Patient Derived Xenograft Models ---Clinical Applications

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UCDAVIS COMPREHENSIVE CANCER CENTER

NCI CCC
A Comprehensive Cancer Center Designated by the National Cancer Institute

DISCLOSURE:

I have financial interest/arrangement or affiliation with

Name of Organization	Relationship
Accelerated Medical Diagnostics Inc	Co-founder and shareholder
LP Therapeutics Inc	Co-founder and shareholder
Pandomedx Inc	Co-founder and shareholder

Four patents

1. Bladder cancer-specific ligand cQDGRMGFc for imaging detection, immunotherapy and targeted therapy of bladder cancer (filed. Inventors: Chong-xian Pan, Hongyong Zhang, Kit Lam and Olulanu Aina). US Patent application No. 61/245,492.
2. Leukemia stem cell-targeting ligand and methods of use. Ligands containing the LR(S/T) amino acid motif for targeted therapy and detection of acute myeloid leukemia. (filed. Inventors: Chong-xian Pan and Hongyong Zhang). Patent application No. 14/130,909.
3. Porphyrin-based cancer-targeting nanometer-scale micelles for photodynamic diagnosis and therapy (Inventors: Yuanpei Li, Kit S. Lam, Chong-xian Pan, Tzu-yin Lin). US Provisional patent Application No. 61/736,067.
4. Treatment of Drug Resistant Metastatic Prostate Cancer Using Niclosamide. (Inventors: Allen Gao, Chengfei Liu, Wei Luo and Chong-xian Pan). U.S. Patent Application No. 15/134,228

Introduction

Tradition Cancer Medicine

Diagnosis and staging

↓

Empirical

↓

One formula fits all

Precision Medicine

State-of-the-art technologies:
-omics

↓

Computational biology to identify targets

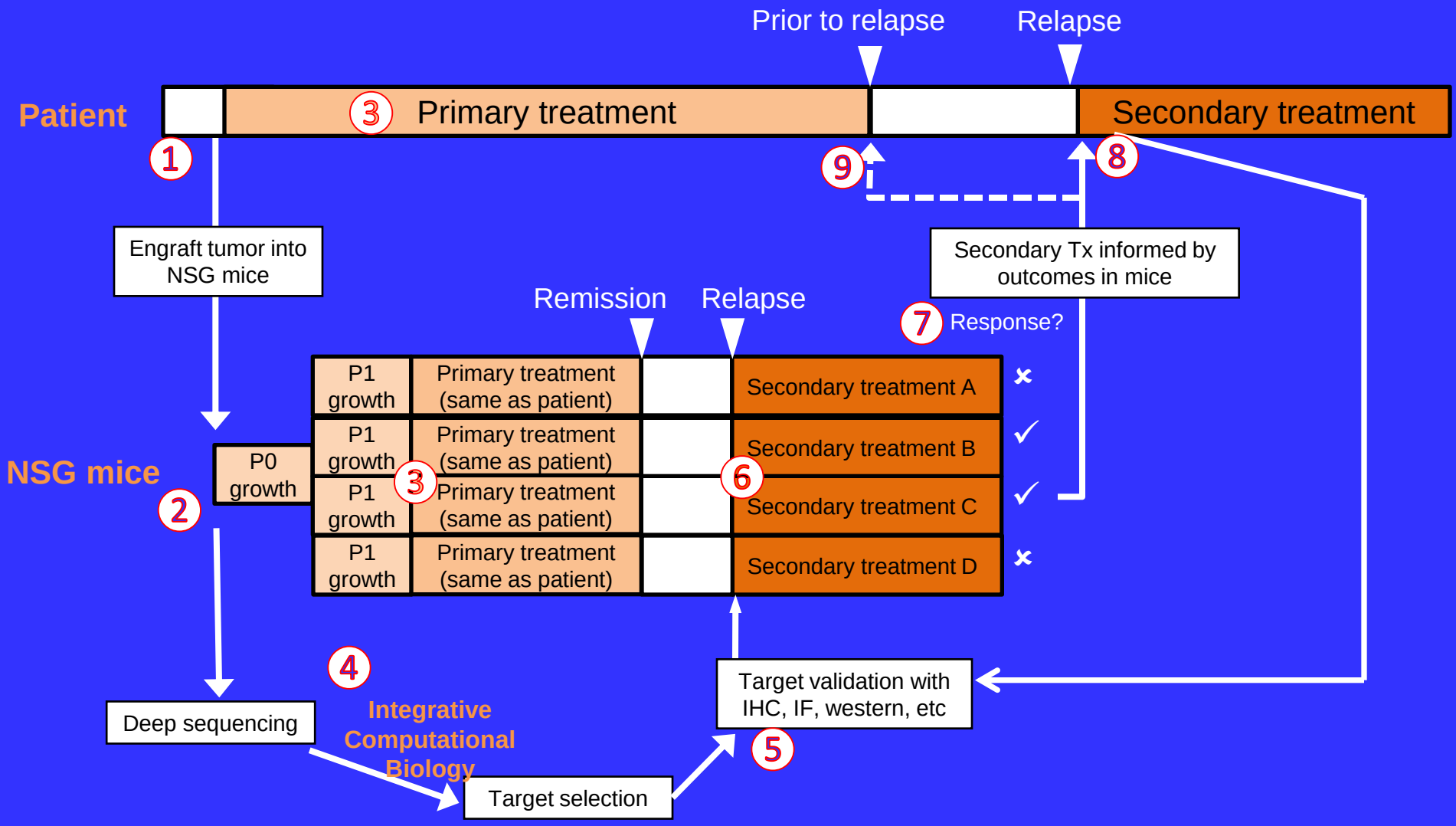
↓

Individualized therapy

Tsimberidou et al: RR 12% with matched targeted therapy vs. 5% with unmatched therapy
(Clin Cancer Res. 2014; 20:4827)

Andre et al. RR 9% plus 21% stable disease with matched targeted therapy in breast cancer
(Lancet Oncology, 2014; 15:267)

Patient-derived models of cancer (PDMCs) for precision medicine



PDXs for precision medicine

Features and applications of PDXs

Special features:

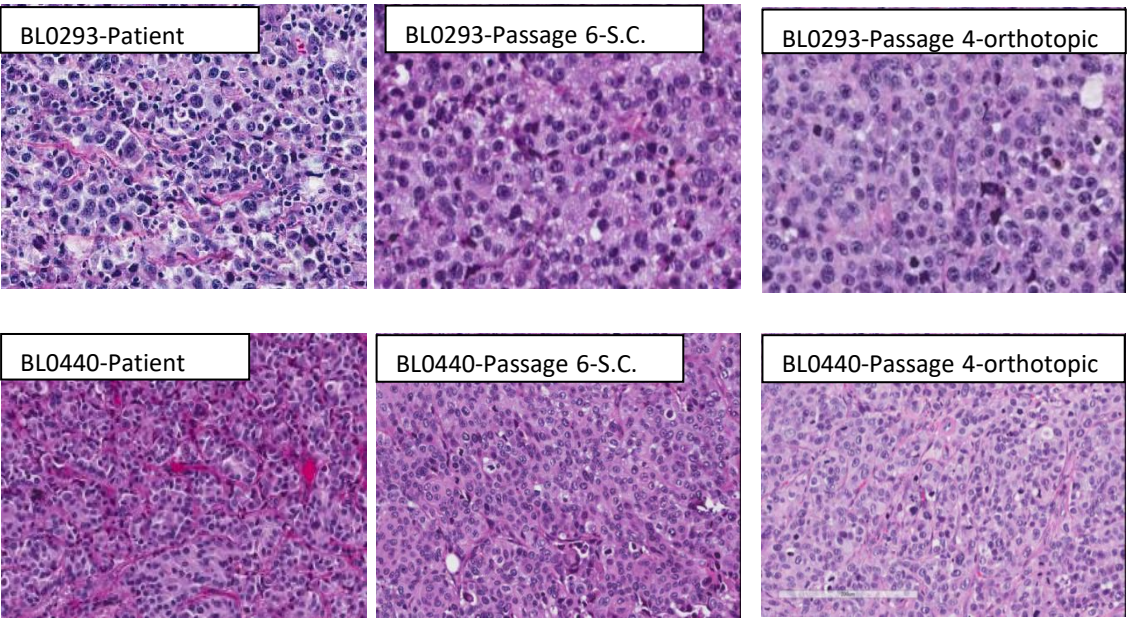
- PDXs are directly derived from unselected uncultured clinical specimens
- PDXs are patient-specific
- PDXs and patient cancers have the same genetic background
- Many identical PDXs can be generated for repeated studies
- Frequent biopsies can be done to study resistance mechanisms

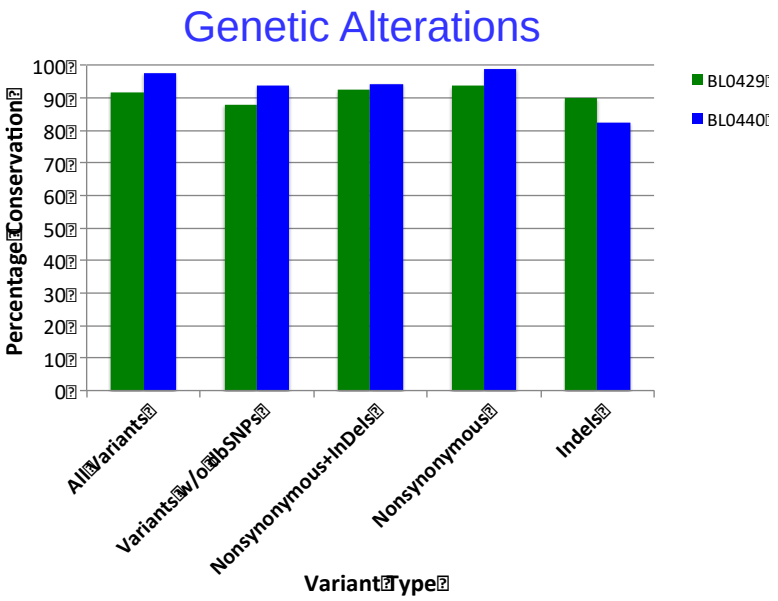
36 bladder cancer PDXs

Clinical Characteristics of the donor patients					
Stages	Tumor ID	Age (yrs)	Stage	Surgery	Prior chemo
Myoinvasive bladder cancer	BL0269F	58	pT4 N0 Mx	Cystectomy	No
	BL0293F	77	pT2a N2 Mx	Cystectomy	No
	BL0307F	78	pT3b N2 Mx	Cystectomy	No
	BL0382F	82	pT2 Nx Mx	TURBT	No
	BL0428F	70	pT2 Nx Mx	TURBT	No
	BL0429F	60	pT4a N3 M1	Cystectomy	No
	BL0479F	78	pT2b Nx Mx	Cystectomy	YES (carbo/gem/PTX)
	BL0440F	71	pT4a N2 Mx	Cystectomy	YES (gem/cis)
	BL0515F	78	pT3bN0Mx	Cystectomy	YES (Gem/Cis)
	BL0545F	70	pT2 N0 Mx	Cystectomy	No
	BL0601F	83	pT3 N0 Mx	Cystectomy	No
	BL0629F	74	pT3 N0 Mx	Cystectomy	No
	BL0645F	75	pT4a N2 Mx	Cystectomy	YES (MVAC)#
	BL0648	71	pT4a N2 Mx	Cystectomy	No. AdenoCa
Non-myo-invasive bladder cancer	BL0262F	64	pTa High	TURBT	No
	BL0364F	76	pTa Low	TURBT	No
	BL0381F *	60	pTa High	TURBT	No
	BL0398F *	60	pT1 No Mx	Cystectomy	No
	BL0470F	55	pTa Nx Mx	TURBT	No
	BL0591F	65	pTis N0 Mx	Cystectomy	No
	BL0606F	77	pT1Nx Mx	TURBT	No
	BL0622F	63	pTis	cystectomy	

