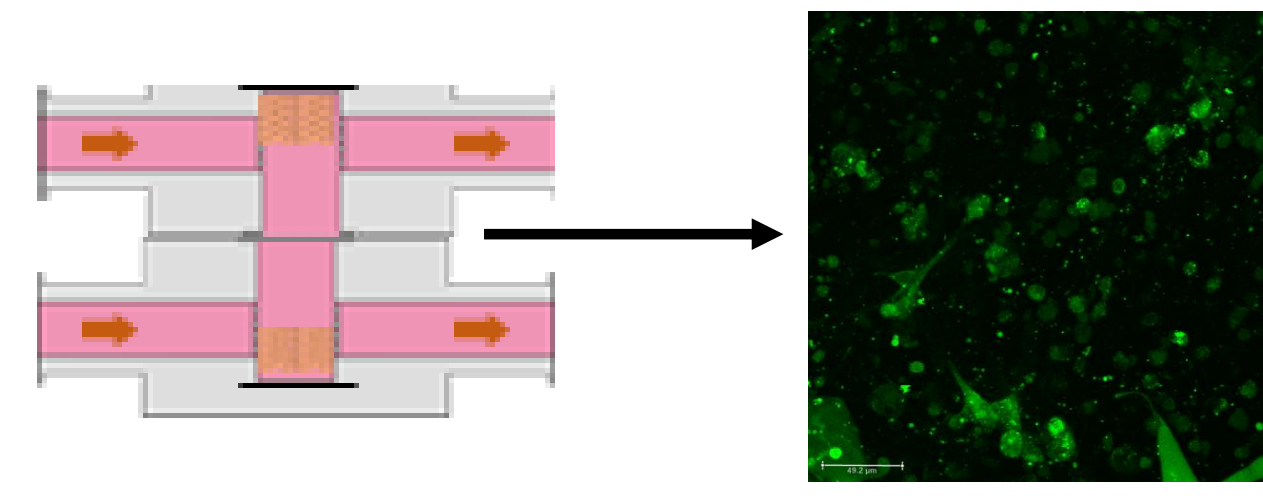| Subject Demographics	
Newly Diagnosed	61%
Relapse	39%
Serous Cell Carcinoma	72%
Clear Cell Carcinoma	14%
high grade	56%

Resected Tumor → Mince & Wash →

1) Molecular Profiling
2) EV3D DRP
3) 2D Comparison
4) Clinical Correlation -CA125
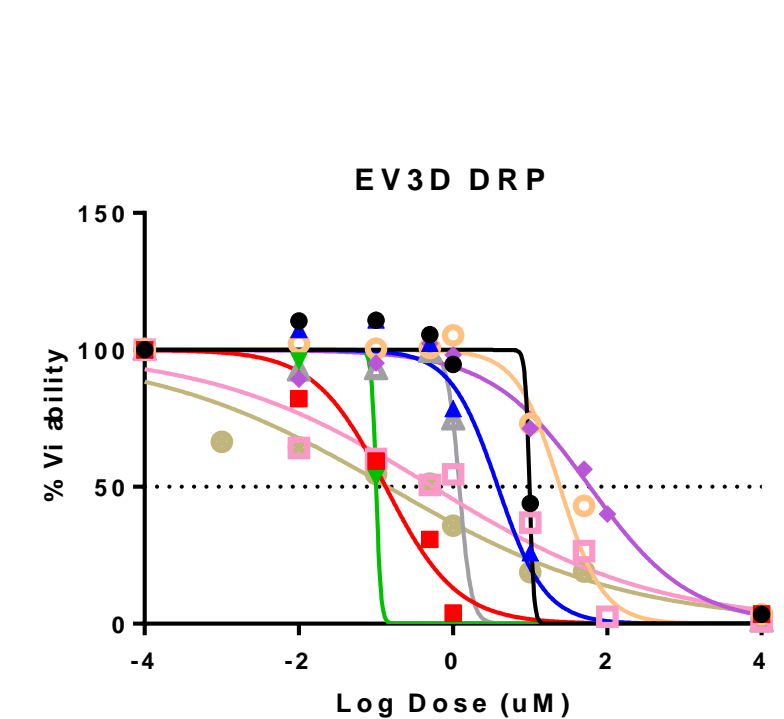
2. EV3D Assay

EV3D DRP Platform
- Mixed Cell Types
- ECM free
- 3D Spheroid
- 2.5k cells/well
- 8 drugs, 7 day turnaround

