Nonclinical Profile of the First in Human Anti-CD38 ADC STI-6129 for Treatment of Hematological Malignancies

Lingna Li[1], Katherine Fells[3], Angel Wei[3], Ernest Kovacs[2],
Zheng Yan[2], Xiaoqing Li[2], Dylan Deng[2], Yue He[3], Henry Ji[3] and Hong Zhang[1*]

[1]Concortis Biotherapeutics, San Diego, CA; [2]Levena Biopharma, San Diego, CA; [3]SORRENTO THERAPEUTICS, INC., San Diego, CA, *Corresponding author

Abstract

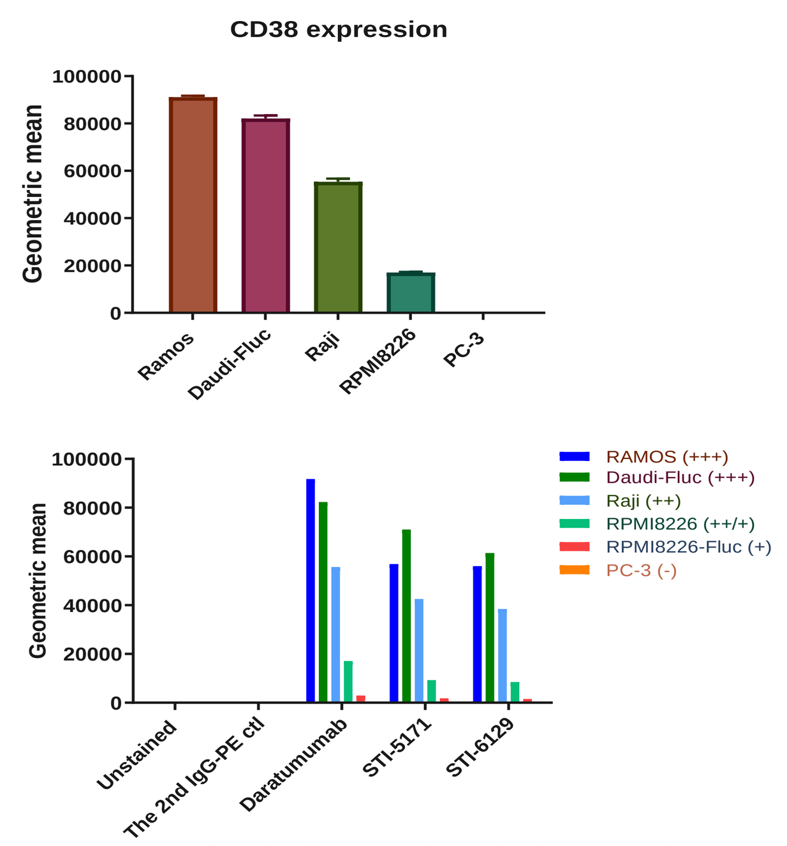
CD38 is a validated target for the treatment of CD38-overexpressing hematologic malignancies such as multiple myeloma (MM). Daratumumab is the first anti-CD38 mAb drug approved by the FDA for use as single agent and in combination with standard therapies for MM. However, resistance to Daratumumab in both primary and refractory MM patients has been reported.

Here we report the nonclinical profile of STI-6129 (also CD38-077), a CD38-targeting antibody-drug conjugate (ADC), as a new therapeutic agent for MM. STI-6129 consists of a fully human anti-CD38 antibody, STI-5171, identified from the Sorrento G-MAB® antibody library, conjugated to the microtubule inhibitor duostatin-5.2 (Duo-5.2) via a non-polyethylene glycol linker using our site-specific proprietary C-LOCK technology. STI-5171 binds specifically to CD38-positive but not CD38-negative tumor cell lines with a KD ~ 1.72E-8 M. STI-6129 is internalized into CD38-positive cells at a rate comparable to that of the unconjugated antibody STI-5171. In cytotoxicity studies, STI-6129 exhibited a CD38-dependent cytotoxic activity against a panel of CD38-expressing tumor cell lines with the range of EC50 from 5-100 nM. Importantly, STI-6129 does not display cytotoxic activity (EC50 > 1 µM) against normal human PBMCs after 120 hr exposure. Further, the anti-tumor activity of STI-6129 showed broad, potent, and CD38-dependent in vivo efficacy in multiple xenograft animal models. More importantly, STI-6129 has demonstrated cytotoxic activity in human multiple myeloma cells isolated from daratumumab-refractory patients. Pharmacokinetic evaluation of STI-6129 in Daudi-Fluc tumor-bearing mice revealed that the ADC is stable with half-life, T1/2, of 7-11 days, comparable to that of the unconjugated antibody. The serum concentration of STI-6129 remained above the therapeutically effective level up to 1 week in mice after a single injection at 10 mg/kg. In cynomolgus monkey, the PK profiles of STI-6129 and STI-5171 were nearly identical, further confirming the stability of the ADC in monkey blood circulation. In the repeat-dose toxicity studies, target organ toxicity of DUO.5.2 and STI-6129 was only observed in bone marrow and rapidly dividing cells derived from the hematopoietic system, and male reproductive organs, changes consistent with the expected activity of dolastatin 10 analog MMAE. All the changes were recoverable at the end of the recovery period

