Click Activated Protodrugs Against Cancer (CAPAC™): A modular platform for tumor-directed oncology therapeutics

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Introduction to the CAPAC™ Platform

The **C**lick **A**ctivated **P**rotodrugs **A**gainst **C**ancer (**CAPAC**) platform is designed to activate powerful cancer therapies at tumor sites while minimizing systemic toxicity. The CAPAC platform utilizes highly efficient reactions (click chemistry) between attenuated drugs (protodrugs) modified with *trans*-cyclooctene (TCO) and a tetrazine-modified tumor-targeting agent (**Figure 1**). This makes the CAPAC platform agnostic to tumor characteristics and interpatient variability. Moreover, the platform is modular and can be applied to a wide variety of therapeutic agents (**Figure 2**) [1].

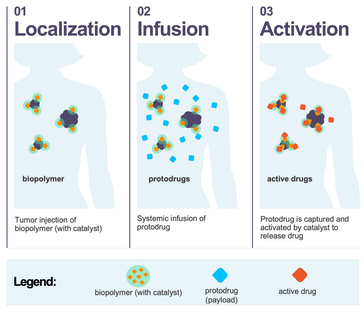


Figure 1. The CAPAC Platform. (**1**) Tumor-targeting agent (e.g. biopolymer) is locally injected at the tumor. (**2**) Protodrug, an attenuated therapy activated by click chemistry, is infused systemically. (**3**) Protodrug is captured by its click chemistry partner through a rapid covalent reaction between tetrazine and TCO moieties, followed by localized release of the active drug.

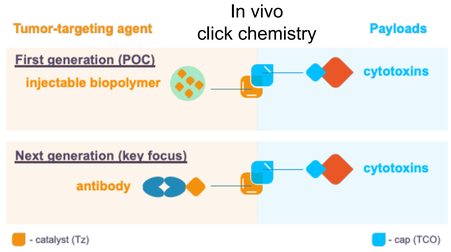


Figure 2. Modularity of the CAPAC platform. The modular design of the CAPAC platform enables the application to multiple drug classes. Targeting agents localize a catalyst (tetrazine, Tz) at the tumor. Upon infusion of payloads bearing the TCO partner, the pairs react. This removes the TCO cap from the payload and releases the active drug, thereby localizing and activating powerful therapies at the tumor.

SQ3370 uses click chemistry to successfully deliver doxorubicin (Dox) to the tumor site to elicit anti-tumor responses (**Figure 3**) [2]. SQ3370 provides enhanced safety and efficacy in preclinical models compared to conventional Dox [1,2] and is currently being tested in the clinic in advanced solid tumors (**Figure 4**).

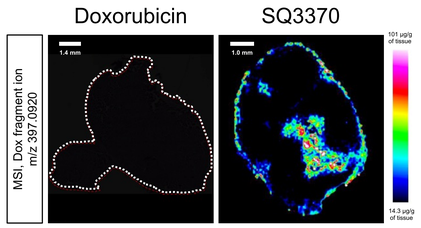
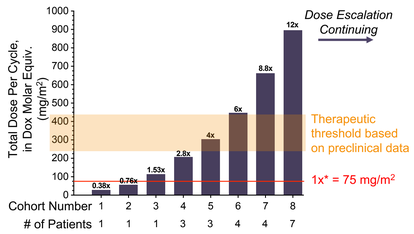


Figure 3. SQ3370 doxorubicin tissue distribution. MALDI-imaging mass spectrometry of SQ3370 and Dox shows activation and distribution in treated tumors. MC38 tumors were treated with Dox (IV, 8.1mg/kg, QDx2) or SQ3370 (SQL70 biopolymer intratumorally, followed by 2 daily IV doses of SQP33 at 3x the molar equivalent dose of Dox). Tumors were harvested 1 hour after the second dose. The CAPAC platform delivers active Dox in the tumor surrounding the advanced necrosis area. In contrast, systemic IV Dox leads to levels below the limit of quantification at the tumor. Limit of detection = 9.1 µg/mg of tissue.