EV3D NGS directed DRP Platform
- Mixed Cell Types
- 3D Microtumor
- Perfusion culture
- Drugs defined by NGS
- 14-19 days in culture

3. Data Analysis

	IC50 (uM)
Drug	EV3D
Cisplatin	9.845
Topotecan	0.1340
LY 294002	3.826
Doxorubicin	0.1009
Gemcitabine	59.52
Carboplatin	24.39
Paclitaxel	0.1429
Trametinib	0.5476
Afatinib	1.212

	Survival Fraction
Drug	EV3D
Cisplatin	0.6
Topotecan	0.2
LY 294002	0.6
Doxorubicin	0.2
Gemcitabine	0.8
Carboplatin	0.7
Paclitaxel	0.3
Trametinib	0.4
Afatinib	0.5

EV3D DRP Correlates with Mutations

A.

Mutated Genes	KIYATEC Rates	Reference Rates
P53	64%	80%
PI3K	29%	8.3%
KRAS	21%	16.7%
CDKN2A	14%	2%
NOTCH	7%	2%
BRAF	7%	4%
MET	7%	4%
EGFR	7%	N/A

B.

LY294002 IC50 vs. Mutation
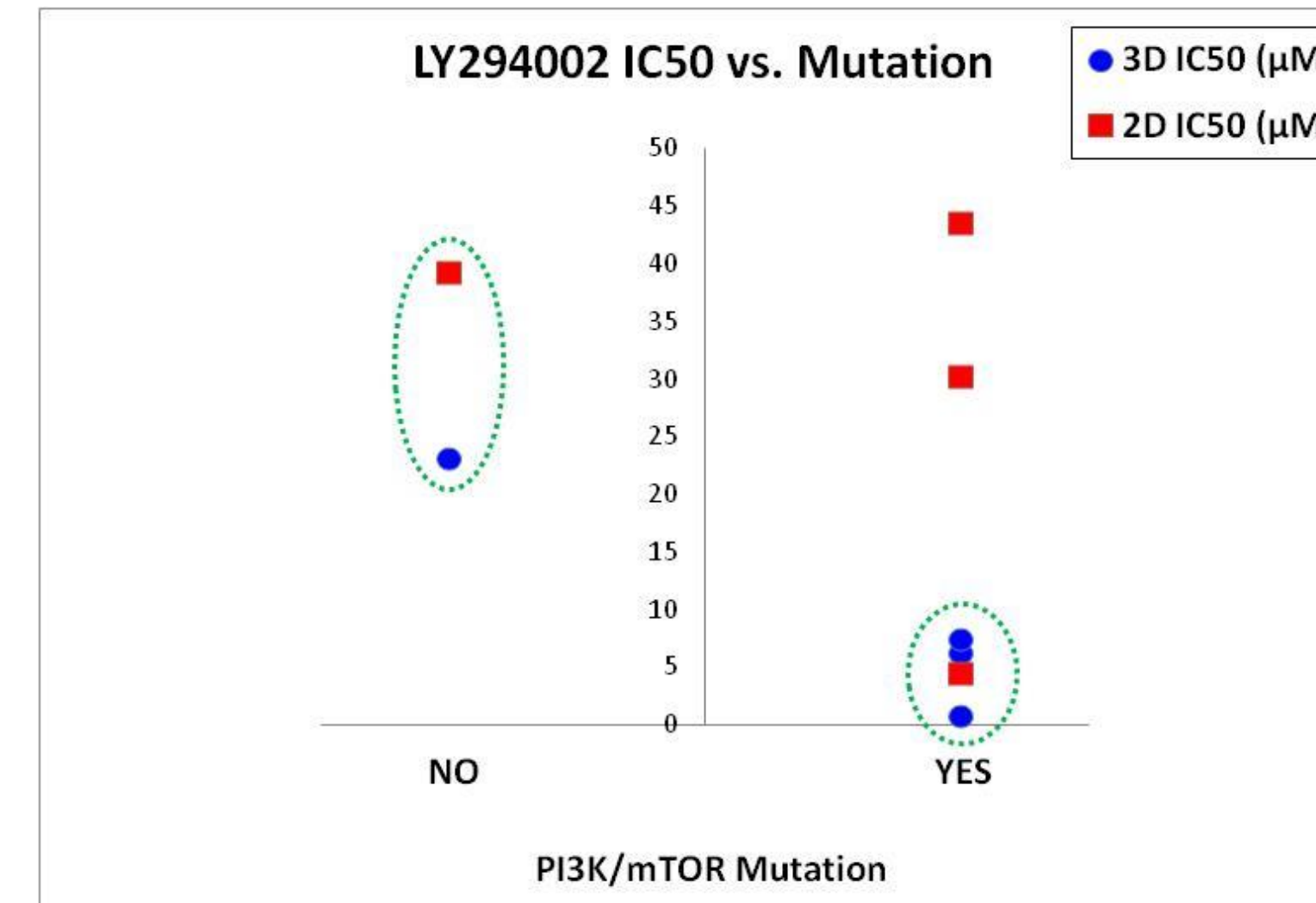
Pearson Correlation Coefficient	
EV3D IC50 (uM)	0.95
2D IC50 (uM)	0.38