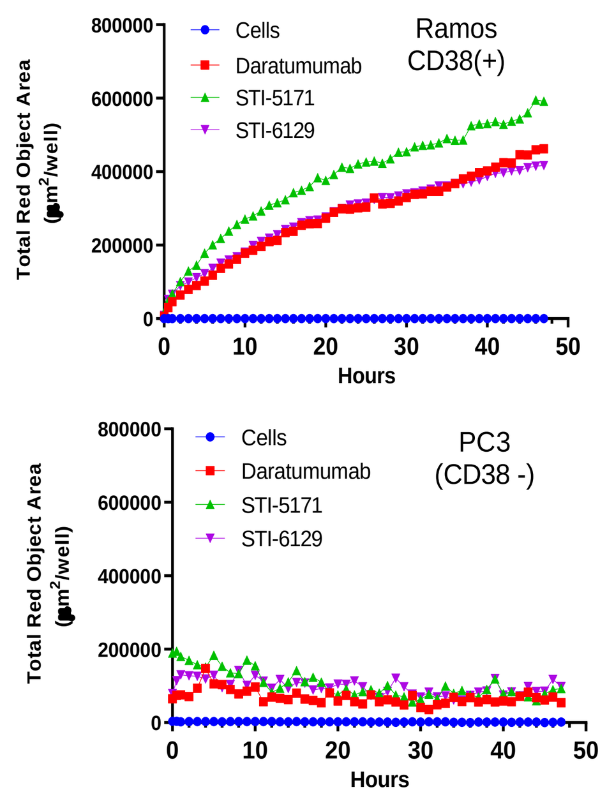
In summary, STI-6129 exhibits potent in vitro and in vivo anti-tumor activities in multiple CD38-positive hematological models with a relative safe and stable profile of toxicity. These results warrant further development of STI-6129, potentially as a better or alternative agent for treatment of multiple myeloma.

Characterization of Anti-CD38 Antibody Drug Conjugates STI-6129

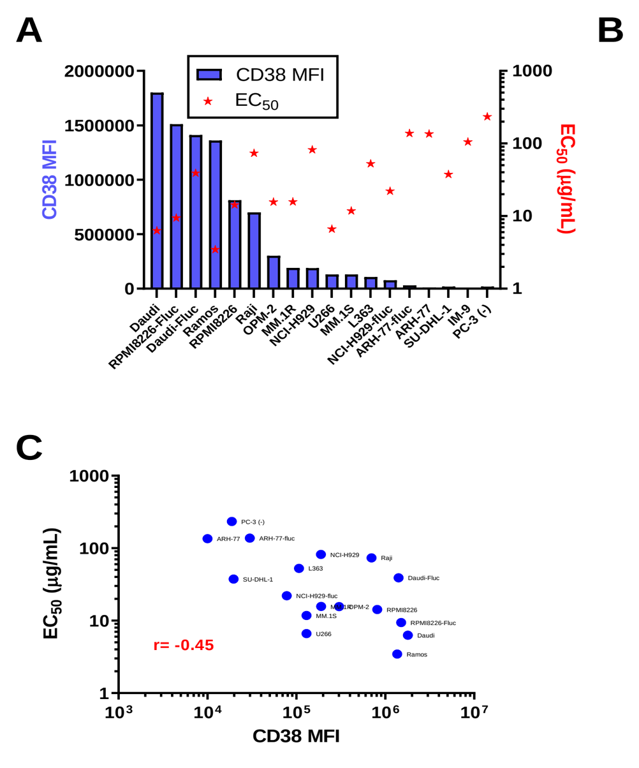
STI-6129 Retained Binding Affinity to CD38-positive Tumor Cells