PDXs for precision medicine

Characterization of PDXs





Fidelity of morphology

Conservation of genetic aberrations (92-97%)

Applications of PDMCs

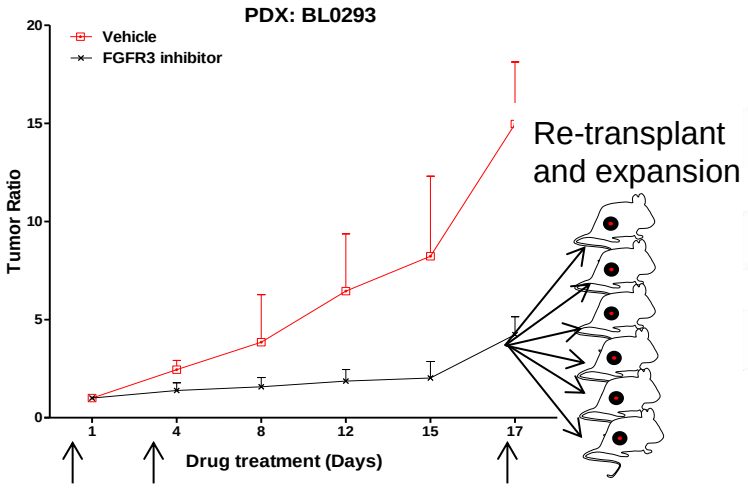
Screening for effective targeted therapy

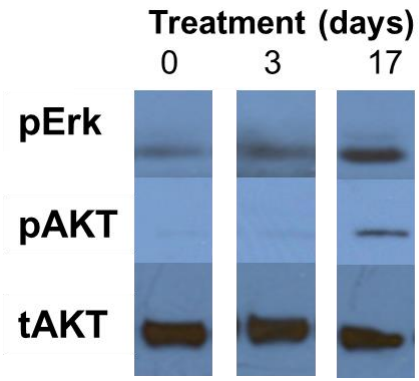
Screening for effective chemotherapy

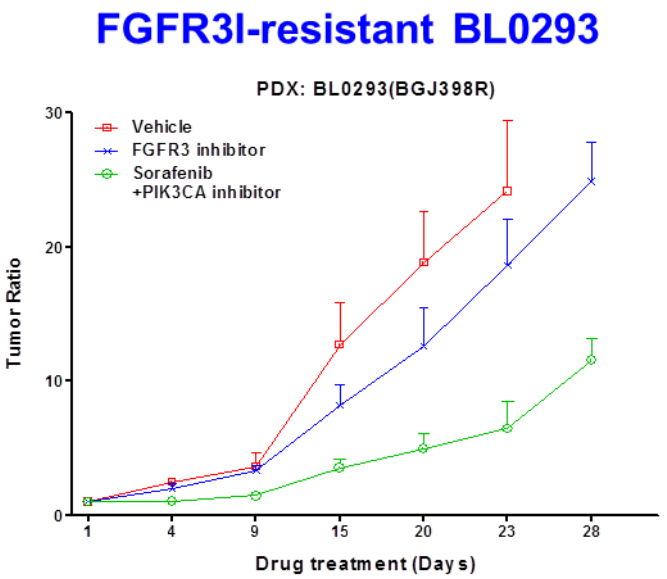
Re-purposing of FDA approved drugs

Elucidation of resistance mechanisms

Overcoming resistance

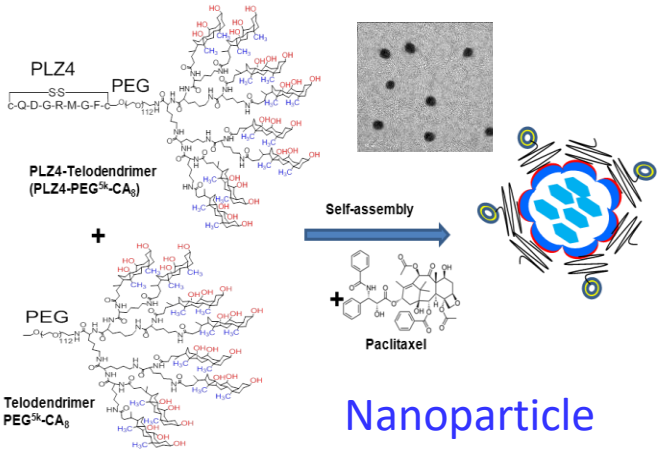




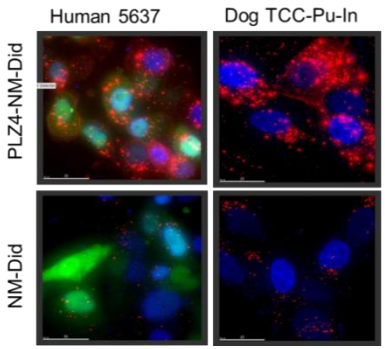


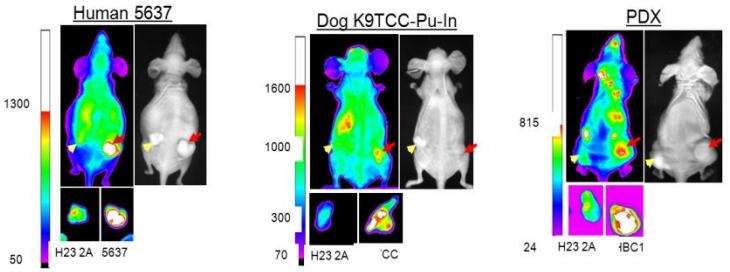
Applications of PDMCs
-Drug development

Bladder cancer-specific PLZ4-nanoparticles (Kit Lam, MD, PhD)



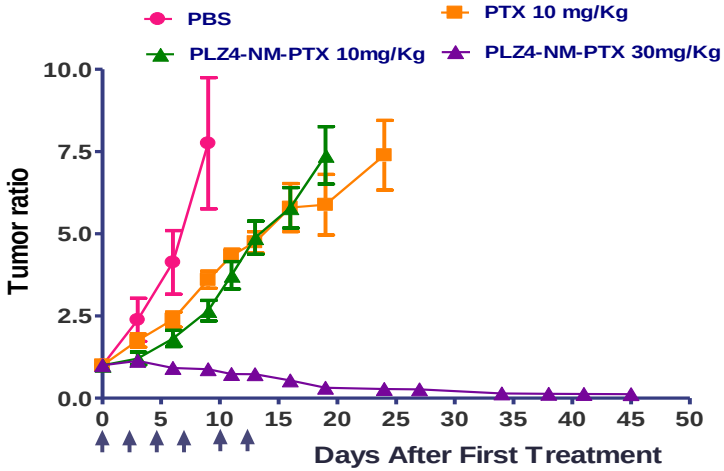
Nanoparticle





In vitro delivery In vivo delivery: left: Lung; Right: bladder

Treatment	OS (days)	WBC (K/ml)
PBS	11	3.96±1.40
PTX 10mg/kg	27	1.16±0.19
PLZ4-NP-PTX 10 mg/kg	24	2.03±0.81*
PLZ4-NP-PTX 30 mg/kg	>70	1.08±0.28

