CAPAC: a modular platform that can improve the safety and efficacy of cancer therapies

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Introduction

- Shasqi is a clinical stage biotech whose mission is to change cancer treatment with click chemistry, a Nobel prize winning technology. **We are the only company using click chemistry in humans.**
- Our aim is to safely activate highly effective treatments at tumors using our **C**lick **A**ctivated **P**rotodrugs **A**gainst **C**ancer (**CAPAC**) platform (**Figure 1**).
- Drug activation relies solely on chemistry and not biology, hence CAPAC is agnostic to tumor characteristics and interpatient variability. Moreover, the platform is modular and can be applied to a wide variety of therapeutic agents with diverse mechanisms of action.
- We have demonstrated clinical proof of concept with SQ3370[1,2], which uses an intratumorally injected biopolymer (SQL70) paired with an anthracycline-based therapy (SQP33) injected systemically (**Figure 2**). In our SQ3370-001 Phase 1 trial (NCT04106492) we were able to administer 15 times (15x) the chemotherapy dose of doxorubicin and no dose limiting toxicity was identified.

Click Chemistry localizes active drug at tumors[1,2]

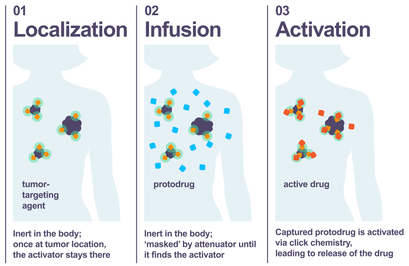


Fig 1. Local tumor targeting using Click Chemistry. An inert, systemically administered protodrug is captured and activated at the tumor by the targeting agent via a covalent click chemistry reaction, followed by chemical rearrangement to release active drug. The targeting agent presented here is a tetrazine (Tz)-modified sodium hyaluronate biopolymer (SQL70) along with several protodrugs.

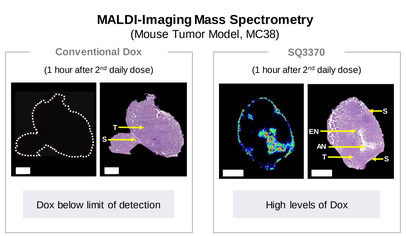
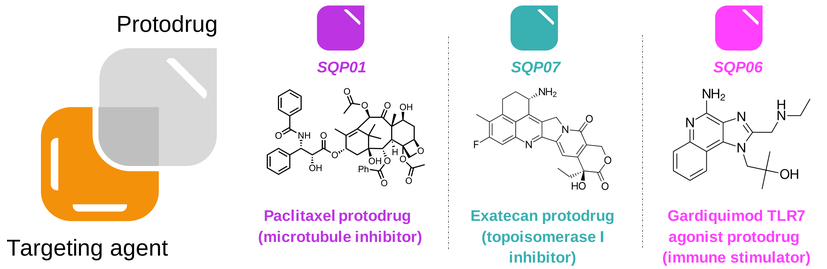


Fig 2. Click Chemistry enables previously unachievable levels of drug exposure leading to necrosis at the tumor. MALDI imaging of tumors 1 hour after conventional Dox (IV, QDx2, 8.1 mg/kg/dose) or SQ3370 (SQL70 biopolymer IT + SQP33 protodrug IV, QDx2, 78.6 mg/kg/dose Dox molar equiv). SQ3370 leads to high Dox levels that correlate with advanced necrosis (AN), while conventional Dox dosing led to levels below the limit of detection at the tumor. EN = early-stage necrosis; S = stroma; T = tumor. Scale bars: 2 mm.

Modular CAPAC Platform Facilitates Activation of Protodrugs With Different Mechanisms of Action (MOA) Using a Single Tumor Targeting Agent



We developed three novel *trans*-cyclooctene (TCO)-modified protodrugs, each with a distinct MOA. Each can be activated by the same tumor targeting agent, creating the ability to use multiple drugs either in combination or sequentially to inhibit tumor growth in different ways.

