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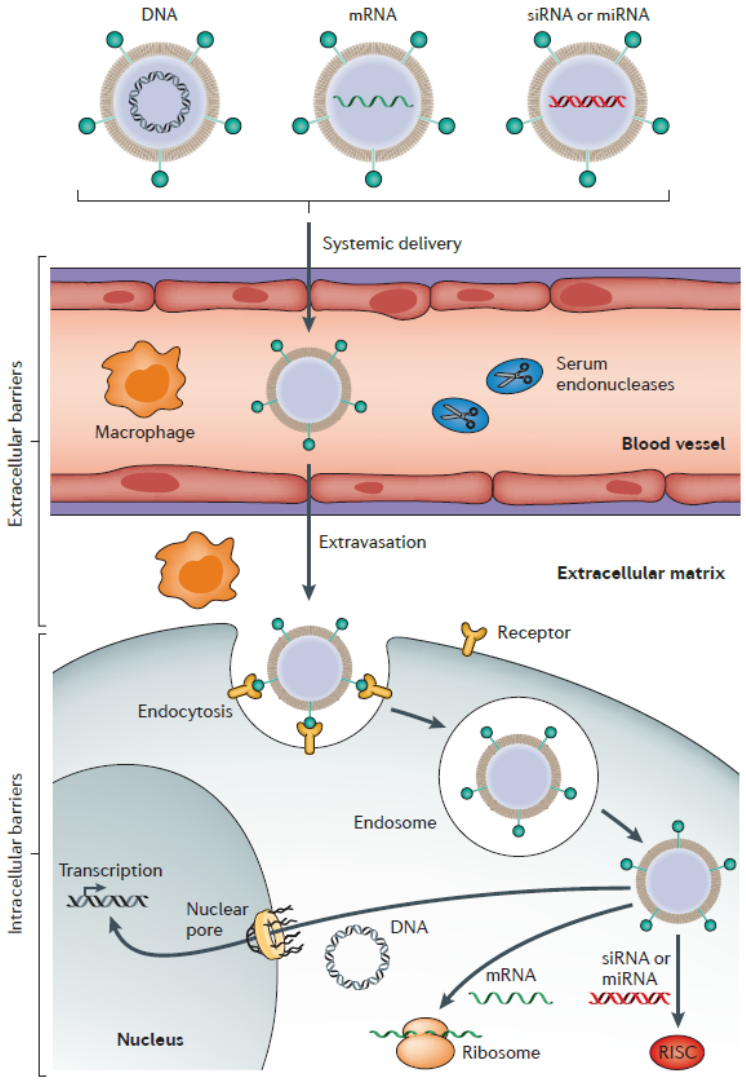
Targeted Delivery Strategies Using Dynamic Polyconjugate™ (DPC™) Technology

Barriers to systemic delivery of nucleic acid-based drugs



H. Yin et al., Nat Rev Genet (2014)

liver

Sinusoidal vasculature (5 μm)

ASGPR

- **Hepatocyte-selective**
- **0.5-1M / cell**
- **Rapidly recycled**
- **GalNAc ligands**

Rapid uptake of GalNAc conjugates

tumor

Enhanced permeability & retention effect

Tumor-selective receptors & ligands

Uptake slower, less efficient

other

- **Continuous endothelium**
- **Endothelial pores (100 nm)**
- **Inflammation EPR**

Tissue-selective receptors & ligands

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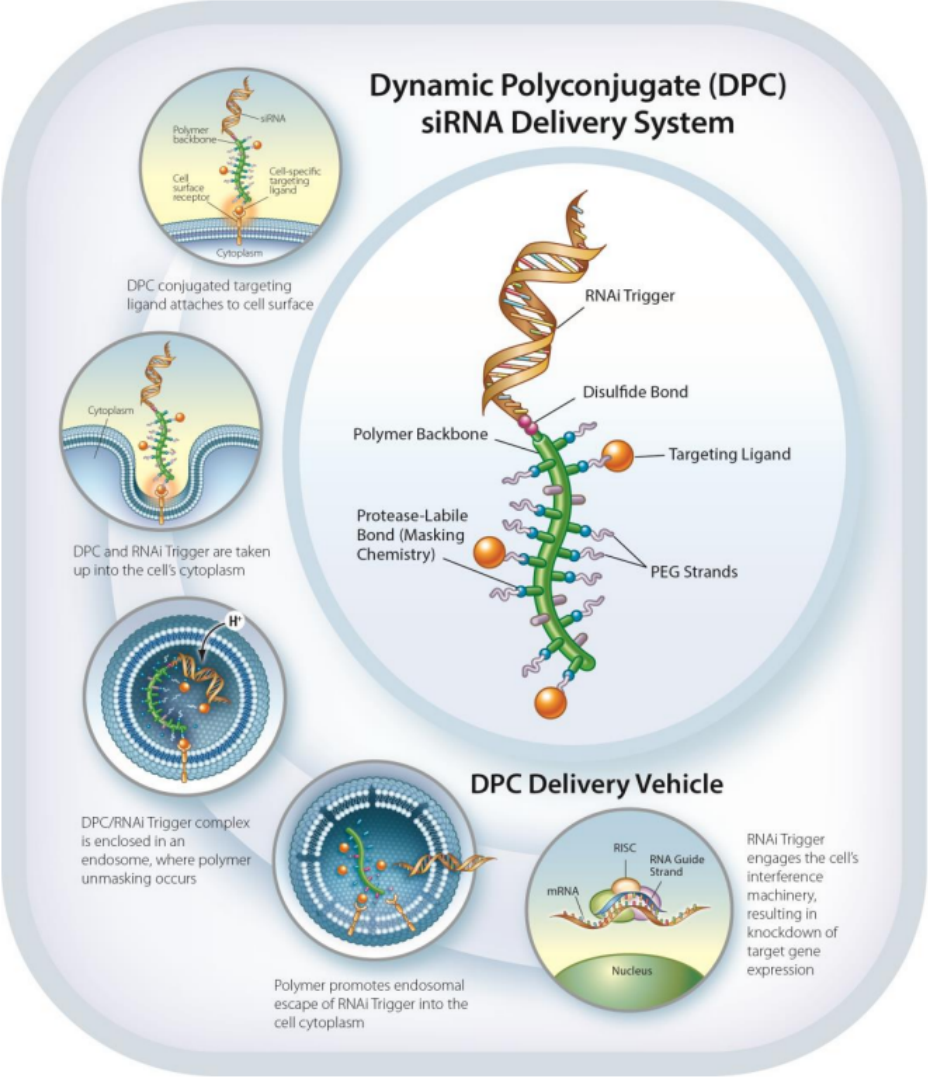
Dynamic Polyconjugate (DPC™) technology

Modular siRNA delivery platform



<u>Polymer backbone</u>

- Scaffold for trigger, ligand
- Extend exposure
  - 5-15 nm size
- Endosomal escape activity