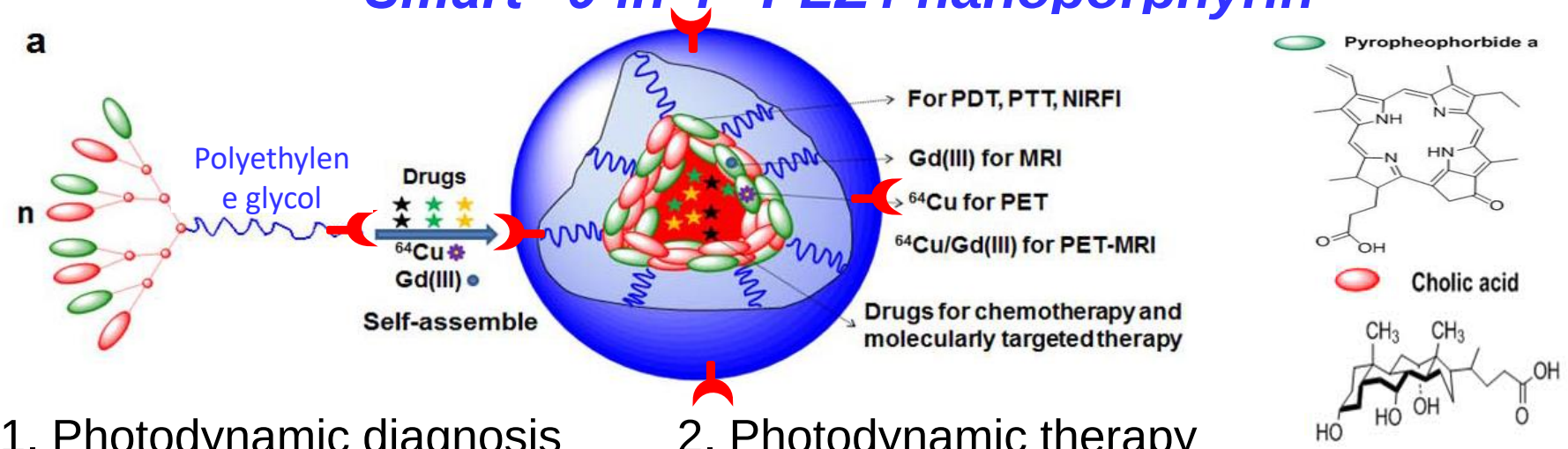


In vivo anti-tumor activity

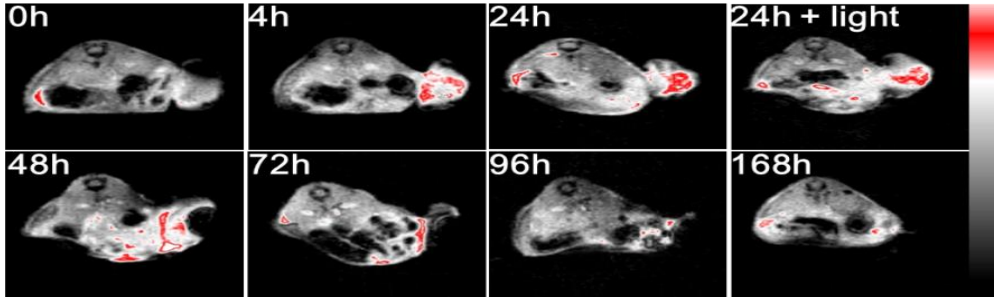
IND No: 117868

Applications of PDMCs
-Drug development

-Smart "9-in-1" PLZ4-nanoporphyrin



1. Photodynamic diagnosis
2. Photodynamic therapy
3. Photothermal therapy
4. Chelation of Gd(III) for MRI
5. Chelation of ^{64}Cu for PET
6. Chelation of ^{67}Cu for radiation therapy
7. Targeted delivery of chemo
8. Chelation of gallium for sonodynamic Tx
9. Near infrared imaging
10. combination of the above



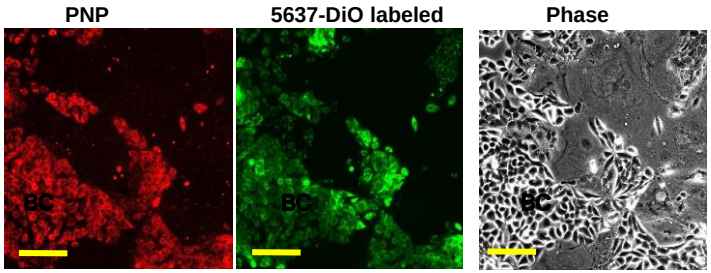
PLZ4-NP targets and deliver chelated metal to the cancer site

Li et al. Nature Communication. 2014
Lin et al. Biomaterials. 2016

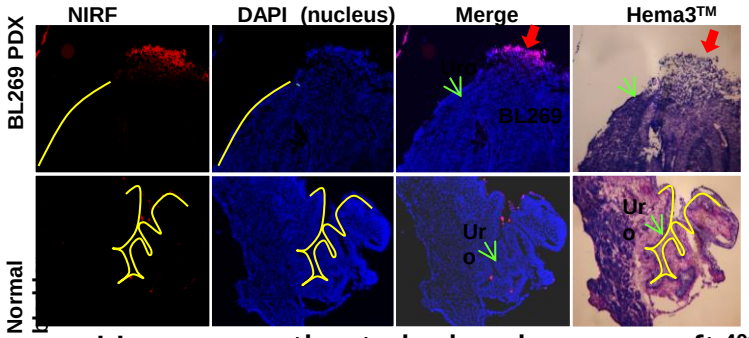
Applications of PDMCs
-Drug development

Pre-IND: 132838

Photodynamic diagnosis

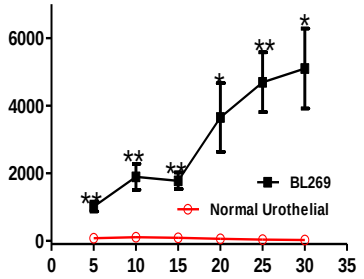


Human bladder cancer cell line 5637

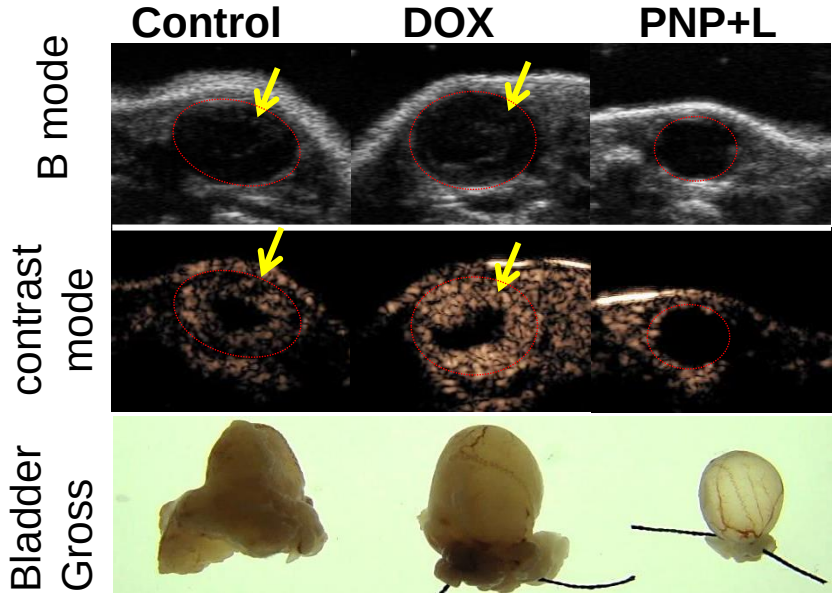


Human patient-derived xenograft 40x

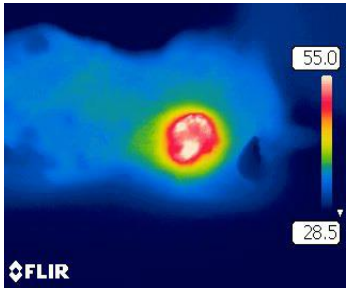
30-40X difference

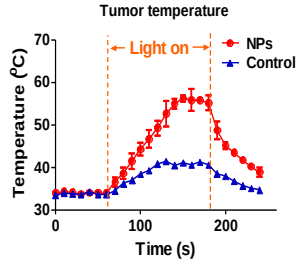


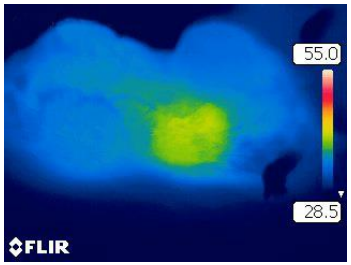
2-3X for 5-ALA

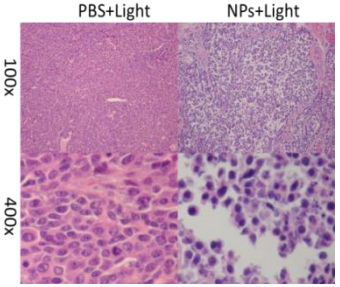


Photodynamic therapy









Photothermal therapy

PLZ4-nanotheranostics

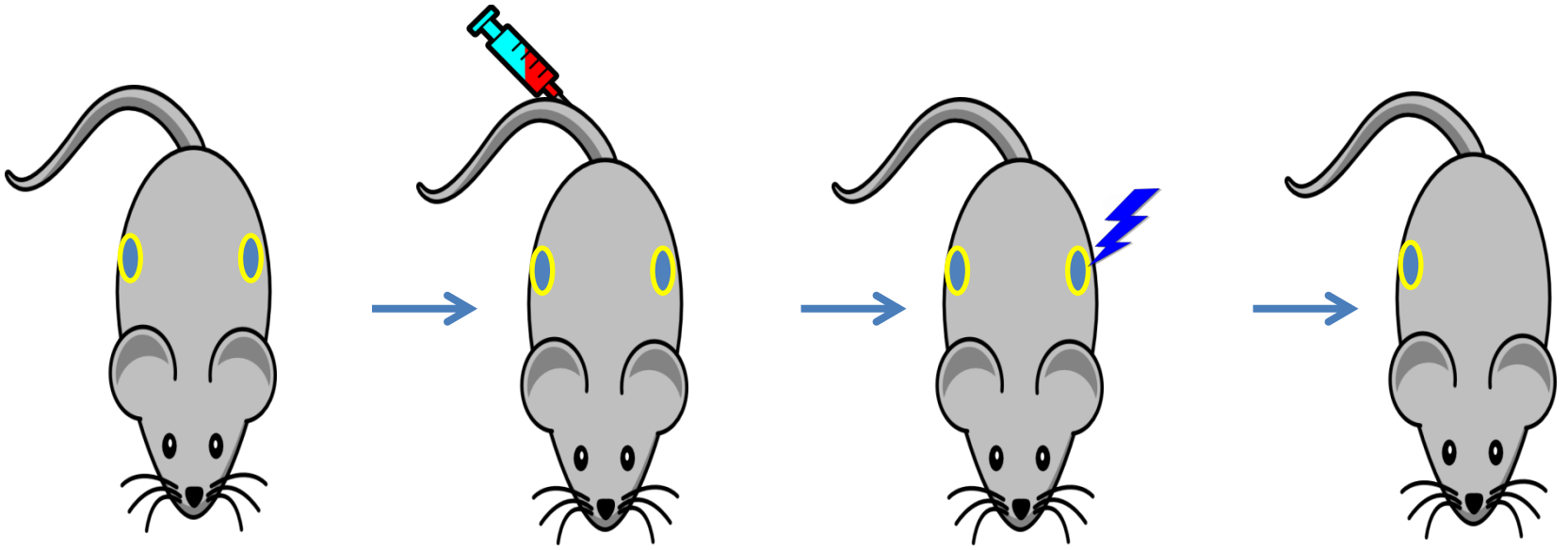
-PLZ4-nanoporphrin

PLZ4-nanoporphrin to potentiate immunotherapy

- PLZ4-nanoporphyrin kills cancer cells and release tumor antigens;
- Photodynamic therapy (PDT) produces reactive oxygen species (ROS) which can modify macromolecules, and make them more immunogenic.
- Heat from photothermal therapy (PTT) denatures macromolecules and makes them more immunogenic.
- PDT is more effective than radiation in potentiate immunotherapy.
- PDT has been used in bladder cancer. But the photosensitizer has low efficiency, low potent, nonspecific (Cancer : normal ratio: 2-3 times), and high toxicity.