STI-6129 is Internalized in CD38-positive Ramos Cells



STI-6129 Showed CD38-Dependent Specific Cytotoxicity in Tumor Cell Lines

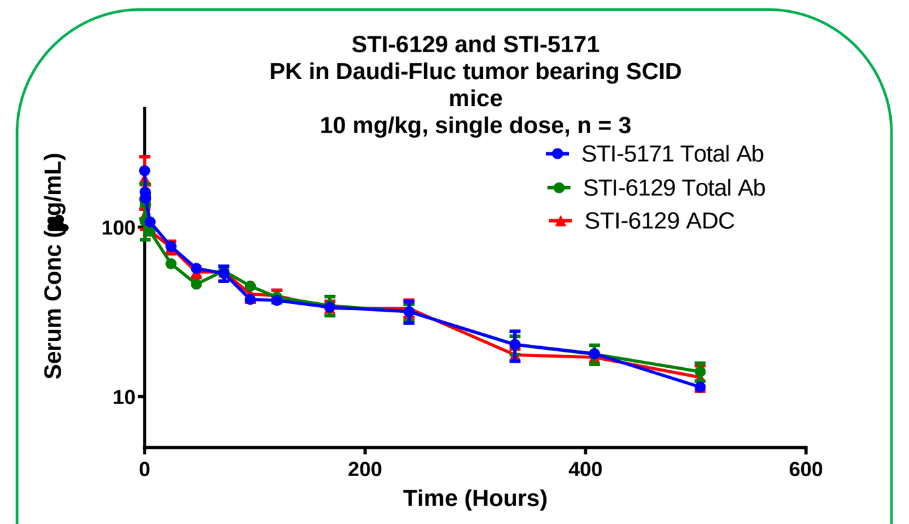


B

Cell Line	EC_{50} (µg/mL)
Daudi	6.29
RPMI8226-Fluc	9.43
Daudi-Fluc	39
Ramos	3.45
RPMI8226	14.3
Raji	73.5
OPM-2	15.6
MM.1R	15.7
NCI-H929	81.9
U266	6.63
MM.1S	11.8
L363	52.5
NCI-H929-Fluc	22.1
ARH-77-Fluc	137
ARH-77	135
SU-DHL-1	37.5
IM-9	105
PC-3 (-)	230

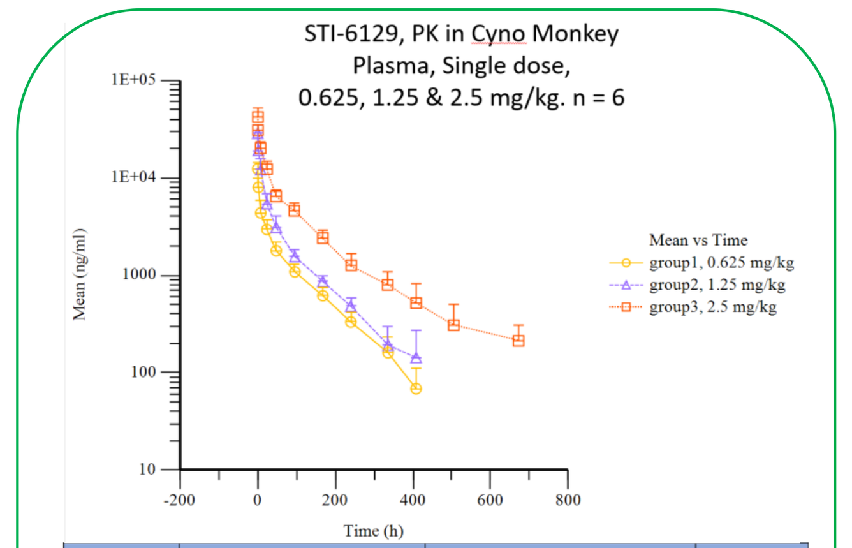
Left panel: Binding of STI-6129 and STI-5171 to CD38(+) tumor cell lines by FACS analysis. **Middle panel**: Internalization of STI-5171 and STI-6129 at 4 µg/ml to Ramos (CD38+) and PC-3 (CD38-) cancer cell lines using the IncuCyte ZOOM real-time monitoring system. **Right panel:** Correlation of CD38 expression vs. responses to STI-6129 in tumor cell lines. (A) Blue bars represent MFI of CD38 expression, while red stars denote (B) EC50 for STI-6129 determined from each cell line. (C) Scatter plot depicting a moderate, inverse Pearson (r) correlation between CD38 expression and STI-6129 EC50 among cell lines

