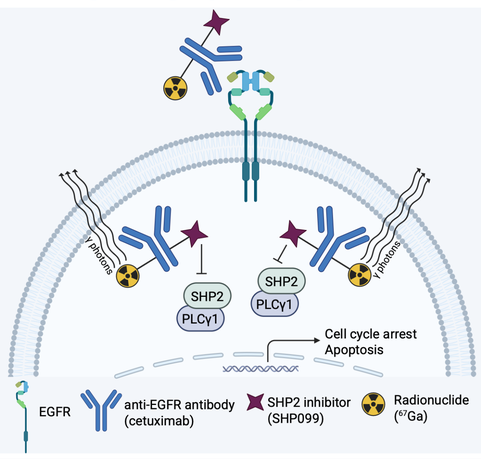
PhD Open Days

Overcoming Cetuximab Resistance in Colorectal Cancer: From PLCγ1–SHP2 Molecular Interactions to Theranostic Strategies

PhD Programme in Biotechnology and Biosciences
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INTRODUCTION



Cetuximab (Ctx) targets the Epidermal Growth Factor Receptor (EGFR) in metastatic colorectal cancer, but resistance limits its efficacy [1,2].

High PLCγ1 levels are linked to Ctx resistance; SHP2 inhibition can re-sensitize resistant cells [3].

Antibody-drug conjugates (ADCs) link a targeting antibody to an anticancer drug for selective delivery.

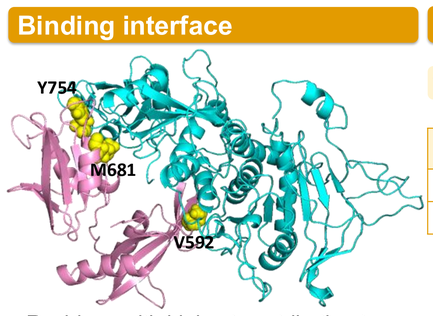
Gallium-67 is a gamma-emitting radionuclide for SPECT imaging (half-life: 3.3 days).

GOAL | Study PLCγ1-SHP2 binding and develop EGFR/SHP2-targeted ADCs for image-guided drug delivery.

1 PLCγ1-SHP2 INTERACTION

PLCγ1 and SHP2 bind directly, without other cell components; Binding only occurs when SHP2 is in its open/active conformation; Interface mutation reduces binding.

Binding interface



Residues with highest contribution to complex's binding free energy:

V592 / M681 / Y754

Effect of alanine mutagenesis

V592A + **M681A** + **Y754A** = **PLCγ1 nSH2-cSH2^{Mut}**

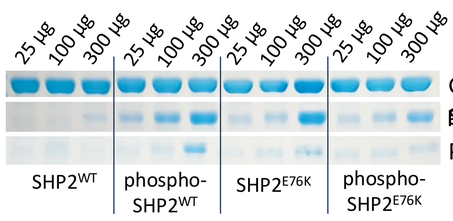
Complex	ΔG (kcal/mol)
SHP2-PLCγ1 nSH2-cSH2^{WT}	-82.4 ± 4.5
SHP2-PLCγ1 nSH2-cSH2^{Mut}	-72.1 ± 14.6

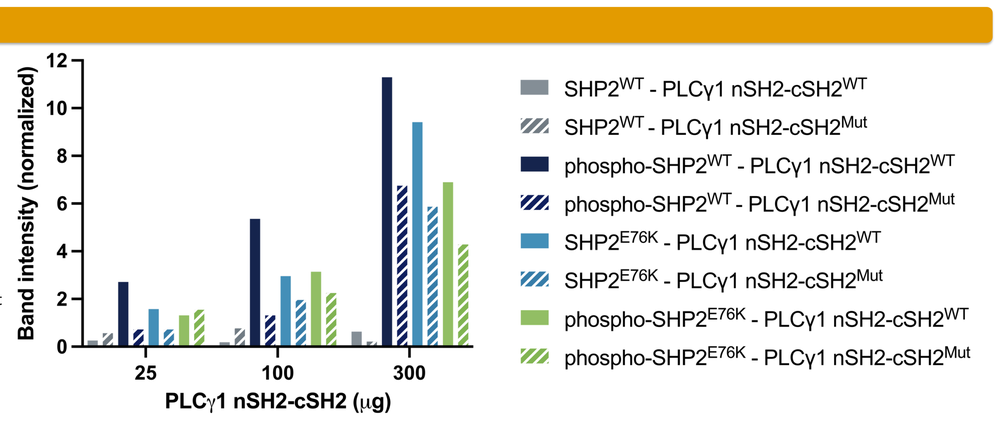
ΔΔG = 10.3 kcal/mol

When the interface residues are mutated, the binding becomes weaker.