<u>Reversible PEG masking</u>

- Improve PK
- Substrate for endosomal proteases
- Endosomal unmasking facilitates trigger release to cytoplasm

<u>Reversible RNAi trigger attachment</u>

<u>Tissue-selective targeting ligand</u>

- N acetyl galactosamine (liver)
  - ARC-520/1: hepatitis B
  - ARC-AAT: alpha-1 antitrypsin deficiency
- αv integrin ligand (tumor)
  - ARC-HIF2: ccRCC
- Other targeting ligands

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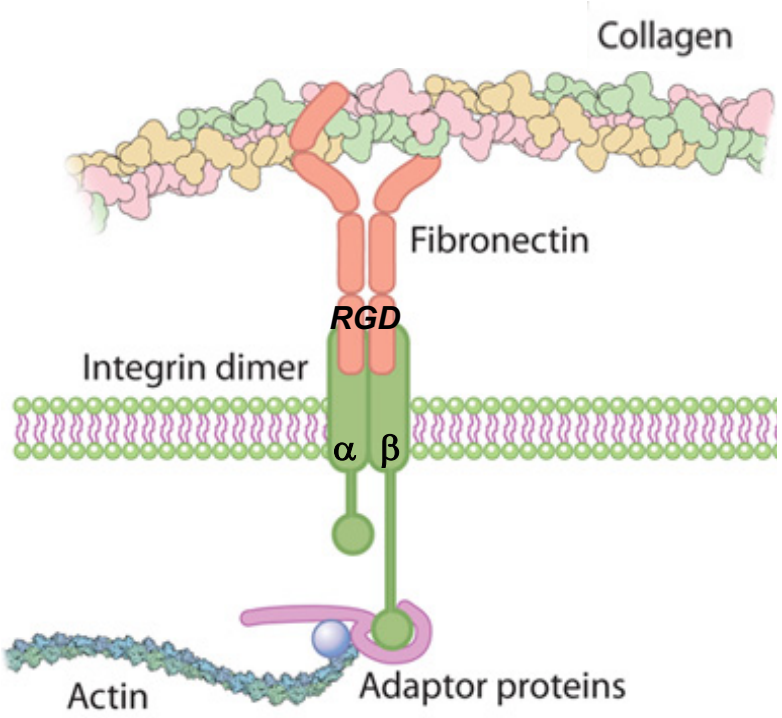
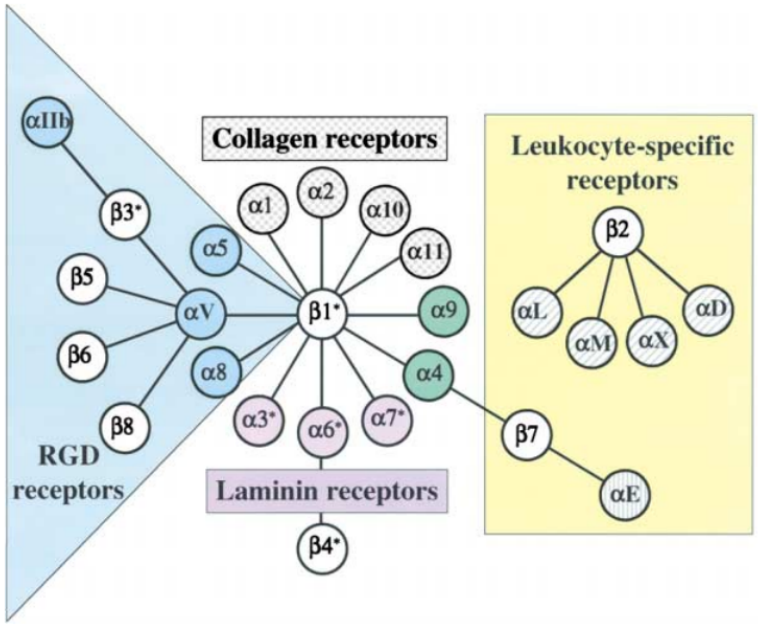
Targeting integrin receptors with proprietary RGD mimetic ligands

- Cell adhesion and signaling
- Heterodimers: 18 α and 8 β subunits
- Diverse natural ligands: ECM, growth factors, etc.
- RGD mimetic: high-affinity, selective αV integrin ligand





- αV integrins overexpressed in many tumors
- Promote angiogenesis and invasiveness
- αV integrin ligands: tumor delivery & imaging

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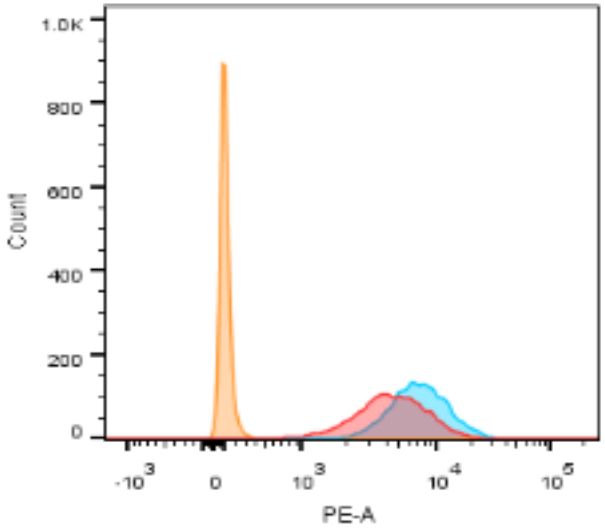
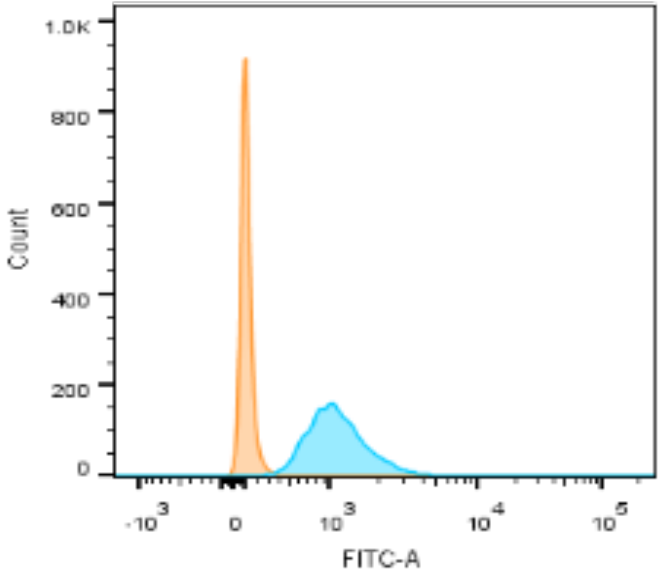
αvβ3 and αvβ5 integrins are expressed on clear cell renal cell carcinoma (ccRCC) tumor line A498