C. KIY OV 028
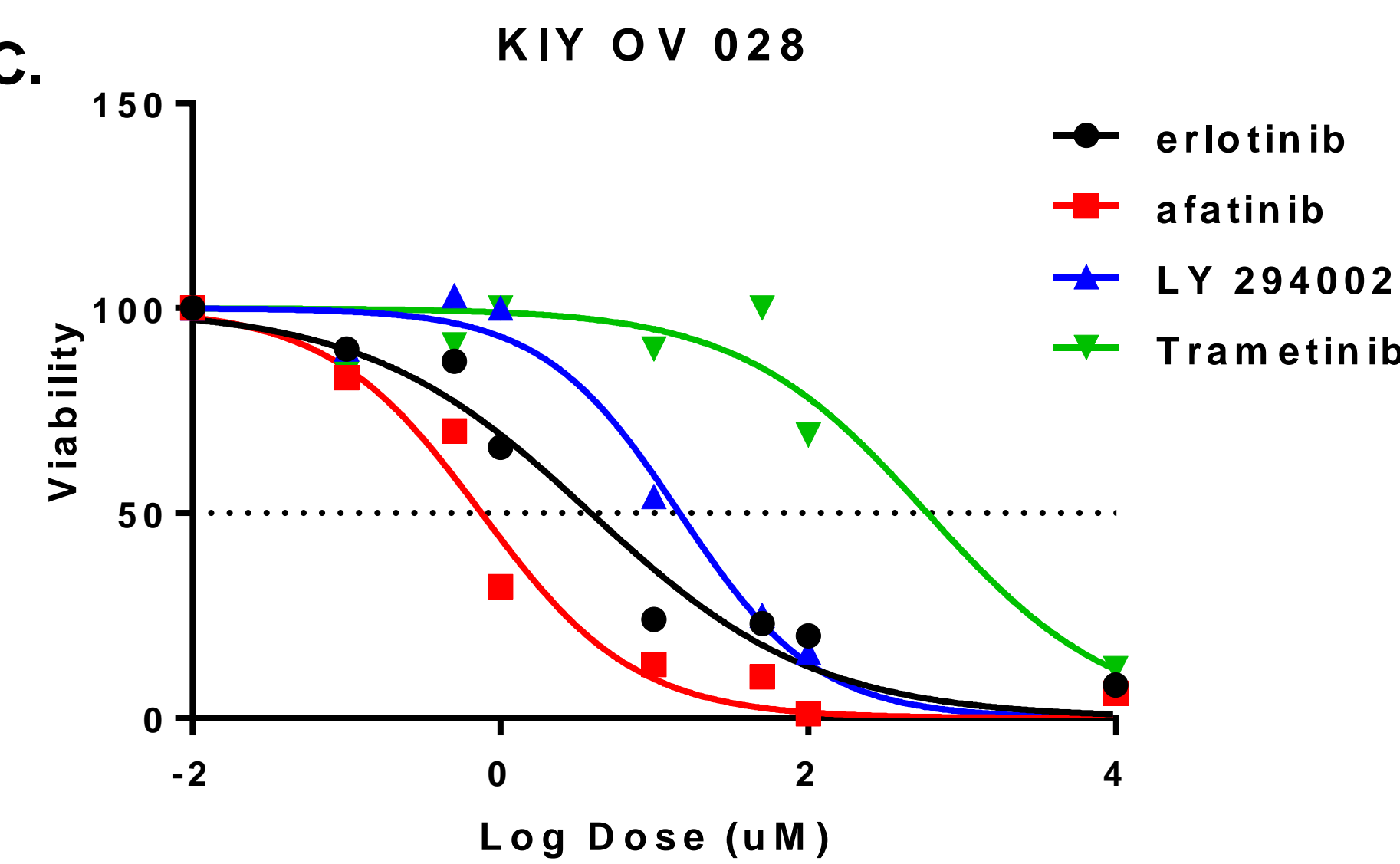
Drug	3D IC50
Erlotinib	2.755
Afatinib	0.6889
LY294002	14.4
Trametinib	>100

