# Generation of site-specific DARPin® drug conjugates using EGFR as a model system

Abstract 215

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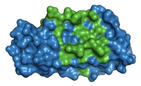
## INTRODUCTION

DARPin® molecules are small engineered proteins, derived from natural ankyrin repeat proteins, that are selected to bind to specific targets with high affinity. Individual DARPin® molecules can be linked together genetically in order to create multi-specific drug molecules. The versatility of DARPin® molecules makes them an attractive alternative to antibodies for the development of drug conjugates. We have developed two DARPin® drug conjugates (DDCs) targeting a known tumor associated antigen, epidermal growth factor receptor (EGFR), as a model system. Two different EGFR DDCs were generated using EGFR-binding DARPin® molecules with different binding affinities. A control DDC using a non-targeting DARPin® molecule was also generated. Each of the multi-DARPin® molecules consisted of four DARPin® modules, including half-life extension domains, and had a total molecular weight of approximately 60kDa. The multi-DARPin® constructs were conjugated to the indolinobenzodiazepine mono-imine DGN549, a potent DNA alkylating payload. DDCs were evaluated for binding and direct cytotoxicity following conjugation. The *in vivo* stability and efficacy of the DDCs were also evaluated. The modularity of DARPin® molecules combined with the potency of the DGN549 payload allows for the production of highly active targeted anti-cancer conjugates.

## Multi-DARPin® constructs utilizing different EGFR binding domains

- EGFR-targeting DARPin® molecules consisting of four mono-DARPin® domains, including serum albumin (SA)-binding DARPin® domains for half-life extension, were generated. A non-targeting DDC was also generated.



Schematic representation of a DARPin® binding domain

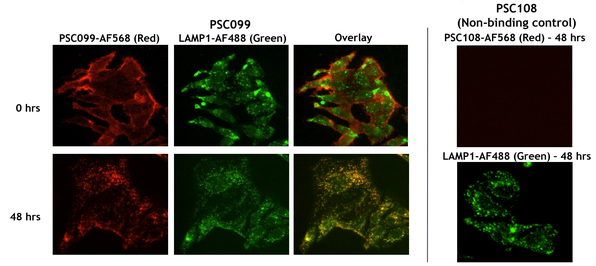
| DARPin® Molecule | | *hEGFR $K_d$ [nM] | *mEGFR $K_d$ [nM] |
|---|---|---|---|
| anti-EGFR DARPin® 1 | PSC099 | 0.02 | 0.4 |
| anti-EGFR DARPin® 2 | PSC106 | 0.08 | 2.3 |
| non-binding (NB) DARPin® | PSC108 | Non-binding | |

*SPR binding of monovalent EGFR molecules

- Two different EGFR targeting DARPins® (red and blue) were evaluated

## EGFR-binding DARPin® molecules internalize and co-localize with the lysosomal marker Lamp1

- EGFR-binding DARPins® bind to EGFR expressing tumor cells, are internalized and delivered to lysosomes
  - DARPin® molecules directly-labelled with AF568 were bound to SKOV3 cells for 1 hr on ice (0 hrs time point) and then incubated at 37°C for 48 hrs. Co-localization with the lysosomal membrane protein, Lamp1, was assessed using an anti-Lamp1 AF488 antibody

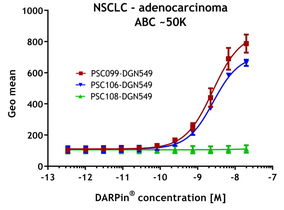



## DARPin® molecules have favorable conjugation properties with the potent DNA alkylator DGN549

- DARPin® molecules were successfully conjugated to the DGN549 payload
- DARPin® DGN549 conjugates bind to EGFR expressing cell lines with high affinity

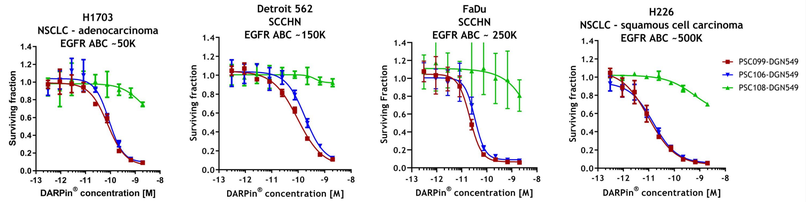
| DDC | DDR | $EC_{50}$ nM | Monomer | Free drug | Yield |
|---|---|---|---|---|---|
| PSC099-DGN549 | 1.5 | 2.5 | >95% | <1% | 75% |
| PSC106-DGN549 | 1.7 | 2.6 | >95% | <1% | 70% |
| PSC108-DGN549 | 2.2 | NA | >95% | <1% | 40% |