FACS analysis with antibodies to αV, αVβ3 and αVβ5

	Sample	Geo. mean
	αVβ3 PE	4508
	αV PE	7468
	Cells	98.3



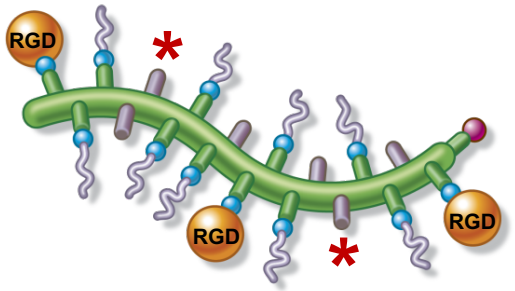
	Sample	Geo. mean
	αVβ5 FITC	1205
	Cells	103



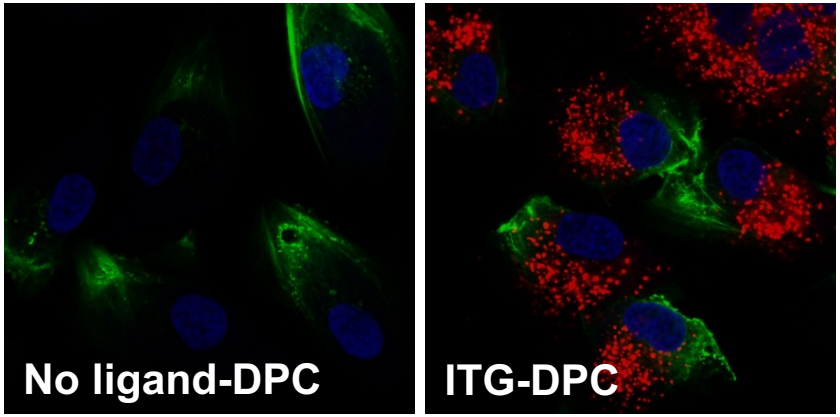
Integrin-targeted DPCs (ITG-DPCs) are internalized by ccRCC cells and tumors

ITG-DPC

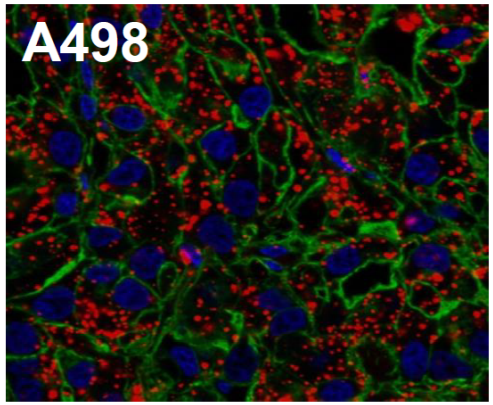
- **Reversibly masked polymer backbone**
- **Multiple RGD mimetic ligands for αV integrin targeting**
- **Cy3 label for tracking**



In vitro A498 cell tracking



In vivo tumor tracking



Green: Actin
Blue: Nuclei
Red: ITG-DPC

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Therapeutic target for ccRCC

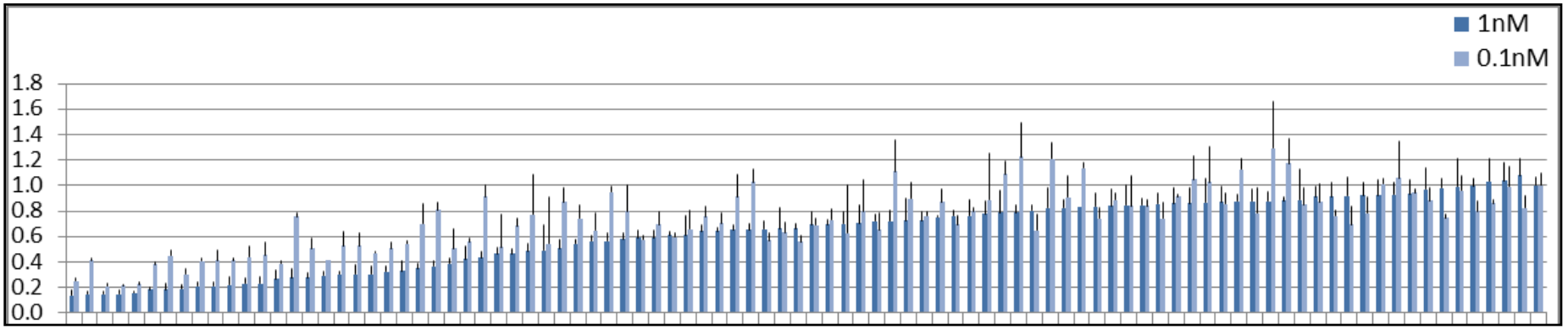
Hypoxia-inducible factor 2 alpha (HIF-2α)

- **Majority of ccRCC tumors (~90%) characterized by inactivation of Von Hippel-Lindau (VHL) ubiquitin ligase tumor suppressor**
  - **Results in HIF-2α stabilization and activation**
- **Strong target validation for HIF-2α in ccRCC**
  - **HIF-2α is an oncogenic transcription factor**
- **Transcription factors difficult to drug with traditional small molecule compounds or antibody therapies**
- **HIF-2α not widely expressed in normal tissues**

Identification of lead HIF-2α RNAi trigger design

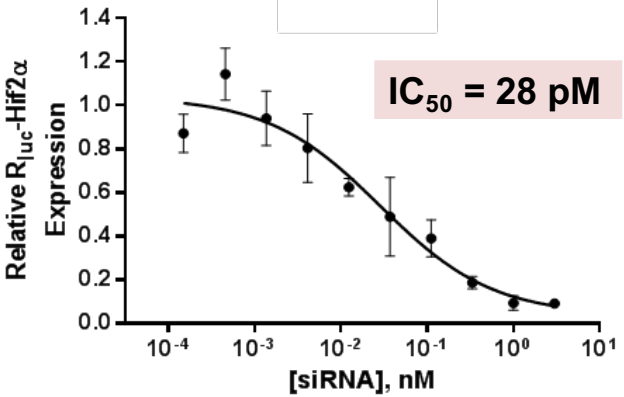
Primary *in vitro* screen and confirmation