PLZ4-nanotheranostics

-PLZ4-nanoporphrin to potentiate immunotherapy



Creation of bilateral syngrafts:
Cells: MB49
Mice: C57BL/6

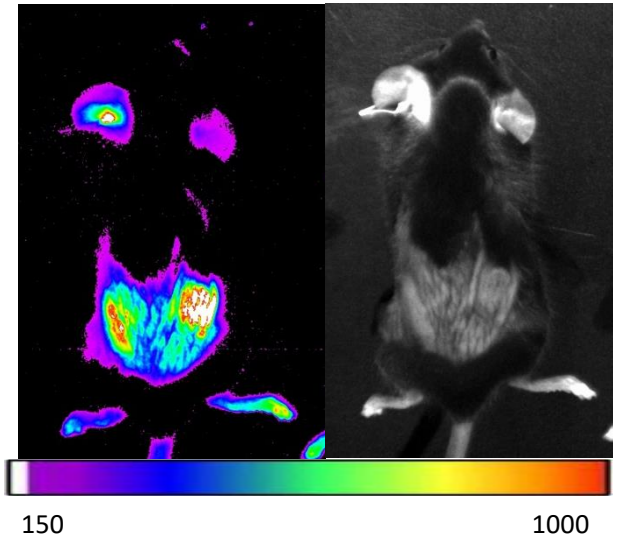
Intravenous injection of PNP

Photodynamic therapy of the **left** tumor

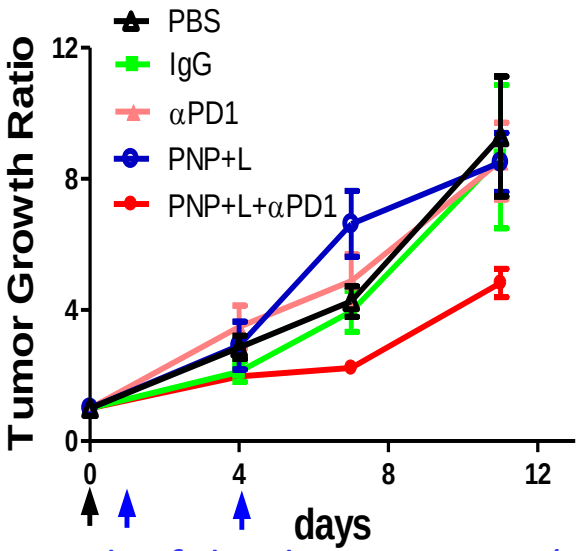
Monitoring growth of the **right** tumor

PLZ4-nanotheranostics

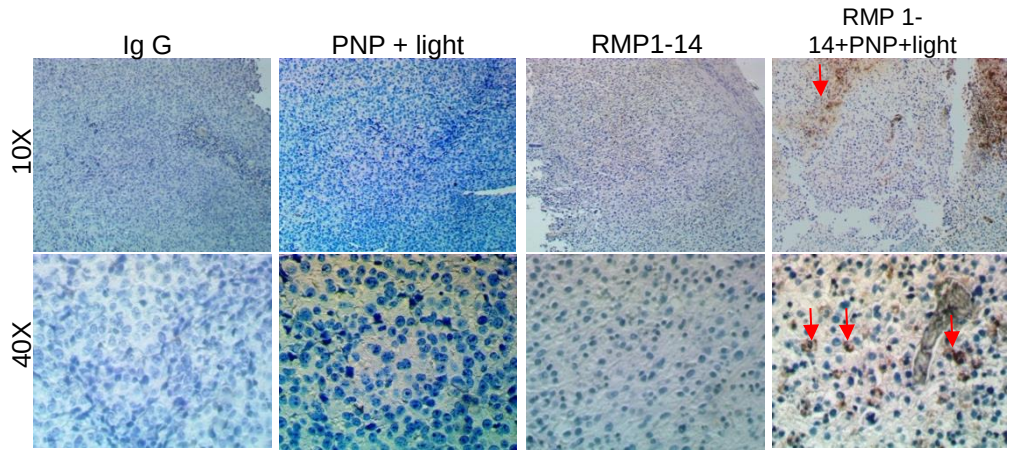
-Photothermal and targeted chemotherapy



Cancer-specific drug delivery



Growth of the distant tumor (not treated with light)



Photodynamic therapy converts “cold” tumor to “hot” tumor

PLZ4-nanotheranostics

-PLZ4-nanoporphrin to potentiate immunotherapy

PLZ4-nanoporphrin to potentiate immunotherapy
-SV40T/Ras double transgenic mice

→

Treatment:
1. Control
2. Anti-PD1 Ab
3. PNP + light
4. Anti-PD1 Ab + PNP + light

→

1. Clinical followup
2. MRI
3. Molecular correlative studies

20-30 days
Palpable mass
MRI to confirm

<u>Treatment:</u>
PD1: 200 µg/mouse, i.p., weekly
PNP: i.v., weekly, plus light
(0.2 w, 3 min)
<u>Tumor measurement</u>: MRI/T2

Mice were obtained from Xue-Ru Wu at New York University.

PLZ4-nanotheranostics

-PLZ4-nanoporphrin to potentiate immunotherapy

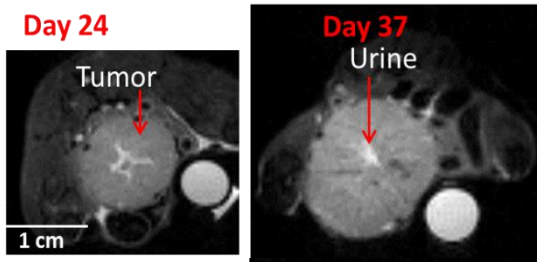
Groups	Ear Tag#	Date of Death	Overall survival	Current Status
Control	#539	01/09/2018	31 days	Dead
	#150	01/10/2018	28 days	Dead
	#1191	02/03/2018	36 days	Dead
	#2032	02/24/2018	23 days	Dead
	#1737	03/22/2018	37 days	Dead
Anti-PD1 antibody	#536	01/16/2018	38 days	Dead
	#542	01/16/2018	38 days	Dead
PDT and PTT	#560	01/07/2018	31 days	Dead
	#581	02/08/2018	34 days	Dead
	#582	03/16/2018	55 days	Dead
	#1791	03/21/2018	45 days	Dead
	#1783	04/12/2018	67 days	Dead
Anti-PD1 antibody + PDT + PTT	#1994	Alive	52 days	Study ongoing
	#535	Alive	150 days	Study ongoing
	#538	03/08/2018	90 days	Dead

Mice were obtained from Xue-Ru Wu at New York Univ.

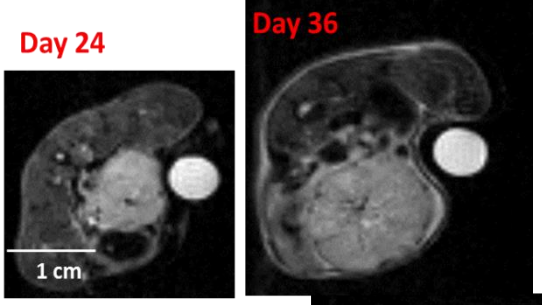
PLZ4-nanotheranostics

-PLZ4-nanoporphrin to potentiate immunotherapy

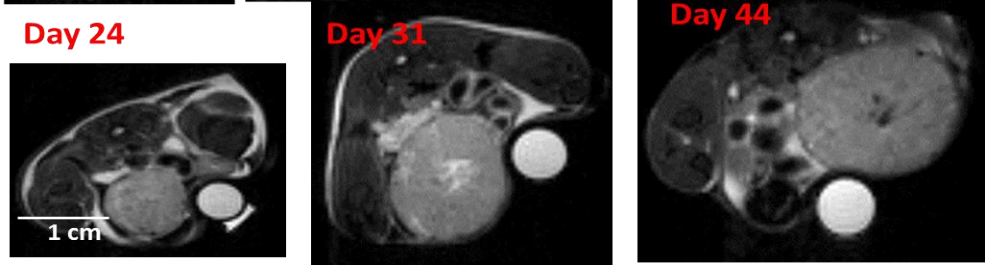
Control



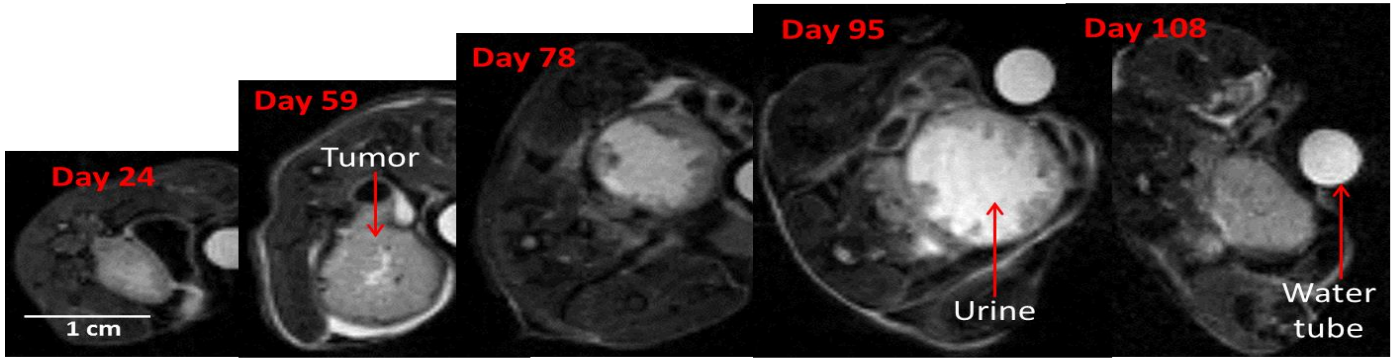
Anti-PD1



PNP PDT



Anti-PD1
+
PNP PDT



MRI to evaluate tumor growth

Applications of PDMCs
-Biomarker development

DNA adducts as a biomarker for chemoresistance to alkylating agents:

- **Platinum agents (cisplatin, carboplatin and oxaliplatin) kill cancer cells through induction of DNA damage (adducts)**
- **Cells with high Pt-DNA adducts will be killed by chemotherapy, and are chemosensitive.**
- **We developed a microdosing approach to measuring DNA adducts after a non-toxic microdose of 14C-drug**