NGS Results
EGFR (P265T)
p53 (copy loss)

D. KIY OV 026
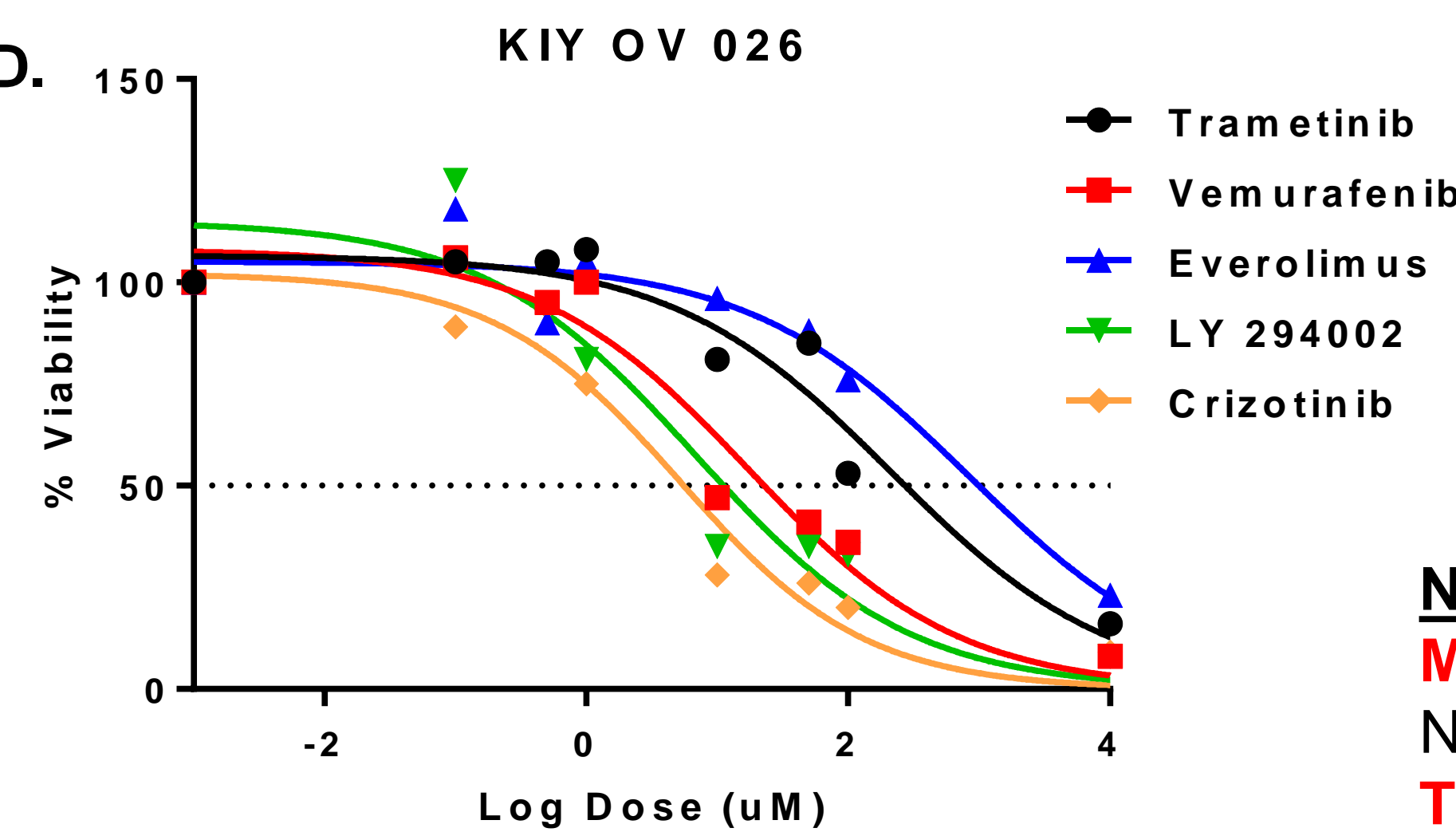
Drug	3D IC50
Trametinib	>100
Vemurafenib	15.99
Everolimus	>100
LY294002	5.547
Crizotinib	4.83