TEAE's in > 25% of patients**				
	12x (N=5)		Total (N=22)	
Preferred Term	All N (%)	Grade≥3 N (%)	All N (%)	Grade≥3 N (%)
Nausea	3 (60.0)	0	13 (59.1)	1 (4.5)
Fatigue	3 (60.0)	1 (20.0)	11 (50.0)	1 (4.5)
Anaemia	1 (20.0)	1 (20.0)	6 (27.3)	3 (13.6)
Constipation	1 (20.0)	0	6 (27.3)	0

Figure 4. SQ3370 Phase 1 shows enhanced exposure and safety at 12x. The clinical proof-of-concept for the CAPAC platform in humans has been achieved. SQ3370 is not a vesicant, and dose-limiting toxicity (DLT) has not been identified to date. Myelosuppression has not been dose limiting. Dose escalation is on-going. *1x equals the molar equivalent dose of 75 mg/m² of conventional Dox. **Treatment emergent adverse events (TEAE) in >25% of patients regardless of causality. Source: SQ3370-001; Data cut: 2022-04-01. ClinicalTrials.gov Identifier: NCT04106492.

Here, we report the expansion of the CAPAC platform to monomethyl auristatin E (MMAE) protodrugs. A **protodrug-activating tetrazine-modified biopolymer (SQL70)** is injected intratumorally, activates a systemically administered **TCO-MMAE protodrug (SQP22)**, and releases active MMAE at the tumor.

Cytotoxicity & Plasma Stability of SQP22

Cell Line	Cytotoxicity IC_{50} (µM)	Fold attenuation
MC38	0.005	704
EMT6	0.003	133
4T1	0.004	>50
B16-F10	0.003	>67
RENCA	0.002	>100

Table 1. TCO-modification of MMAE greatly reduces its cytotoxicity *in vitro*. Cytotoxicity of SQP22 protodrug after activation with tetrazine is shown as IC_{50} across several murine cell lines. Fold attenuation was calculated based on the IC_{50} values in the presence of tetrazine (activated drug) relative to the absence (attenuated protodrug) of tetrazine.

% Protodrug remaining after 4 hours in	
Human Plasma	Mouse Plasma
94%	100%

Table 2. SQP22 protodrug is highly stable in human and mouse plasma. Human and mouse plasma were used to quantify the stability of SQP22 protodrug. Shown are the fractions of the protodrug remaining after 4 hours, quantified by LC-MS.

In Vivo Attenuation of SQP22

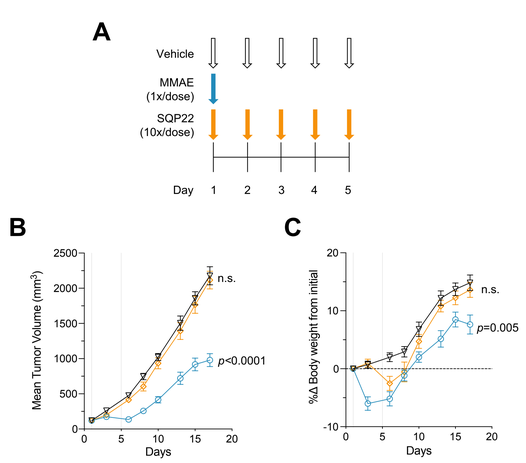


Figure 5. The attenuation of SQP22 protodrug is highly effective as protodrug has no effect in absence of tetrazine catalyst. Female CB17 SCID mice were inoculated with Karpas 299 tumor cells. When tumors reached ~120 mm³, animals were treated with SQP22 protodrug as 5 daily doses in the absence of SQL70 biopolymer or with a single dose of 0.5 mg/kg MMAE (1x) (**A**). No effect on tumor growth (**B**) or body weight (**C**) was observed, even at a cumulative dose of protodrug 50x higher than the molar equivalents of conventional MMAE. This highlights the safety of SQP22 protodrug in absence of a catalyst. Shown are mean ± SEM (*n* = 8 mice/group). *P*-values: Two-Way ANOVA.

References

1. Wu et al., *Chem. Sci.* 2021, DOI: 10.1039/d0sc06099b.
2. Srinivasan et al. *Adv. Therap*. 2021, DOI: 10.1002/adtp.202000243.