Microdosing

-Accelerator Mass Spectrometry (AMS)

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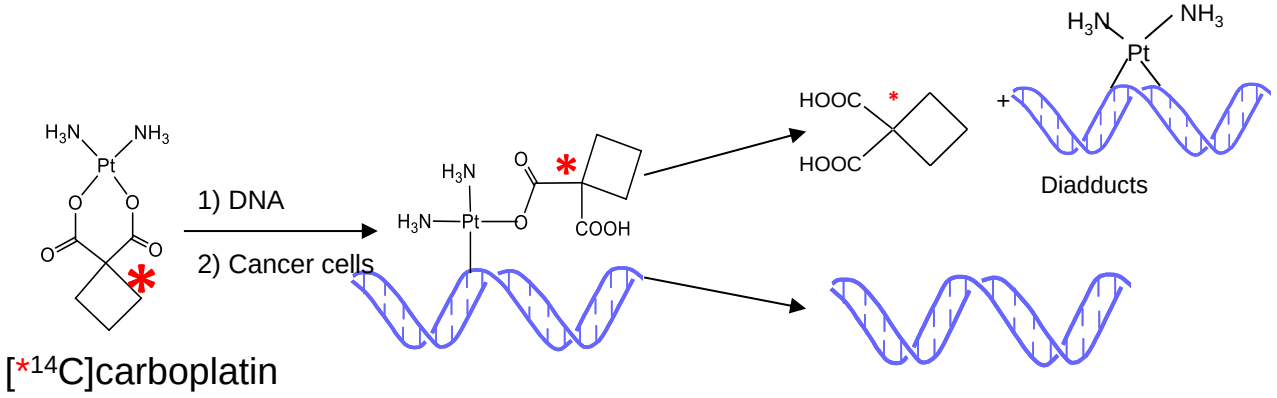
Accelerator Mass Spectrometry (AMS)

- Carbon-14 dating to determine the age of fossils
- Measure ^{14}C at 10^{-21} mole in mg-size specimens
- **^{14}C-labeled drug: one drug molecule per cell in 10^5 cells**
- Because of the ultrasensitivity, cells and patients are treated with one non-toxic **microdose** of ^{14}C-labeled drug to allow the detection of DNA damage and chemoresistance



Study of chemoresistance
-Microdosing technology

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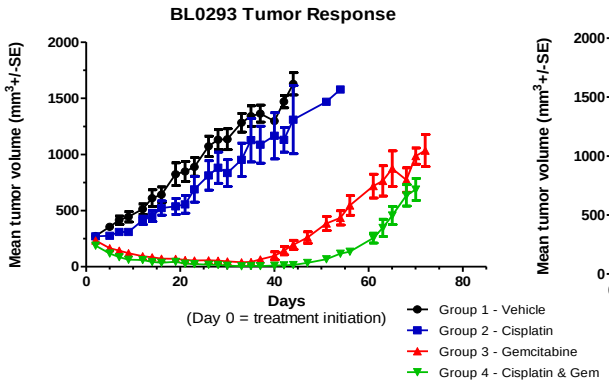
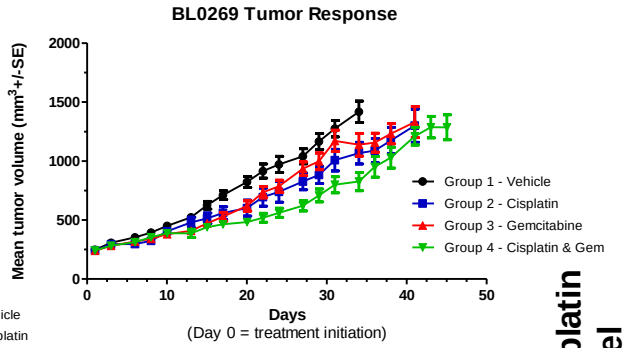
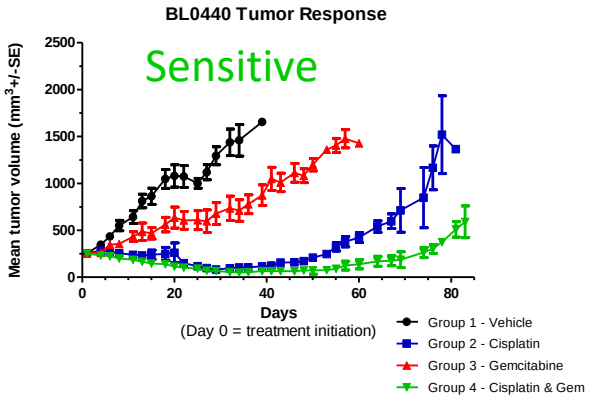
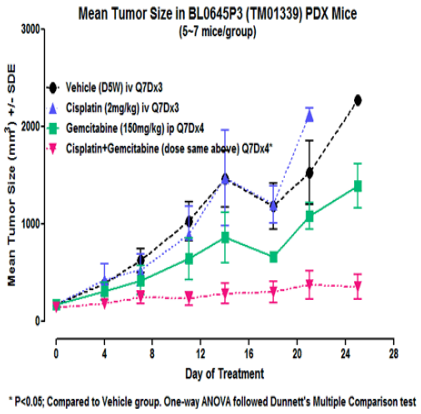
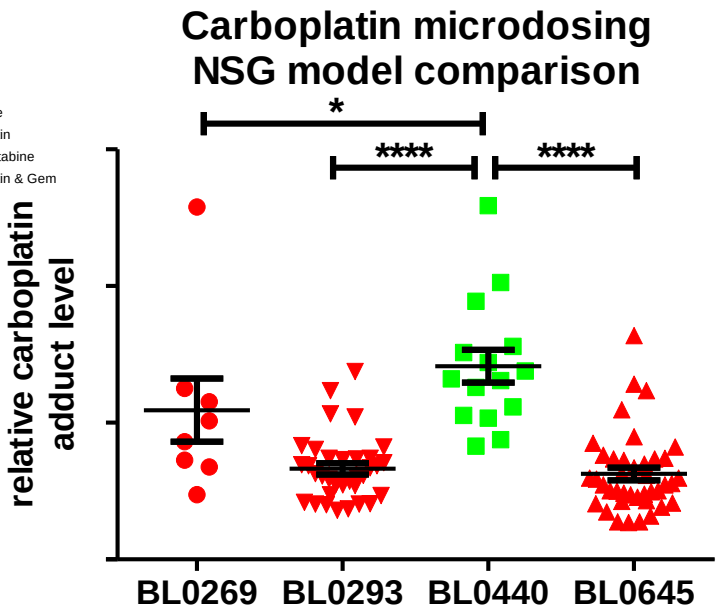
Low DNA adduct levels correlate with chemoresistance
-Bladder cancer patient-derived xenografts (PDX)











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Leading the search for tomorrow's cures

Study of chemoresistance
-Microdosing Clinical Trial

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A Phase 0 microdosing trial

Bladder cancer and NSCLC

↓

Phase 0 study: One microdose (1/100th) of ^{14}C-carboplatin:

1. PK study (drug metabolism);
2. DNA adducts of PBMC.
3. Repair of DNA adducts in cultured PBMC.
4. DNA adducts in bladder cancer specimens from TURBT

↓

Off-study therapeutic chemo with platinum chemotherapy

↓

1. Evaluate response, and correlate with DNA damage and repair, PK, cell uptake and efflux,.

2. Molecular correlation (such as ERCC and XRCC)

Clinicaltrials.gov: NCT01261299; NCT02077998. PIs: Pan

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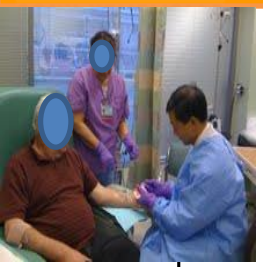
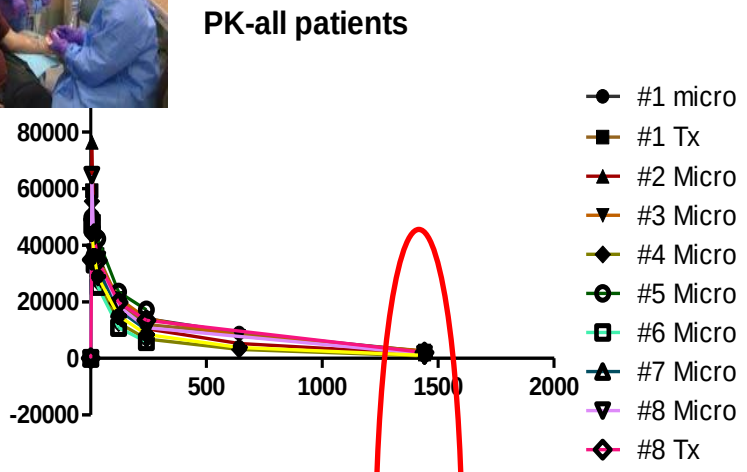
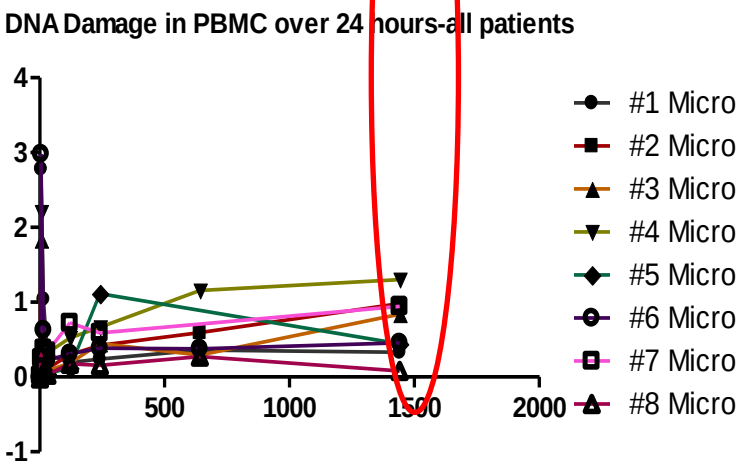
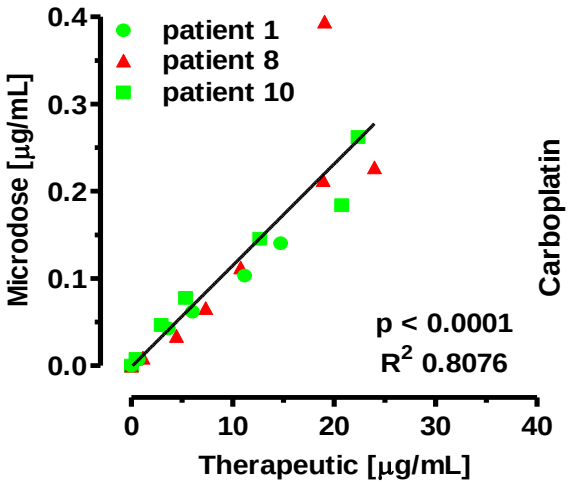
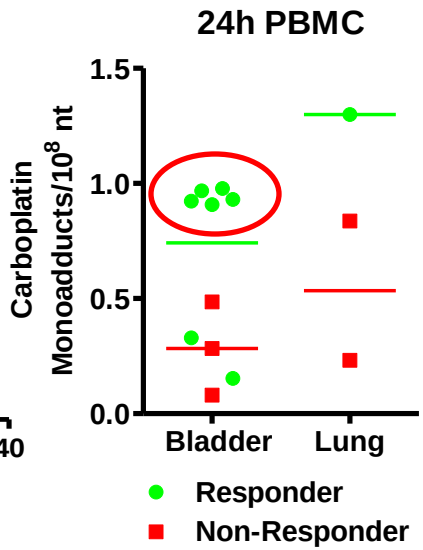










1. 24 hr is the best time for biopsy/sampling
2. Microdosing predicts PK of therapeutic dosing
3. High DNA adduct levels correlate to response

Why two pts with low DNA adducts responded?
Is this because of the chemo partner drug?