Cotransfection of HIF-2α-luciferase construct and candidate HIF-2α triggers

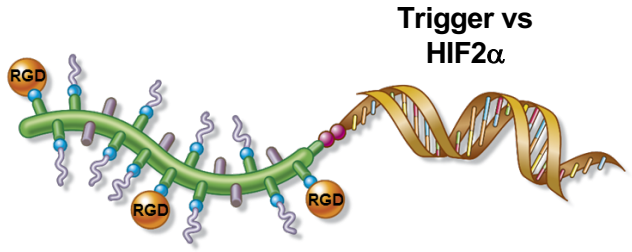


Hits

Lead



IC_{50} = 28 pM



ARC-HIF2

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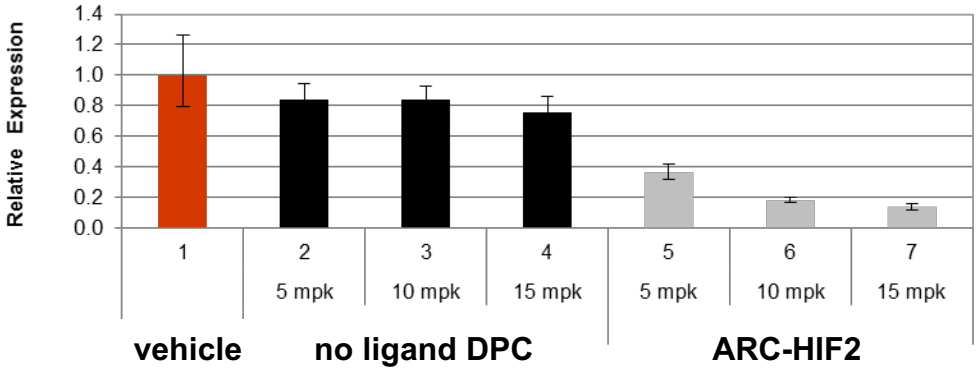
Deep, durable HIF-2α knockdown after single dose

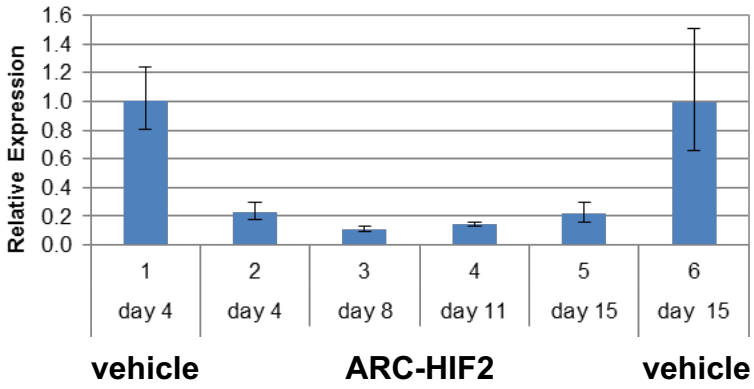
ARC-HIF2 in orthotopic renal A498 ccRCC tumors

- Nude mice, 3-4 week established tumors
- Single IV dose ARC-HIF2 on Day 1

Tumor HIF-2α expression
Day 6



Tumor HIF-2α expression
15 mg/kg



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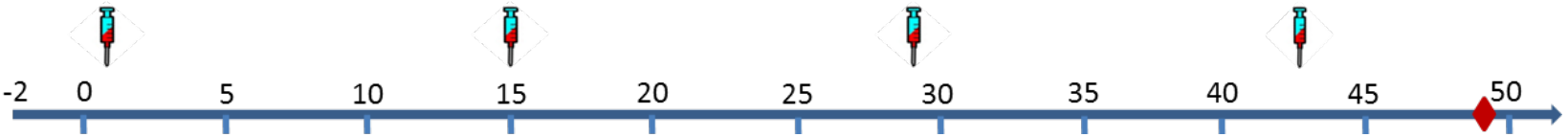
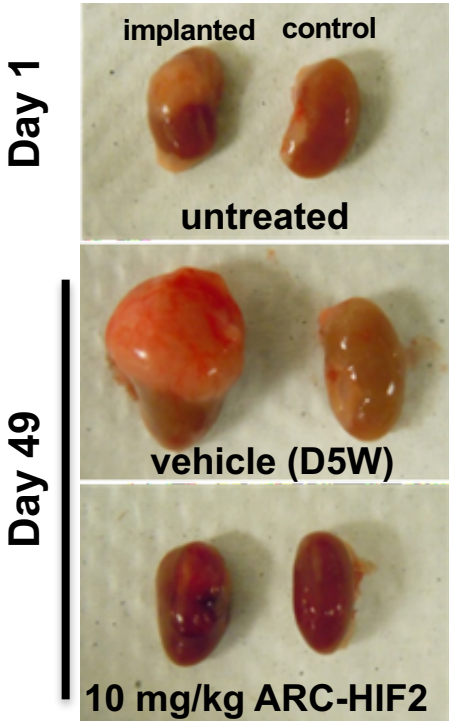
ARC-HIF2 reduces growth of orthotopic A498 tumors

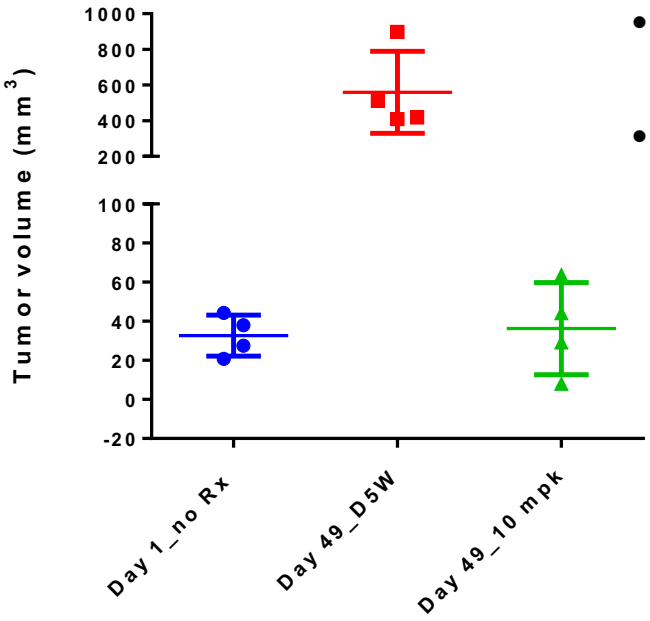
10 mg/kg ARC-HIF2; q2w x4



Tumor morphology



Tumor volume



- ARC-HIF2 treatment produced tumor stasis
- Well-tolerated (no Δ in body weight)