Direct binding and the open SHP2 conformation

SHP2^{WT} Tyr
SHP2^{E76K} Tyr-P
phospho-SHP2^{WT} Tyr
phospho-SHP2^{E76K} Tyr-P

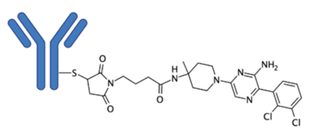




2 EGFR/SHP2-TARGETED ADCs: CELLULAR ACTIVITY

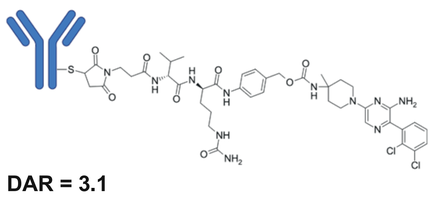
ADCs improve activity in cells expressing high levels of PLCγ1; ADC-2 presents the larger decrease in survival

ADC-1: non-cleavable



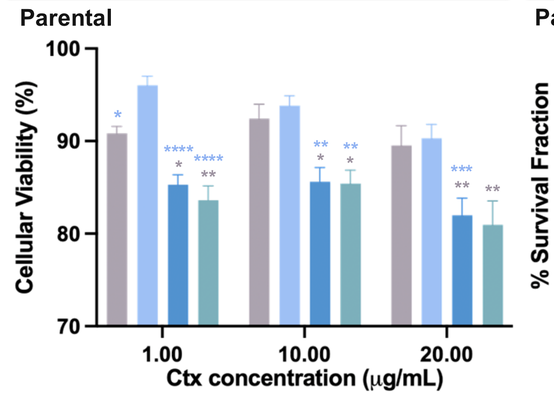
DAR = 3.5

ADC-2: cleavable

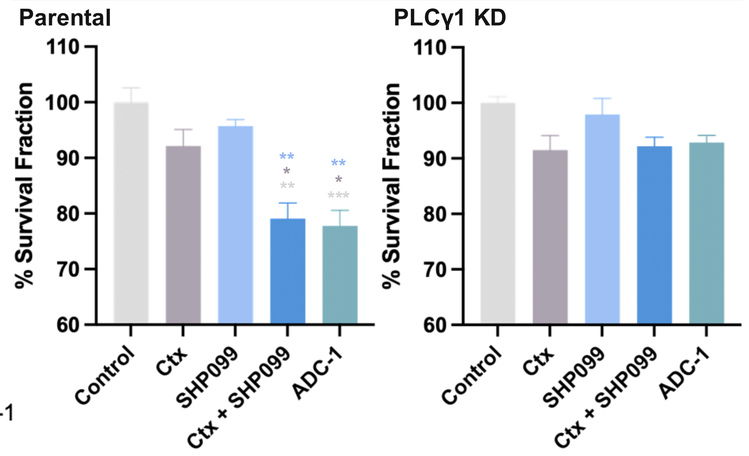


DAR = 3.1

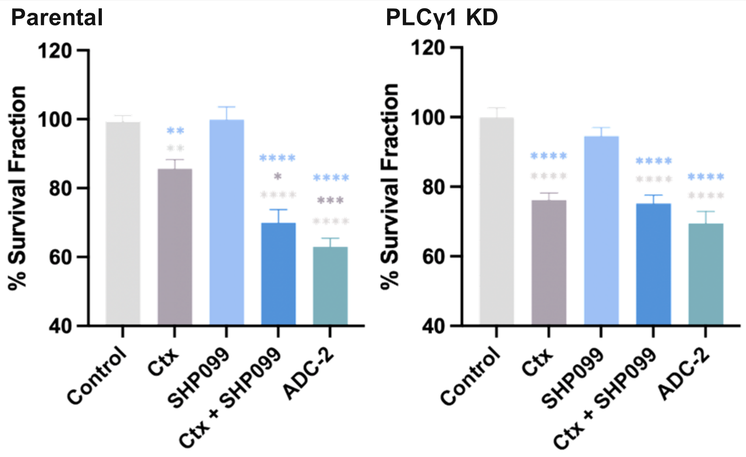
ADC-1: Viability (72 h)