The Jackson Laboratory
Leading the search for tomorrow's cures

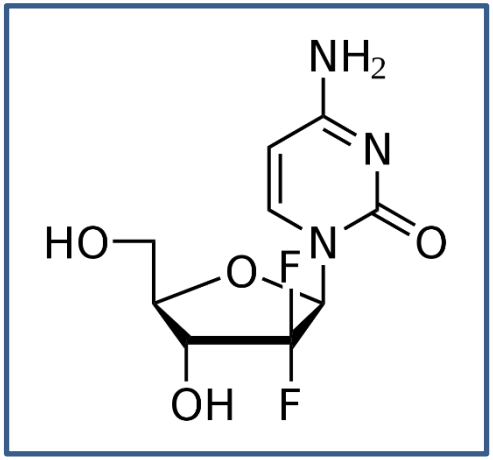
Study of chemoresistance

-Gemcitabine microdosing study

UCDAVIS COMPREHENSIVE CANCER CENTER

Lawrence Livermore National Laboratory

- Nucleoside analog
- Incorporation into DNA and block DNA replication
- Inhibits ribonucleotide reductase
- Combine with platinum for bladder cancer



- The level of gemcitabine in DNA correlates with cellular sensitivity to gemcitabine
- Using ^{3}H- or ^{14}C-labeled gemcitabine, the AMS-based microdosing approach may be able to measure the incorporation of gemcitabine into DNA and identify chemoresistance.

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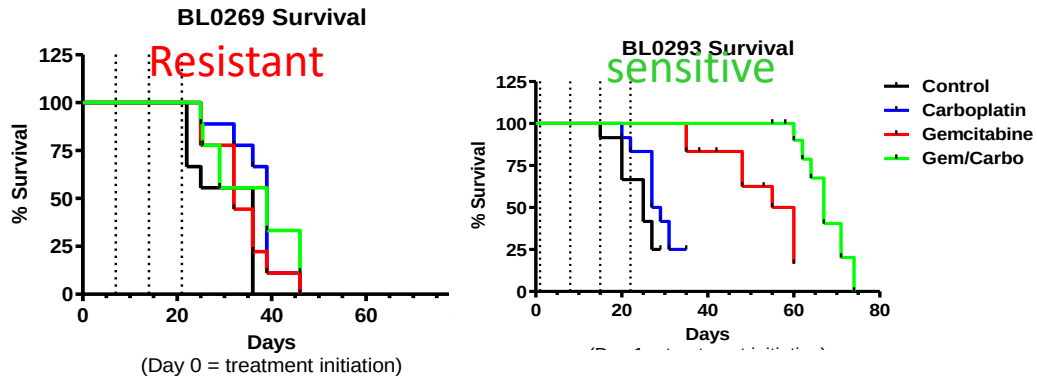
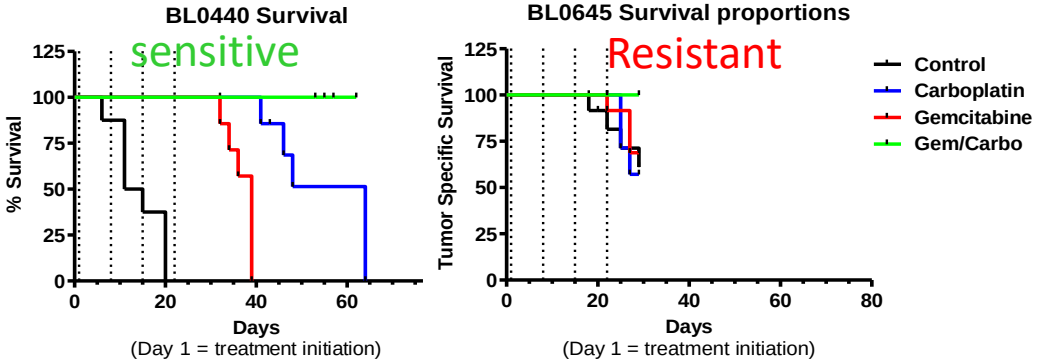
Study of chemoresistance
-Microdosing -Gemcitabine

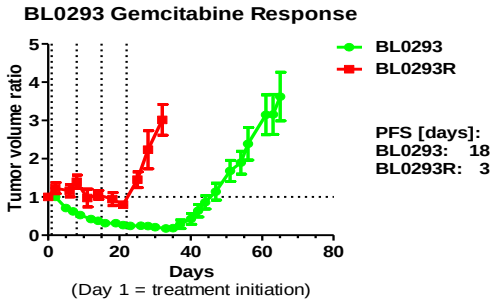
UCDAVIS COMPREHENSIVE CANCER CENTER

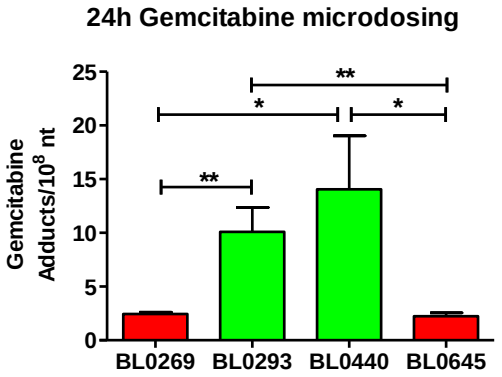
Lawrence Livermore National Laboratory

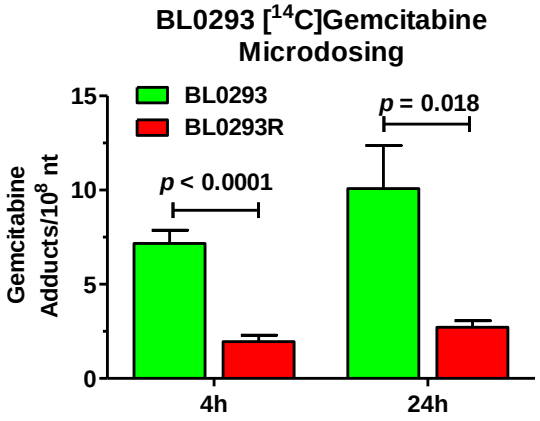
Low gemcitabine incorporation in DNA correlated with resistance











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PDXs for *Personalized therapy*

Summary

1. We have established over 30 Bladder cancer PDX models;
2. PDXs retain the morphology and genetic aberrations of parental patient cancers;
3. Deep sequencing identified multiple druggable targets;
4. PDX platform can **potentially** be used for: screening for effective targeted therapy, chemotherapy, drug re-purposing, replacing the role of serial biopsies to study secondary drug resistance, facilitating drug development, and developing biomarkers;

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Microchamber Project

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PDX Project

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PLZ4 project

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Thank you very much!!!

Questions?

PDXs for *Personalized therapy*

Microchamber to complement PDXs

Drawbacks of PDX:

- Long time to develop P0 PDXs (4-6 mo);
- engraftment rate: 40%
- Expensive

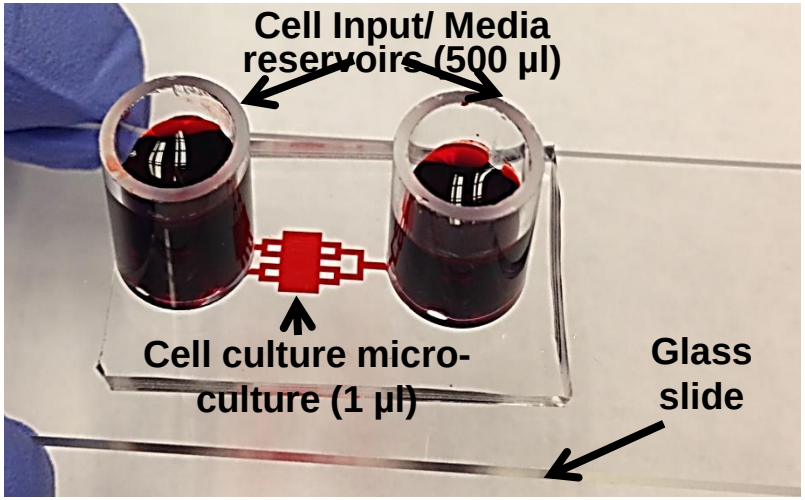
Microchamber organoid cultures to complement PDXs

PDXs for *Personalized therapy*

Microchamber to complement PDXs

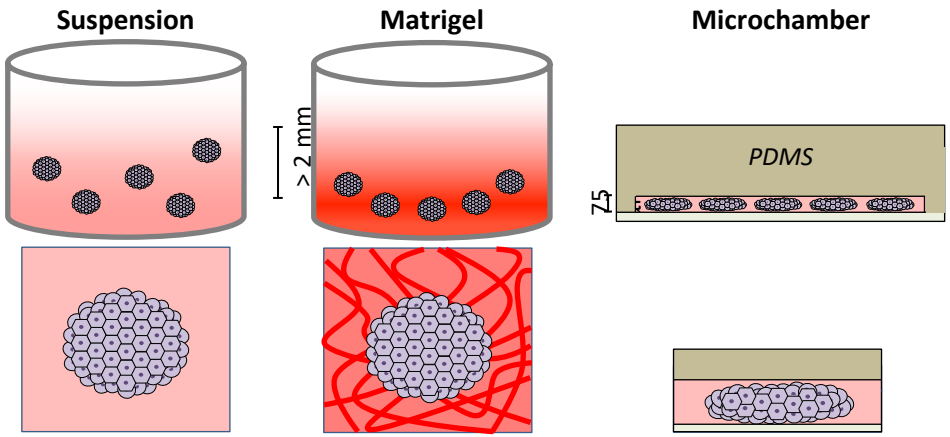
Microchamber culture to complement PDXs

(Originally developed for culture of hepatocytes)