Pharmacokinetic Properties of STI-6129 in Mice and Monkey



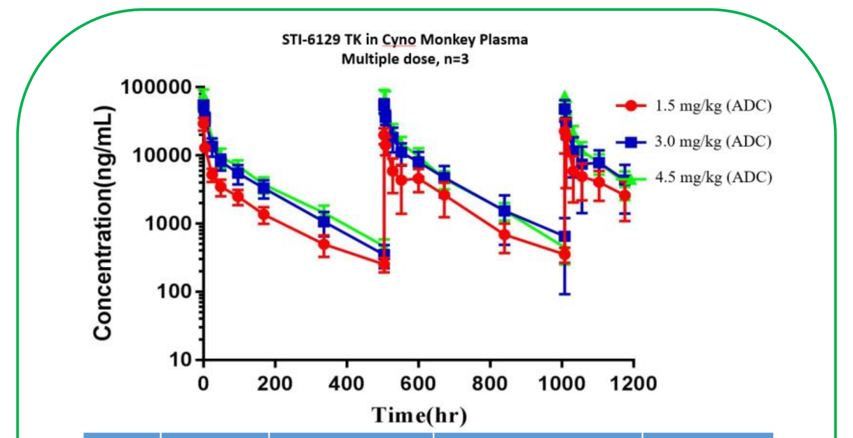
PK parameters	STI-5171 TAb	STI-6129 ADC	STI-6129 TAb
T1/2(h)	175±79	259±147	232±89
C_{max}(µg/mL)	216±14	221±94	167±44
$AUC_{INF,obs}$(h*µg/mL)	19174±2337	20881±3620	20246±4357

Additional Information: The PK profiles for intact ADC and TAb for STI-6129 were similar and comparable to that of the unconjugated antibody STI-5171, suggesting that little or no loss of toxin from the antibody in the general circulation. This also demonstrates that conjugation of the toxin to form STI-6129 does not impact the PK parameters of STI-5171 in mice.



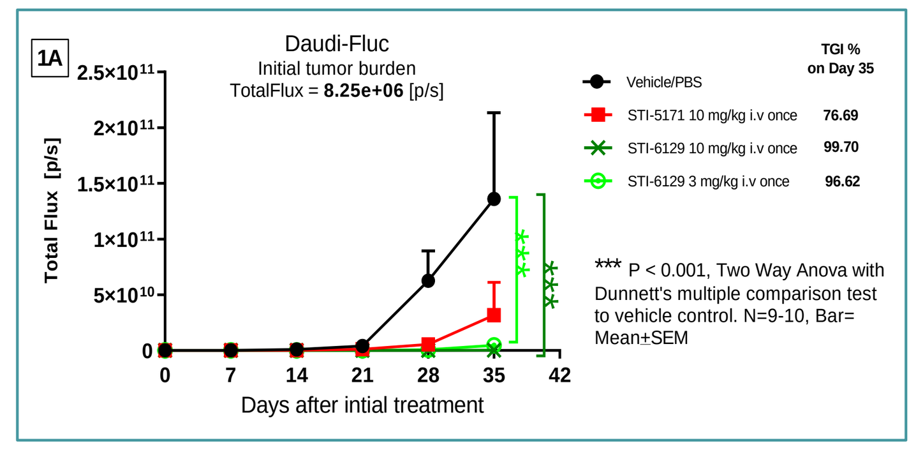
PK parameters	STI-6129 ADC			STI-6129 TAb			STI-5171 TAb
	0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	2.5 mg/kg
T1/2_z(h)	72.8±26.4	85.7±44.7	111±40.9	73.0± 24.3	82.2±30.2	97.1±35.5	65.1±21.2
Cmax(ng/mL)	12400±1820	28600±5820	42500±9240	13300±1680	31200±7210	43500±9920	47800±10100
AUC_{INF_obs}(h*ng/mL)	376000±48700	691000±11200	1590000±230000	341000±42800	676000±142000	1480000±263000	1530000±456000

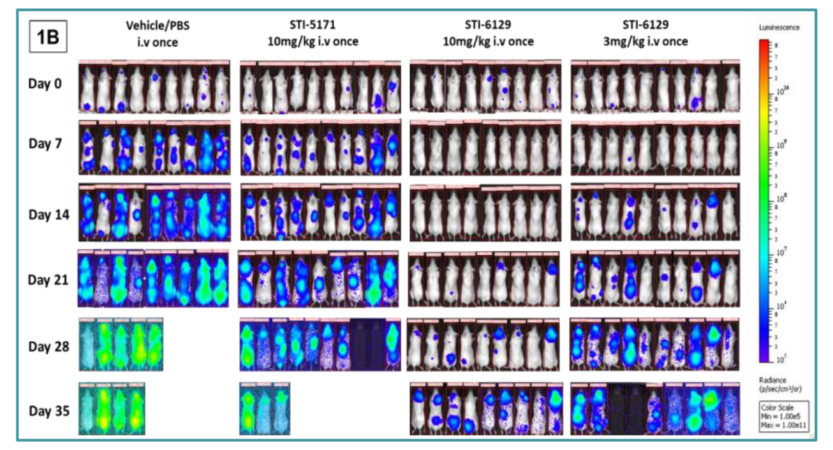
Additional Information: DUO-5.2 levels remained below the level of quantification limit of 0.200 ng/mL in all groups. It was concluded that the pharmacokinetic profiles for STI-6129 and STI-5171 were similar and that the toxin, DUO-5.2, had no impact on the pharmacokinetics of the CD38 antibody.