NGS Results
MET (P1382L)
NF1 (V266F)
TSC2 (S1282_G1285 deletion)
TP53 (C242S)

Figure 1: NGS Identified Mutations Correlate with EV3D DRP. (A) Patient tumors collected by KIYATEC closely mimic global mutation rates (Ross, 2013 Clearity Foundation, Foundation Med). **(B)** Response to LY 294002 correlated with the presence of a PI3K mutation in EV3D DRP better than in 2D cell culture. **(C)** KIY OV 028 was identified as having an EGFR and a p53 mutation. When treated with drugs, it showed a positive response to both Erlotinib and Afatinib along with no response to Trametinib. **(D)** KIY OV 026 was identified by NGS as having a number of mutations including MET, NF1, TSC2, and p53. It responded to both LY294002 and Crizotinib.
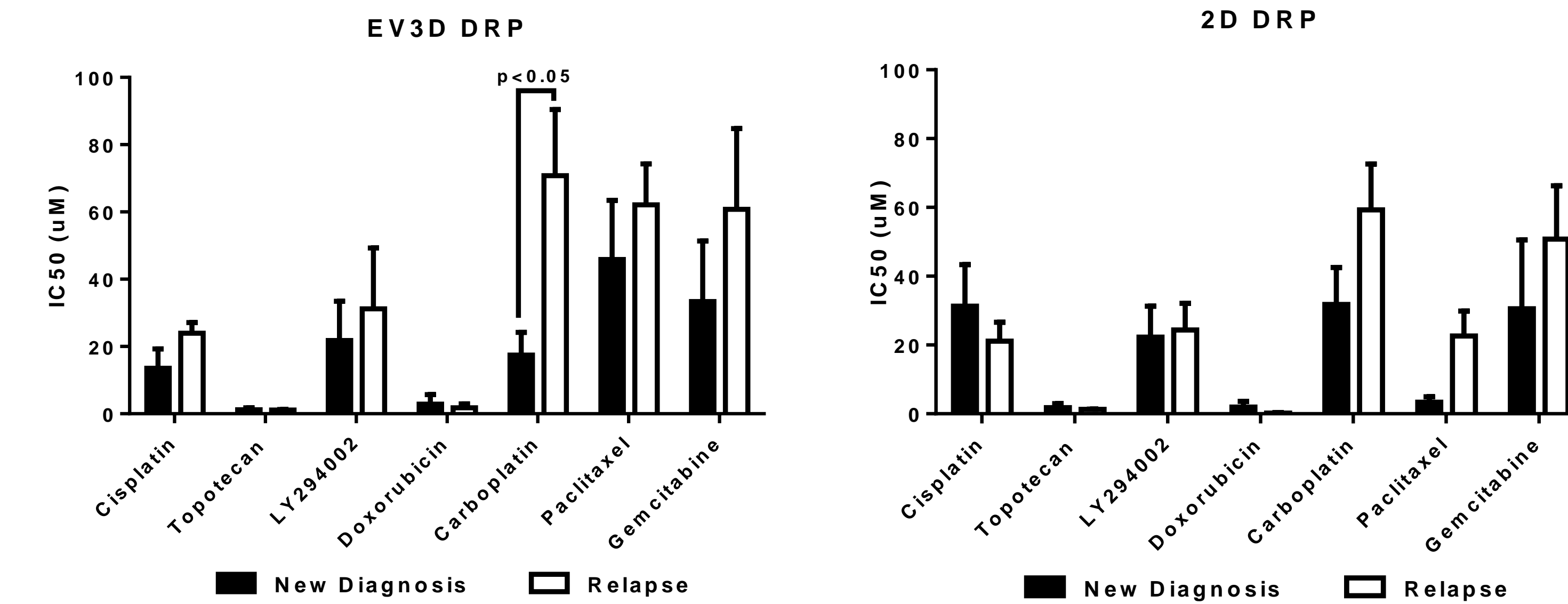
EV3D DRP Correlations