Microchambers (µC, Alex Revzin) :

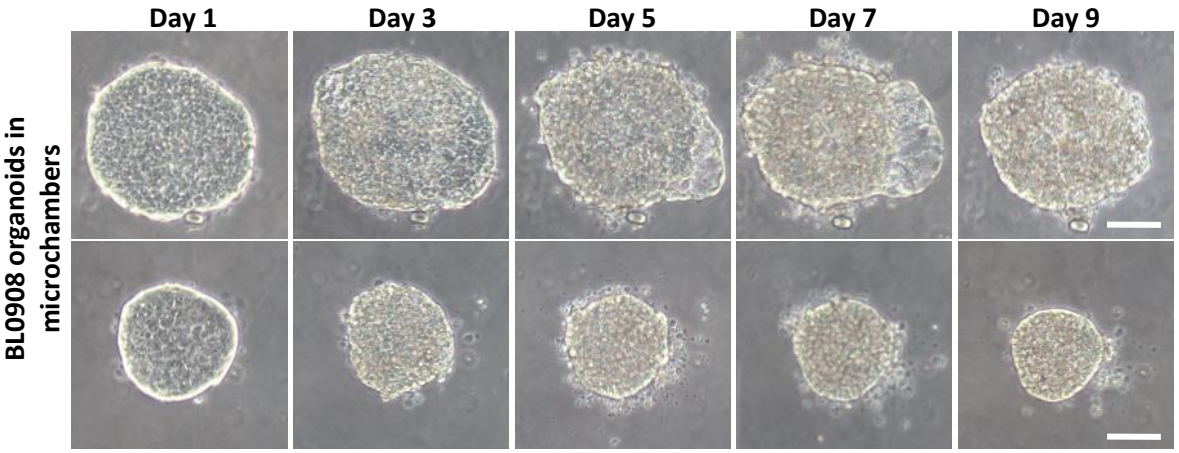
- Biocompatible
- Optically transparent
- Excellent oxygen transport
- Accumulation of endogenous factors
- Enhancement of autocrine and paracrine signals
- Long-term function maintenance of difficult-to-culture cells (ex. Primary hepatocytes and mESC (mouse Embryonic Stem Cells))

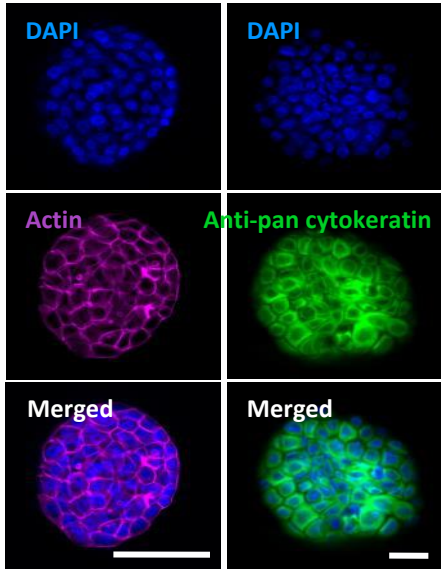


PDXs for *Personalized therapy*

Microchamber to complement PDXs

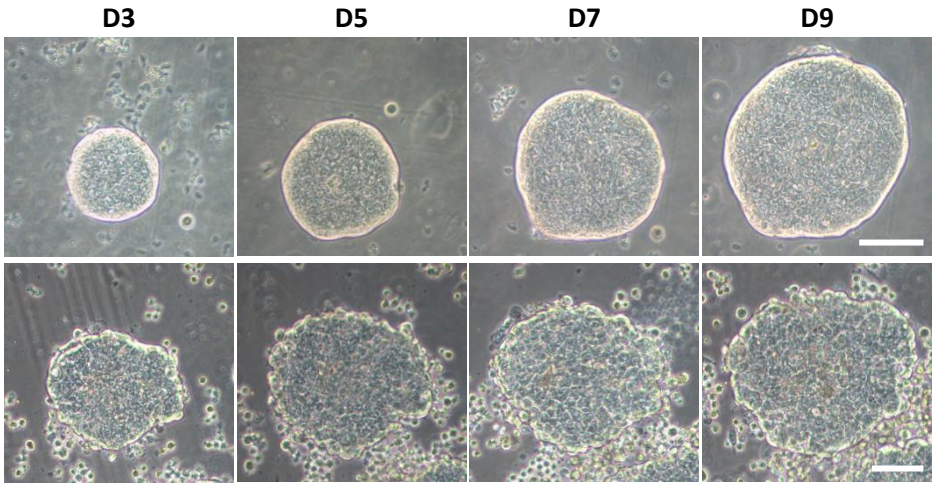
Patient specimen

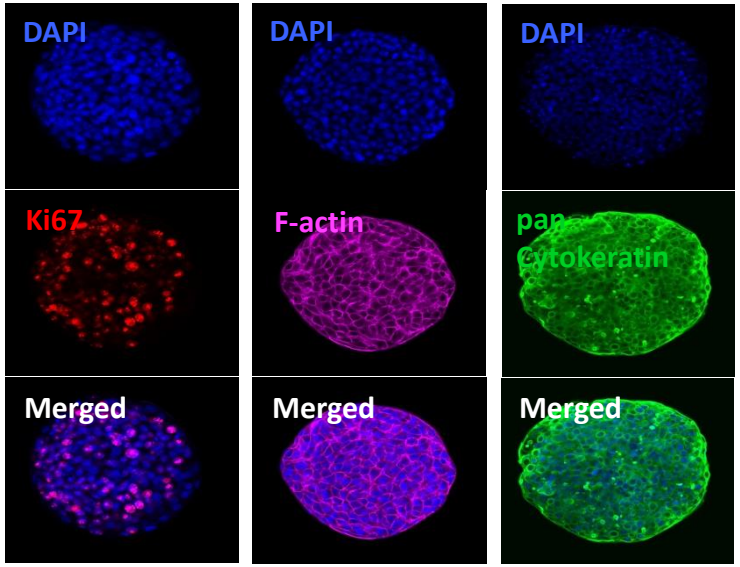




BL0908 organoids on day 9

PDX



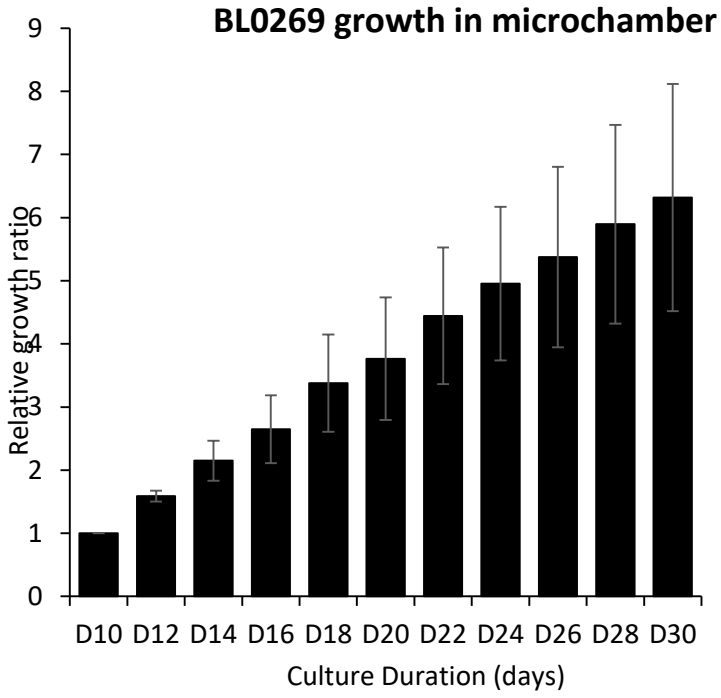


PDXs for *Personalized therapy*

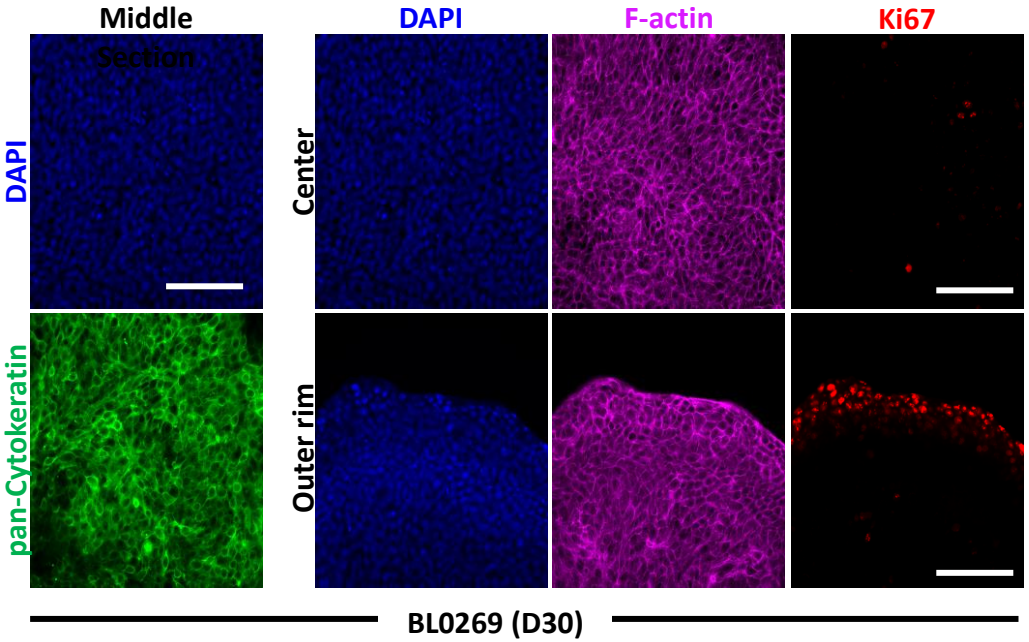
Microchamber to complement PDXs

Cell growth is mainly at the edge of cell mass.

a.



b.