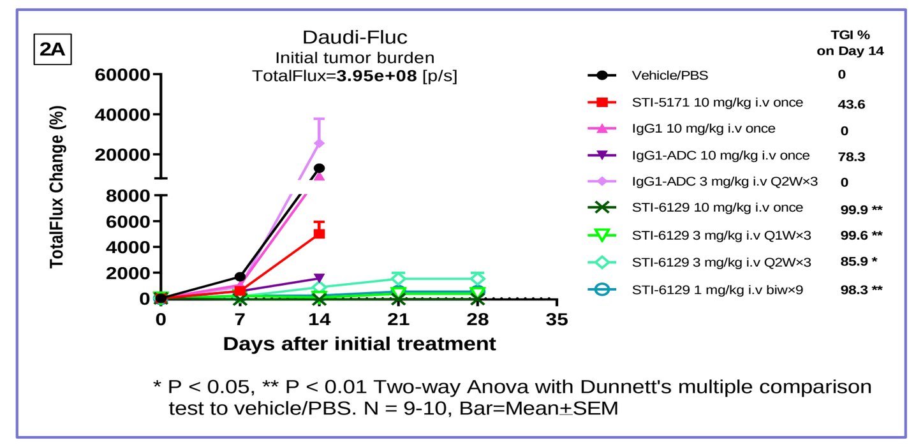


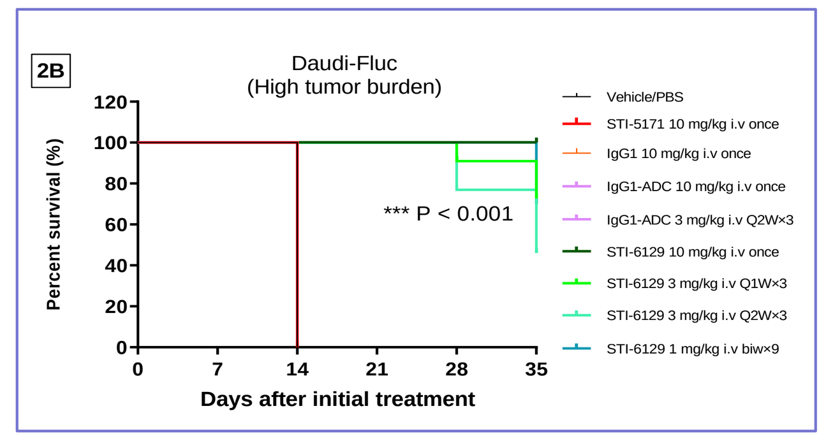
Date	Dose (mg/kg)	C_{max} (ng/mL)	$AUC_{(0-168\ h)}$ (ng*Hours/mL)	T1/2_z (Hours)
D1	1.5	29388±6263	651887±89212	99.5±12.9
	3	56049±8963	1568320±360682	64.4±39.2
	4.5	76557±16291	1973577±312134	-
D22	1.5	19808±5146	746461±436074	96.8±15.2
	3	55104±11583	1998383±615539	98.7±18.3
	4.5	75458±34223	2582444±627896	-
D43	1.5	25340±15240	835481±532454	105±14
	3	51955±11362	1613949±760526	92.6±15.7
	4.5	76220±10899	2264676±445552	59.5±20.3