Figure 2: EV3D DRP response to carboplatin correlates with relapse. When matched EV3D and 2D samples are both treated with carboplatin under the same conditions, a significantly different response is detected between the response of newly diagnosed patients and relapse patients in EV3D tested samples. This significantly different response is not detected in 2D however. This indicates that EV3D may be better able to detect a resistance phenotype than 2D.
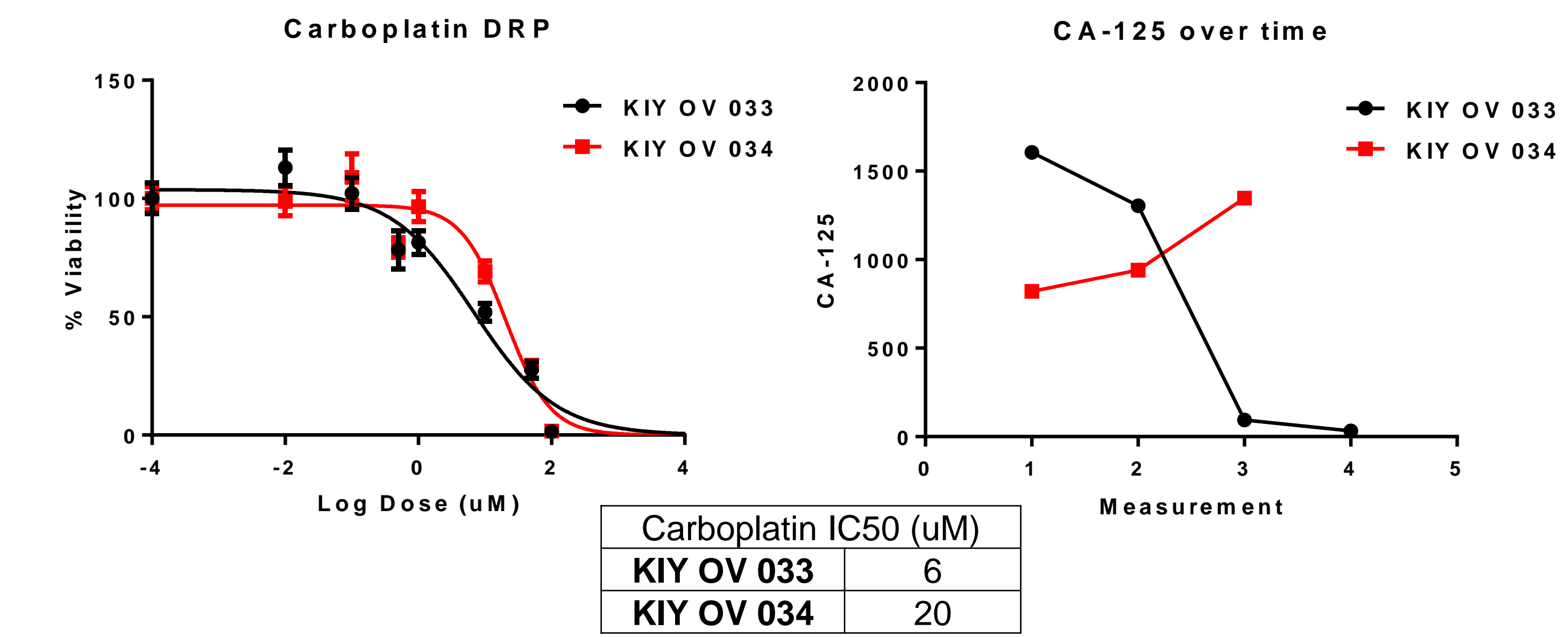

Carboplatin IC50 (uM)	
KIY OV 033	6
KIY OV 034	20

Figure 3: EV3D DRP to Carboplatin correlates with CA-125. Newly diagnosed patient samples were received following 3 cycles of neoadjuvant carboplatin/paclitaxel. Following EV3D DRP, KIY OV 033 showed response to Carboplatin that correlated with CA-125 measured post-treatment while KIY OV 034 had a lower response to Carboplatin, correlating with a lack of CA-125 reduction following neoadjuvant therapy.

Conclusions

- We have successfully isolated cells and performed EV3D DRP on 66% of patient samples
- We can readily identify mutations within the patient samples and our rates mimic those of the general population
- EV3D DRP results for targeted therapies correlates with the presence of known mutations rapidly identified in a CLIA laboratory
- EV3D DRP may predict clinical response to neoadjuvant carboplatin therapy

GREENVILLE HEALTH SYSTEM

iTOR INSTITUTE TRANSLATIONAL ONCOLOGY RESEARCH

KNIGHT CANCER INSTITUTE Oregon Health & Science University

KIYATEC®