Treatment with SQ3370, a doxorubicin-based click chemistry therapeutic, promotes immune activation in the tumor microenvironment

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SQ3370 - First Therapeutic Using Click Chemistry in Humans

SQ3370 localizes active Dox at the tumor using *in vivo* Click Chemtry[1,2]

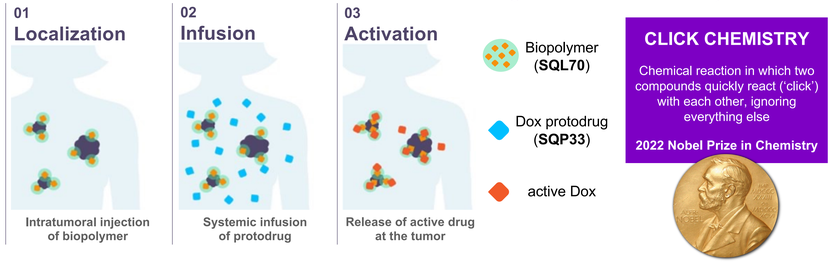


Fig 1. Local tumor targeting using Click Chemistry. An inert, systemically administered protodrug (SQP33) is captured and activated by the biopolymer (SQL70) at the tumor via a covalent click chemistry reaction, which is followed by a chemical rearrangement to release active Dox.

SQ3370 delivers unprecedented levels of active Dox to the tumor[3]

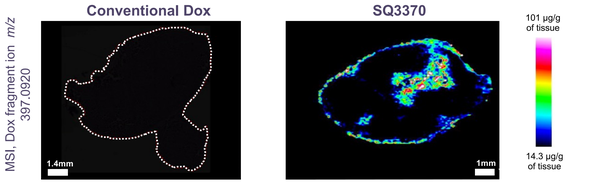
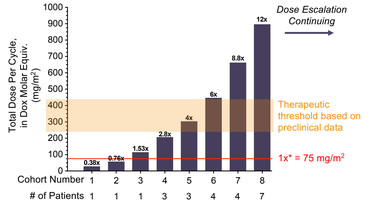


Fig 2. SQ3370 enables previously unachievable levels of Dox exposure at the tumor that can unlock new biological effects[3]. MC38 tumor-bearing mice were treated with conventional Dox (IV, QDx2, 8.1mg/kg/dose) or SQ3370 [SQL70 intratumorally, SQP33 (IV, QDx2, 78.6 mg/kg/dose Dox molar eq)]. MALDI imaging of SQ3370-treated tumors (*right*) at 1h after last dose detected high Dox levels that correlated with advanced necrosis (*not shown*), while that of conventional Dox-treated tumors (*left*) did not.

SQ3370 is the first-in-human Click Chemistry therapeutic



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

Fig 3. 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, 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 examine immune-associated changes in the tumor microenvironment after SQ3370 treatment using patient biopsies and syngeneic tumor models.

SQ3370 Activates Anti-Tumor Immune Responses in Human Tumors

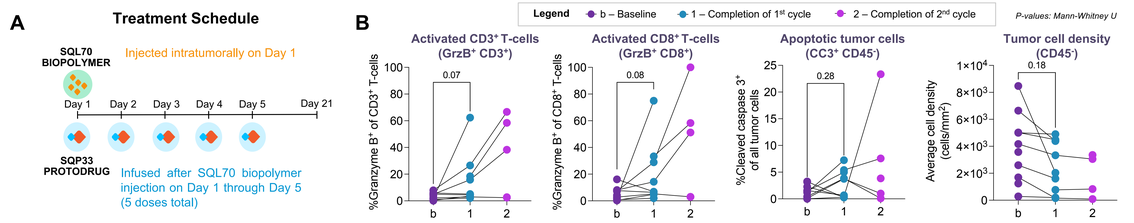
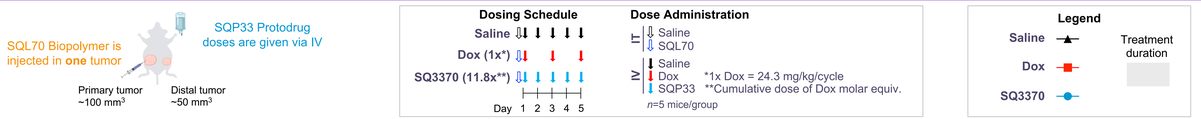
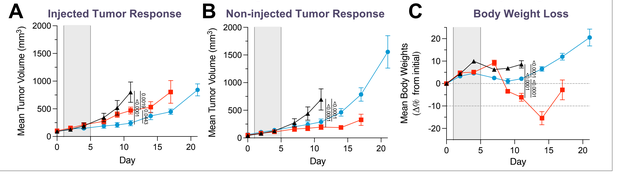


Fig 4. Assessment of anti-tumor immune cell activity and apoptosis in patient biopsies. Tumor biopsies were collected from injected lesions during the Phase 1 clinical trial (**A**) at baseline and after each of the initial two cycles. Tumors from patients [cohort 4x (N=3), 6x (N=3), 8.8x (N=4), and 12x (N=2)] were analyzed by multiplex IHC (**B**). Increased cytotoxic activity of CD3⁺ and CD8⁺ T-cells was assessed by granzyme B (GrzB) expression. GrzB released from T-cells induces apoptotic death of target cells by caspase-dependent mechanisms. Tumor cell apoptosis was confirmed by cleaved caspase 3 (CC3) staining. Tumor cells were defined as CD45⁻ due to lack of cytokeratin expression in many sarcoma biopsies. Tumor cell density decreased after SQ3370, suggesting treatment efficacy in tumors of this highly pre-treated, advanced stage patient population. 1x= 75 mg/m² of conventional Dox.

