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. CAPAC utilizes highly efficient reactions (click chemistry) between attenuated drugs (protodrugs) modified with a *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 [1]. SQ3370, the lead candidate, successfully delivers Doxorubicin (Dox) to the tumor site to elicit anti-tumor responses [2]. SQ3370 consists of a tetrazine-modified sodium hyaluronate biopolymer (SQL70) that is injected intratumorally and captures systemically administered SQP33, a TCO-modified protodrug of Dox, leading to site-specific release of active Dox at the tumor. SQ3370 provides enhanced safety and efficacy in preclinical models compared to conventional Dox [1,2] and is currently in a Phase I study in advanced solid tumors (NCT04106492). Here, we report the expansion of the CAPAC platform to monomethyl auristatin E (MMAE)-based protodrugs in combination with the SQL70 biopolymer.

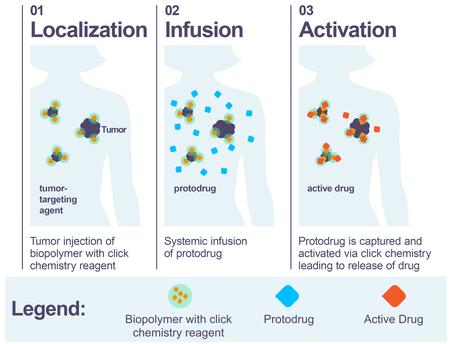

Figure 1. The CAPAC Platform. (**1**) Biopolymer click reagent (e.g. SQL70 biopolymer) is locally injected at the tumor area. (**2**) Protodrug, an attenuated therapy activated by click chemistry, is infused systemically. (**3**) Protodrug is captured by its click chemistry partner through a rapid covalent reaction between tetrazine and TCO moieties, followed by localized release of the active drug.

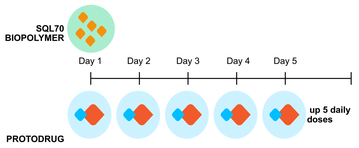

Figure 2. Treatment schematic. SQL70 biopolymer is injected on Day 1 into a single lesion. The protodrug is infused once daily, starting ~1 hour after SQL70 biopolymer injection. The protodrug is given either as 3 or 5 daily doses.

In Vitro Cytotoxicity & Plasma Stability of TCO-MMAE Protodrug

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 SQRD-015 protodrug after activation with tetrazine is shown as IC_{50} across several murine cell lines. Fold attenuation was calculated as based on the IC_{50} values in presence of tetrazine (activated drug) relative to absence (attenuated protodrug) of tetrazine.

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

Table 2. SQRD-015 protodrug is highly stable in human and mouse plasma. Human and murine plasma were used to quantify stability of SQRD-015 protodrug. Shown are the fractions of the protodrug remaining after 4 hours as quantified by LC-MS.

In Vivo Safety of TCO-MMAE Protodrug

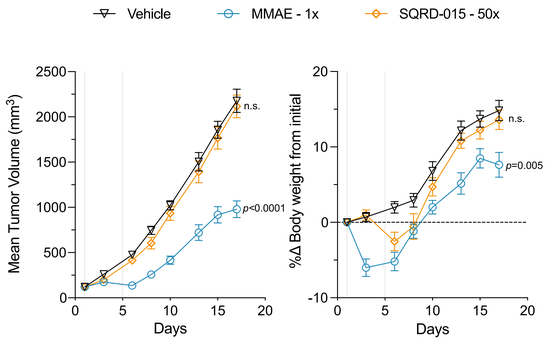

Figure 3. Attenuation of SQRD-015 protodrug is highly effective. Female CB17 SCID mice were inoculated with Karpas 299 tumor cells and tumors were allowed to reach ~120 mm^3. Animals were then treated with SQRD-015 protodrug as 5 daily doses in the absence of SQL70 biopolymer or with a single dose of 0.5 mg/kg MMAE (1x). No effect on tumor growth (*left*) or body weight (*right*) was observed, even at a cumulative dose of protodrug 50x higher than the molar equivalents of conventional MMAE. Shown are mean ± SEM (*n* = 8 animals/group). *P*-values were determined by Two-Way ANOVA.