DDR: Drug to DARPin® molecule ratio




## DARPin® drug conjugates have potent *in vitro* cytotoxicity against cell lines with a range of EGFR expression

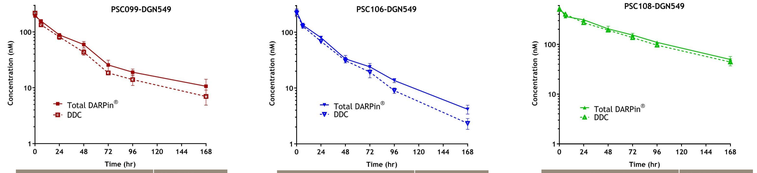
- The *in vitro* potency of the EGFR-targeting DDCs was evaluated in a panel of cell lines expressing EGFR




- The DARPin® DGN549 conjugates displayed pM potency in cell lines ranging in cell surface expression of EGFR from 50K to 500K antibodies bound per cell (ABC)

## DARPin® drug conjugates are stable in circulation

- The pharmacokinetic (PK) profiles of the EGFR-targeting DARPin® drug conjugates were evaluated in CD-1 mice



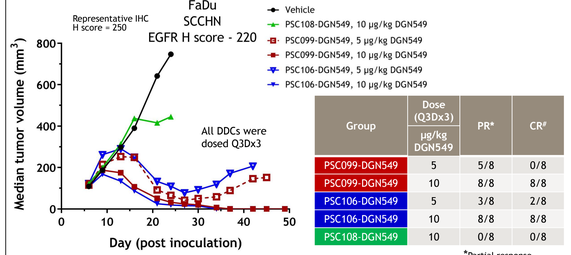

| Molecule | | Half-life (hr) |
|---|---|---|
| PSC099-DGN549 | Total DARPin® | 42 |
| | DDC | 38 |

| Molecule | | Half-life (hr) |
|---|---|---|
| PSC106-DGN549 | Total DARPin® | 33 |
| | DDC | 28 |

| Molecule | | Half-life (hr) |
|---|---|---|
| PSC108-DGN549 | Total DARPin® | 55 |
| | DDC | 52 |

- The PK profiles of intact DDCs and total DARPin® are similar demonstrating that the molecules are stable in circulation
- The non-targeting DDCs showed a terminal half-life of around 50 hrs. Murine cross-reactive EGFR DDCs showed half-lives of 28-38 hrs, indicating reasonable pharmacokinetic behavior in mice.

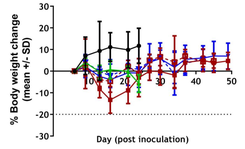
## EGFR DARPin® drug conjugates demonstrate potent and antigen-specific anti-tumor activity *in vivo*




| Group | Dose (Q3Dx3) µg/kg DGN549 | PR* | CR# |
|---|---|---|---|
| PSC099-DGN549 | 5 | 5/8 | 0/8 |
| PSC099-DGN549 | 10 | 8/8 | 8/8 |
| PSC106-DGN549 | 5 | 3/8 | 2/8 |
| PSC106-DGN549 | 10 | 8/8 | 8/8 |
| PSC108-DGN549 | 10 | 0/8 | 0/8 |

*Partial response
#Complete response

The DDCs display dose dependent anti-tumor activity in an EGFR-expressing xenograft model




- The DDCs were well tolerated
- No mice lost >20% body weight and all body weight loss recovered over time

## CONCLUSIONS

- EGFR DARPin® molecules bind to cells with high affinity and are internalized and trafficked to lysosomes
- DARPin® molecules display favorable conjugation properties (yield, % monomer, DDR, free drug) following conjugation to the DNA alkylator DGN549
- EGFR DARPin®-DGN549 conjugates have potent *in vitro* activity in a panel of cell lines expressing a range of EGFR levels
- The DARPin® drug conjugates are stable in circulation and have high anti-tumor activity in an EGFR expressing xenograft model
- DARPin® drug conjugates combine the potency of antibody drug conjugates and the modular DARPin® architecture to create designer therapeutics