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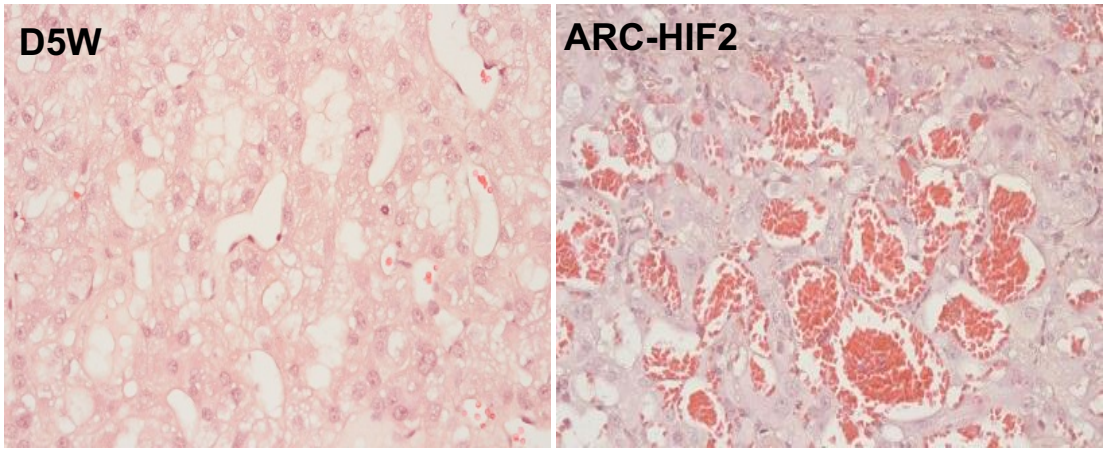
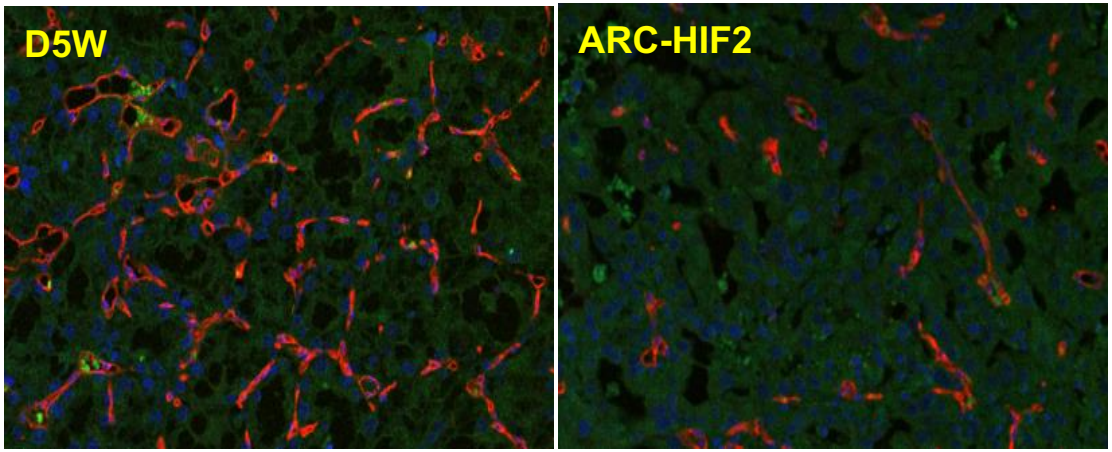
Morphological changes in ARC-HIF2 treated tumors

Histological evidence of tumor degeneration and reduced vascularization

Tumor morphology by H&E (Day 49)



Tumor CD31 IHC (Day 49)



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2064

Novel HIF-2α targeted RNAi therapeutic for renal cell carcinoma

So Wong, Weijun Cheng, Darren Wakefield, Aaron Almeida, Andrei Blokhin, Holly Hamilton, Vladimir Subbotin, Julia Hegge, Zane Neal, Guofeng Zhang, David Rozema, David Lewis, Steven Kanner

Arrowhead Pharmaceuticals, Inc., Madison, WI 53711, USA

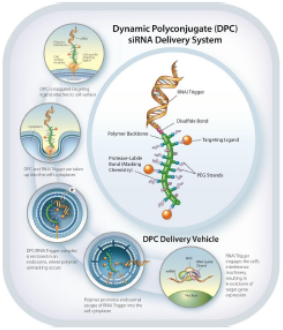
INTRODUCTION

Targeted therapy including VEGF and mTOR pathway inhibitors has dramatically transformed treatment options and outcomes for patients with metastatic clear cell renal cell carcinoma (ccRCC). However, alternate treatments are needed as resistance to these initially promising agents occurs frequently. RNAi interference (RNAi), an innate gene silencing mechanism, has been explored as a new class of therapeutics where conventional treatments are lacking or have failed. The challenge in leveraging this promising approach has been efficient delivery of an RNAi trigger (siRNA) to target tissue. Over 90% of ccRCC tumors express a mutant inactive form of the von Hippel-Landau protein (pVHL), an E3 ubiquitin ligase that promotes target protein degradation. Strong evidence supports the observation that pVHL functional loss leads to the accumulation of the transcription factor hypoxia-inducible factor 2α (HIF-2α), a tumorigenic driver of ccRCC.

METHODS

We have developed a targeted delivery platform called Dynamic Polyconjugate™ (DPC) as an RNAi-based therapeutic targeting HIF-2α for advanced ccRCC. The ccRCC-specific DPC (ITG-DPC) comprises a membrane active polymer to promote RNAi trigger endosomal release, a ligand that binds to αV-containing integrin receptors expressed on tumor cells, reversible masking to prevent polymer activity before reaching the endosomal compartment, and a potent and specific RNAi trigger to HIF-2α. The modular nature of this delivery platform allows for flexibility to optimize each functional component independently. The ligand-dependent delivery of ITG-DPC was first evaluated in cultured tumor cells and then confirmed in ccRCC tumors established in nude mice using fluorescently-labeled ITG-DPC and confocal microscopy. To validate silencing of HIF-2α as an effective therapeutic approach, an inducible shRNA to HIF-2α was expressed in ccRCC tumors established in mice that significantly silenced HIF-2α gene expression and induced tumor regression.

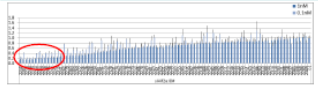
Proof-of-concept functional delivery was then obtained using optimized HIF-2α ITG-DPC in two different orthotopic RCC tumor bearing mouse models.



Schematic of ITG-DPC and target cell delivery

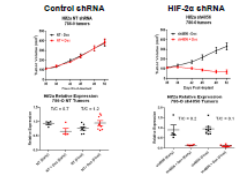
RESULTS

A. HIF-2α RNAi Trigger selection in cultured cell system



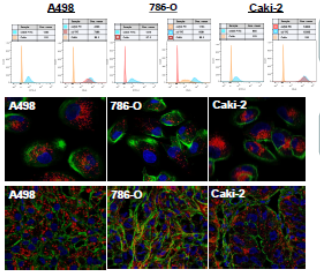
Lead sequences selected for further evaluation

B. *In vivo* target validation in SQ 786-O RCC xenograft expressing Dox inducible HIF-2α shRNA



Induction of HIF-2α shRNA expression resulted in HiF2α mRNA knockdown and tumor growth regression