SQ3370 Activates Anti-Tumor Immune Responses in Animal Tumors



SQ3370 reduces MC38 tumor growth with minimal effects on body weight



SQ3370 reduces B16-F10 tumor growth with minimal effects on body weight

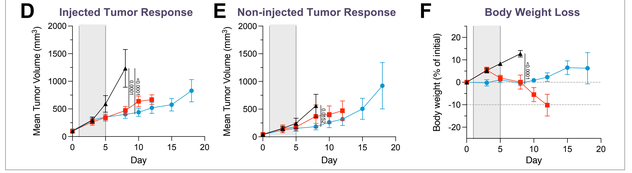


Fig 5. SQ3370 induces an anti-tumor response in primary injected and distal non-injected syngeneic tumors. Mice inoculated at both flanks with either MC38 (**A-C**) or B16-F10 (**D-F**) cells received intratumoral (IT) injections of saline or SQL70 biopolymer into the large tumor (~100 mm³), while the small tumor (~50 mm³) remained non-injected. Saline, Dox or SQP33 were administered intravenously (IV). Significant tumor growth inhibition was observed in injected (**A, D**) and non-injected (**B, E**) tumors in both syngeneic models, suggesting local and systemic SQ3370-mediated anti-tumor activity. Body weight loss (**C, F**) was most severe after Dox treatment (>10% loss from initial), with SQ3370 showing improved safety over Dox. Shown are mean ± SEM (*n* = 5 mice/group). *P*-values: Two-Way ANOVA with Tukey method for multiple comparisons.

SQ3370 induces T-cell infiltration into MC38 tumors

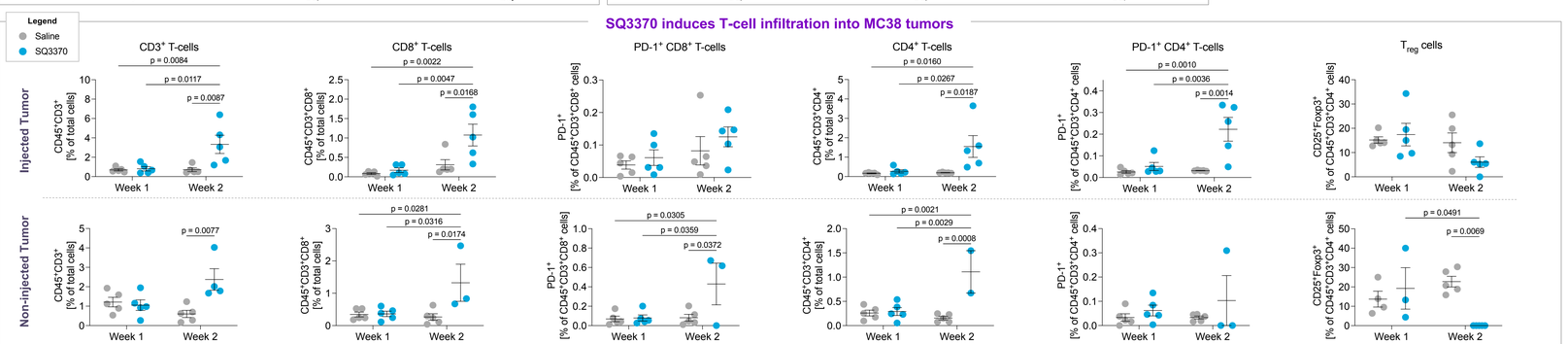


Fig 6. SQ3370 induces distinct T-cell responses in primary and distal syngeneic tumors. Injected and non-injected MC38 tumors (described in Fig. 3) were harvested one or two weeks after treatment completion and analyzed by polychromatic flow cytometry (CD45, CD3, CD4, CD8, PD-1, CD25, Foxp3). SQ3370 significantly increased T-cell infiltration (CD3⁺, CD8⁺, CD4⁺ subsets) after two weeks in injected and non-injected tumors compared to saline and week 1 samples. PD-1 showed distinct expression after two weeks in injected (increased PD1⁺CD4⁺ T-cells) and non-injected (increased PD-1⁺CD8⁺ T-cells) tumors. T regulatory (T_{reg}) cells significantly decreased after two weeks in non-injected tumors compared to saline. Shown are mean ± SEM (*n*=2-5/group) as a percentage of total or marker-gated (CD4⁺ or CD8⁺) cells. *P*-values: Two-Way ANOVA with Tukey method for multiple comparisons.

Conclusions

- SQ3370 is the only click chemistry therapeutic in humans, delivering doxorubicin specifically to tumors.
- SQ3370 reduces growth of primary injected and distal non-injected syngeneic tumors, suggesting that systemic SQ3370-mediated anti-tumor immune activation contributes to its efficacy.
- T-cell infiltration is significantly increased in primary and distal syngeneic tumors two weeks after SQ3370 dosing completion.
- Tumor-infiltrating T-cells express PD-1 two weeks after SQ3370 treatment, suggesting that antigen-specific, potentially tumor-reactive T-cells infiltrate the tumor microenvironment.
- Increased cytotoxic T-cell activity after SQ3370 was confirmed in patient biopsies and indicates an increase in tumor cell death.
- SQ3370 can promote a shift from an immune suppressive towards a T-cell permissive / supportive tumor immune microenvironment in clinical and nonclinical tumors.

References

1. Wu et al., *Chem. Sci.* 2021, DOI: 10.1039/d0sc06099b.
2. Srinivasan et al., *Adv. Therap.* 2021, DOI: 10.1002/adtp.202000243.
3. McFarland et al., World ADC 2022, Poster #21.

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