References

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

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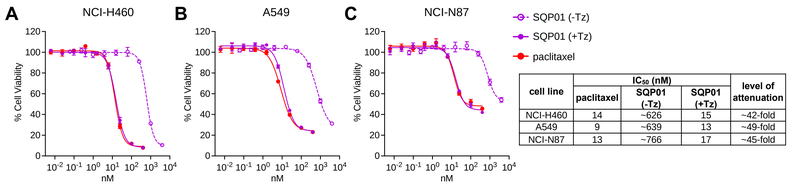
Expanding the Arsenal: Development of Next Generation Click Chemistry Protodrug Classes with Different MOAs

A Paclitaxel Protodrug with Biopolymer Shows Significant Anti-Tumor Efficacy Compared to Paclitaxel in NCI-N87 Tumors



cell line	IC50 (nM) paclitaxel	SQP01 (-Tz)	SQP01 (+Tz)	level of attenuation
NCI-H460	14	~626	15	~42-fold
A549	9	~639	13	~49-fold
NCI-N87	13	~766	17	~45-fold

Fig 3. In vitro cytoxicity activity of paclitaxel protodrug SQP01. Cytotoxicity of paclitaxel and SQP01 as TCO-modified protodrug (-Tz) and click-activated payload (+Tz) was measured across 3 human cancer cell lines: **(A)** NCI-H460 and **(B)** A549 lung carcinoma and **(C)** NCI-N87 gastric carcinoma cell line. Cells were treated with compounds for 3 days and cell viability measured. Results are plotted as mean ± standard deviation (*n*=3) with half-maximal inhibitory concentrations (IC_{50}) reported in the table. SQP01 cytotoxicity is 42- to 49-fold attenuated compared to the Tz-reacted payload based on relative IC_{50} values. Cytotoxic potency of activated SQP01 is similar to paclitaxel.

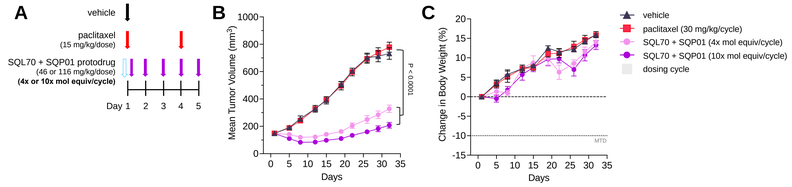
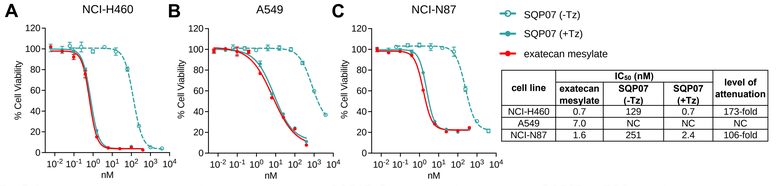


Fig 4. SQP01 causes significant regression of NCI-N87 tumors in the presence of SQL70 biopolymer without body weight loss. A, Schedule of dosing of agents. SQP01 was dosed 1 hour after SQL70 injection *(blue arrow)* at 46 or 116 mg/kg/dose for a total of 5 doses/cycle (equivalent to 4x or 10x paclitaxel molar equivalent dosing, respectively). **B-C,** Tumor volumes of NCI-N87 xenografts **(B)** and body weight changes in Balb/c nude mice **(C)** are shown as mean ± SEM (*n* = 6 mice/group). *P*-values were determined by two-way ANOVA (analysis of variance). Mol equiv, molar equivalent; MTD, maximum tolerated dose; SEM, standard error of mean.

Single-Dose of an Exatecan Protodrug with Biopolymer Causes Complete Tumor Regression of NCI-N87 Tumors



cell line	IC50 (nM) exatecan mesylate	SQP07 (-Tz)	SQP07 (+Tz)	level of attenuation
NCI-H460	0.7	129	0.7	173-fold
A549	7.0	NC	NC	NC
NCI-N87	1.6	251	2.4	106-fold

Fig 5. In vitro cytoxicity activity of exatecan protodrug SQP07. Cytotoxicity of exatecan and SQP07 as TCO-modified protodrug (-Tz) and click-activated payload (+Tz) was measured across 3 human cancer cell lines: **(A)** NCI-H460, **(B)** A549, and **(C)** NCI-N87. Cells were treated with compounds for 3 days and cell viability measured. Results are plotted as mean ± standard deviation (*n*=3) with reported IC_{50} values in the table. SQP07 cytotoxicity is 173- and 106-fold attenuated compared to the Tz-reacted payload based on relative IC_{50} values in NCI-H460 and NCI-N87 cells, respectively. Cytotoxic potency of activated SQP07 is similar to exatecan. NC, not calculable.