C. Targeting ligand validation and tumor model selection

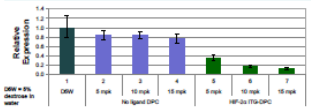


Integrin (αvβ3, αvβ5) expression profiling by FACS

In vitro ITG-DPC cell binding and internalization

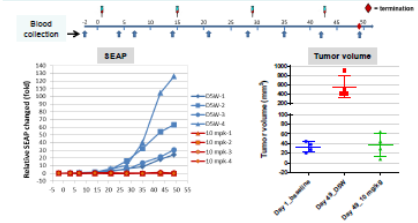
In vivo ITG-DPC tumor delivery

D. Ligand and dose dependent HIF-2α gene silencing in A498-SEAP* orthotopic RCC tumor model

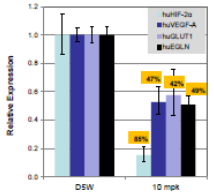


*A498-SEAP cells were stably transfected with a SEAP (secreted embryonic alkaline phosphatase) expression construct. Serum collected from tumor bearing mice can be used to monitor tumor growth

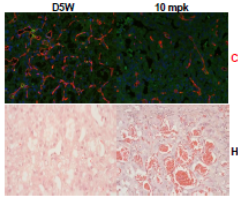
E. Tumor growth inhibition after 4 bi-weekly doses in orthotopic A498-SEAP ccRCC tumor bearing mice



Inhibition of HIF-2α and regulated genes

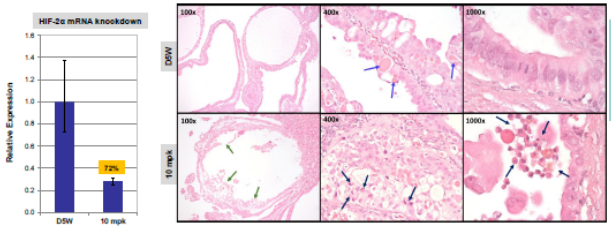


Tumor and blood vessel degeneration



- Tumor volumes (calculated with surface measurements) were mostly unchanged in the treatment group, suggesting tumor stasis
- However, histological examination of tumor sections revealed extensive internal tumor destruction
- Down-regulation of HIF-2α regulated pathway genes including VEGF-A, and the corresponding reduction in tumor-associated CD31 positive neovascularization, corroborated on-target effects of HIF-2α gene silencing.
- Treatment was well tolerated

F. QW x2 HIF-2α ITG-DPC promotes tumor degeneration in orthotopic Caki-2 papillary RCC tumor bearing mice



- Dosed on day 1 and day 8
- Tumor HIF-2α expression level and histopathology on day 14
- Significant tumor degeneration was observed in treatment group

Summary and Conclusions

- *In vivo* ligand dependent delivery of ITG-DPC to RCC tumor bearing mice was demonstrated
- Silencing HIF-2α expression by RNA interference results in reduction of HIF-2α regulated genes, promotes tumor cell death and structural degeneration in two different orthotopic RCC tumor models
- HIF-2α specific RNAi-based-therapeutic has the potential to radically impact the late-stage RCC treatment paradigm

Acknowledgement

- We thank all members of Arrowhead Discovery Biology, Discovery Chemistry and Laboratory Animal Research groups for all their involvement in this work

Extrahepatic targeting discovery

Ligand-receptor pair validation and optimization workflow

Flow cytometry: primary screen
- Receptor binding, competition

Cellular tracking: secondary screen
- Uptake by confocal microscopy

***In vivo* tracking screens**
- Tumor / tissue uptake

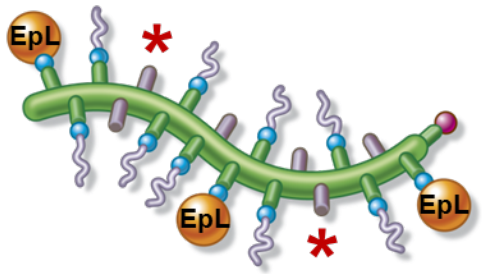
***In vivo* delivery PoC, lead optimization**
- Ligand-dependent functional delivery (KD)
- Targeted DPC paired with therapeutic trigger
- Disease-modifying efficacy studies

Ligand / DPC optimization cycle
- *affinity*
- *selectivity*
- *valency*
- *stability*

Receptor classes
- Integrins
- Receptor tyrosine kinases
- G protein-coupled receptors

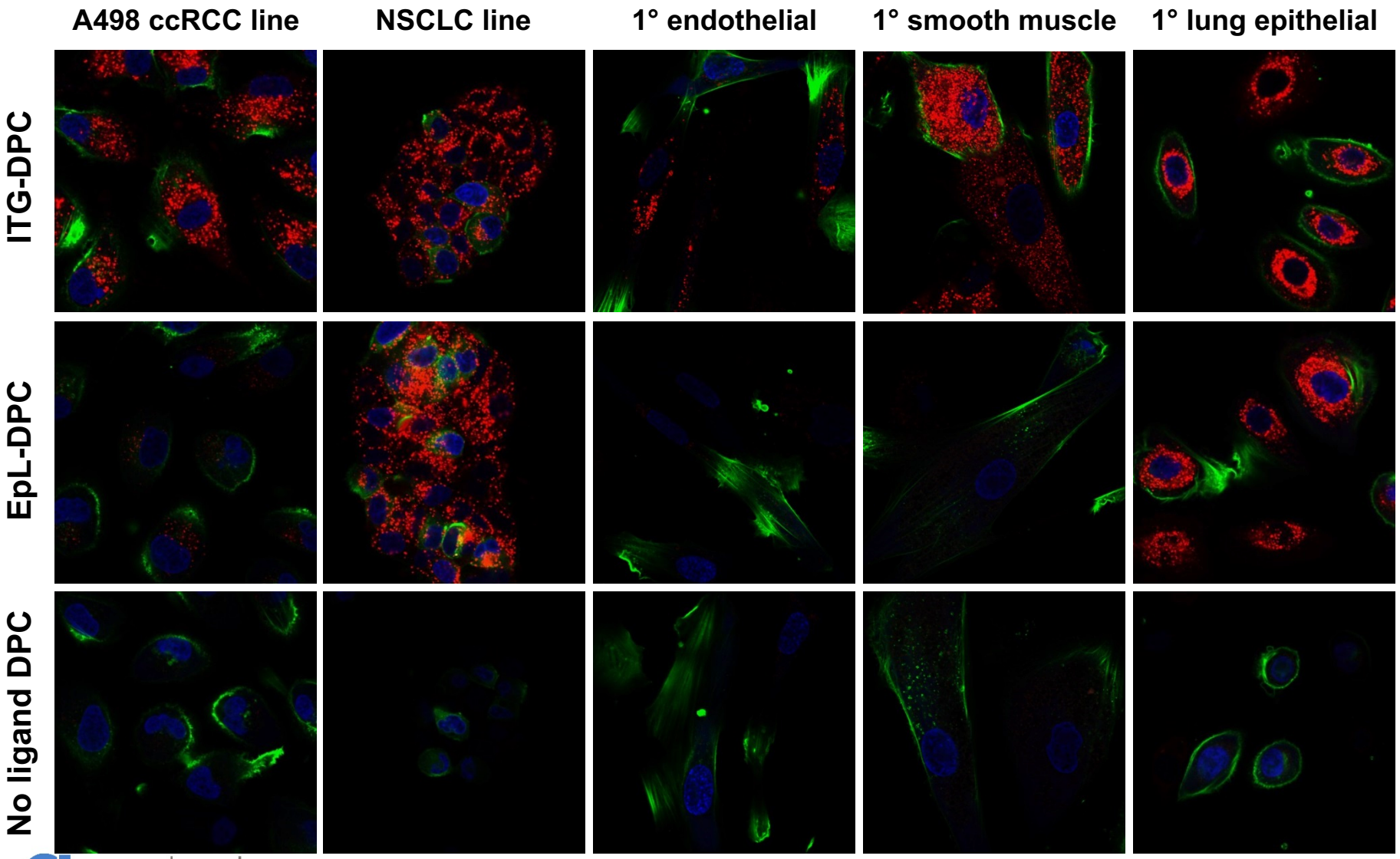
Ligand chemistries
- Peptide
- Small molecule