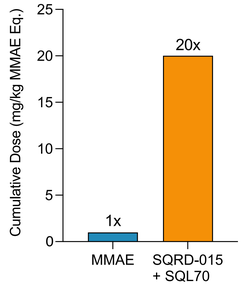

Figure 4. The Maximum Tolerated Dose (MTD) of SQRD-015 protodrug is 20x higher than conventional MMAE. Naive C57BL/6 mice were treated with SQRD-015 protodrug as 5 daily doses after an injection of the SQL70 biopolymer or with a single dose of 1 mg/kg MMAE (1x). Cumulative Dose represents sum of all daily administered doses. Body weight loss of 10% was set as the threshold for MTD (*n* = 5 animals/group).

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 of TCO-MMAE Protodrug

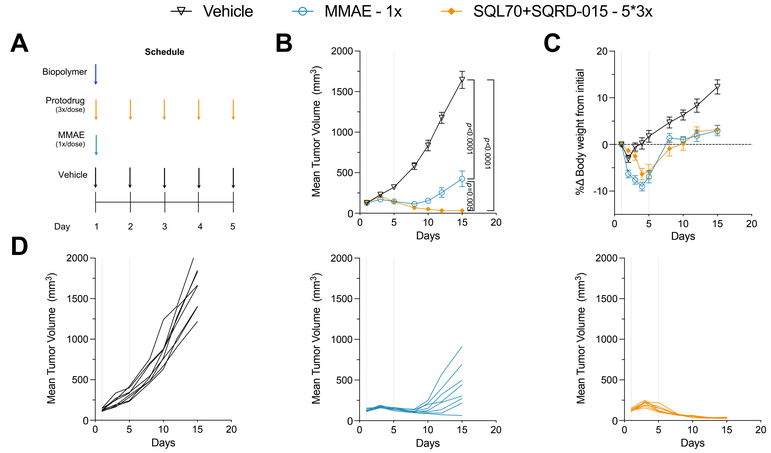

Figure 5. SQRD-015 protodrug reduces tumor growth in combination with SQL70 biopolymer in the Karpas 299 xenograft model. (**A-D**) Anti-tumor efficacy was tested in female CB17 SCID mice using the Karpas 299 tumor model. Animals bearing tumors ~125 mm^3 in size were treated with vehicle, a single dose of 0.5 mg/kg MMAE (1x) or SQL70 biopolymer (intratumorally) followed by 5 daily doses of SQRD-015 protodrug (**A**). Mean tumor volumes (**B**), body weight changes (**C**), and individual tumor volumes (**D**) are shown (*n* = 8 animals/group). The cumulative dose was 15-times the molar equivalents of conventional MMAE, administered as 5 daily doses of 3x. Mean ± SEM. *P*-values were determined by Two-Way ANOVA.

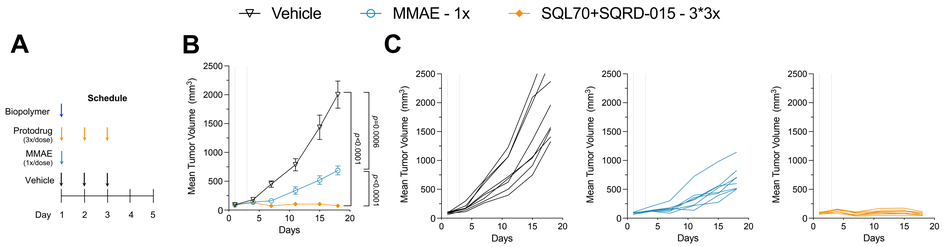

Figure 6. SQRD-015 protodrug reduces tumor growth in combination with SQL70 biopolymer in the RENCA syngeneic tumor model, even at a reduced dosing frequency. Female C57BL/6 mice were inoculated with RENCA tumor cells. Once tumors reached ~100 mm^3, animals were treated with vehicle, a single dose of 1 mg/kg MMAE (1x) or SQL70 biopolymer (intratumorally) followed by SQRD-015 protodrug for 3 daily doses at a cumulative dose of 9x the molar equivalents of conventional MMAE (**A**). Mean tumor volumes per group ± SEM (**B**) and individual tumor volumes within each group (**C**) are shown (*n* = 8 animals/group). *P*-values were determined by 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 molecule (e.g. SQL70 biopolymer)
- The CAPAC platform is modular and can deliver therapies such as Dox (SQP33) or MMAE (SQRD-015) specifically to tumor sites
- SQRD-015 protodrug shows good plasma stability and attenuation of cytotoxicity *in vitro* and *in vivo*
- SQRD-015 protodrug treatment following SQL70 biopolymer injection significantly inhibits tumor growth in syngeneic and xenograft models and significantly outperforms MMAE at MTD