STI-6129 Inhibits Burkitt's Lymphoma Daudi-Fluc Tumor Growth in NOD SCID Mice





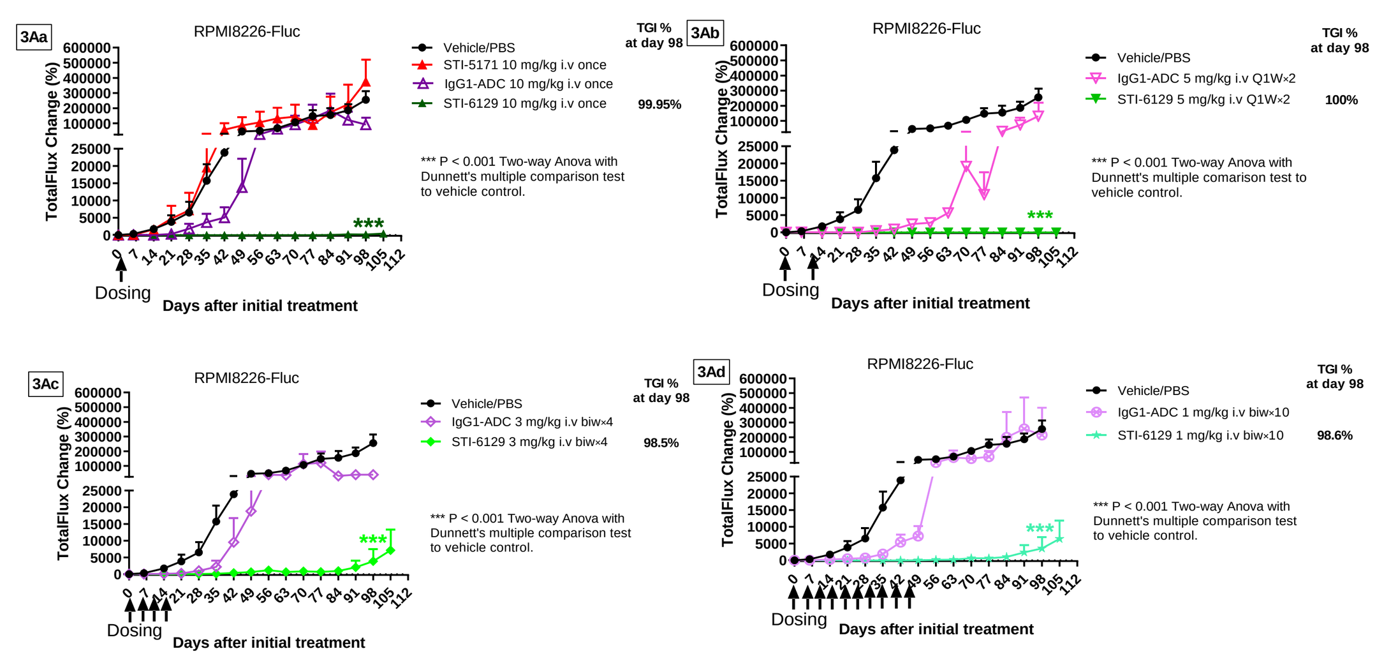


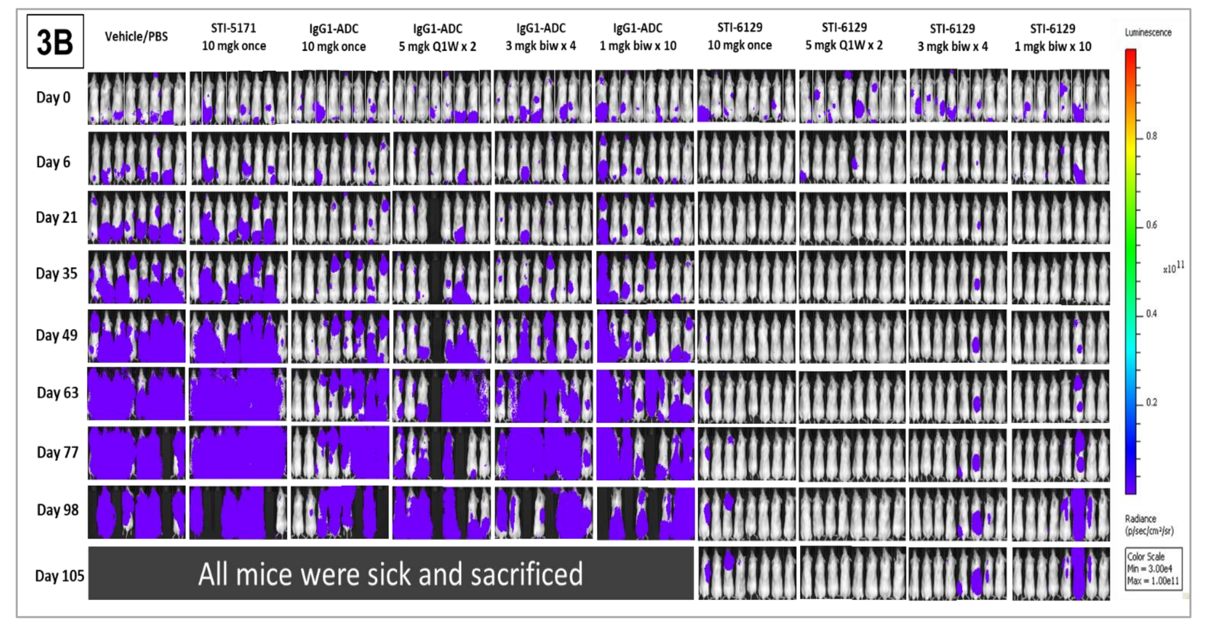


1A: Tumor growth inhibition with single dose of STI-6129 at 3 or 10 mg/kg i.v in NOD SCID Daudi-Fluc xenograft mice. **1B:** Bioluminescence images of individual NOD SCID mice bearing Daudi-Fluc tumor xenografts.