Epithelial cell-targeting ligand (EpL)



EpL-DPC

Selective EpL-DPC internalization by non-small cell lung cancer (NSCLC) tumor cells and epithelial cells



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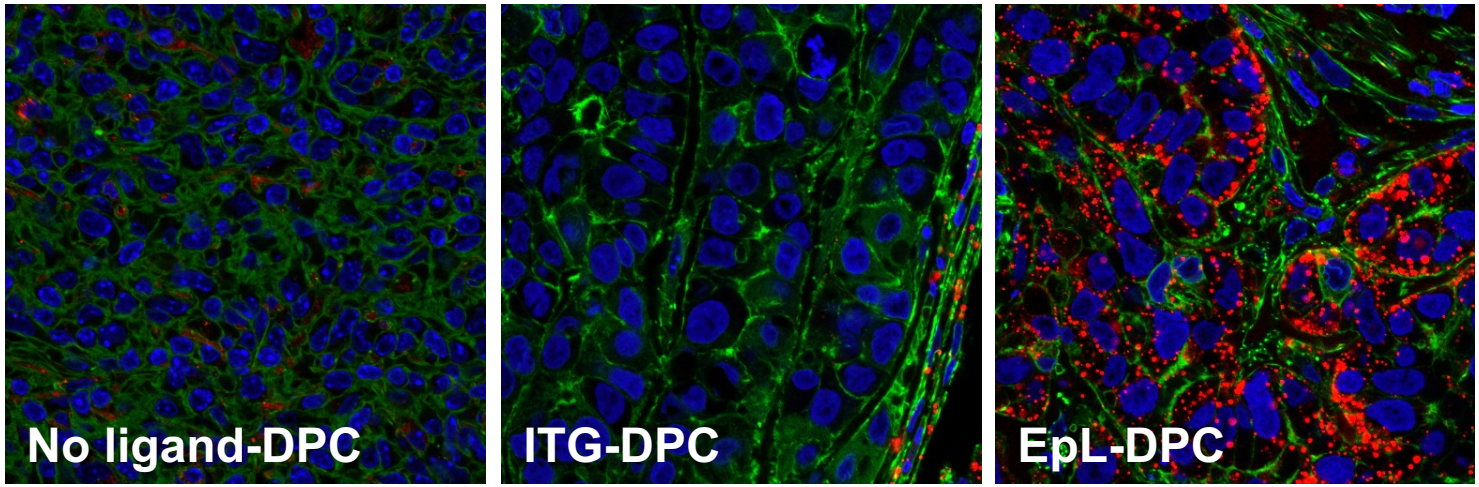
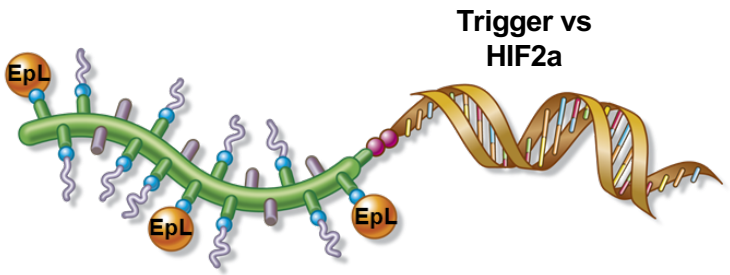
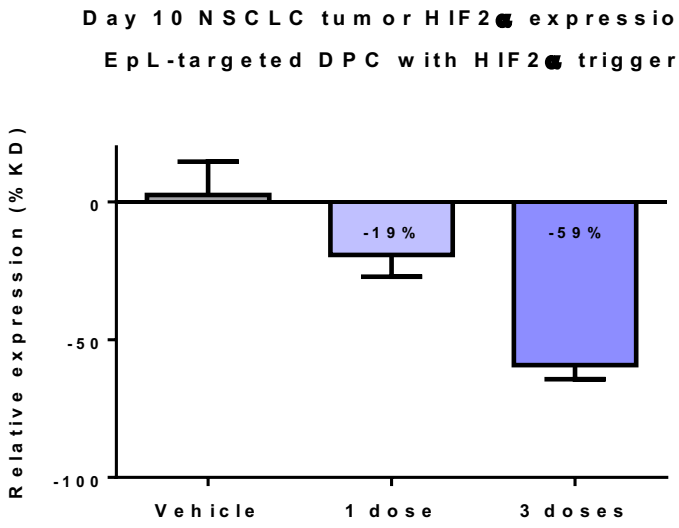
EpL-DPC delivery to subcutaneous NSCLC tumors