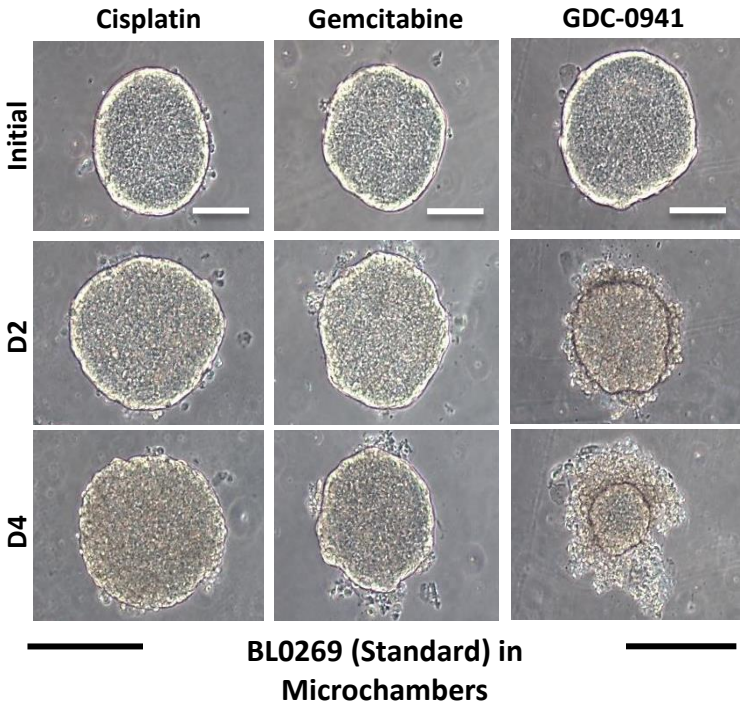


PDXs for *Personalized therapy*

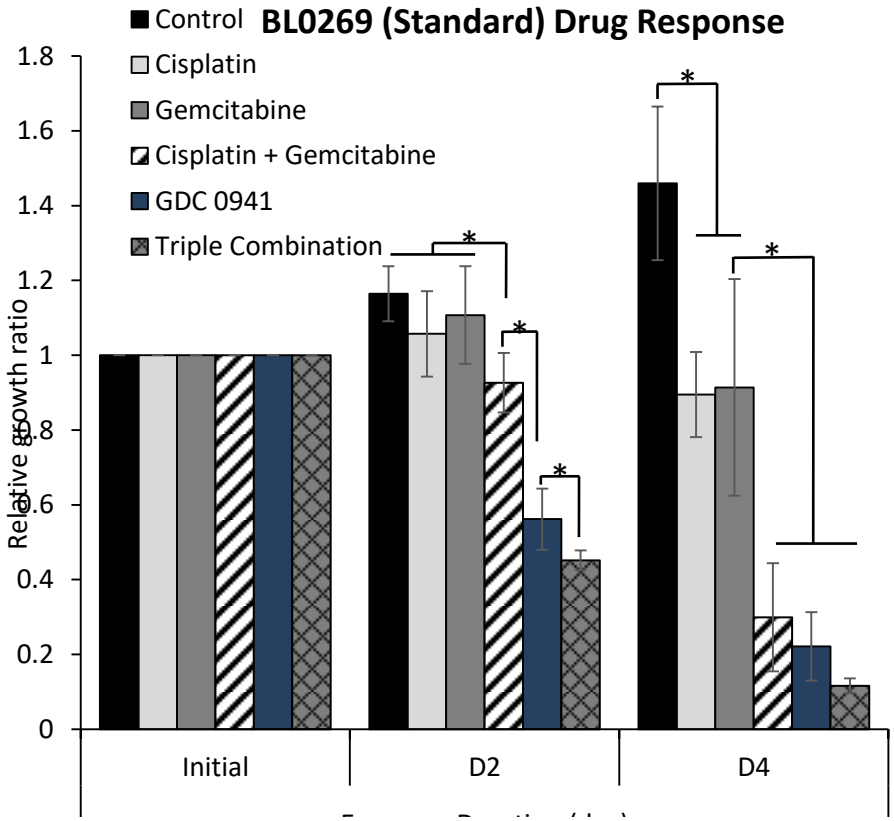
Microchamber to complement PDXs

Efficacy studies
-BL0269 with a PI3K mutation

a.



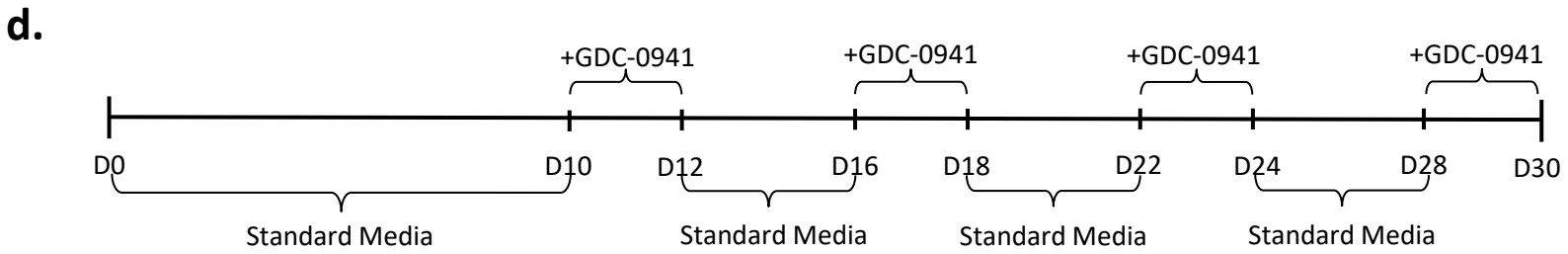
b.

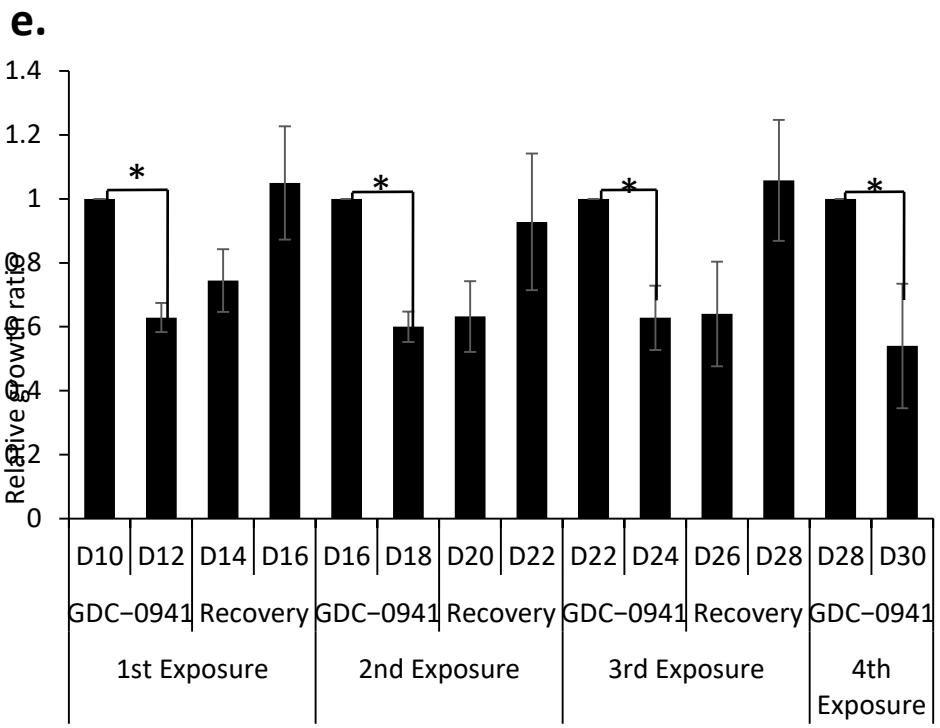


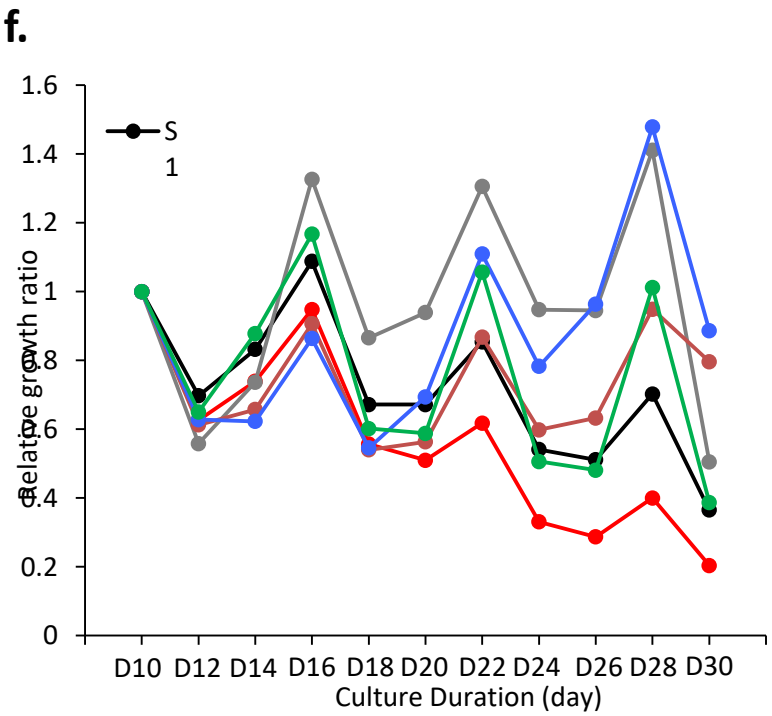
PDXs for *Personalized therapy*

Microchamber to complement PDXs

Tumor heterogeneity







PLZ4-nanotheranostics

-Summary

1. PLZ4 specifically binds to human and dog bladder cancer cells

2. Nanomicelles coated with PLZ4 can specifically deliver the drug load to bladder cancer *in vitro* and *in vivo*.

3. Micelle formulation of PTX significantly decreases the toxicity and prolongs the overall survival in mice carrying PDXs.

4. PLZ4 nanoporphyrin can be potentially used for PDD, PDT, PET, MRI, photothermal therapy, radiation, targeted chemotherapy and combination of the above.

5. Combination of PNP and immunotherapy