In Vivo Anti-Tumor Efficacy and Safety of SQP22 + SQL70

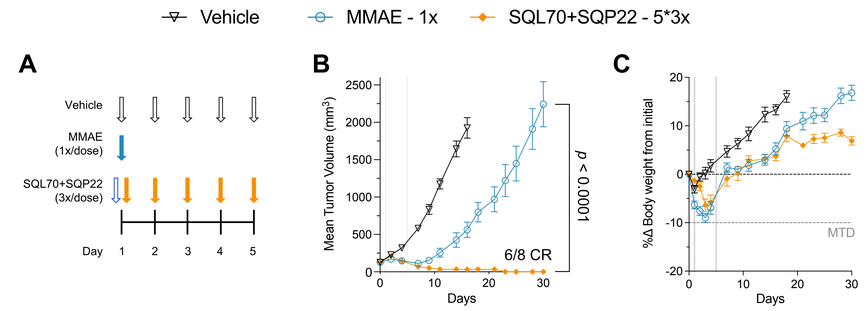


Figure 6. SQP22 with SQL70 biopolymer produces complete responses in 6/8 mice in the Karpas 299 xenograft model, while being well tolerated. Anti-tumor efficacy was tested in female CB17 SCID mice using the Karpas 299 tumor model. Animals bearing tumors ~125 mm³ in size were treated with vehicle, a single dose of 0.5 mg/kg MMAE (1x) or SQL70 biopolymer (intratumorally) followed by 5 daily doses of SQP22 protodrug (**A**). The cumulative dose was 15x the molar equivalents of conventional MMAE, administered as 5 daily doses of 3x. Mean tumor volumes (**B**) and body weight changes (**C**) are shown (*n* = 8 animals/group). Six out of 8 animals in the SQL70+SQP22 group showed complete response (CR). MMAE and SQL70+SQP22 treatments significantly reduced tumor volumes compared to the vehicle control group on day 16 ($p < 0.0001$ for each comparison), but only SQL70+SQP22 led to complete tumor regression during the experiment. Body weight changes were significantly different between vehicle and MMAE ($p = 0.0003$) and vehicle and SQL70+SQP22 ($p < 0.0001$) on day 16, but neither treatment led to body weight loss of >10% (MTD, maximum tolerated dose). Mean ± SEM are shown. *P*-values: Two-Way ANOVA.

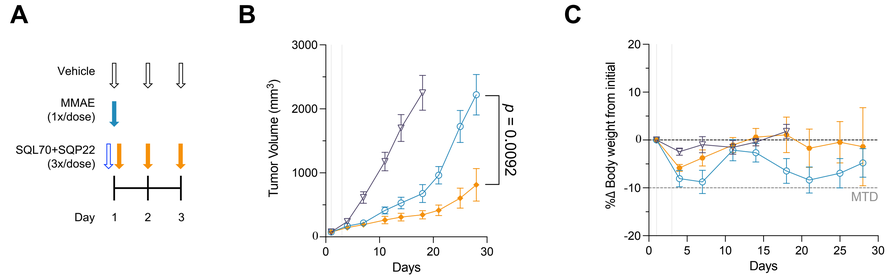


Figure 7. SQP22 protodrug reduces tumor growth in combination with SQL70 biopolymer in the RENCA syngeneic tumor model, even at a reduced dosing frequency. Female C57BL/6 mice were inoculated with RENCA tumor cells. Once tumors reached ~80 mm³, mice were treated with vehicle, a single dose of 1 mg/kg MMAE (1x) or SQL70 biopolymer (intratumorally) followed by SQP22 protodrug for 3 daily doses at a cumulative dose of 9x the molar equivalents of conventional MMAE (**A**). Mean tumor volumes (**B**) and body weight changes (**C**) are shown (*n* = 5 animals/group). MMAE and SQL70+SQP22 treatments significantly reduced tumor volumes compared to the vehicle control group on day 18 ($p = 0.0021$ and 0.0016, respectively). Moreover, SQL70+SQP22 tumors remained significantly smaller compared to MMAE-treated tumors on day 28 ($p = 0.0092$). Body weight loss of SQL70+SQP22 did not drop below the MTD, suggesting limited treatment-induced toxicity. *P*-values: Two-Way ANOVA.

Summary and Conclusion

- The CAPAC platform expands the pharmacological capabilities of cancer drugs through a two-component strategy based on a TCO-modified protodrug and a tetrazine-modified targeting agent (e.g., SQL70 biopolymer).
- Drug release depends on *in vivo* click chemistry, leading to the targeted delivery of therapies such as MMAE (e.g., SQP22 protodrug) to the tumor.
- SQP22 protodrug shows good plasma stability and attenuation of cytotoxicity, both *in vitro* and *in vivo.*
- SQP22 protodrug plus SQL70 biopolymer eliminates tumors in multiple models.
- The CAPAC platform enables the targeting of unique synthetic- or neo-antigens to widen the therapeutic window of potent chemotherapies.