Tumor tracking 24h post IV dose







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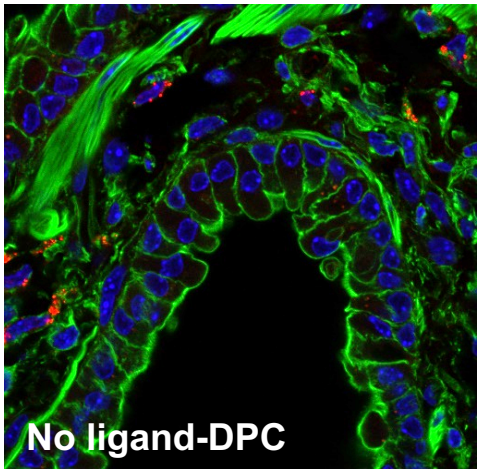
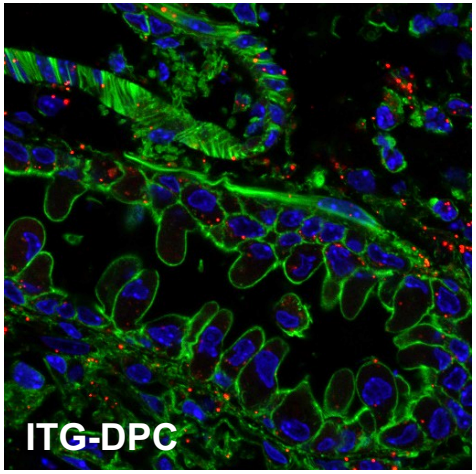
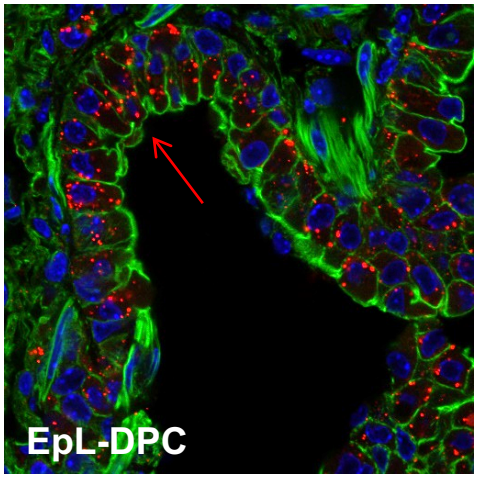
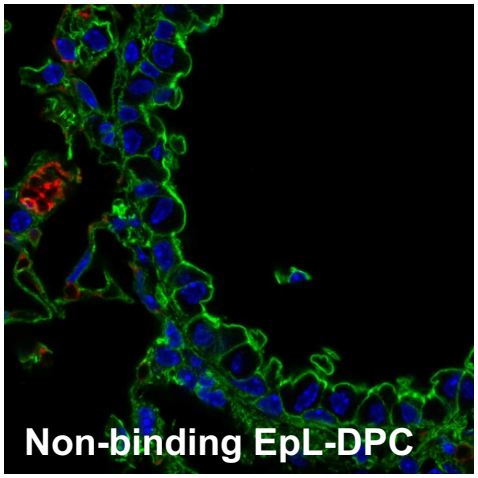
EpL-DPC delivery to pulmonary epithelium

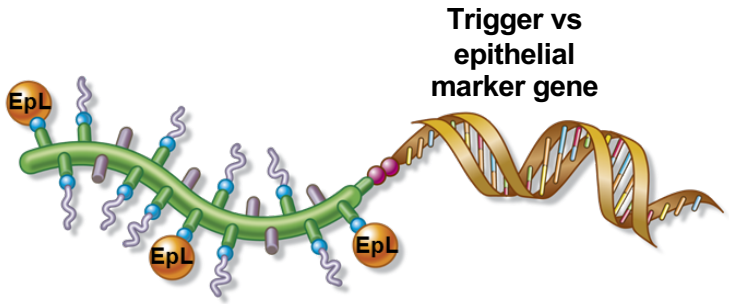
Tissue tracking 24h post IV dose

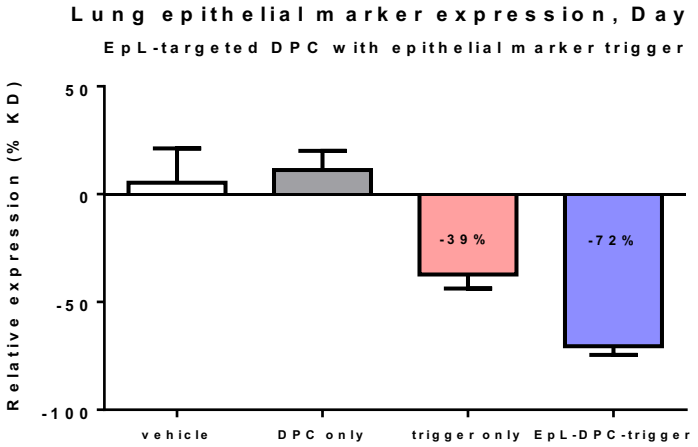












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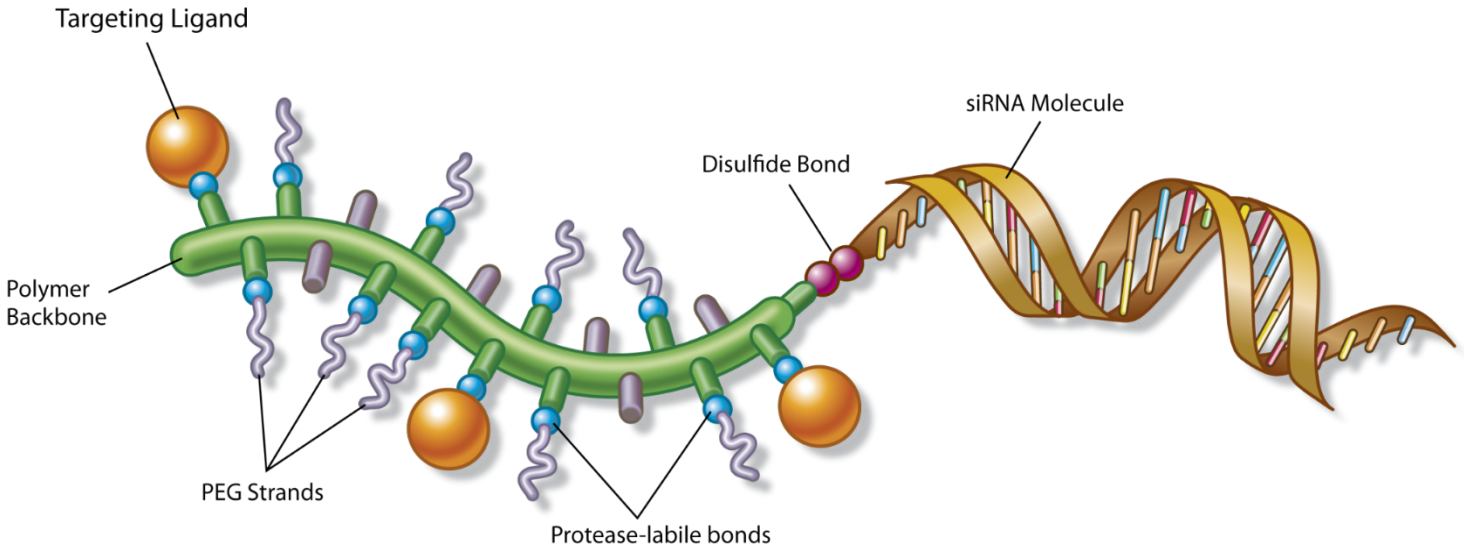
Dynamic Polyconjugate (DPC®) technology

Modular siRNA delivery platform



<u>Reversibly masked polymer</u>

- Manipulate pharmacokinetic properties & endosome escape activity

<u>Targeting ligands</u>

- RGD mimetic for integrin delivery to ccRCC tumors
- EpL for targeted delivery to NSCLC tumors & epithelial tissues

<u>RNAi triggers</u>

- HIF2α for ccRCC
- Therapeutic triggers for NSCLC and epithelial targets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