2A: Tumor growth inhibition with various dosing regimens of STI-6129 in NOD SCID Daudi-Fluc xenograft mice with high initial tumor burden. **2B:** STI-6129 significantly prolonged survival of NOD SCID Daudi-Fluc xenograft mice with high initial tumor burden.

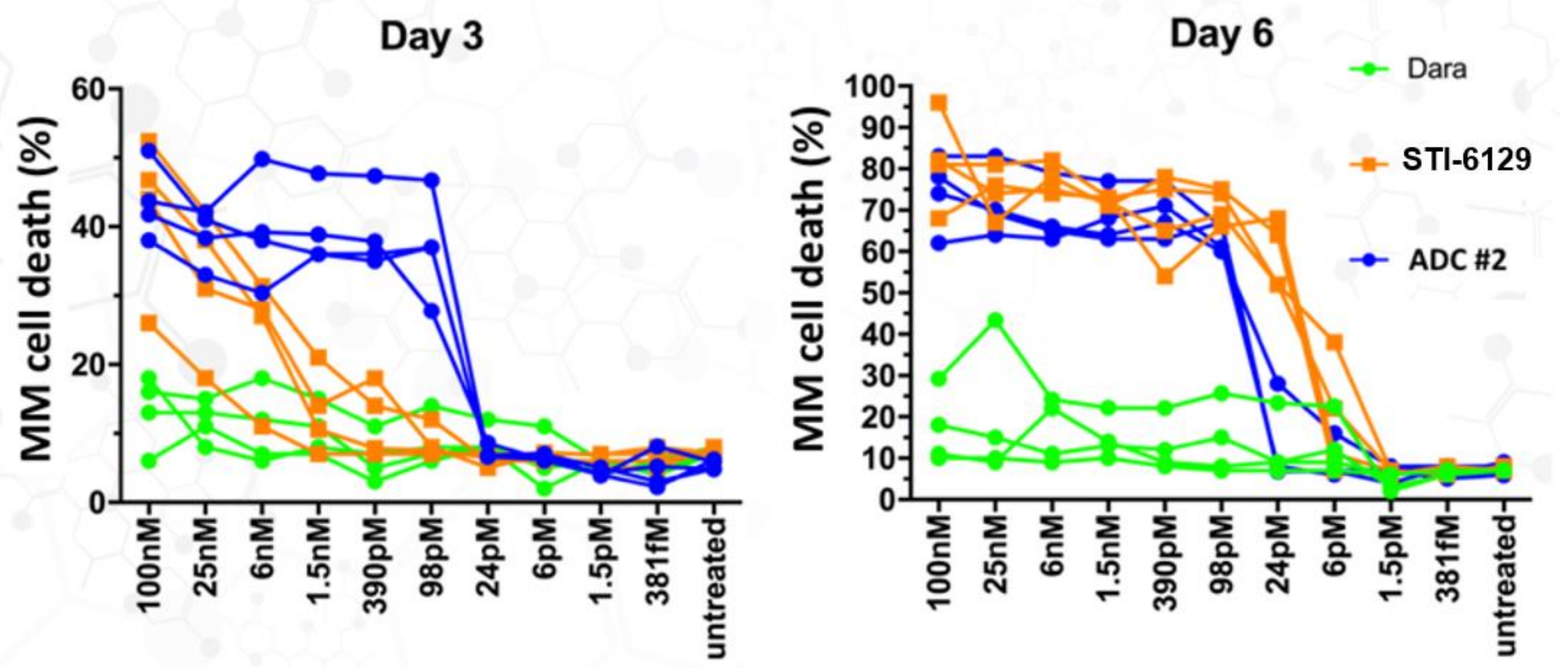
STI-6129 Inhibits Multiple Myeloma RPMI8226-Fluc Tumor Growth in NSG Mice





Tumor growth inhibition of STI-6129 in NSG RPMI8226-Fluc xenograft mice. STI-6129 treatment with (**3Aa**) 10 mg/kg i.v single dose, (**3Ab**) 5 mg/kg i.v Q1W×2, (**3Ac**) 3 mg/kg i.v Q1W×4, and (**3Ad**) 1 mg/kg i.v biw×10 significantly inhibited RPMI8226-Fluc tumor growth through Day 105 after initial treatment. **3B:** Bioluminescence images of individual mice treated with different dosing regimens of STI-6129 in RPMI8226-Fluc model in NSG mice.