ADC-1: Survival (72 h inc + 12 days growth)



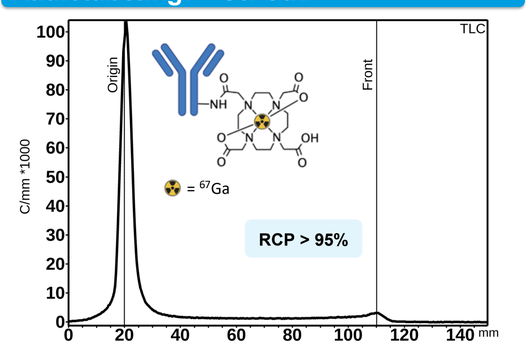
ADC-2: Survival (72 h inc + 12 days growth)



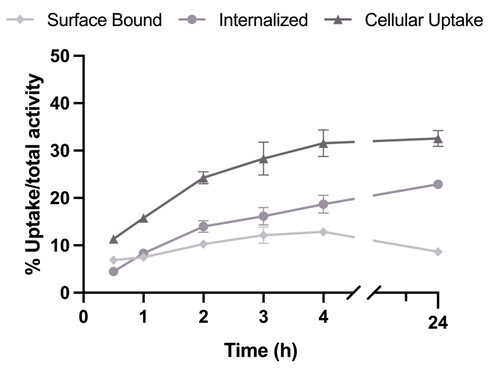
3 ^{67}Ga-ADCs: UPTAKE & EGFR RECOGNITION

Radiochemical purity > 95%; Stable up to 48 h. Uptake, internalization and EGFR recognition are retained.

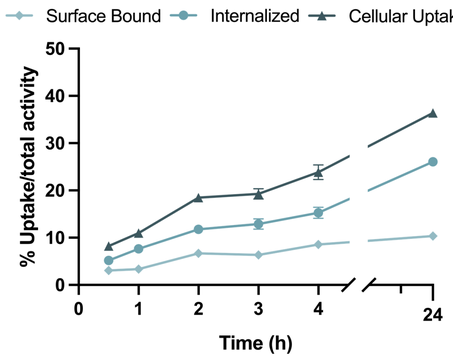
Radiolabeling: ^{67}Ga-Ctx



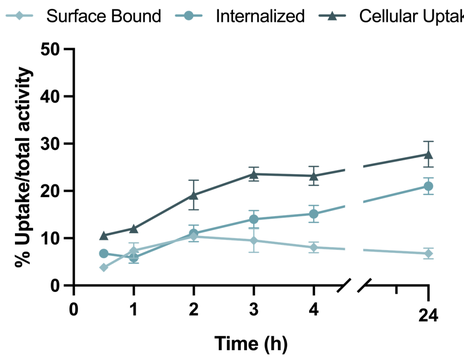
^{67}Ga-Ctx: Uptake



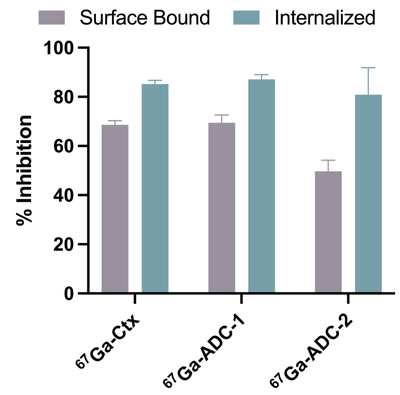
^{67}Ga-ADC-1: Uptake



^{67}Ga-ADC-2: Uptake



EGFR blocking



CONCLUSION

1 PLCγ1 binds to active SHP2 directly

2 EGFR/SHP2 ADCs improve activity in cells with high levels of PLCγ1

3 ^{67}Ga-ADCs retain EGFR recognition and uptake

Future Work: Explore the anti-tumor and cytotoxic effect of the ADCs in vivo, as well as their ability to monitor therapeutic response by molecular imaging.

References: [1] El Guerrab et al., Oncotarget 7 (2016):73618; [2] Zhao et al., Oncotarget 8 (2017):3980; [3] Cruz-Duarte et al., Clin Cancer Res 28 (2022):1203.

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