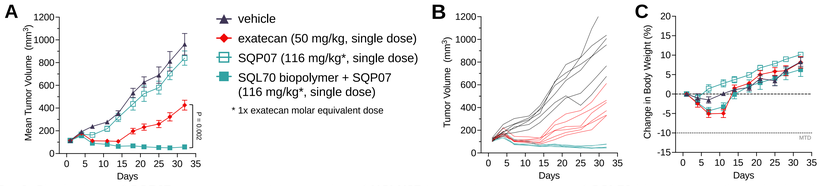
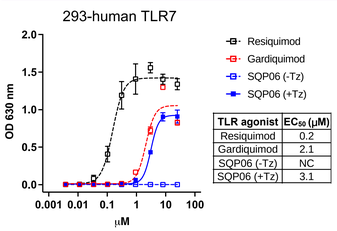


Fig 6. Single dose of SQP07 causes complete regression of NCI-N87 tumors in the presence of SQL70 biopolymer with minimal and transient body weight loss. A, Average tumor growth curves of NCI-N87 xenografts in Balb/c nude mice. Single dose of SQP07 at 116 mg/kg (molar equivalence to 50 mg/kg MTD of exatecan) was given 1 hour after SQL70 injection on day 1. Data shown are mean ± SEM (*n* = 6 mice/group). **B,** Individual tumor growth curves shown in **(A)** for the vehicle, exatecan, and SQP07 protodrug with SQL70 groups. **C,** Percent body weight changes. *P*-values were determined by two-way ANOVA (analysis of variance). MTD, maximum tolerated dose; SEM, standard error of mean.

An Immune-Stimulatory TLR7 Agonist Protodrug Mediates Immune Stimulation Requiring Tz-Mediated Activation



TLR agonist	EC50 (μM)
Resiquimod	0.2
Gardiquimod	2.1
SQP06 (-Tz)	NC
SQP06 (+Tz)	3.1

Fig 7. TLR7 agonist gardiquimod protodrug, SQP06, induces NF-κB signaling in HEK-hTLR7 cells only in its activated form. A, HEK cells stably expressing human TLR7 with a NF-κB-driven secreted embryonic alkaline phosphatase (SEAP) reporter gene were treated with gardiquimod, resiquimod (TLR7/8 agonist), and SQP06 as TCO-modified protodrug (-Tz) and click-activated payload (+Tz) for 18 hours and SEAP activity measured. Data are shown as mean ± standard deviation (*n*=3) with EC_{50} values. NC, not calculable.

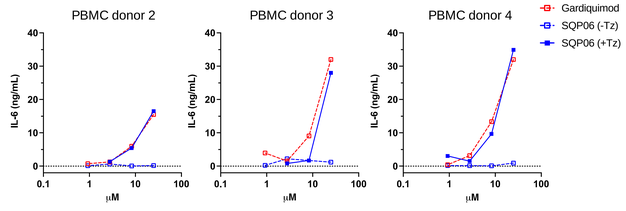


Fig 8. Pro-inflammatory IL-6 secretion in human peripheral blood mononuclear cells (PBMC) cultures is significantly attenuated even at high concentrations of SQP06 until its conversion into the active payload by Tz-mediated click reaction. Human PBMC cultures from *n*=3 donors were treated with 0.9 to 25 μM gardiquimod, SQP06 as protodug (-Tz) or active payload (+Tz) for 18 hours. Cell culture supernatants were collected to measure IL-6 levels. Data shown are averages of pooled samples (*n*=3).

Conclusions

- We developed three new protodrugs with different therapeutic MOAs. We generated protodrugs with significantly attenuated activity both *in vitro* and *in vivo* in the absence of the Tz activator. However, full functional activity is unmasked in the presence of free Tz *in vitro* or a Tz-modified tumor targeting agent *in vivo*. Here, we describe:
  - ➢ SQP01, a paclitaxel protodrug, with ~40-fold reduced cytotoxicity across different cell lines in vitro. SQP01 in the presence of SQL70 biopolymer caused significant tumor growth inhibition compared to paclitaxel with no body weight loss even at 10-fold molar equivalent paclitaxel dose.
  - ➢ SQP07, an exatecan protodrug, is greatly attenuated and in its activated form is potently cytotoxic across 3 different cell lines. A single dose of SQP07 at 116 mg/kg in the presence of SQL70 is sufficient to cause complete regression of NCI-N87 tumors for up to at least 30 days with minimal body weight loss.
  - ➢ SQP06, an immunostimulatory TLR7 agonist protodrug with a non-cytotoxic payload. SQP06 shows greatly attenuated immune activity *in vitro* and upon Tz-mediated activation induces strong cytokine secretion in PBMC cultures.
- All 3 protodrugs are currently in preclinical development with either a biopolymer or systemic tumor targeting agents, such as small antibody fragments (Fabs) and peptides. Other protodrugs with different MOAs are being pursued.
- For information about immune changes in clinical samples after SQ3370 treatment, visit poster #3261; for Shasqi's MMAE protodrug activated by biopolymer or an antigen targeting agent, visit poster #1540.