Effects of STI-6129 on MM cells Isolated from Daratumumab-Relapsed MM Patients



- MM = multiple myeloma
- Dara = daratumumab + PBMC
- ADC#2 = STI-5171 with another Toxin
- 100,1000 cells were used in each experiment

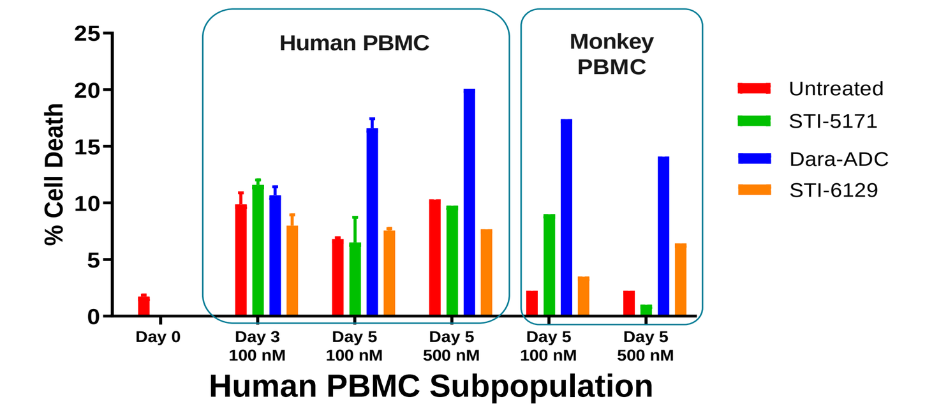
Nonclinical Safety profile of STI-6129

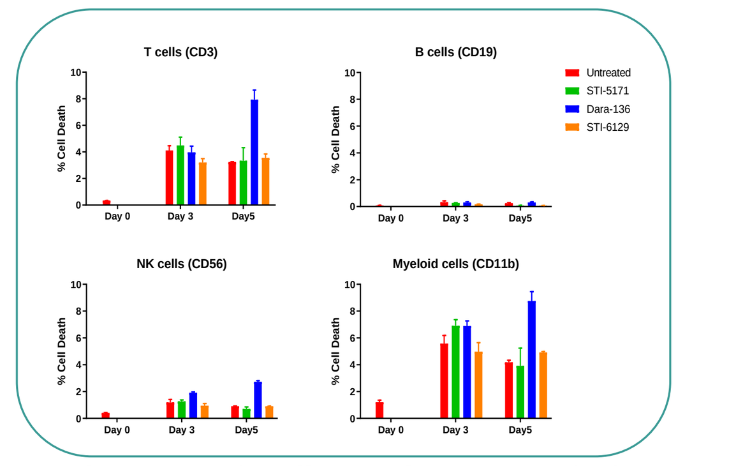
- The in vivo safety pharmacology study was conducted in cynomolgus monkeys, and it was GLP compliant.
- Repeat-dose toxicology studies were conducted in rats for payloads and in monkeys for STI-6129.
- Toxicokinetics was also evaluated in the toxicology studies in monkeys. The pivotal repeat-dose toxicity studies in NHP (non-human primate) were GLP compliant.

Key Findings

- DUO-5.2 HCL and STI-6129 --- Target organs identified in the repeat-dose toxicity studies were the bone marrow, hematopoietic tissues and male reproductive organs, changes consistent with the expected activity of a MMAE analog.
- Reversible, dose-dependent bone marrow hypocellularity with lymphoid organ toxicity characterized by atrophy in the thymus, spleen and or lymph nodes.
- No safety issues on CNS, cardiovascular and respiratory were observed
- All the changes recovered completely at the end of the recovery period, except atrophy in spleen which showed a tendency to recover
- In the pivotal toxicity study, a severely toxic dose (STD10) was not observed therefore 4.5 mg/kg of STI-6129 was considered the highest non-severely toxic dose (HNSTD).

STI-6129 Showed Minimal Cytotoxic Effect on Human & Monkey PBMCs





Upper graph: Cytotoxic effects of STI-6129 in human and monkey PBMCs. **Lower graph:** Cytotoxic activity of 15 µg/mL STI-6129 following 72 or 120 hr treatment to human PBMC subpopulation immune cells including T, B, NK and myeloid cells. STI-6129 showed minimum cytotoxic effect to human and monkey PBMCs.

Summary and Conclusions

- STI-6129 demonstrates strong binding affinity, internalization specificity, and cytostatic and cytotoxic activity against CD38-positive hematopoietic cells. Importantly, it does not prominently affect the viabilities of normal human PBMCs even at high micromolar equivalent concentrations.
- STI-6129 demonstrates target-specific, highly potent, and relatively safe preclinical efficacy to various human hematological disseminating tumor xenograft models.
- STI-6129 demonstrates a stable, and relative safe profiles of PK and toxicity with T1/2 about 7-11 days and undetectable serum level of payload DUO.5.
- STI-6129 has received FDA IND Clearance and become the FIH of anti-CD38 ADC for treatment of Hematological Malignancies.

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